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Critical micellar concentration shifting as a simple tool for evaluating cyclodextrin/enhancer interactions

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Abstract

It has previously been found that the undesirable side effects of enhancers may be reduced, virtually without affecting their promoting absorption actions, when they are used in combination with a cyclodextrin. Many enhancers are amphiphilic moieties able to form micelles in solution. The enhancing effect is ascribed to the free molecules in solution. Their toxic effects seem to be linked to the solubilization of cell membrane components (such as phospholipids or cholesterol), due to micelles formed by the enhancers. A study on the influences of cyclodextrins on critical micellar concentration (cmc) shifting has been carried out by surface tension measurements. The formation of a complex in solution between the cyclodextrin and the enhancer shifts cmc to higher values, and changes the ratio between free and micellized molecules of the enhancer, thus minimizing its toxic effects. Laureth-9, quaternary ammonium salts and cholic acid derivatives were considered as enhancers. Changes in buffering salts and tonicity of the measuring medium were also taken into consideration as formulative variables.

Keywords: Surface tension; Critical micellar concentration; Cyclodextrins; Penetration enhancers; Reduction of toxicity

1. Introduction

Many chemical substances improve the transmucosal absorption of drugs, especially of peptides and proteins. However, some safety concern has been raised, considering that the enhancement action is very often due to reversible or even irreversible mucosal damage (De Ponti and Lardini, 1991). A great deal of work has been done in order to understand the mechanism of action of these molecules, and to establish definite rules for obtaining a safe enhancement. In particular, for penetration enhancers with amphiphilic structure (which are molecules able to form micelles in solution) the enhancing effect could be ascribed to free molecules in solution. The toxic effects could be due to the solubilization of cell membrane components (such as phospholipids and cholesterol) because of micelles formed by the enhancers (Nakanishi et al., 1990). The amphiphilic molecules have a characteristic tendency to self aggregate in aqueous solution, so that their hydrophobic parts are removed from, and their hydrophilic parts are exposed to, the solvent.

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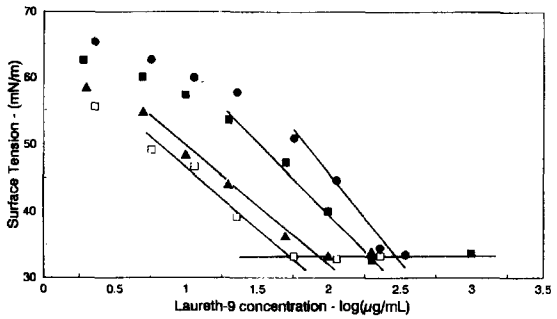

Fig. 1. L9 surface tension in deionized water (□) and with the addition of 0.5 mg/ml of α-CD (●), HP-β-CD (■) or γ-CD (▲).

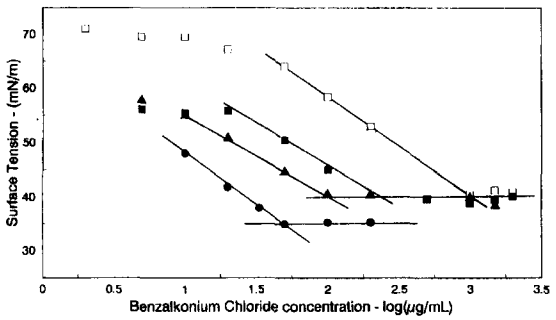

Fig. 2. BC surface tension in different isotonic media: water (□), Sodium chloride 0.9% (●), lactose 14.28% (■) and phosphate buffer pH 6.4 (▲).

Below the critical micellar concentration (cmc), these molecules form a variety of low molecular weight aggregates in solution, and monolayers are formed at the surface of the solvent. This reduces the surface tension. Above the cmc value, micelles coexist in solution with the free monomers.

In principle, it should be possible to destroy the surface monolayers, and to change the properties of the solutions by transferring the amphiphilic molecules to bulk solvents, i.e. by making them more soluble. Based on this assumption, we have added cyclodextrins to solutions of amphiphilic molecules used as penetration enhancers and evaluated their influences by a surface tension measurement technique.

Cyclodextrins are cyclic oligosaccharides with six, seven, or eight α-D-glucose units per macrocycle (α, β, γ-cyclodextrins) able to form inclusion complexes and have been extensively used to enhance the solubility of organic molecules in water (Bekers et al., 1991).Their central cavity is of hydrophobic character and has a diameter from 5 to 8 Å (varying from α to γ), which is ideally suited to accommodate the hydrophobic, aliphatic part of chosen amphiphilic molecules. Moreover, the rims of the cyclodextrins are lined with hydroxyl groups which should be able to form hydrogen bonds with the hydrophilic parts of the enclosed substances, and thus increase the stability of the complexes formed.

