Dissolution of cholesterol–calcium bilirubinate compressed discs by microemulsions

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The purpose of the present work was to characterize the composition and the structure of a monophasic solution able to dissolve cholesterol–calcium bilirubinate compressed discs simulating cholesterol and pigment biliary stones. Thermodynamically stable microemulsions composed of methyl *tert*-butyl ether, sodium dodecyl sulfate, *n*-butanol, and of an aqueous ethylenediaminetetraacetate solution were investigated. Their structure was defined by viscosity and conductivity measurements. The kinetics of compressed disc dissolution served to determine the optimal composition of the mixtures for rapid dissolution of the two types of discs. All the experimental results show that cholesterol and calcium bilirubinate dissolutions occur simultaneously and that the more efficient microemulsions are those exhibiting a bicontinuous structure.

Dissolution de pastilles de cholestérol–bilirubinate de calcium par des microémulsions. Cette étude a pour objet de définir la composition et la structure d'une solution monophasique capable de dissoudre des pastilles de cholestérol et de bilirubinate de calcium simulant les calculs biliaires cholestéroliques et pigmentaires humains. Des microémulsions thermodynamiquement stables composées de méthyl tertio-butyl éther, de dodécylsulfate de sodium, de *n*-butanol et d'une solution aqueuse d'éthylène diamine tétraacétate ont été étudiées. Leur structure a été caractérisée par des mesures de viscosité et de conductivité. L'étude cinétique de la dissolution des pastilles placées au contact de ces microémulsions montre que les dissolutions du cholestérol et du bilirubinate se font simultanément et que les meilleures performances sont obtenues avec des microémulsions à structure bicontinue.

The treatment of biliary lithiasis is essentially surgical to date. However, for high operative risk patients, a nonsurgical alternative is required. Recent advances in endoscopic catheterization now permit the direct instillation of stone solvents into the biliary system. The first clinical studies consisted in injecting methyl *tert*-butyl ether (MTBE) in the gallbladder by percutaneous transhepatic cannulation, allowing at least 95% dissolution of cholesterol stones in 72 of 75 patients.[1,2] On the other hand, we have shown, *in vitro* and in animals, that it was possible to dissolve mixed stones composed of cholesterol and pigments with multicomponent contact solvents.[3,4] These solvents were MTBE–dimethyl sulfoxide mixtures, and a complex aqueous solution intended to dissolve the non-cholesterol part of stones. When applied alternately these solvents led to complete and rapid disappearance of stones. Furthermore, we reported that in patients with large duct stones, contact litholysis therapy contributed to bile duct clearance in 40 of 44 patients.[5] However, alternating infusion with the solvents constitutes a heavy procedure in clinical practice, and it would be useful to dispose of one monophasic solvent efficiently and simultaneously to dissolve the main biliary stone components.

In this work, we have studied the effectiveness of different types of microemulsions to dissolve cholesterol–calcium bilirubinate compressed discs. Viscosimetric and conductometric analysis of these microemulsions allow us to relate their solubilizing power to their structure. Thus, we show that the microemulsions exhibiting a bicontinuous structure are the most efficient for dissolving model biliary stones. This work is a step in the development of new topical solvents for the efficient dissolution of the two major stone types in the human biliary tract.

Experimental

Materials

Sodium dodecyl sulfate (SDS) and MTBE were supplied by Sigma Chemical Co (St Louis, Mo). Ethylenediaminetetraacetic acid (EDTA), bilirubin and anhydrous cholesterol were provided by Fluka (Buchs, Switzerland). Tritiated cholesterol. {[1α, 2α (n) ^{3}H] cholesterol} with a specific activity of 50 μCi mmol^{-1} was a CEA product (France). *n*-Butanol and the kit for cholesterol analysis were purchased from Merck (Darmstadt, Germany) and Boehringer-Mannheim (Mannheim, Germany), respectively. All other products used in this investigation were of A grade.

