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Poloxamer sorption on liposomes: comparison with polystyrene latex and influence on solute efflux

M. Jamshaid, S.J. Farr, P. Kearney and I.W. Kellaway

Welsh School of Pharmacy, UWIST, Cardiff (U.K.)

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Summary

The adsorption characteristics of several polyoxyethylene–polyoxypropylene (POE-POP) block copolymers (Synperonics) were studied on both polystyrene microspheres and small unilamellar vesicles (SUVs) composed of egg phosphatidylcholine (egg PC). Photon correlation spectroscopy (PCS) was used to monitor the increase in hydrodynamic radius following polymer sorption. Apparent adsorbed layer thickness (δ) of all POE-POP block copolymers was shown to be Langmurian. This δ increased non-linearly with the molecular weight of the polymer. Adsorption of Synperonic F108 occurred rapidly onto latex particles with no subsequent change of hydrodynamic radius on storage. For SUVs, δ was significantly less ($P < 0.05$) than that measured for the same polymer with polystyrene microspheres, which may indicate a degree of bilayer penetration by the polymers. The efflux rate of entrapped 6-carboxyfluorescein (6-CF) was considerably lower for SUVs prepared from egg PC and cholesterol (1 : 1 molar ratio) than for those prepared from egg PC alone when suspended in Synperonic F108. Retention of 6-CF was higher for multilamellar vesicles (MLVs) than for SUVs when incubated in the same polymer solution.

Introduction

Compared to other colloidal delivery systems, liposomes have certain advantages: they are easily biodegraded, weakly immunogenic (Van Rooijen and Nieuwmegen, 1980) and possess limited intrinsic toxicity (Campbell, 1983). Homogenous populations of liposomes can be prepared (Zumbuehl and Weder, 1981; Olson et al., 1979) and are preferred to polydispersed systems as drug carriers. A number of approaches have been used in order to achieve liposomal drug targeting such as coating with heat-aggregated immunoglobulin (IgM) (Weissmann et al., 1975) or polysaccharide (Iwamoto and Sunamoto, 1982; Sunamoto et al., 1983), non-covalent association of cell specific antibodies (Gregoriadis and Neerunjun, 1975) and covalent attachment of poly- and monoclonal antibodies (Hashimoto et al., 1983).

A major disadvantage with i.v. administered colloidal carriers such as liposomes, microspheres or nanoparticles is that they are efficiently removed by the mononuclear phagocytic system (MPS) of the liver and spleen (Illum et al., 1982; Poste and Kirsh, 1983). Coating of colloidal particles with amphipathic polymers, especially those

Correspondence: I.W. Kellaway, Welsh School of Pharmacy, UWIST, P.O. Box 13, Cardiff CF1 3XF, U.K.

of high molecular weight, has resulted in prolonged clearance kinetics after intravenous administration (Illum and Davis 1984; Illum et al., 1986). Adsorption of these polymers onto polystyrene latex microspheres has been reported of the Langmurian type with equilibrium achieved within 24 h (Kayes and Rawlins, 1979). Napper and Netschey (1971) have suggested that POE-POP block copolymers stabilise colloidal particles by a steric enthalpic–entropic mechanism, where the POP group acts as an anchoring moiety and the POE segments provide stability in the surrounding medium by a repulsion effect. This orientation of the POE groups would be expected to result in an increase in the hydrophilicity of the colloidal surfaces.

In this work, we have investigated the adsorption characteristics of a wide range of POE-POP block copolymers (poloxamers; Synperonic, ICI, France) by measuring the thickness of the apparent adsorbed layer (δ) on latex particles and on SUVs prepared from egg phosphatidylcholine (PC) by detergent dialysis (Zumbuehl and Weder, 1981). δ was calculated from the increase in hydrodynamic radius as determined by PCS. Stability of coated vesicles was assessed by monitoring the efflux rate of entrapped 6-CF, a solute commonly used to establish liposome stability in vitro and in vivo (Gregoriadis and Senior, 1980; Kirby and Gregoriadis, 1981; Kirti et al., 1986).

