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INVESTIGATION OF PHASE AND STATE RELATIONS
IN COMPLEX LIPID SYSTEMS

R. C. Bean

Philco-Ford Corporation

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Office of Saline Water

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Investigation of Phase and Phase Relations
in Organic-Lipid Systems

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This project was designed to examine the ion and water permeability of lipid phases in various lipid-polymer-water mixtures; the effect that changes in phase structure might have upon that permeability; and whether the permeability relations so elaborated might have some utility in water extractive processes.

Permeability was studied with lipids incorporated into membranes or dispersed in a bulk phase. Membrane forms included: the lipid-loaded membrane, in which membranes were prepared by saturating a microporous, polymeric support with lipids and then treating in various ways; the lipid-skin membrane, formed by compressing a thin film of lipid particles onto one face of a microporous support; the cast-lipid membrane, formed by casting a solution containing both lipid and polymer upon a glass surface; and the bilayer lipid membrane (BLM).

Results of studies on the lipid-loaded membranes demonstrated that they generally had a low resistance at temperatures below a transition temperature near the melting point of the lipid. At this point, an abrupt 100 to 10,000-fold increase in resistance occurred in all cases, except with primary alkylamines. At temperatures below the liquifying transitions, some membranes had excellent ion selective properties with low resistance (10 to 50 ohm cm²).

Keywords

Membranes*, Ion Transport*, Lipids*, Permeability, Reverse Osmosis

Subject Terms

Lipid Membranes*, Water Permeability*, Thermal Transitions*, Amphipathic Lipids*, Photoelectric Reaction, Bilayer Lipid Membrane, Salt Rejection, Lipid-Loaded Membranes, Lipid-Skin Membranes, Cast Lipid Membranes

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A novel photoelectric reaction was observed in the lipid-loaded membranes. Various membranes, supported in cellulose ester microporous filters, produced photo-voltaic and/or photoconductive responses to ultraviolet illumination. Photopotentials ranging up to 150 mv were obtained. Sensitization to visible light could be developed with certain dyes (e.g., methylene blue, rhodamine B).

Certain formulations of cast-lipid membranes showed promise for development of good reverse osmosis properties. Polyamide membranes cast with certain anionic or neutral long alkyl chain lipids incorporated into the casting solutions showed better water permeability and salt rejection than similar membranes prepared without lipids.

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RELATIONS IN COMPLEX LIPID SYSTEMS

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INVESTIGATION OF PHASE AND STATE
RELATIONS IN COMPLEX LIPID SYSTEMS

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FOREWORD

This is one of a continuing series of reports designed to present accounts of progress in saline water conversion and the economics of its application. The data are expected to contribute to the long-range development of economical processes applicable to low-cost demineralization of sea and other saline water.

Except for minor editing, the data herein are as contained in a report submitted by the contractor. The data and conclusions given in the report are essentially those of the contractor and are not necessarily endorsed by the Department of the Interior.

SUMMARY

This project was designed to examine the ion and water permeability of lipid phases in various lipid-water and lipid-polymer-water mixtures; the effect that changes in phase structure might have upon that permeability; and whether the permeability relations so elaborated might have some utility in water extractive processes. Only very simple amphipathic lipids (alkylamines, alkanols, fatty acids, glycerides, alkyl phosphates) were considered in these investigations.

Permeability was studied with lipids incorporated into membranes or dispersed in a bulk phase. Membrane forms included: the lipid-loaded membrane, in which membranes were prepared by saturating a microporous, polymeric support with lipids and then treating in various ways; the lipid-skin membrane, formed by compressing a thin film of lipid particles onto one face of a microporous support; the cast-lipid membrane, formed by casting a solution containing both lipid and polymer upon a glass surface; and the bilayer lipid membrane (BLM).

Results of studies on the lipid-loaded membranes demonstrated that they generally had a low resistance at temperatures below a transition temperature near the melting point of the lipid. At this point, an abrupt 100 to 10,000-fold increase in resistance occurred in all cases, except with primary alkylamines. Other 2 to 5-fold perturbations of resistance with changing temperature, indicated the existence of several different, reproducible phase changes in a number of the composite membranes. At temperatures below the liquifying transitions, some membranes containing dialkyl phosphates or some alkylamines had excellent ion selective properties with low resistance (10 to 50 ohm cm^2).

A novel photoelectric reaction was observed in the lipid-loaded membranes. Various membranes, consisting of alkanols, alkyl esters or aldehydes (but not fatty acids, hydrocarbons, or halogenated hydrocarbons) supported in cellulose ester microporous filters, produced photovoltaic and/or photoconductive responses to ultraviolet illumination. Photopotentials ranging up to 150 mv were obtained. Sensitization to visible light could be developed with certain dyes (e.g., methylene blue, rhodamine B).

Some of the lipid-skin membranes, under conditions used here, had excellent ion-selective permeability properties, suggesting that mechanisms might be developed for applying them in situations where membrane generation and removal in situ might be desirable. Neither the lipid-skin nor lipid-loaded membranes showed desirable properties for reverse osmosis applications.

Certain formulations of cast-lipid membranes, however, showed promise for development of good reverse osmosis properties. Polyamide membranes cast with certain anionic or neutral long alkyl chain lipids incorporated into the casting solutions showed better water permeability and salt rejection than similar membranes prepared without lipids.

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SECTION 1

INTRODUCTION

The investigation of phase relations in complex lipid systems was largely instigated by the realization that very little information was available on the effect of transitions between various phase structures in lipid-water or lipid-water-polymer mixtures upon permeability or sequestering properties of those systems. In particular, the data on ion and water permeability or sequestering in solid lipid phase structures is extremely scanty. This lack of information could be hiding useful properties that might be readily applied to separation processes, especially in the removal of salt and other contaminants from water.

Even simple mixtures of water and amphipathic lipids* may exist in several distinct phase structures, some of which may be rather complex (1-3). Some structures involve sequestering regions, in which both water and ions may be trapped, with spacing dependent upon ion strength as well as the nature of the ion; others include conformations that might provide ion-conducting channels through an otherwise low conductivity barrier. Phase structures in lipid water systems depend not only upon the structure of the lipid, but also on the relative concentrations of water and lipid and upon the concentration and nature of ions in the system.

The introduction of a third component into the lipid-water mixture, further increases the potential complexities of the phase structures. A soluble macromolecule, such as a protein, may be incorporated into the lipid-water

* An amphipathic lipid is a molecule which possesses distinct, separate regions of hydrophilic and hydrophobic character. They have also been referred to as hetero-polar or amphiphilic lipids.

structure in a number of different ways (4). The introduction of the lipid and water into a highly porous, polymeric matrix, having different affinities from those exhibited by the lipids, can serve a similar purpose. Thus, adsorption of lipids onto a microporous filter (5-8), or loading a microporous filter with solvents (9-13) may create membranes having interesting properties reflecting structural and group functional properties of both components.

In our own early studies of lipid-phase relations in membrane applications, we had found mechanisms for preparing low resistance membranes, with high ionic selectivity, using microporous filters as supports for various lipids (14). Low resistances were obtained in the cation selective membranes prepared with dioctadecyl phosphate only after specific aqueous heat treatments altered the phase structure of the membrane to develop conducting channels. Other experiments suggested that phase structures could also be altered as a consequence of applying high potential gradients across a micropore filter-lipid membrane in which the lipids were asymmetrically distributed.

These data, along with other lines of evidence pointing toward permeability control with lipids, suggested that properties of the amphipathic lipids could be usefully applied to water purification processes. This project was initially directed toward developing more information on the permeability of simple lipid-water phases, the ion exchange or sequestering capabilities of these systems, and the changes that might develop in any of these properties as the result in the change of phase structure. This latter interest expressed the view that such alterations might be successfully applied in cyclic processes for water purification.

The initial objectives of the project included:

1. Examination of the exchange and sequestering properties of dispersions of lipids in aqueous media and how these properties are altered through changes in phase relations induced by physical treatments or light.
2. Determining the permeability properties of membranes consisting of lipids supported upon a variety of polymeric films and how permeability is affected by phase transitions.
3. Evaluating the potential for application of lipid systems to water purification processes, either as membranes or in countercurrent extraction mechanisms.

The final objective implies utilization of practical materials. Accordingly, the investigations were carried out with simple lipid substances, avoiding, for the most part, testing exotic or unstable materials. However, some attention was given to stabilized lipid bilayer membranes since previous work (15-22) has established that some properties of these membranes are increasing for selective isolation or purification processes. In these

experiments, materials best suited to lipid bilayer formation were utilized without particular regard to simplicity or practical availability.

In the initial program outline, investigations of properties of bulk phase or disperse phase lipid-water systems were given essentially equal footing with the studies of membrane properties. As indicated in the experimental section, early experiments with the disperse phase relations suggested that the lipid systems would have little advantage in water purification over more common ion exchange or partition processes. Consequently, the major attention was shifted to membrane systems.

SECTION 2

EXPERIMENTAL METHODS AND MATERIALS

The studies in this project encompassed a group of diversified approaches in which methods and materials themselves were under study. Consequently, some of these factors may be discussed specifically under Section 3, Experimental Results. The more generally applicable procedures are given below.

2.1 MATERIALS

2.1.1 LIPIDS AND MISCELLANEOUS CHEMICALS

Most lipids and other chemicals used in these studies were obtained from commercial sources. Some processing was required for some commercial products to provide a better defined material. All glycerol esters were obtained from K & K Laboratories, Plainview, New York, as technical grade materials. These esters were recrystallized from hexane, methanol, or hexane-methanol mixtures. In some cases, several crystalline fractions were obtained with relatively diverse properties, indicating rather heterogeneous composition of the original material. Chromatography indicated that some of the recrystallized fractions used in the experimental work still retained significant amounts (5% to 15%) of esters other than those designated as the major constituent.

Octadecan-1-ol, hexadecan-1-ol, and ethylene glycol monododecanoate were also purchased from K & K.

The 1-n-decylamine, 1-n-dodecylamine, 1-n-tetradecylamine, 1-n-octadecylamine, dodecanoic acid, tetradecanoic acid, octadecanoic acid, mono-octadecylphosphate, and di-octadecyl phosphate were obtained from Aldrich Chemical Company, Milwaukee, Wisconsin. The primary amines were recrystallized for use while the remainder were used as received. Didodecyl phosphite

(dilauryl phosphite), tridodecyl phosphite, dioctadecyl phosphate, octadecyl phosphate and didodecyl phosphate were also obtained from Aldrich. Di-n-octylamine, di-n-decylamine, di-n-dodecylamine, tri-n-decylamine, tri-n-dodecylamine, decan-1-ol, dodecan-1-ol and tetradecan-1-ol were products of Eastman Organic Chemicals Division, Rochester, New York.

Oxidized cholesterol, a mixture of at least four different steroid components, was prepared by refluxing recrystallized cholesterol (Eastman Organic Chemicals Division), 4% in octane, for about 6 hours while bubbling oxygen through the solution. The precipitate formed upon cooling was removed, the octane evaporated in vacuo and solutions prepared from the syrupy material in nonane, decane, or dodecane.

Sphingomyelin was purchased from ICN Nutritional Biochemicals Corporation, Cleveland. Although this material was generally used as received, slow deterioration during storage required occasional refinement through acetone precipitation from a solution in 2:1 chloroform-methanol (10 volumes acetone to 1 volume chloroform-methanol solution) in the cold.

Fatty acid esters of cyclohexanol and of phenol derivatives were synthesized by Dr. J. W. Mason and Mr. R. Salisbury.

"Sorbitan tristearate" was Span 65, an emulsifier product of Hercules Chemical Division, Wilmington. It is actually a complex mixture of esters of the 1-4 anhydro sorbitol, the 1-5 anhydro sorbitol and isosorbide (1-4, 3-6, dianhydro sorbitol).

Solvents and salts used in experiments here were analytical or reagent grade commercial preparations used without additional treatment.

Water, in most experiments, was de-ionized and distilled in glass.

2.1.2 POLYMERS AND POLYMERIC SUPPORTS

Nylon 66, poly-(hexamethylene-diamine adipamide), was the Zytel preparation from E. I. DuPont de Nemours & Company, Wilmington. Nylon 6, polycaprolactam, was Gulf Nylon Resin No. 452, Gulf Oil Corporation, Houston. Nylon 610, poly-(hexamethylenediamine sebacamide), was synthesized from sebacoyl chloride and hexamethylenediamine through interfacial condensation with the amine in aqueous solution and the acid chloride in carbon tetrachloride or ether. A similar synthesis was used for the preparation of Pip-10 polyamide, poly-(piperazyl sebacamide). The synthesized polyamides were exhaustively washed with solvents prior to use. Another polyamide of undefined composition, Emerez 1537, Emery Industries, Cincinnati, was similar to the Pip-10 in solubility properties and may have been related in structure.

Polyphenylene oxide was General Electric preparation No. 691-111. Polysulfone was obtained from Union Carbide Corporation, but had no further designation

as received. Cellulose acetate was Eastman Chemical Products, Inc. (Kingsport, Tennessee) preparation E-398-10. Ethyl cellulose was Ethocel, of medium ethylation, viscosity 50 cps, a product of Dow Chemical Company, Midland, Michigan.

Commercial microporous supports were also tested, in addition to the polymers above, as supporting structures for the lipid phases. These included Millipore filters (Millipore Corporation, Bedford, Massachusetts) type MF, mixed cellulose nitrate and acetate ester, of 0.22 μm , 0.45 μ and 5.0 μm pores; LC, Teflon, with 10 μm pore; EG, cellulose acetate, 0.2 μm pores; NS, nylon polyamide, 7.0 μm pore. Nuclepore membranes (General Electric Corporation, Schenectady), polycarbonate films with 0.5 or 5.0 μm pores, were also used.

2.2 PROCEDURES

2.2.1 MEMBRANE PREPARATION

Several different kinds of membranes have been used in these studies. For convenience in discussion, these may be designated as the lipid-loaded membrane, the lipid-skin membrane, the cast-lipid membrane, and the bilayer lipid membrane, or BLM.

The lipid-loaded membrane was prepared by filling the pores of a microporous support film with the lipid material. The microporous support was allowed to imbibe liquid lipid to fill the pores. The imbibition was carried out on a glass surface (a large microscope slide cover slip) on top of a hot plate, at a temperature above the melting point of the lipid. Excess lipid was removed by pressing the loaded film between smooth filter papers on the surface of the hot plate. The films were then treated to alter phase structure in various ways, most frequently by treating with water or salt solution at a temperature above the melting point of the lipid. This approach initially forms a mosaic type of membrane, with lipid pores surrounded by polymeric regions. However, the lipids may also form monolayers, or multilayers over the polymer surfaces, and complex construction may develop.

The lipid-skin membrane was formed by pressing a colloidal dispersion of the lipid in water or salt solution through a polymeric support so that a thin skin of the lipid was formed on one side of the support. The dispersions were prepared by mechanical homogenization of the lipids in water at a temperature above the melting point of the lipids. After a uniform suspension was obtained, the dispersions were cooled below the lipid melting point. The skins, therefore, were initially formed from solid micelles or crystals. In some cases, properties were modified by heating the lipid-skin membranes above the melting points of the lipids in air or water and then cooling in the same medium.

The initial structure produced by these procedures would be a thin skin of lipid material packed onto the surface and into the pores of the supporting material. The skin, itself, would generally have a porous structure, with aqueous channels formed between the packed micelles or crystals of lipid. The micelles and crystals, themselves, often had complex structures, including multilamellar, tubular, polygonal, spherical, or irregular jagged configurations. Subsequent heat treatments, however, would alter these configurations, ranging from removing the skins to forming more uniform lipid structures on the surface and in the pores of the support. Generally, some degree of asymmetric structure was retained even after heat treatments.

