

Mechanism of mixed liposome solubilization in the presence of sodium dodecyl sulfate

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Abstract

Structural transformations of vesicles to micelles that take place during the interaction of sodium dodecyl sulfate (SDS) with individual and mixed vesicles of phosphatidyl choline (PC) and phosphatidic acid (PA), have been studied by monitoring changes in optical density and in the concentration of free SDS monomer in the respective systems. Incorporation of the surfactant monomers (SDS) in the bilayers was found to result in an initial increase in concentration of the mixed vesicles up to its saturation. Subsequently a progressive relaxation of these structures together with a simultaneous formation of mixed micelles was found to occur. The breakup of bilayer and the formation of mixed micelles were completely dependent on the structure of the individual phospholipid. The solubilization of the anionic phosphatidic acid vesicle was very fast in the presence of SDS, due to its simple structure and its compatibility with the SDS molecule. But solubilization of the zwitter ionic phosphatidyl choline vesicle was very complicated in the presence of SDS, possibly due to its complex structure and the zwitterionic nature. On the other hand, solubilization of the mixed vesicle (mixed liposome) and formation of mixed micelle was found to be relatively easier. This may be attributed to one of the phospholipid component preferentially coming out first through interaction with SDS, thereby making the overall system unstable and enhancing the micellization processes. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Structural transformations; Micellization processes; Vesicles; Zwitterionic nature

1. Introduction

The solubilization and reconstitution of biological membranes using surfactants are currently attracting much attention [1–3]. Surfactant-liposome systems represent good models for

studying solubilization of cell membranes [4–7] and surfactants interactions with such biosystems as skin. The interaction of surfactants with liposomes eventually leads to the rupture of its vesicle structure resulting in the solubilization of phospholipid components. In general, there is agreement that growth of vesicles occurs in the initial stages, followed by the formation of a number of complex lipid-surfactant aggregates associated with the vesicle to micelle transformations. The interactions of sodium dodecyl sulfate (SDS) with

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simplified membrane models of PC liposomes, PA liposomes and PC and PA mixed vesicles have been investigated in this work. The above surfactant was chosen because of its known irritating action on biological surfaces [8–10].

We have characterized in detail the vesicle to micelle structural transitions involved in the interaction of SDS with individual PA, PC and mixed liposomes using a combination of optical density and SDS monomer measurements. The Re (liposome surfactant molar ratio) and K (partition coefficient) values calculated using the monomer SDS concentration left in the supernatant after interaction for 1 h with different liposomes are compared with the optical density data obtained.

2. Experimental

SDS was obtained from Fluka, which is 99% pure, and used as such. Surface tension data give an indirect measure of purity of the SDS sample. We have measured the surface tension of SDS in triple distilled water as well as in phosphate buffer (data is not shown) but no minimum was observed in the surface tension curve. This indicates the SDS sample is pure and without any further purification we used it for our experimental purposes. Phosphatidic acid (PA) from egg yolk lecithin and phosphatidyl choline (PC) also from egg lecithin was purchased from Sigma. The buffer used was phosphate buffer at pH 7.0.

2.1. Liposome preparation

Unilamellar liposomes of uniform size were prepared following the method of Helenius [11]. A lipidic film was formed by removing the organic solvent from the individual lipid (1 mM), and lipid mixtures (lipid compositions PC/PA 1:1 molar ratio) by rotary evaporation. Each lipid film was dispersed in the phosphate buffer and sonicated in a bath sonication unit at 40°C for 1 h. The resulting liposomes were sized through a polycarbonate filter of 0.2 μm pore size.

2.2. Absorbance measurements

Absorbance measurements were made at 25°C with Shimadzu UV-240 spectrophotometer ($\lambda = 350 \text{ nm}$, cell length = 5 cm). The absorbance change of each vesicle suspension (1 mM) was monitored before and after interaction with SDS (1 to 12 mM). The absorbance measurements were taken after surfactant addition and subsequent vigorous agitation for different intervals of time; the values were corrected taking into account the dilution involved.

