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Report

Solubilization of Liposomes by Weak Electrolyte Drugs. I. Propranolol

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The solubilization of dimyristoylphosphatidylcholine (DMPC) liposomes by a weak electrolyte drug, propranolol (PPL) hydrochloride, has been studied as a function of pH, [PPL], [DMPC], and temperature. The solubilization of liposomes at 40°C by 0.2 m*M* PPL occurred at different rates from 2.9 to 14.4 m*M* DMPC but converged at complete solubilization after 13 hr at pH 12.0. At the same [PPL], solubilization was complete after 18 days at pH 11.0, but incomplete solubilization occurred at pH 10.0 and not at all at lower pH's. In 14.4 m*M* DMPC liposomes, solubilization was gradual and proportional to the [PPL] from 0.001 to 0.10 m*M* up to 95 hr, then rapid thereafter. The [PPL] at which the solubilization efficiency began to increase rapidly was determined to be 0.078 m*M*. The rate of solubilization was also influenced by the fluidity of the bilayers, a sevenfold increase in the time for complete solubilization being observed upon cooling from 40 to 20°C. Surface tension (st) data confirmed a low critical micelle concentration (CMC) and continued decrease in the st above the CMC. It is concluded that the critical ratio of PPL to DMPC for solubilization occurs in localized regions of the bilayers, with total solubilization at different rates depending on the [PPL] and the physical properties of the liposomes. The processes may be used advantageously to prepare small vesicles or to extract lipids or proteins, more efficiently than detergents, from biological membranes.

KEY WORDS: solubilization; liposomes; propranolol; dimyristoylphosphatidylcholine.

INTRODUCTION

Studies on the interaction of solutes with liposomes have led to the use of liposomes as a model of biological membranes to determine drug distribution and transport across membranes and as a colloidal drug depot for target-specific delivery. However, the interaction between solutes and the phospholipid bilayers may affect the physical state and integrity of liposomes. Certain solutes affect the phase transitions of phospholipids, altering the rigidity of the formed membrane and, as a consequence, its permeability. In the extreme case, liposomes may be effectively destroyed and converted to mixed micelle systems in the presence of surface-active agents. This possibility is regarded as a serious limitation of the liposome as an oral drug delivery system because of exposure to enzymes and bile acids in the fluids of the gastrointestinal tract (1). Alternatively, advantage is made of the solubilization of membranes by detergents to extract phospholipids and proteins or in the preparation of unilamellar vesicles (see reviews in Refs. 2–4).

The potential solubilization of liposomes by drugs and other solutes which are incorporated in the matrix of these ordered structures requires further study. For example, mixed micelles of chlorpromazine and phospholipid which were formed from liposomes at concentrations of chlorpromazine greater than 1.4×10^{-4} *M* yielded lower partition coefficients than the corresponding liposome system (5). Also, the advantages of the liposome as a drug delivery system disappear when solubilization occurs (6). Drugs that are known to possess high surface activities in aqueous systems would be expected to behave like detergents and solubilize liposomes. Solubilization occurs at a total critical drug concentration, D^c_T, which yields a critical molar ratio of drug to phospholipid (7). In some cases, the concentration in the aqueous phase at equilibrium approximates the CMC (8). The surface activities of different classes of drugs have been previously reviewed (9) but the extent of solubilization of liposomes by drugs has not been systematically studied.

BACKGROUND AND THEORY

Solutes that interact with phospholipid bilayers may do so by either low- or high-affinity binding. Low-affinity binding occurs when there are no significant surface interactions between the solute molecules and the bilayers and binding may be considered as partitioning as a result of hydrophobic characteristics of the solute and van der Waals attractive forces within the bilayer structures. High-affinity binding is manifested when electrostatic forces of attraction dominate the interactions between solute and membrane surfaces. As

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models of biological membranes, liposomes are ideal for studying the incidence and extent of these types of interactions. Highly surface-active agents exert a particularly strong destabilizing influence on liposomes because of their tendencies to bind with these colloidal particles, aggregate, and form mixed micelles which essentially solubilize the liposomes at critical detergent/lipid ratios. However, very few pharmacological agents, i.e., drugs, have the propensity to solubilize membranes at the concentrations and under the conditions generally applied. Weak electrolyte drugs may exhibit unique behavior in this regard because of large differences in the aqueous solubility, partitioning behavior, and surface activity of the ionized and unionized species of the same drug.