The formation of a complex in solution between cyclodextrins and the free molecules of the enhancers shifts the cmc to higher values, by changing the ratio between free and micellized molecules. In that way the formation of micelles is shifted to higher enhancer concentrations, and the toxicity should, for that reason, be reduced. Moreover, at enhancer concentrations below cmc, cyclodextrins are able to reduce the surface properties of the enhancers by destroying the surface monolayer and by transferring the free molecules to bulk solvent.

Table 1
Effects of various CDs on L9 cmc shifting. Measurements carried out in water

CDs concentration (mol/l)			L9 cmc	Δcmc vs. L9 alone
α	HP-β	γ	(mol/l)	(mol/l)
–	–	–	9.28×10^{-5}	–
5.1×10^{-5}	–	–	1.41×10^{-4}	4.82×10^{-5}
5.1×10^{-4}	–	–	4.42×10^{-4}	3.49×10^{-4}
–	3.8×10^{-5}	–	1.36×10^{-4}	4.32×10^{-5}
–	3.8×10^{-4}	–	4.36×10^{-4}	3.43×10^{-4}
–	–	3.9×10^{-4}	1.68×10^{-4}	7.52×10^{-5}

Table 2
Effects of changes in the tonicity of the medium on BC cmc value

Medium (NaCl%)	BC cmc (mol/l)
0.45	1.5×10^{-4}
0.90	1.3×10^{-4}
1.80	1.0×10^{-4}

Recent experimental protocols (Jabbal Gill et al., 1994a; Jabbal Gill et al., 1994b) have confirmed this phenomenon. When an absorption enhancer is used in combination with a cyclodextrin, the undesirable side effects of the enhancer may be reduced, virtually without affecting its absorption promoting activity.

These observations may provide the basis for the formulation of transmucosal dosage forms, using the best enhancer concentration and at the best cyclodextrin/enhancer ratio in order to obtain the highest efficacy in absorption and the safest drug dosage form.

2. Materials and methods

2.1. Materials

Laureth-9 (L9, Mw 582), glicodeoxycholic acid sodium salt (GDC, Mw 471.6), cetylpyridinium chloride (CP, Mw 340), taurodeoxycholic acid sodium salt (TDC, Mw 521.7) were supplied by Sigma; benzalkonium chloride (BC, Mw 353.5) was supplied by Carlo Erba; α- and γ-cyclodextrins (α-CD, Mw 972, γ-CD, Mw 1297) were supplied by Wacker Chemie; and 2-hydroxypropyl-β-cyclodextrin (HP-β-CD, Mw 1300) was supplied by Jannsen Biotech. All the substances were used without further purification.

Table 3
Effects of various isotonic media on BC cmc value compared to deionized water

Isotonic medium	BC cmc (mol/l)
NaCl 0.9%	1.3×10^{-4}
NaCl 0.68% + lactose 3.29%	1.5×10^{-4}
NaCl 0.45% + lactose 6.42%	2.4×10^{-4}
NaCl 0.23% + lactose 9.57%	2.9×10^{-4}
Lactose 14.28%	1.1×10^{-3}
Deionized water	3.0×10^{-3}

2.2. Methods

Experiments were carried out by the ring removal method with an interfacial tensiometer based on the concept of Lecomte Du Noüy (Krüss Digital Tensiometer K10). The solution of the penetration enhancer was contained in a quartz beaker thermostated at 25°C, in which a platinum ring was immersed. The ring was attached to a balance and by lowering the beaker, the surface tension (σ) could be directly measured in mN/m when the surface film broke. Pure water was used for calibration ($\sigma \simeq 72$).

The surface tension of the enhancers diluted in water or in buffers was evaluated with increasing concentration of different CDs. The surface tension values were then plotted versus the logarithms of the enhancer concentrations with the cmc calculated as the intercept of the two straight lines obtained in the range just before and after the cmc value. All the measurements were carried out at least in duplicate and all the linear regression squared values were higher than 0.980.

3. Results and discussion

The addition of CDs to solutions of L9 in deionized water clearly shifts the cmc to higher values. This phenomenon is particularly evident with α- or HP-β-CD (Fig. 1).

As shown in Table 1, when the concentration of α- or HP-β-CD is 0.5 mg/ml (5.1×10^{-4} M and 3.8×10^{-4} M, respectively), the experimental cmc value found is fivefold higher than that of the enhancer alone. The experimental shift of cmc obtained for L9, expressed in molarity, almost corresponds to the molarity of the CD added. For this reason, the formation of a 1:1 complex between the enhancer and the CD can be reasonably hypothesized.