Cast-lipid membranes were made by spreading on glass plates, applying the same techniques described below for preparing polymeric support films, but using a casting solution containing both polymer and lipid. The lipid in the casting mixture can alter phase relations during the gelling and leaching and may create different structures from those obtained in absence of the lipid. The specific structure obtained is dependent upon polymer and lipid interactions as well as lipid solubility and phase relations with the solvent system.

The bilayer lipid membrane (BLM) is formed by brushing a lipid solution across an aperture or apertures in a septum immersed in an aqueous medium (19,22,23). The film initially formed will spontaneously thin to a bilayer configuration (ca 7 nm thick) if certain kinds of amphipathic lipids are utilized and certain conditions are met. The normal mechanisms for BLM preparation have been widely described, but the question of concern in these investigations was the preparation of stabilized BLMs that would not be as fragile as those normally prepared. The specific methods used to this end are described in the experimental results.

2.2.2 FILM AND MEMBRANE CASTING PROCEDURES

Generally, an effort was made to keep films and membranes prepared by casting procedures to thicknesses below 5 μm , with the exception of some asymmetric cellulose acetate membranes used as references. This was done to reduce frictional factors in water transport in the lipid-containing membranes, since their structure usually involved a resistant phase through the entire thickness of the membrane, in contrast with the thin layer at one face that forms the resistant region of the asymmetric, reverse osmosis membrane.

Films were usually spread on glass with a doctor knife set at 25 to 75 μm , with solution concentrations gauged to produce the final thickness of 1 to 5 μm . As indicated in the experimental results, films were spread either at room temperature or with the 1/2-inch glass substrate heated to about 45°. In some cases, solvent was permitted to evaporate completely or nearly completely before stripping or immersing in water to assist in stripping the film from the glass. In others, the membranes were immersed

in water at 0°C at various periods following spreading to leach the solvent rapidly, providing a more asymmetric structure.

Films that were formed with a leaching process were generally stored in water until after testing was finished except in those cases where the film was intended only as a porous support for the lipids. For the latter purpose, the membranes were air-dried before lipid loading was carried out.

2.2.3 MEMBRANE ELECTRICAL CONDUCTIVITY AND PERMEABILITY MEASUREMENTS

Our methods for measuring electrical characteristics of bilayer lipid membranes have been described in some detail previously (14,19,24). Currents and potentials are monitored through agar salt bridges in each electrolyte compartment to calomel electrodes. The electrodes are coupled to a Keithley Electrometer, Model 610B, in the low impedance mode, acting as ammeter, while a second Keithley electrometer, in high impedance mode (10^{14} ohm input impedance), measures potential drop across the membrane. For the normal, high resistance BLM, this simple circuit can be essentially equivalent to a voltage clamp, with a low resistance in series with the membrane, or to a current clamp, with a high series resistance. A dry cell and voltage divider circuit provides the monitoring signal. Outputs of the electrometers are recorded on X-Y recorders to obtain current-voltage characteristics of membranes (with potential on the X-axis, current on the Y-axis), or, using the X-axis in the time mode, simultaneous current-time and potential-time recordings can be obtained on two recorders.

For measurements of polymer or lipid-polymer membrane characteristics, the same monitoring system could be used where membrane resistances remained high (10^5 ohm cm^2 or greater). For lower resistances, the system was modified by introducing a regulated DC power supply, variable to 40 volts, and using platinum current carrying electrodes (inserted directly into the electrolyte compartments) with separate calomel electrodes for potential measurements. The Keithley electrometer was still used for measurement of potential drop, but current was measured with a low resistance microammeter since the lowest impedance of the Keithley was about 10^4 ohm.

Many of the thin polymer and lipid polymer membranes examined here were relatively fragile, requiring some careful handling. Consequently, it was desirable to utilize small membrane areas for electrical measurements, with mounting conditions that would minimize handling. Two systems were used for mounting. One (Figure 1A) was simply a pair of hemispherical glass joints, ground to a flat face, each attached to upright tubes for a current carrying and potential measuring electrodes. The membrane was simply clamped between the two flat faces (sometimes using a small amount of silicone grease on the surface to insure proper sealing), the reservoirs filled, and the electrodes inserted for measurements. Membrane area exposed in these cells was about 0.95 cm^2 . This cell was adequate for measurements made at room temperature, but did not lend itself to measurements at varying temperature.

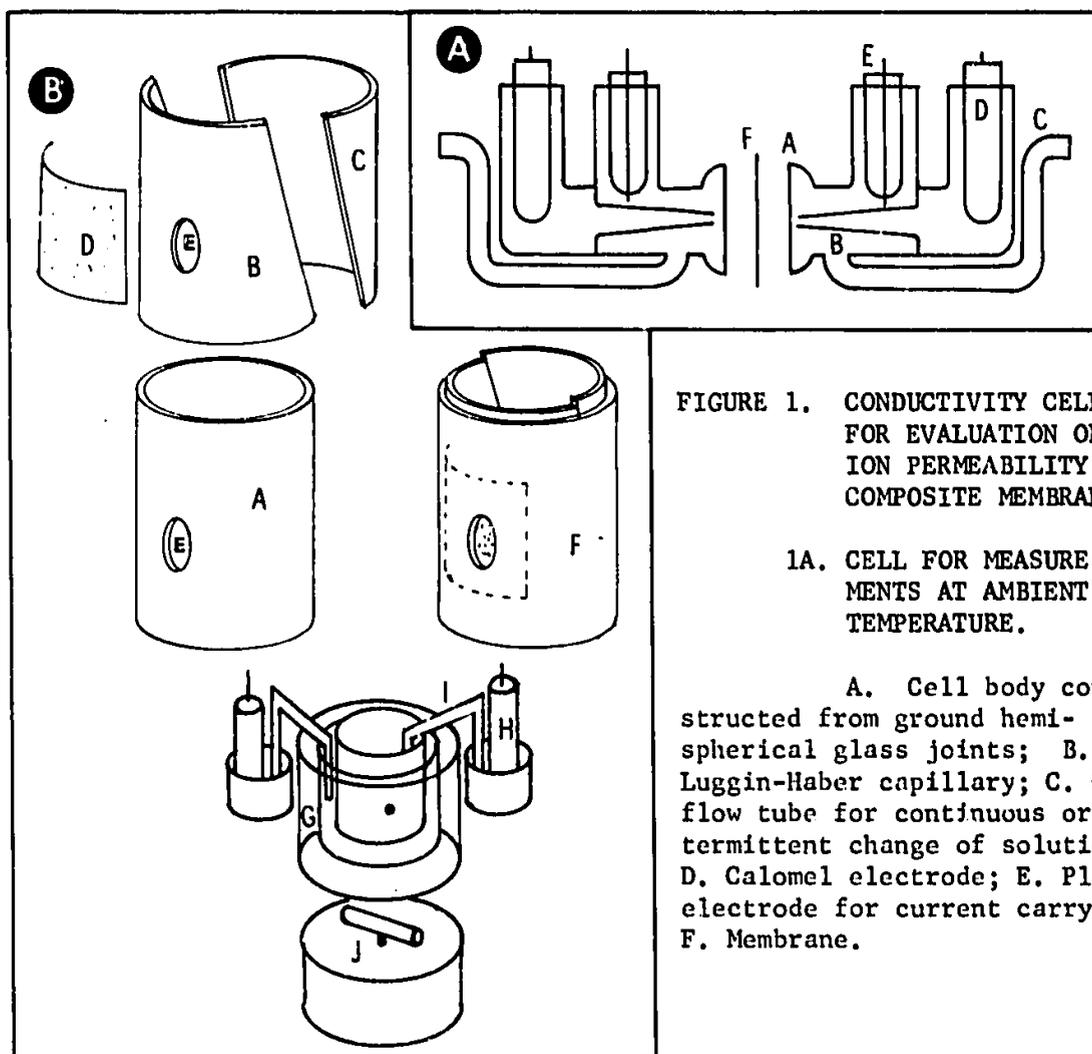


FIGURE 1. CONDUCTIVITY CELLS FOR EVALUATION OF ION PERMEABILITY IN COMPOSITE MEMBRANES.

1A. CELL FOR MEASUREMENTS AT AMBIENT TEMPERATURE.

A. Cell body constructed from ground hemispherical glass joints; B. Luggin-Haber capillary; C. Outflow tube for continuous or intermittent change of solutions; D. Calomel electrode; E. Platinum electrode for current carrying; F. Membrane.

1B. CELL USED FOR CONTROLLED AND VARIABLE TEMPERATURE MEASUREMENTS.

A. Polyethylene vial forming outer section of membrane support and inner electrolyte container; B., C. Polyethylene split ring sections; D. Membrane; E. Apertures in membrane support; F, Membrane support assembled, with split ring wedge pressed in to hold the membrane against the vial; G. Water-jacketed outer electrolyte compartment; H. Calomel electrodes; I. Salt-agar bridges; J. Magnetic stirrer to activate stirring bars in each compartment.

For variable temperature measurements, the cell normally used in BLM studies was adapted for use with solid membranes, as illustrated in Figure 1B. The membrane is clamped tightly in a polyethylene vial, using a diagonally split polyethylene ring as a wedge device to press the membrane against the inside surface of the vial and provide a tight electrical seal. This supporting cup formed one electrolyte compartment of the cell when it was placed inside a water-jacketed glass cup, which acted as the second electrolyte compartment. These compartments were coupled with monitoring instruments, as described above. Temperature was controlled by circulating water from either of two refrigerated constant temperature baths through the jacket. Rapid transitions from one temperature to another could be made simply by switching circulation from one bath to the other or slow transitions could be developed through changing the control temperature in one or the other of the baths.

For some of the more fragile membranes, the potentially disruptive process of clamping the membrane was avoided entirely by using simple adhesion to a film of silicone vacuum grease to seal the membrane to the polyethylene support. This was accomplished by putting a thin film of the grease around the aperture on the outer surface of the polyethylene vial, illustrated in Figure 1, and then rolling this surface over the membrane on a flat surface. The thin membranes would adhere tightly and would remain in place, with good electrical isolation during a long period of immersion in the electrolyte solution. The external solution was generally maintained at a level of about 1 mm higher than the internal solution during tests to avoid the possibility that a small internal hydrostatic pressure would dislodge the membrane.

Electrolyte solution temperatures were monitored as the potential produced by an iron-constantan thermocouple, in a glass shield, placed in the inner compartment near the membrane. The potential was measured with a Hewlett-Packard Microvoltammeter, Model 425A, and the output of this meter could be introduced into the X-axis of the recorders, as described below, for producing current-temperature and potential-temperature recordings.

Measurements for membrane conductance and diffusion potential (ion selectivity) were usually carried out simultaneously. The two compartments were filled with electrolyte solution of two different concentrations, usually with 0.1 M NaCl or KCl in the outer compartment, and 1.0 M salt in the inner compartment. The outer compartment was connected with the reference (ground) electrode. Consequently, negative diffusion potentials would indicate cationic selectivity in the test membranes, positive diffusion potentials would show some anionic selectivity.

A current-voltage curve (outputs of ammeter and voltmeter introduced to Y and X axes of a single recorder) was generally obtained for each membrane as soon as it appeared that the characteristics were reasonably constant after setting up the membrane in the cell. Where thermal transitions were

to be studied, the current and potential outputs were then transferred to Y-axes of separate X-Y recorders with the temperature transducer to the X-axes of both recorders. As temperatures were being programmed, recordings were made alternately on the two recorders, depressing the voltage-temperature recording pen after adjusting a biasing potential to obtain a zero current level through the membrane and then immediately recording the current similarly upon application of a constant 10 mv polarizing potential added to the biasing potential. This produced two point records, one corresponding to the change in "open circuit" potential (zero current), and the other for a short circuit current obtained with constant 10 mv polarization. With the low polarizing potentials, and consequent small currents, the DC resistance observed was generally nearly symmetric, even with asymmetric solutions. The potential recording provided a measurement of the diffusion potential and its change with temperature while the current recording revealed any changes in membrane conductance.

2.2.4 WATER AND SOLUTE PERMEABILITY MEASUREMENTS

The great diversity of membranes tested in this project demanded a large number of tests for water and solute permeability. Since facilities for testing membranes under standard reverse osmosis conditions were generally in demand for other projects and a relatively long test period would be required for each membrane, it was necessary to apply alternative testing procedures that would give the required permeability information more rapidly and conveniently. The approach used was the evaluation of hydraulic conductivity and reflection coefficients with a small apparatus similar to that described by Heyer, et al. (25).

The body of this apparatus (Figure 2) consists of a Swinney Filter holder (Millipore Filter Company) of either stainless steel or plastic construction. This body has Luer-lock threading on the input, or high concentration side of the compartment, permitting attachment to a high pressure system. The membrane is clamped in the body, supported by a perforated disc, sealed between Teflon or silicone rubber gaskets. The normal construction of this system permits application of up to 100 lbs/in² against the membrane and this limit may be raised by introduction of a secondary support beneath the perforated plate. Thus, the system is quite adequate for balancing osmotic pressures due to solutions in the range of 0.1 to 0.2 osmolar.

The lower side of the filter holder is attached, through a three-way stopcock, to a syringe and a calibrated capillary (usually a 50 μ l Microcap pipette). Volume transfer across the membrane was observed by movement of the meniscus in the capillary, with 2 mm movement indicating 1 μ l volume transfer. The syringe was used to adjust meniscus position in the capillary, to withdraw any excess liquid in the lower compartment after initial mounting, and as a volume reservoir to absorb any abrupt changes in the system as the result of applied pressure changes on the top compartment.

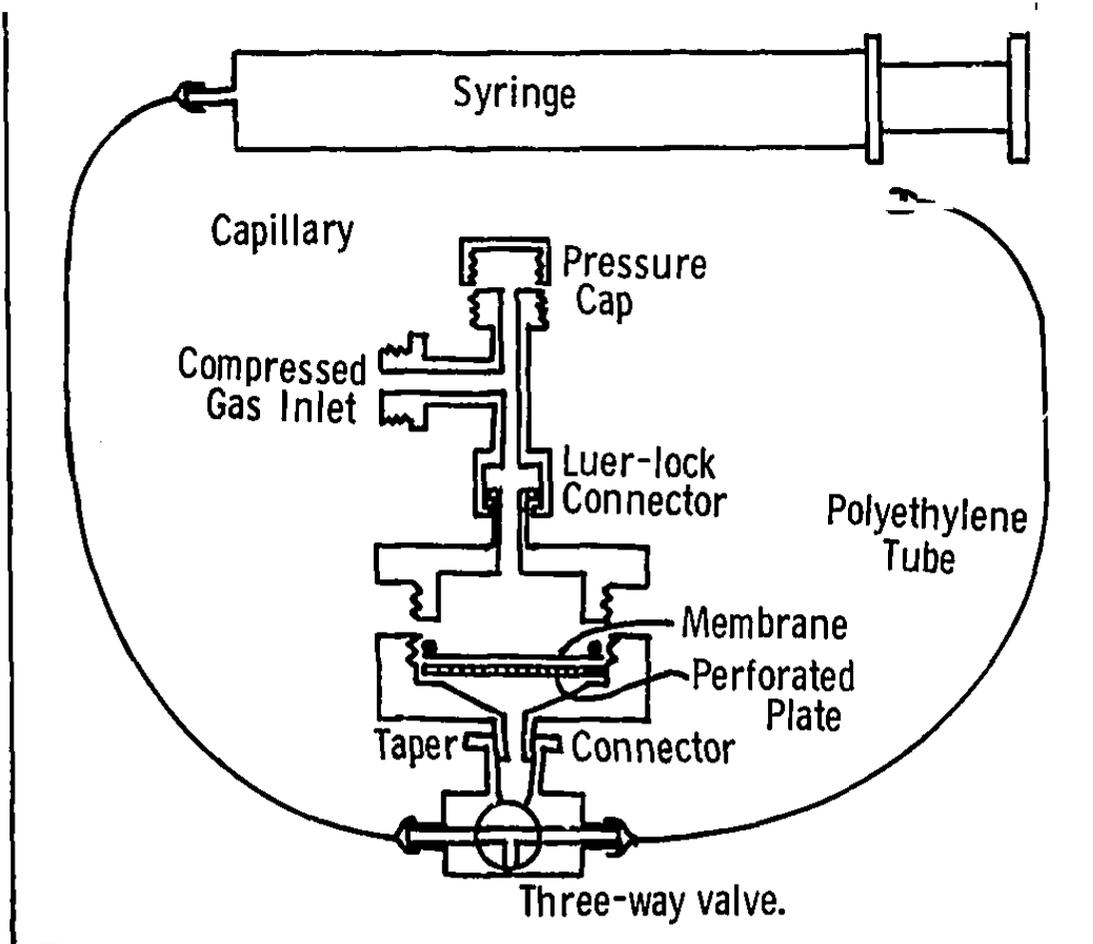


FIGURE 2. APPARATUS FOR OSMOTIC FLOW AND REFLECTION COEFFICIENT MEASUREMENTS.