The structural and kinetic aspects of the solubilization of phospholipids by detergents has been reviewed recently by Lichtenberg *et al.* (4). The mechanism of solubilization of lipid bilayers is not understood but the critical solubilizing molar ratio in the bilayers, R_e^c, is a means of characterizing the surfactant behavior in these systems (7). Considering propranolol as the drug in question and assuming that the concentration of only nonionized propranolol, [PPL], is responsible for the transformation of liposomes to mixed micelles, then (7)

$$R_e = [PPL]_b/L \quad (1)$$

where $[PPL]_b$ and L are the molar concentrations of PPL in the bilayers and phospholipid, respectively, whereas R_e^c represents the critical solubilizing mole ratio of PPL to phospholipid.

Using an approach used by Schurtenberger *et al.* (8) that assumes an equilibrium partition of PPL between the bilayers and the aqueous medium, a modified distribution coefficient, K, was defined where

$$K = [(PPL]_b/L)/[PPL]_w \quad (2)$$

i.e., K is the ratio of the mole ratio concentrations of PPL and phospholipid to the molar [PPL] in the aqueous medium. Furthermore, K (mM^{-1}) may also be derived from (7)

$$K = K_m \cdot M \cdot V \cdot 10^{-6} \quad (3)$$

where K_m is the molar partition coefficient of PPL, M is the average molecular weight of the phospholipid, and V is the specific volume of the liposomes. Since the total drug concentration, $[PPL]_T = [PPL]_w + [PPL]_b$, where $[PPL]_w$ is the aqueous phase PPL concentration, then

$$[PPL]_T = R_e(L + 1/K) \quad (4)$$

and at the critical solubilizing concentration of PPL

$$[PPL]_T^c = R_e^c(L + 1/K) \quad (5)$$

where a plot of $[PPL]_T^c$ versus L enables determination of R_e^c and K. Also, if the phase transformation occurs when $[PPL]_w \simeq$ CMC, then

$$[PPL]_T^c = R_e^c \cdot L + CMC \quad (6)$$

MATERIALS AND METHODS

Propranolol (PPL) hydrochloride (Ayerst Laboratories, Montreal) was dissolved in an aqueous Sorensen glycine–NaOH buffer solution adjusted to the desired pH (10), then added to a dried film of L-α-dimyristoylphosphatidylcholine (DMPC; 99%) (Sigmal Chemical Co., St. Louis, Mo.) in a round-bottom flask, formed by rotary evaporation of its chloroform solution, then overnight drying at 30°C in a vacuum oven, and dispersed by hand-shaking at approximately 40°C. The dispersion was further vortex-mixed for 10 min and the formed liposomes were subsequently equilibrated at the desired temperature. The turbidities of the liposome dispersions were determined from absorbance measurements (Beckman Model 25 or Model DU-7 spectrophotometer) at a wavelength of 520 nm. Initial turbidities were reproducibly obtained using this procedure. Results are shown as the averages of duplicate determinations, which were essentially identical. All experiments involving turbidity measurements were run against a control liposome preparation containing no drug. Glass-distilled water was used in the preparation of all aqueous solutions and all other chemicals and solvents were reagent grade.

Surface tension measurements of PPL at pH 12.0 were conducted at RT (21°C) and 40°C employing the Wilhelmy plate method with the Rosano surface tensiometer as a function of the [PPL].

RESULTS

The solubilization of DMPC liposomes as a function of time can be conveniently followed by turbidity measurements. As shown in Fig. 1, 0.2 mM PPL completely solubilized 14.4 mM DMPC liposomes after about 12 hr at pH 12.0 and after 18 days at pH 11.0 following a lag period of 1 day, but only partial solubilization occurred at pH 10.0 after about a 10-day lag time. At pH 8.0 or 9.0 (or in liposomes without PPL at pH 12.0) no change in turbidity was observed after 1 week. The time-dependent curve at pH 11.0 also indicates two apparent phase transformations during the solubilization process. When solubilization was complete at pH 11.0 or 12.0, the sample had the appearance of a transparent

