JOURNAL OF COLLOID AND INTERFACE SCIENCE **191,** 341–348 (1997)
ARTICLE NO. CS974957

Addition of (Tri-)Block Copolymers to Phospholipid Vesicles: A Study of the Molecular Morphology and Structure by Using Hydrophobic Dye Molecules as Bilayer Probes

K. Kostarelos,*,†,[1] P. F. Luckham,† and Th. F. Tadros*,†

*Zeneca Agrochemicals, Formulation Section, Jealott's Hill Research Station, Bracknell, Berkshire RG12 6EY, United Kingdom; and †Imperial College of Science, Technology and Medicine, Chemical Engineering and Chemical Technology Department, University of London, London, SW7 2BY, United Kingdom

Received December 6, 1996; accepted April 28, 1997

A dispersion of soybean lecithin in water leads to the formation of multilamellar vesicles (MLVs), which on sonication break down into small unilamellar vesicles of approximately 50 nm in diameter. The addition of polymeric molecules in the liposomal system is thought to provide the liposomes with a steric barrier. The molecules used were (tri-) block copolymers (Synperonics) containing a central hydrophobic part (polypropylene oxide) and two hydrophilic chains (polyethylene oxide). The aim of this work was to study whether it was possible to anchor deep inside the lipid bilayer the copolymer hydrophobic block. The exact localization of the copolymer molecules was investigated using a multiprobe technique. The full spectra of two hydrophobic dyes, namely Nile red (NIL) and Pinacyanol chloride (PCYN), were compared while solubilized inside the liposome bilayer. The sensitivity of their spectral characteristics to polarity and self-aggregation produced a monitor of the bilayer microenvironment. The more hydrophobic NIL proved an accurate polarity sensor of the bilayer microenvironment and the formation of PCYN dimers and nonabsorbing aggregates can be directly related to the local (bilayer) concentration of the dye and the volume available to the solubilized dye molecules. Shifts of the maximum absorbance (λ_{max}) for both dyes showed that the bilayer environment was becoming more apolar with increasing copolymer concentration. The absorbance peak of PCYN due to dimer/aggregate formation increased at moderate copolymer concentrations, indicating that the polymer is incorporated inside the lipid bilayer.

Key Words: **liposomes; vesicles; block copolymers; steric effect; dyes.**

INTRODUCTION

Phospholipid vesicles (liposomes) are considered to be extremely advantageous colloidal dispersions, because of their ability to solubilize both hydrophilic and lipophilic substances in their inner aqueous phase and their lipid bilayer, respectively. Their biocompatibility (low toxicity) and biodegradability has attracted numerous applications, mainly in the medical field, as carriers of various drugs. Unfortunately, the limited stability of liposomes may cause a problem for their application as drug carriers and elsewhere, such as in cosmetic formulations. Various attempts have been made to sterically stabilize the phospholipid vesicles, either by adsorption of macromolecules such as glycolipids, proteins, and water-soluble polymers onto the liposome surface (1–3); by incorporation of glycolipids (4) and polyoxyethylene ether surfactants in the vesicles (5, 6); or by chemisorption of polyethylene glycol (PEG) chains on the phospholipid head group (7). The last covalent attachment of polymer chains has proved to be the most successful approach so far, providing the liposome system with prolonged blood circulation half lives.

It was thought that the addition of polymeric surfactant molecules of the (tri-) block type could provide the vesicles with an equally effective steric barrier, as long as the anchoring moiety was deeply buried in the bilayer. For this reason, two methods of copolymer addition were comparatively studied. The first method consists of initial addition of the copolymer, before the formation of the multilamellar (MLVs) or the unilamellar (SUVs) vesicles. This would allow the polymer to participate in the vesiculation process and bury its hydrophobic component in the lipid bilayer with greater ease. According to the second method, the block copolymer is added after formation of the vesicles. In this case, the block copolymer will simply adsorb on the vesicle surface. Such systems have been studied previously from a pharmaceutical point of view (2).

Various techniques have been used for the study of the liposome bilayer microenvironment. The use of small fluorescent molecules has proven particularly advantageous in providing information about the bilayer structure (8). These molecules are either solubilized in the bilayer at opti-

[1] To whom correspondence should be addressed at Farmeco Co., 27 Eleftherias Str., 15123 Athens, Greece. E-mail farmeco@eexi.gr.

