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Charge-transfer chromatographic study of the interaction of antibiotics with sodium dodecylsulfate[1]

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Abstract

The interaction of 29 antibiotics with the anionic surfactant sodium dodecylsulfate (SDS) was studied by charge-transfer reversed-phase chromatography carried out on impregnated silica layers using water–methanol mixtures as eluents. The hydrophobicity of antibiotics and the relative strength of SDS–antibiotic interaction was calculated separately for each antibiotic–SDS pair. SDS interacted with 17 antibiotics where the antibiotic–SDS complex was either more hydrophillic or more hydrophobic than the uncomplexed molecule. The relative strength of interaction depended considerably on the molecular structure of the antibiotics. No significant linear correlation was found between the hydrophobicity parameters of antibiotics and their capacity to interact with SDS. Stepwise regression analysis proved that the inductive effect of substituents, their electron-withdrawing power and proton-acceptor capacity exert a significance influence on the strength of interaction. © 1997 Elsevier Science B.V.

Keywords: Antibiotics; Sodium dodecylsulfate; Charge-transfer chromatography

1. Introduction

Due to its advantageous physicochemical properties sodium dodecylsulfate (SDS) has been extensively used in the formulation of various bioactive compounds such as pesticides [1], disinfectants [2] and pharmaceuticals [3,4]. It has been established that SDS promotes the solubilization of clofazimine analogues in aqueous solutions [5], interacts with griseofulvin [6,7], improves the intestinal absorption of the anthelmintic albendazole [8], improves the dissolution rate of sparingly soluble drugs [9,10], influences the photolytic degradation of gramicidin A [11] and modifies the structure of interleukin-2 [12].

SDS in itself exerts marked biological activities. Thus, it has an immunomodulatory effect [13], causes secondary structural changes in proteins and protein fragments [14,15] and modifies the degree of dissociation of various bioactive complexes [16,17]. Due to its capacity to bind to proteins SDS exert a considerable influence on the activity of a wide variety of enzymes such as latent potato leaf polyphenol oxidase [18], lecithin:cholesterol acyltransferase [19], ATP-ase of *P*-glycoprotein [20] and β-galactosidase [21].

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Besides its beneficial effects SDS has some side effects too. It shows marked cutaneous [22] and ocular irritancy [23] and can cause environmental pollution in rivers and river sediments [24,25]. The hydrophobic or hydrophillic character of the interaction of SDS with other molecules has not been extensively studied and the results strongly depend on the molecules in interaction with SDS. Thus, hydrophillic interaction was found between the polar head groups of SDS and the heterocyclic ring of papaverine [26], whereas the decisive role of hydrophobic forces in the interaction of SDS with nonionic surfactants has been emphasized [27].

Chromatographic methods, especially liquid chromatography, have been frequently used for the study of various molecular interactions. The various applications have been recently reviewed [28,29]. 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 or 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 indication of the strength of interaction. Commonly the retention of the more hydrophobic molecule is determined under reversed-phase conditions and the more hydrophillic interactive partner is added to the eluent in various concentrations.

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

2. Experimental

The common and IUPAC names of antibiotics are shown in Table 1. DC-Fertigplatten KIESELGEL 60 F_{254} (Merck, Germany) were impregnated by overnight predevelopment in *n*-hexane–paraffin oil (95:5, v/v). The plates are prepared from silica (particle size 20–50 μm) containing a dye which fluoresceses at 254 nm. Paraffin oil forms a hydrophobic layer on the plates which is insoluble in the subsequently used eluents of methanol and water. Exhausting extraction of the paraffin coated-silica supports with *n*-hexane indicated that silica adsorbs 5% paraffin oil w/w. The antibiotics were separately dissolved in methanol to give a concentration of 5 mg ml^{-1} and 2 μl of solution was spotted on to the plates. As the object was to study the complex formation between the antibiotics and SDS and not the study of the effect of SDS 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.%. The use of this wide range of methanol concentration was because of the highly different hydrophobicity of the antibiotics. SDS was dissolved in the eluent in the concentration range of 0–15 mg ml^{-1}. Development was performed in sandwich chambers (22 × 22 × 3 cm^3) 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 lipophillicity in reversed-phase thin-layer chromatography was calculated for each antibiotics 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.

