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Inclusion complexes of some non-ionic surfactants with cyclomalto-oligosaccharides

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ABSTRACT

The formation of inclusion complexes of some nonylphenyl and tributylphenyl poly(oxyethylene) surfactants [tensides, R–O–$(CH_2CH_2O)_nH$] with cyclomalto-hexaose, -heptaose, and -octaose (cyclodextrins, CDs), and di- and tri-*O*-methylcyclomaltoheptaose was studied on the basis of changes in surface tension. The relative strength of the interaction of CD and tenside was measured by reverse-phase t.l.c. The CDs increased the surface tension of the more hydrophobic tensides and decreased that of the more hydrophilic tensides. The bulk of the hydrophobic moiety of tensides, the length of the poly(oxyethylene) chain, the CD–tenside molar ratio, and the salt concentration influenced significantly the surface tension. The strengths of the inclusion complexes were influenced significantly by the concentration of the CD, the diameter of the cavity, and the length of the poly(oxyethylene) chain. The formation of inclusion complexes may influence the performance of formulations containing tensides and a CD.

INTRODUCTION

Non-ionic tensides display numerous biological effects. Non-ionic block-polymer surfactants 31R1 and L101 (Laboratorium voor Microbiologie, Rijksuniversiteit Utrecht, The Netherlands; composition not known) stimulate the induction of delayed-type hypersensitivity to a synthetic peptide comprising amino acid residues 9–21 of *Herpes simplex* virus type 1-glycoprotein[1]. Poly(ethylene oxide) and poly(propylene oxide) block copolymers enhance the avidity of antibodies in polyclonal antisera against *Streptococcus pneumoniae* type 3 in normal and Xid mice[2]. Poloxalene (molecular mass, 3 kD), a 30% poly(ethylene oxide) and 70% poly(propylene oxide) copolymer, inhibits the absorption of neutral fat and cholesterol in laboratory animals[3]. Polyethoxylated fatty alcohols influence the loading of low-density lipoprotein with lipid-soluble materials[4]. Poly(ethylene glycols) interact with many natural and synthetic polymers[5]. A mixture of 70% monolinoleic glyceride, 18% mono-oleic glyceride, and 11% saturated monoglycerides decreases the retrogradation of amylopectin[6]. Triton X-100 activates ATP-ase by altering the hydrophobic environment around the enzyme[7]

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and poly(ethylene glycol) 600 increases the production of alpha-amylase by *Bacillus subtilis*[8]. Nonylphenyl and tributylphenyl poly(oxyethylene) surfactants increase the proteolytic activity of papain[9], decrease the activity of horseradish peroxidase[10], and readily form complexes with such drugs as chlorhexidine[11]. Polyoxyethylene-stearat, -sorbitanstearat, and -sorbitanlaurat form complexes with tri- and tetra-cyclic psychiatric drugs[12]. Poly(55)oxypropylene/dipoly(8)oxyethylene increases the neuroleptic activity of haloperidol after its solubilisation in surfactant micelles[13].

Non-ionic tensides also display toxic effects. Thus, poly(ethylene glycol) causes cell fusion[14] and Triton X-100 induces cell death of *Bacillus subtilis* due to autolysis[15]. Poly(ethylene oxide) surfactants with various aromatic and aliphatic hydrophobic parts showed toxicity towards *Mysidopsis bahia*[16]. The non-ionic tensides, Activator N.F. and Ortho X-77 (Loveland Inc., IN, U.S.A., and Chevron Chem., IN, U.S.A.; composition not known) were toxic to larvae of the midge *Chironomus riparius*[17]. Emulgen 913, a poly(ethylene glycol) nonylphenyl ether, decreases the liver weight and microsomal heme content in rats[18] and lessens the heme oxygenase activity in the kidney of red carp[19].

Due to their capacity to form inclusion complexes, cyclomalto-oligosaccharides (cyclodextrins, CDs) are used in the stabilisation and formulation of drugs, flavors, and fragrances, and also in agrochemistry[20]. Methylated CDs, but not CDs themselves, have surface activity[21]. Many surface-active agents can form inclusion complexes with CD, resulting in striking changes in the critical micelle concentrations, surface tension, *etc.*[22,23] The formation of inclusion complexes of some non-ionic tensides with CD derivatives lessens their phytotoxicity[24].

