[Chem. Pharm. Bull. 21(10)2161—2167(1973)]

UDC 615.011.4.014.015.14

Effect of Surfactants on Drug Absorption. IV.[1] Mechanism of the Action of Sodium Glycocholate on the Absorption of Benzoylthiamine Disulfide in the Presence of Sodium Laurylsulfate and Polysorbate 80

Isamu Utsumi, Keiichi Kohno, and Yoshikazu Takeuchi

Products Research and Development Laboratory, Tanabe Seiyaku Co., Ltd.[2]

(Received January 23, 1973)

The mechanism of the action of sodium glycocholate (SGC), which resulted in disappearance of the inhibitory effect of sodium laurylsulfate (SLS) on the absorption of benzoylthiamine disulfide (BTDS) from rat intestine, was investigated.

It was observed that membrane altering action of SGC did not contribute to the antagonistic effect of SGC against SLS, as determined by circulation experiments with BTDS after precirculation with or without SGC in the presence or absence of SLS through rat small intestine. However, studies as to the physico-chemical behaviour of BTDS such as micellar partitioning in the solution of SLS, SGC and their mixture revealed that the effect of SGC on the BTDS absorption in the presence of SLS was due to the formation of the mixed micelles, which resulted in an increase in the amount of BTDS out of the micelles.

In contrast of SLS, polysorbate 80 did not show any apparent changes in the characteristic of the micelles in the presence of SGC. This finding was quite agreeable with the *in situ* absorption experiment of BTDS.

It has been reported[1] by the authors that sodium glycocholate (SGC) had a unique effect on the absorption and metabolism of benzoylthiamine disulfide (BTDS) in synthetic surfactant solutions in *in situ* circulation experiments using rat small intestine.

An addition of SGC in the circulation fluid obviously enhanced the absorption rate of BTDS but synthetic surfactants, sodium laurylsulfate (SLS) and polysorbate 80, decreased its rate in the same system. When SLS and SGC were mixed to make binary system, SGC apparently enhanced the decreased absorption rate up to around the control level. On the other hand, SGC did not influence the absorption inhibitory effect of polysorbate 80, suggesting a possible difference in the role of SGC between the two binary systems.

Since SLS was proved to form complex, [BTDS–LS_2], through ionic and hydrophobic interaction to decrease the absorption rate,[3] the observed result in the SLS–SGC system should be due to a certain change in the mode of interaction between BTDS and SLS. Also, a kind of membrane altering action of bile salts which is noted by several authors[4–8] might be considered as another factor of reducing the action of SLS in such binary systems.

The present study aimed to clarify the mechanism of such effects of SGC noted on the surfactant mixture of SGC with either SLS or polysorbate 80. A rat intestinal tract was pretreated with SGC and then circulated BTDS-containing media so as to evaluate the role of the membrane altering action of SGC, and the *in vitro* partitioning of BTDS between aqueous and micellar phases in the binary systems was examined by several methods. The data obtained may be of value to consider a potency of interaction of biosurfactant with synthetic surfactant in the intestinal tract.

1) Part III: I. Utsumi, K. Kohno, and T. Takeuchi, "to be published".
2) Location: *962 Kashima-cho, Higashiyodogawa-ku, Osaka, 532, Japan.*
3) Part II: I. Utsumi, K. Kohno, and Y. Takeuchi, *Yakuzaigaku*, 33, 49 (1973).
4) C.H. Nightingale, R.J. Wynn, and M. Gibaldi, *J. Pharm. Sci.*, **58**, 1005 (1969).
5) S. Feldman and M. Gibaldi, *J. Pharm. Sci.*, **58**, 425 (1969).
6) S. Feldman and M. Gibaldi, *J. Pharm. Sci.*, **58**, 967 (1969).
7) S. Feldman, M. Salvino, and M. Gibaldi, *J. Pharm. Sci.*, **59**, 705 (1970).
8) K. Kakemi, H. Sezaki, R. Konishi, T. Kimura, and M. Murakami, *Chem. Pharm. Bull.* (Tokyo), **18**, 275 (1970).