2.3. SDS concentration in bilayer

The SDS concentration in lipid bilayers was determined after interactions of individual vesicles with SDS for 1 h. The above mixtures were then filtered through an Amicon filter (Molecular wt cutoff 3000) in order to separate SDS monomers from vesicles and micelles in the bulk. The monomer concentration in each filtrate was determined by a two-phase titration method [12]. The SDS concentration in each bilayer was calculated by subtracting the monomer concentration in the filtrate from the initial concentration. The partition coefficient (K) and effective surfactant to phospholipid molar ratio (Re) were calculated by using the equation described below.

2.4. Solubilization parameters

The perturbation of the phospholipid (PL) bilayer produced by the surfactants leads to the solubilization of the lipid components via mixed micelle formation [13]. This solubilization results in changes in the optical density of the system, which was monitored during the solubilization process.

When defining the parameters related to the solubilization of liposomes, it is essential to consider the fact that mixing of lipids and SDS is not ideal because of specific interactions between both components [14,15]. To evaluate the alterations of lipid bilayers caused by SDS, the effective SDS/PL molar ratio, Re , in an aggregate (liposome or micelle) is defined as follows [13].

$$Re = \frac{(\text{Total surfactant}) - (\text{surfactant monomer})}{(\text{Total PL}) - (\text{PL monomer})} \quad (1)$$

The second term of the denominator is negligible due to the low solubility of PL in water. It is generally believed that partition equilibrium of surfactants between the bilayer and the aqueous medium governs the incorporation of surfactants into liposomes, thereby producing saturation and solubilization of these structures.

Lichtenberg [13] and Almong et al. [16] have shown that for mixing of lipids and surfactant, in dilute aqueous media, the distribution of surfactant between lipid bilayers and aqueous media is given by a partition coefficient K .

$$K = \frac{S_{PL}}{(PL + S_{PL})S_w} \quad (2)$$

Where S_{PL} is the concentration of SDS in the bilayers and S_w is the SDS concentration in aqueous medium. For $PL \gg S_{PL}$, the definition of K , given by Schurtenberger [17] applies:

$$K = \frac{S_{PL}}{(PL \cdot S_w)} = \frac{Re}{S_w} \quad (3)$$

whereas Re is the above mentioned ratio of surfactant to PL in the vesicle bilayer ($Re = S_{PL}/PL$). Under any other conditions, Eq. (2) has to be employed to define K :

$$K = \frac{Re}{S_w(1 + Re)} \quad (4)$$

The overall solubilization process of PL bilayers by SDS can be characterized by two parameters, termed Re_{sat} and Re_{sol} , corresponding to the SDS/lipid molar ratios. These parameters correspond to the Re at which SDS (i) saturates the liposome; and (ii) leads to total solubilization of the liposomes. The determination of these parameters was carried out by measuring SDS monomer concentration in the solution with liposome after 24 h of interactions. Thus, in any liposome sample the necessary SDS concentration needed to saturate the bilayer is given by:

$$\text{Total surfactant} = S_w + S_{PL} \quad (5)$$

Where:

S_w = total monomer concentration in the aqueous medium

S_{PL} = total SDS concentration in lipid bilayer

The partition coefficients of SDS in liposome have been determined by applying Eq. (4). The partition coefficient during solubilization may be regarded as a dynamic equilibrium between the different stages of transition from lipid bilayer to mixed micelles.

3. Results and discussions

Solubilization of liposomes made up of 1 mM phosphatidyl choline only by sodium dodecyl sulfate as measured by optical density is given in Fig. 1. Here, the dependence of liposome solubilization on SDS concentration for different interaction times is shown. After 15 min. of interaction, about 35% of the liposome has been solubilized by 12 mM SDS. As the interaction was allowed to continue the solubilization increased, with complete solubilization observed after 12 h of interaction even with a lower (9 mM) amount of SDS. Optical density data for the solubilization of PA liposome by SDS is given in Fig. 2 for different intervals of time. It is obvious from this figure that solubilization of PA liposome takes place much more rapidly than PC liposome and about 80 to 90% solubilization was observed in the presence of 1 mM and 2 mM of SDS after only 15 min of interaction. Almost complete solubilization of the above liposome was observed in the presence of 6 mM of SDS after 15 min of interaction. As the interaction time increased, solubilization of the above liposome increased rapidly and almost complete solubilization was observed in the presence of 1 mM of SDS after 12 h of interaction. Thus SDS can completely solubilize PA liposome at 1 mM, but it takes longer, whereas at higher concentrations of SDS the solubilization takes place much more rapidly.

