Recent Work

Title
PHOTOSENSITIZED ELECTRON TRANSPORT ACROSS LIPID VESICLE WALLS

Permalink
https://escholarship.org/uc/item/8hj5f9xj

Author
Ford, W.E.

Publication Date
1982-11-01
PHOTOSENSITIZED ELECTRON TRANSPORT ACROSS LIPID VESICLE WALLS

William Ellsworth Ford
(Ph.D. Thesis)

November 1982

TWO-WEEK LOAN COPY
This is a Library Circulating Copy which may be borrowed for two weeks. For a personal retention copy, call Tech. Info. Division, Ext. 6782.

Prepared for the U.S. Department of Energy under Contract DE-AC03-76SF00098
DISCLAIMER

This document was prepared as an account of work sponsored by the United States Government. While this document is believed to contain correct information, neither the United States Government nor any agency thereof, nor the Regents of the University of California, nor any of their employees, makes any warranty, express or implied, or assumes any legal responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by its trade name, trademark, manufacturer, or otherwise, does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency thereof, or the Regents of the University of California. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof or the Regents of the University of California.
PHOTOSENSITIZED ELECTRON TRANSPORT ACROSS LIPID VESICLE WALLS

William Ellsworth Ford

Ph.D. Thesis

CHEMISTRY

November 1982

Chemical Biodynamics Division
Lawrence Berkeley Laboratory
University of California
Berkeley, CA 94720

This research was supported by the Office of Energy Research, Office of Basic Energy Sciences, Chemical Sciences Division of the U.S. Department of Energy under Contract DE-AC03-76SF00098.

This manuscript printed from originals provided by the author.
PHOTOSENSITIZED ELECTRON TRANSPORT ACROSS LIPID VESICLE WALLS

by William Ellsworth Ford

ABSTRACT

This thesis deals with the prospect of using lipid bilayer vesicles in artificial photosynthetic devices which decompose water photochemically as a means of converting and storing solar energy. The vesicles serve to mediate charge separation during photosensitized electron transfer reactions. The vesicle systems of particular interest have amphiphilic dyes dissolved in the walls of the vesicles and aqueous solutions of electron-donating and accepting molecules separated by the walls. Photosensitized electron transfer across the bilayer from the donor to the acceptor results in spatially separated redox products, thereby preventing energy-wasting back-reactions between these intermediates in the decomposition of water.

Chapter 1 outlines the overall strategy and some physicochemical properties of vesicles. Chapters 2 and 3 characterize some electronic spectral and photochemical properties of synthetic amphiphilic dyes, with emphasis on micellar aqueous media. The first class of dyes described is the porphyrins, particularly the compound obtained by quaternizing metal-free meso-tetra(4-pyridyl)porphyrin with one molecule of hexadecylbromide (abbreviated H$_2$TPyP-1). H$_2$TPyP-1 is easily incorporated into egg yolk phosphatidyl-
choline (egg PC) vesicles. Self-aggregation of the porphyrin occurs in vesicle suspensions and other colloidal aqueous media. Aggregation can be controlled to some extent by varying the egg PC:H₂TPyP-1 molar ratio and the temperature of the sample, and possibly also by adding amphiphilic anions. The aggregates of H₂TPyP-1 have a Soret band maximum near 445 nm, which is shifted by about 25 nm to longer wavelength relative to the monomer.

The other dyes studied are di-hexadecyl analogues of the \([\text{tris}-(2,2'-\text{bipyridine})\text{ruthenium}]^{2+}\) complex ([Ru-(bipy)₃]²⁺). The two new amphiphilic complexes which are described in Chapter 3 have two 2,2'-bipyridine ligands and a third 2,2'-diimine ligand with two hexadecyl substituents. Spectral properties of the two analogues are compared to those of the parent complex. The complex with a biimidazole derivative as the third diimine ligand has a 30-fold weaker luminescence and poor photochemical stability compared to [Ru(bipy)₃]²⁺. This difference is probably due to weaker coordinative bonding of the Ru²⁺ ion to the biimidazole ligand compared to the bipyridine ligand. The other analogue with a bipyridine derivative as the third ligand ([bipy)₂Ru(bipy-CONHC₁₆H₃₃)]²⁺) has spectral properties resembling those of [Ru(bipy)₃]²⁺ when it is dissolved in solvents of low polarity. The absorption and emission of [(bipy)₂Ru(bipy-CONHC₁₆H₃₃)]²⁺ are sensitive to increases in solvent polarity. The dependence on solvent leads to the
conclusion that the metal-to-ligand charge-transfer absorption band of the complex is a composite of individual bands due to electron-transfer from Ru$^{2+}$ to each ligand in the complex. These transitions vary independently with solvent changes, and increases in solvent polarity stabilize electron-transfer to the amide-substituted bipyridine ligand more than the unsubstituted ligands.

Chapter 4 describes the evolution of a model system for studying photosensitized electron transport across vesicle walls. The bilayers are composed solely of egg PC and $[(bipy)_2Ru(bipy-CONHC_{16}H_{33})]^{2+}$, which is the photosensitizer. Based on steady-state quantum yield and luminescence quenching measurements, a kinetic model, and measurements reported in the literature for analogous reactions, it is concluded that electron transport across the vesicle wall has a pseudo-first-order rate constant of 10-1000 s$^{-1}$ and an activation energy of 16±5 kcal/mole. These and other observations argue against a mechanism for electron transport requiring the diffusion of the ruthenium complex across the membrane ("flip-flop"), so the direct diffusion of electrons is implicated. Overall, the results are favorable to the prospect of using vesicles in artificial photosynthetic devices. Possible directions for the development of such devices are considered in the final Chapter.
This thesis is based on experimental work that was completed by October of 1980. By necessity, the references cited will be restricted to those published before 1981 except for Laane et al. (1981) and other references cited in Mettee et al. (manuscript in preparation).

I am most grateful to Professor Melvin Calvin for providing me the opportunity to study artificial photosynthesis and a stimulating environment for doing so.
### ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>AQS</td>
<td>1,4-anthraquinone-2-sulfonate</td>
</tr>
<tr>
<td>biim-C_{16}H_{33}</td>
<td>1,1'-di(1-hexadecyl)-2,2'-biimidazole</td>
</tr>
<tr>
<td>biim-H</td>
<td>2,2'-biimidazole</td>
</tr>
<tr>
<td>bipy</td>
<td>2,2'-bipyridine</td>
</tr>
<tr>
<td>bipy-CONHC_{16}H_{33}</td>
<td>N,N'-di(hexadecyl)-4,4'-dicarboxamide-2,2'-bipyridine</td>
</tr>
<tr>
<td>bipy-COOH</td>
<td>4,4'-dicarboxy-2,2'-bipyridine</td>
</tr>
<tr>
<td>bipy-COOR</td>
<td>diester derivative of bipy-COOH</td>
</tr>
<tr>
<td>CPC</td>
<td>cetyl pyridinium chloride</td>
</tr>
<tr>
<td>C_{7}V^{2+}</td>
<td>heptylviologen; 1,1'-diheptyl-4,4'-bipyridinium ion</td>
</tr>
<tr>
<td>C_{16}V^{2+}</td>
<td>hexadecylviologen; 1,1'-dihexadecyl-4,4'-bipyridinium ion</td>
</tr>
<tr>
<td>DHAPS</td>
<td>3-(dimethylhexadecylammonio)propane-1-sulfonate</td>
</tr>
<tr>
<td>DPPC</td>
<td>dipalmitoyl phosphatidylcholine</td>
</tr>
<tr>
<td>EDTA</td>
<td>ethylenediamine-N,N,N',N'-tetraacetate</td>
</tr>
<tr>
<td>egg PC</td>
<td>phosphatidylcholine from hens' egg yolks</td>
</tr>
<tr>
<td>H_{2}TCP P</td>
<td>metal-free meso-tetra(4-carboxyphenyl)porphyrin</td>
</tr>
<tr>
<td>H_{2}TP P</td>
<td>metal-free meso-tetraphenylporphyrin</td>
</tr>
<tr>
<td>H_{2}TPyP</td>
<td>metal-free meso-tetra(4-pyridyl)porphyrin</td>
</tr>
<tr>
<td>H_{2}TPyP-1</td>
<td>5-(1'-hexadecylpyridinium-4-yl)-10,15,20-tris[4'-pyridyl]-21H,23H-porphine</td>
</tr>
<tr>
<td>H_{2}TSPP</td>
<td>metal-free meso-tetra(4-sulfonatophenyl)porphyrin</td>
</tr>
<tr>
<td>kk</td>
<td>kilokayser (1000 cm^{-1})</td>
</tr>
<tr>
<td>MnTPyP-4-CH_{3}</td>
<td>manganese complex of meso-tetra(4-methyl-}</td>
</tr>
</tbody>
</table>
ABBREVIATIONS

pyridinium)porphyrin

MV²⁺ methylviologen; 1,1'-dimethyl-4,4'-bipyridinium ion

NHE normal hydrogen reference electrode

NR nitroso-R-salt; 3-hydroxy-4-nitroso-2,7-naphthalene-
disulfonic acid disodium salt

OAc⁻ acetate ion

PC phosphatidylcholine

phen 1,10-phenanthroline

(Ru²⁺) [(bipy)₂Ru(bipy-COHNH₁₆H₃₃)]²⁺

SDS sodium dodecyl-1-sulfate

VK₁ vitamin K₁ quinone
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Chapter 1. General Introduction</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1. Water Decomposition</td>
<td>2</td>
</tr>
<tr>
<td>1.2. Photosensitized Electron Transfer</td>
<td>2</td>
</tr>
<tr>
<td>1.3. Membranes</td>
<td>6</td>
</tr>
<tr>
<td>1.4. Lipid Bilayer Membranes</td>
<td>9</td>
</tr>
<tr>
<td>1.5. Vesicles - Physical and Chemical Properties</td>
<td>11</td>
</tr>
<tr>
<td>1.6. Vesicles in Solar Water-Decomposing Devices</td>
<td>18</td>
</tr>
<tr>
<td>1.7. Literature Compilation - Photochemical Redox Reactions Mediated by Vesicles</td>
<td>19</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Chapter 2. Electronic Spectra and Photochemistry of Some Amphiphilic Porphyrins</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1. Introduction</td>
<td>21</td>
</tr>
<tr>
<td>2.2. Experimental Section</td>
<td>24</td>
</tr>
<tr>
<td>2.2.1. Measurements</td>
<td>24</td>
</tr>
<tr>
<td>2.2.2. Materials</td>
<td>25</td>
</tr>
<tr>
<td>2.2.3. Methods</td>
<td>26</td>
</tr>
<tr>
<td>2.2.3.1. Extinction Coefficients and Relative Fluorescence Yields of Metal-Free Porphyrins</td>
<td>26</td>
</tr>
<tr>
<td>2.2.3.2. Preparation of Aqueous Samples</td>
<td>27</td>
</tr>
<tr>
<td>2.2.3.3. Quenching of $\text{H}_2\text{TPyP-1}$ Fluorescence by Anthraquinone-2-Sulfonate</td>
<td>28</td>
</tr>
<tr>
<td>2.2.3.4. Capture of MnTPyP-4-CH$_3$ by Egg PC Vesicles</td>
<td>29</td>
</tr>
<tr>
<td>2.2.3.5. EPR of Illuminated Bilayer Suspensions [with Anne McGuire]</td>
<td>30</td>
</tr>
<tr>
<td>2.2.3.6. Steady-State Photolyses</td>
<td>31</td>
</tr>
<tr>
<td>2.3. Results</td>
<td>35</td>
</tr>
<tr>
<td>2.3.1. Electronic Absorption and Emission</td>
<td></td>
</tr>
</tbody>
</table>
TABLE OF CONTENTS

Spectra ................................................. 35

2.3.1.1. Metal-Free Porphyrins in Ethanol-
            Dimethylformamide ......................... 35

2.3.1.2. Solvent Dependence of the Spectra of
            $H_2TPyP-1$ ................................ 38

2.3.1.3. Cu(II)TPyP-1 in Various Solvents .... 49

2.3.1.4. Mn(III)TPyP-1 in Various Solvents .... 55

2.3.2. Addition of Anthraquinone-2-Sulfonate to
            $H_2TPyP-1$ ................................ 58

2.3.2.1. In DPPC Vesicle Suspensions .......... 58

2.3.2.2. In Egg PC Vesicle Suspensions .... 62

2.3.2.3. In Water ................................ 65

2.3.3. Capture of Mn(III)TPyP-4-CH$_3$ by Egg PC
            Vesicles .................................... 65

2.3.4. Photochemistry ............................... 67

2.3.4.1. EPR of $H_2TPyP-1$ in Egg PC Bilayers
            [with Anne McGuire] ......................... 67

2.3.4.2. Zn(II)TPyP-1 in Dodecyl-1-Sulfate
            Micelles .................................. 70

2.3.4.3. Mn(III)TPyP-1 in Vesicle Suspensions
            Containing Vitamin K$_1$ ...................... 70

2.3.4.4. Manganese Porphyrins in Methanol-Water
            Solutions [with Itamar Willner] .......... 76

2.4. Discussion ........................................ 80

2.4.1. Electronic Spectra of Porphyrins ........ 80

2.4.1.1. General Characteristics ................. 81

2.4.1.2. Spectral Perturbations Caused by
            Environmental Factors ..................... 85

2.4.2. Absorption and Fluorescence Spectra of
            $H_2TPyP-1$ ................................ 90

2.4.2.1. Compared to Other Derivatives of
            $H_2TPyP-1$ ................................ 91

2.4.2.2. Characterization of the 445-nm-
            Absorbing Species .......................... 91
TABLE OF CONTENTS

2.4.2.3. pH Dependence ................................................. 98

2.4.2.4. Spectral Changes in the Ultraviolet Region Involving the Pyridine
           Substituents .................................................. 100

2.4.2.5. Fluorescence Quenching by AQS ............................. 104

2.4.3. Cu(II)TPyP-1 Absorption Spectra ............................. 111

2.4.4. Mn(III)TPyP-1 Absorption Spectra ........................... 112

2.4.5. Capture of Mn(III)TPyP-4-CH₃ by Egg PC Vesicles ........... 113

2.4.6. Light-Induced EPR Signals from Bilayer Suspensions
           Containing H₂TPyP-1 ........................................... 115

2.4.7. Photochemistry of Mn(III)TPyP-1 in Vesicle Suspensions ... 117

2.4.8. Photochemistry of Manganese Porphyrins
           in Methanol-Water Solutions .................................. 121

2.5. Conclusions ....................................................... 124

Chapter 3. Preparation and Characterization of Two
           Amphiphilic Analogues of the Tris-(2,2'-Bipyridine)-Ruthenium(II) Complex

3.1. Introduction ..................................................... 126

3.2. Experimental Section .......................................... 132

3.2.1. Measurements ................................................. 132

3.2.2. Materials .................................................... 133

3.2.3. Methods ...................................................... 134

3.2.3.1. Preparation of Samples ................................... 134

3.2.3.2. Electron Microscopy and Dialysis of Aqueous Dispersions
           of 1 [with Agatha Tung] .................................... 136

3.2.4. Syntheses ..................................................... 136

3.2.4.1. cis-[Dichloro-bis-(2,2'-Bipyridine)-
              Ruthenium(II)] Hydrate
              ([(Bipy)₂RuCl₂]•2·5H₂O) .................................. 136

3.2.4.2. 1,1'-Di(1-Hexadecyl)-2,2'-Bimidazole
3.2.4.3. [(1,1'-Di(1-Hexadecyl)-2,2'-Biimidazole)-bis-(2,2'-Bipyridine)Ru(II)]-Chloride Hydrate ([(Bipy)₂Ru(Biim-C₁₆H₃₃)]Cl₂·3H₂O) (1) ........................................ 139

3.2.4.4. "[(Bipy)₂Ru(H₂O)Cl]PF₆" ........................................ 141

3.2.4.5. 4,4'-Dicarbonyl Chloride-2,2'-Bipyridine ............................ 141

3.2.4.6. N,N'-Di(hexadecyl)-4,4'-Dicarboxamide-2,2'-Bipyridine (Bipy-CONHC₁₆H₃₃) .......... 142

3.2.4.7. [(N,N'-Di(hexadecyl)-4,4'-Dicarboxamide-2,2'-Bipyridine)-bis-(2,2'-Bipyridine)Ru(II)]Perchlorate Hydrate ([(Bipy)₂Ru(Bipy-CONHC₁₆H₃₃)]-ClO₄)₂·H₂O) (2) ....................... 143

3.3. Results ........................................................................ 144

3.3.1. Syntheses .................................................................. 144

3.3.1.1. Preparation of [(Bipy)₂Ru(Biim-C₁₆H₃₃)]Cl₂·3H₂O (1) ...................... 144

3.3.1.2. Preparation of [(Bipy)₂Ru(Bipy-CONHC₁₆H₃₃)](ClO₄)₂·H₂O (2) ............. 145

3.3.2. Solubilities of 1 and 2 ........................................... 145

3.3.3. Optical Absorption and Emission Spectra .................... 147

3.3.3.1. [Ru(Bipy)₃]Cl₂·6H₂O ........................................... 147

3.3.3.2. [(Bipy)₂Ru(Biim-C₁₆H₃₃)]Cl₂·3H₂O (1) .................................. 147

3.3.3.3. [(Bipy)₂Ru(Bipy-CONHC₁₆H₃₃)]-ClO₄)₂·H₂O (2) .......................... 152

3.4. Discussion ..................................................................... 157

3.4.1. Syntheses .................................................................. 157

3.4.2. Solubilities of 1 and 2 ........................................... 159

3.4.3. Optical Absorption and Emission Spectra .................... 161

3.4.3.1. Electronic Structure and Spectra of [Ru(Bipy)₃]²⁺ and the Corresponding
# TABLE OF CONTENTS

3.4.3.2. Mixed-Ligand Big-Bipyridyl-Ru(II) Complexes ........................................ 170
3.4.3.3. [(Bipy)\textsubscript{2}Ru(Biim-C\textsubscript{16}H\textsubscript{33})Cl\textsubscript{2}\cdot3H\textsubscript{2}O (1) ........................................... 173
3.4.3.4. [(Bipy)\textsubscript{2}Ru(Bipy-CONHC\textsubscript{16}H\textsubscript{33})-(ClO\textsubscript{4})\textsubscript{2}\cdotH\textsubscript{2}O (2) ...................... 176
3.5. Conclusions ............................................................................................................. 185

Chapter 4. A Model System for Photosensitized Electron Transfer Across Vesicle Walls

4.1. Introduction ............................................................................................................. 187
4.2. Experimental Section ............................................................................................ 192
4.2.1. Measurements ...................................................................................................... 192
4.2.2. Materials ............................................................................................................. 193
4.2.3. Methods .............................................................................................................. 194
4.2.3.1. Preparation of Aqueous Solutions ................................................................. 194
4.2.3.1.a. 0.30 M (NH\textsubscript{4})\textsubscript{3}EDTA, pH 8.6 ............................................. 194
4.2.3.1.b. 0.87 M NH\textsubscript{4}OAc-NH\textsubscript{4}OH Buffer Containing 0.018 M Zn(OAc)\textsubscript{2}, pH 8.6 ............................................. 195
4.2.3.2. Preparation of Vesicle Samples .............................................................. 195
4.2.3.2.a. Ethanol Injection ............................................................................................ 195
4.2.3.2.b. Gel-Filtration ............................................................................................... 197
4.2.3.2.c. Deaeration .................................................................................................. 198
4.2.3.3. Photochemistry ............................................................................................... 199
4.2.3.3.a. Steady-State Illuminations .......................................................................... 199
4.2.3.3.b. Detection of Viologen Radical ...................................................................... 200
4.2.3.3.c. Actinometry ................................................................................................. 202
4.2.3.3.d. Quantum Yield Calculations ....................................................................... 205
4.2.3.3.e. Light Intensity Dependence ......................................................................... 206
4.2.3.3.f. Cobalt(II)-EDTA Complex as Electron Donor ........................................... 207
4.2.3.3.g. Quenching of (Ru\textsuperscript{2+}) Luminescence ........................................... 208
4.2.3.4. Permeability of Egg PC Vesicles .......... 210
  4.2.3.4.a. Permeability to MV2+ ............ 210
  4.2.3.4.b. Permeability to EDTA3- .......... 211
4.3. Results .................................. 213
  4.3.1. Initial Photochemical Experiments at Neutral pH .......... 213
    4.3.1.1. Homogeneous Solutions .......... 213
    4.3.1.2. Vesicle Suspensions ........... 214
  4.3.2. Photochemical Experiments at Basic pH ........... 217
    4.3.2.1. Effect of Increasing the pH .... 217
      4.3.2.1.a. Homogeneous Solutions .... 218
      4.3.2.1.b. Vesicle Suspensions ....... 218
    4.3.2.2. Vesicle Suspensions with Other Compositions .......... 220
      4.3.2.2.a. Cobalt(II)-EDTA Complex Instead of EDTA .......... 226
      4.3.2.2.b. No Effect of Retinyl Acetate .......... 227
  4.3.3. Related Experiments .................. 229
    4.3.3.1. Inhibition by Zn2+ ............... 229
    4.3.3.2. Light Intensity Dependence of the Quantum Yield .......... 229
    4.3.3.3. Aging Effects on the Quantum Yield .... 231
    4.3.3.4. Quenching of (Ru2+) Luminescence .... 231
    4.3.3.5. Effects of Ionophores and Transmembrane Potentials [with Colja Laane] .... 237
    4.3.3.6. Temperature Dependence [with Tetsuya Sakai and Howard D. Mettee] .... 237
  4.3.4. Permeability of Egg PC Vesicles .......... 240
  4.3.4.1. Permeability to MV2+ .......... 240
  4.3.4.2. Permeability to EDTA3- .......... 242
4.4. Discussion ................................ 246
  4.4.1. Rationale for the Design of the Model Vesicle System .......... 246
  4.4.2. Methodology .......................... 250
**TABLE OF CONTENTS**

4.4.3. Homogeneous Solutions .................. 255
  4.4.3.1. Net Reaction and Thermodynamics ... 255
  4.4.3.2. Kinetic Model ....................... 256
4.4.4. Vesicle Systems ......................... 260
  4.4.4.1. Kinetic Model [with John W. Otvos,
             Howard D. Mettee, and Tetsuya
             Sakai] .................................. 261
  4.4.4.2. Electron Transport Across Vesicle
           Walls .................................. 268
    4.4.4.2.a. Rate Constant .................... 268
    4.4.4.2.b. Cation Transport and Transmembrane
                 Potentials [with Colja Laane] .... 273
    4.4.4.2.c. Temperature Dependence [with Howard
                 D. Mettee, Tetsuya Sakai, and
                 John W. Otvos] ....................... 274
    4.4.4.2.d. Mechanisms ....................... 277
  4.4.4.3. Variations in Composition .......... 281
  4.4.4.4. Permeabilities of MV²⁺ and EDTA³⁻ .. 285
4.5. Conclusions ................................ 289

Chapter 5. Prospectus
  5.1. Components .............................. 291
    5.1.1. Vesicles ............................ 291
      5.1.1.1. Improving Stability ............. 291
      5.1.1.2. Improving Quantum Yields ...... 294
    5.1.2. Dyes ................................ 295
    5.1.3. Catalysts ........................... 296
  5.2. Arrangements -- Two Photosystems .... 299
  5.3. Conclusions ............................. 303

References .................................... 306

Appendix: Photosensitized Electron Transport Across Lipid
          Vesicle Walls: Enhancement of Quantum Yield
by Ionophores and Transmembrane Potentials
[Laane et al., 1981] ................. 333
CHAPTER 1. GENERAL INTRODUCTION

The eventual goal of this work is to design and develop artificial photosynthetic devices which achieve the decomposition of water via photosensitized electron transfer reactions using membranes to separate, and thereby inhibit back-reactions between, high-energy intermediates (Calvin, 1978; Calvin, 1979). The primary goal of this thesis was to determine whether vesicular lipid bilayer membranes are, either intrinsically or extraneously, permeable enough to electrons that transmembrane charge separation could compete effectively with energy-wasting back-reactions at the membrane-water interfaces. The conclusions of this thesis are generally favorable for the prospect of using vesicles or other kinds of lipid bilayers in artificial photosynthetic devices.

In this Chapter the strategies and principles for accomplishing the photochemical decomposition of water using pigmented vesicles are outlined, some physicochemical properties of vesicles are reviewed, and a compilation of literature dealing with photosensitized electron transfer reactions mediated by pigmented vesicles is presented. Chapters 2 and 3 describe the synthesis and characterization of two kinds of surfactant dyes intended for use as photosensitizers in vesicle systems. Chapter 4 describes the development and characterization of a model system incorporating one of those dyes for studying photosensitized elec-
tron transport across vesicle membranes. Future prospects for this field are considered in the final Chapter.

1.1. WATER DECOMPOSITION

Basically, water decomposition by sunlight can be viewed as splitting the \( \text{H}_2\text{O} \) molecule into the reducing equivalent of two hydrogen atoms, \([\text{H}]\), and the oxidizing equivalent of an oxygen atom, \([\text{O}]\):

\[
(1-1) \quad \text{H}_2\text{O} + \text{light energy} \rightarrow 2[\text{H}] + [\text{O}]
\]

The \([\text{H}]\) and \([\text{O}]\) can react with organic or inorganic substrates; the amount of light energy stored depends on the nature of the substrates. If there are no substrates, two \([\text{H}]\) combine to form \( \text{H}_2 \) and two \([\text{O}]\) combine to form \( \text{O}_2 \), so the products of water decomposition are \( \text{H}_2 \) and \( \text{O}_2 \):

\[
(1-2) \quad \text{H}_2\text{O} \rightarrow \text{H}_2 + \frac{1}{2} \text{O}_2 \quad (\Delta G^0 = 57 \text{ kcal/mole})
\]

More free energy can be stored if the \([\text{O}]\)-acceptor is water itself, producing hydrogen peroxide:

\[
(1-3) \quad 2\text{H}_2\text{O} \rightarrow \text{H}_2 + \text{H}_2\text{O}_2 \quad (\Delta G^0 = 82 \text{ kcal/mole})
\]

Alternatively, \([\text{H}]\) or \([\text{O}]\) can be used for synthetic purposes, e.g., epoxidation:

\[
(1-4) \quad \text{H}_2\text{O} + \text{R-CH=CH-R'} \rightarrow \text{H}_2 + \text{R-CH-CH-R'}
\]

1.2. PHOTOREACTION TRANSFER

Water decomposition can, in principle, be accomplished by a series of light-driven, coupled oxidation-reduction reactions that ultimately involve the oxidation and reduction of water. In essence, we are attempting to mimic the
process of photosynthesis (Sauer, 1979). During photosynthesis, green plants use the energy of sunlight to decompose water into $O_2$ and $[H]$, the substrate for $[H]$ being carbon dioxide and the product being carbohydrate ($(CH_2O)_n$):

$$H_2O + CO_2 \rightarrow O_2 + \frac{1}{n}(CH_2O)_n$$

The overall water-splitting reaction can be divided into water-oxidizing and water-reducing parts. The two parts are essentially electrochemical half-cells which are connected by a net flow of electrons and protons from the $[O]$-part to the $[H]$-part and a net flow of $H_2O$ in the opposite direction. Three kinds of components are required in the process: a dye ($S$), a catalyst for water oxidation ($C[O]$), and a catalyst for water reduction ($C[H]$). Auxiliary redox reagents, electron donors and acceptors, can be included to mediate the transfer of oxidizing and reducing equivalents between the dye and the catalysts. It is possible for the dye to function as both the light-absorber and the catalyst for decomposing water (e.g., manganese porphyrins, see Chapter 2).

The function of the dye is to absorb sunlight and, via its photoexcited state ($^*S$), catalyze electron transfer reactions against the free energy gradient. $^*S$ is both a stronger reducing agent and oxidizing agent than $S$. The process of dye-catalyzed redox reactions is referred to as photosensitized electron transfer. The redox photosensitization process is the primary light-to-chemical energy-
converting event in natural photosynthesis and in many schemes for artificial water-decomposing devices. For recent reviews of photosensitized electron transfer reactions, the reader is referred to Seely (1978a), Whitten (1980), and Sutin and Creutz (1980).

The primary function of the catalysts \( C\) and \( C\) is to accumulate oxidizing or reducing equivalents. The catalysts are required because, for example, the oxidation of water to \( O\) requires the transfer of two electrons while the redox reactions of \( S\) are single-electron events. The secondary function of the catalysts is to pass \( O\) or \( H\) on to suitable substrates.

Two routes to the photosensitized decomposition of water to \( H_2\) and \( O_2\) are diagrammed in Fig. 1-1. In the first case, \( S\) reduces \( C\) to \( C\), and \( S\) is regenerated from \( S\) by oxidizing \( C\) to \( C\). The catalysts are regenerated in dark steps by reducing or oxidizing water. In the second case, \( S\) oxidizes \( C\) and \( S\) is regenerated by reducing \( C\).

The oxidized or reduced forms of \( S\), \( C\), and \( C\) which are produced during the water-splitting cycles are high-energy intermediates that can react with one another, in competition with the water-splitting reactions:

\[
(1-5) \quad S^+ + C^- \longrightarrow S + C \\
(1-6) \quad C^- + C^{+} \longrightarrow C + C
\]

These back-reactions result in the loss of chemical poten-
Figure 1-1. Two cycles that accomplish photosensitized decomposition of water using a dye (S), a catalyst for water reduction (C[H]), and a catalyst for water oxidation (C[O]). The product of water reduction is generally represented as the equivalent of a hydrogen atom ([H]), and the product of water oxidation is generally represented as the equivalent of an oxygen atom ([O]). In the top photocycle, the photoexcited state of S (*S) reduces C[H] by one electron to produce S⁺. S⁺ subsequently oxidizes C[O] to regenerate S. The catalysts subsequently pass the redox equivalents on to water. Two light-initiated cyclic events are required to oxidize H₂O to [O]. Analogous events occur in the bottom photocycle except that *S is reduced in the primary redox reaction. Intermediary electron acceptors or donors may be used as redox mediators between the dye and the catalysts.
1.2 Potential energy. To inhibit energy-wasting back-reactions such as (1-5) and (1-6), phase boundaries can be introduced as barriers to keep the intermediates separated. One arrangement which is especially attractive has the water-oxidizing and water-reducing parts separated by a dye-containing membrane.

1.3. MEMBRANES

Pigmented membranes which separate two aqueous compartments can, in principle, be used to photosensitize water decomposition by mediating the transport of electrons and protons from the [O] side of the membrane to the [H] side as indicated in Fig. 1-2. This process requires vectorial charge transfer across the two opposing interfaces and across the membrane phase between them. Electron transport across the membrane has to be accompanied by ion transport for charge balance; proton transport in the same direction as electron transport, or hydroxide ion transport in the opposite direction, are appropriate in the present case since these ions are involved in the decomposition of water (Fig. 1-1).

The major role of the membrane is to keep separate the redox intermediates of the water-splitting process, but it can also serve to organize and orient the various components of the system and to influence the reactions through surface or transmembrane electrostatic potentials thereby potentially improving the efficiency of energy conversion. Back-
Figure 1-2. The use of a pigmented membrane to mediate photosensitized decomposition of water is illustrated. Either of the two photocycles in Fig. 1-1 could be involved. The main function of the membrane is to physically separate the water-oxidizing and reducing parts of the cycle to avoid recombination between reactive intermediates. The overall reaction requires the net transport of two electrons and two protons from the water-oxidizing side to the water-reducing side of the membrane. In the example shown, the products of water decomposition are hydrogen peroxide and molecular hydrogen.
\[ \text{water} \rightarrow \text{membrane} \rightarrow \text{water} \]

\[ H_2O_2 + 2H^+ + C_{[0]} \rightarrow 2H_2O + C_{[H]} \]

\[ S \]

\[ 2e^- + 2H^+ \]

\[ \rightarrow H_2 + 2OH^- \]

\[ 2H_2O \]

XBL 809-4410
reactions between \( C_{[\mathbf{H}]}^- \) and \( C_{[\mathbf{O}]}^+ \) in Fig. 1-2 are prevented because the two catalysts are physically separated by the membrane. Inhibition of the recombination between \( S^+ \) and \( C_{[\mathbf{H}]}^- \), or between \( S^- \) and \( C_{[\mathbf{O}]}^+ \), by the membrane relies on two factors: i) the interface separates the reactants, and ii) electron transport through the membrane competes with the recombination. Therefore the quantum efficiencies of devices that decompose water photochemically using the membrane principle depicted in Fig. 1-2 will depend critically on the relative rates of back-reactions at the interfaces and charge transport across the membrane. These considerations make lipid bilayer membranes attractive as mediators of the photosensitized decomposition of water.

1.4. LIPID BILAYER MEMBRANES

Amphiphilic lipids are molecules with distinctly polar and non-polar parts. Often the polar parts are ionic (anionic, cationic, or zwitterionic) and the non-polar parts are aliphatic hydrocarbon chains. When dispersed in aqueous media, these lipids tend to self-aggregate, oriented so that their polar parts are in contact with the aqueous phase and their non-polar parts are directed away from the aqueous phase. The structures of the lipid aggregates, or micelles, depend on the lipid concentration and molecular geometry, among other things (Adamson, 1967; Fendler and Fendler, 1975; Israelachvili et al., 1980; Tanford, 1980). Detergent molecules (e.g., fatty acids) typically have one polar group
and one aliphatic hydrocarbon substituent and form globular micelles with hydrocarbon cores consisting of 20-100 molecules (Fendler and Fendler, 1975; Tanford, 1980). By contrast, lipids with two hydrocarbon substituents commonly form bilayered micelles consisting of thousands of molecules when dispersed in water.

Lipid bilayers are essentially two opposed lipid monolayers which separate two aqueous phases. The bilayers have hydrocarbon-like interiors whose physicochemical properties resemble those of biological membranes (Jain, 1972; Papahadjopoulos and Kimelberg, 1974; Tien, 1974; Jain and Wagner, 1980) (see next Section). Lipids that are dispersed in water tend to form closed bilayer vesicles or liposomes (Papahadjopoulos and Kimelberg, 1974; Tanford, 1980; Szoka and Papahadjopoulos, 1980) which separate the inner aqueous phase from the outer one in which they are suspended. A cross-sectional view of a vesicle is shown in Fig. 1-3. The term "vesicle" will be reserved for unilamellar (single-walled) bilayers while the term "liposome" will refer to concentric multilamellar bilayers. Vesicles are apparently thermodynamically favored over liposomes whenever conditions for dynamic equilibration prevail (Tanford, 1980).

1.5. VESICLES - PHYSICAL AND CHEMICAL PROPERTIES

The properties of lipid bilayer vesicles that will be reviewed in this Section fall into three categories: structure, dynamics, and permeability.
Figure 1-3. Cross-sectional view of a phospholipid bilayer vesicle. The head-groups of the lipid molecules are represented by ellipses and the fatty alkyl substituents are represented by wavy lines. The vesicle is shown in a biconcave conformation. The thickness of the vesicle wall is about 40 Å. The vesicle encapsulates an aqueous phase and is surrounded by a separate, continuous aqueous phase.
The best characterized vesicles and liposomes are those composed of phosphatidylcholines (PC) (lecithins) whose general formula is shown below, where the fatty acid substituents \( R_1 \)-COOH and \( R_2 \)-COOH vary.

\[
\begin{align*}
\text{H}_2\text{COCOR}_1 \\
\text{R}_2\text{OCO} &\text{C} < \text{H} \\
\text{H}_2\text{CO} &\text{-FO-OC}\text{H}_2\text{CH}_2\text{N(CH}_3\text{)}_3^+ \\
\text{O} &\text{-}
\end{align*}
\]

The polar part of the PC molecule contains phosphorylcholine, which is a zwitterion over the pH range 2-11 (Papahadjopoulos and Miller, 1967). The PC mainly used in this work was extracted from hens' egg yolks. Like most naturally-occurring phosphoglycerides, the fatty acyl group \( R_1 \) tends to be saturated and \( R_2 \) tends to be unsaturated (Tattrie et al., 1968; van Deenen, 1971; Tanford, 1980). The saturated component of egg PC is predominantly either stearic or palmitic acid and the unsaturated component is either oleic or linoleic acid; the average molecular weight of egg PC is about 770 g/mole (Singleton et al., 1965; Tattrie et al., 1968).

Lipid bilayers such as vesicles are classified as smectic liquid-crystals (Fergason and Brown, 1968). Bilayers composed of naturally occurring lipids are generally in their fluid (or "liquid crystalline") phase at room temperature due to the presence of unsaturated or branched aliphatic hydrocarbon substituents (van Deenen, 1971; Chap-
man, 1975; Jain and Wagner, 1980; Szoka and Papahadjopoulos, 1980). A transition from the fluid phase to the solid (or "gel") phase occurs when the temperature is decreased below a characteristic temperature, \( T_C \). The \( T_C \) for egg PC bilayers occurs over the range \(-7 \) to \(-15 \) °C (Papahadjopoulos and Kimelberg, 1974; Chapman, 1975).

The sizes of vesicles can be controlled to some extent by the method by which the lipid is dispersed into the aqueous phase (Szoka and Papahadjopoulos, 1980). The method of dispersion used most in this work involved the addition of egg PC dissolved in ethanol to a vigorously stirred aqueous phase (e.g., see Section 4.2.3.1). This method is known to give vesicles whose largest dimensions range from about 200 to 1000 Å, depending on conditions like the concentration of the egg PC in ethanol (Batzri and Korn, 1973; Kremer et al., 1977; Burnell et al., 1980). Vesicle suspensions are usually polydisperse with respect to size and often contain multilamellar liposomes unless the suspensions have been fractionated; the suspensions are considered homogeneous when the size distribution is a single Gaussian about an average diameter (Mason and Huang, 1978).

The dimensions that will be assumed representative of the vesicles which were used in this work are listed in Table 1-1 and are based on a spherical geometry with a radius of 360 Å. This radius was determined by Dr. Alan Benesi (1979) by laser fluorescence correlation spectroscopy.
Table 1-1. Vesicle dimensions

**Intrinsic values**
- outer radius\(^b\) = 360 Å
- thickness = 40 Å
- surface area per egg PC molecule = 70 Å\(^2\)
- molecular weight per egg PC molecule = 770 g/mole

**Extrinsic values**
- Per vesicle
  - outer surface area = \(1.6 \times 10^6 \text{ Å}^2\)
  - inner surface area = \(1.3 \times 10^6 \text{ Å}^2\)
  - internal volume = \(1.4 \times 10^{-16} \text{ cm}^3\)
  - number of egg PC molecules = \(4.2 \times 10^4\) molecules
  - molecular weight = \(3.2 \times 10^7\) g/mole
- Per cm\(^3\) of vesicle suspension when egg PC conc. = 0.0020 M
  - concentration of vesicles = \(4.8 \times 10^{-8}\) M
  - = \(2.9 \times 10^{13}\) vesicles/cm\(^3\)
  - (surface area)/(suspension volume) = \(8.3 \times 10^3\) cm\(^2/\text{cm}^3\)
  - (internal volume)/(suspension volume) = \(4.0 \times 10^{-3}\) cm\(^3/\text{cm}^3\)

---

\(^a\) Spherical shape assumed

\(^b\) ±150 Å, as measured by laser fluorescence correlation spectroscopy (Benesi, 1979)
and has an uncertainty of ±150 Å. The two head-group layers contribute about 10 Å to the thickness of the bilayer, so the thickness of the hydrocarbon interior is about 30 Å (Papahadjopoulos and Kimelberg, 1974).

Some of the dimensions listed in Table 1-1 (e.g., thickness of the bilayer and surface area per molecule) are essentially intrinsic properties of egg PC vesicles which do not depend greatly on the size of the vesicles. The rest of the properties listed (e.g., aggregation number and surface area per vesicle) are extrinsic in that they depend on the size and topology of the vesicles. For simplicity, the extrinsic properties listed were calculated on the basis of spherically shaped vesicles. In fact, the shapes of vesicles, which depend on the minimal surface energy, are expected to be non-spherical with shapes including biconcave discs like red blood cells (Fergason and Brown, 1968; Kornberg and McConnell, 1971) (e.g., Fig. 1-3).

The mobilities of lipid molecules in bilayers such as vesicles change during the phase transition (Sackmann, 1978; Jain and Wagner, 1980). Lipid molecules can diffuse either laterally or transversely relative to the plane of the bilayer-water interface. The lateral diffusion coefficients of lipids in liposomes range from $10^{-8}$ to $10^{-7}$ cm$^2$/s when the bilayers are in their fluid phase ($T > T_C$), and are about two orders of magnitude lower in the gel phase ($T < T_C$) (Fahey and Webb, 1978; Crawford et al., 1980). A diffu-
sion coefficient of $5 \times 10^{-8}$ cm²/s corresponds to a mean displacement (radius) of the lipid molecule of $10^4$ Å/s (Razi Naqvi, 1974). The lateral diffusion coefficient of egg PC in liposomes increases with increasing temperature with an Arrhenius activation energy of about 9 kcal/mole (Kuo and Wade, 1979).

The transverse diffusion of lipids across bilayer membranes ("flip-flop") is slow compared to the lateral diffusion (Rothman and Lenard, 1977; Sackmann, 1978; Thompson, 1978). The most probable half-time for the flip-flop of egg PC is of the order of weeks or months while for cholesterol it is probably in excess of 6 days (Thompson, 1978). Activation energies for flip-flop of PC analogues in vesicles above their $T_c$ have been determined to be 19.4 kcal/mole (Kornberg and McConnell, 1971) and 23.7±2.0 kcal/mole (deKruijff and Van Zoelen, 1978).

A third kind of motion which characterizes the dynamics of lipid molecules in vesicle suspensions is the exchange of molecules between individual vesicles. In this respect, vesicles are relatively stable entities compared to detergent micelles. Detergent micelles are dynamic in the sense that the monomers exchange between micelles with relaxation times of the order of $10^{-6}-10^{-3}$ s (Fendler and Fendler, 1975; Thomas et al., 1978; Aniansson, 1978; Lindman and Wennerström, 1980). By contrast, relaxation times for the exchange of monomers between vesicles are of the order of
This difference in relaxation times is reflective of the fact that the concentration of monomers in detergent micelle suspensions, about $10^{-5}$-$10^{-2}$ M (Fendler and Fendler, 1975; Sackmann, 1978; Lindman and Wennerström, 1980), is much greater than the concentration of monomers in vesicle suspensions composed of amphiphilic lipids with two long-chained hydrocarbon substituents, about $10^{-10}$-$10^{-7}$ M (Sackmann, 1978; Tanford, 1980).

The final physicochemical property of vesicles considered in this Section is the permeability of the vesicle wall to extraneous species, including neutral molecules, ions, and electrons. The permeability of liposomes and vesicles to small neutral molecules generally decreases as the size of the molecule and its hydrogen-bonding capacity increases, and increases as the partition coefficient of the molecule between non-polar solvents (e.g., octanol) and water increases (Stein, 1967; Jain, 1972; Cohen, 1975; Jain and Wagner, 1980; Defrise-Quertain et al., 1980). Macromolecules like proteins, DNA, and colloidal metal oxides are essentially impermeable (Szoka and Papahadjopoulos, 1980; Hutchison et al., 1980). The permeabilities are generally lower at temperatures below $T_c$ than above $T_c$ and have maxima at temperatures close to $T_c$, when the fluid and gel phases of the bilayer coexist (Papahadjopoulos and Kimelberg, 1974; Marsh et al., 1976; Jain and Wagner, 1980; Szoka and Papa-
The permeabilities of vesicles and liposomes to small nonionic or ionic molecules are often characterized by permeability coefficients ($P$) which have the units of velocity (cm/s) (Jain, 1972; Papahadjopoulos and Kimelberg, 1974; Jain and Wagner, 1980; see Section 4.4.8 for further discussion). The value of $P$ for water crossing egg PC liposomes is about 0.0045 cm/s (Jain and Wagner, 1980), which means that water is one of the most permeable molecules known. The high permeability of water partly reflects the penetration of water molecules into the lipid bilayer (Papahadjopoulos and Kimelberg, 1974; Griffith et al., 1974). Values of $P$ for highly polar, neutral molecules like glucose and for ions with localized charges like $K^+$ and $Cl^-$ fall below $10^{-9}$ cm/s (Jain, 1972; Papahadjopoulos and Kimelberg, 1974; Jain and Wagner, 1980).

In general, electrons cross lipid bilayer membranes either by the diffusion of electron-carrying molecules (Hinkle, 1973; Hong and Mauzerall, 1974; Hauska, 1977; Futami et al., 1979; Matsuo et al., 1980) or by purely electronic processes in which the charge carriers are electrons (or holes) (Rosenberg, 1971; Ilani and Berns, 1973; Tien, 1974; Mangel, 1976; Tien and Karvaly, 1976; Seefeld et al., 1977; Kuhn, 1979; Ford et al., 1979). Electron transport mechanisms in vesicle systems are discussed in Chapter 4 (Section 4.4.4.2).
1.6. VESICLES IN SOLAR WATER-DECOMPOSING DEVICES

Four aspects of vesicle suspensions that make them attractive for use in solar energy converting devices follow:

i) Vesicle walls are extremely thin membranes which separate two aqueous phases. Thus the water-oxidizing and reducing components in the device can be dissolved in separate aqueous compartments, but close enough to one another to facilitate electron and proton transport between them.

ii) The surface area per gram of lipid is high, being several hundred square meters per gram. The importance of interfaces for charge separation (as mentioned in Section 1.3) makes a high specific surface area desirable for efficient utilization of materials.

iii) The lipid components are in an organized state. Order at a molecular level can be an important factor for energy conversion because energy and charge transfer processes are sensitive to the relative orientations of donors and acceptors.

iv) The compositions of vesicle walls can be varied easily. The search for useful dye-catalyst combinations is facilitated by the ease with which the various components can be substituted for one another. Components can be made to dissolve in the vesicle walls by synthesizing their amphiphilic analogues.

Some of the disadvantages of using vesicles in solar
energy harnessing devices and possible modifications to circumvent those problems are considered in Chapter 5.

1.7. LITERATURE COMPILATION - PHOTOCHEMICAL REDOX REACTIONS MEDIATED BY VESICLES

The use of vesicle or liposome suspensions as media for photosensitized electron transfer reactions is a relatively recent innovation. The investigation of photoredox reactions in other colloidal aqueous media such as detergent micelle and polymer solutions was pioneered by, for example, Vernon, 1961; Oster (Bellin, 1965), Massini and Voorn (1968), Krasnovsky (1972), Matsuo (Kano and Matsuo, 1974), and Seely (1977). Interest in photoredox reactions in planar bilayer membranes (Tien, 1974; Hong and Mauzerall, 1974; Berns, 1976) and lipid multilayer assemblies (Costa and Porter, 1974; Seefeld et al., 1977; Kuhn, 1979) roughly paralleled the rising interest in vesicle suspensions.

Photosensitized electron transfer reactions in vesicle systems can be grouped into two categories: (i) systems in which electron transfer at vesicle-water interfaces were being examined (Chapman and Fast, 1968; Nicholls et al., 1974; Tomkiewicz and Corker, 1975; Oettmeier et al., 1976; Escabi-Perez et al., 1979; Infelta et al., 1979; Hurley et al., 1980; Infelta and Fendler, 1980; Pileni, 1980; Nagamura et al., 1980; Infelta et al., 1980; Takayanagi et al., 1980; Matsuo et al., 1980a) and (ii) systems in which electron transport across the vesicle walls was being examined
1.7

(Mangel, 1976; Toyoshima et al., 1977; Ford et al., 1978; Kurihara et al., 1979a,b; Sudo and Toda, 1979; Ford et al., 1979; Matsuo et al., 1980a,b; Sudo et al., 1980). In the latter systems, coupled redox reactions at the two opposing vesicle-water interfaces took place.
CHAPTER 2. ELECTRONIC SPECTRA AND PHOTOCHEMISTRY OF SOME AMPHIPHILIC PORPHYRINS

This Chapter deals with spectroscopic and photochemical properties of some synthetic porphyrins, in particular the long-chained amphiphilic one derived from meso-tetra(4-pyridyl)porphyrin whose structural formula is represented by that of the metal-free derivative (abbreviated \( \text{H}_2\text{TPyP-1} \)) shown in Fig. 2-1.

2.1. INTRODUCTION

Porphyrrins have a number of characteristics that make attractive for use as catalysts in water-decomposing solar energy converters. Porphyrrins can serve as catalysts for a variety of photochemical or thermal chemical reactions which involve electron or atom-transfer (Calvin, 1961; Vernon, 1961; Mauzerall, 1962; Tollin and Green, 1963; Seely, 1966; Sidorov, 1968; Whitten et al., 1971; Krasnovsky, 1972; Carapellucci and Mauzerall, 1975; Castro et al., 1977; Harel and Manassen, 1977; Groves and Kruper, 1979; Harriman and Porter, 1980; Willner et al., 1980b).

Different types of catalytic properties can be obtained from the same porphyrin ligand by varying the central metal ion. As photosensitizers, the porphyrins are attractive because their molar extinction coefficients (\( \varepsilon \)) are high \((10^4-10^5 \text{ M}^{-1}\text{cm}^{-1})\) over a wide range of the visible spectrum and they undergo oxidation-reduction reactions reversibly.

Porphyrrins are notorious for their tendencies to self-
Figure 2-1. $\text{H}_2\text{TPyP-1 (5-(1'-Hexadecylpyridinium-4'-yl)-10,15,20-tris[4'-pyridyl]-21H,23H-porphine bromide).}$
H₂TPyP-1

XBL 8010-4437
aggregate or to form complexes with exogenous molecules (Mauzerall, 1965; Bergeron et al., 1967; Boucher and Katz, 1967; Cann, 1967; Heathcote et al., 1968; Pasternack, 1973; Krishnamurthy et al., 1975; Fuhrhop, 1976; White, 1978). In addition, metalloporphyrins can coordinate exogenous ligands in one or both of the axial positions perpendicular to the porphyrin plane (Corwin et al., 1963; McLees and Caughey, 1968; Pasternack et al., 1974; Nappa and Valentine, 1978).

Earlier work (Ford, 1975; Ford, 1976) using chlorophyll a as photosensitizer in aqueous micellar solutions of detergents was hampered by the instability of chlorophyll to "allomerization" and loss of the magnesium ion ("pheophytinization") (Seely, 1966). Porphyrins that would be stable compared to chlorophyll and could be dissolved in detergent micelles or phospholipid vesicles were attractive alternatives to chlorophyll. Amphiphilic porphyrins which are either cationic or anionic with one or more long-chained alkyl substituents were prepared (Okuno et al., 1980) from metal-free or metallated meso-tetra(4-pyridyl)porphyrin or meso-tetra(4-carboxyphenyl)porphyrin, which can be readily synthesized (Longo et al., 1969) or obtained from commercial sources. The synthetic surfactant porphyrins were expected to be oriented when dissolved in micelles or vesicles with the charged porphyrin rings located near the interface and the alkyl substituent(s) pointed away from the water phase, like chlorophyll (Steinemann et al., 1971; Steinemann et
al., 1972; Lauger et al., 1974; Oettmeier et al., 1976; Podo et al., 1976; Fragata, 1977).

2.2. EXPERIMENTAL SECTION

2.2.1. MEASUREMENTS

Unless otherwise noted, all measurements were performed at room temperature (23±2 °C) and the samples were saturated with air.

Ultraviolet and visible absorption spectra were recorded on a Cary model 118 spectrophotometer. Quartz cuvettes with 1.00 or 0.30 cm path lengths, or a glass cuvette for anaerobic work (Section 2.2.3.6), were used. The porphyrin concentrations were normally between 4 x 10^{-6} and 2 x 10^{-5} M. Extinction coefficients of the porphyrins in turbid samples were approximately corrected by subtracting the estimated turbidity from the absorbance.

Luminescence spectra were recorded on a Perkin-Elmer model MPF-2A fluorescence spectrophotometer equipped with a 150 watt xenon arc lamp and a red-sensitive, type R-136, photomultiplier tube (Hamamatsu TV Co.). Luminescence was detected at right angles to the excitation, and was not corrected for the wavelength dependence of the excitation and photomultiplier response. A red cut-off filter (either Corning #2-61 or #2-69) was placed between the cuvette and photomultiplier tube when aqueous suspensions which were light-scattering were used.

EPR spectra were recorded on a Varian model E-9 spec-
trometer with a TE-102 microwave cavity operated at 9.5 GHz (X-band). A flat sample cell made of quartz was used.

2.2.2. MATERIALS

Unless otherwise noted, chemicals were obtained from commercial suppliers and were used without further purification. Water was deionized, then distilled in a glass apparatus, or was twice deionized.

H₂TPyP and H₂TCP (from Strem) were purified by chromatography over either talc or charcoal, respectively, and their hexadecyl derivatives and metallo-complexes were prepared by Dr. Yohmei Okuno (Okuno, 1976; Okuno et al., 1980). Manganese meso-tetra(4-N-methylpyridyl)porphyrin perchlorate (MnTPyP-4-CH₃) and manganese(III)-meso-tetra(4-sulfonatophenyl)porphyrin, tri-sodium salt, (Mn(III)TSPP) were samples supplied by Dr. E. B. Fleischer. Zinc(II)-meso-tetrphenylporphyrin (Zn(II)TPP) (from Strem) was used as received.

Phosphatidylcholine extracted from hens' egg yolks (egg PC) according to the procedure of Singleton et al. (1965) was supplied by Dr. Susan Kohler. Stock solutions of the egg PC in ethanol (fraction C, 28.5 mg/ml, and fraction D, 18.9 mg/ml) were stored under N₂ at -20 °C. Synthetic dipalmitoyl phosphatidylcholine (DPPC) was obtained from Sigma (grade I, approximately 99%).

Sodium dodecyl-1-sulfate (SDS) was "specially pure" from BDH. Cetyl pyridinium chloride (CPC) was from
Schwarz/Mann. 3-(Dimethylhexadecylammonio)propane-1-sulfonate (DHAPS) was synthesized by the method of Clunie et al. (1967). Vitamin K$_1$ quinone (VK$_1$) (from Sigma) was stored desiccated in the refrigerator. Dialyzed catalase (from Boehringer) was supplied by Lydia Chang as a 0.2 mg/ml solution in 0.030 M phosphate buffer. The catalase solution was stored in the refrigerator and was diluted to 0.02 mg/ml with water before use.

The sodium salt of 1,4-anthraquinone-2-sulfonate (AQS) (from Eastman) was recrystallized twice from hot water. 1,4-Benzquinone-2,5-disulfonate was prepared by oxidizing an aqueous solution of the corresponding hydroquinone (di-potassium salt, from Eastman) with Ag$_2$O. The crystals that precipitated from the filtered suspension were recrystallized from water.

2.2.3. METHODS

2.2.3.1. EXTINCTION COEFFICIENTS AND RELATIVE FLUORESCENCE YIELDS OF METAL-FREE PORPHYRINS

To 100 ml volumetric flasks were added small portions (0.61-0.75 μmole) of the porphyrins weighed to ± 0.00001 g, followed by 50-50% (by volume) ethanol-dimethylformamide mixed solvent to 100 ml. The absorption and emission spectra were recorded of the solutions in 1 x 1 cm cuvettes. The estimated uncertainties in the extinction coefficients were between 10% and 15%. Fluorescence excitation was centered at the maximum of the visible band near 515 nm.
2.2.3.1

(absorbance = 0.10-0.20). Fluorescence intensities were corrected approximately for internal filter effects using equation 235 (p. 222) of Parker (1968), using $d_1 = 0.1$ cm and $d_2 = 0.9$ cm. Relative quantum yields of fluorescence were calculated as the corrected fluorescence peak intensity divided by the excitation peak absorbance.

2.2.3.2. PREPARATION OF AQUEOUS SAMPLES

$10^{-3}$ M stock solutions of $H_2TPyP-1$ (bromide salt) in methanol or ethanol, Cu(II)TPyP-1 and Zn(II)TPyP-1 (perchlorate salts) in dimethylformamide, Mn(III)TPyP-1 (bromide, perchlorate salt) in either dimethylformamide or 0.01 M aqueous HCl, and Zn(II)TPP in tetrahydrofuran were kept in sealed glass vials in the dark. The concentrations of $H_2TPyP-1$ stock solutions were regularly checked by measuring the absorbance of the solution diluted with methanol.

The porphyrins were suspended in aqueous solutions or pure water by injecting the organic stock solutions via syringe into the aqueous phase while it was being stirred vigorously with a vortex-mixer. The porphyrins were similarly dispersed into aqueous detergent solutions except in a few cases (Section 2.3.1.2).