Experimental

Materials——BTDS and surfactants. They were the same preparations used in our previous studies.[1,2,9] Sephadex G-25 fine grade (Pharmacia) was used.

Drug-surfactant Solutions——Solutions of BTDS (10 μg/ml) containing surfactant were prepared by the same way described in the first report[9] of this series. SGC was used in concentration ranging from 0.05—0.5% (w/v). The concentration of SLS and polysorbate 80 was held at 0.1% (w/v) and 0.5% (w/v) respectively when each of them was mixed with SGC to make binary system.

Measurement of Absorption and Metabolism of BTDS——The procedure employed in this study was the same with that used in the previous report,[1] the third of this series.

Solubility Measurement——The solubilities of BTDS in surfactant solutions were determined by the thiochrome method as previously reported.[9]

Ultraviolet (UV) Absorption Measurement——The absorption spectrum for BTDS in surfactant solutions was determined by using Hitachi 124 spectrophotometer. The concentration of BTDS was held at 10 μg/ml in solutions where SLS, SGC or their mixture was added to give a desirable concentration at 37° and pH 6.4. The reference cell contained a surfactant solution without BTDS to correct a possible absorbance of the surfactant.

Determination of Micellar Partitioning of BTDS——Molecular sieve technique[10,11] using sephadex G-25 was applied with a little modification. This method was preliminary evidenced to be sufficient in this study for determining the partitioning of BTDS between aqueous and micellar phases in surfactant solution. Five hundred mg of sephadex G-25 in test tube with stopper was added to 2 ml of buffer solution, pH 6.4, and was allowed to swell over night. One ml of the same buffer solution containing BTDS in the amount ranging from 0—80 μg (under coexistence of surfactant, the amount of BTDS was 40 μg) plus 1 ml of buffer or buffered surfactant solution was then added so that total volume of liquid added to the gel was 4 ml. The test tube was shaken for 2 hours at 37°. After equilibration was attained, the solution was gently filtered by Tōyō Filter paper, No. 5A, and the filtrate was analysed for BTDS as previously reported.[9]

The amount of BTDS involved in micelles can be calculated by equation (I):

$$c = b - k(a-b) \qquad (1)$$

Where a and b designated respectively the total amount of BTDS added to the system and amount existing in the external phase of gel after equilibration. c is the total amount of BTDS involved into micelles. k is a partition coefficient for BTDS between the internal and external phase.

Result and Discussion

1. Precirculation with Surfactants

The membrane permeability enhancing effect of bile salts has been reported. For example, Nightingale, *et al.*,[4] have reported the effectiveness of sodium taurodeoxycholate on reducing the time required to produce the overturn of goldfish in a medium containing pentbarbital. Bile salt has also been shown to produce a statistically significant increase in the intestinal transfer rate of certain drugs, including salicylate[5] salicylamide,[6] phenol red, 4-aminoantipyrine[7] and some sulfonamides[8] with both the *in vitro* and *in situ* model systems.

Accordingly, our *in situ* data[1] on the increase in the absorption rate for BTDS in the presence of SGC may also be the result of an alteration in the permeability. Similarly, the characteristic effect of SGC in the SLS–SGC binary mixture, where SGC reduces the inhibitory effect of SLS on BTDS absorption, could be largely due to the same direct action of SGC on the intestinal membrane. To examine this possibility, precirculation experiments were carried out, in which the circulation media containing only the surfactants was circulated through the intestinal tract for 30 minutes before circulation of BTDS.

Figure 1 shows a typical example of a time course of BTDS absorption from the intestine pretreated with isotonic buffer solution with or without 0.5% SGC. Ten minutes circulation

9) I. Utsumi, K. Kohno, and Y. Takeuchi, *Chem. Pharm. Bull.* (Tokyo), **21**, 1727 (1973).
10) M.S. Parker, M. Barnes, and T.J. Badley, *J. Pharm. Pharmacol.*, **18**, 103s (1966).
11) W.H. Pearlaman and O. Crépy, *J. Biol. Chem.*, **242**, 162 (1967).

with BTDS after the precirculation of SGC revealed an increase in the absorption rate. Since such action was no longer observed with a longer circulation, the increase in the absorption rate due to precirculation was evaluated by the value of a 10 minutes circulation.

