AN INVESTIGATION OF SOME REDOX COMPOUNDS ON THE PHOTOEFFECT OF A BILAYER LIPID MEMBRANE CONTAINING CHLOROPLAST EXTRACTS

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ABSTRACT

AN INVESTIGATION OF SOME REDOX COMPOUNDS ON
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By

Paul K. Shieh

Following Braun's idea of redox reaction in a glass test tube, Tien has successfully demonstrated that a Chl-BLM non-metallic substance in aqueous environment was capable of effecting a redox reaction across the membrane. When light is directed onto the Chl-BLM, the pigment (Chl) in the membrane will be excited and will dissociate into electron and hole (or positive charge). This electron will be captured by the electron acceptor on one side of the membrane/solution interface and, respectively, the hole will move across the membrane and be caught by the electron donor on the opposite side of the membrane/solution interface. As a result, a light-induced electric motive force (EMF) can be detected by a pair of calomel electrodes and an electrometer. From the functional membrane point of view, Chl-BLM functions as a photovoltaic cell and is capable of absorbing and releasing electrons in light to facilitate the redox reaction across the membrane/solution interface.

The present work focuses on the survey of the detailed mechanism of this redox reaction across the Chl-BLM. In particular are the followings factors: the pH gradient across the BLM; the membrane potential in dark; the presence of redox compounds to the aqueous phases which can be of importance in effecting this membrane associated redox reaction when carrying out photo-emf measurements.

Results for the present study were: 1) the discovery of the maximum BLM photo-emf enhancement and the possible reaction mechanism under proper conditions, such as good redox compounds, the proper pH of aqueous solution, the membrane potential in dark, and the combination of one or two of the above circunstances; 2) the determination of the electron donating or accepting power of redox compounds presented in the Chl-BLM system by applying the method of BLM reference electrode photo-emf technique; 3) the establishment of a simple equation and parameters which correlate with the Chl-BLM photo-response.

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CHAPTER I

INTRODUCTION

It is well known that, in biological systems, the most important structures are membranes, which constitute the large part of cytoplasmic organelles as well as surface barriers between cells. The membranes are important not merely in their structures but also in their functions through the biological processes. For instance, as Muehlethaler [1966] has pointed out, the photochemical reactions of photosynthesis in chloroplast take place in lamellar systems. These lamellar systems are constructed by many structure units such as the so-called "thylakoid membrane".

Even though the biological membranes are believed to be crucial in the function of biological processes, the direct investigation into the function of biological membranes is still not well developed due to their structural complexity.

Since the concept of bilayer in biological membrane was established by Gorter and Grendel [1925] in the early twentieth century, the relevant model systems have been studied instead of the biological membrane itself. The search for a better membrane model has developed a method for the formation of bimolecular lipid membrane (or BLM) in aqueous solution by Mueller, Rudin, Tien, and Wescott [1962, 1963].

The bimolecular leaflet model has been extended to the structure basis of practically all types of biological membranes that have been studied. These membranes include the plasma membrane of erythrocyte, the nerve membrane of axon, the cristae membrane of mitochondrion, the thylakoid membrane of chloroplast, and the outer segment sac membrane of retinal rod. A schematic representation of these basic units visualized under the electron microscope has been provided by Tien [1971]. It has been evident for many years that if the bimolecular lipid layer were indeed the major structural component of biological membranes, knowledge concerning the properties and the formation of such a structure in vitro would be of both experimental and theoretical significance.

Excellent review articles in the general field of BLM can be found elsewhere. The general surveys from 1962 to 1967, including the techniques of membrane formation, stability, and the physico-chemical characteristic comparison of BLM with those of natural membranes, were carefully reviewed by Tien and Diana [1968]. It seems that the BLM possess certain dimensional, electrical, permeability, and "excitability" characteristics which closely resemble those of biological membranes. The major discrepancy between the properties of model systems and those of natural membranes has been electrical resistance. In some cases, bilayer resistances may exceed those of natural membranes by a factor of more than 10^6. For this reason, therefore, it has been argued that the bimolecular leaflet model (or Davson-Danielli model) is incorrect [Korn, 1966]. It has been found that bilayer resistances can be varied over a wide range by the addition of simple components to the systems. Mueller et al. [1964] have found that a protein obtained from a variety

of sources can lower the membrane resistance by a factor of 10^4 or more. Similar results were obtained with the cyclic polypeptide, alamethicin by Mueller et al. [1968]. In spite of the electric resistance difference in BLM from that of natural membrane, investigations of bilayer lipid membranes have both extended and served hitherto to emphasize the limitations of membrane models. The specificity and variety of reactions which occur at membrane interfaces are far greater than would have been anticipated for structures which serve only to define the interface between two compartments, thus making BLM useful for the study of membrane-associated phenomena near membrane interfaces at the molecular level.

The most intensive studies of the BLM system hitherto are the light-induced phenomena which were first reported by Tien [1968 a, b, c], where the process of charge-carrier generated by light can be observed in BLM containing photoactive pigments such as chlorophyll and its derivatives. The two most commonly studied photoelectric phenomena are the photovoltaic effect and photoconduction. It was suggested [Tien, 1968b] that when BLM was exposed to light, electrons and holes were generated in the membrane with the illuminated side becoming negatively charged. These photoelectric effects produced by the ultrathin membrane clearly demonstrate the existence of mobile electrons and holes in the membrane structure. Similar evidence of electronic conductance in BLM was reported by Jain et al. [1970] from the proper redox reaction of I_2 near the aqueous phases. Most recently, the electronic conduction process of light in Chl-BLM type membrane has been reported [Tien, 1972]. Enhancement of Chl-BLM photo-emf has been found in the presence of some proper dyes and ferric chloride. It is suggested that one side of

Chl-BLM is oxidized and the other side is reduced when light is turned on. The result is quite agreeable with that of Beguslavsky et al. [1972], where photopotential of BLM in the presence of Fe salts and thionine dye can be explained on the basis of redox reaction taking place in aqueous and lipid phases.

Study of the BLM light-induced phenomenon and its relation to biological function has only begun, however. The most important works have been those of reconstructing the vital biological functions, such as the problems of the visual process, using a carotenoid-BLM [Tien and Kobamoto, 1969], and photosynthesis and its related phenomena [Clayton, 1965; Franck, 1957; Mitchell, 1961], where the BLM can serve as a model of the photosynthetic apparatus to study the detailed mechanism of photosynthesis in plants. However, up to now full information of the detailed mechanism of the light-induced electronic process of BLM still is needed in order to survey those light events which occur in biological systems.

Statement of the Problem:

Following Braun's idea of redox reaction in glass test tube, Tien has successfully demonstrated that a Chl-BLM non-metallic substance in aqueous environment, was capable of effecting redox reaction across the membrane. When light is directed onto Chl-BLM, the pigment (Chl) in the membrane will be excited and dissociated into electron and hole (or positive charge). This presence of electron acceptor on one side of the membrane/solution interface will then capture this electron and the hole will move across the membrane and be caught by electron donor on the opposite side of the membrane/solution interface. From the

functional membrane point of view, it functions just like a photovoltaic cell (or photobattery) and is capable of absorbing and releasing electrons by light to facilitate the redox reaction across the membrane/solution interface.

Recently the research interest in this area has been focused on the detailed mechanism of this redox reaction across Chl-BLM. Several factors can be of importance in effecting this membrane associated redox reaction when carrying out photo-emf measurements: (1) the light intensity, (2) duration time, (3) the amount of chlorophyll in BLM, (4) the pH gradient across the BLM, (5) the membrane potential in dark, and (6) the presence of redox compounds to the aqueous phases. The first three factors of Chl-BLM photo-emf response have been studied intensively and reported elsewhere. This work will mainly concern the last three factors of the Chl-BLM photo-emf response, which are still not quite known.

This research has three objectives. They are: (1) finding good redox compounds, the proper pH gradient across the membrane, the membrane dark potential, and the combination of one or two above conditions on the maximum BLM photo-emf enhancement and the possible reaction mechanism; (2) determining the electron donating or accepting power of redox compounds presented in the BLM aqueous phases by using the method of BLM reference electrode photo-emf technique; and (3) establishing a simple and well-defined equation and parameters which correlate with the Chl-BLM photo-response. Hopefully these parameters might inform us of some meanings for the phenomenon itself.

Chapters IV and V will describe the pH dependent and membrane dark potential effect on Chl-BLM photo-response and the detailed mechanism.

A complete description of the behavior of $FeCl_3$ on Chl-BLM photoeffects is given in Chapter IV, before we can use this compound with Chl-BLM as a reference electrode system for later investigation. Chapter IV gives a complete description of the redox compounds investigated. The leveling of these compounds' electron donating or accepting powers in the BLM system are presented in Chapter V. Furthermore, the Chl-BLM photo-emf enhancement in the presence of proper redox couples are also discussed in Chapter IV. An equation to express this Chl-BLM photo-response, which is based on the so-called "relaxation process" has been established and described in Chapter V.

CHAPTER II

LITERATURE REVIEW

DEVELOPMENT OF BIOLOGICAL MEMBRANE MODELS

Historically, the existence of lipids in the biological membrane was first found by Overton in 1899. His experimental evidence is that lipids or lipid-like materials could diffuse across plant plasma membranes. Then, in the early twentieth century, a simple bilayer concept was developed by Gorter and Grendel. The extracted lipids from red blood cells were spread on a Langmuir trough [Gaines, 1966]. They found that the measured area occupied by the lipid monolayer was about twice that of the interfacial area for the intact red blood cells. It was suggested that red blood cells are enclosed by lipid membranes of bimolecular thickness [Gorter and Grendel, 1925].

Owing to the complexity of biological membranes, people began to study them in model systems instead. Very often, earlier models were criticized either because they did not meet the required bimolecular thickness of cell membranes, or because they had quite different properties and environment compared with natural membranes.

By the late 1950's, as revealed by electron microscopy, the bimolecular thickness of most biological membranes had been ascertained.

It appears that all biological membranes possess a common structure consisting of a bimolecular lipid leaflet covered on both sides by a layer of protein or other nonlipid material [Robertson, 1967]. Five types of biological membranes which possess this bimolecular leaflet have been diagrammatically shown [Tien, 1971]. All of them possess a bimolecular leaflet unit 50 to 100 Å thick.

FORMATION OF BILAYER (OR BIMOLECULAR) LIPID MEMBRANES

The search for a good membrane model to study those of biological membrane events has finally been completed. Mueller, Rudin, Tien and Wescott announced the formation of BLM (bimolecular of black lipid membrane) in aqueous solution in 1962. They first studied lipid monolayers and Langmuir-Blodgett multilayers, and then played with soap bubbles and films. Two early publications appeared to influence their approach toward BLM formation. One was a reprint of Boys' classic book on soap subbles and the other was a volume dedicated to N.K. Adam in which A.S.C. Lawrence recounted some highlights in the development of monolayer, soap films, and colloid chemistry [Lawrence, 1968]. A soap film in air in its final stage of thinning has a structure which may be pictured as two monolayers sandwiching an aqueous solution. Once they recognized this structure, together with its molecular orientation, Rudin and co-workers simply proceeded to make a film of two monolayers sandwiching an organic phase in aqueous solution. As far as forming a BLM is concerned, it is easier than spreading a monolayer at an air/water interface. By preparing electron micrographs, they estimated the thickness to be between 60 and 90 Å. They also found that when certain

proteins were allowed to absorb onto the BLM this lipo-protein system could be made electrically excitable.

General Properties of BLM and Their Similarity to Biological Membranes

Within the past decade, many projects of BLM, such as basic structure of BLM, its formation technique, its chemical and physical properties, were under investigation. An excellent review article for the technique of membrane formation and some basic physical-chemical properties of BLM was published by Tien and Diana in 1968. A comparison of known properties of BLM with those of natural membranes has been made by Tien and Diana [1968]. It seems that the BLM possess certain dimensional, electrical, permeability, and "excitability" characteristics which closely resemble those of biological membranes.

HISTORICAL DEVELOPMENT OF REDOX REACTION (ELECTRONIC CONDUCTION PROCESSES) IN BIOLOGICAL SYSTEMS

It is understood from an electrochemical point of view, that when an electric current passes through a phase which is impermeable to ions or electrons, a coupled redox reaction must take place. It is due to this redox reaction that the movement of electronic charges across this phase from one system to another is made possible.

The term "redox reaction" occurring in the living system was first postulated by Lund [1928] in the early twentieth century. In the experiment of the onion root, he found the potential difference occurring between two points on the root was simply the algebraic sum of individual emf's from these two points in the cell. The magnitude of the single electric potential in any locus of the cell is primarily

determined by the ratio of the concentration of oxidant to reductant, or the reaction rate which constitutes the oxidation mechanism in this region of the cell. The continuous bio-electric currents resulting from the oxidation-reduction potentials were from positive (or higher) potential point to the negative (or lower) potential point. A great success of his experiment is the mechanism of this redox reaction consistent with Clark's and Wieland's classical concepts of the essential step in oxidation-reduction. But this finding of transmembrane potentials caused by the redox reaction across the membrane has not been investigated in depth until quite recently.

In crystals and metals, atoms are arranged in very close proximity packing with electrons fused into common bands or low energy levels. If, however, one of these electrons is raised by the absorption of energy to a higher energy level so called "excited" state, it will move and transport its energy freely. It will then give off its excess energy by falling back to the lower energy level. A similar mechanism in living system was first stated by Szent-Gyorgyi [1941]. In the study of photosynthesis, he proposed that the electrons raised to a higher energy level by the observed light could move and transport their energy freely through the system of chlorophyll molecules assuming those chlorophylls were packed very closely in the system analogous to those molecules in crystal and metals.

Hitherto, many studies of biological processes, including photosynthesis, vision, and nerve excitation, can be essential due to this electronic conduction mechanism. In photosynthesis, for example, it is now believed that two kinds of mechanisms are possible in the primary process of light energy conversion. First is the energy

migration mechanism proposed by Olson in 1967, in which pigments in thylakoid membrane absorbed light transferred to a "reaction center". This excitation energy arrived at the reaction center and was then separated into two stages; one was reducing stage and the other oxidizing stage. Second is the charge separation mechanism, where electrons and holes traveled between different reaction centers. These ideas were originally proposed by Van Niel [1941]. He postulated the process of photosynthesis in terms of the oxidant [OH] and reductant [H]. Katz in 1949 explained this oxidant as "hole" and the reductant as "electron". Experimental observations in support of Katz's terminology were given by Arnold and Sherwood [1957] and Nelson [1957]. The results of their experiments indicate that dried chlorophyll and chlorophyll film have organic semiconductor characteristics.

However, the redox concept in bio-membrane was not popular until 1962. Jahn [1962] proposed that there were two redox enzyme systems existing on both sides of a bio-membrane. Later Digby [1965] observed that silver or copper were deposited on one side of the membrane surface of Crustecea following a direct current flow across it. It was presumed that redox reaction occurring at this membrane surface was the driving force for this deposition. More recently, Mitchell [1966] developed a chemiosmotic hypothesis of phosphorylation based on a redox mechanism on membrane, which attracted much attention.

HISTORICAL DEVELOPMENT OF REDOX REACTION IN ARTIFICIAL MEMBRANE (INCLUDING BLM)

The movement of electron flow in artificial system was first explained by Coehn [1898] in terms of "electrostenolysis". The

subject has been briefly reviewed by Tien [1972]. It was suggested that a reduction reaction occurred at the side of the barrier where the anode was situated, and an oxidation reaction took place on the other side of the barrier. Two experimental observations to support this explanation were provided by Becquerel [1867, 1877] and Braun [1891 a, b]. Becquerel found that metallic crystals were deposited on the inner side and a dark yellowish layer of liquid could diffuse from the outside surface to solution, when a copper nitrate solution in a test tube with cracks was placed in a sodium sulfide solution. It was assumed that copper ions were deposited from the reduction of copper nitrate, and from the dark yellowish liquid polysulfide which was oxidized from sulfide ion on the other side. Braun [1891] used a glass tube with fine cracks filled with a diluted chloroplatinic acid, which was then immersed in a beaker containing the same solution. After passing a direct current of sufficient voltage through platinum electrodes which were immersed in both sides of barrier, a metallic mirror was deposited on the side facing the anode and a gaseous product was placed on the opposite side.

There had been a number of studies of electrostenolysis since Coehn had his terminology of electrostenolysis. In 1961, Kallman and Pope observed a number of redox reactions as well as photoconductivity through a thin crystal anthracene layer as a barrier. They found that this photocurrent could be enhanced in the presence of oxidizing agents such as Ce^{+4} and I_2.

The most recent artificial systems used as models of biological membranes are bilayer lipid membranes. Experimental evidence for electron conduction in BLM has been provided by many investigators

[Tien, 1968, 1971; Tien and Verma, 1970; Jain et al., 1970; Pant and Rosenberg, 1971].

Tien [1972] has extensively studied the electronic conduction process in photosensitized Chl-BLM membrane. Enhancement of Chl-BLM photo-emf was found in the presence of some dyes and ferric chloride. It was suggested that Chl-BLM functions as an energy barrier where one side is oxidized and the other side reduced upon illumination. The result was quite agreeable with that of Boguslavsky et al. [1972] where photopotential of BLM in the presence of Fe salts and thionine dye was explained as the basis of redox reaction taking place at BLM/aqueous interfaces. Ilani and Berns [1972] also gave evidence to support the electronic conduction in BLM system and, furthermore, predicted that only a fraction of the difference between the standard redox potential of redox couples was effective as the driving force of the BLM photoresponse.

CHAPTER III

EXPERIMENTAL

1. Materials Used and Solution Preparation

a. Chl-BLM Forming Solution Extraction

The chlorophyll pigments used in this investigation were prepared from a bag of fresh spinach leaves obtained from a super market. A standard procedure for preparing Chl-BLM extracts was described by Tien et al. [1968]:

1. Remove the ribs and stalks from spinach leaves then wash and dry the leaves.
2. Add leaves slowly to the blender which contains 300 ml of (0.5 M sucrose + 0.05 M $KHCO_3$) buffer solution at pH 7.5. First run them in the blender with low speed, then with high speed for 30 seconds after all leaves have been added.
3. Filter the mixture through 4 layers of cheesecloth. Centrifuge the filtrate in 40 ml quantities (8 tubes) at low speed (Variac at 40 volts) for 5 minutes. Discard the supernatant.
4. Distribute another 100 ml of buffer solution to these eight tubes and transfer all of them into 4 tubes. Centrifuge at 40 volts for 5 minutes, then discard the supernatant.

5. Wash the residue with 50 ml H_2O totally and let stand for 5 minutes before another centrifugation. Now centrifuge them at high speed (Variac at 45 volts) for 10 minutes, then discard the supernatant.
6. Extract the residue from above with 90 ml of 2:1 petroleum ether and methanol solution in the blender at medium speed for 1 minute. This is the procedure to separate pigments and lipids from water and protein.
7. Add above mixture to 2 tubes and centrifuge at low speed (Variac at 30 volts) for 5 minutes.
8. Pipette off the top layer from the tube into a round flask and evaporate to dryness at 40°C.
9. Add 5 ml of 1:1 n-butanol and dodecane to the residue. This is the Chl-BLM extract.

This Chl-BLM extract will not change its properties for at least 2 to 3 months, if it is stored in the refrigerator and protected in the dark.

b. Chemical Solution Preparation

All compounds used either for bathing solution or other purposes were obtained directly from chemical companies and prepared without further purification.

Two types of concentrations for solution were selected, for completely H_2O soluble compounds. The concentration was 10^{-1} (M/1). For H_2O insoluble compound, the concentration was made at saturated level. Most solutions had pH at 4 to 5, except those made for other purposes.

c. Techniques of Chl-BLM Formation

About 0.002 ml of BLM forming solution was injected into the hole in the teflon chamber with the aid of a microsyringe (Hamilton Co., PB-600, 0.1 cc). Formation of the membrane was observed visually through a telescope with the aid of a low intensity green light directed onto the Chl-BLM. Such Chl-BLM have a diameter of about 0.1 cm and thickness of 60 to 90 Å. A stable Chl-BLM can last for several hours.

2. Apparatus and Electrical Measurements

The experimental set-up for observing Chl-BLM electric properties is illustrated in Figure 1. The components in the set-up include:

a. Light source -- Keystone movie projector (Model K-525) and the projector lamp (DFC 120V, 150 watts)

b. Shutter -- A shutter, used automatically or manually, functions to control the duration of the illumination.

c. Filters -- The light beam emerging from the aperture of the lamp location passes through a heat absorbing filter (a water bath) and a focus lens before reaching the chamber of membrane formation.

d. BLM inner chamber -- Made from a 10 ml teflon cup. A portion of the cup was machined down to about 0.0025 cm, and a hole of 0.1 cm diameter was punched through it.

e. BLM outer chamber -- It was made of glass, the front portion of which was flattened to facilitate the observation of the membrane.

f. Stirring -- Two magnetic stirrs, one for each chamber, were stirred through the aid of an electric motor. Proper control

of rate of stirring seems to be important for black membrane formation.

g. Calomel electrodes -- Electric contact with BLM was made through a pair of calomel electrodes which were immersed in the BLM aqueous solutions. For convenience, the electrode in the inner chamber was anode (or active electrode) and that in the outer chamber was cathode (or reference electrode).

h. Electrometer -- BLM potential difference can be read from this electrometer which was connected to calomel electrodes through a connection box.(Keithley Model 610B).

i. External variable voltage source -- An external current can be passed to BLM by an external battery through an external resistor in series with the BLM. This external set-up is from Heath, Model EUA 20-12.

j. Resistance substitution box -- This was used to connect electrometer and recorder. This box functioned as a transducer to reduce high voltage (3 volts) output from the electrometer before reaching the recorder. This consists of two boxes together; one has reading of 10,000 x 10^3 Ω, the other 470 x 10^3 Ω.

k. Recorder -- This was used to draw the response automatically. It came from Servo-Recorder, Model EUW-20A.

3. Procedure

a. Photo-emf Measurement

The Chl-BLM photo-emf was studied in a set-up as illustrated in Figure 1. The cell arrangement is represented as follows.

Figure 1. Set-up for BLM photo-response measurement.

L = light source, projector lamp

S = shutter

HF = heat absorbing filter

F = focus lens

I = BLM inner chamber

O = BLM outer chamber

ES = electric stirring

M = stirring motor

C = calomel electrodes

CB = connection box

E = electrometer

B = variable voltage source

RS = resistance substitution box

R = recorder

saturated calomel electrode	aqueous solution	BLM	aqueous solution	saturated calomel electrode

The membrane potential was measured with an electrometer (Keithley, Model 610 B) through the connection of a pair of calomel electrodes via saturated KCl salt bridge. The output of the electromoter was fed into a chart recorder (Servo-Recorder, Model EUW-20A). An electric circuit for this measurement is illustrated in Figure 2. A BLM cell can best be described as a parallel connection of a resistance and capacitance. V_p and R_p were BLM photobattery and photo-generated resistance. When light was on, a switch S_1 was connected to the position Z, and V_p and R_p were connected parallel to R_m and C_m as suggested by Ilani et al. [1972]. When Chl-BLM photo-emf was measured in the absence of external voltage sources, the switch S_2 was opened. However, the switch could be closed when external voltage sources were applied. When an external current was passed to the BLM by an external battery (E_E), through an external resistor (R_E) which was in series with the BLM, the switch S_3 was closed at position X. If only the external battery (E_E) was applied, the switch S_3 was closed at position Y.

b. Membrane Resistance Measurements

A dc membrane resistance was obtained by applying external voltage and external resistor in series with the BLM. The polarization of applied voltage could be controlled through the switch of the voltage divide. This input external resistance could be varied from 10^5 to 10^9 ohms.

Figure 2. Electric circuits for BLM photo-response

C_m = membrane capacitance

E = electrometer

E_E = external battery

P = voltage divider and switch for applying polarizing potentials

R_m = membrane resistance

R_p = light generated resistance

R_E = input resistance

R_o = internal resistance of applying voltage source

S = switches 1, 2, and 3

V_p = BLM photo-battery

The membrane resistance was calculated according to Ohm's law for the circuit shown in Figure 2; it was

$$R_m = R_E \cdot \frac{V_m}{E_E - V_m} ,$$

where R_E was the input resistance, E_E was the applied voltage and V_m was membrane potential. For the best result, R_E was adjusted so that V_m/E_E could lie between 0.1 and 0.8.

c. BLM Photo-Conductivity Measurement

BLM photo-conductivity ($\Delta\delta$) can be measured and calculated as follows:

1) $$R_D = \frac{\Delta V_D}{E_E - \Delta V_D} \cdot R_E$$

where $\Delta V_D = (V_{D_{close}} - V_{D_{op}})$

= membrane dark potential difference in the presence and absence of external voltage and resistor.

2) $$R_L = \frac{\Delta V_L}{E_E - \Delta V_L} \cdot R_E$$

where $\Delta V_L = (V_{L_{close}} - V_{L_{op}})$

= membrane light potential difference in the presence and absence of external voltage and resistor.

3) P_D = membrane resistivity in the dark

$= R_D \cdot 1$

where 1 is the diameter of membrane ($\sim$0.1 cm) and $P_L = R_L \times 1$.

4) δ_D = membrane dark conductivity

$$= \frac{1}{r_D}$$

5) $\Delta\delta$ = membrane photoconductivity

$$= \delta_L - \delta_D$$

CHAPTER IV

RESULTS

1. Basic Properties

a. Chl-BLM Photo-emf as pH Dependence Measurement

The survey of the effect of aqueous solution pH on BLM electric properties has been begun recently. Ohki [1969] reported that phospholipid bilayers exhibit their minimum value of electric capacitances around pH 4 of aqueous solution. Tien [1971] also found that BLM formed from chloroplast extracts differ from lecithin or oxidized cholesterol BLM in that they are sensitive to hydrogen ion (H^+). In the pH range of 4 to 6, a membrane potential of 50 to 58 mV per 10-fold H^+ ion concentration gradient of aqueous solution was observed. It is presumed that membrane dark potential is created by the diffusion of hydrogen ion from one side of the cell to the other.

Now there are two questions to be asked: (1) What is the effect of pH on the potentials in the dark? (2) Does the pH of aqueous solution affect BLM light-induced electro-motive force? If so, what then will be the possible mechanism of this phenomenon?

The pH gradient between the inner and outer KCl aqueous solutions can be made by adding H^+ or OH^- to the inside of BLM cell, while the

pH of KCl (10^{-1} M) in the outer solution is fixed at 5.5. The open-circuit membrane potential in the dark under a pH gradient can be read from a Keithley 610B electrometer. After the steady membrane potential in the dark is reached, a 6 second light duration is then applied for the measurement of BLM photo-emf.

Figure 3 is the plot of BLM dark and light-induced membrane potential versus the pH of KCl aqueous solutions. Chl-BLM exhibit three flat portions of dark potential in the whole pH range; these are lower than pH 3.7, between pH 5 and 6, and higher than pH 8. There are two points where membrane potentials change very rapidly, which are at pH 4.5 and 6. When the inner aqueous solution pH is below 4.5, membrane potential in the inner chamber appears to rapidly become very negative with respect to the outer chamber before a saturated dark potential is reached at pH 3.7. However, when the inner aqueous solution pH is higher than 6, a rapidly increasing positive membrane dark potential can be seen before its saturated value at pH 8. Figure 3 and the above description indicate that the resulting curve of this experiment is a typical titration curve. There are two probable pKa values existing for this curve; one is 4 and the other 6.7.

H^+ ion dependence on Chl-BLM photo-emf has been measured through the whole pH range of KCl aqueous solution. This light-induced membrane potential is shown as a solid line in Figure 3. Figure 4 is the graph of Chl-BLM photo-emf versus concentrations of H^+ (10^{-2} M) added to the inner chamber. Figure 5 is an alternative graph from Figure 4, obtained by plotting BLM photo-emf versus the pH difference of outer KCl aqueous solution from the inner. It is found that Chl-BLM

has a maximum photo-emf at pH 4 or ΔpH 1.35; this photo-emf is about 9 to 12 mV, with H^+-containing side becoming more negatively charged.

b. The General Characteristics of $FeCl_3$ in the Chl-BLM Photoeffects

The study of the electrical properties of bimolecular lipid membrane in the presence of $FeCl_3$ in aqueous phase has attracted increased attention recently. The importance of this study is not only due to its existence in the biological significance [MacDonald & Thompson, 1972], but also its facilitation of membrane stability [Tien & Kobamoto, 1969]. Chlorophyll itself has its hydrophilic group set in the hydrophilic portion of lipid BLM, and its hydrophobic group in the hydrophobic region. The addition of Fe^{+3} probably makes this charge fixation more exact. BLM made from lecithin-cholesterol-decane has been reported by MacDonald et al. [1972] and shows the resistance drop by a factor of 10^5 to 10^6 with the addition of micrograin quantities of $FeCl_3$ to the aqueous phase. It is suggested that a drop in membrane resistance might be due to the decrease of the interaction strength between $FeCl_3$ and the membrane/solution interface when iron hydrolysis takes place. Loxsom and Tien [1972] observed that in the light-induced event, a Chl-BLM photo-emf with 60 to 70 mV can be produced as light is exposed to the Chl-BLM, where $FeCl_3$ is set in one side of the membrane.

Since BLM with $FeCl_3$ has gradually become well known in the study of membrane associated phenomena, a better understanding of the general properties of $FeCl_3$ in the bimolecular lipid membrane system is necessary before such a system can be used to further investigations and applications.

