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SHORT COMMUNICATION

P.C. Schulz
S. Moya

Study of the required HLB for the solubilization of cholesterol in aqueous solution

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P.C. Schulz (✉) · S. Moya
Departamento de Quimica e lng. Qca
Universidad Nacional del Sur
Avda. Alem 1253
8000 Bahia Blanca
Argentina

Abstract The solubilization of cholesterol by anionic surfactant mixtures was studied as a function of their HLB values. The relationship between the logarithm of the critical micelle concentration and the HLB value of the mixtures was not linear, which was attributed to a lack of strict additivity of the HLB values. The solubilized cholesterol/surfactant ratio was determined and it was found to be higher than that in bile salts in all the studied surfactant mixtures. Below HLB = 24, emulsions were obtained, and the remaining cholesterol was solid. Above that value, limpid solutions were obtained, giving a solubility maximum at HLB ≈ 35. The non-solubilized cholesterol was mainly in the form of lamellar mesophase.

Key words Cholesterol – solubilization – HLB – mixed micelles – emulsions – critical micelle concentration

Introduction

The deposition of platelets containing cholesterol in the blood vessels, which is known as artheriosclerosis, is a significant cause of death in industrialized societies [1]. Cholesterol is solubilized in bile by mixed micelles of bile salts and phospholipids. Under normal conditions, cholesterol precipitates in the gallblader as gallstones [2–4]. It is commonly accepted that cholesterol gallstones formation occurs in two steps. The former occurs when the amount of cholesterol exceeds the solubilizing capacity of the bile salts. Cholesterol starts to precipitate partially as monocrystals. Secondly, these microcrystals join together and then grow to form macroscopic gallstones [2].

Current therapies are focused to the enzymatic inhibition of the endogenic cholesterol biosynthesis [5] or to bile salts reduction to decrease the blood cholesterol content [6]. The cholesterol deposit solubilization by the use of synthetic substances might provide a new, alternative therapeutic approach. Emphasis was rencently put on the in vivo solubilization of cholesterol as a therapy [6]. The complexation of cholesterol by some synthetic receptors enhanced the solubility by a factor of 190 [7]. Therefore, it is also of interest to study the dissolution of cholesterol by micelles of surfactants [8–13].

A major amount of research on the solubilization of cholesterol was made with bile salts and phospholipids [14–18]. These are either twin-tailed surfactants or those with atypic apolar groups. Bile salts are the most important naturally occurring biosurfactants in living beings. Their principal action is to solubilize in micellar solution fats, monoglycerides, fatty acids and cholesterol [19, 20]. Aqueous bile salts have micellar properties which are different to that of common surfactants, because of their stiff, bulky hydrophobic structure, which has strong affinity to the steroid structure of cholesterol [21–26].

To design biocompatible synthetic surfactants, it is necessary to determine the surfactant solubilizing power, defined as cholesterol molecules per surfactant molecule, and the type of fluid structure formed (emulsion, liquid crystals, microemulsion or micellar solubilization) vs. the Hydrophile–Lipophile Balance (HLB), especially at low

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total concentration of surfactant, because the use of surfactants in biological media may cause undesirable effects. This may occur even with biocompatible surfactants.

Some biocompatible surfactants are phospholipids, lecithins, glycerol monolauryl ether, and N-lauroyl arginine methyl ether [27].

Cholesterol is essentially insoluble in water, but it may be solubilized via an appropriate surfactant. This solubilization may be in the form of mixed micelles, microemulsions or by the formation of a liquid crystal which can be emulsified. The solubilization in the form of mixed micelles or microemulsions may be useful in the therapy of artheriosclerosis, by eliminating the excess of cholesterol. The elimination of cholesterol gallstones is also possible by solubilization. In this case, the conditions are less critical, and liquid crystals and emulsions may also be useful.

Surfactants used in blood vessels must fulfill some conditions: they must be innocuous, and must not react (or react innocuously) with the blood components (platelets, cells, proteins and the walls of veins and arteries); they must not vary the blood osmotic pressure, pH and electrolytic content. The solubilized systems must be fluid with structures as small as possible. This requires a very careful design of the surfactant on the basis of biological considerations and by the investigation in vitro and in vivo, which is outside of the scope of this work. This problem demands the colaboration of organic chemists and biologists. However, to design this kind of surfactant, it is necessary to obtain knowledge of the required physicochemical properties required to solubilize cholesterol: the appropriate HLB value, the solubilizing capacity and the conditions to obtain a given microstructure (liquid crystal, microemulsion, mixed micelle or emulsion) with the smallest surfactant concentration.

