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Interaction of some steroid drugs with β-cyclodextrin polymer

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Abstract

The interaction of 15 steroidal drugs with a water-soluble β-cyclodextrin polymer was studied by reversed-phase thin-layer chromatography in the absence and in the presence of 0.1 *M* sodium chloride. The relative strength of interaction was calculated and the relationship between the hydrophobicity parameters of the drugs and the strength of the drug–β-cyclodextrin polymer was elucidated by principal component analysis. Drugs readily formed inclusion complexes with the cyclodextrin derivatives; the strength of the interaction was higher in the presence of sodium chloride. It was assumed that the formation of inclusion complexes may influence the behaviour of the drugs resulting in modified biological efficacy. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Complexation; Principal component analysis; Pharmaceutical analysis; Steroids; Drugs, steroidal; Cyclodextrins

1. Introduction

Cyclodextrins (CDs) can complex a wide variety of organic compounds [1–3] modifying their physicochemical parameters. Thus, the formation of the inclusion complexes of antimycotic agents [4], insulin [5], and anticancer drugs [6] has been reported. The physicochemical and pharmacological characteristics of drug–cyclodextrin 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], increases the stability of the guest molecule [9], enhances the peak concentration of the drugs in blood [10], and improves bioavailability [11].

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 [12], and hydrogen bond formation [13] may influence the strength of the drug–CD interaction. Over the past decade chromatographic methods have been extensively used to study the interactions between bioactive compounds [14]. These methods use a low quantity of compounds and the interacting molecules need not be very pure because the impurities are readily separated during the chromatographic process. Reversed-phase thin-layer chromatography (RP–TLC) has been successfully used to study many biologically important interactions [15,16]. The method is rapid and does not need complicated instrumentation, however, the stoichiometry of the complex cannot be determined and only the relative strength of interaction can be calculated.

The objectives of this work were to study the interaction of steroidal drugs with water-soluble β-CD polymer by means of RP–TLC, to calculate the

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relative strength of interaction, 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, Düren, Germany) silica plates were impregnated by overnight predevelopement in *n*-hexane–paraffin oil (95:5, v/v). The chemical structures and the IUPAC names of steroidal drugs are shown in Fig. 1 and in Table 1, respectively. 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 and 2 μl of the solutions were plotted on the plates. Methanol was chosen as the organic solvent miscible with water because it forms only a weak inclusion complex with CD [17]. It was applied in the concentration range of 20–27.5% (v/v) in steps of 2.5%. The use of this narrow range of methanol concentration was motivated by the fact that each drug showed acceptable mobility only in this narrow concentration range. At higher and lower concentrations some drugs remained at the start or were very near the solvent front making the calculation of the spot position difficult. The water-soluble β-CD polymer (weight-average molecular mass 4500, β-CD content 64.2%, intrinsic viscosity $5.7 \cdot 10^{-3}$ l/g) was the product of the Cyclolab Research and Development Laboratory (Budapest, Hungary). It was prepared by cross-linking the monomer with epichlorohydrin. The β-CD polymer was dissolved in the methanol–water eluent systems in the concentration range of 0–15 mg/ml in steps of 5 mg/ml. Developments were carried out in sand-