The cell body consisted of a Swinney filter holder. Membrane diameters of either 0.5 inch or 1.0 inch could be used with appropriate filter holders. The body was either stainless steel or plastic, with little difference in handling or behavior in either case. The membrane was introduced and clamped in the holder with the latter submerged in water to facilitate handling fragile membranes and to avoid air bubbles. The holder was then mounted in the valve and the top attached (for pressure experiments). The syringe was used to adjust meniscus position in the flow-measuring capillary. The syringe and capillary were mounted on a stand above the water level of the controlled temperature bath surrounding the rest of the apparatus.

The upper compartment was fitted with couplings to permit application of pressure from compressed nitrogen. Simple liquid reservoirs (syringe tubes) could also be attached.

The membranes were generally inserted and mounted with the body totally immersed in distilled water so that no air was trapped in the system. The body was then mounted on the syringe-capillary fitting, meniscus and internal volumes adjusted with the syringe, and the pressure fitting attached for a determination of hydraulic conductivity (water flow through the membrane under specified transmembrane pressure conditions) of the membrane.

Following this determination, the distilled water in the upper compartment was replaced with a test solution by flushing the 2 ml volume of that compartment with about 20 ml of the test solution, through a polyethylene tube inserted through the neck of the compartment to the membrane level in the compartment.

Two kinds of experiments could then be carried out. With both chambers open to atmospheric pressure, the rate of transfer of solution or water volume due to osmotic pressure was measured. With pressure applied to the upper chamber, accurate evaluations of osmotic pressure and of reflection coefficients could be obtained.

In these experiments, the permeability characteristics of the membrane for water and a given solute can be expressed in terms of K , the hydraulic conductivity coefficient and σ , the reflection coefficient for the solute. The latter coefficient may be calculated from the relation:

$$J_v = K(\Delta P - \sigma\pi) \quad (1)$$

in which J_v is net volume flux, ΔP is the pressure differential across the membrane, and π is the osmotic pressure. The value of σ may vary from 1.0, for a completely impermeant (ideal) solute, to zero, for a solute that flows through the membrane as readily as water (or other solvent). Negative values of σ may be obtained under certain circumstances, indicating a membrane structure that develops preferential transport of the solute over the solvent.

Unfortunately, the derivation of σ is subject to a number of errors in dynamic measurements. While a value for σ may be obtained from simple measurements of fluxes at atmospheric pressures with a concentration differential, these values may be significantly distorted by concentration gradients developing at the membrane interface as a consequence of the flow through the membrane. In experiments in which a significant volume flux occurs, one may expect, with a low permeability solute, that some dilution of the solution will develop in the stagnant layers at the membrane surface as water flows into the high concentration side from the low concentration side, resulting in some decrease in the apparent driving force

across the membrane. Correspondingly, if the solute is permeable in the membrane, to some degree, its concentration at the low concentration interface may not accurately reflect the concentration in the bulk solution on that side of the membrane. For reflection coefficients calculated from flux data gained under these conditions, both factors will act to reduce the observed value below the true value. On the other hand, with applied pressures greater than the osmotic pressure, the concentration of a low permeability solute will increase at the membrane interface as solvent selectively flows outward, increasing the effective osmotic pressure, reducing the difference between applied pressure and osmotic pressure and consequently, the volume flux. This, once more, would result in calculated reflection coefficients lower than the true values.

Consequently, reflection coefficients should be determined under conditions approaching as closely as possible to equilibrium conditions, either with applied pressures very close to the osmotic pressure to obtain low volume flux or at very low solute concentrations to produce low osmotic volume fluxes. Either procedure actually requires numerous determinations at varying pressure or concentrations in order to obtain curves that may be extrapolated to zero conditions, where flow rates should be undisturbed by concentration polarizations. In view of the large number of samples to be tested in this program, this procedure would also have been excessively time consuming, and it was applied only for a relatively small number of samples.

The alternative, rapid analysis, utilized a simple comparison of reflection coefficients determined from fluxes at atmospheric pressure under specified, constant conditions. As indicated above, the apparent reflection coefficient will generally be considerably lower than the true value. In addition, the distortion will be greater as the hydraulic conductivity coefficient increases so that the reflection coefficient obtained for a membrane with a very low hydraulic conductivity cannot be compared directly with that for a membrane with a high conductivity.

Despite these problems, the data derived from simple osmotic flux and hydraulic conductivity measurements can be valuable guides to the potential utility of a novel membrane. Consequently, most of the data generated on membrane systems has utilized only single concentration osmotic flux measurements. Selected membranes have been tested subsequently under more precise conditions to obtain more properly comparable relations.

For the latter measurements, determinations of osmotic flux were made with several different concentrations of NaCl and curves extrapolated to zero concentration. In calculating reflection coefficients, the osmotic pressures were derived from the relation,

$$\pi = RT\phi C_s \quad (2)$$

in which R and T have their usual meanings, ϕ is the osmotic coefficient, and C_3 is the concentration of the solute. Values of ϕ for salts were taken from tables in Robinson and Stokes (26).

In the tabulations of data on permeability in this report, those values obtained from single concentration measurements will be labeled as "apparent reflection coefficients" while those obtained from multiple concentration observations and extrapolation will be given the designation of reflection coefficients.

2.2.5 PERMEABILITY MEASUREMENTS AT VARYING TEMPERATURES

The small apparatus described above was readily immersed in a water bath, with capillary tube and syringe mounted above water level, to provide temperature control. A small circulating thermoregulator immersed in the same water bath provided a simple mechanism for study of relation of permeability to temperature. Unfortunately, relatively few experiments on temperature relations could be accomplished due to press of other experiments.

2.2.6 PHOTOELECTRIC MEASUREMENTS

The photoelectric responses of various membranes were measured with the same systems described under the section on electrical conductivity. A quartz window in the water jacketed outer compartment permitted illumination of the membranes with ultraviolet light from a Mineralight Model R-51 (Ultra-violet Products Corporation, San Gabriel, California) shortwave mercury vapor lamp or with visible light from a 15 watt, focused microscope lamp. Light intensities were varied simply by varying the light distance to the membrane.

Most measurements of light response were made under low external resistance conditions (potential control), but these observations were frequently supplemented with current control measurements, utilizing a high series resistance or open circuit.

2.2.7 ACID-BASE AND ION EXCHANGE CAPACITY TITRATIONS

Various lipid dispersions were analyzed for acidic or basic equivalence, above and below the melting points of the lipids. Titrations were carried out using a pH meter and glass electrode to follow change of pH with acid or base additions and determine the proper regions of inflection for optimal accuracy in determining equivalence points. Titrations were carried out with 0.100 N NaOH or HCl on samples containing 1 to 10 mg lipid per ml water.

Ion exchange capacities were evaluated by mixing a neutral salt solution with a suspension of a known quantity of lipid in water, and mixing thoroughly in a homogenizer at a temperature above the melting point of the lipid.

A pH measurement was made at the elevated temperature and then the sample was allowed to cool in a water bath and the pH taken once more. The excess lipid was removed by filtration or centrifuging and a portion of the supernatant solution was titrated back to neutrality. The titration was taken as the exchange capacity of the sample although it would be subject to some error where the lipid had a finite solubility in the salt solutions. This was a significant factor for some of the amines, but does not really have any effect upon the conclusions derived from these data.

SECTION 3

EXPERIMENTAL RESULTS

3.1 BULK PHASE PROPERTIES OF LIPID-WATER AND LIPID-POLYMER-WATER MIXTURES

3.1.1 PHASE STRUCTURES

Although an extensive analysis of gross phase structure of a large number of lipid-water systems was out of the scope of this project, it was desirable to determine the main characteristics of some of the systems with which extensive work might be done. Therefore, for a limited series of neutral, basic and acidic lipids, aqueous dispersions were prepared and observed on a gross and microscopic basis.

For most observations, each lipid was mixed with water in two proportions, a concentrated system, in which lipid was 20 to 30 percent of the total, and a dilute system, in which the lipid constituted only around 1 percent to 2 percent of the total. Mixtures were prepared in both distilled water and in 1M NaCl to determine the effect of salt on the phase relations. In these studies, only the free amines and acids were utilized, rather than salts which generally form stable disperse phases very readily. In order to give the lipids adequate opportunity to form disperse phases, each aqueous-lipid mixture contained in a conical glass tissue homogenizer, was warmed in a water bath to liquify the lipid. A rotating plunger was then used to homogenize the mixture. The plunger action was usually maintained during cooling in a room temperature water bath. Despite this action, many of the materials simply reaggregated on the water surface or precipitated on the surfaces of the homogenizer and pestle, rather than maintaining a disperse phase. In other cases, micro-crystalline forms were produced. Many systems, though, produced complex phase structures.

TABLE 1
PHYSICAL STRUCTURES OF LIPID-WATER
HOMOGENATES AND MIXTURES

Lipid and Melting Point	Structure in Water ^a	Structure in 1M NaCl ^b
n-decylamine liquid	<p><u>Conc.</u>-Viscous gel, lamellar sheets. Poor birefring.; <u>Dilute</u>- Viscous, stable susp. of 1-20 μm spherical to longstring micelles. Concentric ring structure in polar. light.</p>	<p><u>Conc.</u>-Viscous semi-gel of 10-20 μ spherical micelles. Birefring.; <u>Dilute</u>- Unstable susp. irregular 1-20 μ micelles.</p>
n-dodecylamine 28-30°	<p><u>Conc.</u>-Stable solid gel, lamellar sheets. <u>Dilute</u>- Irregular 0.5-50 μm micelles in stable dispersion.</p>	<p><u>Conc.</u>-Close-packed, birefring. micelles to lamellar sheets. <u>Dilute</u>- Unstable susp. of 5-25 μm irreg. micelles.</p>
n-tetradecylamine 69-76°	<p><u>Conc.</u>-Folded lamellar and 1-50 μm micelles in stable, solid gel, poorly birefring.; <u>Dilute</u>- Stable susp. of 1-10 μm micelles and micelle aggregates.</p>	<p><u>Conc.</u>-Solid gel, irregular micelles and lamellar with high birefring. <u>Dilute</u>- Unstable susp. of irreg. micelles to crystalline aggregates.</p>
n-octadecylamine 53°	<p><u>Conc.</u>-Stable, solid gel of linearly folded lamellar or long needle crystals, highly birefring. <u>Dilute</u>- Stable susp. of irreg. micelles with near crystalline appearance.</p>	
d-n-octylamine liquid 15°	<p>Separates rapidly into two liquid phases above room temp. Forms solid water in oil gel separating from water at room temp. or below.</p>	<p>Will not emulsify.</p>
di-n-decylamine 33-36°	<p>Precipitates on homogenizer surfaces during cooling. No disperse phase.</p>	<p>Same as water.</p>

TABLE 1 (Continued)

Lipid and Melting Point	Structure in Water ^a	Structure in 1M NaCl ^b
di-n-dodecylamine 44-46°	As above.	As above.
tri-decylamine	Separates to two clear phases.	Same as in water.
tri-dodecylamine 16°	As above.	As above.
Octanoic Acid	Conc.-0.5-100 μ m droplets in partly stable emulsion. Dilute- Similar but with more of small droplets.	Fails to emulsify.
Dodecanoic Acid 44-45°	Conc. and Dilute- Forms separate lipid phase on top of aqueous phase. Lipid phase has large, irregular sac-structures.	Poor emulsification. Forms separate solid lipid phase.
Hexadecanoic 56-58°	As above.	As above.
Octadecanoic	As above.	As above.
n-dodecan-1-ol 25°	Emulsions separate rapidly to clear liquid lipid and aqueous phases.	Same as in water.
n-hexadecan-1-ol 48-49°	Alcohol crystallizes and separates upon cooling. No stable dispersion.	Same as in water.
di-dodecyl-phosphite liquid	Separates to nearly clear water phase (few 0.5-20 μ droplets) with upper emulsion layer of close-packed droplets in water.	Separates as in water system but large sac-like micelles, 20-50 μ , with spike or hair-like protuberances constitute oil phase.

TABLE 1 (Continued)

Lipid and Melting Point	Structure in Water ^a	Structure in 1M NaCl ^b
tri-dodecyl phosphite liquid	Separates slowly to two clear phases.	Same as for water.
Di-dodecyl phosphate 42°	Irregular microcrystals and some micellar structures in semi-stable suspension.	Same as in water. More micellar structures.
Di-octadecyl phosphate 78°	Needle microcrystals, 0.5 x 2 to 1 x 20 μ m, and aggregates of crystals. Crystals slowly separate to form close-packed phase at top.	Same as water, but crystals more irregular.
Ethylene glycol mono-dodecanoate 40°	Rough micro-crystals and aggregates.	Same as in water.
Glyceryl mono-dodecanoate	Rough plate micro-crystals, rectangular to rhombic. Tendency to smaller crystals in dilute system.	Same as in water.
Glyceryl di-dodecanoate 45°	Irregular microcrystals and aggregates.	Same as in water.
Span 65 (Sorbitan tristerate) 60°	Solid phase separates during cooling. Solid phase traps some aqueous micelles.	Same as in water.

^a Lipids were homogenized with distilled water at two different concentrations. Mixtures designated by Conc. were about 20-30% lipid. Those indicated by Dilute were around 1%.

^b Mixtures in 1M NaCl were prepared as indicated in (a) and in text.

3.1.2 BULK PHASE TITRATIONS

One of the questions of some importance to this investigation was whether a phase transition in aqueous lipid dispersions could affect ion exchange or acid-base equivalence properties sufficiently to permit application of such transitions in cyclic regeneration processes for water desalting. This question was approached through the evaluation of normal acid-base equivalence of lipid dispersions and ion exchange properties of some of these dispersions at temperatures above and below the melting point of the lipids. Only a few materials were tested, since it became apparent that practical applications would be rather difficult and would compare poorly with normal ion exchange processes. Even where significant differences did appear in solid and liquid disperse phases, the differences were largely apparently due to rate limitations, rather than equivalence relations, with permeability into solid dispersed material limiting the observed reactions. Thus, titrations of solid phase materials generally indicate degree of exposure of titratable groups.

General results are indicated in Table 2. In general, the amines tested formed clear or nearly clear solutions or sols during titration to the equivalence point. Carboxylic acids formed turbid colloidal sols when titrations were carried out at temperatures permitting total titrations. Similarly, the phosphates titrated to uniform micellar dispersions at temperatures above their melting points and would remain disperse upon cooling, but the samples titrated at room temperature showed little change and only a small part of the total acidity was titrated within a reasonable period.

