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Interaction of Some Antibiotics with Hydroxypropyl-β-cyclodextrin*

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Abstract. The interaction between 19 antibiotics and hydroxypropyl-β-cyclodextrin (HPβCD) was studied by reversed-phase thin-layer chromatography. HPβCD formed inclusion complexes with 16 compounds, the complex always being more hydrophilic than the uncomplexed drug. The intensity of interaction significantly increased with increasing specific hydrophobic surface area of the guest molecule proving the preponderant role of hydrophobic interactions in inclusion complex formation. The intensity of the HPβCD-drug interaction significantly decreased with increasing concentration of methanol in the environment indicating that methanol can also enter the cyclodextrin cavity and inhibits competitively the inclusion complex formation or the free energy of transfer from water to the HPβCD cavity should be less negative at higher concentration of methanol in the aqueous medium.

Key words: Hydroxypropyl-β-cyclodextrin, antibiotics, hydrophobic interactions.

1. Introduction

Cyclodextrins (CDs) are cyclic oligosaccharides which have the ability to form inclusion complexes with many organic and inorganic compounds of various chemical structures [1, 2]. CDs readily form inclusion complexes with many drugs such as steroids [3, 4], antimycotic agents [5], insulin [6, 7], anticancer drugs [8] etc. The inclusion complex formation modifies the physicochemical characteristics of guest molecules, it improves the performance of intravenous formulation [9], prolongs the pulmonary absorption of sulbutanol [10], sustains the release rate of drugs [11], increases the stability of the guest molecule [12], enhances the peak concentration of drugs in blood [13] and improves bioavailability [14] etc.

The character of binding between the host and guest molecules is extensively discussed. It is assumed that van der Waals dipole-dipole and hydrophobic interactions [15, 16] as well as hydrogen bond formation [17] are involved in the inclusion complex formation.

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Reversed-phase-thin-layer chromatography has been successfully applied to study many biologically important interactions [18, 19]. The method is rapid and does not need complicated instrumentation.

The objectives of this work were to study the interaction of some antibiotics with hydroxypropyl-β-cyclodextrin (HPβCD) by means of reversed-phase chromatography, and to elucidate the role of environmental conditions and molecular parameters in the inclusion complex formation. The choice of HPβCD for this study was motivated by the fact that the hydroxypropyl CD derivatives are more soluble in water than the unmodified CDs and, furthermore, their complexes do not precipitate.

2. Materials and Methods

The chemical names of the antibiotics investigated are listed in Table I. Nigericin was purchased from BIOGAL Pharmaceutical Works (Debrecen, Hungary) whereas the other antibiotics were the products of Sigma Chemie GmbH (Deisenhofen, Germany). Polygram UV_{254} plates (Machery-Nagel, Dürren, Germany) were impregnated by overnight predevelopment in n-hexane-paraffin oil (95 : 5l v/v). The solutes were separately dissolved in methanol to give a concentration of 5 mg/mL and 3 μL of solution was spotted on to the plates. As the object was to study the complex formation between the solutes and HPβCD and not the study of the effect of HPβCD on the separation of solutes, they were separately spotted on the plates. In this way the ratio HPβCD : solute was the same for each compound. Methanol was chosen as the organic solvent miscible with water because it forms only weak inclusion complexes with β-cyclodextrins [20, 21]. Methanol was incorporated in the eluent in the concentration range 0–45 vol. % in steps of 5 vol. %. HPβCD was dissolved in the eluent in the concentration range of 0–12.5 mg/mL in steps of 2.5 mg. Development was performed in sandwich chambers (22 × 22 × 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 at 254 nm and by iodine vapour. Each determination was run in quadruplicate. The R_M value given by $\log(1/R_f - 1)$, which characterizes the molecular lipophilicity in reversed-phase-thin-layer chromatography was calculated for each drug and eluent system.