0021-9797/97 $25.00

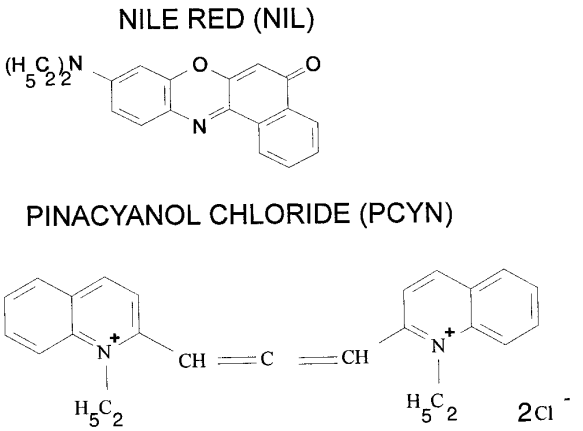

FIG. 1. Molecular structure of the chromophores NIL and PCYN.

mum concentrations or are covalently bonded to the phospholipids forming the bilayer. Investigation of the fluorescence and UV/visible spectra of such hydrophobic molecules has provided valuable information about the electric, dynamic, conformational, and structural properties of the vesicle bilayer.

In order to monitor the vesicle bilayer structure in the present study, two hydrophobic dyes, Nile red (NIL) and Pinacyanol chloride (PCYN), were used. These dyes show marked solvatochromic behavior, sensitively indicating changes in solvent polarity (9). In addition, the formation of PCYN dimers and polyaggregates can be used to further characterize the dye environment (10). By monitoring the changes in the respective UV/visible absorption patterns of the particular chromophores, information was obtained about their direct microenvironment.

The work described in this paper uses the spectral characteristics of these dyes as an indication of whether the block copolymer is incorporated inside the lipid layer or simply adsorbed at the vesicle surface, depending on the method of vesicle preparation.

MATERIALS AND METHODS

Soybean lecithin was purchased by Sigma (approximately 50% *d*-α-phosphatidylcholine). The sonication technique (11) was carried out using a Kerry ultrasonic bath (50 Hz) for the preparation of the vesicles. A block copolymer of the Synperonic PE family was supplied by ICI Surfactants, Belgium; namely the Synperonic PF127 was used. This polymer has the structure

$$(EO)_{99} - (PO)_{65} - (EO)_{99},$$

where EO refers to ethylene oxide and PO to propylene oxide.

Nile red and Pinacyanol chloride were purchased from Sigma, and their chemical structures are shown in Fig. 1. All concentrations are referred to as % wt/wt of the total sample weight. All materials were used without further purification.

Preparation of the Vesicles

As mentioned above, two methods have been used to prepare the vesicles. For ease of presentation, the samples denoted (I) refer to vesicle systems where the block copolymer was added initially, i.e., let to participate in vesicle formation. The samples denoted (A) refer to the systems where addition of the polymer took place after the liposomes had been formed.

The dye-containing vesicles were formed by preparing stock solutions of NIL and PCYN in a $CHCl_3$:CH_3OH (4:1) solvent mixture. The required amount of lipid was also dispersed in the solvent mixture and rotary evaporated at 40°C and under 30 mm Hg pressure. The dry lipid–dye film formed in the round bottomed flask was left under pressure for at least 1 hr to ensure the complete removal of solvent traces.

In the case of initial addition, the copolymer was added to the dry lipid–dye film and the mixture was redispersed in water. Sonication followed using a sonibath and the mean vesicle diameter was monitored using dynamic light scattering measurements (PCS). All (I) samples were prepared separately.

In the case of addition of the polymer after vesicle formation, a stock lecithin-dye mixture was dispersed in solvents, rotary evaporated, and resuspended in water. This was followed by sonication leading to the formation of small unilamllar vesicles. Various amounts of Synperonic PEF127 solutions were added to the liposome system to reach the required concentrations.

All procedures were performed with minimum exposure to light, keeping the dye solutions wrapped in aluminum foil at all times, to avoid any photodegradability complications. The final concentration of lipid was 2% (wt/wt) and the lipid:dye molar ratio was kept constant for all preparations at 500:1. At this molar ratio, the partition coefficient between membrane-bound and free dye molecules in solution ensures that no significant interference of the spectral data occurs. As has been previously shown (9) not more than 5% of the cyanide dye (PCYN) and only traces of the highly hydrophobic NIL are dissolved in buffer. Also, preliminary PCS measurements were performed for the small unilamellar vesicles to ensure that no significant increase in size was caused by solubilization of the dye molecules inside the liposomal bilayer. Liposome dispersions of 42.4 and 53.9 nm were used (by controlling the duration of sonication). After solubilizing NIL and PCYN molecules inside the lipid bilayer, the mean vesicle sizes were 42.6 and 52 nm, respectively. Therefore, the inclusion of the probe molecules inside the bilayers caused no significant changes of the liposome structure.

Dynamic Light Scattering Measurements

The mean diameter of the vesicles was monitored using the Malvern (UK) 4700 PCS, with an Argon laser beam of 488 nm wavelength. All measurements were carried out at a 90° angle and at 25°C. Great care was taken to minimize vesicle–vesicle interactions and multiple scattering effects, by diluting the samples 100 times immediately prior to measurement, therefore resulting in a final lipid concentration of 0.02% (wt/wt). A more detailed description of the light scattering technique can be found elsewhere (12).