Vesicle suspensions composed of either egg PC or DPPC and porphyrin were usually prepared by injecting solutions of the phospholipids and porphyrin codissolved in ethanol into vigorously stirred aqueous solutions (Batzri and Korn,
1973). Sonicated vesicle suspensions containing DPPC and H$_2$TPyP-1 were prepared as follows. A stock solution of DPPC in chloroform (0.013 M) was stored under argon at -20 °C. Stock suspensions containing 1 x 10^{-3} M DPPC and 1.0 x 10^{-4} M H$_2$TPyP-1 were prepared by adding appropriate volumes of the stock solutions to a thin-walled (0.3 mm) polyethylene tube (1.5 cm diameter) and removing the solvent by evaporation under a stream of N$_2$ or argon. 3.0 ml of aqueous phase, which was previously deaerated (boiled, bubbled with argon), was added under argon and the tube was sealed with a rubber serum stopper. The mixture was vortex-stirred to yield a turbid suspension which was then sonicated for 2 minutes at >45 °C in a bath-type sonicator (Branson model W185, operated at 120 watts). The sonicated mixture was stored in the refrigerator. Samples for spectra were prepared by diluting the stock suspensions by about 10-fold with deaerated aqueous phase, followed by a second sonication for 1 minute.

2.2.3.3. QUENCHING OF H$_2$TPyP-1 FLUORESCENCE IN VESICLES BY ANTHRAQUINONE-2-SULFONATE

The vesicle suspensions with egg PC:H$_2$TPyP-1 molar ratios of 50:1 and 5:1 contained the same concentrations of the porphyrin (6.7 x 10^{-6} M) and different concentrations of egg PC (3.6 x 10^{-4} and 3.6 x 10^{-5} M, respectively). The suspensions were prepared in 0.05 M NaCl, 0.005 M ammonium acetate aqueous solution (pH 6.5) by the ethanol injection
method with final concentrations of ethanol of 3.6% (by volume) in each.

The suspensions were transferred to 1 x 1 cm cuvettes with four polished sides. The absorption, emission, and emission excitation spectra were recorded. The emission intensity at 650 nm (excitation at 588 nm) was monitored for quenching by AQS. The AQS was added via syringe as its aqueous solution (0.0010 M) to the vesicle suspension in the cuvette. The cuvette contents were shaken well before the emission intensity at 650 nm was measured again. The intensities were corrected for dilution of the samples.

2.2.3.4. CAPTURE OF MnTPyP-4-C\textsubscript{3} BY EGG PC VESICLES

Egg PC and MnTPyP-4-C\textsubscript{3} were codissolved in ethanol before the vesicles were prepared by the injection method. The porphyrin that was in the continuous aqueous phase was removed by gel-filtration. The details of this experiment follow.

A stock solution of MnTPyP-4-C\textsubscript{3} perchlorate was prepared by dissolving 0.00766±0.00002 g of the porphyrin in 0.200 ml of dimethylformamide. 0.020 ml of this solution was transferred to a small test tube with a conical tip. To that aliquot was added 0.13 ml of egg PC solution in ethanol (28.5 mg/ml, approximately 37 mM). A precipitate was produced which was redissolved upon warming the mixture. The warm solution was drawn into a syringe and injected into 3.00 ml of vortex-stirred basic aqueous solution containing
0.113 M Na$_2$SO$_4$, 0.113 M K$_2$SO$_4$, and 0.0125 M Na$_4$P$_2$O$_7$ (pH 9.6), yielding a clear, cola-colored suspension.

A pipette-sized column containing 60 x 5 mm of Sephadex G-25-medium (from Pharmacia) swollen in water was equilibrated with the aqueous sulfate-pyrophosphate solution. To the column was applied 0.3 ml of the vesicle suspension. The vesicles were eluted with the aqueous solution. The first three 0.4 ml fractions that were colored were collected (0.6 ml of buffer eluted before the first colored fraction). The gel bed remained uniformly stained (pale green). The absorption spectra of the collected fractions were recorded.

After 1 hour, the first fraction was gel-filtered a second time over another column of Sephadex (55 x 5 mm). The first three 0.5 ml fractions that were colored were collected. The gel bed remained colorless afterwards. The absorption spectra of the collected fractions were recorded.

2.2.3.5. **EPR OF ILLUMINATED BILAYER SUSPENSIONS** [with Anne McGuire]

The samples for electron spin resonance (EPR) experiments were bilayer dispersions containing 0.045 M egg PC and 0.0028 M H$_2$TPyP-l in aqueous 0.05 M NaCl, 0.005 M ammonium acetate (pH 6.5) which contained either no AQS, 2.0 x 10$^{-3}$ M, or 4.4 x 10$^{-3}$ M AQS. To prepare the samples, a solution of egg PC in ethanol was added to a solution of the porphyrin in chloroform, and the solvent was removed by rotary
evaporation. The aqueous phase was added to the lipid film, the flask was capped with a serum stopper under argon, and the mixture was stirred with a vortex mixer until the dispersion had a homogeneous texture.

The bilayer dispersion was transferred to a flat quartz EPR cell, care being taken to keep it under argon. The cell was capped at both ends with teflon plugs.

The samples were illuminated while they were in the EPR cavity with either continuous or flashed visible light. The continuous light source was a microscope lamp (American Optical) with a tungsten bulb filtered with a Corning glass cutoff filter #3-75 (>370 nm) in the first experiment ([AQS] = $2 \times 10^{-3}$ M). In the second continuous photolysis experiment ([AQS] = 0 or $4.4 \times 10^{-3}$ M), the light source was the visible lamp of a Cary model 14 spectrophotometer (500 watt quartz-halogen bulb); the light passed through a #3-75 cutoff filter, a single convex focusing lens, and 10 cm of water. In the flash photolysis experiments, repetitive xenon flashes 12 s long at half-height and 0.80 s apart were obtained from an ICL model L-391 programmable flash lamp system. The flash light was directed to the EPR cavity through a 2 ft light pipe and was filtered with a #3-75 cutoff filter, a #1-69 infrared filter, and a 50% transmission grid. EPR signals due to flash illumination were signal-averaged.

2.2.3.6. STEADY-STATE PHOTOLYSES
The light source for all steady-state illuminations, except during EPR experiments (Section 2.2.3.5), was a 1000 watt xenon arc lamp (Oriel Universal Arc Lamp Source, model C-60-50) operated at about 900 watts. The light was filtered through 4.2 cm of aqueous cupric sulfate solution (100 g/l) and a Corning #3-73 glass cutoff filter to transmit light with wavelengths between about 420 and 590 nm, or through 9.5 cm of the aqueous cupric sulfate solution to transmit light with wavelengths between about 330 and 560 nm. The filter absorbances are shown in Fig. 2-2.

The samples were housed in a glass cuvette with a stopcock so that the photolyses could be carried out anaerobically and the absorption spectral changes could be monitored between intervals of photolysis. The cuvette design and dimensions are shown in Fig. 2-3. The sample (usually about 3 ml) was placed in the lower chamber of the cuvette which contained a small teflon-coated magnet so the sample could be stirred during photolysis. The upper chamber of the cuvette was sealed with a rubber serum stopper (†19u). Apiezon N grease was used to seal the stopcock.

Vesicle suspensions to be photolyzed were deaerated in the cuvette by stirring the sample while it was under reduced pressure for 10 minutes, then bubbling it with N₂ or argon for 5-10 minutes. The vacuum was applied to the sealed cuvette by puncturing the serum stopper with a syringe needle attached to a rubber hose connected to the
Figure 2-2. Absorption spectra of three light filters used for steady-state photolysis experiments. Solid line: aqueous cupric sulfate solution (concentration = 100 g/l, path length = 9.5 cm). Dashed-dotted line: Corning cut-off filter #3-73. Dashed line: a combination of Corning filters #3-72 and #5-57.
Figure 2-3. Glass cuvette used for anaerobic steady-state photolysis experiments. The optical path length is 1.0 cm. Samples were stirred magnetically with a small teflon-coated magnet in the lower chamber of the cuvette. The upper chamber was capped with a rubber serum stopper so that solutions could be added to the cuvette under an inert atmosphere.
vacuum line. N₂ or argon were likewise introduced via syringe needles. Anaerobic additions of solutions to the cuvette were made by passing argon through the upper chamber while the solution was added via syringe.

2.3. RESULTS

2.3.1. ELECTRONIC ABSORPTION AND EMISSION SPECTRA

2.3.1.1. METAL-FREE PORPHYRINS IN ETHANOL-DIMETHYLFORMAMIDE

The visible absorption spectra of the metal-free porphyrins dissolved in ethanol-dimethylformamide (50-50%, by volume) mixed solvent are summarized in Table 2-1. Alkylation of the pyridine substituents of H₂TPyP caused the positions of the band maxima to move to lower energies. The extinction coefficients were also affected. Alkylation caused the extinction coefficients of the Soret band near 420 nm and the visible bands near 515 and 650 nm to decrease while the remaining two visible bands near 550 and 590 nm increased in intensity. The decreases in intensities of the Soret bands were accompanied by increases in the band half-widths.

The porphyrin solutions had a red luminescence which was not quenched by air (O₂). The emission spectra had two maxima, one at about 650 nm and a weaker one around 705-710 nm. The peak positions and relative intensities of the bands near 650 nm are listed in Table 2-2. Based on the lack of quenching by O₂, the mirror image relationship between the absorption and emission maxima, and a comparison
Table 2-1. Absorption spectra of derivatives of H$_2$TPyP and H$_2$TCP P dissolved in ethanol-dimethylformamide

<table>
<thead>
<tr>
<th>Porphyrin</th>
<th>Soret band</th>
<th>Visible bands</th>
</tr>
</thead>
</table>
| H$_2$TPyP         | $\lambda_{\text{max}}$ ($\varepsilon$) | 414 (--

|          | rel.int. | 510 543 587 643 | 1.00 0.28 0.30 0.13 |
| H$_2$TCP P-1     | width$_{1/2}$ | 14.8 |                |
| H$_2$TCP P-2     | $\lambda_{\text{max}}$ ($\varepsilon$) | 421 (2.4x10$^5$) | 514 551 590 646 | 1.00 0.43 0.36 0.13 |
| H$_2$TCP P-3     | width$_{1/2}$ | 24.6 |                |
| H$_2$TCP P-4     | $\lambda_{\text{max}}$ ($\varepsilon$) | 425 (2.3x10$^5$) | 516 553 590 647 | 1.00 0.45 0.38 0.12 |
| H$_2$TCP P-1     | width$_{1/2}$ | 25.6 |                |
Table 2-2. Fluorescence spectra of derivatives of $H_2TPyP$ and $H_2TCPP$ dissolved in ethanol-dimethylformamide

<table>
<thead>
<tr>
<th>Porphyrin</th>
<th>$\lambda_{\text{max}}$ (nm)</th>
<th>rel. quantum yield(^a)</th>
<th>width(1/2) (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$H_2TPyP$</td>
<td>644</td>
<td>1.15</td>
<td>21</td>
</tr>
<tr>
<td>$H_2TPyP-1$</td>
<td>648</td>
<td>1.00</td>
<td>25</td>
</tr>
<tr>
<td>$H_2TPyP-2^b$</td>
<td>652</td>
<td>0.97</td>
<td>27</td>
</tr>
<tr>
<td>$H_2TPyP-3$</td>
<td>652</td>
<td>0.89</td>
<td>27</td>
</tr>
<tr>
<td>$H_2TPyP-4$</td>
<td>653</td>
<td>0.64</td>
<td>28</td>
</tr>
<tr>
<td>$H_2TCPP-1$</td>
<td>650</td>
<td>2.19</td>
<td>21</td>
</tr>
</tbody>
</table>

\(^a\) Photomultiplier response at $\lambda_{\text{max}} \pm 2.5$ nm divided by the sample absorbance at the excitation wavelength

\(^b\) Mixture of two isomers
to the literature (Gouterman, 1978), the emission can be assigned as fluorescence. Alkylation of the pyridine substituents of $H_2TPyP$ caused the fluorescence maxima to move to lower energies. The peak intensities decreased with increasing degree of quaternization, but the widths of the bands increased, so the integrated intensities were approximately the same for the four $H_2TPyP$ derivatives.

2.3.1.2. SOLVENT DEPENDENCE OF THE SPECTRA OF $H_2TPyP-1$

The absorption and fluorescence spectra of $H_2TPyP-1$ dissolved in organic solvents or in aqueous micellar dispersions varied widely; the spectra of the aqueous dispersions often depended on the age of the samples. Spectral data for fourteen samples are summarized in Table 2-3. In all samples examined, the fluorescence excitation spectra had maxima corresponding to those in the absorption spectra.

The spectral features of $H_2TPyP-1$ did not vary much in the different organic and mixed-organic solvents examined. $H_2TPyP$ was dispersed into aqueous detergent solutions either i) by injecting a solution of the porphyrin in ethanol or methanol into the aqueous detergent solution or ii) by pouring the detergent solution into a solution of the porphyrin in methanol. The addition of 0.10 M SDS solution to a solution of $H_2TPyP-1$ in methanol (method (ii)) resulted in a sample whose absorption spectrum had a split Soret band, with band maxima at 420 and 445 nm of nearly equal intensities, as shown in Fig. 2-4. Warming the sample to 80
### Table 2-3. Absorption spectrum of H$_2$TPyP-1 dissolved in various solvents

<table>
<thead>
<tr>
<th>Solvent*</th>
<th>Soret band</th>
<th>Visible bands</th>
<th>Width$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. MeOH</td>
<td>415 (240)</td>
<td>511 548 588 646</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>d14.1</td>
<td>1.00 0.36 0.33 0.13</td>
<td></td>
</tr>
<tr>
<td>2. 90-10 MeOH-H$_2$O (0.01 M NH$_4$OAc)</td>
<td>415 (250)</td>
<td>511 548 588 645</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>14.3</td>
<td>1.00 0.37 0.34 0.13</td>
<td></td>
</tr>
<tr>
<td>3. 60-40 CH$_3$CN-H$_2$O (0.01 M NH$_4$OAc)</td>
<td>415 (260)</td>
<td>512 549 587 644</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>15.7</td>
<td>1.00 0.38 0.35 0.14</td>
<td></td>
</tr>
<tr>
<td>4. 0.10 M SDS</td>
<td>422 (190)</td>
<td>518 556 590 646</td>
<td>31</td>
</tr>
<tr>
<td></td>
<td>12.8</td>
<td>1.00 0.50 0.40 0.19</td>
<td></td>
</tr>
<tr>
<td>5. 0.020 M CPC</td>
<td>423 (240)</td>
<td>517 555 591 648</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>15.3</td>
<td>1.00 0.44 0.35 0.17</td>
<td></td>
</tr>
<tr>
<td>6. DPPC 10:1, A</td>
<td>421 (130)</td>
<td>517 553 592 647</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td>10.3</td>
<td>1.00 0.43 0.37 0.17</td>
<td></td>
</tr>
<tr>
<td>7. egg PC 50:1, B</td>
<td>420 (280)</td>
<td>514 550 590 646</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>15.8</td>
<td>1.00 0.37 0.33 0.15</td>
<td></td>
</tr>
<tr>
<td>8. egg PC 10:1, B</td>
<td>421 (210)</td>
<td>515 551 590 647</td>
<td>25</td>
</tr>
<tr>
<td>1 hr</td>
<td>13.8</td>
<td>1.00 0.39 0.34 0.15</td>
<td></td>
</tr>
<tr>
<td>9. egg PC 10:1, B</td>
<td>421 (180)</td>
<td>516 552 592 647</td>
<td>25</td>
</tr>
<tr>
<td>7 hr</td>
<td>12.1</td>
<td>1.00 0.41 0.36 0.14</td>
<td></td>
</tr>
<tr>
<td>10. 1.0 M HCl</td>
<td>443 (280)</td>
<td>505 550 590 642</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>17.0</td>
<td>0.17 0.23 0.77 1.00</td>
<td></td>
</tr>
<tr>
<td>11. 0.10 M NaCl pH 2.1</td>
<td>422 (170)</td>
<td>517 555 588 643</td>
<td>26</td>
</tr>
<tr>
<td></td>
<td>15.1</td>
<td>1.00 0.38 0.41 0.11</td>
<td></td>
</tr>
<tr>
<td>12. 0.10 M NaCl pH 10.8, 12 hr</td>
<td>447 (40)</td>
<td>528 565 600 651</td>
<td>57</td>
</tr>
<tr>
<td></td>
<td>3.4</td>
<td>1.00 0.76 0.48 0.19</td>
<td></td>
</tr>
<tr>
<td>13. B, 5 min</td>
<td>444 (90)</td>
<td>524 558 595 649</td>
<td>43</td>
</tr>
<tr>
<td></td>
<td>4.9</td>
<td>1.00 0.40 0.36 0.11</td>
<td></td>
</tr>
</tbody>
</table>

---

*a* PC:porphyrin molar ratios are given for vesicle suspensions; buffer A is 0.010 M NH$_4$OAc, pH 6.5; buffer B is 0.050 M NaCl, 0.0050 M NH$_4$OAc, pH 6.8  

*b* Full width at half-maximal intensity of Soret band (nm)  

*c* Band maximum, nm (extinction coefficient, mM$^{-1}$cm$^{-1}$)  

*d* Relative intensity
Figure 2-4. Absorption spectra of $\text{H}_2\text{TPyP-1}$ in aqueous SDS micellar solutions. Solid line: sample prepared by adding an aqueous 0.1 M solution of SDS to a methanolic solution of $\text{H}_2\text{TPyP-1}$. Broken line: same sample after warming to 80 °C for 15 minutes.
°C for 15 minutes increased the intensity of the 420 nm band at the expense of the one at 445 nm (see Fig. 2-4). By contrast, when the porphyrin was solubilized in 0.10 M SDS by the first method, the absorption spectrum had a single Soret band at 422 nm (§4 of Table 2-3). The absorption spectrum of H₂TPyP-1 dissolved in a cationic micellar solution (0.020 M CPC) did not depend on the method of preparation (§5, Table 2-3).

Except in special cases like the sample with SDS described above, the general spectral characteristics of H₂TPyP-1 dissolved in detergent micellar solution resembled those of the porphyrin dissolved in organic solvents, although the band maxima of the detergent sample spectra were shifted by several nanometers to longer wavelengths relative to the organic solution spectra. The extinction coefficients of the Soret bands in SDS samples with single maxima near 420 nm were noticeably smaller and the band widths were greater than were the corresponding values for the CPC sample and the organic solutions.

The preparation of phospholipid vesicles containing H₂TPyP was first attempted by sonicating for several minutes mixtures of dipalmitoyl phosphatidylcholine (DPPC) and the porphyrin which were dispersed in unbuffered or buffered aqueous media. The buffer used was either 0.010 M sodium phosphate (pH 7.0) or 0.010 M ammonium acetate (pH 6.5). In all the samples the DPPC:porphyrin molar ratio was
10:1. The absorption spectra of the samples depended to some extent on the aqueous phase. Generally, compared to the organic solution spectra, i) the extinction coefficients of the bands were about one-quarter as great and ii) the Soret bands were single-peaked with half-widths that were greater by between 1.6 and 1.9 in the vesicle samples. Spectral characteristics for a representative case are given in Table 2-3 (#6).

Generalizations can be made regarding the time dependence of the DPPC-H$_2$TPyP-1 vesicle absorption spectra:

i) The extinction coefficients of the Soret and visible bands decreased by 5-10% during the first 100 minutes following sonication, and by similar amounts during the next 1000 minutes.

ii) The intensities of the bands at 555, 590, and 650 nm increased relative to the band at 520 nm. These changes were more pronounced in the unbuffered samples than they were in the buffered ones.

iii) The peak positions of the bands shifted to longer wavelengths. The shifts during the first 1000 minutes were about 1 nm in the ammonium acetate buffered samples and 5 nm or less in the unbuffered and phosphate buffered samples.

iv) The Soret band widths increased. The width at half-maximum intensity increased 15-20% during 1000 minutes in the unbuffered sample and 5-10% in the buffered samples.

The Soret bands in the spectra of samples prepared in
phosphate buffer often had prominent shoulders at around 450 nm which were not obvious in the spectra of the samples prepared in water or ammonium acetate buffer.

There were time-dependent changes in the fluorescence spectra which paralleled the changes in absorption. The fluorescence intensities decreased and the band maxima near 650 nm shifted to longer wavelengths and became broader with time. The wavelength shifts were comparable in magnitude to accompanying shifts in the Soret absorption bands.

The absorption and emission spectra of H$_2$TPyP-1 dissolved in egg PC vesicle suspensions, and the time-dependence of the spectra, depended on the egg PC-to-porphyrin molar ratio. Samples with molar ratios of 5:1 and 50:1 were examined. The spectra of the 50:1 molar ratio vesicles shown in Fig. 2-5 ($^7$ of Table 2-3) closely resembled the organic solution spectra with respect to extinction coefficients, relative intensities of the bands, and band widths. The wavelengths of the band maxima for the vesicle samples were shifted several nanometers to lower energies relative to the maxima for the organic solutions; the magnitudes of the band shifts decreased as the energies of the bands decreased.

The spectra of the 5:1 molar ratio vesicles differed from the 50:1 molar ratio vesicles mainly in the Soret band region. The extinction coefficient of the Soret band at 420 nm was 35% less and the band width was 20% greater when the
Figure 2-5. Absorption (solid line) and fluorescence (broken line) spectra of H$_2$TPyP-1 dissolved in egg PC vesicles with an egg PC:H$_2$TPyP-1 molar ratio of 50:1. Porphyrin concentration = 6.7 x 10$^{-6}$ M. Path length = 1.00 cm. Buffer = 0.05 M NaCl, 0.005 M NH$_4$OAc, pH = 6.8.
ratio was 5:1 than when it was 50:1 and there were redshifts in the band maxima of about 1 nm. There were clear changes in the absorption spectrum of the 5:1 molar ratio sample after 6 hours following preparation; the absorbance of the Soret band decreased by 12% and a shoulder near 450 nm appeared (Table 2-3, #8 and #9). By comparison, the spectrum of the 50:1 molar ratio vesicle suspension was practically unaffected by storage for 6 hours.

The fluorescence intensity at 650 nm was about 70% less when the egg PC:porphyrin molar ratio was 5:1 than when the ratio was 50:1.

The absorption spectrum of 5:1 molar ratio egg PC:H₂TPyP-1 vesicles that had been stored refrigerated (°C) for 4 weeks, before and after addition of HCl, is shown in Fig. 2-6. Compared to the spectrum of the freshly prepared sample, the absorbance at 420 nm had dropped to about 85% of its original value and a small shoulder at about 450 nm had appeared. More dramatically, the absorption contributed by H₂TPyP-1 in the ultraviolet region between 230 and 270 nm had increased about 20-fold. The addition of HCl to the sample (to a pH of about 1.5) caused the absorbance at 256 nm to increase by a factor of 1.8 while the shoulder near 450 nm became less distinct, with a concurrent increase in absorbance at 420 nm. A drop in the sample turbidity due to HCl addition was evidenced by a 50% decrease in the absorbance at 750 nm, where the porphyrin does not absorb. A
Figure 2-6. Absorption spectrum of H$_2$TPyP-1 dissolved in a 1 month old vesicle suspension composed of H$_2$TPyP-1 and egg PC with an egg PC:H$_2$TPyP-1 molar ratio of 5:1. Solid line: pH = 6.8. Broken line: after acidification with 12 M HCl to pH = 1.4. The left-hand panel shows the ultraviolet region on an expanded wavelength scale. The vesicle suspension was stored at 4 °C. Porphyrin concentration = 6.7 x 10$^{-6}$ M. Path length = 1.00 cm.
subsequent addition of NaOH to the sample reversed the effect of HCl on the spectrum.

H₂TPyP-1 dissolved readily in 1.0 M aqueous HCl, giving a bright green solution with a weak luminescence or none at all when viewed under a "black light". The absorption spectrum had a Soret band at 444 nm and a progression of four visible bands whose peak intensities increased with increasing wavelength (Table 2-3, #10), which is opposite to the trend shown by the porphyrin in neutral media.

The absorption and fluorescence spectra of H₂TPyP-1 dispersed in water had a marked pH dependence. To determine the pH-dependence, the porphyrin was dissolved in 0.10 M HCl and the pH was raised by adding 1.0 M NaOH. The absorption spectra at pH 1.6, 2.1, and 10.8 are shown in Fig. 2-7, and the spectra at pH 2.1 and 10.8 are summarized in Table 2-3 (#11 and #12). The colloidal nature of the sample at pH 10.8 was apparent from the turbidity of the sample; the absorbance at 446 nm dropped by 15% in 1 hour. The fluorescence intensity at 650 nm dropped by 55% and the peak shifted from 651 to 654 nm when the pH was raised from 2.1 to 10.8. An emission band with a maximum at 618 nm with about 20% the height of the 650 nm band was present at pH 10.8, but was absent in the fluorescence spectrum of the sample at pH 2.1. The excitation spectrum for the fluorescence at 654 nm when the pH was 10.8 had peaks at about 450 nm (shoulders at 420 and 460 nm), 525, 560, and 595 nm.
Figure 2-7. pH dependence of the absorption spectrum of H$_2$TPyP-1 in aqueous 0.1 M HCl-NaCl. The pH was raised from its initial value in 0.1 M HCl by the addition of aqueous 1 M NaOH. Porphyrin concentration = 7 x 10$^{-6}$ M. Path length = 1.00 cm.
Unfortunately, the intensity of the emission at 618 nm was too low to obtain a meaningful excitation spectrum for that band. The excitation spectrum for fluorescence at 651 nm from the pH 1.6 sample had maxima at 517, 550, and 587 nm which corresponded to the band maxima in the absorption spectrum of the sample.

A sample with absorption and emission patterns like those of the sample at pH 10.8 was obtained by injecting a solution of H$_2$TPyP-1 in ethanol into a 0.05 M NaCl aqueous solution buffered at pH 6.8 with 0.005 M ammonium acetate. The absorption spectrum is reproduced in Fig. 2-8 and summarized in Table 2-3 (§13). The absorption band intensities decreased as time passed.

2.3.1.3. Cu(II)TPyP-1 IN VARIOUS SOLVENTS

The absorption spectrum of Cu(II)TPyP-1 (perchlorate salt) dissolved in organic solvents, and in aqueous dispersions with detergent micelles or egg PC vesicles had an intense ($\varepsilon > 10^5$ M$^{-1}$cm$^{-1}$) Soret band and two weaker visible bands. The lower wavelength visible band appeared as a shoulder on the other; in addition, there was a third, weaker visible band near 500 nm. In general, the Soret band of Cu(II)TPyP-1 was more sensitive to changes in solvent than were the visible bands. The solvent dependence of the Soret band is summarized in Table 2-4.

The absorption spectrum of Cu(II)TPyP-1 dissolved in egg PC vesicles depended on the egg PC:porphyrin molar
Figure 2-8. Absorption spectrum of $\text{H}_2\text{TPyP-1}$ dispersed in neutral aqueous buffer by the ethanol-injection method. Buffer = 0.05 M NaCl, 0.005 M NH$_4$OAc, pH = 6.8. Porphyrin concentration = $7.9 \times 10^{-6}$ M, ethanol concentration = 0.4% (by volume). Path length = 1.00 cm.
Table 2-4. Soret absorption band of Cu(II)TPyP-1 dissolved in various solvents

<table>
<thead>
<tr>
<th>Solvent</th>
<th>$\lambda_{\text{max}}$ (nm)</th>
<th>$\varepsilon$ (mM$^{-1}$cm$^{-1}$)</th>
<th>Width$_{1/2}$ (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>methanol</td>
<td>414</td>
<td>230</td>
<td>22</td>
</tr>
<tr>
<td>vesicles$^a$, 100:1</td>
<td>416</td>
<td>280</td>
<td>17</td>
</tr>
<tr>
<td>*</td>
<td>416</td>
<td>210</td>
<td>20</td>
</tr>
<tr>
<td>micelles$^b$, pH 1.15</td>
<td>425</td>
<td>200</td>
<td>29</td>
</tr>
<tr>
<td>*</td>
<td>423</td>
<td>200</td>
<td>29</td>
</tr>
<tr>
<td>*</td>
<td>422</td>
<td>200</td>
<td>28</td>
</tr>
<tr>
<td>*</td>
<td>421</td>
<td>200</td>
<td>27</td>
</tr>
<tr>
<td>*</td>
<td>418</td>
<td>210</td>
<td>25</td>
</tr>
<tr>
<td>*</td>
<td>417</td>
<td>220</td>
<td>23</td>
</tr>
<tr>
<td>*</td>
<td>416</td>
<td>240</td>
<td>22</td>
</tr>
<tr>
<td>water$^c$, pH 1.20</td>
<td>424</td>
<td>190</td>
<td>26</td>
</tr>
<tr>
<td>*</td>
<td>423</td>
<td>190</td>
<td>26</td>
</tr>
<tr>
<td>*</td>
<td>424</td>
<td>170</td>
<td>26</td>
</tr>
<tr>
<td>*</td>
<td>424</td>
<td>160</td>
<td>26</td>
</tr>
<tr>
<td>*</td>
<td>422</td>
<td>90</td>
<td>38</td>
</tr>
<tr>
<td>*</td>
<td>424</td>
<td>50</td>
<td>55</td>
</tr>
<tr>
<td>*</td>
<td>424</td>
<td>100</td>
<td>29</td>
</tr>
<tr>
<td>water</td>
<td>411</td>
<td>80</td>
<td>54</td>
</tr>
</tbody>
</table>

$^a$ Egg PC vesicles in 0.050 M NaCl, 0.0050 M NH$_4$OAc, pH 6.5; molar ratio egg PC:porphyrin
$^b$ 0.0090 M DHAPS in 0.10 M HCl, titrated with 1.0 M NaOH
$^c$ In 0.10 M HCl, titrated with 1.0 M NaOH
$^d$ Reacidified with 1.0 M HCl
ratio. Decreasing the ratio from 100:1 to 10:1 caused a 25% drop in the peak extinction coefficient and a 15% increase in the width of the Soret band, without shifting the peak position. Parallel changes occurred in the main visible band at 542 nm, but they were of smaller relative magnitude than were the changes in the Soret band.

The absorption spectrum of Cu(II)TPyP-1 dissolved in an aqueous solution of a zwitterionic detergent (DHAPS) depended on the pH in the range 1 to 11. The spectra at three pH's are reproduced in Fig. 2-9. The band pattern itself was unchanged, but the Soret peak blue-shifted from 425 to 416 nm while the band width dropped, and parallel changes occurred in the main visible band (550 to 542 nm). The peak extinction coefficient of the Soret band remained constant up to pH 2.2, then it increased (Table 2-4).

The spectrum of the porphyrin dispersed alone into water (containing 0.1 M hydrogen/sodium chloride) without detergent was also pH-dependent, but the effect of pH differed greatly from its effect on the spectrum in the presence of detergent. The spectra at three pH's are shown in Fig. 2-10. In the absence of detergent, there was little change of the peak positions while the shape of the spectrum was markedly affected by pH. From pH 1.2 to 2.2, the Soret band width was constant and the extinction coefficient dropped slightly (Table 2-4). Above pH 2.2, shoulders appeared on the Soret band, so the half-width increased, and
Figure 2-9. pH dependence of the absorption spectrum of Cu(II)TPyP-1 in aqueous detergent solution containing 0.1 M HCl-NaCl. The detergent was the zwitterionic DHAPS, whose concentration was 0.009 M. The pH was raised from its initial value of 1.15 by the addition of aqueous 1 M NaOH. Porphyrin concentration = 6 x 10^{-6} M. Path length = 1.00 cm.
Figure 2-10. pH dependence of the absorption spectrum of Cu(II)TPyP-1 in aqueous 0.1 M HCl-NaCl. The pH was raised from its initial value of 1.2 by the addition of aqueous 1 M NaOH. Porphyrin concentration = 6 x 10^-6 M. Path length = 1.00 cm.
the extinction coefficient at 424 nm dropped sharply. The samples became increasingly turbid with increasing pH, as reflected by the increases in absorbance above 650 nm, where Cu(II)TPyP-1 itself was transparent (Fig. 2-10). There were slow decreases in the absorbancies of the turbid samples with time. Reacidification of the sample from pH 2.9 to 2.2 partially restored the original spectrum at that pH (Table 2-4).

Dispersion of the porphyrin into pure water (with no salt) via injection of its dimethylformamide solution gave an opalescent, nearly clear sample whose Soret band peaked at 411 nm which had a prominent shoulder near 390 nm. The main visible band peaked at 549 nm (ε = 9000 M⁻¹cm⁻¹).

2.3.1.4. Mn(III)TPyP-1 IN VARIOUS SOLVENTS

The absorption spectra of Mn(III)TPyP-1 dissolved in either 50-50% (by volume) ethanol-dimethylformamide mixed solvent, in egg PC vesicles, or in water had similar patterns, differing mainly in the positions of the band maxima. The extinction coefficients of the porphyrin dispersed in water were uniformly considerably smaller than in the other two solvent systems, but the band peak positions and their relative heights were close to those of the ethanol-dimethylformamide solution. The spectra are compared in Table 2-5. The absorption spectrum of Mn(III)TPyP-1 dissolved in egg PC vesicles, in which the egg PC:porphyrin molar ratio was 30:1, is reproduced in Fig. 2-11.
Table 2-5. Absorption spectrum of Mn(III)TPyP-1 (bromide)

In ethanol–dimethylformamide (50–50%, by volume)

| \( \lambda_{\text{max}} \) (nm) | 325 | 373 | 396 | 462 | 507 | 561 | 595 | 680 | 770 |
| \( \varepsilon \) (mM\(^{-1}\)cm\(^{-1}\)) | sh  | 40  | 41  | 130 | 6.2 | 12  | sh  | 1.7 | 1.1 |
| width\(_{1/2}\) (nm) | 16  |     |     |     |     |     |     |     |     |

In egg PC vesicles\(^a\)

| \( \lambda_{\text{max}} \) (nm) | 324 | 371 | 391 | 459 | 510 | 566 | 600 | 675 | 760 |
| \( \varepsilon \) (mM\(^{-1}\)cm\(^{-1}\)) | 30  | 34  | 34  | 130 | 6.2 | 11  | sh  | 1.5 | 1.1 |
| width\(_{1/2}\) (nm) | 18  |     |     |     |     |     |     |     |     |

In water (2.2% dimethylformamide)

| \( \lambda_{\text{max}} \) (nm) | 325 | 375 | 397 | 462 | 508 | 561 | 590 | 683 | 765 |
| \( \varepsilon \) (mM\(^{-1}\)cm\(^{-1}\)) | sh  | 32  | 33  | 78  | 4.7 | 8.6 | sh  | 1.0 | 0.9 |
| width\(_{1/2}\) (nm) | 19  |     |     |     |     |     |     |     |     |

\(^a\) Egg PC:porphyrin molar ratio = 30:1; the aqueous phase was buffered at pH 8.3 with 0.225 M (Na,K)SO\(_4\) and 0.0125 M Na\(_4\)P\(_2\)O\(_7\)
Figure 2-11. Absorption spectrum of Mn(III)TPyP-1 dissolved in egg PC vesicles with an egg PC:porphyrin molar ratio of 30:1. Porphyrin concentration = $2 \times 10^{-5}$ M. Path length = 1.00 cm. Buffer = $0.225 \text{ M } (\text{Na, K})\text{SO}_4$, $0.0125 \text{ M } \text{Na}_4\text{P}_2\text{O}_7$, pH = 8.3.
The color of micellar detergent solutions containing the zwitterionic detergent DHAPS and Mn(III)TPyP-1 changed from yellow-brown to pale green when the solution was made basic (from pH 4.4 to 10). The absorption spectrum shifted from one that resembled the ones in Table 2-5 to one with a blue-shifted and broader Soret band ($\lambda_{\text{max}} = 418$ nm, $\varepsilon = 5.3 \times 10^4$ M$^{-1}$cm$^{-1}$) and red-shifted visible bands (maxima at 576 and 615 nm).

2.3.2. ADDITION OF ANTHRAQUINONE-2-SULFONATE TO H$_2$TPyP-1

2.3.2.1. IN DPPC VESICLE SUSPENSIONS

Anthraquinone-2-sulfonate (AQS) perturbed the absorption spectrum and quenched the fluorescence of H$_2$TPyP-1 dissolved in DPPC vesicle suspensions. The Soret band of the porphyrin was particularly affected by AQS. Three general statements can be made about this system: i) AQS affected both the absorption and fluorescence of the samples at low concentrations ($10^{-6}$-$10^{-5}$ M), when the AQS:H$_2$TPyP-1 molar ratio was less than 1. ii) The ratio of fluorescence intensity at 653 nm in the presence of AQS to the intensity in the absence of AQS had a limiting value between 0.25 and 0.30. iii) Under some conditions, porphyrin species with Soret band maxima at 445 nm were formed.

As a result of adding AQS to previously prepared DPPC vesicles containing H$_2$TPyP-1 (DPPC:porphyrin molar ratio = 10:1, DPPC concentration = $1 \times 10^{-4}$ M), the most obvious spectral perturbations were i) a decrease in the Soret band
height, i) a decrease in the fluorescence intensity at 653 nm, and iii) shifts of the Soret and visible absorption bands to longer wavelengths by several nanometers. For example, when $2 \times 10^{-5}$ M AQS was added to vesicles prepared in 0.010 M ammonium acetate buffer (pH 6.5), the Soret band shifted from 422 to 425 nm, the peak height dropped by 40%, the band width at half-height increased by 35%, and the fluorescence intensity at 655 nm decreased by 75%. The sample turbidities also increased.

The spectral perturbations induced by AQS were more pronounced when AQS was present during vesicle formation than when it was added afterwards. Five vesicle suspensions containing $1.9 \times 10^{-5}$ M $H_2TPyP-1$ and $1.0 \times 10^{-4}$ M DPPC were prepared by sonicating the lipids in 0.10 M ammonium acetate (pH 6.6) containing either 0, $0.2 \times 10^{-5}$, $0.5 \times 10^{-5}$, $1.0 \times 10^{-5}$, or $10 \times 10^{-5}$ M AQS. The absorption and fluorescence spectra of the samples are summarized in Table 2-6; spectra of the first four samples are shown in Fig. 2-12. In the absence of AQS, the Soret band of $H_2TPyP-1$ peaked at 424 nm. In the presence of $0.5 \times 10^{-5}$ M AQS, the Soret band peaked at 444 nm and the fluorescence at 653 nm was quenched to 28% of its value without AQS. At an intermediate concentration of $0.2 \times 10^{-5}$ M, the porphyrin had a split Soret band with maxima near 440 and 425 nm (Fig. 2-12). Increasing the concentration of AQS above $0.5 \times 10^{-5}$ M had little further effect on the absorption and fluorescence of the porphyrin.
Table 2-6. Effect of anthraquinone-2-sulfonate on the absorption and fluorescence of H$_2$TPyP-1 in DPPC bilayer suspensions$^a$

<table>
<thead>
<tr>
<th>AQS conc., M $\times 10^5$</th>
<th>Absorption $\lambda_{\text{max}}$, nm</th>
<th>Fluorescence $\lambda_{\text{max}}$, nm</th>
<th>$F/F_0^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>424$^c$</td>
<td>518 555 592 648 653</td>
<td>1.00</td>
</tr>
<tr>
<td>0.2</td>
<td>441$^d$</td>
<td>524 561 595 650 653</td>
<td>0.43</td>
</tr>
<tr>
<td>0.5</td>
<td>444$^e$</td>
<td>526 562 596 650 654</td>
<td>0.28</td>
</tr>
<tr>
<td>1.0</td>
<td>443</td>
<td>526 562 596 650 653</td>
<td>0.29</td>
</tr>
<tr>
<td>10</td>
<td>445</td>
<td>527 563 598 650 654</td>
<td>0.27</td>
</tr>
</tbody>
</table>

$^a$ Sonicated suspensions containing $1.8 \times 10^{-5}$ M H$_2$TPyP-1 and $1 \times 10^{-4}$ M DPPC. AQS was added to the aqueous phase (0.1 M NH$_4$OAc, pH 6.6) before sonication.

$^b$ Peak fluorescence intensity relative to the intensity in the absence of AQS. Excitation was at 590 nm and slit widths were 8 nm.

$^c$ $\varepsilon = 6.9 \times 10^4$ M$^{-1}$cm$^{-1}$, half-width = 39 nm

$^d$ Shoulder at about 430 nm

$^e$ $\varepsilon = 7.1 \times 10^4$ M$^{-1}$cm$^{-1}$, half-width = 34 nm
Figure 2-12. Absorption (solid lines) and fluorescence (broken lines) spectra of H₂TPyP-1 dissolved in DPPC vesicles prepared in aqueous buffer containing 0, 0.2, 0.5, or 1.0 mM AQS. The relative fluorescence intensities are indicated by the signal heights. The DPPC:porphyrin molar ratio was 5.6:1. Porphyrin concentration = 1.8 x 10⁻⁵ M. Buffer = 0.1 M NH₄OAc, pH = 6.6.
In another experiment, a vesicle suspension containing $8 \times 10^{-6}$ M $H_2TPyP-1$ and $4 \times 10^{-5}$ M DPPC was prepared by the ethanol injection method into a hot (60 °C) aqueous solution of $4 \times 10^{-6}$ M AQS, 0.05 M NaCl, and 0.005 M ammonium acetate (pH 6.8). The vesicle suspension was quickly cooled to room temperature, and the absorption spectrum was monitored as a function of time while the sample was kept at 23 °C. 10 minutes after vesicle preparation, the Soret maximum was at 424 nm. After 40 minutes, the Soret band had two maxima (428 and 443 nm) of roughly equal absorbances. After 70 minutes, the Soret band peaked at 444 nm and had a shoulder at about 430 nm. The shape of the band remained that way after 3 hours. Subsequent incubation of the suspension at 47 °C for 15 minutes caused the absorbance of the shoulder to increase at the expense of the band at 444 nm, so two peaks were present (422 and 444 nm). An additional 15 minutes at 48 °C left the spectrum unchanged.

2.3.2.2. IN EGG PC VESICLE SUSPENSIONS

Changes effected by AQS on the absorption spectrum of $H_2TPyP-1$ dissolved in egg PC vesicle suspensions were qualitatively like the changes in DPPC vesicle suspensions. The effectiveness of a given concentration of AQS to reduce the height of the Soret peak at 420 nm, and to quench the porphyrin fluorescence at 650 nm, was greater when the egg PC:porphyrin molar ratio was 5:1 than when it was 50:1.

Two suspensions of vesicles were prepared with the same
concentration of H₂TPyP-1 (7.2 x 10⁻⁶ M) but 10-fold different egg PC concentrations so that the egg PC:porphyrin molar ratios were 5:1 and 50:1. The buffer was 0.05 M NaCl, 0.005 M ammonium acetate (pH 6.8). The spectra of the suspensions and their time dependence were described previously in Section 2.3.1.2. As a result of adding 1.6 x 10⁻⁴ M AOS to the 5:1 molar ratio sample, the peak absorbance of the Soret band dropped by 40% and a pronounced shoulder near 450 nm appeared, the Soret and visible band maxima shifted by 3-8 nm to longer wavelengths, and the fluorescence intensity at 650 nm dropped by 76%. In addition, the optical density at 800 nm, which reflected the turbidity of the suspension, doubled. When 3.8 x 10⁻⁴ M AOS was added to the 50:1 molar ratio sample, the Soret band absorbance dropped by 30%, the Soret and visible bands red-shifted by 2-6 nm, and the fluorescence intensity at 650 nm dropped by 64%. The optical density at 800 nm was unaffected.

Stern-Volmer plots for the quenching of the fluorescence of H₂TPyP-1 at 650 nm by AOS in the vesicle suspensions curved downward, that is, the initial slopes were the highest. The curves for vesicles with 5:1 and 50:1 molar ratios are plotted in Fig. 2-13. The AOS concentration required to quench half of the fluorescence intensity ([Q]₁/₂) was 1 x 10⁻⁵ when the molar ratio was 5:1 and 9 x 10⁻⁵ M when it was 50:1. By comparison, [Q]₁/₂ was 2 x 10⁻³ M when the system was homogeneous (60-40% (by volume) acetato-
Figure 2-13. Stern-Volmer plots for the quenching of the fluorescence of H₂TPyP-1 by AQS in a homogeneous solution and two egg PC vesicle suspensions. Also shown are the concentrations of AQS required to reduce the fluorescence intensity to half its value without AQS ([Q₁/₂]). Squares: Homogeneous solution. Solvent = 60-40% (by volume) acetonitrile-water. Triangles: Vesicle suspension; egg PC:H₂TPyP-1 = 50:1; egg PC conc. = 3.6 x 10⁻⁴ M. Circles: Vesicle suspension; egg PC:H₂TPyP-1 = 5:1; egg PC conc. = 3.6 x 10⁻⁵ M. AQS was added to the pre-formed vesicles. Buffer = 0.05 M NaCl, 0.005 M NH₄OAc, pH = 6.8.
Quenching of mono n-alkyl tetrapyridyl porphyrin fluorescence by anthraquinone-2-sulfonate vesicles.

\[ \frac{[Q]}{[Q]_{1/2}} = 1 \times 10^{-5} \text{ M} \]

\[ [Q]_{1/2} = 9 \times 10^{-5} \text{ M} \]

\[ [Q]_{1/2} = 2 \times 10^{-3} \text{ M} \]

60% CH₃CN - 40% H₂O

Vesicles, phospholipid:porphyrin = 50:1

Vesicles, phospholipid:porphyrin = 5:1
nitrile-water) and the Stern-Volmer plot was linear (Fig. 2-13).

2.3.2.3. IN WATER

The addition of AQS to samples of H₂TPyP-1 dispersed alone in aqueous electrolyte solutions, without detergents or PC, had relatively little effect on the absorption and fluorescence of the porphyrin.

AQS (3 x 10⁻⁵ and 9 x 10⁻⁵ M) was added to a dispersion of H₂TPyP-1 (6 x 10⁻⁶ M) in 0.1 M NaCl at pH 10 (prepared by titration, Section 2.3.1.2). The fluorescence intensity at 653 nm was unchanged (± 6%) when 9 x 10⁻⁵ M AQS was added, and the absorption spectrum of the porphyrin did not change when 3 x 10⁻⁵ M AQS was added. Similarly, the absorption spectrum of the porphyrin dispersion (7 x 10⁻⁶ M) prepared by ethanol injection (Fig. 2-8) was unaffected by the addition of up to 1 x 10⁻⁴ M AQS, and the fluorescence at 654 nm was quenched by 11%, 14%, and 14% when the AQS concentrations were 0.2 x 10⁻⁴, 0.5 x 10⁻⁴, and 1.0 x 10⁻⁴ M, respectively.

2.3.3. CAPTURE OF Mn(III)TPyP-4-CH₃ BY EGG PC VESICLES

Mn(III)TPyP-4-CH₃ was captured by egg PC vesicles when a solution of the porphyrin and egg PC in ethanol-dimethylformamide was added to stirred aqueous buffer (Section 2.2.3.4). Capture of the porphyrin was tested by gel-filtration of the vesicle suspension twice over Sephadex G-25, once soon after the vesicles were prepared and the
second time 1 hour after the first gel-filtration. The gel bed in the first column was uniformly colored after the vesicle fraction had eluted. (The adsorbed porphyrin was not readily eluted with buffer solution.) All of the porphyrin in the vesicle fraction remained with the vesicles during the second gel-filtration, and the gel bed remained colorless.

Estimation of the fraction of MnTPyP-4-CH$_3$ that had been captured by the vesicles was complicated by the fact that the absorption spectrum of the porphyrin dissolved in the buffer or in organic solvents was atypical of a manganese(III)-porphyrin, while the spectrum of the porphyrin associated with the gel-filtered vesicle fraction resembled the spectrum of Mn(III)TPyP-1 dissolved in egg PC vesicles (e.g., Fig. 2-11). The MnTPyP-4-CH$_3$ sample used was a powder obtained from Prof. E. B. Fleischer that was originally Mn(III)TPyP-4-CH$_3$ perchlorate and was between 5 and 15 years old (personal communication). It was soluble in water, dimethylformamide, and acetone, and was insoluble in chloroform, tetrahydrofuran, and ethyl acetate. The spectrum of the porphyrin in aqueous buffer had a Soret band peak at 439 nm, in contrast to the usual "split Soret band" pattern of manganese(III)-porphyrins (Boucher, 1972).

The fraction of MnTPyP-4-CH$_3$ that had been captured by the vesicles was estimated by assuming that the molecular weight of the solid was that of [Mn(III)TPyP-4-CH$_3$]-
(ClO₄)₄·3H₂O (1300 g/mole) and that the extinction coefficient at 460 nm for the vesicle-captured porphyrin was the same as that for Mn(III)TPyP-1 dissolved in egg PC vesicles (1.3 x 10⁵ M⁻¹cm⁻¹), so that both the number of moles of porphyrin added to the system and the number that remained after gel-filtration could be calculated. Doing so, it was found that approximately 10% of the MnTPyP-4-CH₃ had been captured by the egg PC vesicles.

2.3.4. PHOTOCHEMISTRY

2.3.4.1. EPR OF H₂TPyP-1 IN EGG PC BILAYERS [with Anne McGuire]

Visible illumination of bilayer suspensions containing egg PC and H₂TPyP-1 (molar ratio 30:1) gave rise to an EPR signal with a g-value near 2.00, whether or not AQS was dissolved in the suspensions. The light and dark spectra (first derivative) are shown in Fig. 2-14. There was no EPR signal from unilluminated samples. In the absence of AQS, the light-induced signal was 6.5±0.5 gauss wide (peak-to-peak). Light-dark cycles showed that the signal reversibly appeared and disappeared. In the presence of 4.4 x 10⁻³ M AQS, the signal was narrower, 3.7±0.5 gauss, and asymmetric (Fig. 2-14). The kinetics of the signal decay in a sample containing 2.0 x 10⁻³ M AQS was examined by flash photolysis (Fig. 2-15). The signal appeared simultaneously with the flash (12 s duration) and disappeared with a half-time of 6 ms. A second-order plot for the signal decay (signal⁻¹ vs.
Figure 2-14. Visible light-induced EPR signals in anaerobic aqueous egg PC bilayer suspensions containing H$_2$TPyP-1 without (solid line) or with (dash-dotted line) 4.4 x 10^{-3} M AQS. Also shown is the baseline signal of an unilluminated sample (dashed line). The illumination was with continuous visible light (>370 nm). Egg PC:porphyrin molar ratio = 16:1. Porphyrin concentration = 2.8 x 10^{-3} M. Buffer = 0.05 M NaCl, 0.005 M NH$_4$OAc, pH = 6.5. Microwave frequency = 9.421 GHz. Modulation amplitude = 5 gauss. Time constant = 0.5 s. Receiver gain = 6.3 x 10^4. Microwave power = 25 milliwatts.
Figure 2-15. Rise and decay of the flash light-induced EPR signal at 3386 gauss in an anaerobic aqueous egg PC bilayer suspension containing H$_2$TPyP-1 and 2 x 10$^{-3}$ M AQS. The signal is the average for 250 flashes. The xenon arc flash lamp was filtered to pass only visible light (>370 nm). Other conditions were as given in Fig. 2-14.
time) was linear.

2.3.4.2. Zn(II)TPyP-1 IN DODECYL-1-SULFATE MICELLES

Matsuo et al. (1976) examined the photosensitized reduction of AQS with ascorbic acid using Zn(II)TPP solubilized in SDS micelles as photosensitizer. We compared Zn(II)TPP and the surfactant analogue Zn(II)TPyP-1 in this role (Calvin, 1979).

AQS was reduced when anaerobic 0.05 M SDS solutions containing 5 x 10^{-4} M AQS, 0.010 M ascorbic acid, and either of the Zn(II)-porphyrins were illuminated with visible light (370-600 nm). The solutions were buffered at pH 4.2 with 0.05 M sodium acetate. The reduction of AQS was indicated by increases in absorbance below 500 nm. The peak position of the product was 380 nm, which corresponds to the hydroquinone of AQS (ε = 6700 M^{-1}cm^{-1} (Cooper, 1966)). The initial absorption spectra of the samples were restored when air was admitted due to the oxidation of reduced AQS by O_2.

The quantum yield of AQS fell as the hydroquinone accumulated. The initial quantum yield of quinone reduction was approximately 1.8 times greater with Zn(II)TPyP-1 as photosensitizer than with Zn(II)TPP. This difference might reflect a greater accessibility of Zn(II)TPyP-1 to AQS and ascorbic acid compared to Zn(II)TPP (Calvin, 1979).

2.3.4.3. Mn(III)TPyP-1 IN VESICLES CONTAINING VITAMIN K_1

Mn(III)TPyP-1 which was dissolved in egg PC vesicles containing vitamin K_1 quinone (VK_1) was reduced to Mn(II)-
TPyP-1 when the anaerobic vesicle suspensions were illuminated with visible light. Details of this line of experiments are considered below.

The visible absorption spectrum of a vesicle suspension composed of egg PC, VK₁, and Mn(III)TPyP-1 (egg PC:VK₁:porphyrin molar ratios were 30:2:1) was practically identical to the spectrum of vesicles without VK₁. The suspensions were prepared by adding a solution of the vesicle-forming components in a mixed ethanol-dimethylformamide solvent to stirred aqueous buffer. The final concentration of Mn(III)-TPyP-1 was 2 x 10⁻⁵ M.

Illumination with visible light (420-600 nm) of vesicle suspensions which had been gel-filtered to remove the organic solvents resulted in spectral changes typified by those in Fig. 2-16. The split Soret band pattern characteristic of Mn(III)-porphyrins (Loach and Calvin, 1963; Boucher, 1972) became less complicated, with a single band at 448 nm which was more intense than the original band at 460 nm. The visible band at 561 nm shifted to 575 nm, and its shoulder at about 600 nm shifted to 627 nm and intensified. The relatively weak absorption bands at 680 and 770 nm disappeared. There were isosbestic points at 672, 600, 568, 493, 456, and 400 nm. The quantum yield of the reaction did not depend on the pH in the range 7.0 to 8.8. Admission of air to the vesicle suspension resulted in the conversion of the absorption spectrum to its original form within minutes.
Figure 2-16. Absorption spectral changes upon photoreduction of Mn(III)TPyP-1 dissolved in egg PC vesicles containing vitamin K₁ quinone. The anaerobic vesicle suspension was illuminated with continuous visible light (420-600 nm). Cumulative illumination times in minutes: 0 (solid line); 2 (dash-dotted line); 4 (long-dashed line); 8 (short-dashed line). Egg PC:porphyrin:quinone molar ratios = 30:1:2. Porphyrin concentration = 1.3 x 10⁻⁵ M. Path length = 1.0 cm. Buffer = 0.225 M (Na,K)SO₄, 0.0125 M Na₄P₂O₇, pH = 8.8.
The changes in the absorption spectrum of Mn(III)TPyP-1 during illumination can be attributed to reduction of the porphyrin to Mn(II)TPyP-1 because: i) Chemical reduction of Mn(III)TPyP-1 dissolved in egg PC vesicles with sodium borohydride results in similar spectral changes (see below). ii) The spectral changes are similar to those observed when Mn(III)-porphyrins are reduced to Mn(II)-porphyrins (Loach and Calvin, 1963; Boucher, 1972; Harriman and Porter, 1979). iii) The regeneration of Mn(III)TPyP-1 when air is introduced into the cuvette is consistent with the porphyrin having been a Mn(II)-porphyrin (Loach and Calvin, 1963; Boucher, 1972; Harriman and Porter, 1979).

In a particular experiment at pH 8.8 (Fig. 2-16), the Mn(III)-to-Mn(II) conversion was complete after 6 minutes of illumination. Further illumination of the vesicle suspension for 2 minutes did not lead to further spectral changes. At this point in the experiment, $3 \times 10^{-4}$ M of AOS was added to the sample anaerobically. The absorption spectrum of the manganese porphyrin was unchanged (except for dilution), but the absorbance at 330 nm, where AOS has a peak ($6000 \text{ M}^{-1}\text{ cm}^{-1}$), only increased by 0.1 (per cm of sample) instead of 1.8, which would have been the case if the AOS had not been altered by the pre-illuminated sample. Subsequent illumination resulted in the growth of the absorption band at 330 nm due to AOS without changing the spectrum of the porphyrin until the increase in absorbance amounted to 1.7.
When a few grains of sodium borohydride were added in the dark to a suspension of egg PC vesicles containing Mn(III)TPyP-1 (molar ratio 30:1), the color of the suspension changed from brown to green, as in the photochemical experiments. The resultant absorption spectrum had maxima at 613 nm \((1.0 \times 10^4 \text{ M}^{-1}\text{cm}^{-1})\), 572 nm \((1.5 \times 10^4 \text{ M}^{-1}\text{cm}^{-1})\), 440 nm \((1.8 \times 10^5 \text{ M}^{-1}\text{cm}^{-1})\), 370 nm \((2.8 \times 10^4 \text{ M}^{-1}\text{cm}^{-1})\), and 327 nm \((2.8 \times 10^4 \text{ M}^{-1}\text{cm}^{-1})\), where the extinction coefficients given in parentheses are based on the assumption that the Mn(III)TPyP-1 in the sample had been converted entirely to Mn(II)TPyP-1. The admission of air eventually restored the original spectrum due to Mn(III)TPyP-1.