To separate the effects of methanol and HPβCD on the lipophilicity of solutes and to take into consideration the possible interaction between methanol and HPβCD, the following equation was fitted to the experimental data:

$$R_M = R_{M0} - b_1 \cdot C_1 - b_2 \cdot C_2 + b_3 \cdot C_1 \cdot C_2 \quad (1)$$

where R_M is the R_M value for a solute determined at given methanol and HPβCD concentrations; R_{M0} is the R_M value extrapolated to zero methanol and HPβCD concentrations; b_1 is the decrease in the R_M value caused by a 1% increase in

TABLE I. Chemical names of antibiotics.

Number	Common name	Chemical name
1	Ampicillin	6-[(Aminophenylacetyl))amino]-3,3-dimethyl-7-oco-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid
2	Cephalothin	3-[(Acetyloxy)methyl]-8-oxo-7-[(2-hienylacetyl)amino]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid
3	Chloramphenicol	2,2-Dichloro-*N*-[2-hydroxy-1-(hydroxymethyl)-2-(4-nitrophenyl)ethyl]acetamide
4	Cycloheximide	4-[2-(3,5-Dimethyl-2-oxocyclohexyl)-2-hydroxyethyl]-2,6-piperidinedione
5	Doxycycline	4-(Dimethylamino)-1,4,4α,5,5α,6,11,12α-octahydro-3,5,10,12,12α-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide monohydrate
6	Erythromycin	14-Ethyl-7,12,13-trihydroxy-3,5,7,9,11,13-hexamethyl-2,10-dioxo-6-[[3,4,6-trideoxy-3-(dimethylamino)-β-xylohexopyranosyl]oxy]-oxacyclotetradec-4-yl-2,6-dideoxy-3-*C*-methyl-3-*O*-methyl-α-L-ribohexopyranoside
7	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
8	Kasugamycin	3-*O*-[2-Amino-4-[(carboxyiminomethylamino]-2,3,4,6-tetradeoxy-α-D-arabinohexapyranosyl]-D-chiroinositol
9	Methycillin	6-(2,6-Dimethoxybenzamido)-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo-[3.2.0]-heptane-2-carboxylic acid
10	Nalidixic acid	1-Ethyl-1,4-dihydro-7-methyl-4-oxo-1,8-naphthyridine-3-carboxylic acid
11	Nigericin	Tetrahydro-6-[[9-methoxy-2,4,10-trimethyl-2-[octohydro-2,3-dimethyl-5-[tetrahydro-6-hydroxy-6-(hydroxymethyl)-3,5-dimethyl-2H-pyrane-2-yl][2,2-bifurane]-5-yl]-1,6-dioxaspiro[[4.5]dec-7-yl]methyl]-α,3-dimethyl-2*H*-pyrane-2-acetic acid
12	Novobiocin	*N*-[7-[[3-*O*-(Aminocarbonyl)-6-deoxy-5-*C*-methyl-4-*O*-methyl-β-L-lyxopyranosyl]oxy]-4-hydroxy-8-methyl-2-oxo-2*H*-1-benzopyran-3-yl]-4-hydroxy-3-(3-methyl-2-butenyl)benzamide
13	Oxacillin	[[(5-Methyl-3-phenyl-4-isoxazolyl)carbonyl]-amino]-33,-dimethyl-6-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid
14	Oxolinic acid	5-Ethyl-5,8-dihydro-8-oxo-1,3-dioxolo[4,5-*g*]-quinoline-7-carboxylic acid

TABLE I. Continued.

Number	Common name	Chemical name
15	Oxytetracyclin	4-(Dimethylamino)-1,4,4α,5,5α,6,11,12α-octahydro-3,5,6,10,12,12α-hexahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide
16	Penicillin G	3,3-Dimethyl-7-oxo-6-[(phenylacetyl)amino]-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid
17	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)naphthol[2,1-*b*]furan-1,11(2*H*)-dione-21-acetate
18	Streptomycin	*O*-2-deoxy-2-(methylamino)-α-L-glucopyranosyl--(1-2)-*O*-5-deoxy-3-*C*-formyl-α-L-lyxofuranosyl-(1-4)-*N*,*N*,bis(aminoiminomethyl)-D-streptamine
19	Tetracyclin	4-(Dimethylamino)-1,4,4α,5,5α,6,11,12α-octahydro-3,6,10,12,12α-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide

methanol concentration in the eluent (related to the specific hydrophobic surface area of drugs) [22]; b_2 is the decrease in the R_M value caused by a 1 mg/mL concentration change of HPβCD in the eluent (related to the relative strength of interaction) [23]; b_3 is the indicator of the impact of the methanol-HPβCD interaction on the R_M value; C_1 and C_2 are the concentrations of methanol and HPβCD, respectively. Equation 1 was applied separately for each compound. When the coefficient of variation of the parallel determinations was higher than 6%, the data were omitted from the calculations.

In order to test the validity of the hypothesis that in the case of homologous series of solutes the slope and intercept values (b_1 and R_{M0} in Equation 1) are strongly intercorrelated [24, 25] a linear correlation was calculated between the two physicochemical parameters:

$$R_{M0} = A + B \cdot b_1 \,. \tag{2}$$

For the elucidation of the role of molecular hydrophobicity in the inclusion complex formation of solutes a linear correlation was calculated between the lipophilicity (R_{M0}), specific hydrophobic surface area (b_1) and the relative strength of interaction (b_2):

$$b_2 = A + B_1 \cdot R_{M0} + B_2 \cdot b_1 \,. \tag{3}$$

The influence of the chromatographic parameters on the impact of methanol on the complex formation (b_3) was calculated by the following equation:

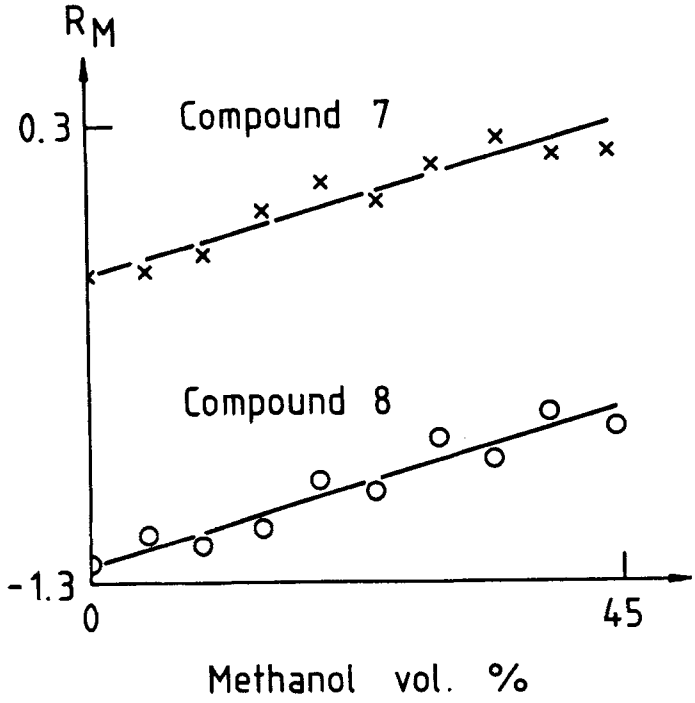

Fig. 1. Relationship between the lipophilicity (R_M) of compounds **7** and **8** and the methanol concentration in the eluent. See Table I for the chemical structures.

$$b_3 = A + B_1 \cdot R_{M0} + B_2 \cdot b_1 + B_3 \cdot b_2 \,. \tag{4}$$

The parameters of Equations 3 and 4 were calculated by stepwise regression analysis [26]. In the common multivariate regression analysis the presence of independent variables exerting no significant influence on the change of dependent variable considerably decreases the significance level of the equation. Stepwise regression analysis eliminates from the selected equation the dependent variables having no significant impact on the dependent variable thus increasing the reliability of the calculation.

3. Results and Discussion

Nigericin remained on the start in each eluent system, indicating that this compound is highly lipophilic and its interaction with HPβCD can be determined under the experimental conditions used.

