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Simultaneous interaction of steroidal drugs with γ- and hydroxypropyl-β-cyclodextrin studied by charge-transfer chromatography

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Abstract

The simultaneous interaction of 15 steroidal drugs with τ-cyclodextrin (τCD) and hydroxypropyl-β-CD (HPβCD) was determined by charge transfer chromatography and the relative strength of interaction was calculated for each drug–τCD–HPβCD ternary complex. The mixture of CDs interacted with each steroidal drugs decreasing the lipophilicity of the guest molecules. The chemical structure of steroidal drugs markedly influenced their capacity to interact with the mixture of CDs, the more lipophilic compounds formed stronger complexes with CDs. In the overwhelming majority of cases the stability of drug–τCD–HPβCD system was higher than those of binary (drug–τCD and drug–HPβCD) system indicating the probability of ternary complex formation. The data indicated that the ternary complex formation has to be taken into consideration in pharmaceutical formulations containing more than one type of CD or CD derivatives.

Keywords: Steroidal drugs; τ-Cyclodextrin; Hydroxypropyl-β-cyclodextrin; Ternary complexes

1. Introduction

Cyclomalto-oligosaccharides (cyclodextrins, CDs) are ring structures formed of six to eight glucopyranose monomers. The cavity of the CD ring can accommodate a wide variety of organic and inorganic compounds of various dimensions [1,2]. Due to the formation of inclusion complexes the physicochemical parameters and, consequently, the biological activity of the guest molecule may considerably deviate from those of uncomplexed bioactive compound. Thus, cholesterol–CD complex induced the high affinity binding of human oxytocin receptor in virus-infected insect cells [3], and was frequently employed as carrier for pharmaceuticals such as paclitaxel (taxol) [4] and antisense oligonucleotides [5]. CDs can improve the delivery of drugs (that of acitrecin through hairless mouse skin [6], and that of hydrocortisone into excised human skin) [7]. CDs further facilitated the release of hydrocortisone

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from ointments [8], and modified degradation rate of pharmaceuticals [9,10].

Due to the practical and theoretical importance of the formation of inclusion complexes the character and relative impact of various interactive forces on the complex formation has been vigorously discussed. Thus, the influence of hydrophobic forces [11], dipole–dipole and van der Waals interactions [12,13] and the formation of intermolecular hydrogen bonds [14,15] were reported.

Due to their considerable pharmaceutical importance the interaction of steroidal drugs with CDs has been intensively studied [16,17].

Besides other physicochemical methods various chromatographic techniques have also been used for the determination of molecular interactions [18]. Chromatographic methods show advantageous characteristics: the compound to be complexed need not to be very pure because the impurities are readily separated during the chromatographic process. Chromatographic methods have also been employed for the study of the interactions of steroidal drugs with CDs [19,20].

The objectives of the study were the determination of the simultaneous interaction of steroidal drugs with τCD and hydroxypropyl-β-CD (HPβCD) (drug–τCD–HPβCD) by means of charge transfer chromatography carried out on reversed-phase thin-layer chromatographic plates, the calculation of the relative strength of interaction, the comparison of the relative strength of interaction with those determined for drug–τCD and drug–HPβCD molecular pairs and the elucidation of the relationship between the capacity of steroidal drugs to form inclusion complexes and their hydrophobicity parameters. The study was motivated by the assumption that the bulky structure of this class of drugs offers more than one molecular substructure to be included in the cyclodextrin cavity, therefore, the formation of ternary complexes (two different CDs and one steroidal drug) is feasible. The ternary complex formation may result in a pharmaceutical formulation of different biological activity. To the best of our knowledge the simultaneous interaction of steroidal drugs with more than one CDs has never been studied in detail.