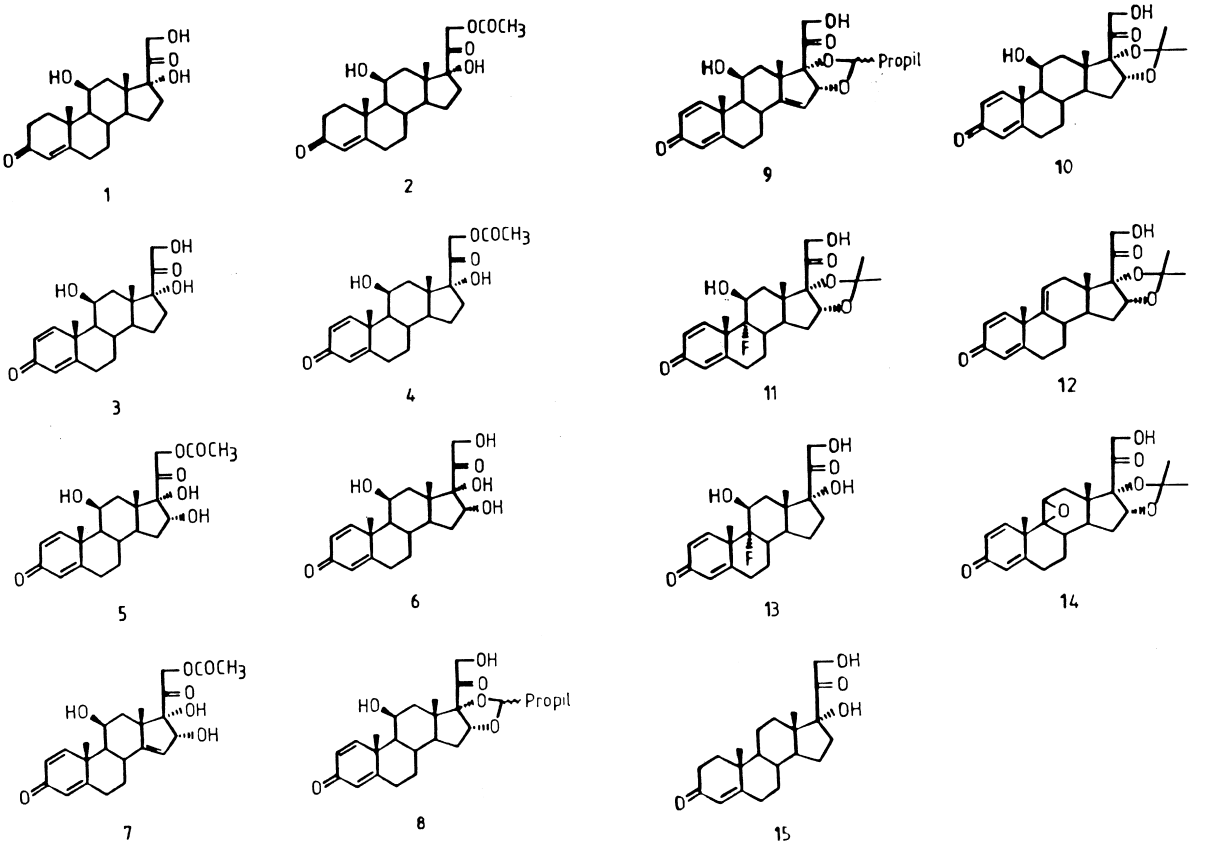

Fig. 1. Chemical structures of steroidal drugs.

Table 1
IUPAC name of steroidal drugs

No. of drug	IUPAC name
1	11β,17α,21-Trihydroxypregn-4-ene-3,20-dione
2	11β,17α-Dihydroxypregn-4-ene-3,20-dione-21-acetate
3	11β,17α,21-Trihydroxypregna-1,4-diene-3,20-dione
4	11β,17α-Dihydroxypregna-1,4-diene-3,20-dione-21-acetate
5	11β,16α,17α-Trihydroxypregna-1,4-diene-3,20-dione-21-acetate
6	11β,16α,17α,21-Tetrahydroxypregna-1,4-diene-3,20-dione
7	11β,16α,17α-Trihydroxypregna-1,4,14-triene-3,20-dione-21-acetate
8	16α,17α-Butylidenebis(oxy)-11β,21-dihydroxypregna-1,4-diene-3,20-dione
9	16α,17α-Butylidenebis(oxy)-11β,21-dihydroxypregna-1,4,14-triene-3,20-dione
10	11β,21-Dihydroxy-16α,17α-[methylethylidenebis (oxy)]pregna-1,4-diene-3,20-dione
11	9-Fluoro-11β,21-dihydroxy-16α,17α-[1-methylethyli-denebis(oxy)]pregna-1,4-diene-3,20-dione
12	21-Hydroxy-16α,17α-[1-methylethylidenebis(oxy)] pregna-1,4,9(11)-triene-3,20-dione
13	9-Fluoro-11β,17α,21-trihydroxypregna-1,4-diene-3,20-dione
14	9β,11β-Epoxy-21-hydroxy-16α,17α-[1-methylethyli-denebis(oxy)]pregna-1,4-diene-3,20-dione
15	17α,21-Dihydroxypregn-4-ene-3,20-dione

wich chambers (22×22×3 cm) at room temperature, the distance of development being about 16 cm. After development the plates were dried and the spots were detected under UV light. To study the effect of salt on the strength of the interaction between the drugs and the β-CD polymer each experiment was also carried out in the presence of 0.1 *M* sodium chloride. Each experiment was run in quadruplicate. The R_M value characterizing the molecular lipophilicity in RP–TLC 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 determination was higher than 8% the R_M value was omitted from the following calculations.