Compounds **7** and **8** showed anomalous retention behaviour (Figure 1); their retention increased with increasing concentration of methanol in the eluent. This phenomenon can be tentatively explained in terms of a silanophilic effect: at higher methanol concentrations, the solute molecules have an enhanced probability of

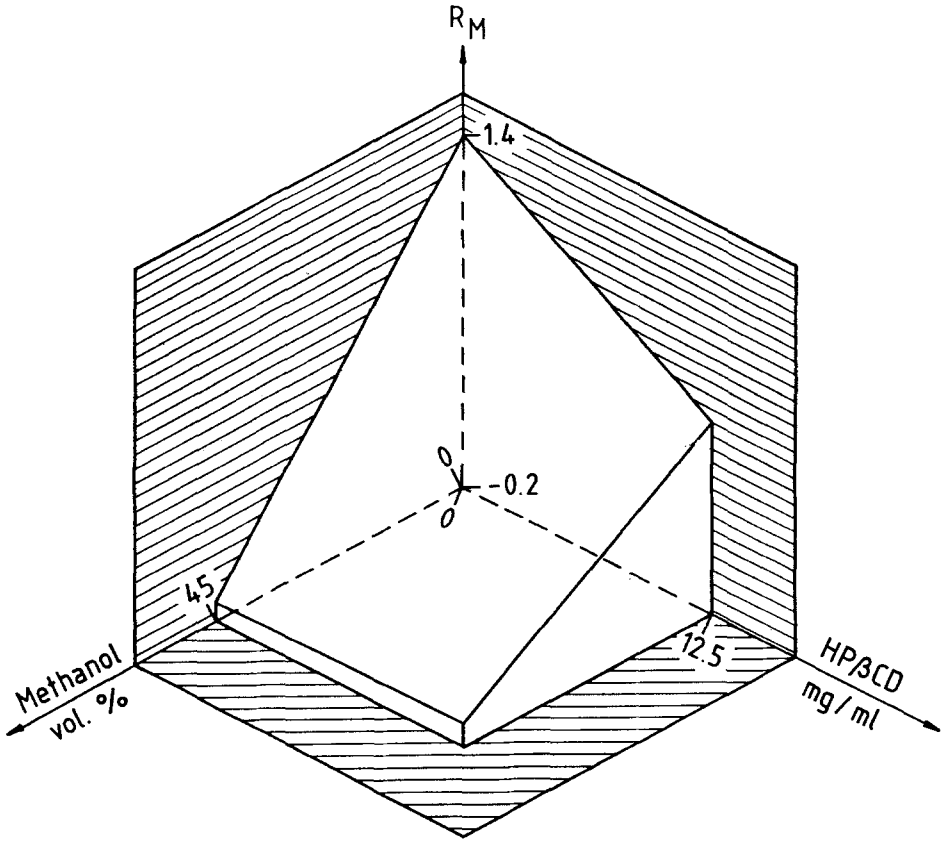

Fig. 2. The effect of methanol and hydroxypropyl-β-cyclodextrin concentration on the R_M value of compound **4**. See Table I for the chemical structures.

access to the silanol groups uncovered by the impregnating agent. The interaction with the free silanol groups results in an increased retention and an increased apparent lipophilicity [27]. Due to the anomalous retention behaviour of **7** and **8** their interaction with HPβCD cannot be determined with reversed-phase chromatography.

The simultaneous effects of methanol and HPβCD concentrations on the R_M values of compound **4** are shown in Figure 2. The R_M values decrease in each instance with increase in methanol concentration, i.e., this compound does not show any anomalous retention behaviour in this concentration range that would invalidate the evaluation using Equation 1. An increase in HPβCD concentration also caused a decrease in R_M values, indicating complex (probably inclusion complex) formation. Interaction of the more hydrophilic HPβCD with the antibiotic reduces the lipophilicity of the latter. This finding suggests that the biological

TABLE II. Parameters of multilinear correlations between the R_M values of antibiotics and the concentrations of methanol (C_1) and hydroxypropyl-β-cyclodextrin (C_2) in the eluent. Numbers refer to the antibiotics in Table I. n.s. = not significant.