As the practice of including both tensides and CDs in pharmaceutical or agrochemical formulations continues to increase, studies of the interaction of CDs and their derivatives and non-ionic tensides is of practical and theoretical importance.

We now report on the effect of the formation of inclusion complexes, on the surface activity of some tensides, the relative strengths of interaction of tensides and CD derivatives, and correlation of the effects with the physicochemical parameters of the interacting molecules.

EXPERIMENTAL

Cyclomalto-hexaose (αCD), -heptaose (βCD), and -octaose (γCD), heptakis(2,6-di-*O*-methyl)-βCD (DIMEB), and heptakis(2,3,6-tri-*O*-methyl)-βCD (TRIMEB) are products of CHINOIN Pharmaceutical and Chemical Works (Budapest, Hungary). Their purity, when checked by h.p.l.c., was >98%. The non-ionic tensides studied (Table I) were commercial products purchased from Hoechst AG (Germany), and each contained a hydrophobic moiety and a hydrophilic poly(oxyethylene) chain. Each nonylphenol derivative is a mixture of compounds with various lengths of poly(oxyethylene) chain[25]. Moreover, the hydrophobic moiety of the tributylphenol derivatives contained various isomers. Although the inhomogeneity of the tensides lessens the validity of the conclusions drawn from the measurement of surface tension, the results do promote a better understanding of tenside–CD interactions.

TABLE I

Chemical structures of the non-ionic tensides, $R-(CH_2CH_2O)_nH$

No.	*R*	*Average value of* n	*No.*	*R*	*Average value of* n
1	Nonylphenyl	4	11	Tributylphenyl	4
2		5	12		6
3		6	13		8
4		8	14		10
5		9	15		11
6		10	16		13
7		11	17		18
8		15	18		30
9		23	19		50
10		30			

The contribution of the poly(oxyethylene) chain to the lipophilicity of the tensides is low[26] and the isomers of tributylphenol have similar lipophilicities and behavior in reverse-phase t.l.c. Since these non-ionic tensides are always used as mixtures of isomers, they were used without further purification in the following studies.

Determination of surface tension. — The concentration of tenside was usually 10^{-4} M and each (except 4, 15, and 19, see Table I) was mixed with the CD in the molar ratio tenside–CD in the range 1:0.2–1:100 severally in twice-distilled water, M KCl, M $MgCl_2$, and M $CaCl_2$, also prepared from twice-distilled water. The surface tension of each solution was determined in triplicate at 25° by a thermostated stalagmometer (5-mL vol.) constructed at the Plant Protection Institute of the Hungarian Academy of Sciences (Budapest, Hungary). The drops were detected by a photocell and counted with a microprocessor; the coefficient of variation was kept below 2%. The stalagmometer was washed with acetone, dried in a stream of air, and washed three times with the sample solution.

The surface tension data were subjected to stepwise regression analysis[27] in order to select the parameters that were correlated with this property. The independent variables were the bulk of the hydrophobic moiety of the tenside[28], the diameter of the cavity of the CD, the number of oxyethylene groups per molecule, the CD–tenside molar ratio, the salt concentration, and the charge and radius of the cation. The last five independent variables were included in the calculations because the ionic environment may influence the formation of complexes[29]. Quadratic functions were included since the linear character of the correlation has not been proved.

Determination of formation of inclusion complexes — The determination of the relative stability of CD inclusion complexes by reverse-phase t.l.c. has been described[30]. T.l.c. was performed on Kieselgel 60 plates (Merck) impregnated with paraffin oil as described[29], with aliquots (4 μL) of solutions (20 mg/mL) of non-ionic tensides in methanol, a solvent that reacts only weakly with CDs[30].

