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## INTERACTION OF CHANNEL-BLOCKING BISPYRIDINIUM COMPOUNDS WITH SUPPORTED PHOSPHOLIPID LAYERS

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**Abstract:** The interaction of phospholipid monolayers of dioleoyl phosphatidylcholine (DOPC) on mercury electrodes with bispyridinium compounds is reported in this paper. The interaction is related to the modification of the capacitance-potential plot and in particular of two well defined phase transitions of the phospholipid monolayer. The order of the extent of interaction of the test compounds with the monolayer can be related to their structure and is:- P65>Toxogonin>BPE>HS6>TMB4>HI6>BPT>P2S. The penetration of the compound into the monolayer depends on potential. At potentials more negative than the occurrence of the two phase transitions, the test compounds penetrate further and disrupt the monolayers. At more positive potentials this effect is reversed.

**Key Words:** Phospholipid Monolayer, Bispyridinium Compounds, Capacitance, Potential, Phase Transitions

### INTRODUCTION

In the past decade a powerful membrane model [1,2] has been developed and perfected. The membrane model consists of phospholipids deposited as a monolayer on a mercury electrode. This membrane model has many advantages over existing membrane models. The technique of deposition is simple and it requires no specialised depositional techniques. In fact the structure of the monolayer on the electrode depends on the intermolecular forces which hold the monolayer together as well as on molecule-solution and molecule-mercury interactions. The monolayer has been shown to represent exactly half a bilayer [3] and the structure of the lipid-water interface is precisely the same as that of the bilayer-water interface where many reactions of physiological interest take

place. The membrane model is quite unique in that the potential and current can be very precisely controlled and measured. The fluid nature of the mercury support allows the formation of a perfect monolayer impermeable to ions with no imperfections and defects. The renewable nature of the mercury in the electrode enables mercury drops with monolayers to be discarded and new drops to be formed and fresh monolayers to be deposited.

A basic measurement of the monolayer properties is the capacitance [1,2]. This is very sensitive to the structure and nature of the dielectric. Figure 1 shows the capacitance of a monolayer of dioleoyl phosphatidylcholine (DOPC) plotted as a function of the applied potential ( $-E/V$ ). Up to 400 mV negative of the Position of Zero Charge (PZC) of the electrode of  $\sim -0.4$  V, the capacitance maintains a constant value of  $1.75 \mu\text{F cm}^{-2}$  (capacitance minimum). This corresponds to the monolayer behaving as a perfect capacitor where the dielectric constant of the hydrocarbon of thickness 1.3 nm of the phospholipid is  $\sim 2.2$  [4].

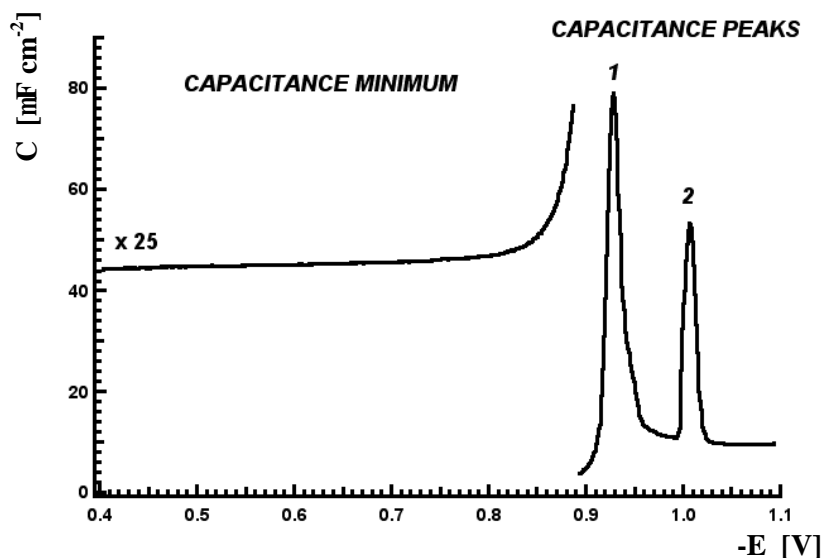


Fig. 1. Capacitance ( $C$ ) –potential ( $-E$ ) plot of DOPC monolayers in  $0.1 \text{ mol dm}^{-3}$  KCl solution at pH 6.8.