Materials and Methods

Polystyrene latex microspheres (10% w/v suspension, 0.091 μm mean diameter) were purchased from Dow Chemicals, U.K. POE-POP block copolymers (Synperonics F68, F87, F88, F127, F98 and F108) were supplied by ICI, France. Egg lecithin approximately 90%, aluminium oxide (active, neutral), disodium hydrogen orthophosphate, potassium dihydrogen orthophosphate, sodium chloride, Triton X-100, acetone, chloroform and absolute ethanol were obtained from BDH Chemicals, U.K. Cholesterol 99% from porcine liver, phosphomolybdic acid crystalline, sodium cholate and Sephadex G-25(M) were purchased from Sigma Chemical Co. UK. 6-CF was obtained from Eastman Kodak, U.S.A. and purified by the method of Ralston et al. (1981). Silica gel plates (Polygram sil N-HR) were from Camlab, U.K.

Coating and characterisation of polystyrene microspheres

Polystyrene microspheres (0.002% w/v suspension) were suspended in filtered (0.22 μm pore size membrane filter) solutions containing various concentrations (0.001, 0.005, 0.010, 0.030, 0.050, 0.080 and 0.100% w/v) of a series Synperonic polymers at room temperature for 24 h. δ was calculated from the increased hydrodynamic diameter of the particles as determined by PCS. The PCS system (Malvern Instruments, U.K.) consisted of a laser (Spectra Physics He/Ne 35 mW) spectrophotometer (R 144), a 64 channels multibit correlator (K7025) and a 32k Commodore PET microcomputer. Sizes were calculated using the Malvern proprietary software to give a simple *Z*-average value or alternatively, the correlation data were inverted using the programme CONTIN (Provencher, 1982) to give a complete size distribution. The Rayleigh-Debye form factors for solid spheres were used for the latex data and the corresponding form factors for hollow spheres of shell thickness 5 nm used for analysis of SUVs.

Purification of egg PC

Egg PC was purified from 90% lecithin by the method described by Martin et al. (1978). The pure egg PC was stored under acetone at $-20\,^{\circ}C$ and dried under a stream of O_2-free N_2 before use.

Preparation and sizing of liposomes

Egg PC/cholate molar ratios of 0.3–0.8 were chosen for the production of mixed micellar solutions where in all cases the concentration of egg PC was kept at 13 mg/ml. The required amount of egg PC was dissolved in a minimum volume of chloroform and evaporated to a thin film at $37\,^{\circ}C$ by means of a rotary vacuum evaporator (Buchi, Switzerland) and flushed twice with O_2-free N_2 to ensure complete removal of traces of the solvent. The appropriate quantity of sodium cholate in 0.85% w/v NaCl was added and the lipid film dispersed by shaking followed by incubation at $37\,^{\circ}C$ until a clear solution was obtained. The

micellar solution was injected into the cells of the dialysis apparatus (Lipoprep; Diachema AG, F.R.G.) and dialysed against 0.85% w/v saline for 24 h at 37 °C. High-permeability cellulose membranes were used having a molecular weight cut off of 10,000. The resulting liposomes of each batch were filtered through a 0.22 μm pore size membrane filter and dispersed in filtered 0.85% w/v NaCl to give a final lipid concentration of 0.5 mg/ml prior to PCS analysis.

Coating of liposomes

SUVs prepared with an egg PC/cholate molar ratio 0.4 were dispersed in filtered solutions (0.001, 0.005, 0.010, 0.050 and 0.100% w/v) of the various Synperonics in 0.85% w/v saline to give a final lipid concentration of 0.5 mg/ml. These were incubated at room temperature for 24 and 48 h. The apparent adsorbed layer thickness (δ) was determined as before and the electrophoretic mobility of uncoated and F108 coated liposomes compared using a Zetasizer IIc (Malvern Instruments, UK).

