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Interaction of some anticancer drugs with carboxymethyl-β-cyclodextrin

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Abstract

The interaction between 23 anticancer drugs and carboxymethyl-β-cyclodextrin (CM-β-CD) was studied by reversed-phase charge-transfer thin-layer chromatography and the relative strength of interaction was calculated. CM-β-CD formed inclusion complexes with 13 compounds, the complex always being less hydrophobic than the uncomplexed drug. The inclusion-forming capacity of drugs differed considerably depending on their chemical structures. Principal component analysis indicated that the hydrophilic parameters (hydrophobicity, specific hydrophobic surface area) of drugs exert the greatest influence on the stability of CM-β-CD-drug inclusion complexes.

Keywords: Carboxymethyl-β-cyclodextrin; Anticancer drug; Hydrophobic interaction

1. Introduction

Cyclodextrins (CDs) and various CD derivatives form inclusion complexes with a wide variety of drugs such as cephalotin and aztreonam (Loftsson and Johannesson, 1994), various barbituric acid derivatives (Csabai et al., 1993) and non-steroidal anti-inflammatory drugs (Loftsson et al., 1993). The formation of inclusion complexes considerably modifies the pharmacological and pharmacokinetical behaviour of the original drug. Thus, CDs proved to be good protection agents against enhanced damage in nasal delivery systems (Gill et al., 1994a,b). Hydroxypropyl-β-CD (HP-β-CD) enhanced the extent and rate of absorption of carbamazepine in dogs (Betlach et al., 1993), and glucosyl-α-CD and maltosyl-α-CD improved bioavailability of *p*-boronophenylalanine (Hatanaka et al., 1993). Dimethyl-β-CD largely improved the nasal absorption of two peptide drugs (Verhoef et al., 1994), and increased the absorption of insulin in the lower jejunal/upper ileal segments of the rat (Shao et al., 1994).

The role of the various molecular forces in the strength of the host-guest interaction has been vigorously discussed. It is generally accepted that hydrophobic forces (Ramusino and Pichini, 1994) as well as polar and steric factors (Davies and Savage, 1994) are involved in complex formation. However, it has also been established that dipole-dipole, ion-dipole, van der Waals and hydrogen-bonding interactions may have a considerable impact on the formation of inclusion complexes (Inoue et al., 1993). Another study indicated that the inclusion process is mainly, but not exclusively, controlled by van der Waals attractions (Fathallah et al., 1994).

Chromatographic methods have been fre-

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quently used for the study of molecular interactions (Cserháti and Valkó, 1993). Charge-transfer chromatography carried out on reversed-phase thin-layer chromatographic plates has been recently used to study the interaction of some anticancer drugs with amino acids (Cserháti and