UV/Vis Measurements

All absorbance measurements were carried out using a Lamda 2 Perkin–Elmer UV/Vis spectrometer, as 400–700 nm wavelength sweeps, recording the absorbance spectra directly. The corresponding dye-free preparation was used as a reference sample to compensate for turbidity effects.

RESULTS

Absorbance Behavior of Free Dye Molecules

Nile red. Nile red is an uncharged benzophenoxazone dye, poorly soluble and strongly quenched in water, with a high partition coefficient from water to hydrophobic solvents. In chloroform it gives a light red color, while solubilized in vesicles it shows a deep red color. It is photochemically stable. NIL has been used as a fluorescent stain for intracellular lipids (13, 14) and as a polarity sensor deducing protein structure and conformation (15). Its structure (Fig. 1) reveals widely separated potential electron donor and acceptor groups, suggesting that its spectroscopic properties may be sensitive to the polarity of the solvent (16).

The absorbance spectrum of NIL in the visible region shows a single broad peak at 556 nm. Preliminary studies were carried out to elucidate the dependence of NIL's maximum absorption (λ_{max}) on the polarity of the solvent. Increasing the polarity of the solvent mixture chloroform/methanol causes a moderate increase in the λ_{max} of NIL (Fig. 2). This finding correlates well with previous work (15) in which NIL λ_{max} shifted to higher wavelength as the polarity of dioxane/water solvent mixtures was increased.

Attempts to solubilize NIL in 1% wt/wt block copolymer F127 aqueous dispersion failed. The observed insolubility of NIL molecules (Fig. 3) can be interpreted as the inability to solubilize very hydrophobic moieties (NIL molecules) inside the more hydrophilic, relative to the chloroform/methanol solvent environment, PPO micelle core.

Pinacyanol chloride. Pinacyanol chloride was also used to prove the vesicle bilayer. PCYN is a cationic cyanine dye, and less hydrophobic than NIL. It is a large molecule (Fig. 1) compared to other cyanines like methylene blue, MER 540 (the latter being the most extensively studied cyanine dye). PCYN, similarly to NIL, shows solvatochromic sensitivity. In the preliminary study undertaken (Fig. 2), its λ_{max} drops as polarity of the solvent mixture chloroform:methanol is increased.

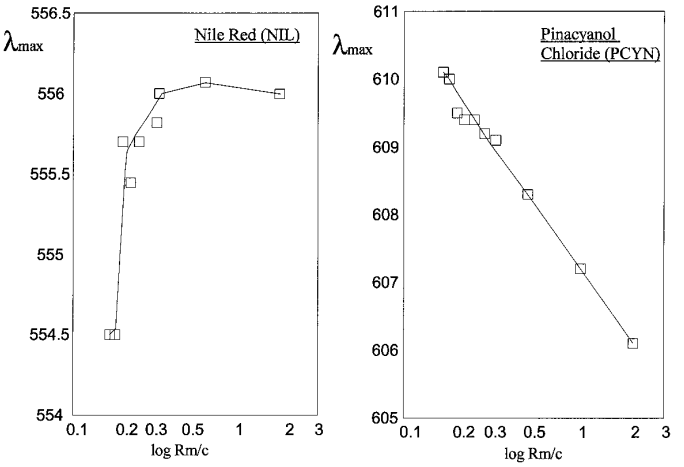

FIG. 2. Solvatochromic behavior of NIL and PCYN when increasing the polarity of the solvent mixture methanol:chloroform. $R_{m/c}$ is the ratio (w/w) of methanol:chloroform.

PCYN's absorbance spectrum exhibits a strong dependence of its spectroscopic properties on the extent of aggregation occurring between the dye molecules (10). The sharp peak at 600 nm is assigned to the absorbance of monomer dye molecules. The peak at 560 nm is due to absorbance of the dimeric form of PCYN. The third component, usually observed as a shoulder to the dimer peak, at about 525 nm is thought to be due to the polyaggregate form of the chromophore. Even though the absorbance spectral characteristics of PCYN have not been examined extensively, there have been reports in the past, mainly by the group of Pal and Gosh (10, 17, 18), that this particular dye has the tendency to homoaggregate under certain conditions, such as when bound to polyelectrolytes. These PCYN self-aggregates are almost nonabsorbing, exhibiting a low wavelength multipeak visible spectrum of extremely low intensity, a phenomenon described as metachromasia. Other reports suggest that the accumulation of bound probe will apparently raise the local dye concentration such that the formation of bound dye self-aggregates is favored. Such aggregates are either weakly or nonabsorbing (19).

Figure 4 shows the absorbance spectrum of PCYN in the solvent mixture $CHCl_3$:CH_3OH (4:1). The solubilization of the dye molecules in block copolymer F127 aqueous dispersions was achieved. The ratio between the dimeric:monomeric absorbance components increases evidently at 1% F127, when compared with the absorbance patterns produced in solvents. Also, at 1% F127, λ_{max} (602 nm) decreases considerably, compared to the λ_{max} value obtained when PCYN was solubilized in the solvent mixture (609.4 nm).