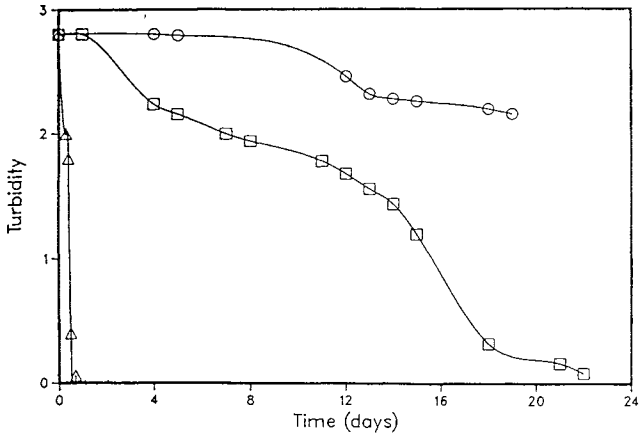

Fig. 1. Time-dependent solubilization of DMPC liposomes by propranolol hydrochloride (PPL) at 40°C as a function of the pH of the medium. (○) pH 10.0; (□) pH 11.0; (△) pH 12.0. The initial concentrations of PPL and DMPC were 0.2 and 14.4 mM, respectively. Turbidities were recorded as absorbances at 520 nm. Solubilization was considered complete at a turbidity of 0.2.

solution. In each case, the transition from turbidity to transparency was reversible by adjusting the pH, i.e.,

$$\text{turbid} \underset{\text{acid}}{\overset{\text{alkali}}{\rightleftharpoons}} \text{transparent}$$

The results of a systematic study of the solubilization of DMPC liposomes at pH 12.0 by various concentrations of PPL are shown in Fig. 2. It is apparent that, also under these conditions, there are clearly two phase transformations which have occurred during the solubilization process—a slower initial phase which continues for approximately 95 hr, then a rapid terminal phase which varies from 8 to 20 hr depending on the [PPL]. The beginning of the initial phase is also characterized by a lag time at lower [PPL]s which becomes a rapid equilibration phase transformation at 0.1 m*M* PPL. In addition, the terminal phase begins at the same time at all [PPL]s (the parbolic shape of the curves at this point was due to computer curve-fitting of the data). Furthermore, a dramatic change in the rate of solubilization was observed after an increase in the [PPL] from 0.1 to 0.2 m*M* compared to rate changes observed from concentrations below 0.1 m*M* PPL.

The solubilization of liposomes by 0.2 m*M* PPL as a function of the [DMPC] is described in Fig. 3. As expected, the lower the liposome concentration, the greater the rate of change of turbidity. Only at 8.64 and 14.4 m*M* DMPC were there noticeable lag times in the solubilization process, whereas at 2.88 m*M* DMPC a rapid decline in turbidity occurred over the first 2 hr and, subsequently, a slower rate of change prevailed. In these systems, after about 2 hr the rate of solubilization was uniform at each [DMPC]; however, the rate profiles at each [DMPC] converged at 13 hr, at which time solubilization was complete.

The solubilization of DMPC liposomes at three different temperatures is shown in Fig. 4. It is evident that DMPC liposomes in the rigid gel state below the phase transition temperature at 20°C (T_c = ca. 23°C) exhibit a much slower rate of solubilization in the presence of 0.2 m*M* PPL than when the bilayers are in the fluid, liquid crystalline state at

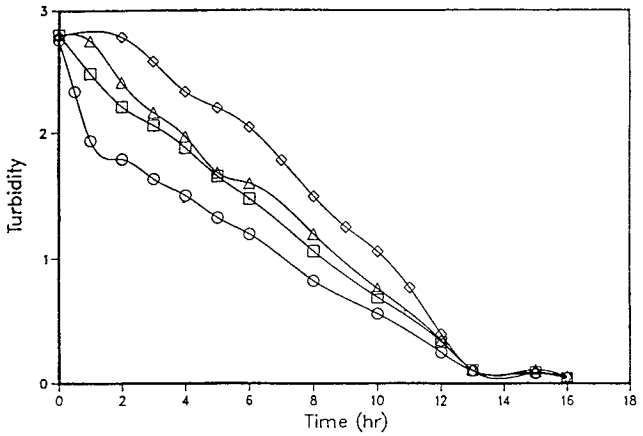

Fig. 3. Time-dependent solubilization of DMPC liposomes by 0.2 m*M* PPL at 40°C and pH 12.0 as a function of [DMPC]: (○) 2.88 m*M*; (□) 5.76 m*M*; (△) 8.64 m*M*; (◇) 14.4 m*M*.