Spectral changes in the ultraviolet region from 220 to 300 nm which involved \(VK_1\) were observed during the photo-reduction of Mn(III)TPyP-1 in vesicles, but the fate of the quinone in the reaction could not be established based on the spectral changes alone. \(VK_1\) dissolved in ethanol had absorption maxima at 247 nm \((2.0 \times 10^4 \text{ M}^{-1}\text{cm}^{-1}, \text{shoulder near 243 nm})\), 269 nm \((1.7 \times 10^4 \text{ M}^{-1}\text{cm}^{-1}, \text{shoulder near 262 nm})\), and 329 nm \((3.3 \times 10^3 \text{ M}^{-1}\text{cm}^{-1})\). The absorption spectrum of a vesicle suspension containing egg PC, \(VK_1\), and Mn(III)TPyP-1 (molar ratios 30:2:1) had maxima at 238 and 262 nm (with a shoulder at about 270 nm) on top of a relatively large absorbance due to light scattering by the vesicles. Illumination of the vesicle suspension was carried out in a quartz cuvette with a teflon plug stopper,
instead of the usual glass cuvette with attached stopcock, so that spectral changes below 300 nm could be monitored. Concurrent with the photoinduced spectral changes in the visible region attributed to the conversion of Mn(III)TPyP-1 to Mn(II)TPyP-1, the absorption bands at 238 and 270 nm lost intensity and became rounded, while the absorbance increased around 257 nm. The absorbance changes at these wavelengths amounted to 0.15, 0.034, and 0.13 (per cm of sample), respectively. Therefore it appears that VK₁ was altered by some photochemical process, but the spectral changes associated with the reaction are uncertain because the reduction of the porphyrin contributed to the changes in an unknown way.

A vesicle suspension (pH 7.5) which had been gel-filtered was tested for O₂ evolution during illumination using a Clark-type teflon membrane covered electrode. The vesicle suspension contained p-benzoquinone-disulfonate (0.0010 M) to act as a pool of hydrogen atom acceptor. During illumination with white light for 10 minutes, the O₂ concentration in the 1 ml vesicle suspension changed by less than 10⁻⁵ M according to the recorder pen deflection, which was calibrated by replacing deaerated water with air-saturated water.

A previously illuminated vesicle suspension was tested for H₂O₂ by adding catalase and monitoring changes in O₂ levels with the Clark-type oxygen electrode. As in the
2.3.4.3  

experiment to test for O₂ production, 0.0010 M of p-benzoquinone-disulfonate was added to the water phase to act as a hydrogen atom sink. The H₂O₂ concentration in the illuminated sample was estimated to be less than 10⁻⁵ M, according to the recorder pen deflection, which had been previously calibrated with standardized H₂O₂.

2.3.4.4. MANGANESEPORPHYRINS IN METHANOL-WATER SOLUTIONS  

[with Itamar Willner]

Either Mn(III)TPyP-1 or two water-soluble manganese porphyrins, dissolved in methanol-water mixed solvent, photosensitized the reduction of methylviologen (MV²⁺) to MV⁺ when phthalic acid was present. The reduction of MV²⁺ was monitored at 603 nm (ε = 1.24 \times 10^4 \text{ M}^{-1}\text{cm}^{-1} for MV⁺ (Steckhan and Kuwana, 1974)). Mn(III)TPyP-1 was superior as photosensitizer compared to both Mn(III)TSPP and MnTPyP-4-CH₃. Phthalic acid could be replaced by diphenic acid, but the quantum yield of MV²⁺ reduction was about one-half the yield with phthalic acid. No MV⁺ (<10⁻⁶ M) was accumulated when benzoic acid, maleic acid, salicylic acid, or diphenylsulfide were used instead of phthalic acid. The results of the experiments are summarized in Table 2-7.

When Mn(III)TPyP-1 and either phthalic acid or diphenic acid were in the aqueous methanol system, the reduction of Mn(III)TPyP-1 to Mn(II)TPyP-1 preceded the accumulation of MV⁺. The reduction reaction was detected by the spectral changes described previously in Section 2.3.4.3. The pre-
Table 2-7. Photosensitized reduction of $\text{MV}^{2+}$ by Mn(III)-porphyrins in the presence of potential oxygen-atom acceptors$^a$

<table>
<thead>
<tr>
<th>porphyrin</th>
<th>$[\text{O}]$-acceptor</th>
<th>relative quantum yield of $\text{MV}^+$ production</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mn(III)TPyP-1</td>
<td><img src="image1" alt="Structure" /></td>
<td>100</td>
</tr>
<tr>
<td><img src="image2" alt="Structure" /></td>
<td></td>
<td>48</td>
</tr>
<tr>
<td><img src="image3" alt="Structure" /></td>
<td></td>
<td>&lt;2</td>
</tr>
<tr>
<td><img src="image4" alt="Structure" /></td>
<td></td>
<td>&lt;0.2</td>
</tr>
<tr>
<td><img src="image5" alt="Structure" /></td>
<td></td>
<td>&lt;0.2</td>
</tr>
<tr>
<td><img src="image6" alt="Structure" /></td>
<td></td>
<td>&lt;0.2</td>
</tr>
<tr>
<td>MnTPyP-4-CH$_3$</td>
<td><img src="image7" alt="Structure" /></td>
<td>~ 23</td>
</tr>
<tr>
<td>Mn(III)TSPP</td>
<td><img src="image8" alt="Structure" /></td>
<td>~ 10</td>
</tr>
</tbody>
</table>

$^a$ The solvent consisted of a homogeneous mixture containing methanol (2.75 ml) and aqueous 0.36 M $\text{KH}_2\text{PO}_4$ (0.25 ml, pH = 4.5). $\text{MV}^{2+}$ conc. = 0.0015 M. $[\text{O}]$-acceptor conc. = 0.03 M.
The assumption that phthalic and diphenic acids functioned as oxygen atom acceptors (to produce the peroxyacids) will be discussed in Section 2.4.8. The oxidation of Mn(II)TPyP-1 and MV⁺ with air following the experiments regenerated >95% of the Mn(III)TPyP-1, judging from the absorption spectra. When phthalic acid was used as the oxygen atom acceptor, 3.8 x 10⁻⁷ moles of MV⁺ were produced when the solution contained 3.2 x 10⁻⁸ moles of porphyrin, representing a turnover of 12 MV⁺ per Mn(III)TPyP-1.

The "blank" experiment without Mn(III)TPyP-1 was positive, that is, MV⁺ was produced when a solution of MV²⁺ in the methanol-water solvent was illuminated. The absorbance increase (per cm of sample) at 603 nm, which was proportional to the MV⁺ concentration, was 0.057 per minute of illumination. The addition of 0.050 M phthalic acid did not affect the rate of absorbance increase at 603 nm (0.055 per minute). The comparable rate in the system containing MV²⁺, phthalic acid, and Mn(III)TPyP-1 (Table 2-7) was 0.15 per minute of illumination.

The anionic porphyrin Mn(III)TSPP could be used as photosensitizer in place of Mn(III)TPyP-1, but the quantum yield of MV²⁺ reduction was about 10 times lower with Mn(III)TSPP when phthalic acid was used as oxygen atom acceptor (Table 2-7). The experiment with Mn(III)TSPP differed from the one with Mn(III)TPyP-1 in two other ways: i) the reduction of Mn(III)TSPP to Mn(II)TSPP was concurrent
with the reduction of \( \text{MV}^2+ \) and ii) \( \text{Mn(III)TSPP} \) was only partially (80%) regenerated after the experiment when the solution was exposed to air.

In contrast to the experiments with \( \text{Mn(III)TPyP-1} \) in which the reduction of the porphyrin was essentially complete before \( \text{MV}^+ \) was accumulated, when \( \text{Mn(III)TSPP} \) was the photosensitizer, \( \text{MV}^+ \) was detectable when only a small fraction of the porphyrin \( (\lambda_{\text{max}} = 467 \text{ nm}) \) had been reduced to \( \text{Mn(II)TSPP} \) \( (\lambda_{\text{max}} = 436 \text{ nm}) \). For example, when the concentrations of \( \text{MV}^+ \) were \( 3 \times 10^{-6} \) and \( 6 \times 10^{-6} \) M, the fractions of \( \text{Mn(III)TSPP} \) reduced were 5\% and 8\%, respectively. Besides the appearance of absorption bands near 400 and 600 nm belonging to the viologen radical and the band at 436 nm belonging to \( \text{Mn(II)TSPP} \), a band at 415 nm appeared during the illumination. The species responsible for the 415 nm band was stable to aeration of the sample and had absorption bands near 515 and 645 nm which were weaker than the one at 415 nm. The spectral characteristics of this product resembled those of the metal-free porphyrin \( (\text{H}_2\text{TSPP}) \), which suggested that \( \text{Mn(III)TSPP} \) had demetallated at some stage of the overall process. The fraction of \( \text{Mn(III)TSPP} \) that decomposed during the accumulation of \( 1 \times 10^{-7} \) moles of \( \text{MV}^+ \) was 20\%, based on the absorbance of the porphyrin at 460 nm before and after the experiment.

\( \text{MnTPyP-4-CH}_3 \) photosensitized the reduction of \( \text{MV}^2+ \) when phthalic acid was present with a quantum yield approximately
25% of the yield with Mn(III)TPyP-1 as photosensitizer (Table 2-7). The absorption spectrum of the porphyrin both before and after illumination was similar to its spectrum in ethanol-dimethylformamide, so, as mentioned in Section 2.3.3, the oxidation state of the manganese ion could not be assigned with certainty. Illumination of the solution first resulted in an intensification of the Soret band at 443 nm (by about 15%) without shifting the peak position, and the visible bands red-shifted several nanometers (from 566 to 569 nm and from -610 to -615 nm). Further illumination led to the accumulation of MV+. When the solution was later exposed to air, the absorption spectrum reverted to the spectrum before illumination. These results suggest that at least part of the MnTPyP-4-CH₃ was reduced photochemically and reoxidized by O₂, so the oxidation state of the Mn ion was probably not 2+.

2.4. DISCUSSION

2.4.1. ELECTRONIC SPECTRA OF PORPHYRINS

The absorption and emission spectra of the surfactant porphyrins described above were found to vary considerably in various colloidal aqueous media. Much of this discussion will be devoted to interpreting these spectral variations. The spectra reflect the electronic structures of the porphyrins and are therefore important in understanding their photochemical properties. This first section describes some general features of the spectra of porphyrins and ways that
the spectra depend on the media in which the porphyrins are dispersed.

2.4.1.1. GENERAL CHARACTERISTICS

Some peculiarities of the molecular structures of meso-tetraphenylporphyrin (H₂TPP) and analogous meso-substituted porphyrins should be pointed out before discussing their electronic absorption spectra. X-ray crystallography shows that the phenyl rings in the meso-positions are not in the same plane as the porphyrin ring, but are tilted at an angle between 40 and 85 degrees (Fleischer, 1970; Scheidt, 1978). Therefore the meso-substituents can not be considered part of the \( \pi \)-electron systems of the porphyrins (Fleischer, 1970). The porphyrin ring systems themselves are relatively flexible to deviations from planarity. When in solution, there is probably an equilibrium between planar and buckled forms of the porphyrins in which the planar conformation predominates, and there is a low energy barrier to buckling (Fleischer, 1970; Pasternack et al., 1976). Protonation of the pyrrole nitrogen atoms of H₂TPP also causes a loss of planarity of the porphyrin ring (Fleischer, 1970; Scheidt, 1978).

The basic features of the visible and near-ultraviolet absorption spectra of metal-free porphyrins and metalloporphyrins with closed-shell metal ions can be explained by a model which treats the porphyrin chromophore as a cyclic polyene (Gouterman, 1961; Gale et al., 1972;
The major bands in the electronic spectra down to 200 nm are interpreted as predominantly singlet ($\pi$, $\pi^*$) in origin. The principle resonance electronic structures for the ground states of porphyrins are conjugated rings with 18 $\pi$-electrons and either 18 or 16 atoms for the metal-free or metallated porphyrins, respectively (Gouterman, 1961; Fleischer, 1970). The 18-atom rings of metal-free porphyrins, ignoring the ring substituents and assuming planarity, have $D_{2h}$ symmetry (e.g., Fig. 2-1), while the replacement of the two centrally-located protons with a metal ion imparts a square-planar, $D_{4h}$, symmetry (Gouterman, 1961). By convention, the x-y plane is the one containing the porphyrin ring system and the x-axis in metal-free porphyrins is parallel to the inner proton axis (e.g., Fig. 2-1).

The electronic spectra of most porphyrins contain an intense band ($\varepsilon = 10^5 \text{ M}^{-1}\text{cm}^{-1}$) in the near-ultraviolet or blue visible part of the spectrum (350-450 nm) and weaker bands ($\varepsilon = 10^3-10^4 \text{ M}^{-1}\text{cm}^{-1}$) in the rest of the visible part of the spectrum (500-700 nm). The former band is known as the "Soret" or "B" band and the ones in the visible region are known as the "visible" or "Q" bands. The visible and Soret bands are due to electronic transitions from the singlet ground state to the first and second singlet excited states, respectively. The absorption bands are not single-peaked, but have associated peaks or shoulders which
are attributed to the first vibrationally excited states of the Q and B bands (Gale et al., 1972). The relative intensities of the origin of the visible band (Q(0-0)) and its vibrational component at higher energy (Q(1-0)) vary widely with different central metal ions and with the porphyrin ring substituents (Gouterman, 1978). For example, in the absorption spectrum of Cu(II)TPyP-1 dissolved in detergent solution (Fig. 2-9), Q(0-0) appears near 585 as a shoulder on its more intense vibrational component, Q(1-0), at 550 nm. The B(1-0) vibrational component of the Soret band usually appears as a weak shoulder on the B(0-0) component (Gouterman, 1978).

Metal-free porphyrins generally have twice as many Q bands as their closed-shell metallo-derivatives. The lower symmetry of the primary resonance structure of metal-free porphyrins (D$_{2h}$) compared to most metalloporphyrins (D$_{4h}$) causes the first excited state to split into two components, thus doubling the number of Q bands (Gouterman, 1961). The Q$_x$ component, whose transition moment is parallel to the proton axis, is lower in energy than the Q$_y$ component (Gale et al., 1972; Gouterman, 1973). Both the Q$_x$ and Q$_y$ bands of metal-free porphyrins have additional bands attributed to their first vibrationally excited states. Accordingly, in the absorption spectrum of H$_2$TPyP-1 dissolved in egg PC vesicles (Fig. 2-5), the visible bands at 646, 590, 550, and 514 nm are the Q$_x$(0-0), Q$_x$(1-0), Q$_y$(0-0), and Q$_y$(1-0) bands,
respectively. The $Q_x(1-0)$ and $Q_y(1-0)$ bands actually have mixed polarizations (Anex and Umans, 1964; Gale et al., 1972).

The absorption maxima of $H_2$TPP derivatives with para-substituents on the phenyl rings generally shift to longer wavelengths as the electron-donating character of the substituent increases (Meot-Ner and Adler, 1975). The red-shifts are accompanied by increases in the transition dipoles of the $Q(0-0)$ bands relative to the $Q(1-0)$ bands; the four-orbital model of Gouterman satisfactorily accounts for the substituent effects (Meot-Ner and Adler, 1975).

Absorption bands that are weaker and at higher energies than the Soret band have been labeled, in order of increasing energies, the N, L, and M bands by Gouterman (1978).

Porphyrins exhibit both fluorescence and phosphorescence. The quantum yields of the emissions from metallo-porphyrins vary widely with the metal ions (Becker and Allison, 1963a,b; Seybold and Gouterman, 1969; Gouterman, 1973; Hopf and Whitten, 1975; Gouterman, 1978). As the atomic numbers of diamagnetic metal ions, which have either empty or full d-orbitals, are increased, fluorescence yields decrease and phosphorescence yields increase. Paramagnetic metal ions, which have partly filled d-orbitals, have a quenching effect on porphyrin luminescence. In some cases this quenching is due to the presence of low energy excited
2.4.1.1 states of d-d character lying between the lowest-lying triplet π-π* excited state and the ground state of the porphyrin (Gouterman, 1978). The presence of such states can also perturb the absorption spectra of metalloporphyrins (Gouterman, 1978). As pointed out earlier, the emission bands at about 650 and 710 nm from solutions of H₂TPyP-l and H₂TCPP-l can be assigned as fluorescence due to the mirror-image relationship between these bands and the Qₓ(0-0) and Qₓ(1-0) absorption bands, and the lack of significant quenching of this emission by O₂ (Gouterman, 1978).

2.4.1.2. SPECTRAL PERTURBATIONS CAUSED BY ENVIRONMENTAL FACTORS

Porphyrin spectra are sensitive to a wide variety of environmental conditions. Five ways that the solvent environment can affect the electronic spectra of porphyrins follow: i) The central nitrogen atoms of metal-free porphyrins can become protonated or deprotonated. ii) The axial positions of metalloporphyrins can be ligated. iii) Porphyrins can form molecular complexes with other molecules. iv) A non-homogeneous distribution of porphyrins can distort their absorption spectra. v) The environment can be conducive to porphyrin self-aggregation. These effects are further considered below. In addition, the peak positions and relative intensities of porphyrin absorption and emission bands respond to changes in solvent polarity (Seely and Jensen, 1965).
Metal-free porphyrins are considered ampholytes in which two nitrogen atoms (=N-) of imino (pyrrolenine) type are each capable of accepting a proton, while the other two nitrogen atoms (HN<) of pyrrole type are each capable of donating or accepting a proton (Gurinovich et al., 1968). These acid-base reactions significantly alter the porphyrin spectra. Protonation of both of the inner ring imino nitrogen atoms to form the di-acidic porphyrins increases the symmetry of the ring system from point group D$_{2h}$ to D$_{4h}$, which is analogous to the effect of metallation. Consequently, the four-banded visible spectra of metal-free porphyrins is expected to collapse to two-banded spectra when the porphyrins are di-protonated, and this change is usually observed (Gurinovich et al., 1968; Gouterman, 1978). The deprotonated forms exist in alkaline media. The acid-base equilibria of H$_2$TPyP-4-CS$_3$ in aqueous solutions have been described by Hambright and Fleischer (1970). Acid-base titrations showed only the di-protonated/free base equilibrium (pK$_a$ = 2.2±0.2) and the free base/mono-deprotonated equilibrium (pK$_a$ = 12.9±0.2) in the pH range 0-14.

The absorption spectra of metalloporphyrins depend on the presence or absence of metal-coordinated ligands in the axial positions, and on the chemical nature of the axial ligands (Corwin et al., 1963; McLees and Caughey, 1968; Pasternack et al., 1974; Nappa and Valentine, 1978). The absorption peaks of Zn(II)-meso-tetraphenylporphyrin red-
shift and the intensity of the Q(0-0) band increases relative to the Q(1-0) band when axial ligands are added to solutions of the porphyrin in non-coordinating solvents (Nappa and Valentine, 1978). Axially coordinated water molecules can be deprotonated. Spectral changes involved in the acid-base equilibria of water coordinated to Mn(III)-porphyrins were described by Loach and Calvin (1963) and by Harriman and Porter (1979).

The absorption and emission of porphyrins are sometimes significantly altered by the presence of electron-donating or accepting molecules (Mauzerall, 1965; Cann, 1967; Heathcote et al., 1968, Barry et al., 1973). The formation of 1:1 molecular complexes was established in some cases. Complexation generally results in a broadening and red-shifting of the absorption bands of the porphyrins, and quenching of the fluorescence is usually observed.

The absorption spectra of dyes that are dispersed heterogeneously in a medium often appear "flattened" compared to their spectra in homogeneous solutions (Gledhill and Julian, 1963; Britz et al., 1977). For example, in a closed-packed array of dye molecules, complete absorption of light by the initial dye layer at a particular wavelength prevents the remaining dye molecules in the array from contributing to the absorbance at that wavelength. This effect is greatest in the spectral regions where the extinction coefficients are greatest, which results in a
rounding out of spectral features. Consequently, a particu-
late distribution of porphyrins can lead to an apparent loss
of optical density, particularly in the Soret band region.

Porphyrin self-aggregation is usually accompanied by a
perturbation of the absorption spectra. Porphyrins are
notorious for their propensity for aggregating (Blumberg and
Peisach, 1965; Bergeron et al., 1967; Boucher and Katz,
1967; Pasternack et al., 1972; Zachariasse and Whitten,
1973; Pasternack, 1973; Krishnamurthy et al., 1975; Fuhrhop,
1976; White, 1978). Theoretical treatments for the spectra
of dye aggregates predict a variety of wavelength shifts and
band broadening or narrowing upon aggregation, due to exci-
ton coupling of the transition dipoles of the monomers
(Kasha, 1963; Becker, 1969). The predicted spectral changes
depend on the strengths of the interaction and on the rela-
tive orientations of the transition moments.

Most instances of aggregation of either metal-free or
metalloporphyrins reported in the literature resulted in
either red or blue shifts of the Soret bands, and red shifts
and broadening of the visible bands, but the magnitudes of
the changes varied widely. Some examples are described
below.

The dimerization of Cu(II)-uroporphyrin III in aqueous
solution results in a 6 nm blue shift of the Soret band and
2-3 nm blue shifts of the visible bands (Blumberg and
Peisach, 1965). Polymerization of the dimers causes the
Soret band to further blue shift by 15 nm and the visible bands to red shift by 10-20 nm. EPR spectra of the dimers show that the copper ions are dipole-coupled, about 3.5 Å apart, and that many copper ions interact in the polymers.

The absorption spectra of monolayers of metal-free methyl esters of protoporphyrin IX, hematoporphyrin IX, coproporphyrin III, coproporphyrin I, and uroporphyrin III, spread at N₂-water interfaces, have Soret bands that are broadened and either red-shifted or blue-shifted, and visible bands that are red-shifted, compared to the spectra of chloroform solutions of the porphyrins (Bergeron et al., 1967). The porphyrin films have molecular areas of 50-70 Å², which are between the minimal areas of 45 and 145 Å²/molecule expected if the porphyrin planes are perpendicular or parallel, respectively, to the water-N₂ interface.

The dimerization of ethylenediamine-substituted metal-free, Zn(II), Cu(II), or Ni(II)-protoporphyrin IX in aqueous solutions results in blue shifts of the Soret bands by 12-15 nm, and red shifts of the visible bands by 2-5 nm (Das, 1975). The porphyrin monomer-dimer equilibrium is shifted toward the dimer by high porphyrin concentrations and by low temperatures, and is shifted toward the monomer by the addition of ethanol.

The synthesis of covalently linked porphyrins (Anton et al., 1976; Chang, 1977; Kagan et al., 1977; Ichimura, 1977) offers an opportunity to examine the effect of orientation
and distance of separation on the spectra of porphyrin dimers (Anton et al., 1976). The Soret band of a doubly-bridged cofacial porphyrin is blue-shifted 15 nm and the extinction coefficient per porphyrin is 44% less compared to the monomer spectrum in dichloromethane when the porphyrin-porphyrin separation is 6.4 Å (Anton et al., 1976). Further reduction of the distance between porphyrins to 4.2 Å further blue-shifts the Soret band by 2 nm with little effect on the molar extinction coefficient. The quantum yields of fluorescence are about 1/3 and 1/10 those of the monomer when the porphyrins are separated by 6.4 and 4.2 Å, respectively (Anton et al., 1976).

A quadruply-bridged cofacial derivative of metal-free meso-tetra(4-carboxyphenyl)-porphyrin with a three-atom bridge has a Soret band that is broadened (a 40% increased half-width) relative to the appropriate monomer bands, but the peak position is unshifted (Chang, 1977). The Soret band of the dimer has a prominent shoulder at around 450 nm, and the visible bands are broadened and red-shifted. Qualitatively similar differences exist between the cofacial Zn(II)-porphyrin dimer and the Zn(II)-porphyrin monomer. The fluorescence band of the metal-free dimer is red-shifted and broadened relative to the monomers, but the quantum yield is approximately the same in the dimer as in a mixture of its monomeric components.

2.4.2. ABSORPTION AND FLUORESCENCE SPECTRA OF H₂TPyP-1
2.4.2.1. COMPARED TO OTHER DERIVATIVES OF H₂TPyP-1

The absorption and fluorescence spectra of solutions of H₂TPyP and its mono-, di-, tri-, and tetra-alkylated derivatives showed definite trends with respect to the degree of alkylation (Table 2-2). The band shifts and broadenings and changes in relative band intensities were of greater magnitude in the first part of the series (from H₂TPyP to H₂TPyP-2) than in the second part (from H₂TPyP-2 to H₂TPyP-4). The shifts in peak positions to lower energies with increasing degree of alkylation contrasts with the trend observed by Meot-Ner and Adler (1975) in which peak positions shifted toward higher energies as the para-substituents of the phenyl rings of H₂TPP derivatives became better electron acceptors. Some measure of the increase in electron-accepting character of the pyridyl substituents upon quaternization is given by the fact that the reduction potential of H₂TPyP-4-CH₃ is 0.37 volts higher than that of H₂TPyP (Shamim et al., 1979).

2.4.2.2. CHARACTERIZATION OF THE 445-NM-ABSORBING SPECIES

A number of samples containing H₂TPyP-1 in aqueous media were prepared that contained forms of the porphyrin with Soret bands maxima near 445 nm. Reasons for attributing the 445 nm band of the aqueous samples to aggregates of H₂TPyP-1 will be considered below.

There were three situations that led to forms of H₂TPyP-1 with a Soret band maximum between 440 and 445 nm:
i) when the porphyrin was dispersed in water (buffer), in the absence of other surfactants (e.g., Figs. 2-7 and 2-8), ii) when DPPC and H$_2$TPyP-1 were dispersed, together with AQS, in water by sonication (e.g., Fig. 2-12), and iii) when aqueous SDS was added to a solution of H$_2$TPyP-1 in methanol (e.g., Fig. 2-4). Besides having a Soret band with a peak between 440 and 445 nm, which often had a shoulder at about 420 nm, the spectra also had a second band of lower intensity with a peak between 360 and 370 nm. The Soret band half-widths and, to a lesser extent, the positions of the visible band maxima differed from one sample to another, but, because of similarities in the spectra, it will be assumed that the same form of H$_2$TPyP-1 was present in each case.

The heating of samples in which the Soret band of H$_2$TPyP-1 had two distinct maxima caused the absorption band near 420 nm to increase in intensity at the expense of the band near 445 nm (Sections 2.3.1.2 and 2.3.2.1); the coincidence of the disappearance of the bands at 365 and 445 nm was evident (e.g., Fig. 2-4). Thus heating favors the porphyrin monomer over the aggregate. This temperature dependence is consistent with the 445-nm-absorbing species having been the porphyrin aggregate since aggregates of both chlorophyll a and a cyanine dye in phospholipid vesicles are likewise disfavored by increases in the temperature (Lee, 1975; Kurihara et al., 1977).

The H$_2$TPyP-1 species absorbing maximally at around 445
nm in egg PC vesicle suspensions was favored by increasing the porphyrin:egg PC molar ratio, i.e., by increasing the concentration of $\text{H}_2\text{TPyP-1}$ in the vesicle wall. The difference absorption spectrum obtained by subtracting the spectrum of the 50:1 egg PC:$\text{H}_2\text{TPyP-1}$ molar ratio vesicle suspension (1-3 hours old) from the spectrum of the 5:1 molar ratio suspension (7 hours old) has maxima at about 360, 450, 530, 567, and 610 nm, and minima at about 418, 513, 548, 585, and 643 nm. The positions of the minima match the maxima in the absorption spectra of $\text{H}_2\text{TPyP-1}$ under conditions where the porphyrin can be presumed to be predominantly monomeric (see Table 2-3). The absolute differences in absorbance are uncertain because the turbidities of the two samples differed, but similarities between the difference spectrum and the spectral changes associated with the conversion of the porphyrin monomer to its aggregate indicate that the vesicles with the lower egg PC:$\text{H}_2\text{TPyP-1}$ molar ratio contained a higher proportion of the porphyrin aggregate than did the vesicles with the higher molar ratio. The assignment of the 445-nm-absorbing form of $\text{H}_2\text{TPyP-1}$ to its aggregate is thus supported by the way that the spectrum of the porphyrin depends on its concentration in the vesicle wall.

A number of environmental factors can cause spectral changes like band shifts and broadenings, but, of the five factors listed in Section 2.4.1.2, all but porphyrin self-
aggregation could be eliminated in present examples. Acid-base reactions were unlikely because the samples were in most cases buffered near pH 7. Axially coordinated ligands need not be considered because $\text{H}_2\text{TPyP-1}$ is a metal-free porphyrin. The particulate nature of the samples, compared to homogeneous solutions, could account for some band broadening, but not for the shifts in band maxima. A number of components in the samples could conceivably have formed molecular complexes with $\text{H}_2\text{TPyP-1}$, but the fact that the 445-nm-absorbing form of the porphyrin was observed when the porphyrin was dispersed alone in aqueous buffer indicates that complexation by exogenous agents is not required. The assignment of the source of this spectral perturbation to aggregation, in which the porphyrin rings interacted, is consistent with the fact that these species were observed in colloidal aqueous media, in which the "local" porphyrin concentrations were relatively high. The turbidities of the aqueous dispersions of $\text{H}_2\text{TPyP-1}$ indicates the presence of colloidal particles, which were presumably composed of micellar $\text{H}_2\text{TPyP-1}$ and associated counterions.

It is surprising that aggregates of $\text{H}_2\text{TPyP-1}$ could exist in aqueous 0.10 M SDS solutions because, assuming that the aggregation number of the detergent micelles is 100 or less (Fendler and Fendler, 1975; Tanford, 1980; Lindman and Wennerström, 1980), the concentration of micelles ($>10^{-3}$ M) far exceeded the concentration of porphyrin (about $10^{-5}$ M),
so the statistical probability of having more than one porphyrin per micelle was very small. The SDS samples containing porphyrin aggregates were prepared by adding solutions of SDS in water to solutions of H$_2$TPyP-1 in methanol. Conceivably, the porphyrin aggregates were formed at the beginning of the mixing process, when the volume of the added water phase was comparable to the volume of the methanol phase and SDS micelles were not present.

A sample prepared by Okuno (1977) that had a single Soret peak at 441 nm attributed to aggregated H$_2$TPyP-1 eventually (within weeks) converted to one consistent with monomeric H$_2$TPyP-1 (e.g., #4 of Table 2-3) when the sample was stored in the dark at room temperature. Thus the aggregates were only slowly dissociated by the SDS micelles in spite of the statistical improbability of finding more than one porphyrin molecule per micelle. In the egg PC-H$_2$TPyP-1 vesicle systems examined, it was likely that most vesicles contained hundreds of porphyrin molecules. In contrast to the micellar SDS samples, the vesicular samples showed increasing proportions of aggregated porphyrin with time.

H$_2$TPyP-1 was apparently monomeric when the solution of porphyrin in methanol and the solution of SDS in water were mixed the other way, that is, by adding the methanolic solution to the aqueous one. In this case, the porphyrin was dispersed into an aqueous solution already containing SDS micelles which could solubilize the porphyrin before it
could self-aggregate. Chlorophyll-\textsubscript{a} had a similar behavior (Ford, 1975; Ford, 1976). Thus, when a solution of chlorophyll-\textsubscript{a} in acetone was diluted with aqueous SDS solution, the hydrated chlorophyll aggregate absorbing near 740 nm (Ballschmiter and Katz, 1972) was obtained, whereas addition of a solution of chlorophyll in methanol to aqueous SDS solution resulted in the monomeric form of the porphyrin.

The absorption band at about 365 nm was evidently associated with the Soret band at 445 nm in samples containing aggregated H\textsubscript{2}TPyP-1 (e.g., Figs. 2-4, 2-7, and 2-12) was possibly a second component of the aggregate Soret band, or else a component of another electronic transition such as the N-band. If the Soret band of the aggregate were split into components at 445 and 365 nm, then the exciton coupling energy between porphyrin ring systems, which equals about half the frequency separating the two Soret peaks (Chang, 1977), is about 2.3 kK. Estimates for exciton couplings in other porphyrin aggregates (Bergeron \textit{et al.}, 1967) or covalently linked porphyrin dimers (Chang, 1977) are of similar magnitude.

Band shifts in the fluorescence excitation spectra which accompanied the aggregation of H\textsubscript{2}TPyP-1 paralleled band shifts in the absorption spectra, so it is likely that the aggregates were fluorescent. The emission spectra from the aggregate samples were not much different from the
monomer spectra, except that a peak near 618 nm was often present and the main peak near 650 nm was red-shifted by 3-5 nm. The origin of the emission at 618 nm was not determined.

During the titration of H$_2$TPyP-1 in 0.1 M HCl with NaOH (Section 2.3.1.2), the intensity of the fluorescence at pH 10.8, when the porphyrin was predominantly aggregated, was not much less than the fluorescence intensity from the sample between pH 1.6 and 2.1, in which the porphyrin was predominantly monomeric, so, at least in this instance, the fluorescence quantum yields of the two forms of H$_2$TPyP-1 were approximately the same.

AQS apparently induces the aggregation of H$_2$TPyP-1 in aqueous media. AQS could be expected to form molecular complexes with H$_2$TPyP-1, as does VK$_1$ with chlorophyll a in lipid monolayer assemblies (Costa et al., 1972). The result depicted in Fig. 2-12 is reminiscent of a titration, indicating the formation of a stoichiometric complex between H$_2$TPyP-1 and AQS. The observed stoichiometry of 1 AQS per 4 H$_2$TPyP-1 seems inconsistent with a distinct molecular complex having been formed, however, but it is possible that molecular complexes between AQS and the porphyrin were intermediates in a mechanism by which AQS induced the aggregation of H$_2$TPyP-1. This hypothesis that AQS catalyzed the aggregation of H$_2$TPyP-1, but was not part of the final aggregate, is supported by the observation that the addition
of AQS to dispersions of H₂TPyP-1 in 0.1 M NaCl (pH 10), in which the porphyrin was already in an aggregated state, had little effect on the absorption and fluorescence of the porphyrin (Section 2.3.2.3).

2.4.2.3. pH DEPENDENCE

H₂TPyP-1 (Fig. 2-1) has five nitrogen atoms which can become protonated in moderately acidic media (pH 1-3), three of which are the N-atoms of the pyridine meso-substituents and the other two are the pyrrotenine N-atoms of the porphyrin ring itself. The phenyl groups of meso-tetraphenylporphyrin are not considered to be conjugated with the π-electron system of the porphyrin chromophore due to non-planarity (Fleischer, 1970), so it is reasonable to expect the same argument holds for the pyridyl groups of H₂TPyP-1. Thus the acid-base reactions of the pyridyl groups should not strongly perturb the absorption spectrum of the porphyrin.

The absorption spectrum of H₂TPyP-1 dissolved in aqueous (or methanolic) 1 M HCl (Table 2-3, #10) was similar to the spectra of the di-acidic forms of H₂TPyP (Fleischer, 1962) and H₂TPyP-4-CH₃ (Hambright and Fleischer, 1970) in which all four nitrogen atoms of the porphyrin rings are protonated. The visible absorption pattern of the di-acidic form of H₂TPyP-1 is four-banded, like that of the free-base form, but the band intensities increase with decreasing energy and the spectrum is dominated by the two lowest-
energy bands instead of the opposite trend seen in neutral media. A two-banded pattern is predicted to result from the increase in symmetry of the porphyrin chromophore from $D_{2h}$ to $D_{4h}$ (Section 2.4.1). Actually, the symmetry of the di-acidic porphyrin is probably not square planar due to buckling (Fleischer, 1970; Scheidt, 1978), but a two-banded visible pattern is still expected because four-fold symmetry is maintained (Gouterman, 1978). The green color of the di-acidic forms of meso-substituted porphyrins (Meot-Ner and Adler, 1975) is unusual, as most di-acidic porphyrins are violet-colored (Gurinovich et al., 1968; Fleischer, 1970). The suggestion that buckling of $H_2TPP$ upon protonation results in a resonance between the $\pi$-electron systems of the phenyl groups and porphyrin ring thereby imparting the green color (Gurinovich et al., 1968; Fleischer, 1970) is inconsistent with the fact that di-acidic forms of porphyrins with alkyl instead of aryl meso-substituents are also green in acidic media (Meot-Ner and Adler, 1975).

The absorption spectrum of $H_2TPyP-1$ dispersed alone in water (containing 0.1 M hydrogen/sodium chloride) was sensitive to increases in the pH above 2, where protonation of the pyrrole nitrogen atoms was negligible. Raising the pH resulted in spectral changes that could be attributed to aggregation and increases in the sample turbidity. These changes were probably linked to deprotonation of the pyridine substituents of $H_2TPyP-1$, which occurs for the
parent porphyrin (H$_2$TPyP) at pH's between 2 and 3 (Fleischer, 1962; Fleischer and Webb, 1963; Pasternack, 1973). This acid-base equilibrium determines the solubility of H$_2$TPyP-1 in water. At pH 2 or lower, when the pyridine substituents are predominantly protonated, H$_2$TPyP-1 is soluble in water.

Whether or not micelles of the protonated porphyrin were present under the conditions of the experiment, when the porphyrin concentration was <10$^{-5}$ M, depends on its "critical micelle concentration." The critical micelle concentrations of other surfactants with single hexadecyl chains like 1-hexadecyltrimethylammonium bromide and 1-hexadecylpyridinium chloride are about 1 x 10$^{-3}$ (Fendler and Fendler, 1975), so micelles of H$_2$TPyP-1 were probably absent. At pH > 3, H$_2$TPyP-1 was insoluble in water, but initially it remained in suspension as colloidal particles.

2.4.2.4. SPECTRAL CHANGES IN THE ULTRAVIOLET REGION INVOLVING THE PYRIDINE SUBSTITUENTS

There was a remarkable 20-fold enhancement in the absorbance of the UV band near 255 nm of H$_2$TPyP-1 when egg PC vesicles containing the porphyrin were stored at 4 °C for 4 weeks (Section 2.3.1.2, Fig. 2-6). Two features of the spectra identify this absorption band as belonging to the pyridine meso-substituents of the porphyrin: i) Both the spectrum of pyridine in water, shown in Fig. 2-17 (Krumholz, 1951; DMS UV Atlas of Organic Compounds, 1971), and of the
Figure 2-17. Absorption spectra of solutions of the free-base and protonated forms of pyridine in water. Taken from the DMS UV Atlas of Organic Compounds (1971).
aged vesicle sample, shown in Fig. 2-6, have maxima at 251, 257, and 262 nm, with associated shoulders on the low-wavelength side of the 251 nm peak. ii) The nearly doubling in peak height at 257 nm when acid was added to the vesicle suspension (Fig. 2-6) is analogous to the spectral change that accompanies the protonation of pyridine, as shown in Fig. 2-17 (Krumholz, 1951; DMS UV Atlas of Organic Compounds, 1971).

The absorption spectra of H$_2$TPyP-1 in organic solvents and in freshly-prepared vesicle suspensions (e.g., Fig. 2-5) had relatively weak absorption bands centered near 255 nm with extinction coefficients of about 9000 M$^{-1}$cm$^{-1}$, which corresponds to 2300 M$^{-1}$cm$^{-1}$ per pyridine substituent, including the alkylated one. By comparison, the corresponding extinction coefficients for pyridine dissolved in water and in cyclohexane are 3200 and 1900 M$^{-1}$cm$^{-1}$, respectively (DMS UV Atlas of Organic Compounds, 1971). This correspondence indicates that the electronic transitions of the pyridine substituents are normally essentially independent of those of the porphyrin chromophore.

The aggregation of H$_2$TPyP-1 appeared to be linked to the enhancement of the UV absorption band intensity since the enhancement was only observed in colloidal aqueous samples containing the aggregates. Also, the addition of HCl to the aged vesicle sample affected both the UV and Soret bands of the porphyrin (Fig. 2-6).
The theory of the ultraviolet spectra of benzene and mono-substituted benzenes (Jaffé and Orchin, 1962; Becker, 1969) could provide some clues as to the cause of the intensification of the pyridyl absorption band of $H_2TPyP-1$. The (0-0) vibrational band of the lowest-energy transition of benzene ($B_{2u}$) is electronically forbidden, but the vibrationally excited states that distort the hexagonal geometry are not, so the $B_{2u}$ band appears in the spectrum of benzene, but it is relatively weak (-200 $M^{-1}cm^{-1}$). Mono-substitution of benzene formally makes the transition allowed, but the band intensities are still low ($10^3-10^4$ $M^{-1}cm^{-1}$) compared to allowed transitions ($10^4-10^5$ $M^{-1}cm^{-1}$) because, even though the molecular symmetry is $C_{2v}$, the symmetry of the $\pi$-electron system is still approximately $D_{6h}$. The extent of perturbation of the hexagonal electronic symmetry by substituents depends on "inductive" and "conjugative" effects (Becker, 1969). The theory for benzene similarly applies to pyridine (Jaffé and Orchin, 1962).

The porphyrin moiety of $H_2TPyP-1$ can be considered essentially a para-substituent for the pyridine groups of the porphyrin. Consequently, by analogy to mono-substituted benzenes, changes in the electronic structure of the porphyrin could affect the intensity of the pyridine-localized absorption bands. Such changes could be caused by the aggregation of $H_2TPyP-1$ since it was clear from the visible absorption spectra of the porphyrin that aggregation
significantly alters its electronic structure (Section 2.4.2.2).

2.4.2.5. FLUORESCENCE QUENCHING BY AQS

The Stern-Volmer plots for the quenching of the fluorescence of H₂TPyP-1 by AQS in homogeneous solutions were linear (e.g., Fig. 2-13), so the quenching process required the diffusion of \(^1\text{H}_2\text{TPyP-1}^*\) and AQS. The corresponding plots for vesicle suspensions were non-linear, where the capacity for AQS to quench the porphyrin fluorescence appeared to saturate. As will be shown below, this saturation can be interpreted in terms of the Langmuir adsorption equation (Langmuir, 1918; Adamson, 1967; Van Holde, 1971; Tinoco et al., 1978), assuming that AQS could bind to a fixed number of identical and independent sites on the vesicles. It is reasonable that the adsorption of AQS to the vesicle surface was involved in the fluorescence quenching reaction since the porphyrin is constrained to the membrane phase, due to its low solubility in water (at neutral pH's), while AQS is an amphiphilic molecule with appreciable solubility in water. Since the quenching reaction required encounters between AQS and photoexcited \(\text{H}_2\text{TPyP-1}\), only surface-bound AQS could quench the porphyrin fluorescence.

The Langmuir equation was originally applied to describing the equilibrium adsorption of a gas on a solid surface, but the theory is equally applicable to adsorption...
from solution (Adamson, 1967; Van Holde, 1971; Tinoco et al., 1978) and has been used to interpret the binding of aqueous solutes to macromolecules (Klotz, 1946; Oster, 1955) and to lipid bilayers (McLaughlin, 1977; Fukushima et al., 1980). The Langmuir adsorption isotherm has the general form of Eq. 2-1 below, where the parameter $\theta$ is the fraction of sites that are occupied, $\nu$ stands for the amount adsorbed, $P$ is the pressure of the adsorbate in the continuous phase, and $b$ is the equilibrium constant for adsorption.

$$\theta = \nu/\nu_{\text{max}} = bP/(1 + bP)$$  \hspace{1cm} (2-1)$$

In the case of the binding of an aqueous solute to a macromolecule, the solute concentration in the water phase, $[Q]_w$, replaces $P$ in Eq. 2-1, and the association constant $K_a$ replaces $b$:

$$\theta = K_a [Q]_w/(1 + K_a [Q]_w)$$  \hspace{1cm} (2-2)$$

(The sub- or super-scripts $w$ and $m$ will denote whether the parameters are their water or membrane phase values, respectively.) $K_a$ in Eq. 2-2 is an average equilibrium constant for binding; in spite of the assumption that the binding sites are identical, the individual binding constants differ in magnitude for entropic reasons (Van Holde, 1971; Tinoco et al., 1978). Mathematical treatment of binding according to the law of mass-action leads to an expression of similar form to the Langmuir equation (Klotz, 1946; Thulborn and Sawyer, 1978).

The adsorption equilibrium being considered is repre-
sent by Eq. 2-3 below, where $S_m$ stands for vacant sites for AQS on the membranes, $Q_w$ for AQS dissolved in the water phase, and $S\cdot Q_m$ for membrane-bound AQS.

(2-3) $S_m + Q_w === S\cdot Q_m$

If the binding sites are identical and independent of one another, the following relationship holds:

(2-4) $K_a = [S\cdot Q]_m/[S]_m[Q]_w$

The connection between the parameters characterizing adsorption and those characterizing fluorescence quenching is made by assuming that: i) quenching only occurred in the membrane phase (including the interface), ii) the distribution of AQS between the bulk aqueous and membrane phases was determined by the Langmuir equation (2-2), and iii) the quenching of the fluorescence of $H_2TPyP-1$ by AQS within the membrane phase obeyed Stern-Volmer kinetics. Under these conditions, Eq. 2-5 below holds, where $F$ is the intensity of fluorescence with AQS present, $F_0$ is the intensity in the absence of AQS, $k_q^m$ is the bimolecular rate constant for quenching within the membrane phase, $\tau_0$ is the lifetime of the excited state in the absence of quencher, and $K_{S-V}^m$ is the Stern-Volmer constant.

(2-5) $F_0/F - 1 = k_q^m\tau_0[S\cdot Q]_m = K_{S-V}^m[S\cdot Q]_m$

The maximal value for $(F_0/F - 1)$, $(F_0/F - 1)_{\text{max}}$, is determined by the "local" concentration of binding sites in the vesicles, $[S\cdot Q]_m^{\text{max}}$. Thus

(2-6) $(F_0/F - 1)_{\text{max}} = K_{S-V}^m[S\cdot Q]_m^{\text{max}}$
and

\[(2-7) \quad \Theta = \left(\frac{F_0}{F} - 1\right) \left(\frac{F_0}{F} - 1\right)_{\text{max}} = \left(\frac{F_0}{F} - 1\right) \left(\frac{K_m}{S \cdot Q}\right)_{\text{max}}\]

Eq. 2-8 follows from Eqs. 2-2 and 2-7:

\[(2-8) \quad \frac{F_0}{F} - 1 = \frac{K_m}{S \cdot Q}\left(\frac{1}{1 + K_m[S \cdot Q]}\right)\]

Eq. 2-8 is equivalent to an expression given by Thulborn and Sawyer (1978) which they derived from the mass-action law rather than the Langmuir adsorption equation. They concluded that the fluorescence quenching of anthracene derivatives dissolved in egg PC vesicles by Cu^{2+} obeyed Eq. 2-8.

Eq. 2-7 can be rearranged to express a linear dependence of \((\Theta - 1)\) on \([Q]_w^{-1}:

\[(2-9) \quad (\Theta - 1) = \left(\frac{K_a}{[Q]_w}\right)^{-1}\]

Experimentally, only the overall, "bulk", concentration of AQS, \([Q]_T\), was known. In this Section, concentrations that are "primed", like \([Q]_m\), will be relative to the total volume of the sample ("bulk" concentrations) while concentrations that are unprimed, like \([Q]_m\) and \([Q]_w\), will be relative to the volume of either the membrane (m) or water (w) phases ("local" concentrations). By this convention, \([Q]_T = [Q]_m + [Q]_w\). A plot of \((\Theta - 1)\) versus \([Q]_w^{-1}\) would be linear only if \([Q]_w \gg [Q]_m\) so that \([Q]_m = [Q]_w\). Then

\[(2-10) \quad (\Theta - 1) = \left(\frac{K_a}{[Q]_T}\right)^{-1}\]

Plots of \((\Theta - 1)\) versus \([Q]_w^{-1}\) for vesicle suspensions with egg PC:H\textsubscript{2}TPyP-1 molar ratios of 5:1 and 50:1 (the
latter sample contained 10 times more egg PC than the former) are shown in Fig. 2-18. The values of \((F_0/F - 1)_{max}\) which were used for that plot were estimated from the Stern-Volmer plots in Fig. 2-13; values of 4.7 and 2.5 were estimated for the 5:1 and 50:1 samples, respectively. The full range of the data was covered by plotting the logarithms of both functions. The data fit Eq. 2-10 reasonably well in both cases, but the fit was better in the 50:1 molar ratio case. The corresponding values for \(K_a\), which equaled the inverse slopes of the plots, were \((4.1\pm0.5) \times 10^4\) and \((0.9\pm0.1) \times 10^4\) M\(^{-1}\) when the egg PC:porphyrin molar ratios were 5:1 and 50:1, respectively.

The 5-fold difference in \(K_a\) could reflect the greater positive surface charge density of the 5:1 molar ratio vesicles compared to the 50:1 vesicles due to the cationic charge of \(H_2TPyP-1\). A more positive vesicle surface would favor adsorption of the negatively charged AQS and disfavor desorption, thereby increasing \(K_a\) as observed (McLaughlin, 1977). The increase in \((F_0/F - 1)_{max}\), which is proportional to concentration of binding sites, with the increase in porphyrin concentration likewise could reflect an influence of surface charge density on the adsorption of AQS.

The fact that the plots in Fig. 2-18 are nearly linear indicates that a relatively small fraction of the total amount of AQS was bound. The bound fraction bound can be estimated by assuming values for the volume fraction of the
Figure 2-18. Log-log plot of the inverse of the relative fluorescence intensity of H₂TPyP-1 dissolved in egg PC vesicles versus the inverse of the concentration of anthraquinone-2-sulfonate added to the continuous aqueous phase. The two vesicle suspensions contained $5 \times 10^{-5}$ M H₂TPyP-1 and either $1 \times 10^{-3}$ M egg PC (50:1 molar ratio) (triangles) or $1 \times 10^{-4}$ M egg PC (5:1 molar ratio) (circles). The solid lines are solutions to Eq. 2-9, with $(F_0/F - 1)_{\text{max}}$ and $K_a$ equal to 2.5 and $0.9 \times 10^4$ M⁻¹ (50:1 molar ratio sample), or 4.7 and $4.1 \times 10^4$ M⁻¹ (5:1 molar ratio sample).
\[ 1 - \left\{ \left( 1 - \frac{\phi}{\phi^0} \right) / x^{\text{DW}} \left( 1 - \frac{\phi}{\phi^0} \right) \right\} \]
sample occupied by vesicles, \( V_m/V_T \), and for the Stern-Volmer constant for quenching within the vesicle phase, \( K^m_{S-V} \). The bulk value for the maximal concentration of bound AQS, \([S\cdot Q]^m_{\text{max}}\), equals its local value times \( V_T/V_m \), so it follows from Eq. 2-6 that

\[
([S\cdot Q]^m_{\text{max}}) = (V_m/V_T)(F_0/F - 1)_{\text{max}}/K^m_{S-V}
\]

The volume fraction \( V_m/V_T \) was approximately \( 10^{-3} \) when the egg PC concentration was \( 10^{-3} \) M (50:1 molar ratio) and \( 10^{-4} \) when the concentration was \( 10^{-4} \) M (5:1 molar ratio). It was assumed that \( K^m_{S-V} \) was approximately the same as the value in homogeneous solution, 500 M\(^{-1}\). With these numbers it is calculated from Eq. 2-11 that no more than 6% of the total amount of AQS was adsorbed when the egg PC:H\(_2\)TPyP-l molar ratio was 50:1, and no more than 3% was adsorbed in the 5:1 case. These calculations support the previous presumption that the plots of \((\Theta - 1)^{-1} \) versus \([Q]^l_T\) were linear because \([Q]^l_T \gg [Q]^m\).

Apparently, possible sources of deviations from Langmuir adsorption behavior such as i) a dependence of \( K_a \) on \( \Theta \) due to the negative charge that bound AQS imparts to the vesicle surface, which would make the plots in Fig. 2-18 curve downward (McLaughlin, 1977), and ii) changes in the absorption spectrum of \( H_2\)TPyP-l due to increasing concentrations of AQS (Section 2.4.2.2) did not contribute significantly to the fluorescence quenching measurements.

Phenomena other than binding can lead to Stern-Volmer
plots that saturate with increasing quencher concentrations. In the case examined by Lehrer (1971), a fraction of the population of fluorescent molecules was inaccessible to the quencher. This case could apply to the present vesicle system if AQS were impermeable to the vesicle walls on the time scale of the quenching measurements (tens of minutes). The vesicle data did not conform to the modified Stern-Volmer equation given by Lehrer, however. In the case examined by Bieri and Wallach (1975), saturation was due to the formation of clusters between fluorophore and quencher in vesicles when the quencher concentration exceeded a critical value. This example could be relevant to the present system since AQS appeared to induce aggregation of H₂TPyP-1, but the version of the Stern-Volmer equation proposed by Bieri and Wallach did not fit the AQS data.

2.4.3. Cu(II)TPyP-1 ABSORPTION SPECTRA

As with H₂TPyP-1, there was optical evidence for the self-aggregation of Cu(II)TPyP-1 in some aqueous media. In particular, the pH dependence of the absorption spectrum of Cu(II)TPyP-1 dispersed alone in water (containing 0.1 M HCl/NaCl) suggested that porphyrin-porphyrin ring interactions perturbed the usual (monomer) spectrum as the solubility of the porphyrin decreased with increasing pH. In contrast to H₂TPyP-1, the Soret band maximum of Cu(II)TPyP-1 did not shift upon aggregation, but a band appeared near 380 nm together with a broad tail on the low-energy side of the
2.4.3

Soret band (see Fig. 2-10). These new bands possibly correspond to the ones at 365 and 445 nm previously attributed to the aggregates of $\text{H}_2\text{TPyP-1}$ (Section 2.4.2.2). As with $\text{H}_2\text{TPyP-1}$, the spectrum of $\text{Cu(II)TPyP-1}$ varied most between pH 2 and 3, so deprotonation of the pyridine substituents with increasing pH was implicated (Section 2.4.2.3).

To differentiate between spectral changes caused by the deprotonation of the pyridine substituents from changes due to aggregation, the pH titration of $\text{Cu(II)TPyP-1}$ was repeated in an aqueous detergent solution so the porphyrin would remain monomeric. Raising the pH of the detergent solution caused the Soret band to sharpen and blue shift. This trend was analogous to the effect of quaternization on the Soret band of $\text{H}_2\text{TPyP-1}$ (Section 2.4.2.1), and was opposite to the trend when there was no detergent present.

When $\text{Cu(II)TPyP-1}$ was dissolved in egg PC vesicles, the dependence of the Soret band extinction coefficient and half-width on the egg PC:porphyrin molar ratio paralleled the $\text{H}_2\text{TPyP-1}$ case (Sections 2.3.1.2 and 2.3.1.3). As before, the dependence on molar ratio can be attributed to an increased probability of porphyrin-porphyrin ring interactions when the porphyrin concentration within the bilayers is increased.

2.4.4. $\text{Mn(III)TPyP-1}$ ABSORPTION SPECTRA

In contrast to the $\text{H}_2\text{TPyP-1}$ and $\text{Cu(II)TPyP-1}$ cases, aqueous dispersions of $\text{Mn(III)TPyP-1}$ had absorption spectra
that were undistorted compared to its organic solution spectra, except that the molar extinction coefficients were less in the aqueous samples. Mn(III)TPyP-1 was insoluble in water, so it was likely that the porphyrin was in a colloidal state in its aqueous dispersion. Thus it appeared that the kinds of porphyrin-porphyrin interactions that perturbed the electronic spectra of \( \text{H}_2\text{TPyP-1} \) and Cu(II)TPyP-1 were absent with Mn(III)TPyP-1. This difference could be linked to the fact that Mn(III)-porphyrins usually coordinate one or two axial ligands (Boucher, 1972; Boucher, 1973), while metal-free and Cu(II)-porphyrins usually have none (Scheidt, 1978). Axial ligands would sterically interfere with stacking of the porphyrin rings.