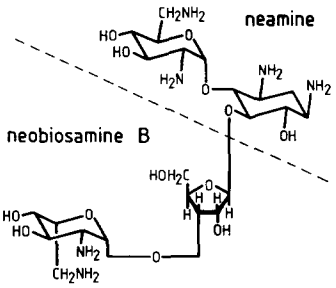
To separate the effects of methanol and SDS on the lipophillicity of antibiotics the following equation was fitted to the experimental data:

$$R_M = R_{M0} + b_1 \cdot c_1 + b_2 \cdot c_2 \quad (2)$$

where $R_M = R_M$ value for an antibiotics determined at given methanol and SDS concentrations; $R_{M0} = R_M$ value extrapolated to zero methanol and SDS concentrations (related to the hydrophobicity of antibiotics) [30,31]; b_1 = decrease in the R_M value caused by 1% increase in methanol concentration in the eluent (related to the specific hydrophobic surface area of antibiotics) [32]; b_2 =

Table 1
Common and IUPAC names of antibiotics

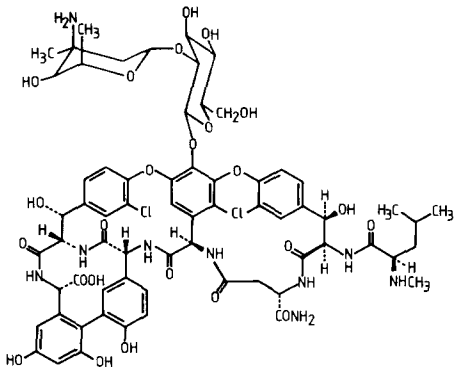
No.	Common name	IUPAC name
1	Ampicillin	6-[(Aminophenylacetyl)amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid
2	Antimycin	4,6-Dihydro-8-hydroxy-3,4,5-trimethyl-6-oxo-3H-2-benzopyran-7-carboxylic acid
3	Cefotaxime	3-[(Aceyloxy)methyl]-7-[[(2-amino-4-thiazolyl) (methoxyimino)acetyl]amino]-8-oxo-5-thial-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid
4	Cephalexin	7-[(Aminophenylacetyl)amino]-3-methyl-8-oxo-5-thia-1-azabicyclo[4.3.0]oct-2-ene-2-carboxylic acid
5	Cephalotin	3-[(Acetyloxy)methyl]-8-oxo-7-[(2-thienylacetyl) amino]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-carboxylic acid
6	Chloram-phenicol	2,2-Dichloro-*N*-[2-hydroxy-1-(hydroxymethyl)-2-(4-nitrophenyl)ethly]acetamide
7	Cycloheximide	4-2-(3,5-Dimethyl-2-oxocyclohexyl)-2-hydroxyethyl]-2,6-piperidinedione
8	Doxycycline	4-(Dimethylamino) -1,4,4α,5,5α,6,11,12α-octa-hydro-3,4,10,12,12α-pentahydroxy-6-methyl-1,11-dioxo-2-naphtacenecarboxamide monohydrate
9	Erythromycin	14-Ethyl-7,12,13-trihydroxy-3,4,7,9,11,13-hexamethyk-2,10-dioxo-6-[[3,4,6-trideoxy-3-(dimethylamino)-β-xylohexopyranosyl]oxy] -oxacyclotetradec-4-yl-2,6-dideoxy-3-C-methyl-2-*O*-methyl-α-L-ribohexopyranoside
10	Gentamycin	*O*-2-Amino-2-deoxy-α-D-glucopyranosyl-(1,4) -*O*-[3-deoxy-3-(methylamino)-α-D-xylopyranosyl-(1,6)-2-deoxy-D-streptamine
11	Gramicidin	HCO-L-Val-Gly-L-Ala-D-Leu-L-Ala-D-Val-L-Val-D-Val-[L-Trp-D-Leu]$_3$-L-Trp-NHCH$_2$CH$_2$OH
12	Griseofulvin	7-Chloro-2,3,6-trimethoxy-6-methylspiro-[benzofuran-2(3H),1-[2]cyclohexene]-3,4-dione
13	Kanamycin	*O*-3-Amino-3-deoxy-α-D-glucopyranosyl-(1-6)-*O*-[6-amino-6-deoxy-α-D-glucopyranosyl-(1-4)] -2-deoxy-D-streptamine
14	Kasugamycin	3-*O*-[2-Amino-4-[carboxyiminomethylamino)-2,3,4,6-tetradeoxy-α-D-arabinohexapyranosyl] -D-chiroinositol
15	Methycillin	6-(2,6-Dimethoxybenzamido)-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo-[3.2.0]-heptane-2-carboxylic acid
16	Nalixidic acid	1-Ethyl-1,4-dihydro-7-methyl-4-oxo-1,8-naph-tyridine-3-carboxylic acid
17	Nalixidic acid ethylester	1-Ethyl-1,4-dihydro-7-methyl-4-oxo-1,8-naph-tyridine-3-carboxylic acid ethylester
18	Neomycin	