The ion exchange measurements here do not really give an indication of the probable differences in exchange capacity in two phase structures since the titrations are only for the low temperature system. They do indicate, however, that the exchange capacities are really too low for practical application even if there were some significant changes between liquid and solid states. Even if these lipids were to approach their theoretical ion exchange capacity, which they do not, the best ones would be no better than normal strong base or strong acid resin ion exchangers. Consequently, there appeared to be little purpose in pursuing this area of investigation further.

Some tests were also made to determine whether neutral lipid dispersions could affect salt concentrations in salt solutions as a consequence of differential sequestering of the salts and water, through homogenizing the neutral lipids in a salt solution and determining change in pH or conductivity between the original dispersing solution and the supernatant from the homogenates. Practically no effect was observed with dodecanol, several glycerides, or Span 65, at any temperature tested.

Some titrations and ion sequestering experiments were also carried out with lipids in association with polymers, either by making thin films containing the lipid and polymer, or homogenizing the polymer solution (in a water

TABLE 2
ACID-BASE TITRATIONS AND ION EXCHANGE CAPACITIES
OF LIPID-WATER MIXTURES

Lipid	Temperature °C	Titer eq./mole	Specific Titer meq./gm	Ion Exchange Capacity meq./gm
Dodecanoic Acid	24	0.42	2.1	0.08
	50	0.96	4.8	
n-dodecylamine	24	0.93	5.5	0.21
	40	0.95	5.6	
di-n-dodecylamine	24 (liquid)	0.92	2.8	0.05
di-dodecyl phosphate	24	0.20	0.49	0.11
	50	1.78	4.4	

soluble solvent) and lipid together while adding water to precipitate the mixture (for octanoic acid plus nylon 66). The polymer reduced both ion exchange and free acid or base titers to insignificant values.

3.2 CHARACTERISTICS OF LIPID-LOADED MEMBRANES

3.2.1 THERMAL TRANSITIONS IN LIPID-LOADED MICROPOROUS FILMS

The measurement of the changes in conductivity and ion selectivity (diffusion potential) of composite membranes as a function of temperature was one of the most interesting aspects of this investigation. Some of the results are illustrated in Figures 3-10.

It may be noted that each lipid membrane (with one exception) shows a very large change in conductance (note that conductance scale is logarithmic) near but not always at the melting point of the lipid. There is also generally a similar abrupt change in diffusion potential at the same point. In addition, many of the membranes demonstrate further smaller, but still significant fluctuations in conductance or diffusion potential at temperatures well below the melting points.

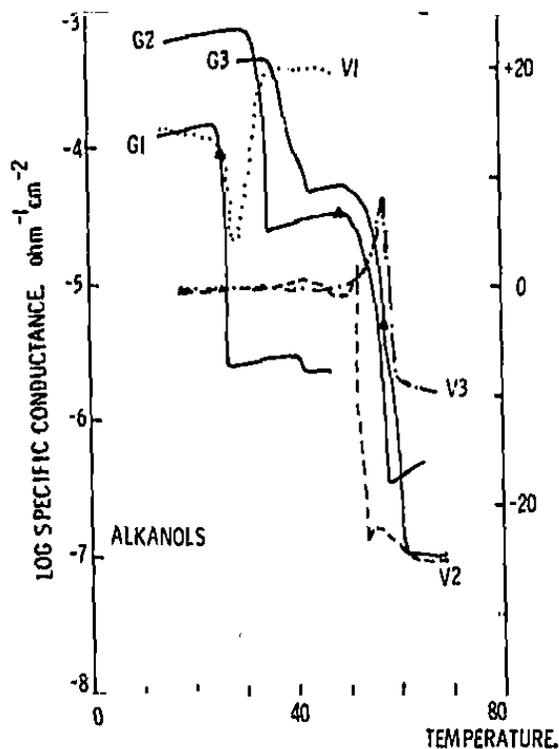


FIGURE 3

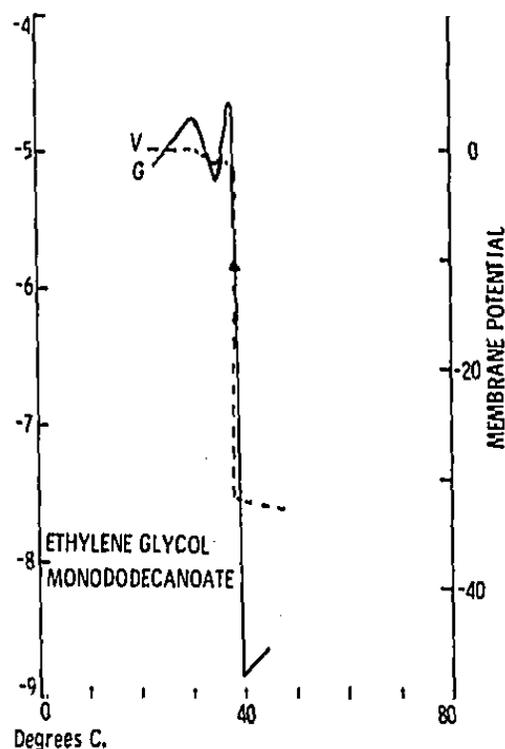


FIGURE 4

FIGURE 3. THERMAL TRANSITIONS IN ALKANOL-CELLULOSE ESTER LIPID-LOADED MEMBRANES.

Measurements were made with 0.1 M KCl in outer (reference electrode) compartment and 1.0 M KCl in the inner (active electrode) compartment. After loading the membrane with lipid (as described in Section 2) and mounting, temperature was programmed upward or downward at 1° to 2° per minute, recording conductance and diffusion potentials simultaneously as previously described. Vertical scale on the left side is \log_{10} of membrane conductance in $\text{ohm}^{-1}\text{cm}^{-2}$ (or mhos/cm^2) with membrane diffusion potential in millivolts indicated by right hand scale. Horizontal scale is temperature in degrees centigrade. The observed melting point of each lipid in air is indicated by the triangle on the conductance curve. Curves for Figures 3-10 are presented for the second increasing temperature cycle, except where otherwise noted.

Curve G1, membrane conductance with dodecan-1-ol on a Millipore HA support; V1, membrane potentials for the same membrane; G2, conductance for hexadecan-1-ol in Millipore HA; G3, conductance for octadecan-1-ol in Millipore HA; V3, diffusion potential for same membrane.

FIGURE 4. THERMAL TRANSITIONS IN ETHYLENE GLYCOL MONODODECANOATE-CELLULOSE ESTER MEMBRANE.

Curve G, conductance for ethylene glycol monododecanoate in a Millipore HA filter; V, diffusion potential for the same membrane. Conditions as for Figure 3.

With two exceptions, only the curves for a rising temperature program are shown here. In almost all cases, some hysteresis was evident between rising and falling temperature curves, but the curves representing the total cycle were fairly well reproducible beyond the first warming cycle, where some irreversible changes might occur. The hysteresis in subsequent cycling was probably partly due to failure to develop true equilibrium conditions since the temperature program of 1-2° per minute would create some thermal gradients across the membranes. There may also be some lag due to time required for transitions between phases.

While it might be possible to attribute some of the multiple fluctuations to the heterogeneity of the samples, particularly in the case of glycerides where compound definition remained rather poor, the multiple transitions can still be observed regularly with materials known to have a relatively high degree of purity, as for the alkanols. In the latter cases, double transitions occur with all three lipids.

It should also be noted that the multiple transitions or fluctuations were either much subdued or absent with the same lipids in Teflon supports rather than the mixed cellulose ester supports used in most of the illustrated experiments. The effect of support is also indicated by the change in thermal curves for glycerol monododecanoate in Figure 5. In this case, a distinct, low temperature conductance fluctuation is apparent in the cellulose ester support, but in neither the polysulfone nor the Nuclepore membrane support. On the other hand, the polysulfone curve does maintain the secondary inflection in the main transition region and develops an inverted transition not found with the other supports.

None of the membranes concerned in these illustrations appeared to have a very low resistance in their most conductive state. However, as demonstrated later, some lipid loaded membranes could develop very low resistances (less than 10 ohm cm²) when slight changes were made in preparative conditions. This applied especially to the alkylamines on Teflon and to the dialkyl phosphates (not illustrated in these Figures) on various supports.

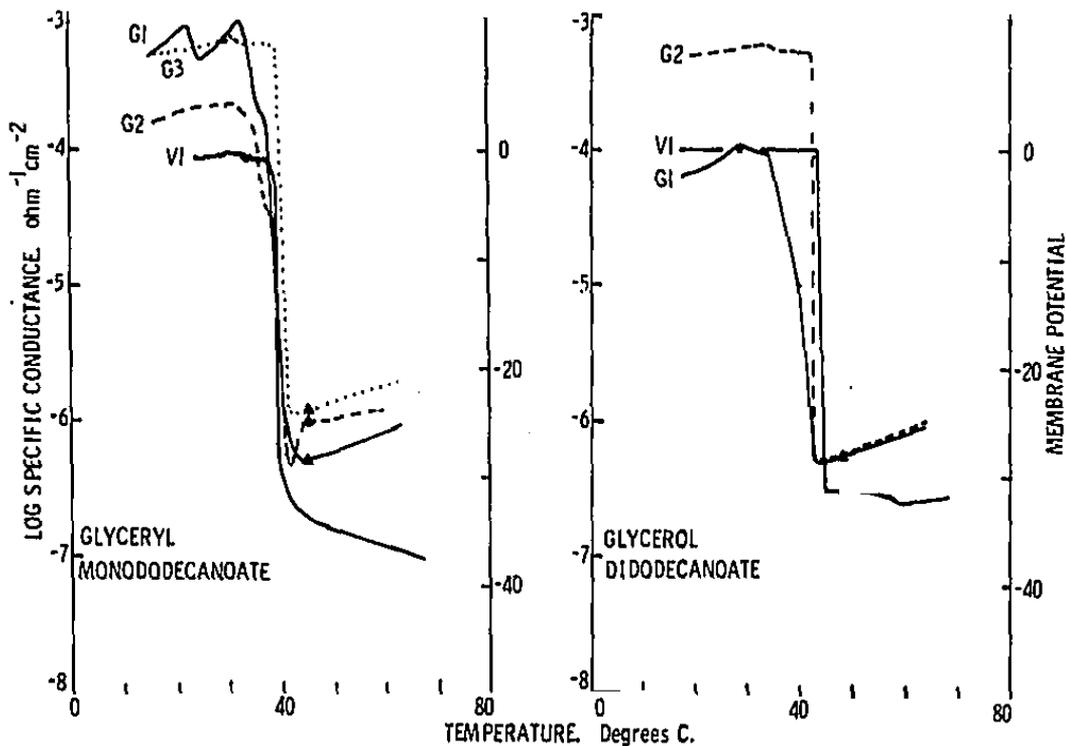


FIGURE 5

FIGURE 6

FIGURE 5. THERMAL TRANSITIONS FOR GLYCEROL MONODODECANOATE MEMBRANES IN VARIOUS SUPPORTING MEDIA.

Curve G1, conductance for glycerol monododecanoate in a Millipore HA filter; VI, diffusion potential for same membrane; G2, conductance with lipid in a porous polysulfone support having pores less than 1 μm diameter and thickness of about 10 μm ; G3, conductance for lipid in Nuclepore filter, 0.5 μm pore, 11 μm thickness.

See Figure 3 for conditions.

FIGURE 6. THERMAL TRANSITIONS FOR GLYCEROL DIDODECANOATE MEMBRANES.

Curve G1, conductance for glycerol didodecanoate in Millipore HA filter; Curve VI, diffusion potential for the same membrane on a descending temperature cycle; G2, conductance for same lipid in Nuclepore, 0.5 μm pore, membrane.

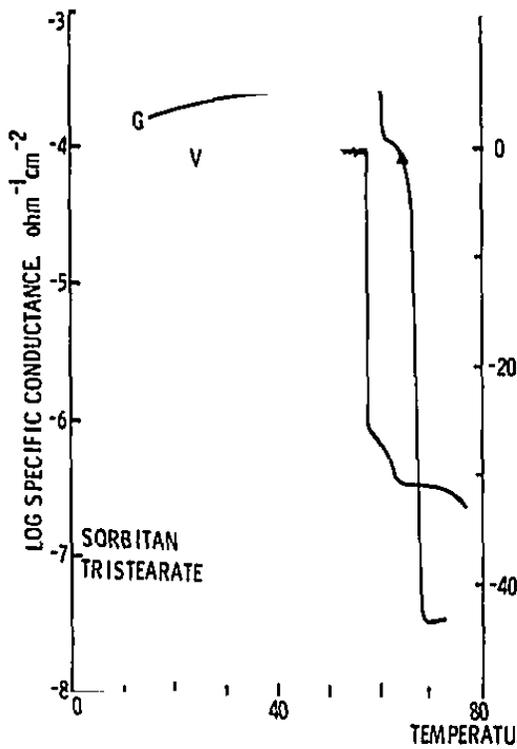


FIGURE 7

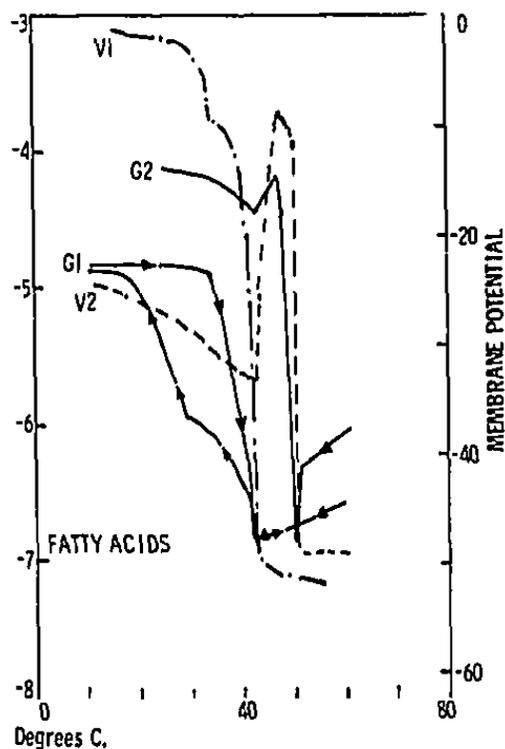


FIGURE 8

FIGURE 7. THERMAL TRANSITIONS FOR SPAN 65 MEMBRANES.

Curve G, conductance for membrane with Span 65 ("sorbitan tristearate") in Millipore HA support, for descending temperature cycle. Curve V, membrane potential for same membrane, descending temperature. The Span 65 was recrystallized once before preparation of the membrane.

See Figure 3 legend for conditions.

FIGURE 8. THERMAL TRANSITIONS IN MEMBRANE CONTAINING FATTY ACIDS.

Curve G1, conductance for dodecanoic acid in Millipore HA support. Ascending and descending temperature cycles are indicated by arrows on curves. The fatty acid membranes demonstrated large thermal hysteresis effects, as illustrated, in contrast with most other systems where small hysteresis effects could be attributed almost entirely to thermal gradients. Curve V1, diffusion potential for same membrane, but ascending temperature only. Curve G2, octadecanoic acid in Millipore HA, ascending temperature; V2, membrane potential for octadecanoic acid membrane.

See Figure 3 legend for conditions.