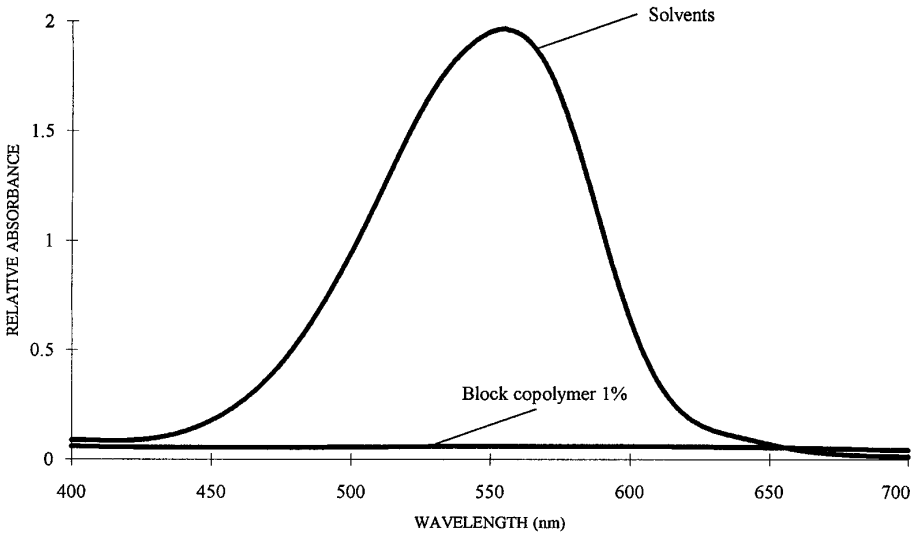

FIG. 3. Absorbance spectrum of NIL in the solvent mixture $CHCl_3$:CH_3OH (4:1) compared with the unsuccessful attempt to solubilize NIL in 1% (w/w) PF127 aqueous dispersion.

This indicates that the F127 micelle core is more polar than the solvent environment (which explains why the more hydrophobic dye—NIL—could not solubilize inside the copolymer micelles in Fig. 3).

This considerable increase in PCYN dimer population when interacting with 1% F127 (Fig. 4) has not been reported before. Dimer formation is generally attributed to increases in the collision rate of the molecules, which can be caused by decreasing the viscosity of the solvent, increasing the temperature, or increasing the dye local concentration (e.g., by decreasing the volume in which the dye molecules can solubilize) (20). According to recently reported studies (21), the microviscosity of the triblock copolymer micellar core is independent of the total copolymer concentration.

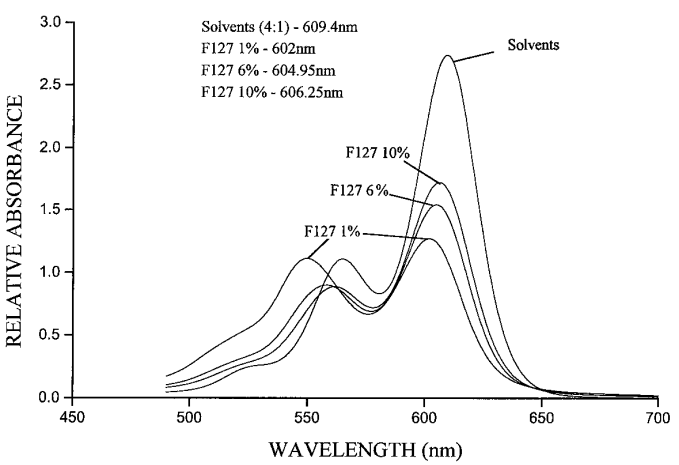

FIG. 4. Absorbance spectrum of PCYN in solvent mixture $CHCl_3$:CH_3OH (4:1) compared with the spectra obtained when solubilizing PCYN in increasing concentration PF127 aqueous dispersions. The inset table depicts the λ_{max} of the dye monomer peak in the different preparations.

Also, the temperature was kept constant throughout the present work. Therefore, PCYN dimerization is attributed to the restriction in the volume available to the dye molecules (micelle size) and their specific interactions with the polypropylene (PPO) chains, which will comprise the copolymer micelle core.

As the block copolymer concentration increases, the dimer:monomer ratio decreases, followed by an increase in the maximum absorbance values. The observed dependence of PCYN dimerization and maximum absorbance (λ_{max}) on copolymer concentration is indicative of, but does not provide definite evidence of block copolymer micellization. Further dynamic light scattering experiments were carried out[2] to provide (in accordance with the observations described above) confirmation of the presence of F127 micelles. Figure 5 shows the variation in micelle diameter and polydispersity index of aqueous F127 dispersions at 1 and 6% (wt/wt), with increasing temperature (25–65°C). As can be seen, the micelle radius obtained at 25°C increases as copolymer concentration is increased (from 16.3 ± 0.75 nm at 1% F127 to 25 ± 1.125 nm at 6% F127), indicating micellar swelling as more molecules are present. These findings agree almost perfectly with previously reported values of triblock copolymer micelle size and its variation with copolymer concentration (22–24). For both concentrations studied, micelle size and polydispersity index decreased with increasing temperature, suggesting more monodisperse, uniformly sized micelle dispersions of approximately 14 nm radius at the plateau reached. Similar trends have been reported for 15% F127 dispersion (22).