19	Nigericin	Tetrahydro-6-[[9-methoxy-2,4,10-trimethyl-2-[octohydro-2,3-dimethyl-5-[etrahydro-6-hydroxy-6-(hydroxymthyl)-3,5-dimethyl-2H-pyrane-2-yl][2,2-bifurane]-5-yl]-1,6-dioxaspiro[[4.5]dec-7-yl]methyl] -α,3-dimethyl-2H-pyrane-2-acetic acid
20	Novobiocin	*N*-[7-[[3-*O*-(Aminocarbonyl)-6-deoxy-5-C-methyl-4-*O*-methyl-β-L-lyxopyranosyl]oxy] -4-hydroxy-8-methyl-2-oxo-2H-1-benzopyran-3-yl]-4-hydroxy-3-(3-methyl-2-butenyl)benzamide

Table 1 (continued)

No.	Common name	IUPAC name
21	OCT	4-(Dimethylamino)
		-1,4,4α,5,5α,6,11,12α-octa-hydro-3,5,6,10,12,12α-hexahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide
22	Oxytetracycline	4-(Dimethylamino)
		-1,4,4α,5,5α,6,11,12α-octa-hydro-3,5,6,10,12,12α-hexahydroxy-6-methyl-1,11-dioxo-2-naphtacenecarboxamide
23	Penicillin G	3,3-Dimethyl-7-oxo-6-[(phenylacetyl)amino]-4-thia-1-azabicyclo[3.2.0]heptane-2-caroxylic acid
24	Polymixin B	(+)-6-Methyloctanoyl-L-DAB(τ-NH_2)-L-Thr-L-DAB-L-DAB-L-DAB(τ-NH_2)
		-D-Phe-L-Leu-L-DAB(τ-NH_2)-L-DAB(τ-NH_2)-L-Thr DAB = α, τ-diaminobutyric acid
25	Puromycin	3-[[Amino-3-(4-methoxyphenyl)-1-oxopropyl]amino]-3-deoxy-*N*,*N*-dimethyladenosine
26	Rifamycin SV	5,6,9,17,19,21-Hexahydroxy-23-methoxy-2,4,12,16,18,20,22-heptamethyl-2,7-(epoxypentadeca[1,11,13]
		trienimino)naphtol[2,1-b]furan-1,11(2H)-dione-21-acetate
27	Tobramycin	*O*-3-Amino-3-deoxy-α-D-glucopyranosyl-(1-6)
		-O-[2,6-diamino-2,3,6-trideoxy-α-D-ribohexopyra-nosyl-(1-4)]-2-deoxy-D-streptamine
28	Trichotecin	12,13-Epoxy-4-[(1-oxo-2-butenyl)-oxy]tricho-tech-9-en-8-one
29	Vancomycin	



decrease in the R_M value caused by 1 mg ml^{-1} concentration change of SDS in the eluent (related to the relative strength of interaction); and C_1 and C_2 are the concentration of methanol and SDS, 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.

To find the physicochemical parameters of antibiotics significantly influencing their interaction with SDS, stepwise regression analysis was applied [33]. The relative strength of antibiotics–SDS interaction (b_2) was the dependent variable, whereas the hydrophobicity (R_{M0}) and specific hydrophobic surface area (b_1) of Eq. (2) were the independent variables. The number of acceptance limit was set to the 95% significance level. As no significant correlation was found between the measured hydrophobicity parameters of antibiotics and their capacity to interact with SDS some polarity parameters of the hydrophillic substructures of the antibiotics were calculated and correlated with the relative strength of interaction using stepwise regression analysis under the same conditions as mentioned above. The polarity parameters included in the calculation were H–

AC and H–Do, indicator variables for proton acceptor and proton donor properties, respectively; F and R are electronic parameters characterizing the inductive and resonance effect, respectively and σ is Hammett's constant, characterizing the electron-withdrawing power of the substituent.

3. Results and discussion

The spot of neomycin (compound **18**) was very near to the eluent front even in water as eluent indicating that neomycin is a highly hydrophillic drug and this method is not suitable for the determination of its interaction with SDS. Polymixin B (compound **24**) remained at the start on each eluent system therefore the SDS–polymixin interaction cannot be determined under the chromatographic conditions applied.