To separate the effects of methanol and β-CD polymer on the lipophilicity of steroidal drugs the following equation was fitted to the experimental data:

$$R_M = R_{M0} + b_1C_1 + b_2C_2 \quad (2)$$

where $R_M = R_M$ value for a drug determined at a given methanol and β-CD polymer concentration; $R_{M0} = R_M$ value extrapolated to zero methanol and β-CD polymer concentrations (best estimate of the lipophilicity of the drug); b_1 = decrease in the R_M value caused by a 1% increase in methanol concentrations in the eluent (related to specific hydrophobic surface area of drugs [18]); b_2 = decrease in the R_M value caused by 1 mg/ml concentration change of β-CD polymer in the eluent (related to the relative strength of interaction); C_1 and C_2 = concentrations of methanol and β-CD polymer, respectively. Eq. (2) was applied separately for each steroidal drug in both eluent systems.

To find the physicochemical parameters of steroidal drugs that were significantly influencing their complex-forming capacity, principal component analysis (PCA) [19] was applied. The observations were the parameters of Eq. (2) calculated in the presence and in the absence of sodium chloride (R_{M0}, b_1, b_2, R_{M0salt}, b_{1salt}, b_{2salt}) and the steroidal drugs were the variables. The variance explained by the PCA components was set to 99%. To facilitate the visual evaluation of the resulting data matrices, two-dimensional nonlinear mapping [20] was carried out on both the PC loadings and variables. The iteration was carried out to the point where the difference between the last two iterations was lower than 10^{-8}.

3. Results and discussion

The parameters of Eq. (2) are compiled in Tables 2 and 3. Blank sites in Table 2 indicate that in the presence of sodium chloride the interaction between drug 12 and the β-CD polymer cannot be established. The equations fits the experimental data well, the significance levels in each instance being over

Table 2
Relationship between the R_M values of steroidal drugs and the concentrations of methanol (C_1) and polymer (C_2) in ion-free eluent. Numbers refer to steroidal drugs in Fig. 1. $R_M = R_{M0} + b_1C_1 + b_2C_2$

Parameter	No. of steroidal drug														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
R_{M0}	1.84	1.96	2.21	2.74	2.13	2.31	2.14	2.70	2.74	2.35	2.89	2.54	2.36	2.59	2.31
$-b_1 \cdot 102$	3.61	4.00	2.69	4.82	4.79	3.46	5.81	4.03	4.29	2.48	5.73	3.22	4.72	4.53	3.22
$s_{b1} \cdot 10^3$	6.53	44.96	6.36	6.61	5.64	9.33	9.21	4.26	5.12	7.34	9.15	6.50	6.36	4.96	6.49
$-b_2 \cdot 10^2$	1.24	2.30	2.97	1.31	3.32	2.25	2.07	1.87	1.52	2.17	3.62	1.05	3.50	2.25	4.52
$s_{b2} \cdot 10^3$	3.26	2.97	1.31	3.32	2.25	2.07	2.05	2.92	3.51	5.02	6.26	4.54	4.36	3.39	4.44
b_1 %	59.17	38.26	66.56	49.78	51.33	66.76	55.66	66.88	65.84	43.84	46.73	67.78	47.95	57.97	32.80
b_2 %	40.83	61.73	38.83	50.32	48.66	33.24	44.34	33.12	34.16	56.16	53.27	32.22	52.05	42.03	67.20
R^2	0.876	0.924	0.958	0.921	0.888	0.845	0.875	0.935	0.908	0.970	0.889	0.971	0.929	0.934	0.937
$F_{calc.}$	52.57	55.43	54.86	53.14	35.80	24.66	53.59	65.18	44.49	26.06	36.33	35.07	59.70	63.74	64.13