Preparation of liposomes containing 6-CF

The advantage of using 6-CF as a model solute in liposome studies is that at concentrations ≥ 0.25 M, the dye is self-quenched. This allows fluorometric estimation of free solute levels without prior separation from the liposomally associated fraction. Encapsulation of 6-CF was achieved as follows: egg PC (15 mg/ml) was dissolved in chloroform with and without the addition of an equimolar amount of cholesterol. The solvent was evaporated using a rotary vacuum evaporator at 37 °C and the lipid film flushed twice with O_2-free N_2 as before. The resultant film was dispersed in the required volume of 0.1 M phosphate buffered saline (PBS), pH 7.4, containing 0.25 M 6-CF and the suspension sonicated for 40–600 s using a titanium ultrasonic probe (cycles of 60 s sonication followed by a 30 s cooling period) under a N_2 stream to prepare MLVs and SUVs respectively. Each suspension was then centrifuged at 195,000 g for 1 h. The pellet was redispersed in PBS to yield MLVs, or for SUVs the supernatant was taken and passed through a Sephadex G-25(M) column equilibrated with PBS. Liposomal 6-C F was measured in the absence (free 6-CF) and presence (total 6-CF) of Triton X-100 (1% w/v final concentration) using a Perkin Elmer LS-5 fluorescent spectrometer adopting excitation and emission wavelengths of 490 and 520 nm, respectively. The samples were diluted to fall within the concentration range of 4–50 ng/ml. Retention of 6-CF in the various liposome types following dilution with PBS or 0.01% w/v Synperonic F108 in PBS was calculated by the following equation:

$$\% \text{ retention} = (C_t - C_f) \times 100/C_0 \quad (1)$$

where C_t, C_f are the total and free 6-CF concentrations at various time intervals respectively, and C_0 is the entrapped concentration at time zero.

To examine pH effects on the efflux rate of 6-CF, SUVs prepared from egg PC and cholesterol containing entrapped 6-CF were suspended in solutions of PBS of varying pH and the free 6-CF percentage determined every 60 min using the equation:

$$\% \text{ efflux} = C_f \times 100/C_0 \quad (2)$$

Results and Discussion

Adsorbed layer thickness of Synperonic polymers at the polystyrene / water interface

In order to fully characterize the system, preliminary analysis of the PCS data consisted of a comprehensive size distribution analysis using CONTIN. Fig. 1 shows the size distribution of the latex microspheres in water and in various concentrations of F127. In 0.001% w/v F127, the mean size is shifted to slightly higher values due to the presence of the adsorbed layer. At a higher concentration, 0.050% w/v, a second peak centered around 20 nm is evident and attributable to micelles of F127. As the concentration of Synperonic is increased further, the intensity of the second peak increases until at 1.0% w/v, the latex signal is a minor component. This illustrates a limiting concentration above which the simple unimodal Z-average analysis is inappropriate as it

will be a weighted average of both the true latex microsphere peak and the secondary micelle peak. However, for each of the Synperonics studied equilibrium layer thicknesses were achieved at concentrations below that at which the micelle contribution was significant.

Fig. 2 illustrates the concentration-dependent thickness of adsorbed layers of the various Synperonics on latex micropheres. All polymers resulted in an increase in the hydrodynamic diameter consistent with the previously reported Langmurian adsorption (Kayes and Rawlins, 1979). After an initial increase in δ with increasing concentration of polymer, no further change resulted over the concentration range studied (0.001–0.100% w/v). Equilibrium values of δ are given in Table 1. As in previous studies (Doroszkowski and Lambourne, 1968; Kayes and Rawlins, 1979), δ increased in a non-linear manner with molecular weight of the polymer. Kayes and Rawlins (1979) reported the need for a 24 h equilibration period for adsorption of POE-POP block copolymers to

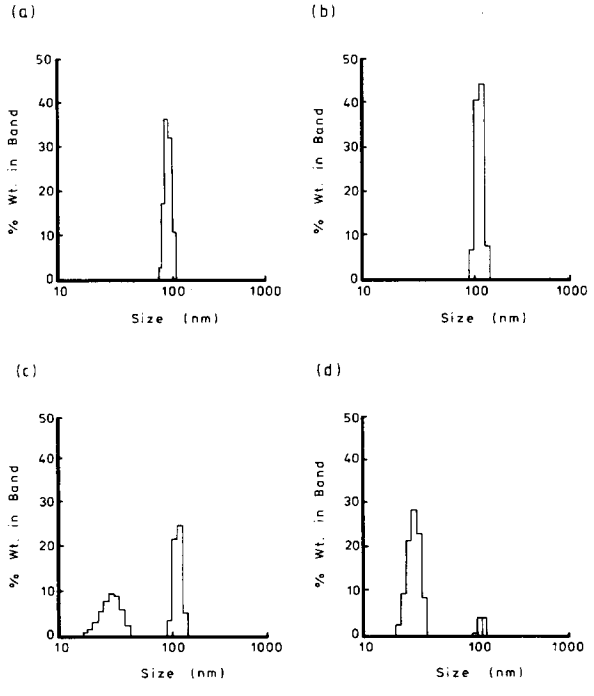

Fig. 1. Size distribution analysis (CONTIN) of polystyrene latex microspheres following 24 h incubation in (a) water, (b) 0.001, (c) 0.05 and (d) 1.0% w/v Synperonic F127.