At more negative potentials the increase in capacitance corresponds to the development of defects in the monolayer as evidenced by the increased permeability to ions [5]. Two sharp phase transitions are characterised (capacitance peaks 1 and 2) which correspond to a reorientation of the structure of the monolayer to form a pored bilayer at more negative potentials [5]. The occurrence of these phase transitions has been mathematically modelled [3] and

experimentally characterised [5] and is due to the changing affinity of the phospholipid hydrocarbon tails and head groups and water for an increasingly polarised mercury surface. The phase transition corresponding to capacitance peak 1 represents the conversion of the monolayer into two phases of thick and thin monolayer. The phase transition corresponding to capacitance peak 2 relates to this inhomogeneous monolayer structure converting to a pored bilayer. Since the occurrence of the phase transitions depends on the intermolecular forces between the phospholipid segments and water, the phospholipid segments and mercury and the individual phospholipid segments, the phase transitions are highly sensitive to any change in organisation of the monolayer [3,6]. The dependence of the phase transitions on the change in the order of the monolayer renders the system ideal for testing the biological membrane perturbing properties of biologically active compounds [6]. A degradation of the monolayer organisation leads to the phase transitions taking place over a wider range of potential and is seen as a depression and broadening of the capacitance peaks 1 and 2 [6]. Such an effect is also indicated by an increase in the capacitance between potentials  $-0.4$  and  $-0.8V$ .

In this study the effect of bispyridium compounds on the organisation of phospholipid monolayer was assessed. Bispyridinium oximes are used in the treatment of poisoning by organophosphorus anticholinesterases and are generally believed to exert their therapeutic effect by reactivating the inhibited acetylcholinesterase (AChE). Other observations indicate that such compounds have an additional therapeutic action which is not related to the regeneration of AChE. One mechanism of action is that the bispyridinium oximes block the ion channel associated with the nicotinic acetylcholine receptor and thus counteract the effects of excessive cholinergic stimulation. This finding has been supported by experiments of *in vitro* channel blocking studies [7]. As a result of this interest in these compounds, a study was initiated to investigate the biological membrane activity of these bispyridinium compounds. The bispyridinium compounds represent a very interesting set of compounds to study because almost all of the compounds are based on the two ring structure shown in Figure 2. One of the instructive deductions from this investigation would be to see how the substitution of one group would alter the interaction of the compound with the phospholipid layer. Such studies are directly relevant to the activity of these compounds at the biological membrane level.

## MATERIALS AND METHODS

Electrolytes were fully deaerated with special grade argon before each experiment and a blanket of argon was maintained above the electrolyte during the experiment. This was done to avoid any introduction of oxygen during the experiment which interacts with the lipid layers. Monolayers of DOPC (semi-synthetic grade, Lipid Products, UK) were prepared as before [1,2] by adding

the phospholipids to pentane and spreading at the gas-water interface (area = 28 cm<sup>2</sup>) in the electrochemical cell (volume = 50 cm<sup>3</sup>). A fresh mercury drop of area A = 0.0088 cm<sup>2</sup> was coated with the monolayer from the gas-water interface prior to each experiment. The electrolyte used was 0.1 mol dm<sup>-3</sup> KCl prepared from precombusted KCl (BDH Chemicals Ltd). The test bispyridinium compounds were dissolved in water to give 4 g dm<sup>-3</sup> working solutions and stored at 4°C in darkened glass bottles.