99.9% (see calculated F values). The ratios of variance explained were about 74–98% (see r^2 values). The parameters of Eq. (2) show high variations between the steroidal drugs proving that the lipophilicity (R_{M0}), specific hydrophobic surface area (b_1) and the capacity of steroidal drugs to form inclusion complexes with β-CD polymer (b_2) differ considerably. These differences are due to the different chemical structure of the drugs. This finding also suggests that the inclusion complex formation may influence the biological effect differently from the individual steroidal drugs. The path coefficients (b% values) indicate that the impact of the change of the concentrations of methanol and β-CD polymer concentrations on the RP–TLC mobility of steroidal drugs is commensurable with the retention of steroidal drugs which can be equally modified by changing either the methanol or the β-CD polymer concentration in the eluent. The relative strength of interaction was higher in the presence of salt (compare b_2 values in Tables 2 and 3). This phenomenon can be explained by the assumption that the ions suppress the dissociation of the polar groups of steroidal drugs enhancing in this manner their apparent lipophilicity (salting-out effect). As the inner wall of the cyclodextrin cavity is hydrophobic the increased lipophilicity of the drugs facilitates their binding to the apolar inner wall increasing the strength of interaction.

The results of the PCA are compiled in Table 4. Three principal components explain the majority of the total variance (91.31%). The hydrophobicity parameters (R_{M0}, b_1, R_{M0salt}, b_{1salt}) are widely distributed between the PC components indicating that their information content is markedly different and the presence of sodium chloride considerably

Table 3
Relationship between the R_M values of steroidal drugs and the concentrations of methanol (C_1) and polymer (C_2) in the presence of 0.1 M sodium chloride. Numbers refer to steroidal drugs in Fig. 1. $R_M = R_{M0} + b_1C_1 + b_2C_2$

Parameter	No. of steroidal drug														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
R_{M0}	1.87	2.42	1.99	2.13	2.00	1.77	2.05	2.34	2.32	2.28	2.07	1.79	2.22	2.43	2.53
$-b_1 \cdot 10^2$	4.06	3.44	3.66	2.95	3.05	3.73	3.57	2.73	2.70	2.44	2.87	1.54	4.28	4.01	4.21
$s_{b1} \cdot 10^3$	4.92	77.98	1.01	9.09	6.63	4.07	6.53	4.82	4.94	6.68	5.08	4.57	1.21	4.80	4.21
$-b_2 \cdot 10^2$	1.18	2.99	2.17	1.83	1.52	1.28	2.07	1.25	1.20	1.26	1.66	–	3.20	4.80	12.90
$s_{b2} \cdot 10^3$	2.46	3.99	5.11	4.99	3.31	2.03	3.26	2.41	2.47	3.34	2.54	–	6.25	2.40	6.47
b_1 %	63.24	68.11	46.38	44.56	51.75	59.20	46.24	52.03	52.94	49.19	46.28	–	59.86	50.57	61.85
b_2 %	36.76	31.89	53.62	55.44	48.25	40.80	53.76	47.97	47.06	50.81	53.72	–	40.14	49.43	38.15
R^2	0.875	0.851	0.733	0.739	0.876	0.904	0.844	0.819	0.904	0.879	0.952	0.962	0.943	0.923	0.982
$F_{calc.}$	45.58	37.31	15.41	15.09	20.83	61.81	35.08	39.54	36.65	33.74	37.45	41.32	38.86	50.13	57.34

Table 4
Relationship between the hydrophobicity parameters and complex forming capacity of steroidal drugs. Results of principal component analysis

No. of principal component	Eigenvalue	Variance explained (%)	Total variance explained (%)
1	2.70	45.12	45.12
2	1.43	23.82	68.94
3	1.34	22.37	91.31

	Principal component loadings		
Parameters	No. of principal components		
	1	2	3
R_{M0}	−0.22	0.96	−0.12
b_1	−0.09	0.48	0.86
b_2	0.82	0.31	0.06
R_{M0salt}	0.67	0.32	−0.56
b_{1salt}	0.76	−0.25	0.52
b_{2salt}	0.96	−0.02	−0.01

modifies both the lipophilicity and specific hydrophobic surface area of steroidal drugs. The values of the strengths of interaction (b_2, b_{2salt}) have high loadings in the first component. This finding suggests that sodium chloride similarly influences the strength of interaction of each steroidal drug with β-CD polymer. The two-dimensional non-linear map of principal component loadings is shown in Fig. 2. The parameters can be classified in two different manners. The lipophilicity, specific hydrophobic surface