2. Materials and methods

2.1. Theoretical background

The mobility of solutes in reversed-phase thin-layer chromatography (RP-TLC) depends on the lipophilicity, more lipophilic solute shows lower mobility under RP-TLC conditions. This phenomenon can be exploited for the determination of molecular lipophilicity. As the majority of solutes remained on the start in water as mobile phase, the elution strength of mobile phase generally has to be enhanced by the addition of an organic modifier miscible with water. As the R_f values do not depend linearly on the concentration of organic modifier in the mobile phase the term R_M value was introduced for the characterisation of solute lipophilicity in RP-TLC:

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

When a hydrophilic additive is mixed into the mobile phase and it interacts with the solutes resulting in the decrease of apparent lipophilicity. The effects of organic modifier and eluent additive on the lipohpilicity of solutes can be separated by the following equation:

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

where $R_M = R_M$ value for a solute determined at given organic modifier and eluent additive concentrations; $R_{M0} = R_M$ value extrapolated to zero organic modifier and eluent additive concentrations; b_1 = decrease in the R_M value caused by 1% increase in the concentration of organic modifier in the eluent (related to the specific hydrophobic surface area of drugs [21]; b_2 = decrease in the R_M value caused by 1 mg/ml concentration change of eluent additive in the eluent (related to the relative strength of interaction); C_1 and C_2 = concentrations of organic modifier and eluent additive, respectively.

The relationship between chromatographic parameters and physicochemical characteristics of solutes can be easily calculated by stepwise regression analysis [22]. In the common multivariate regression analysis the presence of independent variables exerting no significant influence on the change of dependent variable considerably de-

creases 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 increasing in this manner the reliability of the calculation. Software of stepwise regression analysis was prepared by CompuDrug Ltd, Budapest, Hungary.

2.2. 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. Drugs were the gift of Professor Sándor Görög, Gedeon Richter, Ltd, Budapest, Hungary. The drugs were separately dissolved in methanol at a concentration of 3 mg/ml and 2 μl of the solutions were plotted on the plates. Water–methanol mixtures were used as eluents, the methanol concentration ranging from 20 to 80 vol.%. As the object was to study the complex formation between the solutes and τCD and HPβCD and not the study of the effect of CDs on the separation of solutes, they were separately spotted on the plates. In this way the competition between the steroidal drugs for the binding sites of CDs was excluded. Methanol was chosen as the organic solvent miscible with water because it

