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Charge-transfer chromatographic study of the interaction of antibiotics with cetyltrimethylammonium bromide

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The interaction of 20 antibiotics with the cationic surfactant cetyltrimethylammonium bromide (CTAB) was studied by charge-transfer reversed-phase chromatography carried out on impregnated alumina layers using water-methanol mixtures as eluents. The lipophilicity and specific hydrophobic surface area of antibiotics and the relative strength of their interaction with CTAB was calculated. CTAB interacted with 10 antibiotics the antibiotic – CTAB complex generally being more hydrophilic than the uncomplexed molecule. The relative strength of interaction depended considerably on the molecular structure of the antibiotics. Significant linear correlation was found between the lipophilicity of antibiotics and their capacity to interact with CTAB indicating the involvement of hydrophobic forces in the interaction.

1. Introduction

Because of their advantageous physicochemical properties cationic surfactants have been extensively used in both pharmaceutical and pesticide formulations [1] and cosmetics [2]. They markedly increase the transdermal flux of active ingredients, e.g. the effect of cationic and anionic surfactants on the transdermal flux of methyl nicotinate was higher than that of a nonionic surfactant (Brij® 36T) [3]. Ionic surfactants readily bind to proteins modifying protein structure and enzymatic activity. Thus, ionic surfactants SDS (sodium dodecyl sulfate) and CTAB (cetyltrimethylammonium bromide) were effective whereas Tween® 80 and polyoxyethylene 9 laurylether have negligible effect on the dissociation, α-chymotryptic degradation, and enteral absorption of insulin hexamers [4]. It was also reported that enzyme activity increased in aqueous cetyltrimethylammonium ion micelles [5]. Anionic (SDS) and cationic surfactants (dodecyl trimethylammonium bromide) modified the structure and enzymatic activity of jack bean urease [6]. Ionic surfactants interact not only with proteins but also with membrane phospholipids: cetyl pyridinium chloride caused mechanical rupture of diphytanoylphosphatidylcholine membranes in high electrical fields [7], and SDS increased the surface tension of phosphatidylcholine monolayers whereas CTAB inhibited the film formation below the critical micelle concentration [8].

Anionic surfactants also show marked toxicity [9]. The order of toxicity of surfactants measured on ocular lens organ culture was: benzalkonium chloride > cetylpyridinium bromide > Triton-X-100 > SDS > Geropon AC-78 > Tween 20 [10]. A study of the uptake of neutral red by rabbit corneal cells revealed that nonionic surfactants have a smaller toxic effect than cationic, anionic and amphoteric ones [11]. Another study comparing two cytotoxicity tests for predicting ocular irritancy established that the red blood cell lysis test was predictive. Surfactants caused membrane disruption, anionic and cationic surfactants being more toxic than nonionic ones [12].

Chromatographic methods, specially liquid chromatography have frequently been used for the study of various molecular interactions. These applications have recently been reviewed [13, 14]. The principle of the determination of the strength of interaction is based on the measurement of the hydrophobicity of one of the interacting molecular species in the absence and in the presence of the other interacting molecular species. As the hydrophobicity of the complex is different from that of the uncomplexed molecule the difference in hydrophobicity is an indicator of the strength of interaction. Commonly the retention of the more hydrophobic molecule is determined under reversed-phase conditions and the more hydrophilic interactive partner is added to the eluent in various concentrations.

The objectives of this work were the determination of the interaction of CTAB with some antibiotics, the calculation of the relative strength of the interaction, and the elucidation of the involvement of hydrophobic and hydrophilic forces in the CTAB – antibiotics interaction.

2. Investigations, results and discussion

The simultaneous effect of methanol and CTAB concentrations on the R_M values of the antibiotics puromycin and gramicidin (compounds **17** and **9** in Table 1) are shown in Figs 1 and 2, respectively. CTAB in the eluent may decrease (Fig. 1) or increase (Fig. 2) the retention of individual antibiotics. This phenomenon suggests that the drug – CTAB complex can be more or less hydrophobic than the uncomplexed drug molecule. Modification of the hydrophobicity of an antibiotic may result in changes to the penetration rate, mobility, adsorption capacity, and decom-

Table 1: Common names of antibiotics

Compd.	Common name
1	Ampicillin
2	Antimycin
3	Cefotaxime
4	Cephalexin
5	Cephalotin
6	Chloramphenicol
7	Cycloheximide
8	Erythromycin
9	Gramicidin
10	Griseofulvin
11	Kanamycin
12	Methycillin
13	Nalixidic acid ethylester
14	Novobiocin
15	Oxacillin
16	Penicillin G
17	Puromycin
18	Rifamycin SV
19	Tobramycin
20	Trichotecin