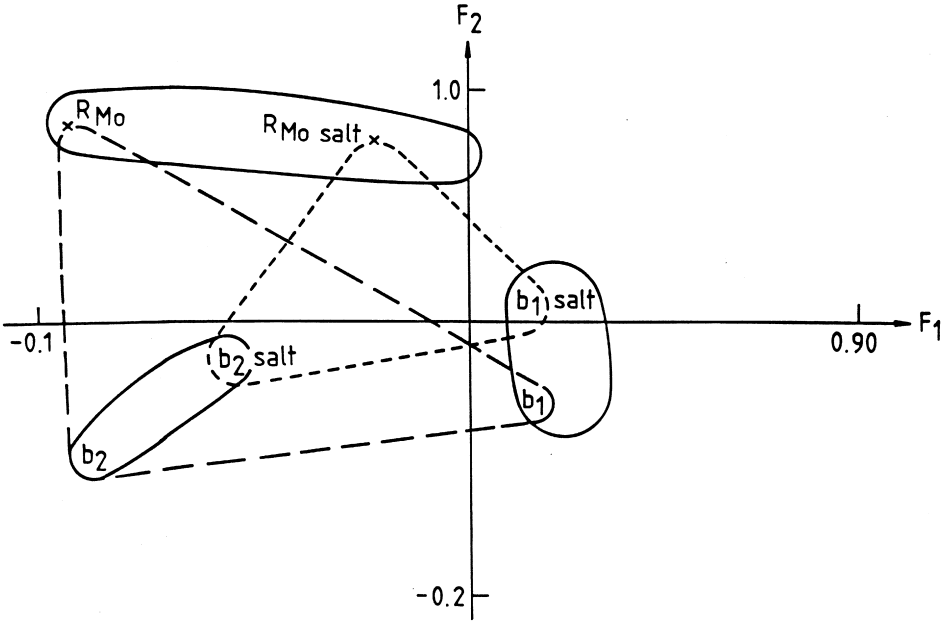

Fig. 2. Similarities and dissimilarities between the hydrophobicity parameters and the capacity of steroidal drugs to interact with β-cyclodextrin polymer. Two-dimensional non-linear map of principal component loadings. Number of iterations: 69. Maximum error: $1.5 \cdot 10^{-2}$. For symbols see Experimental.