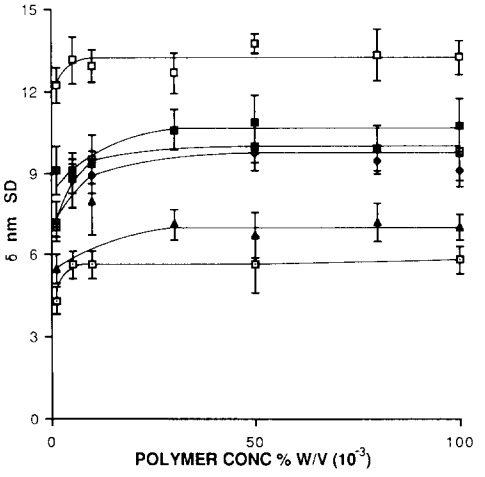

Fig. 2. Adsorbed layer thickness of various Synperonic polymers onto polystyrene latex microspheres after 24 h incubation. (⊟), F68; (▲), F87; (◇), F88; (⊞), F127; (■), F98; (■), F108.

TABLE 1

Equilibrium values of δ on polystyrene latex microspheres as a function of Synperonic type

Synperonic type	δ (nm ± S.D.)
F68	5.70 ± 0.09
F87	7.21 ± 0.46
F88	9.18 ± 0.41
F127	9.77 ± 0.25
F98	10.50 ± 0.42
F108	13.20 ± 0.38

polystyrene latex. However, it was shown that the adsorption of Synperonic F108 (0.05% w/v) onto polystyrene latex was rapid as no significant change in δ occurred over a 24 h period; δ at zero and 24 h were 12.63 ± 0.70 nm and 12.93 ± 1.16 nm respectively. However, it is conceivable that molecular reorientation within the adsorbed film may occur during this period, a change which need not be detectable by measurement of δ.

Characterisation and coating of liposomes

It was first reported by Kagawa and Racker (1971) that removal of detergent from mixed micellar solutions results in the formation of liposomes. Several workers (Schwendener et al., 1981;

TABLE 2

Mean size and polydispersity of SUVs prepared by surfactant dialysis as a function of egg PC/cholate molar ratio

Egg PC/cholate (Molar ratio)	Mean size (nm ± S.D.)	Polydispersity index (± S.D.)
0.3	70.73 ± 0.62	0.06 ± 0.01
0.4	73.20 ± 0.85	0.06 ± 0.01
0.5	72.28 ± 1.10	0.05 ± 0.02
0.6	72.76 ± 0.34	0.04 ± 0.02
0.7	90.77 ± 2.10	0.06 ± 0.02
0.8	88.78 ± 1.02	0.07 ± 0.02

Rhoden and Goldin, 1979; Milsmann et al., 1978) have prepared liposomes from various lipids and detergents by using the same technique.

The mean size of SUVs prepared by dialysis (Table 2) increased with egg PC/cholate molar ratio. All populations were monodisperse as indicated by both the size distribution analysis (CONTIN) and the polydispersity index of the unimodal analysis. With the higher surfactant concentrations, shorter times were needed for clarification of mixed micellar solutions due to a solubility effect. As shown in Fig. 3, vesicles incubated in the various Synperonic solutions resulted in an apparent adsorbed polymer film. The PCS signal of each suspension retained the full intensity during incubation indicating that the vesicular structure remained intact. Concentration dependence was similar to that shown for the latex microspheres; δ increased with Synperonic concentration until a plateau level was reached. Equilibration was slow however, with δ after 48 h greater than that after 24 h (Table 3). In each case, δ (48 h) was slightly less than that measured for the latex microspheres which may be attributed to the penetration of the polymer chains into the outer