Figure 3. Chl-BLM light and dark potentials versus the pH of KCl (10^{-1} M/l) aqueous solution in the inner chamber, while the pH of KCl aqueous solution in the outer chamber is held at constant value of 5.5

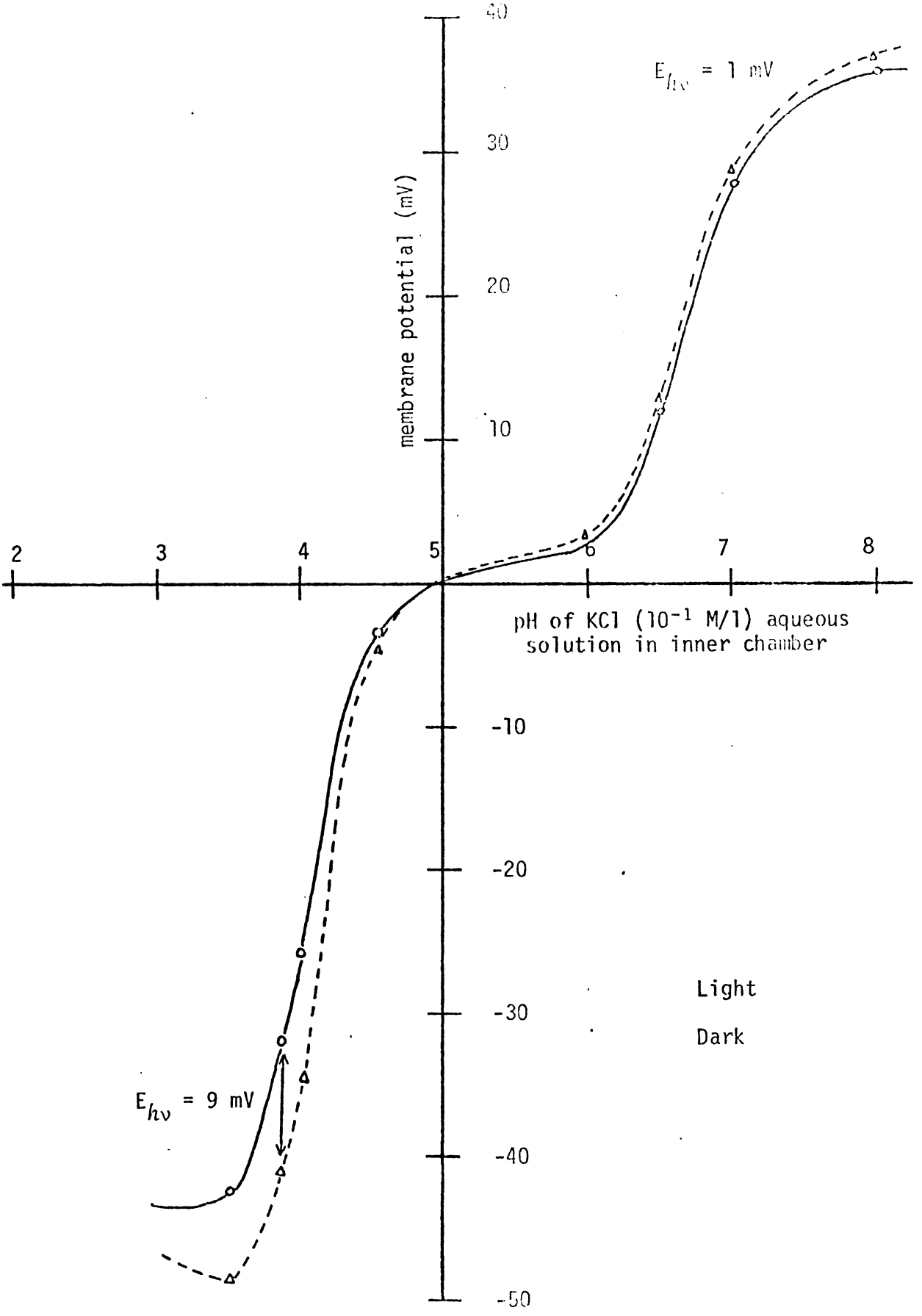

40
30
20
10
-10
-20
-30
-40
-50
membrane potential (mV)
2
3
4
5
6
7
8
pH of KCl (10^{-1} M/l) aqueous solution in inner chamber
$E_{h\nu}$ = 1 mV
$E_{h\nu}$ = 9 mV
Light
Dark

Figure 4. Chl-BLM photo-emf (mV) versus HCl concentration which has been added to the BLM inner chamber. The KCl (10^{-1} M/l) aqueous solution in BLM outer chamber has a constant pH of 5.5. Here 6 second light illumination is applied. Chl-BLM photo-emf is obtained by subtracting Chl-BLM light potential from the dark potential.

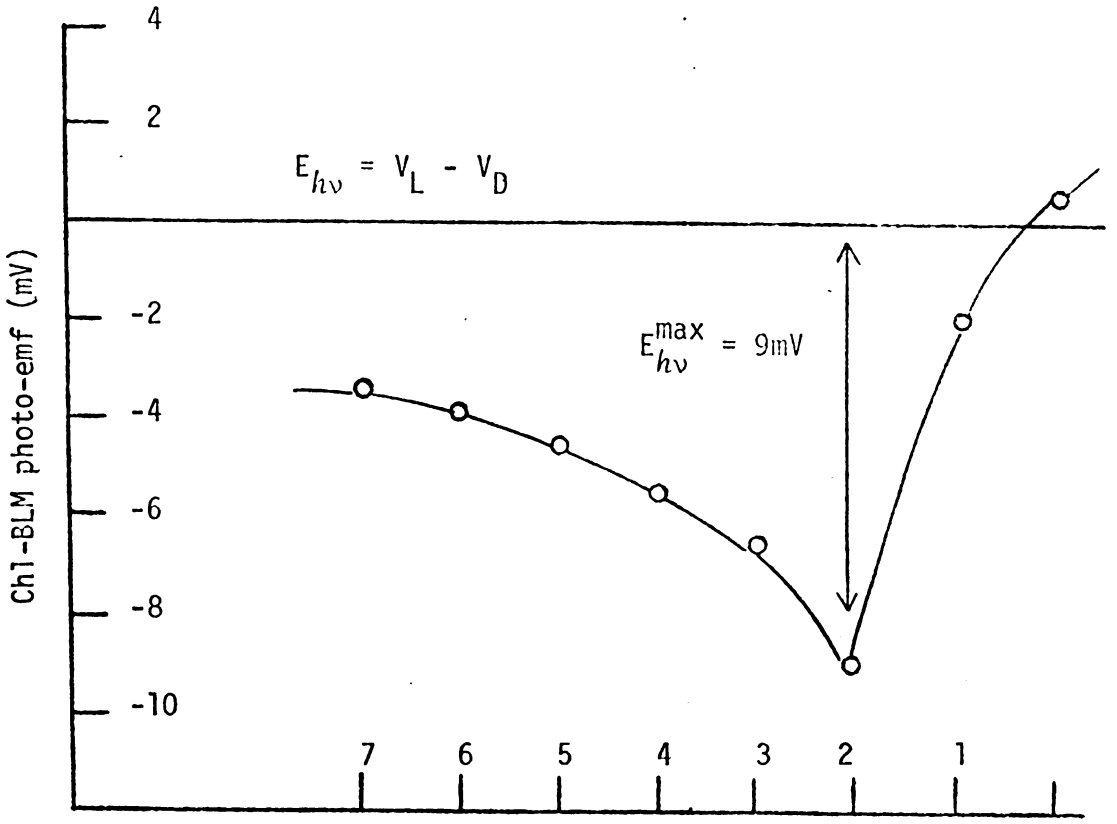
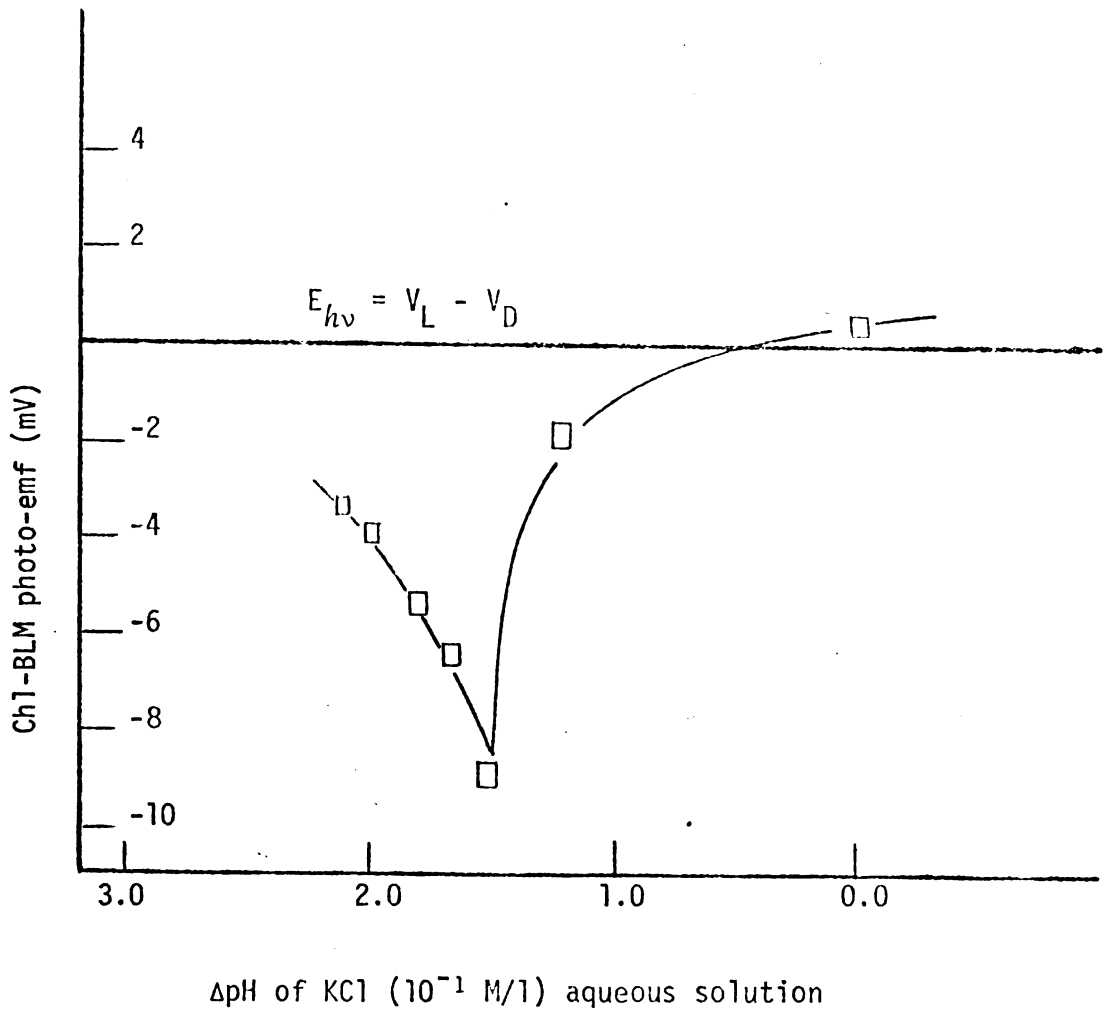

4
2
-2
-4
-6
-8
-10
$E_{h\nu} = V_L - V_D$
$E_{h\nu}^{max}$ = 9mV
Chl-BLM photo-emf (mV)
7
6
5
4
3
2
1
HCl concentration (x 10^{-4} M/l) in inner chamber
while outer chamber at constant pH.

Figure 5. Chl-BLM photo-emf (mV) versus the change of KCl pH in the inner chamber by the addition of HCl (10^{-4} M/l), while the pH of KCl solution in outer chamber has the constant value of 5.5. The maximum Chl-BLM photo-response is about 9 to 12 mV when the KCl solution in inner chamber has pH around 3.85 to 4.


4
2
$E_{h\nu} = V_L - V_D$
-2
-4
-6
-8
-10
Chl-BLM photo-emf (mV)
3.0
2.0
1.0
0.0
ΔpH of KCl (10^{-1} M/l) aqueous solution

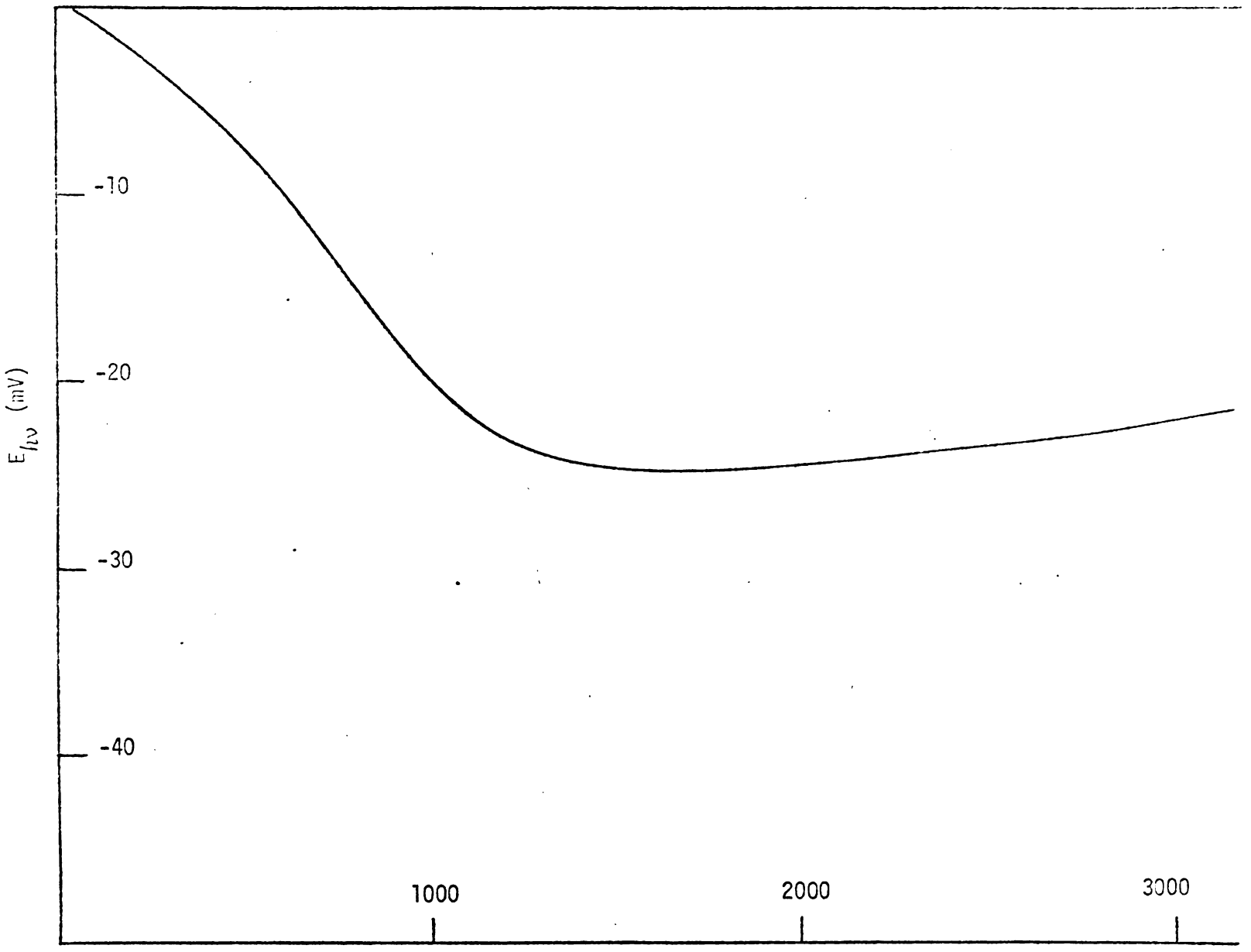
The general properties of $FeCl_3$ in Chl-BLM system which still are not well known will be included such as: (1) how long it will take the Chl-BLM to reach its maximum photo-emf response after $FeCl_3$ has been added to the system; (2) what the alternate light and duration time effects upon Chl-BLM light-induced emf are; (3) what the Chl-BLM photo-emf will be when either buffer acetate of KCl is the aqueous phase of the system. (Do both cases of Chl-BLM photo-emf have different response values? What does $FeCl_3$ concentration dependence on Chl-BLM photo-emf response look like?); (4) how to obtain the Chl-BLM absolute photo-emf which is merely due to Fe^{+3} ion itself and; (5) whether Chl-BLM dark resistance also is dependent on the amount of $FeCl_3$ presented. All these properties will be investigated in this section.

1) Time dependent measurement upon Chl-BLM photo-response in the presence of $FeCl_3$. The purpose of this study is to find the equilibrium time for the photo-response in this particular system; i.e., the time of maximum photo-emf response in the system.

$FeCl_3$ (10^{-3} M/l) was added to the inner chamber of BLM cell with NaAc as aqueous solution. At the same time, an equal amount of solution was removed from the inner chamber in order to maintain the same hydrostatic pressure on both sides of the cell. Time was counted by a chart recorder, and six second light duration was applied to the system.

Figure 6 shows Chl-BLM photo-emf versus the time after $FeCl_3$ had been introduced. It was found that the system takes about 1,000 seconds after $FeCl_3$ has been added to reach its equilibrium time of photo-response. This maximum photo-emf is approximately -21 to -26 mV;

Figure 6. Chl-BLM photo-emf as a function of time (second) in dark after $FeCl_3$ (10^{-3} M/l) has been added to the inner chamber. NaAc with pH of 5 was added to both sides of BLM interface.


-10
-20
-30
-40
$E_{h\nu}$ (mV)
1000
2000
3000
Time (second) after Fe^{-3} has been added to NaAc buffer acetate

the negative sign of photo-emf represents the Fe^{+3}-containing side becoming negatively charged in the light. It is also seen from the figure that the photo-emf responses maintain almost constant value at about 1,000 to 3,000 seconds after $FeCl_3$ has been added.

2) Alternate light and duration time onto the Chl-BLM photo-emf in the presence of Fe^{+3} (10^{-3} M) near KCl and NaAc aqueous solution.

i) Duration time measurement

It is known that there are three factors which must determine the quantity of BLM photo-emf response. They are: (1) the light intensity; (2) the distance between light source and the membrane and; (3) time of illumination upon membrane. The first two factors have been measured elsewhere [Tien, 1968b]. Now it seems to be important to know the maximum time of illumination for Chl-BLM with $FeCl_3$ as a system. This experiment has been done by shining light with different times of illumination onto Chl-BLM where $FeCl_3$ (10^{-3} M/l) is set in the inner chamber of BLM cell, and when the aqueous solutions are KCl (10^{-1} M), pH of 5.5. Six seconds, 18 seconds, 30 seconds and 60 seconds as times of illumination are used in this measurement. Figure 7 shows several different photo-emf waveforms with respect to different times of illumination. It is indicated that each waveform, even though it has a different time of illumination, reaches the same amount of maximum photo-emf response. Figure 8 shows the extended waveform with 6 second illumination from Figure 7. This figure clearly indicates that Chl-BLM in the presence of $FeCl_3$ near KCl aqueous phase will reach its maximum photo-emf within 1.5 seconds of illumination time.

Figure 7. Chl-BLM photo-emf patterns in the presence of $FeCl_3$ (10^{-3} M/l) as function of illuminated time (seconds). Every photo-emf seems to reach the same maximum response even though each has different time of illumination.

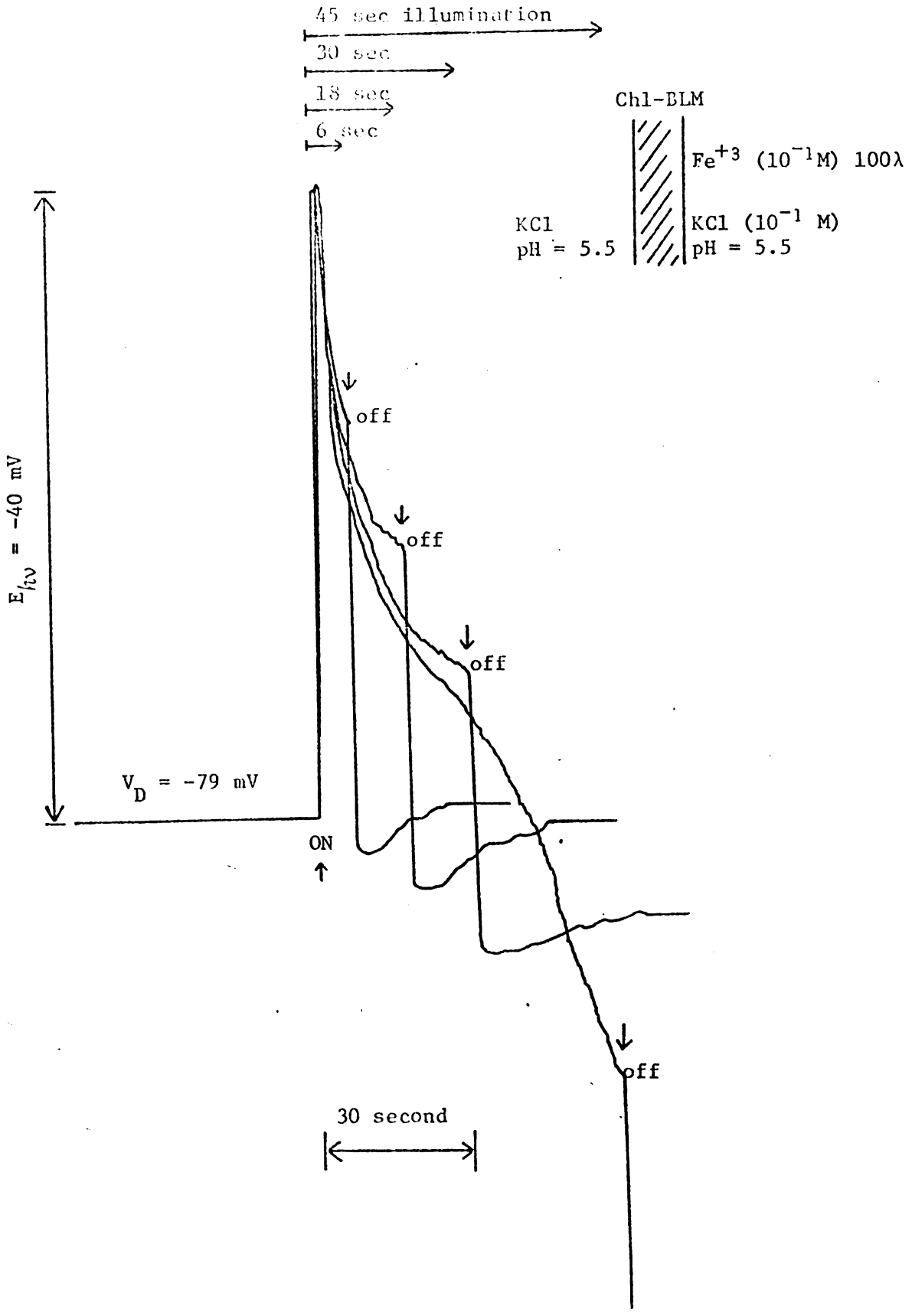

45 sec illumination
30 sec
18 sec
6 sec
Chl-BLM
Fe^{+3} (10^{-1}M) 100λ
KCl
pH = 5.5
KCl (10^{-1} M)
pH = 5.5
$E_{h\nu}$ = -40 mV
off
off
off
off
V_D = -79 mV
ON
30 second

Figure 8. Chl-BLM photo-emf pattern in the presence of $FeCl_3$ (10^{-3} M/l) in inner chamber. KCl (10^{-1} M), pH 5.5 was present in both sides of BLM chamber. This is a 6 second light illumination and the maximum response seems to be reached within 1.5 seconds of light illumination.

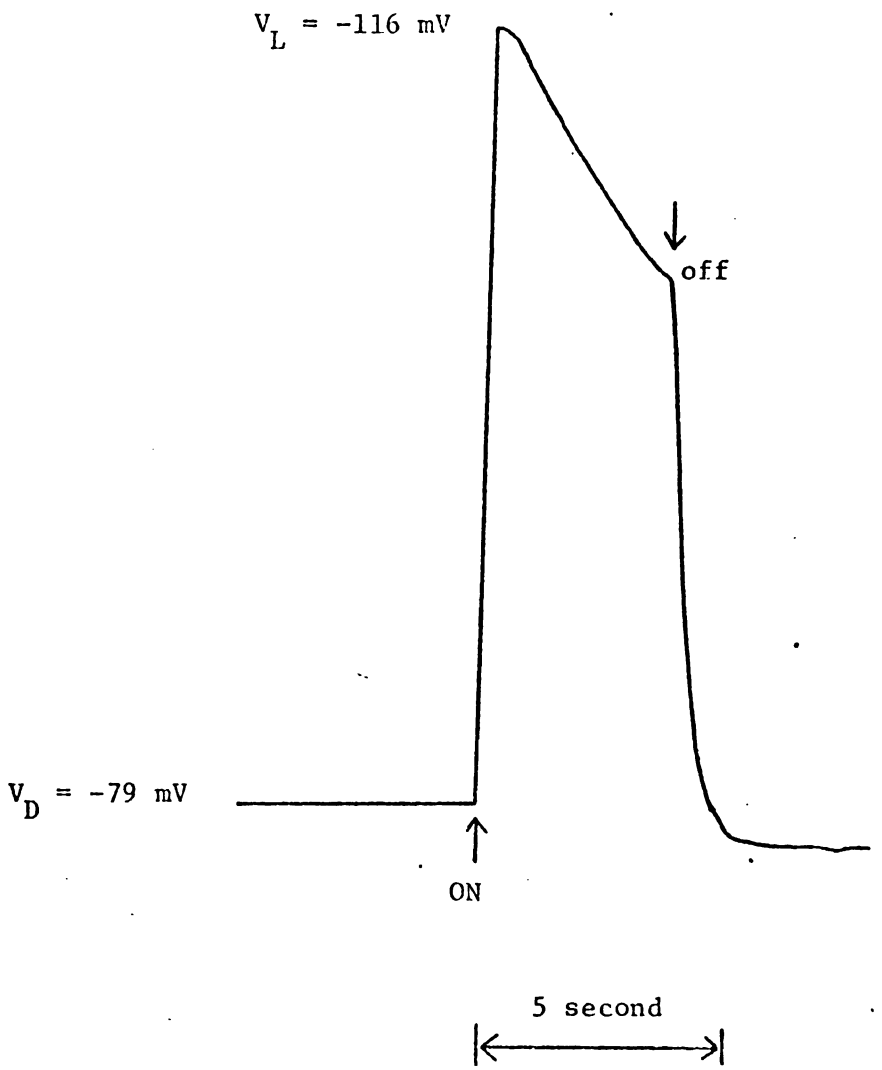

V_L = -116 mV
off
V_D = -79 mV
ON
5 second

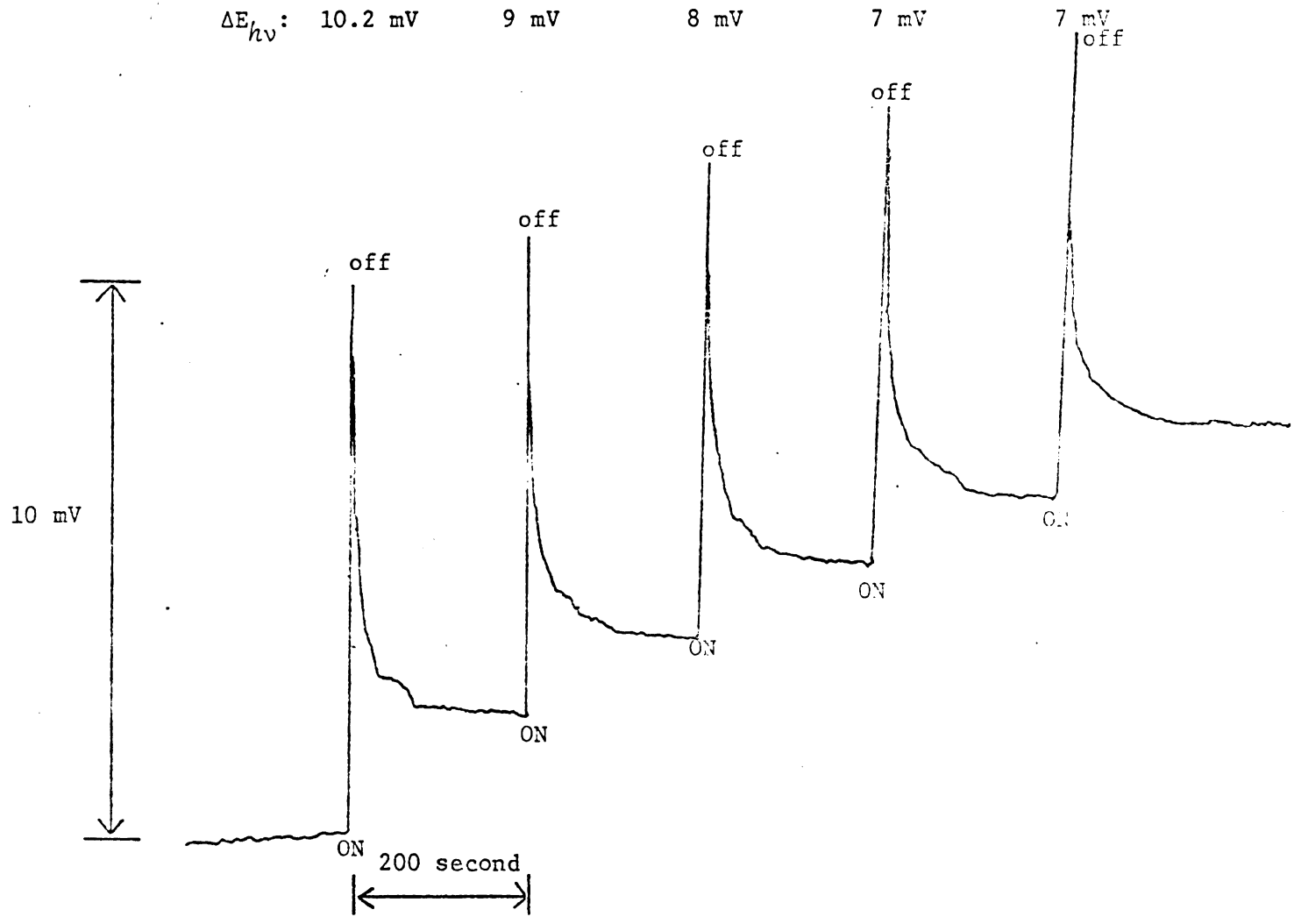
Figure 7 also demonstrates that the slow component of photo-emf waveform increases its rate as the time of illumination increases. It is found that the waveform of Chl-BLM potential after light is off will drop and pass the base line of dark potential toward a less negative value. The longer the illumination time, the greater the potential drop. 60 second illumination time of BLM photo-emf waveform has the fast rate of slow component and the membrane will quite often break near 60 seconds of illuminated time.

ii) Alternate light effect on Chl-BLM photo-emf measurement

Two observations of alternate light effect on Chl-BLM photo-emf have been made, one in KCl aqueous solution phase and the other in sodium acetate buffer solution phase. In the KCl system, light is directed onto Chl-BLM with 6 second illumination. The time interval between two lights is 200 seconds. Figure 9 shows the result of this observation.

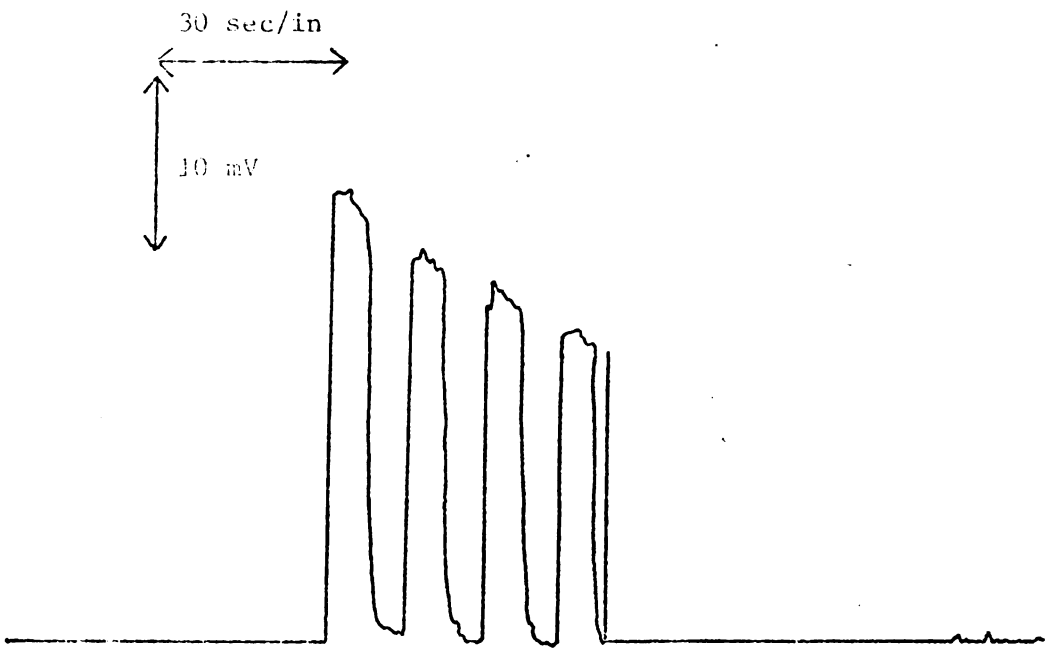
In NaAc system, light is directed onto Chl-BLM with 6 second illumination. The time interval between two lights is 6 seconds. Figure 10 shows the result of this second observation. The main difference between these two measurements is the behavior of dark potential when light is "off". In NaAc system, when light is "off", the potential will drop back almost to the starting line within 6 seconds and prepare for another light duration. In the KCl system, when light is "off", the potential will not drop back down to the starting line; instead, it will stay somewhat higher than the starting potential line for at least 200 seconds. The possible explanation is that, when light is "off", the positive charge (hole) and the negative charge (electron) produced by light will completely recombine in the

Figure 9. Chl-BLM photo-emf as a function of alternate light in the presence of $FeCl_3$ (5×10^{-3} M/l) in the inner chamber near KCl (10^{-1} M/l) aqueous solutions. Six second illumination is applied and 200 seconds in dark is allowed between illuminations.