[2] The appropriate values for medium viscosity and the water refractive index were input for each temperature at which a measurement was performed.

Therefore, from the above, it can be concluded that F127 micelles are indeed formed at the concentrations studied, and PCYN molecules can solubilize inside the PPO core (which is expected, since PCYN is less hydrophobic than NIL). Increasing the F127 concentration from 1 to 6 and 10%, the volume available to dye molecules increases since the copolymer micelle size has increased, hence the decrease in PCYN dimerization manifested by the observed decrease of the dimer peak and the concomitant increase of the monomer peak. Moreover, the dye microenvironment becomes more apolar as the number of PPO chains in each micelle increases, exactly as the λ_{max} shifts to higher values dictated (Fig. 4).

Absorbance Behavior of Bilayer-Bound Dye Molecules

MLVs. When NIL and PCYN are solubilized in the chloroform:methanol (4:1) solvent mixture, they give strong sharp spectra peaking at 556 and 609 nm, respectively. When solubilized inside multilamellar vesicle (MLV) bilayers, the maximum absorbance is shifted, for both dyes, in directions indicating more apolar microenvironments (Table 1). In NIL's case λ_{max} drops about 10 nm, while for PCYN λ_{max} shifts 6 nm higher. These trends, compared with the solvatochromic study (Fig. 2), suggest that the bilayer lipid phase is more apolar than the solvent mixture used.

TABLE 1
Maximum Absorbance (λ_{max}) of Chromophores in Multilamellar Vesicles (MLVs)

	Nile red (NIL) (nm)	Pinacyanol chloride (PCYN) (nm)
Solvent mixture		
Chloroform:methanol (4:1)	556	609.4
MLVs	546.5	615.15
MLVs + F127 1% (I)	546.5	615.4
MLVs + F127 1% (A)	545.5	615.2
MLVs + F127 6% (I)	547.4	615.5

The effect of block copolymer addition was investigated using multilamellar vesicles. Table 1 shows that there is not any significant shift of λ_{max} when F127 is added to the liposome dispersions. Also, the absorbance spectra for the two dyes seems to be completely unaffected whether the copolymer addition was made before or after the MLV formation. It has to be noted that the MLV samples were extremely turbid. This excess turbidity was due to an increase in the scattering of the incident beam from the sample, leading to poor sensitivity and introducing larger errors.

SUVs. Dye molecules were solubilized inside the lipid bilayer of small unilamellar vesicles (SUVs), following the procedure described previously. The transparency of the latter systems renders them as a sensitive tool for absorbance studies, overcoming the large errors that were introduced using the multilamellar structures.

NIL solubilized inside the bilayer of SUVs (Fig. 6). The addition of copolymer initially or after liposome formation seems to cause a shift to lower values of absorbance by 3–4 nm, indicating that the lipid bilayer polarity is slightly decreasing in the presence of copolymer, irrespective of the way the F127 was added.

PCYN was used to systematically study the interaction between the block copolymer molecules and the SUVs, comparing the two different ways of copolymer addition, i.e., before and after vesicle formation. The sensitivity of its visible absorbance toward self-aggregation provided strong evidence of direct interaction between the copolymer and dye molecules only when adding the F127 initially. The initial addition of F127 at low concentrations (0.05, 0.5, 1, 2%) causes a considerable depression in the total absorbance (Figs. 7a–7e). When increasing the molar ratio copolymer:lipid even further, the absorbance and the spectral features recover (Figs. 7f–7i). Note that when the copolymer is added following vesicle formation, there is hardly any difference between any of the curves (Fig. 7j), indicating that, in this case, the polymer is not