Table 1
Commercial and IUPAC names of anticancer drugs

Number of drugs	Commercial name	IUPAC name	Source
1	ftorafur	*N*-(2-furanidyl)-5-fluorouracil	Medexport (Russia)
2	bicnu	*N*,*N*-bis(2-chloroethyl)-*N*-nitrosourea	Laboratoire Bristol (France)
3	leukeran	4-[bis(2-chloroethyl)amino]benzenebutanic acid	Wellcome Foundation Ltd (UK)
4	vincristine	22-oxo-(3α,14β,16α)-14,15-dihydro-14-hydroxy-eburnamenine-14-carbocyclic acid methyl ester	Richter Gedeon Ltd (Hungary)
5	vinblastine	(3α,14β,16α)-14,15-dihydro-14-hydroxyeburnamenine-14-carbocyclic acid methyl ester	Richter Gedeon Ltd (Hungary)
6	vumon	4′-*O*-demethyl-1-*O*(4,6-*O*-2-thenylidene-β-D-glucopyranosyl) epipodophyllotoxin	Bristol-Arzneimittel (Germany)
7	provera	17-α-acetoxy-6-α-(methyl)progesterone	Upjohn Ltd (UK)
8	bleogin	N^1-[3-dimethyl(sulfonio)propyl]bleomycin amide	Nippon Kayaku (Japan)
9	paraplatin	9,11,15-trihydroxy-15-methylprosta-5,13-dienoic acid	Bristol-Arzneimettel (Germany)
10	zitazonium	2-[4-(2-chloro-1,2-difenilethynile)fenoxi]-*N*,*N*-diethyl ethamine citrate	Egis Pharm. Works (Hungary)
11	farmorubicin	(8*S-cis*)-10-[(3-amino-2,3,6-trideoxy-α-L-arabinohexopyranosyl)oxy]-7,8,9,10-tetrahydro-6,8,11-trihydroxy-8-(hydroxyacetyl)-1-methoxy-5,12-naphthacenedione	Farmitalia (Italy)
12	adriblastine (doxorubicin)	10-[3-(amino-2,3,6-trideoxy-α-L-hexapyranosyl)oxy]-7,8,9-tetrahydro-6,8,11-trihydroxy-8-(hydroxyacetyl)-1-methoxy-5,12-naphthacenedione	Farmitalia (Italy)
13	natulan	*N*-(1-methylethyl)-4-[(2-methylhydrazino)methyl]benzamide	Roche (Switzerland)
14	alexan	4-amino-1-β-D-arabifuranosyl-2(14)-pyrimidine	Mack (Germany)
15	mitomycin C Kyowa	[1-*aR*]-6-amino-8-[(aminocarbonyl)oxymethyl]-1,1*a*,2,8,8*a*,8*b*-hexahydro-8*a*-methoxy-5-methyl-azirino-[2′,3′ : 3,4]pyrrolo[1,1*a*]indole-4,7-dione	Kyowa (Japan)
16	cytoxan	2-[bis(2-chloroethyl)amino]tetrahydro-2H-1,3,2-oxazaphosphorine 2-oxide monohydrate	Bristol-Myers (Germany)
17	estracyt	estra-1,3,5-(10)-triene-3,17-diol-3-[bis-chloroethyl)carbamate	Aktiebolaget (Sweden)
18	deticene	5-(3,3-dimethyl-1-triazenyl)-1H-imidazole 4-carboxamide	Rhone-Poulenc (France)
19	methotrexate	2,4-diamino-10-methylpteroylglutamic acid	Lachema (Czech Republic)
20	myelobromol	1,6-dibromo-1,6-bis(deoxy)-D-mannitol	Chinoin (Hungary)
21	zitostop	1,2,5,6-tetramesyl-D-mannitol	EGIS Pharm. Works (Hungary)
22	elobromol	1,6-dibromo-1,6-bis(dezoxy)-D-dulcitol	Chinoin (Hungary)
23	taxol	[2*aR*-[2*a*α,4β,4*a*β,6β,9α(*aR**,β*S**),11α,-12α,12*a*α,12*b*α]]-β-(benzoylamino)-α-hydroxybenzenepropanoic acid 6,12*b*-bis(acetoxy)-12-(benzoyloxy)-2*a*,3,4,4*a*,5,6,9,10,11,12,12*a*,12*b*-dodecahydro-4,11-dihydroxy-4*a*,8,13,13-tetramethyl-5-oxo-7,11-methano-14-cyclodeca[3,4]benz[1,2-*b*]oxet-9-yl ester	Sigma Chemie GmbH (Germany)

Holló, 1994a) and with HP-β-CD (Cserháti and Holló, 1994b).

The objectives of this work were to study the interaction of some commercial anticancer drugs with carboxymethyl-β-cyclodextrin (CM-β-CD), to determine the relative strength of interaction with the aid of reversed-phase chromatography, and to assess the role of various molecular parameters in the formation of inclusion complexes. The elucidation of the formation of inclusion complexes between the anticancer drugs and CM-β-CD may promote the development of new, more effective anticancer drug formulations with higher biological efficiencies and lower toxic side effects.

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 IUPAC names of anticancer drugs are listed in Table 1. 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 0 to 70 vol%. As the object was to study complex formation between the solutes and CM-β-CD and not to study the effect of CM-β-CD on the separation of solutes, they were separately spotted on the plates. Methanol was chosen as the organic solvent miscible with water because it forms only weak inclusion complexes with CDs (Buvári et al., 1983/1984; Harada and Takahashi, 1984). The application of this wide range of methanol concentration was motivated by the highly different hydrophobicity of anticancer drugs. CM-β-CD was the product of Cyclolab Research and Development Laboratory, Budapest, Hungary; it contained 3.6 carboxymethyl groups/CD ring. CM-β-CD was added to the eluents at 0, 10, 20, 30 and 40 mg/ml concentrations. 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 anticancer drugs were revealed by their visible and UV spectra, iodine vapour and phosphomolybdenic acid reagent. 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:

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

where R_f is the distance of the solute from the origin divided by the distance of the eluent front from the origin.

When the coefficient of variation of the parallel determinations was higher than 8%, the R_M value was omitted from the following calculations.