Time after Fe^{+3} has been added in KCl (10^{-1} M)	200 sec	400 sec	600 sec	800 sec	1000 sec

Figure 10. Chl-BLM photo-emf as a function of alternate light in the presence of $FeCl_3$ (5×10^{-3} M/l) in inner chamber. NaAc (10^{-1} M/l) aqueous solution is used. Thirty seconds of illumination and 30 seconds in dark between lights.


30 sec/in
10 mV

NaAc system, but not in the KCl system. As has been described before, in the KCl system the H^+ in the Fe^{+3}-containing side always tries to some degree to attract the electrons produced in light and prevents the complete recombination of these electrons with light-generated H^+ in the other side.

3) $FeCl_3$ concentration effect on Chl-BLM photo-emf measurement.

$FeCl_3$ was introduced into one of the two aqueous KCl (10^{-1} M) solutions separated by Chl-BLM, with both sides at pH 5. Six seconds of illumination was directed onto the Chl-BLM for every 500 second interval in the dark between two different amounts of $FeCl_3$. Figure 11, curve a gives the relation between $FeCl_3$ concentration and Chl-BLM photo-emf response. It shows that the Chl-BLM will have the maximum photo-emf of -53 mV at Fe^{+3} (2×10^{-4} M/l). The negative sign of photo-emf here means that the Fe^{+3}-containing side becomes more negatively charged in light.

Two important phenomena have been observed in this experiment: (1) the H^+ in $FeCl_3$ solution can cause the additional Chl-BLM photo-emf for the system. Figure 11, curve b is the Chl-BLM photo-emf versus pH change of the aqueous solution in BLM inner chamber caused merely by the addition of H^+. Figure 12 is the comparable graph from Figure 11, which represents Chl-BLM photo-emf versus the concentration of $FeCl_3$ and eliminates the amount of Chl-BLM photo-emf due to H^+ ion. (2) A significant Chl-BLM dark potential can be created after the addition of $FeCl_3$ and a negative sign of potential on the $FeCl_3$-containing side. This resulting dark potential gradient is presumably due to the diffusion of Chl-BLM sensitive H^+ from the $FeCl_3$-containing side to the other side. The driving force is the pH gradient of two

Figure 11. Chl-BLM photo-emf as a function of $FeCl_3$ concentration (10^{-5} M/l). The pH of inner chamber which corresponds to each $FeCl_3$ concentration is shown in the bottom scale. Curve b is Chl-BLM photo-emf versus pH change of KCl aqueous solution in inner chamber when H^+ replaced $FeCl_3$.

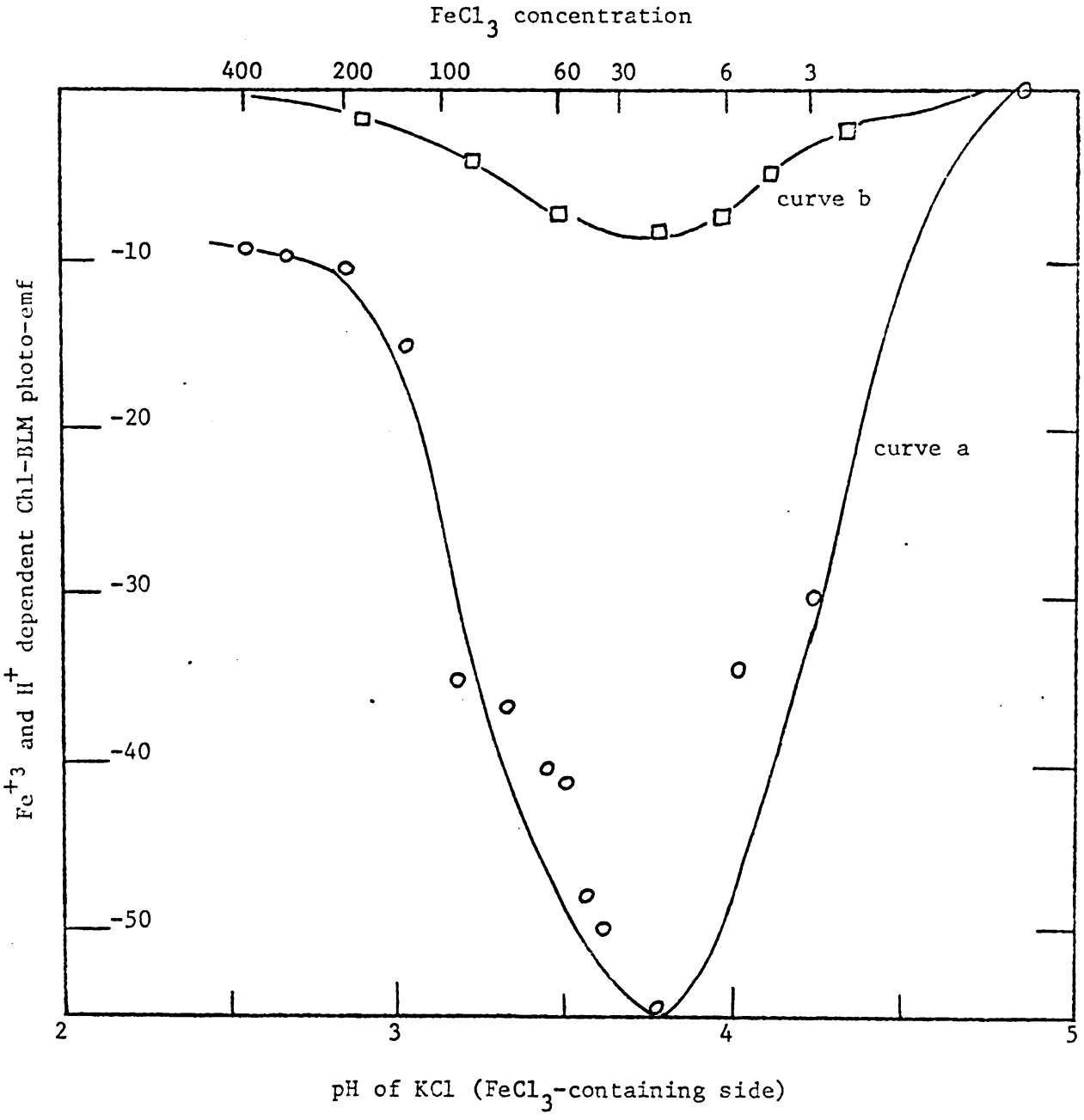
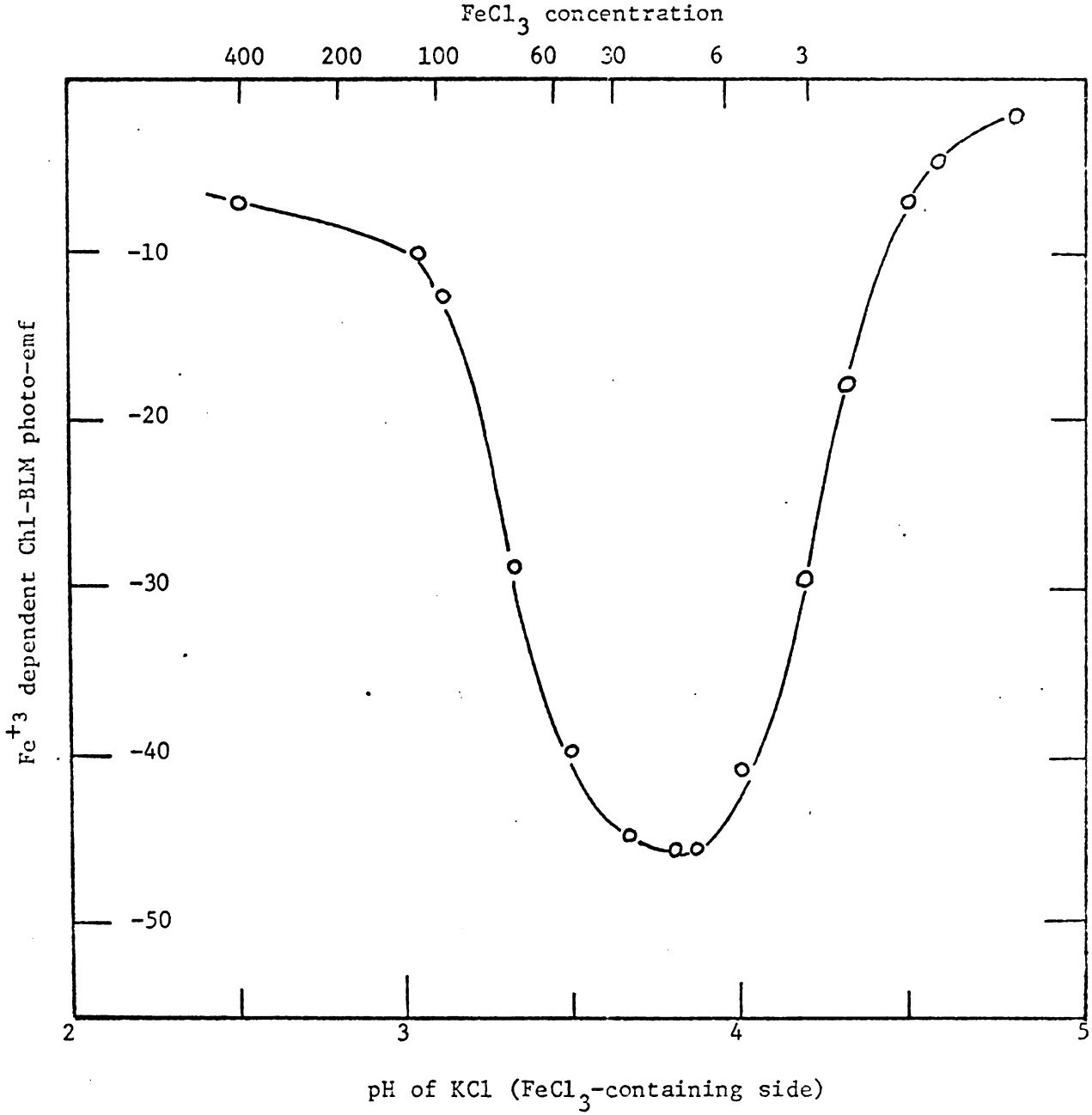

FeCl3 concentration
400
200
100
60
30
6
3
curve b
curve a
-10
-20
-30
-40
-50
Fe+3 and H+ dependent Chl-BLM photo-emf
2
3
4
5
pH of KCl (FeCl3-containing side)

Figure 12. Chl-BLM photo-emf as a function of $FeCl_3$ concentration in KCl aqueous solution only, while H^+ (contained in $FeCl_3$ solution) dependent photo-responses are eliminated. This graph is obtained simply by the subtraction of curve a in Figure 12 from curve b.


FeCl3 concentration
400
200
100
60
30
6
3
-10
-20
-30
-40
-50
Fe+3 dependent Chl-BLM photo-emf
2
3
4
5
pH of KCl (FeCl3-containing side)

aqueous phases of Chl-BLM. The pH of the inner BLM chamber becomes lower with the addition of $FeCl_3$ to this side. Figure 13 is a curve which shows this non-linear relationship between Chl-BLM dark potential and the pH change of the aqueous solution in the inner chamber, after the addition of $FeCl_3$. The maximum dark membrane potential is about 85 mV and lies near pH 3.8. The importance of this dark potential lies in its field direction opposite to the facilitation of negative charge which moves toward Fe^{+3}. This results in the apparent magnitude of Chl-BLM photo-emf being much less than the true photo-emf. One could obtain the absolute Chl-BLM photo-emf which is merely due to $FeCl_3$ concentration by reducing this dark potential toward zero under the application of some external voltages. Figure 14 represents the membrane dark potential independent Chl-BLM photo-emf versus $FeCl_3$ concentration curve. Chl-BLM exhibits a maximum photo-emf of 107 mV near $FeCl_3$ (2×10^{-4} M/l). It seems that this photo-emf is simply the summation of Chl-BLM apparent photo-emf and the Chl-BLM photo-emf induced by the $FeCl_3$ created dark potential.

4) Chl-BLM dark resistance in the presence of $FeCl_3$ near KCl aqueous solution. The effect of $FeCl_3$ concentration on BLM dark resistance has been reported. The experimental evidence indicates that BLM resistance drops in the presence of $FeCl_3$ concentration around 10^{-7} to 10^{-5} M. It also has been found that oxidized cholesterol BLM membrane resistance has the lower value around the aqueous solution with pH from 4 to 6, and increases the magnitude when pH is above 6 or below 4. There are two questions still unanswered: (1) What does the BLM, especially Chl-BLM, dark resistance look like in the entire wide range of $FeCl_3$ concentrations? (2) How does one interpret it?

Figure 13. Chl-BLM dark potential as a function of KCl pH change in inner chamber in the presence of various amounts of $FeCl_3$. The original pH of KCl in inner chamber before the addition of $FeCl_3$ was 5.3. The maximum membrane dark potential was 85 mV at KCl pH of 3.85 to 4.

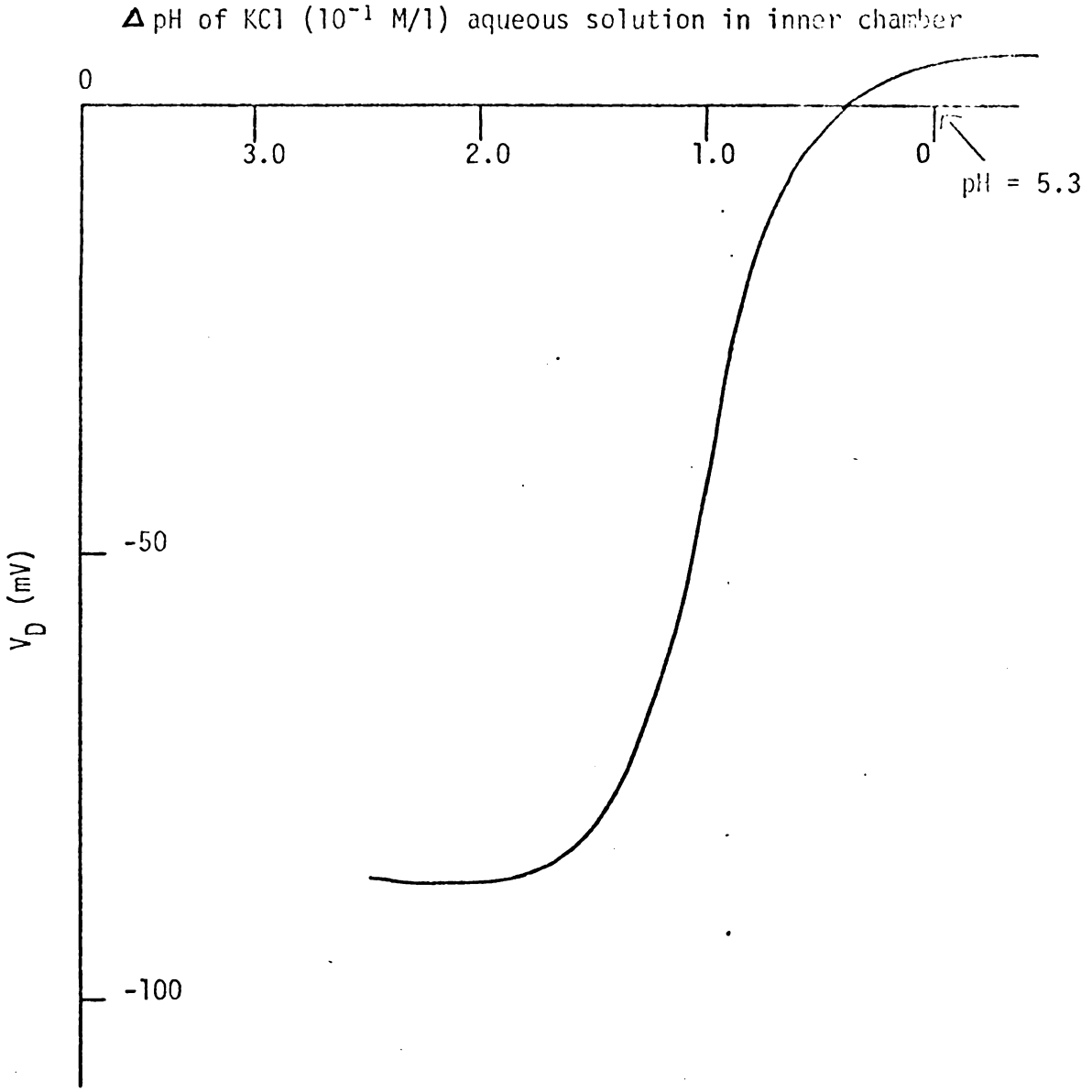
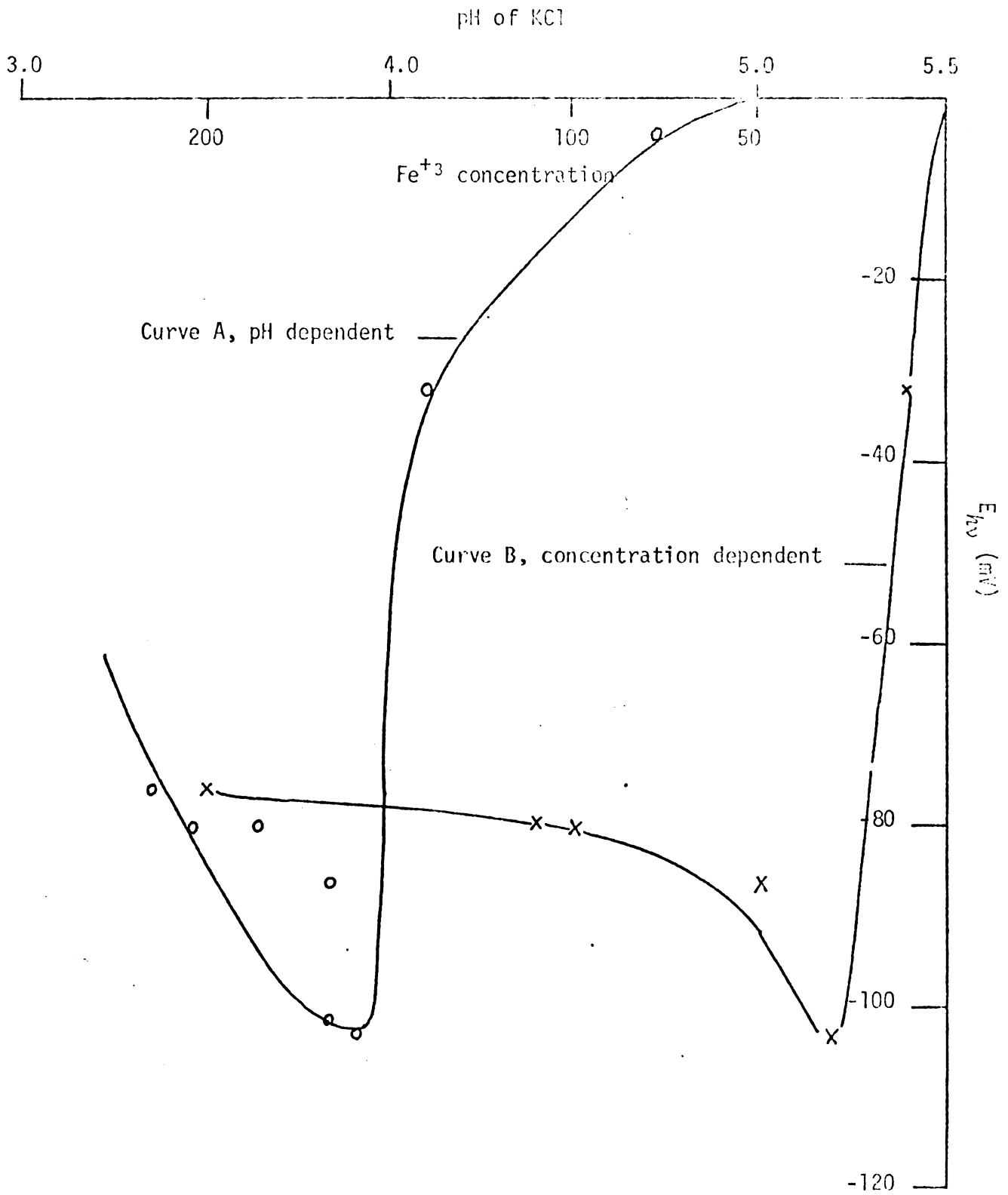

Δ pH of KCl (10^{-1} M/l) aqueous solution in inner chamber
0
3.0
2.0
1.0
0
pH = 5.3
-50
-100
V_D (mV)

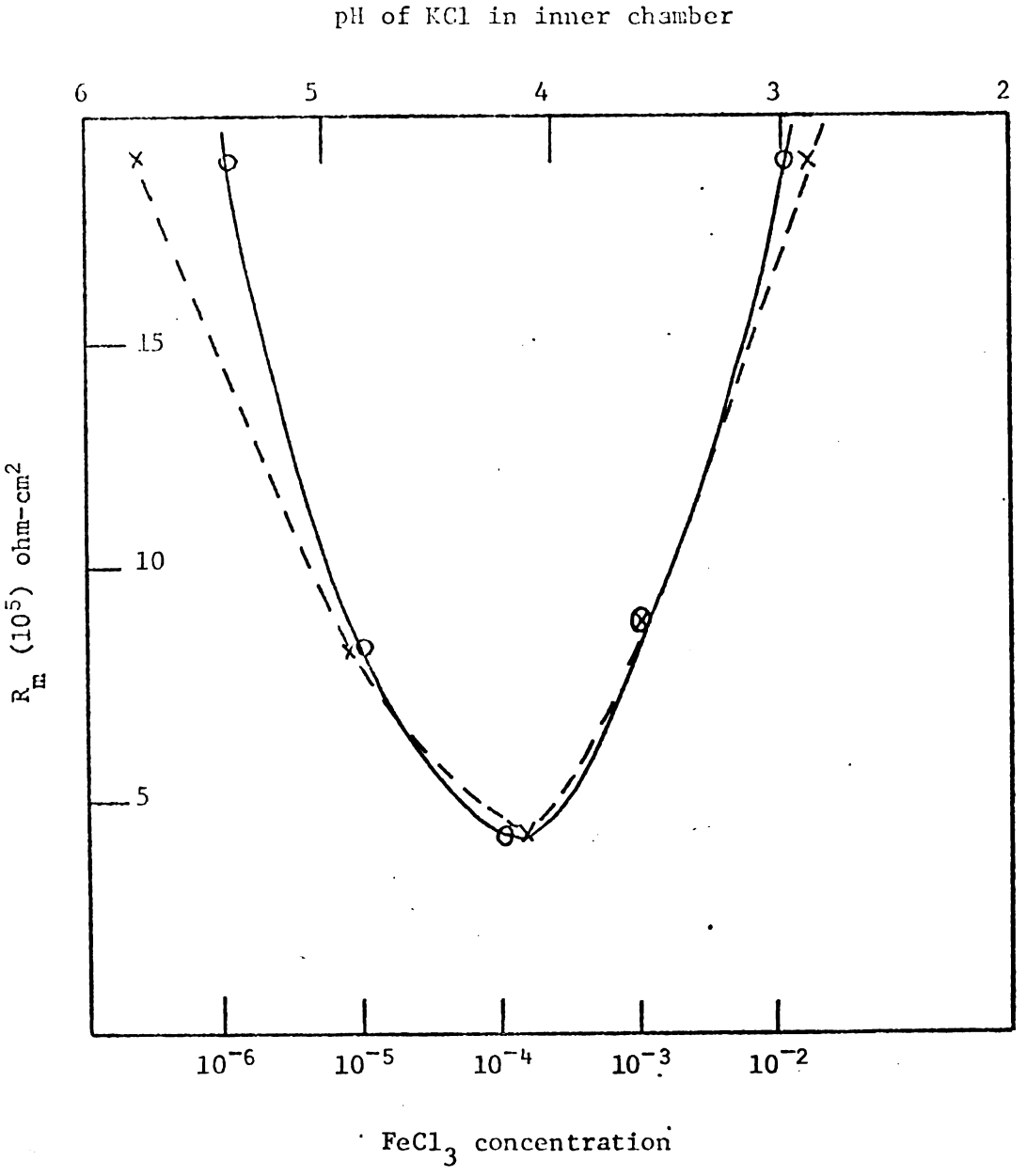
Figure 14. Membrane dark potential independent Chl-BLM photo-emf as a function of $FeCl_3$ (10^{-5} M/l) concentration in inner chamber (as shown in curve B). Curve A is the membrane dark potential independent Chl-BLM photo-emf versus pH of KCl in inner chamber by the addition of $FeCl_3$ to this chamber.


pH of KCl
3.0
4.0
5.0
5.5
200
100
50
Fe^{+3} concentration
Curve A, pH dependent
Curve B, concentration dependent
-20
-40
-60
-80
-100
-120
E_{h_v} (mV)

In this experiment, the Chl-BLM dark resistances have been observed in the wide range of $FeCl_3$ concentration (from 10^{-2} to 10^{-6} M). Figure 15 is the plot of Chl-BLM resistance versus $FeCl_3$ concentration. The result of Chl-BLM resistance versus the pH change of KCl in the inner chamber by the addition of $FeCl_3$ solution has been plotted in the same figure. This indicates that the Chl-BLM resistance, when $FeCl_3$ (10^{-4} M) is introduced, has the lowest resistance (4.4×10^4 ohm-cm^2). This is a resistance value about one hundred times lower than that in the absence of $FeCl_3$, where Chl-BLM resistance is 1.1×10^6 ohm-cm^2. In the case where $FeCl_3$ (10^{-3} M) is present, Chl-BLM has tenfold lower resistance than that in the absence of $FeCl_3$. Chl-BLM dark resistance decreases with the addition of $FeCl_3$ in the range of 10^{-6} M to 10^{-4} M, but will increase with $FeCl_3$ concentrations from 10^{-4} to 10^{-2} M. The plot of Figure 15 also indicates that the Chl-BLM resistance exhibits the lowest value when the inner chamber KCl solution has its pH reduced to 4 by the addition of $FeCl_3$ to this chamber. The Chl-BLM resistance drops from pH 6 to 4, but increases again from pH lower than 4. These results are quite consistent with those of MacDonald et al. It is suggested that high membrane resistance formed from the strong binding of Fe^{+3} and negative polar group of p-lipid or pigment will drop if this binding complex is hydrolyzed near the solution/BLM interface. The quantity of resistance drop will depend upon the degree of binding of the complex and its hydrolysis. From pH 6 to 4, the more complex present, the more chance of hydrolysis, and the larger the decrease in BLM resistance. Therefore, this hydrolysis is written as

$$Fe^{+3}(H_2O)_4 \xrightarrow{\text{hydrolysis}} Fe^{+2}(H_2O)_3(OH) + H^+$$

Figure 15. Chl-BLM dark resistance versus the concentration of $FeCl_3$ in the inner chamber. The upper scale is the relative pH of KCl aqueous solution in inner chamber after the addition of $FeCl_3$ to this chamber. pH of KCl solution in outer chamber is kept at constant value around 6.

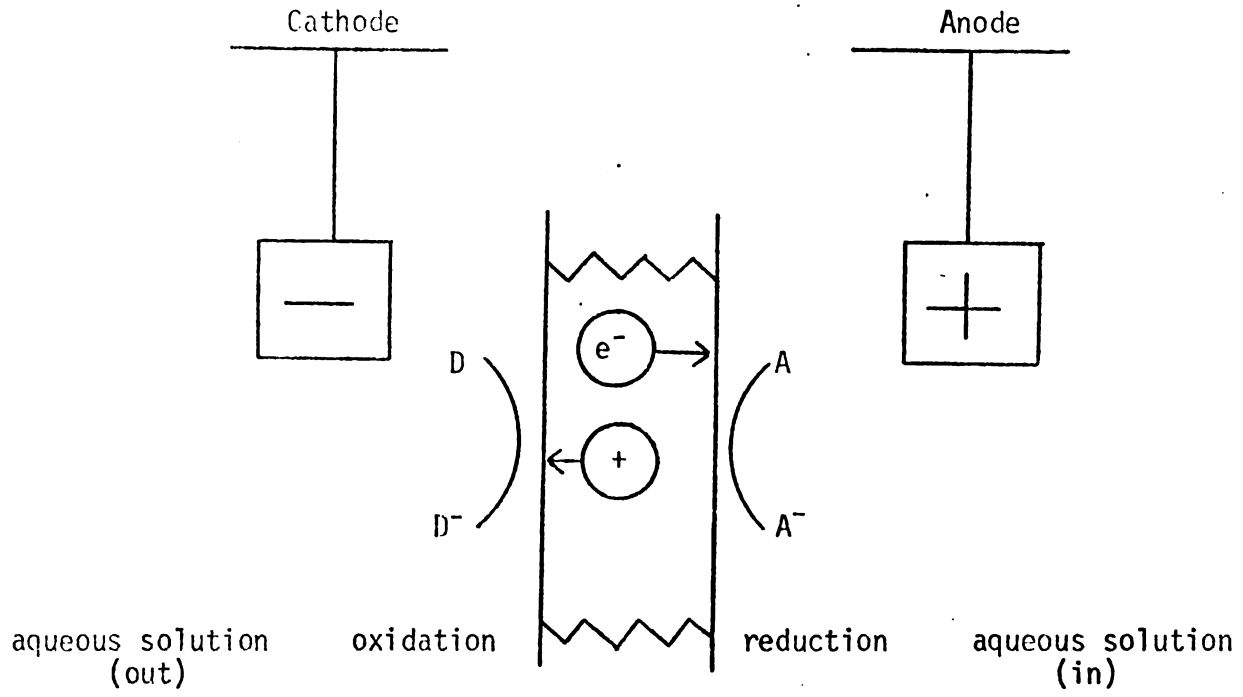

pH of KCl in inner chamber
6
5
4
3
2
15
10
5
R_m (10^5) ohm-cm^2
10^{-6}
10^{-5}
10^{-4}
10^{-3}
10^{-2}
$FeCl_3$ concentration

It seems that this hydrolyzed rate will decrease in the low pH of solution/BLM interface, the BLM resistance increasing in the low pH below 4. When pH is above 6, $FeCl_3$ will be hydrolyzed before it has the chance to form a complex and this $Fe(OH)_3$ will not affect BLM resistance to any degree.

c. The Importance of Chl-BLM Dark Potentials and Their Effect Upon its Photo-emfs

It is known that Chl-BLM illuminated by light can generate charges and also make charge separations when an electric field exists. Such a field can be either externally applied or chemically induced. The direction of the polarity of this charge separation will depend on the direction of the electric field. Figure 16 is a schematic diagram to show this relationship in Chl-BLM system. The stronger the positive sign in anode, the greater the tendency of negative charges to move toward the membrane/solution interface facing this anode. Similarly, the stronger the negative sign in cathode, the greater is the tendency of positive charges to move toward the membrane/solution interface facing the cathode. It is strongly suggested that the direction of the polarity of this charge separation will depend mainly on the direction of the electric field. When the chemicals, which are not only capable of diffusion through the membrane to create the electric field but also capable of electron donating or accepting, are present, then the polarity of this charge separation may vary to some degree or even change its direction as compared to that merely due to the electric field. For example, the pH gradient in aqueous phases across Chl-BLM and the presence of NaI asymmetrically in Chl-BLM aqueous phase are two representative cases which will be discussed in this section.