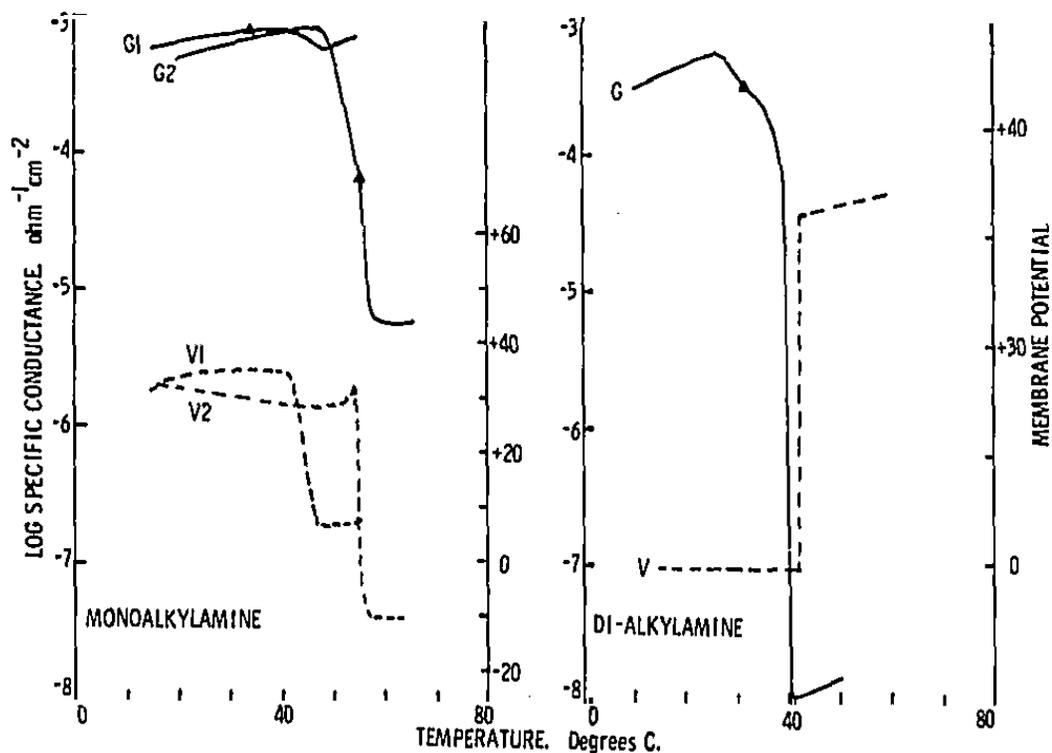


FIGURE 9

FIGURE 10

FIGURE 9. MONOALKYLAMINE MEMBRANE THERMAL TRANSITIONS.

Curve G1, 1-tetradecylamine in Millipore LC, Teflon filter with 8 μ m pores; V1, diffusion potential for the same membrane; G2, 1-octadecylamine on Millipore LC; V2, diffusion potential for the same membrane.

Conditions as in Figure 3.

FIGURE 10. THERMAL TRANSITIONS FOR DIALKYLAMINE MEMBRANES.

Curve G, di-n-decylamine on Nuclepore, 0.5 μ m pore; V, diffusion potential for the same membrane.

See Figure 3 legend for conditions.

Almost all membranes demonstrate a significant ion selectivity, as indicated by diffusion potentials. Unfortunately, in most cases, maximal ion selectivity is developed only at higher temperatures where conductivity is extremely low. The exceptions are the monoalkylamines (Figure 9) and dialkyl phosphates (not shown). In these cases, the maximal conductance and ion selectivity are found below the melting points for the lipids with resistances as low as 10 ohm cm^2 and selectivity is more than 90 percent effective in blocking the co-ion transport. Thus, the selectivity and conductivity appear to be rather strongly dependent upon both specific lipid properties as well as support interaction with the lipids.

3.3.2 ION AND WATER PERMEABILITY OF LIPID-LOADED MEMBRANES

In addition to ion permeability or electrical conductivity, another important parameter to determine for the lipid-loaded membranes was water permeability. A summary of comparisons of conductivity, ion selectivity, and water permeability for selected membranes is given in Table 3. These membranes do not constitute the total tested but may represent the characteristics of the interesting systems.

It may be noted, first, that several membranes have ion selectivities and resistances well within the range found with commercial ion exchange membranes. In most instances, the high permeability to ions is not accompanied by a similar permeability to water. In the thick (more than 100 μm) cellulose ester membranes, water permeability tends to be extremely poor, as indicated by the low hydraulic conductivity coefficient. The water permeability is improved, however, in the much thinner polyamide films (less than 10 μm) in which the resistant path may be much shorter with consequent reduction of frictional resistance. There is some possibility, however, that some of the increased conductivity of the thinner membranes may be due to flaws in the membranes. Regions of high water flux and low ion selectivity may be caused either from excessively large pores developed in casting, in which lipid loading is faulty, or from extrusion of lipid from pores under the pressure exerted in the hydraulic conductivity testing procedure. Under controlled conditions, with pores of appropriate diameter, the latter mechanism might produce high water flux without interfering significantly with ionic selectivity or salt rejection, where Donnan exclusion was the major factor in salt rejection rather than hyperfiltration. In addition, hindered diffusion in micropores may create significant molecular permeability discrimination, even though the pore diameters may be several-fold the diameters of the diffusing molecules (27).

TABLE 3

ION PERMEABILITY AND SELECTIVITY AND
WATER PERMEABILITY IN SELECTED LIPID-LOADED MEMBRANES^a

Lipid	Support ^b	R_s^c Ωcm^2	τ_s^{+d}	R_l^c $M\Omega\text{cm}^2$	τ_l^{+d}	Transition ^e $^{\circ}\text{C}$	K^f $\mu\text{l}/\text{mm}/\text{atm}/\text{cm}^2$
Ethylene glycol monododecanoate	CE	2500	0.5	500	0.80	30,35, <u>38</u>	<0.1
	PCA	6700	0.5	500		39, <u>43</u>	<0.1
	PA610P	2500	0.69	4.0	0.85	<u>39</u>	
Glyceryl monododecanoate	CE	2000	0.5	8.9	0.81	20,24, <u>30</u> <u>38</u>	<0.1
	PCA	2000	0.5	2.0	0.81	<u>43</u>	<0.1
	Pip-10	4000	0.5	0.5	0.62	<u>41</u>	<0.1
Glyceryl didodecanoate	CE	10000	0.5	100.	0.89	<u>43</u>	<0.1
	PCA	2500	0.5	10.	0.88	<u>44</u>	<0.1
	PSul	3300	0.5	500.	0.88	35-40	
Span 65	CE	1000	0.5	10.	0.80	<u>58</u>	
	PCA	8000	0.5	2.	0.89	<u>58</u>	
Dodecanol	CE	8000	0.65	0.5	0.7	<u>25</u>	<0.1
	Pip-10	2000	0.60	0.5	0.7	<u>25</u>	0.8
	PSul	15000	0.65	0.01	0.8	<u>22</u>	
Dodecanoic Acid	CE*	50000	0.54	10.	0.94	10,34, <u>41</u>	
	CE**	59	0.75	6.0	0.92		<0.1
	Pip-10	320	0.60	40	0.95	35-38	1.8
Dodecylamine	Teflon	14	0.08	0.1	0.45	<u>45</u>	0.19
	PCA	70	0.15	800.	0.48	<u>45</u>	
Didecylamine	PCA	1400	0.5	90.	0.08	<u>40</u>	
Tridecylamine	Nylon 66	70	0.10	0.3	0.05	<u>25</u>	0.5
Didodecyl phosphate	CE	8	0.95	0.11	0.95		<0.1
	Nylon 66	11	0.89	2.1	0.90		0.3
Didodecyl phosphate	CE	14	0.94	0.2	0.89	78	<0.1
	Pip-10	68	0.92	8	0.95	78	0.7

TABLE 3 (Continued)

- ^a The tabulated values may be considered as representative of class behaviors to some degree. Many other membranes were tested, but either showed inferior characteristics or properties similar to those already tabulated here.
- ^b CE = mixed cellulose ester Millipore filter HA or GS, 110 μm thick; PCA = Nuclepore polycarbonate membrane filter, 0.5 μm pore, 11 μm thickness; PA610P = 1:1 mixture of nylon 610 polyamide with Pip-10 polyamide in porous film cast from formic acid, with most pores less than 1 μm , 1-2 μm thick; Pip-10 = polyamide film cast from formic acid, 1-2 μm thick, with pores less than 1 μm in diameter; PSul = polysulfone film, cast from formamide, 3 μm thick, pores less than 1 μm ; CE*, Millipore HA filter loaded with fatty acid with strong heating during loading resulting in large change in filter structure before further processing; CE**, Millipore HA filter loaded with fatty acid just at melting point; Teflon = Millipore LC Teflon filter with 6 μm pores, 125 μm thick; nylon 66 = membrane 3 μm thick with pores less than 2 μm diameter, cast from trifluoroethanol containing small amounts of sodium borate and washed after casting to remove borate crystals.
- ^c R_S and R_L , specific resistances of membranes measured at temperatures below and above, respectively, the main transition points for these membranes. Note that resistance values for R_S are given in ohm cm^2 while those for R_L are given in megohm cm^2 giving a factor of 10^6 difference in tabulations.
- ^d τ_S^+ and τ_L^+ , cation transport numbers below and above, respectively, the transition points of the membranes. Determined with a 10:1 salt gradient across the membrane and calculated from the Nernst relation,

$$E_D = (\tau^+ - \tau^-) \frac{RT}{R} \ln \frac{a_1}{a_2}, \text{ or}$$

$$E_D = (2\tau^+ - 1) \frac{RT}{R} \ln \frac{a_1}{a_2},$$

where E_D is the measured diffusion potential and a_1 and a_2 are activities of the electrolyte on the two sides of the membrane, and τ^- is the anion transport number. A value for τ^+ of 0.5 indicates no selectivity ($E_D = 0$ due to equal anion and cation transport) while a value of 1.0 indicates perfect cation selectivity and 0.0 shows a perfect anion selectivity.

TABLE 3 (Continued)

^e Temperatures of conductivity and/or selectivity transitions for the membranes. The underlined value indicates the major transition or only transition; smaller transitions are indicated by values without underlining.

^f Hydraulic conductivity coefficients, determined as outlined in methods section. Reflection coefficients were not generally determined for these membranes, since the small hydraulic conductivity normally found made reflection coefficient measurements difficult as well as of little practical value.

3.3.4 PROPERTIES OF MEMBRANES FORMED BY DYNAMIC LOADING PROCESSES

Materials adsorbed to a support as micelles or microparticles from a liquid suspension may develop a different membrane structure from that obtained in a process involving direct incorporation or application, as in the previous section. "Dynamic" formation of a membrane by such mechanisms has received extensive attention, particularly with polymers introduced onto a porous matrix to form a barrier layer with desired selectivity (e.g., ref. 28) or surfactants adsorbed onto an already active membrane to improve rejection properties (e.g., 29).

In the present investigation, the dynamic formation was accomplished by expressing various lipids, in dilute suspensions, from a syringe onto the porous film, supported in a Swinney syringe filter unit. This compact unit permitted carrying out the coating operation at any desired temperature simply by submerging the filter holder and part of the syringe body in a bath of the desired temperature. Application pressures were simply controlled manually, avoiding exertion of pressure that would puncture the membranes.

Since the suspensions used in these operations were, again, formed from unneutralized acids or amines and alcohols or esters without additional surfactants, particles in the suspensions were frequently rather heterogeneous in size. Excessive heterogeneity was avoided by using only the stable suspension obtained after prolonged settling of the initial homogenates.

The results with the materials actually tested here (Table 4) suggest that these substances are not remarkably well adapted to this kind of application. In general, there is no improvement in ionic selectivity of the dynamically loaded membranes over the membranes loaded by the melting procedures. In fact, several membranes fail to demonstrate significant ion selectivity in the dynamic system where the normal loading techniques were quite effective in developing selectivity. Only water permeability relations are improved, but this is of little value without ion retention mechanisms.

There is some redemption, however, in the behavior of the dynamically loaded membranes after cycling through a melting sequence. In several instances (dioctadecyl phosphate and the monoamines) this treatment produces ion selectivity quite comparable with that of the melt-loaded membranes. This might make it possible to consider utilization of a dynamic loading process with lipids as a mechanism for making and regenerating membranes in electrodialysis operations where fouling might be a significant factor in membrane life.

Unfortunately, no tests were made using the more stable and highly dispersed neutralized amines, acids, or phosphates. Such neutralized lipids generally exist in highly ionized micellar dispersions in which micelle sizes are much more uniform and smaller than those encountered with the substances used here. Such structures might have produced tighter pore structures with much greater ion exchange and selectivity properties.

3.3 PROPERTIES OF CAST-LIPID MEMBRANES

The presence of a lipid in the polymer solution used for casting a membrane may have a significant effect on the phase relations of the polymer in the gelling process, leading to potential alterations of the membrane structure from that expected in absence of lipid. Such structural alterations may persist, with effects on permeability relations of the membranes, even though preparative conditions may eventually totally remove the lipid from the final membrane. Consequently, the study of effects upon structure and permeability of lipids present during casting of polymeric membranes could have far reaching consequences. A study of the effect of simple triglycerides on permeability of asymmetric membranes has already suggested that lipids may have beneficial effects upon the water permeability and salt rejection of cellulose membranes (30).

Water and solute permeability of numerous cast-lipid membranes were examined in our project. Most of these membranes were very thin, less than 5 μm in thickness, with only a few approaching the normal thickness of the cellulose acetate, asymmetric membrane used as standard. Many of these membranes must still be considered as asymmetric in many respects, even though their physical description and properties may not correspond entirely to those expected in asymmetric membranes for reverse osmosis. This may be best illustrated by the electrical conductance curves for one of the high

TABLE 4
 PROPERTIES OF MEMBRANES FORMED BY DYNAMIC
 LOADING OF POLYMERIC FILMS WITH LIPID DISPERSIONS

Lipid ^{a)}	Support	R (μcm^2)	τ^+	Hydraulic Conductivity, $\mu\text{l}/\text{min}/\text{atm}/\text{cm}^2$	Apparent σ NaCl
Ethylene Glycol Monododec- anoate	N-610,P-10	2,500	0.69	-	-
Glyceryl Monododec- anoate	CE-HA	2,000	0.50	18.7	0.0
	CE-HA**	80×10^6	0.83	-	-
	PCA		0.50		
Glyceryl Didodec- anoate	CE-HA	2,000	0.50	5.3	-
	CA	400	0.50	2.2	0.35
	PCA	2,000	0.50	-	-
Span 65	PCA	7,700	0.50	0.1	-
	PCA**	500,000	0.85	-	-
Hexadecanol	CE-GS	800	0.50	12.8	0.05
Dodecanoic Acid	CE-GS	180	0.78	7.7	0.09
	CE-GS**	1,000	0.80	0.2	0.21
	PCA	5,000	0.60	-	-
Dioctadecyl Phosphate	CE-GS	48	0.89	6.2	0.12
	CE-GS**	36	0.92	<0.1	-
	CE-HA**	95	0.85	<0.1	-
Didecylamine	Teflon	85	0.35	12.2	0.1
Dodecylamine	Teflon	100	0.40	3.3	0.0
	Teflon**	88	0.12	0.8	0.2

^a See Table 3 for general notations. The lipids were generally applied near, but below their melting points until flow of the suspension through the filter (under hand pressure in a 10 ml syringe) decreased substantially or stopped.

TABLE 4 (Continued)

* These values were obtained for membranes at temperatures above the melting point of the lipids contained in them.

** These values were obtained at about room temperature but after going through a warming and cooling cycle in which a high resistance was obtained in the warmer state.

resistance membranes containing a combination of polymer and lipid, as in Figure 11. This current-voltage curve shows a distinct rectification, indicating that the energy barrier for current carrier passage through the membrane is greater in one direction than the other. Such asymmetry does not only appear with the cast-lipid membranes, but may be obtained also with polymer membranes that have had one face coated with a lipid film, or even in membranes containing only a single polymer or mixture of polymers. The critical factor appears to be the development of an asymmetric structure as a consequence of forming the membrane with opposing faces in contact with two media of varying dielectric and functional properties, glass and air in these instances. If conditions are utilized that reduce the differences between the two interface contacts, the asymmetry may be greatly reduced or disappear entirely.