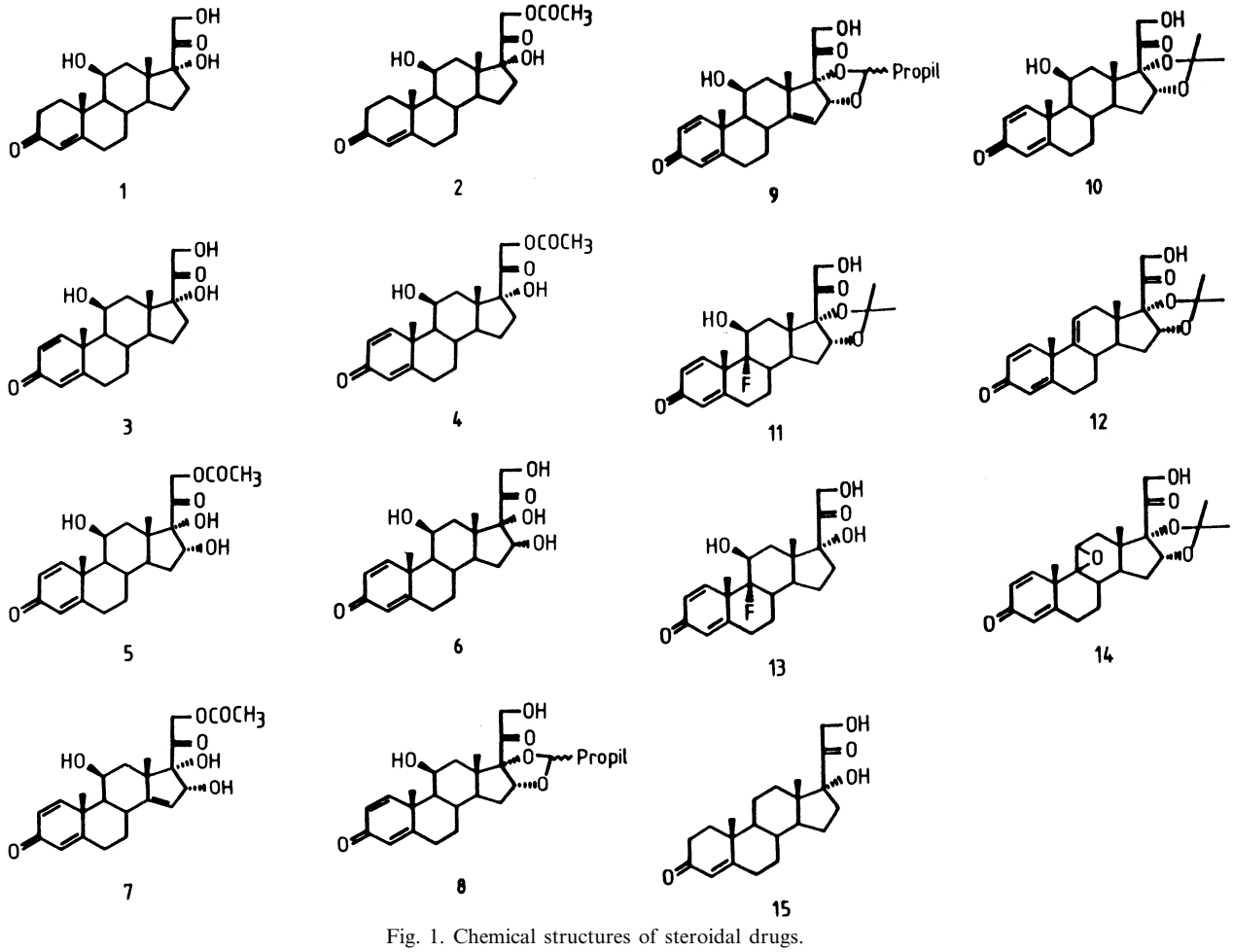

Fig. 1. Chemical structures of steroidal drugs.

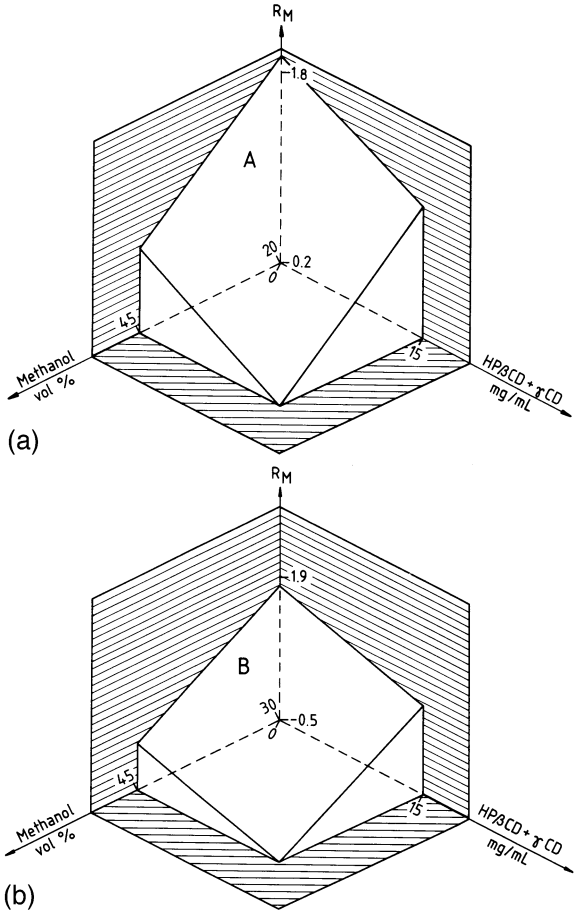

Fig. 2. Effects of methanol, τ- and hydroxypropyl-β-cyclodextrin (τCD and HPβCD) concentrations on the R_M value of steroidal drugs 9 (a) and 12 (b) in Fig. 1.

forms only weak inclusion complexes with CDs [23,24]. The application of this wide range of methanol concentration was motivated by the highly different lipophilicity of steroidal drugs. HPβCD and τCD were purchased from CYCLOLAB Research and Development Laboratory (Budapest, Hungary) and were added to the eluents in the concentration range of 0–15 mg/ml concentrations in the molar ratio of 1:1. Developments were carried out in sandwich chambers (22 × 22 × 3 cm) at room temperature, the distance of development being about 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 was calculated for each drug in each eluent by Eq. (1). When the coefficient of variation of the parallel determinations was higher than 6% the R_M value was omitted from the following calculations. The lipophilicity, specific hydrophobic surface area and the relative strength of drug–HPβCD–τCD interaction were calculated by Eq. (2). Eq. (2) was applied separately for each steroidal drug.