Figure 16. Mechanism of redox reactions across BLM. A - metal ion or electron acceptor, A^- - reduced, D - anion or electron donor, D^+ - oxidized, e^- - electron, + - positive hole; where anode is the electrode with positive sign which is the result of oxidation reaction in electrode. Cathode is the electrode with negative sign which is the result of reducing reaction in electrode. The charges generated by light in membrane have the tendency to move with electrons toward positive electrode and positive holes toward negative electrode. The side electrons move toward will be reduced and the side positive holes move toward will be oxidized.


Cathode
Anode
D
D⁻
e⁻
+
A
A⁻
aqueous solution
(out)
oxidation
reduction
aqueous solution
(in)

1) Electric field induced by externally applied voltages. The presence of an electric field across the Chl-BLM caused by externally applied voltages has been found to make the membrane more photo-responsive. The experimental conditions chosen were as follows. The Chl-BLM was formed in 10^{-1} M acetate buffer with both sides at pH 5. The alternate sign of external voltages was applied and the external resistor was set at 10^7, 10^8 and 10^9 Ω. Six second light duration was directed onto the Chl-BLM after the membrane dark potential had been steady. Figure 17 is the plot of Chl-BLM photo-emf versus membrane potentials. A general conclusion from Figure 17 may be noted.

i) At the same value of membrane dark potential, the amount of Chl-BLM photo-response will depend on the application of external resistor. The magnitude of this response follows the order:

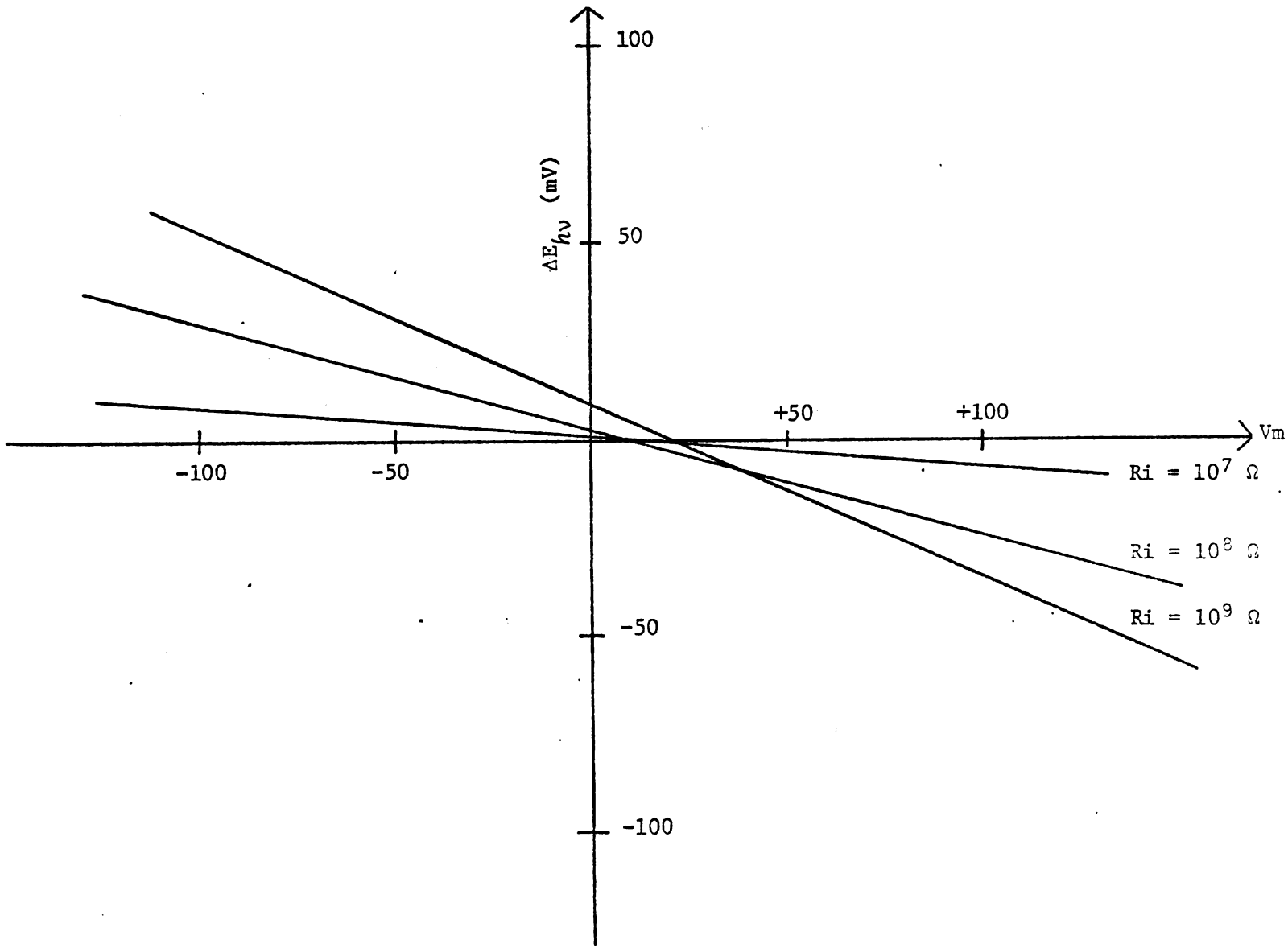
Chl-BLM with Ri = 10^9 Ω > Chl-BLM with Ri = 10^8 Ω > Chl-BLM with Ri = 10^7 Ω

ii) At applied voltages of 20 to 30 mV and above, or below zero mV, the photoresponses are all monophasic. The response always seems to reduce this electric field in such a way that, when the external electrode is anode, the side of the membrane facing the anode is then negative and vice versa.

iii) The magnitude of Chl-BLM photo-emf in the presence of the electric field seems to be independent of the age of the spinach chloroplast extracts.

2) Membrane potential induced by pH gradient across the membrane. The pH gradient in aqueous solutions across the Chl-BLM that induced the membrane dark potential was found and is shown in Figure 3. This

Figure 17. Chl-BLM photo-emf (mV) versus membrane dark potential (mV). Six second light illumination was used. Sodium acetate buffer (10^{-1} M/l) pH 5, was used as aqueous solutions. Each curve corresponds to a different value of shunt resistance.


100
50
$\Delta E_{h\nu}$ (mV)
+50
+100
Vm
-100
-50
Ri = 10^7 Ω
Ri = 10^8 Ω
Ri = 10^9 Ω
-50
-100

pH gradient is thought to be the driving force of H^+ diffusion across the membrane and, the sign of membrane potential is negative with H^+-containing side. It has been suggested that the moving of negative charges (or electrons) toward the H^+-containing side has been reduced to some extent and this reduction is due to the field direction of the dark potential which has the negative polarity in the side to which this negative charge moves. A general characteristic of Chl-BLM photoresponse in the presence of membrane potential generated by pH gradient can be described as follows.

i) The photoresponses are all monophasic and the H^+-containing side always becomes more negatively charged. This indicates that the electron accepting strength of H^+ is much stronger than that of the electric field.

ii) The hyperpolarization of membrane light potential indicates that H^+ is a sufficiently strong electron acceptor so as to be able to avoid the depolarization of membrane in light by the dark membrane electric field.

iii) It has been shown that the Chl-BLM H^+ dependent photo-emf would be enhanced if one could diminish this dark membrane potential to zero mV.

iv) Chl-BLM dark potential induced by a pH gradient is not linearly related to the pH change, but increases exponentially and reaches saturated values near pH of 3 to 4.

3) Electric field induced by chemical diffusion across the Chl-BLM. The Chl-BLM dark potential (or electric field) can also be induced by the diffusion of chemicals. Sodium iodide was used as a representative chemical compound in this experiment. NaI was added to the

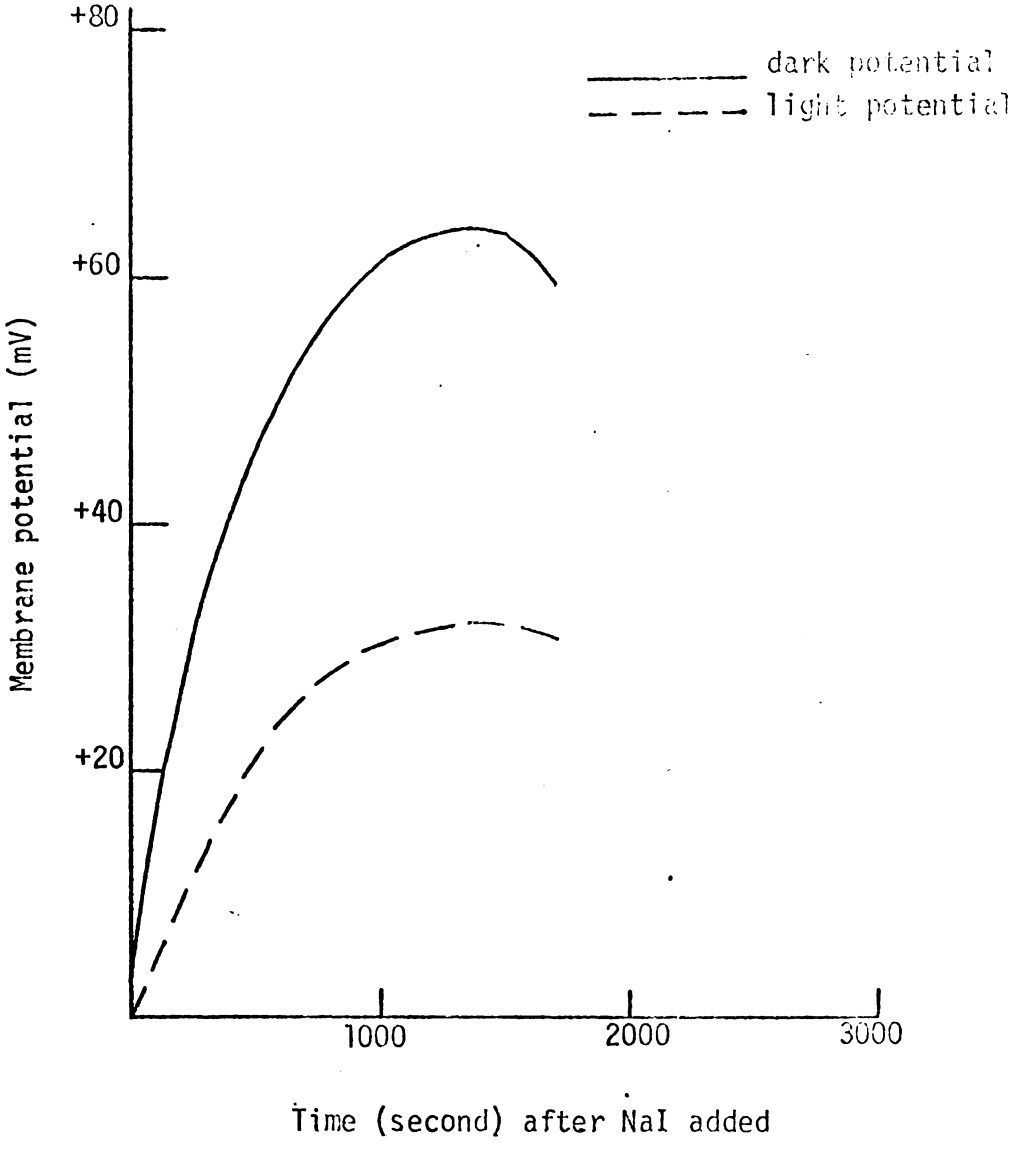
aqueous phase of the inner chamber while buffer acetate was added to both sides of the BLM cell. The sign of dark potential, with the NaI-containing side positive, indicates that this potential is generated by the diffusion of iodide ion (negative charge) from inner to outer chamber. No observable pH change in the inner chamber was found with the addition of NaI. Figure 18 is the plot of Chl-BLM dark and light potential versus time (second) after NaI (10^{-3} M) was introduced. It indicates that the Chl-BLM dark and light potentials both arrive at their maximum values 1,000 seconds after the addition of NaI compound. NaI concentration effect on Chl-BLM photo-emf measurement also shows that Chl-BLM has a maximum photo-response of about 40 mV in the presence of NaI (5×10^{-4} M/1).

2. Investigation of Chemical Compounds' Electron Donating and Accepting Power by the Measurement of Chl-BLM Photo-responses and Their Enhancements

Light induced photo-emf on certain bilayer lipid membranes containing chlorophylls and/or xanthophylls has been reported [Tien, 1968 b, c]. These light-induced photo-emfs have been explained by means of charge carrier (electrons and holes) production and separation in the BLM by light. It was assumed, under certain circumstances, that one side of the biface would be oxidizing and the other reducing [Tien, 1968 b, c]. The experimental evidence and further work of enhancing BLM photo-emf have recently been reported [Tien and Verma, 1970].

There are two important questions in this area remaining to be answered: a) From a theoretical point of view, if some chemical

Figure 18. Chl-BLM light and dark potential as a function of time in dark after the addition of NaI (10^{-3} M/l) to inner chamber. Sodium acetate buffer (10^{-1} M/l) with pH of 5 was used as aqueous solution.


+80
+60
+40
+20
Membrane potential (mV)
dark potential
light potential
1000
2000
3000
Time (second) after NaI added

elements can enhance BLM photo-response, can they be systemized into a table like that of standard redox potential table of chemical compounds in aqueous solution? b) How can the most efficiency be attained in coupling a good electron donor and acceptor in order to have the maximum Chl-BLM photo-emf enhancement. It has been known that Chl-BLM is simply a barrier, separated by two aqueous media, which is designed to facilitate energy conversion such as occurred in nature. It can be used to convert light energy into electrical energy, or chemical energy. This is the energy needed for the chemical event occurring near the BLM surface. Now the question is, if the chemical event is a type of redox reaction, in the presence of chemical species, then is it possible to systemize the electron donating or accepting power of these chemical species? The next question is how to obtain the most efficient use of light energy. This question can be answered as soon as the chemicals' redox power table, mentioned above, has been set up. Then, by the proper coupling of a good electron donating compound with a good electron accepting compound, a significant enhancement of Chl-BLM photo-emf can be expected. The higher BLM photo-response, the more efficient the system is. The present chapter summarizes the experimental evidence and attempts to answer the questions raised above.

The experimental set-up for observing this photovoltaic effect and photo-emf enhancement is similar to that previously described (see Figure 1). An external battery and shunt resistor are also used in the close-circuit photo-emf measurement. In addition, a reference electrode was chosen as a standard to detect the redox power of test compounds present near the membrane surface. This standard system consists of a

pair of calomel electrodes across the BLM which separates two aqueous phases of buffer acetate (10^{-1} M) at pH 5. The outside chamber contained (5×10^{-4} M/l) ferric chloride fresh solution. In more detail, the characteristics of this reference electrode system are as follows:

i) aqueous phases -- buffer acetate (10^{-1}) pH at 5;

ii) membrane composition -- spinach chloroplast extracts;

iii) outside chamber containing -- $FeCl_3$ (5×10^{-4} M/l);

iv) time needed in dark before $[E_{h\nu}]_{Fe^{+3}}$ measurement -- 1000 seconds;

v) time interval in dark between $[E_{h\nu}]_{Fe^{+3}}$ and $[E_{h\nu}]_{Fe^{+3}+X}$ -- 500 seconds;

vi) time interval in dark for each additional measurement -- 200 seconds;

where $[E_{h\nu}]_{Fe^{+3}}$, the standard Chl-BLM photo-emf for this reference electrode, is about 15 ± 3 mV with $FeCl_3$-containing side negative. $[E_{h\nu}]_{Fe^{+3}+X}$ is the Chl-BLM photo-emf for this reference electrode, plus the chemical species present in the opposite side of $FeCl_3$.

The general procedure for the experiment (survey the electron-donating or accepting power of chemical compounds) was: i) measure Chl-BLM open-circuit photo-emf 1,000 seconds after the addition of $FeCl_3$ (5×10^{-4} M/l) to the outside BLM chamber. This is $[E_{h\nu}]_{Fe^{+3}}$; ii) add the test compound to the inner BLM chamber. The concentration of test compound will be considered for only two cases, for complete water soluble compound which concentration will be made up at 10^{-1} M, and for water insoluble compound which will be made up at saturated level; iii) measure Chl-BLM photo-emf 500 seconds after the addition of the test compound. This is $[E_{h\nu}]_{Fe^{+3}+X}$; iv) measure Chl-BLM

photo-emf 200 seconds after every additional amount of test compound;
v) subtracting $[E_{h\nu}]_{Fe^{+3}} + X$ from $[E_{h\nu}]_{Fe^{+3}}$, one obtains $\Delta[E_{h\nu}]$ which is the photo-emf with respect to the reference redox potential $[E_{h\nu}]_{Fe^{+3}}$ as zero.

The electron donating or accepting power of the test compound will be determined by the above measurement. The test compound will be an electron donor when Chl-BLM has a photo-emf larger than that of the reference electrode or $\Delta[E_{h\nu}] > 0$. Alternately, the test compound will be an electron acceptor when Chl-BLM has a photo-emf less than that of the reference electrode, or $\Delta[E_{h\nu}] < 0$.

An attempt at Chl-BLM photo-emf enhancement can be made by coupling some good electron donors with ferric chloride in the opposite side and by studying the maximum value of Chl-BLM photo-emf response.

The Chl-BLM dark membrane potential created either by the presence of a chemical compound in the aqueous phase, or applied externally, has been found to have a very crucial influence upon Chl-BLM photo-response. The detail of this phenomenon has been found and described in the previous section. The purpose of this section is to look at the Chl-BLM photo-emf in the presence of chemical compound at membrane dark potential which has been reduced to zero by a properly applied external source. Additionally, a maximum enhancement of Chl-BLM photo-emf in the presence of a chemical compound under the externally applied source will also be discussed in this section.

The electron-donating or accepting power of a chemical compound can be determined through the measurement of Chl-BLM photo-response. This investigation has been systematically conducted from inorganic to organic compounds, then through some biochemical compounds. Inorganic

compounds will be picked up, in order, from the chloride form of elements in every group of the periodic table.

a. Inorganic Compound Investigations

Group I: All solutions of HCl, LiCl, KCl, NaCl, RbCl and CsCl have been prepared with (10^{-1} M). CuCl and $CuCl_2$ solutions were made in the saturated state.

Results of Chl-BLM photo-emf measurement for elements in this group are collected into Table 1. The order of electron accepting power in group IA is $Rb^+ > Li^+ > Cs^+ > H^+ > K^+ > Na^+$.

Both $CuCl_2$ and CuCl are apparent electron donors in the reference electrode of Chl-BLM system owing to the positive value of $\Delta E_{h\nu}$. This is due to the presence of $CuCl_2$ or CuCl to Chl-BLM which will create quite large dark potential, with Fe^{+3}-containing side becoming more positive, resulting in the facilitation of light-induced negative charge toward the Fe^{+3}-containing side. However, CuCl and $CuCl_2$ have been shown to be electron acceptors when they are present asymmetrically in Chl-BLM system alone, with acetate buffer as aqueous phases at pH 5. In the absence of reference electrode, Chl-BLM will create a large dark potential with the $CuCl_2$-containing side negative or the CuCl-containing side positive.

Group III: Only the chloride of Tl element in this group shows significant Chl-BLM photo-emf enhancement in BLM reference electrode. TlCl appears to be an electron donor in the BLM reference electrode due to the large dark BLM potential generated with the TlCl-containing side negative, which once again causes the system to more readily absorb light-generated negative charges toward Fe^{+3} side. $\Delta E_{h\nu}$ for TlCl case is +46 mV, where BLM dark potential is -23 mV. Some compounds of

TABLE 1

The Chl-BLM Photo-emf in the Presence of Inorganic Substances

Classification	outside compd.	/BLM/inside compd.	V_D (mV)	$E_{h\nu}$ (mV)	$(\Delta E_{h\nu})_x$ (mV)	E^o_{redox} (volt)
Group I	$FeCl_3$	HCl (2×10^{-3}M/1)	12	16	-3	0.00
	(5×10^{-4}M)	LiCl (10^{-3}M/1)	15	10.5	-5.5	-3.046
		KCl (10^{-3}M/1)	3	16	-1	-2.924
		RbCl (10^{-3}M/1)	12	10.5	-8.5	-2.925
		CsCl (10^{-3}M/1)	13.5	12.5	-5	-2.923
		CuCl (2×10^{-2}M/1)	6	53	38	0.522
		$CuCl_2$ ($6\times10^{-}$ M/1)	-16	72	54	0.158
Group III	$FeCl_3$	TlCl (18×10^{-3}M/1)	-23	67	46	0.3363
	(5×10^{-4}M)	Ce^+ (4×10^{-3}M/1)	-13	31	16	1.443
		$LaCl_3$ (5×10^{-3}M)	2	40	14	-----
Group V	$FeCl_3$	NaN_3(13×10^{-3}M)	12	66	46	-----
	(5×10^{-4}M)	Na_2HAsO_4 (15×10^{-3}M/1)	14	44	29	0.58
Group VI	$FeCl_3$	H_2O_2 (30%)	---	---	---	-----
	(5×10^{-4}M)	Na_2S (3×10^{-3}M/1)	-25	-7	-30	-----
		$Na_2S_2O_4$ (5×10^{-3}M/1)	-5	44	15	-----
		$Na_2S_2O_3$ (20×10^{-3}M/1)	9	93	74	0.10
		$CrCl_3$ (3×10^{-3}M/1)	5	13.5	-3.5	-0.41
		$(NH_4)_6Mo_7O_{24}$ (20×10^{-3}M/1)	-8	81	64	-----
Group VII	$FeCl_3$	NaF (10×10^{-3}M/1)	29	9.5	-7.5	-----
	(5×10^{-4}M)	NaBr (3×10^{-3}M/1)	19.5	9.5	-8	-----
		NaCl (3×10^{-3}M/1)	10	11	-5	-----
		NaI (5×10^{-3}M/1)	13	127	114	-----
		I_2(6×10^{-3}M/1)	58	-8	-31	-----
		NaI-I_2 (10^{-3}M/1)	93	-3	-17	-----
Group VIII	$FeCl_3$	$Co(NH_3)_6Cl_3$ (2×10 M/1)	9	7	-15	1.842
	(5×10^{-4}M)	$FeCl_2$ (4×10^{-3}M/1)	8	86	74	-----

elements in Lanthanide series are chosen as representatives for our measurement, such as $Ce(NH_3)(NO_3)_4$ and $LaCl_3$. Ce^{+4} is a strong acidic solution and has a pH of 3.6. Chl-BLM shows a photo-emf of about 25 ± 1.4 mV in the presence of Ce^{+4} (10^{-3} M/l) near buffer acetate aqueous phase. This result indicates that Ce^{+4} performs similarly to Fe^{+3} toward Chl-BLM. However, a positive value of $[\Delta E_{h\nu}]_x$ for Chl-BLM reference electrode in the presence of Ce^{+4} (10^{-3} M/l) has been observed. This is due to the BLM reference electrode dark potential generated from the H^+ ion in Ce^{+4} which causes light-induced negative charges to more easily move toward Fe^{+3}. $LaCl_3$ (5×10^{-3} M/l) has been found to result in a Chl-BLM photo-emf of about 5.3 mV. The polarity of this photo-response indicates that $LaCl_3$ functions as an electron donor. It is a fast response and requires only 1 sec light illumination to reach saturated response. A small negative membrane dark potential with $LaCl_3$-containing side is gradually generated in the presence of $LaCl_3$ to Chl-BLM inner chamber. This similar phenomenon will also be found in the Chl-BLM reference electrode dark potential in the presence of $LaCl_3$. The positive $[\Delta E_{h\nu}]_x$ value with respect to the Chl-BLM reference electrode indicates that $LaCl_3$ is an electron donor to the BLM system. Table 1 lists the numerical values of this $[\Delta E_{h\nu}]_x$.

Group V: NaN_3, $Na_2HAsO_4 \cdot 7H_2O$ are compounds among those elements in Group V which were selected for our measurement. Both compounds exhibit very similar behavior: 1) both give high pH in solution; NaN_3 has pH of 7, Na_2HAsO_4 has pH at 9; 2) both compounds give Chl-BLM reference electrode a positive $[\Delta E_{h\nu}]_x$ value; $[\Delta E_{h\nu}]_x$ = +46 mV in the case of NaN_3 (13×10^{-3} M/l) and $[\Delta E_{h\nu}]_x$ = +29 mV in the case of Na_2HAsO_4 (15×10^{-3} M/l); 3) Chl-BLM reference electrodes show no change

in dark potential in the presence of either NaN_3 or Na_2HAsO_4 solution. The only difference is that, when Fe^{+3} and NaN_3 react, a deep red precipitate with a strong odor can be observed. However, when Fe^{+3} reacts with Na_2HAsO_4, only yellow-white precipitate occurs. Data from these results are listed in Table 1.

Group VI: Compounds of elements O, S, Cr and Mo in this group are listed in our measurement. Compounds Na_2S, $Na_2S_2O_3$, $Na_2S_2O_4$, $CrCl_3$ and $(NH_4)_6Mo_7O_{24}4H_2O$ were prepared with all concentrations at 10^{-1} M in solution, except H_2O_2 (30%) which is directly obtained commercially as solution. Chl-BLM reference electrode photo-emf, in the presence of H_2O_2 measurement, gives no significant variance in its response, which indicates neither electron donating or accepting of this compound. Na_2S is a high pH (=12) solution which was prepared at 10^{-1} M for our measurement. The presence of Na_2S (10^{-3} M/1) on the buffer acetate (pH = 5) of the inner chamber of Chl-BLM will generate a negative membrane dark potential. Figure 19 is a plot of membrane photo-emf versus time (second) at which Na_2S (10^{-3} M/1) has been introduced into BLM. It indicates that membranes composed of either chlorella or spinach all exhibit a maximum photo-response of about 13 to 15 mV between 600 and 1,000 sec after Na_2S has been added. The concentration effect of Na_2S on Chl-BLM photo-emf has been drawn in the Figure 20 which indicates the maximum Chl-BLM photo-response around 20 mV in the presence of Na_2S (3×10^{-3} M/1). The polarity of Chl-BLM photo-emf indicates that Na_2S functions as an electron acceptor.

$Na_2S_2O_4$ (S.D.T.) is a very unstable solution which changes its color very frequently. The following Table lists this solution color change with time after the solution has been prepared.

Figure 19. Chl-BLM or Chlorella-BLM photo-emf as a function of time (second) in dark with Na_2S (10^{-3} M/l) present in the inner chamber. Sodium acetate buffer used as aqueous solutions.

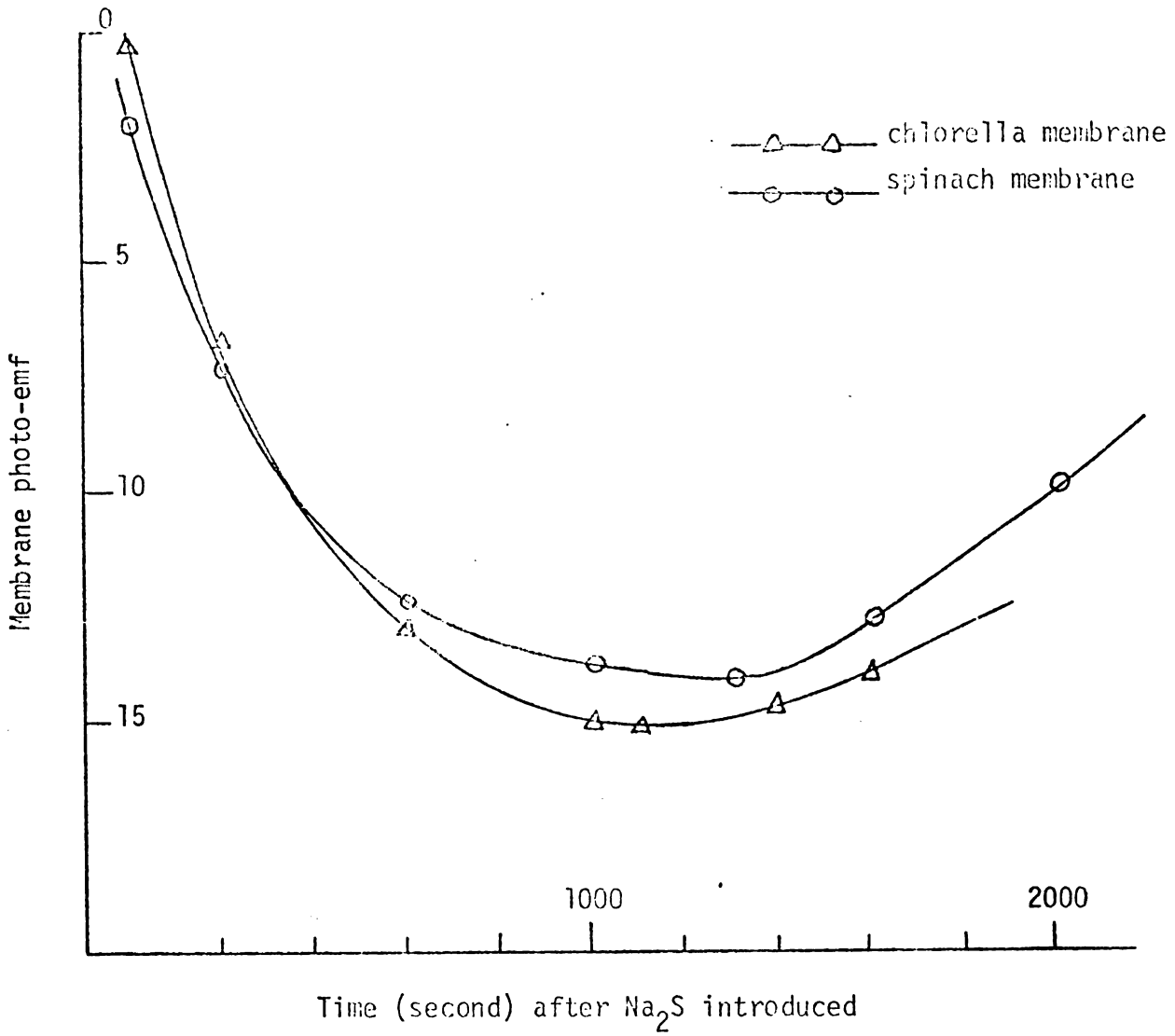

0
5
10
15
Membrane photo-emf
chlorella membrane
spinach membrane
1000
2000
Time (second) after Na_2S introduced

Figure 20. Chl-BLM photo-emf as a function of Na_2S (10^{-3} M/l) concentration near NaAc pH 5. Six second light illumination, and a period of 600 second in dark between two concentration effect measurements.