Methods

Preparation of the disc components. The anhydrous cholesterol was recrystallized three times from an ethyl alcohol solution at 333 K. Monohydrate cholesterol was prepared as described by Igimi and Carey.[6] Calcium bilirubinate (6.4% Ca) was synthesized from diacidic bilirubin according to Leuschner *et al.*[7]

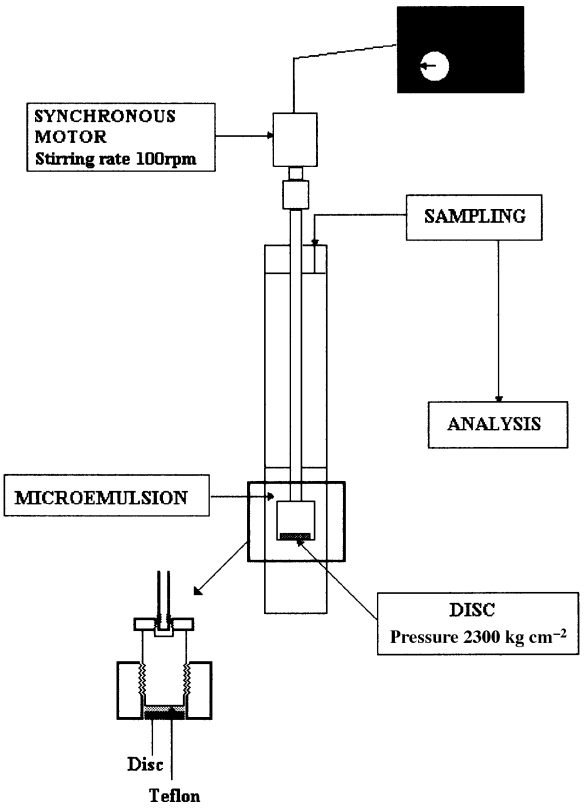

Fig. 1 Diagrammatic representation of rotating disc apparatus for dissolution rate studies.

Microemulsion preparation. Microemulsions consisted of MTBE, of a 1% (w/w) aqueous solution of EDTA buffered at pH 9.4 with 50 mM NaOH–glycine, of an anionic surfactant (SDS) and of a cosurfactant (*n*-butanol). The SDS + *n*-butanol mixture was prepared to achieve a mass ratio of 1 to 2.

The pseudoternary diagram of this system has been plotted in order to delimit the monophasic region. This diagram was determined by adding increasing amounts of the SDS–*n*-butanol mixture to the EDTA aqueous solution. Different quantities of MTBE were then introduced into each of these solutions. The resulting solution was equilibrated by gentle rotation in a thermostat at a constant temperature of 310 ± 0.5 K and the occurrence of a possible phase separation was observed by nephelometry. The monophasic region being defined, different microemulsions were then prepared to investigate their viscosity and conductivity.

Viscosity study. Viscosity measurements were performed using a Couette viscosimeter to ensure the Newtonian flow of the microemulsions. The kinematic viscosities (ν) were evaluated by means of an Ubbelohde tube (Viscosimetric MS, Fica) placed in a thermostated bath whose temperature was regulated at 310 ± 0.5 K. Densities (ρ) of the solutions were measured with a PAAR DMA 602 density meter, at 310 ± 0.5 K.

Conductivity study. All conductometric analyses were performed with a Knick 702 conductometer at 310 ± 0.5 K and in a closed-circuit device to avoid evaporation of the organic phase. The microemulsions were obtained as follows: mixtures of (SDS + *n*-butanol)–EDTA aqueous solution were prepared to achieve mass ratios of 0.33, 0.54, 0.82, 1.00, 1.22, 1.86 and 3.00. Known volumes of MTBE were then progressively added to each of the previous mixtures using a dilution device in order to cover all the monophasic range. The medium was continuously homogenized by means of a peristaltic pump and the conductivity measurements were accomplished after each addition of MTBE.

Solubility of cholesterol and calcium bilirubinate. The solubility of cholesterol monohydrate in the above described microemulsions has been evaluated as follows: each microemulsion containing a monohydrate cholesterol excess was maintained under agitation in a thermostat at 310 ± 0.5 K. The supernatants collected from each microemulsion were analysed for the solubilized cholesterol content using the enzymatic kit: the concentration of the lutidine dye (3,5-diacetyl-1,4-dihydrolutidine) formed is stoichiometric to the amount of cholesterol and is measured by the increase of light absorbance at 405 nm.