The tensides were spotted separately on the plates and the tenside–CD ratio was

identical for each tenside. The eluent was aqueous methanol with the methanol increased from 45 to 80% (v/v) in steps of 5%. The concentration of α-, β-, and γ-CD in the eluent was in the range 0–13.22mM. The plates were dried at 105° and the tensides were detected with modified Burger reagent[32]. Each determination was run in quadruplicate. The R_M value, which characterises the molecular lipophilicity in reverse-phase t.l.c., was calculated for each tenside and eluent, and is given by $(1/R_F - 1)$.

The influence of the various parameters on the R_M values was also calculated with stepwise regression analysis. The R_M values were the dependent variables. The independent variables were the average number of oxyethylene groups per molecule (x_1), the methanol concentration (%, x_2), the CD concentration (mM, x_3), and the diameter of the CD cavity (x_4). The derived variables x_3,x_4, x_1,x_3, x_1,x_4, and x_1,x_3,x_4 were included in the calculation in order to determine the interaction of the components. The other conditions were as described above.

RESULTS AND DISCUSSION

The addition of a CD to an aqueous solution of a tenside modifies the surface tension which, together with other observations in the literature, indicates at least partial inclusion of the tenside in the cavity of the CD. Three effects are discernible in the interaction of βCD and the tributylphenyl tensides (Fig. 1). (*a*) βCD reduces the surface tension of aqueous solutions of the tensides with shorter poly(oxyethylene) chains (*i.e.*, in the more hydrophobic tensides) and improves their weak effect (curve $n = 6$ in Fig. 1) presumably because the tenside–βCD complex is more hydrophilic than the tenside. The effect occurs over the whole range of molar ratios. (*b*) As the length of the poly(oxyethylene) chain increases (*i.e.*, in the more hydrophilic tensides), there is a minimum in the plot of the surface tension versus concentration of βCD (curve $n = 8$ in Fig. 1). At a low concentration of βCD, effect (*a*) dominates but, at higher concentrations, the βCD shields the hydrophobic moiety of the tenside and enhances the solubility of the tenside–βCD complex. (*c*) When the poly(oxyethylene) chain is large (*i.e.*, in the hydrophilic tensides), an increase in the concentration of βCD progressively decreases the surface tension (curves $n = 18$ and 30 in Fig. 1).

The effect of αCD on the surface tension of the nonylphenyl tensides was less then that of βCD (Fig..2), which suggests that more stable complexes are formed with βCD. The effect of βCD increases with increase in the concentrations of the nonylphenyl tensides (*i.e.*, with decrease in the surface tension) (Fig. 3). Since tensides are generally used in this range of concentrations in order to effect the required decrease of the surface tension, their performance may be markedly reduced by the presence of CDs.

DIMEB has marked surfactant activity[21], but its effect in aqueous solutions of DIMEB–tenside mixtures depends on the length of the poly(oxyethylene) chain (Table II). Thus, the surface tension of the more hydrophobic tensides is reduced and that of the more hydrophilic tensides is increased, which reflects a strong antagonistic effect.

The factors that influence significantly the surface tension of tenside–CD solutions are compiled in Table III. The high F value ($F_{99.9\%} = 4.42$) indicates a close fit of the

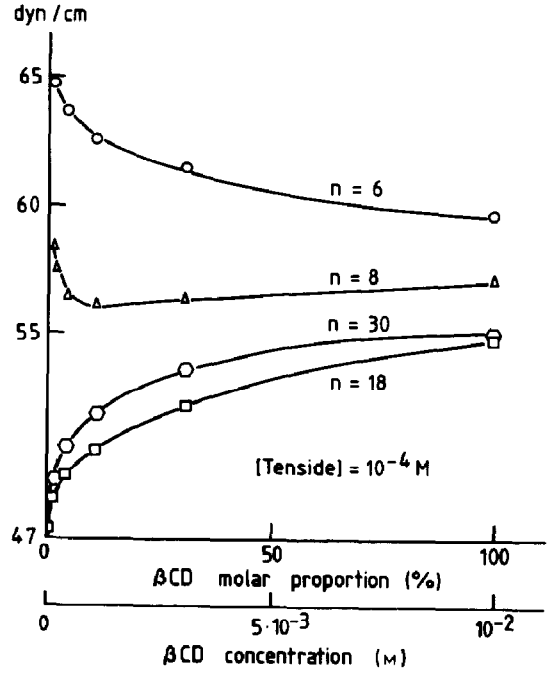

Fig. 1. Effect of βCD on the surface tension of 10^{-4}M tributylphenyl tenside: n = number of oxyethylene groups per molecule.