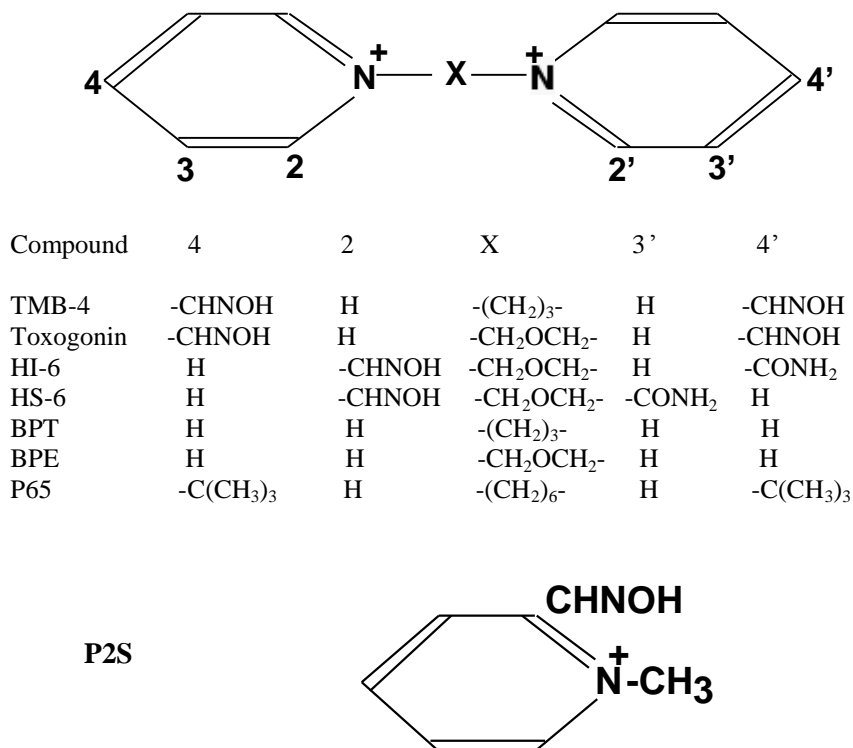


Fig. 2. Structures of bispyridinium compounds investigated.

To assess the effect of the bispyridinium compounds on the capacitance of the monolayers, the following procedure was carried out. Monolayers of DOPC were deposited on mercury. Control measurements of the capacitance of these monolayers were done. Following this, aliquots of the test compound were added to the electrolyte to give the required solution concentration. Capacitance measurements of the monolayer in contact with the test compound in solution were carried out during the first potential scan. The electrode used for the deposition of the lipid layers was a Kemula type Hanging Mercury Drop Electrode (HMDE) where the drops can be repeatedly renewed. Experiments

were carried out using a Metrohm potentiostat (E506 Polarecord) and data were recorded with a Maclab (16 bit, 100 kHz) data acquisition system. The Maclab system was also used to stimulate the cell potential. The Metrohm potentiostat in combination with a PAR 5110 lock-in amplifier was employed to measure the capacitance of the coated electrodes. All potentials in this study are quoted versus the Ag/AgCl: 3.5 mol dm<sup>-3</sup> KCl reference electrodes.

## RESULTS

Figures 3 and 4 show the order of the effects of the bispyridinium compounds on the capacitance-potential profile of the DOPC coated electrodes.

Figure 3 shows that toxogonin has a greater effect on the capacitance-potential profile than TMB4 in depressing the capacitance peak. P2S has an insignificant influence on the shape of the capacitance-potential plot and P65 has the strongest effect at the lowest solution concentration in that the depression of the capacitance peaks was noted with a very much lower concentration of P65 in solution (2 μmol dm<sup>-3</sup> as compared with 20 and 10 μmol dm<sup>-3</sup> of TMB4 and toxogonin respectively). Figure 4 shows that HS6 and BPE have a greater effect on the capacitance of the DOPC coated electrode than HI6 and BPT respectively. The effect of contact of DOPC with BPT is to shift the second capacitance peak to more positive potentials.