The solubility of calcium bilirubinate was measured in the dark because of the high photosensitivity of bilirubin, which easily oxidizes to biliverdin. The microemulsions were placed in the presence of calcium bilirubinate powder, under gentle agitation, in a thermostat at 310 ± 0.5 K for 24 h. Supernatants collected from each microemulsion were diluted with methyl alcohol, and the calcium bilirubinate concentrations assayed spectrophotometrically at 453 nm.

Preparation of the discs. Compressed discs composed of 80% cholesterol and 20% calcium bilirubinate w/w or 80% calcium bilirubinate and 20% cholesterol w/w, simulating mixed cholesterol stones and pigment stones, respectively, were prepared. Calcium bilirubinate-rich discs contained additional tritiated cholesterol. The mixtures were homogenized by using an agate mortar, then an aliquot of about 350 mg was compressed at 2300 Kg cm^{-2} for 1 min to form a 1.30 cm diameter disc.

Dissolution kinetics. Dissolution kinetics were studied by using the rotating disc procedure.[6,8] This method consists in immersing a disc, initially fixed at the end of a rod, in 25 ml of microemulsion (Fig. 1). Teflon was applied to the upper disc face to ensure good waterproofing and to avoid wetting. The rod was rotated at 100 rpm. During the dissolution process (lasting 2–8 h) 25 μl of microemulsion was withdrawn every 5 min and assayed for bilirubin and cholesterol. All dissolution kinetics measurements were performed at 310 ± 0.5 K in the dark.

Chemical analyses of bilirubin and cholesterol. The dissolved bilirubin was diluted with methyl alcohol prior to analysis by spectrophotometry at 453 nm. Cholesterol was assayed with the enzymatic method and by radioactivity determination, for high- and low-cholesterol discs, respectively.

Results and discussion

Phase diagram

The pseudoternary diagram SDS–aqueous phase–MTBE exhibited a small monophasic region (Fig. 2). This region increased significantly when a mixture of SDS + *n*-butanol was substituted for SDS. Consequently, *n*-butanol as cosurfactant is necessary to obtain a large monophasic zone. This zone includes (SDS + *n*-butanol)–aqueous phase mixtures with mass ratios between 0.33 and 3.00.

Microemulsion viscosity

Fig. 3 shows the viscosity of the microemulsions as a function of the amount of MTBE for different (SDS + *n*-butanol)–aqueous phase mass ratios.

Without MTBE, increasing the (SDS + *n*-butanol)–aqueous phase mass ratio by ten led to a twofold higher viscosity. With progressive addition of MTBE, the viscosity of mixtures with the highest (SDS + *n*-butanol) contents regularly decreased. Moreover, for MTBE proportions higher than 30%, the microemulsions exhibited similar viscosities for all (SDS + *n*-

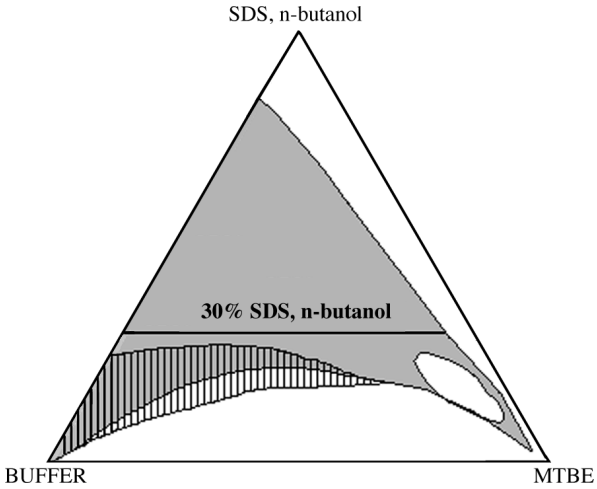

Fig. 2 Pseudoternary phase diagrams: The hatched area corresponds to the monophasic region of the SDS–aqueous phase–MTBE diagram. The grey area corresponds to the monophasic region of the (SDS + *n*-butanol)–aqueous phase–MTBE diagram. The horizontal line represents microemulsions containing 30% (SDS + *n*-butanol).