In the gallblader, the conditions are less strict, but still the condition of low biological activity remains as a restriction. The solubilizate system may be an emulsion, a micellar solution or a lamellar liquid crystal. All these structures might produce the fluidification of cholesterol and its eventual elimination. The surfactant concentration is not so critical as in blood.

In this paper we study the interaction of cholestrol with one-tail surfactants because their HLB is well known. Anionic surfactants were selected, because of their low specific biological activity. Cationic surfactants have specific biological activity, which exceeds the effects of the surface tension reduction.

Experimental

Cholesterol was Volco (puriss.). The surfactants used to obtain different HLB values were oleic acid (Raudo, analytical grade), decanoic acid (Fluka) and sodium dodecylsulfate (SDS, Aldrich). Sodium soaps were prepared by neutralizing the respective acids with NaOH in ethanol, and then elimination of the solvent by distillation. Their HLB was computed by the method of Davies and Rideal [28], and the HLB values of the mixtures of surfactants were computed in the usual form. Because of the low water solubility of mixtures with low HLB values, we explored the HLB range between 14 and 40.

The critical micelle concentration (cmc) of the mixtures was measured by conductivity using a CRIBABB conductimeter, and by the change in color and fluorescence of Rhodamine 6G.

The cholesterol solubility was measured by weighing about 50 mg of cholesterol in vials and adding 10 ml of each surfactant mixture, with a concentration equal to one, two or three times the cmc. The samples were left for one week with daily sonication for 1 h. If all the cholesterol solubilizated, we added more to obtain saturation. Then they were centrifuged for 3 hs. The solid residue was studied by polarized light microscopy. The supernatant was also studied by polarized and normal light microscopy. Then, 3 ml of the supernatant was dried to determine the amount of solubilized cholesterol, taking into account the contribution to the weight of the surfactant mixture in the sample. All the determinations were performed in duplicate.

Results

The determined cmc values are given in Table 1 and shown in Fig. 1. The cholesterol solubility (in mol of cholesterol/mol of surfactant) vs. surfactant HLB is shown in Fig. 2. Figure 3 shows the concentration of solubilized cholesterol vs. surfactant HLB and concentration.

Table 1 cmc values of different mixtures of anionic surfactants, as a function of the HLB

Composition	HLB	cmc [mol dm^{-3}]
0.6 g OA + 0.4 g SD	14.7	0.00044
0.16 g OA + 0.84 g SD	20.2	0.00091
SD	23.8	0.10
0.68 g SD + 0.32 g SDS	29.3	0.023
0.432 g SD + 0.568 g SDS	33.0	0.018
0.29 g SD + 0.71 g SDS	35.5	0.013
0185 g SD + 0.815 g SDS	37.0	0.0083
SDS	40.0	0.00865

OA: oleic acid; SD: sodium decanoate; SDS: sodium dodecyl sulfate

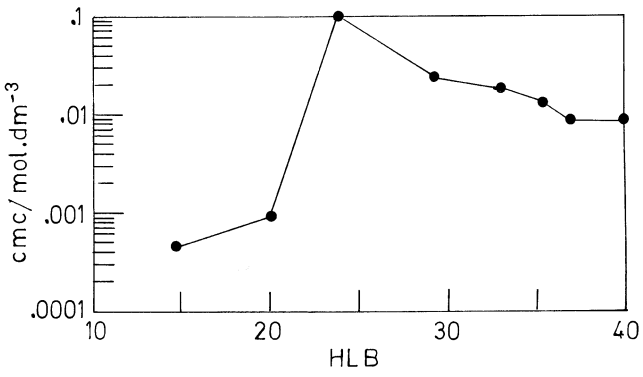

Fig. 1 Critical micelle concentration of anionic surfactant mixtures vs. HLB values