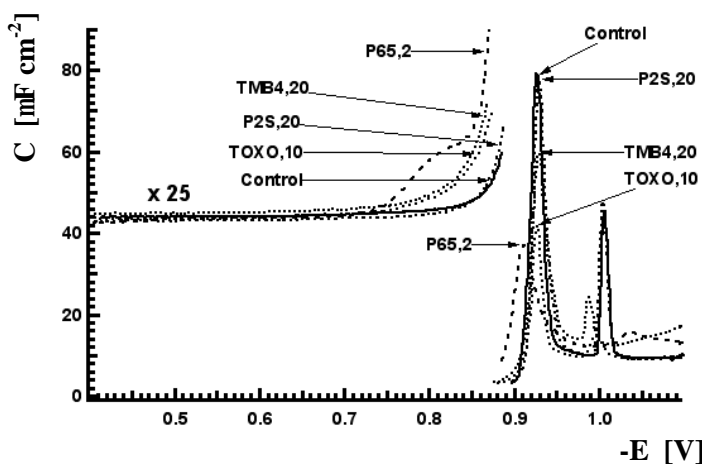


Fig. 3. Effect of bispyridinium compounds (compound, concentration in mol dm<sup>-3</sup>) on the capacitance (C)-potential (-E) plot of DOPC monolayers (control) in 0.1 mol dm<sup>-3</sup> KCl solution.

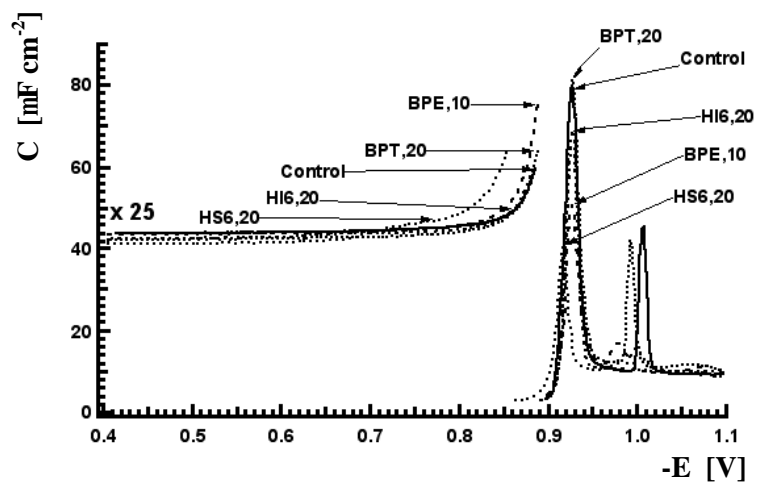


Fig. 4. Effect of bispyridinium compounds (compound, concentration in  $\text{mol dm}^{-3}$ ) on the capacitance (C)-potential (-E) plot of DOPC monolayers (control) in  $0.1 \text{ mol dm}^{-3}$  KCl solution.