Table 4
Effects of various CDs on BC cmc shifting. Measurements carried out in an isotonic solution with NaCl 0.68% and lactose 3.29%

CDs concentration (mol/l)			BC cmc	Δcmc vs. BC alone
α	HP-β	γ	(mol/l)	(mol/l)
–	–	–	1.5×10^{-4}	–
5.1×10^{-4}	–	–	5.9×10^{-4}	4.4×10^{-4}
1.0×10^{-3}	–	–	1.2×10^{-3}	1.1×10^{-3}
–	3.8×10^{-4}	–	5.9×10^{-4}	4.4×10^{-4}
–	7.6×10^{-4}	–	7.9×10^{-4}	6.4×10^{-4}
		3.9×10^{-4}	2.5×10^{-4}	1.0×10^{-4}
–	–	7.8×10^{-4}	3.4×10^{-4}	1.9×10^{-4}

γ-CD has less influence on cmc shift, its cavity being too large to form a stable inclusion compound with the hydrophobic moiety of L9. Other kinds of interactions, such as hydrogen bond, could be hypothesized as responsible for the slight increase in the cmc for the L9/γ-CD systems.

A deeper insight into the interaction phenomena between enhancers and CDs has been obtained by analyzing BC/CD systems. In this case, a preliminary evaluation of the influence of different solvents on the behaviour of the enhancer in solution has been carried out by simply comparing isotonic solutions made up with different solutes (sodium chloride, lactose or phosphate buffer). The results show that ionic species are more effective in inducing the micellization of BC at lower concentrations than non-ionized ones (Fig. 2).

For the isotonic medium prepared with sodium chloride, an increase in the surface activity of the enhancer is also evident. The surface tension values at the cmc pass from about 40 mN/m to about 35 mN/m. The higher ability of sodium chloride versus other salts (e.g. phosphates) in reducing the cmc values for BC, which is a quaternary ammonium salt, could be ascribed to the counterion effect of chlorine which induces easier formation of micelles.

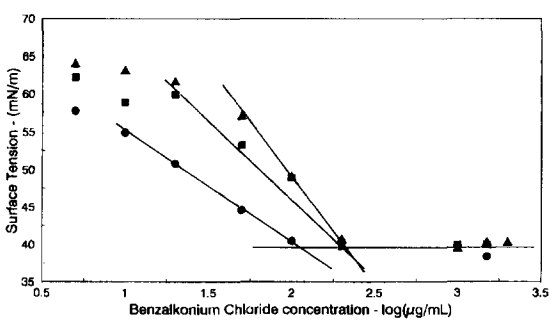

Fig. 3. BC surface tension in water (●) and with the addition of HP-β-CD at 0.03% (■) or at 0.05% (▲).

The tonicity of the medium also has an influence on cmc. Increasing the quantity of sodium chloride in solution decreases the cmc, as shown in Table 2. Under isotonic conditions, the effects of sodium chloride on cmc can easily be decreased (Table 3) by simply preparing isotonic solutions with a mixture of appropriate quantities of the salt and of another isotonic agent, such as lactose. The right quantities of sodium chloride and lactose have been calculated according to the Mellen–Seltzer sodium chloride equivalent method (Martin et al., 1983).

The effects of CDs on cmc shifting for BC are almost the same as those already shown for L9. The cmc value for solutions containing 0.5 mg/ml of α- or HP-β-CD is four times higher than that of pure BC. For both CDs, the molarity shift of cmc is almost proportional to the moles of CDs added (Table 4). Moreover, a more complex interaction is hypothesized with HP-β-CD as hydrogen bonding has a great relevance in describing HP-β-CD/BC interactions. This fact is clearly evident from the effects of the HP-β-CD at very low concentrations of the enhancer, where the decrease of surface tension value is minimized (Fig. 3).

Table 5
Effects of various CDs on CP cmc shifting. Measurements carried out in phosphate buffer pH 6.4 with 2% lactose added

CDs concentration (mol/l)			BC cmc	Δcmc vs. BC alone
α	HP-β	γ	(mol/l)	(mol/l)
–	–	–	1.5×10^{-4}	–
5.1×10^{-4}	–	–	6.1×10^{-4}	4.6×10^{-4}
1.0×10^{-3}	–	–	1.0×10^{-3}	8.5×10^{-4}
–	3.8×10^{-4}	–	5.8×10^{-4}	4.3×10^{-4}
–	7.6×10^{-4}	–	2.6×10^{-3}	2.5×10^{-3}
–	–	3.9×10^{-4}	3.0×10^{-4}	1.5×10^{-4}

All these results can be extremely useful for the transmucosal delivery of low absorbance drugs. In fact, if the toxic effects of enhancers are only due to their micellar structure, it appears possible to magnify their enhancing properties by choosing a concentration below their cmc value, or, if it is necessary to use higher concentrations, by changing and/or by increasing the cmc by using an appropriate medium.