In order to find the relationship between the hydrophobicity of steroidal drugs and their complex forming capacity stepwise regression analysis was applied. The relative strength of interaction (b_2) was the dependent variable whereas the lipophilicity (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.

The relative strength of interaction of the ternary drug–τCD–HPβCD ($b_{\text{drug}-\tau\text{CD}-\text{HP}\beta\text{CD}}$) system was compared with those of drug–τCD ($b_{\text{drug}-\tau\text{CD}}$) and drug–HPβCD ($b_{\text{drug}-\text{HP}\beta\text{CD}}$) systems using linear regression analysis:

$$b_{\text{drug}-\tau\text{CD}-\text{HP}\beta\text{CD}} = A + B \cdot b_{\text{drug}-\tau\text{CD}} \quad (3)$$

$$b_{\text{drug}-\tau\text{CD}-\text{HP}\beta\text{CD}} = A + B \cdot b_{\text{drug}-\text{HP}\beta\text{CD}} \quad (4)$$

The values of $b_{\text{drug}-\tau\text{CD}}$ and $b_{\text{drug}-\text{HP}\beta\text{CD}}$ were taken from Ref. [25] and [26], respectively.

3. Results and discussion

The simultaneous effect of methanol and the concentrations of CDs on the lipophilicity of drugs 9 and 12 are shown in Fig. 2. The lipophilicity decrease in each instance with increase in the concentration of organic modifier, i.e. these solutes do not show any anomalous retention behaviour in this concentration range that would invalidate the evaluation using Eq. (2). An increase in the concentration of CDs also caused a decrease in lipophilicity, indicating

molecular interaction (probably inclusion complex formation). The results suggests that the biochemical characteristics (adsorption, uptake, half-life etc.) of drug–τCD–HPβCD system may be different from that of uncomplexed drug molecule resulting in modified effectiveness.

The parameters of Eq. (2) are compiled in Table 1. The equation fits the experimental data well, the significance level in each instance being over 99.9% (see calculated F values). The ratios of variance explained were between 90 and 98% (see r^2 values). Each steroidal drug interacts with CDs (b_2 values differ significantly from zero) that means that in pharmaceutical formulations containing both steroidal drugs and CDs 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 CDs (b_2) differ considerably. The result also suggests that the inclusion complex formation may influence differently the biological effect of individual steroidal drugs. The path coefficients (b' values) indicates that the impact of the change of methanol and the concentration of CDs on the reversed-phase mobility of steroidal drugs is commensurable that is the retention of steroidal drugs can be equally modified by changing either the methanol or the concentration of CDs in the mobile phase.

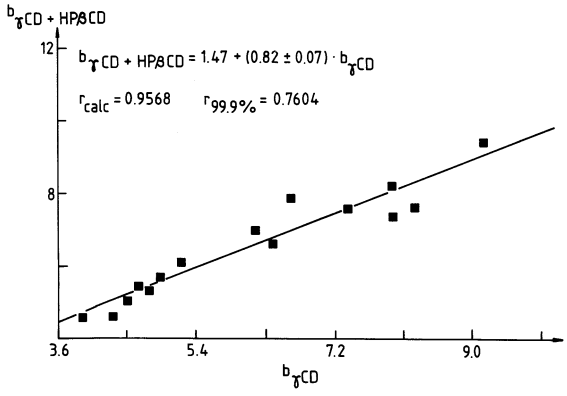

Fig. 3. Linear relationship between the relative strength of drug–τCD–HPβCD ($b_{\text{drug–τCD–HPβCD}}$) and drug–τCD ($b_{\text{drug–τCD}}$) interaction.

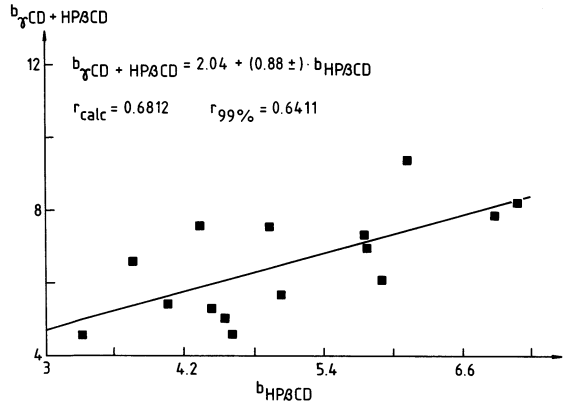

Fig. 4. Linear relationship between the relative strength of drug–τCD–HPβCD ($b_{\text{drug–τCD–HPβCD}}$) and drug–HPβCD ($b_{\text{drug–HPβCD}}$) interaction.