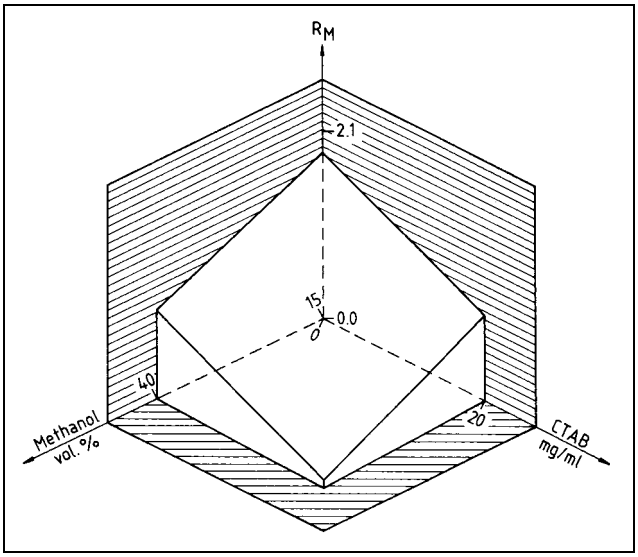

Fig. 1: Effect of methanol and CTAB concentrations in the eluent on the R_M value of puromycin (compound **17**)

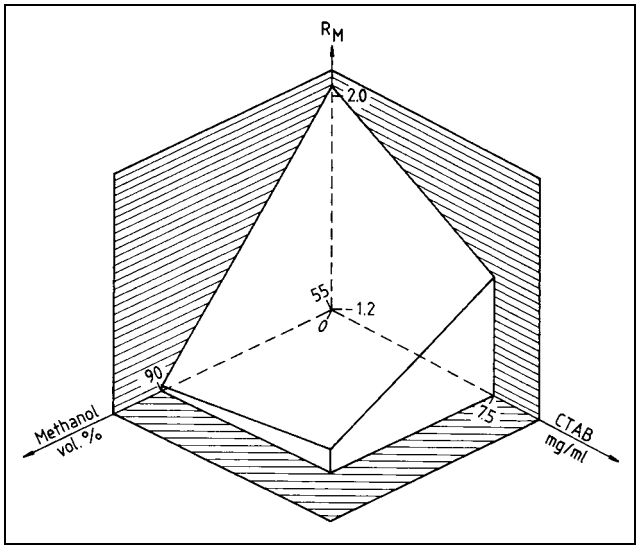

Fig. 2: Effect of methanol and CTAB concentrations in the eluent on the R_M value of gramicidin (compound **9**).

position rate of the drug, thereby enhancing or lessening its biological efficiency.

The parameters of Eq. 2 are given in Table 2. Blank entries in the table indicate that in these instances the effect of the corresponding independent variable on the mobility of the antibiotics cannot be established. The equation fits the experimental data well (see $F_{calc.}$ values), the significance level in each instance being over 95%. The ratios of variance explained were between 32–97% (see r^2 values). The parameters of Eq. 2 differ considerably, demonstrating that the lipophilicity (R_{M0}), specific hydrophobic surface area (b_1) and capacity of antibiotics to form complexes with CTAB (b_2) differ considerably. In most cases methanol has a greater impact than CTAB on the mobility of antibiotics (see path coefficient, b′% values). Ten antibiotics interacted with CTAB (the b_2 values differ significantly from zero), however, the relative strength of interaction differs markedly. This finding suggests that the interaction of antibiotics with CTAB may influence the biological efficiency of the individual drugs in different ways.

Significant linear correlation was found between the lipophilicity and specific hydrophobic surface area of antibiotics (Fig. 3). This finding indicates that from a chro-

Table 2: Relationship between the R_M values of antibiotics and the concentrations of methanol (C_1) and cetyltrimethylammonium bromide (C_2) in the eluent

Parameter	Compd.									
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
n	23	19	24	19	21	20	20	14	14	16
R_{M0}	1.63	1.72	1.05	1.08	1.12	0.68	1.28	1.84	5.85	2.53
$-b_1 \cdot 10^2$	2.55	1.72	2.64	1.48	1.69	1.95	2.95	3.08	7.80	4.73
$s_{b1} \cdot 10^3$	3.29	5.96	7.02	2.98	4.97	1.92	2.67	6.44	0.72	2.33
$-b_2 \cdot 10^2$	3.89	–	–	–	–	–	1.31	–	−3.67	–
$s_{b2} \cdot 10^3$	6.34	–	–	–	–	–	5.20	–	15.40	–
b_1'%	55.92	–	–	–	–	–	81.45	–	82.02	–
b_2'%	44.08	–	–	–	–	–	18.55	–	17.98	–
r^2	0.8092	0.3294	0.3920	0.5928	0.3777	0.8513	0.8784	0.6564	0.9282	0.9671
$F_{calc.}$	42.47	8.35	14.19	24.74	11.53	103.09	61.39	22.92	71.05	411.37

