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ADDITION OF BLOCK COPOLYMERS TO LIPOSOMES PREPARED USING SOYBEAN LECITHIN. EFFECTS ON FORMATION, STABILITY AND THE SPECIFIC LOCALIZATION OF THE INCORPORATED SURFACTANTS INVESTIGATED.

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ABSTRACT

Soybean lecithin disperses into water forming multilamellar liposomes, which on sonication produce vesicles of the order of 40-50nm (diameter), as determined by Photon Correlation Spectroscopy (PCS). The effect of concentration of lecithin and sonication time was systematically investigated. Vesicles were then prepared by incorporation of A - B - A block copolymers of polyethylene oxide (PEO) and polypropylene oxide(PPO), i.e.(PEO-PPO-PEO), in order to construct systems of increased steric stability. The effect of the molecular weight of the PEO and PPO chains on the vesicle size was systematically studied by using various molecules to prepare the vesicles. Initial addition of these (tri-)block copolymers causes an increase in the size of the vesicles. This increase continues until a certain concentration of block copolymer is reached, after which a decrease in size is observed. The initial increase was thought to be due to the incorporation of the block copolymer onto the vesicle bilayer. The reduction at high surfactant concentration is thought to be due to solubilization of the bilayer and the ultimate breakdown of the vesicles. Electrophoresis experiments showed a reduction in the ζ-potential of the vesicles on incorporation of the block copolymer which can be

attributed to the shift of the shear plane. Various models are presented to describe this incorporation. The vesicles prepared using the block copolymers are believed to enhance the steric effects and so lead to more stable and pharmaceutically optimum systems.

INTRODUCTION

When phospholipids are dispersed in water they form vesicular bilayer structures best known as liposomes. Since their discovery (1) they have been the subject of considerable research because of the special features they acquire such as: a) the ability to carry both hydrophillic and lipophilic molecules, b) the variety of sizes and structures of liposomes depending on the method of preparation selected, c) their biocompatibility and biodegradability. Their applications include pharmaceuticals (drug carriers), cosmetics, agrochemicals, research tools (e.g. as model cell membranes). The major drawback of these vesicular colloids is their limited physical and biological stability. Indeed serious attempts have been made in order to increase their physical stability, for e.g. by freeze thawing techniques(2), and mainly their biological stability i.e. increase their blood circulation and serum stability, inhibit protein and macrophage binding etc. The strategies proposed include: **a)** stiffening of the lipid bilayer by either selection of phospholipids with high transition temperatures (T_c), so that the gel-packing of the lipid chains induces rigidity at low temperatures(3), inclusion of cholesterol to reduce the mobility of the lipid chains(4), or by polymerization of specific polymerizable groups included in the phospholipid molecules(5, 6); **b)** modification of the liposome surface to avoid attraction or recognition, by using phospholipids containing charged or uncharged head groups; adding molecules with hydrophillic chains able to extend in the aqueous phase (gangliosides(7), various surfactants(8, 9)); and grafting polyethylene glycol on the phospholipid head group(10).

The main objective of this work is to study the role of added A-B-A block copolymers of the Polyethylene(PEO)-Polypropylene(PPO)-Polyethylene(PEO) type on the formation and stability of the vesicles prepared using soybean lecithin. These block copolymers are well known to sterically stabilize other colloidal dispersions like carbon black, silica particles, e.t.c. (11-14). It is likely that the hydrophobic component, namely the PPO, will be strongly adsorbed and/or incorporated inside the vesicle, leaving the PEO chains dangling in solution, thus providing an effective steric barrier. Incorporation of the anchoring group in the bilayer will cause tension on the geometric and mechanical constraints that are thought to govern the aggregation process of surfactants into vesicles as well as micelles, microemulsions, etc.(15).

MATERIALS AND METHODS

Liposomes were prepared with the sonication method, using a Kerry ultrasonic bath. L-α-Dimiristroylphosphatidyl choline (Sigma, ~48% DMPC purity) was mixed with the surfactants (ICI Surfactants, Belgium, Synperonics) and both dispersed in water. The Synperonics are block (triblock) copolymers of the following family structure:

(Polyethylene oxide)$_X$		(Polypropylene oxide)$_Y$		(Polyethylene oxide)$_X$
(PEO)$_X$	-	(PPO)$_Y$	-	(PEO)$_X$
A	-	B	-	A

The triblock copolymers used are shown in Table 1 with some of their characteristic values and the numbers of PEO and PPO units that constitute their lipophilic and hydrophillic chains. All materials were used without any further purification.