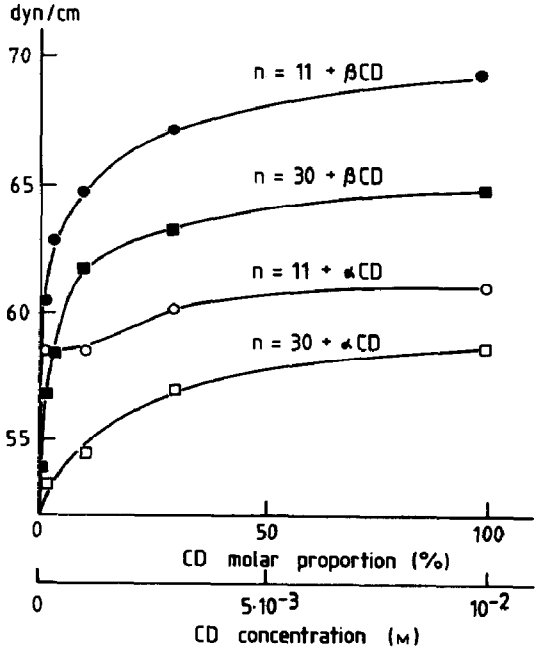

Fig. 2. Effect of αCD and βCD on the surface tension of 10^{-4}M nonylphenyl tensides: n = number of oxyethylene groups per molecule.

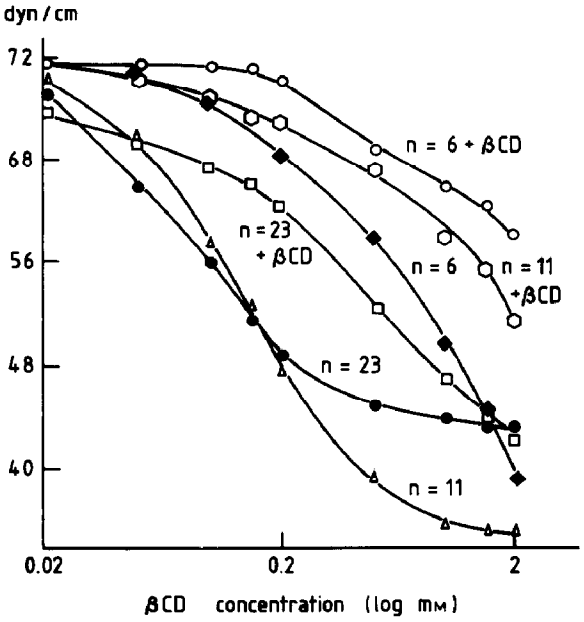

Fig. 3. Effect of βCD on the surface tension of 10^{-4}M nonylphenyl tensides: n = number of oxyethylene groups per molecule.

TABLE II

Effect of heptakis(2,6-di-O-methyl)-βCD (DIMEB) on the surface tension (dyn/cm) of 10^{-4}M solutions of some non-ionic tensides. See Table I

Tenside	*DIMEB–tenside molar ratio*			
	0:1	*1:1*	*3:1*	*10:1*
5	62.5	61.4	58.5	58.5
6	61.8	59.1	59.1	57.8
7	59.7	59.8	57.2	56.9
10	53.9	54.9	55.5	56.3
13	58.5	57.8	57.2	56.4
16	54.4	54.9	55.8	55.8
17	47.5	49.2	49.2	49.5
18	48.2	50.4	50.9	51.6

experimental data to the calculated equation. Only five of the twelve independent variables influence significantly the surface tension of tenside–CD, the significance level being 99.9% for each variable ($t_{99.9\%}$ = 3.35). The major effect on surface tension is related to the number of oxyethylene groups per molecule, probably because only the hydrophobic moiety of a tenside molecule is included (at least partly) in the cavity of CDs. The fact that the bulk of the hydrophobic moiety of the tenside molecule has a significant influence on the surface tension also indicates that this moiety is involved in

TABLE III

Effect of various physicochemical parameters[a] (independent variables) of cyclodextrins and tensides on the surface tension of their solutions. Results of stepwise regression analysis.