Fig. 3 illustrates solubilization of 1 mM of liposome (1PA:1PC) by SDS at different intervals of time. After 15 min of interaction, in the presence of 1 mM of SDS, an increase in optical density from 1.2 to 1.25 was observed. This increase in the optical density is attributed to the

adsorption of SDS onto the lipid vesicle and the resultant increase in the size of the vesicle. At higher concentrations of SDS also, the same processes may be occurring, but it is difficult to observe the intermediate process because the reaction is very fast due to the availability of the high

concentration of surfactant molecules. As the interaction time increased at 1 mM of SDS the optical density passed through a maximum and then gradually decreased indicating that solubilization is occurring. Complete solubilization of 1 mM of liposome by 10 mM of SDS was observed

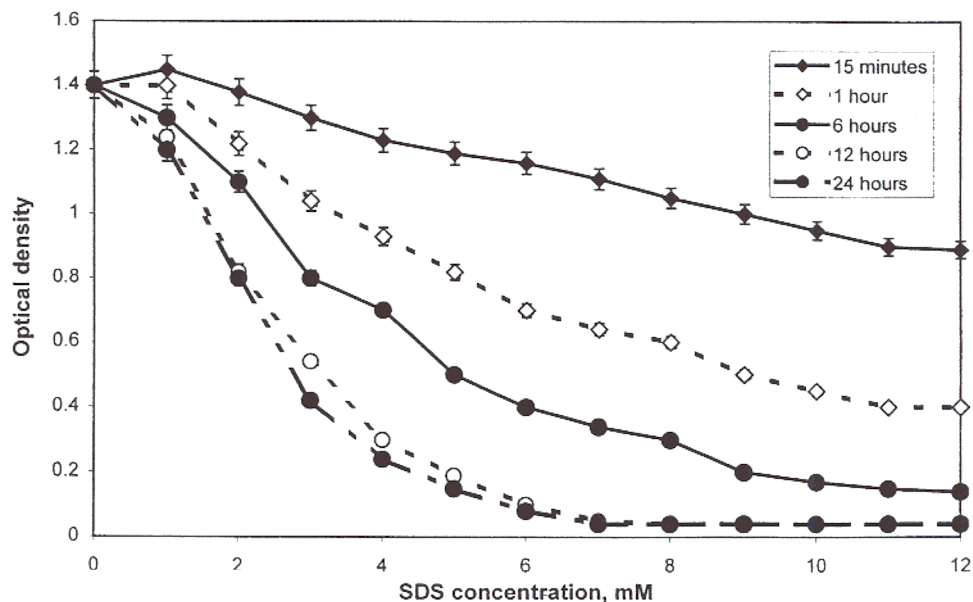


Fig. 1. Solubilization of phosphatidyl choline liposome (1 mM) by sodium dodecyl sulfate at different intervals of time.

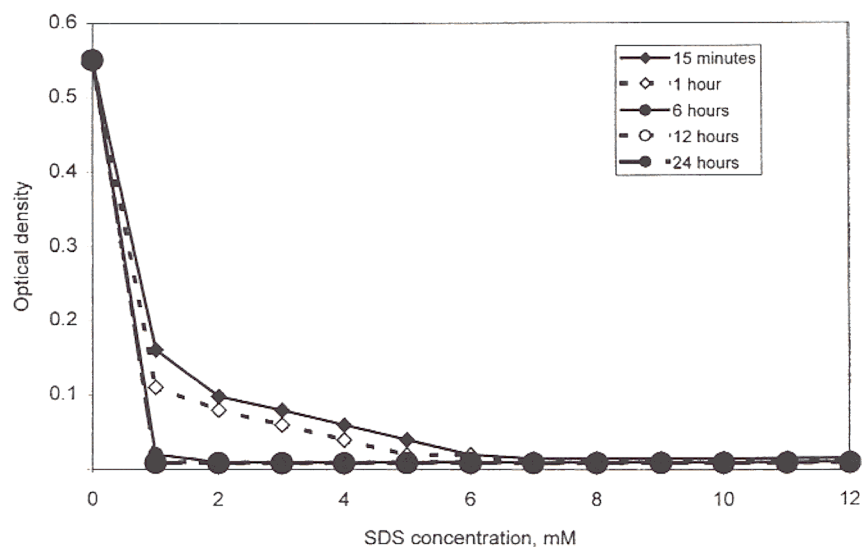


Fig. 2. Solubilization of phosphatidic acid liposome (1 mM) by SDS at different intervals of time.

