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Inclusion complex formation of steroidal drugs with hydroxypropyl-β-cyclodextrin studied by charge-transfer chromatography[1]

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Abstract

The interaction between 17 steroidal drugs and hyroxypropyl-β-cyclodextrin (HPβBCD) was determined by charge-transfer chromatography and the relative strength of interaction was calculated. HPβCD interacted with each steroidal drugs decreasing the hydrophobicity of the guest molecules. The relative strength of interaction considerably depended on the structure of the drug molecule. Hydrophobicity parameters of drugs significantly influenced the strength of interaction indicating the involvement of hydrophobic forces in the binding of drugs to HPβCD. The marked influence of HPβCD on the hydrophobicity of drugs suggests that this interaction may modify the biological properties (adsorption, uptake, half-life etc.) of drug-HPβCD complexes drug resulting in modified efficacy.

Keywords: Steroidal drugs; Hydroxypropyl-β-cyclodextrin; Complex formation

1. Introduction

Cyclodextrins (CDs) are cyclic oligosaccharides built up from six to eight glucopyranose units. Due to their ring structures CDs have the capacity to form inclusion complexes with a wide variety of organic and even inorganic compounds [1,2]. The formation of various drug–CD inclusion complexes has been extensively studied. Thus, the formation of the inclusion complexes of antimycotic agents [3], insulin [4,5], anticancer drugs [6] etc. has been reported. The physicochemical and pharmacological characteristics of drug–CD inclusion complexes deviate considerably from those of uncomplexed drug molecules. Due to this modification the formation of inclusion complexes improves the performance of intravenous formulation [7], prolongs the pulmonary absorption [8], sustains the release rate [9], increases the stability of the guest molecule [10], enhances the peak concentration of drugs in blood [11], improves bioavailability [12,13], and enhances the extent and rate of absorption in organs [14].

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Much effort has been devoted to the elucidation of the involvement of various binding forces in the drug–CD interaction. It was assumed that dipole–dipole, van der Waals and hydrophobic interactions [15,16] and hydrogen bond formation [17,18] may influence the strength of the drug–CD interaction.

Various chromatographic techniques can be successfully used for the study of molecular interactions [19]. The advantage of the chromatographic techniques are that they use a low quantity of compounds, and the interacting molecules need not to be very pure because the impurities are separated during the chromatographic process. Charge-transfer reversed-phase thin-layer chromatography has been previously used to study the formation of the inclusion complexes of anticancer drugs [20], barbituric acid derivatives [21] and anti-hypoxia drugs [22].

Due to their considerable pharmaceutical importance the interaction of steroidal drugs with CDs has been extensively studied [23,24]. Chromatographic methods have also been used for the study of such interactions [25,26].

The objectives of this work were to study the interaction of steroidal drugs with hydroxypropyl-β-cyclodextrin (HPβCD) by means of charge transfer chromatography, to compare their inclusion forming capacity and to elucidate the role of molecular parameters in the inclusion complex formation.

2. Experimental

Polygram UV_{254} (Macherey-Nagel, Dürren, Germany) plates were impregnated by overnight predevelopment in *n*-hexane-paraffin oil 95:5 (v/v). The chemical structures of steroidal drugs are shown in Fig. 1. The drugs were the gift of Professor Sándor Görög, Gedeon Richter, Budapest, Hungary. The drugs were separately dissolved in methanol at a concentration of 3 mg ml^{-1} and 2 μl of the solutions were plotted on the plates. Water–methanol mixtures were used as eluents, the methanol concentration ranging from 0 to 90 vol.%. Methanol was chosen as the organic solvent miscible with water because it forms only weak inclusion complexes with β-cyclodextrins [27,28]. Hydroxypropyl-β-cyclodextrin (CYCLOLAB Research and Development Laboratory, Budapest, Hungary) was added to the eluents in the concentration range 0–15 mg ml^{-1}. Developments were carried out in sandwich chambers (22 × 22 × 3 cm) at room temperature, the distance of development being ~16 cm. After development the plates were dried at 105°C and the spots of steroidal drugs were revealed by their UV spectra and by iodine vapour. Each experiment was run in quadruplicate.