The effect may be attributed to the different energies arising from polarization within the asymmetric layers of solution and membrane. Thus, a particular casting process provides, at one face of the membrane, a narrow region of low dielectric (e.g., lipid) that comprises the major charge transport barrier, together with a second region of distinctly higher dielectric with a relatively sharp boundary between these regions. This asymmetric structure may create a large energy differential between transport of a charge carrier in the sequence from aqueous interface into and through the high dielectric membrane region, into and through the low dielectric region, and into the aqueous interface on the other side, and its transport in the opposite sense. The process may be analogous to, and in some cases, quite similar mechanistically, to the semiconductor diode in which a charge depletion region adjacent to the interface may control passage of charge carriers through the diode.

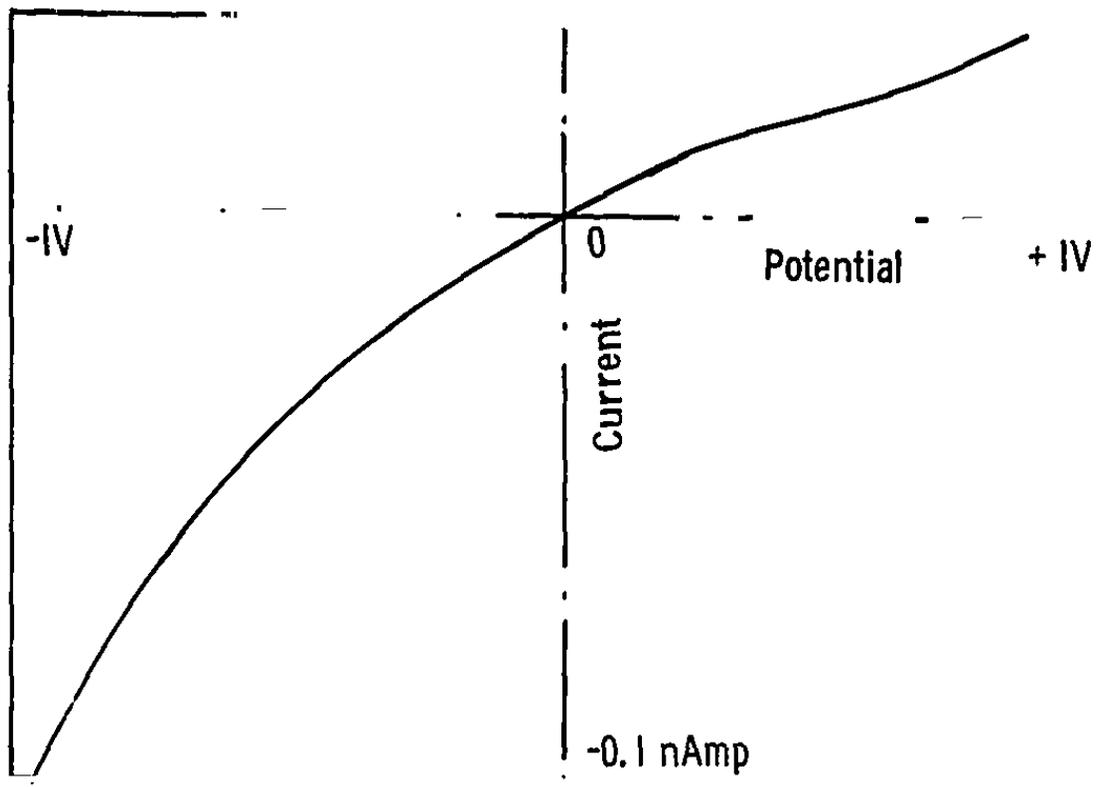


FIGURE 11. ASYMMETRIC RESISTANCE IN CAST-LIPID MEMBRANES.

The figure shows current-potential curves for a thin membrane (ca 2 $m\mu$) cast from a solution of 5% Ethocel plus 0.05% tocopherol succinate in 2:1 chloroform-methanol. Positive currents (cation flow from glass contact side of membrane to air interface side) are smaller than negative currents.

Lipids are not entirely essential in producing similar curves. Even greater asymmetry may be obtained with some combinations of polymers only (e.g., Pip-10 with Ethocel) and appears to depend upon differences in composition or group orientation on two sides of the membrane.

Table 5 presents a summary of water permeability (hydraulic conductivity coefficient) and apparent reflection coefficients for NaCl for a number of cast-lipid membranes. It may be reiterated here that the "apparent" reflection coefficients listed in Table 5 must be used only as comparison standards since they were determined essentially on single point estimates (one solute concentration, one pressure differential) subject to the probable distortions discussed in the section on procedures. A more accurate reflection coefficient for NaCl in a few selected membranes is given in Table 6, where values were obtained through evaluation of osmotic flow at several solute concentrations and utilizing the slope at zero solute concentration from extrapolated curves. This mechanism avoids the severe concentration polarization problems found with the single point analysis, but requires a substantial amount of time for each analysis. Therefore, it could be utilized only on a few systems that demonstrated promise under the single point evaluation.

The results indicate that none of the samples tested so far can match the hydraulic conductivity along with the salt rejection characteristics of the standard asymmetric cellulose acetate membrane normally used for reverse osmosis. On the other hand, the relative water permeability and salt rejection characteristics of several lipid-polymer membranes are greatly improved over those found for membranes containing only the corresponding polymer. Consequently, it appears likely that some lipid-polymer combinations might be developed into excellent reverse osmosis membranes if some attention were given to formulating optimal casting concentrations and conditions.

3.4 POLYMER STABILIZED BILAYER LIPID MEMBRANES (BLM)

The BLM was not a primary concern of this project, despite interesting permeability characteristics, since fragility makes it of little practical value. However, studies on stabilization of BLMs with polymeric supports were in progress in another project in this laboratory (31) and some of the polymers and techniques for membrane preparation developed in the project on phase relations were of value in developing mechanisms for significant BLM stabilization. These studies were of note in demonstrating, first, that a BLM may be formed in pores of the order of $1 \mu\text{m}$ in diameter under proper conditions, and also in showing that membranes with BLM properties may be formed from combined lipid and polymer solutions with added stability due to an internally generated polymeric matrix. These relations are clearly concerned with phase relations in composite lipid membranes and are presented here as supplemental information on the behavior of complex lipid systems.

The experimental BLM is normally formed by stretching a film of lipid (usually in solution in some solvent) across an aperture in a relatively, thin, hydrophobic septum, submerged in an aqueous solution. The aperture area generally ranges from about 0.2 mm^2 upward, depending upon the

TABLE 5
PERMEABILITY CHARACTERISTICS OF SELECTED CAST-LIPID MEMBRANES

Membrane Polymer	Composition(a) Lipid	Hydraulic Conductivity $\mu\text{l}/\text{atm}/\text{cm}^2/\text{min}$	Apparent σ_{NaCl}	Approximate Thickness μm
CA	Standard R.O. Membrane	2.62-3.0	0.26-0.35	
Nylon 66(b)	-	1.53	0.13	7
(c)	-	0.25	0.15	4
(d)	Dodecanol	2.1	0.13	3
(d)	Tetradecanoic Acid	2.1	0.24	
(d)	Glyceryl Monododecanoate	13	0.0	
(d)	Glyceryl Didodecanoate	0.9	0.07	3
(d)	Span 65	5.2	0.01	
(e)	Tetradecanoic Acid	3.1	0.08	4
(e)	Dodecanol	1.15	0.20	
(e)	Glyceryl Monododecanoate	15	0.0	
Nylon 6 (f)	-	0.25	(0.05)	~2
(f)	Dodecanol	Open	-	~3
(f)	Dodecanoic Acid	0.8	0.35	
(f)	Glyceryl Monododecanoate	Open	-	-
(f)	Diocetyl Phosphate	1.5	0.32	
(f)	Dodecylamine	1.2	0.28	Fragile
Pip-10 (g)	-	0.08	(0.6)	2
(g)	Dodecanol	0.8	0.21	3
(g)	Tetradecanoic Acid	0.45	0.43	3
(g)	Glyceryl Monododecanoate	8.2	0.02	

TABLE 5 (Continued)

Membrane Polymer	Composition(a) Lipid	Hydraulic Conductivity $\mu\text{l}/\text{atm}/\text{cm}^2/\text{min}$	Apparent σ_{NaCl}	Approximate Thickness μm
Pip-10 (h)	-	2.0	0.05	4
Polyphenylene Oxide (i)	-	<0.1	-	2
(i)	Benzoic Acid	0.2	0.8	2

^a Membranes were cast on glass from solutions and under conditions indicated in the footnote corresponding to the letter in parenthesis in the membrane designation.

^b Nylon 66 (DuPont), 25%, in 90% formic acid, cast at 1 mil at 45° with a 10 sec. air cure, 0° water gel bath.

^c As in b, but with 60 sec. air cure.

^d Nylon 66, 15%, plus 0.2 to 1.0% of lipid cast at 1 mil on 45° plate, 30-60 sec. cure, 0° water gel bath.

^e As in d, but cast at room temperature.

^f Nylon 6, 3 to 10% in trifluoroethanol, cast at 1 to 2 mils, 15 to 30 sec. cure at room temperature, 0° water leach bath. Lipid added at 0.1 to 2% depending upon lipid.

^g Pip-10, 5% in chloroform-methanol, cast at 2 to 3 mils at room temperature. Lipids incorporated at 0.1 to 0.5%. Castings essentially fully-dried at 30-60 seconds. Water bath used to lift thin films from glass.

^h Pip-10, 10% in formic acid, was cast at 3 mils at room temperature, air-cured 30 seconds, gelled in 0° water.

ⁱ Polyphenylene oxide, 5%, in 1:1 methylene chloride-benzene, cast at 2 mils at 45°, air-dried and lifted from the glass with water. Benzoic acid added at about 0.5%.

TABLE 6
SALT AND WATER PERMEABILITY OF CAST-LIPID MEMBRANES

Membrane Composition ^(a)		Hydraulic Conductivity	σ_{NaCl} ^(b)
Polymer	Lipid		
Cellulose Acetate	-	2.58	0.95
Nylon 66	-	0.25	0.39
Nylon 66	+ Tetradecanoic Acid	2.1	0.84
Nylon 66	+ Dodecanol	2.1	0.42
Nylon 6	-	0.20	(0.05)
Nylon 6	+ Dodecanoic Acid	0.8	0.75
Nylon 6	+ Dodecylamine	1.2	0.70
Nylon 6	+ Dioctadecyl Phosphate	1.5	0.79
Pip-10	-	0.08	(0.6)
Pip-10	+ Tetradecanoic Acid	0.45	0.70
Pip-10	+ Dodecanol	0.8	0.55

^a Casting conditions and proportions were those listed for corresponding systems in Table 5 footnotes.

^b Reflection coefficient for NaCl, σ_{NaCl} was determined through observation of osmotic flux at several σ_{NaCl} concentrations and extrapolating curves to zero concentration to evaluate true osmotic flux.

mechanisms being used for spreading the film and the purpose to which the membrane is to be applied. Under these conditions, the thick film normally obtained upon initial stretching will spontaneously thin to a film consisting largely of two monomolecular leaflets of lipid, less than 10 nm in thickness. With smaller apertures, and the usual septum thickness of about 0.1 to 0.5 mm, it may be difficult to obtain spontaneous thinning to a bilayer. This is due to opposition from capillary forces within the aperture to the forces stimulating bulk phase compression and spontaneous collapse of the two monolayers bounding the bulk phase to a bilayer with extrusion of excess lipid to the aperture walls. Successful thinning in small openings must depend upon development of appropriate interfacial relations between the lipid and support, and a favorable relation of aperture depth to diameter, as well as appropriate phase relations that promote active extrusion of solvent with development of ordered structure in the stretched films.

It has been apparent, however, that BLM stability to mechanical and electrical stress is improved by reducing the ratio of membrane area to circumferential support structure. Consequently, it has been desirable to attempt to produce stable BLMs through formation in microporous supports, or through generation of a strong polymeric network simultaneously with the formation of the BLM. Both mechanisms have now been achieved here, with existence of the BLM configuration proven through analysis of the interaction of certain materials that will create changes only in the bilayer structure and not in thicker films.

The first mechanism was demonstrated on microporous films prepared from Pip-10 polyamide. Castings were prepared with films having thicknesses of about 1 μm with pores of a similar or smaller diameter. The Pip-10 film was mounted in the standard thin film holding device (Figure 1B) and spread with oxidized cholesterol in nonane solution. The spreading of the lipid solution greatly increased the film resistance but, after a few minutes, resistance decreased slightly again. Tests of current-voltage relations showed, at this point, a non-linear curve, with some asymmetry, which, except for the asymmetry, was rather characteristic of thin lipid films stretched across wide apertures. Various other properties (lability to high potential stress, photoelectric responses with iodide ion) also helped to suggest that some areas of bilayer configuration existed.

Mechanisms for unequivocally demonstrating the existence of a BLM configuration in a membrane of this structure are limited. Normally, thickness of the stretched black films across wider apertures may be estimated through measurements of capacitance or through optical reflection techniques. Otherwise, one may resort to difficult and uncertain fixing, imbedding and sectioning for electron microscopy. Neither capacitance nor optical reflection could be applied to the membranes here, since the pores were too small for optical observations and it was impossible to estimate area of lipid membrane to obtain values of unit area capacitance. Consequently, a

convenient alternative was utilized, the interaction of EIM, or "excitability inducing material" (23), with BLMs. It has been demonstrated (32, 33) that EIM changes the conductance of BLMs by penetration of a protein substance through the membrane to form a long-lived conducting channel. These channels can be formed only if the membrane is actually in a bilayer configuration or differs from a bilayer by a very small thickness. Consequently, the development of a conductance characteristic of the EIM, with typical reversible negative resistance regions, must demonstrate existence of significant regions of bilayer thicknesses.

As indicated in Figure 12, the reaction of EIM with a Pip-10 supported lipid film results not only in the lowering of resistance, but development of a region of transition (negative) resistance, corresponding to the characteristics expected for a small negative resistance shunted by the relatively large linear resistance of the rest of the membrane area. Thus, the existence of a bilayer structure in very small pores has been demonstrated for the first time.

Although bilayer stability was significantly increased for both mechanical and electrical stress in these configurations, it was not by a spectacular margin. For electrical stress, the breakdown potentials appeared to be increased often by a factor of about 50 to 150 percent. Mechanical strength was not measured by any objective quantitative mechanisms in this case, but there was a substantial degree of improvement in stability to disturbance of the electrolyte solution in the vicinity of the membrane.

The Pip-10 supports are not compatible with lipid solutions containing benzene or halogenated hydrocarbons, which are components of lipid solutions that have been most versatile in BLM preparations. Consequently, a search was made for supports with a greater adaptability. Nylon 66, 610, and 6 have very little solubility in chloroform so these were tested in microporous supports for BLM stabilization. Although bilayers could apparently be formed, BLM stability seemed lower than normally obtained with large aperture membranes. This appeared to be due to poor interaction of lipids with the supporting material. It may be mentioned that the Pip-10 BLMs showed some evidence of self-healing characteristics. Under high potential stress membrane resistance would decrease abruptly, indicating rupture of BLMs in some region. However, the resistance would return to original values upon lowering the potential gradient, indicating repair of the region of electrical rupture.

Even though the total area of membrane in these micropore systems was relatively large, it is obvious that only a very small percentage of the area ever attained BLM status. Consequently, much further work may be required to obtain large area, stable BLMs.