30 and 40°C. In addition, it was determined that upon cooling to 10°C, no solubilization occurred over a period of 3 weeks. In summary, cooling the liposomes from 40 to 30°C approximately doubled the time required for complete solubilization. In contrast, cooling from 40 to 20°C increased the time for solubilization by approximately sevenfold.

Plots of the surface tension data in Fig. 5 show that the CMC of unionized PPL occurs at 0.05 and 0.03 m*M* at 25 and 40°C, respectively, a behavior consistent with that observed generally for nonionized surface-active monomers (9a). Thus, nonionized PPL is much more surface-active than ionized PPL [CMC = 95 mmol/kg (11)]. Above the CMC, the surface tension continues to be lowered, indicative of a mass action model of micellization which predicts a decreasing monomer activity above the CMC (9b).

DISCUSSION

Previous results of the partitioning of PPL in the *n*-octanol–water system as a function of pH indicated that almost total extraction of PPL into the organic phase occurs well below its pK_a (=9.45) (12,13). Also, the ion-corrected partitioning of PPL in DMPC liposomes has been reported as

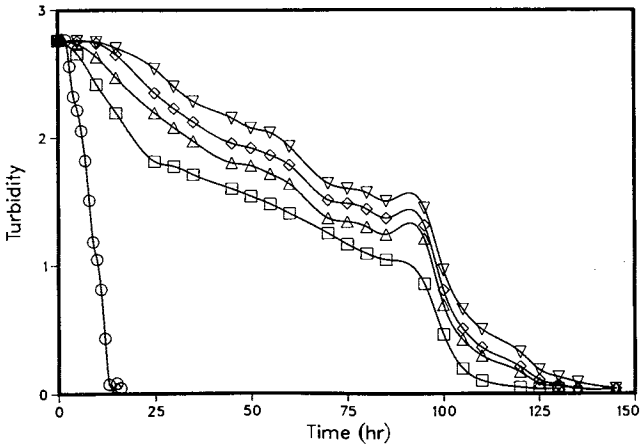

Fig. 2. Time-dependent solubilization of 14.4 m*M* DMPC liposomes by PPL at 40°C and pH 12.0 as a function of [PPL]: (▽) 0.001 m*M*; (◇) 0.01 m*M*; (△) 0.05 m*M*; (□) 0.1 m*M*; (○) 0.2 m*M* PPL.

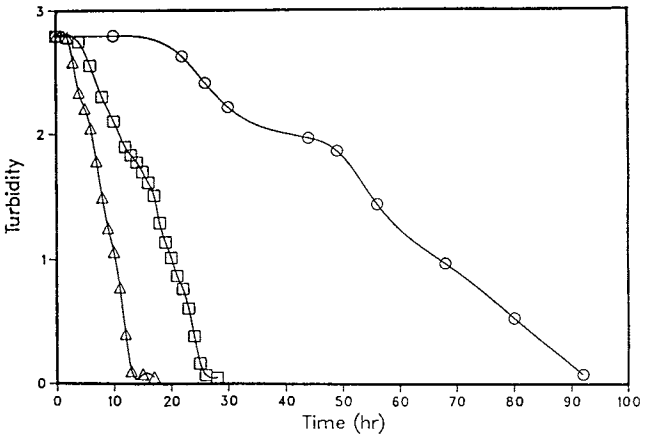

Fig. 4. Time-dependent solubilization of 14.4 m*M* DMPC liposomes by 0.2 m*M* PPL at pH 12.0 as a function of the temperature: (○) 20°C; (□) 30°C; (△) 40°C.

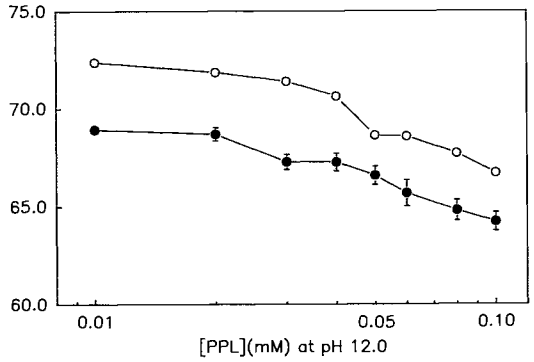

Fig. 5. Surface tensions of PPL solutions at pH 12.0 at two temperatures: (○) 21°C (RT); (●) 40°C.