From the formulation point of view, CDs can be used for both these purposes. The proper CD can significantly increase the cmc value or, simply, can avoid the surface properties of enhancers by increasing their surface tension value. In general, the concentration range for use of enhancers can easily be enlarged, maintaining in solution the free molecules not organized in micelles by coupling the effects of CDs with the proper choice of the buffering salts, the tonicity of the medium and the pH, if the enhancers have ionizable moieties.

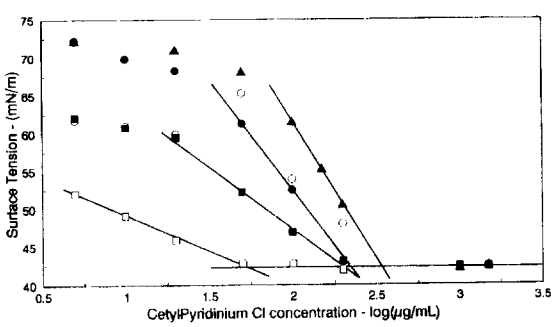

Fig. 4. CP surface tension in water (□) and with the addition of α-CD at 0.05% (●) or 0.1% (▲), and HP-β-CD at 0.05% (■) or 0.1% (○).

Confirmation of the studies performed with BC was obtained with another quaternary ammonium salt, CP. α and HP-β-CDs are quite effective in shifting the cmc value (Table 5). Also with CP, hydrogen bonding has a great importance in describing its interactions with both CDs (Fig. 4).

Other sets of experiments have been performed with cholic acid derivatives, GDC and TDC, since the effectiveness of CDs in reducing their mucosal toxicity was known from in vitro and in vivo testing (Jabbal Gill et al., 1994a; Jabbal Gill et al., 1994b). For GDC, only the interaction with γ-CD has been evaluated. Although no changes in cmc values were evident (Table 6), a range of mutual concentrations of GDC and γ-CD effective in reducing the surface properties of the enhancer (between 0.1 and 0.8 mg/ml) could be obtained. These results confirm that in order to obtain a safer formulation, it is important not only to shift the cmc value, but also to consider the possible decrease of surface properties of the enhancer itself.

Similar results have been obtained with TDC (Fig. 5). No changes in cmc value due to the addition of γ-CD were detected (Table 6). When

Table 6
Effects of γ-CD on GDC and TDC cmc. Measurements carried out in deionized water

	GDC cmc (mol/l)	TDC cmc (mol/l)
Without γ-CD	3.5×10^{-3}	5.9×10^{-3}
With 7.7×10^{-4} mol/l γ-CD	3.9×10^{-3}	5.8×10^{-3}

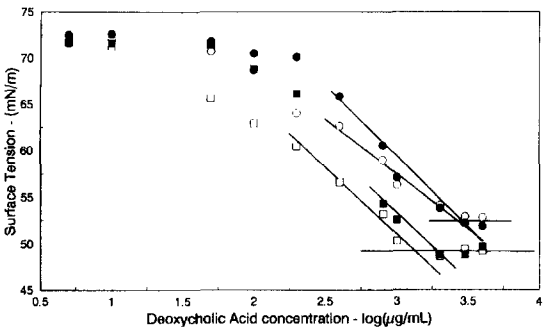

Fig. 5. Surface tension of GDC in water (□) and with the addition of γ-CD at 0.2% (■), and of TDC in water (○) and with the addition of γ-CD at 0.2% (●).

the data are compared with those obtained with GDC, the only difference is that the cmc value for TDC is higher, probably due to the different structure of the micelles and the different hydration volume for the charged species (sulphate for TDC vs. carboxylate for GDC). The hydrogen bonding effect is particularly evident in the initial part of the curves.

4. Conclusions

Surface tension measurements can be used as a quick and predictive method to evaluate the ability of cyclodextrin to make complexes with penetration enhancers and thus reduce the toxic effects of enhancers on cell membranes. This method can be proposed as a potential screening test for amphiphilic molecules, before their in vitro or in vivo biological evaluation.

It is possible to find the right enhancer dosage in a formulation to obtain good absorption and to minimize the toxic effect after establishing a suitable enhancer/cyclodextrin ratio by first evaluating the effects of different ionic strength, different buffering salts, etc. on critical micellar concentration shift.

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