2.4.5. CAPTURE OF Mn(III)TPyP-4-CH\(_3\) BY EGG PC VESICLES

Mn(III)TPyP-4-CH\(_3\) was captured by egg PC vesicles when the porphyrin and egg PC were added together in ethanol to aqueous buffer (Section 2.3.3). The criterion used for capture was that some of the porphyrin eluted with the vesicles in the void volume of the Sephadex column. The MnTPyP-4-CH\(_3\) that remained associated with the vesicles was presumably located inside the vesicles, either dissolved in the aqueous compartments or bound to the surface. The fact that essentially all of the porphyrin remained with the vesicles during the second gel-filtration 1 hour later showed that its release from the vesicle interior was relatively slow.
The purpose of this experiment was to test the method of adding the vesicle-forming component (egg PC) and the component to be entrapped together during ethanol-injection as a means of entrapment. The rationale behind the method used was that, if MnTPyP-4-CH\textsubscript{3} and egg PC were present together during vesicle formation, the porphyrin might become enclosed within the vesicle shell before it had time to become dispersed in the entire aqueous phase. This way, the amount of MnTPyP-4-CH\textsubscript{3} captured would be greater than the amount which could be captured if it were dissolved in the aqueous phase prior to vesicle formation. The latter method would presumably have been successful, but of the order of 100 times as much porphyrin would probably have been required to entrap an equivalent amount. It was estimated that 10\% of the MnTPyP-4-CH\textsubscript{3} was captured when it was added with the egg PC; the generality of this technique to trapping other molecules remains to be determined.

The absorption spectrum of the MnTPyP-4-CH\textsubscript{3} from the bottle was unusual, so the oxidation state of the manganese could not be assigned on that basis. The spectrum of the porphyrin in solution more resembled that of a Mn(II) porphyrin than a Mn(III) one, but Mn(II)TPyP-4-CH\textsubscript{3} would not have been stable to air oxidation (E. B. Fleischer, personal communication, 1978; Harriman and Porter, 1979a). Curiously, the spectrum of the porphyrin captured by the egg PC vesicles was characteristic of Mn(III)TPyP-4-CH\textsubscript{3} while the
2.4.5 115

spectrum of the untrapped portion was not. This transforma-
tion happened during the experiment and required egg PC
since the porphyrin from the bottle was stable when dis-
solved in buffer alone or with ethanol.

2.4.6. LIGHT-INDUCED EPR SIGNALS FROM BILAYER SUSPENSIONS
CONTAINING H₂TPyP-1

The isotropic light-induced EPR signal observed in
bilayer suspensions without AQS (Section 2.3.4.1, Fig. 2-14)
was similar to the EPR spectra of the one-electron oxidation
or reduction products of H₂TPP under a variety of condi-
tions, which have g-values of 2.00 to 2.01 and peak-to-peak
line widths between 3.8 and 5.2 gauss (Lexa and Reix, 1974;
Fajer and Davis, 1979), so the corresponding radicals of
H₂TPyP-1 (H₂TPyP-1⁺ and H₂TPyP-1⁻) were likely sources of
the observed signal. (The shape of the spectrum alone was
sufficient to discount the possibility that the signal was
due to the triplet state of the porphyrin (Thurnauer et al.,
1975).) This suggests that electron transfer between two
H₂TPyP-1 molecules to produce H₂TPyP-1⁺ and H₂TPyP-1⁻, was
the source of the radicals. In principle, the reaction
could be between a photoexcited porphyrin molecule and a
ground state one,

(2-12) *H₂TPyP-1 + H₂TPyP-1 → H₂TPyP-1⁺ + H₂TPyP-1⁻

or between two photoexcited porphyrins molecules,

(2-13) *H₂TPyP-1 + *H₂TPyP-1 → H₂TPyP-1⁺ + H₂TPyP-1⁻

For discussions of such processes see Carapellucci and
Mauzerall (1975), Mauzerall (1976), Seely (1978a,b), and Ballard and Mauzerall (1980). Based on the electrochemical potentials and excited state energies of $\text{H}_2\text{TPP}$ (Seely, 1978a), Eq. 2-12 was not possible if the excited state were the lowest excited triplet state, and was possible via the lowest excited singlet state only if the separated radicals were solvent-stabilized relative to the contact ion pair by $\geq 0.3$ electron volts. The disproportionation of photoexcited molecules (Eq. 2-13) would be energetically favorable via either the singlet or triplet excited states.

AQS could influence the EPR behavior of the egg PC-$\text{H}_2\text{TPyP-1}$ bilayer dispersions by acting as an electron acceptor, oxidizing either $^*\text{H}_2\text{TPyP-1}$ or $\text{H}_2\text{TPyP-1}$.

$\begin{align*}
(2-14) & \quad ^*\text{H}_2\text{TPyP-1} + \text{AQS} \rightarrow \text{H}_2\text{TPyP-1}^+ + \text{AQS}^- \\
(2-15) & \quad \text{H}_2\text{TPyP-1}^- + \text{AQS} \rightarrow \text{H}_2\text{TPyP-1} + \text{AQS}^- 
\end{align*}$

Evidence for the former reaction is the quenching of the fluorescence of $\text{H}_2\text{TPyP-1}$ by AQS (Section 2.4.2.5). Based on the reduction potentials for the $\text{H}_2\text{TPP}/\text{H}_2\text{TPP}^-$ and AQS/AQS$^-$ couples (Seely, 1978a; Farrington et al., 1978), Eq. 2-15 is a spontaneous reaction. By either process, the contribution of $\text{H}_2\text{TPyP-1}^-$ to the EPR spectrum would be smaller compared to the spectrum obtained without AQS. The observed effect of AQS seen in Fig 2-14 is consistent with this picture if the spectrum without AQS is assumed to be the superposition of signals due to both $\text{H}_2\text{TPyP-1}^+$ and $\text{H}_2\text{TPyP-1}^-$, and the spectrum with AQS contains only the higher field (lower $g$-
value) component presumably belonging to H\textsubscript{2}TPyP-1⁺. The "toe" near 3395-3400 gauss on the latter signal, which makes it asymmetric, could then be attributed to AQS⁻ (Kano and Matsuo, 1974). On the other hand, the narrowing of the EPR signal of illuminated bilayers could have been due to the aggregation of H\textsubscript{2}TPyP-1, which was induced by AQS (Section 2.4.2.2).

The flash-induced EPR signal decayed according to second-order kinetics. AQS was present in the sample, so the decay mechanism was presumably either by Eq. 2-16 or 2-17:

\[
\begin{align*}
\text{(2-16)} & \quad \text{H}_2\text{TPyP-1}^+ + \text{H}_2\text{TPyP-1}^- \rightarrow 2 \text{H}_2\text{TPyP-1} \\
\text{(2-17)} & \quad \text{H}_2\text{TPyP-1}^+ + \text{AQS}^- \rightarrow \text{H}_2\text{TPyP-1} + \text{AQS}
\end{align*}
\]

The fact that the signal decay was second-order by a graphical criterion does not prove that the radical decay mechanism was by a second-order process. The order of the reaction could be tested by varying the incident light intensity, which determines the initial concentration of radicals (Hurley et al., 1980). For example, the radical decay in illuminated vesicle suspensions containing chlorophyll-a and quinones occurs by a first-order process, but the heterogeneous nature of the sample makes first-order plots non-linear (Hurley et al., 1980).

2.4.7. PHOTOCHEMISTRY OF Mn(III)TPyP-1 IN VESICLE SUSPENSIONS

The photoreduction of Mn(III)TPyP-1 to Mn(II)TPyP-1 in vesicle suspensions containing vitamin \( K_1 \) quinone (VK\(_1\))
probably took place by one of two mechanisms in which
photoexcited Mn(III)TPyP-1 (*Mn(III)TPyP-1) was either
oxidized by VK$_1$ (Eq. 2-18) or reduced by an electron donor
in the vesicle suspension (Eq. 2-19):

\[(2-18) \quad *\text{Mn(III)TPyP-1} + \text{VK}_1 \rightarrow \text{Mn(IV)TPyP-1}^+ + \text{VK}_1^-
\]
\[(2-19) \quad *\text{Mn(III)TPyP-1} + \text{donor} \rightarrow \text{Mn(II)TPyP-1}^- + \text{donor}^+
\]

(Electronic charges on the porphyrins will be included in
equations in this Section and the following one for the sake
of balancing the reactions, and do not necessarily reflect
the actual charges on the molecules. Also, axial ligands
are ignored.) In the second case, Mn(II)TPyP-1 is produced
directly, while in the first it is produced during subse­
quent dark reactions (see below).

Reactions analogous to Eq. 2-18 occur in homogeneous
aqueous solutions with water-soluble Mn(III)-porphyrins and
quinones (Harriman and Porter, 1979b). Mn(IV)-porphyrins
are potent oxidizing agents capable of oxidizing water to O$_2$
or H$_2$O$_2$ (Eqs. 2-20 and 2-21) (Loach and Calvin, 1963; Calvin
\textit{et al}., 1964; Tabushi and Kojo, 1975; Harriman and Porter,
1979a,b; Harriman and Porter, 1980).

\[(2-20) \quad 2 \text{Mn(IV)TPyP-1}^+ + 2 \text{H}_2\text{O} \rightarrow 2 \text{Mn(II)TPyP-1}^- + \text{O}_2 + 4\text{H}^+
\]
\[(2-21) \quad 2 \text{Mn(IV)TPyP-1}^+ + 2 \text{H}_2\text{O} \rightarrow 2 \text{Mn(III)TPyP-1} + \text{H}_2\text{O}_2 + 2\text{H}^+
\]

The fact that Mn(II)TPyP-1 was produced during illumination
of the vesicle system does not eliminate the occurrence of
Eq. 2-21 because VK$_1^-$ produced in Eq. 2-18 could reduce Mn(III)TPyP-1 to Mn(II)TPyP-1:

(2-22)    Mn(III)TPyP-1 + VK$_1^-$ $\rightarrow$ Mn(II)TPyP-1$^-$ + VK$_1$

The stoichiometric amount of O$_2$ or H$_2$O$_2$ that would have corresponded to a single turnover (reduction) of Mn(III)-TPyP-1 via Eqs. 2-18 and 2-22 and either 2-20 or 2-21 would have been undetectable by the analytical methods employed, but through the photosensitized reduction of VK$_1$ or additional oxidants (e.g., p-benzoquinone-disulfonate) the process could have cycled enough times to accumulate measurable quantities of O$_2$ or H$_2$O$_2$.

Besides oxidizing water to O$_2$ or H$_2$O$_2$, Mn(IV)TPyP-1 could, in principle, have oxidized the carbon-carbon double bonds of the fatty acid substituents of egg PC. A possible net reaction would be the transfer of an oxygen atom from water to an olefin:

(2-23)    H$_2$O + R-C=C-R' + Mn(IV)TPyP-1$^+$ $\rightarrow$

\[
2 \text{H}^+ + \text{R-C-C-R'} + \text{Mn(II)TPyP-1}^-
\]

Oxygen atom transfers will be considered in the next Section.

An alternative mechanism for the photochemical reduction of Mn(III)TPyP-1 to Mn(II)TPyP-1 is reductive quenching by an irreversibly oxidizable electron donor (Eq. 2-19). Possible candidates for donor were ethanol or dimethylformamide, which were introduced during the preparation of the
vesicle suspensions, or axially ligated OH⁻ (Harriman and Porter, 1979b). The fact that the photoreduction was observed even after gel-filtration, which removed most of the organic solvents, argues against their participation in the photochemistry. The photochemistry of Mn(III)TPyP-1 in aqueous methanol solutions, discussed in the next Section, also indicated that alcohols did not reduce *Mn(III)TPyP-1.

Spectral changes in the ultraviolet region caused by illumination indicated that VK₁ was changed, but assignment of the changes to reduction of the quinone was complicated by several factors. One problem was that the spectrum of the quinone appeared atop a relatively large background absorption. Secondly, the conversion of Mn(III)TPyP-1 to Mn(II)TPyP-1 contributed in an unknown way to to the spectral changes in the ultraviolet. A third problem was that several reduced quinone species were possible, particularly the hydroquinone and its deprotonated form, which have different spectra. The spectral changes clearly showed the disappearance of the absorption bands around 240 and 270 nm belonging to the quinone itself. Reduction of VK₁ to the hydroquinone should have caused a drop in absorbance around 270 nm and an increase around 240 nm (Crane and Sun, 1972).

The absence of absorption bands due to AQS in the spectrum of vesicle suspensions that had been illuminated prior to the addition of AQS was a curious result. The subsequent appearance of absorption bands due to AQS upon
illumination showed that the molecule was not irreversibly destroyed, and suggests that the AQS was reduced by some product that had accumulated during the initial illumination. However, the usual reduced forms of AQS, its hydroquinone or semiquinone forms, are highly colored (Cooper, 1966; Carlson and Hercules, 1973; Kano and Matsuo, 1974) and would have been obvious in the absorption spectrum of the sample. Conceivably, AQS was reduced to the corresponding anthrone, which is the keto form of a tautomeric pair whose enol form is the anthrol, and which is less colored than the quinone (Fieser and Fieser, 1956).

2.4.8. PHOTOCHEMISTRY OF MANGANESE PORPHYRINS IN METHANOL-WATER SOLUTIONS

Calvin and Willner recognized in 1979 that Mn(IV)-porphyrins could be intercepted by nucleophilic compounds to effect oxygen atom transfer from water to the nucleophile. The oxygen atom would originate from a water molecule originally coordinated to the manganese atom in the axial position of the porphyrin (Calvin et al., 1964). The advantage of oxygen atom transfer compared to O₂ evolution would be two-fold: i) O₂ evolution would represent a substantial loss in free energy relative to the high-energy oxo-Mn(IV)-porphyrin intermediate. For example, about 25 kcal/mole more energy can be stored if water is decomposed to H₂ and H₂O₂ (Eq. 1-3) than if it is decomposed to H₂ and O₂ (Eq. 1-2). ii) The oxygen atom transfer reaction could be used to
produce commercially important compounds (e.g., epoxides and peroxides).

Willner et al. (1980b) obtained spectroscopic evidence for an oxo-species of Mn-TPP, formulated as O=Mn(IV)-TPP, which was active as an oxygen transfer agent.

Willner et al. (1980a) found that illumination of solutions of Mn(III)TPyP-1 aqueous methanol in the presence of methylviologen (MV²⁺), as electron acceptor, and triphenylphosphine, as nucleophile, resulted in reduction of Mn(III)TPyP-1 to Mn(II)TPyP-1, followed by reduction of MV²⁺ to MV⁺. It was clear that Mn(II)TPyP-1 as well as Mn(III)-TPyP-1 had to be photoactive in order for the porphyrin to recycle:

\[ *\text{Mn(II)TPyP-1}^- + \text{MV}^{2+} \rightarrow \text{Mn(III)TPyP-1} + \text{MV}^+ \]

Decreasing the concentrations of either MV²⁺ or triphenylphosphine caused the quantum yield of MV²⁺ reduction to decrease. When platinum oxide (PtO₂) was added to the system to catalyze the reduction of water by MV⁺ (Eq. 2-25), triphenylphosphine oxide was accumulated.

\[ 2 \text{MV}^+ + 2 \text{H}_2\text{O} \rightarrow 2 \text{MV}^{2+} + \text{H}_2 + 2 \text{OH}^- \]

These results were taken as evidence for light-driven oxygen atom transfer from water to triphenylphosphine photosensitized by MnTPyP-1. Willner (unpublished results) also found that triphenylphosphine could be replaced with certain olefins, iodobenzene, or phthalic acid. In none of the latter cases were oxidation products identified, however.
Phthalic acid was of interest as an oxygen atom acceptor because the presumed product, phthalyl peroxide, is essentially equivalent to hydrogen peroxide with respect to chemical reactivity. The carboxylic acid substituent in the ortho position of the molecule could have a stabilizing effect on the peroxide moiety via hydrogen bonding or mixed anhydride formation. The present effort was to test the generality of the reaction using other carboxylic acids instead of phthalic acid, and to see if other manganese porphyrins could replace Mn(III)TPyP-1. The results are summarized in Table 2-7.

Other than phthalic acid, only diphenic acid was active with respect to light-induced reduction of Mn(III)TPyP-1 and MV²⁺. This result supports the contention that the ortho carboxylic acid group of phthalic acid was functional in stabilizing the peracid moiety. The negative results with maleic, benzoic, and salicylic acids in a sense served as "blank" experiments which show that the photosensitized reduction of MV²⁺ took place only in the presence of suitable nucleophiles. These negative results also show that the reduction of MV²⁺ that occurred in samples without Mn(III)TPyP-1 (Section 2.3.4.4) did not contribute significantly to the experiments in Table 2-7.

The photosensitized reduction of MV²⁺ in the presence of phthalic acid was accomplished with Mn(III)TSPP and MnTPyP-4-CH₃ as well as Mn(III)TPyP-1, but the latter
compound was superior. The tetrasulfonated porphyrin became demetalated during the reaction. This probably happened when the manganese atom was in its divalent state since Mn(II)-porphyrins are known to be relatively acid labile (Boucher, 1972). Apparently, the porphyrin ring substituents influence the acid lability of the Mn(II)-porphyrins since no demetallation was observed with the two pyridinium-substituted porphyrins. The meso-substituents also influence the Mn(III)/Mn(II) reduction potentials, judging from the proportions of the two oxidation states that were in equilibrium with a given concentration of MV·. Mn(III)TPyP-1 was essentially completely reduced before any MV· was accumulated, while both Mn(III)TSPP and Mn(II)TSPP were present after some MV· had been accumulated. Thus Mn(III)-TPyP-1 is more easily reduced than Mn(III)TSPP, which is consistent with the simple expectation that making the porphyrin peripheral charge more negative should make the metal center more difficult to reduce. Electrochemical measurements by Harriman and Porter (1979a) support this contention.

2.5. CONCLUSIONS

H₂TPyP-1 and its metallo-derivatives are easily incorporated into vesicle systems. This fact, together with the other advantages of porphyrins as photocatalysts that were mentioned in Section 2.1, make these compounds promising as components of solar energy-converting devices using vesi-
H₂TPyP-1 self-aggregates in phosphatidylcholine vesicles. Some control over the extent of aggregation can be exerted by varying the PC:porphyrin molar ratio and the temperature of the sample, and possibly also by adding amphiphilic anions. It would be interesting to compare the monomeric and aggregated porphyrins as photosensitizers in the vesicle systems. Based on the pH dependence of the solubility of H₂TPyP-1 in aqueous media, it is likely that the monomer-aggregate equilibrium in vesicle suspensions could also be controlled by varying the pH, which would determine the net electronic charge on the porphyrin molecules. Such equilibria could perhaps be monitored and structural information could be obtained from EPR measurements using Cu(II)-TPyP-1 instead of H₂TPyP-1 (Blumberg and Peisach, 1965; Smith and Pilbrow, 1974; Lin, 1979).

Manganese porphyrins show great promise as water-decomposing photosensitizers that are capable of passing on the oxygen atom of water to Lewis bases.
CHAPTER 3. PREPARATION AND CHARACTERIZATION OF TWO
AMPHIPHILIC ANALOGUES OF THE TRIS-
(2,2'-BIPYRIDINE)-RUTHENIUM(II) COMPLEX

3.1. INTRODUCTION

The tris-(2,2'-bipyridine)ruthenium(II) complex (abbreviated [Ru(bipy)₃]²⁺), whose structure is illustrated in Fig. 3-1, is, like the porphyrins, a widely used photosensitizer for energy transfer (Gutierrez et al., 1970; Wrighton and Markham, 1973; Bolletta et al., 1976) and electron transfer (Meyer, 1976/77; Ballardini et al., 1978; Bock et al., 1979; Nagle et al., 1979) reactions (for recent reviews, see Balzani et al., 1979; Whitten, 1980; Sutin and Creutz, 1980). The photoluminescence of the complex in fluid solutions was first reported in 1959 (Paris and Brandt); interest in the photochemistry of [Ru(bipy)₃]²⁺ and related compounds has expanded rapidly during the past 10 years.

The simplicity of the chemistry of [Ru(bipy)₃]²⁺-type dyes makes them attractive as photosensitizers in vesicle systems. The spherical, "three-bladed propeller" shape of [Ru(bipy)₃]²⁺ (Fig. 3-1) makes the dye less likely to associate with itself or other molecules compared to more planar dyes, like porphyrins (see Chapter 2), although the V-shaped gaps between the bipyridine ligands can accommodate small molecules such as H₂O and ClO₄⁻ (Jensen et al., 1958; Van Meter and Neumann, 1976; Rillema et al., 1979. The tendency
Figure 3-1. Octahedral conformation of tris-2,2'-bipyridyl metal ion (M) complexes such as [Ru(bpy)$_3$]$^{2+}$. 
of porphyrins to form molecular complexes in colloidal aqueous media complicates their photochemistry since several light-absorbing species exist simultaneously. The kinetic inertness of \([\text{Ru(bipy)}_3]^{2+}\) (and its one-electron reduced or oxidized forms) to ligand loss or substitution (Brandt et al., 1954) is another attractive aspect of that class of dyes. Thus the synthesis of long-chained analogues of \([\text{Ru(bipy)}_3]^{2+}\) for use in vesicle suspensions was undertaken.

Amphiphilic derivatives of \([\text{Ru(bipy)}_3]^{2+}\) with long-chained hydrocarbon substituents were first prepared by Sprintschnik et al. (1976). The complexes have the formulae \([([\text{bipy})_2\text{Ru(bipy-COOR})]^{2+}\), where bipy-COOR is a substituted 2,2'-bipyridine with two octadecyl or dihydrocholesteryl groups attached by carboxylic acid ester linkages in the para (4,4') positions. The ester linkages were found to be susceptible to hydrolysis, especially at basic pH's (Valenty and Gaines, 1977; Sprintschnik et al., 1977; Gaines et al., 1978), so we sought other amphiphilic analogues of \([\text{Ru(bipy)}_3]^{2+}\) which might be more stable.

Attempts to prepare \(\text{Ru}^{2+}\) complexes with two bipy ligands and a third Schiff base-derived diimine ligand with a dodecyl substituent were unsuccessful (Ford, 1976). The rationale behind this approach was based on the literature of Fe(II)-diimine complexes (Busch and Bailar, 1956; Krumholz, 1965; Lindloy and Livingstone, 1967; Ito and Tanaka, 1970; Krumholz, 1971) which shows that ligands with
the 2,2' -diimine group (-N=C-C=N-) give rise to metal-to-ligand charge-transfer absorption bands similar to those responsible for the colors of [Fe(bipy)$_3$]$^{2+}$ and [Ru(bipy)$_3$]$^{2+}$. Reacting [(bipy)$_2$RuCl$_2$] with pyridine-2-aldehyde followed by 1-dodecylamine failed to yield the desired bis-bipyridine complex with the Schiff base 2-pyridylmethyleneamino-dodecane as third ligand, as pure materials were not isolated (Ford, 1976/77). Nor was the complex isolated after reacting [(bipy)$_2$RuCl$_2$] with the preformed Schiff base. Dose and Wilson (1978) later reported failure to prepare the analogous 2-p-tolylcarboxaldime complex.

We prepared two new amphiphilic analogues of [Ru(bipy)$_3$]$^{2+}$ with hexadecyl (-C$_{16}$H$_{33}$) substituents (Okuno et al., 1978). One of them is formulated [(bipy)$_2$Ru(biim-C$_{16}$H$_{33}$)]$^{2+}$ (1), in which the long-chained diimine ligand is 1,1'-di(1-hexadecyl)-2,2'-biimidazole (biim-C$_{16}$H$_{33}$) (Fig. 3-2). Like bipy, the biimidazole ligand contains the diimine group, so 1 was expected to have properties similar to those of [Ru(bipy)$_3$]$^{2+}$. The other complex is formulated [(bipy)$_2$-Ru(bipy-CONHC$_{16}$H$_{33}$)]$^{2+}$ (2), which is analogous to the ester derivatives of Sprintschnik et al. (1976) except that the hydrocarbon substituents are attached via amide rather than ester bonds (Fig. 3-3). It was hoped that the amide bonds of 2 would be less susceptible to hydrolysis than ester bonds.
Figure 3-2. \(((\text{Bipy})_2\text{Ru(biim-C}_{16}\text{H}_{33})\text{)}^{2+}\) (1) \(((1,1'\text{-di(1-hexadecyl)-2,2'-biimidazole})\text{-bis-(2,2'-bipyridine)ruthenium-}(\text{II})\text{]}\text{ dichloride).\)
\[ [\text{bpy}]_2 \text{Ru(biim-C}_{16}\text{H}_{33})] \text{Cl}_2 \]
Figure 3-3. \([\text{[(Bipy)_2Ru(bipy-CONH}_16\text{H}_{33}]^{2+}}\) (2) \(\text{[(4,4'-di-}\) (1-hexadecyl)carboxamide-2,2'-bipyridine)-\text{bis-}(2,2'-bipyridine)ruthenium(II)]^{2+}\).
$$\left[ \text{bpy}_2 \text{Ru(bpy-CONHC}_{16}\text{H}_{33}) \right]^{2+}$$
In this Chapter, the preparation and characterization of complexes 1 and 2 are described. The optical properties of 1 and 2 are compared to those of the parent [Ru-(bipy)$_3$]$^{2+}$, which has been extensively investigated. An understanding of the electronic spectra of [Ru(bipy)$_3$]$^{2+}$ and its analogues is important because the optical properties of the complexes are intimately related to their photoredox chemistry. This knowledge will be useful in designing new photosensitizers.

3.2. EXPERIMENTAL SECTION

3.2.1. MEASUREMENTS

Unless otherwise noted, all measurements were performed at room temperature (23±1.5 °C) and samples were air-saturated. Ultraviolet and visible absorption spectra were recorded on either Cary Model 118 or 14 spectrophotometers. Quartz cuvettes with 1.00 or 0.30 cm path lengths were used, and the reference cuvettes contained the same solvent as the sample cuvettes.

Luminescence and luminescence excitation spectra were recorded on a Perkin-Elmer Model MPF-2A fluorescence spectrophotometer equipped with a 150 watt xenon arc lamp and red-sensitive, type R-136, photomultiplier tube (Hamamatsu TV Co.). The emission was detected at right angles to the excitation, and was not corrected for the wavelength dependencies of the excitation source and photomultiplier response. Emission slit widths were about 6 nm and excita-
tion slit widths were about 10 nm. Quartz cuvettes with four polished sides and 1.00 by 0.30 cm path lengths were used; the cuvettes were oriented so the excitation beam struck the 1.00 cm face. Relative luminescence intensities were measured at the excitation and emission maxima of solutions with the same optical densities (0.46±0.02 per cm) at their excitation maxima. The relative intensities were corrected approximately for the drop in sensitivity of the photomultiplier by interpolating the manufacturer's response curves (Murov, 1973).

Infrared spectra were recorded on a Perkin-Elmer Model 283 spectrometer. The compounds were suspended in pressed KBr pellets. Melting points were determined with a capillary melting point apparatus. Elemental analyses were performed by the Microanalytical Laboratory operated by the College of Chemistry, University of California, Berkeley.

3.2.2. MATERIALS

Unless otherwise noted, materials were obtained from commercial suppliers and were used without further purification. Water was deionized, then distilled in a glass apparatus, or was twice deionized (Millipore Milli-Q water purification system). N₂ used was the house-line supply which was evaporated from a liquid nitrogen source.

1-Bromohexadecane was synthesized (Okuno, 1976/77) from hexadecyl alcohol and hydrogen bromide. 2,2'-Biimidazole (biim-H) was prepared (Okuno, 1976/77) according to litera-
ture procedures (Debus, 1858) from 40% aqueous glyoxal (from Aldrich) and ammonia. 1-Hexadecylamine (from Aldrich) was converted to its hydrochloride salt and recrystallized several times from ethanol. 2,2'-Bipyridine (bipy) (from Aldrich) was recrystallized twice from hexane. 4,4'-Dicarboxy-2,2'-bipyridine (bipy-COOH) was prepared according to a literature procedure (Case, 1946) by permanganate oxidation of 4,4'-dimethyl-2,2'-bipyridine (from G. F. Smith Co.). [Ru(bipy)$_3$]Cl$_2$·6H$_2$O (from G. F. Smith Co.) was crystallized from water by addition of saturated aqueous KCl. 3-(Dimethylhexadecylammonio)propane-1-sulfonate (DHAPS) was synthesized according to the literature method (Clunie et al., 1967) from N,N-dimethylhexadecylamine (from K and K) and 1,3-propane sultone (from Aldrich). Phosphatidylcholine from hens' egg yolks (egg PC) was extracted and purified according to Singleton et al. (1965) and stored as a solution in ethanol at -20 °C. Methylene chloride was dried by distillation over phosphorus pentoxide. Triethylamine and tetrahydrofuran were kept over KOH pellets. Infrared quality KBr was supplied by the Harshaw Chemical Co.

Dialysis tubing (Size 8 from Union Carbide Corp. Food Products Division) was washed with EDTA and ethanol before use (McPhie, 1971).

3.2.3. METHODS

3.2.3.1. PREPARATION OF SAMPLES
0.010 M stock solutions of [Ru(bipy)₃]Cl₂ in water, 1 in ethanol, and 2 in dimethylformamide were stored in sealed vials in the dark. Extinction coefficients for 1 and 2 were determined by transferring aliquots of the stock solutions to test tubes, removing the solvent with a stream of N₂ or under reduced pressure, and adding the new solvent via pipette. The complexes were dissolved in 0.010 M aqueous DHAPS solution by adding aliquots of the stock solutions directly to the vortex-stirred detergent solution.

The chloride salt of 1 was dispersed into water simply by adding water to the solid and warming the mixture slightly (−40 °C) with stirring. The perchlorate salt of 2 was dispersed into water by heating a suspension of the solid in water to boiling, vortex-stirring the hot suspension, then cooling the suspension to room temperature and centrifuging it with a clinical-type centrifuge to sediment the suspended solids. The compound was dispersed into 1.5 ml of hot (−60 °C) 0.10 M aqueous ammonium acetate solution by adding 0.050 ml of an ethanol solution of the complex to the vigorously stirred buffer.

A small amount of 2 was converted to its chloride salt by stirring a 67-33% (by volume) methanol-water solution of 2 with anion exchange resin (Bio-Rad AG1-X2, Cl⁻ form), which had been previously washed with the methanol-water solvent, filtering the mixture to remove the resin, and evaporating the solution to dryness with a stream of N₂.
3.2.3.1

The resulting Cl\textsuperscript{-} salt of 2 dispersed readily into warm water, like 1 did. Vesicle suspensions composed of egg PC and 2 were prepared by the ethanol injection method (Batzri and Korn, 1973).

3.2.3.2. ELECTRON MICROSCOPY AND DIALYSIS OF AQUEOUS DISPERSIONS OF 1 [with Agatha Tung]

A drop of an aqueous dispersion of 1 (1.0 \times 10^{-3} \text{ M}) was applied to an electron microscope grid coated with a parlodion film, and the excess liquid was removed with tissue paper. The film was negatively stained with 2% phosphotungstic acid solution (neutralized to pH 7.2) and examined with a Siemens Elmskop I electron microscope. An unstained sample was likewise prepared and examined.

Approximately 0.5 ml of the aqueous dispersion of 1 was placed into a washed dialysis bag whose ends were tied with string. The dispersion was dialyzed against 50 ml of water for 24 hours in the dark.

3.2.4. SYNTHESSES

3.2.4.1. \textit{cis-[DICHLORO-bis-(2,2'-BIPYRIDINE)RUTHENIUM(II)] HYDRATE ([(bipy)\textsubscript{2}RuCl\textsubscript{2}] \cdot 2.5\text{H}_2\text{O})}

The procedure described by Sprintschnik et al. (1977) (kindly provided by Dr. G. L. Gaines, Jr. prior to its publication) was modified slightly and scaled down. Details are given below.

To a 250 ml round-bottomed flask were added 2.515 g (0.0103 mole) of RuCl\textsubscript{3}\cdot1-3\text{H}_2\text{O} (from Alfa; elemental analysis
best fit the dihydrate), 3.351 g (0.0215 mole) of 2,2'-bipyridine, and 100 ml of dimethylformamide. The mixture was refluxed and stirred (magnetically) under a stream of N₂ for 3.5 hr. Air was excluded to avoid oxidation of Ru²⁺ complexes to their Ru³⁺ forms. Next the reflux condenser was removed so the solvent vapors could escape in the N₂ stream. Heating was stopped 2 hr later when the volume of the reaction mixture was about 15 ml. After the mixture had cooled to room temperature, 100 ml of acetone was added. The flask was sealed and stored in a cold room overnight.

The following day the mixture was filtered in the cold room. The dark crystals were washed with cold water until the filtrate was pale pink colored (the absorption spectrum of the initially orange aqueous wash showed predominantly [Ru(bipy)₃]²⁺). (The mother liquor probably contained the complex [(bipy)₂Ru(CO)Cl]⁺ (Clear et al., 1980) which photocatalyzes the water-gas shift reaction (Cole-Hamilton, 1980).)

To a 500 ml round-bottomed flask were added the washed crystals, 200 ml of water, 200 ml of ethanol, and 53 g of LiCl. The deep purple solution was bubbled briefly with argon, then it was refluxed with stirring under a stream of N₂ for 1 hr. The reflux condenser was removed and the mixture was allowed to evaporate under N₂ to half-volume during the next 2 hr. The flask was sealed under argon and stored in the cold room overnight.
The once-recrystallized solid was filtered and washed twice with cold water in the cold room. To a 500 ml round-bottomed flask were added the washed crystals, 200 ml of water, and 200 ml of ethanol. The mixture was bubbled briefly with argon, then it was refluxed and stirred for 1 hr under N₂. The resultant solution was hot-filtered into a 500 ml round-bottomed flask. 53 g of LiCl was added. The mixture was bubbled with argon briefly, then refluxed under N₂ for 10 min. The reflux condenser was removed and the solvent was evaporated under N₂ to half-volume during the next 2 hr. The flask was sealed under argon and stored overnight in the cold room.

The twice-recrystallized product was filtered and washed twice with cold water in the cold room. The purple crystals, which reflected green light, were dried under reduced pressure over phosphorous pentoxide to give 3.86 g of [(bipy)₂RuCl₂]·2.5H₂O (73% yield based on Ru).

Anal. Found: C, 45.06; H, 4.04; N, 10.48. Calculated for [(C₁₀H₈N₂)₂RuCl₂]·2.5H₂O: C, 45.37; H, 3.97; N, 10.59.

3.2.4.2. 1,1'-DI(1-HEXADECYL)-2,2'-BIIMIDAZOLE

(BIIM-C₁₆H₃₃) [Okuno, 1976/77]

To a 250 ml round-bottomed flask were added 1.34 g (0.010 mole) of 2,2'-biimidazole and 100 ml of dimethylformamide. The suspension was stirred well (magnetically) while 2.5 ml of an aqueous 35% NaOH solution (0.022 mole) was added. After the mixture became homogeneous (green
colored), 6.71 g (0.220 mole) of 1-bromohexadecane and another 2.5 ml of aqueous 35% NaOH were added. The mixture was stirred at room temperature for 3 hr. The resultant precipitate was filtered and saved. Water was added to the filtrate and the mixture was extracted with diethyl ether. The filtered precipitate was dissolved in ether and combined with the ether extracts. The combined ether solution was washed several times with water, and dried over Na$_2$CO$_3$. The ether solution was concentrated, then chromatographed on a silica gel column (1.8 x 17 cm). The fraction that eluted with 20-80% (by volume) methanol-benzene was recrystallized from ethanol. The yield was 0.59 g (10% based on bimidazole) of a pale pink powder.

Mp 58-59 °C. NMR (220 MHz) in CDCl$_3$, ppm: 0.85 (6 H, triplet, $J = 6$ Hz), 1.24, 1.66, 2.00 (ca. 56 H), 4.36 (4 H, triplet, $J = 8$ Hz), 6.92 (2 H, singlet).

Anal. Found: C, 77.96; H, 12.00; N, 9.44. Calculated for C$_{38}$H$_{70}$N$_4$: C, 78.29; H, 12.10; N, 9.61.

3.2.4.3. [(1,1'-DI(1-HEXADECYL)-2,2'-BIIMIDAZOLE)-bis-(2,2'-BIPYRIDINE)Ru(II)]CHLORIDE HYDRATE

([[(BIPY)$_2$Ru(BIIM-C$_{16}$H$_{33}$)]Cl$_2$]3H$_2$O) (I)

To a 100 ml round-bottomed flask fitted with a reflux condenser were added 0.0560 g (1.04 x 10$^{-4}$ mole) of [[(bipy)$_2$RuCl$_2$]2H$_2$O, 5 ml of ethanol, and 5 ml of water. The mixture was stirred magnetically at 80 °C for 20 min under N$_2$. To the resultant hot solution were added 0.731 g
(1.25 x 10^{-4} \text{ mole}) of biim-C_{16}H_{33} dissolved in 10 ml of 1,4-dioxane. Heating under N_2 was continued at 90 °C for 6 hr, the volume being maintained at ca. 20 ml by occasionally adding ethanol. The resulting dull orange solution was evaporated to dryness in a rotary evaporator. The brown solid was washed with diethyl ether to remove the unreacted ligand, then transferred to a porous glass filter. The solid was carefully extracted by gravity filtration with a limited volume (ca. 2 ml) of cold 95% ethanol, leaving a dark violet solid (presumably [(bipy)_2RuCl_2]).

The ethanol extract of the crude product was purified by two passes through a Sephadex LH-20 column (2 x 43 cm), using 50-50% (by volume) acetone-95% ethanol as the eluting solvent. The desired product eluted first as a red-orange band. That band was followed by a purple band (probably [(bipy)_2RuCl_2]), a brown band (probably [(bipy)_2RuCl-(solvent)]^+), and an unidentified yellow band. The desired product fraction was rechromatographed and the middle portion of the second pass was evaporated to dryness, crystallized once from hot acetone, washed with cold acetone, suspended in diethyl ether, and collected by filtration. The brick-red solid was dried over phosphorus pentoxide under reduced pressure for 12 hr. The yield was 0.043 g (40% based on Ru).

Anal. Found: C, 61.95; H, 7.91; N, 9.94. Calculated for [(C_{10}H_8N_2)_2Ru(C_{38}H_{70}N_4)]Cl_2·3H_2O: C, 62.14; H, 8.21; N,
3.2.4.4. "[(Bipy)₂Ru(H₂O)Cl]PF₆"  

To a 250 ml round-bottomed flask were added 0.114 g (2.16 x 10⁻⁴ mole) of [(bipy)₂RuCl₂]·2.5H₂O, 35 ml of water, and 45 ml of ethanol. The mixture was refluxed and stirred magnetically under N₂ for 1 hr to convert the complex to [(bipy)₂Ru(H₂O)Cl]⁺. The reflux condenser was removed and the solution was allowed to evaporate under the N₂ stream to half-volume. The concentrated brown solution was passed through a column (1.7 x 6 cm) containing 2.5 g (dry weight) of anion exchange cellulose (Bio-Rad Cellex-D) that had been converted to its PF₆⁻ form; water was the elution solvent. The eluted solution was lyophilized to yield 0.10 g of dark brown powder. The powder was stored in a sealed glass vial at room temperature in the dark. The color of the powder darkened gradually over the period of weeks, and eventually became nearly black (solutions in acetone were blue). The darkened powder was submitted for analysis.

Anal. Found: C, 33.53; H, 2.77; N, 7.84. Calculated for [(C₁₀H₈N₂O)₂Ru(H₂O)Cl]PF₆: C, 39.25; H, 2.94; N, 9.16. Molar ratios found: C, 4.99; H, 4.95; N, 1.00 (expected for 2,2'-bipyridine: C, 5.00; H, 4.00; N, 1.00).

3.2.4.5. 4,4'-DICARBONYL CHLORIDE-2,2'-BIPYRIDINE  

To a 250 ml round-bottomed flask fitted with a reflux condenser were added 0.597 g (2.45 x 10⁻³ mole) of 4,4'-dicarboxy-2,2'-bipyridine and 15 ml of thionyl chloride
under argon. \( \text{N}_2 \) was passed through the assembly while the mixture was refluxed for 4.5 hr, giving a yellow solution. The solution was rotary evaporated to near dryness. The pale yellow solid was dried and stored over NaOH in vacuo. IR (KBr pellet): 1755 cm\(^{-1}\) (\(-\text{COCl}\)). (A shoulder on the 1755 cm\(^{-1}\) band near 1730 cm\(^{-1}\) (\(-\text{COOH}\)) grew noticeably over the 30 min period that the KBr pellet was in the IR spectrometer.)

### 3.2.4.6. \( \text{N,N'-(HEXADECYL)-4,4'-DICARBOXYAMIDE-2,2'-BIPYRIDINE} \) (\(\text{BIKY-CONH}\text{C}_{16}\text{H}_{33}\))

The flask containing 4,4'-dicarbonyl chloride-2,2'-bipyridine was flushed with argon while 1.648 g (5.93 \( \times \) 10\(^{-3}\) mole) of 1-hexadecylamine hydrochloride, 50 ml of methylene dichloride, and 1.0 ml of triethylamine were added. A precipitate formed immediately. The flask was fitted with a reflux condenser and the mixture was kept at reflux temperature under \( \text{N}_2 \) for 2.5 hr. Following the reaction, the precipitate was collected by filtration, and was washed consecutively with diethyl ether, aqueous sodium bicarbonate solution, water, ethanol, and acetone. The off-white solid was dried at room temperature under reduced pressure, yielding 0.82 g (49\% based on the 4,4'-dicarboxy-2,2'-bipyridine used to prepare 4,4'-dicarbonyl chloride-2,2'-bipyridine).

The ligand was recrystallized with difficulty from hot tetrahydrofuran. Mp 216-218 °C; IR (KBr pellet): 1630 cm\(^{-1}\) (\(-\text{CONH}\)\(-\)).
3.2.4.6

Anal. Found: C, 76.47; H, 10.59; N, 7.98. Calculated for \( \text{C}_{44}\text{H}_{74}\text{N}_{4}\text{O}_{2} \): C, 76.52; H, 10.72; N, 8.17.

3.2.4.7. \([\text{N,N'-DI(HEXADECYL)-4,4'-DICARBOXAMIDE-2,2'-BIPYRIDINE)}\text{-bis-(2,2'-BIPYRIDINE)Ru(II)}]\text{-PERCHLORATE HYDRATE}

\([\{(\text{BIPY})_{2}\text{Ru(BIPY-CONHC}_{16}\text{H}_{33})\}](\text{ClO}_{4})_{2}\cdot\text{H}_{2}\text{O}) \quad (2)

To a 250 ml round-bottomed flask fitted with a reflux condenser were added 0.120 g (1.74 \times 10^{-4} \text{ mole}) of bipy-CONHC_{16}H_{33}, 60 ml of chloroform, and 0.0993 g of "\[(\text{bipy})_{2}\text{-Ru(H}_{2}\text{O)Cl}]\text{PF}_{6}\)" (containing 2.8 \times 10^{-4} \text{ mole of bipy}) in 25 ml of acetone. The mixture was refluxed under \( \text{N}_{2} \) for 3 hr, then the solvent was removed by rotary evaporation. The solid was suspended in cold chloroform and the suspension was gravity-filtered through porous glass to extract the product from unreacted bipy-CONHC_{16}H_{33}. Chloroform was removed from the filtrate by rotary evaporation, and the brown solid was redissolved in 25 ml of acetone. 2 precipitated upon addition of 21 ml of 0.30 M aqueous sodium perchlorate solution. The rust-colored precipitate was collected by filtration, washed four times with water, and dried in vacuo at 50 °C over phosphorus pentoxide for 16 hr. The yield was 0.129 g (70\% based on the bipy content of "\[(\text{bipy})_{2}\text{Ru(H}_{2}\text{O)Cl}]\text{PF}_{6}\)". IR (KBr pellet): 1660 \text{ cm}^{-1}

(-CONH-).

Anal. Found: C, 58.26; H, 6.85; N, 8.29. Calculated for \([(\text{C}_{10}\text{H}_{8}\text{N}_{2})_{2}\text{Ru(C}_{44}\text{H}_{74}\text{N}_{4}\text{O}_{2})]\text{(ClO}_{4})_{2}\cdot\text{H}_{2}\text{O}: C, 58.18; H, 6.97;
3.3.1. SYNTHESES

3.3.1.1. PREPARATION OF [(BIPY)$_2$Ru(BIIM-$C_{16}H_{33}$)]$Cl_2$·$3H_2O$ (1)

2,2'-Biimidazole was alkylated with 1-bromohexadecane in alkaline dimethylformamide at room temperature. This procedure was developed and carried out by Dr. Yohmei Okuno (1976/77), and is included above for completeness. Biim-$C_{16}H_{33}$ reacted with cis-[(bipy)$_2$Ru(H$_2$O)Cl]$^+$,Cl$^-$ in dioxane-water-ethanol at 90 °C to produce 1. The product was separated from most of the remaining starting materials by ethanol extraction. Column chromatography over Sephadex LH-20, which separates primarily by molecular exclusion, yielded analytically pure material after two passes.

3.3.1.2. PREPARATION OF [(BIPY)$_2$Ru(BIPY-CONHC$_{16}H_{33}$)]$^-$

$(ClO_4)_2$·$H_2O$ (2)

The long-chained bipyridine derivative, bipy-CONHC$_{16}H_{33}$, was prepared via the acid chloride of 4,4'-dicarboxy-2,2'-bipyridine. The ligand (50% excess) failed to react with [(bipy)$_2$Ru(H$_2$O)Cl]$^+$,Cl$^-$ in refluxing ethanol-water (70-30%, by volume) within 2 hours, as judged by the absorption spectrum of the diluted reaction mixture and its lack of luminescence. On the other hand, bipy-CONHC$_{16}H_{33}$ did react with the air-altered PF$_6^-$ salt of [(bipy)$_2$Ru(H$_2$O)$^-$-Cl]$^+$ in a refluxing mixture of chloroform and acetone to yield the desired complex. Chloroform extraction of the
dried reaction mixture separated the product from most of the starting materials. Precipitation of the partially purified product from acetone with aqueous sodium perchlorate solution yielded analytically pure 2.

3.3.2. SOLUBILITIES OF 1 AND 2

Both 1 (chloride salt) and 2 (perchlorate salt) were readily soluble in chloroform, ethanol, acetone, and tetrahydrofuran, and were insoluble in benzene and diethyl ether. Dimethylformamide was the best solvent found for 2.

1 dissolved easily (>10% by weight; >0.1 M) in warm water to give optically clear preparations which were indefinitely stable at room temperature. The addition of salts such NaCl caused the complex to precipitate from water. When an aqueous dispersion of 1 was placed in cellophane dialysis tubing and dialyzed against water for 24 hours, the complex remained inside the tubing. Electron microscopic examination of a dispersion which had been negatively stained revealed a nearly uniform population of round particles with diameters of about 200 Å as well as much larger filamentous particles (Fig. 3-4). The unstained preparation was not visible in the microscope.

2 dissolved slightly in boiling water, and some of it (10^-5-10^-4 M) remained in solution at room temperature. The material obtained by exchanging the perchlorate counterion of 2 with chloride dispersed readily into warm water to form optically clear preparations, like the chloride salt of 1.
Figure 3-4. Electron micrograph of a dispersion of [(bipy)$_2$Ru(biim-C$_{16}$H$_{33}$)]Cl$_2$ in water, which was negatively stained with 2% aqueous phosphotungstate. Ruthenium complex concentration = 0.0010 M. Magnification = 43,500 x.
3.3.3. OPTICAL ABSORPTION AND EMISSION SPECTRA

3.3.3.1. [Ru(BIPY)_3]Cl_2 \cdot 6H_2O

The absorption and emission spectra of a solution of [Ru(bipy)_3]Cl_2 in water are reproduced in Fig. 3-5. Four of the absorption bands, whose assignments have been established (see Section 3.3.4.1), are labeled I to IV for purposes of discussion. Band maxima and extinction coefficients are listed in Table 3-1. The excitation spectrum for the emission at 605 nm peaked at 465 nm, with shoulders on the high-frequency side at about 450, 425, and 400 nm, and a structureless tail on the low-frequency side.

The absorption spectrum of the complex varied little with the solvents examined; band I peaked at 453, 451, and 453 nm when the solvents were water, ethanol, and dichloromethane, respectively.

3.3.3.2. [(BIPY)_2Ru(BIIM-C_{16}H_{33})]Cl_2 \cdot 3H_2O (1)

The absorption and emission spectra of [(bipy)_2Ru(biim-C_{16}H_{33})]Cl_2 dissolved in 50-50% (by volume) ethanol-water are reproduced in Fig. 3-6 and summarized in Table 3-1. The absorption bands are labeled by analogy to those of [Ru(bipy)_3]^{2+}. Generally, the spectrum of 1 resembled that of [Ru(bipy)_3]^{2+} except that band I was red-shifted by 21 nm to 474 nm, and band II was much more prominent. The emission maximum was likewise red-shifted. The quantum yield for the luminescence of 1 was about 3% of that of [Ru(bipy)_3]^{2+}. 
Figure 3-5. Absorption (solid lines) and emission (broken line) spectra of $[\text{Ru(bipy)}_3]\text{Cl}_2$ dissolved in water.
### Table 3-1. Absorption and emission of $[\text{Ru(bipy)}_3]\text{Cl}_2$, $[(\text{bipy})_2\text{Ru(biim-}C_{16}\text{H}_{33})]\text{Cl}_2$ (1), and $[(\text{bipy})_2\text{Ru(bipy-CONHC}_{16}\text{H}_{33})](\text{ClO}_4)_2$ (2)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Absorption</th>
<th>Emission</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\lambda_{\text{max}}, \text{nm}$ $\lambda_{\text{max}}$</td>
<td>$\text{rel. quantum yield}$</td>
</tr>
<tr>
<td></td>
<td>$\varepsilon, \text{mM}^{-1}\text{cm}^{-1}$</td>
<td></td>
</tr>
</tbody>
</table>

#### [Ru(bipy)$_3$]Cl$_2$

- **in water**
  - $\lambda_{\text{max}}$: 453 nm
  - $\varepsilon$: (14) mM$^{-1}$cm$^{-1}$
  - **Emission**
    - $\lambda_{\text{max}}$: 600 nm
    - rel. quantum yield: 100

- **in ethanol-water**
  - $\lambda_{\text{max}}$: 474 nm
  - $\varepsilon$: (8.9) mM$^{-1}$cm$^{-1}$
  - **Emission**
    - $\lambda_{\text{max}}$: 637 nm
    - rel. quantum yield: 3

- **in chloroform**
  - $\lambda_{\text{max}}$: 463 nm
  - $\varepsilon$: (14) mM$^{-1}$cm$^{-1}$
  - **Emission**
    - $\lambda_{\text{max}}$: 615 nm
    - rel. quantum yield: 230

#### $[(\text{bipy})_2\text{Ru(biim-}C_{16}\text{H}_{33})]\text{Cl}_2$

- **in ethanol-water**
  - $\lambda_{\text{max}}$: 484 nm
  - $\varepsilon$: (12) mM$^{-1}$cm$^{-1}$
  - **Emission**
    - $\lambda_{\text{max}}$: 660 nm
    - rel. quantum yield: 60

---

*a* Aqueous detergent solution; DHAPS is $\text{CH}_3(\text{CH}_2)_{15}\text{N}^+\text{(CH}_3)_2(\text{CH}_2)_3\text{SO}_3^-$
Figure 3-6. Absorption (solid lines) and emission (dashed line) spectra of [(bipy)$_2$Ru(biim-C$_{16}$H$_{33}$)]Cl$_2$ dissolved in ethanol-water (50-50%, by volume).
[(bipy)$_2$Ru(biim-C$_{16}$H$_{33}$)]Cl$_2$

in 50%-50% CH$_3$CH$_2$OH-H$_2$O
The absorption spectrum of 1 varied little with the solvents examined, but the position of the emission maximum was solvent-dependent. The solvents examined were chloroform, ethanol, methanol, ethanol-water mixtures, water, and aqueous detergent solution (0.010 M DHAPS). The positions of the absorption bands I and III varied by less than 2 nm, with the exception of the chloroform solution, which had maxima at 484 and 292 nm, respectively.

The emission spectrum of 1 in 50-50% ethanol-water (Fig 3-5) peaked at 640 nm and had shoulders at about 655 and 690 nm. Dispersions of 1 in water had emission maxima at 658 nm with prominent shoulders at about 643 nm. The emission maximum for the aqueous detergent solution of 1 was at 662 nm, with a shoulder near 640 nm and a more prominent one near 680 nm. When the solvent was 50-50% ethanol-water, the excitation spectrum for the emission at 640 nm peaked at 467 and 365 nm, with shoulders at about 480, 450, and 430 nm.

The quantum yield for the luminescence of 1 was about 5% greater when the solvent was 70-30% ethanol-water than when it was pure water, when both samples were saturated with argon. The emission intensity at 650 nm dropped reversibly to a limiting 90% of its initial value within 25 s when the solvent was 50-50% ethanol-water, and dropped reversibly to a limiting 80% of its initial value within 50 s when the solvent was water. The admission of air reduced the luminescence intensity from the samples containing etha-
nol by 5%, and increased the intensity from the samples in pure water by 5%.

3.3.3.3. \([(BIPY)_2 Ru(BIPY-CONHC_{16}H_{33})](ClO_4)_2 \cdot H_2O \quad (2)\]

The absorption spectrum of 2 had a marked dependence on solvent compared to the spectra of \([Ru(bipy)_3]^2+\) and 1. The two extreme spectral patterns observed for 2 are represented by solutions of 2 in chloroform and in aqueous detergent (DHAPS) solution. These spectra are reproduced in Fig. 3-7 and summarized in Table 3-1. The spectrum of the chloroform solution closely paralleled that of \([Ru(bipy)_3]^2+\) except for a red-shift of band I by 10 nm to 463 nm. The weak shoulder near 300 nm on band III, which is absent in the spectrum of \([Ru(bipy)_3]^2+\), was due to the bipy-CONHC_{16}H_{33} ligand since it appears in the spectrum of the ligand alone. The visible spectrum of 2 in aqueous detergent solution was more complex than that of the chloroform solution.

Absorption spectra of 2 dissolved in various polar organic solvents had shapes that were intermediate to the chloroform and detergent solution spectra. In general, as the longest wavelength peak of band I red-shifted, both band II and the shoulder on band III near 305 nm became more prominent, while the peak intensity of band III dropped. There was a roughly linear correlation between the frequency of band I and the solvent polarity (as indexed by the Hildebrand solubility parameter (Hildebrand and Scott, 1964; Snyder, 1979)) (see Section 3.4.3.3).
Figure 3-7. Absorption (solid lines) and emission (dashed lines) spectra of \([(\text{bipy})_2\text{Ru(\text{bipy-CONHC}_16\text{H}_{33})})(\text{ClO}_4)_2\cdot\text{H}_2\text{O}\]
dissolved in chloroform (upper panel) or aqueous detergent solution (lower panel). The detergent is DHAPS (0.010 M).
[(bipy)$_2$Ru(bipy-CONHC$_{16}$H$_{33}$)]($ClO_4$)$_2$·H$_2$O in CHCl$_3$

and

[(bipy)$_2$Ru(bipy-CONHC$_{16}$H$_{33}$)]($ClO_4$)$_2$ in aqueous detergent solution
The absorption spectra of 2 dispersed in various aqueous media had the characteristic pattern of the detergent solution spectrum shown in Fig. 3-7. Egg PC vesicle suspensions containing 2 had band I maxima near 480 nm. The other aqueous samples examined were those obtained by injecting an ethanolic solution of 2 into aqueous 0.10 M ammonium acetate solution, by adding warm water to the chloride salt, or by boiling a suspension of the perchlorate salt in water. In the latter samples, two kinds of spectra were obtained. In one case, the spectral pattern was similar to the ones mentioned above, with the peak of band I at 482 nm; this sample was noticeably light-scattering. In the other case, the spectral pattern of the complex was unusual in that band I peaked at 469 nm, and there were maxima near 570 and 610 nm on the low energy tail of band I (Fig. 3-8).