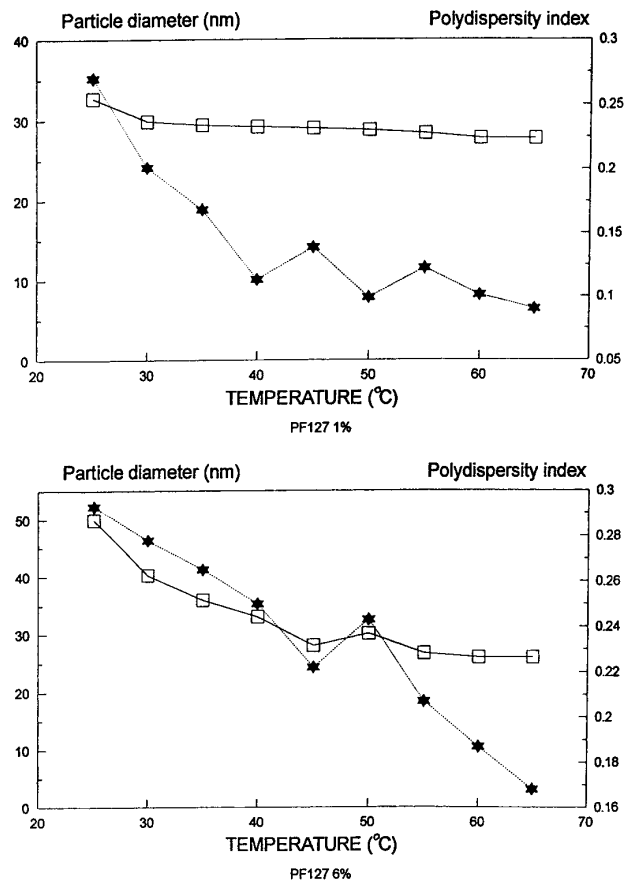

FIG. 5. Dynamic light scattering (DLS) measured particle diameter (□) and polydispersity index (★) of a 1 and 6% (wt/wt) PF127 aqueous dispersions as a function of temperature.

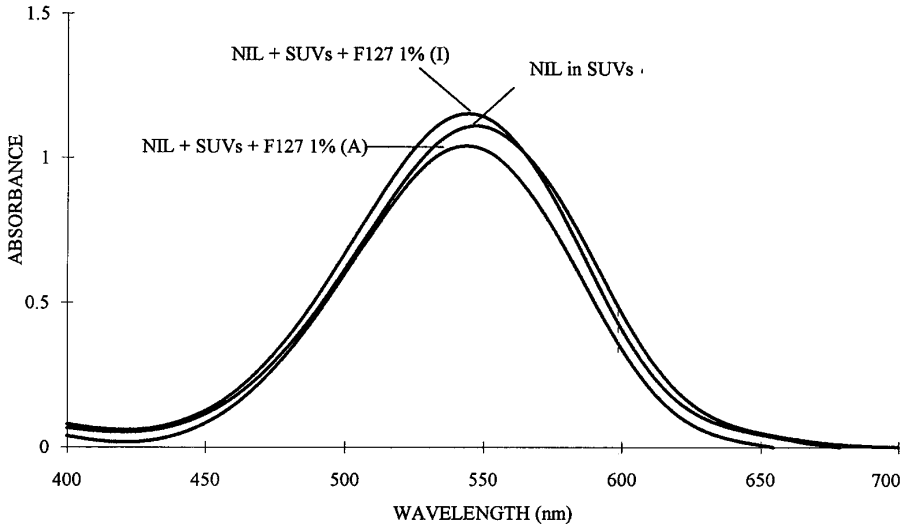

FIG. 6. Absorbance spectra of NIL when bound to small unilamellar vesicles (SUVs). Note the slight shift of λ_{max} when the block copolymer is added, irrespective of the way of addition. Bulk lipid concentration, 2% (wt/wt).

interacting with the dye and therefore is not incorporated into the lipid bilayer. These dramatic effects of copolymer addition are most evident in the samples of PCYN containing vesicles. The dye's deep blue color disappears in the (I) samples for low polymer concentrations and is recovered for higher concentrations, while the (A) samples retain their color throughout the copolymer concentration increase.