The R_M value characterizing the molecular hydrophobicity in reversed-phase thin-layer chromatography was calculated for each drug in each eluent:

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

When the coefficient of variation of the parallel determinations was >8% the R_M value was not taken into consideration in the following calculations.

To separate the effects of methanol and HPβCD on the hydrophobicity of steroidal drugs 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 a drug determined at given methanol and HPβCD concentrations; $R_{M0} = R_M$ value extrapolated to zero methanol and HPβCD concentrations; 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 drugs [29]; b_2 = decrease in the R_M value caused by 1 mg ml^{-1} concentration change of HPβCD in the eluent (related to the relative strength of interaction); C_1 and C_2 = concentrations of methanol and HPβCD, respectively. Eq. (2) was applied separately for each steroidal drug.

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 Eq. (2)) are strongly intercorrelated [30,31] linear correlation was calculated between the two physicochemical parameters:

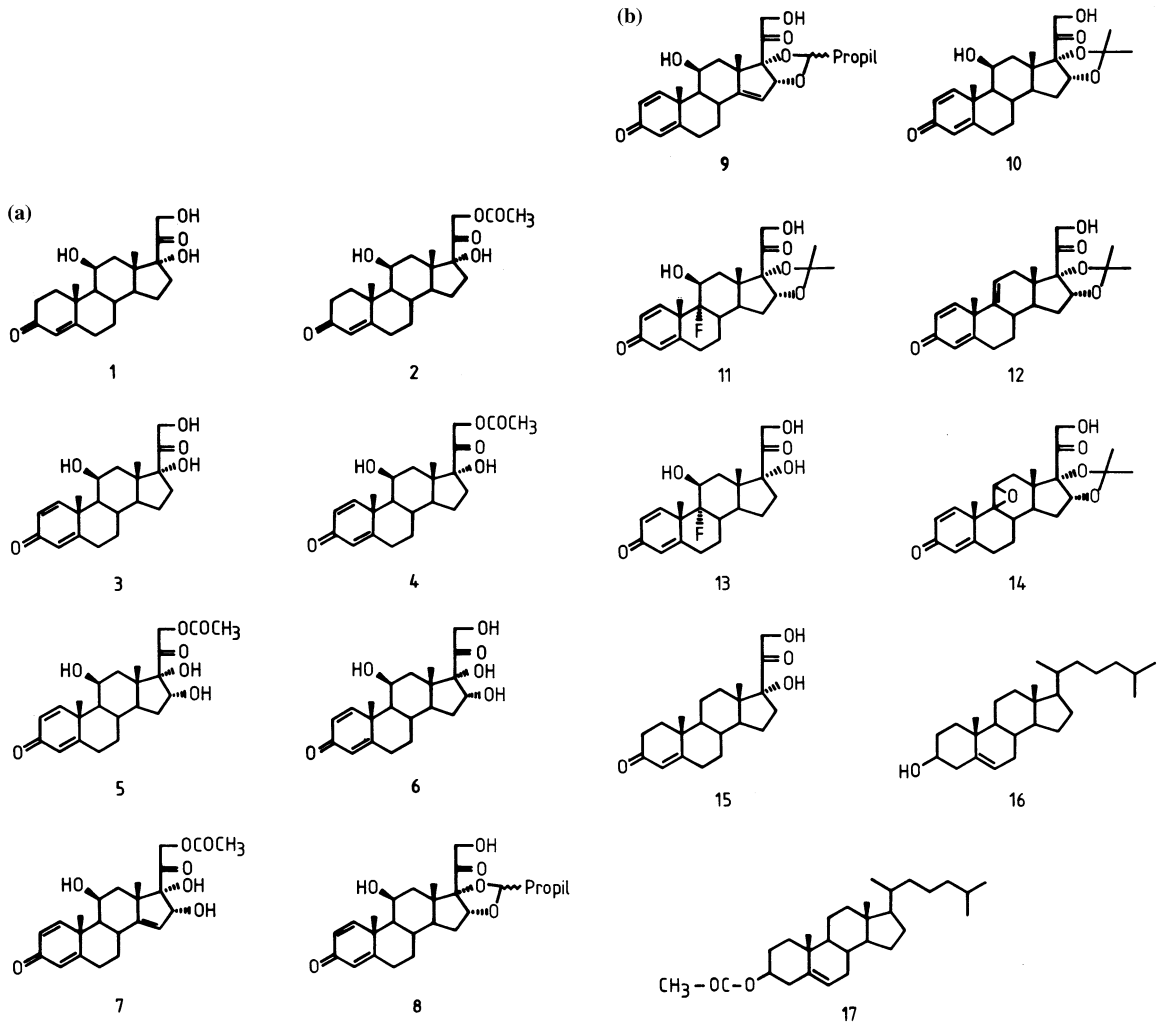

Fig. 1. Chemical structures of steroidal drugs.

$$R_{M0} = A + B \cdot b_1 \quad (3)$$

where A and B values are the constants (intercept and slope value) of the linear relationship between R_{M0} and b_1.