The excitation spectrum for the emission at 615 nm from 2 dissolved in chloroform had a peak at 466 nm with shoulders at about 450 and 430 nm (Fig. 3-9). The excitation spectrum for the emission at 660 nm from the detergent solution had similar peak and shoulder positions, but, in addition, there was a prominent shoulder at about 480 nm. This shoulder appeared as a relatively weak inflection in the excitation spectrum for the chloroform solution. In contrast to the chloroform and aqueous detergent solutions, the excitation spectrum for the emission at 660 nm from 2
Figure 3-8. Absorption spectrum of [(bipy)$_2$Ru(bipy-CONHC$_{16}$H$_{33}$)](ClO$_4$)$_2$ dispersed in water by boiling a suspension of the solid. Path length = 1.00 cm.
Figure 3-9. Emission excitation spectrum of [(bipy)$_2$Ru-
(bipy-CONHC$_{16}$H$_{33}$)](ClO$_4$)$_2$·H$_2$O dissolved in egg PC vesicles
(solid line) or chloroform (broken line). Egg PC:ruthenium
complex molar ratio = 20:1; buffer = 1 M NH$_4$OAc, 0.1 M KCl,
0.1 M NaCl, pH = 7.
dissolved in egg PC vesicles had six distinct maxima above 400 nm (Fig. 3-9). The excitation maxima were at 491, 481, 467, 450, 440, and 400 nm, with the 467 nm peak being the highest one. There were shoulders at about 500, 460, 430, and 410 nm.

3.4. DISCUSSION
3.4.1. SYNTHESSES

\[(\text{Bipy})_2\text{Ru(H}_2\text{O)Cl}]^+\text{} \] is a useful intermediate for the preparation of \(\text{bis-bipyridyl Ru(II)}\) complexes in which the coordinated water and chloride ion are replaced by two monodentate or one bidentate ligand (Dwyer et al., 1963; Crosby and Elfring, 1976; Staniewicz et al., 1977; Dose and Wilson, 1978; Sullivan et al., 1978; Sullivan et al., 1979). \[(\text{Bipy})_2\text{Ru(H}_2\text{O)Cl}]^+\text{} \] is prepared by dissolving \[(\text{bipy})_2\text{RuCl}_2\] in refluxing water or aqueous alcohol, and is usually used without isolation:

\[
\[(\text{bipy})_2\text{RuCl}_2\] + \text{H}_2\text{O} \rightarrow \[(\text{bipy})_2\text{Ru(H}_2\text{O)Cl}]^+ + \text{Cl}^- 
\]
The reaction is best carried out under an inert atmosphere to avoid the oxidation of Ru(II) to Ru(III) by air. Continued refluxing after the addition of excess ligand is usually sufficient for the preparation of mixed-ligand complexes. The relative proportions in the reaction mixture of \[(\text{bipy})_2\text{Ru(H}_2\text{O)Cl}]^+, \text{ which is brown, and } \[(\text{bipy})_2\text{RuCl}_2]\text{, which is purple, can be judged from the color of the solution before adding the ligand. When the solvent is aqueous ethanol, the cationic aquated form is favored by increasing} \]
the proportion of water in the solvent. My experience with this system indicates that \([(\text{bpy})_2\text{Ru}(\text{H}_2\text{O})\text{Cl}]^+\) is more readily substituted than \([(\text{bpy})_2\text{RuCl}_2]\).

The limited solubilities of the ligands biim-C\(_{16}\)H\(_33\) and bipy-CONHC\(_{16}\)H\(_33\) in refluxing aqueous-ethanol made the usual procedure unsatisfactory for the preparation of complexes 1 and 2. Biim-C\(_{16}\)H\(_33\) was sufficiently soluble in 1,4-dioxane that the addition of the ligand as its dioxane solution to \([(\text{bpy})_2\text{Ru}(\text{H}_2\text{O})\text{Cl}]^+\) in ethanol-water, followed by refluxing, yielded 1. Purification of the complex by careful extraction of the dried reaction mixture, followed by gel-permeation chromatography over Sephadex LH-20, was straightforward. It was clear from the purification and low yield that a considerable fraction of the starting materials were unreacted. The yields could undoubtedly be improved by using different reaction conditions.

The ligand bipy-CONHC\(_{16}\)H\(_33\) was practically insoluble in ethanol and most organic solvents examined, but it was slightly soluble in hot tetrahydrofuran or chloroform. The hexafluorophosphate salt of \([(\text{bpy})_2\text{Ru}(\text{H}_2\text{O})\text{Cl}]^+\) provided a source of this substitution-active bis-bipyridyl complex for the preparation of the mixed-ligand complexes in non-aqueous solvents. The hexafluorophosphate salt reacted with bipy-CONHC\(_{16}\)H\(_33\) in refluxing acetone-chloroform solvent to yield \([(\text{bpy})_2\text{Ru}(\text{bipy-CONHC}_{16}\text{H}_{33})]^2+\), which was isolated as its perchlorate salt. (The photolysis of the parent compound,
[Ru(bipy)₃]Cl₂, in chlorinated solvents produces [(bipy)₂Ru-Cl₂] and bipy (Gleria et al., 1978; Durham et al., 1980), so this reaction should be carried out in dim light.)

The nature of the slow conversion of "[(bipy)₂Ru(H₂O)-Cl]PF₆" from the brown form to the dark blue form in the solid state (Section 3.3.4.4) was not investigated. However, the absorption spectrum of the darkened material dissolved in acetone had a maximum at 665 nm, resembling the spectra of oxo-bridged Ru(III) complexes with the general formula given below,

\[
[(\text{bipy})_2\text{Ru-O-Ru(bipy)}_2]^n+
\]
\[
\begin{array}{c}
\text{X} \\
\text{X}
\end{array}
\]

where X = Cl⁻ (n = 2) or H₂O (n = 4) (Weaver et al., 1975). These complexes are formed by air-oxidation of [(bipy)₂Ru-(S)Cl]⁺ (S = acetone) in acetone solution (Weaver et al., 1975). The darkened solid therefore possibly contained the oxo-bridged dimer, although elemental analysis of the substance was not consistent with it having been the pure dimer (as its PF₆⁻ salt). The material was readily converted to [(bipy)₂Ru(H₂O)Cl]⁺ by dissolving it in water or adding water to the acetone solutions.

3.4.2. SOLUBILITIES OF 1 AND 2

1 dispersed relatively easily into water compared to other compounds with two long hydrocarbon chains and charged polar groups, such as dipalmitoylphosphatidylcholine, didodecyldimethylammonium bromide, and 1,1'-dihexadecyl-4,4'-
bipyridinium dichloride. Since the solubilities (critical micelle concentrations) of these kinds of molecules are of the order of $10^{-7}$–$10^{-10}$ M (Sackmann, 1978; Tanford, 1980), it is likely that micellar aggregates of 1 were present in the aqueous dispersions. The optical clarity of the dispersions indicates that the aggregates were smaller than 1000 Å. The impermeability of the complex to dialysis tubing supports this contention and indicates the presence of aggregates with a dimension greater than 48 Å, the approximate average pore diameter of the tubing (McPhie, 1971). Direct evidence for colloidal aggregates of 1 was obtained by electron microscopy, by which round particles with diameters of about 200 Å were seen (Fig. 3-4). It is likely that these were vesicles, that is, hollow bilayer spheres, since bilayers are the usual micellar form of amphiphilic molecules with two long aliphatic chains (Section 1.4). The dispersions of 1 were unstable to added electrolytes, which caused them to become turbid and precipitate.

The chloride salt of 2 was considerably more "soluble" in water than the perchlorate salt, presumably because the chaotropic, "water structure-breaking" nature of perchlorate ion compared to chloride ion (Hatefi and Hanstein, 1974) causes dehydration of the polar region of the complex, thereby making it more difficult to disperse in water than the chloride form. Counterions greatly influence the surface pressure-area characteristics and luminescence of
monolayers of di-ester analogues of 2 (Gaines et al., 1978). Compared to chloride ion, perchlorate ion has a condensing effect on monolayers of the complexes and increases the luminescence intensity by a factor of 20 (Gaines et al., 1978). Counterions probably influence the micelle-forming properties of such amphiphilic complexes as well, and could account for the variability in the optical properties of the aqueous dispersions of the perchlorate salt of 2.

3.4.3. OPTICAL ABSORPTION AND EMISSION SPECTRA

3.4.3.1. ELECTRONIC STRUCTURE AND SPECTRA OF [Ru(bipy)₃]²⁺ AND THE CORRESPONDING COMPLEXES OF Fe(II) AND Os(II)

The electronic properties of [Ru(bipy)₃]²⁺ are reviewed below to provide a basis for interpreting the spectral properties of the mixed-ligand analogues, 1 and 2. A comparison of [Ru(bipy)₃]²⁺ to the Fe(II) and Os(II) tris-bipyridyl complexes emphasizes the effect of the atomic number of the metal ion on electronic structure, as reflected by spectral and photophysical properties (Felix et al., 1979; Felix et al., 1980; Sutin and Creutz, 1980).

The tris-bipyridyl complexes of Fe(II), Ru(II), and Os(II) are all diamagnetic with (nd)⁶ electron configurations in their ground electronic states, and they have trigonal geometries (D₃ symmetry, see Fig. 3-1). The ground states are singlets, with the spectroscopic notation ¹A₁. The molecular geometries are approximately octahedral without a center of symmetry (point group O). The geome-
tries are probably not much different when the complexes are in their electronic excited states or their trivalent or monovalent oxidation states (Rillema et al., 1979; Sutin and Creutz, 1980). The tris-(1,10-phenanthroline) metal complexes resemble their bipy counterparts.

The lowest-lying electronic excited states of tris-(2,2'-bipyridine) metal complexes are classified into three types (DeArmond, 1974; Crosby, 1975): (i) metal-localized excited states in which an electron is promoted within the metal ion from a "non-bonding" $d$ orbital ($d_{xy}$, $d_{yz}$, or $d_{zx}$) orbital to an "antibonding" $d_{o}^{*}$ ($d_{x^2-y^2}$ or $d_{z^2}$) orbital, (ii) ligand-localized excited states in which an electron is promoted within a bipy ligand from a bonding $\pi$-orbital to an antibonding $\pi^{*}$-orbital, and (iii) metal-to-ligand charge-transfer (MLCT) excited states in which an electron is promoted from a non-bonding $d_{\pi}$-orbital on the metal ion to an antibonding $\pi^{*}$-orbital on bipy.

In the following discussion, transitions between electronic states and their corresponding absorption bands will be classified according to the nature of the excited state produced by the transition. The symbols ($d_{\pi}$, $d_{o}^{*}$), ($\pi$, $\pi^{*}$), and ($d_{\pi}$, $\pi^{*}$) will be used to designate the metal-localized ("ligand-field"), ligand-localized, and MLCT excited states, respectively.

Absorption bands due to transitions between the ground state ($^{1}A_{1}$) and either the singlet $^{1}(d_{\pi}, \pi^{*})$ or $^{1}(\pi, \pi^{*})$
excited states dominate the spectrum of [Ru(bipy)$_3$]$^{2+}$ in the visible and ultraviolet regions, respectively. "Low resolution" band assignments for the spectrum of [Ru(bipy)$_3$]$^{2+}$ (Fig. 3-5) are listed in Table 3-2, where the two highest filled $\pi$-orbitals and the two lowest empty $\pi^*$-orbitals of bipy are labeled, in order of increasing energy, $\pi_2$, $\pi_1$, $\pi_1^*$, and $\pi_2^*$. Bands I and II are predominantly MLCT transitions, while bands III and IV are predominantly ligand-localized transitions (Palmer and Piper, 1966; Mason, 1968; Lytle and Hercules, 1969; Bryant et al., 1971; Harrigan and Crosby, 1973; Van Houten and Watts, 1976; Mayoh and Day, 1978; Felix et al., 1979; Wallace and Bard, 1979; DeArmond et al., 1980; Felix et al., 1980). The emission originates from the lowest MLCT excited states (Lytle and Hercules, 1969; Demas and Crosby, 1971; Harrigan and Crosby, 1973; Fujita and Kobayashi, 1973; Van Houten and Watts, 1976; Mayoh and Day, 1978; Wallace and Bard, 1979; DeArmond et al., 1980; Hipps, 1980). The emission spectrum has shoulders which are resolved in rigid matrices (Lytle and Hercules, 1969; Crosby, 1975; DeArmond et al., 1980; Felix et al., 1980).

Spin-allowed ($d_\pi$, $\pi^*$) transitions, which are polarized in the metal-ligand direction (perpendicular to the $C_3$ axis, see Fig. 3-1), account for the peak and shoulder of band I of [Ru(bipy)$_3$]$^{2+}$ (Mason, 1968; Lytle and Hercules, 1969; Felix et al., 1979; DeArmond et al., 1980; Felix et al.,
Table 3-2. Assignments for bands I to IV in the visible and ultraviolet absorption spectrum of \([\text{Ru(bipy)}_3]^{2+}\)

<table>
<thead>
<tr>
<th>electron distribution</th>
<th>state</th>
<th>band assignment ((1A_1 \rightarrow) excited state)</th>
</tr>
</thead>
<tbody>
<tr>
<td>((\pi 1)^2(d_\pi)^6)</td>
<td>(1A_1)</td>
<td>I</td>
</tr>
<tr>
<td>((d_\pi)^5(\pi 1^*)^1)</td>
<td>(1(d_\pi, \pi 1^*))</td>
<td>II</td>
</tr>
<tr>
<td>((d_\pi)^5(\pi 2^*)^1)</td>
<td>(1(d_\pi, \pi 2^*))</td>
<td>III</td>
</tr>
<tr>
<td>((d_\pi)^5(d_\sigma^*)^1)</td>
<td>(1(d_\pi, d_\sigma^*))</td>
<td>IV</td>
</tr>
<tr>
<td>((\pi 1)^1(\pi 1^*)^1)</td>
<td>(1(\pi 1, \pi 1^*))</td>
<td></td>
</tr>
<tr>
<td>((\pi 1)^1(\pi 2^*)^1)</td>
<td>(1(\pi 1, \pi 2^*))</td>
<td></td>
</tr>
</tbody>
</table>

\(d_\pi = d_{xy}, d_{yz}, d_{zx}; d_\sigma^* = d_{x^2-y^2}, d_z^2\)

bipy orbital energies: \(\pi 2 < \pi 1 < \pi 1^* < \pi 2^*\)
1980). The "tail" on the low-energy side of band I arises in part from the corresponding spin-forbidden (singlet-to-triplet) transitions, whose intensities are derived mainly from a spin-orbit coupling mechanism (Felix et al., 1979; Felix et al., 1980). The orange-colored emission of [Ru(bipy)₃]²⁺ is attributed to the radiative decay of the lowest-lying triplet MLCT excited states (Lytle and Hercules, 1969; Demas and Crosby, 1971; Wallace and Bard, 1979), although, strictly, strong spin-orbit coupling prohibits the assignment of purely singlet or triplet character to the MLCT excited states of the complex (Crosby et al., 1974).

Band II, which is rather obscure in the absorption spectrum of [Ru(bipy)₃]²⁺, shows up in the excitation wavelength dependence of the luminescence of the complex (Fujita and Kobayashi, 1973; DeArmond et al., 1980) and is more prominent in the absorption spectra of mixed-ligand complexes (Bryant et al., 1971) (see next Section). The assignment for band II as a MLCT transition (Bryant et al., 1971) is supported by semi-empirical molecular orbital calculations (Mayoh and Day, 1978). The ligand-field (dₓ, dᵧ*) absorption band, which is expected to be relatively weak (<100 M⁻¹cm⁻¹), is probably masked by the MLCT bands (Palmer and Piper, 1966).

Absorption bands III and IV are assigned to (π, π*) transitions localized on the bipy ligands, mainly because
similar bands are observed in the spectra of mono-protonated bipy and other metal complexes with bipy (Nakamoto, 1960; Kiss and Császár, 1963; Mason, 1968; McWhinnie and Miller, 1969; Lytle and Hercules, 1969; Bryant et al., 1971; DeArmond et al., 1980).

Qualitative orbital and state energy diagrams applicable to [Ru(bipy)₃]²⁺, which were deduced from the spectral assignments in Table 3-2 and photophysical measurements in the literature, are shown in Fig. 3-10. The frequencies of the absorption and emission band maxima fix the energies of the excited states relative to the ground state. Consequently, the approximate energy levels of the orbitals relative to the dₓ orbital can be deduced.

The positions of the (dₓ, dₒ*) states relative to the (dₓ, π*) states greatly influence the excited state decay processes of the [Ru(bipy)₃]²⁺ and analogous complexes (Fink and Ohnesorge, 1969; Van Houten and Watts, 1976; Durham et al., 1980). The (dₓ, dₒ*) states appear to be non-radiative and are primarily responsible for ligand-substitution photochemistry while the (dₓ, π*) states are radiative and are primarily responsible for electron-transfer photochemistry (Van Houten and Watts, 1976; Durham et al., 1980). As mentioned above, the (dₓ, dₒ*) bands are "invisible" in the absorption spectrum of [Ru(bipy)₃]²⁺, but, based on the temperature dependence of the luminescence of the complex, Van Houten and Watts (1976) concluded that the (dₓ, dₒ*)
Figure 3-10. Orbital and state energy diagram for [Ru-(bipy)$_3$]$^{2+}$ (after Crosby (1975), Van Houten and Watts (1976), and Wallace and Bard (1979)). The orbital diagram indicates the splitting of the five d-orbitals of the Ru$^{2+}$ ion by the octahedral ligand field, as well as the relative energies of the d-orbitals and the $\pi$ and $\pi^*$ orbitals of the bipyridine ligands. The left-hand side of the state diagram indicates the electron configurations and relative energies of the ground and five lowest excited states of the complex, before electron configuration interaction. The right-hand side of the state diagram indicates the relative energies of the ground state and lowest excited singlet and triplet states. Some of the rate constants by which the excited states decay to the ground state are shown.
states lie about 3.6 kK above the radiative \((d_\pi, \pi^*)\) states. According to their model, radiative \((7 \times 10^4 \text{ s}^{-1})\) and non-radiative \((1.2 \times 10^6 \text{ s}^{-1})\) decay from the \((d_\pi, \pi^*)\) manifold, and non-radiative decay \((10^{13} \text{ s}^{-1})\) from the \((d_\pi, d_\sigma^*)\) manifold are the two main pathways for deexcitation of photoexcited \([\text{Ru(bipy)}_3]^{2+}\) in aqueous solution at room temperature. The probability that an excited state is of \((d_\pi, \pi^*)\) or \((d_\pi, d_\sigma^*)\) parentage is assumed to be determined by the Boltzmann distribution equilibrium between the states. Non-radiative decay from the \((d_\pi, d_\sigma^*)\) states contributes significantly to the deexcitation of photoexcited \([\text{Ru(bipy)}_3]^{2+}\) at 25 °C, even though the Boltzmann factors heavily favor the \((d_\pi, \pi^*)\) states, because of the large difference in rate constants for decay from the two states (Van Houten and Watts, 1976).

Detailed descriptions of the lowest excited \((d_\pi, \pi^*)\) configuration of \([\text{Ru(bipy)}_3]^{2+}\) lead to the question of whether the optically promoted electron is best represented as being localized on one of the bipy ligands, or as being delocalized over all three ligands, or whether the electron hops within the complex from ligand to ligand (Mason, 1968; Crosby and Elfring, 1976; Dallinger and Woodruff, 1979; DeArmond et al., 1980). Distinctions between these cases depends on the time scale being considered. Models based on luminescence quantum yields, lifetimes, and polarization support a delocalized representation (Crosby and Elfring,
1976; DeArmond et al., 1980), while resonance Raman studies support a localized representation (Dallinger and Woodruff, 1979). The time resolution of the resonance Raman spectra was of the order of nanoseconds (Dallinger and Woodruff, 1979), while the luminescence occurs in the microsecond time domain, so the two views could be compatible if the electron hops between bipy ligands on the sub-microsecond time scale. In mixed-ligand bis-bipy complexes containing third ligands that are better electron acceptors than bipy, luminescence polarization results suggest that the optically promoted electron is localized on the third ligand (Ferguson et al., 1979) (Sections 3.4.3.2 and 3.4.3.4).

Like [Ru(bipy)₃]²⁺, [Fe(bipy)₃]²⁺ and [Os(bipy)₃]²⁺ are highly colored complexes due to MLCT absorption bands in the visible region. There are notable differences in the photophysical properties of the three complexes reflecting differences in spin-orbit coupling and ligand field stabilization energies (10 Dq), which determines the energy gap between the dₓ and dₒ* orbitals. The lowest excited state of [Fe(bipy)₃]²⁺ appears to be a metal-localized (dₓ, dₒ*) state (Creutz et al., 1980; Sutin and Creutz, 1980). [Fe(bipy)₃]²⁺ does not luminesce and has a short excited-state lifetime in water (0.8 ns) compared to [Ru(bipy)₃]²⁺ (600 ns). The reversal of the order of the (dₓ, π*) and (dₓ, dₒ*) states in the Fe(II) and Ru(II) complexes is consistent with the empirical expectation that 10 Dq for [Fe-
(bipy)$_3^{2+}$ should be about 30% less than 10 Dq for [Ru-(bipy)$_3^{2+}$] (Jørgensen, 1969). A 9-fold higher oscillator strength for the spin-forbidden MLCT absorption bands of [Os(bipy)$_3^{2+}$] compared to [Ru(bipy)$_3^{2+}$] is attributed to the stronger spin-orbit coupling in the Os(II) complex (Felix et al., 1979; Felix et al., 1980). Spin-orbit coupling is probably also responsible for the 30-fold shorter excited-state lifetime of the emission of [Os(bipy)$_3^{2+}$] in water (19 ns (Creutz et al., 1980)) compared to that of [Ru(bipy)$_3^{2+}$] (Demas and Crosby, 1971).

3.4.3.2. MIXED-LIGAND BIS-BIPYRIDYL Ru(II) COMPLEXES

The replacement of one of the bipy ligands of [Ru-(bipy)$_3^{2+}$] with a symmetric bidentate ligand, or two identical monodentate ligands, can affect the electronic structure of the complex in several ways, including: (i) the molecular symmetry is reduced from $D_3$ to $C_2$, (ii) the unique ligand(s) have $\pi$ and $\pi^*$ orbital energies different from those of bipy, and (iii) the ligand field stabilization energy (10 Dq) is different, so the $d_\pi$ and $d_\sigma^*$ orbital energies are different. If the unique ligand is a 2,2'-diimine, as is the case with complexes 1 and 2, the approximately octahedral microsymmetry of [Ru(bipy)$_3^{2+}$] is conserved (Hipps and Crosby, 1975).

Generalizations can be made about the absorption and emission spectra of mixed-ligand complexes with formulae cis-[(bipy)$_2$Ru(L)], where L stands for a bidentate ligand or
two monodentate ligands, compared to the spectra of the parent \([\text{Ru(bipy)}_3]^{2+}\) complex. (The net charge on \([\text{Ru(bipy)}_2^-\text{Ru(L)}]\) depends on the charge on \(L\).) In general, the maxima of both MLCT bands I and II shift to lower frequencies, and the extinction coefficient for band II increases relative to that of band I as the ligand field strength (or "pi-acidity" (McWhinnie and Miller, 1969; Cotton and Wilkinson, 1972)), of \(L\) decreases relative to that of bipy (Bryant et al., 1971; Mahoney and Beattie, 1973; Brown et al., 1975; Brown et al., 1976; Staniewicz et al., 1977; Dose and Wilson, 1978; Sullivan et al., 1978; Sullivan et al., 1979; Haga and Tanika, 1979; Durham et al., 1980). The red-shifts of the absorption maxima are accompanied by red-shifts of the emission maxima, and the emission quantum yields and lifetimes decrease (Demas and Crosby, 1971; White and Ohnesorge, 1972).

The electron distribution in the \(d_{\pi}, \pi^*\) excited state of \([\text{Ru(bipy)}_2\text{Ru(L)}]\) should differ from that of \([\text{Ru(bipy)}_3]^{2+}\) since \(L\) will generally be either a poorer or a better electron acceptor than bipy. As was mentioned in the previous Section, the optically promoted electron in \(\text{Ru(bipy)}_3\) could hop between bipy ligands. By making the complex electronically asymmetric upon replacing a bipy ligand with \(L\), the electron should become more localized on either bipy or \(L\), since the probability for an electron to hop from bipy to \(L\) should differ from the probability for the reverse
process.

For the series of four complexes \([\text{Ru(bipy)}_m\text{(phen)}_n]^{2+}\), where phen is 1,10-phenanthroline and \(m + n = 3\), the emission frequencies, quantum yields, and lifetimes have values that are weighted averages of the corresponding values for the parent complexes \((m = 3 \text{ or } n = 3)\) (Crosby and Efring, 1976). In addition, the frequencies and extinction coefficients for band I of the complexes follow this trend (Staniewicz et al., 1977). Thus, for this series of complexes, the lowest MLCT excited state configuration is best viewed as a weighted average property of individual bipy---Ru\(^{2+}\) and phen---Ru\(^{2+}\) interactions. This implies that the photoexcited electron in the complexes is delocalized over all three ligands (Crosby and Efring, 1976). By contrast, when one of the bipy ligands of \([\text{Ru(bipy)}_3]^{2+}\) is made more electron-accepting with carbonyl substituents in the positions para to the nitrogen atoms of bipy, the electron resides mainly on the substituted ligand (Ferguson et al., 1979), or perhaps on a single pyridine ring of the substituted ligand (Seefeld et al., 1977). The contrast between these example could be due to the similarities between bipy and phen as ligands. Electronic asymmetry in mixed-ligand complexes and their excited states should become more pronounced as \(L\) and bipy become more dissimilar.

It will be assumed in the following discussion of the optical properties of 1 and 2 that the ligand field strength
of mixed-ligand complexes, which is determined by the entire coordination sphere about the Ru$^{2+}$ ion, is a weighted-average value of the field strengths of the parent tris-complexes, while the ligands retain their individual $\pi$ and $\pi^*$ orbitals. Thus, the orbital energy diagram for a mixed-ligand complex is assumed to have $d_\pi$ and $d_\sigma^*$ orbitals, whose energy of separation ($10D_q$) is an average value determined by the entire ligand field, and to have $\pi$ and $\pi^*$ orbitals due to each ligand. Intraligand ($\pi$, $\pi^*$) absorption bands due to both bipy and L, as well as MLCT ($d_\pi$, $\pi^*$) bands due to electron transfer from the metal center to each ligand, are thus expected to appear in the absorption spectrum of [(bipy)$_2$Ru(L)].

3.4.3.3. [(BIPY)$_2$Ru(BIIM-C$_{16}$H$_{33}$)]Cl$_2$·3H$_2$O (1)

Overall, the spectral features of 1 parallel those of [Ru(bipy)$_3$]$^{2+}$, so analogous assignments for bands I to IV will be assumed in the following discussion.

The red shift of band I, prominence of band II, and weak emission of 1 compared to [Ru(bipy)$_3$]$^{2+}$ (Figs. 3-4 and 3-6, Table 3-1) indicate that biim-C$_{16}$H$_{33}$ lies below bipy in the "spectrochemical series" of ligand field strengths. This conclusion is based on correlations with other bis-bipyridyl Ru(II) complexes (see Section 3.4.3.2). The lower value of $10D_q$ for biim-C$_{16}$H$_{33}$ is probably largely due to geometric factors. The nitrogen atoms of bidentate ligands with five-membered rings like biim-C$_{16}$H$_{33}$ are less favorably
positioned for octahedral coordination to metal ions than are the nitrogen atoms of ligands with six-membered rings like bipy (Brandt et al., 1954; Lindoy and Livingstone, 1967; Abushamleh and Goodwin, 1979).

The reduction in $10D_q$ caused by replacing a bipy ligand of $[Ru(bipy)_3]^{2+}$ with biim-$C_{16}H_{33}$ accounts for the red-shifted MLCT absorption and emission maxima, as well as the lower emission quantum yield of $\mathcal{L}$ compared to $[Ru(bipy)_3]^{2+}$. The reduction in $10D_q$ decreases the $d_\pi - \pi^*$ orbital energy separations (Fig. 3-10), so the MLCT absorption and emission bands red-shift. There is also a decrease in the energy gap between the radiative ($d_\pi, \pi^*$) and the non-radiative ($d_\sigma, d_\sigma^*$) excited states which, according to the model of Van Houten and Watts (1976), reduces the luminescence quantum yield (Section 3.4.3.1).

Biim-H is a considerably poorer ligand than bipy for tris-coordination to either Fe$^{2+}$ or Ni$^{2+}$ (Abushamleh and Goodwin, 1979). $[Fe(biim-H)_3]^{2+}$ is pale yellow in color and paramagnetic (high-spin), in contrast to $[Fe(bipy)_3]^{2+}$, which is intensely violet colored and diamagnetic. The spectroscopic value for $10D_q$ in $[Ni(biim-H)_3]^{2+}$ is 10.8 kK while $10D_q$ in $[Ni(bipy)_3]^{2+}$ is 12.7 kK (Abushamleh and Goodwin, 1979).

Surprisingly, there is no band in the ultraviolet spectrum of $\mathcal{L}$ that can be attributed to the $(\pi l, \pi l^*)$ transition localized on biim-$C_{16}H_{33}$. The shape of the spectrum of $\mathcal{L}$
below 300 nm resembles that of [Ru(bipy)$_3$]$^{2+}$ (compare Figs. 3-5 and 3-6). The bands at 288 and 243 nm (Table 3-1) can be attributed to the $^1(\pi_1, \pi_1^*)$ and $^1(\pi_1, \pi_2^*)$ transitions that are localized on bipy, by analogy to the assignments for [Ru(bipy)$_3$]$^{2+}$ (e.g., Tables 3-1 and 3-2). The ultraviolet spectrum (400-220 nm) of biim-C$_{16}$H$_{33}$ in ethanol solution has a single peak at 260 nm ($\varepsilon = 10,800 \pm 1000$ M$^{-1}$cm$^{-1}$), so this band is as intense as that for bipy ($\varepsilon = 10,200$ M$^{-1}$cm$^{-1}$ at 283 nm (Nakamoto, 1960)).

By contrast to 1, the ultraviolet spectra of [Ru-(bipy)$_2$(phen)]$^{2+}$ and [Ru(bipy)(phen)$_2$]$^{2+}$ contain bands due to both bipy and phen (Crosby and Elfring, 1976). On the other hand, the 1,8-naphthyridine ligands in bis-bipyridyl Ru(II) complexes analogous to 1 are "invisible" in the ultraviolet spectra of the complexes (Staniewicz et al., 1977).

The absorption and emission spectra of [(bipy)$_2$Ru(biim-H)]$^{2+}$ reported by Dose and Wilson (1978) differ significantly from the spectra for 1 reported here (Table 3-1). Band I of their compound peaks at 448 nm (9,500 M$^{-1}$cm$^{-1}$), and there is no maximum corresponding to band II; the emission maximum is at 595 nm. The replacement of hydrogen by hexadecyl substituents is not be expected to affect the spectra to that extent. During the course of this work, it was found that refluxing a mixture of [(bipy)$_2$RuCl$_2$] and biim-H in 50-50% (by volume) ethanol-aqueous 1.0 N HCl
yields an orange product whose visible absorption spectrum in ethanol resembles that of \( \lambda \) (maxima at 342 and 478 nm).

The source of the differences between these results and those of Dose and Wilson is not obvious. A possible complication is that Dose and Wilson used acetonitrile solutions for their spectra. Acetonitrile can replace weakly bound ligands such as the 1,8-naphthyridines in bis-bipyridyl Ru(II) complexes (Brown et al., 1975; Dose and Wilson, 1978). Another complication is the fact that biim-H can coordinate to metal ions through the \(-\text{NH}-\) groups instead of the diimine nitrogen atoms (Lindoy and Livingstone, 1967), so various isomers are possible for \([(\text{bipy})_2\text{Ru(biim-H)}]^2+\).

There was no evidence for a concentration dependence of the optical properties of \( \lambda \). In particular, the absorption spectrum and luminescence quantum yield of \( \lambda \) dispersed in water, when it was apparently aggregated as vesicles (Section 3.4.2), were similar to those of the complex dissolved in ethanol or aqueous ethanol, when it was presumably monomeric. Gaines et al., (1978) likewise found no evidence for a concentration quenching of the luminescence of the long-chained esters \([(\text{bipy})_2\text{Ru(bipy-COOR)}]^2+\) in monolayer films.

3.4.3.4. \([(\text{BIPY})_2\text{Ru(BIPY-COOC}_{16}\text{H}_{33})](\text{ClO}_4)_2 \cdot \text{H}_2\text{O} \) (2)

Compared to \([\text{Ru(bipy)}_3]^2+\), the absorption spectrum of \( \text{2} \) is sensitive to solvent changes (Ford and Calvin, 1980). Similar changes are exhibited by di-carboxylic acid ester
derivatives (Sprinthschnik et al., 1976; Valenty and Gaines, 1977; Sprinthschnik et al., 1977; Seefeld et al., 1977; Harriman, 1977; Yellowlees et al., 1978; Gaines et al., 1978; Ferguson et al., 1979; Johansen et al., 1979; Gaines, 1980) and di-ketone derivatives (Johansen et al., 1979) which are analogous to 2. Transferring these dicarbonylated complexes, abbreviated to [(bipy)$_2$Ru(bipy-COR)]$^{2+}$ (where R = -NHC$_{16}$H$_{33}$, -OC$_{18}$H$_{37}$, or -C$_{18}$H$_{37}$), from aqueous solvents to purely organic solvents causes the visible MLCT absorption band (I) with two maxima to collapse into bands with single peaks and the emission maxima to blue-shift (e.g., Fig. 3-7). Johansen et al. (1979) suggested that the spectral changes reflected changes in the state of aggregation of the complexes. It will be argued below that the changes were due the solvent dependence of the MLCT transition corresponding to electron transfer from Ru$^{2+}$ to the dicarbonylated bipy ligand in the mixed-ligand complexes. Similar views were expressed by Ferguson et al. (1979) and by Gaines (1980).

The absorption spectra of 2 in various solvents had characteristics intermediate to the two extremes shown in Fig. 3-7. There appeared to be a correlation between the spectral patterns and the solvent polarity. A plot of the frequency of the long-wavelength peak of band I ($\nu_\text{max}$), as a spectral parameter, versus the dielectric constant of the solvent, as a measure of solvent polarity, was linear for
organic solvents, but points for micellar aqueous systems fell well below the line determined by organic solvents when the dielectric constant of bulk water (80) was used. Using estimates for the dielectric constant of micelle/water interfaces of 10-40 (Fernández and Fromherz, 1977; Bellamare and Fragata, 1980), instead of the value for bulk water, made the deviation even greater. There was a poor correlation between $\bar{\nu}_{\text{max}}$ and either Kosower's (1958) $Z$-values or Reichardt's (1965) $E_T$-values, for both the organic and aqueous solvent systems. However, a plot of $\bar{\nu}_{\text{max}}$ versus the Hildebrand solubility parameter ($\delta$), which is another measure of solvent polarity (Hildebrand and Scott, 1964; Snyder, 1979) was roughly linear for all solvents examined, including points corresponding to aqueous media. The plot is shown in Fig. 3-11. The solubility parameter equals the square root of the energy of vaporization divided by the molar volume. The value of $\delta$ for the aqueous dispersions (points a to e) was assumed to be 23, which is the value for bulk water (Snyder, 1979).

Unfortunately, no single parameter can be used to characterize solvent polarity (Reichardt, 1965). Nevertheless, the linear correlation in Fig. 3-11 indicates that the effect of solvent on the spectrum of 2 was mainly one of polarity, with no specific interactions between the complex and the solvent (e.g., hydrogen-bonding) being obvious. A plot analogous to Fig. 3-11 for [Ru(bipy)$_3$]$^{2+}$, using the
Figure 3-11. Plot of the peak frequency of the visible charge-transfer absorption band (I) of \([(\text{bipy})_2\text{Ru(bipy-CONH-C}_{16}\text{H}_{33})](\text{ClO}_4)_2\cdot\text{H}_2\text{O}\) versus the Hildebrand solubility parameter of the solvent \((\delta)\). Points \(a\) to \(e\) correspond to various aqueous media; the \(\delta\)-value of 23 for these points is the value for pure water. The \(\delta\)-values for the mixed solvents are the weighted average values for the pure solvents on a volume basis (Snyder, 1979). The least-squares straight line for all points except for \(a\) to \(e\) is shown.
data of Harriman (1977) with water, ethanol, and ether-pentane-alcohol as solvents, shows a similar trend, with a slope that is about 60% smaller than that for 2.

The decrease in $\tilde{v}_{\text{max}}$ with increasing solvent polarity implies that the lowest photoexcited state of 2 has a greater dipole moment than does the ground state of the complex (Reichardt, 1965). This conclusion is consistent with the MLCT assignment for the visible absorption bands of [Ru(bipy)$_3$]$^{2+}$ and related complexes (Section 3.4.3.1). The charge-transfer configuration of [Ru(bipy)$_3$]$^{2+}$ is viewed as having a Ru$^{3+}$ core coordinated to a bipyridine anion radical (Hipps and Crosby, 1975; Creutz and Sutin, 1980; Sutin and Creutz, 1980). The optically transferred electron in photoexcited [(bipy)$_2$Ru(bipy-CONHC$_{16}$H$_{33}$)]$^{2+}$ can presumably reside on either the bipy or bipy-CONHC$_{16}$H$_{33}$ ligands. In the latter case, electron configurations can be drawn in which the electron is located on the oxygen atom of one of the amide substituents. These configurations, which are indicated in Fig. 3-12, make the dipole moment of the $(d_\pi, \pi^*(\text{bipy-CONHC}_{16}\text{H}_{33}))$ excited state greater than that of the $(d_\pi, \pi^*(\text{bipy}))$ excited state. Thus, increasing the solvent polarity causes the $(d_\pi, \pi^*(\text{bipy-CONHC}_{16}\text{H}_{33}))$ contribution to band I to red shift more than the $(d_\pi, \pi^*(\text{bipy}))$ contribution. If it is assumed that the MLCT bands are, approximately, composites of bands due to individual Ru$^{2+}$-ligand interactions (Section 3.4.3.2), then band I should split as
Figure 3-12. Ground and charge-transfer excited state distributions in \([(\text{bipy})_2\text{Ru(bipy-CONH}C_{16}\text{H}_{33})]\)^{2+} involving the carboxamide-substituted ligand. Only a portion of the complex, the ruthenium ion and one ring of bipy-CONHC_{16}H_{33}, is shown. The excited state is envisaged as having the ruthenium ion in its 3+ oxidation state and the optically-promoted electron on one of the ligands. Only excited state configurations involving the carboxamide substituent are shown. Resonance contributions with the electron on the ring systems of the ligands are not included.
the solvent polarity increases. The spectral changes seen with 2 (Fig. 3-7) are consistent with this interpretation; similar arguments apply to other \([(\text{bipy})_2\text{Ru(bipy-COR)}]^2^+\) complexes as well (Ferguson et al., 1979; Ford and Calvin, 1980). The spectrum of \([\text{Ru(bipy)}_3]^2^+\) is relatively insensitive to solvent polarity because the three \(d_{\pi}, \pi^*(\text{bipy})\) transitions are equally affected, so band I does not split.

The spectral changes associated with the transfer of 2 from aqueous detergent solution to chloroform (Fig. 3-7) bear a striking resemblance to the changes associated with deprotonation of the carboxylic acid substituents of \([(\text{bipy})_2\text{Ru(bipy-COOH)}]^2^+\) (Valenty and Gaines, 1977; Giordano et al., 1977; Sprintzschnik et al., 1977; Gaines et al., 1978; Ferguson et al., 1979). The spectra of the protonated and deprotonated forms of that complex are shown in Fig. 3-13. The correspondence between the spectral patterns of the two complexes indicates a correspondence between their electronic structures. In the case of \([(\text{bipy})_2\text{Ru(bipy-COOH)}]^2^+\), deprotonation of the carboxylic acid substituents should disfavor excited-state configurations with the optically-promoted electron located on the carbonyl oxygen atoms of the bipy-COOH ligand (Ferguson et al., 1979; Ford and Calvin, 1980) (e.g., Fig. 3-12). Thus deprotonation is analogous to decreasing the solvent polarity in the case of \([(\text{bipy})_2\text{Ru(bipy-COR)}]^2^+\).

The increased prominence of the shoulder near 305 nm on
Figure 3-13. Absorption spectra of the deprotonated and protonated forms of [(bipy)$_2$Ru(bipy-COOH)]$^{2+}$ dissolved in 1.0 M KCl solution. Upper panel: the deprotonated form, pH = 5.84. Lower panel: the protonated form, pH = 1.05 (the pH was adjusted with concentrated HCl).
$[\text{bipy}_2 \text{Ru(bipy-COO}^-\text{)}]^{0}$ in water

$[\text{bipy}_2 \text{Ru(bipy-COOH)}]^{2+}$ in water
band III, which accompanies the red shifts of the MLCT bands I and II shown in Figs. 3-7 and 3-13, further implicates a role of the carbonyl substituents in the spectral changes because the shoulders are due to the carbonyl substituents of bipy-CONHCH_{16}H_{33} and bipy-COOH (Ferguson *et al.*, 1979; Ford and Calvin, 1980).

The interpretation presented above implies that the electron distributions of the MLCT excited states of [(bipy)$_2$Ru(bipy-COR)]$^{2+}$ are asymmetric, with the optically-promoted electron having a greater probability of residing on the bipy-COR ligand than on the bipy ligands. Thus a vectorial redistribution of charge during photoexcitation is an intrinsic property of the complexes. Such a directionality to electron transfer might be advantageous in effecting efficient photoinduced charge separation in micellar media in which amphiphilic derivatives would be naturally oriented at micelle-water interfaces.

A practical consequence of the solvent dependence of the spectrum of 2 is that the polarity of the environment of the chromophore can be assessed from the shape of the spectrum. The absorption spectrum of 2 dissolved in egg PC vesicle suspensions resembles that of the complex dispersed alone, or with detergents, in other aqueous media, so it can be inferred that the dye molecules are oriented in the vesicles with the charged chromophores located near the bilayer-water interface. This information is useful in
interpreting the dynamics of electron transfer across vesicle walls which is photosensitized by 2 (Chapter 4).

The excitation spectrum for the luminescence from 2 in vesicle suspensions shows considerably more structure in band I than the corresponding absorption spectrum. Nor is this structure resolved in the excitation spectra of chloroform solutions (Fig. 3-9). This latter difference was not a solvent effect alone since the excitation spectra of aqueous detergent solutions are more like those of chloroform solutions than vesicle suspensions. Perhaps the spectrum for the vesicle sample reflects an environment for 2 that was more viscous than its environment in the other two samples.

3.5. CONCLUSIONS

Two surfactant *tris*-bipyridyl-Ru(II) analogues can be prepared in a straightforward manner by reacting *bis*-bipyridyl-Ru(II) complexes with dihexadecyl-substituted diimine ligands. Complex 2, which contains a di-substituted bipyridine derivative (bipy-CONHCl₆H₃₃) as third ligand, is more attractive as a photosensitizer than is complex 1, which contains a di-substituted biimidazole derivative (biim-C₁₆H₃₃), because 2 has a greater stability to photodecomposition and higher luminescence quantum yield than 1. In designing new dyes of this class, attention should be paid to the strengths of the Ru(II)-ligand coordinate bonds, which can greatly influence the photochemical properties of the complexes by affecting the relative positions of the
MLCT and metal-localized excited states.

The chloride salts of the two new complexes (1 and 2) disperse readily into warm water, which is unusual for amphiphiles with two hexadecyl substituents. The resulting dispersions certainly contain micellar aggregates of the complexes, and vesicular structures are likely. The dispersions should be further characterized to determine the structures of the micelles. In contrast to the amphiphilic porphyrins discussed in Chapter 2, there is little or no effect of self-aggregation on the optical properties of either 1 or 2.

An interpretation for the solvent-dependence of the absorption spectrum of 2 leads to the conclusion that the metal-to-ligand charge-transfer bands are composites of bands due to charge-transfer transitions from Ru²⁺ to each ligand in the complex. These metal-to-ligand charge-transfer transitions vary independently with solvent changes, with increases in solvent polarity favoring transitions to \((d_\pi, \pi^*(\text{bipy-CONHC}_{16}\text{H}_{33}))\) excited states over \((d_\pi, \pi^*(\text{bipy}))\) excited states.

Luminescence excitation spectra provide spectral information not provided by the absorption spectra.
CHAPTER 4. A MODEL SYSTEM FOR PHOTOSENSITIZED ELECTRON TRANSFER ACROSS VESICLE WALLS

4.1. INTRODUCTION

The primary goal of this work was to develop a model system for studying dye-photosensitized electron transfer across vesicle walls. Ultimately we should be able to use membrane suspensions of this sort in solar energy conversion and storage devices which operate on the principle of water decomposition (see Chapter 1). Once a suitable model vesicle system was found, it was intended to characterize the mechanism of charge transport through the bilayer, and to improve the rates of transmembrane charge transport processes. These goals have been partly accomplished, inasmuch as a model system has been found, information about the locations of the various components has been obtained, a mechanism for the process has been proposed, and several ways for improving quantum yields of electron transport through the vesicle walls have been found.

Several arrangements for photosensitizing dyes (S) which are dissolved in vesicle walls were considered for accomplishing electron transfer from an electron donor (D) in one aqueous phase to an electron acceptor (A) in the other. Three such arrangements are depicted in Fig. 4-1. In all three cases, the membranes are asymmetric with respect to the aqueous solutions of D and A. Arrangement (1) is symmetric with respect to the dye S, which is amphi-
Figure 4-1. Three arrangements in which pigmented lipid bilayer membranes separate aqueous solutions of electron donor (D) and acceptor (A). In arrangement (1), the amphiphilic dye (S) is dissolved in each monolayer of the membrane. In (2), the membrane is asymmetric with respect to two kinds of amphiphilic dyes ($S_1$ and $S_2$). In (3), the dye ($S_3$) is free to diffuse within the membrane.
1) Water membrane water

D S S A

2) Water membrane water

D S₁ S₂ A

3) Water membrane water

D S₃ A

XBL 8010-4115
philic so that the position of the chromophore is close to the membrane-water interface. Arrangement (2) differs from (1) in that, in (2), there are two different photosensitizers ($S_1$ and $S_2$) and the membrane itself is asymmetric. In arrangement (3) the dye $S_3$, which is not necessarily amphiphilic, is dissolved in the membrane and is free to diffuse between the interfaces (the "location" of $S_3$ may change upon its photooxidation or reduction, however). All three arrangements are technically feasible, but (1) and (3) are probably generally simpler to prepare than is (2).

The vesicle systems described in this Chapter were prepared to be symmetric with respect to the photosensitizer, so they conformed to arrangement (1) of Fig. 4-1. The dye used was $[(bipy)_2Ru(bipy-CONHCH_3)]^{2+}$, which was described in Chapter 3; this dye will hereafter be abbreviated to (Ru$^{2+}$). The (Ru$^{2+}$) was co-dissolved in vesicle walls with egg yolk phosphatidylcholine (egg PC), which was the major component of the walls. Ethylenediamine-
N,N,N',N'-tetraacetate (EDTA) was dissolved in the interior aqueous compartments as the ultimate electron donor, and a water-soluble viologen such as methylviologen (MV$^{2+}$) was dissolved in the continuous aqueous phase as the ultimate electron acceptor. A schematic cross-section of the vesicle wall showing the arrangement of EDTA, (Ru$^{2+}$), and MV$^{2+}$ is shown in Fig. 4-2. The orientation of the (Ru$^{2+}$) molecules shown is inferred from spectral studies which indicate that
Figure 4-2. Probable locations of the electron donor (EDTA$^{3-}$), photosensitizer ([[bipy]$_2$Ru(bipy-CONHC$_{16}$H$_{33}$)]$^{2+}$), and electron acceptor (MV$^{2+}$) in a model system for studying photosensitized electron transport across vesicle walls.
the chromophore is located in an aqueous environment (Chapter 3).

Upon illumination with blue light, electron transfer across the vesicle walls from EDTA to MV$^{2+}$ was photosensitized by (Ru$^{2+}$). The progress of the reaction can be monitored spectrophotometrically because the blue methylviologen radical (MV$^+$) accumulates irreversibly. This system was modeled after an analogous homogeneous system in which [Ru-(bipy)$_3$]$^{2+}$ is the photosensitizer (Takuma et al., 1977; Takuma et al., 1978).

When this work was begun, it was not clear from the literature whether vesicle walls were intrinsically semiconducting to electronic charge carriers, or whether membrane-bound electron carriers, which pick up electrons at one interface and transfer them to the opposing interface by diffusion, were necessary to achieve transmembrane electron transport at sufficient rates to compete with back-reactions (Chapter 1). Originally we included vitamin K$_1$ (VK$_1$), which is a long-chained naphthoquinone, and decachlorcarborane to assist the transport of electrons and protons through the vesicle walls (Liberman et al., 1970; Anderson et al., 1976; Futami et al., 1979), in case they were needed (Ford et al., 1978). Later, we found that these two components were not required, and proposed that the mechanism of electron transfer across the vesicle walls involved electron-exchange between (Ru$^{2+}$) and (Ru$^{3+}$) dissolved in opposing monolayers
of the bilayer membranes (Ford et al., 1979). The effects of temperature (Mettee et al., manuscript in preparation) and ionophores and transmembrane electrochemical potential gradients (Laane et al., 1981; see Appendix 1) on the photosensitization reaction were also examined. These experiments will be discussed in detail in this Chapter.

4.2. EXPERIMENTAL SECTION

4.2.1. MEASUREMENTS

Unless otherwise noted, all measurements were carried out at room temperature (22.5±1.5 °C) and the samples were deaerated by bubbling with either N₂ or argon.

Ultraviolet and visible absorption spectra were measured with either Cary model 118 or 14 spectrophotometers; cuvettes with path lengths of either 1.00 or 0.30 cm were used.

Luminescence quenching was measured with a Perkin-Elmer model MPF-2A fluorescence spectrophotometer equipped with a 150 watt xenon lamp and a red-sensitive, type R-136, photomultiplier (Hamamatsu TV Co.). Luminescence was detected at right angles to the excitation. A red cut-off filter (Corning #2-69) was placed between the cuvette and photomultiplier tube. Quartz cuvettes with four polished sides and 1.00 by 0.30 cm path lengths were used; the cuvettes were oriented so the excitation beam struck the 1.00 cm face. Excitation was at 460 nm (slit width = 10 nm) and emission was detected at 660 nm (slit width = 6 nm).
4.2.2. MATERIALS

Unless otherwise noted, chemicals used were obtained from commercial suppliers and were used without further purification. Water was twice deionized. N₂ or argon gases for sample deaeration were passed through a column of a copper catalyst to remove O₂.

Egg PC was extracted and purified by Dr. S. Kohler according to the procedure described by Singleton et al. (1965). A stock solution of the egg PC in ethanol (fraction C, 28.5 mg/ml, which corresponds to a concentration of 0.037 M based on an average molecular weight of 770 g/mole), was stored under N₂ at -20 °C. Smaller portions of the stock solution were routinely transferred to a glass container, sealed under N₂, and stored in the freezer compartment of a refrigerator. Thin layer chromatography on silica (Analtech Inc., precoated with Silica Gel GF) of the egg PC, developed with 65-25-4% (by volume) chloroform-methanol-water and visualized with ultraviolet light, gave a single spot (Rf = 0.45).

The perchlorate salt of (Ru²⁺) was synthesized as described in Chapter 3. 1,1'-Dihexadecyl-4,4'-bipyridinium (hexadecylviologen, C₁₆V₂⁺) dichloride was synthesized by Dr. Y. Okuno (1977). Reinecke salt (NH₄[Cr(NH₃)₂(SCN)₄]·H₂O) from Eastman was converted to the potassium salt and recrystallized (Wegner and Adamson, 1966). 3-(Dimethylhexadecylammonio)propane-1-sulfonate (DHAPS) was synthesized by
the procedure of Clunie et al. (1967). Anthraquinone-2-sulfonate (AQS) from Eastman was recrystallized twice from hot water.

\( \text{VK}_1 \) (from Sigma) and all-trans retinyl acetate (type I, crystalline, synthetic, from Sigma) were stored desiccated in a refrigerator. 1,1'-Dimethyl-4,4'-bipyridinium (methylviologen, MV\( ^{2+} \)) dichloride (from Sigma), 1,1'-diheptyl-4,4'-bipyridinium (heptylviologen, C\( _7V^{2+} \)) dibromide (from Aldrich), decachloro-m-carborane (from PCR Research), 3-hydroxy-4-nitroso-2,7-naphthalenedisulfonic acid disodium salt (Nitroso-R-salt, NR) (from Fisher), and dioctadecyldimethylammonium bromide (from Eastman) were used as received.

Stock solutions of (Ru\( ^{2+} \)) (0.010 M) in dimethylformamide, \( \text{VK}_1 \) (0.020 M) in ethanol, decachloro-m-carborane (0.0010 M) in ethanol, C\( _{16}V^{2+} \) (0.010 M) in ethanol, C\( _7V^{2+} \) (0.036 M) in water, and MV\( ^{2+} \) (0.20 M) in water, were stored in the dark in glass vials at room temperature

4.2.3. METHODS

4.2.3.1. PREPARATION OF AQUEOUS SOLUTIONS

4.2.3.1.a. 0.30 M (NH\(_4\))\(_3\)EDTA, pH 8.6

Approximately 20 ml of water was added to 2.19±0.01 g (7.49 mmole) of ethylenediamine-\( N,N,N',N' \)-tetraacetic acid. Concentrated ammonium hydroxide (approximately 2.2 ml) was added while the mixture was being stirred, until the EDTA had dissolved and the pH had reached 8.55±0.05. The solution was transferred to a graduated cylinder and diluted
with water to a volume of 25.0±0.1 ml. The EDTA solution was stored in a glass container in the refrigerator.

To determine the stoichiometry of the preparation, the same procedure was followed using 4.0 N KOH instead of NH₄OH. At pH 8.7, the K⁺ concentration was 0.91 M, so the ammonium EDTA solution was best formulated as 0.30 M (NH₄)₃EDTA.

4.2.3.1.b. 0.87 M NH₄OAc-NH₄OH BUFFER CONTAINING 0.018 M Zn(OAc)₂, pH 8.6

18.74±0.02 g of NH₄OAc was dissolved in about 150 ml of water. 1.4 ml of concentrated ammonium hydroxide was added to give a pH of 8.5, and the solution was diluted to 200 ml. The final pH was 8.5.

0.612±0.001 g of Zn(OAc)₂·2H₂O was added to 100 ml of the NH₄OAc-NH₄OH solution. The pH was 7.9. 0.85 ml of ammonium hydroxide was added until the pH was 8.6. The solution was diluted to 158±2 ml and filtered. The final pH of the solution was 8.60±0.05.

4.2.3.2. PREPARATION OF VESICLE SAMPLES

4.2.3.2.a. ETHANOL INJECTION

Vesicle suspensions were prepared by adding the vesicle-forming components, dissolved in a water-miscible organic solvent (mainly ethanol), to vigorously stirred aqueous (NH₄)₃EDTA solution (Batzri and Korn, 1973). The following procedure for making vesicles whose walls contained egg PC, (Ru²⁺), and C₁₆V²⁺ (molar ratios 200:10:1; [egg PC] = 2 x
10^{-3} M) was typical.

3.00\pm0.04 \text{ ml of 0.30 M (NH}_4\text{)_3EDTA solution (Section 4.2.3.1.a) was pipetted into a glass test tube, and the top of the tube was sealed with parafilm to prevent splashing losses. The tube was held with a clamp, and plastic foam was placed between the tube and the clamp so that the tube could be swirled mechanically while its vertical motion was restricted. (Alternatively, the test tube could be hand-held.) The clamped test tube was inserted maximally into the rubber cavity of a Vortex Jr. Mixer (Scientific Industries, Inc.) with deep (about 4 cm) walls.

Room lights were kept dim when possible. Aliquots of stock solutions (Section 4.2.2) of (Ru^{2+}) in dimethylformamide (0.0300\pm0.0003 \text{ ml}), C_{16}V^{2+} in ethanol (0.0030\pm0.0003 \text{ ml}), and egg PC in ethanol (0.175\pm0.003 \text{ ml}) were transferred in that order, via syringes, into a small glass test tube with a conical tip. The mixture was swirled to insure its homogeneity. Each stock solution was sealed and returned to the dark immediately after use. The egg PC stock solution was flushed briefly with N_2 before the flask was sealed and replaced in the refrigerator freezer compartment.

The solution mixture was drawn into a 0.25 ml syringe (with about 0.05 ml of air between the plunger and solution), leaving no more than about 0.002 ml of solution in the test tube. The organic solution was injected (2-3 s duration) into the test tube containing vortex-stirred EDTA
solution. The tip of the syringe needle was not necessarily submerged, but care was taken that the organic solution struck the aqueous phase without first contacting the glass tube wall. (About 0.002 ml of the organic solution unavoidably remained in the syringe.) Agitation of the mixture was discontinued after 5-10 s, then stopped. N₂ was passed over the vesicle suspension while the tube was sealed with paraffin film. The suspension was stored in a refrigerator overnight before gel-filtration.