$n = 189$ $a = 76.41$ $r = 0.8790$ $F = 124.6$				
Independent variable	b	s_b	$t_{calc.}$	p.c.%
Square of the width of the hydrophobic moiety	-2.54×10^{-4}	-1.3×10^{-5}	19.11	12.73
Number of oxyethylene groups per molecule (n)	−1.61	0.17	9.52	43.64
n^2	3.51×10^{-2}	4.4×10^{-3}	7.99	36.36
CD–tenside molar ratio	2.83×10^{-2}	5.5×10^{-3}	5.10	3.63
Salt concentration (M)	−2.30	0.44	5.24	3.64

[a] n = Number of observations; a = intercept; b = slope, and indicates the change of surface tension caused by unit change of the independent variable; s_b = standard deviation of the slope; r = coefficient of correlation, its square indicates the ratio of variance explained; F = calculated value of "F" test, and indicates the fitting of the equation to the experimental data; $t_{calc.}$ = calculated value of "t" test, and indicates the significance level of the individual independent variables; p.c.% = path coefficient or normalised slope value, and indicates the relative impact of the individual independent variables on the surface tension, independently of their dimensions.

TABLE IV

Effect of various parameters (x_1–x_4) of CDs and tensides on the lipophilicity (R_M) of tensides; result of stepwise regression analysis. For symbols, see TABLE III.

$$R_M = a + b_1.x_1.x_3.x_4 + b_2.x_2 + b_3.x_3.x_4$$

where x_1 = number of oxyethylene groups per molecule; x_2 = concentration of methanol in the eluent (vol.%); x_3 = concentration of CD in the eluent (mM); and x_4 = diameter of the cavity of the CD

Parameter	*Nonylphenyl derivatives*	*Tributylphenyl derivatives*
n	132	200
a	387	361
b_1	8.14×10^{-3}	-1.70×10^{-3}
s_{b1}	2.25×10^{-3}	5.11×10^{-4}
Path coefficient (%)	9.18	8.15
b_2	−5.43	−4.52
s_{b2}	0.14	0.13
Path coefficient (%)	74.67	80.38
b_3	−0.27	−0.11
s_{b3}	0.04	0.02
Path coefficient (%)	16.15	11.47
r^2	0.9264	0.8730
F	557.35	448.97

the formation of complexes with CDs. The surface tension of tenside–CD mixtures is reduced significantly as the concentration of salt is increased, but neither the charge nor the radius of the cation has a significant effect.

The effects of various parameters on the lipophilicity of tensides are compiled in Table IV. The two equations selected by stepwise regression analysis fit well the experimental data [the significance levels (F values) were >99.9% and are similar, which indicates that complex-forming capacities of both types of tensides are similar]. The change in the concentrations of methanol and CD, the number of oxyethylene groups per molecule, and the diameter of the cavity of the CD account for 90% of the change of the R_M value of tensides (see r^2 values). The lipophilicity of the tensides significantly decreases with increasing concentration and diameter of the cavity of the CD, and indicates that γCD forms the most stable complexes. As noted above, the hydrophobic moiety of the tenside is probably inserted into the cavity of CD, thereby decreasing the hydrophobicity. The effect of the number of oxyethylene groups on the lipophilicity of tensides is low, which suggests that the polar poly(oxyethylene) chains may interact with the hydrophilic outer surface of the CDs.

Thus, CDs readily form inclusion complexes with tensides, which modifies their surfactant activity and decreases their lipophilicity. Hence, there may be synergistic or antagonistic effects in the performance of pharmaceutical and agrochemical formulations that contain both CDs and tensides.

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