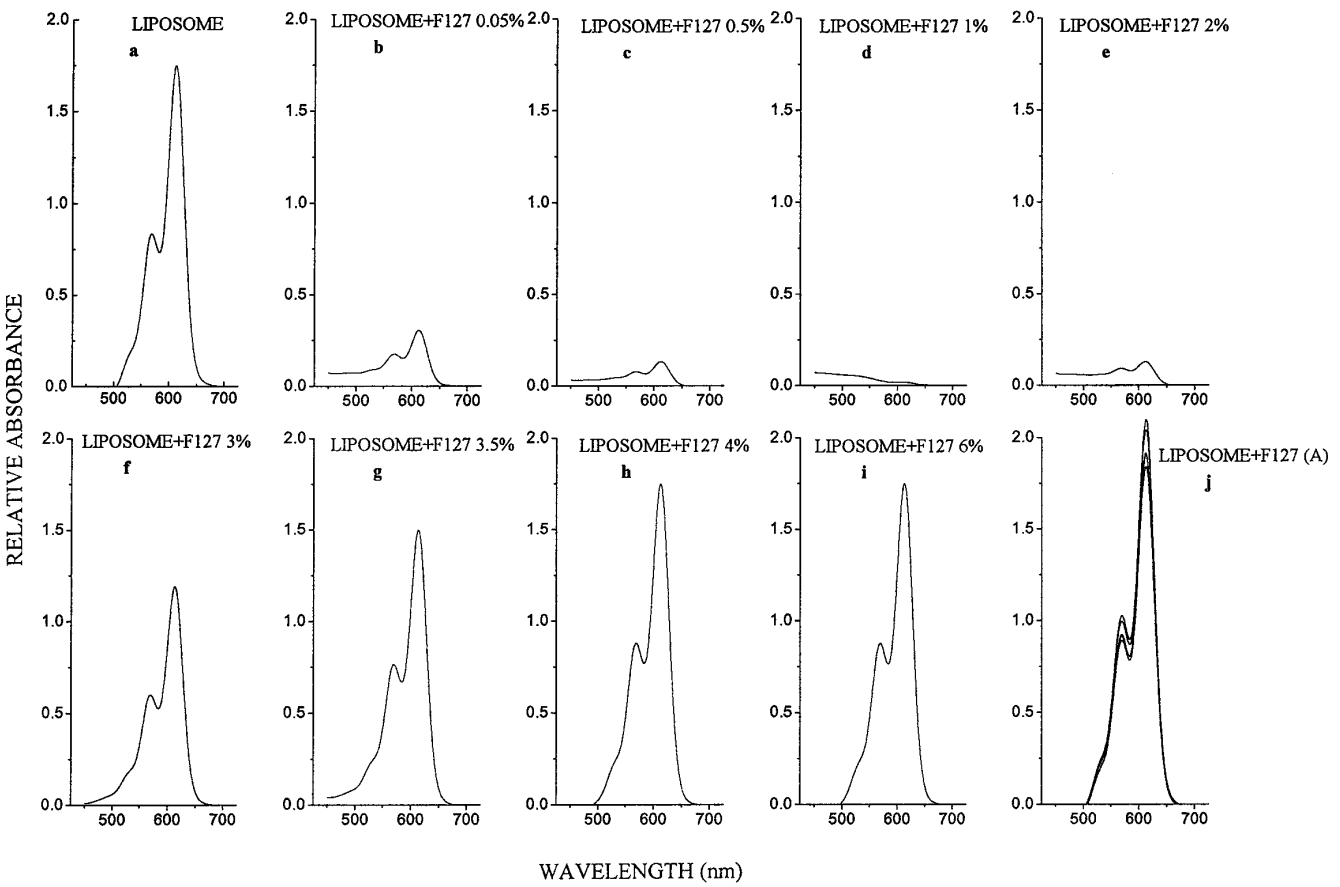

FIG. 7. Absorbance spectra obtained when PCYN was bound to SUVs. Spectra a–i refer to the (I) vesicle systems, where absorbance intensity almost disappears at 1% (wt/wt) of added copolymer and gradually recovers for higher concentrations. The last graph (j) is a compilation of the PCYN absorbance spectra obtained, when bound to the (A) vesicle systems. Note that in this case intensity fluctuates only marginally. Bulk lipid concentration, 2% (wt/wt).

DISCUSSION

(A) Vesicles

From the above results, information about the specific location of the copolymer molecules after their addition to pre-formed vesicles—(A) systems—was obtained. Both the NIL and PCYN dyes, when solubilized in the bilayer of MLVs and SUVs, provided only a crude indication that the polarity of the bilayer was slightly decreased when adding F127 molecules. Such an effect can be due to (a) the attraction of the water molecules that resided at the liposome surface to the large block copolymer chains, leading to an increase in the apolarity of the liposome microenvironment, and/or (b) the movement of the dye molecules slightly deeper into the bilayer, as the copolymer molecules approach in their vicinity. Similar effects have been reported after studying and characterizing NIL and PCYN bound to the lipid bilayer (9), for moderate concentrations of added Triton X-100 to pre-formed MLVs. In the case of PCYN–bilayer-bound molecules, for which a more systematic study was undertaken (Figs. 7j), the absorbance pattern exhibited by the dyes was almost completely unchanged for increasing amounts of F127 added, thus providing conclusive evidence that for these systems, i.e., the (A) vesicles, the block copolymer molecules do not interfere with the lipid membrane microenvironment, therefore being present only at the surface of the liposomes.

(I) Vesicles

The formation of vesicles (MLVs or SUVs) in the presence of block copolymers when dyes are solubilized in their bilayer results in a more apolar bilayer (at least the microenvironment in the vicinity of the dye probes), exactly as mentioned above for the (A) systems. However, the systematic study using PCYN-bound molecules to SUV's bilayer exhibited a gradual decrease in intensity of absorbance until it almost disappeared at an F127 concentration of 1% (wt/wt), while the smooth recovery of absorbance intensity was achieved at higher copolymer concentrations (Figs. 7a–7i). This was attributed to the previously reported self-aggregation of the PCYN molecules, leading to the almost nonabsorbant polyaggregate form (10, 17, 18).