TABLE 1 : CHARACTERISTICS OF THE SYNPERONICS

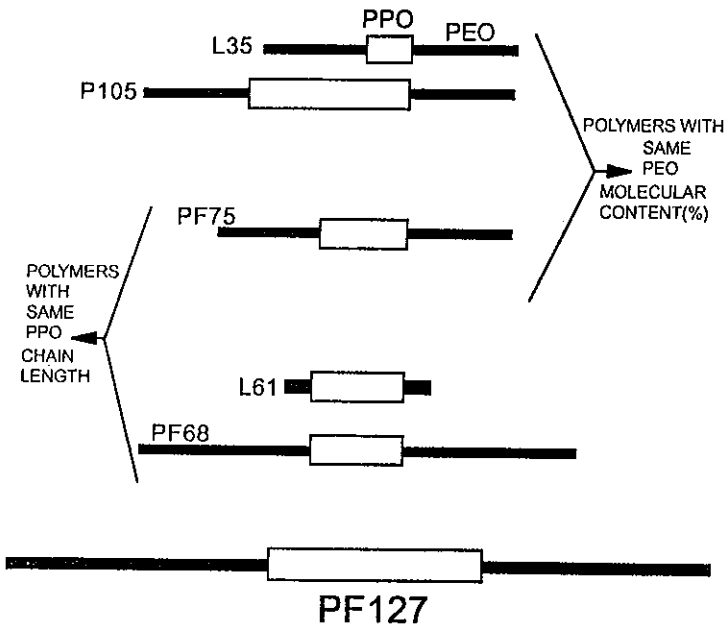
Synperonic PE Grade	Molecular Weight	Cloud point, °C (10% aqueous)	Structure
L35	1900	78-82	$(EO)_{12}(PO)_{16}(EO)_{12}$
L61	2090	15-19	$(EO)_3\ (PO)_{30}\ (EO)_3$
P75	4150	84-90	$(EO)_{26}(PO)_{35}(EO)_{26}$
P105	6500	-	$(EO)_{38}(PO)_{54}(EO)_{38}$
PF68	8350	-	$(EO)_{75}(PO)_{30}(EO)_{75}$
PF127	12000	-	$(EO)_{98}(PO)_{67}(EO)_{98}$

PREPARATION OF THE VESICLES

Vesicles can be formed using a wide variety of techniques and methods already established (16). In this study the sonication method was used as it was found to produce after approximately 240 mins of sonication time, liposomes of 40-50 nm mean diameter. Each dispersion after sonication was filtered through 0.2 μm pore size filters (Millipore). The optimum concentration of phospholipids for producing the liposomes was found to be 2%(w/v). The pH of the dispersions was monitored during sonication without any considerable changes, fluctuating between 5 and 6. Therefore after systematic study the standard vesicle preparation procedures adopted were 240 mins sonication time, and 2%(w/v) of lecithin.

The samples containing the copolymers were prepared exactly as above. The method of preparation chosen was designed so that the polymers could be able to

SCHEME 1.



of synperonics, where each member follows certain physicochemical attributes (e.g. solvency, micellisation, e.t.c.), dependent on which homopolymer chain exerts more influence on the molecule.

Initially, the largest molecule (PF127, m.wt.~11900) was used to examine the effects of the addition. Vesicle diameter and ζ-potential were monitored with increasing polymer concentration. The initial negative ζ-potential value of the liposome surface (Fig.1) is attributed to the presence of various phospholipid molecules of anionic character, namely phosphatidylserine, phosphatidylinositol, phosphatidylglycerol.

The extension of the copolymer's polyethylene oxide chains from the bilayer to the aqueous medium was considered responsible for the observed decrease in