4.2.3.2.b. GEL-FILTRATION

The vesicle suspensions were gel-filtered (Flodin, 1961) to replace the EDTA solution in the continuous aqueous phase with ammonium acetate buffer containing Zn²⁺. The column used for gel-filtration was a 1.0 cm (internal diameter) by 20 cm Econo-Column (Bio-Rad Laboratories) packed to a height of 17.5 cm with Sephadex G-25-medium (Pharmacia Fine Chemicals) dextran gel. The column was fitted at the bottom with a stopcock. Solutions were applied to the top of the column via disposable pipettes, and were eluted by gravity feed.

Before applying the vesicles, the gel bed was equilibrated with 0.87 M NH₄OAc-NH₄OH + 0.018 M Zn(OAc)₂ aqueous buffer (Section 4.2.3.1.b). Excess buffer above the gel bed was removed by allowing it to pass into the gel, and the column stopcock was closed. A 10 ml graduated cylinder was placed under the column to collect the eluent. The vesicle
suspension was pipetted onto the top of the gel bed, and the column stopcock was reopened. After all the vesicle suspension (3.2 ml) had entered the gel, buffer was added to the top. The position of the vesicles in the column was followed by their yellow color; the colored fraction passed through as a single band. 5.75±0.05 ml of buffer eluted before the vesicle fraction began to elute (the first few drops containing vesicles, which were noticeably dilute, were not collected). The column stopcock was closed and the 10 ml graduated cylinder was replaced with a 5 ml one. Elution was continued until 3.00±0.05 ml of the vesicle fraction was collected. Later, the column was washed with water, aqueous EDTA solution, and again with water.

4.2.3.2.c. DEAERATION

The vesicle suspension obtained by gel-filtration was immediately transferred, via disposable pipette, to a glass cuvette with stopcock (described in Section 2.2.3.6). 0.0150±0.0003 ml of aqueous 0.20 M MV²⁺ solution, sufficient to give a final concentration of MV²⁺ of 0.0010 M, had previously been transferred to the cuvette. With the stopcock of the cuvette open, and a rubber serum stopper sealing the antechamber of the cuvette, the vesicle suspension was stirred magnetically under reduced pressure (applied via hose and syringe needle) for 10 minutes. The stopcock was closed, and the cuvette was transferred to a Schlenk line. There the vesicle suspension was bubbled with scrubbed N₂.
(via syringe needle) for 10 minutes. Following deaeration, the stopcock of the cuvette was kept tightly closed with rubber bands.

4.2.3.3. PHOTOCHEMISTRY

4.2.3.3.a. STEADY-STATE ILLUMINATIONS

The light source for steady-state illuminations was a 1000 watt xenon arc lamp (Oriel Universal Arc Lamp Source, model C-60-50) operated at 45 volts and 20 amps. The light passed first through a quartz condensing lens in the lamp housing, then through a combination of filters which absorbed infrared and ultraviolet radiation and transmitted blue light. The filter combination was an aqueous cupric sulfate solution and two Corning glass filters, #3-72 and #5-57. The peak transmission of the combination was 60% at 460 nm (see Fig. 2-2). Illumination of the sample was initiated and stopped with a manual shutter.

In most cases, the cuvette was illuminated through the window of a blackened plexiglas box containing about 10 ml of water as a constant temperature bath (22±1.5 °C). The window was 1.0 cm wide by 2.0 cm high. The cuvette was positioned 40 cm from the light source. The vesicle suspensions were stirred magnetically during illumination with a small teflon-coated magnet in the cuvette. The temperature of the bath, as measured with an iron-constatan thermocouple, varied by less than 0.5 °C during 15 min of continuous illumination.
For temperature-dependence studies, the temperature of the sample was maintained at either 18.0, 28.0, or 38.1 °C (all ±0.1°C) during the illuminations and the spectral measurements by keeping the cuvette in a large brass block (6 x 6 x 6 cm) with window openings and a channel through which coolant was circulated. A Haake model FE-2 thermo-regulator which was heat-sinked with tap water controlled the temperature of the block. An Electrometrics model ITS 600 digital thermister probe inserted into the brass block was used to monitor the temperature.

4.2.3.3.b. DETECTION OF VIOLOGEN RADICAL

The absorption spectrum (800-330 nm) of the vesicle suspension was recorded after deaeration, before the sample was illuminated. Then the optical density at 602 nm (OD·602), where MV⁺ has an absorbance maximum, was recorded as a function of time for about 30 s to establish an initial baseline. Next, the cuvette was taken to the lamp and illuminated for 10-60 s, then taken back to the spectrophotometer to record the change in OD·602. This procedure was continued until the rate of MV⁺ production fell to a value that was small to the initial rate.

The gas phase trapped in the cuvette above the vesicle suspension acted as a reservoir for a small amount of residual O₂, which cause a reoxidation of the viologen radical which was initially formed. The teflon coating of the magnetic stir bar and the cuvette walls also appeared to be
sources of residual $O_2$. The amount of $O_2$ initially present was typically $10^{-8}$ moles, assuming that each molecule of $O_2$ removed 2 $MV^+$ according to Eqs. 4-1 and 4-2 (Farrington et al., 1978):

\begin{align}
(4-1) \quad O_2 + MV^+ & \rightarrow O_2^- + MV^{2+} \\
(4-2) \quad O_2^- + MV^+ & \rightarrow O_2^{2-} + MV^{2+}
\end{align}

The diffusion of residual $O_2$ from the gas phase to the aqueous phase was rapid enough compared to the time scale of the absorbance measurement that the OD-602's measured after illuminations were time-dependent. OD-602 was monitored as a function of time and extrapolated back to "zero" time to estimate its value immediately after illumination. After the first several illumination intervals, the levels of $O_2$ were low enough that OD-602 was relatively time-independent, so extrapolation was not required.

The following procedure was adopted to alleviate the uncertainty caused by residual $O_2$. Within 30-40 s following illumination, OD-602 was recorded as a function of time. The cuvette contents were then shaken vigorously and OD-602 was again monitored. This step was repeated until successive shakings resulted in an absorbance drop of less than 0.01 and the absorbance was practically constant. Initially, the shaking resulted in complete oxidation of viologen radical. Sample shaking between illuminations was discontinued after it was apparent that the $O_2$ level in the cuvette was low enough to be neglected.
The number of moles of viologen radical produced during illumination was calculated from the sample volume (3.02 ml) and the increase in OD\textsuperscript{602}, using an extinction coefficient at 602 nm of $1.24 \times 10^4$ M$^{-1}$cm$^{-1}$, which is the value for MV$^+$ in water given by Steckhan and Kuwana (1974):

\begin{equation}
\text{(moles of radical produced)} = \frac{(\text{OD\textsuperscript{602}} \text{ cm}^{-1})(3.02 \times 10^{-3} \text{ l})}{(1.24 \times 10^4 \text{ M}^{-1}\text{cm}^{-1})}
\end{equation}

4.2.3.3.c. ACTINOMETRY

The light intensity of the lamp and filter arrangement described in Section 4.2.3.3.a was measured by Reinecke salt solution actinometry (Wegner and Adamson, 1966). Reinecke salt, which is the Cr$^{3+}$ complex $[\text{Cr(NH}_3)_2(\text{SeN})_4]^-$ (K$^+$ salt), decomposes when its aqueous solutions are illuminated. The photoreaction results in the release of thiocyanate ion (SCN$^-$) from the complex (Eq. 4-4) and the quantum yield (0.305) is nearly independent of the wavelength of light from 390 to 750 nm (Wegner and Adamson, 1966).

\begin{equation}
[\text{Cr(NH}_3)_2(\text{SCN})_4]^- + \text{H}_2\text{O} + h\nu \rightarrow [\text{Cr(NH}_3)_2(\text{SeN})_3(\text{H}_2\text{O})]+ \text{SCN}^-
\end{equation}

The concentration of released SCN$^-$ was determined spectrophotometrically as its red ferric complex, presumably $[\text{Fe(H}_2\text{O})_5(\text{SCN})]^{2+}$ (Eq. 4-5):

\begin{equation}
[\text{Fe(H}_2\text{O})_6]^{3+} + \text{SCN}^- \rightarrow [\text{Fe(H}_2\text{O})_5(\text{SCN})]^{2+} + \text{H}_2\text{O}
\end{equation}

The following procedure was used to make the measurements. Five 1.80±0.02 ml aliquots of aqueous 0.10 M Fe(NO$_3$)$_3$·9H$_2$O in 0.5 M HClO$_4$ were pipetted into five labeled
test tubes. Next, 0.132 g of recrystallized Reinecke salt was weighed in dim light. Then, in a dark room with dim red lighting, the tared Reinecke salt was transferred to a 10 ml graduated cylinder. Water was added to a volume of 5.0 ml. The mixture was shaken well. Almost all of the solid dissolved. Undissolved material was removed by filtering the mixture through a filter disc with a pore size of 0.45 μm (Millipore catalogue number HAWP01300). The filtered solution was collected in a 5 ml graduated cylinder. 3.50±0.05 ml of the filtered solution was transferred to the same cuvette that was used during illumination of vesicle suspensions (Section 4.2.3.3.a).

The cuvette was illuminated for four 60 s intervals while the solution was stirred magnetically. The incident light intensity was attenuated by placing a Balzers 18% neutral density filter between the Corning filters and the cuvette. After each interval, 0.20±0.01 ml aliquots were removed with a 0.25 ml syringe and added to one of the test tubes containing ferric nitrate solution. The syringe was rinsed with water after each transfer. The initial volume of the solution was sufficient to keep the level of the solution from falling below that being illuminated.

A "dark" blank sample was prepared by transferring 0.20 ml of the filtered Reinecke salt solution remaining in the graduated cylinder to the fifth aliquot of ferric nitrate solution. The blank was required because the Reinecke salt
decomposes thermally as well as photochemically (Wegner and Adamson, 1966).

The absorption spectra (800-350 nm) of the five samples were recorded (0.30 cm path length) soon after the illumination was completed. The concentration of \([\text{Fe(H}_2\text{O)}_5\text{(SCN)}]^{2+}\) in each solution was calculated by dividing the absorbance at 456 nm (per cm of solution) by 4280 ± 110 M\(^{-1}\)cm\(^{-1}\), the extinction coefficient for the ferric complex at that wavelength. This value was determined in a separate measurement using a KSCN solution of known concentration, and is close to the value determined by Wegner and Adamson (1966) (4.30 x 10\(^3\) M\(^{-1}\)cm\(^{-1}\) at 450 nm). The concentration of \([\text{Fe(H}_2\text{O)}_5\text{-(SCN)}]^{2+}\) in the blank was subtracted from the other four values. The number of moles of SCN\(^-\) released during each interval of illumination was obtained by multiplying these concentration differences by the volume of the Reinecke salt solution in the cuvette during illumination. The average of these four values was used to calculate the incident light intensity, \(I_0\).

\(I_0\) (einstein/min) was calculated from the rate of release of SCN\(^-\) by assuming that all of the incident light was absorbed by the Reinecke salt solution and taking the average quantum yield for Reinecke salt decomposition to be 0.305 in the range of 440-550 nm (Wegner and Adamson, 1966):

\[
(4-6) \ I_0 = \frac{\text{moles of SCN}^- \text{ released per min}}{0.305}
\]

The approximation that essentially all of the light was
absorbed was justified by the fact that the optical density (per cm) of the Reinecke salt solution was 2.6 at 460 nm, where the transmission of the filter was maximal, and only about 8% of the Reinecke salt was decomposed during the experiment.

On two separate occasions, $I_0$ was found to be $4.3 \pm 1.1 \times 10^{-5}$ and $3.2 \pm 0.4 \times 10^{-5}$ einsteins/min-cm$^2$; the xenon lamp was replaced during the time interval between these two measurements. The relatively large uncertainty in $I_0$ was probably due to inaccurate sampling of the photolyzed Reinecke salt solution, which was made difficult by the necessity for dim lighting conditions.

4.2.3.3.d. QUANTUM YIELD CALCULATIONS

The quantum yield of viologen radical production ($\varphi$) was calculated from the rate of radical production (Eq. 4-3) and the rate of light absorption by the sample, $I_{abs}$ (einsteins/min):

$$\varphi = \frac{\text{moles of radical produced per min}}{I_{abs}}$$

$I_{abs}$ was calculated from $I_0$ (Eq. 4-6) and the fraction of $I_0$ that was absorbed by the sample ($f_{abs}$):

$$I_{abs} = I_0 \times f_{abs}$$

The fraction $f_{abs}$ was calculated as a weighted average value for the fraction absorbed in each of the eleven 10 nm wavelength intervals spanning the range transmitted by the filter combination (440-550 nm):

$$f_{abs} = \frac{\sum_i (f_i \times T_i)}{\sum_i T_i}$$
In Eq. 4-9, $f_i$ denotes the fraction of incident light absorbed by the sample in the wavelength interval $i$, and was calculated from the absorption spectrum of the sample. $T_i$ denotes the average filter transmission in interval $i$, and was calculated from the absorption spectrum of the filter. Eq. 4-9 does not take into account the wavelength dependence of the lamp output, but, judging from output spectra for xenon lamps (Murov, 1973), the approximation that the distribution was flat between 440 and 550 nm was satisfactory for present purposes.

The estimated value of $f_{abs}$ was 0.87 for a typical vesicle suspension containing egg PC and (Ru$^{2+}$) (molar ratios 200:10) with a maximal absorbance of 0.93 (per cm) at 480 nm.

4.2.3.3.e. LIGHT INTENSITY DEPENDENCE

To determine the light intensity dependence of viologen radical production, $I_0$ was attenuated with neutral density filters. The percent transmission at 480 nm of the filters (metallic film type from Balzers) were measured by placing them in the sample beam of a spectrophotometer.

The vesicle suspensions contained egg PC, (Ru$^{2+}$), and C$_{16}$V$^{2+}$ (molar ratios 200:10:1), and MV$^{2+}$ (0.0010 M) was in the continuous aqueous phase. The suspensions were illuminated with full (100%) intensity until the concentration of viologen radical was about $2 \times 10^{-5}$ M. Subsequent illuminations were with either attenuated or full light intensities.
The effect of light attenuation was determined by comparing the $\phi$'s during alternate intervals with and without attenuation. The reported relative $\phi$'s represent the average values for at least four ratios.

4.2.3.3.f. COBALT(II)-EDTA COMPLEX AS ELECTRON DONOR

Cobaltous-EDTA complex was entrapped inside vesicles instead of EDTA$^{3-}$ to determine how the cobaltous complex compared to uncomplexed EDTA$^{3-}$ as an electron donor. Details of this experiment follow.

An aqueous solution containing 0.30 M [Co(II)-EDTA](NH$_4$)$_2$ and 0.60 M NH$_4$OAc was prepared as follows: 1.969±0.003 g (6.73 x 10$^{-3}$ mole) of ethylenediamine-N$_2$N,N',N'-tetraacetic acid and 2.128 g (26.9 x 10$^{-3}$ mole) of NH$_4$HCO$_3$ were suspended in about 5 ml of water. The mixture was stirred with warming for 20 minutes, until the solids dissolved and CO$_2$ evolution ceased. 1.678 g (6.73 x 10$^{-3}$ mole) of Co(OAc)$_2$·4H$_2$O was dissolved in about 6 ml of warm water, and the solution was slowly added to the EDTA solution. The resulting solution was diluted with water in a graduated cylinder to 22.4±0.2 ml, then stored refrigerated in a glass container. The pH was 6.7. The absorption spectrum of the solution had maxima at 465 and 620 nm and shoulders at 480 and 500 nm, indicating that the cobalt ion was in its divalent state (Srinivasan and Rabinowitch, 1970).

Vesicles composed of egg PC, (Ru$^{2+}$), and C$_{16}V^{2+}$ were
prepared as usual (Section 4.2.3.2.a) in 3.0 ml of the 0.30 M Co(II)-EDTA solution. The vesicle suspension was gel-filtered on a Sephadex G-25 M column to replace the continuous aqueous phase with buffer solution (1.2 M NH₄OAc, adjusted to pH 6.8 with acetic acid). MV²⁺ (0.0030 M) was added to the vesicle fraction, and the suspension was de-aerated and illuminated with 420-590 nm light as usual (Section 4.2.3.3.a).

4.2.3.3.g. QUENCHING OF (Ru²⁺) LUMINESCEENCE

Experiments with VK₁ or C₁₆V²⁺ as quenchers were carried out with vesicles composed of egg PC and (Ru²⁺) (molar ratio 200:10, [egg PC] = 0.0016 M). The vesicles were prepared by the injection method in 4.0 ml of an aqueous buffer containing 1.0 M NH₄OAc, 0.10 M KCl, and 0.10 M NaCl, pH 7. The suspension was diluted to 7.0 ml with buffer and used without gel-filtration. VK₁ and C₁₆V²⁺ are insoluble in water, so they were added to the vesicle suspensions as their solutions in ethanol (Section 4.2.2). The luminescence measurements were conducted with aerated samples.

Quenching by VK₁ was examined first. 1.0 ml of the diluted vesicle suspension was transferred to a quartz cuvette for reference, and 3.0 ml was stored in a refrigerator for later quenching by C₁₆V²⁺. An aliquot (about 1 ml) of the remaining suspension was pipetted to a sample cuvette, and the luminescence intensities at 660 nm for both the
reference and sample were recorded. Then the aliquot in the sample cell was returned to the remaining 2 ml portion. This suspension was stirred magnetically while an aliquot of VK₁ solution was added, and stirring was continued for an additional 2 minutes. An aliquot of the vesicle suspension was transferred back to the sample cuvette, and the luminescence intensities of the sample and reference were recorded. This process was repeated several times.

The quenching experiment was repeated using the second 3.0 ml portion of vesicle suspension, but this time aliquots of a solution of C₁₆V²⁺ in ethanol were added.

For quenching by MV²⁺ and C₇V²⁺, vesicles containing egg PC and (Ru²⁺) (molar ratio 200:10, [egg PC] = 0.0020 M) were prepared by the injection method in 10.0 ml of an aqueous buffer containing 0.91 M NH₄OAc-NH₄OH, pH 8.5 and were used without gel-filtration. The MV²⁺ and C₇V²⁺ were added as aqueous solutions (Section 4.2.2).

The temperature dependence of the quenching by C₇V²⁺ was examined with vesicles that had been gel-filtered to remove ethanol. The suspensions were kept at either 18.0, 28.1, or 38.0 °C (all ±0.1 °C) using a Haake model FE-2 thermoregulator to circulate water through the sample cell block supplied with the spectrofluorimeter.

The quenching of the luminescence of (Ru²⁺) was characterized by making Stern-Volmer plots, in which the ratio of the luminescence intensity without quencher to that with
4.2.3.3

quencher is plotted as a function of quencher concentration. The intensities were corrected for dilution of the samples caused by the addition of quencher solution. The luminescence of the reference samples was monitored so that corrections for variations in the performance of the instrument during the experiments (e.g., in the intensity of the excitation lamp) could be made.

4.2.3.4. PERMEABILITY OF EGG PC VESICLES

4.2.3.4.a. PERMEABILITY TO MV²⁺

The permeability of egg PC vesicles to entrapped MV²⁺ was determined by removing the untrapped MV²⁺ from the vesicle suspension at different times by gel-filtration, then measuring the remaining amount of entrapped MV²⁺ by spectrophotometry. Details of the experiment are given below.

Egg PC vesicles were prepared by ethanol injection into 3.0 ml of a stirred aqueous buffer (1.0 M NH₄OAc, 0.10 M KCl, 0.10 M NaCl, pH 7) containing 0.20 M MV²⁺ (Cl⁻ salt). The concentration of egg PC in the suspension was 0.0012 M. The vesicle suspension was stored in a refrigerator for 5 hours. The suspension was initially gel-filtered through a column packed with Sephadex G-25 M (18.5 x 1.1 cm) and equilibrated with the buffer. 4.0 ml of the vesicle fraction was collected after 6.5 ml of buffer had eluted. This process removed 99.5% of the untrapped MV²⁺ (see Results, Section 4.3.4.1).
1.5 ml of this gel-filtered suspension was filtered again through a second column (15.5 x 1.1 cm) within 5 minutes after the first. 2.0 ml of the vesicle fraction was collected after 6.2 ml of buffer had eluted. Meanwhile, the remaining 2.5 ml of once gel-filtered vesicle suspension was kept in the dark at room temperature. The second Sephadex column was washed well with water, then with the buffer solution. 2 hours after the first gel-filtration, 1.5 ml of the once gel-filtered suspension was passed through the second column, which had been washed and reequilibrated with buffer. 6.2 ml of buffer was eluted before the following 2.0 ml containing vesicles was collected.

The amount of MV$_2^+$ in the vesicle suspensions was determined spectrophotometrically, based on the ultraviolet absorption band of MV$_2^+$, which peaks at 256 nm (ε = 2.05 x 10$^4$ M$^{-1}$cm$^{-1}$ (Krumholz, 1951)).

4.2.3.4.b. PERMEABILITY TO EDTA$^{3-}$

The permeability of egg PC vesicles to entrapped EDTA$^{3-}$ was determined by using a vesicle-impermeant Cu(II)-complex in the continuous aqueous phase to monitor the concentration of untrapped EDTA$^{3-}$ as a function of time. A cationic surfactant was co-dissolved with egg PC in the vesicle wall to simulate the positive charge imparted by (Ru$^{2+}$). Experimental details are given below.

The aqueous stock solution of the Cu$^{2+}$ complex used to analyze EDTA$^{3-}$ concentrations contained equimolar concentra-
tions (0.010 M) of cupric acetate and the di-sodium salt of 1-nitroso-2-naphthol-3,6-disulfonate (also known as nitroso-R salt, which will be abbreviated NR). To prepare the solution, 0.0188 g (9.4 x 10^{-5} mole) of Cu(OAc)₂·H₂O and 0.0353 g (9.4 x 10^{-5} mole, based on the anhydrous molecular weight) of NR were separately dissolved in approximately 0.5 ml of water. The cupric acetate solution was added slowly to the yellow-green NR solution, with intermittent vortex stirring, resulting in the formation of a brown solution and precipitate. The mixture was diluted to about 6 ml with water and warmed. The undissolved precipitate was removed by filtering the mixture through a 0.45 μm pore-sized disc (Millipore). The filtered solution was stored in the dark at room temperature. The concentration of complexed NR salt in this solution was 0.010 M. This value was determined spectrophotometrically by diluting the solution and adding excess Na₂EDTA to give the uncomplexed form of NR, which has an absorption band maximum at 420 nm (ε = 6100 M⁻¹cm⁻¹).

Vesicles were prepared in 0.30 M (NH₄)₃EDTA solution as described in Section 4.2.3.2, except that dioctadecyldimethylammonium ion instead of (Ru²⁺) was co-dissolved with egg PC. 1.6 ml of the vesicle fraction which eluted from the Sephadex G-25 column was passed through a second column (0.7 x 5 cm) containing DEAE anion exchange gel (Pharmacia) which had been preequilibrated with the buffer solution. 1.4-1.5 ml of the vesicle fraction was collected after 0.7-
0.8 ml of buffer had eluted. Two procedures were used to determine the rate of release of EDTA$^{3-}$ from these vesicles. One sample was prepared by adding 0.048 ml of the 0.010 M CuNR solution to 1.2 ml of the vesicle suspension, and the spectrum of the CuNR complex was measured periodically. This sample will be referred to as the "undiluted" sample. The other procedure was to titrate a $5 \times 10^{-5}$ M solution of CuNR with aliquots of the vesicle suspension.

Spectral changes for CuNR due to the release of EDTA by the vesicles were calibrated by titrating the complex in the presence of vesicles with an aqueous solution of Na$_2$EDTA of known concentration (0.0162 M). The vesicles were composed of egg PC and dioctadecyldimethylammonium ion, as before, and had been prepared in buffer without EDTA$^{3-}$ and gel-filtered. The addition of EDTA caused an absorption band at 392 nm and shoulder near 470 nm to drop in intensity, while a band at 423 nm grew in intensity. The ratio of the absorbance at 392 nm to the absorbance at 423 nm, and the ratio of the absorbance at 473 nm to the absorbance at 423 nm, were plotted as functions of the EDTA concentration, yielding two smooth sigmoidal curves.

4.3. RESULTS

4.3.1. INITIAL PHOTOCHEMICAL EXPERIMENTS AT NEUTRAL pH

4.3.1.1. HOMOGENEOUS SOLUTIONS

The vesicle systems were modeled after the photosensitized reduction of MV$^{2+}$ using EDTA as electron donor and
[Ru(bipy)$_3$]$^{2+}$ as photosensitizer, which occurs in homogeneous aqueous solution (Takuma et al., 1977; Takuma et al., 1978). This experiment was repeated for comparison to the vesicle systems and to test for inhibition of the reaction by Zn$^{2+}$.

MV$^+$ was produced when an aerobic aqueous solution containing [Ru(bipy)$_3$]$^{2+}$ (5 x 10$^{-5}$ M), MV$^{2+}$ (5 x 10$^{-4}$ M), and EDTA (0.0010 M), buffered at pH 7.1 with 1.0 M ammonium acetate, was illuminated with visible light (420-590 nm). The dependence of the concentration of MV$^+$ on the cumulative illumination time was probably sigmoidal, beginning to saturate after >20% of the MV$^{2+}$ was reduced (see Fig. 4-3a).

When the experiment was repeated with 0.0020 M Zn$^{2+}$ added to the solution, no MV$^+$ was detected after 15 minutes of illumination (Fig. 4-3b). An increase in the concentration of MV$^+$ of less than 1 x 10$^{-6}$ M could have been detected. Based on the concentrations of MV$^+$ in the solutions with and without Zn$^{2+}$ after 10 minutes of illumination, the presence of Zn$^{2+}$ resulted in a decrease in the quantum yield of viologen radical by a factor of 100 or more. However, because residual O$_2$ in the cuvette could have reoxidized up to about 2 x 10$^{-8}$ mole of MV$^+$ before it was detected (Section 4.2.3.3.b), the inhibition factor could have been as low as 20.

4.3.1.2. VESICLE SUSPENSIONS

In the first vesicle system examined, C$_{16}$V$^{2+}$, VK$_1$, and
Figure 4-3. Photosensitized electron transfer from EDTA to MV$^{2+}$ in homogeneous aqueous solutions and vesicle suspensions at pH 7. The concentration of MV$^+$ is plotted as a function of the cumulative number of minutes of illumination. (a): Homogeneous solution containing 0.0010 M EDTA, 5.0 x 10$^{-4}$ M MV$^{2+}$, and 5 x 10$^{-5}$ M [Ru(bipy)$_3$]$^{2+}$. (b): Same conditions as in (a) except that 0.0020 M Zn$^{2+}$ was also present. (c): Egg PC vesicle suspension with 0.5 M EDTA inside the vesicles, 0.0010 M MV$^{2+}$ and 0.010 M Zn$^{2+}$ outside, and 1 x 10$^{-4}$ M (Ru$^{2+}$) dissolved in the vesicle walls. 1 x 10$^{-5}$ M C$_{16}$V$^{2+}$, 1 x 10$^{-4}$ M VK$_1$, and 1 x 10$^{-5}$ M decachlorocarborane were also dissolved in the vesicle walls. The concentration of egg PC was 0.002 M. (d): Same conditions as in (c) except that 0.018 M DHAPS detergent was added to disrupt the vesicles.
PHOTO-INDUCED ELECTRON TRANSFER ACROSS VESICLE WALLS

- **a**: EDTA, MV$^{2+}$, (Ru$^{2+}$)
- **b**: EDTA, MV$^{2+}$, (Ru$^{2+}$), Zn$^{2+}$
- **c**: EDTA, MV$^{2+}$, Zn$^{2+}$
- **d**: EDTA, MV$^{2+}$, Zn$^{2+}$, detergent

**Legend:**
- EDTA: Ethylenediaminetetraacetic acid
- MV$^{2+}$: Methylene blue
- Ru$^{2+}$: Ruthenium(II) ion
- Zn$^{2+}$: Zinc(II) ion

**Graph:**
- Time (min): x-axis
- [MV$^+$] $\times 10^5$ M: y-axis

**Note:**
- The graph shows the concentration of MV$^+$ over time under different conditions.
- The diagrams illustrate the photo-induced electron transfer process across vesicle walls with different metal ions present.
decachlorocarborane were included in the vesicle wall with egg PC and (Ru$^{2+}$) to possibly assist electron transport. The inner aqueous phase contained EDTA (0.50 M) at pH 6.6 and the outer one contained MV$^{2+}$ (0.0010 M) at pH 7.1. Zn$^{2+}$ (0.010 M) was added to the continuous phase to insure that only EDTA located inside the vesicles was the reducing agent for MV$^{2+}$ (see Discussion). For further experimental details, the reader is referred to the original reference (Ford et al., 1978) and to the legend in Fig. 4-3.

The absorbance of the vesicle suspensions at 480 nm due to the visible maximum of (Ru$^{2+}$) ranged from 0.8 to 0.9 (per 1.0 cm path length) after gel-filtration. These values were corrected approximately for light-scattering. The absorbances of the suspensions due to light-scattering at 800 nm, where (Ru$^{2+}$) does not absorb, were about 0.01 (per cm).

Viologen radical was produced when the anaerobic vesicle suspensions were illuminated with visible light (420-590 nm). As with the homogeneous samples, the concentration of radical had a sigmoidal dependence on the extent of illumination, beginning to saturate after about 10% of the viologen was reduced (Fig. 4-3c). The maximal rate of increase in the radical concentration was $2 \times 10^{-6}$ M/min, which corresponded to a quantum yield of the order of $10^{-4}$. MV$^+$ could not be distinguished spectroscopically from C$_{16}$V$^+$, but MV$^{2+}$ was clearly being reduced since the analytical concentration of C$_{16}$V$^{2+}$ in the system was $1 \times 10^{-5}$ M while greater
than 1 x 10^{-4} M of viologen radical was produced (Fig. 4-3c). Bleaching of the (Ru^{2+}) absorbance at 480 nm during the experiment amounted to 3\pm2\%, the uncertainty being due to slight increases in light-scattering.

A "blank" experiment without EDTA was performed. When the vesicles were prepared with ammonium acetate solution entrapped instead of ammonium EDTA solution, no viologen radical (<10^{-6} M) was detected after 60 minutes of illumination.

When the zwitterionic detergent DHAPS (0.018 M) was added to disrupt the vesicles, the concentration of viologen radical produced after 60 minutes of illumination was less by a factor of 25 compared to the experiment without detergent (Fig. 4-3d). A product with a maximal absorbance at 365 nm of about 0.18 (per cm path length) (shoulder at about 350 nm) was produced within the first 10 minutes of illumination, and remained unchanged afterward. When this experiment was repeated without added Zn^{2+}, no viologen radical (<10^{-6} M) was detected after 60 minutes of illumination. The formation of a product absorbing maximally at 365 nm was again observed. These experiments demonstrated that the integrity of the vesicles was required for viologen radical production.

4.3.2. PHOTOCHEMICAL EXPERIMENTS AT BASIC pH

4.3.2.1. EFFECT OF INCREASING THE pH

The low quantum yield in the vesicle system at pH 7.1
(previous Section) amplified the problem with residual O\textsubscript{2} in the sample. It was expected that raising the pH would raise the quantum yield by making the EDTA easier to oxidize (see Discussion, Section 4.4.3.2). The effect of raising the pH was first tested with the homogeneous system.

4.3.2.1.a. HOMOGENEOUS SOLUTIONS

Under homogeneous conditions using, [Ru(bipy)	extsubscript{3}]\textsuperscript{2+} as the photosensitizer, the maximal quantum yield of MV\textsuperscript{2+} reduction (\(\phi_{\text{max}}\)) was about 8 times greater at pH 9.1 (1.0 M ammonium borate buffer) than at pH 7.1 (Section 4.3.1.1).

4.3.2.1.b. VESICLE SUSPENSIONS

The pH of the vesicle system was raised from 7 to 8.6. Vesicles were prepared as before (Section 4.3.1.2) except that the encapsulated aqueous phase contained 0.30 M (NH\textsubscript{4})\textsubscript{3}EDTA and the continuous aqueous phase contained 0.9 M NH\textsubscript{4}OAc-NH\textsubscript{4}OH, 0.018 M Zn(OAc)\textsubscript{2}, and 0.0010 M MVCl\textsubscript{2} at pH 8.6. The pH of both phases was 8.6. Experimental details of the preparation and illumination of the suspensions are given in Sections 4.2.3.2 and 4.2.3.3. Plots of the amount of viologen radical accumulated versus the amount of light absorbed were sigmoidal for this system (Fig. 4-4), as they were at pH 7.1. \(\phi_{\text{max}}\) was 6 times greater at pH 8.6 than at 7.1. This improvement in quantum yield improved the reproducibility of the experiments to about 10\% (Ford, 1978), mainly by reducing the uncertainty that arose from residual amounts of O\textsubscript{2} in the cuvette (Section 4.2.3.3.b). A slight
Figure 4-4. Photosensitized electron transfer from EDTA$^{3-}$ to MV$^{2+}$ across egg PC vesicle walls at pH 8.6. The moles of MV$^+$ and C$_{16}$V$^+$ produced is plotted as a function of the moles of photons absorbed by (Ru$^{2+}$) for three vesicle systems. Each system had 0.30 M EDTA$^{3-}$ inside the vesicles, 0.0010 M MV$^{2+}$ and 0.018 M Zn$^{2+}$ outside, and 1 x 10$^{-4}$ M (Ru$^{2+}$) dissolved in the vesicle walls; the concentration of egg PC was 0.002 M. Squares: 1 x 10$^{-5}$ M C$_{16}$V$^{2+}$, 1 x 10$^{-4}$ M VK$_1$, and 1 x 10$^{-5}$ M decachlorocarborane were dissolved with (Ru$^{2+}$) in the vesicle walls. Triangles: 1 x 10$^{-5}$ M C$_{16}$V$^{2+}$ was dissolved with (Ru$^{2+}$) in the vesicle walls. Circles: Vesicle walls composed of egg PC and (Ru$^{2+}$) only.
inflection which occurred after about $20 \times 10^{-8}$ moles ($7 \times 10^{-5} \text{ M}$) of viologen radical had accumulated (Fig. 4-4) was a reproducible feature.

4.3.2.2. VESICLE SUSPENSIONS WITH OTHER COMPOSITIONS

The time courses for viologen radical production during the photolyses of vesicle suspensions with six different compositions (labelled A to F) were determined. The compositions are listed in Table 4-1 along with relative and absolute quantum yields. Most of the experiments were repeated; the data for individual experiments are listed in Table 4-2. Typical spectral changes observed are reproduced in Fig. 4-5, where the product with absorption maxima near 600 and 395 nm is the blue viologen radical ($\text{C}_{16}\text{V}^+$ and/or $\text{MV}^+$). Representative plots for the build-up of viologen radical in the six vesicle systems are shown in Figs. 4-4 and 4-6, where the slopes of the curves equal the quantum yield ($\phi$). The curves are sigmoidal for all compositions, except perhaps F in which the induction period is not obvious. The quantum yields listed in Table 4-1 are the maximal observed values ($\phi_{\text{max}}$). The $\phi$'s in Table 4-1 represent average values for the individual experiments listed in Table 4-2. Relative values of $\phi_{\text{max}}$ in experiments performed with "white" light were related to the values in experiments with "blue" light through experiments with system B, which were performed under both conditions.

The removal of VK$_1$ and decachlorocarborane from the
Table 4-1. Dependence of the quantum yield of viologen radical on composition

<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>egg PC</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>(Ru^{2+})</td>
<td>0.10</td>
<td>0.10</td>
<td>0.10</td>
<td>0.10</td>
<td>0.28</td>
<td>0.10</td>
</tr>
<tr>
<td>C_{16}V^{2+}</td>
<td>0.01</td>
<td>0.01</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.20</td>
</tr>
<tr>
<td>vitamin K_{1}</td>
<td>0.10</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>carborane</td>
<td>0.01</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>MV^{2+}</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>C_{7}V^{2+}</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1.0</td>
<td>1.0</td>
<td>0</td>
</tr>
</tbody>
</table>

rel. \( \phi \)^b 80±8 100±5 15±4 120±40 260±80 1600±500
\( \phi \times 10^{4} \)^b 3.1±1.0 3.8±0.7

---

^a^ Temperature = 22.5±1.5°C, pH = 8.6±0.1. Concentrations listed are the bulk values, and were actually 7±3% lower due to dilution during gel-filtration. All vesicle suspensions had 0.30 M (NH\(_{4}\))\(_{3}\)EDTA inside the vesicles and 0.01 M Zn\(^{2+}\) outside.

^b^ Maximal observed quantum yields, relative or absolute values.
Table 4-2. Supplemental data for Table 4-1

<table>
<thead>
<tr>
<th>expt&lt;sup&gt;a&lt;/sup&gt;</th>
<th>date</th>
<th>volume&lt;sup&gt;b&lt;/sup&gt;, l</th>
<th>1-T&lt;sub&gt;480&lt;/sub&gt;&lt;sup&gt;c&lt;/sup&gt;</th>
<th>max. rate&lt;sup&gt;d&lt;/sup&gt; M/min x 10&lt;sup&gt;6&lt;/sup&gt;</th>
<th>light&lt;sup&gt;e&lt;/sup&gt; source</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>06-05-78</td>
<td>3.02</td>
<td>0.84</td>
<td>10.2</td>
<td>white</td>
</tr>
<tr>
<td>A2</td>
<td>06-07-78</td>
<td>*</td>
<td>0.90</td>
<td>11.5</td>
<td>*</td>
</tr>
<tr>
<td>B1</td>
<td>06-13-78</td>
<td>*</td>
<td>0.90</td>
<td>13.9</td>
<td>*</td>
</tr>
<tr>
<td>B2&lt;sup&gt;f&lt;/sup&gt;</td>
<td>06-16-78</td>
<td>*</td>
<td>0.88</td>
<td>12.6</td>
<td>*</td>
</tr>
<tr>
<td>B3</td>
<td>06-18-78</td>
<td>*</td>
<td>0.91</td>
<td>14.2</td>
<td>*</td>
</tr>
<tr>
<td>B4</td>
<td>10-19-78</td>
<td>*</td>
<td>0.93</td>
<td>7.6</td>
<td>blue&lt;sup&gt;g&lt;/sup&gt;</td>
</tr>
<tr>
<td>C1</td>
<td>06-11-78</td>
<td>*</td>
<td>0.90</td>
<td>1.9</td>
<td>white</td>
</tr>
<tr>
<td>D1</td>
<td>02-19-79</td>
<td>3.09</td>
<td>0.90</td>
<td>6.6</td>
<td>blue&lt;sup&gt;h&lt;/sup&gt;</td>
</tr>
<tr>
<td>D2</td>
<td>04-10-79</td>
<td>*</td>
<td>0.93</td>
<td>6.1</td>
<td>*</td>
</tr>
<tr>
<td>E1</td>
<td>04-09-79</td>
<td>*</td>
<td>1.00</td>
<td>14.5</td>
<td>*</td>
</tr>
<tr>
<td>F1</td>
<td>11-25-78</td>
<td>*</td>
<td>0.90</td>
<td>98.4</td>
<td>*</td>
</tr>
</tbody>
</table>

<sup>a</sup> Refer to Table 4-1 for compositions

<sup>b</sup> Sample volume, ±0.03 ml

<sup>c</sup> Fraction of 480 nm light absorbed by (Ru<sup>2+</sup>), corrected for turbidity, ±0.02

<sup>d</sup> Maximal rate of increase in viologen radical concentration per minute of illumination, ±0.4 x 10<sup>-6</sup> M/min

<sup>e</sup> 1000 watt xenon arc source; white: filtered through aqueous cupric sulfate solution and Corning filter #3-73 (420 < λ < 590 nm); blue: filtered through aqueous cupric sulfate sol'n and filters #3-72 and 5-57 (440 < λ < 540 nm)

<sup>f</sup> Gel-filtered twice

<sup>g</sup> I<sub>0</sub> = (4.3±1.1) x 10<sup>-5</sup> einstein/min·cm<sup>2</sup>

<sup>h</sup> I<sub>0</sub> = (3.2±0.4) x 10<sup>-5</sup> einstein/min·cm<sup>2</sup>
Figure 4-5. Spectral changes during the photosensitized reduction of MV$^{2+}$ and C$_{16}$V$^{2+}$ in egg PC vesicle suspensions. The initial absorption spectrum with a maximum near 480 nm is due to (Ru$^{2+}$) (1 x 10$^{-4}$ M). The photoproducts with maxima near 600 and 395 nm are MV$^+$ and C$_{16}$V$^+$. The composition of the vesicle suspension is labelled B in Table 4-1. Path length = 1.0 cm.
Figure 4-6. Photosensitized electron transfer from EDTA$^{3-}$ to either C$_7$V$^{2+}$ or C$_{16}$V$^{2+}$ across egg PC vesicle walls at pH 8.6. The moles of viologen radical produced is plotted as a function of the moles of photons absorbed by (Ru$^{2+}$) for three vesicle systems. Each system had 0.30 M EDTA$^{3-}$ inside the vesicles and 0.018 M Zn$^{2+}$ outside; the concentration of egg PC was 0.002 M. Squares: 0.0010 M C$_7$V$^{2+}$ was outside the vesicles and 1 x 10$^{-4}$ M (Ru$^{2+}$) was dissolved in the vesicle walls. Circles: 0.0010 M C$_7$V$^{2+}$ was outside the vesicles and 2.8 x 10$^{-4}$ M (Ru$^{2+}$) was dissolved in the vesicle walls. Triangles: 2 x 10$^{-4}$ M C$_{16}$V$^{2+}$ and 1 x 10$^{-4}$ M (Ru$^{2+}$) were dissolved in the vesicle walls; there was no water-soluble viologen outside the vesicles.
original composition (A), giving composition B, had a relatively small effect on $\phi_{\text{max}}$ (Table 4-1), so those components were not required for electron transport across the vesicle wall. The absolute value of $\phi_{\text{max}}$ in system B was $(3.1 \pm 1.0) \times 10^{-4}$. Further removal of $\text{C}_{16}\text{v}^{2+}$ from the vesicle wall, giving system C, reduced $\phi_{\text{max}}$ by a factor of 7, but the replacement of $\text{MV}^{2+}$ with $\text{C}_{7}\text{v}^{2+}$, giving system D, more than compensated that loss (Table 4-1). Thus no components other than egg PC and $(\text{Ru}^{2+})$ were required for electron transport across the bilayer.

The dependence of $\phi$ on the concentration of $(\text{Ru}^{2+})$ in the vesicle wall was examined by comparing vesicles with an egg PC:$(\text{Ru}^{2+})$ molar ratio of 200:28 (composition E) to those with the usual molar ratio of 200:10 (D). The increase in molar ratio by a factor of 2.8±0.2 resulted in an increase in $\phi_{\text{max}}$ by a factor of 2.2±0.3 (Fig. 4-6).

Increasing the concentration of $\text{C}_{16}\text{v}^{2+}$ by a factor of 20 (composition E versus B) increased $\phi_{\text{max}}$ by a factor of 16 (Table 4-1).

The shape of the absorption spectrum (800-330 nm) of $(\text{Ru}^{2+})$ in the vesicles suspensions was unchanged during the photolysis experiments. Hydrolysis of the amide bonds in the molecule would have caused the absorption maximum at 480 nm to blue-shift (see Chapter 3). The absorbance at 480 nm was bleached by less than 3% in all runs.
sitions and light sources was good. Relative values of $\phi_{\text{max}}$ in experiments A1 and A2 differed by $\pm 1\%$ from the average value, and the yields in experiments B1, B2, and B3 differed by $\pm 5\%$ from the average (Table 4-2). The uncertainties in the maximal rates of viologen radical production were under 4% for all cases except C and D, where they were 20% and 6%, respectively.

Besides $\phi_{\text{max}}$, two other aspects of the time course viologen radical production that were affected by variations in vesicle compositions were i) the induction period and ii) the fraction of total viologen that was reduced at saturation. All of the curves except for E clearly had sigmoidal shapes. The induction periods in cases A, D, and E were more distinct than they were in the other cases. In all cases, $\phi$ decreased after reaching a maximal value. The fraction of viologen that was reduced when $\phi$ was half its maximal value was approximately 0.055, 0.025, 0.015, 0.05, 0.04, and 0.40 for compositions A to E, respectively.

4.3.2.2.a. COBALT(II)-EDTA COMPLEX INSTEAD OF EDTA

When the cobaltous complex of EDTA, which is a reversible electron donor (Srinivasan and Rabinowitch, 1970), was trapped inside the vesicles instead of EDTA, no viologen radical was detected (<10$^{-6}$ M) after 15 minutes of illumination, meaning that $\phi_{\text{max}}$ was less than 10$^{-6}$. The system was analogous to the one labelled C in Table 4-1 in that the vesicle walls contained $\text{C}_{16}\text{V}^2+$, and $\text{M}\text{V}^2+$ (0.0030 M) was in
the continuous aqueous phase (Section 4.2.3.3.f).

The reversibility of Co(II)-EDTA as an electron donor was tested in homogeneous solution. Illumination with blue light for 10 minutes of an anaerobic solution containing $5 \times 10^{-5}$ M $[\text{Ru(bipy)}_3]^{2+}$, 0.0010 M $\text{MV}^{2+}$, 0.0010 M Co$^{2+}$, 0.0010 M EDTA, and 0.90 M $\text{NH}_4\text{OAc}$ at pH 6.4 produced no detectable $\text{MV}^+$ ($<1 \times 10^{-6}$ M). This result can be compared to the experiment with EDTA at pH 7.1 (Section 4.3.1.1) in which the concentration of $\text{MV}^+$ was $1.2 \times 10^{-4}$ M after 10 minutes of illumination.

4.3.2.2.b. NO EFFECT OF RETINYL ACETATE

Incorporation of the polyene retinyl acetate into vesicles composed of egg PC, (Ru$^{2+}$), and $\text{C}_{16}\text{V}^{2+}$ with entrapped EDTA$^{3-}$ solution had little effect on the quantum yield of $\text{C}_{16}\text{V}^+$ production at pH 8.6.

The photolysis of the vesicle suspension containing retinyl acetate (egg PC:(Ru$^{2+}$):$\text{C}_{16}\text{V}^{2+}$:retinyl acetate molar ratios = 200:10:20:37) was compared to the photolysis of a suspension of similar composition without retinyl acetate (labelled $\mathcal{F}$ in Table 4-1). The time courses of $\text{C}_{16}\text{V}^+$ production upon illumination were practically identical in the two cases. The value of $\overline{\varphi}_{\text{max}}$ for the system with retinyl acetate was less by 8%, and the amount of viologen radical produced after 4 minutes of illumination was less by 6%, compared to the system without it. These differences are probably within the experimental uncertainty.
The stability of retinyl acetate dissolved in egg PC vesicles (egg PC:retinyl acetate molar ratio 200:24) to oxidation by atmospheric O₂ was tested by monitoring the absorption spectrum (800-230 nm) of a vesicle suspension that was kept exposed to air at room temperature in the dark. The spectrum of the freshly prepared (within 15 minutes) sample had a single peak at 328 nm ((2.8±0.4) x 10⁴ M⁻¹cm⁻¹) with a shoulder at about 318 nm, which is similar to the absorption spectrum of retinol in ethanol (Baxter and Robeson, 1942). The absorbance at 328 nm decreased with time, being 1% lower after 1.6 hours and 4% lower after 15 hours. The decreases in absorbance at 328 nm were accompanied by increases in absorbances at around 265 and 400 nm of much smaller magnitude, with isosbestic points near 293 and 378 nm.

A portion of the above vesicle suspension was stored in a refrigerator after preparation and submitted to gel-filtration 23 hours later. Following gel-filtration, the absorbance at 328 nm was 18% lower than that of the freshly prepared vesicle suspension, more than the approximately 10% decrease expected as a result of dilution during gel-filtration. The gel-filtered suspension had visible absorption bands that were not in other spectra. In particular, there was a relatively narrow band at 458 nm (band width at half-maximum of 19±1 nm) whose height was about 9% that of the band at 328 nm, as well as weaker bands near 560 and 600 nm.
This spectrum is reproduced in Fig. 4-7.

4.3.3. RELATED EXPERIMENTS

4.3.3.1. INHIBITION BY Zn$^{2+}$

The inhibition by Zn$^{2+}$ of viologen radical production at neutral pH was described in Section 4.3.1.1. To test for inhibition at pH 8.6, the following experiment was carried out.

Vesicles composed of egg PC, (Ru$^{2+}$), and C$_{16}$v$^{2+}$ (molar ratios 200:10:20, [egg PC] = 0.0020 M) were prepared in 0.91 M NH$_4$OAc-NH$_4$OH buffer (pH 8.6) and gel-filtered with that buffer. 0.30 M (NH$_4$)$_3$EDTA solution was added to give an EDTA$^{3-}$ concentration of 0.0030 M in the aqueous phase outside the vesicles. The vesicle suspension was deaerated as usual and illuminated with blue light. The time course of the accumulation of C$_{16}$v$^+$ was sigmoidal, with a $\Phi_{\text{max}}$ of 1300 relative to those in Table 4-1. $\Phi$ reached its half-maximal value after 15% of the viologen was reduced.

Zinc acetate solution (1.0 M) was added to the vesicle suspension to give a Zn$^{2+}$ concentration of 0.018 M. The suspension was bubbled with N$_2$ which contained enough O$_2$ that the C$_{16}$v$^+$ was slowly reoxidized. When the concentration of radical remaining was about 10$^{-6}$ M, the suspension was illuminated again. No radical was produced in 15 minutes, corresponding to an inhibition by Zn$^{2+}$ of >100.

4.3.3.2. LIGHT INTENSITY DEPENDENCE OF THE QUANTUM YIELD

There was a linear dependence of $\Phi$ on $I_0$ over a 10-fold...
Figure 4-7. Absorption spectrum of retinyl acetate dissolved in aged egg PC vesicles. The egg PC:retinyl acetate molar ratio was 100:12. The vesicle suspension was aged for 1 day at 4 °C.
4.3.3.2 attenuation of $I_0$ for vesicle suspensions containing both $C_{16}V^2+$ and $MV^2+$ (Section 4.2.3.3.e). The suspensions used were those labelled B1, B2, and B3 in Table 4-2. The plot of the relative value of $\phi$ versus $I_0$ is shown in Fig. 4-8. The least-squares slope for the experimental points is $0.96\pm0.15$.

4.3.3.3. AGING EFFECTS ON THE QUANTUM YIELD

A noticeable reduction in $\phi$ occurred if the vesicle suspensions were stored in the dark at ambient temperature for a day or longer. The experiments listed in Table 4-1 were usually completed within 2 hours following gel-filtration. In some cases, the vesicle suspensions were stored in the sealed cuvette after the initial photochemical experiment and reexamined later. There was a slow reoxidation of the viologen radical during storage at a rate of 5-10% per day. Usually the suspensions were bubbled with $N_2$, which contained a trace of $O_2$, to further reduce the viologen radical concentration before they were photolyzed again.

The $\phi$'s for aged samples were compared to the $\phi$'s before sample storage. The storage time required to reduce $\phi$ to 50% of its original value was roughly 20 hours for vesicles composed of egg PC, $(Ru^{2+})$, and $C_{16}V^2+$ (composition B of Table 4-1). Storage of vesicle suspensions in a refrigerator for 2 days prior to gel-filtration had little or no effect on $\phi$.

4.3.3.4. QUENCHING OF $(Ru^{2+})$ LUMINESCEENCE
Figure 4-8. Linear dependence of the relative quantum yield of MV* production on light intensity. Neutral density filters were used to attenuate the intensity of the actinic light. The experimental points have a least-squares slope of 0.96±0.15 and are compared to the dashed line whose slope is 1.0. The composition of the vesicle system is labelled B in Table 4-1.
Rate w/ filter
Rate w/o filter

% Tof filter

0.5
1.0

XBL 786-4031
The luminescence at 660 nm of (Ru$^{2+}$) dissolved in egg PC vesicles was quenched by C$_{16}$V$^{2+}$, VK$_1$, C$_7$V$^{2+}$, anthraquinone-2-sulfonate (AQS), and O$_2$ (air). Quenching by MV$^{2+}$ was practically undetectable for concentrations up to 0.015 M. The Stern-Volmer plots were linear within uncertainty in the concentration ranges examined, except in the case of C$_{16}$V$^{2+}$ in which there was a downward curvature. Stern-Volmer quenching constants, $K_{SV}$, were obtained from the initial slopes of the plots. The results are summarized in Table 4-3. The values for $K_{SV}$ fell in the sequence: C$_{16}$V$^{2+}$ = anthraquinone-2-sulfonate $\gg$ VK$_1$ $\gg$ C$_7$V$^{2+}$ = O$_2$ $\gg$ MV$^{2+}$. C$_{16}$V$^{2+}$ and VK$_1$ were introduced to the vesicle suspensions as their ethanol solutions (Section 4.2.3.3.g); the addition of ethanol alone had no detectable effect on the luminescence intensity.

$K_{SV}$ for quenching by C$_7$V$^{2+}$ increased with increasing temperature with an Arrhenius activation energy of 11.7±2.5 kcal/mole in the range 18-38 °C. The data are listed in Table 4-4 and plotted in Fig. 4-9 (bottom). It should be noted that the unquenched luminescence intensity of (Ru$^{2+}$) in vesicle suspensions decreased with increasing temperature, corresponding to an activation energy of -2.6±0.2 kcal/mole (figure not shown). This negative contribution to the activation energy for quenching by C$_7$V$^{2+}$ probably represents the thermally-activated transition from the luminescent charge-transfer excited state of (Ru$^{2+}$) to the non-
Table 4-3. Quenching of the luminescence of \((\text{Ru}^{2+})\) in vesicle suspensions\(^a\)

<table>
<thead>
<tr>
<th>quencher</th>
<th>(K_{S-V}), M(^{-1})</th>
<th>egg PC conc., mM</th>
<th>buffer(^c) conc., mM</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\text{C}_{16}\text{V}^{2+})</td>
<td>(7400 \pm 1000) (6)</td>
<td>0.9</td>
<td>W</td>
</tr>
<tr>
<td>VK(_1)</td>
<td>(780 \pm 100) (3)</td>
<td>0.9</td>
<td>W</td>
</tr>
<tr>
<td>MV(^2+)</td>
<td>&lt; 2 (11)</td>
<td>2</td>
<td>X</td>
</tr>
<tr>
<td>(\text{MV}^{2+})</td>
<td>(1.1 \pm 0.5) (6)</td>
<td>2</td>
<td>Y</td>
</tr>
<tr>
<td>(\text{C}_7\text{V}^{2+})</td>
<td>(55 \pm 5) (9)</td>
<td>2</td>
<td>Y</td>
</tr>
<tr>
<td>(\text{AQS})</td>
<td>(110 \pm 20) (1)</td>
<td>0.2</td>
<td>Z</td>
</tr>
<tr>
<td>(\text{O}_2)</td>
<td>(5600 \pm 500) (6)</td>
<td>0.2</td>
<td>X</td>
</tr>
</tbody>
</table>

\(^a\) Temperature = 24±2 °C; suspensions were aerobic except where noted

\(^b\) From slopes of Stern-Volmer plots; number of points for the plots (excluding the origin) are given in parentheses

\(^c\) W : 1.0 M NH\(_4\)OAc, 0.1 M NaCl, 0.10 M KCl, pH = 7.2±0.2
X : 0.125 M (Na,K)\(_2\)SO\(_4\), 0.05 M Na phosphate, pH = 6.4±0.1
Y : 0.91 M NH\(_4\)OAc-NH\(_4\)OH, pH = 8.6±0.1
Z : 0.94 M NH\(_4\)OAc-NH\(_4\)OH, 0.016 M Zn(OAc)\(_2\), pH = 8.5±0.1

\(^d\) From initial slope; plot was linear up to 0.03 mM, then the slope decreased

\(^e\) Sample was deaerated by bubbling with argon

\(^f\) Based on the solubility of \(\text{O}_2\) in water at 25 °C (Murov, 1973)
Table 4-4. Quenching of (Ru$^{2+}$) luminescence by C$_7$V$^{2+}$ in egg PC vesicle suspensions: Temperature dependence$^a$

<table>
<thead>
<tr>
<th>$T$, $^\circ$C</th>
<th>$P_0$ (arbitrary units)$^b$</th>
<th>$K_{S-V}$, M$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>18.0±0.1</td>
<td>0.685±0.005</td>
<td>20.5±4.4</td>
</tr>
<tr>
<td>28.1±0.1</td>
<td>0.599±0.004</td>
<td>35.3±5.7</td>
</tr>
<tr>
<td>38.0±0.1</td>
<td>0.515±0.003</td>
<td>73.2±6.7</td>
</tr>
</tbody>
</table>

$^a$ Vesicle suspensions were anaerobic; buffer: 0.90 M NH$_4$OAc-NH$_4$OH with 0.018 M Zn(OAc)$_2$, pH 8.6

$^b$ Luminescence intensity in the absence of C$_7$V$^{2+}$
Figure 4-9. Arrhenius plots of $\ln \phi$ and $\ln K_{s-y}$ versus $1/T$. The composition of the vesicle system is labelled $\Phi$ in Table 4-1.
$E_\phi = 28 \text{kcal} \cdot \text{mol}^{-1}$

$E_{S \cdot V} = 12 \text{kcal} \cdot \text{mol}^{-1}$

$(1/T) \times 10^3 (\text{K}^{-1})$
luminescent metal-localized excited state, by analogy to the parent compound, [Ru(bipy)$_3$]$^{2+}$ (Van Houten and Watts, 1976).