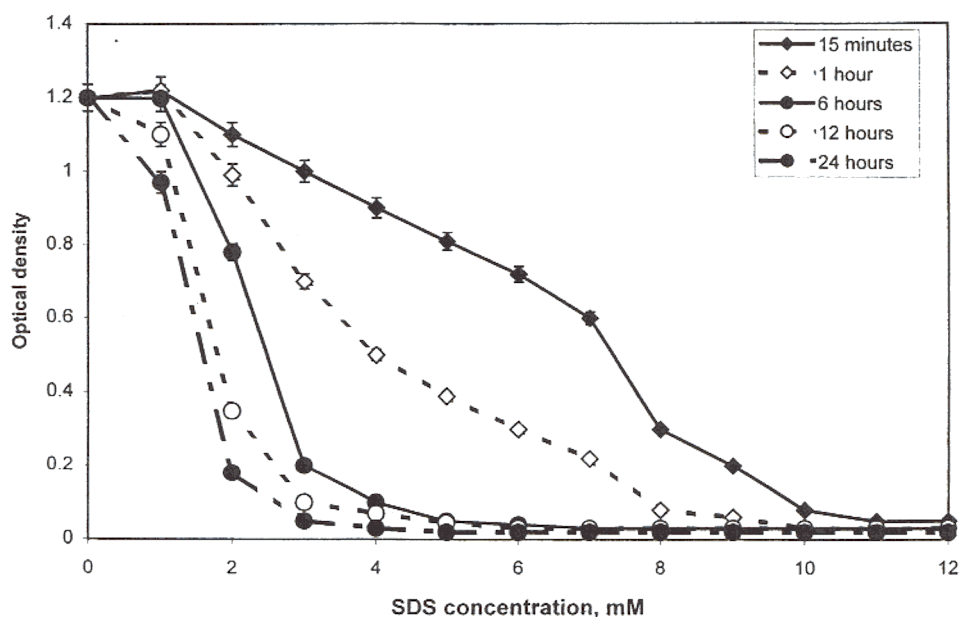


Fig. 3. Solubilization of 1 mM of liposome(1PA:1PC) as a function of SDS concentration at different intervals of time.

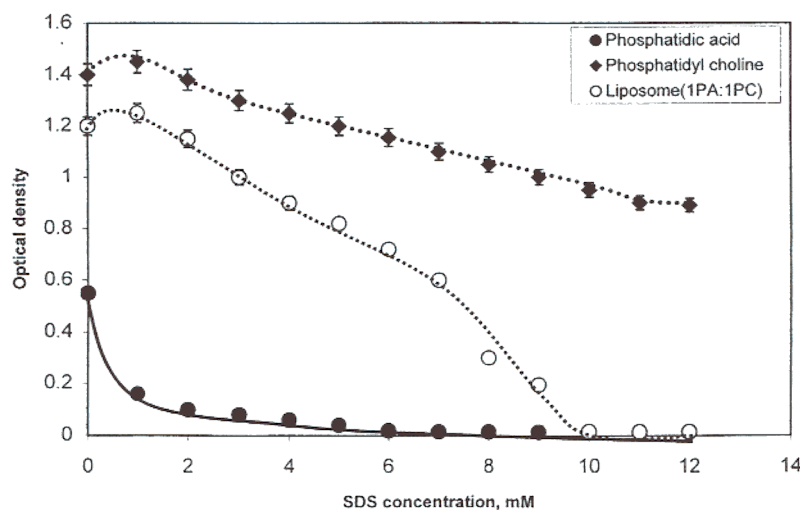


Fig. 4. Solubilization of 1 mM of different liposomes by SDS after 15 min of interaction.

after 15 min of interaction whereas in the presence of 4 mM of SDS, complete solubilization was observed after 6 h. This indicates that solubilization of liposome depends on both the concentration of the surfactant as well as time of interaction with liposome. More interaction time is needed at low surfactant concentrations, for

complete solubilization.