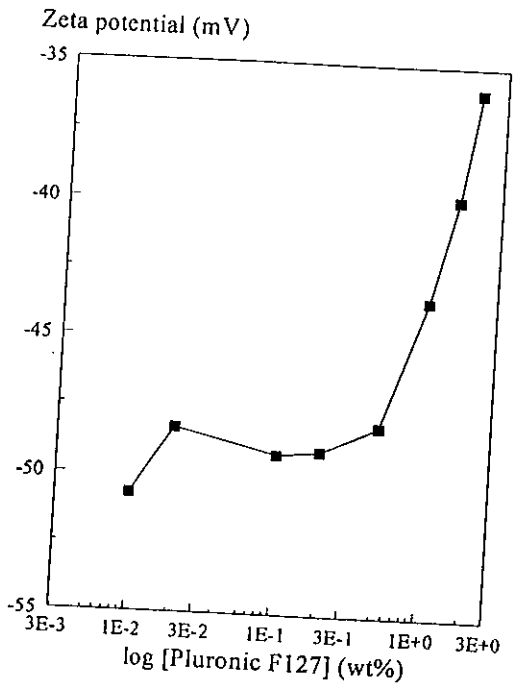


Figure 1: Changes in the vesicle zeta-potential with increasing F127conc.(wt%). The smooth decrease indicates the shift of the shear plane.

the vesicle ζ-potential. Therefore, clear evidence was provided of the presence of the polyethylene oxide chains onto the surface of the vesicles.

The photon correlation spectroscopy (PCS) results indicated a gradual increase in the mean diameter of the vesicles with increasing synperonic concentration, until a plateau value was reached (Fig.2). Further increase of the surfactant concentration above a certain value (~5wt% for the F127) lead to reduction of the liposome diameter. The increase in vesicle size may be due to adsorption onto the liposome bilayer and/or incorporation of the block copolymer molecules into the bilayer. The decrease above a certain concentration of block copolymer may be attributed to solubilization of the bilayer with the consequent formation of mixed

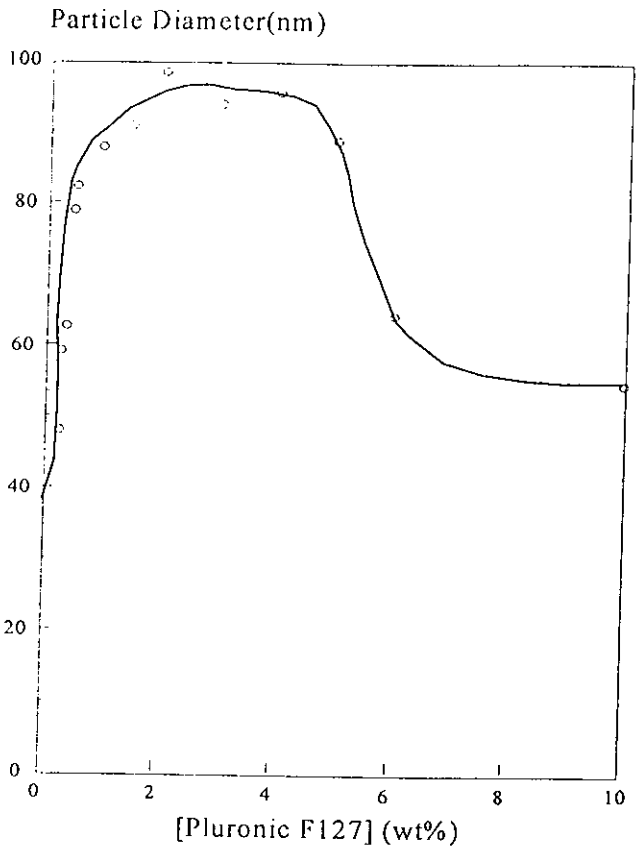


Figure 2: Mean particle diameter of the vesicle (nm) when adding synperonic PF127, against conc.(wt%). All samples were sonicated for ~240 mins and the lecithin conc. kept constant at 2(wt%).

micelles(17, 18, 19). Recent cryo-TEM experiments showed this solubilization effect. It is also likely that the reduction in mean vesicle diameter could be due to the formation of separate synperonic micelles.

EXPERIMENTS USING SERIES OF TRIBLOCK COPOLYMERS (SYNPERONICS)

The patterns of incorporation were studied as a function of the copolymer molecule size and concentration. The possibility of dependencies existing