$$R_M = R_{M0} - b_1 \cdot C_1 - b_2 \cdot C_2 + b_3 \cdot C_1 \cdot C_2$$

Parameter	No of Antibiotics				
	1	**2**	**3**	**4**	**5**
n	16	15	17	26	29
R_{M0}	0.78	0.76	0.75	1.41	1.95
$b_1 \cdot 10^{-2}$	2.87	3.36	2.18	3.40	2.96
$s_{b1} \cdot 10^{-3}$	1.68	1.53	1.29	1.21	1.47
$b_2 \cdot 10^{-2}$	3.41	3.19	3.13	5.73	7.91
$s_{b2} \cdot 10^{-3}$	5.38	5.14	4.13	3.81	5.17
$b_3 \cdot 10^{-3}$	1.10	1.43	7.18	13.42	1.75
$s_{b3} \cdot 10^{-4}$	2.49	4.97	1.72	1.51	2.06
b_1 %	60.45	68.72	57.68	54.40	44.88
b_2 %	23.43	22.68	26.52	26.79	32.80
b_3 %	16.12	8.60	15.80	18.81	22.32
$F_{calc.}$	125.25	189.82	139.74	508.99	222.73
r^2	0.9666	0.9794	0.9677	0.9852	0.9626
	6	**7**	**8**	**9**	**10**
n	28	15	15	15	28
R_{M0}	2.03	−0.23	−1.26	0.90	1.80
$b_1 \cdot 10^{-2}$	3.02	−1.13	−1.17	3.22	3.09
$s_{b1} \cdot 10^{-3}$	1.42	2.12	1.85	1.25	1.31
$b_2 \cdot 10^{-2}$	3.17	n.s.	n.s.	4.22	2.22
$s_{b2} \cdot 10^{-3}$	5.16	–	–	4.20	4.75
$b_3 \cdot 10^{-3}$	n.s.	n.s.	n.s.	0.98	n.s.
$s_{b3} \cdot 10^{-4}$	–	–	–	4.05	–
b_1 %	77.59	–	–	64.69	83.54
b_2 %	22.41	–	–	29.54	16.46
b_3 %	–	–	–	5.77	–
$F_{calc.}$	228.55	28.62	40.03	238.36	280.36
r^2	0.9462	0.6715	0.7409	0.9835	0.9557

properties (adsorption, uptake, half-life, etc.) of antibiotic-HPβCD complexes may be different from that of uncomplexed antibiotics resulting in modified effectivity.

The parameters of Equation 1 are compiled in Table II. Blank sites in Table II indicate that these independent variables did not influence significantly the R_M value of the surfactant. The equation fits the experimental data well, the significance levels in each instance being over 99.9% (see calculated F values). The ratios of variance explained were about 67–98% (see r^2 values). The majority of antibiotics interact with HPβCD (b_2 values differ significantly from zero) indicating that in

pharmaceutical formulations containing both antibiotics and HPβCD their possible interaction has to be taken into consideration. The parameters of Equation 1 show high variations between the antibiotics proving that the lipophilicity (R_{M0}), specific hydrophobic surface area (b_1) and the capacity of antibiotics to form inclusion complexes with HPβCD (b_2) differ considerably. This finding suggests also that the inclusion complex formation may influence differently the biological effect of individual antibiotics. The complex forming capacity of antibiotics with HPβCD decreases considerably with increasing concentration of methanol in the eluent (see b_3 values). This result can be explained by the suggestion that methanol also forms inclusion complexes with HPβCD. This complex is probably very weak, however, methanol is present in higher concentration than the antibiotics influencing the competition for the HPβCD cavity. This competition results in decreasing stability of antibiotic-HPβCD complexes at higher methanol concentrations. However, the data can also be explained by the supposition that the free energy of transfer of an antibiotic from water to the cavity of HPβCD could be less negative at higher concentrations of methanol in the eluent. The path coefficients (b_i % values) indicate that the change of methanol and HPβCD concentrations has similar impact on the retention behaviour of antibiotics.