The solubilization of 1 mM of different liposomes by SDS, after 15 min interaction time, is shown in Fig. 4. It is evident that solubilization of PA liposome is very fast and complete solubilization takes place at 6 mM of SDS during the above interval. Whereas complete solubilization of 1mM

liposome (1PA:1PC) was observed in the presence of 10 mM of SDS, only 35% solubilization of PC liposome was observed under the same conditions. This suggests that the PC liposome structure is more rigid so the interaction of SDS and PC liposome is hindered. The solubilization rate of liposome (1PA:1PC) is faster than that of PC even though it is composed of both PC and PA. This may be due to the exiting of one of the phospholipid components preferentially first during surfactant liposome interaction, which can destabilize the system and enhance the solubilization process.

Fig. 5 represents the SDS monomer present in the supernatant after 1 h of interaction with different liposomes. In the case of phosphatidic acid liposome, the monomer concentration of SDS remains constant after interaction with 6 mM of SDS whereas in the case of the mixed liposome the same effect was observed at around 10 mM of SDS. This indicates complete solubilization of liposome and onset of formation of micelles. In contrast to the above, in the case of PC liposome the monomer concentration of SDS continues to increase in the concentration range studied. Thus

solubilization of PC liposome is not complete during the 1 h of interaction with different SDS concentrations, suggesting that the system did not yet reach mixed CMC under these conditions.

The variation in SDS monomer concentration (S_w) versus SDS/phospholipid molar ratio (Re) for different liposomes is illustrated in Fig. 6. It is clear from the figure that the monomer concentration left in the supernatant increases with increase in surfactant concentration. Here a preferential incorporation of surfactant species into liposome governs the initial stages of this interaction, leading to the beginning of bilayer saturation at surfactant concentrations lower than its CMC. Additional amounts of surfactant increases the free surfactant until the surfactant CMC is reached and solubilization starts to occur at this point. Thus, the Re saturation parameter corresponds to the surfactant/phospholipid molar ratio needed for the initiation of the bilayer solubilization via mixed micelle formation. This surfactant concentration may be considered to be the CMC of the new surfactant/phospholipid mixed system. The phosphatidic acid vesicle and mixed liposomes reach the Re saturation at 6 and 10 mM

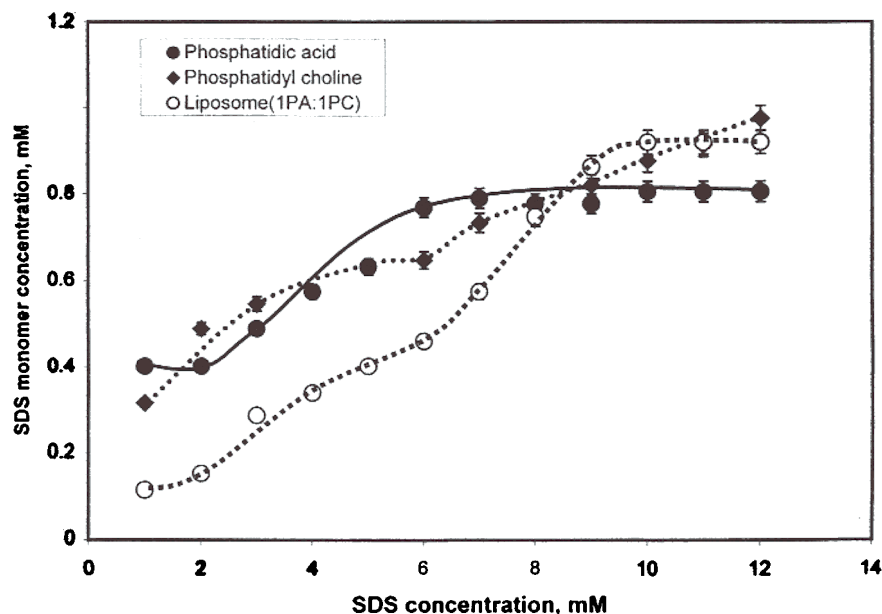


Fig. 5. SDS monomer concentration in the supernatant after 1 h of interactions with 1 mM of different liposomes.