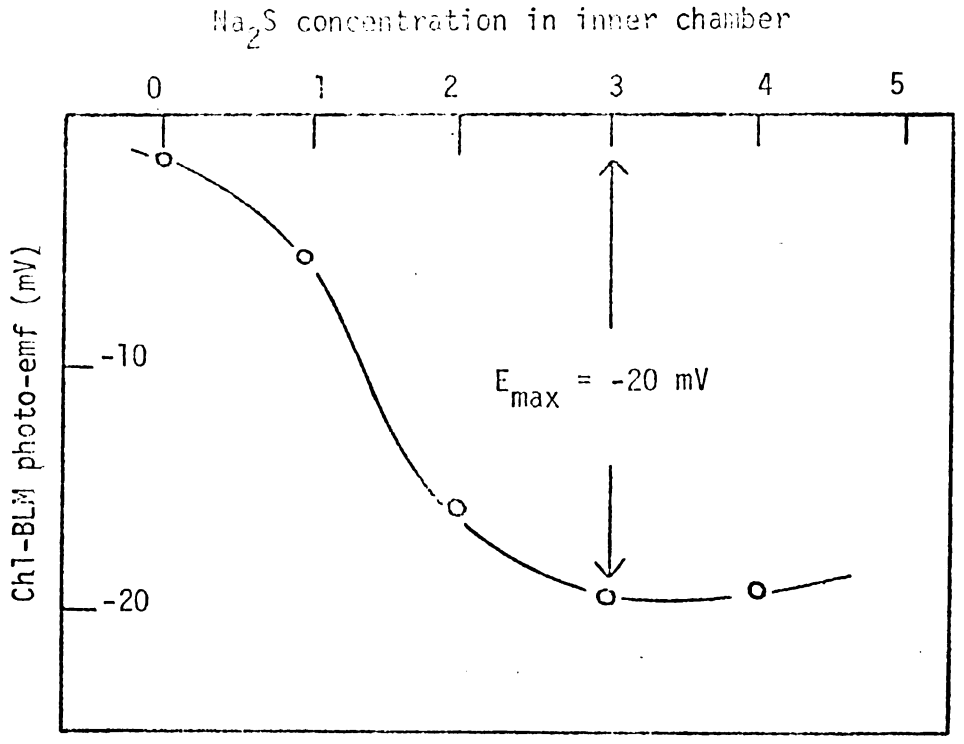
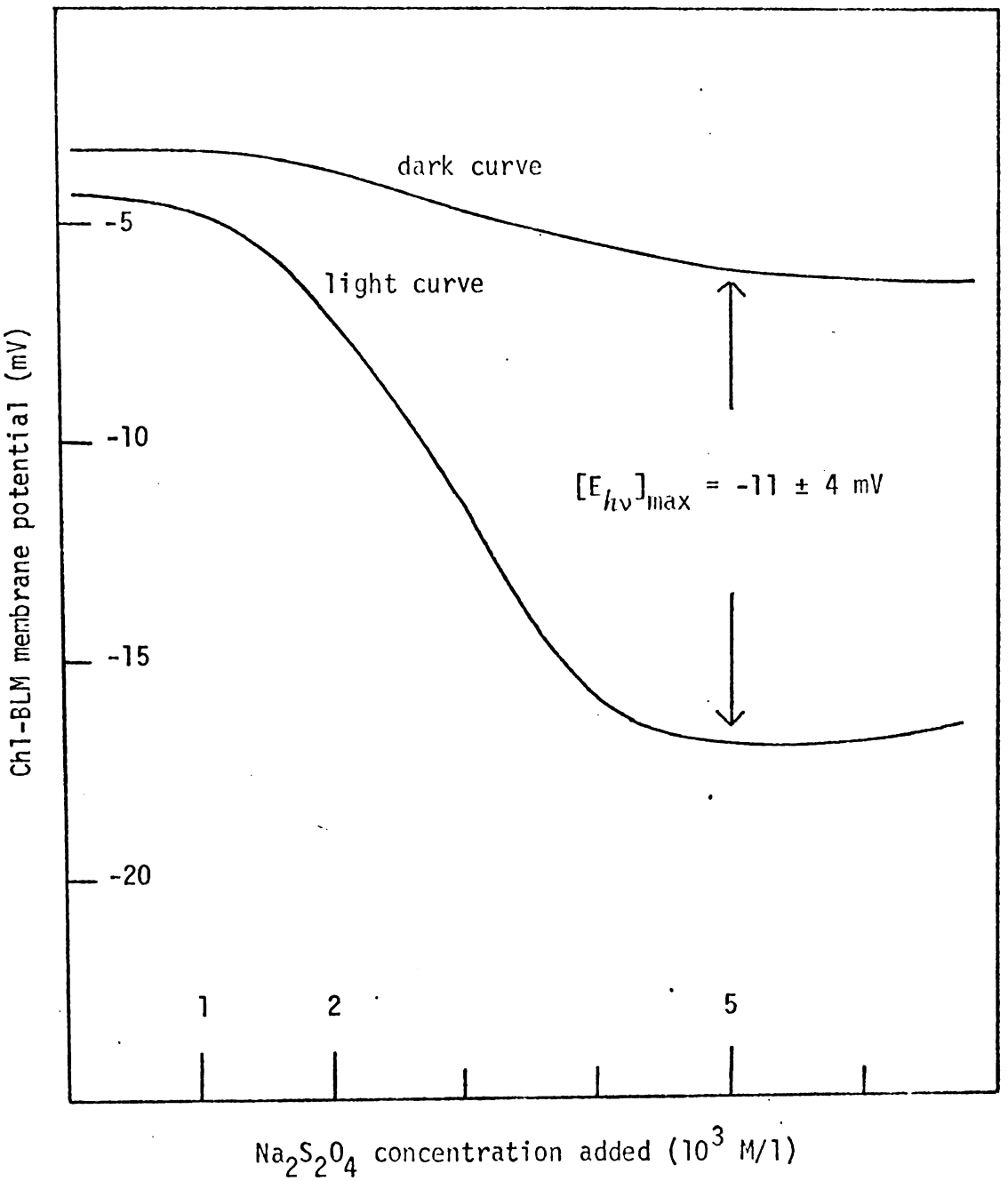

Na_2S concentration in inner chamber
0
1
2
3
4
5
Chl-BLM photo-emf (mV)
-10
-20
E_{max} = -20 mV

TABLE 2

Time (min) after solution has been prepared	Solution color
0 - 0.5	colorless
0.5 - 3	brown
3 - 10	brown - white aqueous
10 - 15	white - cloudy
15 - 60	strong white - cloudy
60 - 80	light white - cloudy
80 - 120	colorless

$Na_2S_2O_4$ solution, in the white-cloudy state only, will cause a Chl-BLM photo-response. Little or no Chl-BLM photo-response can be observed in the other color range. A maximum Chl-BLM photo-emf of about 11 ± 4 mV at $Na_2S_2O_4$ (5×10^{-3} M/l) has been observed. Figure 21 is a plot of this response versus concentration of $Na_2S_2O_4$. The polarity of Chl-BLM photo-emf in the presence of $Na_2S_2O_4$ indicates that $Na_2S_2O_4$ functions as an electron acceptor. This compound is very unstable and is very readily oxidized in the air to give sulphite ions [Wood & Holliday, 1967). The white-cloudy color of $Na_2S_2O_4$ solution is thought to be the existence of these sulphite ions. It is this sulphite ion which absorbs light-generated negative charges from Chl. This experiment also found that Chl-BLM generates a negative dark potential in the $Na_2S_2O_4$-containing side. A positive $[\Delta E_{h\nu}]_x$ value of +15 mV in the presence of $Na_2S_2O_4$ to BLM reference electrode may be due to the polarity of this dark potential which will facilitate the attraction of light-generated negative charges toward Fe^{+3}. $Na_2S_2O_3$ (10^{-3} M/l) is a colorless solution and has a pH of 6.6. A positive

Figure 21. Chl-BLM light and dark potentials as functions of $Na_2S_2O_4$ (10^{-3} M/l) concentration. Sodium acetate buffer was used as aqueous solutions. The maximum photo-response of about 11 ± 4 mV can be observed near $Na_2S_2O_4$ (5×10^{-3} M/l).


dark curve
light curve
-5
-10
-15
-20
$[E_{h\nu}]_{max}$ = -11 ± 4 mV
1
2
5
Chl-BLM membrane potential (mV)
$Na_2S_2O_4$ concentration added (10^3 M/l)

$[\Delta E_{h\nu}]_x$ value of 74 mV has been found for the BLM reference electrode in the presence of $Na_2S_2O_3$ (20×10^{-3} M/l). This demonstrates that $Na_2S_2O_3$ functions as an electron donor in the BLM reference electrode system. $CrCl_3$ (10^{-3} M/l) is a green color solution. A negative $[\Delta E_{h\nu}]_x$ value of about -3.5 mV from BLM reference electrode in the presence of $CrCl_3$ (3×10^{-3} M/l) may indicate that $CrCl_3$ functions as an electron acceptor in the BLM reference electrode system. $(NH_4)_6Mo_7 \cdot 4H_2O$ (10^{-3} M) solution has pH of 5.8. A maximum Chl-BLM photo-emf near buffer acetate in the presence of this solution of about 17 mV has been observed, where $(NH_4)_6Mo_7 \cdot 4H_2O$ solution functions as an electron donor. A positive $[\Delta E_{h\nu}]_x$ value of BLM reference electrode in the presence of $(NH_4)_6Mo_7O_{24}$ of about +64 mV also strongly supports the electron-donating property of $(NH_4)_6Mo_7O_{24}$ solution. Negative BLM reference electrode dark potential in the presence of $(NH_4)_6Mo_7O_{24}$ has been found. General conclusive results of compounds of this group in the BLM system are listed in Table 1.

Group VII: Sodium salts of F, Cl, Br, and I in this group were prepared at 10^{-3} M/l. The importance of membrane dark potential to Chl-BLM photo-response is very significant, especially when compounds of this group are present in the BLM system. The presence of NaF (3×10^{-3} M/l) gives a Chl-BLM photo-emf of about 2 to 3 mV. The polarity of this Chl-BLM photo-emf demonstrates that this compound functions as an electron donor. A membrane dark potential (F^--containing side positive) of approximately 0 to 10 mV has been observed in the presence of this compound. A negative $[\Delta E_{h\nu}]_x$ value of about 7.5 mV in the presence of this compound to BLM reference electrode may indicate the electron accepting property of this NaF. This result

is just opposite to the above finding (NaF functions as an electron donor). The difference is due to the generated BLM reference electrode dark potential in the presence of NaF which polarity is against the facilitation of light-generated negative charges being attracted by Fe^{+3}. NaBr (10^{-3} M/l), when present alone in Chl-BLM, gives Chl-BLM maximum photo-emf of about 2 mV in acetate buffer system. NaBr here functions as an electron donor. However, a negative $[\Delta E_{h\nu}]_x$ value of 8 mV from the BLM reference electrode in the presence of NaBr indicates that NaBr functions as an electron acceptor which is opposite to the above finding. This again is due to the large BLM reference electrode dark potential (Br^--containing side positive) generated by the presence of NaBr solution, which is present in such a way as to be against the facilitation of light-generated negative charges being attracted by Fe^{+3} (similar to NaF case). A similar result has been found for NaCl compound. This $[\Delta E_{h\nu}]_x$ is 5 mV (negative value) in the presence of NaCl (3×10^{-3} M/l) to BLM reference electrode. NaI (10^{-3} M) exhibits completely different behavior compared to other halogen compounds (F^-, Br^-, Cl^-). This shows electron accepting property in the measurement of Chl-BLM photo-emf near NaAc when NaI is present alone. This photo-emf is 10 to 20 mV in the presence of NaI (10^{-3} M/l), where membrane dark potential is around 50 to 60 mV with the NaI-containing side positive. However, the positive $[\Delta E_{h\nu}]_x$ value of about 114 mV for BLM reference electrode in the presence of NaI (5×10^{-3} M/l) indicates that NaI functions as an electron donor in the BLM reference electrode, even though the large membrane dark potential (NaI-containing side positive) is generated. I_2 solution is prepared at saturated state. A negative $[\Delta E_{h\nu}]_x$ value of about -31 mV has been

found in the presence of I_2 to BLM reference electrode, which demonstrates the electron accepting property of I_2 in BLM reference electrode system. General characteristics of compounds in this group toward BLM reference electrode are listed in Table 1.

Group VIII: $Co(NH_3)_6Cl_3$, and $FeCl_2$ are among compounds of this group which have been tested. $Co(NH_3)_6Cl_3$ is a water-soluble compound and has pH of 6.6. It is known to have electron accepting properties similar to $FeCl_3$: 1) time dependent measurement - Chl-BLM shows maximum photo-response 600 seconds after the addition of $Co(NH_3)_6^{+3}$ to BLM cell; 2) concentration effect measurement - a measurement of Chl-BLM photo-emf in the presence of varying concentrations of $Co(NH_3)_6^{+3}$ and, the maximum response is about 21 mV, while $Co(NH_3)_6^{+3}$ is (2×10^{-3} M/1); 3) rise-time of photo-response - Chl-BLM photo-emf will reach its maximum response rapidly in 1 1/2 seconds of light illumination; 4) Chl-BLM photo-emf enhancement - over 100 mV of Chl-BLM photo-emf can also be obtained by coupling $Co(NH_3)_6^{+3}$ (10^{-3} M/1) and Oolong tea, catechin and tannic acid near NaAc (10^{-1} M) at pH 5; 5) extreme unstability of Chl-BLM in the presence of $Co(NH_3)_6^{+3}$ and tannic acid in the light. Chl-BLM will break immediately in 1 to 3 second light illumination in the presence of $Co(NH_3)_6^{+3}$ and tannic acid. This breakdown light potential is near 120 mV. When Chl-BLM dark potential, in the presence of $Co(NH_3)_6^{+3}$ and tannic acid, is raised to 160 mV by external variable voltage, immediate rupture of the Chl-BLM can be observed. Both cases imply that Chl-BLM has a break-down voltage of approximately 120 to 160 mV (This is the voltage at which BLM can be maintained before light-generated species in membrane rupture it). A negative $[\Delta E_{h\nu}]_x$ value of about -15 mV from BLM reference electrode

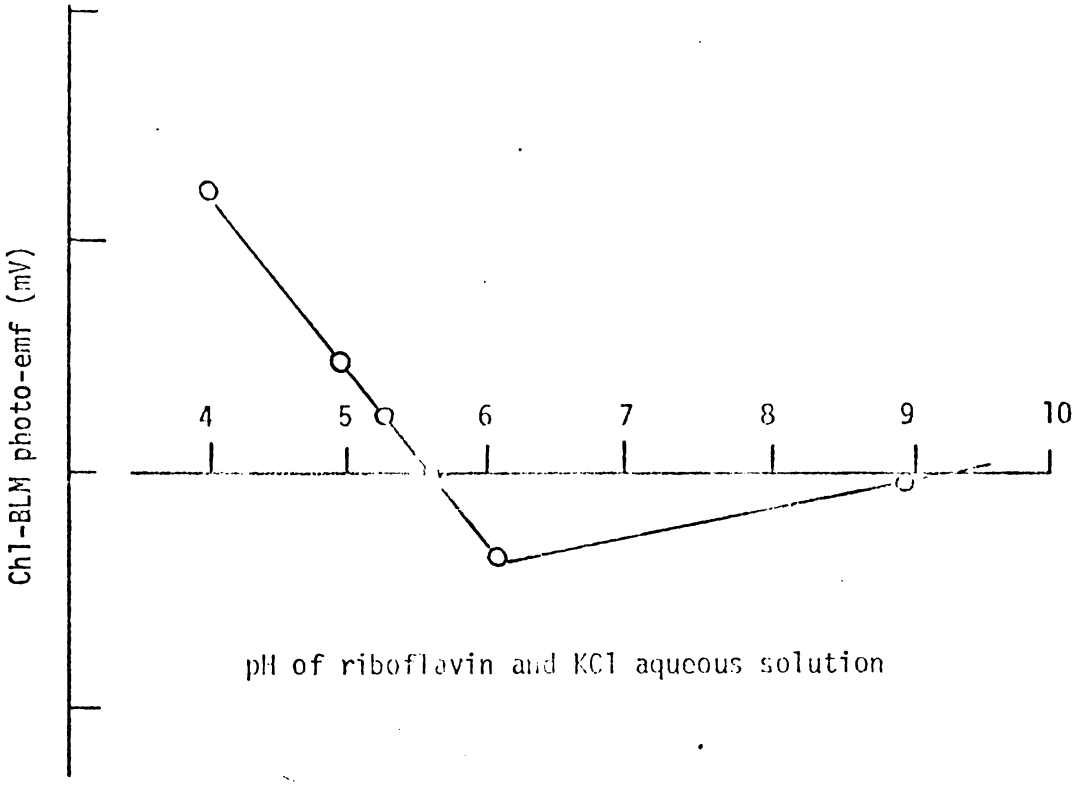
in the presence of $Co(NH_3)_6^{+3}$ (10^{-3} M/1) implies that $Co(NH_3)_6^{+3}$ functions as an electron acceptor in the BLM reference electrode system. No varying of BLM reference electrode dark potential has been observed in the presence of this compound. $FeCl_2$ (10^{-3} M/1) is the reduced form of $FeCl_3$ and is relatively unstable; the measurement has to be done within 2 hours after this fresh solution has been made. Concentration effect of $FeCl_2$ on Chl-BLM photo-emf near NaAc pH 5 measurement implies that $FeCl_2$ functions as an electron donor and that Chl-BLM has a maximum photo-response of about 6 mV in the presence of $FeCl_2$ (10^{-3} M/1). General characteristics of this group of compounds in Chl-BLM reference electrode are listed in Table 1.

b. Organic Compound Investigations; Quinone-like (Wurster salt) Compounds: Riboflavin, Benzoquinone, Hydroquinone and Quinhydrone

Riboflavin is only partially soluble in H_2O (1,2 mg/10 ml at 27.5°C and 1,9 mg/10 ml at 40°C); the riboflavin solution for our measurement was prepared by dissolving 2 mg riboflavin powder in 10 ml KCl at various pH values (4, 5, 6, 7 and 9). Results of pH dependent riboflavin electron-donating-accepting property on Chl-BLM photo-emf measurement are plotted as Chl-BLM photo-emf versus pH of KCl aqueous phase in the presence of riboflavin (Figure 22). It is found that Chl-BLM has positive photo-emf (riboflavin-containing side positive) when riboflavin and KCl aqueous phase pH is below 5.5, and has negative photo-response when riboflavin and KCl aqueous phase pH is above 5.6. This implies that riboflavin functions as an electron donor when pH is below 5.5 and as an electron acceptor when pH is above 5.6.

Quinhydrone should have behavior analogous to that of riboflavin. In acidic solution, it dissociates into a mixture of benzoquinone and

Figure 22. Chl-BLM photo-emf as a function of the pH of riboflavin and KCl aqueous solution. Riboflavin solution is prepared by dissolving 2 mg into 10 ml KCl at each desired pH.


Chl-BLM photo-emf (mV)
4
5
6
7
8
9
10
pH of riboflavin and KCl aqueous solution

hydroquinone. At high pH, benzoquinone dominates the mixture and Chl-BLM photo-emf measurement results in the benzoquinone-containing side having negative polarity. At low pH, hydroquinone dominates the mixture and Chl-BLM photo-emf measurement results in the hydroquinone-containing side having positive polarity. Nevertheless, the existing membrane dark potentials in the presence of quinhydrone solution or hydroquinone solution sometimes have made this determination difficult. A Chl-BLM reference electrode was necessary for our determination. A negative $[\Delta E_{h\nu}]_x$ value of about 4 mV for BLM reference electrode in the presence of benzoquinone (2.8×10^{-4} M/l) implies that benzoquinone functions as an electron acceptor. A positive $[\Delta E_{h\nu}]_x$ value of about 45 mV for the BLM reference electrode in the presence of quinhydrone (7×10^{-4} M/l) solution implies that this solution functions as electron donor. This result is consistent with our assumption that, at low pH ($\sim$5), hydroquinone will dominate the mixture to function as an electron donor. A large positive $[\Delta E_{h\nu}]_x$ value of about 79 mV for BLM reference electrode in the presence of hydroquinone (7.3×10^{-4} M/l) implies that pure hydroquinone solution functions as an electron donor. General characteristics of the quinone-like group on Chl-BLM photo-response are listed in Table 3.

Poly-phenolic substances

This group of compounds includes Oolong tea, tannic acid, catechin, gallic acid and their derivatives. The most important and characteristic compounds of tea leaf are the polyphenols in the cell sap, which undergo a series of chemical changes when the leaf is macerated during manufacture. Polyphenols include a wide range of organic compounds of the aromatic or benzene series, which make up about 30% of solid matter in

TABLE 3

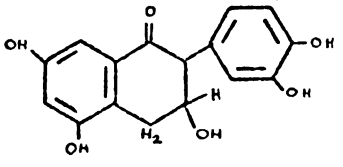
Numerical Values of Chl-BLM Photo-emf in the Presence of Organic and Miscellaneous Compounds

Classification	Compound/BLM/Compound outside	inside	V_D(mV)	$E_{h\nu}$ (mV)	$\Delta E_{h\nu x}$	E^o_{redox}
Quinone-like compounds	$FeCl_3$ ($5x10^{-4}$M/l)	Riboflavin ($8x10^{-5}$M/l)	8	99	79	-0.208
		Benzoquinone ($2.8x10^{-4}$M/l)	13	16	-4	0.293
		Hydroquinone ($7.3x10^{-4}$M/l)	9	99	79	-----
		Quinhydrone ($7x10^{-4}$M/l)	8	65	45	-----
Poly-phenolic compounds	$FeCl_3$ ($5x10^{-4}$M/l)	Oolong tea ($4x10^{-3}$g/ml)	10	132	111	-----
		Tannic acid ($4.5x10^{-7}$M/l)	7	133	113	-----
		Catechin ($3.5x10^{-3}$M/l)	7	121	100	-----
		Gallic acid (10^{-2}M/l)	6	84	72	-----
Vitamins	$FeCl_3$ ($5x10^{-4}$M/l)	Ascorbic acid ($4x10^{-3}$M/l)	13	144	125	-----
		Riboflavin ($8x10^{-5}$M/l)	8	99	79	-0.208
		Thiamine (10^{-2}M/l)	-9	40	24	-----
		Vitamin K_1 ($2.4x10^{-2}$g/ml)	7	37	26	-----
		Nicotinic acid (10^{-2}M/l)	4	30	16	-----
Biocompounds	$FeCl_3$ ($5x10^{-4}$M/l)	Flavin mono-nucleotide ($7x10^{-3}$M/l)	10	150	138	-----
		β-NAD ($6x10^{-4}$g/ml)	-2	67	45	-----
		Cytochrome C ($2x10^{-5}$g/ml	-4	48	28	0.254
		Ferrozine	-15	85	73	-----

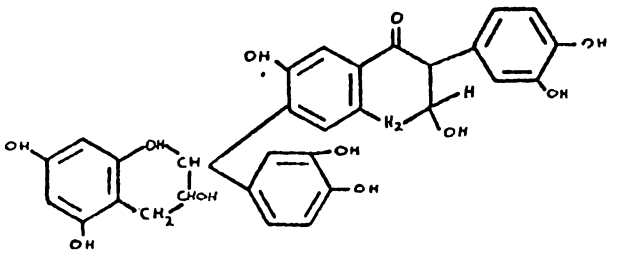
a tea shoot. Knowledge of polyphenols in tea began with the isolation of l-epicatechin and l-epicatechin gallate from leaf in Java. Now people know those occurring in tea are derivatives of gallic acid and catechin [Eden, 1958], so called "tea-tannins" which are more similar to tannins. These include (+)-catechin, (+)-gallocatechin, (-)-epicatechin and (-)-epigallo-catechin. Our finding from Chl-BLM measurement is that only the polyphenol group and vitamins in tea constitution affect Chl-BLM photo-response. The effect of vitamins on Chl-BLM photo-response will be discussed in a later section. Here we will concentrate on the polyphenol group effect.

Concentration effect of Oolong tea ($\frac{4 \times 10^{-2} g}{30 \text{ ml}}$) on Chl-BLM photo-emf measurement near NaAc with a pH of 5 shows a maximum Chl-BLM photo-emf of about 10 to 20 mV, 500 seconds after the presence of Oolong tea can be obtained. The polarity of this photo-response implies that Oolong tea functions as an electron donor. It has been found that Chl-BLM photo-response in the presence of Oolong tea is also dependent upon the pH of aqueous phase. The optimum pH for this photo-response is approximately 4 to 5 and the response decreases as pH increases. At pH = 9, there is little or no photo-response that can be observed. Surveys of components in tea constitution which are responsible for this photo-response have been carried out for most compounds in the polyphenolic group, nonphenolic group and aromatic group, such as caffeine, theobromine, theophylline, queicitin, phenol, tannic acid and catechin. It was found that only those components in the polyphenolic group offer Chl-BLM significant photo-responses. Tannic acid (1.5×10^{-5} M/l) is one of the basic structures of tea tannin and has pH of 2.85. Chl-BLM reaches its maximum photo-response (10 to 15 mV) in the presence

of tannic acid (7.5×10^{-7} M/1). The polarity of this photo-response implies that tannic acid functions as an electron donor in the BLM system. A positive $[\Delta E_{h\nu}]_x$ value of about 113 mV for BLM reference electrode in the presence of tannic acid (4.5×10^{-7} M/1) also indicates that this compound functions as electron donor. D-catechin and its derivatives have been found to be major components in the tea polyphenolic group [Shalamberidze, 1969). This solution was made at a concentration of 2.2×10^{-2} M and had pH 4. The maximum Chl-BLM photo-response is about 10 mV in the presence of this compound. The polarity of photo-response indicates that catechin functions as an electron donor in the BLM system. A positive $[\Delta E_{h\nu}]_x$ value of about 100 mV for BLM reference electrode in the presence of catechin (3.5×10^{-3} M/1) also implies that it functions as an electron donor. The structure basis for electron donating character for either tannic acid or catechin and their derivatives may be due to the existence of a double bond connecting oxygen near the center of the molecule.



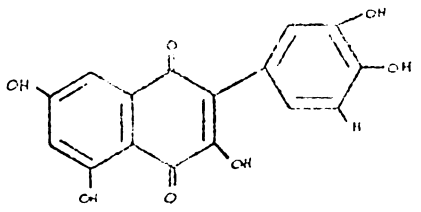
Catechin



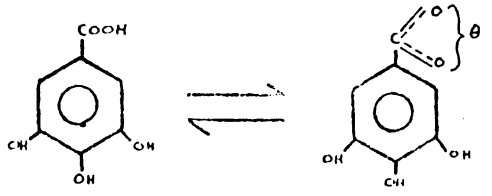
Tannic acid (di-catechin)

The existence of those electron releasing OH^- groups in the molecule will contribute and make this oxygen connecting double bond electron rich. As a result, this oxygen contained site in the molecule will make them electron donating in BLM photo-response measurement.

Quercitin (6.5×10^{-3} M/l) functions as an electron acceptor in Chl-BLM system and a Chl-BLM photo-emf around 5 to 10 mV can be obtained. The electron accepting property of this compound may be due to its structure which contains quinone base.



Gallic acid is another basic structure of those components in the tea polyphenolic group. A positive $[\Delta E_{h\nu}]_x$ value of about 72 mV for BLM reference electrode in the presence of gallic acid (10^{-2} M/l) implies that it functions as an electron donor. The basis for its electron donating character may be due to the existence of its resonance structure.



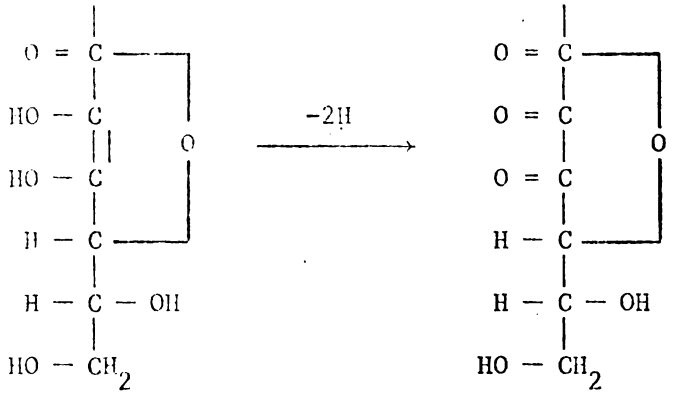
gallic acid

In addition, three hydroxyl groups in the benzene ring are an electron releasing group which may enhance the electron rich character in carboxylic acid site. General characteristics of those compounds from tea polyphenols in BLM system are listed in Table 3.

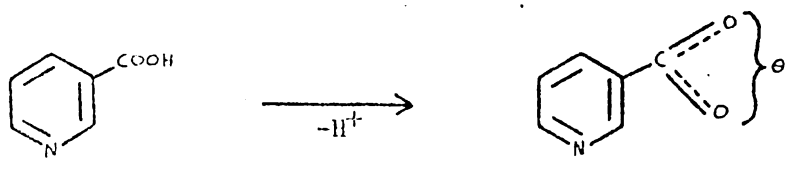
Vitamins

Only those vitamins which are soluble in water are considered here, e.g., vitamin C (ascorbic acid), vitamin B_1 (thiamine chloride), vitamin PP (nicotinic acid), vitamin B_2 (riboflavin) and vitamin K_1

($C_{32}H_{46}O_2$). Ascorbic acid (10^{-1} M/l), an acidic substance, has pH as low as 2.6. A Chl-BLM photo-emf of 10 mV can be observed 400 seconds after ascorbic acid (4×10^{-3} M/l) has been added. It functions as an electron donor in the BLM system. This electron donating character is due to the loss of two hydrogen atoms from its reduced form as shown in the following equation.



A positive $[\Delta E_{h\nu}]_x$ value of about 125 mV for BLM reference electrode in the presence of ascorbic acid (4×10^{-3} M/l) also implies that it functions as an electron donor. This is the best electron donor, hitherto, which can couple with Fe^{+3} to give the ultimate Chl-BLM photo-emf response in our laboratory. Nicotinic acid (10^{-1} M/l), a colorless solution, has pH of 3. A positive $[\Delta E_{h\nu}]_x$ value of about 16 mV for BLM reference electrode in the presence of this compound (10^{-2} M/l) implies that it functions as an electron donor in the BLM system. This may be due to its resonance structure which exhibits an electron-rich site.



Nicotinic acid

Thiamine (vitamin B_1) (10^{-1} M/l), a colorless solution, has pH of 2.7. A positive $[\Delta E_{h\nu}]_x$ value of about 24 mV for the case of thiamine (10^{-2} M/l) BLM reference electrode is obtained. The polarity of this photoresponse implies that thiamine functions as an electron donor. The result of its electron donating property in BLM reference electrode is due to the existing membrane dark potential (near -10 mV) which will facilitate movement of the light-generated negative charges toward Fe^{+3}. Vitamin K_1 (liquid form obtained commercially) is an orange solution. When it is present in BLM reference electrode, a positive $[\Delta E_{h\nu}]_x$ value of about 26 mV can be obtained. It is suggested that this compound functions as an electron donor in BLM reference electrode system. General characteristics of vitamins in the BLM system are listed in Table 3.

c. Miscellaneous Studies

Some important biochemical redox compounds, such as flavin mononucleotide, β-NAD, cytochrome C and ferrozine were selected for our measurement. FMN, an orange solution, is the derivative of riboflavin and has pH of 6.2. It is shown to be an electron donor owing to a positive $[\Delta E_{h\nu}]_x$ value of about 138 mV that is observed in the presence of this compound to BLM reference electrode. The electron donating property of this compound is thought to be due to a riboflavin basis in the structure. β-NAD (or DPN) is commercially obtained in liquid form (2 mg/ml) and has a pH of 3.3. A positive $[\Delta E_{h\nu}]_x$ value of about 45 mV for β-NAD present to the BLM reference electrode implies that it functions as an electron donor. A gradual decrease in membrane dark potential has been found. Cytochrome C (liquid form obtained

commercially) functions as an electron donor in BLM reference electrode since a positive $[\Delta E_{l/v}]_x$ value of 28 mV is observed. Since a drastic decrease in membrane dark potential in the presence of cytochrome C is seen, the resulting photo-emf enhancement for BLM reference electrode may be due to this potential which facilitates the attraction by Fe^{+3} of light-generated electrons. Ferrozine is a colorless but strong smelling solution which is shown to function as an electron donor in BLM reference electrode. A positive $[\Delta E_{l/v}]_x$ of about 73 mV is obtained. A drastic decrease in membrane dark potential may result in easier electron attraction toward Fe^{+3}. General characteristics of bio-redox compounds in the BLM reference electrode system are listed in Table 3.