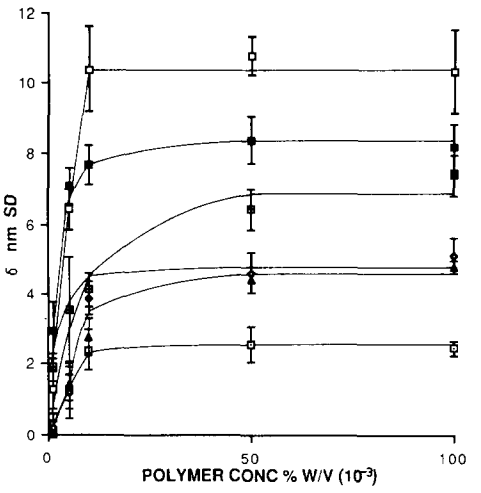

Fig. 3. Adsorbed layer thickness of various Synperonic polymers onto egg PC SUVs after 48 h incubation. (⊟), F68; (▲), F87; (◇), F88; (⊞), F127; (■), F98; (□), F108.

phospholipid bilayer. Indeed, this could well explain the small increase in δ over 48 h. There could well be an element of absorption of the polymer into the vesicle itself.

Electrophoretic mobility of uncoated and F108-coated SUVs of egg PC were of values -0.3487 and -0.2750 μm cm s^{-1} V^{-1} respectively. This small but significant difference ($P = 0.0024$) may be ascribed to the projecting polyoxyethylene groups from the vesicle surfaces.

Stability of liposomes in Synperonic polymers

6-CF efflux rate was found to be pH-dependent from SUVs (egg PC : cholesterol, 1 : 1) as previously reported (Weinstein et al., 1977; Szoka et al., 1979). After 5 h incubation in buffer at 37°C, 4, 5, 6 and 58% of the entrapped solute was lost at pH 8, 7, 6 and 5 respectively. This is due to

TABLE 3

The influence of polymer concentration, incubation time and colloid type on δ (nm ± S.D.) for Synperonic F108

F108%	0.001	0.005	0.01	0.05	0.10
SUVs					
24 h	0.62 ± 0.39	5.63 ± 0.48	8.41 ± 0.57	9.13 ± 0.66	8.47 ± 0.48
48 h	1.27 ± 0.51	6.42 ± 0.57	10.41 ± 1.14	10.80 ± 0.51	10.33 ± 1.14
Latex					
24 h	12.22 ± 0.60	13.15 ± 0.81	12.92 ± 0.57	13.77 ± 0.33	13.27 ± 0.60

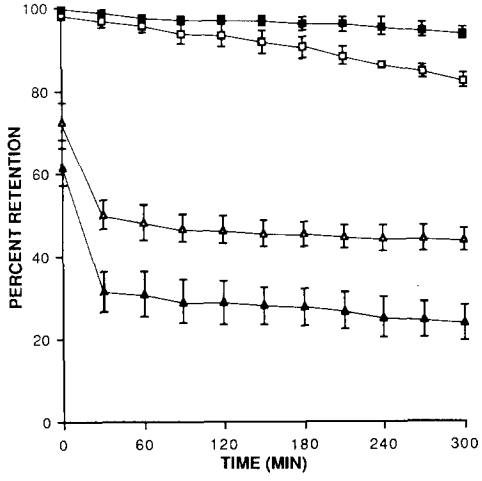

Fig. 4. Liposomal retention of 6-CF in SUVs in the presence and absence of Synperonic F108 (0.01% w/v) in PBS at 37 °C. (□), Egg PC; (■), egg PC; cholesterol, 1 : 1 in absence of F108; (▲), egg PC; (△) egg PC/cholesterol, 1 : 1 in presence of F108.

protonation of the molecule which is more readily transferred through the phospholipid bilayer. Accordingly, stability experiments were conducted at pH 7.3–7.4.

Incubation of SUVs and MLVs in F108 resulted in higher efflux rates compared to those in PBS alone (Figs. 4 and 5). The percentage retained at the start of the incubation was 62% for SUVs prepared from egg PC and 73% for those prepared from egg PC and cholesterol (1 : 1). This reduced to 24% and 44%, respectively, after 5 h. Similarly, for MLVs of egg PC the percentage retained at the start of incubation was 77% and for those prepared from egg PC and cholesterol (1 : 1) 83%. This decreased to 35% and 60% respectively after 5 h. The increased leakage of 6-CF from liposomes in the presence of F108 could be due to the ingression of the polymer into the phospholipid bilayer leading to the eventual formation of pores or regions of greater membrane fluidity through which 6-CF passed. The presence of cholesterol leads to the well-documented condensing effect on the acyl chain of the phospholipid (Ladbrooke et al., 1968; Demel and Kruyff, 1976) thus reducing the penetration of the polymer into the bilayer. For MLVs, it is likely that the effect of F108 is predominantly confined to the outermost bilayer, therefore the ingression of polymer was insufficient to affect all concentric bilayers. Consequently, the percentage of 6-CF retained was significantly higher than with SUVs where all solute is entrapped by a single bilayer.