Parameter	Compd.									
	11	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**
n	12	24	20	16	24	24	17	17	15	20
R_{M0}	1.04	1.37	1.60	1.90	1.74	1.10	2.42	2.32	1.81	1.96
$-b_1 \cdot 10^2$	2.23	2.92	3.11	3.04	3.33	1.70	3.58	3.82	1.63	3.34
$s_{b1} \cdot 10^3$	5.96	2.50	3.46	2.60	2.33	3.12	4.58	1.72	0.67	4.74
$-b_2 \cdot 10^2$	–	3.29	2.78	–	4.38	2.51	4.49	–	4.56	3.97
$s_{b2} \cdot 10^3$	–	5.02	6.75	–	4.68	6.25	8.36	–	13.58	9.23
b_1'%	–	64.04	68.61	–	65.73	57.53	59.28	–	41.87	62.15
b_2'%	–	35.96	31.39	–	34.27	42.47	40.72	–	58.13	37.85
r^2	0.5836	0.8891	0.8316	0.9075	0.9284	0.6696	0.8201	0.9704	0.5066	0.7680
$F_{calc.}$	14.02	84.19	41.97	137.35	136.06	21.28	31.81	492.07	6.16	28.13

n is the number of data points; R_{M0} is related to the hydrophobicity of the antibiotics; b_1 is related to the specific hydrophobic surface area of the antibiotics; b_2 is related to the relative strength of CTAB – antibiotic interaction; s_{b1} and s_{b2} are the standard deviations of b_1 and b_2; b_1'% and b_2'% are standard partial regression coefficients of b_1 and b_2 which are normalized to unity; r^2 coefficient of determination; $F_{calc.}$ calculated F value indicating the fitness of eq. 2 to the experimental data. Blank sites in Table indicates that no significant interaction was found between CTAB and these antibiotics

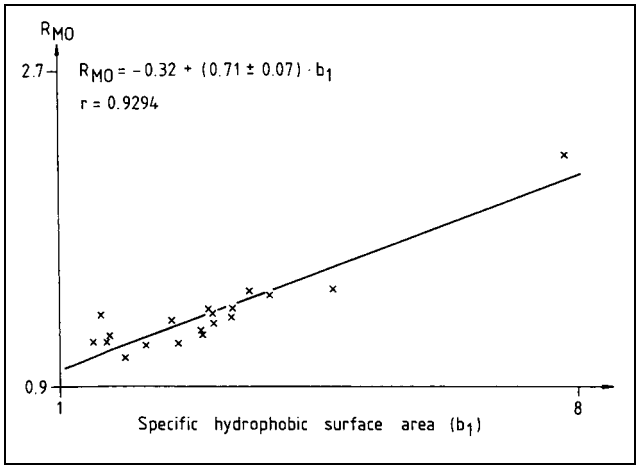

Fig. 3: Relationship between the lipophilicity (R_{M0}) and specific hydrophobic surface area (b_1) of antibiotics

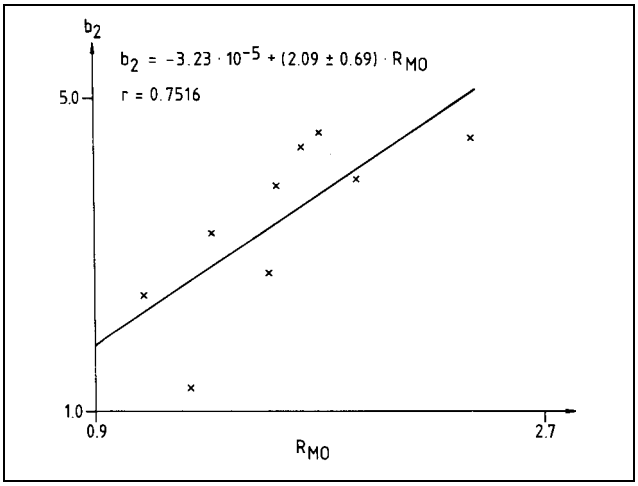

Fig. 4: Relationship between the lipophilicity (R_{M0}) of antibiotics and their capacity to interact with CTAB (b_2)

matographic point of view these solutes behave as a homologous series of compounds, although they are structurally different.