The results, summarized in Table I showed about a 2-fold increase in the absorption rate of BTDS after precirculation with 0.5% SGC, 0.1% SLS and 0.1% SLS–0.5% SGC mixture, respectively. However, the pretreatment with these surfactants exerted no effect when BTDS was circulated in SLS solution. Thus, the precirculation with SGC, per se, had no influence on reducing the inhibitory action of SLS on the absorption rate of BTDS.

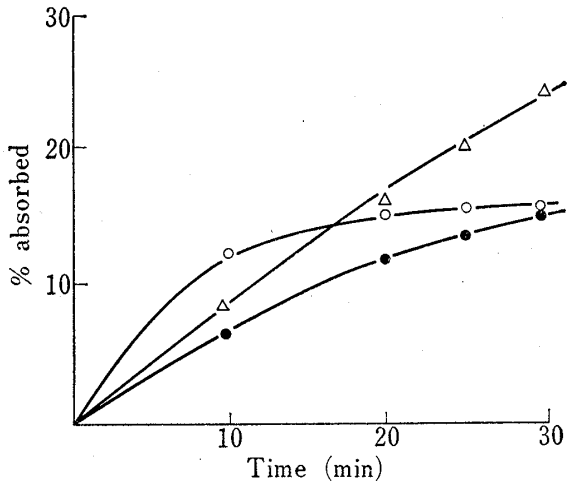

Fig. 1. Effect of Precirculation with SGC on the Absorption of BTDS

BTDS: 10 μg/ml (50 ml), pH 6.4, 37°
●: control, △: 0.5% SGC, ○: precirculation with 0.5% SGC

This suggest that the permeability enhancing action of SGC is not a dominant factor in controlling the characteristic absorption behaviour of BTDS in the binary mixture of 0.1 % SLS with 0.5% SGC.

TABLE I. Effect of Precirculation with Surfactants on the Absorption of BTDS

System	Precirculation	% absorbed during first 10 min
Control[a)]	without surfactant	6.8±1.0
Control[a)]	0.5% SGC	12.6±1.3
Control[a)]	0.1% SLS	12.1±2.5
Control[a)]	0.1% SLS—0.5% SGC	10.3±3.7
0.1% SLS	without surfactant	1.6±1.0
0.1% SLS	0.5% SGC	2.0±0.9
Control[a)]	0.5% polysorbate 80	7.9±3.2
Control[a)]	0.5% polysorbate 80—0.5% SGC	9.8±0.5
0.5% polysorbate 80	0.5% SGC	4.2±1.8
0.5% polysorbate 80	without surfactant	3.0±0.6

a) BTDS containing media without surfactant

Based on this consideration, there is a possibility that the addition of SGC inhibits the formation of the BTDS–LS complex, thus changing the absorption behaviour of BTDS by forming a new mixed micelles through the interaction of SGC, SLS and BTDS. This possibility will be detailed in a later section, based on the observed alteration in the physicochemical properties of the binary surfactant mixtures.

2. Solubility of BTDS in the Mixture of SGC with SLS and with Polysorbate 80

Figure 2 represents the saturated solubility of BTDS in solutions of SLS, SGC and their mixtures at 37° and pH 6.4. The concentration of SLS in the mixtures was held at 0.1%.

