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INTERACTION OF SOME NON-IONIC TENSIDES WITH DIOLEOYL PHOSPHATIDYL CHOLINE, STUDIED BY CHARGE-TRANSFER CHROMATOGRAPHY

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SUMMARY

The interaction of the membrane phospholipid, dioleoyl phosphatidyl choline (DOPC), with 23 non-ionic tensides belonging to four homologous series was studied by charge-transfer reversed-phase thin-layer chromatography. The dependence of the lipophilicity of DOPC on the tenside concentration of the eluent was considered to be linearity related to the stability of the complex. The polyethoxylated nonylphenyl and tributylphenyl derivatives showed similar behaviour patterns. They formed complexes with DOPC, but the strength of complexation depended neither on the type of hydrophobic moiety nor on the number of ethylene oxide groups per molecule. However, for polyethoxylated oleyl alcohol derivatives, the stability of the complex depended considerably on the number of ethylene oxide groups, thus suggesting a different form of interaction. The length of the alkyl chain in the hydrophobic part of the tensides also influenced the strength of complex formation.

INTRODUCTION

Pesticide formulations generally contain non-ionic tensides, which can modify not only the application parameters of a formulation but also its biological effect[1] and selectivity[2,3]. The tensides themselves may have phytotoxic action and microbiocidal effects. These biological activities depend considerably on the chemical structure of the tensides. In the case of homologous series, they depend on the number of ethylene oxide groups per molecule[4,5]. Non-ionic tensides increase membrane permeability, decease the resistance of the lipid bilayer and enhance the glucose and potassium efflux of liposomes[6–1]. Quantitative structure–activity relationship (QSAR) studies suggest that, structually, membrane-damaging effects depend on the presence of bulky hydrophobic parts (built into the hydrophobic alkyl chains of fatty acids)

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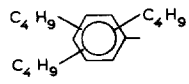
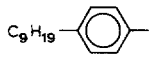
and the number of ethylene oxide groups per molecule[11]. Membranes contain a mixture of various phospholipids. Therefore, it is highly probable that non-ionic tensides interact with phospholipids, forming organic molecular complexes.

As charge-transfer chromatography proved to be suitable for studying such complex formation[12,13], this method has been applied in our work to investigate the interactions of membrane phospholipid, dioleoyl phosphatidyl choline (DOPC), with some non-ionic tensides. However, charge-transfer chromatography suffers some drawbacks. In most cases, purely organic eluents are used. The correlation between the binding constant proposed[14] and the interactive forces is not clear; the binding constants determined in organic eluents cannot easily be related to the interactions taking place in aqueous biological systems.

In our experiments, we used an improved version of charge-transfer chromatography. To avoid the application of purely organic eluents, we used reversed-phase thin-layer chromatography (RP-TLC) instead of adsorption TLC. The tensides were mixed in the eluent at various concentrations, and the dependence of the R_M (lipophilicity) value of DOPC on tenside concentration was considered to be related to the interactive forces. The theoretical basis of this procedure is the fact that the more hydrophobic tensides lessen the lipophilicity of the more hydrophobic DOPC by complexation. The degree of lipophilicity decrease corresponds to the stability of

TABLE I

CHEMICAL STRUCTURE OF NON-IONIC TENSIDES OF GENERAL STRUCTURE Q-O-$(CH_2CH_2O)_n$H

No. of compound	*Hydrophobic part, Q*	*Number of ethylene oxide groups per molecule, n*
1		4
2		6
3		8
4		10
5		13
6	Tributylphenyl	30
7		4
8		6
9		9
10		10
11		11
12		13
13		15
14	Nonylphenyl	30
15		2
16	$CH_3(CH_2)_7CH=CH(CH_2)_8$-	5
17		8
18		15
19	Oleyl alcohol	20
20	Sorbitan monolaurate	20
21	Sorbitan monopalmitate	20
22	Sorbitan monostearate	20
23	Sorbitan mono-oleate	20

the complex[15]. This method has been successfully applied to studies of the cyclodextrin inclusion complex formation of polymyxine[16], substituted symmetric triazine[17], and triphenylmethane derivatives[18]. Similar experimental conditions have also been applied in reversed-phase ion-pair system in TLC[19–21].

We are well aware that our data does not prove that the interaction of DOPC and non-ionic tensides is due to charge-transfer phenomena, therefore application of the expression "charge-transfer chromatography" is perhaps misleading. We consider charge-transfer chromatography in a more general sense, as a method suitable for detecting any type of weak interaction between two molecular species.

EXPERIMENTAL

The chemical structures of the tensides are given in Table I. DOPC was purchased from Sigma and used without purification. DC Cellulose Alufolien (Merck, Darmstadt, F.R.G.) were impregnated in 5% paraffin oil in *n*-hexane overnight. After evaporating the *n*-hexane at room temperature, 4 μl of solution of 5 mg DOPC/ml chloroform were spotted onto the plates. Water–ethanol (12:13) was used as eluent. Tensides were dissolved in the eluent in a concentration range of 1–150 mM, depending on the strength of interactive forces. After development, the plates were dried at 105°C, and DOPC spots were detected by iodine vapour. All R_F values were the mean of five independent determinations.