butanol)–aqueous phase mass ratios. For the lowest (SDS + *n*-butanol) contents (mass ratios 0.33 and 0.54), the dynamic viscosity first increased and then reached a maximum with a 15% and 10% MTBE fraction, respectively. These mixtures correspond to points **A** and **B** in Fig. 5 (shown later). This viscosity pattern suggests the occurrence of micellar aggregates whose size increases with the addition of MTBE. Moreover, bringing the aggregates closer together favours their mutual interactions, which might consequently increase the viscosity. The decrease in the viscosity pattern with the subsequent addition of MTBE occurs from a particular MTBE weight fraction, which may indicate a structure transition from the aqueous to the bicontinuous medium.

For the mixtures with large surfactant–cosurfactant contents in which the viscosity continuously decreased as the amount of MTBE increased, it seems that the *n*-butanol concentration was high enough to play the role of an organic phase. Thus, the continuously declining dynamic viscosity may already indicate the occurrence of bicontinuous structure systems. This supports the results of Kathopoulis[9] and Lindman *et al.*[10] on the octylbenzenesulfonate–*n*-pentanol–*n*-decane–water system.

Conductivity of microemulsions

Fig. 4 represents the conductivity of (SDS + *n*-butanol)–aqueous phase mixtures to which were added increasing

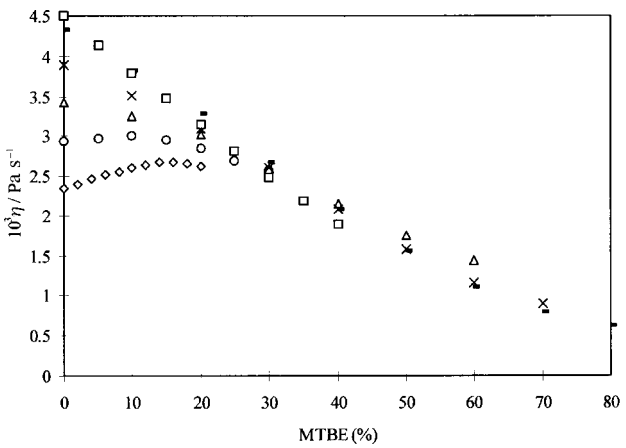

Fig. 3 Dynamic viscosity of microemulsions as a function of the amount of MTBE for various (SDS + *n*-butanol)–buffer mass ratios: (◇) 0.33, (○) 0.54, (△) 0.82, (×) 1.22, (—) 1.86, (□) 3.

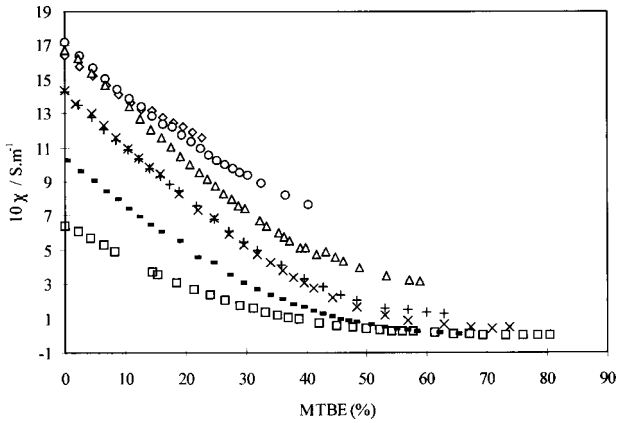

Fig. 4 Conductivity of microemulsions as a function of MTBE percentage for various (SDS + *n*-butanol)–buffer mass ratios: (◇) 0.33, (○) 0.54, (△) 0.82, (+) 1, (×) 1.22, (—) 1.86, (□) 3.

amounts of MTBE. Microemulsions containing large proportions of water had the highest conductivity. The conductivity decreased continuously when the microemulsions were enriched with MTBE and tended to very low values. Considering the microemulsions with (SDS + *n*-butanol)–aqueous phase mass ratios of 1.22, 1.86 and 3.00, one observes that the conductivity becomes extremely low for microemulsions containing more than 45% MTBE. The portion of the diagram corresponding to the transition between the bicontinuous and the reverse medium was determined by the composition of mixtures for which the slope of the curves χ *vs.* % MTBE became nearly constant. This transition area is reported in Fig. 5 and passes through points **C** (60% MTBE), **D** (52% MTBE) and **E** (45% MTBE).