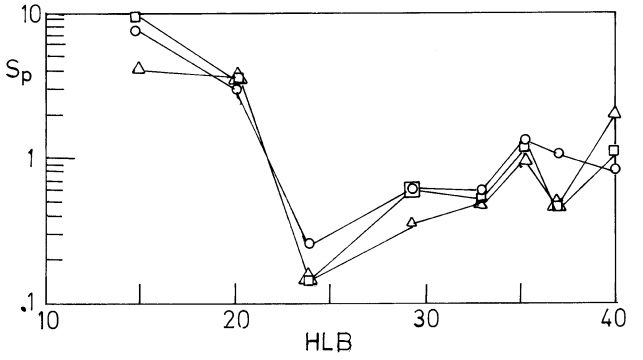

Fig. 2 Moles of solubilized cholesterol per surfactant molecule, vs. the HLB value and concentration of the surfactant (○) cmc, (△) 2cmc, (□) 3cmc

Microscopic results will be analyzed in the discussion section.

Discussion

Cmc

There are several theories relating the HLB value with the cmc. In particular, there is commonly a linear relation between the HLB value and the logarithm of the cmc [29–33]. However, these research works were made usually on a homologous series of surfactants. Becher found that the relation between HLB and log cmc is not strictly correct, especially for high HLB values [34]. In this work, we worked on mixtures of surfactants with different structure. As it may be seen clearly in Fig. 1, there is not a linear relation between HLB and log cmc. This dependence is strongly non-linear in oleic acid–sodium decanoate mixtures. The causes may be the difference in chain structure, because the double bond at the middle of the oleic acid tail has some affinity with water, and the different interaction

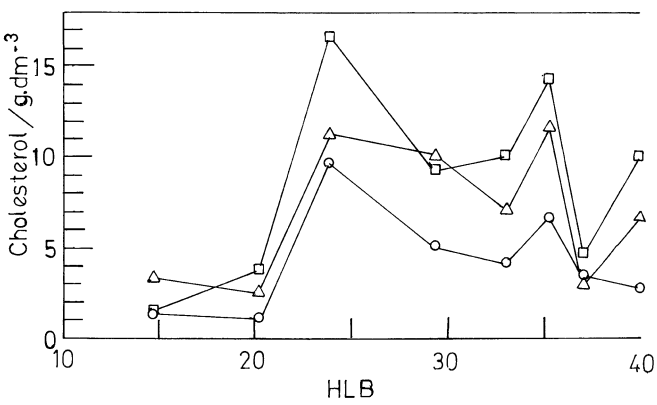

Fig. 3 Cholesterol solubilized ($g\,dm^{-3}$) vs. the HLB and concentration of the surfactant (○) cmc, (△) 2cmc, (□) 3cmc

of the polar heads with water: The acid group may form hydrogen bonds with water, other acid groups and carboxylate groups, and has a small effective charge. The main carboxylate group behavior is that of an ion. The tails of sodium decanoate and SDS are both saturated, and the sulphate group also behaves as an ion, which explains the almost linear relation between HLB and log cmc in their mixtures. We believe that our data support the opinion that HLB values are not strictly additive, as it is commonly supposed. Schott arrived at the same conclusion [35].

Solubilizing power

The cholesterol solubility in water was found to be below $10^{-7}\,mol\,dm^{-3}$ ($3.87\times10^{-5}\,g\,dm^{-3}$) [36] or $4.7\times10^{-6}\,mol\,dm^{-3}$ ($1.82\times10^{-3}\,g\,dm^{-3}$) [37].

In Fig. 2 the solubilizing power S_p (=molecules of cholesterol solubilized by molecule of surfactant) was plotted vs. the HLB value of the surfactant mixture, for concentrations of one, two and three times the cmc. It may be seen that the more effective surfactants are those of low HLB values. There is a minimum at HLB = 23.8, which coincides with the lowest surfactant cmc (Fig. 1). At low HLB values, the fluid phase was a white, turbid emulsion which was stable for at most three months. This may explain the high S_p value of these surfactants, because surfactants were at the surface of the particles of emulsified cholesterol. The most effective solubilizer is that of HLB = 14.7. However, due to its low cmc value, the amount of solubilized cholesterol is also low. At twice the cmc, this is 3.32 g of cholesterol per liter. Nevertheless, this is beteween 1800 and 86 000 times that of cholesterol in pure water.