The simultaneous effect of methanol and SDS concentrations on the R_M values of antibiotics gramicidin and cepaphalotin (compounds **11** and **5** in Table 1) are shown in Figs. 1 and 2, respectively. SDS in the eluent may decrease (Fig. 1) or increase (Fig. 2) the retention of individual antibiotics. This phenomenon suggests that the drug–SDS complex can be more or less hydrophobic than the uncomplexed drug molecule. The modification of the hydrophobicity of antibiotics may result in different penetration rate, mobility, adsorption capacity, and decomposition rate of the drug, thereby enhancing or lessening its biological efficiency.

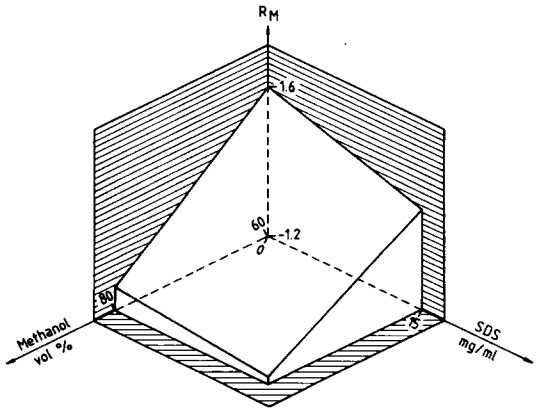


Fig. 1. Effect of methanol and SDS concentrations in the eluent on the R_M value of gramicidin (compound **11**).

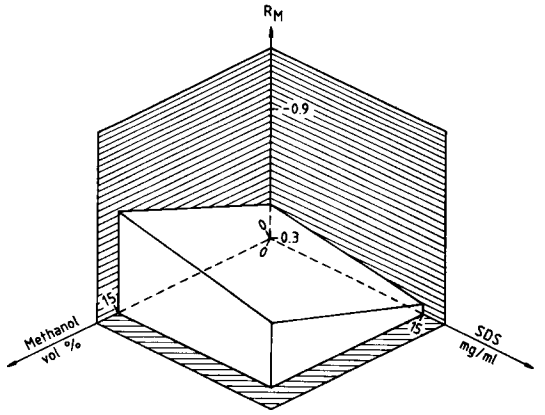


Fig. 2. Effect of methanol and SDS concentrations in the eluent on the R_M value of cephalotin (compound **5**).

The parameters of Eq. (2) are compiled 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 well to the experimental data (see $F_{calc.}$ values), the significance level in each instance being over 95%. The ratios of variance explained were about 37–98% (see r^2 values). The parameters of Eq. (2) differ considerably, demonstrating that the lipophillicity (R_{M0}), specific hydrophobic surface area (b_1) and the capacity of antibiotics to form complexes with SDS (b_2) differ considerably. In most cases methanol has a higher impact than SDS on the mobility of antibiotics (see path coefficient, b% values). The majority of antibiotics interacts with SDS (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 SDS may influence the biological efficiency of the individual drugs in different ways. No significant linear correlation was found between the hydrophobicity parameters of antibiotics and their capacity to interact with SDS indicating that other than hydrophobic forces are involved in the inter-

Table 2
Relationship between the R_M values of antibiotics and the concentrations of methanol (C_1) and sodium dodecylsulfate (C_2) in the eluent