Moreover, concerning the conductivity values *versus* the weight fraction of MTBE with microemulsions containing 30% (SDS + *n*-butanol) (Fig. 6), one notes that the decrease in conductivity is more marked for MTBE proportions higher than about 15% (Fig. 5, point **F**). Such a percentage was also found in the viscosity pattern. For weight fractions higher than 55% MTBE (Fig. 5, point **G**), the slope of the curve tends to stabilize. This limiting point is located around the area separating bicontinuous and reverse media. Concerning the microemulsions without MTBE, maximum conductivity was reached with about 35–40% (SDS + *n*-butanol) (Fig. 5, point **H**); for larger percentages, the micellar structure no longer occurs. These data are in accordance with the transitions previously described on the basis of viscosity measurements between the aqueous and the bicontinuous medium.

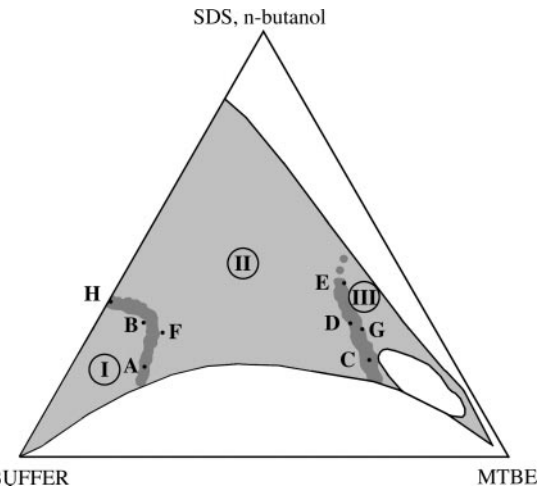

Fig. 5 Delineation of boundaries between aqueous (I), bicontinuous (II) and reverse (III) media. Changes in structure were in fact gradual.

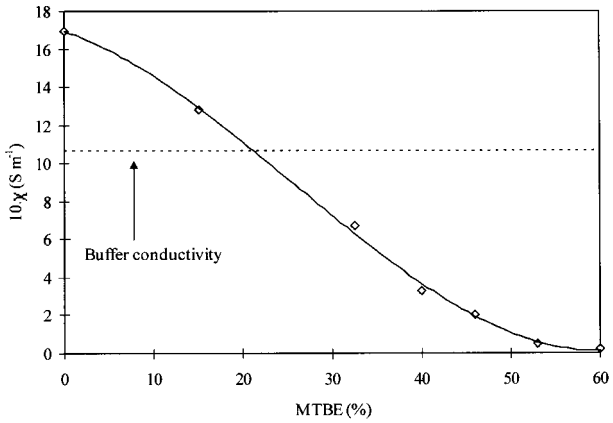

Fig. 6 Conductivity of microemulsions containing 30% (SDS + *n*-butanol) as a function of MTBE. Buffer conductivity (1.071 S m^{-1}) is indicated by the horizontal line.

The boundaries between the various media are reported in Fig. 5.

Solubility of cholesterol monohydrate and calcium bilirubinate in microemulsions

The solubility of cholesterol monohydrate was evaluated in microemulsions containing (SDS + *n*-butanol)–aqueous phase mixtures with mass ratios between 0.33 and 3.00 (Fig. 7). As expected, the cholesterol solubility increased with concentration of MTBE in the microemulsions. In some cases, this solubility was even higher than the solubility of cholesterol monohydrate in pure MTBE (0.418 mol l^{-1}). This clearly indicates that (SDS + *n*-butanol) is actually involved in the dissolution of cholesterol. Likewise, given the experimental results with increasing quantities of surfactants, it is evident that SDS micelles act as a solubilizing agent. The most efficient microemulsions, which exhibited a cholesterol solubility higher than that obtained in pure MTBE, correspond to media with a bicontinuous structure.