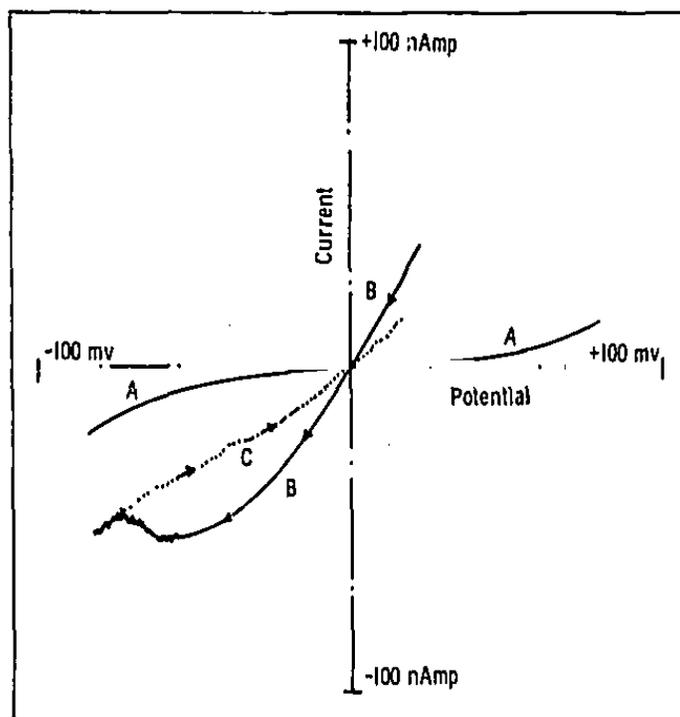


FIGURE 12. DEMONSTRATION OF INTERACTION OF EXCITABILITY INDUCING MATERIAL (EIM) WITH BLMs FORMED IN A MICROPOROUS SUPPORT.

The Pip-10 polyamide film was cast on glass from a chloroform solution containing formamide to produce porosity. Film thickness was probably less than $1 \mu\text{m}$ while pores of about $1 \mu\text{m}$ were visible under phase contrast microscopy.

After mounting and immersing this film, oxidized cholesterol in no.ane was brushed across one face. After 10 minutes standing at 25° , current-voltage curve A was obtained. EIM was added to one side and Curve B and C were obtained after standing for 20 minutes. The increase in conductance at low potentials together with the negative resistance at higher levels, indicate existence of bilayer regions where EIM could penetrate.

Simultaneous generation of BLMs with an integral support network provided relatively large areas of membrane having good bilayer characteristics and with better stability than the BLMs in micropore films. Furthermore, these membranes demonstrated a pronounced self-healing capability in which steep increases in conductivity at high potential gradients could be reversed, without apparent permanent alteration of the membrane structure, by return to low stress conditions.

Three polymers were found effective in creating some degree of stabilization of the BLM when incorporated as part of the membrane generating solution. These were ethyl cellulose (Ethocel), Pip-10, and polyphenylene oxide. The first two were approximately equivalent in developing electrical and mechanical stabilization, usually providing a two- to three-fold increase in both factors. The latter polymer was less effective, partly as the result of requiring solvents in the lipid-polymer mixtures that were not normally used in generation of BLMs.

Solutions containing 2-3 percent sphingomyelin and about 20 percent tocopherol and 2 percent of Ethocel or Pip-10 in chloroform-methanol solvent were used for generating the BLMs. The thinning process was generally somewhat inhibited by the polymer and care was required in stretching the solutions or little or no thinning would develop. With polyphenylene oxide, it was necessary to replace the chloroform-methanol solvent with benzene.

In contrast with the analysis of BLMs in the micropore support systems, the normal indicators of bilayer configuration, increase in capacitance or optical reflection, could be used for demonstrating approach to a bilayer configuration. Capacitances, determined from charging time constants with a high resistance in series with the membrane, were generally in the range of 0.5 to 1.0 $\mu\text{F}/\text{cm}^2$, which is compatible with the 0.25 to 1.0 $\mu\text{F}/\text{cm}^2$ obtained normally with BLMs. However, the thickness cannot be calculated accurately since the average dielectric constant of the mixture of lipids and polymer is unknown.

These membranes reacted readily with EIM to develop negative resistance regions, as shown in Figure 13, but they also showed some characteristics in the EIM interaction that suggested that a substantial part of the membrane area was in some state of organization that was rather different from that of the normal BLM in absence of the polymer. Thus, it was presumed that a fair proportion of the lipid in the black area must be immobilized through association with a polymeric matrix interwoven through the system.

The stabilizing capacity of this supporting matrix was best demonstrated by the apparent self-healing characteristics of the potential-stress injury to the membrane. As illustrated in Figure 14, when a Pip-10 membrane is subjected to an increasing ramp potential (curve A) a point is reached at which conductance abruptly increases by several orders of magnitude.

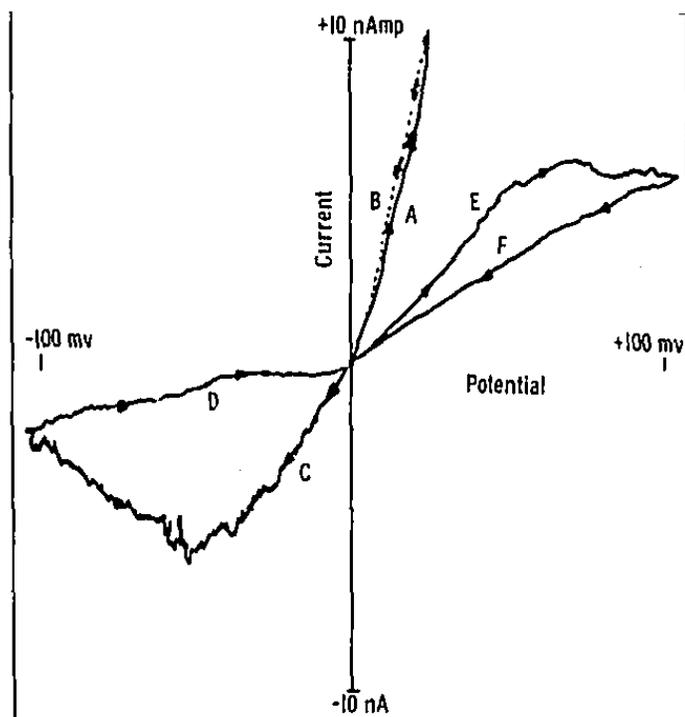


FIGURE 13. EIM NEGATIVE RESISTANCE IN A LIPID BILAYER INTERNALLY STABILIZED WITH ETHYL CELLULOSE.

The membrane was generated in 1 M NaCl on a polyethylene aperture, from a solution containing about 1.5% sphingomyelin, 20% tocopherol and 2% ethylcellulose in chloroform-ethanol solution. EIM was present during the generation of the lipid bilayer, resulting in rapid reaction as thinning occurred. Resistance was monitored with a +10 mv potential during thinning and EIM interaction.

After a reasonably steady resistance was attained, the potential-current scans illustrated were made. An initial positive ramp potential caused recording of curve A with the decreasing ramp creating line B (dashed line). The negative ramp, immediately following, showed the steep increase in resistance above 50 mv, in curve C. After return to zero potential (curve D), subsequent positive ramps (E,F) showed that a negative resistance region could also be obtained in the positive sector, but the open state did not return completely even with a long rest at zero or moderate positive polarizing potentials. It may be noted that the region of initiation of the increase in resistance in the positive sector occurs at somewhat lower potentials than would normally be expected for the same type of membrane without the stabilizing polymer.

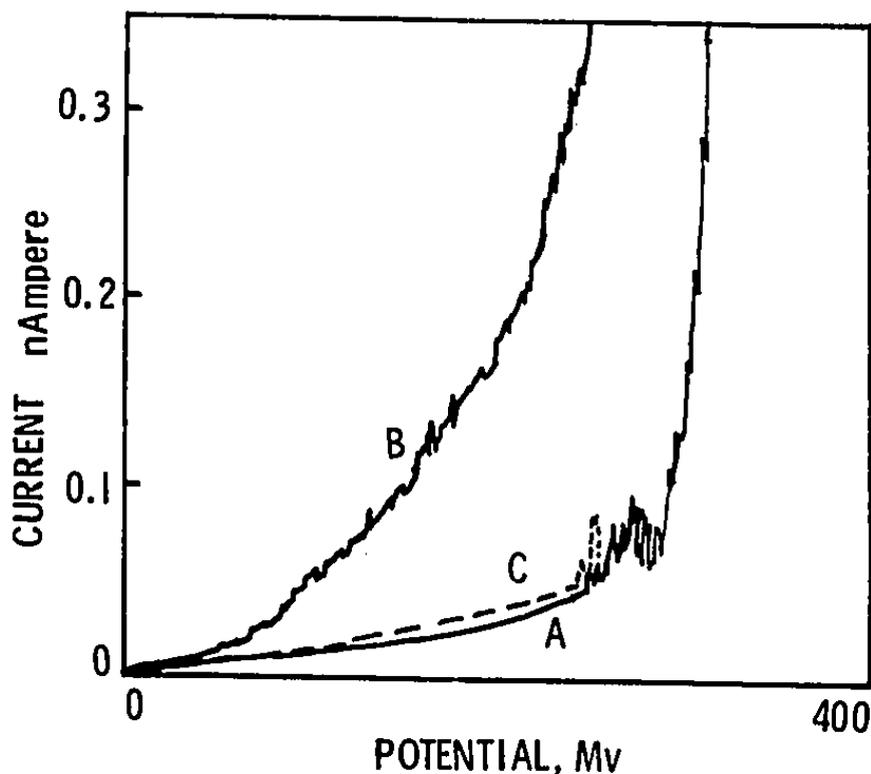


FIGURE 14. INTERNAL STABILIZATION AND SELF-REPAIR OF MEMBRANE PUNCHTHROUGH REGIONS IN A BLM CONTAINING A POLYAMIDE MATRIX.

Curves are tracings of current-voltage recordings obtained with a triangular (ramp) potential applied across a BLM stabilized with Pip-10 polyamide. The membrane was prepared from a solution containing 2% sphingomyelin, 20% tocopherol and 2% Pip-10 in chloroform-methanol. Shortly after thinning to a full black state was achieved, Curve A was obtained with a rising ramp to 325 mv under potential control. Instabilities begin to appear at 250 mv, but the membrane does not rupture even though conductance is increased over twenty-fold according to separate measurements. As the potential gradient is reduced, conductance approaches normal values again (Curve B) and, after a short rest at zero polarization, another increasing ramp shows that the membrane is essentially totally repaired.

Although conductance may fluctuate wildly in this region, the membrane is generally still stable and will remain intact (still higher potentials may cause rupture with the rupture most frequently occurring at the edges where discontinuities may exist). Upon reduction of the potential gradient (curve B), the conductance gradually returns toward the original value. A subsequent increasing ramp potential (curve C) differs only slightly from the original curve A.

These phenomena suggest that at potential gradients of about 400,000 to 600,000 volts/cm (250 mv to 300 mv across a 50 to 70 Å barrier) the lipid bilayer regions may be broken, creating areas of high conductance. However, the polymer matrix is apparently sufficiently strong to keep such breakdown regions from propagating in a zippering reaction and maintain each rupture within a limited region over a long period of time. Upon removal of the stress, the lipids that were displaced in the rupture must return to the conformation that is most stable for those conditions, the bilamellar structure characteristic of the BLM, with consequent increase in resistance as the ruptured areas are healed.

While these results may not be of great immediate significance in present membrane technology, there is a strong suggestion that mechanisms may be developed in the future for formation of stabilized BLMs under conditions where their particular properties may be of real advantage.

3.5 PHOTOELECTRIC RESPONSES OF COMPLEX LIPID MEMBRANES

In the previous sections of this report, phase transitions have been induced either by thermal changes or by alterations of the medium in contact with the lipids. Another mechanism for introducing energy into a complex lipid structure in a manner that might stimulate phase transitions would be the absorption of radiant energy in the membranes. Prior to initiation of this project, some experimental evidence had been obtained that the absorption of near ultraviolet light by lipid-loaded membranes could produce significant photovoltaic or photoconductive responses under circumstances that suggested that ionic injection or ejection mechanisms were involved rather than electronic processes. Since there was a distinct possibility that the photoelectric responses could be due to phase transitions that caused a change in sequestering activity of the lipid structures in the membranes, this phenomenon was pursued further here.

The photoelectric reactions of lipid-loaded membranes were first observed in systems consisting of a Millipore GS filter containing tocopherol. This system was tested because it had previously been observed that a film of tocopherol (possibly about 1 μ m thick) stretched across an aperture in an insulating septum produced a photovoltaic response with an action spectrum corresponding to the absorption peak of the tocopherol at 294 nm (22). Subsequent tests, however, demonstrated that even greater photoelectric responses could be obtained with other lipids, some of which have relatively

poor absorption bands in the region of illumination. Consequently, a survey of photoelectric reactions with simple lipids in Millipore mixed ester filters (Millipore GS) was carried out. Results of this survey are summarized in Table 7.

Although these tests do not exhaust the possible combinations, they do seem to indicate some interesting requirements for photoelectric activity in this kind of response. First, it may be noted that best responses were generally obtained with alcohols, particularly phytol, while little or no response was derived from carboxylic acids. This is particularly significant since the electronic absorption bands of the acids are much more effective in the region of the maximum energy from the light source than are those of the alcohols. If the response were simply related to electronic activation, one might expect the opposite magnitude of response. Thus, even though the electronic excitation may be, and probably must be, involved, it cannot be the only limiting factor.

Some care was exerted to differentiate photoconductive and photovoltaic responses, in contrast with some studies of photoelectric effects in membranes where the two terms are frequently confused or used interchangeably. The two processes are really quite distinct, with the photoconduction developing simply as the result of increased numbers and/or mobility of carriers in the barrier region and photovoltaic effects developing due to vectorial separation of charges across an energy boundary with consequent development of a potential across that boundary. In most instances, the degree of contribution of PC and PV effects may be determined through an examination of current-voltage curves in the light and dark (i.e., variation of current under varying applied potentials under potential control conditions or variation of potential with current under current control). A reaction that is largely photovoltaic will be indicated by essentially parallel light and dark curves, with the light curve simply displaced along the potential axis. A photoconductive reaction will generally be indicated by an increase of the slope of the current-voltage curve in the light over that in the dark. While a number of complications may arise in interpretations, particularly as the result of non-linearity of current-voltage relations, the analysis of these curves may be of some assistance in determining whether the observed photoelectric phenomena are largely due to bulk phase relations (PC) or to interfacial reactions (PV).

TABLE 7

PHOTOELECTRIC RESPONSES OF LIPID-LOADED MICROPORE FILTERS

Lipid ^{a)}	V _{oc} ^{b)} mv	I _{sc} ^{c)} n-amp/cm ²	Light ^{d)} Distance Inches	Type of ^{e)} Response
<u>Alcohols</u>				
Hexanol	Low resistance, no response			
Cyclohexanol (25°)	2	60	6	PC
1-methyl cyclohexanol (25°)	3	12	6	PC
1-menthol (35°)	2	0.3	6	PC
1-octanol (35°)	4.5	20	6	PC & PV
1-decanol (35°)	5	25	6	PC & PV
1-decanethiol (35°)	3.5	0.8	6	PC
1-dodecanol (52°)	9	10	6	PV & PC
1-hexadecanol	15-20	15	6	PV & PC
9-octadecene-1-ol	42	3.5	12	PV(& PC)
Geraniol	20	39	12	PV
Linalool	15	15	12	PV
Phytol	120	15	6	PV
<u>Acids</u>				
9-octadecenoic acid (oleic acid)	0	0	6	-
Linoleic acid	2	0.01	6	PC
Decanoic acid	0	0	6	-

TABLE 7 (Continued)

Lipid ^{a)}	\overline{V}_{oc} ^{b)} mv	i ^{c)} n-amp/cm ²	Light ^{d)} Distance Inches	Type of ^{e)} Response
<u>Esters</u>				
Methyl oleate	0.5	0.9	6	PC
Butyl oleate	0.6	1.5	6	PC
Butyl stearate	0	0.24	6	-
Glyceryl monooleate	8	4.5	6	PV(& PC)
Glyceryl dioleate	22	2.1	6	PV(& PC)
Cyclohexyl oleate	9	0.7	6	PV & PC
Span 80 ("sorbitan mono- oleate") ^{f)}	20	75	6	PC(& PV)
Span 85, fraction B ^{g)}	12	2.8	6	PV & PC
Span 85 (sorbitan trioleate)	2	0.03	6	PC
Phenyl oleate	5	0.3	6	PV
p-methyl phenyl oleate	7	25	6	PV
m-methyl phenyl oleate	8	1.2	6	PV
p-methoxy phenyl oleate	15	4.5	6	PV
<u>Aldehydes</u>				
Decanal	6	18	6	PV

a) Millipore GS (0.22 μ m pore, cellulose ester) was saturated with the liquid or molten lipid, blotted to remove excess lipid and mounted in the cell with 0.1 M NaCl both sides. All measurements here were taken at 50°C unless otherwise specified.

b) \overline{V}_{oc} = maximum open circuit potential for the illuminated membrane.