4.3.3.5. EFFECTS OF IONOPHORES AND TRANSMEMBRANE POTENTIALS

[with Colja Laane]

$\varphi_{\text{max}}$ was influenced by ionophores such as valinomycin (with $K^+$) and electrostatic potentials across the membrane. For details of the experiments, the reader is referred to the original manuscript (Laane et al., 1981), which is given in the Appendix.

4.3.3.6. TEMPERATURE DEPENDENCE [with Tetsuya Sakai and Howard D. Mettee]

The effect of temperature on $\varphi$ in the range 18-38 °C was examined using the vesicle system with composition D in Table 4-1. Curves for the build-up of $C_7V^+$ during illumination at 18.0, 28.0, and 38.1 °C are shown in Fig. 4-10. A kinetic model which is presented later in the Discussion (Section 4.4.4.1) predicts that the curves are parabolas. Fig. 4-10 includes the least-squares parabolas that were calculated from the experimental points. The initial quantum yields for the accumulation of $C_7V^+$ ($\varphi_0$) can be calculated from the linear coefficients of the parabolas (see Discussion). The least-squares values of the coefficients for the parabolas and the values of $\varphi_0$ at the three temperatures are listed in Table 4-5, where it is seen that $\varphi_0$ increased by a factor of 20 upon warming from 18 to 38 °C. An Arrhenius plot of $\ln \varphi_0$ versus $1/T$ is shown in Fig. 4-9.
Figure 4-10. Temperature dependence of photosensitized electron transfer from EDTA$^{3-}$ to C$_7$V$^{2+}$ across egg PC vesicle walls. The concentration of C$_7$V$^+$ is plotted as a function of the cumulative number of minutes of illumination. The composition of the vesicle system is labelled D in Table 4-1. The lines drawn are parabolas obtained from least-squares fits of the data. Circles: T = 18.0±0.1 °C. Squares: T = 28.0±0.1 °C. Triangles: T = 38.1±0.1 °C.
Table 4-5. Least-squares coefficients\textsuperscript{a} for the equation
\[ t = a_0 + a_1[C_7V^+] + a_2[C_7V^+]^2 \]

<table>
<thead>
<tr>
<th>$T$, °C</th>
<th>egg PC: (Ru\textsuperscript{2+}) molar ratio</th>
<th>$a_0$, s</th>
<th>$a_1$, s/M</th>
<th>$a_2$, s/M\textsuperscript{2}</th>
<th>$\rho_0$</th>
</tr>
</thead>
<tbody>
<tr>
<td>18.0±0.1</td>
<td>100:5</td>
<td>8±140</td>
<td>250±50</td>
<td>37±13</td>
<td>2.4±0.6</td>
</tr>
<tr>
<td>28.0±0.1</td>
<td>*</td>
<td>16±78</td>
<td>61±14</td>
<td>17±2</td>
<td>10±3</td>
</tr>
<tr>
<td>38.1±0.1</td>
<td>*</td>
<td>5±26</td>
<td>10.4±4.7</td>
<td>8.0±0.6</td>
<td>58±29</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Stated uncertainties are the standard deviations (90\% confidence limits). $a_0$ was made relatively small by subtracting the induction periods from the illumination times. Values for $a_2$ were not corrected for attenuation of actinic light by $C_7V^+$. 
(top), from which an activation energy for $\phi_0$ of $28.0 \pm 4.5$ kcal/mole is obtained.

Theoretically, $\phi_0$ is equivalent to $\phi_{\text{max}}$, which was introduced in Section 4.3.2.2, since the initial quantum yield is the maximal one when the induction period is excluded. This distinction is made here because the two values differ somewhat due to the different methods of obtaining them.

4.3.4. PERMEABILITY OF EGG PC VESICLES

4.3.4.1. PERMEABILITY TO MV$^2+$

Gel-filtration was used to remove untrapped MV$^2+$ from vesicles with entrapped MV$^2+$, and the absorbance at 256 nm was measured to determine the amount of entrapped MV$^2+$ (Section 4.2.3.4.a). The spectral data are summarized in Table 4-6. The absorbance at 400 nm where MV$^2+$ does not absorb was due to light-scattering by the vesicles and was assumed to be proportional to the concentration of vesicles. Light-scattering was estimated to contribute less than 5% to the absorbance at 256 nm, so the latter values were nearly proportional to the amount of entrapped MV$^2+$ in the samples. Vesicles that were gel-filtered 2 hours after the first gel-filtration contained 6±2% less entrapped MV$^2+$ than ones that were gel-filtered 5 minutes after the first. This difference is taken to represent the rate of escape of MV$^2+$ from the vesicles (see Discussion, Section 4.4.4.4) and corresponds to a rate of $2 \times 10^{-12}$ moles/s.
Table 4-6. Release of MV$_2^+$ from egg PC vesicles$^a$

<table>
<thead>
<tr>
<th>Sample treatment</th>
<th>Absorbance at 256 nm$^b$</th>
<th>Absorbance at 400 nm$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>gel-filtered once</td>
<td>4.62±0.09</td>
<td>0.100±0.003</td>
</tr>
<tr>
<td>second gel-filtration</td>
<td></td>
<td></td>
</tr>
<tr>
<td>after 5 minutes</td>
<td>2.04±0.03</td>
<td>0.050±0.003</td>
</tr>
<tr>
<td>second gel-filtration</td>
<td></td>
<td></td>
</tr>
<tr>
<td>after 2 hours</td>
<td>1.92±0.03</td>
<td>0.050±0.003</td>
</tr>
</tbody>
</table>

$^a$ 0.20 M MV$_2^+$ entrapped; T = 22.5±1.5 °C; egg PC conc. after second gel-filtration = 0.9 mM

$^b$ Absorbance per cm path length, including contribution from turbidity
The data can also be used to estimate the efficiency of the removal of untrapped MV$^{2+}$ by gel-filtration. The first gel-filtration of the vesicle suspension with 0.20 M MV$^{2+}$ located inside and outside reduced the bulk concentration of MV$^{2+}$ to less than $2.2 \times 10^{-4}$ M. About half of this value was due to the entrapped MV$^{2+}$. Thus gel-filtration removed about 99.5% of the MV$^{2+}$ that was originally in the continuous aqueous phase.

4.3.4.2. PERMEABILITY TO EDTA$^{3-}$

A Cu$^{2+}$ complex with 1-nitroso-2-naphthol-3,6-disulphonate (nitroso-R salt, NR) was used as an analytical reagent for detecting EDTA$^{3-}$ in the continuous aqueous phase of vesicle suspensions. The absorption spectrum of uncomplexed NR ligand dissolved in aqueous ammonium acetate buffer (pH 7.1) had band maxima at 370 and 422 nm ($\varepsilon = 6100$ M$^{-1}$cm$^{-1}$ for both bands). The addition of an equimolar amount of CuBr$_2$ caused the maxima to shift to 390 and 465 nm. Subsequent addition of di-sodium EDTA restored the original spectral pattern. Thus the 1:1 CuNR complex could be used for spectrophotometric analysis of EDTA.

The spectral changes were calibrated by titrating a buffered solution of CuNR with an EDTA solution of known concentration (Section 4.2.3.4.b). When the CuNR and lipid concentrations were $3.8 \times 10^{-4}$ and $0.0020$ M, respectively, increasing the concentration of EDTA from 0 to $3.43 \times 10^{-4}$ M caused the absorbance ratio OD$\cdot$392/OD$\cdot$423 to decrease...
smoothly from 1.56 to 0.80 and the ratio OD·475/OD·423 to decrease from 0.90 to 0.16. When the CuNR and lipid concentrations were 0.46 x 10^{-4} M and 0.15 x 10^{-3} M, respectively, increasing the concentration of EDTA from 0 to 1.73 x 10^{-4} caused OD·392/OD·423 to decrease from 1.28 to 0.77 and OD·475/OD·423 to decrease from 0.61 to 0.14.

The absorption spectrum of CuNR in the presence of vesicles with entrapped EDTA^3^- solution varied with time following gel-filtration in a way that was consistent with a gradual increase in the concentration of EDTA^3^- available to the complex. The spectral changes for the 'undiluted' vesicle sample are reproduced in Fig. 4-11. The apparent concentrations of EDTA^3^- were estimated from absorbance ratios using the calibration curves. The results for the undiluted and diluted vesicle samples are listed in Table 4-7.

The time dependence of the increase in the concentration of EDTA^3^- was tested graphically for first-order kinetic behavior according to Eq. 4-10, where [EDTA]_{t=0} stands for the EDTA^3^- concentration immediately after gel-filtration, [EDTA]_{t=\infty} for the limiting concentration at long times, [EDTA]_t for the concentration at time t, and k is the first-order rate constant:

\[(4-10) \frac{([EDTA]_{t=\infty} - [EDTA]_t)}{([EDTA]_{t=\infty} - [EDTA]_{t=0})} = e^{-kt}
\]

The values of [EDTA]_{t=0} and [EDTA]_{t=\infty} were extrapolated from plots of [EDTA]_t vs. time and are given in Table 4-7.
Figure 4-11. Changes in the absorption spectrum of the Cu\(^{2+}\)-nitroso-R complex due to the release of EDTA\(^3-\) from the inner aqueous compartments of egg-PC vesicles. The arrows indicate absorbance increases or decreases with time. The spectral changes are due to the conversion of nitroso-R from its Cu\(^{2+}\) complex, which is brown, to the uncomplexed form, which is yellow-green. The individual spectra were recorded 19.8, 31.2, 43.7, 54.2, 68.1, 92.2, 116.1, and 259.5 hours after the addition of 3.8 x 10\(^{-4}\) M Cu\(^{2+}\)-nitroso-R complex to a vesicle suspension with entrapped 0.30 M (NH\(_4\))\(_3\)EDTA solution (egg PC conc. = 0.002 M). Path length = 0.30 cm.
Table 4-7. Release of EDTA from egg PC vesicles\(^a\)

<table>
<thead>
<tr>
<th>Time, hr(^d)</th>
<th>[EDTA], M (\times 10^4)</th>
<th>Time, hr(^d)</th>
<th>[EDTA], M (\times 10^4)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(e)</td>
<td>(f)</td>
<td>(e)</td>
</tr>
<tr>
<td>2.7</td>
<td>1.22</td>
<td>1.27</td>
<td>2.0</td>
</tr>
<tr>
<td>6.7</td>
<td>1.34</td>
<td>1.38</td>
<td>5.9</td>
</tr>
<tr>
<td>19.8</td>
<td>1.60</td>
<td>1.64</td>
<td>19.5</td>
</tr>
<tr>
<td>31.2</td>
<td>1.80</td>
<td>1.87</td>
<td>31.0</td>
</tr>
<tr>
<td>43.7</td>
<td>2.03</td>
<td>2.10</td>
<td>43.5</td>
</tr>
<tr>
<td>54.2</td>
<td>2.21</td>
<td>2.28</td>
<td>54.0</td>
</tr>
<tr>
<td>68.1</td>
<td>2.38</td>
<td>2.49</td>
<td>67.8</td>
</tr>
<tr>
<td>92.2</td>
<td>2.65</td>
<td>2.77</td>
<td></td>
</tr>
<tr>
<td>116.1</td>
<td>2.80</td>
<td>3.00</td>
<td></td>
</tr>
<tr>
<td>259.5</td>
<td>3.45</td>
<td>3.45</td>
<td></td>
</tr>
</tbody>
</table>

---

\(^a\) 0.30 M \((NH_4)_3\)EDTA entrapped; concentration of EDTA in the continuous aqueous phase was determined photometrically with 1:1 Cu(II)-nitroso-R salt complex; \(T = 22.5\pm1.5 \, ^\circ C\)

\(^b\) Egg PC conc. = 2 mM; CuNR conc. = 0.38 mM

\(^c\) Egg PC conc. = 0.3 mM; CuNR conc. = 0.046 mM

\(^d\) Number of hours (\(\pm0.1 \, \text{hr}\)) following gel-filtration and anion exchange

\(^e\) Based on OD \(\cdot 392/OD \cdot 423\)

\(^f\) Based on OD \(\cdot 475/OD \cdot 423\)
Plots of the logarithm of the fraction on the left side of Eq. 4-10 vs. time are linear for both the undiluted and diluted samples (Fig. 4-12). The corresponding k's and half-times given in Table 4-8. The value of k for the release of EDTA$^{3-}$ is 5 times greater in the diluted sample than in the undiluted one.

The efficiency of the removal of EDTA$^{3-}$ from the continuous aqueous phase by gel-filtration can be estimated from the value of $[\text{EDTA}]_{t=0}$. $(1.15\pm0.05) \times 10^{-4}$ M EDTA$^{3-}$ remained in the continuous phase when the vesicles, which were originally suspended in 0.30 M EDTA$^{3-}$, were gel-filtered through a Sephadex G-25 column (1.0 x 18 cm) (Table 4-8). Thus gel-filtration removed all but about 0.4% of the untrapped EDTA$^{3-}$. Subsequent passage of the vesicles through a shorter column of anion exchange gel further reduced the EDTA$^{3-}$ concentration by a factor of 10 (Table 4-8).

4.4. DISCUSSION

4.4.1. RATIONALE FOR THE DESIGN OF THE MODEL VESICLE SYSTEM

The primary goal of this work was to develop a model system for studying dye-photosensitized redox reactions across vesicle walls. The vesicle system described in this Chapter is analogous to the homogeneous photosystem developed by Takuma et al. (1977, 1978) in which redox products can be accumulated because the reducing agent, EDTA, is oxidized irreversibly. Extension of the homo-
Figure 4-12. First-order plots of the data in Table 4-7 for the release of EDTA\textsuperscript{3-} from inside egg PC vesicles. The first-order rate constants which are based on least-squares linear fits are shown. Circles: undiluted sample; egg PC conc. = 0.002 M, CuNR conc. = $3.8 \times 10^{-4}$ M. Squares: diluted sample; egg PC conc. = $3 \times 10^{-4}$ M, CuNR conc. = $4.6 \times 10^{-5}$ M.
Table 4-8. First-order kinetic analysis of the results of Table 4-7

<table>
<thead>
<tr>
<th>sample</th>
<th>[EDTA]_{t=0} M x 10^4</th>
<th>[EDTA]_{t=\infty} M x 10^4</th>
<th>k, hr^{-1}</th>
<th>t_{1/2}, hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>undiluted</td>
<td>1.15±0.05</td>
<td>3.45±0.05</td>
<td>0.012±0.002</td>
<td>58±5</td>
</tr>
<tr>
<td>diluted</td>
<td>0.15±0.03</td>
<td>0.85±0.03</td>
<td>0.057±0.014</td>
<td>12±3</td>
</tr>
</tbody>
</table>
geneous system to the heterogeneous one required selection of the components in the vesicle wall and decisions about the concentrations of the various components of the system.

The use of EDTA as electron donor and MV\(^{2+}\) as electron acceptor satisfied two criteria:

i) Both compounds are polar and ionic, so they were expected to be sufficiently impermeable that the vesicle walls could be made asymmetric with respect to solutions of EDTA and MV\(^{2+}\) and kept that way during the experiment. This expectation was confirmed by permeability measurements (Section 4.4.4.4).

ii) The progress of the reaction could be easily monitored spectrophotometrically because one of the products, MV\(^{+}\), is blue (\(\varepsilon = 1.24 \times 10^4 \text{ M}^{-1}\text{cm}^{-1}\) at 602 nm (Steckhan and Kuwana, 1974)) while the reactants are colorless.

Either EDTA or MV\(^{2+}\) could be located in the interior aqueous compartments of the vesicle suspension, with the redox partner located in the continuous aqueous phase, but EDTA was chosen because of the availability of the compound compared to MV\(^{2+}\).

Zn\(^{2+}\) was added to the continuous phase with MV\(^{2+}\) to insure that only EDTA located inside the vesicles was the reducing agent for MV\(^{2+}\). Some EDTA could be located outside the vesicles as a result of its escape from the interior or incomplete removal of EDTA by gel-filtration. The complexation of EDTA by metal ions inhibits the oxidation of the
4.4.1

molecule by coordinating to the lone pair electrons of the nitrogen atoms of EDTA (see Section 4.4.3.1). Zn\(^{2+}\) was selected as inhibitor because its stability constant with EDTA is high (3.2 x 10\(^{16}\)) and because Zn\(^{2+}\) is diamagnetic and difficult to oxidize or reduce. These latter properties made it likely that the Zn(II)-EDTA complex would interfere with the photochemistry of the vesicle system.

The long-chained dye (Ru\(^{2+}\)) was selected as the vesicle-bound photosensitizer instead of the long-chained porphyrins synthesized by Okuno (Okuno et al., 1980) for this initial work. The tendency of the porphyrins to self-aggregate (Chapter 2) could complicate the interpretation of the photochemistry of the system since there could be several possible photosensitizing species. A preliminary experiment using Zn(II)TPyP-1 instead of (Ru\(^{2+}\)) in a vesicle system analogous to system \(Q\) in Table 4-1 indicated that the two dyes are equally effective as photosensitizers.

Phosphatidylcholine from hens' eggs (egg PC) was selected as the major vesicle wall component. Egg PC vesicles have been well characterized (Szoka and Papahadjopoulos, 1980; also see Chapter 1, Section 1.5). Unlike vesicles composed of cationic or anionic lipids, egg PC vesicles are not very subject to aggregation (e.g., flocculation) in aqueous solutions of high ionic strength.

4.4.2. METHODOLOGY
The vesicle suspensions used in this work were prepared by a modification of the ethanol-injection method originally described by Batzri and Korn (1973). This method is an attractive alternative to ultrasonic disruption (Szoka and Papahadjopoulos, 1980), which is probably the most commonly used method for preparing unilamellar vesicles, mainly because it is simpler and seems likely to be more reproducible. A disadvantage of ethanol injection compared to ultrasonic disruption is an upper limit to the concentration of egg PC of about 0.002 M (Batzri and Korn, 1973). This limit was not a problem in the present experiments because the concentration of (Ru$^{2+}$) could be high enough ($10^{-4}$ M) for the sample to absorb most of the actinic blue light while the vesicles were still composed mostly of egg PC (e.g., an egg PC:(Ru$^{2+}$) molar ratio of 20:1). Most of the ethanol which was introduced during the preparation of the vesicles was probably removed during the gel-filtration step.

In Batzri and Korn's original procedure, an ethanol solution of egg PC was rapidly injected through a syringe into 0.16 M KCl. My own experience with this method indicates that the rate of injection of the egg PC solution is relatively unimportant as long as the aqueous phase is stirred vigorously during the injection. Actually, the lipid solution can be dropped slowly into (e.g., via pipette) into the stirred aqueous phase instead of being injected without affecting the appearance of the resultant
suspension. Vortex-stirring of the aqueous phase, as described in Section 4.2.3.2.a, turned out to be the simplest way of achieving vigorous agitation; most of the vesicle suspensions used in this work were prepared this way.

The vesicle walls were "doped" with other components by adding solutions of those compounds to the ethanolic egg PC solution before injection. It was not necessary to use purely ethanol as the organic solvent when preparing the vesicles, but the other organic solvents used were miscible with water, like ethanol. Ethanol was the solvent of choice when it was practical. For example, the perchlorate salt of \((\text{Ru}^{2+})\) was much more soluble in dimethylformamide than in ethanol, so stock solutions \((0.010 \text{ M})\) of the complex were prepared with dimethylformamide as solvent, thereby minimizing the final organic solvent content of the vesicle suspension.

Gel-filtration was the method chosen to replace the continuous aqueous phase with another one. Gel-filtration is a rapid, convenient alternative to dialysis (Flodin, 1961). The principle of the method is molecular exclusion chromatography. The pores of the water-swollen dextran gel beads in the column exclude the vesicles but not the aqueous phase in which they are suspended, so the vesicles elute first. By collecting only the main part of the vesicle fraction, ignoring the leading and trailing edges, the same
volume of suspension could be collected as was applied with less than 10% dilution if columns with little "dead volume" beneath the gel bed (such as Econo Columns from Bio-Rad) were used. The vesicle suspensions at pH 7.1 (Section 4.3.2) were gel-filtered through homemade columns with a larger dead volume, resulting in 30% dilution.

The deaeration procedures used were not sufficient to avoid leaving enough O₂ to reoxidize 10⁻⁸ moles of viologen radical. Submitting the suspensions to reduced pressure prior to bubbling with N₂ or argon was more effective for the removal of O₂ than was bubbling alone. The reduced pressure probably helped remove O₂ from the walls of the cuvette and from the teflon coating of the magnetic stirring bar. The freeze-thaw method of degassing was not attempted because it was feared that the process could disrupt the vesicles, causing the continuous and discontinuous aqueous phases to mix.

The initially photo-produced viologen radical was sacrificed in order to reduce the amount of O₂ in the cuvette to a negligible level. The cuvette contents were shaken to facilitate the diffusion of O₂ into the vesicle suspension from the gas phase above, as the partitioning of O₂ between the aqueous suspension and the gas phase favors the latter by a factor of 30 (Murov, 1973).

It was assumed that the extinction coefficient for the radicals of methyl-, heptyl-, and hexadecylviologen was 1.24
x \(10^4\) \(\text{M}^{-1}\text{cm}^{-1}\) at 602 nm, which is the value determined for 
\(\text{MV}^+_1\) by Steckhan and Kuwana (1974). Assignment of the same 
extinction coefficient to all three viologen radicals is 
justified by the similarities in their spectral patterns. 
However, there is considerable discrepancy in the literature 
regarding the extinction coefficient for \(\text{MV}^+_1\) (Trudinger, 
1970). Another source of uncertainty in the assumption is 
that two other reduced forms of viologens are possible. 
These are the radical dimers (Eq. 4-11) and the doubly-
reduced species (Eq. 4-12): 
\[
\begin{align*}
\text{(4-11)} & \quad \text{MV}^+_1 + \text{MV}^+_1 \rightarrow (\text{MV}^+_1)_2 \\
\text{(4-12)} & \quad \text{MV}^+_1 + e^- \rightarrow \text{MV}^0 \quad (E_0 = -0.77 \text{ volts vs. NHE})
\end{align*}
\]

Viologen radicals can dimerize reversibly (Kosower and 
Cotter, 1964; Evans et al., 1976). The dimers have ab-
sorption maxima at about 550 and 367 nm, making them purple. 
The extinction coefficient at 602 nm of the radical dimers, 
per monomer, is about 25% less than the value for the mono-
mer itself. There was no spectral evidence for the forma-
tion of dimers of \(\text{MV}^+_1\) in the photolysis experiments. In 
experiments with either \(\text{C}_7\text{V}^{2+}\) or \(\text{C}_{16}\text{V}^{2+}\) and no \(\text{MV}^2+\) (compo-
sitions \(D, E, \) and \(F\) in Table 4-1), it was clear from the 
spectra that the radicals were predominantly monomeric. 
However, the absorbances between 600 and 400 nm in these 
cases were higher relative to their 602 nm maxima than was 
observed with \(\text{MV}^+_1\), suggesting that up to 15% of the radicals 
were dimeric.
4.4.2 The reduction of $\text{MV}^+$ to $\text{MV}^0$ has a potential of -0.77 volts vs. the normal hydrogen electrode (NHE) (Steckhan and Kuwana, 1974). The doubly-reduced viologen is yellow, with maxima at about 378 and 400 nm, and it is not readily reoxidized by $O_2$ (Carey and Case, 1977), in contrast to $\text{MV}^+$. There were absorbance changes during illumination that were consistent with the production of doubly-reduced viologen, but that form represented only a small proportion of the reduced viologen.

4.4.3. HOMOGENEOUS SOLUTIONS

4.4.3.1. NET REACTION AND THERMODYNAMICS

The photosensitized oxidation of EDTA and reduction of $\text{MV}^{2+}$ in aqueous solution gives mainly $\text{MV}^+$ and fragmented products of oxidized EDTA. At pH 5, in the presence of a platinum catalyst so that $H_2$ accumulates instead of $\text{MV}^+$, the oxidation products of EDTA are mainly (75-80%) ethylenediamine-$N,N,N'$-triacetate and glyoxylate (Keller et al., 1980), but formaldehyde and carbon dioxide could be produced instead of glyoxylate (Enns and Burgess, 1965). The net reaction at pH 8.5 is presumed to be Eq. 4-13:

\[
(4-13) \quad R'R''\text{NCH}_2\text{COO}^- + \text{OH}^- + 2 \text{MV}^{2+} \rightarrow R'R''\text{NH} + \text{O} = \text{CHCOO}^- + 2 \text{MV}^+
\]

Ethylenediamine-$N,N,N'$-triacetate ($R'R''\text{NH}$) formed in Eq. 4-13 can likewise be oxidized to ethylenediamine-$N,N'$-diacetate, which is inert because it contains no tertiary amine (Enns and Burgess, 1965; Keller et al., 1980).
By analogy to the path proposed for the oxidation of triethylamine (Smith and Mann, 1969; Whitten, 1980), EDTA first loses an electron, then a proton, then another electron, producing an iminium ion which hydrolyzes to ethylenediamine-N,N,N'-triacetate and glyoxylate:

\[
\begin{align*}
&\text{R'}\text{R''NCH}_2\text{COO}^- + e^- + H^+ \\
&\rightarrow R'\text{R''NCH}_2\text{COO}^- \\
&\rightarrow R'\text{R''NCHCOO}^- + H_2O \\
&\rightarrow R'\text{R''NH} + \text{O=CHCOO}^-
\end{align*}
\]

The standard free energy change \(\Delta G^0\) for Eq. 4-13 at pH 8.5 is estimated from tables of free energies of formation (Krebs and Kornberg, 1957) using glycine as reactant instead of EDTA and ammonia as product instead of ethylenediamine-triacetate (Eq. 4-15), and from the electrochemical potentials of the \(\text{MV}^{2+}/\text{MV}^+\) couple (-0.45 volts vs. NHE, pH independent (Steckhan and Kuwana, 1974)) and the \(H^+/H_2\) couple \((0.00 - 0.059(pH)\) volts vs. NHE) (Eq. 4-16):

\[
\begin{align*}
&\text{H}_3\text{NCH}_2\text{COO}^- + \text{OH}^- \rightarrow \text{NH}_3 + \text{O=CHCOO}^- + \text{H}_2 \quad \Delta G_1^0 \\
&\text{MV}^{2+} + \text{H}_2 \rightarrow 2 \text{MV}^+ + 2 \text{H}^+ \quad (\text{pH} 8.5) \quad \Delta G_2^0
\end{align*}
\]

Based on these reactions, \(\Delta G^0 = \Delta G_1^0 + \Delta G_2^0 = +8.5 - 2.8 = +5.7 \text{ kcal/mole}\). The estimated free energy change \(\Delta G\) for 10% conversion in a solution containing \(10^{-3} \text{ M EDTA and } 10^{-3} \text{ M MV}^{2+}\) is \(-4.6 \text{ kcal/mole}\) using the Nernst equation, so the reaction is thermodynamically "downhill". Similar calculations for the reaction at pH 7.0 give \(\Delta G^0 = +15.9 \text{ kcal/mole}\) and \(\Delta G = +5.6 \text{ kcal/mole}\.

4.4.3.2. KINETIC MODEL
A model for the kinetics of the photosensitized reduction of MV$_2^+$ using [Ru(bipy)$_3$]$^{2+}$ as the dye is summarized by Eqs. 4-17 to 4-25, where the unprimed k's represent first-order rate constants and the primed k's represent second-order rate constants.

\begin{align*}
(4-17) & \quad [\text{Ru(bipy)}_3]^{2+} + \text{hv}_1 \rightarrow *[\text{Ru(bipy)}_3]^{2+} \\
(4-18) & \quad *[\text{Ru(bipy)}_3]^{2+} \rightarrow [\text{Ru(bipy)}_3]^{2+} + \text{heat} \\
(4-19) & \quad *[\text{Ru(bipy)}_3]^{2+} \rightarrow [\text{Ru(bipy)}_3]^{2+} + \text{hv}_2 \\
(4-20) & \quad *[\text{Ru(bipy)}_3]^{2+} + \text{MV}_2^+ \rightarrow \{[\text{Ru(bipy)}_3]^{3+}, \text{MV}_t\} \\
(4-21) & \quad \{[\text{Ru(bipy)}_3]^{3+}, \text{MV}_t\} \rightarrow [\text{Ru(bipy)}_3]^{2+} + \text{MV}_2^+ \\
(4-22) & \quad \{[\text{Ru(bipy)}_3]^{3+}, \text{MV}_t\} \rightarrow [\text{Ru(bipy)}_3]^{3+} + \text{MV}_t^+ \\
(4-23) & \quad [\text{Ru(bipy)}_3]^{3+} + \text{MV}_t^+ \rightarrow [\text{Ru(bipy)}_3]^{2+} + \text{MV}_2^+ \\
(4-24) & \quad [\text{Ru(bipy)}_3]^{3+} + \text{EDTA} \rightarrow [\text{Ru(bipy)}_3]^{2+} + \text{EDTA}_t^+ \\
(4-25) & \quad \text{EDTA}_t^+ \rightarrow \text{products} \\
\end{align*}

According to this scheme, the absorption of light by [Ru(bipy)$_3$]$^{2+}$ populates the luminescent charge-transfer excited state, *[Ru(bipy)$_3$]$^{2+}$ with a quantum yield of close to 1.0 (Bolletta et al., 1976). * [Ru(bipy)$_3$]$^{2+}$ can disappear in three ways: (i) it can decay to the ground state without luminescing, $k_s = 1.2 \times 10^6$ s$^{-1}$ (Van Houten and Watts, 1976); (ii) it can decay to the ground state with the emission of orange light, $k_t = 7 \times 10^4$ s$^{-1}$ (Van Houten and Watts, 1976); or (iii) it can be quenched by MV$_2^+$ through an electron transfer mechanism, $k_u = 1.0 \times 10^9$ M$^{-1}$s$^{-1}$ (Keller, 1976).
et al., 1980). Quenching of *\([\text{Ru(bipy)}_3]^{2+}\) by EDTA is negligible (Takuma et al., 1977; Sutin, 1979; Keller et al., 1980).

By analogy to other systems (Tollin, 1976; Seely, 1978; Meyer, 1978; Balzani et al., 1979; Whitten, 1980; Sutin and Creutz, 1980; Masuhara and Mataga, 1981), electron transfer quenching is here assumed to produce a solvent-encaged radical ion-pair (\([\text{Ru(bipy)}_3]^{3+}, \text{MV}^+\)) which can either recombine without net electron transfer, \(k_V\), or separate to give the individually solvated products, \(k_W\). The fraction of quenching events that yield individually solvated products, \(\text{MV}^+\) and \([\text{Ru(bipy)}_3]^{3+}\) ions, is approximately 0.2 (Kiwi and Gratzel, 1978; Sutin and Creutz, 1980; Juris et al., 1981), so \(k_W/(k_V + k_W) = 0.2\).

\([\text{Ru(bipy)}_3]^{3+}\) formed in Eq. 4-22 can be reduced either by \(\text{MV}^+\), \(k_X = 2.8 \times 10^9 \, \text{M}^{-1}\text{s}^{-1}\) (pH independent) (Keller et al., 1980), or by EDTA, \(k_Y\). The value of \(k_Y\) is uncertain. Sutin (1979) reported that \(k_Y\) is \(2 \times 10^6 \, \text{M}^{-1}\text{s}^{-1}\) at pH 8.2, Keller et al. (1980) reported a value of \(1.1 \times 10^8 \, \text{M}^{-1}\text{s}^{-1}\) at pH 5, and Miller and McLendon (1981) reported a value of \(2 \times 10^6 \, \text{M}^{-1}\text{s}^{-1}\). The oxidized EDTA radical undergoes a series of reactions that result in fragmentation of the acetate side chain, \(k_Z\) (Enns and Burgess, 1965; Keller et al., 1980) (see previous Section). \(\text{MV}^+\) accumulates during illumination of the solution because of the irreversibility of the oxidation of EDTA. The quantum yield of the reaction drops as the \(\text{MV}^+\)
concentration increases mainly because MV\textsuperscript{+} becomes increasingly more competitive with EDTA as the reducing agent for [Ru(bipy)\textsubscript{3}]\textsuperscript{3+} (see Section 4.4.4.1 for a discussion of the analogous vesicle system).

The net reaction (Eq. 4-13) requires that two molecules of MV\textsubscript{2+} be reduced for each molecule of EDTA oxidized. Only one quantum event is required because the EDTA radical produced by Eq. 4-24 becomes, upon deprotonation, a reducing agent capable of reducing MV\textsubscript{2+} (Whitten, 1980; Keller \textit{et al.}, 1980). Keller \textit{et al.} (1980) determined a pseudo-first-order rate constant of 2.6 \times 10\textsuperscript{5} s\textsuperscript{-1} for that reaction at pH 7. Such reactions subsequent to the one-electron oxidation of EDTA are not included in the kinetic scheme.

The 8-fold increase in the quantum yield (\(\Phi\)) of the production of MV\textsuperscript{+} that resulted when the pH was raised from 7 to 9 was probably largely due to making more favorable both the reduction of [Ru(bipy)\textsubscript{3}]\textsuperscript{3+} by EDTA (Eq. 4-24) and the subsequent decomposition of EDTA\textsuperscript{+} (Eq. 4-14). Presumably, the electron that is removed when EDTA reduces [Ru-(bipy)\textsubscript{3}]\textsuperscript{3+} originates from a lone pair of electrons on one of the nitrogen atoms of EDTA. Protonation of the nitrogen atoms disfavors oxidation. Raising the pH increases the proportion of the nitrogen atoms that are unprotonated, thereby increasing \(\Phi\). The pK\textsubscript{a}'s for EDTA are 2.0, 2.67, 6.16, and 10.26 (Skoog and West, 1976).

The drastic drop in \(\Phi\) caused by the presence of Zn\textsubscript{2+} at
twice the concentration of EDTA was presumably because complexation by Zn\(^{2+}\), like protonation, makes the removal of electrons from EDTA more difficult. Ca\(^{2+}\) was similarly used by Oster and Oster (1959) to retard the dye-photosensitized reduction of ferric ion with EDTA.

4.4.4. VESICLE SYSTEMS

Initially, C\(_{16}\)V\(^{2+}\), VK\(_1\), and decachlorocarborane were included with egg PC and (Ru\(^{2+}\)) in the vesicle wall to possibly assist charge transfer through the membranes and/or across the membrane-water interfaces (Systems labelled A and B in Table 4-1). Subsequent experiments showed that the additional components were not required (System C), but \(\phi\) was considerably less (Table 4-1). System C with MV\(^{2+}\) in the external aqueous phase was attractive as a model system because the vesicle walls were composed solely of egg PC and (Ru\(^{2+}\)), but, as with the original vesicle system at pH 7, the low value of \(\phi\) amplified problems caused by residual O\(_2\) in the cuvette. The luminescence quenching experiments indicated that the poor efficiency of the quenching of \(*\)(Ru\(^{2+}\)) by MV\(^{2+}\) was a primary cause of the low value of \(\phi\). C\(_7\)V\(^{2+}\) was a more effective quencher than MV\(^{2+}\), so this substitution was made (System D). Substituting MV\(^{2+}\) with C\(_7\)V\(^{2+}\) more than compensated the loss in \(\phi\) caused by removing C\(_{16}\)V\(^{2+}\) from the vesicles (Table 4-1). Therefore mechanistic aspects of this simpler system will be considered in detail below (Section 4.4.4.2). The effects of other components...
will be considered separately (Section 4.4.4.3). First, a kinetic model for interpreting the results will be developed.

4.4.4.1. KINETIC MODEL [with John W. Otvos, Howard D. Mettee, and Tetsuya Sakai]

In considering the dynamics of photosensitized electron transport across vesicle walls, it is probably misleading to use conventional rate laws which are based on observations of fluid homogeneous solutions to derive a set of differential equations relating the concentrations of reactants, intermediates, and products, and the time dependencies of these concentrations. The vesicle suspension is a heterogeneous, anisotropic system and many of the reaction steps take place at the lipid bilayer-water interface, so the dimensionality of the reactions is reduced compared to the situation in homogeneous systems (Adam and Delbruk, 1968; Razi Naqvi, 1974; Hardt, 1979). Furthermore, our only measurements so far have been bulk spectroscopic properties like absorbance and luminescence. Therefore it is not necessarily true that the observables in our system for a reaction such as

\[ P + Q \rightarrow R \]

will behave according to the equation

\[ \frac{d[R]}{dt} = k[P][Q] \]

It is more appropriate in describing the dynamics of our system to define a set of states each having character-
istic geometric relations among the components, and then to study the specific rates of interconversion of these states. Representations of the individual states and an interconversion diagram for them are shown in Fig. 4-13, where the k's are pseudo-first-order rate constants and \( I_{\text{abs}} \) is the rate of absorption of photons by (Ru\(^{2+}\)).

State A is the initial configuration with (Ru\(^{2+}\)) in the vesicle wall, which separates the ultimate reactants EDTA and C\(_7\)V\(^{2+}\).

State B is the same as A after optical excitation of (Ru\(^{2+}\)) to (Ru\(^{2+}\)*).

State C results from electron-transfer quenching of (Ru\(^{2+}\)*) by C\(_7\)V\(^{2+}\) at the outer surface of the vesicles. The resultant (Ru\(^{3+}\)) could be (i) in a "solvent cage" with its C\(_7\)V\(^{+}\) partner, (ii) partially separated from but correlated with its C\(_7\)V\(^{+}\) partner, or (iii) separated from and uncorrelated with its C\(_7\)V\(^{+}\) partner. At present there is insufficient information to distinguish between these possibilities, so State C is regarded as a composite of all three. State C's important property with respect to the dynamic scheme is that the active entity (Ru\(^{3+}\)) is located at the outside surface of the vesicle wall, insulated from EDTA on the inside.

State C contains (Ru\(^{3+}\)) at a steady-state concentration. State C is produced from State B with a pseudo-first-order rate constant \( k_1 \). This process competes with internal
Figure 4-13. Kinetic model for photosensitized electron transport across vesicle walls from EDTA to C$_7$V$^{2+}$. Upper panel: Five states of the system from the initial ground state (A) to the final state (E). Lower panel: Interconversion diagram for the five states defining the pseudo-first-order rate constants.
conversion of (Ru\(^{2+}\))^* to (Ru\(^{2+}\)), \(k_i\), with luminescence of (Ru\(^{2+}\))^*, \(k_f\), and with a quenching of (Ru\(^{2+}\))^* by C\(_7\)V\(^{2+}\) that does not result in charge separation, \(k_q\).

The (Ru\(^{3+}\)) in State C can disappear in three ways: (i) it can recombine with its geminate C\(_7\)V\(^{+}\) partner which is held near the outer interface by surface forces, \(k_b\), (ii) it can recombine with a C\(_7\)V\(^{+}\) from the bulk aqueous phase, or with a surface-adsorbed C\(_7\)V\(^{+}\), with which it is uncorrelated, \(k_2\), or (iii) it can disappear by a process that results in transporting the active (Ru\(^{3+}\)) species across the vesicle wall to the inside, \(k_3\). The mechanism of this latter process will be considered below (Section 4.4.4.2.d). Of these three pathways for the disappearance of State C, only the second one, the reaction between (Ru\(^{3+}\)) and an uncorrelated C\(_7\)V\(^{+}\), should be affected by the analytical (bulk) concentration of C\(_7\)V\(^{+}\), [C\(_7\)V\(^{+}\)]. In the dynamic scheme we assume that

\[(4-26) \quad k_2 = k_2'[C_7V^+]\]

where \(k_2'\) is a bimolecular rate constant. This linear dependence of \(k_2\) on [C\(_7\)V\(^{+}\)] is a reasonable assumption, even though [C\(_7\)V\(^{+}\)] includes both the reactive adsorbed C\(_7\)V\(^{+}\) as well as the presumably non-reactive C\(_7\)V\(^{+}\) that is dissolved in the bulk aqueous phase.

State D, with the active (Ru\(^{3+}\)) on the inner surface of the vesicle wall, is also at steady-state, being produced in the step characterized by \(k_3\) and disappearing either by
back-reacting to State C, $k_3$, or by reacting with EDTA, $k_4$. It is only when EDTA is irreversibly oxidized, producing State E, that a net increment of detectable $C_7V^\dagger$ is produced. It is this bulk $C_7V^\dagger$ that we use as the measure of the progress of the overall reaction.

Thus States B, C, and D exist at steady-state during illumination while A is converted to E as a final product. The steady-state concentrations have the following values:

$$[B]_{ss} = \frac{I_{abs}}{(k_i + k_f + k_q + k_1)}$$

$$[C]_{ss} = \frac{k_1[B]_{ss}}{(k_b + k_2 + k_3)}$$

$$[D]_{ss} = \frac{k_3[C]_{ss}}{(k_3 + k_4)}$$

Since $\frac{d[E]}{dt} = k_4[D]_{ss}$ and $\frac{d[C_7V^\dagger]}{dt} = \frac{d[E]}{dt}$, it follows that

$$\frac{d[C_7V^\dagger]}{dt} = I_{abs}\frac{k_1}{(k_i + k_f + k_q + k_1)} \times \frac{k_3}{(k_b + k_2 + k_3)} \frac{k_4}{(k_3 + k_4)}$$

The only rate constant in Eq. 4-27 that depends on the bulk concentration of $C_7V^\dagger$ is $k_2$, which is assumed to have the linear dependence given by Eq. 4-26. Making that substitution, Eq. 4-27 can easily be integrated to give the expected dependence of $[C_7V^\dagger]$ on $t$, the cumulative time of photolysis:

$$k_b + k_3) [C_7V^\dagger] + (1/2)k_2'[C_7V^\dagger]^2 = Ct$$

where $C = I_{abs}k_3k_4/(k_i + k_f + k_q + k_1)(k_3 + k_4)$. Thus the relationship between $[C_7V^\dagger]$ and $t$ is predicted to be parabolic. This relationship was observed experimentally (e.g., Fig. 4-10).
Defining the initial quantum yield, $\phi_0$, as the initial rate of the accumulation of $C_7V^+$ divided by $I_{abs}$, and noting that initially $k_2 = 0$ since $[C_7V^+] = 0$, it follows from Eq. 4-27 that

$\phi_0 = \frac{k_1}{(k_i + k_f + k_q + k_l)} \times \frac{k_3}{(k_b + k_3)} \times \frac{k_4}{(k_3 + k_4)}$

Thus $\phi_0$, which is determined experimentally, can be considered to be the product of the probabilities of transitions from State B to C, from State C to D, and from State D to E. We assume that the probability of the transition from the ground state of (Ru$^{2+}$) (State A) to its charge-transfer excited state (Ru$^{2+}$)* is probably close to 1.0, by analogy to the case with [Ru(bipy)$_3$]$^{2+}$ (Bolletta et al., 1976; Sutin and Creutz, 1980).

The kinetic model given above does not treat possible complicating side reactions involving EDTA$^+$, which exists in State D. One of these is the back-reaction between EDTA$^+$ and (Ru$^{2+}$):

$$\text{EDTA}^+ + \text{(Ru}^{2+}\text{)} \rightarrow \text{EDTA} + \text{(Ru}^{3+}\text{)}$$

It assumed that this reaction is not important because the release of a proton from the EDTA$^+$ to the medium, which converts the radical from an oxidizing agent to a reducing agent (Whitten, 1980; Keller et al., 1980), is expected to occur rapidly at pH 8.5.

Subsequent reactions involving the deprotonated EDTA radical as a reducing agent could influence the dynamics of
the system, however. In the homogeneous solutions, MV$^{2+}$ is
the likely electron acceptor (Section 4.4.3.2). In the
vesicle system, C$_7$V$^{2+}$ is not available as the electron
acceptor because the vesicle wall separates the EDTA radical
from the viologen. If the reduction potential for the EDTA
radical is sufficiently low, (Ru$^{2+}$) in the vesicle wall
could be reduced to (Ru$^{+}$) (the analogous reduction of [Ru-
(bipy)$_3$]$^{2+}$ requires a potential of -1.28 volts vs. NHE
(Sutin and Creutz, 1980); the reduction potential for (Ru$^{2+}$)
should be more positive than that value by up to several
tenths of a volt due to the electron-accepting nature of the
substituents on (Ru$^{2+}$) (Delaive et al., 1978)). If (Ru$^{+}$) is
produced, then reactions involving that species need to be
considered in the kinetic model. Lacking evidence for the
production of (Ru$^{+}$), this complication is best omitted from
the model.

Another limitation of the model described above is that
the attenuation of $I_{abs}$ by C$_7$V$^\dagger$, whose absorption spectrum
overlaps that of (Ru$^{2+}$), is not included. Like MV$^\dagger$ (Fig. 4-5),
C$_7$V$^\dagger$ has no distinct absorption bands near 460 nm where
$I_0$ was maximal, but its extinction coefficient at 460 nm is
moderate (ε = 2100± M$^{-1}$cm$^{-1}$). The attenuation of $I_{abs}$ by
C$_7$V$^\dagger$ caused the observed parabolas for the build-up of C$_7$V$^\dagger$
(e.g., Fig. 4-10) to have greater curvature than the true
ones. Thus the quadratic coefficients $a_2$ describing the
parabolas in Table 4-5 are greater than their true values.
For the experiment at 38 °C, the integrated effect of attenuation on the accumulation of $C_7V^+$ is estimated to be 20%, so the stated value of $a_2$ is probably about 20% higher than its true value; the effect of attenuation on the $a_2$'s for the other experiments were less than 20%. The linear coefficient $a_1$ from which the values of $\bar{\theta}$ were calculated should be close to their true values, however.

### 4.4.4.2. ELECTRON TRANSPORT ACROSS VESICLE WALLS

An understanding of the mechanism of electron transport across the vesicle wall is a primary goal of this work. Some properties of the electron transport step will be evaluated below in terms of the kinetic model described in the previous Section, then mechanisms for electron transport will be considered in light of these properties.

#### 4.4.4.2.a. RATE CONSTANT

One property of electron transport through the vesicle wall that can be evaluated is the magnitude of the rate constant for the process. It will be assumed throughout this discussion that the rate constants for transport in the forward and reverse directions ($k_3$ and $k_{-3}$) are equal in magnitude. Eq. 4-29 will be used to make estimates for these rate constants from $\bar{\theta}$ and the other rate constants characterizing the kinetic model.

Values for $\bar{\theta}$ were obtained from least-squares fits of the data in Fig. 4-10. According to Eq. 4-28, the time-dependence of the accumulation of $C_7V^+$ is expected to be
parabolic:

\[ t = a_1 [C_7V^+] + a_2 [C_7V^+]^2 \]

By this convention, it follows from Eq. 4-28 that the linear coefficient \( a_1 \) has the following value:

\[ a_1 = (k_i + k_f + k_q + k_1)(k_b + k_3) \times \frac{(k_3 + k_4)}{(I_{abs}k_1k_3k_4)} \]

Comparing Eqs. 4-29 and 4-31, it is concluded that \( \varphi_0 \) equals the reciprocal of the product of \( a_1 \) and \( I_{abs} \):

\[ \varphi_0 = (I_{abs}a_1)^{-1} \]

Values for \( a_1 \), \( a_2 \), and \( \varphi_0 \) based on least-squares fits are given in Table 4-5. Correspondence between the experimental data and the least-squares parabolas is satisfactory (Fig. 4-10).

The first term in the expression for \( \varphi_0 \) in Eq. 4-29 represents the probability that \((Ru^{2+})^*\) is quenched by \(C_7V^{2+}\) with the formation of \((Ru^{3+})\) and \(C_7V^+\) (State B to C) (Fig. 4-13). This term can be expanded to two:

\[ k_l/(k_i + k_f + k_q + k_1) = \]

\[ \{(k_q + k_1)/(k_i + k_f + k_q + k_1)\}(k_1/(k_q + k_1)) \]

This expansion is made because the steady-state luminescence quenching measurements presented earlier (Section 4.3.3.4) do not distinguish between \( k_q \) and \( k_1 \) (see Fig. 4-13). Since less than 10% of the luminescence from \((Ru^{2+})^*\) was quenched by 0.0010 M \(C_7V^{2+}\), the following approximation holds:

\[ k_i + k_f + k_q + k_1 \approx k_i + k_f \]

Furthermore, the Stern-Volmer quenching constant is defined
as:

\[(4-35) \quad K_{s-v}[C_7V^{2+}] = \frac{(k_q + k_1)}{(k_i + k_f)}\]

It follows from Eqs. 4-29, 4-33, 4-34, and 4-35 that

\[(4-36) \quad \phi_o = K_{s-v}[C_7V^{2+}] \frac{k_1}{(k_q + k_1)} \times\]

\[\{\frac{k_3}{(k_b + k_3)}\} \frac{k_4}{(k_3 + k_4)}\]

It will be assumed that \(\frac{k_1}{(k_q + k_1)}\), the efficiency of radical formation per quenching event (State B to C), is 0.2, the value for the quenching of \(*[Ru(bipy)_3]^{2+}\) by MV²⁺ in homogeneous solution (Kiwi and Grätzel, 1978; Sutin and Creutz, 1980 (Section 4.4.3.2)). Thus Eq. 4-36 becomes

\[(4-37) \quad \phi_o = (0.2)(K_{s-v}[C_7V^{2+}]) \times\]

\[\{\frac{k_3}{(k_b + k_3)}\} \frac{k_4}{(k_3 + k_4)}\]

Assuming that \(k_3 = k_3\), the magnitude of \(k_3\) can be estimated using Eq. 4-37 if the values of \(k_b\) and \(k_4\) are known. As will be discussed below, estimates for these latter two rate constants can be made based on literature values.

An estimate for \(k_b\) is obtained from values for analogous radical recombination reactions in PC vesicle systems, in which either chlorophyll a (Hurley et al., 1980) or a dodecyl homologue of (Ru²⁺) (Nagamura et al., 1980; Takayanagi et al., 1980) were used as photosensitizers. In each case, the pseudo-first-order rate constant fell in the range \(10^2-10^4\) s⁻¹, including fast and slow components. Also, the apparent bimolecular rate constants in the vesicle systems were 0.01-1 times their corresponding values in homogeneous solutions. Thus it is reasonable to assume that \(k_b\) falls
between $10^2$ and $10^4$ s$^{-1}$.

The reduction of $[\text{Ru(bipy)}_3]^{3+}$ by EDTA has a bimolecular rate constant of $>10^6$ M$^{-1}$s$^{-1}$ in homogeneous aqueous solutions at pH $> 8$ (Sutin, 1979; Keller et al., 1980; Miller and McLendon, 1981) (Section 4.4.3.2). ($\text{Ru}^{3+}$) is at least as strong an oxidant as $[\text{Ru(bipy)}_3]^{3+}$ due to the electron-accepting nature of the amide substituents (Delaive, et al., 1978), so the bimolecular rate constant for the reduction of ($\text{Ru}^{3+}$) by EDTA$^{3-}$ in homogeneous solution is probably $>10^6$ M$^{-1}$s$^{-1}$. Drawing from the empirical observation mentioned above, that rate constants in vesicle systems are 0.01-1 times their values in homogeneous solutions, the bimolecular rate constant for the reduction of ($\text{Ru}^{3+}$) by EDTA$^{3-}$ in the vesicle system is probably $>10^4$ M$^{-1}$s$^{-1}$. Based on this estimate, and assuming that the "local" concentration of EDTA at the vesicle surface is of the same magnitude as its "bulk" concentration in the inner aqueous compartment (0.3 M), it is likely that $k_4 > 10^4$ s$^{-1}$, so $k_4 > k_b$.

The magnitude of $k_3$ relative to $k_4 > k_b$ is not known, but, again assuming that $k_3 = k_3^-$, there are only five possibilities to consider:

Case 1: $k_4 > k_b = k_3$
Case 2: $k_4 > k_b > k_3$
Case 3: $k_3 = k_4 > k_b$
Case 4: $k_3 > k_4 > k_b$
Case 5: $k_4 > k_3 > k_b$

Under these different conditions, the expression \( \{k_3/(k_b + k_3)\}\{k_4/(k_3 + k_4)\} \) on the right-hand side of Eq. 4-36 reduces approximately to the following terms:

- Case 1: \( \{k_3/(k_b + k_3)\}\{1\} \)
- Case 2: \( \{k_3/k_b\}\{1\} \)
- Case 3: \( \{1\}\{k_4/(k_3 + k_4)\} \)
- Case 4: \( \{1\}\{k_4/k_3\} \)
- Case 5: \( \{1\}\{1\} \)

In Cases 1 and 2, \( \phi_0 \) increases with increases in \( k_3 \), while the opposite is true in Cases 3 and 4; \( \phi_0 \) is nearly independent of \( k_3 \) in Case 5.

The experimental observations discriminate between the five possibilities outlined above. The argument rests mainly on the observed effects of ionophores and transmembrane electrostatic potentials on \( \phi_0 \) (Laane et al., 1981; see the Appendix). In the absence of transmembrane potentials, ionophores such as valinomycin are expected to have positive influences on \( k_3 \) (and \( k_{-3} \)) (see next Section). None of the other rate constants included in the kinetic model are expected to be sensitive to ionophores. The fact that ionophores have a positive influence on \( \phi_0 \) (Laane et al., 1981) therefore indicates that \( \phi_0 \) increases with \( k_3 \), meaning that either Case 1 or 2 prevail.

Based on the argument presented above, the expression for \( \phi_0 \) further reduces to Eq. 4-38:
\[ \phi_0 = \{K_{S-V}[C_7V^{2+}]\}\left[\frac{k_1}{(k_q + k_1)}\right] \left[\frac{k_3}{(k_b + k_3)}\right] \]

\[ \phi_0 \text{ ranged from } 10^{-4} \text{ to } 10^{-3}, \quad K_{S-V}[C_7V^{2+}] \text{ ranged from } 0.01 \text{ to } 0.1, \quad \text{and } \frac{k_1}{(k_q + k_1)} = 0.2 \text{ (see above), so } \frac{k_3}{(k_b + k_3)} \text{ was probably between 0.01 and 1. Since } k_b \text{ was probably between } 10^2 \text{ and } 10^4 \text{ s}^{-1} \text{ (see above), } k_3 \text{ probably fell within the range } 10-1000 \text{ s}^{-1}. \]

The significance of the magnitude of \( k_3 \), with regard to the mechanism of electron transport across the vesicle wall, will be discussed in Section 4.4.4.2.d.

4.4.4.2.b. CATION TRANSPORT AND TRANSMEMBRANE POTENTIALS

[with Colja Laane]

Electroneutrality requires that the net flow of electrons from the vesicle interior to the continuous aqueous phase be accompanied by a compensating flow of either cations in the same direction or anions in the opposite direction (Hinkle, 1973; Green, 1975; Hauska, 1977; Kurihara et al., 1979b). An uncompensated flow of electrons would produce a potential gradient across the membrane, impeding further electron migration in that direction. Thus an enhancement of the co-transport of ions should have a positive effect on \( \phi_0 \), which is a measure of the efficiency of electron transport across the vesicle wall. Conversely, a pre-existing transmembrane potential should be able to influence charge transport across vesicle walls. These expectations were tested and realized using potassium ion gradients and valinomycin to generate transmembrane potentials (Cafiso and Hubbell, 1978). The reader is referred to the original
manuscript (Laane et al., 1981), which is given in the Appendix, for details and discussion.