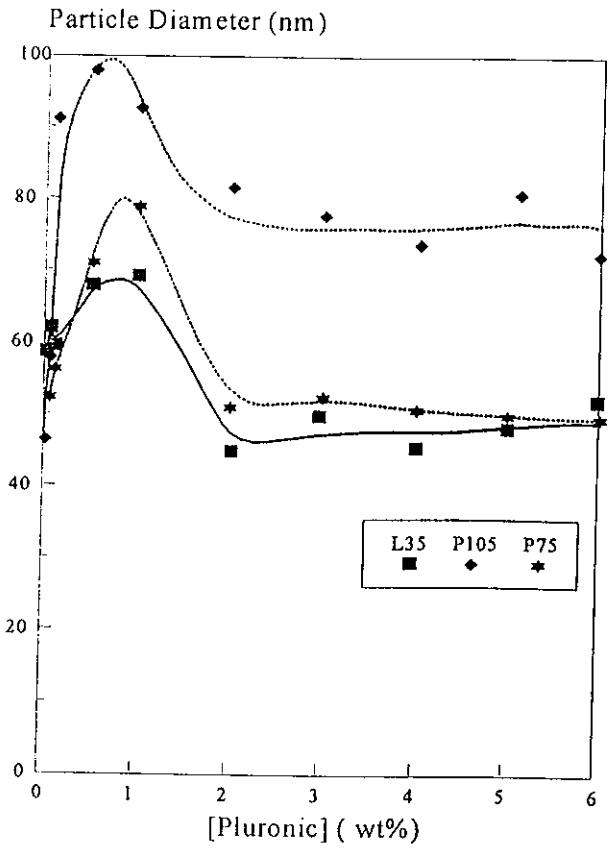


Figure 3: Vesicle size (nm) as measured by the PCS when the L35, P75 and P105 synperonic molecules are included in the dispersion mixture against their concentration. The synperonics used contain the same percentage of PEO in the total m.wt. (% of PEO=~50%)

between these patterns and the PEO or PPO polymer chain length was examined. In the first case (Fig.3) the PEO content of the synperonics used (L35, P75, P105) was maintained at around the same percentage of the total molecular weight. The peak in mean particle diameter at about 1%(w/v) and the reduction with further increases of synperonic concentration for all three molecules, describe an identical incorporation pattern taking place. Note that the vesicle diameter differences between each graph seem to be in accordance with the total molecular weight of the corresponding synperonics.

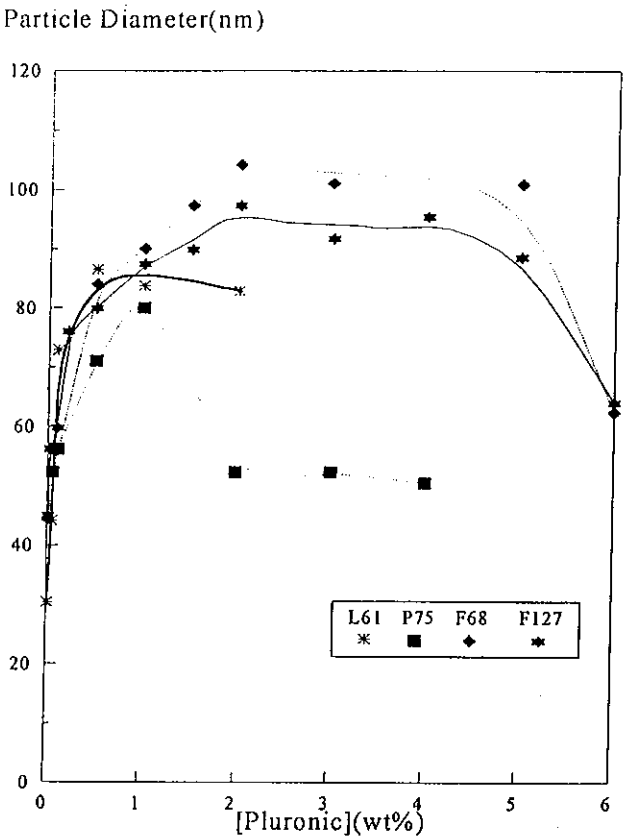


Figure 4: Vesicle diameter (nm) depicted against synperonic concentration(wt%). In this figure, L61, P75 and F68 have approx. the same PPO units/chain = 34, while differing in the PEO units/chain : 3, 26 and 75 respectively. The turbidity of the dispersions containing > 0.5-1(wt%) L61 increases dramatically (extremely high scattering), indicating the immiscibility of the molecule. Note that F68 and F127 show similarincorporation patterns (F127: PEOunits/chain=98 and PPOunits/chain=65)

In the next case (Fig.4), the selected synperonic molecules (L61, P75, F68) contained the same PPO homopolymer chain of 30 units (M.wt.~1740). Their incorporation patterns do not show any similarities. But when comparing the incorporation patterns of molecules containing similar hydrophillic amount in their molecules (F68 and F127), the similarities are evident. Therefore, speculations can

be made about a relationship between the copolymer's polyethylene oxide content and its concentration required to solubilize the liposomal bilayer, but this is yet to be proved.