being an order of magnitude greater than in the *n*-octanol/buffer system (14,15), which indicates that the extraction is even more pronounced by liposomes. Thus, with liposomes containing 14.4 m*M* DMPC and 0.2 m*M* PPL, the extraction of PPL would be expected to result in a relatively high ratio of PPL to lipid in the bilayer, R_e. Also, at pH 11.0 or 12.0 the liposomes were solubilized but at different rates, indicating that a critical ratio for solubilization, R_e^c, was attainable but the equilibration time was a function of the concentration of unionized PPL present. In contrast, at pH's lower than 10.0 (i.e., pH 8 or 9) there were insufficient unionized PPL molecules to attain the R_e^c in the bilayers since no change in turbidity was observed in the liposomal suspension after 3 weeks. At pH 10.0 only partial solubilization occurred, i.e., there were too few nonionized PPL molecules available to attain R_e^c in all of the bilayers. At pH 10.0, 11.0, and 12.0, PPL is calculated as being 78, 97.4, and 99.7% nonionized, respectively, which approximately corresponds to 1.08, 1.35, and 1.39 mol nonionized PPL/100 mol DMPC, respectively, in the total system. Lichtenberg has argued that the product, $K \cdot$ CMC, approximates R_e^c, and therefore, at pH 12.0, $R_e^c \approx 1.03$, which is comparable to 0.98 for octyl glucoside but somewhat higher than 0.5 for the nonionic detergent $C_{12}E_8$ (7). At this pH, PPL has a CMC = 0.03 m*M* (Fig. 5) and $1/K = 0.029$ m*M*[5] [cf. 22 and 22.5 m*M*, respectively, for octyl glucoside, and 0.09 m*M* (17) and 0.175 m*M*, respectively, for $C_{12}E_8$]. Thus, it is concluded from these results that extraction by the outer lamellae of the liposomes occurs initially, the R_e^c is reached and solubilization occurs, then the process continues with the next lamellae, and so on until solubilization is complete. The continued lowering of the surface tension above the CMC of PPL is indicative of an increasing number of PPL monomers available for partitioning and, subsequently, to participate in mixed micelle formation with phospholipid molecules. Hence, the slower rates of solubilization at pH 10.0 and 11.0 indicate that the extraction process is a function of the thermodynamic activity of nonionized PPL monomers in solution. At pH 12.0 the activity of PPL was found to be greater than the required minimum for solubilization at [PPL]s as low as 10^{-13} *M* (determined from an independent experiment).

[5] K was determined from Eq. (3) using $K_m = 47{,}863$ (15), $M = 676$, and $V = 1.06$ (16).

The time for complete liposome solubilization is dependent on several factors as previously alluded to (4) and as seen from the evidence of the present study at pH 12.0. The observed differences in the time periods required for complete solubilization and the apparent phase transformations which the liposomes undergo during these time periods (Fig. 2) support the argument that solubilization is dependent on the attainment of the R_e^c, which occurs over longer periods of time at the lower [PPL]s. Over the range 0.001 to 0.05 m*M* PPL the rate at which solubilization occurred (i.e., turbidity decreased) up to 95 hr is shown in Fig. 6. It is apparent that log [PPL] had only a small effect on the rate up to 0.05 m*M* but a large effect at higher concentrations. The intersection of the two curves yielded a value of 0.078 m*M*, which may be referred to as the threshold concentration for rapid solubilization. In comparison, at a constant [PPL] of 0.2 m*M*, an increased rate of solubilization of liposomes occurred at higher [DMPC]s because of the greater extraction behavior of liposomes for PPL of higher phospholipid contents. A positive linear relationship was found between the rate of solubilization and the [DMPC] (derived from Fig. 3) as shown in Fig. 7, and accordingly, the time to solubilize all liposome concentrations was identical, i.e., 13 hr. However, in an experiment at 10^{-10} *M* PPL the extent of solubilization decreased as the [DMPC] increased up to 57.6 m*M* DMPC (at which no solubilization occurred), indicating that because an insufficient number of PPL monomers partitioned in the bilayers, R_e^c was not attained.