To separate the effects of methanol and CM-β-CD on the hydrophobicity of anticancer 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 is the R_M value for a drug determined at given methanol and CM-β-CD concentrations, R_{M0} denotes the R_M value extrapolated to zero methanol and CM-β-CD concentrations, b_1 is the 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) (Horváth et al., 1976), b_2 represents the decrease in the R_M value caused by 1 mg/ml concentration change of CM-β-CD in the eluent (related to the relative strength of interaction), and C_1 and C_2 are the concentrations of methanol and CM-β-CD, respectively. Eq. 2 was applied separately for each anticancer drug.

To determine the physicochemical parameters of anticancer drugs related to their capacity to form inclusion complexes, principal component analysis (PCA) (Mardia et al., 1979) was applied, the explained variance being set to 99%. The parameters determined using Eq. 2 (R_{M0}, b_1 and b_2) and the following calculated physicochemical were the variables: π Hansch-Fujita's substituent constant characterizing hydrophobicity; H-Do, indicator variable for proton donor properties; M-RE, molar refractivity; F and R, electronic parameters characterizing the inductive and reso-

nance effect, respectively; σ, Hammett's constant, characterizing the electron-withdrawing power of the substituent; E_s, Taft's constant, characterizing steric effects of the substituent; B_1 and B_4, Sterimol width parameters determined by distance of substituents at their maximum point perpendicular to attachement.

Anticancer drugs were considered as variables. Drugs showing no significant interaction with CM-β-CD were not included in the calculation. To facilitate the visual evaluation of the resulting data matrices two-dimensional nonlinear mapping (Sammon, 1969) was carried out on the principal component loadings and variables. The iteration for the calculation of nonlinear maps was continued as the difference between the last two iterations was lower than 10^{-8}.

To compare the complex forming capacity of CM-β-CD and HP-β-CD (Cserháti and Holló, 1994b) with anticancer drugs linear correlation

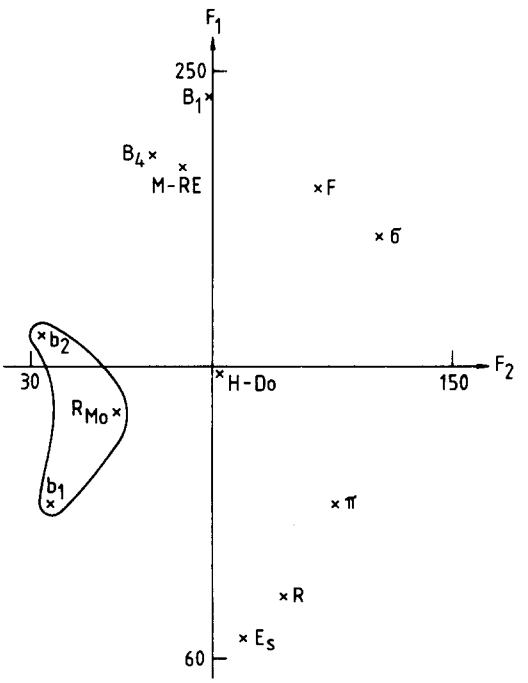

Fig. 1. Relationship between the physicochemical parameters of anticancer drugs. Two dimensional non-linear map of principal component loadings. Number of iterations, 104; maximal error, 3.96×10^{-2}. For symbols see Section 2.

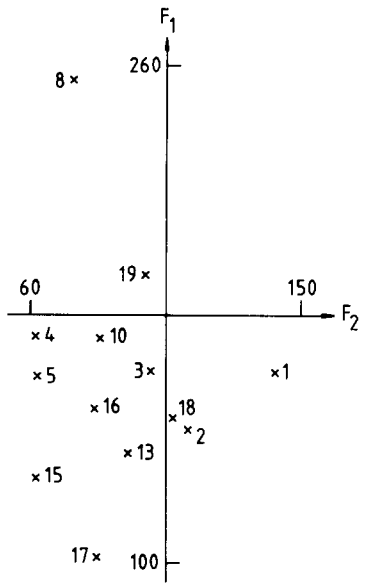

Fig. 2. Similarities and dissimilarities between the anticancer drugs according to their physicochemical parameters. Two dimensional non-linear map of principal component variables. Number of iterations; 105; maximal error; 3.78×10^{-2}. Numbers refer to anticancer drugs in Table 1.

was calculated between the corresponding b_2 (relative strength of interaction) values.

3. Results and discussion

Compounds **9** and **19–21** were near to the front in each eluent system and over the CM-β-CD front, which means that these drugs are highly hydrophilic and their interaction with CM-β-CD cannot be determined under the experimental conditions used.