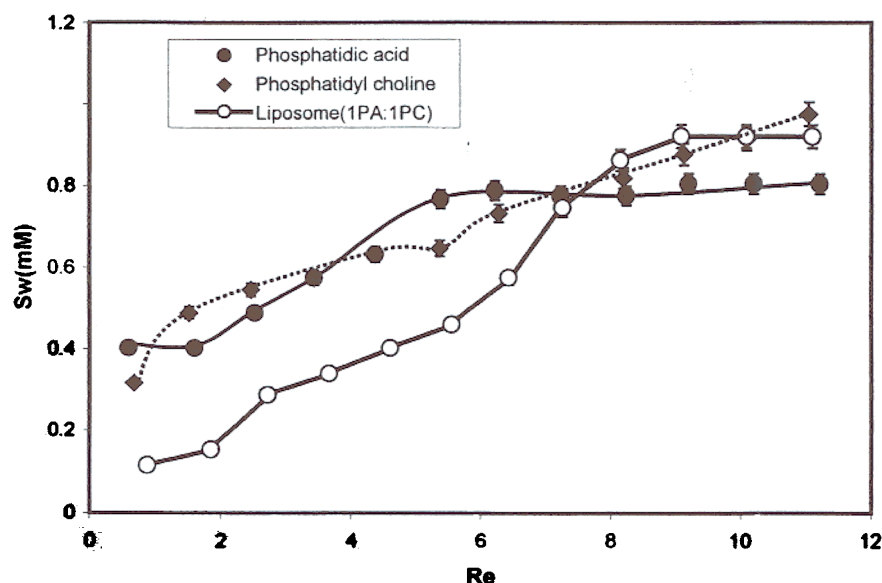


Fig. 6. Variation of SDS monomer concentration (S_w) with SDS/Phospholipid molar ratio (Re) for different liposomes

SDS, respectively, whereas in the case of phosphatidylcholine, the Re saturation has not yet been reached. Thus the phosphatidyl choline requires more SDS for complete solubilization, even though the molar concentrations of both phosphatidic acid and phosphatidyl choline are the same. This is attributed to the rigidity of the phosphatidyl choline structure. Even though the molar concentration of mixed (PA:PC) liposome is almost double than the individual phospholipid liposomes the Re saturation of the mixed liposome is reached at around 9 to 10 mM of SDS. As indicated earlier this is attributed to the preferential exiting of one of the liposome components during the interaction with SDS and the resultant destabilization, which enhances the solubilization processes. This is also possibly the reason as to why the Re_{sat} of liposome (1PA:1PC) lies between that of phosphatidic acid and phosphatidyl choline liposome.

The proposed mechanism of interaction of SDS with individual phospholipids is shown in Fig. 7. The interaction of phosphatidic acid with SDS is straightforward due to its simpler molecular structure (Fig. 7a). Because of the anionic nature of both SDS and PA, head on interaction is not

expected due to coulombic repulsion. On the other hand, the hydrophobic dodecyl group can easily interact with the hydrophobic chain of the phosphatidic acid to form a mixed micelle. This is considered to be the reason for the faster rate of solubilization of PA liposome in the presence of SDS. In contrast to PA, the molecular structure of phosphatidyl choline is complex as it contains two types of charge groups. When SDS molecule interacts with PC liposomes it can be expected to experience the coulombic attraction due to the presence of $N^+(CH_3)_3$ but since the nitrogen is bonded to three bulky $(CH_3)_3$ groups, the interaction between SO_4^{2-} and $N^+(CH_3)_3$ may not be very strong. On the other hand, the hydrophobic chain is not compatible with the aqueous medium and hence association can be expected between the hydrophobic chains as shown in Fig. 7b, but this may not be energetically feasible due to the bulky structure of the phosphatidyl choline molecule. SDS and PC under these conditions will interact to arrive at a compatible rearrangement among them selves. Another possible way of interaction of SDS with PC molecule is through hydrophobic chain-chain interaction as shown in Fig. 7c. Since the PC molecule does carry a

positive charge group, it will definitely try to aggregate with some anionic molecules and this complicates the process, leading to slower solubilization than in the case of PA liposome. Due to the above structural complications the PC molecule may not be able to form mixed micelles with SDS molecules easily. In contrast, phosphatidic acid does not have such structural problems, the micellization and solubilization hence being faster.