TABLE 7 (Continued)

- c) I_{sc} = short circuit current for the illuminated membrane.
- d) Distance of Mineralight R-51 from the experimental membrane.
- e) The abbreviations PV and PC indicate the predominant photoelectric response, photovoltaic or photoconductive. Parentheses enclosure indicates relatively minor response characteristics.
- f) Span 85 ("sorbitan trioleate") and Span 80 ("sorbitan monooleate") are mixtures of a number of compounds, each containing, apparently, relatively large quantities of both mono- and tri-esters. Span 85 fraction B is a silicon gel fractionation product, probably mainly a di-oleate.

The differences observed in photoconductivity and photovoltaic responses, then, appear to have some significance in the interpretation of the photoelectric phenomena of the composite membranes. Again, it is rather interesting that materials with closely related functional groups (and, therefore, similar light absorption characteristics) varied greatly in the type of response given. This suggests, again, that some factor other than the energy absorption in the functional group is responsible for determining the nature and degree of response.

It seemed likely that structural organization of the composite membrane might be responsible for many of the differences. Such organization may have significant effects in many areas, ranging from direct effects upon the electronic absorption spectra and movement of absorbed energy in exciton transfer mechanisms, to variation in interface area and structure as well as development of penetrating regions of hydration through the membrane that would provide regions of ionic conduction (possibly variable).

Further studies were carried out to evaluate some of the organizational relations. These included determining relation of response to supporting material, effect of temperature (particularly at a phase transition) upon response characteristics, the change in response upon dilution of an active

lipid with an inactive material. These studies used dodecanol as the active lipid, since it had a reasonably good response, showed both photovoltaic and photoconductive components in the response, had a melting point within easily controlled temperature ranges, and could be handled readily in liquid, solid, or solution forms.

The results of tests of a variety of supports indicated that only the cellulose esters produced good responses, although smaller effects could be obtained in a few others such as polysulfone and polyamides. Even in the Millipore supports, however, there was a surprising difference in the behavior with the mixed cellulose esters (acetate and nitrate), as represented by the GS filter, and a pure cellulose acetate filter (Millipore EG) of similar structure. The dodecanol-EG membrane produced a bimodal response, in which the early current response (at zero external polarization) was directly opposite to that normally observed with the GS membrane, and then reversed itself upon longer exposure to develop a similar polarity. Further investigation suggested that the reason for the difference was probably the independent development of photovoltaic and photoconductive responses in these membranes. In the GS membrane, an immediate photovoltaic response developed, driving a current through the external circuit in a corresponding direction. A photoconductive effect developed essentially simultaneously. On the other hand, a marked photoconductivity developed before the photovoltaic response in the EG-membrane. Consequently, the small currents across the membrane, always present due to slight asymmetries in the system, were increased upon lowering of membrane resistance. Then, as a potential developed across the membrane, opposing the small endogenous potentials, the current reversed itself. A unidirectional response could be obtained simply through applying a small polarizing potential to counteract the normal asymmetries. However, a bimodal response still existed due to the differences in rate of development of PC and PV effects. The photoconductivity remained a greater factor in the EG than in the GS membrane, while very poor photovoltaic responses occurred in the EG as compared with the GS system.

In view of their marked dependence upon the support, some experiments were also carried out to determine whether the photoelectric reactions could be attributed to impurities in the commercial filters. To this end, both GS and EG filters were extracted exhaustively with several solvents, dried and retested with dodecanol or phytol for photoelectric reactions. Extraction with alcohols, hydrocarbons, or halogenated hydrocarbons had little or no effect upon the responses in the GS filter, but the alcohol or halogenated hydrocarbon extraction destroyed the response with the EG filter. However, this effect may be due to structural alterations of the filter since it was apparent that these solvents had swelled and distorted the filter.

The investigation of change of response with temperature showed that there was no photoelectric reaction below 24°, the approximate melting point of

the dodecanol, but a strong reaction could be obtained within a fraction of a degree above that temperature. In fact, an interesting delayed reaction could be developed in membranes thermostatted just below the transition point, with the energy of the light absorbed apparently being sufficient to cause a transition to the active (liquid) state after a short period of illumination. Above this temperature, there was a small increase in magnitude of response with increasing temperature until a maximum was reached around 40 to 45° for the photovoltaic response and around 50° for the photoconduction. Above these temperatures, both reactions decreased rather rapidly. Rates of development of maximal responses showed a regular increase with increasing temperature.

It must be emphasized here that the abrupt transition to an observable photoelectric response at the melting point of the lipid coincides with a tremendous change in resistance of the membrane (cf. Figure 3). Consequently, the lack of an observable photoelectric reaction may not entirely preclude the possible existence of an effective light-stimulated driving force across the membrane. It may be possible that a similar light response develops across the membrane, but cannot be monitored on the external circuit due to internal short-circuiting through transport of ions across the membrane in the low resistance channels of the solid lipid system. Unfortunately, this possibility was not considered until it was too late to carry out the relatively difficult analyses to detect changes of ionic concentrations across the membrane due to such transport. If this reaction existed, however, it could provide a potentially useful mechanism for light-stimulated ion transport.

With dilution of the dodecanol by an inactive, but similar hydrocarbon, hexadecane or tetradecane, the photoelectric response decreased according to a first power relation at higher concentrations of the dodecanol, but approaching a second power relation at lower concentrations (Figure 15). These relations would be compatible with a surface-related mechanism, in which the limiting factor would be the adsorption of a regular array of the dodecanol to an interface, either of its own micelles or at the cellulose ester surface.

All the studies above were carried out with ultraviolet light stimulation, using a lamp with maximal energy in the region of 250 nm. Since studies with lipid bilayers had demonstrated that photoelectric reactions of tocopherol or iodide ion could be sensitized to visible light with various dyes, tests were also made for sensitizing the composite membrane to visible light. A number of dyes (rhodamine B, methylene blue, crystal violet, etc.) were mixed with the dodecanol and introduced into the filter in the usual manner, and then tested for light response. None of these dye-lipid combinations demonstrated significant light responses with either visible or ultraviolet light stimulus (again, it may be pointed out that these membranes were generally low resistance compared with the normal dodecanol membranes and short circuiting could have occurred). On the other hand,

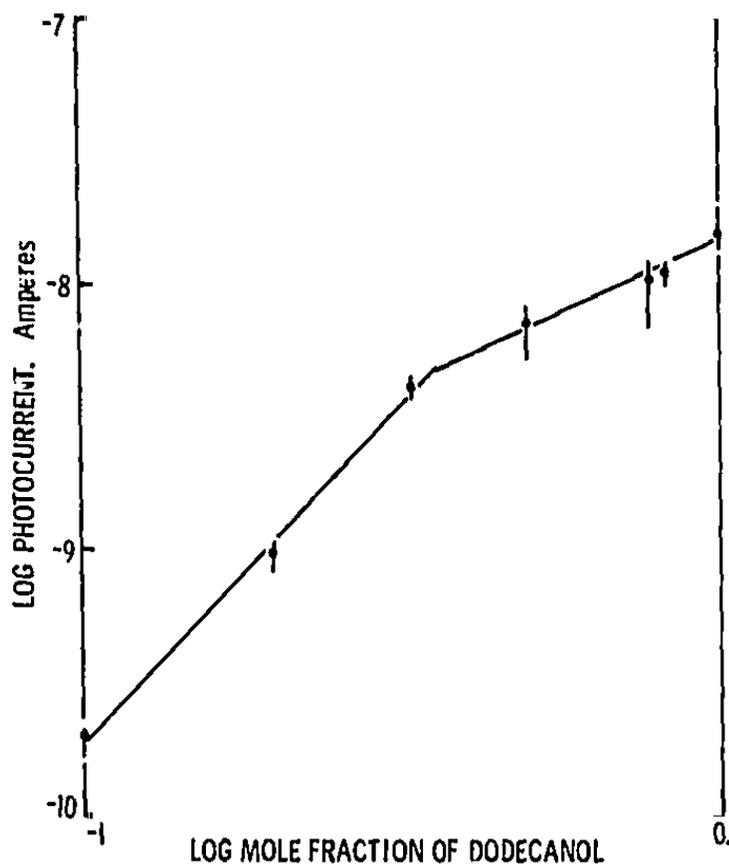


FIGURE 15. DILUTION-RESPONSE RELATIONS FOR PHOTOCURRENT IN A DODECANOL-CELLULOSE ESTER MEMBRANE.

The membranes were loaded with mixtures of dodecanol in hexadecane and the steady state photocurrent (constant potential conditions) determined for each concentration of dodecanol. Each point represents the average of two to six experiments with the bars indicating the range of current values obtained. Hexadecane alone gave an imperceptible response.

when a dodecanol-GS membrane was prepared and then exposed to a solution of either rhodamine B or methylene blue in water, these membranes, with adsorbed and absorbed dyes, remained responsive to ultraviolet light and also gained a significant response to visible light (comparisons are not possible at this time since neither light source was calibrated so relative incident energy is unknown). This demonstrated not only that the composite membrane light reaction could be sensitized to a wide range of the light spectrum, but also that the structure of the dodecanol membrane is important in maintaining the response.

Through a related mechanism of sensitization, some of the otherwise photoelectrically inactive membranes could be activated. Millipore-hydrocarbon membranes were normally totally insensitive to light, but a tetradecane-GS membrane would show a strong photoelectric response to ultraviolet light when KI was added to the electrolyte on the dark side, or to visible light when KI and ferricyanide both were added.

Similarly, some cast membranes could be made photoelectrically active through introduction of photoactive ions. Ethocel membranes containing either tocopherol or octadecanol had very little or no light response until KI and/or ferricyanide was introduced into the solution on the dark side of the membrane. Then large responses were obtained with photovoltaic reactions predominating with KI and photoconduction being the major process with ferricyanide. Other cast films, utilizing several different polymers or lipids, also behaved similarly. However, in the cast films, the lipids were not generally essential to the development of a photoelectric reaction (photoactive ions were essential in all cases) although the responses were normally increased or modified through incorporation of the lipid.

These studies demonstrate that composite membranes can be used to convert some forms of light energy to potentially useful electrical energy. While power yields are still small, even in comparison with those obtained from BLMs under proper conditions, the potential seems unrestricted. It may be noted that photoelectric cells, utilizing thin gel films of dye, with semiconductor electrodes replacing the aqueous electrodes of these experiments, have yielded light energy to electrical power conversion with efficiencies in excess of 0.5 percent (R. E. Kay, Aeronutronic Division, unpublished results), bringing the thin organic film photocell within range of the inorganic semiconductor photocells. Further studies on the mechanisms responsible for the composite membrane photoelectric reactions, together with mechanisms for improving the aqueous electrode interaction, might permit application of such membranes to salt extraction processes utilizing solar power.

SECTION 4

CONCLUSIONS

One of the major points of interest in the present study of composite membranes was to evaluate differences between the solvent-filter membranes previously studied extensively (9-13) and similar lipid-loaded filter membranes in which the lipids could exist in solid or various micellar forms in addition to simple liquid phases. The results of experiments with the lipid-loaded membranes demonstrate that their properties may be as diversified as the lipids utilized in them and the various phase relations that can exist in the membranes.

In this class of membrane, the liquid state for the lipid almost invariably imposes a high resistance structure upon the membrane, with the exceptions existing in the case of some of the amines. Some of the mono-alkylamines manage to maintain a high conductivity above the solid to liquid transition point. On the other hand, ionic selectivity was generally much greater in the high resistance state above the liquid transition, but exceptions occurred with alkylamines and alkyl phosphates. In the latter cases, low resistance membranes, with excellent ion selectivity, were obtained through relatively simple procedures. Such membranes, with further development, might be considered for special purpose applications in place of normal polymeric ion exchange membranes. Some of the experiments with dynamic loading of porous supports suggests that such membranes may be formed and renovated in situ, a factor that might be of significant advantage in conditions of high contamination.

There was little expectation that the normal lipid-loaded membranes, with a region resistant to water penetration extending clear through the full thickness of the membrane, should be very useful in reverse osmosis applications. Hope in this area was reserved for membranes in which the resistant

layer would be a very thin film produced by dynamic loading processes or in films in which the casting procedure produced a very thin barrier region. The lipids utilized in these investigations did not prove readily adaptable to formation of tight, thin skins under the conditions utilized. However, the possible mechanisms for developing good barrier skins through dynamic processes have not been fully explored. The materials used here suffered from formation of relatively unstable, large particle dispersions in aqueous media. The salts of the acids and bases, on the other hand, form stable suspensions of micelles that would have the potential for forming rather tight skins, with high ionic selectivity. Further studies should be made in those directions.

A more promising area was uncovered in the investigation of characteristics of cast films in incorporating lipids in the casting media. The incorporation of lipids into cast films resulted, in a number of cases, in substantial improvement of salt rejection or water permeability over those characteristics in the asymmetric membranes cast from polymer alone. There appears to be substantial basis for assuming that novel membranes with good reverse osmosis properties could be developed, utilizing a variety of different polymers and lipids.

A question inherent in the investigation of effects of phase transitions on permeability of lipid systems was whether such transitions could be applied to transport processes in a cycling process for efficient salt or water extraction in water desalination. Such processes might be considered an "active transport" in the sense that they would involve an introduction of energy at some point to alter the system so that material taken up at low concentration could be discharged in a region of high concentration. In this sense, of course, even reverse osmosis may be considered as active transport, with the energy imparted in the form of a high pressure counter-acting the normal osmotic relations.

The most obvious mechanism for utilization of phase transitions in useful transport would be a counter-current operation in which water or salt was adsorbed or absorbed by micelles or particles of lipid in one portion of the cycle and then discharged in another portion of the cycle as a consequence of a phase transition that destroyed the structure essential to retention of the transported material. Initial experiments along this line were not promising since capacities of lipid systems were low and little difference in capacities could be detected between different phase structures.

A more promising mechanism may be derived from the apparent photosensitivity of the lipid membranes, particularly the lipid-loaded systems. A wide variety of lipids in cellulose ester membranes demonstrated capabilities for developing photoconductivity or photovoltaic potentials upon exposure to an ultraviolet exciting light. Through introduction of an appropriate sensitizing dye, the response could be extended to the visible regions.

Present power factors and efficiencies are very low in the lipid membranes, but there is substantial promise of increasing the yield into useful ranges. This has already been accomplished in "membrane" photocells in which the lipid membrane has been replaced with a gel containing the dye and the aqueous electrode with a semiconductor (tin oxide) electrode. Such organic photocells are capable of producing micro-watts per square centimeter with conversion efficiencies ranging up to 0.5 percent of incident light. This compares with the normal efficiencies of about 5 percent conversion for good inorganic semiconductor photocells.

Finally, experiments with bilayer lipid membranes have demonstrated mechanisms for substantial stabilization of these fragile, but useful systems. While it is unlikely that the present degree of stabilization will permit significant application of the BLMs, further progress may permit wider utilization of their unique permeability properties.

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