3. Investigation of Chl-BLM Photo-emf Enhancement by Redox Compounds in the Absence and Presence of Applied Voltages

Many compounds, such as ascorbic acid, tannic acid, Oolong tea, catechin, NaI and FMN are found to be good electron donors in BLM reference electrode system. It is suggested that under proper arrangement of this electron donating compound with a strong electron accepting compound ($FeCl_3$ was used in this experiment), a significant Chl-BLM photo-enhancement can be expected.

Oolong tea/Fe^{+3} system

The electrode reactions for this particular system are: aqueous solution/Oolong tea/BLM/$FeCl_3$/aqueous solution. Five second light illumination was used. The maximum Chl-BLM photo-emf of about 162 mV was obtained, three hours after both compounds had been added.

Tannic Acid/Fe^{+3} system

The expression of electrode reactions for this system is: aqueous solution (NaAc pH = 5)/tannic acid (4×10^{-7} M/l)/BLM/$FeCl_3$ (5×10^{-4} M/l)/aqueous solution (NaAc pH = 5). Five second light illumination was used. The maximum Chl-BLM photo-emf response was 140 mV, 50 minutes after both compounds had been added.

Catechin/Fe^{+3} system

Electrode reaction expression for this system is: aqueous solution (NaAc pH = 5)/catechin (1.4×10^{-2} M/l)/BLM/$FeCl_3$ (5×10^{-4} M/l)/ aqueous solution (NaAc pH = 5). Five second light illumination was used. The maximum Chl-BLM photo-emf was 135 mV, 90 minutes after both compounds had been added.

FMN/Fe^{+3} system

Electrode reaction expression for this system is: aqueous solution (NaAc pH = 5)/FMN (7×10^{-3} M/l)/BLM/$FeCl_3$ (5×10^{-4} M/l)/aqueous solution (NaAc pH = 5). Six second light illumination was used. The maximum Chl-BLM photo-emf was 167 mV, 66 minutes after both compounds had been added.

Ascorbic Acid/Fe^{+3} system

Electrode reaction expression for this system is: aqueous solution (NaAc pH = 5)/Ascorbic acid (1.5×10^{-3} M/l)/BLM/$FeCl_3$ (10^{-3} M/l)/ aqueous solution (NaAc pH = 5). Five second light illumination was used. The maximum Chl-BLM photo-emf was 188 mV, 100 minutes after both compounds had been added. This is the most significant value of Chl-BLM photo-emf, in the absence of external electric field, that has been found so far.

Table 4 lists data obtained from each BLM photo-emf enhancing measurement.

It has been postulated that a membrane dark potential with Fe^{+3}-containing side being of negative polarity tends to draw Fe^{+3} ions away from Chl-BLM surface thus, not only decreasing the total number of ferric ions near BLM/solution interface, but also decreasing the binding strength between ferric ions and Chl-BLM. If an external variable voltage source could be supplied to eliminate this dark potential, a much greater photo-emf enhancement would be found. For example, a result of BLM reference electrode photo-emf versus its membrane dark potential is shown as a red curve in Figures 23 and 24. The linear relationship between BLM reference electrode photo-emf and its dark potential can be as high as V_D = +150 mV and as low as V_D = -150 mV. BLM reference electrode photo-response will be cancelled out by membrane dark potential at V_D = +24 mV. This curve also shows that the polarity of photo-response may change its sign from negative to positive as the membrane dark potential passes from higher than +24 mV to lower than +24 mV. At V_D = -100 mV, the maximum Chl-BLM reference electrode photo-response will be 93 mV, this is because membrane dark potential now has a field direction the same as Fe^{+3} ion so as to direct light-generated negative charges more easily toward Fe^{+3}.

After some selected compounds have been introduced individually to the BLM reference electrode inner chamber, the measurement of Chl-BLM photo-emf response at various membrane dark potentials controlled by external variable voltage sources and external resistance shunt $R_i = 10^9\ \Omega$ is performed. Figures 23 and 24 give those plots of BLM reference electrode photo-emf in the presence of a selected compound

TABLE 4

Significant Chl-BLM Photo-emf Enhancement in the Presence of Redox Coupling Systems

Compound in Outer/BLM/Inner side		Without external electric field		With external electric field		Time to det. needed for max. response (min)
		V_D(mV)	$E_{h\nu}$(mV)	V_D(mV)	$E_{h\nu}$(mV)	
Oolong tea (4×10^{-3}g/cc)	$FeCl_3$ (5×10^{-3}M/1)	2 - 3	-165 to -160	150	-200	100
$FeCl_3$ (5×10^{-4}M/1)	Tannic acid (4.5×10^{-7}M/1)	15	140 to 145	-160	240	50
$FeCl_3$ (5×10^{-4}M/1)	Catechin (1.4×10^{-2}M/1)	16	135 to 133]	-145	210	90
$FeCl_3$ (5×10^{-4}M/1)	FMN (7×10^{-3}M/1)	10	167 to 165	-150	208	66
Ascorbic acid	$FeCl_3$	-11	-188 to -192	200	-300 to -287	100

Figure 23. Linear relationship of Chl-BLM photo-emf with its dark potential for several representative inorganic redox coupling systems.

1. $FeCl_3$(5×10^{-4} M/l)/BLM/NaI
2. $FeCl_3$(5×10^{-4} M/l)/BLM/$Na_2S_2O_3$
3. $FeCl_3$(5×10^{-4} M/l)/BLM/$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$
4. $FeCl_3$(5×10^{-4} M/l)/BLM/$LaCl_3$
5. $FeCl_3$(5×10^{-4} M/l)/BLM/Reference electrode
6. $FeCl_3$(5×10^{-4} M/l)/BLM/TlCl
7. $FeCl_3$(5×10^{-4} M/l)/BLM/Ce^{+4}
8. $FeCl_3$(5×10^{-4} M/l)/BLM/$Co(NH_3)_6^{+3}$
9. $FeCl_3$(5×10^{-4} M/l)/BLM/Na_2S

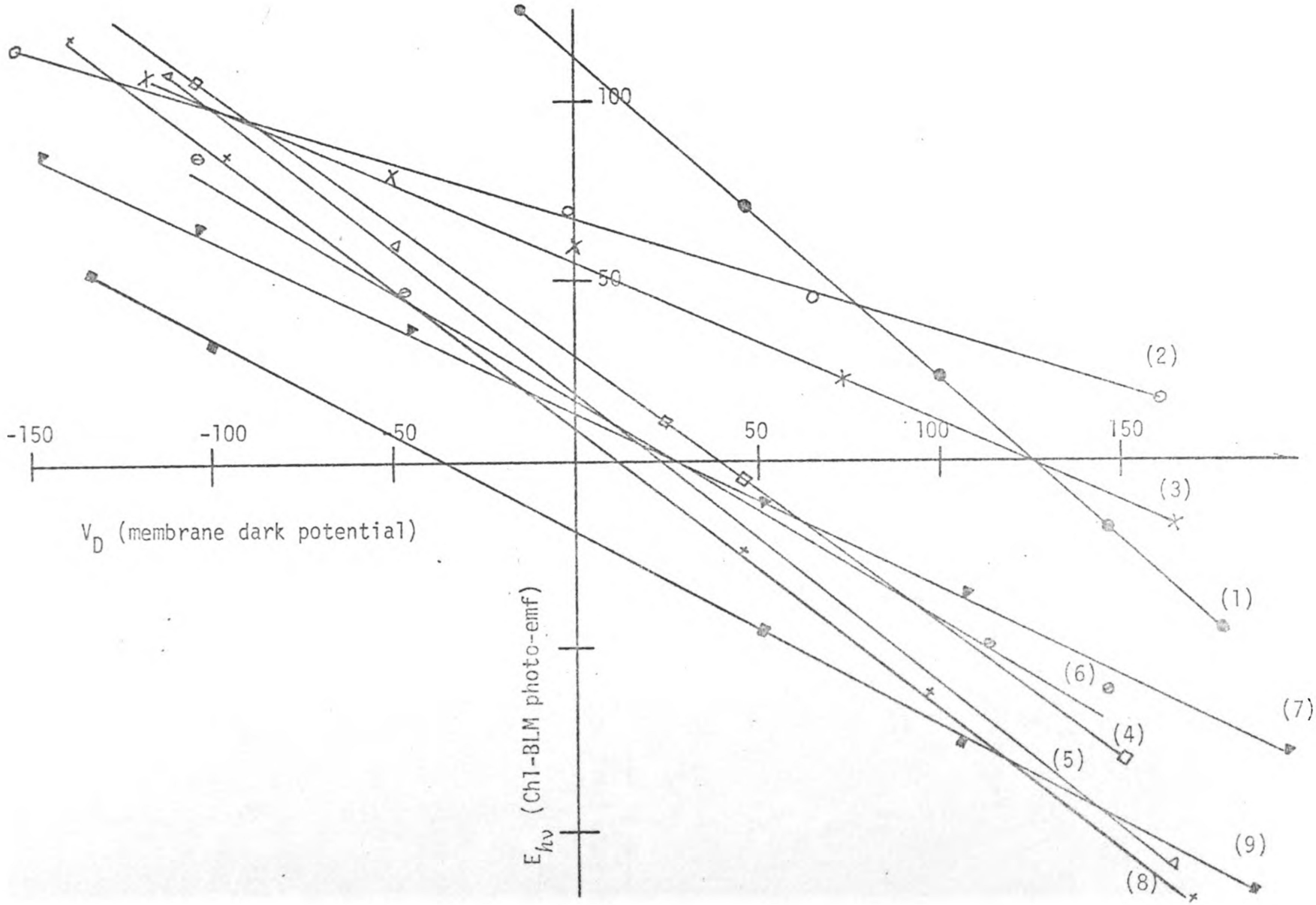
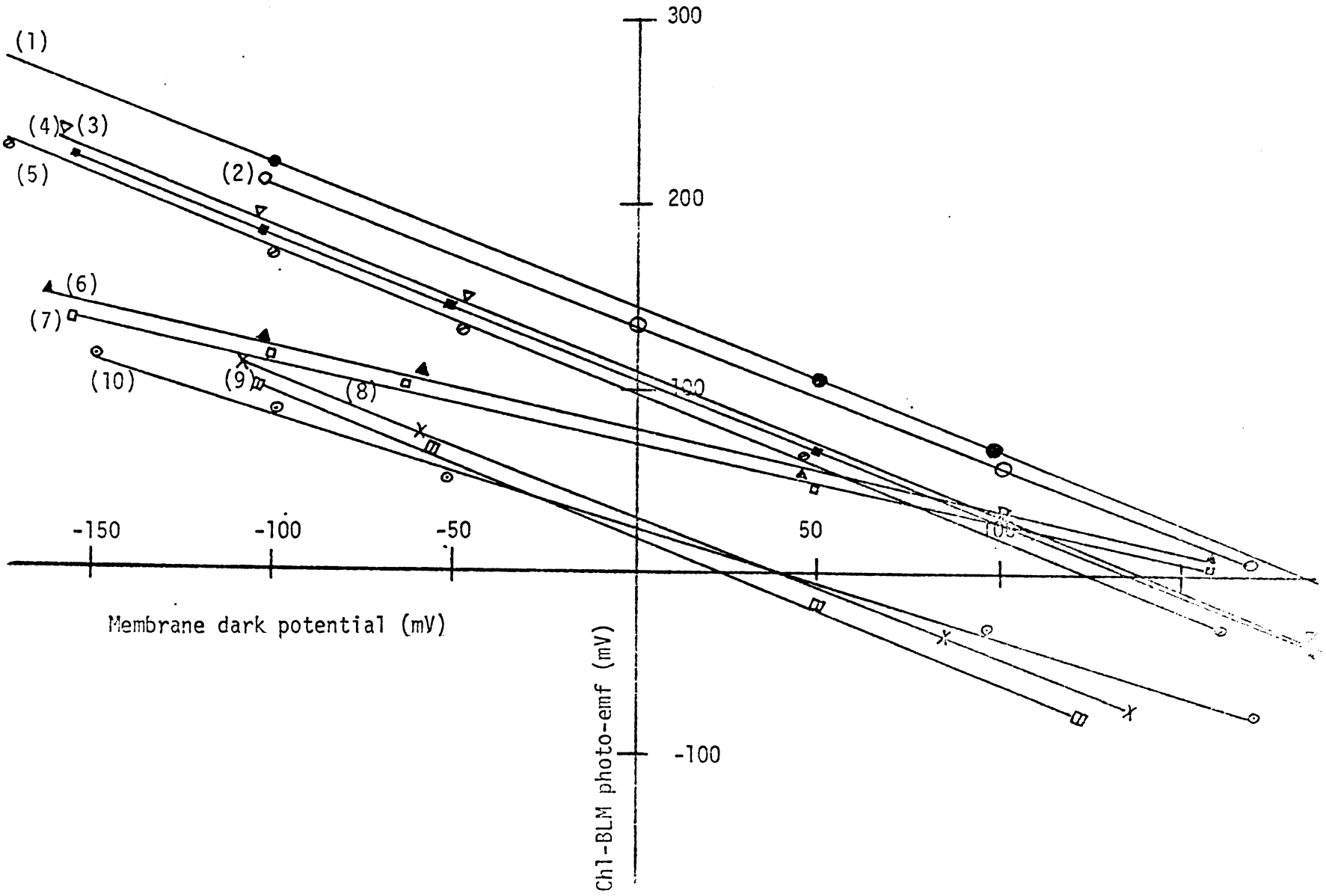

100
50
-150
-100
50
50
100
150
(1)
(2)
(3)
(4)
(5)
(6)
(7)
(8)
(9)
V_D (membrane dark potential)
$E_{h\nu}$ (Chl-BLM photo-emf)

Figure 24. Linear relationship of Chl-BLM photo-emf with its dark potential for several representative organic redox coupling systems.

1. $FeCl_3$(5×10^{-4} M/l)/BLM/Ascorbic acid (3.0×10^{-3} M/l)
2. $FeCl_3$(5×10^{-4} M/l)/BLM/FMN (7×10^{-3} M/l)
3. $FeCl_3$(5×10^{-4} M/l)/BLM/Tannic Acid (4×10^{-7} M/l)
4. $FeCl_3$(5×10^{-4} M/l)/BLM/Oolong tea (4×10^{-3} M/l)
5. $FeCl_3$(5×10^{-4} M/l)/BLM/Catechin (7×10^{-4} M/l)
6. $FeCl_3$(5×10^{-4} M/l)/BLM/Hydroquinone
7. $FeCl_3$(5×10^{-4} M/l)/BLM/Riboflavin
8. $FeCl_3$(5×10^{-4} M/l)/BLM/Cytochrome C
9. $FeCl_3$(5×10^{-4} M/l)/BLM/
10. $FeCl_3$(5×10^{-4} M/l)/BLM/β-NAD
11. Ascorbic Acid (1.5×10^{-4} M/l)/BLM/$FeCl_3$ (10^{-3} M/l)


(1)
(2)
(3)
(4)
(5)
(6)
(7)
(8)
(9)
(10)
300
200
100
-100
-150
-100
-50
50
100
Membrane dark potential (mV)
Chl-BLM photo-emf (mV)

versus the membrane dark potential. Two important pieces of new information are gained: (1) BLM membrane dark potential independent electron donating or accepting characteristic of compound in origin can be observed. Since BLM reference electrode has a photo-emf of 18 mV at $V_D = 0$, the compound will be an electron donor in origin if the BLM reference electrode, in the presence of this compound, has a photo-emf greater than 18 mV. Otherwise, it will be an electron acceptor. The order of electron donating power for inorganic compounds in Chl-BLM at $V_D = 0$ is NaI > $Na_2S_2O_3$ > $(NH_4)_6Mo_7O_{24}\cdot 24H_2O$ > $LaCl_3$ > $FeCl_3$. Respectively, the order of electron accepting power for inorganic compounds in Chl-BLM at $V_D = 0$ is Na_2S > $Co(NH_3)_6^3$ > $(NH_4)_2Ce(NO_3)_6$ > TlCl > $FeCl_3$. For organic compounds, the order of their electron donating power at $V_D = 0$ is ascorbic acid > FMN > tannic acid > Oolong tea > catechin > hydroquinone > riboflavin > cytochrome C > β-NAD^+ > $FeCl_3$. (2) The Chl-BLM photo-emf can be more than 200 mV in the presence of ascorbic acid, tannic acid, FMN, catechin and NaI with $FeCl_3$ and large membrane dark potential. Among them, ascorbic acid/Fe^{+3} coupling system can enhance Chl-BLM photo-emf near 300 mV.

CHAPTER V

DISCUSSION

1. Chl-BLM Photo-emf as pH Dependence

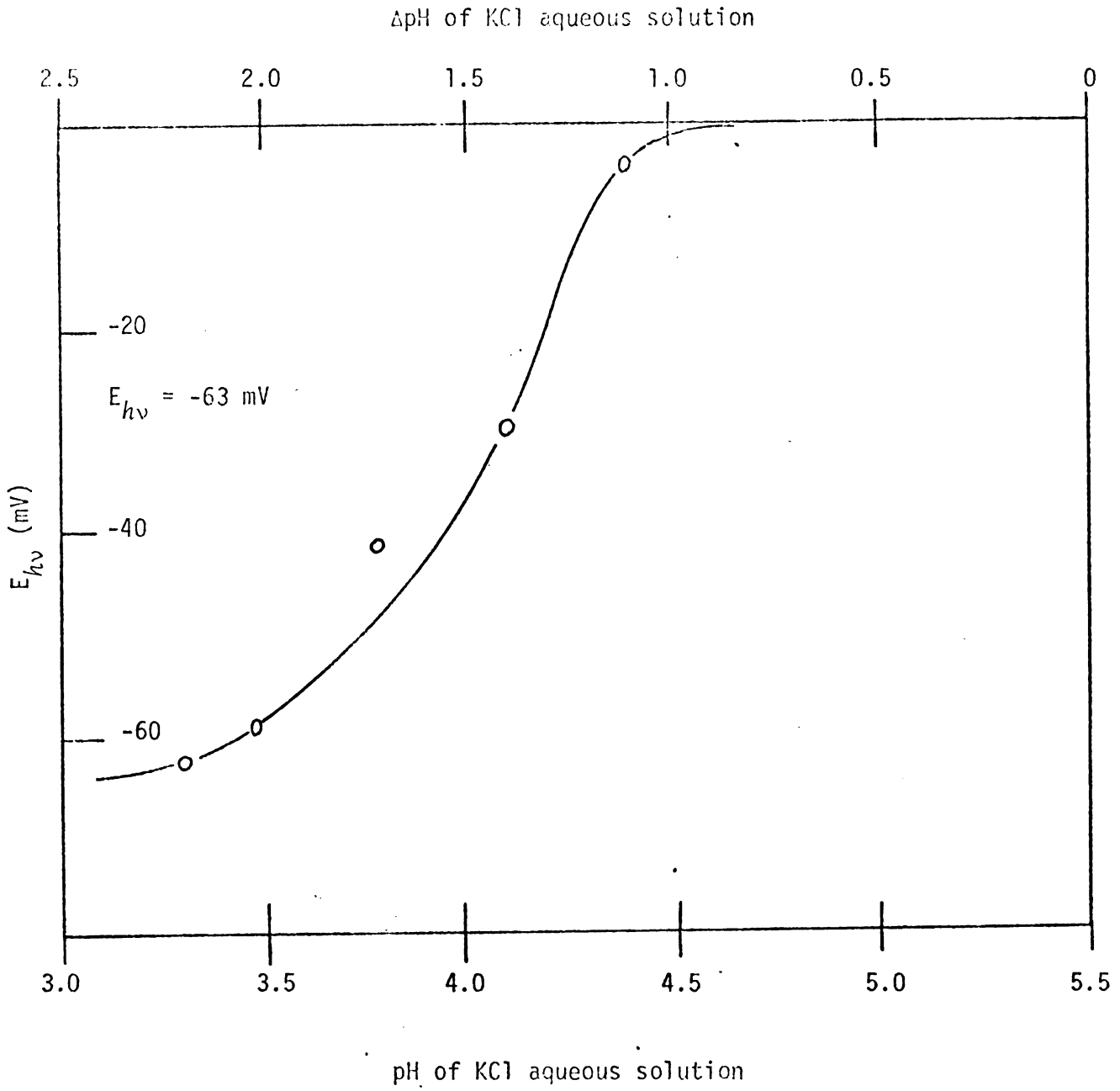
The curve of Chl-BLM membrane dark potential versus pH appears as a typical titration curve. This titration curve exhibits two pKa values: one of 4 and the other of 6.7. Since the pH in membrane usually has a value 1 to 2 units lower than that of bulk phase [Kobamoto, 1970; Hartley and Roe, 1970], these two pKa values in membrane phase should be 2 to 3 and 4.7 to 5.7. Owing to the lack of detailed composition of membrane structure, one cannot point out exactly which components in membrane these two pKa values belong to. However, since p-lipids (or phosphoglycerides which include phosphatidyl ethanolamine, phosphatidyl choline, phosphatidyl serine) are the major components in membrane structure and have pKa around 1 to 2, one might say that the first pKa of 2 to 3 can be one of the pKa values of these p-lipids, especially where phosphatidyl serine has a pKa = 3 (due to carboxyl group). The second pKa value of 4.7 is most likely the pKa of saturated fatty acids which may also be included in the membrane structure.

There is also one possibility that cannot be ruled out; i.e., the case where some p-lipids are hydrolyzed, thus yielding saturated fatty acids which have pKa values around 1 to 2. Since Chl-BLM has its

maximum photo-emf in the region of pH = pKa_{bulk} = 4, one may ask what the relation between this pKa and photo-emf is. When the substance in membrane has a pH near its pKa value, as much as half of this weak acid has been dissociated and, as a result, will facilitate some electric events. This is evidence that Chl-BLM drops its electric resistance about 10-fold when the pH in the inner chamber is lowered from 5 to 4. Bamberg and Neumcke [1972] also reported that the conductivity of bilayer membrane exhibited the maximal value near the pK value of the uncoupler in the membrane. In addition, at pH = pKa, the large amount of H^+ produced in the inner chamber will function as an electron acceptor for the light event in the membrane system.

It was found that a large membrane dark potential was created by the diffusion of hydrogen ion in this system, with the hydrogen ion-containing side becoming negatively charged. As long as the light was maintained on the Chl-BLM, this dark potential presented a field in such a direction as to draw the light-induced electrons toward the hydrogen ion-containing side. As a result, one observes a Chl-BLM apparent photo-emf instead of the true (or absolute) BLM photo-emf due to hydrogen ion alone as an electron acceptor. In order to observe this true BLM photo-emf, one can simply apply some external voltages to reduce this dark potential before the photo-emf measurement. The resistance of external resistor has a value about 10^9 Ω which is a higher value than that of the membrane itself. Figure 25 is a plot of the membrane dark potential independent Chl-BLM photo-emf versus pH change of KCl solution. The pH of KCl solution in the outer chamber has a constant value of 5.5. It is seen from the figure that Chl-BLM has a maximum photo-emf of 63 mV at ΔpH = 2.2. An interesting phenomenon is that the

Figure 25. The membrane dark potential independent and H^+ dependent Chl-BLM photo-emf. The pH of KCl solution in inner chamber was varied by the addition of HCl (10^{-4} M/l), while the outer chamber pH was kept constant at 5.5. The Chl-BLM dark potentials were reduced to zero by the externally applied voltage sources.


ΔpH of KCl aqueous solution
2.5
2.0
1.5
1.0
0.5
0
-20
$E_{h\nu}$ = -63 mV
-40
-60
$E_{h\nu}$ (mV)
3.0
3.5
4.0
4.5
5.0
5.5
pH of KCl aqueous solution

Chl-BLM open-circuit photo-emf as the pH dependence is simply the subtraction of the Chl-BLM photo-emf with corresponding externally applied voltage from the membrane dark potential independent and H^+ dependent Chl-BLM photo-emf. For example, at KCl pH = 4, the open-circuit Chl-BLM H^+ dependent photo-emf has the value of 12 mV, and Chl-BLM photo-emf under the externally applied voltage is 30 mV. The sum of these two photo-emfs is exactly equal to the membrane dark potential independent and H^+ dependent Chl-BLM photo-emf; i.e., 42 mV.

2. Determination of Electron Donating or Accepting Power of Redox Compounds in BLM System

A graph has been constructed showing the tendency of various redox compounds to donate or accept electrons in light, with respect to the BLM reference electrode, by using measurements of $[\Delta E_{h\nu}]_x$. As shown in Diagrams A and B, the farther up this diagram a compound is, the greater is its tendency to donate electrons (relative to Fe^{+3}) and the farther down this diagram a compound is, the greater is its tendency to accept electrons. Among them, NaI (inorganic compound), FMN, and ascorbic acid (organic and biocompound) are the strongest electron donors. However, I_2, Na_2S and Benzoquinone are strongest electron acceptors.

3. Mechanisms of Chl-BLM Photo-emf

General reaction mechanisms of Chl-BLM light-induced photo-emf in the presence of electron acceptor and electron donor near KCl aqueous solution, as shown in the following figure, were: (i) Light caused the excitation of chlorophyll (Chl) molecule. This excited Chl then dissociated into a positive charge and a negative charge; (ii) This negative charge moved toward the side of existing positive electric field

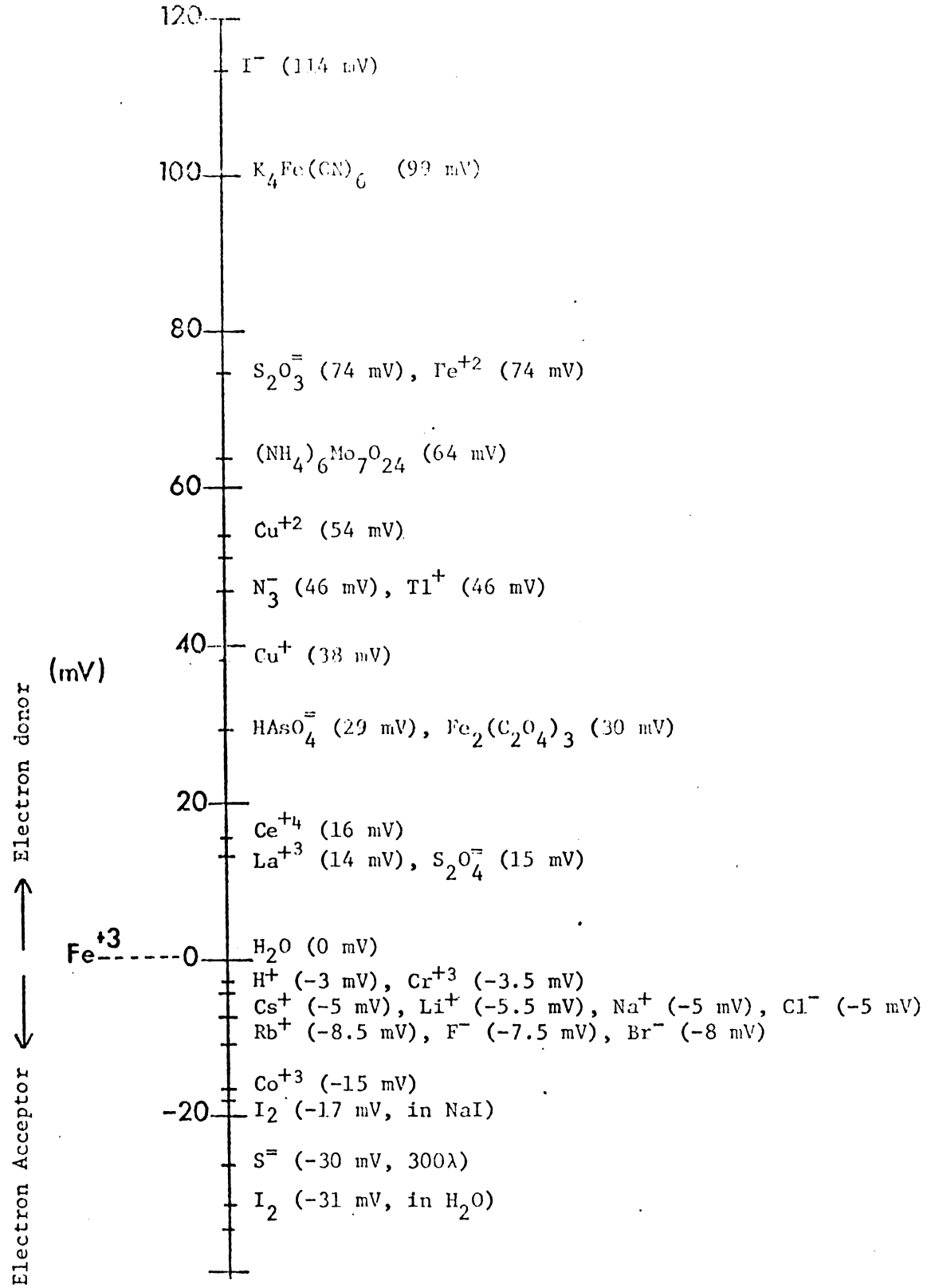


Diagram A. Redox power of inorganic compounds

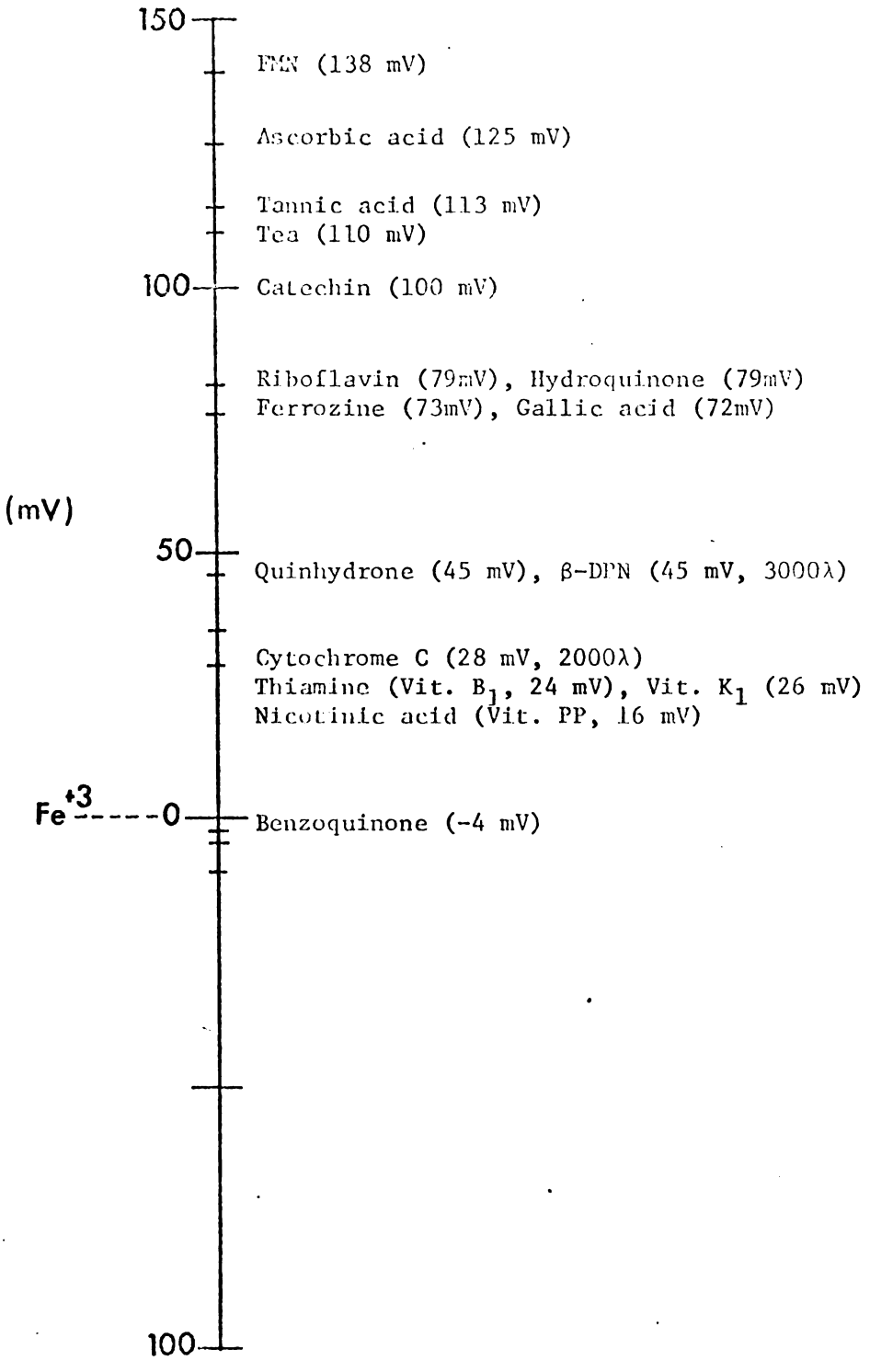
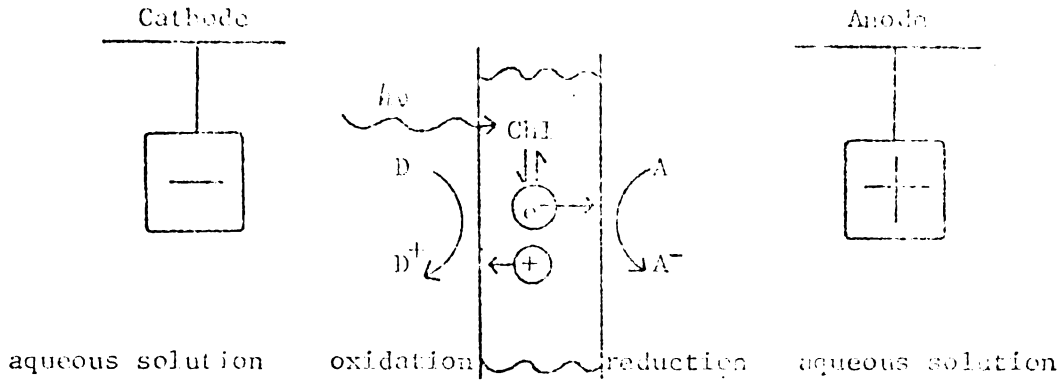


Diagram B. Redox strength of organic and biochemical compounds



or was absorbed by an electron acceptor (A), while the positive charge of Chl moved toward the opposite side and was caught by the electron donor (D) [Tien, 1968]. Based on this postulation, the quantity of this photo-emf seems to depend on the number of generated charge carriers and their separations. Therefore, any factor which can effect this charge generation and separation would cause the varying of the photo-response. These factors will be discussed in more detail in a later section.