Significant linear correlation was found between the lipophilicity of antibiotics and their capacity to interact with CTAB indicating that hydrophobic forces are involved in the interaction of antibiotics with CTAB (Fig. 4). However, the ratio of variance explained was relatively low (56.49%) suggesting that physicochemical parameters other than the molecular lipophilicity may exert a considerable effect on the interaction of antibiotics with CTAB. The interaction between CTAB and antibiotics suggests that this interaction may have a marked influence on the biological efficiency of any pharmaceutical formulations simultaneously containing CTAB and antibiotics.

It can be concluded from the data that charge-transfer chromatography carried out on reversed-phase thin-layer chromatographic layers is a suitable method to study the interaction of antibiotics with CTAB. The large differences between the relative strengths of interaction indicate that the impact of the binding of antibiotics to CTAB on the biological efficiency of the pharmaceutical formulations may be different and it has to be determined separately for each antibiotic.

3. Experimental

The common names of the antibiotics are shown in Table 1. DC-Alufolien Aluminiumoxide 60 F_{254} (Merck, Darmstadt, Germany) were impregnated by overnight predevelopment in n-hexane paraffin oil (95 : 5, v/v). Paraffin oil forms a hydrophobic layer on the plates which is insoluble in the methanol and water, eluents which were used. Exhaustive extraction of the paraffin coated-alumina supports with n-hexane indicated that alumina adsorbs 2.5% paraffin oil w/w. The antibiotics were separately dissolved in methanol to give a concentration of 5 mg/ml and 2 µl of solution was spotted on to the plates. As the object was to study complex formation between the antibiotics and CTAB and not the effect of CTAB on the separation of antibiotics, the antibiotics were separately spotted on the plates. Methanol-water mixtures were used as eluents with the methanol concentration varying between 0–85 vol.% in steps of 5 vol.%. This wide range of methanol concentration was used because of the highly different hydrophobicity of the antibiotics. CTAB was dissolved in the eluent in the concentration range of 0–15 mg/ml. Development was performed in sandwich chambers ($22 \times 22 \times 3$ cm) at room temperature, and the running distance was ca 15 cm. The chambers were not presaturated. After development the plates were dried at room temperature and the spots were detected under UV light or by iodine vapour. Each determination was run in quadruplicate. The R_M value characterizing the molecular lipophilicity in reversed-phase thin-layer chromatography was calculated for each antibiotic in each eluent:

$$R_M = \log (1/R_f - 1) \quad (1)$$

where R_f is the distance of the solute from the start divided by the distance of the eluent front from the start.

To separate the effects of methanol and CTAB on the lipophilicity of antibiotics the following equation was fitted to the experimental data:

$$R_M = R_{M0} + b_1 \cdot C_1 + b_2 \cdot C_2 \quad (2)$$

where $R_M = R_M$ value for an antibiotic determined at given methanol and CTAB concentrations; $R_{M0} = R_M$ value extrapolated to zero methanol and CTAB concentrations (related to the hydrophobicity of antibiotics) [15, 16]; b_1 = change in the R_M value caused by 1% increase in methanol concentration in the eluent (related to the specific hydrophobic surface area of antibiotics) [17]; b_2 = change in the R_M value caused by 1 mg/ml concentration change of CTAB in the eluent (related to the relative strength of interaction); and C_1 and C_2 = concentrations of methanol and CTAB, respectively. Eq. 2 was applied separately for each drug. When the coefficient of variation of the parallel determinations was higher than 6%, the data were omitted from the calculations.

Similar methods have recently been employed for the determination of the interaction of anionic surfactants with hydroxypropyl-β-cyclodextrin [18], antibiotics with sodium dodecylsulfate [19], antidepressant drugs and metabolites with cyclodextrins [20], etc.

To test the validity of the hypothesis that for homologous series of solutes the lipophilicity (R_{M0}) and the specific hydrophobic surface area (b_1) are strongly intercorrelated [21, 22] linear correlation was calculated between these parameters.

To find which physicochemical parameters of antibiotics significantly influence their interaction with CTAB, stepwise regression analysis was applied [23]. The relative strength of antibiotic – CTAB interaction (b_2) was the dependent variable, while the hydrophobicity (R_{M0}) and specific hydrophobic surface area (b_1) of eq. 2 were the independent variables. The number of independent variables accepted was not limited and the acceptance limit was set to the 95% significance level.

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