CHAPTER V

CONCLUSION

BCP was used throughout the experiment to be an optical probe in membrane bilayers made not only from various pure phospholipids but also from the mixtures of phospholipids and other components such as cholesterol, cholesterol derivatives as well as charged amphiphiles. The objective of addition of these components was to modify membrane bilayer fluidity and to confirm that BCP, if possible, could be used to investigate fluidity on these model bilayers which were constructed by various components. If BCP could detect fluidity in various conditions occurred; thus BCP could also be used as an optical probe in other biological membranes . However, cautions should be taken when external probes were used due to membrane perturbation. This may be ratified or supported by other methods such as differential scanning calorimetry (DSC) or circular dichroism spectroscopy, etc .

The experimental results presented here should be summarized in the following manners.

1. One of the important factors that governs characteristic of membrane is acyl chain length of phospholipid. At experimental temperature, 25 ^{o}C , DLPC (C 12) and DMPC (C 14) are in fluid state while the gel or solid state is obtained from the longer one, DPPC (C 16) and DSPC (C 18). BCP binding to phospholipids with difference in lipid state shows less penetration of BCP into gel state membrane by diminution of membrane-monomer band whereas dimer band enlarges. On the other hand, larger monomer bands are observed in fluid state membrane.

2. Temperature has a marked influence on the state of membrane. At temperature above phase transition temperature (T_C) of lipid, membrane is in fluid state whereas solid state exists at temperature lower than T_C. When DMPC liposomes are used as model membrane bilayers and experimental temperature is decreased from 32^{o}C through T_C of DMPC (23 ^{o}C) to 15^{o}C, the results illustrate that at temperature above T_C, BCP binding to bilayers orients in monomer conformation and locates in hydrocarbon region deep in membrane owing to great shift of monomer-λ_{max} to longer wavelength. Meanwhile when temperature is gradually decreased, monomer' s position in membrane changes to outer surface layer; thus wavelength of maximum absorption shiftes to shorter

wavelength and remaines constant when reached T_C or lesser. Declination of monomer band is observed with the growth of dimer band, attributed to reorientation of monomer to dimer conformations at low temperature.

3. Cholesterol is one component that influences integrity of membrane. It has a dual effect on phospholipid, depending on its T_C. A fluidizing efffect is shown at temperature below T_C, however, as temperature is increased above T_C, a condensing effect is presented. Cholesterol is added in various % mole fraction to phospholipids both in liquid crystalline phase (DLPC and DMPC) and in gel phase (DPPC) and liposomes formed by these components are used as membrane bilayers to bind BCP. In the case of liquid crystalline phase lipid, cholesterol shows condensing effect; thus, BCP binds to membrane in lesser extent compared to those pure phospholipids. Moreover, major form is dimer showing λ_{max} at about 575 nm. In contrast, cholesterol makes gel phase lipid more fluid, consequently, BCP binds to membrane in greater extent, however, dimer forms still remains dominant, possibly by formation of dimer obtained from two monomer molecules come close together.

4. When phospholipids in which acyl chains differ in length of 4 carbon atoms (DLPC and DPPC) are used to prepare membrane bilayers in various molar ratios. These two lipids do not mix ideally; thus domains on membrane readily occur and their properties depend on their composition. On membrane

bilayers, the mixture portion of DLPC and DPPC in molar ratio equivalence is surrounded by another excess phospholipid or distributes throughout liposomal membranes. BCP binding depended on that encircled lipid. As shown, the excess of DLPC and DPPC illustrate different dye binding.

5. Charged amphiphiles (positively and negatively charged amphiphile) have a significant mean on arrangement in lipid bilayer and may be affected binding of dye in membrane. When BCP binds to phospholipid bilayers, it is observed that λ_{max} of monomer shifts to longer wavelength, due to hydrophobic property and electrostatic interaction. Repulsion between charged molecules existing in membrane enlarged the space, resulted in loose packing. Subsequently, BCP binds to membrane in greater extent and dimer forms preferentially occur; regarding to high concentration of monomer in restricted area that permits reorientation. Charged repulsion between dyes and negatively charged amphiphiles on phospholipid membrane hinders dye binding, in contrast to positively charged one. Monomer band is also dominant with positively charged membrane.

6. Cholesterol derivatives differ from cholesterol in 3 β - OH group, possessing longer side chain containing propoxy (derivative I) or ethoxy (derivative O and II) group. Inclusion of hydroxy cholesterol derivatives to membrane produces fluidizing effect , due to head group disturbance

of membrane bilayer, therefore ,condensing effect of cholesterol can be modified by addition of all cholesterol derivatives (derivative O, I and II). From acquired results, there are shown that derivative II illustrates the highest fluidizing effect. In membrane bilayers which absent or present cholesterol, all cholesterol derivatives show fluidizing effect, but these derivatives make membrane more fluid in the presence of cholesterol because of more fragile property of membrane at very high concentration of cholesterol.

Bromcresol purple belongs to the class of sulfonphthalein dye that has found widespread application as an acid dye for analyzing amine containing drugs in various preparations.From the studies the effects of bilayer phase transitions, domain formation and molecular packing on visible absorption characteristics of BCP, it has been clarified that BCP has been able utilized successfully as a useful optical probe to sense the degree of lipid packing of bilayers. Since its absorption spectra are clear, simple and easy to interprete and change depending on membrane structures and compositions, then the informations so obtained from the experiments on lipid bilayer membranes may be applicable to the study of the structural and functional events occurring in biological membrane. However, an appropiate concentration of BCP in each investigation should be carefully explored since aggregation of dye obtaining from nonsuitable concentration may lead to misguided results.