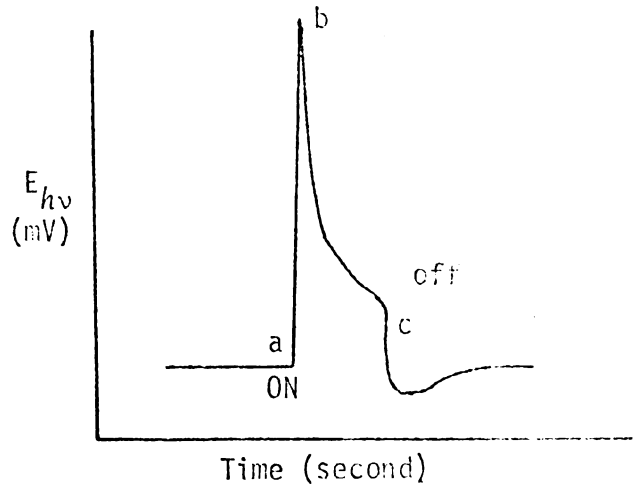
a. Charge Separation

The polarity and the amount of charge separation have been found to depend on the direction and the strength of the electric field or the side on which the electron acceptor or electron donor is present. The side on which the electron acceptor was reduced had negative polarity and the side where the electron donor was oxidized had positive polarity. The stronger the electron donating or accepting power of the compound, the larger the photo-emf response. Several systems are chosen here to support this hypothesis.

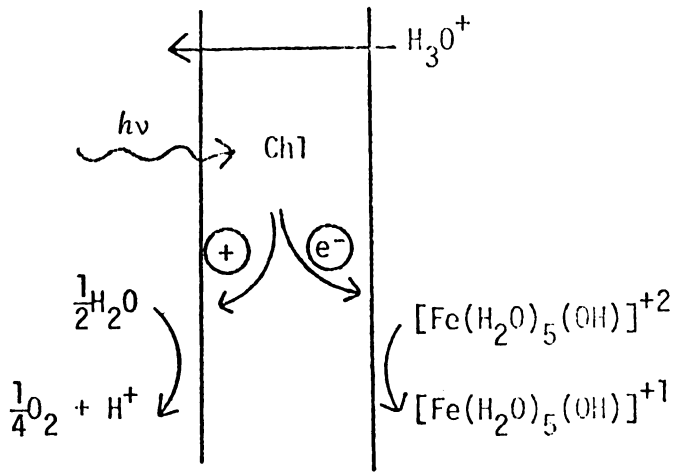
1) $FeCl_3$ as an electron acceptor. Figure 26 indicates the waveform of this photo-emf and the reaction mechanism. When $FeCl_3$ is

Figure 26. A possible interpretation for $FeCl_3$ in Chl-BLM light-induced interface reaction mechanism, its equations and its photo-emf pattern.

Chl-BLM photo-emf pattern



Reaction mechanism



Equations for reaction

$$Chl + h\nu \longrightarrow Chl^*$$

$$Chl^* \longrightarrow Chl^+ + e^-$$

$$[Fe(H_2O)_6]^{+3} \longrightarrow [Fe(H_2O)_5(OH)]^{+2} + H_3O^+ \text{ (in dark)}$$

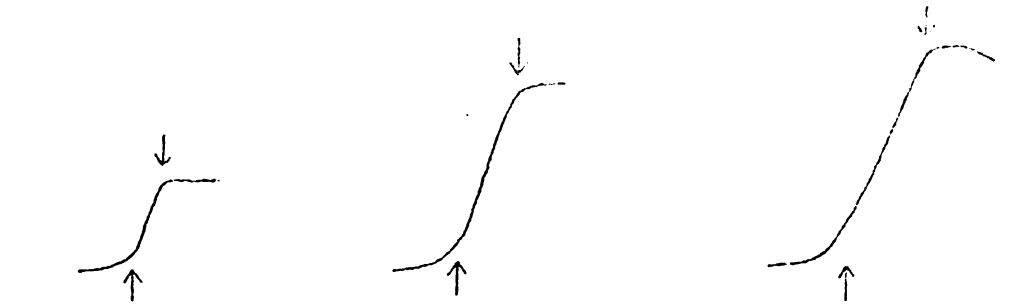
$$[Fe(H_2O)_5(OH)^{+2} + e^- \longrightarrow [Fe(H_2O)_5(OH)]^{+1}$$

$$Chl^+ + \frac{1}{2} H_2O \longrightarrow Chl + H^+ + \frac{1}{4} O_2$$

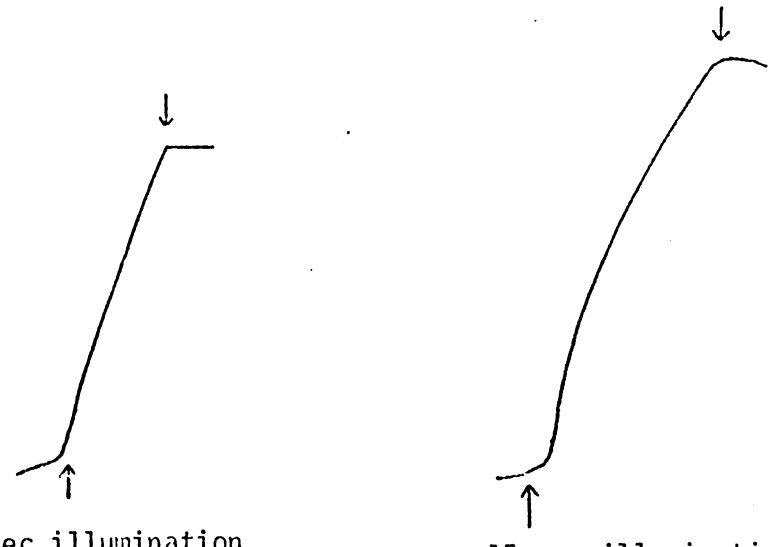
reduced the side becomes negatively charged. This is shown by the portion ab in the photo-emf waveform. Shortly after the maximum response, the slow component begins to affect the negative polarity which is the result of the H^+ diffusion from the other side to the Fe^{+3}-containing side (see portion bc, Figure 26).

Varying the substance in the aqueous solution results in changing the Chl-BLM photo-emf waveform. A comparison is made by replacing $FeCl_3$ with H^+. Figure 27 shows the Chl-BLM photo-emf versus different times of illumination where H^+ (2×10^{-4} M/l) was set in the inner chamber. The main difference in photo-emf waveform between these two systems can be divided into two categories: (1) the rate of rising in photo-emf response is quite different in the two systems; (2) the slow component in photo-emf which is due to H^+ diffusion can be seen in the system with $FeCl_3$ present, but not in the system with H^+. The rate of rising in photo-emf curve in Figure 27 is much slower than in Figure 7. The system, as shown in Figure 7, takes only 1.5 seconds of illumination to reach its maximum photo-response. However, the system in Figure 27 needs at least 30 seconds of illumination to reach its maximum photo-response. In the $FeCl_3$-containing Chl-BLM system, the slow component of photo-emf appears right after the system has reached its maximum response and, the longer the light duration the more significant is this slow component. In the H^+-containing Chl-BLM system, the appearance of this slow component is not significant even with longer time of light duration. It is likely that the H^+ production by the oxidation of water in the H^+-containing Chl-BLM system is not as efficient as that of the $FeCl_3$-containing Chl-BLM system. This inefficiency in H^+ production causes the slowdown of H^+ diffusion from the left to the

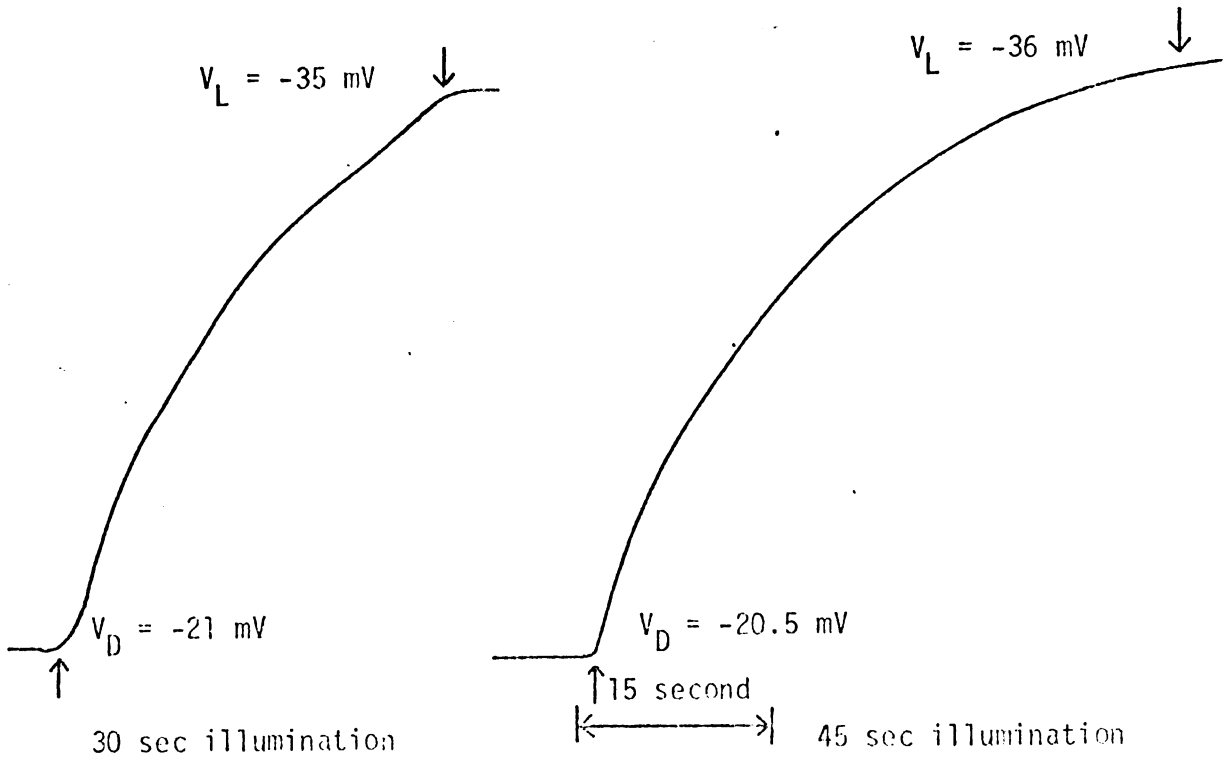
Figure 27. Chl-BLM photo-emf as a function of light duration in the presence of HCl (2×10^{-4} M/l) in the inner chamber. KCl (10^{-1} M/l), pH at 5.5, was the aqueous phase. It takes 30 seconds of light duration for this system to reach its maximum photo-response. The slow component of photo-response cannot be seen for this system even when light is "off".



3 sec illumination 4.5 sec illumination 6 sec illumination



9 sec illumination 15 sec illumination



30 sec illumination 45 sec illumination

right side. In addition, the inner chamber with a large amount of H^+-containing side has a strong attraction to the electron and reduces the rate of recombination of electron with positive charge (hole) to some degree when light is "off". It is shown in Figure 27 that photo-emf waveform remains in the region of the maximum response point and will not immediately go back to the base line when light is "off".

2) Na_2S solution as an electron acceptor. Figure 28 lists the postulated mechanism and equations for the Chl-BLM photo-response in the presence of Na_2S solution. Na_2S compound has four components, Na^+, OH^-, and $S^=$, when it is dissolved in water. This $S^=$ is a very unstable component which will be oxidized into insoluble S in the presence of daylight, oxygen and water. The evidence for the existence of this insoluble S in the BLM cell is the gradual increasing of a white cloud in the Na_2S-containing chamber after several lights act on the system. Light has two effects on the BLM system; one is supplying enough energy to activate the reaction $S^= \xrightarrow[H_2O]{O_2} S + 2OH^-$; the other is to excite Chl in BLM. Light-generated negative charge from excited Chl is then absorbed by this insoluble S and the positive charge (or hole) then diffuses to the other side to oxidize water. The producing proton (H^+) after all, will diffuse back to interact with $S^=$ to produce H_2S. The existence of H_2S in BLM inner chamber is evident when the strong smell of H_2S is produced in the chamber after the measurement. Additional evidence in support of the above postulation comes from the measurement of Chl-BLM reference electrode photo-emf in the presence of Na_2S solution. A large negative value of $[\Delta E_{h\nu}]_x$ with 30 mV in the presence of Na_2S (3×10^{-3} M/l) on BLM reference electrode indicates that Na_2S functions as an electron acceptor instead of as an electron donor. A

Figure 28. Postulated reaction mechanism and equations for BLM/solution interfaces interaction in light in the presence of Na_2S solution.

Reaction Mechanism

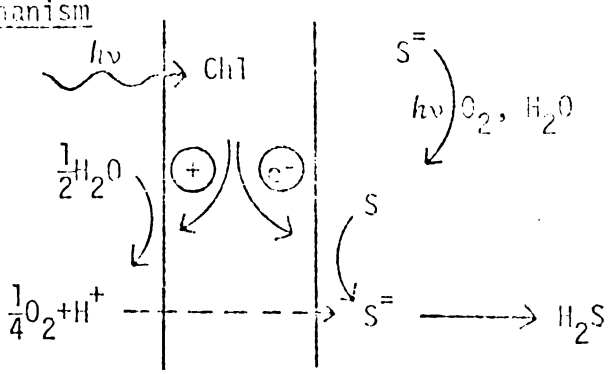
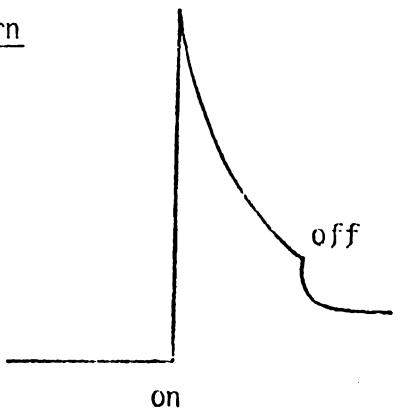


Photo-emf Pattern



Equations for Reaction

$$Na_2S + 2H_2O \longrightarrow 2Na^+ + 2OH^- + 2H^+ + S^=$$

$$S^= \xrightarrow[h\nu]{\frac{1}{2}O_2,\ H_2O} S + 2OH^-$$

$$2Chl + 2h\nu \longrightarrow 2Chl^*$$

$$2Chl^* \longrightarrow 2Chl^+ + 2e^-$$

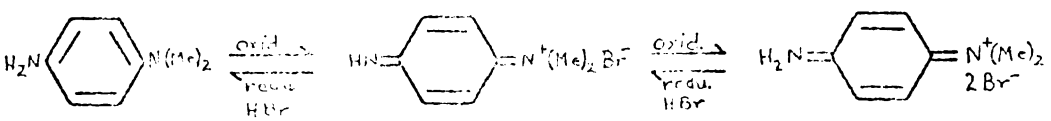
$$S + 2e^- \longrightarrow S^=$$

$$2Chl^+ + H_2O \longrightarrow 2Chl + 2H^+ + \frac{1}{2}O_2$$

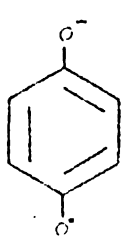
$$2H^+ + S^= \longrightarrow H_2S$$

large negative dark potential of the BLM reference electrode generated by the addition of H_2S is observed. White cloudiness and strong H_2S smell have also been found in the inner chamber of the BLM reference electrode.

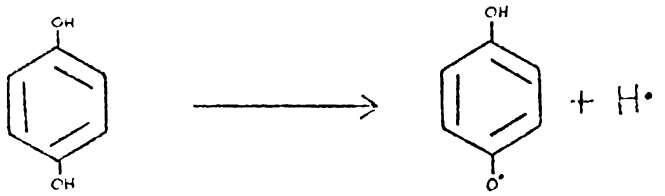
3) Chl-BLM photocurrent response in the presence of riboflavin (electron accepting or donating compound) at various pH values. Two decades after Wurster [1879], Willstatter [1904-1909] found Wurster salt actually was an intermediate of the reaction:



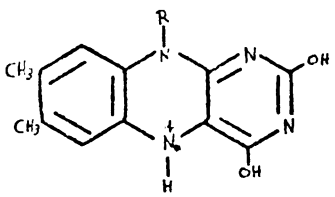
This finding has been extended to the study of quinone and riboflavin relative compounds. For quinone, Millar & Springall [1969] found that quinhydrone was also an intermediate state of benzoquinone and hydroquinone, which has been satisfactorily represented as a molecular complex, held together by unknown forces. Later investigators found crystalline quinhydrone to consist of long chains of alternated quinone and hydroquinone molecules linked by O-H-O hydrogen bond. In neutral or acidic solution it dissociates into an equimolecular mixture of quinone and hydroquinone, but in alkali solution it exists as a free radical semiquinone anion.



This is a stage which corresponds to the loss of one electron from the hydroquinone.

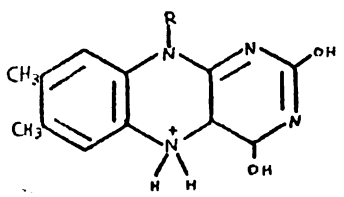


An analogous situation is also obtained in the riboflavin molecule. In alkali solution, most riboflavin will form as

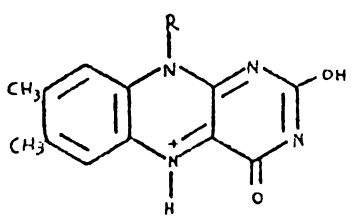


R = CH_2OH | CHOH | CHOH | CHOH | CH_2

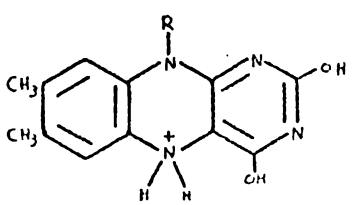
which can be reduced to become



In acidic solution, riboflavin will dissociate into the mixture of RF-A and RF-B (as indicated below).



(RF - A)



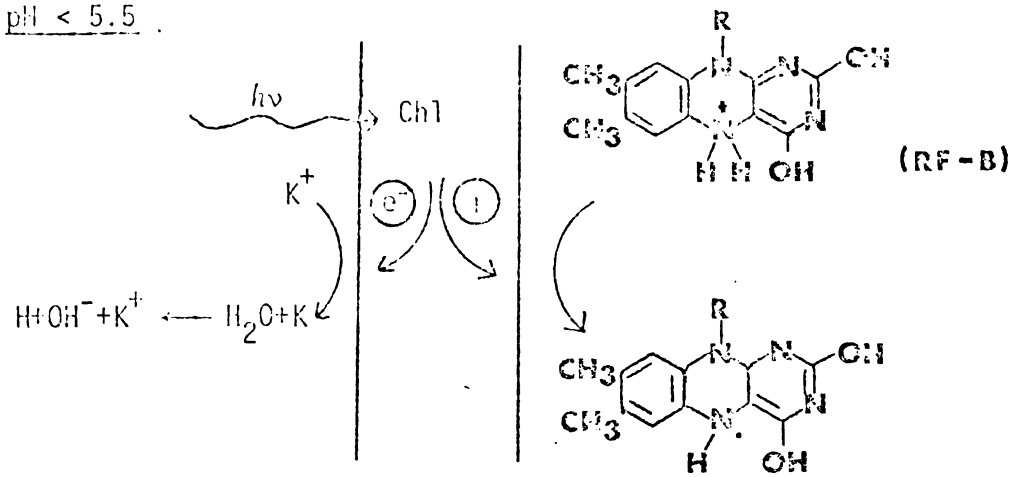
(RF - B)

The ratio of the amount of RF-A to that of RF-B will vary at different solution pH values. The above findings indicate that riboflavin (vitamin B_2) and quinhydrone have generally analogous behavior. Their behaviors are quite pH dependent. Similar behaviors of riboflavin and quinhydrone in the Chl-BLM system are found in some experimental results. A postulated interpretation for the light generated mechanism of Chl-BLM in the presence of riboflavin at various pH values is described in Figure 29. A positive $[\Delta E_{h\nu}]_x$ value of about 79 mV for the BLM reference electrode in the presence of riboflavin (8×10^{-5} M/1, pH = 5) implies that riboflavin functions as an electron donor. This is because most of riboflavin mixture at NaAc pH 5 is of the RF-B form.

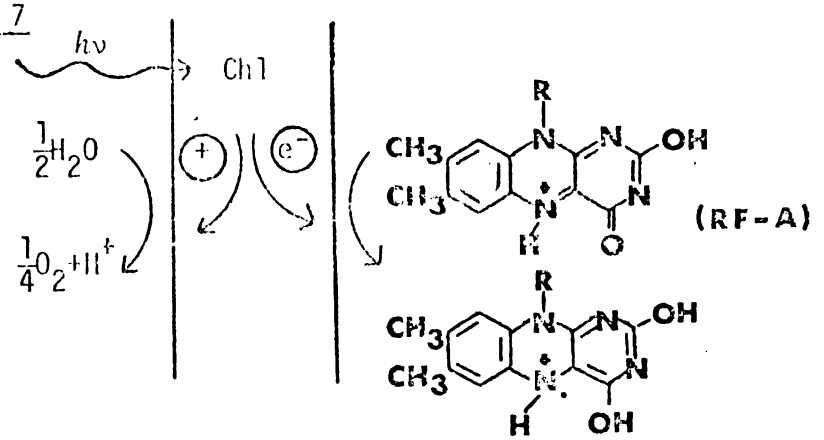
Biphasic Chl-BLM photo-emf form at various pH of riboflavin and KCl aqueous solution (as drawn in Figure 30) strongly suggest that this photo-response will depend on the ratio of the amount of RF-A to RF-B in riboflavin solution. At low pH such as 4 to 5, the amount of riboflavin in form RF-B is greater than in form RF-A; the positive value of Chl-BLM photo-emf will dominate this Chl-BLM photo-emf biphasic form. At high pH such as 6, since the amount of riboflavin in form RF-A is greater than in form RF-B, the negative value of Chl-BLM photo-emf will take over and dominate this Chl-BLM photo-emf biphasic form. Additional experimental evidence to support this postulation is the Chl-BLM photo-emf measurement in the presence of Fe^{+3} and riboflavin near KCl pH of 4 and pH of 6. Results are shown in Figures 31 and 32. They are plots of Chl-BLM photo-emf versus time (second) in dark in the presence of Fe^{+3} (1.6×10^{-4} M/1) and riboflavin (6×10^{-5} M/1) at pH = 4 and 6. Comparable Chl-BLM photo-emf responses with only Fe^{+3} present are also shown in the figures. It is found that at pH = 4,

Figure 29. A possible BLM/solution interface interaction mechanism in light, in the presence of riboflavin at various solution pH.

pH < 5.5



5.5 < pH < 7



pH > 7

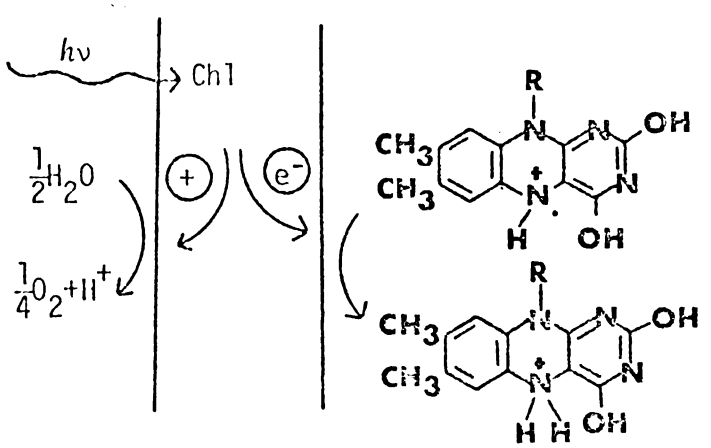


Figure 30. Biphasic Chl-BLM photo-response in the presence of riboflavin and KCl in pH range of 4 to 6. The magnitude of biphasic photo-response will depend upon the pH of riboflavin and KCl. In low pH, the biphasic photo-response will be dominated in positive photo-response portion, and at high pH, the biphasic photo-response will be dominated in negative photo-response portion.

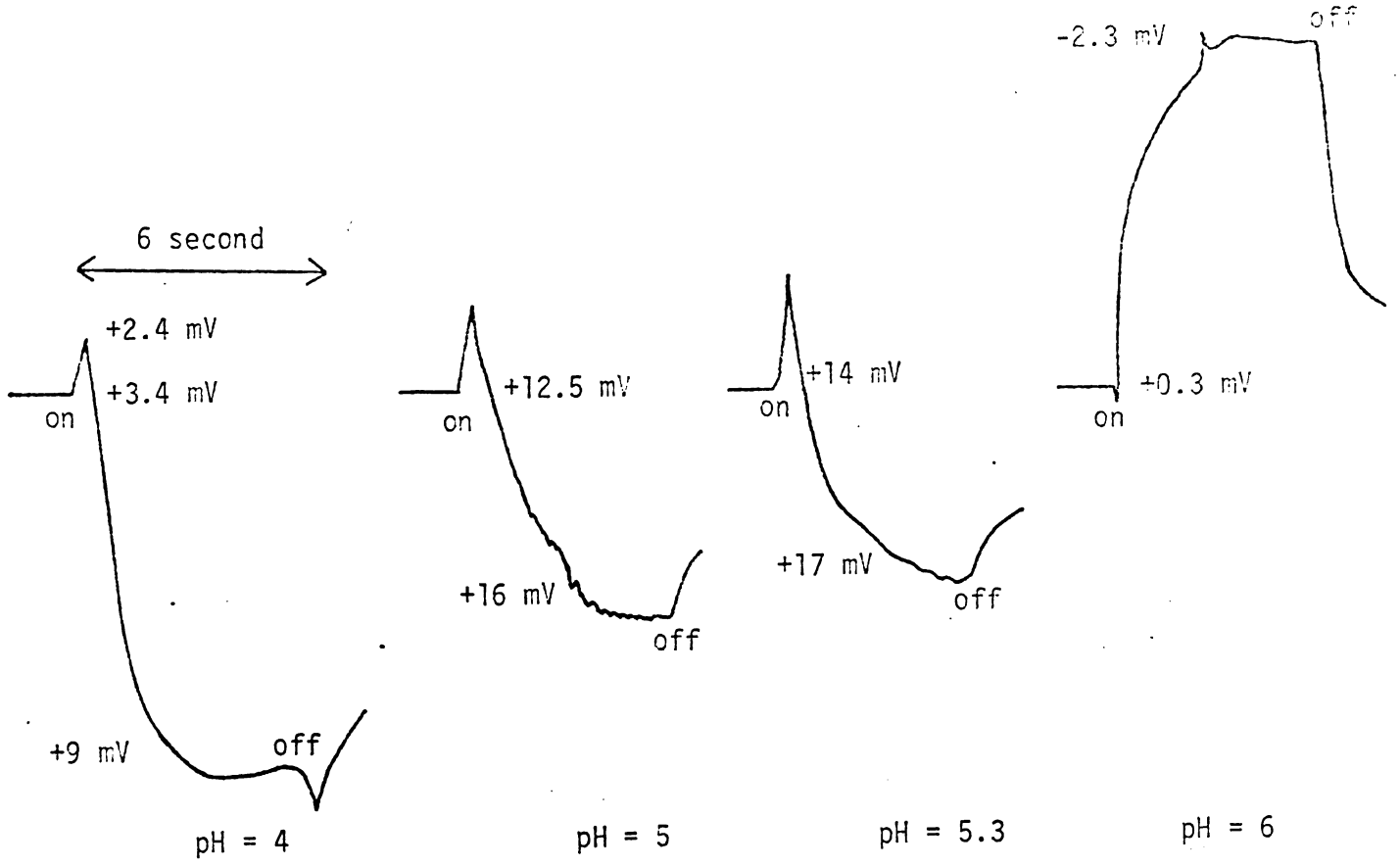

6 second
+2.4 mV
+3.4 mV
on
+9 mV
off
pH = 4
on
+12.5 mV
+16 mV
off
pH = 5
on
+14 mV
+17 mV
off
pH = 5.3
-2.3 mV
off
on
+0.3 mV
pH = 6

Figure 31. Chl-BLM photo-emf as a function of time in dark in the presence of $FeCl_3$ and $FeCl_3$ - riboflavin in KCl, pH of 4. It was found that Chl-BLM photo-response in the presence of Fe^{+3}-riboflavin is higher than that in the presence of Fe^{+3} alone. This implies that riboflavin functions as electron donor at low solution pH.