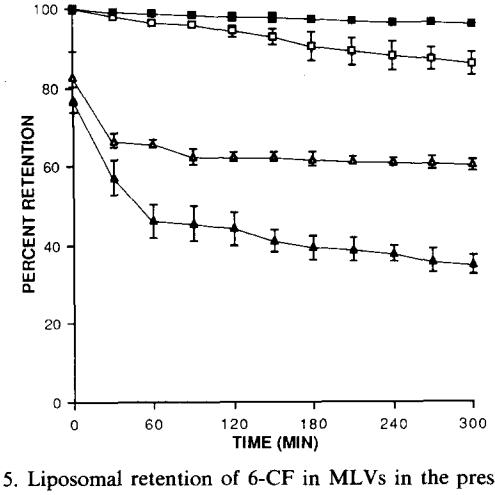

Fig. 5. Liposomal retention of 6-CF in MLVs in the presence and absence of Synperonic F108 (0.01% w/v) in PBS at 37 °C. (□), Egg PC; (■) egg PC : cholesterol, 1 : 1 in absence of F108; (▲), egg PC; (△) egg PC/cholesterol, 1 : 1 in presence of F108.

The present results indicate that coating of liposomes with POE-POP block copolymers is possible. However, the stability data shows that the solute efflux rate is at present unacceptably high in SUVs. If such a coating is to become a viable method of promoting sustained blood levels of these carriers, further developments are necessary in order to reduce this loss due to leakage. Current studies are directed towards elucidation of the nature of the molecular orientation of the Synperonic at the vesicle surface and the extent of bilayer penetration.

References

Campbell, P.I., Toxicity of some charged lipids used in liposome preparations. *Cytobios*, 37 (1983) 21–26.

Demel, R.A. and Kruyff, B.D., The function of sterols in membranes. *Biochim. Biophys. Acta*, 457 (1976) 109–132.

Doroszkowski, A. and Lambourne, R., A viscometric technique for determining the layer thickness of polymer adsorbed on titanium dioxide. *J. Coll. Interface Sci.*, 26 (1968) 214–221.

Gregoriadis, G. and Neerunjun, E.D., Homing of liposomes to target cells. *Biochem. Biophys. Res. Commun.*, 65 (1975) 537–544.

Gregoriadis, G. and Senior, J., The phospholipid component of small unilamellar liposomes controls the rate of clearance of entrapped solutes from the circulation. *FEBS Lett.*, 119 (1980) 43–46.

Hashimoto, Y., Sugawara, M. and Endoh, H., Coating of liposomes with subunits of monoclonal IgM antibody and targeting of liposomes. *J. Immunol. Methods*, 62 (1983) 155–162.

Illum, L. and Davis, S.S., The organ uptake of intravenously administered colloidal particles can be altered using a non-ionic surfactant (poloxamer 338). *FEBS Lett.*, 167 (1984) 79–82.

Illum, L., Davis, S.S., Wilson, C.G., Thomas, N.W., Frier, M. and Hardy, J.G., Blood clearance and organ deposition of intravenously administered colloidal particles: the effect of particle size, nature and shape. *Int. J. Pharm.*, 12 (1982) 135–146.

Illum, L., Hunneyball, I.M. and Davis, S.S., The effect of hydrophilic coating on the uptake of colloidal particles by the liver and by peritoneal macrophages. *Int. J. Pharm.*, 29 (1986) 53–65.

Iwamoto, K. and Sunamoto, J., Liposomal membranes. XII. Adsorption of polysaccharides on liposomal membranes as monitored by fluorescence depolarisation. *J. Biochem.*, 91 (1982) 975–979.

Kagawa, Y. and Racker, E., Partial resolution of the enzymes catalysing oxidative phosphorylation. *J. Biol. Chem.*, 246 (1971) 5477–5487.

Kayes, J.B. and Rawlings, D.A., Colloid and adsorption characteristics of certain polyoxyethylene–polyoxypropylene block copolymers on polystyrene latex. *Colloid Polymer Sci.*, 257 (1979) 622–629.