The solubilization process is also influenced by the physical state of the molecules in the liposomal bilayers based on the data in Fig. 4. Hence, mixed micelle formation is a time-dependent function of the ability of PPL to distribute into the bilayers and reach the R_e^c. This process appears to be dependent on the membrane fluidity, which is considerably reduced below the phase transition temperature of the phospholipid. At 20°C, DMPC liposomes exist in the more viscous gel state, consequently, the rate of solubilization is sevenfold less than liposomes in the fluid, liquid crystalline state at 40°C. In this regard, a decrease in log K'_m of PPL

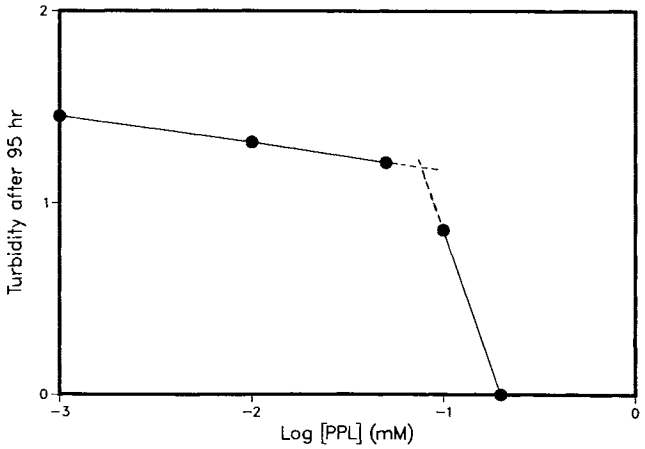

Fig. 6. Plot of turbidity of 14.4 m*M* DMPC liposomes after 95 hr against the corresponding log[PPL] at 40°C and pH 12.0. The intersection of the two extrapolated lines indicates the concentration of PPL at which the solubilization efficiency increases, after which solubilization occurs more rapidly.

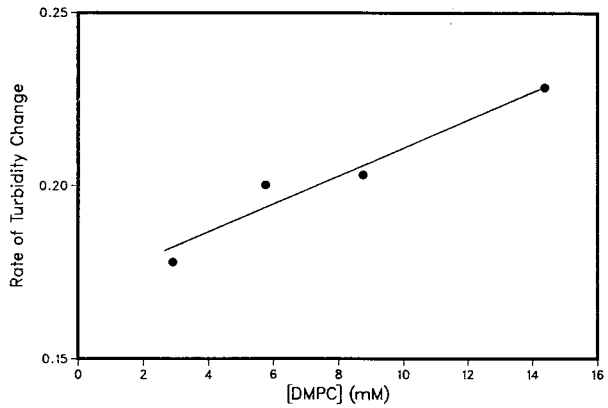

Fig. 7. Plot of the rate of turbidity change of liposomes by 0.2 m*M* PPL at 40°C and pH 12.0 from 2 to 13 hr as a function of the [DMPC]. The correlation coefficient, $r = 0.971$.

below the T_c of DMPC has been reported (14). Again, it is evident that as the solubilization process is slowed down, a lag time and phase transformation become more pronounced prior to complete solubilization.

The solubilization of liposomes by weak electrolyte drugs has several implications in liposome research and possibly in certain applications. First, if at the given pH, the drug possesses surface activity and partitions to a large extent in the bilayers, some degree of or complete solubilization of the liposomes may occur. The process may be slow, taking days or weeks, and may not be noticed during the preparation and characterization stages of product development. Proper adjustment of the pH, liposome concentration, or temperature can help to overcome this problem. Once solubilized the turbidity of the system is zero but the system can be made turbid again by pH adjustment, which for PPL means acidifying the solution. However, the particle size undergoes a change as a result of this treatment. For instance, the average particle size of each of the liposome preparations containing varying amounts of DMPC prior to solubilization was 700 nm (Coulter counter light scattering, Model N4MD, 90°) with a particle size distribution ranging from 673 to 737 nm. After solubilization and readjustment of the pH to restore the turbidity, particle size analysis revealed an average particle size of 40 nm, with a size distribution of 20–55 nm. This suggests a possible convenient means of preparing a homogeneous liposome formulation of small particle size after which the drug may be removed by dialysis. Also, because of the extremely low [PPL]s which solubilize DMPC liposomes at pH 12.0, delipidation or deproteinization of membranes by PPL, by forming mixed micelles which can then be separated according to size by gel permeation chromatography, may be a useful process. In any case, weak electrolyte drugs should not necessarily be considered inert after their incorporation into liposomes and, in the nonionized state at very low concentrations, may exert a solubilizing influence on the liposomes over a period of time.

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