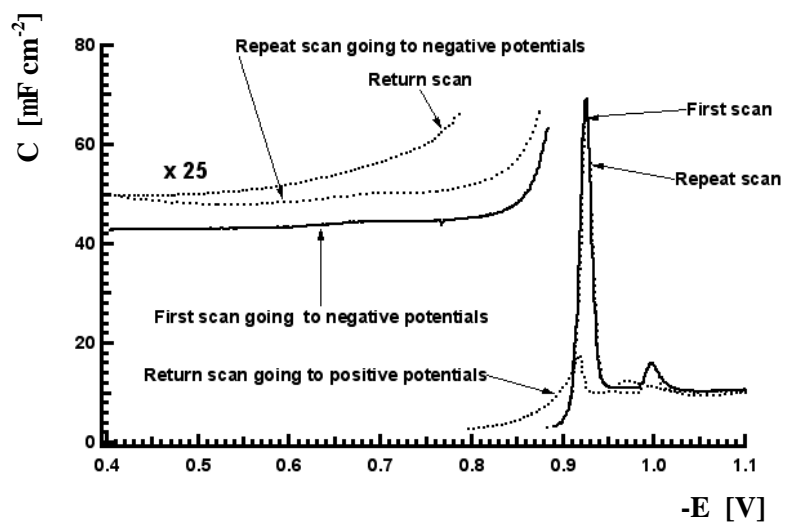


Fig. 5. Effect of HI6 ( $20 \mu\text{mol dm}^{-3}$ ) on the capacitance (C)-potential (-E) plot of DOPC monolayers in  $0.1 \text{ mol dm}^{-3}$  KCl solution.

Figure 5 shows the effect of one of the bispyridinium compounds, HI6, on the capacitance-potential scan of a DOPC monolayer. Both the capacitance peaks representing the phase transitions are suppressed. It is interesting that when the potential is return scanned in the positive potential direction, the capacitance peaks exhibit an almost complete suppression followed by an increase in the capacitance in the capacitance minimum region. On repeating the voltage scan to negative potentials, the capacitance in the capacitance minimum region decreases and the capacitance peaks recover.

## DISCUSSION

A comparison of the different test compound's effects on the lipid layer capacitance is interesting. The extent of interaction is related to the extent of the suppression of the capacitance peaks. Generally aromatic structures on molecules enhance interaction of the molecules with phospholipid layers [6]. Other properties such as hydrophobicity, molecular shape and charge also influence the extent of the interaction.. Figure 3 shows that out of all the bispyridinium compounds, P65 shows the strongest interaction with the lipid layer. This can be explained as due to the longer chain  $-(\text{CH}_2)_6-$  joining the two aromatic groups which allows for greater flexibility of the molecule and a greater chance for interaction of the two aromatic rings with the DOPC layer. In addition the presence of the two terminal  $-\text{C}(\text{CH}_3)_3$  groups enhances the aromaticity of the aromatic rings increasing interaction. Toxogonin shows a stronger interaction with the DOPC layer than TMB4. This is itself interesting and shows that the  $-\text{O}-$  atom as opposed to the  $-\text{CH}_2-$  group effects an increasing interaction with the lipid. This effect is also evident from the results in Figure 4 where BPE shows a stronger interaction with DOPC than BPT, an effect also attributed to the presence of the  $-\text{O}-$  atom compared to the  $-\text{CH}_2-$  group (see Figure 2). HS6 shows a greater interaction with DOPC than HI6, an observation related to the position of the amide group on one of the aromatic rings. The one-ringed P2S in comparison with the two-ringed compounds has no influence on the capacitance-potential profile which is consistent with the fact that aromaticity is one of the main factors determining the extent of interaction of compounds with phospholipid layers.

The influence of compound HI6 on the lipid layer capacitance in Figure 5 which is characteristic of the effects of the test compounds in general is interesting. The initial capacitance scan to negative potentials shows that the compound through its adsorption on the lipid surface influences the lipid reorientation during the two phase transitions. At more negative potentials the lipid layer becomes permeable to ionic species [5]. This enables the compound to fully penetrate the disrupted lipid layer. On the return scan the greater penetration and interaction of the compound in and with respectively the lipid layer is indicated by the almost complete suppression of the capacitance peaks and the

increased values of the capacitance at potentials less negative than  $-0.75$  V. Expulsion of the compound from the monolayer as the potential is scanned to more positive values is shown by the decrease in the capacitance. This is confirmed by the result that the repeat scan to negative potentials shows a similar capacitance-potential profile as obtained from the first voltage scan to negative potentials. These results show a clear mobility of the doubly positively charged bispyridinium compound in the lipid layer. In the absence of applied potential it adsorbs on the surface of the monolayer. At the negative potentials following the two stage reorientation and permeabilisation of the monolayer it penetrates the monolayer. Having penetrated the monolayer at negative potentials, it is expelled from the monolayer system at more positive potentials.

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