To find the physicochemical parameters of steroidal drugs significantly influencing their complex forming capacity, stepwise regression analysis was applied [32]. The relative strength of interaction (b_2) was the dependent variable whereas the hydrophobicity (R_{M0}), specific hydrophobic surface area (b_1) of Eq. (2) and the complex hydrophobicity parameter R_{M0}/b_1 were the independent variables, respectively. The number of accepted independent variables was not limited and the acceptance limit was set to the 95% significance level. Software of stepwise regression analysis was prepared by CompuDrug, Budapest, Hungary.

3. Results and discussion

The simultaneous effect of methanol and HPβCD concentrations on the R_M values of drugs 3 and 15 are shown in Figs. 2 and 3, respectively. The R_M value decrease in each in-

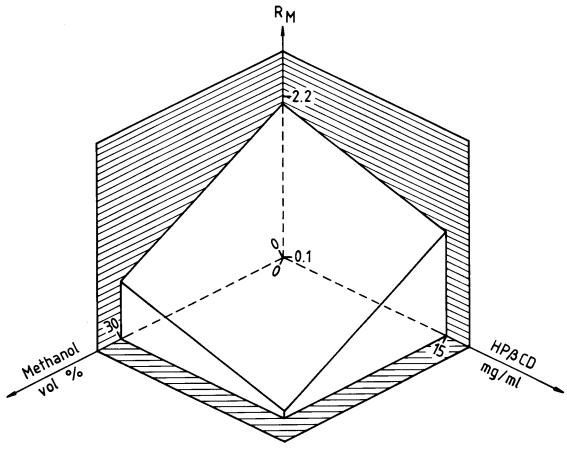

Fig. 2. Effects of methanol and hydroxypropyl-β-cyclodextrin (HPβCD) concentrations on the R_M value of steroidal drug 3 in Fig. 1.

stance with increase in methanol concentration, i.e. these compounds do not show any anomalous retention behaviour in this concentration range that would invalidate the evaluation using Eq. (2). An increase in the HPβCD concentration also caused a decrease in R_M values, indicating complex (probably inclusion complex) formation. Interaction of the more hydrophilic HPβCD with the steroidal drugs decreases the hydrophobicity of the latter. This finding suggests that the biological properties (adsorption, uptake, half-life etc.)