The solubility data of calcium bilirubinate (Fig. 8) show that the higher the aqueous phase in the microemulsion, the higher the solubility. The presence of EDTA, a powerful chelating agent at alkaline pH, accounts for this result. Sodium bilirubinate, formed as a result of the cationic exchange, is soluble in water at pH 9.4. Its solubility in (SDS + *n*-butanol)–aqueous phase mixtures reached a limiting value at about 8×10^{-3} mol l^{-1} a concentration higher than that obtained with an alkaline solution of EDTA (6.41×10^{-3} mol l^{-1}).

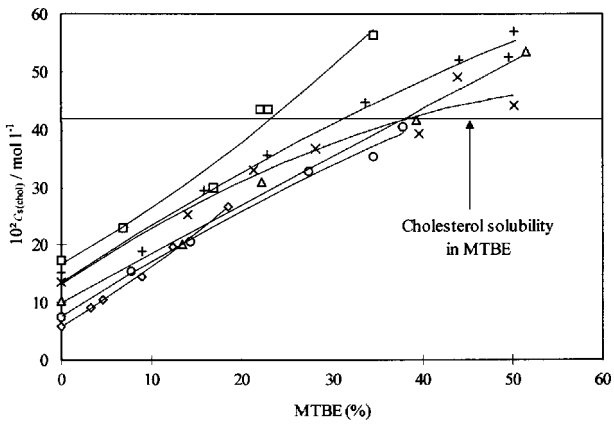

Fig. 7 Cholesterol solubility in (SDS + *n*-butanol)–aqueous phase–MTBE microemulsions as a function of MTBE percentage for various (SDS + *n*-butanol)–buffer mass ratios: (◇) 0.33, (○) 0.54, (△) 0.82, (×) 1.22, (+) 1.86, (□) 3. Cholesterol monohydrate solubility in MTBE (0.418 mol l^{-1}) is indicated by the horizontal line.

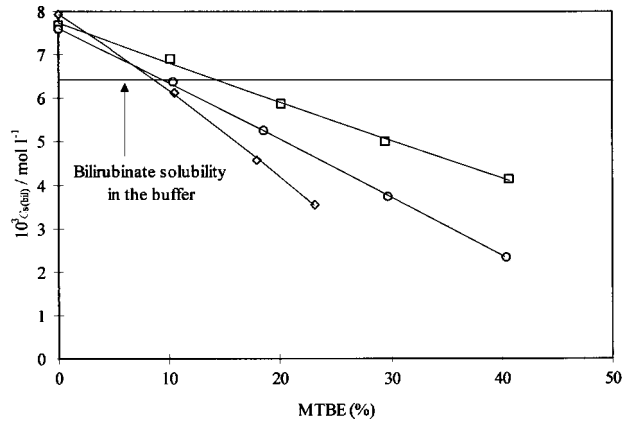

Fig. 8 Calcium bilirubinate solubility in (SDS + *n*-butanol)–aqueous phase–MTBE microemulsions as a function of MTBE percentage for various (SDS + *n*-butanol)–buffer mass ratios: (◇) 0.33, (○) 0.54, (△) 0.82. Calcium bilirubinate solubility (6.41×10^{-3} mol l^{-1}) in EDTA aqueous solution (pH 9.4) is indicated by the horizontal line.

Therefore, the surfactant improves the EDTA solubilizing action.