Chl-BLM photo-emf curve in the presence of Fe^{+3}-riboflavin system.

Chl-BLM photo-emf curve in the presence of Fe^{+3} alone.

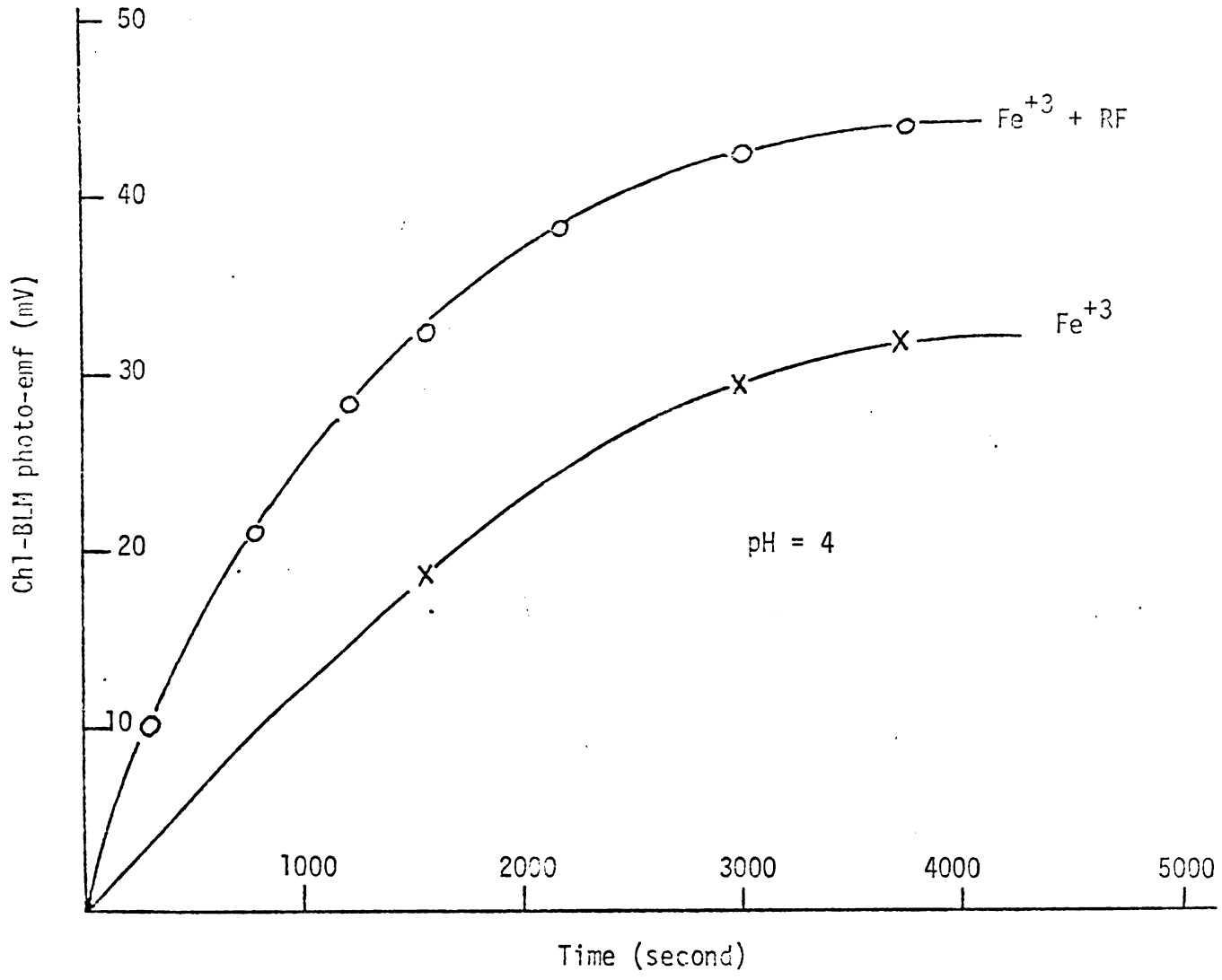
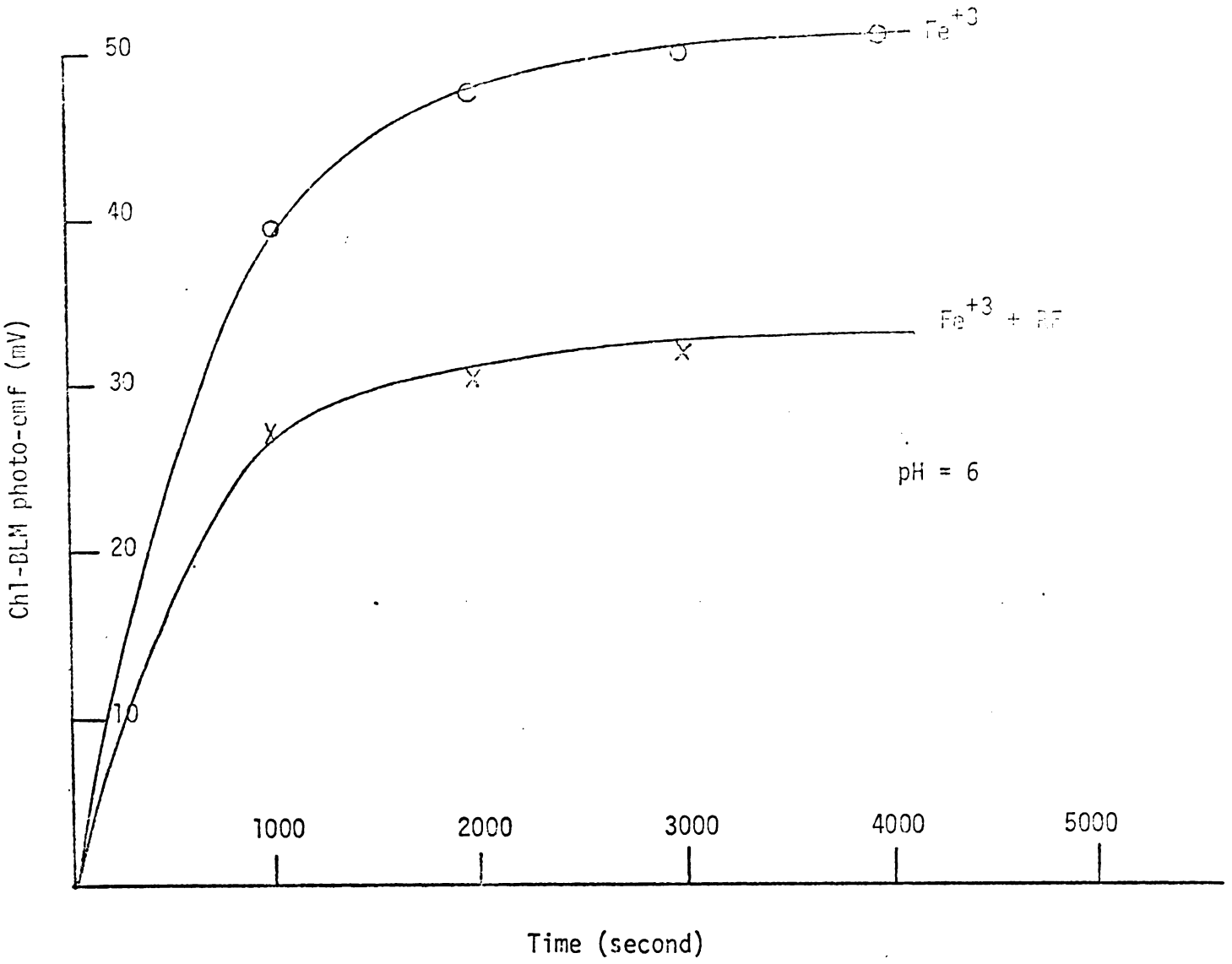

50
40
30
20
10
Chl-BLM photo-emf (mV)
Fe+3 + RF
Fe+3
pH = 4
1000
2000
3000
4000
5000
Time (second)

Figure 32. Chl-BLM photo-emf as a function of time in dark in the presence of $FeCl_3$ and $FeCl_3$-riboflavin in KCl pH of 6. It was found that Chl-BLM photo-response in the presence of Fe^{+3}-riboflavin is lower than that in the presence of Fe^{+3} alone. This implies that riboflavin functions as electron acceptor at high solution pH.

Chl-BLM photo-emf curve in the presence of Fe^{+3}-riboflavin system.

Chl-BLM photo-emf curve in the presence of Fe^{+3} alone.


50
40
30
20
10
Chl-BLM photo-emf (mV)
Fe^{+3}
Fe^{+3} + EF
pH = 6
1000
2000
3000
4000
5000
Time (second)

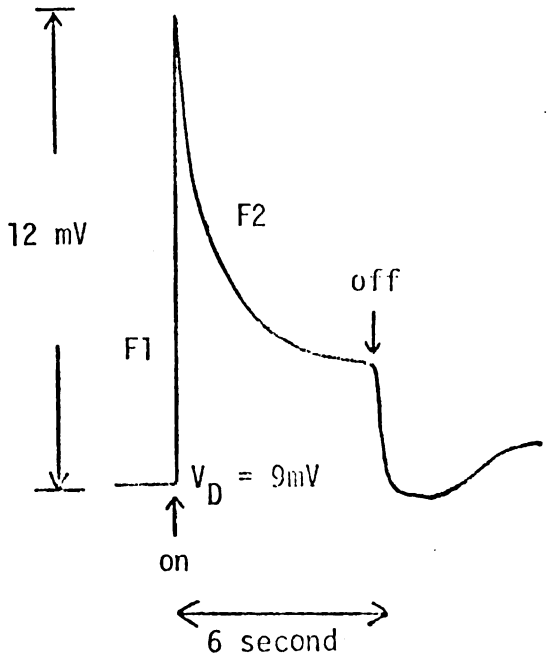
most RF are RF-B (electron donor) forms which couple with Fe^{+3} and will enhance Chl-BLM photo-emf as compared with Fe^{+3} present alone. On the contrary, at pH = 6, most RF are RF-A (electron acceptor) forms which couple with Fe^{+3} and should reduce Chl-BLM photo-emf response as compared with Fe^{+3} alone.

4) Enhancement of Chl-BLM photo-emf response by $FeCl_3$ and $FeCl_2$.

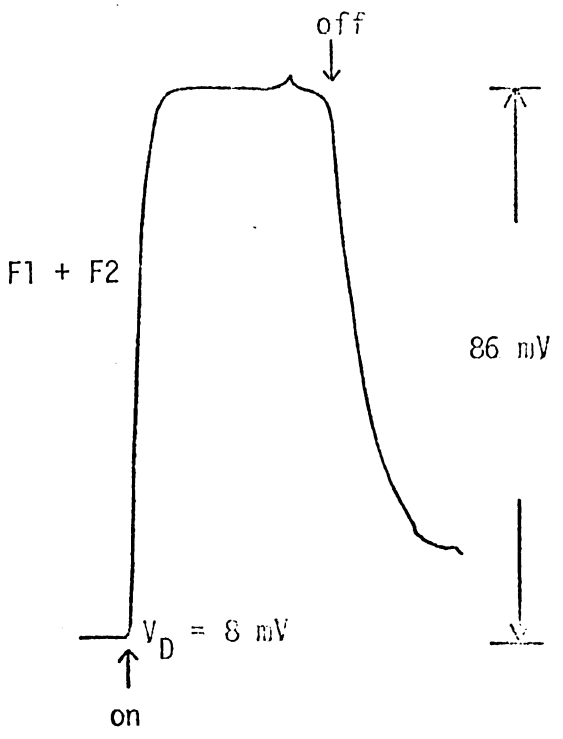
An increment of about 74 mV photo-response with $FeCl_2$ present in the BLM reference electrode has been observed. Two indications are: 1) Fe^{+2} functions as an electron donor in the BLM system; 2) electron donating and electron accepting compounds must both be present in the system to create a photo-response which is much larger than that of either electron donor or acceptor present alone. In addition, the photo-response form of the system with both electron donor and acceptor may change as compared to that in which the electron acceptor is present alone. Figure 33 indicates the difference of Chl-BLM photo-emf form before and after the presence of Fe^{+2} to BLM reference electrode. It seems that the enhancement of Chl-BLM reference electrode photo-emf in the presence of Fe^{+2} resulted in the F2 component (photo-response produced by the attraction of light-generated Chl positive charges or exciton toward electron donor or electric field) which then began to have the same response direction as the F1 component (photo-response produced by the attraction of electron toward electron acceptor).

5) Chl-BLM photo-emf induced by membrane potential in dark plus electron accepting compound. Out experimental evidence shows that Chl-BLM photo-emf in the presence of NaI has the greater value compared to that of photo-emf, with each corresponding dark potential induced

Figure 33. Chl-BLM photo-response pattern "before" and "after" the addition of $FeCl_2$ (10^{-} M/l) to BLM reference electrode. F1 component of photo-response results from the absorption of light-generated negative charges (electrons) toward electron acceptor ($FeCl_3$) and, F2 component of photo-response is produced by the movement of light-generated positive charges or exciton toward electron donor or electric field. In this particular case, F2 has the same direction as F1 which indicates that the light-generated positive charges or exciton are attracted by electron donor ($FeCl_2$) to enhance the charge separation which is created by the electron acceptor.



"Before"



"After"

by a simple externally applied source. It may suggest that NaI-containing I_2 molecules have entered into the membrane and will function as an I_2 electrode. I_2 will be reduced by the electrons produced by Chl absorbing the light. The direction of movement of this reduced Iodine in membrane will then depend on the direction of the electric field induced by NaI diffusion. The experimental evidence to support this postulate is the measurement of photo-emf for Chl-BLM formed by spinach extract mixing varying amounts of I_2. For each 0.25 ml spinach chloroplast extract, very dilute (5λ, 10λ, 20λ, 40λ, 80λ) I_2 solutions (which are made by dissolving 0.0019 gram I_2 into 1 ml of 1:1 ratio buranol-dodecane mixture) have been added to make up Chl-BLM-I_2 forming solutions. Figure 34 is the Chl-BLM-I_2 photo-emf versus the externally applied field. Each photo-emf curve in Figure 34 has greater value than that in Figure 17 which is induced by a simple applied field. Also, the Chl-BLM containing I_2 (5λ) has greater photo-emf than any other I_2-containing Chl-BLM.

Biphasic photo-response can be seen by properly controlling the electric field in the presence of NaI through externally applied voltages. Figure 35 shows this controlled experiment. The light-on and light-off are indicated, respectively, by upward and downward pointing arrows. The events shown in Figure 35 may be noted.

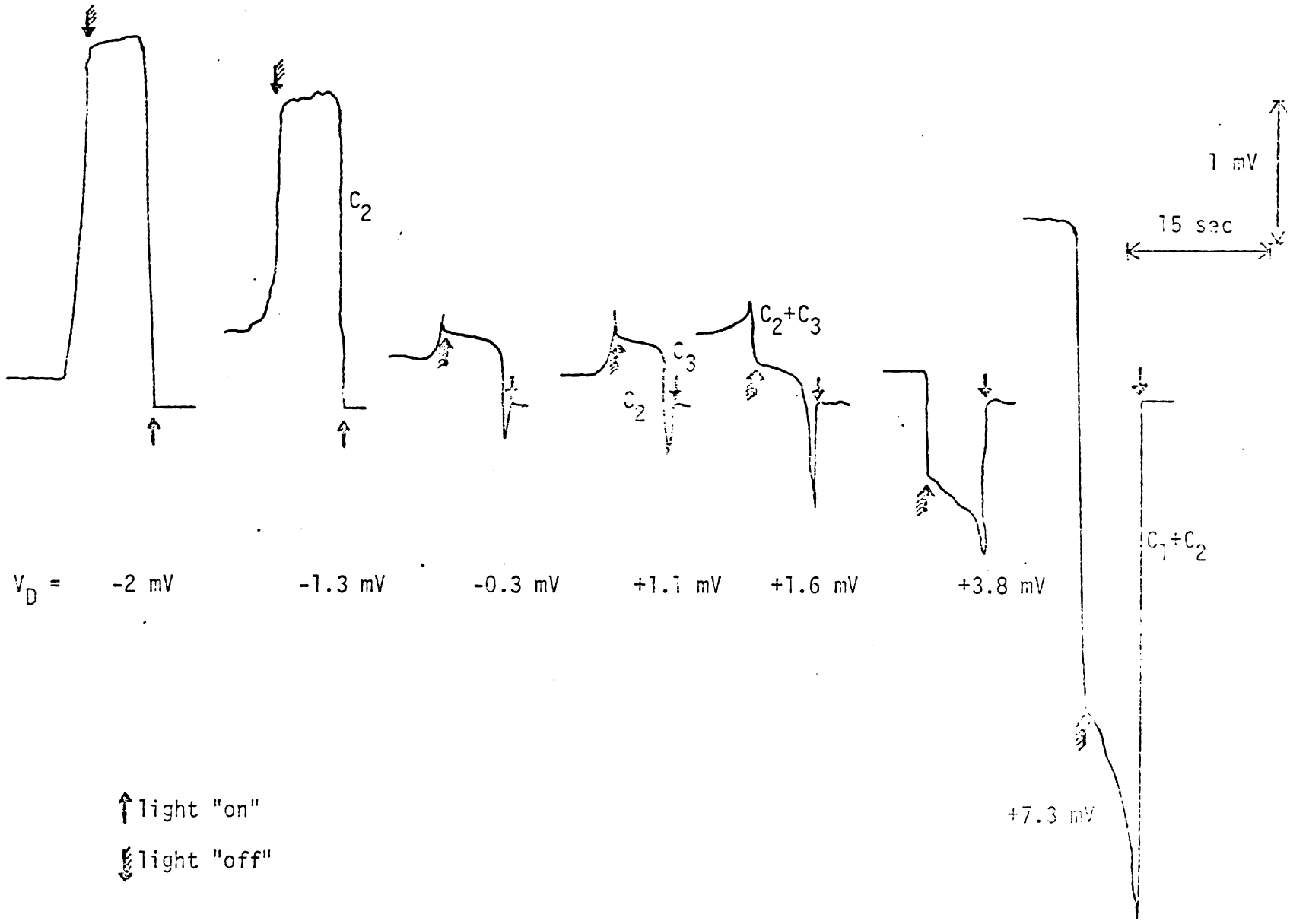
i) The photo-responses consist of two components; an initial fast one followed by a slower component;

ii) The biphasic responses are observed at applied voltages in the range of +3.8 mV to -0.3 mV;

iii) The initial component of the biphasic response is always negative (the side facing the positive electrode);

Figure 34. Chl-BLM-I_2 photo-emf as a function of membrane dark potential. 10^8 ohm of external shunt resistor is applied. Sodium buffer acetate (10^{-1} M), pH 5, was used as aqueous solution. Each curve corresponds to varying amounts of I_2 in Chl-BLM forming solution.

Figure 35. Biphasic response of Chl-BLM photo-emf in the presence of NaI (10^{-} M/l), and membrane dark potentials between +3.8 and -0.3 mV.


1 mV
15 sec
C_2
C_2+C_3
C_3
C_2
C_1+C_2
+7.3 mV
V_D = -2 mV
-1.3 mV
-0.3 mV
+1.1 mV
+1.6 mV
+3.8 mV
light "on"
light "off"

iv) The electron donating property of NaI can be seen only within the range of dark potential of +1.6 mV to -0.3 mV. The electron donating of this NaI will reduce the initial fast component and increase the slow component.

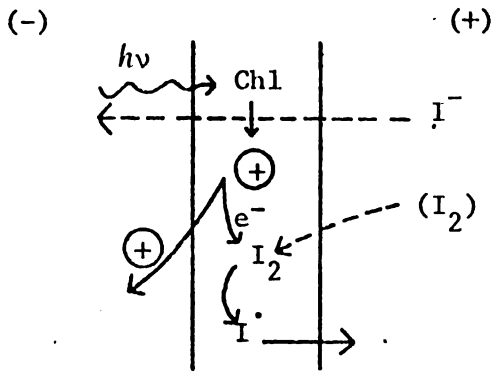
A general mechanism for Chl-BLM photo-emf can be described as follows:

i) The diffusion of I^- from the inner chamber toward the outer chamber will induce a dark potential with I^--containing side becoming positive.

ii) It is possible that the NaI-containing I_2 will enter into the membrane accompanying the diffusion of I^-.

iii) The charges generated by the light absorption of Chl in membrane will cause the reduction of I_2.

iv) This reduced I_2 (or $I\cdot$) will move toward the inner chamber where the positive electric field is.



b. Charge Carrier Generation

Chl-BLM photo-response from the charge separation point of view has been described and detailed in the previous section of this chapter. It is found that the electron donating and accepting power of the redox compound, or the electric field, will directly determine this charge

separation. The amount of Chl-BLM photo-response can also be analyzed from the charge carrier generation point of view. It is found that these light-generated charge carriers will be governed by three factors: 1) the amount of chlorophyll in BLM. This amount of chlorophyll is expressed by an absorption coefficient (i.e., number of moles of chlorophyll per liter of solvent); 2) light intensity; and 3) light duration time.

An equation to express the relation described above can be derived as follows. Let the light intensity be expressed as I. Then the amount of light energy absorbed per unit time in unit cm^2 of a layer of thickness dX is

$$-dI = k \cdot I \cdot dX \qquad (1)$$

where k is the optical absorption coefficient. Now the light energy absorbed per unit time in unit volume will be

$$-\frac{dI}{dX} = kI. \qquad (2)$$

Since the number of charge carriers (electrons and holes) generated per unit time per unit volume is proportional to the light energy absorbed in that time in the unit volume, the above equation will be expressed as

$$\begin{aligned} \Delta n &= \Delta p \ \alpha \ kI \\ &= \beta \cdot kI \end{aligned} \qquad (3)$$

where β is defined as "the quantum yield", i.e., number of pairs charge formed by a single quantum, if I represented the number of quanta per second.

If no other processes took place except carrier liberation, then this number of charge carriers generated per unit volume would increase with time; the expression is

$$\Delta n = \Delta p = \beta k I \cdot t. \tag{4}$$

1) Charge recombination or absorption process. It is known from experiments that, after a certain period of illumination, a maximum photo-response is reached. This follows the process of charge generation and, there must be a converse process of charge carrier annihilation. This converse process may be either charge carrier absorption by electron acceptor and donor, or the charge carrier recombination. The steady state is defined as the state at which the rate of charge carrier generation is equal to the rate of charge carrier recombination or absorption, or

$$\frac{d\Delta n}{dt} = 0. \tag{5}$$

2) Relaxation time of charge carriers. The relaxation time of charge carrier is defined as the time that the light-generated charge carrier is in a free state before absorption or recombination.

The relationship between Δn, Δp at steady state and the relaxation time is expressed as

$$\text{at } \frac{d\Delta N}{dt} = 0$$

$$\Delta n_{st} = (\beta k I) \cdot \tau. \tag{6}$$

3) Relaxation process. The rate of net charge carrier production in BLM, in unit volume $\frac{d\Delta N}{dt}$, can be expressed as

$\frac{d\Delta N}{dt}$ = no. of carrier generated in unit volume unit time − no. of carrier recombined in unit time in unit volume

$$= \frac{\beta k I t}{t} - \frac{\Delta n}{\tau} \quad . \tag{7}$$

By integrating equation (7), the following is obtained:

$$\int \frac{d\Delta N}{\beta k I \tau - \Delta N} = \int \frac{dt}{\tau} \quad -\ln(\beta k I \tau - \Delta N) = \frac{t}{\tau} + K. \tag{8}$$

At $t = 0$, $\Delta N = 0$, therefore, $K = -\ln(\beta k I \tau)$. Inserting K into equation (8), we obtain

$$-\ln(\beta k I \tau - \Delta N) = \frac{t}{\tau} - \ln(\beta k I \tau)$$

$$\ln\left(\frac{\beta k I \tau - \Delta N}{\beta k I \tau}\right) = -\frac{t}{\tau}$$

$$\Delta N = \beta k I \tau \, (1 - e^{-t/\tau})$$

$$= \Delta N_{st} \, (1 - e^{-t/\tau}) \tag{9}$$

At saturating light intensity, BLM photo-emf depends solely upon the number of available traps across the biface (or ΔN) as described by Tien [1971]. Equation (9) can be replaced by the expression of photo-emf E_{op}

$$E_{op} = [E_{op}]_{max} \cdot (1 - e^{-t/\tau}). \tag{10}$$

Since conductivity is the produce of $e \cdot u \cdot \Delta N$, equation (9) can also be expressed in terms of photo-conductivity:

$$\Delta\delta = \Delta\delta_{st} \, (1 - e^{-t/\tau}). \tag{11}$$

When light is "off", equation (7) becomes

$$\frac{d}{dt} \, (\Delta n) = 0 - \frac{\Delta n}{\tau} \quad . \tag{12}$$

Integrating equation (12), we obtain

$$\Delta V = \beta \Delta I_{ss} e^{-t/\tau}$$

$$= \Delta V_{ss} \cdot e^{-t/\tau} \qquad (13)$$

where t is the time that BLM remains in darkness after illumination.

Some experimental results were found to support the above theoretical approach. They are: 1) The τ value, obtained from either the photo-emf measurement of from the photo-conductivity measurement, is 0.4 second for the BLM system alone, 0.35 second for the BLM with $FeCl_3$ and 0.30 second for the BLM with $FeCl_3$ and ascorbic acid. This τ value will vary with the system used. 2) At constant amount of Chl and light intensity, the variable BLM photo-emf is the exponential function of light duration time. Figure 27 demonstrates this. 3) At constant duration time and light intensity, this variable BLM photo-emf is proportional to the amount of Chl contained. Our experimental data from the measurement for the Fe^{+3}/ascorbic system indicate this linear relationship between the photo-response and chlorophyll concentration (in butanol-dodecane mixture), at least in the chlorophyll concentration range of 5×10^{-4} M/l to 4×10^{-3} M/l. 4) At constant duration time and Chl concentration, this variable BLM photo-emf is directly proportional to light intensity. For the Fe^{+3}/ascorbic system, the experimental results indicate the linear relationship between the BLM photo-emf response and the light intensity, at least in the range of 5% to 100% light intensity (100% light intensity is counted in our system as the full light directed on the BLM from Keystone movie projector).

4. Significance of This Study

The primary processes of photosynthesis are still unknown, but there are some hints that the first chemical reaction after the absorption of the light quantum is a redox process in which the excited chlorophyll molecule exchanges an electron with its environment. With respect to this hypothesis, model studies in which photosynthetic pigments are incorporated into artificial lipid layer membranes are of great value. Especially our evidence of redox reaction, in terms of photo-emf technique, in the membrane surface should make the study os these redox events in photosynthesis possible.

CHAPTER VI

SUMMARY

1. General Properties of Chl-BLM Photo-emf

The general properties of Chl-BLM photo-emf which can be observed are the following.

a) The maximum photo-emf occurs near pH 5;

b) The Chl-BLM photo-response is time (in dark) dependent. In the case of $FeCl_3$, the system has to wait for 1000 seconds in dark before a saturated photo-emf can be reached;

c) Duration time is also very important for the photo-response. 1.5 second duration time is needed, at least in the case of $FeCl_3$, to have the maximum photo-response. It also shows that the longer the duration time, the larger the slow component of photo-response;

d) The concentration of chemical compound affects this photo-response substantially. A photo-emf of about 53 mV can be obtained when the concentration of $FeCl_3$ is 2×10^{-4} M/l. In order to have the photo-response, which is dependent on chemical compound concentration only, we must consider the effect of membrane dark potential on the photo-response which is created by the diffusion of $FeCl_3$ containing H^+ from one side to the other. This dark potential always has field

direction opposite to the facilitation of negative charge which moves toward $FeCl_3$. Our evidence is that when one reduces this membrane dark potential to zero, one can enlarge the photo-response to 107 mV in this $FeCl_3$ system;

e) The Chl-BLM membrane resistance can be varied, not only by different chemical species, but also by their concentrations and pH. The membrane resistance in the case of $FeCl_3$ (10^{-4} M/1) is ten times lower than in $FeCl_3$ (10^{-3} M/1). It is suggested that high membrane resistance formed from the strong binding of Fe^{+3} and negative polar group of p-lipid or pigment will drop if this binding complex is hydrolyzed near the solution/membrane interface. Above pH of 6, $FeCl_3$ will be hydrolyzed before it can form a complex and thus, there will be no effect on R_m. The quantity of drop in R_m will depend on the degree of binding of the complex and its hydrolysis;

f) The membrane dark potential (V_D) can be created by H^+ gradient, chemical diffusion or externally applied field. The hyperpolarization of membrane light potential indicates that H^+ is a sufficiently strong electron acceptor to avoid the depolarization of membrane potential in light by the electric field.

2. Determination of Electron Donating and Accepting Strength of Chemical Compounds

A BLM reference electrode technique has been developed to test the electron donating or accepting power of redox compounds. This investigation has been systematically conducted from inorganic to organic compounds, then through some biochemical compounds. Two Diagrams of these compounds' electron donating or accepting power with respect to $FeCl_3$ have been established. The further up this diagram

a compound appears, the greater is its tendency to donate electrons. The further down this diagram a compound appears, the greater is its tendency to accept electrons. It is seen then that NaI, FMN, ascorbic acid are the strongest electron donors, and I_2, Na_2S and benzequinone are the strongest electron acceptors.

The detailed reaction mechanism for the case of each compound is also discussed. For example: Chl-BLM break-down voltage of approximately 120 to 160 mV was observed either by externally applied voltage or light illumination onto the BLM in the presence of $Co(NH_3)_6^{+3}$ and tannic acid.

3. High Quantum Efficiency in Photo-effect

High quantum efficiency in photo-effect by means of the enhancement of Chl-BLM photo-emf in the presence of redox compounds has been obtained. Among them, as shown in Table 4, the largest photo-emf is for the case of $FeCl_3$ and ascorbic acid. An open-circuit photo-emf of about 188 to 192 mV and a closed-circuit emf of about 287 to 300 mV were observed.

4. Chl-BLM Photo-emf Responses Determined by Charge Generation and Separation

Based on the postulation of Tien [1968], the quantity of the Chl-BLM photo-emf seems to depend on (1) the number of generated charge carriers, and (2) their separations. An in depth discussion of them separately would facilitate understanding of the detailed mechanism of this photo-emf. A photo-emf equation based on this charge generation has been derived and its fixation to the experimental results are discussed.

Since the charge separation is mainly determined by the presence of electron donor and acceptor, several typical redox compounds are chosen to support this idea. $FeCl_3$ is a strong electron donor which is reduced by absorbing electrons from the solution/membrane interface. The larger the amount of $FeCl_3$ present, the larger the membrane charge separation. Riboflavin functions as either an electron donor or as an electron acceptor depending on its pH value. As a result, the polarity and the membrane charge separation will depend upon the pH at which the riboflavin solution is made. A proper coupling of $FeCl_3$ and $FeCl_2$ has enlarged this membrane charge separation.

5. Advantages of This Project

It is found that (i) Chl-BLM is a good model system for studying the reaction mechanism of redox reactions occurring at the membrane/solution interface; (ii) Chl-BLM functions as an electrode to transfer the electronic charge from one membrane/solution interface to the other interface; (iii) It seems possible to use Chl-BLM to study the energy conversion process, such as in photosynthesis.

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