4. Conclusions

From these findings we may conclude that the

solubilization of phosphatidic acid liposome is very fast in presence of sodium dodecyl sulfate, while solubilization of zwitterionic phosphatidylcholine liposome is very slow under similar conditions. In the initial interaction stages a direct relationship was established between the growth of vesicles (PC liposome and mixed liposome), and monolayer formation, which is directly dependent on the liposome:SDS molar ratio. However, under similar conditions complete breakdown of PA liposome was observed. Mixed micelles begin to form only when the phospholipid bilayer becomes saturated with SDS, which is clear from the monomer study.

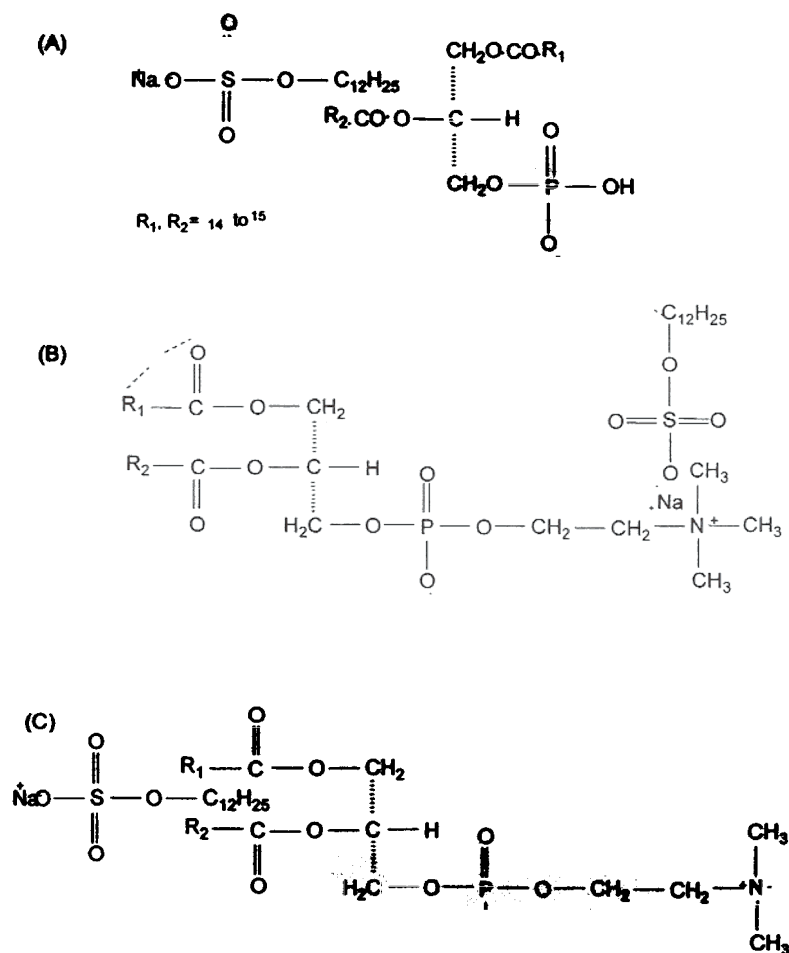


Fig. 7. Probable mechanisms of interaction of sodium dodecyl sulfate with different lipids.

The monomer concentrations of SDS in the supernatant increases until complete solubilization of liposome, after which no further increase in SDS monomer was observed with further increase in SDS concentration. This confirmed that solubilization of liposome by SDS is a micellization process. The monomer measurement in the supernatant gives direct evidence of Lichtenberg's three-stage model. The anionic charge on SDS does not affect its adsorption onto anionic liposome surfaces; indicating that hydrophobic and Vanderwals forces play the major role in the SDS/liposome interaction processes.

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