The R_M values characterizing molecular lipophilicity in RP-TLC were calculated from eqn. 1[22]:

$$R_M = \log\left(\frac{1}{R_F} - 1\right) \tag{1}$$

Linear correlations were calculated separately for each tenside, between the lipophilicity (R_M) value of DOPC and the concentration of tenside in the eluent:

$$R_M = R_{MO} + bC \tag{2}$$

where R_M = actual R_M value of DOPC, determined in the presence of a concentration C of tenside in the eluent; R_{MO} = R_M value of DOPC, determined in tenside-free eluent; b = change of lipophilicity caused by a unit change of tenside concentration in the eluent (considered to be related to complex stability); C = concentration of tenside in the eluent (mM).

RESULTS AND DISCUSSION

Each tenside decreased the lipophilicity of DOPC. Hence, we infer that each tenside forms complexes with this phospholipid (Fig. 1). In the case of an identical number of ethylene oxide groups per molecule, the length of the hydrophobic alkyl chain in sorbitan derivatives influences significantly the strength of complex formation. The longer the alkyl chain of the sorbitan derivatives, the higher the complex stability. As the alkyl chain of fatty acids in DOPC contains 18 carbon atoms, it is

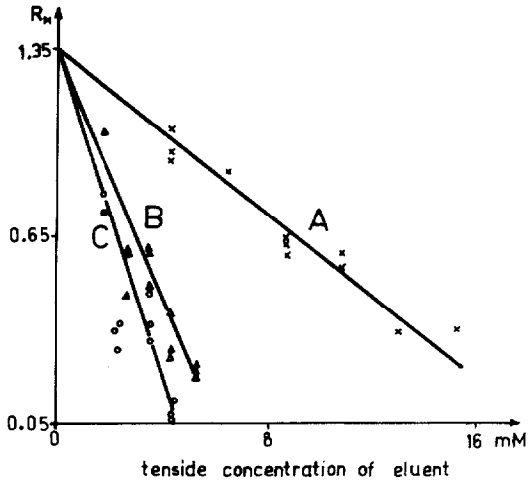


Fig. 1. Dependence of the lipophilicity of DOPC on the tenside concentration of eluent. (A) Sorbitan monolaurate; (B) sorbitan monooleate; (C) sorbitan monostearate.

TABLE II

PARAMETERS OF LINEAR CORRELATION BETWEEN THE LIPOPHILICITY OF DOPC (R_M) AND THE TENSIDE CONCENTRATION OF ELUENT (C)

$R_M = R_{MO} + bC$; s_b = standard deviation of b.

No. of tenside	*n*	*b*	s_b	$r_{calculated}$
1	16	− 4.23	0.22	0.9808
2	18	− 3.90	0.12	0.9919
3	16	− 4.96	0.55	0.9225
4	18	− 3.37	0.14	0.9860
5	16	− 3.92	0.19	0.9951
6	10	− 3.90	0.35	0.9691
7	40	− 3.42	0.16	0.9613
8	16	− 3.90	0.13	0.9924
9	76	− 3.21	0.09	0.9763
10	16	− 3.44	0.12	0.9916
11	12	− 3.84	0.67	0.9985
12	16	− 3.70	0.15	0.9969
13	16	− 3.61	0.07	0.9977
14	24	− 3.62	0.08	0.9897
15	24	− 1.19	0.05	0.9806
16	18	−11.00	0.22	0.9968
17	30	−12.13	0.39	0.9861
18	10	− 8.66	0.62	0.9800
19	22	− 6.20	0.25	0.9838
20	24	− 7.66	0.32	0.9664
21	22	−17.02	0.96	0.9697
22	22	−31.79	1.67	0.9729
23	26	−24.03	0.83	0.9861

understandable that alkyl chains of similar length in tensides are more suitable for forming complexes. The presence of double bonds in the alkyl chain lessens the interactive forces. This may be due to the fact that the double bond modifies the shape of the alkyl chain, sterically hindering complex formation.

The parameters of eqn. 2 are shown in Table II. In all cases, the significance level of linear correlations was >99.9%. The calculated coefficients of regression were higher, in all cases, than the tabulated regression coefficients corresponding to a 99.9% significance level[23]. This finding proves the validity and applicability of eqn. 2. The nonylphenyl and tributylphenyl exert a similar influence on complex stability. The *b* values of tensides 2–8, 4–10, 5–12 and 6–14 did not differ considerably. In these two homologous series, the number of ethylene oxide groups per molecule did not modify complex stability. The values of the slopes of linear correlations between complex stability values and the number of ethylene oxide groups per molecule did not deviate significantly from zero.

Tensides containing oleyl alcohol as the hydrophobic moiety showed a very different behaviour pattern. Except for tenside 15, the others formed more stable complexes with DOPC than tensides 1–14. This finding indicates that oleyl alcohol forms complexes more readily with DOPC than nonylphenyl and tributylphenyl groups. This is understandable when one considers that DOPC contains identical alkyl chains. In the case of tensides 1–6 and 7–14, the number of ethylene oxide groups per molecule exerted a negligible effect on complex stability. The situation was very different for tensides 15–19, where the number of ethylene oxide groups per molecule influenced the interaction forces considerably. It seems that, in this case, there is an optimal number of ethylene oxide groups. This observation suggests that the character of complex formation between DOPC and tensides 1–14 and 15–19 is markedly different from that of the previously mentioned compounds.

We have to stress that the *b* value calculated by our method is not numerically equal to the common complex stability coefficient, although it is linearly correlated with it. The exact complex stability coefficient can be determined by RP-TLC only after calibration by another method. Summarizing our data, we assume that this version of charge-transfer chromatography is adequate for studying complex formation between organic molecules when one of the molecules is more hydrophilic than the other and does not interfer with detection of the other.

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