The formation of dye polyaggregates when the polymer is present during vesiculation can be explained by the overcrowding of the large PCYN cations, caused by the incorporation of the copolymer inside the lipid bilayer. The PPO group of the copolymer, by incorporating inside the lipid bilayer, could force an increase in the packing density of the phospholipid fatty acid chains, where the dye molecules are expected to reside. This will lead to PCYN overcrowding and the subsequent formation of nonabsorbing aggregates. The latter proposed model is particularly valid when considering the unchanged absorbance features of PCYN molecules bound to SUVs, when block copolymer is added after vesicle formation (Fig. 7j). The possibility of PCYN aggregate formation due to interaction with the F127 outside the vesicle bilayer environment is discarded because (as shown in Fig. 4) an increase in the dimer peak would be anticipated in that case. Therefore, it can also be claimed that the present studies were carried out in the absence of block copolymer micelles.

The incorporation of the block copolymer's PPO chain inside the bilayer results in a strongly attached moiety, able to act as a steric barrier. As further studies on the stability of these vesicle systems indicate (25), the incorporation of the copolymer inside the lipid bilayer is indeed preferred to adsorption, offering increased protection against flocculation and eventual destruction of the vesicle dispersion.

CONCLUSIONS

The present paper offers a detailed study of the morphological and structural characteristics of vesicle systems after the addition of block copolymers in two different ways to act as steric barriers. Using two hydrophobic dye molecules to monitor the lipid bilayer microenvironment, it has been demonstrated that the structural features of the (I) vesicles have been altered by allowing the polypropylene oxide (PPO) block of the copolymer to participate in the vesicle formation and thus form an integral component of the bilayer. These resulting vesicles are believed to offer improved anchoring of the polyethylene (PEO) chains onto the vesicle surface, as opposed to the weak binding of the adsorbed copolymer molecules in (A) vesicles, the bilayer of which does not interact with the copolymer.

REFERENCES

1. Jones, M. N., *Adv. Coll. Int. Sci.* **54,** 93 (1995).
2. Jarnsaid, M., Farr, S. J., Kearney, P., and Kellaway, I. W., *Int. J. Pharm.* **48,** 125 (1988).
3. Moghimi, S. M., Porter, C. J. H., Illurn, L., and Davis, S. S., *Int. J. Pharm.* **68,** 121 (1991).
4. Allen, T. M., and Chonn, A., *FEBS Lett.* **223,** 42 (1987).
5. Kronberg, B., Dahlman, A., Carlfors, J., Karlsson, J., and Artursson, A., *J. Pharm. Sci.* **79,** 667 (1990).
6. Virden, J. W., and Berg, J. C., *J. Colloid Interface Sci.* **153**(2), 411 (1992).
7. Gabizon, A., and Papahadjopoulos, D., *Proc. Natl. Acad. Sci.* **85,** 6949 (1988).
8. Borenstain, G., and Barenholtz, Y., *Chem. Phys. Lipids* **64,** 117 (1993).
9. Kaschny, P., and Goñi, F. M., *J. Colloid Interface Sci.* **160,** 24 (1993).
10. Pal, M. K., and Gosh, B. K., *Makromol. Chem.* **180,** 959 (1979).
11. Huang, C., *Biochemistry* **8**(1), 344 (1969).
12. Ostrowsky, N., *Chem. Phys. Lipids* **64,** 45 (1993).
13. Greenspan, P., Mayer, E. P., and Fowler, S. D., *J. Cell Biol.* **100,** 965 (1985).
14. Greenspan, P., and Fowler, S. D., *J. Lipid Res.* **26,** 781 (1985).
15. Sackett, D. L., and Wolff, J., *Anal. Biochem.* **167,** 228 (1987).

16. Weber, G., and Farris, F. J., *Biochemistry* **18**(14), 3075 (1979).
17. Pal, M. K., Ash, S. K., and Gosh, B. K., *Makromol. Chem.* **183,** 229 (1981).
18. Pal, M. K., and Gosh, J. K., *J.M.S.-Pure Appl. Chem. A* **29**(11), 1057 (1992).
19. West, W., and Pierce, S., *J. Chem. Phys.* **69,** 1894 (1965).
20. Lelkes, P. I., and Miller, I. R., *J. Membr. Biol.* **52,** 1 (1980).
21. Nivaggioli, T., Tsao, B., Alexandridis, P., and Hatton, T. A., *Langmuir* **11,** 119 (1995).
22. Attwood, D., Collett, J. H., and Tait, C. J., *in* ''Surfactants in Solution'' (K. L. Mittal and P. Bothorel, Eds.) Vol. 4, p. 419. Plenum Press, New York, 1986.
23. Almgren, M., Brown, W., and Hvidt, S., *Coll. Polym. Sci.* **273,** 2 (1995).
24. Alexandridis, P., Athanassiou, V., Fukuda, S., and Hatton, T. A., *Langmuir* **10,** 2604 (1994).
25. Kostarelos, K., Luckham, P. F., and Tadros, Th. F., submitted for publication.