In each single surfactant system, the curve, in which the solubility was plotted against the concentration of SLS and SGC, showed a typical form of solubilization. Exceeding c.m.c, where the each curve showed a sharp inflexion over a narrow concentration range,

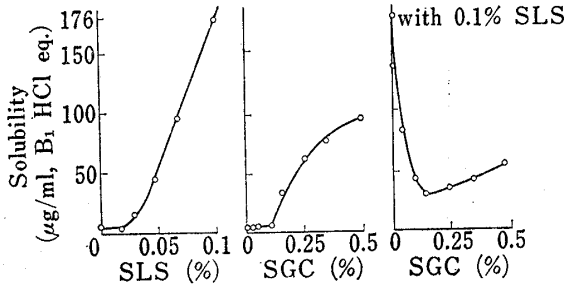

Fig. 2. Solubility of BTDS in the Presence of SLS, SGC and Their Mixtures at 37° and pH 6.4

B_1 HCl: thiamine hydrochloride

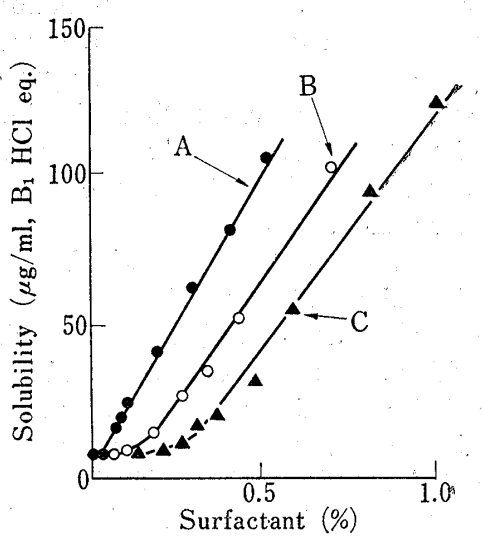

Fig. 3. Solubilizing Power of Various SLS-SGC Mixtures at 37° and pH 6.4

B_1 HCl: thiamine hydrochloride
weight fraction of surfactants
A: SLS: SGC=1: 1
B: SLS: SGC=1: 2.5
C: SLS: SGC=1: 5
solute: BTDS

the solubility increased with concentration of added surfactant, namely the added surfactant formed additional micelles in which BTDS was solubilized. It was apparent that the solubilizing ability of SLS was much higher than that of SGC at the same concentration above the c.m.c.

Figure 2 also showed that when SGC, in various concentrations, was mixed with 0.1% SLS, the amount of BTDS solubilized was distinctly lowered as compared with that in 0.1% SLS-containing system. This substantial decrease can be refered to as an increase in the concentration of BTDS out of micelles.

Figure 3 shows the solubility curves plotted against the concentration of the surfactant mixtures composed of SGC to SLS with their weight ratios of 1:1, 2.5:1 and 5:1. The solubility increased linearly above the c.m.c in all the systems and the one to one mixture showed largest solubilization, followed in the decreasing order in which the mixing ratio of SGC to SLS increased.

On the other hand, as shown in Table II, the c.m.c given was increased with increasing the weight fraction of SGC in the mixture. The increase in the ratio also resulted in a decrease in the micellar partition coefficient, K_m, calculated by the slope of the solubility cruve in Fig. 2 and 3 using an equation (2):[12]

$$C_M/C_F = K_m C_{micelles} \qquad (2)$$

Where C_M and C_F are the concentration of solubilized and unsolubilized BTDS in surfactant solutions, respectively. $C_{micelles}$ is the surfactant concentration above the c.m.c. K_m is defined as an apparent micellar partition coefficient.

These solubilizing properties indicated that the ability to create micelles in the binary system was reduced in the increasing order of mixing ratio of SGC to SLS.