The solubilization in micelles or microemulsions may be related to an interaction between the surfactant polar

heads and cholesterol molecules. Cholesterol is capable of forming relatively strong hydrogen bonds, similar to secondary alcohols [38]. Cholesterol in contact with aqueous sodium oleate give myelin figures, corresponding to the formation of a lamellar mesophase [39]. The IR and Raman analyses indicated the formation of strong hydrogen bonds in the bilayer–water interfaces, between the cholesterol 3β hydroxyle and the oleate polar head. The formation of myelin figures occurred with monohydrated cholesterol, but did not occur with anhydrous cholesterol because the high compactivity of their crystals does not allow the interaction with the surfactant [40]. Sodium oleate does not produce myelin figures without cholesterol. In the study of monolayers of dipalmitoylphosphatidylcholine–oleic acid–cholesterol, strong interactions were not found and the presence of cholesterol did not affect the phospholipid phase transitions [41]. The above information seems to indicate that there is an interaction between cholesterol and carboxylate groups, but it does not exist with carboxyl groups.

This may explain the emulsion formation with the surfactant mixtures containing oleic acid, and the very curious behavior of the solutions with HLB = 23.8. At the cmc the fluid phase was an emulsion, whereas at two and three times the cmc the solution was limpid. This may be related to the fact that soaps have a hydrolysis maximum at the cmc [42, 43], giving an increase in fatty acid content, which may not form hydrogen bonds with cholesterol. The hydrolysis diminishes at concentrations higher than the cmc, thus changing the interaction with cholesterol.

At high HLB values the fluid phase was limpid, giving a maximum in S_p at HLB $\approx$ 35 at the cmc, or at HLB = 40 at 3cmc. The fluid phase is either a micellar solution or a microemulsion. Between HLB = 29.3 and 40, the amount of solubilized cholesterol at the explored concentrations was between 6 and 8 g dm^{-3}. At HLB > 30, the sediment showed textures which corresponded to lamellar mesophases, whereas at lower HLB it was composed of solid crystals.

The value of S_p changed in a different manner with surfactant concentration in different surfactant compositions, and seemed not to be related to the HLB value (Fig. 2). There is a maximum at 2cmc in the HLB = 14.7 mixture, whereas it increased monotonically with increasing concentration in SDS solutions. There was a tendency to decrease with increasing concentration in mixtures containing sodium decanoate. This phenomenon may be related to the formation of hydrogen bonds between carboxylate and hydroxyl groups, and to changes in aggregates structure.

In comparison, the biological surfactants which usually solubilize the cholesterol in bile have a much smaller S_p value. Cholesterol solubilization experiments by bile salts gave $S_p = 0.06760$ (with sodium deoxycholate), 0.0714 (with sodium chenodeoxycholate), 0.0027 (with sodium ursodeoxycholate) and 0.0334 (with sodium cholate) [44]. Nagadome et al. [45] have studied the solubilization and precipitation of cholesterol in aqueous solutions of bile salts. The largest S_p value found was 0.045. Ueno et al. studied the S_p value in mixed micelles of sodium cholate and octaoxyethyleneglycol monododecyl ether. The S_p values were conspicuously modified with the change of the mixed micelle composition, rising with the surfactant concentration. The largest value was about 0.08 [46].

Conclusions

All the S_p values found in this work were higher than that of common biosurfactants (0.08 in the best case of biosurfactants against 0.14 in the worst case of our mixtures). This means that low concentrations of the studied surfactants may lead the total cholesterol concentration (including the deposits) below the solubility limit easier than natural biosurfactants. This means that the modification of the biological conditions by introduction of strange substances may be less drastic.

To fluidize cholesterol gallstones, it seems appropriate to use surfactants with HLB $\approx$ 35, and concentrations of about 0.026 mol dm^{-3}; which give micellar solutions or microemulsions. This gives about 11.6 g dm^{-3} of cholesterol. If the solubilizing capacity was not enough, much of the remaining cholesterol will form a low-viscosity lamellar mesophase which may be eliminated from the gallblader. Low HLB values will give emulsions, whose residue is solid cholesterol.

The value of HLB $\approx$ 35 also seems useful to solubilize cholesterol in blood, using lower concentrations, since the blood cholesterol content is lower than in bile.

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