Kirby, C. and Gregoriadis, G., Plasma-induced release of solutes from small unilamellar liposomes is associated with pore formation in the bilayers. *Biochem. J.*, 199 (1981) 251–254.

Kirti, A., Bali, A. and Gupta, C.M., Influence of the phospholipid structure on the stability of liposomes in serum. *Biochim. Biophys. Acta*, 856 (1986) 36–40.

Ladbrooke, P.D., Williams, R.M. and Chapman, D., Studies on lecithin-cholesterol-water interactions by differential scanning calorimetry and X-ray diffraction. *Biochim. Biophys. Acta*, 150 (1968) 333–340.

Martin, G.P., Kellaway, I.W. and Marriot, C., The solubilisation of progesterone by mixed bile salt–phospholipid solutions. *Chem. Phys. Lipids*, 22 (1978) 227–237.

Milsmann, M.H.W., Schwendener, R.A. and Weder, H.G., The preparation of large single bilayer liposomes by a fast and controlled dialysis. *Biochim. Biophys. Acta*, 512 (1978) 147–155.

Napper, D.H. and Netschey, A., Studies of the steric stabilisation of colloidal particles. *J. Coll. Interface Sci.*, 37 (1971) 528–535.

Olson, F., Hunt, C.A., Szoka, F.C., Vail, W.J. and Papahadjopoulos, D., Preparation of liposomes of defined size distribution by extrusion through polycarbonate membranes. *Biochim. Biophys. Acta*, 557 (1979) 9–23.

Poste, G. and Kirsh, R., Site specific (targeted) drug delivery in cancer therapy. *Biotechnology*, 1 (1983) 869–878.

Provencher, S.W., CONTIN: a general purpose constrained regularisation program for inverting noisy linear algebric and integral equations. *Comp. Phys. Commun.*, 27 (1982) 229–242.

Ralston, E., Hjelmeland, M.L., Klausner, R.D., Weinstein, J.N. and Blumenthal, R., Carboxyfluorescein as a probe for liposome-cell interaction. Effects of impurities, and purification of the dye. *Biochim. Biophys. Acta*, 649 (1981) 133–137.

Rhoden, V. and Goldin, S.M., Formation of unilamellar lipid vesicles of controllable dimensions by detergent dialysis. *Biochemistry*, 18 (1979) 4173–4176.

Schwendener, R.A., Asanger, M. and Weder, H.G., *N*-alkyl glucosides as detergent for the preparation of highly homogenous bilayer liposomes of variable sizes (60–240 nm) applying defined rates of detergent removal by dialysis. *Biochem. Biophys. Res. Commun.*, 100 (1981) 1055–1062.

Sunamoto, J., Iwamoto, K., Takada, M., Yuzuriha, T. and Katayama, K., Improved drug delivery to target specific organs using liposomes as coated with polysaccharides. *Polym. Sci. Technol.*, 23 (1983) 157–168.

Szoka, F.C., Jacobson, K. and Papahadjopoulos, D., The use of aqueous space markers to determine the mechanism of interaction between phospholipid vesicles and cells. *Biochim. Biophys. Acta*, 551 (1979) 295–303.

Van Rooijen, N. and Nieuwmegen, V.R., Liposomes in immunology: Multilamellar phosphatidylcholine liposomes as a simple biodegradable and harmless adjuvant without any immunogenic activity of its own. *Immunol. Commun.*, 9 (1980) 243–256.

Weinstein, J.N., Yoshikami, S., Henkart, P., Blumenthal, R. and Hagins, W.A., Liposome-cell interactions: transfer and intracellular release of a trapped fluorescent marker. *Science*, 195 (1977) 489–492.

Weissmann, G., Bloomgarden, D., Kaplan, R., Cohen, C., Hoffstein, S., Collins, T., Gotleib, A. and Nagle, D., A general method for the introduction of enzymes by means of immunoglobulin-coated liposomes into lysosomes of deficient cells. *Proc. Natl. Acad. Sci. U.S.A.*, 72 (1975) 88–92.

Zumbuehl, O. and Weder, H.G., Liposomes of controllable size in the range of 40–180 nm by defined dialysis of lipid/detergent mixed micelles. *Biochim. Biophys. Acta*, 640 (1981) 252–262.