Generally speaking, the amount of saturated solubility of a compound in solutions of mixed surfactant, which form mixed micelles, is not equal to the sum of the amounts which would be solubilized by a solution of each single species of surfactant, due to different and peculiar micellar characteristic of surfactant solution.[13–18] Above results on Fig. 2 and

12) L.J. Winters and E. Grunwald, *J. Am. Chem. Soc.*, **87**, 4608 (1965).
13) H. Narasaki, *Kogyo Kagaku Zasshi*, **64**, 1955 (1961).
14) H. Narasaki and H. Suzuki, *Kogyo Kagaku Zasshi*, **65**, 801 (1962).
15) S. Saito and H. Hirata, *Kolloid-Z.*, **165**, 162 (1959).
16) J.H. Schunlman, R. Matalon, and M. Cohen, *Discussions Faraday Soc.*, **11**, 117 (1951).
17) K. Kuriyama, *Kolloid-Z.*, **181**, 144 (1962).
18) L. Osipow, F.D. Snell, and J.L. Hickson, *J. Am. Oil Chemist's Soc.*, **35**, 127 (1958).

Table II. c.m.c and Micellar Partition Coefficients (K_m) in SLS–SGC Mixtures at 37° and pH 6.4

Weight fraction of SLS to SGC	CMC (%)	K_m
1:1	0.02	28.6
1:2.5	0.21	10.5
1:5	0.31	8.5

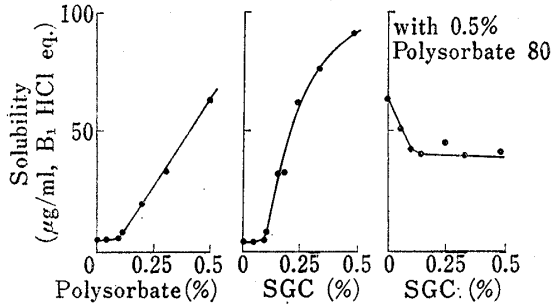

Fig. 4. Solubility of BTDS in the Presence of Polysorbate 80, SGC and Their Mixtures at 37° and pH 6.4

B_1 HCl: thiamine hydrochloride

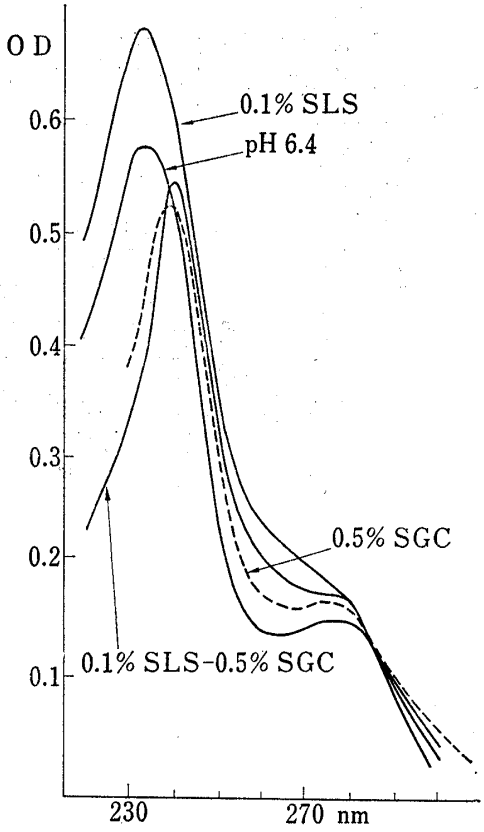

Fig. 5. UV Absorption Spectra of BTDS in Various Solvents

3 can be quite agreeable to these general concept and therefore offer an evidence for a formation of different type of mixed micelles in the mixture of SLS and SGC.

In the solution composed of SGC and polysorbate 80, a possible formation of mixed micelles might also be expected, however, the decrease in the solubility of BTDS by the addition of SGC in the solution of 0.5% polysorbate 80 was found much weaker than that observed in the SGC–SLS system as shown in Fig. 4.

It may thus be deduced that the formation of the mixed micelles consisting of BTDS, SLS and SGC, leading a large decrease in the solubilizing capacity, can be a cause for the specific antagonistic effect of SGC against SLS in the absorption and metabolism of BTDS.

3. UV Spectrum of BTDS in SLS, SGC and Their Mixed Solutions

Figure 5 represents ultraviolet (UV) absorption curves of BTDS at pH 6.4 and 37° in buffer and buffered surfactant solution, there appeared to be two peaks at 231 mμ and 275 mμ.