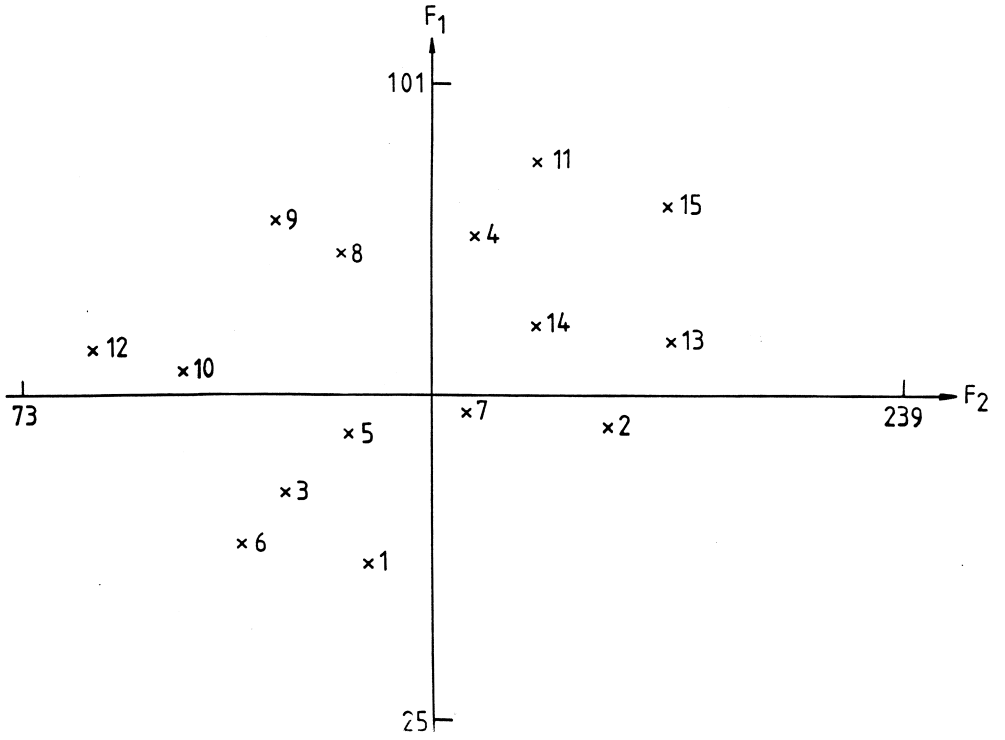

Fig. 3. Similarities and dissimilarities between the steroidal drugs. Two-dimensional non-linear map of principal component variables. Number of iterations: 95. Maximum error: $4.2 \cdot 10^{-2}$. Numbers refer to steroidal drugs in Fig.1.

area and the strength of interaction form clearcut separate doublets proving again their different physicochemical character. However, the parameters determined in the presence and in the absence of sodium chloride also form loose clusters. This result indicates that the dissociated ions of sodium chloride also influence each physicochemical parameter.

The two-dimensional non-linear map of principal component variables is shown in Fig. 3. The steroidal drugs do not form clusters neither on the basis of the nature of substituents nor on the basis of substituent position. This finding suggests that more than one substituent of the steroidal drugs has a marked impact on the capacity to form inclusion complexes with the β-CD polymer.

It can be concluded from the data that steroidal drugs form complexes (probably inclusion complexes) with the β-CD polymer. The strength of complex formation markedly depends on the structure of the drug molecule. It can be assumed that the complex formation of drugs with the β-CD polymer may modify the various biological parameters (uptake, transfer etc) and the biological efficacy of steroidal drugs in the living organism.

Acknowledgements

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References

[1] J. Szejtli, in: Cyclodextrin and Their Inclusion Complexes, Akadémia Kiadó, Budapest, Hungary, 1982.
[2] J. Szejtli, in: Cyclodextrins and Their Inclusion Complexes, Akadémia Kiadó, Budapest, 1982, p. 204.
[3] D.W. Armstrong, F.-Y. He, S.M. Han, J. Chromatogr. 448 (1988) 345.
[4] M. Pederson, M. Edestein, M.V.F. Nielsen, A. Sarpellini, S. Skytte, C. Slot, Int. J. Pharm. 90 (1993) 247.

[5] Y. Watanabe, Y. Matsumoto, M. Seki, M. Takase, M. Matsumoto, Chem. Pharm. Bull. 40 (1992) 3042.
[6] W. Distelmans, R. van Ginckel, W. Vanherck, R. Willebrords, L. Wouters, M. de Brabander, J. Mesens, Anticancer Res. 11 (1991) 253.
[7] H.M.C. Marques, J. Hadgraft, I.Y. Kellaway, G. Taylor, Int. J. Pharm. 77 (1991) 303.
[8] K.S. Estes, M.E. Brewster, A.I. Webb, N. Bodor, J. Pharm. 5 (1990) 101.
[9] F. Djedainipilard, B. Perly, S. Dupas, M. Miocque, M. Galons, Tetrahedr. Lett. 34 (1993) 1145.
[10] J.S. Hostetler, L.H. Hanson, D.A. Stevens, Antimicrob. Agents Chemother. 36 (1992) 477.
[11] B.V. Müller, E. Albers, J. Pharm. Sci. 80 (1991) 599.
[12] B.V. Müller, E. Albers, Int. J. Pharm. 79 (1992) 273.
[13] J.H. Park, M.D. Jang, M.J. Sain, J. Chromatogr. 595 (1992) 45.
[14] T. Cserháti, K. Valkó, Chromatographic Determination of Molecular Interactions, CRC Press, Boca Raton, FL, 1994.
[15] E. Forgács, Biochem. Mol. Biol. Int. 30 (1993) 1.
[16] T. Cserháti, Fresenius' J. Anal. Chem. 345 (1993) 541.
[17] A. Harada and S. Takashi, Chem. Lett. (1984) 2089.
[18] C. Horváth, W. Melander, I. Molnár, J. Chromatogr. 125 (1976) 129.
[19] K.V. Mardia, J.T. Kent, J.M. Bibby, in: Academic Press, London, 1979, p. 213.
[20] J.W. Sammon Jr., IEEE Trans. Comput. C18 (1969) 401.