Parameter	$R_M = R_{M0} + b_1 \times c_1 + b_2 \times c_2$ No. of antibiotics							
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
n	11	12	14	10	13	18	14	13
R_{M0}	0.51	1.69	−0.33	0.41	−0.02	0.44	0.94	2.20
$-b_1 \times 10^2$	4.90	−1.73	—	4.91	4.63	1.85	2.47	2.21
$s_{b1} \times 10^3$	15.44	6.20	—	13.97	9.11	2.61	3.95	5.39
$-b_2 \times 10^2$	—	—	−3.43	—	−1.77	1.56	—	2.03
$s_{b2} \times 10^3$	—	—	12.88	—	7.36	6.73	—	5.90
b_1%	—	—	—	—	67.87	75.52	—	54.30
b_2%	—	—	—	—	32.13	24.48	—	45.70
r^2	0.5019	0.4386	0.3707	0.6066	0.8409	0.7921	0.7652	0.6671
F_{calc}	10.07	7.81	7.07	12.33	26.07	26.67	39.12	10.02
	9	**10**	**11**	**12**	**13**	**14**	**15**	**16**
n	11	8	16	13	9	11	9	15
R_{M0}	1.74	0.58	7.99	2.71	−0.04	0.80	0.15	1.06
$-b_1 \times 10^2$	—	3.57	10.67	4.53	3.98	—	4.53	4.10
$s_{b1} \times 10^3$	—	3.57	10.67	4.53	3.98	—	4.53	4.10
$s_{b1} \times 10^3$	—	12.13	3.48	6.32	15.06	—	7.86	4.95
$-b_2 \times 10^2$	7.46	—	3.87	7.48	—	2.78	—	—
$s_{b2} \times 10^3$	3.66	—	4.97	6.91	—	3.37	—	—
b_1%	—	—	79.80	39.80	—	—	—	—
b_2%	—	—	20.20	60.20	—	—	—	—
r^2	0.9788	0.5903	0.9885	0.9254	0.4989	0.8830	0.8059	0.8300
F_{calc}	414.78	8.65	559.48	62.07	6.97	67.93	33.21	68.35
	17	**19**	**20**	**21**	**22**	**23**	**25**	
n	13	11	17	13	14	9	12	
R_{M0}	1.40	1.56	1.17	2.67	1.82	0.00	2.74	
$-b_1 \times 10^2$	2.54	—	3.80	5.18	3.75	3.38	3.76	
$s_{b1} \times 10^3$	3.18	—	4.79	7.25	6.99	10.23	10.23	
$-b_2 \times 10^2$	2.26	3.94	2.15	—	−2.14	—	−3.91	
$s_{b2} \times 10^3$	3.48	8.20	9.31	—	9.14	—	11.15	
b_1%	55.08	—	77.49	—	69.61	—	51.16	
b_2%	44.92	—	22.51	—	30.39	—	48.84	
r^2	0.8817	0.7193	0.8344	0.8232	0.7852	0.6092	0.6429	
F_{calc}	37.27	23.06	37.79	51.22	20.10	10.90	8.10	
	26	**27**	**28**	**29**				
n	18	11	13	13				
R_{M0}	1.40	1.79	2.06	0.42				
$-b_1 \times 10^2$	5.57	—	3.44	−1.78				
$s_{b1} \times 10^3$	9.16	—	5.10	7.76				
$-b_2 \times 10^2$	6.37	7.30	5.26	5.06				
$s_{b2} \times 10^3$	18.52	10.38	5.57	8.48				
b_1%	63.90	—	42.45	27.81				
b_2%	36.10	—	57.55	72.19				
r^2	0.6987	0.4386	0.9080	0.8683				
F_{calc}	18.55	7.81	49.36	32.96				

Numbers refer to antibiotics in Table 1. 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 SDS—antibiotic interaction.

action of antibiotics with SDS. Stepwise regression analysis selected three polarity parameters influencing significantly SDS–antibiotic interaction:

$$b = 10.54 - (3.73 \pm 1.22) \cdot \mathrm{H-Ac} - (17.20 \pm 2.75) \cdot F + (4.05 \pm 1.06 \cdot \sigma)$$

$$r^2 = 0.8298 \quad F_{calc} = 17.88 \quad F_{99.9\%} = 11.56 \qquad (3)$$

The equation fits well to the experimental data, the significance level being over 99.9% (compare calculated and tabulated F values). The change of the three independent variables explains about 83% of the change of the SDS–antibiotic interaction (see r^2 value). The values of path coefficients indicated that the inductive effect of substituents (F) has the highest impact on the strength of interaction (52.39%) followed by the electron-withdrawing power of the substituents (σ, 31.96%) and by their proton-acceptor capacity (H–Ac, 15.65).

It can be concluded from the results of stepwise regression analysis that the polar head group of SDS can interact with the hydrophillic substructures of the antibiotics and the binding of the apolar hydrocarbon chain to the hydrophobic parts in the antibiotic molecules has a negligible effect on the interaction. The effect observed is probably the result of the interplay of the various polar interactive forces such as electrostatic interactions and hydrogen bond formation. The interaction between SDS and antibiotics suggests that this interaction may have a marked influence on the biological efficiency of any pharmaceutical formulations simultaneously containing SDS 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 for studying the interaction of antibiotics with SDS. The big differences between the relative strengths of interactions indicate that the impact of the binding of antibiotics to SDS on the biological efficiency of the pharmaceutical formulations may be different and has to be determined separately for each antibiotics.

Acknowledgements

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