The spectrum of BTDS in SLS solution of 0.1%, however, possesed a little large extinction coefficient and the effect of micellar entrapping action was noted. When BTDS dissolved in SGC solution of 0.5%, the absorption maximum was shifted to a higher wave length to result in the maximum value of 237 mμ with a slight decrease in extinction ceofficient. When SLS and SGC were mixed up at their concentration of 0.1% and 0.5% respectively in BTDS solution, the spectrum of BTDS was similar with that in the solution of 0.5% SGC, having an absorption maximum of 236 mμ. This result indicated disappearance in the effect of SLS on the spectrum of BTDS in this mixed solution.

These spectrum study also supports the possible formation of mixed micelles of SLS and SGC.

4. Gel Filtration

With a view to getting a detail data on the canceling action of SGC against SLS in an unsaturated system, in which the circulation experiment was done, partitioning of BTDS between an aqueous phase and a micellar phase was determined in this system by a molecular sieve technique.

Figure 6 represents changes in ratio of the amount of BTDS uninvolved in micelles to that in micelles in percent as a function of SLS concentration. There found a growing ratio of total/unbound drug with an increase of the added SLS over the concentration exceeding around 0.02% which was close to the c.m.c value previously determined.[1] Large quantities of BTDS entrapped by SLS micelles were also indicated. When BTDS dissolved in SGC solution, SGC entrapped BTDS into its micelles as well as SLS, but showed a lower entrapping capacity (Fig. 7). Such entrapping characteristic observed in the both surfactants, SLS and SGC, were well correlate with the result obtained in the solubility experiment in the saturated system. Figure 8 represents the results plotting the amount of BTDS out of micelles in

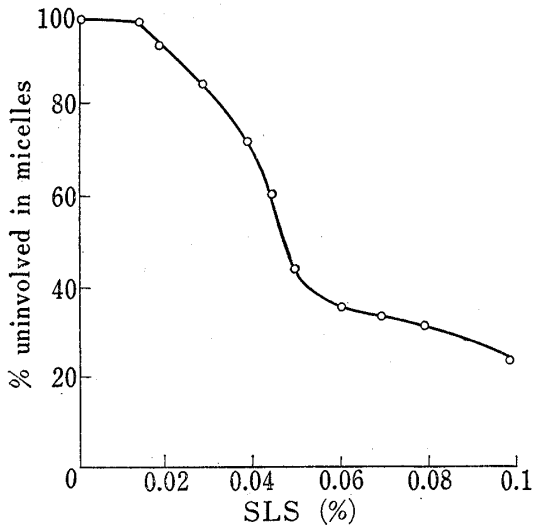

Fig. 6. Micellar partitioning of BTDS in SLS Solution at 37° and pH 6.4

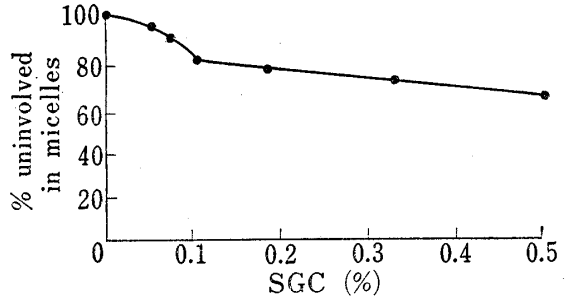

Fig. 7. Micellar partitioning of BTDS in SGC Solution

Table III. Micellar Partitioning of BTDS in Polysorbate 80 Solution with or without SGC at 37° and pH 6.4

System	% uninvolved in micelles
Control	100
0.5% polysorbate 80	41
0.5% polysorbate 80 −0.5% SGC	41