L61 is so hydrophobic, it can hardly disperse alone in water. However, amounts up to about 1%(w/v) could be dispersed using the liposomes, presumably by solubilization of the polymer molecules inside the lipid bilayer. The latter observation alone strongly suggests that some form of incorporation is taking place. When attempting to increase the concentration of L61, the copolymer phase separates; this noted as a considerable increase in the turbidity of the sample.

The above experiments provide clear evidence that when block copolymers are added to liposomal dispersions there is an apparent increase in vesicle size and a decrease in the vesicle ζ-potential. Also, when trying to assess the most influential homopolymer part of the synperonics as to the incorporation patterns followed, the PEO hydrophillic chains seem to be a deciding factor of this process, probably by attracting phospholipid molecules in the formation of mixed micelles rather than vesicles leading to the disruption of the bilayer.

Three different models of incorporation may be introduced and these are schematically shown in Figure 5. In model A, the PPO is assumed to become sandwiched between the lipophilic layers, leaving the PEO chains at the outside and inside, respectively, of the vesicle. In model B, the PPO chain is assumed to become incorporated inside the bilayer in a flat configuration, leaving the PEO chain dangling in solution. These two models present an incorporation that takes place following the physical anchoring mechanism onto fluid surfaces (20). In model C, the molecules are simply adsorbing on the surface of the vesicle.

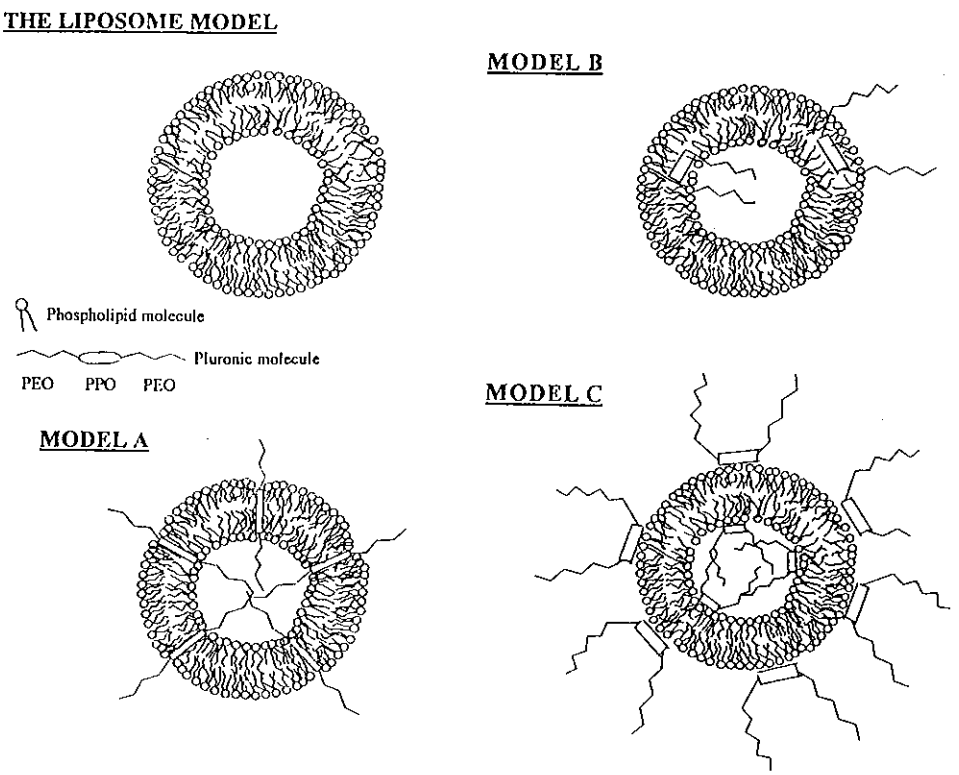


Figure 5: Models of triblock copolymer (synperonic) incorporation onto liposomal surfaces.

The results presented cannot give any concrete evidence if one of the models, or any of their combinations, is the correct one. Recent experiments have shown that a combination of these models is likely to happen. Experiments are being carried out to: elucidate if any of the models is favourable, and accurately localize the incorporated polymer molecules in terms of their conformation and orientation inside the bilayer.

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