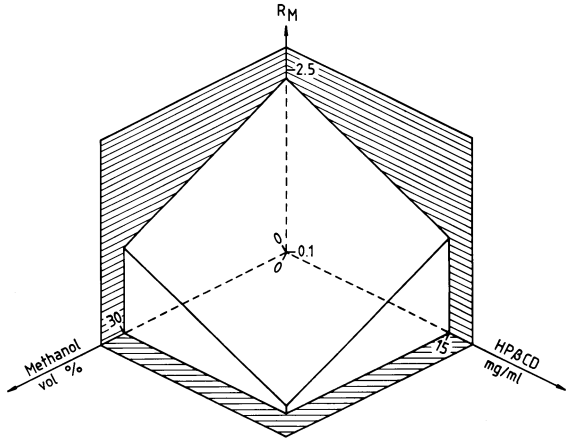

Fig. 3. Effects of methanol and hydroxypropyl-β-cyclodextrin (HPβCD) concentrations on the R_M value of steroidal drug 15 in Fig. 1.

of drug–HPβCD complexes may be different from that of uncomplexed drug resulting in modified effectiveness.

The parameters of Eq. (2) are compiled in Table 1. The meaning of the parameters R_{M0}, b_1 and b_2 values are explained in Section 2; s_{b_1} and s_{b_2} are the standard deviations of the coefficient of regression 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 is the coefficient of determination and $F_{calc.}$ is the calculated F value indicating the fitness of Eq. (2) to the experimental data. The equation fits the experimental data well, the significance level in each instance being $>99.9\%$ (see calculated F values). The ratios of variance explained were ~ 80–99% (see r^2 values). Each steroidal drug interacts with HPβCD (b_2 values differ significantly from zero) that means that in pharmaceutical formulations containing both steroidal drugs and HPβCD their possible interaction has to be taken into consideration. The parameters of Eq. (2) show high variations between the drugs proving that the hydrophobicity (R_{M0}), specific hydrophobic surface area (b_1) and their capacity to form inclusion complexes with HPβCD (b_2) differ considerably. This finding also suggests that the inclusion complex formation may have a different influence on the biological effect of individual steroidal drugs. The path coefficients (b' values) indicates that the impact of the change of methanol and HPβCD concentrations on the reversed-phase mobility of steoidal drugs is commensurable, that is the retention of steroidal drugs can be equally modified by changing either the methanol or the HPβCD concentration in the eluent.

Significant linear correlation was found between the intercept (hydrophobicity) and slope (specific hydrophobic surface area) values of steroidal drugs (Fig. 4). This finding indicates that from a chromatographic point of view these drugs behave as a homologous series of compounds, although their chemical structures are different.

Significant relationships (significance level $>95\%$) were found between the hydrophobicity parameters of steroidal drugs and their capacity to interact with HPβCD. The parameters of linear correlations selected by stepwise regression

Table 1
Relationship between the R_M values of steroidal drugs and the concentrations of methanol (C_1) and hydroxypropyl-β-cyclodextrin (C_2) in the eluent

Parameter	No of steroidal drugs																
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
R_{M0}	2.03	2.90	2.14	2.73	2.49	1.95	2.40	2.79	2.93	3.60	2.35	3.70	2.10	2.75	2.41	7.68	6.19
$-b_1 \cdot 10^2$	4.07	4.89	4.28	4.72	5.11	4.81	4.74	3.89	4.58	5.59	3.98	5.53	4.08	4.96	3.83	8.31	5.16
$s_{b1} \cdot 10^3$	1.95	5.94	2.28	1.44	4.85	2.84	2.87	3.17	2.60	1.72	2.45	2.19	2.77	2.54	5.31	7.91	6.65
$-b_2 \cdot 10^2$	4.55	6.91	4.44	5.78	5.91	4.61	5.04	4.07	3.33	4.94	4.35	6.15	5.76	3.77	7.10	3.34	2.09
$s_{b2} \cdot 10^3$	3.24	7.39	3.78	4.95	6.41	4.72	4.20	3.94	3.24	5.80	3.24	7.38	4.05	3.16	6.61	9.44	7.44
$b_1'\%$	59.73	46.84	61.48	73.72	53.31	63.36	57.90	54.32	63.11	79.26	54.68	75.21	50.88	62.08	40.13	74.73	73.44
$b_2'\%$	40.27	53.16	38.52	26.28	46.69	36.64	43.10	45.68	36.89	20.75	45.53	24.79	49.12	37.92	59.87	25.27	26.56
r^2	0.9768	0.9013	0.9702	0.9836	0.9223	0.9621	0.9638	0.9383	0.9637	0.9881	0.9643	0.9789	0.9638	0.9707	0.9119	0.8526	0.8043
$F_{calc.}$	315.33	54.78	244.58	571.34	77.18	190.50	187.27	91.26	159.34	704.24	175.31	393.75	186.40	198.46	62.08	57.85	30.82