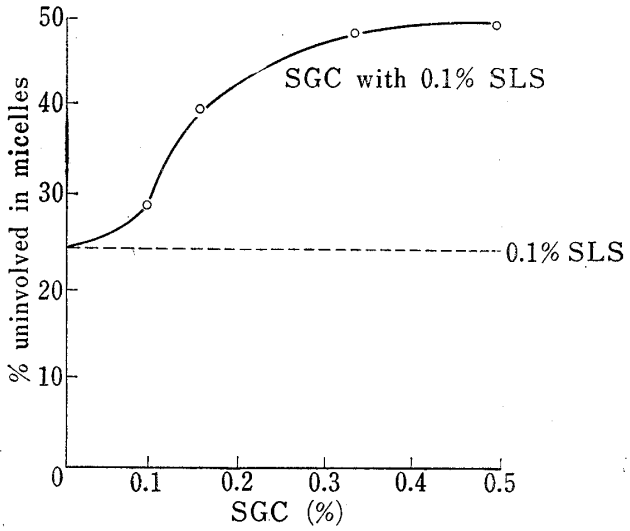

Fig. 8. Effect of SGC Concentration on the Micellar partitioning of BTDS in the Solution of 0.1% SLS at 37° and pH 6.4

total volume including external and internal phases against SGC concentration in the mixed surfactant system consisting of SLS and SGC. The concentration of SLS in this system was fixed at 0.1% against various concentrations of SGC. In the mixture, partitioning of BTDS into the aqueous phase was increased with increasing concentration of SGC and it indicated lowering capacity to entrap BTDS into micelles in the higher concentration of SGC.

Such increases in the amount of BTDS out of micelles should be the convincing results for predicting the changes in the mode of the interaction between BTDS and SLS in the presence of SGC, formation of new mixed micelles. On the other hand, SGC was not remarkably efficient in releasing BTDS from polysorbate 80 micelles (Table III). Of special interest is that such specific antagonistic effect of SGC against SLS are found in the unsaturated system, with respect to BTDS, where the *in situ* absorption experiment was carried out. Accordingly, in relation to the absorption mechanism of BTDS in the binary surfactant mixtures, a biosurfactant, SGC, should reduce the inhibitory action of SLS in a way to form the mixed micelles, but not reduce the action of polysorbate 80.

Conclusion

When BTDS was circulated in a SGC- pretreated rat small intestine, so-called permeability enhancing action of SGC was observed over the concentration above 0.17%, however, this action was supposed to be independent of the antagonistic effect of SGC against SLS in their mixed system since these two different actions were not observed simultaneously.

The determination of the saturated solubility of BTDS informed that the solubility of BTDS in 0.1% SLS solution was remarkably reduced by mixing SGC. In the binary surfactant mixture an increase in the c.m.c value with decreasing micellar partition coefficient was also evidenced in proportion to the increase in the ratio of SGC to SLS.

Thus, it may be concluded that SGC contributes to creating new mixed micelles having a different solubilizing power from that of SLS micelles.

Such SGC effects were further supported by the observed changes in the UV absorption spectrum of BTDS. Namely, the absorption spectrum of BTDS in solution containing both SLS and SGC, at the concentration of 0.1% and 0.5% respectively, was different from that obtained in the solution of 0.1% SLS but was similar to that in the solution of 0.5% SGC, suggesting disappearance of SLS micelles in the presence of SGC.

By using a gelfiltration, the large increase in the amount of BTDS uninvolved in micelles was indicated with increasing the amount of SGC added in the 0.1% SLS-containing BTDS solution. In contrast to this result, SGC, even in the higher concentration, was not remarkably efficient in releasing BTDS from polysorbate 80 micelles.

All these data were paralleled well with those obtained in the *in situ* absorption experiments to give an explanation of the absorption characteristic of BTDS in the solution containing both synthetic and biosurfactants.

In conclusion, the formation of mixed micelles and the ensuing increase in the amount of BTDS out of micelles are evidenced to be responsible for the SGC effect which cancels the action of SLS in the absorption of BTDS. These experimental results may be worthy to be considered from the view point of drug availability because of the wide use of the synthetic surfactants as solubilizer or dispersing agent in pharmaceutical preparations.