4.4.4.2.c. TEMPERATURE DEPENDENCE [with Howard D. Mettee, Tetsuya Sakai, and John W. Otvos]

There is a relatively large thermal activation energy ($E_\theta = 28.0 \pm 4.5$ kcal/mole) associated with the overall process of photosensitized electron transport across the walls of vesicles in the present model system. This observation is favorable to the prospect of using vesicles to mediate charge separation in artificial photosynthetic devices because increases in temperature, which will occur during the normal operation of such systems, will be beneficial, so a means of cooling them will not be necessary or even desirable. As will be argued below, the activation energy associated with the transmembrane electron transport step itself ($E_3$) contributes about 16 kcal/mole to the overall value, so the beneficial aspect of heating may be generally true for other vesicle systems. This point needs to be tested with vesicle systems composed of other components (e.g., other photosensitizers, electron donors or acceptors).

It is of interest to obtain an estimate for the activation energy of $k_3$, one reason being that the value could help to discriminate between possible electron transport mechanisms (see next Section). According to Eq. 4-38, $\phi_0$ can be separated into the product of three fractions, so the
Arrhenius activation energy for $\Phi_0$ ($E_\Phi$) is the sum of the activation energies for quenching ($E_{S-V}$), radical ion-pair separation ($E_{Sep}$), and the probability that ($\text{Ru}^{3+}$) at the outer surface of the vesicle (State C of Fig. 4-13) reacts to produce ($\text{Ru}^{3+}$) at the inner surface (State D) rather than recombining with its geminate $C_7V^+$ partner to produce the ground state of the system (State A). It was determined in Section 4.4.4.2.a that this latter probability, $k_3/(k_b + k_3)$, ranged between 0.01 and 1, so $k_3/(k_b + k_3) = k_3/k_b$. Under this condition, the expression for $E_\Phi$ becomes

$$E_\Phi = E_{S-V} + E_{Sep} + E_3 - E_b$$

where $E_3$ and $E_b$ stand for the Arrhenius activation energies of $k_3$ and $k_b$, respectively.

The value of $E_{S-V}$ was found to be 11.7±2.5 kcal/mole (Section 4.3.3.4). With regard to $E_{Sep}$, there is evidence that the probability for dissociation of radical ion-pairs is determined mainly by the polarity of the medium (Orbach et al., 1972; Seely, 1978; Cheddar and Tollin, 1980; Cheddar and Tollin, 1981; Masuhara and Mataga, 1981), so other physical properties such as viscosity can be considered relatively unimportant. This assumption is reinforced by the results of Kamat and Lichtin (1982), who found that the efficiency of the dissociation of the (methylene blue):$^-\text{Fe}^{3+}$ radical ion-pair has a weak temperature dependence in the range 18-48 °C. It is reasonable to assume that, within the temperature range examined (18-38 °C), which is well above
the phase transition of pure egg PC bilayers (-15 to -7 °C (Chapman, 1975)), the polarity of the vesicle-water interface did not change significantly, so $E_{\text{sep}}$ is small enough to be neglected in Eq. 4-39.

It is also reasonable to assume that $E_b$, the activation energy for the geminate recombination between (Ru$^{3+}$) and $C_7V^+$, is small enough to be neglected in Eq. 4-39, because the reaction is highly exoergic ($-\Delta G^0 > 1$ volt) and the barriers associated with the one-electron redox chemistry of molecules like these are small (Balzani et al., 1979; Sutin and Creutz, 1980). This latter point is exemplified by the fact that the rate constants for electron self-exchange for both kinds of compounds are large ($\geq 10^8$ M$^{-1}$s$^{-1}$) (Sutin and Creutz, 1980; Takuma et al., 1981). Furthermore, Takayanagi et al. (1980) found that the activation energy for a reaction analogous to the one being considered, the recombination between a homologue of the reduced form of (Ru$^2+$) and a phenothiazyl radical cation in dimyristoylphosphatidylcholine vesicle suspensions, is nearly zero.

Based on the approximations that $E_{\text{sep}} = 0$ and $E_b = 0$, Eq. 4-39 becomes

$$E_\phi = E_{S-V} + E_3$$

Since $E_\phi = 28.0 \pm 4.5$ kcal/mole and $E_{S-V} = 11.7 \pm 2.5$ kcal/mole, it is concluded that $E_3 = 16 \pm 5$ kcal/mole. This is the first such estimate for the activation energy associated with electron transport across lipid bilayer vesicles that is not
mediated by the diffusion of electron-transporting molecules like quinones (Futami et al., 1979).

It remains to be considered how much of the 16±5 kcal/mole of $E_3$ is associated with the co-transport of ions, which is required to maintain electroneutrality (see previous Section). The experiments of Laane et al. (1981) (Appendix) give some indication of the contribution of ion transport. It was found that the ionophore valinomycin increased $\phi_0$ by a factor of up to 6.5. Taking this limiting value to represent the removal of the intrinsic barrier to ion transport, then the activation energy difference $\Delta E = RT \ln 6.5$, or about 1.1 kcal/mole, is the estimated contribution of ion transport to $E_3$. Thus co-ion transport does not appear likely to contribute significantly to $E_3$.

4.4.4.2.d. MECHANISMS

The three previous Sections described some properties of electron transport across the vesicle walls. The mechanism of electron transport is considered in this Section. It is important to understand the details of transmembrane electron transport so that improvements in the process can be made rationally.

Two possible electron transport mechanisms will be considered here. These are either (i) the transmembrane diffusion ("flip-flop") of (Ru$^3+$) or else (ii) the direct diffusion of electrons between (Ru$^2+$) on one side of the bilayer and (Ru$^3+$) on the other side (Ford et al., 1979).
The characteristics of electron transport across the vesicle wall that were derived in previous Sections support the electron-exchange mechanism over the flip-flop mechanism. These points are considered below.

The magnitude of $k_3$ is the property that best discriminates between the two mechanisms, mainly because its value ($10^{-1000}$ s$^{-1}$) is orders of magnitude greater than rate constants for the flip-flop of amphiphilic molecules across egg PC vesicles. Pseudo-first-order rate constants for flip-flop range from $10^{-8}$ s$^{-1}$ for egg PC itself (Thompson, 1978) to 1 s$^{-1}$ for the neutral forms of fatty acids (Sackmann, 1978). The value for (Ru$^{3+}$) is expected to fall within this range because the molecule carries a charge of 3+, but the charge is partially delocalized over the bipyridyl ligands. Thus, the magnitude of $k_3$ is probably at least three orders of magnitude too high to be consistent with the flip-flop mechanism. Whether or not this rate constant is consistent with electron-exchange is unknown because there is little or no precedence for electron-exchange across vesicle walls (however, see Kuhn (1979) for a discussion of relaxation times for electron tunnelling across lipid monolayers).

The stimulation of $\Phi_0$ ionophores and transmembrane potentials (Laane et al., 1981) is consistent with the diffusion of electrons being the mode of charge transport. While the rate constants for flip-flop could be influenced
by transmembrane potentials (McLaughlin, 1977), it seems unlikely that the flip-flop of (Ru$^{3+}$) would be significantly influenced by ionophores such as valinomycin.

The activation energy for electron transport, $E_3 = 16\pm5$ kcal/mole, provides further argument against the flip-flop mechanism. The activation energy for the flip-flop of amphiphilic molecules like (Ru$^{3+}$) is expected to be in excess of 20 kcal/mole (Jain and Wagner, 1980). Measured activation energies for the flip-flop of phospholipids across vesicle walls are 19.4 kcal/mole (Kornberg and McConnell, 1971) and 23.7$\pm$2.0 kcal/mole (deKruijff and Van Zoelen, 1978). On the other hand, there is little precedence for activation energies of direct diffusion of electrons across vesicle walls. Tien (1974) compiled activation energies for the dark conductivities of planar lipid bilayer membranes. The average value for "unmodified" bilayers is about 18 kcal/mole. Bilayers that are modified with molecules capable of forming charge-transfer complexes with the lipids (e.g., I$_2$ or Fe$^{3+}$) have activation energies that are about 5 kcal/mole lower. The identity of the charge carriers in these experiments is uncertain, however. While there is direct evidence for electron transport in the vesicle systems, the conductivities measured in the planar bilayer systems could be either ionic or electronic in nature (Tien, 1974). Therefore, while the value for $E_3$ of 16$\pm$5 kcal/mole does not necessarily support the electron-
exchange mechanism, it is further evidence against a flip-flop mechanism for electron transport.

Two ways of viewing direct electron transfer across lipid bilayer membranes involve the treatment of lipid bilayers as either (i) liquid-crystalline semiconductors (Rosenberg, 1971; Tien, 1974; Tien and Karvaly, 1976), or (ii) thin insulators through which electrons can tunnel between dye molecules located in the opposing layers (Ilani and Berns, 1973; Mangel, 1976; Seefeld et al., 1977; Kuhn, 1979; Ford et al., 1979). In either case, it is reasonable that the electron is transferred from (Ru$^{2+}$) in one lipid layer to (Ru$^{3+}$) in the other. This process is indicated by Eq. 4-40, where the subscripts denote the inner and outer layers of the vesicle wall.

$$\text{Eq. 4-40} \quad (\text{Ru}^{2+})_{\text{in}} + (\text{Ru}^{3+})_{\text{out}} \rightleftharpoons (\text{Ru}^{3+})_{\text{in}} + (\text{Ru}^{2+})_{\text{out}}$$

Eq. 4-40 is analogous to the electron self-exchange reaction in homogeneous solution (Sutin and Creutz, 1980). The rate constant $k_3$ is expected to be proportional to the concentration of (Ru$^{2+}$) in the vesicle wall if Eq. 4-40 is important. It was found that $\theta_{\text{max}}$ increased by 2.2±0.3 when the egg PC:(Ru$^{2+}$) molar ratio was increased by 2.8±0.2 (Section 4.3.2.2). This result is consistent with there being a proportionality between $k_3$ and the concentration of (Ru$^{2+}$) in the membrane if none of the other terms in Eq. 4-38 (i.e., $K_{S-V[C7V^{2+}]}$, $k_1$, $k_q$, and $k_b$) are significantly affected. While it is expected that this latter condition
is upheld, it should be pointed out that the quenching studies by Baker (1981) indicate that exciton coupling between \( \text{Ru}^{2+} \) molecules makes \( K_{s-v} \) dependent on the concentration of \( \text{Ru}^{2+} \).

4.4.4.3. VARIATIONS IN COMPOSITION

The discussion so far has been limited to the vesicle system with walls composed solely of \( \text{Ru}^{2+} \) and egg PC and with \( \text{C}_7\text{V}^{2+} \) in the external aqueous phase and \( \text{EDTA}^{3-} \) in the internal one. Experiments with other systems will now be considered.

The quantum yields of viologen radical production were especially sensitive to the type of viologen used (Table 4-1). The differences in \( \emptyset \) can largely be attributed to differences in the partitioning of the viologens between the membrane and aqueous phases. Electron transfer reactions like the quenching of \( \text{Ru}^{2+}* \) by viologens are characterized by critical separation distances of the order of \( 10^{-2} \)\( \text{R} \) (Mauzerall, 1976; Seefeld et al., 1977). The probability of electron transfer decreases exponentially with increasing distance between the two reactant molecules once the critical distance is exceeded. The fact that during gel-filtration of \( \text{Ru}^{2+} \)-egg PC mixed vesicles, \( \text{Ru}^{2+} \) remained associated with the vesicles (Section 4.2.3.2.b) shows that \( \text{Ru}^{2+} \) is confined to the lipid bilayer, so only viologen molecules that are located in or close to the vesicle wall are capable of quenching \( \text{Ru}^{2+}* \).
Steady-state luminescence quenching measurements such as those described in Section 4.3.3.4 provide information about the distribution of viologens between the bilayer and aqueous phases since, for example, a viologen which has no tendency to dissolve in or bind to the surface of vesicles will not be a quencher, even if its concentration in the vesicle suspension is high. This condition is nearly met by MV^{2+}, which barely quenches the luminescence of (Ru^{2+})^* (Table 4-3). By comparison, C_{16}V^{2+} is nearly $10^4$ times more effective a quencher than MV^{2+}. The electron-accepting moieties of the two molecules are nearly identical, so the differences between their effectiveness as quencher must be determined by their distributions in the vesicle suspension. C_{16}V^{2+} is probably almost entirely located in the membrane phase, in view of the fact that the dichloride salt of the compound is practically insoluble in water. Assuming that MV^{2+} and C_{16}V^{2+} molecules that are associated with vesicles are approximately equally effective as quenchers, it can be estimated that only 0.01-0.1% of the MV^{2+} molecules in the vesicle suspensions are close enough (i.e., <20 Å) to the vesicle surface to quench (Ru^{2+})^* during its lifetime (0.6 μs (Matsuo et al., 1980a)). By the same argument, roughly 1% of C_{7}V^{2+} molecules are close enough (Table 4-3). Thus the heptyl chains of C_{7}V^{2+} make that viologen 100 times more effective a quencher than MV^{2+} in vesicle suspensions.

Long-chained quinones like VK_{1} are known to catalyze
electron transport across lipid bilayer membranes (Anderson et al., 1976; Futami et al., 1979) and decachlorocarborane is known to transport protons (Liberman et al., 1970), so the small negative effect of these compounds on $\Phi_{\text{max}}$ (Table 4-1) indicates that other transport mechanisms dominated. Presumably, the dominant electron transport process was the electron-exchange mechanism discussed in the previous Section.

The incorporation of VK$_1$ and the borane did have a marked effect on the induction period, however, being much more prominent when those compounds were present (Fig. 4-4). The lengthening of the induction period was probably caused by the fact that VK$_1$ is more readily reduced than C$_{16}$V$_2^+$ or MV$_2^+$, so most of it would have to be reduced before the viologen radicals could accumulate. The reduction potential for VK$_1$ to its hydroquinone form (VK$_1$H$_2$) is about $-0.12$ volts vs. NHE at pH 7 (Ksenzhek et al., 1977) while the reduction potential for MV$_2^+$ and its homologues like C$_{16}$V$_2^+$ to the radical form is about $-0.45$ volts vs. NHE (Michaelis and Hill, 1933; Steckhan and Kuwana, 1974), so the following reaction is spontaneous under standard conditions, as is the analogous reaction with C$_{16}$V$_2^+$ instead of MV$_2^+$:

\begin{equation}
2M^{+} + VK_{1} + 2H^{+} \rightarrow 2MV_{2}^{+} + VK_{1}H_{2} \quad \Delta G^{0} = -0.33 \text{ volts}
\end{equation}

Absorbance increases in the near UV region with a maximum near 335 nm ($\Delta \text{OD} = 0.1$ per cm path length) which were observed during the induction period were consistent with
the reduction of VK₁ to VK₁H₂ (Crane and Sun, 1972) (Ford, 1978).

Another membrane additive which was tested for an effect on Φ was retinyl acetate. A polyene like retinyl acetate could increase the probability of electron transport across the vesicle wall, thereby increasing Φ, by lowering the barrier to the tunnelling of electrons (Ilani and Berns, 1973; Mangel, 1976; Kuhn, 1979). The slightly negative effect of the retinyl acetate (about 1 molecule per 5 egg PC molecules) on Φ_max for the reduction of C₁₆V²⁺ (Section 4.3.2.2.c) indicates that the polyene had little influence on electron transport. Also, there was probably little energy transfer quenching of the excited state of (Ru²⁺) by retinyl acetate, which occurs between *[Ru(bipy)₃]²⁺ and retinol in homogeneous solution (Bensasson et al., 1976).

The experiment with Co(II)EDTA instead of EDTA was performed to test the effect of replacing an irreversible electron donor with a reversible one (Section 4.3.2.2.b). When Co²⁺ ions are complexed to EDTA, the oxidation of EDTA becomes reversible with a reduction potential (Co(III)EDTA⁺ + e⁻ → Co(II)EDTA) of +0.6 volts vs. NHE (Rock, 1968; Srinivasan and Rabinowitch, 1970). This potential is low enough for Co(II)EDTA to be oxidized by either (Ru³⁺) or [Ru(bipy)₃]³⁺:

(4-42) Co(II)EDTA + [Ru(bipy)₃]³⁺ →

Co(III)EDTA⁺ + [Ru(bipy)₃]²⁺ \( \Delta G^0 = -0.6 \) volts
and high enough for Co(III)EDTA to be reduced by MV⁺:

\[ (4-43) \quad \text{Co(III)EDTA}^+ + \text{MV}^+ \rightarrow \text{Co(II)EDTA} + \text{MV}^2+ \quad \Delta G^0 = -1.0 \text{ volts} \]

The back-reaction in Eq. 4-43 would prevent MV⁺ from accumulating in homogeneous solution, as was observed. In principle, the vesicle walls could prevent the reaction by keeping Co(III)EDTA and MV⁺ separated. Two possible reasons why MV⁺ was not accumulated in the vesicle system are (i) the reduction of (Ru³⁺) by Co(II)EDTA is much slower than the reduction by EDTA, so \( k_3 \gg k_4 \) (see Fig. 4-13), and (ii) Co(III)EDTA quenches \( \text{(Ru}^2+) \) effectively to produce Co(II)EDTA and \( \text{(Ru}^3+) \), thereby making the recombination between \( \text{(Ru}^3+) \) and MV⁺ more likely. On the other hand, the vesicle walls, which contained both \( \text{(Ru}^2+) \) and \( \text{C}16\text{V}^2+ \), could be sufficiently permeable in the dark, either to electrons or to MV⁺, to prevent the accumulation of Co(III)EDTA and MV⁺. Kinetic measurements such as flash photolysis are required to determine the actual situation.

4.4.4.4. PERMEABILITIES OF MV²⁺ AND EDTA³⁻

The conclusions drawn in this Chapter about the photochemistry of the vesicle systems rely on the vesicle walls being asymmetric with respect to EDTA³⁻ and either MV²⁺ or C7V²⁺. Based on the relative impermeability of lipid vesicles to molecules with localized charges and/or extensive hydrogen bonding (see Chapter 1, Section 1.5), it was expected that the vesicle systems would remain asymmetric for
the duration of the photolysis experiments. Permeability measurements for EDTA$^{3-}$ and MV$^{2+}$ (Section 4.3.4) supported this expectation.

The permeabilities of molecules crossing membranes can be compared by comparing their permeability coefficients (P). Applying Fick's law, the rate ($\Delta n/\Delta t$, moles/s) that a substance crosses the vesicle wall is proportional to the diffusion coefficient ($cm^2/s$) of the substance within the membrane phase, the concentration difference across the membrane, and the surface area of the membrane ($a$, $cm^2$), and it is inversely proportional to the membrane thickness ($cm$). The surface area is determined by the dimensions of the vesicles. Experimentally, the concentration of the permeant substances in the aqueous phases are known, but it is the value of the concentration difference across the membrane proper that determines the rate of permeation. It can be assumed that the concentration in the membrane phase equals the concentration in the aqueous phase ($c$, $M$) times an equilibrium constant ($K$) for the partitioning of the substance between the two phases. Furthermore, $K<<1$ for ionic species with localized charges like MV$^{2+}$ and EDTA$^{3-}$, so the actual value of $c$ is essentially the same as if the vesicles were absent. $P$ is defined as the diffusion coefficient divided by the membrane thickness, and has the units of velocity ($cm/s$). Therefore $P$ is related to $\Delta n/\Delta t$, $\Delta c$, $a$, and $K$ by Eq. 4-44:
Consequently, an apparent permeability coefficient, the product $K \cdot P$, can be calculated from the concentration difference across the vesicle wall and the measured rate of escape from the vesicle interior if the dimensions of the vesicles are known.

In the vesicle systems examined, the internal concentration of permeant ($c_i$) was much greater than the external concentration, so $\Delta c \approx c_i$. This condition held approximately during the course of the permeability measurements because the volume of the internal phase was much less (of the order of $1/1000$) than the volume of the external phase. Noting that $c_i = n_i/V$, where $n_i$ is the number of moles of entrapped ion, Eq. 4-44 can be expressed as follows:

$$\Delta n_i/\Delta t = -K \cdot P \cdot a \cdot n_i/V$$

Rearrangement of this equation gives a predicted first-order relationship between $\Delta n_i$ and $\Delta t$:

$$\Delta n_i/n_i = -(K \cdot P \cdot a/V) \Delta t$$

The first-order rate constant ($k$, s\(^{-1}\)) thus equals the term in parentheses:

$$k = K \cdot P \cdot a/V$$

If the vesicles are spheres with radii $r$, the area to volume ratio equals $3/r$, so

$$k = 3K \cdot P/r$$

Using the value for $k$ of 0.012 hr\(^{-1}\) determined from Fig. 4-10 and assuming that the vesicles were spheres with
radii of 340 Å (Table 1-1), K•P for EDTA$^{3-}$ is calculated to be $4 \times 10^{-12}$ cm/s. This value falls between those for glucose ($2.5 \times 10^{-10}$ cm/s at 23 °C) and Na$^+$ ($1.2 \times 10^{-14}$ cm/s at 4 °C) (Papahadjopoulos and Kimelberg, 1974). The value for EDTA$^{3-}$ can be considered an upper limit since the experimental method could not distinguish between the release of EDTA$^{3-}$ and the uptake of the CuNR complex.

It is not clear as to why the measured rate of release of EDTA$^{3-}$ was a factor of 5 greater for diluted vesicles than for undiluted ones (Table 4-7). One explanation is that the vesicles were extensively flocculated in the undiluted suspension, reducing the exterior surface area per vesicle exposed to the CuNR complex in the bulk aqueous phase. Dilution would favor dissociation of the vesicles, leading to a greater apparent rate constant.

The apparent permeability coefficient for MV$^{2+}$ can be estimated if it is assumed that the rate of release of MV$^{2+}$ from the vesicles decayed exponentially with time, as was the case with EDTA$^{3-}$. It was found that $6\pm2\%$ of the entrapped MV$^{2+}$ escaped in 2 hours (Section 4.3.4.1), so the term ($\Delta n_1/n_1$)/$\Delta t$ in Eq. 4-46 equals $-(9\pm3) \times 10^{-6}$ s$^{-1}$. Again assuming that the vesicles were spheres with radii of 340 Å, the value for K•P is calculated as $(1.0\pm0.3) \times 10^{-11}$ cm/s. Thus MV$^{2+}$ is about twice as permeable as EDTA$^{3-}$. Both compounds are sufficiently impermeable that vesicle suspensions with EDTA$^{3-}$ inside and MV$^{2+}$ outside (e.g.,
systems A, B, and C of Table 4-1) remained asymmetric during the photochemical experiments, which took about 2 hours to complete. The twelve additional methylene groups of $C_7V^{2+}$ should make it more permeable than $MV^{2+}$, but the molecule is still expected to be relatively impermeable due to its localized positive charges.

4.5. CONCLUSIONS

The model system described in this Chapter is suitable for studying photosensitized electron transport across vesicle walls. A strong argument can be made for a direct electron transfer process, as opposed to electron transport mediated by diffusional carriers. Individual aspects of this argument rely heavily on order-of-magnitude estimates for reaction rate constants which are based on rate constants for analogous reactions in other systems. An effort should be made to extend this work to other techniques such as flash photolysis so that the rate constants can be measured directly.

The results of this Chapter are generally favorable for the prospect of using vesicles in artificial photosynthetic devices. Electron transport across vesicle walls occurs sufficiently rapidly (with a pseudo-first-order rate constant of $10^{-1000}$ s$^{-1}$ in the present system) to compete effectively with back-reactions at the interfaces, so charge separation by vesicle walls could be an efficient process. The low overall quantum yield in the present system ($\varnothing =$
$10^{-4}-10^{-3}$) is due mainly to a poor efficiency of the quenching of $(\text{Ru}^{2+})^*$ by $C_7\text{V}^{2+}$ (0.01-0.1) and the competition between electron transport across the membrane and the back-reaction between $(\text{Ru}^{3+})$ and $C_7\text{V}^{+}$ (0.01-1).
CHAPTER 5. PROSPECTUS

Some prospects for the use of vesicles in photochemical water-decomposing devices are considered in this Chapter. Ultimately these devices will have to be durable and easy to mass-produce. These factors are not of primary importance now because some chemical principles still need to be established, but practicality should guide long-range research directions. With regard to durability, practical devices need not last for, say, 10 years as long as it were convenient to replace the decomposed parts and recycle the materials (e.g., metals).

Some possible trends in the development of artificial photosynthetic devices containing vesicles are considered in Section 5.1, and two designs in which photosensitive half-cells are connected electrochemically are described in Section 5.2.

5.1. COMPONENTS

5.1.1. VESICLES

5.1.1.1. IMPROVING STABILITY

One problem with using vesicle suspensions in practical devices is that they are intrinsically unstable. The exchange of molecules between vesicles and collisions between the vesicles themselves can lead to flocculation and a loss of asymmetry. Such dynamic processes could be avoided by polymerizing the vesicle suspensions. Immobilization of vesicles within the pores of polymer hydrogels should
prevent collisions between the vesicles, thereby preventing their aggregation. Polymerization of the the components of the vesicle wall should hinder changes in the integrity of the bilayer, thereby making it possible to maintain asymmetry. These modifications are further discussed below.

Hydrogels can immobilize vesicles and prevent their coagulation. Ideally, the pores of gels are too small for the diffusion of vesicles but are large enough for the free diffusion of small molecules about the vesicles. Some preliminary experiments with immobilized vesicles were carried out during the course of this work. Vesicles composed of the cationic didodecyldimethylammonium ion precipitated upon the addition of salts (e.g., NaCl) but did not precipitate if they were embedded in agarose gel (there was no change in the turbidity of the gel). Likewise, a vesicle dispersion composed of the anionic dihexadecylphosphate ion doped with \([(\text{bipy})_2\text{Ru(\text{bipy-CONHC}_6\text{H}_3})]\) became turbid when \([\text{Co(NH}_3)_5\text{Cl}]^{2+}\) (10^{-3} M) was added, but the addition of the cobalt complex did not affect the turbidity of a polyacrylamide gel containing the vesicles. The red luminescence of the ruthenium complex in the gel sample was quenched by the cobalt complex and could be restored by washing the gel with water.

The immobilization of positively or negatively charged vesicles also makes it possible to exchange the counterions. For example, when an agarose gel containing vesicles
composed of dioctadecyldimethylammonium bromide was exposed to an aqueous solution of tetrasulfonated-Cu(II)-phthalocya-
nine, the vesicles became stained blue by the dye, which did not wash out with water (nor with salt solutions); the control experiment with agarose and no vesicles left the gel colorless after washing with water. Presumably, the stained vesicles were left asymmetric, with the phthalocyanine ad-
sorbed to the outer surfaces but not the inner ones.

The polymerization of lipids comprising vesicles has only recently been investigated. In these cases, the groups that were polymerized were either diacetylenes (Day et al., 1979; Johnston et al., 1980) or vinyl (Regen et al., 1980) located on the hydrocarbon "tails" of the lipids. Alterna-
tively, the functional groups could be attached to the polar "heads"; in this case, catalysts and dyes with functional groups could be covalently linked to the surfaces of vesi-
cles during polymerization. Related to interfacial poly-
merization is the stabilization of planar bilayer membranes by treating them with polylysine and glutaraldehyde (King and Steinrauf, 1972; Luschow and Schulz-Harder, 1978). Polymerizing the lipids of vesicles should make the walls less permeable to aqueous solutes and also hinder the flip-
flop of the lipids.

Another way to keep aqueous solutes from crossing vesi-
cle walls is to attach them to water-soluble polymers (e.g., poly(ethylene oxide) (Haehnel et al., 1978)).
The instability of unsaturated lipids to autooxidation (Klein, 1970; Barclay and Ingold, 1980) could be avoided by using saturated, branched hydrocarbons instead of unsaturated ones (Redford et al., 1971; Johnson et al., 1973). By using branched instead of normal saturated hydrocarbons, the vesicle walls will be in their fluid phases at ambient temperatures.

5.1.1.2. IMPROVING QUANTUM YIELDS

Two general ways of improving quantum yields of photosensitized redox reactions across vesicle walls are (i) to decrease the probability of back-reactions at the interfaces and (ii) to increase the probability of electron transfer across the membrane, which competes with the back-reactions. The first approach can make use of surface potentials (Infelta et al., 1980; Infelta and Fendler, 1980; Nagamura et al., 1980). Surface potentials are especially advantageous if the charge of the component dissolved in the water phase changes from zero to that opposite the surface charge. Charged interfaces have adjacent Stern layers which are enriched in ions with the opposite sign and depleted of ions with the same sign (Fendler and Fendler, 1975; McLaughlin, 1977). Thus, for example, if a neutral or zwitterionic molecule accepts an electron from a photoexcited dye that is dissolved in an anionic vesicle, the resultant anionic acceptor radical will be "repulsed" from the interface, thereby impeding the reverse electron transfer reaction (Ford,
The second approach to improving quantum yields can utilize ionophores, electron carriers, and transmembrane potentials (Hinkle, 1973; Hauska, 1977; Futami et al., 1979; Kurihara et al., 1979; Matsuo et al., 1980; Laane et al., 1981). The ionophores used in water-decomposing membrane systems will eventually have to be proton (or hydroxide ion) carriers because of mass balance requirements (Section 1.3). Transmembrane electric potential gradients can be generated either with ion gradients and selective ionophores (Cafiso and Hubbell, 1978) or by making the membranes asymmetric with respect to surface potentials (Ohki, 1973). Membrane potentials produced by K\(^+\) concentration gradients and valinomycin would not last because K\(^+\) transport would accompany electron transport across the membrane and dissipate the concentration gradient. Potentials due to pH gradients could last, however, because protons would be produced and consumed on opposite sides of the membrane during water oxidation and reduction reactions. Membrane potentials due to an asymmetry of surface charges could be made long-lasting by using adsorbed polyions (Ohki, 1973) or by polymerizing the interfaces after making them asymmetric.

5.1.2. DYES

Vesicles whose walls are asymmetric with respect to photosensitizer (e.g., arrangement (2) in Fig. 4-1) could be conveniently prepared by using dyes that adsorb to the walls
from aqueous solution (as opposed to dyes that are dissolved in the walls themselves). Water-soluble dyes will bind to vesicles if they are amphiphilic and/or attracted electrostatically (e.g., Section 5.1.1.1).

Interfacial intermolecular complexes with visible absorption bands to charge-transfer transitions could be useful photosensitizers in vesicle systems. The complex would exist before photoexcitation, so every photon adsorbed would result in electron transfer, in contrast to the usual case of dye and quencher in which there is competition between electron transfer and other modes of deexcitation.

5.1.3. CATALYSTS

The catalysts that mediate the oxidation or reduction of water, designated C[O] and C[H], could be either heterogeneous or homogeneous catalysts. Heterogeneous catalysts have been most widely used; these are usually metals or metal oxides which are in a particulate or colloidal state (Kiwi, 1979). Two ways that heterogeneous catalysts could be incorporated into vesicle systems, close to membrane-bound photosensitizers (e.g., Fig. 1-2), are by (i) preparing the vesicles in the presence of previously-prepared colloidal catalyst, and (ii) precipitating the catalyst within the vesicle by chemically treating a vesicle suspension containing the catalyst precursor (e.g., a metal salt). These methods are considered below.

Lipid bilayers could be supported on particles of cata-
lysts whose dimensions are similar in magnitude to those of vesicles (200–2000 Å) to produce vesicle-encapsulated heterogeneous catalysts. Mann et al. (1979) encapsulated 100–200 Å particles of magnetite (Fe₂O₃) by sonicating a suspension of egg PC with colloidal magnetite. This approach could be extended to other kinds of colloidal inorganic polymers which could be used as the catalysts themselves or as supports for catalysts (Iler, 1955; Cocco and Gottardi, 1978; Matijević, 1978). Alternatively, the dimensions of the catalyst particles could be small (Hirai et al., 1978; Cocco and Gottardi, 1978) compared to those of the vesicles, which can be made with diameters of up to several thousand Ångstroms (Szoka and Papahadjopoulos, 1980).

Instead of encapsulating preformed heterogeneous catalysts, the catalysts could be prepared in situ by first entrapping a vesicle-impermeable precursor (e.g., a metal salt) within the vesicles, then adding a vesicle-permeable precipitating agent. This method was used to precipitate CoS (Skarnulis et al., 1978) and Ag₂O (Hutchison et al., 1980) inside vesicles. Preliminary experiments along this line indicated that ruthenium oxide (RuO₂) can be supported on cationic vesicles of didodecyldimethylammonium ion by exchanging the Br⁻ counterions of the vesicles with [Ru-(H₂O)Cl₅]²⁻, then adding BH₄⁻ to convert the salt to hydrous RuO₂ (Fletcher, 1968). RuO₂ deposited this way catalyzes
the reduction of water to $H_2$ using $[(bipy)_2Ru(bipy-CONHC_{16}H_{33})]^{2+}$ as the photosensitizer and a phenothiazine derivative and Fe$^{2+}$ as electron donors (Park, 1980 [with Tetsuya Sakai, and W. E. Ford]), and RuO$_2$-coated vesicles could catalyze the oxidation of water as well (Kiwi, 1979). Ruthenium or osmium oxides precipitated by the reaction of RuO$_4$ or OsO$_4$ with the double bonds of egg PC vesicles (Chapman and Wallach, 1968; Nielson and Griffith, 1979) could likewise be catalysts for the oxidation or reduction of water (besides stabilizing the vesicles by polymerizing them).

A great disadvantage of using metals or heavy metal oxides as catalysts for the "dark" reactions in photochemical devices is the fact that they have large extinction coefficients in the visible spectrum. This disadvantage makes homogeneous catalysts attractive alternatives to heterogeneous catalysts. Unfortunately, there has been relatively little effort made to search for homogeneous catalysts for the oxidation or reduction of water. Cobalt complexes with tetradeutate macrocycles (Schrauzer et al., 1965; Costa et al., 1970; Brown et al., 1979) or CN$^-$ as ligands (Pringer and Farcas, 1965; Burnett et al., 1967; Wymore, 1968) are promising as homogeneous water-reducing catalysts. Brown et al. (1979) used a tetra-aza cobalt complex in homogeneous systems that produced $H_2$ photochemically. Schiff-base ligands (Costa et al., 1970) are attrac-
tive in that they could be easily functionalized to modify the solubilities of the catalysts. Manganese porphyrins are promising as homogeneous water-oxidizing catalysts (Calvin et al., 1964; Willner et al., 1980; Harriman and Porter, 1980). The solubilities of these compounds can easily be controlled by chemical modification of the porphyrin ring substituents.

5.2. ARRANGEMENTS -- TWO PHOTOSYSTEMS

The single-membrane system depicted in Fig. 1-2 has the catalysts $C[H]$ and $C[O]$ on opposite sides of the membrane. In a vesicle suspension this configuration would mean that the products of reduction and oxidation of water would be produced in the same vessel. If $H_2$ and $O_2$ were being produced, $C[H]$ and $C[O]$ would be exposed to both gases (since gases diffuse freely across lipid bilayers (Whitten et al., 1980; Simon and Gutknecht, 1980)), leading to unwanted back-reactions (Chapter 1). In addition, the $H_2$ and $O_2$ would have to be separated afterward if they were produced in the same container. (If instead a gas and a liquid were produced from [H] and [O] (e.g., $H_2$ and $H_2O_2$, or HCOOH and $O_2$), the problem of separating the products would be alleviated, but, depending on the membrane permeabilities of the products, back-reactions at the catalytic sites could still be a problem.) Hence it would be better to have two kinds of vesicles, one for the reduction of water and the other for the oxidation of water, that were physically
separate but connected so that electrons and protons could move from the water-oxidizing compartment to the water-reducing compartment. Baehnel et al. (1978) designed a photocell that operated on similar principles using two chloroplast suspensions connected by electrodes and a salt bridge composed of an ultrafiltration membrane.

(Natural photosynthesis accomplishes $O_2$ evolution and $CO_2$ reduction simultaneously on a microscopic scale apparently without significant interference, and utilizes the equivalent of the electric and ionic current that connects the two reactions to phosphorylate adenosine diphosphate.)

The problem of interfacing the microscopic system (the photochemical reactions that take place in individual vesicles) with the macroscopic system (the dark electrochemical reactions that couple the two photosystems) is a crucial one. Immobilization of the vesicles should be considered not only for the enhanced stability mentioned in Section 5.1.1.1 but also because a flow system could then be used to move water-soluble electron mediators between the electrodes and the vesicles without moving the vesicles themselves. Several designs utilizing immobilized vesicles are considered below.

The basic arrangement and chemical reactions being considered is shown in Fig. 5-1. Here one kind of vesicle suspension catalyzes the photosensitized oxidation of water and the reduction of mediator $A$, while another kind of
Figure 5-1. Arrangement of the essential components of an artificial photosynthetic device with two kinds of hydrogel-immobilized vesicles as the light-absorbing parts. The geometric configurations shown are meant to be symbolic. The vesicles contain dyes and catalysts for the oxidation or reduction of water as depicted in Fig. 1-2. The redox mediators \( A/A^- \) and \( D/D^+ \) are mobile in both the hydrogel and bulk aqueous phases and serve to couple the two vesicle systems electrochemically via electrodes.
suspension catalyzes the photosensitized reduction of water and the oxidation of mediator D. The A/A\(^-\) and D/D\(^+\) redox couples are reversible ones, and the molecules remain in the continuous aqueous phases of the two vesicle systems. C\(_{[O]}\) and C\(_{[\text{H}]}\), which mediate the irreversible oxidation and reduction of water, are sequestered in the internal aqueous compartments of the vesicles. Among other requirements (Haehnel et al., 1978), A\(^-\) must be inert to the product of water oxidation and D\(^+\) must be inert to the product of water reduction. C\(_{[O]}\) and C\(_{[\text{O}]}\) could be buried within the vesicle walls instead of being in the internal aqueous compartments.

The parts of the device in Fig. 5-1 containing the vesicles need to be thin to facilitate the diffusion of A/A\(^-\) and D/D\(^+\) between the vesicles and the electrodes. A thickness of the order of 0.1 cm would be sufficient for the pigmented vesicle suspensions to absorb 99% of the incident sunlight of a particular wavelength since (bulk) dye concentrations of \(10^{-4}-10^{-3}\) M and extinction coefficients of \(10^4-10^5\) M\(^{-1}\)cm\(^{-1}\) are easily attained. Alternatively, sunlight could be made to pass through several less-absorbent layers of immobilized vesicles.

Two general designs that allow for electrochemical coupling between two immobilized vesicle suspensions that will be considered below. These are based on either (i) columnar or (ii) sandwich configurations.

(i) Columnar configurations. In one type of column,
an aqueous solution of the electron acceptor A is flowed past immobilized vesicles where the reduction of A and oxidation of water take place photochemically; the oxidation of D and reduction of water take place analogously in a second type of column. The aqueous solutions then flow past electrodes and salt bridges to regenerate the solutions of A and D, which are recycled past the vesicle suspensions. The vesicle-containing hydrogel could be shaped either as spherical beads (Kitajima and Butler, 1970; Ekman et al., 1976; Willner et al., 1980) or as thin "leaves". Another possibility is to make use of anisotropic hollow fibers as in immobilized-enzyme technology (Chambers et al., 1976). In this case, the vesicles are immobilized in the outer layer of "sponge" of the fibers, separated by the semipermeable "skin" from the aqueous solutions of A/A⁻ or D/D⁺ which are pumped through the cores of the fibers.

(ii) Sandwich configuration. A possible arrangement is depicted in Fig. 5-2. The sandwich configuration allows the two hydrogel photosystems to be coupled electrochemically without the interposition of bulk aqueous solutions, thereby avoiding the recirculation of the solutions which is required in the columnar configurations. An inert gas such as N₂ is passed through the channels to remove O₂ and H₂.

5.3. CONCLUSIONS

It should be possible to fabricate artificial photosynthetic devices based on vesicles which will be of commercial
Figure 5-2. Prototype of a sandwich configuration for an artificial photosynthetic device -- cross-sectional view. Two layers of porous hydrogel are separated by a porous glass plate which is coated with transparent semiconductor films. Pigmented vesicles which are embedded in the hydrogel layers photosensitize the oxidation and reduction of water (to $O_2$ and $H_2$ in this case) using catalysts and redox mediators as depicted in Figs. 1-7 and 5-1. The two gel layers are connected electrochemically through the semiconductor films on the glass, which conduct electrons, and the pores in the glass, which conduct protons. $N_2$ flows through networks of pores in the gel layers to remove $O_2$ and $H_2$ and supply water vapor.
significance, but a good deal more fundamental research is required first. Two areas of research which should be addressed are the stabilization of asymmetric vesicles via polymerization and the development of homogeneous catalysts for the oxidation and reduction of water. The ability to synthesize new compounds and derivatives of existing ones for incorporation into vesicle systems will greatly facilitate such work.
REFERENCES

REFERENCES

102, 7792-7794.
Blumberg, W. E. and J. Peisach (1965) J. Biol. Chem. 240,
REFERENCES

870-876.
REFERENCES


REFERENCES


REFERENCES

Fahey, P. P. and W. W. Webb (1978) Biochemistry 17, 3046-
REFERENCES


Fleischer, E. B. and L. E. Webb (1963) J. Phys. Chem. 67,
REFERENCES

1131-1133.


Gurinovich, G. P., A. N. Sevchenco, and K. N. Solov'yev...


REFERENCES


REFERENCES

Am. Chem. Soc. 29, 5484-5486.
Krebs, H. A. and H. L. Kornberg (1957) Energy Trans-
New York.
McLaughlin, S. (1977) In Current Topics in Membranes and


Michaelis, L. and E. S. Hill (1933) J. Gen. Physiol. 16, 859-873.


REFERENCES

5075-5080.
REFERENCES

Seely, G. R. (1977) In Primary Processes of Photosynthesis


REFERENCES

173-278.

Snyder, L. (1979) Chemtech, 750-754.
REFERENCES

REFERENCES

Acta 511, 125-140.
Natl. Acad. Sci. USA 72, 3270-3274.
Dekker, New York.
Tien, H. T. and B. Karvaly (1976) In Solar Power and Fuels
(Edited by J. R. Bolton), Chap. 7, pp. 167-223. Academic
Press, New York.
Tinoco, Jr., I., K. Sauer, and J. C. Wang (1978) Physical
Chemistry, pp. 483-489. Prentice-Hall, Englewood Cliffs,
New Jersey.
Tollin, G. and G. Green (1963) Biochim. Biophys. Acta 66,
308-318.
22, 249-256.
Toyoshima, Y., M. Morino, H. Motoki, and M. Sukigara (1977)
Soc. 99, 1285-1287.
Van Houten, J. and R. J. Watts (1976) J. Am. Chem. Soc. 98,
4853-4858.
REFERENCES


APPENDIX

Photosensitized electron transport across lipid vesicle walls: Enhancement of quantum yield by ionophores and transmembrane potentials

(Colja Laane, William E. Ford, John W. Otvos, and Melvin Calvin)

Melvin Calvin Laboratory, Lawrence Berkeley Laboratory, University of California, Berkeley, California 94720

Contributed by Melvin Calvin, January 9, 1981

ABSTRACT The photosensitized reduction of heptylviologen in the bulk aqueous phase of phosphatidylcholine vesicles containing EDTA inside and a membrane-bound tris(2,2'-bipyridine)ruthenium(2+) derivative is enhanced by a factor of 6.5 by the addition of valinomycin in the presence of K+. A 3-fold stimulation by gramicidin and carbonyl cyanide m-chlorophenylhydrazone is observed. The results suggest that, under these conditions, the rate of photoinduced electron transfer across vesicle walls in the absence of ion carriers is limited by cotransport of cations. The rate of electron transfer across vesicle walls could be influenced further by generating transmembrane potentials with K+ gradients in the presence of valinomycin. When vesicles are made with transmembrane potentials, interior more negative, the quantum yield is doubled, and, conversely, when vesicles are made with transmembrane potentials, interior more positive, the quantum yield is decreased and approaches the value found in the absence of valinomycin.

In recent years, light-induced electron-transfer processes have been extensively investigated with the aims of understanding the mechanism of natural photosynthesis and of designing artificial systems that will decompose water by sunlight to produce chemical energy in the form of H2 (1-3). A basic concept in the design of such systems is the use of dyes to photosensitize electron-transfer reactions that produce chemical species capable of oxidizing and reducing water. A major problem accompanying the dissociation of water by sunlight involves the back reactions of the intermediary redox species, whereby the potential energy of the photochemical process is degraded. One way to control the forward and backward reactions is to separate electron mediators such as 1,3-dibutylalloxazine and 1,3-diodoanthraquinone 2,6-disulfonate is stimulated by the addition of proton carriers (11). In Zn-porphyrin-containing vesicles, the photoreduction of 9,10-anthraquinone 2,6-disulfonate is stimulated by the addition of electron mediators such as 1,3-dibutylalloxazine and 1,3-didodecylalloxazine (12). In chlorophyll-containing bilayer lipid membranes (13) or in cyanine dye-containing monolayer assemblies (14), vectorial electron transport across the lipid barrier is enhanced by applying transmembrane electric fields by means of electrodes. In monolayer assemblies, a chainlike pi-electron system facilitates transmembrane electron transport (14). In this paper we report the effects of several ionophores and K+-diffusion potentials on the quantum yield of heptylviologen reduction in the model system described above.

MATERIALS AND METHODS

Materials. Phosphatidylcholine from hen egg yolks was purified by the method of Singleton et al. (15). As sensitizer, the Ru(II) complex [N,N'-di(1-hexadecyl)-2,2'-bipyridine-4,4'-dicarboxamide]-bis(2,2'-bipyridine)ruthenium(2+) was used (10). 1,1'-Diheptyl-4,4'-bipyridinium dibromide (heptylviologen, C7VBr2) was purchased from Aldrich, and EDTA was from Calbiochem, respectively. Carbonyl cyanide m-chlorophenylhydrazone (CCCP) was a generous gift of W. Hubbell.

Vesicle Preparation. Vesicle dispersions containing phosphatidylcholine and the Ru(II) complex at a molar ratio of 200:1 were prepared by the injection method (16) according to Ford et al. (10). Vesicle suspensions were freshly prepared before gel filtration and illumination. The vesicle concentration was estimated to be 0.13 μM, assuming a mean vesicle diameter of 700 Å (17). For the generation of transmembrane potentials, vesicles were prepared in 0.3 M EDTA/50 mM sodium glycine (pH 8.5) containing a high concentration of either K+ or Na+.

Generation of Transmembrane Potentials. The procedure used to obtain vesicles having a potential difference across their membrane was analogous to that described by Cafiso et al. (18). Vesicles having transmembrane K+ gradients with ratios K+*/K+ outward of 1:1, 3:1, 10:1, 45:1, and 90:1, or vice versa, and EDTA trapped inside were obtained by passing vesicles prepared by the injection method through a Sephadex G-25 column. The column was equilibrated with a buffer containing the desired concentration of (K+SO4)outward and sufficient Na2SO4 to make the ionic strength and the osmotic pressure of the continuous

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "advertisement" in accordance with 18 U. S. C. §1734 solely to indicate this fact.
aqueous phase equal to that of the internal vesicle solution. MgSO₄ (2 mM) was added to the medium to ensure that any EDTA leaking from inside to outside the vesicle was not the source of electrons for the CᵥV²⁺ reduction. For example, to obtain Kᵥ/Kₒ = 10:1, vesicles containing 0.9 M K⁺/0.3 M EDTA/50 mM sodium glycine buffer were passed through the column, which was equilibrated with 90 mM K⁺/0.81 M Na⁺/2 mM Mg²⁺/0.452 M SO₄²⁻/50 mM sodium glycine buffer. After the vesicles had passed the column, transmembrane potentials were generated by the addition of valinomycin. The time between the addition of valinomycin and illumination was at least 45 min (18). In this way, vesicles for which Kᵢ/Kₒ > 1 establish a more negative potential inside and for which Kᵢ/Kₒ < 1 establish a more positive potential inside. Gramicidin and CCCP were added only to vesicles for which Kᵢ/Kₒ = 1.

Transmembrane equilibrium potentials were measured as described (18).

Illumination. After addition of CᵥV²⁺ to a final concentration of 1 mM, the vesicle suspension was transferred to a gas-tight cuvette and deaerated with scrubbed argon. The cuvette was then irradiated with blue light (440–550 nm) using a 1000-W xenon arc lamp, according to Ford et al. (10). The temperature was 25.0 ± 0.2°C, and the incident photon flux was (1.67 ± 0.10) × 10⁻¹⁰ einstein min⁻¹ cm⁻², as determined by Reinecke salt actinometry (19). The formation of viologen radical (CᵥV⁺) was monitored at 602 nm after intervals of illumination. The concentration of CᵥV⁺ was calculated by assuming the extinction coefficient of the radical to be the same as that for methylviologen radical, 12,400 M⁻¹ cm⁻¹ (20). The initial time slope quantum yield (φₚ₀) was calculated by dividing the maximal rate of CᵥV⁺ formation by the rate of quanta absorbed.

RESULTS

Effect of Ionophores on Quantum Yield. In vesicle suspensions containing equimolar concentrations of K⁺, Na⁺, and H⁺ on both sides of the membrane, the quantum yield of heptylviologen reduction was enhanced by the addition of CCCP, gramicidin, and valinomycin (Fig. 1). A common feature of these compounds is that they make the membrane more permeable to certain cations (21). However, the transport mechanisms and the selectivity for cations are different. CCCP carries only H⁺ and valinomycin carries mainly K⁺ from one side of the membrane to the other. The pore-forming ionophore gramicidin has, in contrast to CCCP and valinomycin, a fixed position in the membrane and facilitates the movement of several univalent cations (e.g., H⁺, K⁺, and Na⁺).

When the CCCP concentration in the vesicle suspension was increased, the quantum yield increased from 4 × 10⁻⁴ to a constant level of 1.2 × 10⁻³. Maximal stimulation was already observed at a CCCP concentration of 0.13 μM; i.e., approximately one CCCP molecule per vesicle. Recently, comparable effects of CCCP on the rate of Fe(CN)₆³⁻ reduction in chlorophyll-containing liposomes were observed by Kurihara et al. (11). Our results, therefore, substantiate their conclusion that transmembrane electron transfer is facilitated by cation carriers when it is coupled to cation transport in the same direction.

Although less pronounced at relatively low concentrations, the enhancing effect of gramicidin on the quantum yield was similar to that of CCCP. In the case of gramicidin, about 10 molecules per vesicle were necessary to obtain maximal stimulation. When, gramicidin and CCCP together were added in excess to the vesicle suspension, the quantum yield hardly increased further. This suggests that the ion-carrying capacity of either gramicidin or CCCP alone is sufficient to allow for charge neutrality during transmembrane electron transfer. Furthermore, the nature of the cation does not seem to be important.

With valinomycin, the quantum yield could be increased even more (6.5-fold). Valinomycin appeared to be very active; one valinomycin per 10 vesicles (13 nM) was sufficient to stimulate transmembrane electron transport to the same extent as found for CCCP and gramicidin at much higher concentrations. This result is in agreement with the observation that one valinomycin per 30 vesicles is sufficient to make all vesicles permeable to K⁺ (22). Apparently, valinomycin can hop from one vesicle to another. Addition of excess CCCP or gramicidin did not further affect the quantum yield. The presence of K⁺ appeared to be necessary for the action of valinomycin. In vesicle suspensions in which K⁺ was replaced by Na⁺, valinomycin did not influence the quantum yield, a result consistent with the fact that the permeability of Na⁺ is hardly affected by valinomycin (23).

Effect of Transmembrane Potentials on Quantum Yield. The fact that electrons can cross vesicle walls implies that the rate of electron transfer should be influenced by a transmembrane electric field. Fig. 2 shows that the quantum yield of heptylviologen reduction responds strongly to changes in the magnitude and direction of an applied transmembrane electric field. Long-lasting transmembrane potentials were developed by the addition of valinomycin to vesicles having K⁺ gradient across their phospholipid wall. K⁺-diffusion potentials were estimated by measuring the distribution of a hydrophobic nitroxide cation between aqueous and membrane phases (18). The values determined by this method were in good agreement with those calculated by using the Nernst equation. For example, the experimentally determined potential difference generated in vesicles having a Kᵢ/Kₒ of 90 is 115 mV interior more negative, and the calculated value is 117 mV. At a valinomycin concentration of 10 molecules per vesicle (1.3 μM), the quantum yield was approximately doubled by increasing the transmembrane potential to −115 mV. Similar results were found at a much lower valinomycin concentration (one molecule per 10 vesicles). Conversely, the quantum yield could be decreased by reversing the direction of the electric field. With both the high and low valinomycin concentrations, a limiting quantum yield was reached at 4 × 10⁻⁴, which is similar to values obtained in the absence of valinomycin. A quantum yield of 4 × 10⁻⁴ represents the minimum rate of transmembrane electron transfer, that can-

Fig. 1. Effect of ionophores on quantum yield of heptylviologen reduction. Vesicle suspensions were prepared and illuminated with blue light as described in Materials and Methods. ○, Valinomycin plus K⁺; ●, valinomycin plus Na⁺; ▲, CCCP; □, gramicidin; ×, CCCP plus gramicidin.
DISCUSSION

Transmembrane Transport of Cations and Electrons. The results obtained with ionophores extend an earlier study (11) on the coupling between ion transport and photoinduced electron transfer across lipid bilayers. The charge imbalance is shown to be counteracted effectively by enhancing the cation permeability of the membrane by ionophores (see Fig. 1). For valinomycin in the presence of K\(^+\), it has been shown that the transmembrane potential scale does not apply for the data obtained without valinomycin. The highest quantum yield obtained in our experiments was 4.4 \(\times 10^{-3}\). The transmembrane potential exerts a constant effect on the magnitude of the quantum yield even when the size of the electron donor remains constant. The observed ion fluxes are at the upper limit of what could be expected for the applied membrane potentials. This minimum seems to be determined by the intrinsic ability of the membrane to transport cations.

The highest quantum yield obtained in our experiments was 4.4 \(\times 10^{-3}\). The combination of valinomycin (see Fig. 1) and a transmembrane electric field (see Fig. 2) on photoinduced electron transfer, therefore, resulted in an 11-fold increase of the quantum yield.

Transmembrane Potential and Electron Transfer. Our results show that photoinduced electron transfer can be influenced by applying an electric field across the membrane. For vesicles having K\(^+\)/K\(^-\) > 1, transmembrane electron transfer is enhanced and, conversely, for vesicles having K\(^+\)/K\(^-\) < 1, electron transfer is inhibited. In a previous paper (10), evidence was presented suggesting that the most likely mechanism for transmembrane electron transfer is electron exchange between the Ru\(^{3+}\) and Ru\(^{3+}\) complexes at opposing sides of the lipid bilayer. The electrons probably cross the potential barrier of the hydrocarbon portion of the membrane by tunneling. Our results are consistent with an electron-exchange mechanism for electron transfer because transmembrane electric fields are known to affect the tunneling rate by changing the barrier height of the membrane (14).

The highest quantum yield for the reduction of heptylviologen that could be obtained in our model system was 4.4 \(\times 10^{-3}\). In a comparable homogeneous system with Ru(bipy)\(^{3+}\) as sensitizer, the overall quantum yield for the reduction of viologen was 5 \(\times 10^{-3}\). It appeared, however, that the luminescence quantum yield in the vesicle system was about half that of the homogeneous system. Furthermore, in the vesicle system, only 20% of the photoexcited Ru-complex could be quenched by 1 mM viologen although, in the comparable homogeneous system, 50% of the photoexcited Ru(bipy)\(^{3+}\) was quenched. Thus, the highest quantum yield that can be expected under these circumstances for our vesicle system is about 1 \(\times 10^{-3}\). Fig. 2 (highest \(\phi_m = 4.4 \times 10^{-3}\)) shows that this limit is approached by enhancing the cation permeability of the membrane and by applying a transmembrane electric field of 115 mV, interior more negative.

Attempts to increase the quantum yield further either by decreasing the back reaction of the initial photoproduc by using excess methylviologen as a sink for the electron or by adding EDTA to the aqueous bulk phase as an electron source were unsuccessful. Apparently a substantial fraction of the absorbed light is thermally degraded and has no opportunity to produce photoreduction. Parts of the overall photochemical reaction are now being investigated by flash photolysis. These studies may lead to greater insight into the photochemical reactions that take place in a vesicle system.

We wish to thank Mr. G. Karzmar for his help in determination of transmembrane potentials. This investigation was supported by the Netherlands Organization for the Advancement of Pure Research (Z.W.O.) and by the Division of Chemical Sciences, Office of Basic Energy Sciences, U.S. Department of Energy under Contract W-7405-ENG-48.

This report was done with support from the Department of Energy. Any conclusions or opinions expressed in this report represent solely those of the author(s) and not necessarily those of The Regents of the University of California, the Lawrence Berkeley Laboratory or the Department of Energy.

Reference to a company or product name does not imply approval or recommendation of the product by the University of California or the U.S. Department of Energy to the exclusion of others that may be suitable.