Stepwise regression analysis found a significant linear relationship between the relative strength of interaction (b_2) and the lipophilicity of steroidal drugs (R_{M0}):

$$b_2 = 2.30 + (1.67 \pm 0.77) \cdot R_{M0} \quad (5)$$

$$r_{\text{calc.}} = 0.5133 \quad r_{95\%} = 0.4973.$$

The significant impact of molecular lipophilicity on the relative strength of interaction indicates the involvement of hydrophobic binding forces in the interaction of steroidal drugs with CDs. It can be assumed that the gust molecules enter into the cavity of CDs, and they are bound by hydrophobic forces. However, the lipophilicity explain only a fairly low ratio of the total variance indicating than other molecular parameters may also exert a considerable impact on the strength of interaction.

Significant linear relationships were found between the relative strength of drug–τCD–HPβCD interaction and the drug–τCD (Fig. 3) and drug–HPβCD interactions (Fig. 4). Figs. 3 and 4 indicate that the relative strength of interaction of the ternary system similar but not identical with those of binary systems.

The fact that the slope values are higher than zero indicates that the stability of the ternary system is higher than those of binary systems suggesting ternary complex formation. The highest coefficient of regression can be tentatively

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

Parameter	Number of steroidal drugs														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
R_{M0}	2.04	2.93	2.14	2.71	2.52	1.98	2.40	2.71	2.80	3.37	2.25	3.39	2.08	2.49	2.35
$-b_1 \times 10^2$	4.12	5.00	4.33	4.69	5.18	4.89	4.74	3.71	4.24	5.23	3.88	5.04	4.10	4.29	3.76
$s_{b1} \times 10^3$	1.72	5.98	2.20	1.58	4.68	2.44	3.13	3.60	4.12	2.53	5.63	3.41	3.75	4.93	6.51
$-b_2 \times 10^2$	5.02	7.85	5.32	6.96	6.09	4.61	5.68	5.43	4.58	7.67	7.61	9.41	7.34	6.59	8.21
$s_{b2} \times 10^3$	2.85	7.45	3.65	5.38	6.18	4.05	4.58	4.49	5.13	8.53	7.43	11.51	5.49	6.13	8.11
b_1%	57.30	44.20	57.40	69.77	52.92	63.74	54.96	45.97	53.53	69.71	40.21	64.40	44.95	44.74	36.27
b_2%	42.70	55.80	42.60	30.23	47.08	36.26	45.04	54.03	46.47	30.29	59.79	35.60	55.05	55.26	63.73
r^2	0.9833	0.9152	0.9756	0.9797	0.9299	0.9724	0.9606	0.9371	0.9159	0.9655	0.9043	0.9298	0.9499	0.9191	0.8977
$F_{calc.}$	442.80	64.74	300.53	458.63	86.26	264.35	170.88	89.42	65.36	238.16	61.43	112.63	132.84	68.20	52.64

[a] Numbers refer to steroidal drugs in Fig. 1. Concentration range of methanol (C_1) 20–80 vol.%. Concentration range of HPβCD:τCD (molar ratio 1:1) (C_2) 0–15 mg/ml. $R_M = R_{M0} + b_1 \cdot C_1 + b_2 \cdot C_2$.

explained by the supposition tha τCD plays a decisive role in the ternary complex formation too, and the role of HPβCD is of secondary importance.

4. Conclusion

It can be concluded from the data that charge transfer chromatography carried out on reversed-phase TLC plates can be successfully used for the determination of the simultaneous interaction of steroidal drugs with hydrpxypropyl-β- and τ-cyclodextrins. CD mixtures decreased in each instance the apparent lipophilicity of the drugs indicating interaction, probably the formation of inclusion complexes. The comparison of the relative strength of interaction of drug–HPβCD, drug–τCD and drug–HPβCD–τCD mixtures suggested the formation of ternary complexes. The data indicate that the effect of this complex formation on the biological activity of the active ingredient has to be taken into consideration in pharmaceutical formulations containing more than one type of CDs or CD derivatives.

Acknowledgements

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