Significant linear correlation was found between the intercept (lipophilicity) and slope (specific hydrophobic surface area) values of antibiotics:

$$R_{M0} = -1.06 + (0.80 \pm 0.18) \cdot b_1 \cdot 10^2$$
$$n = 17 \quad r_{calc.} = 0.7430 \quad r_{99.9\%} = 0.7246 \tag{5}$$

where 1.06 and 0.80 are the A and B values of Equation 2.

The significant relationship between the two parameters indicates that, from a chromatographic point of view, these antibiotics behave as a homologous series of compounds, although their chemical structures are considerably different. This somewhat surprising result cannot be explained by the structural similarity of the solutes, and the elucidation of the molecular basis of the relationship discussed above need further investigation.

A significant linear relationship was found between the lipophilicity (R_{M0}) and complex forming capacity of antibiotics (b_2):

$$b_2 \cdot 10^2 = 1.63 + (2.54 \pm 1.16) \cdot R_{M0}$$
$$n = 15 \quad r_{calc.} = 0.5197 \quad r_{95\%} = 0.5139 \tag{6}$$

where 1.63 and 2.54 are the A and B_1 values of Equation 3 (B_2 was not significant).

The fact that more lipophilic antibiotics form more stable complexes with HPβCD proves that hydrophobic forces are involved in the binding of these antibiotics to the inner wall of the cyclodextrin cavity.

A significant linear correlation was found between the relative strength of antibiotic-HPβCD interactions (b_2) and the stability decreasing effect of methanol (b_3):

TABLE II. Continued.

Parameter	No of Antibiotics				
	12	**13**	**14**	**15**	**16**
n	25	28	26	28	15
R_{M0}	2.21	1.29	2.05	1.45	0.78
$b_1 \cdot 10^{-2}$	2.51	3.33	3.79	2.59	2.86
$s_{b1} \cdot 10^{-3}$	1.35	1.26	2.02	2.72	0.98
$b_2 \cdot 10^{-2}$	7.41	6.16	3.11	7.09	3.82
$s_{b2} \cdot 10^{-3}$	5.24	4.40	6.88	9.47	3.29
$b_3 \cdot 10^{-3}$	ns.s	1.38	0.72	1.46	0.76
$s_{b3} \cdot 10^{-4}$	–	1.75	2.50	3.76	3.18
b_1 %	56.66	53.81	71.25	44.89	64.89
b_2 %	43.34	27.39	16.68	33.78	30.07
b_3 %	–	18.80	12.07	21.33	5.04
$F_{calc.}$	201.50	408.93	337.34	53.44	306.23
r^2	0.9460	0.9800	0.9778	0.8651	0.9871

	17	**18**	**19**
n	2815	27	
R_{M0}	2.35	2.06	1.45
$b_1 \cdot 10^{-2}$	4.85	3.29	2.54
$s_{b1} \cdot 10^{-3}$	2.41	2.71	2.80
$b_2 \cdot 10^{-2}$	12.93	n.s.	6.90
$s_{b2} \cdot 10^{-3}$	8.38	–	9.56
$b_3 \cdot 10^{-3}$	2.50	n.s.	1.52
$s_{b3} \cdot 10^{-4}$	3.21	–	3.80
b_1 %	45.95	–	43.47
b_2 %	33.71	–	33.94
b_3 %	20.34	–	22.59
$F_{calc.}$	255.14	147.22	44.74
r^2	0.9684	0.9132	0.8483

$$b_3 = 0.39 + (0.16 \pm 0.02) \cdot b_2$$
$$n = 11 \quad r_{calc.} = 0.9187 \quad r_{99.9\%} = 0.8471 \tag{7}$$

where 0.30 and 0.16 are the A and B_2 values of Equation 4 (B_1 and B_3 were not significant).

This result indicates that the more stable the inclusion complex the more rapidly its stability deteriorates with increasing concentration of methanol in the environment.

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