$R_M = R_{M0} + b_1 \cdot C_1 + b_2 \cdot C_2$
Numbers refer to steroidal drugs in Fig. 1.

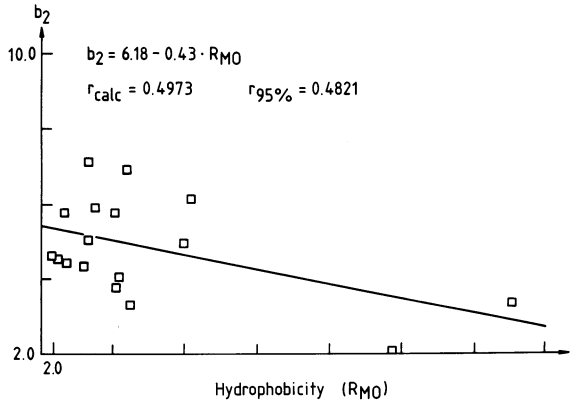

Fig. 4. Relationship between the hydrophobicity (R_{M0}) and specific hydrophobic surface area (b_1) of steroidal drugs.

analysis are compiled in Table 2. The fact that the hydrophobicity parameters exert a considerable influence on the strength of interaction indicates that hydrophobic forces are involved in the interaction. This result supports the assumption that steroidal drugs enter into the lipophilic cyclodextrin cavity, and they are retained by hydrophobic forces. However, these two parameters explain only a fairly low ratio of the total variance indicating than other molecular parameters may account for the strength of interaction. Unfortunately, other molecular parameters of steroidal drugs have never been determined, therefore it was impossible to include them in the calculation.

Table 2
Parameters of linear correlations between the hydrophobicity parameters of steroidal drugs (R_{M0} and b_1) and their capacity to interact with hydroxypropyl-β-cyclodextrin (b_2)

Parameters	No of equation	
	I	II
A	6.18	7.28
B	−0.43	−3.91
s_B	0.18	1.53
$r_{calc.}$	0.4973	0.5498
Significance level %	95	95

$n = 17$.
I. $b_2 = A + B \cdot R_{M0}$.
II. $b_2 = A + B \cdot R_{M0}/b_1$.

Charge-transfer chromatography carried out on reversed-phase thin-layer plates is a versatile and elegant method for the determination of molecular interactions between a wide variety of compounds. The method offers some advantages: it is rapid, does not need pure compounds because the impurities are separated during the chromatographic process and a high number of compounds can be simultaneously investigated. The drawback of the method is that high differences between the lipophilicities of the interacting molecules are necessary and only the relative strength of the interaction can be determined.

It can be concluded from the data that steroidal drugs form complexes (probably inclusion complexes) with HPβCD. The strength of complex formation markedly depends on the structure of the drug molecule and is significantly influenced by the hydrophobicity parameters of the drugs. It is probable that the complex formation of drugs with HPβCD modifies the various biological parameters (uptake, transfer, decomposition rate, etc.) and consequently, the biological efficacy of steroidal drugs in living organisms.

Acknowledgements

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