Dissolution of cholesterol-rich and calcium bilirubinate-rich discs. Investigations of the initial flow

To dissolve the two types of compressed discs, microemulsions with (SDS + *n*-butanol)–aqueous phase ratios of 0.33, 0.54 and 0.82 were selected. From a medical point of view, these microemulsions have two advantages: (1) their low viscosity allows for better homogenization with bile during their *in vivo* instillation and (2) they have a low (SDS + *n*-butanol) content and, in consequence, they are potentially less toxic. The dissolution kinetics of the discs in microemulsions were evaluated at 310 ± 0.5 K in the dark using the rotating disc method. Initially, the surface of the disc is macroscopically homogeneous. During a kinetic experiment, the most soluble compound is preferentially dissolved leading to a surface essentially composed of the least soluble compound. Therefore, the relative surfaces of both solids in contact with the solvent are modified as the solubilization process is occurring. As a result, the permeabilities towards both constituents, related to both the surface composition and the surface area, also vary. Moreover, the solvent was not renewed during the experiments. Consequently, its structure, composition and viscosity are continuously changing as the concentration gradient in the diffuse layer and thereby the mutual diffusion coefficient vary. Hence, the solubility of each compound of the disc, in the presence of the other, can be subsequently modified during the kinetic process. To be free from the simultaneous variations of all these parameters, the experimental results were considered only at very short times. Then, for each dissolution study[11] we report the initial dissolution rates (Fig. 9). This procedure mimics the clinical case, in which the solvent instilled is continuously changing.[12]

When the disc is introduced into solvents, a very thin film consisting of a saturated layer appears at the solid–liquid interface where the interfacial reaction is occurring. A difference of concentration ($C_{s_1} - C_{s_2}$) takes place on both sides of the film. Then, solute will flow into the bulk solution across a diffusion layer of thickness h, which is not stirred and directly in contact with the previous saturated film. The difference of concentration within the diffusion layer is ($C_{s_2} - C_b$).

From Berthoud's law the flow of matter going through a surface of section A as a function of the difference of concentration between the solid surface and the bulk phase of volume V can be expressed[13] as:

$$G = \mathrm{d}n/A\mathrm{d}t = (C_{s_1} - C_b)/R \qquad (1)$$

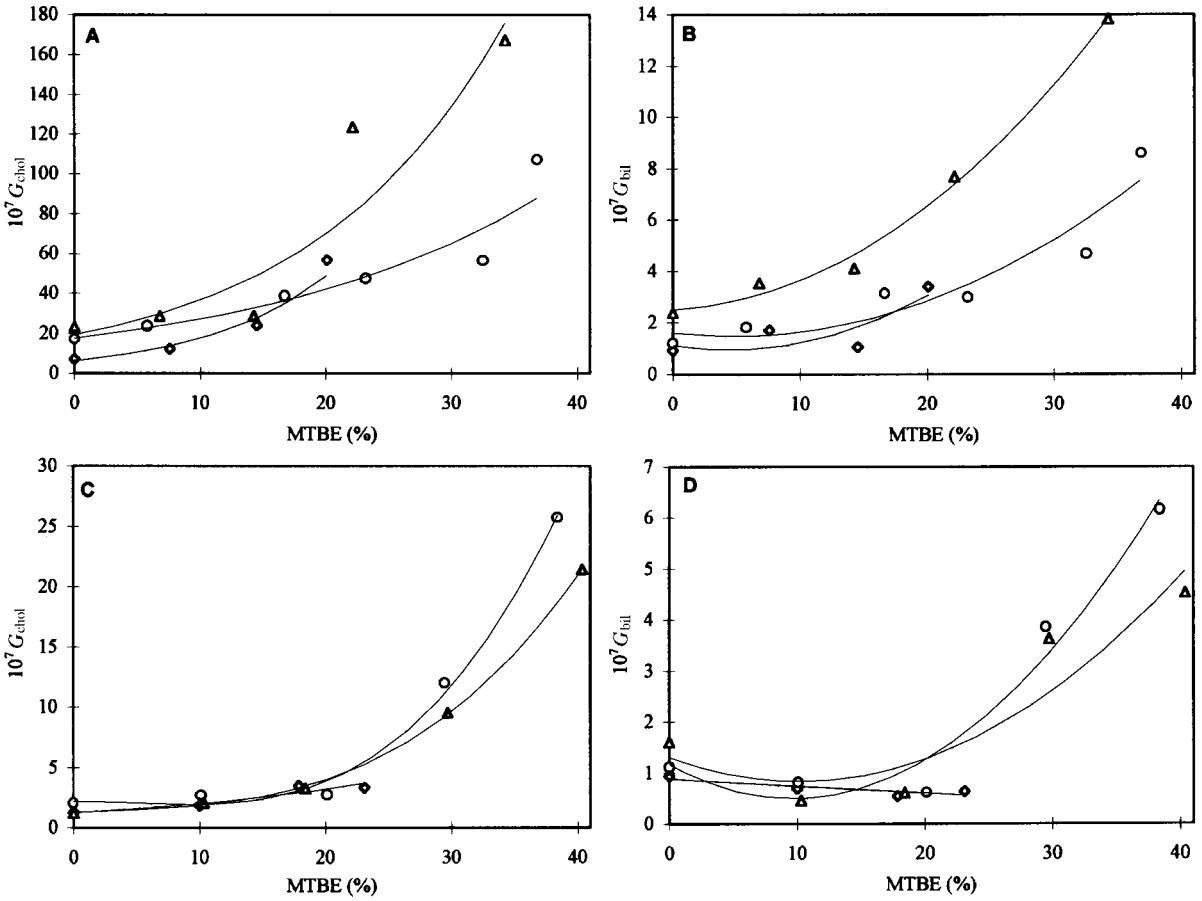

Fig. 9 Initial flows (mol cm^{-2} min^{-1}) of cholesterol and of calcium bilirubinate from cholesterol-rich discs (panels A and B) and calcium bilirubinate-rich discs (panels C and D). The (SDS + *n*-butanol)–aqueous phase ratios were: (◇) 0.33, (○) 0.54, (△) 0.82.

where R is the sum of the diffusional resistance (h/D) and the interfacial resistance ($1/P$). D is the diffusion coefficient of the diffusing species and P is the permeability coefficient related to the solid surface.

The following differential equation is obtained:

$$[(dC_b/dt) + (A/VR)]C_b - (A/VR)C_{s_1} = 0 \quad (2)$$

where C_{s_1} and R are assumed to be constant. The solution of eqn. (2) is written as:

$$C_b = C_{s_1}[1 - \exp[(-A/VR)t]] \quad (3)$$

Eqn. (3) represents the law describing the variation of solute concentration as a function of time at the beginning of the kinetics. During the dissolution process C_{s_1} and R may vary with the composition of the medium. R may also change with the surface composition.

For the previously set out reasons, we have reported the initial dissolution rates *versus* the weight fraction of MTBE (Fig. 9). This plot allows us to define the microemulsion composition that is optimal for dissolving the disc constituents. For very short dissolution times, one observes a linear variation of C_b with time:

$$C_b = C_{s_1}[(A/VR)t] \quad (4)$$

which gives the same expression of the initial flow.

The variation of these flows as a function of the (SDS + *n*-butanol)–aqueous phase ratio and the percentage of MTBE is displayed in Fig. 9. It appears that the initial flows of cholesterol and calcium bilirubinate from cholesterol-rich discs (Fig. 9, A and B) increased to a large extent above 15–25% MTBE. For calcium bilirubinate-rich discs (Fig. 9, C and D), the increase starts abruptly around 20% MTBE. In both cases, these MTBE percentages correspond to the location of the bicontinuous medium in the pseudoternary diagram. Therefore, solvents that may be used to dissolve both the cholesterol-rich and the pigment-rich discs require a weight fraction of MTBE larger than 25%.

Conclusions and physiological implications

We have established in this study that the solubilizing power of (SDS + *n*-butanol)–aqueous phase–MTBE microemulsions depends on their composition and their structure, the most efficient exhibiting a bicontinuous structure. These microemulsions are able to dissolve cholesterol and calcium bilirubinate conglomerates such as those found in human biliary lithiasis.

This first approach provides experimental results that do not take into account the potential toxicity of the solvents. Although animal and human studies with MTBE and alkaline EDTA[14,15] have shown resistance of the gallbladder to these solvents, investigations have to be carried out to evaluate the toxic effect of the described microemulsions.

Acknowledgements

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