The R_M values of drugs decreased in each instance with increase in methanol concentration, i.e., these compounds do not show any anomalous retention behavior in this concentration range that would invalidate the evaluation using Eq. 2. In most instances, an increase in CM-β-CD concentration also caused a decrease in R_M values, indicating complex (probably inclusion complex) formation. Interaction of the more hydrophilic CM-β-CD with the anticancer drugs reduces the hydrophobicity of the latter. This finding suggests

that the biological properties (adsorption, uptake, half-life, etc.) of drug-CM-β-CD complexes may be different from that of uncomplexed drug resulting in modified effectivity.

The parameters of Eq. 2 are compiled in Table 2. Blank sites in Table 2 indicate that these independent variables did not influence significantly the R_M value of the anticancer drug. The equation fits the experimental data well, the significance levels in each instance being over 99%

Table 2
Parameters of linear correlations between the hydrophobicity (R_M) of anticancer drugs and the methanol (C_1) and carboxymethyl-β-cyclodextrin concentration (C_2) in the eluent ($R_M = R_{M0} + b_1 \cdot C_1 + b_2 \cdot C_2$)

Parameter	Compound no.				
	1	**2**	**3**	**4**	**5**
n [a]	21	22	17	14	15
R_{M0}	0.36	1.10	1.92	2.41	2.10
$-10^2 \times b_1$	1.67	2.03	1.45	3.12	2.56
$10^3 \times s_{b1}$ [b]	1.11	0.87	2.54	3.52	3.37
$-10^2 \times b_2$	1.13	1.39	3.43	3.61	1.77
$10^3 \times s_{b2}$ [b]	2.48	1.91	3.61	5.03	6.51
b'_1 (%) [c]	76.82	76.36	37.45	55.26	73.72
b'_2 (%) [c]	23.18	23.64	62.55	44.74	26.28
$F_{calc.}$ [d]	113.66	274.41	48.19	40.36	34.76
r^{2e}	0.9229	0.9651	0.8653	0.8709	0.8425
	6	**7**	**8**	**10**	**11**
n [a]	20	14	21	15	18
R_{M0}	1.65	2.19	1.37	2.25	1.99
$-10^2 \times b_1$	1.63	2.56	1.70	2.17	2.62
$10^3 \times s_{b1}$ [b]	1.78	4.09	4.34	3.25	3.70
$-10^2 \times b_2$	–	–	2.84	3.75	–
$10^3 \times s_{b2}$ [b]	–	–	9.19	7.39	–
b'_1 (%) [c]	–	–	55.83	56.81	–
b'_2 (%) [c]	–	–	44.17	43.19	–
$F_{calc.}$ [d]	83.91	39.87	9.58	22.60	50.67
r^{2e}	0.8154	0.7541	0.5020	0.7767	0.7473
	12	**13**	**15**	**16**	**17**
n [a]	17	17	21	21	12
R_{M0}	2.13	1.47	1.25	1.30	3.36
$-10^2 \times b_1$	2.87	3.28	3.61	2.52	4.32
$10^3 \times s_{b1}$ [b]	3.88	3.23	2.06	8.43	6.41
$-10^2 \times b_2$	–	2.97	1.69	2.05	5.69
$10^3 \times s_{b2}$ [b]	–	3.16	4.58	1.99	16.65
b'_1 (%) [c]	–	61.03	80.13	74.35	66.32
b'_2 (%) [c]	–	38.97	19.87	25.65	33.68
$F_{calc.}$ [d]	54.77	117.71	111.59	448.07	35.16
r^{2e}	0.7739	0.9401	0.9216	0.9792	0.8755

Table 2 (continued)

	18	**19**	**23**
n [a]	22	22	13
R_{M0}	0.95	0.90	4.11
$-10^2 \times b_1$	2.00	2.69	6.33
$10^3 \times s_{b1}$ [b]	1.91	3.68	2.96
$-10^2 \times b_2$	2.23	4.24	–
$10^3 \times s_{b2}$ [b]	4.21	8.12	–
b'_1 (%) [c]	66.36	58.36	–
b'_2 (%) [c]	33.67	41.70	–
$F_{calc.}$ [d]	56.75	31.07	458.10
r^{2e}	0.8502	0.7565	0.9745

[a] Number of data points.
[b] Standard deviations of b_1 and b_2.
[c] Standard partial regression coefficients of b_1 and b_2, which are normalized to unity.
[d] Calculated F value indicating the quality of fit of Eq. 2 to the experimental data.
[e] Coefficient of determination.

(see calculated F values). The ratios of variance explained were about 50–98% (see r^2 values). The majority of anticancer drugs interact with CM-β-CD (b_2 values differ significantly from zero) which means that in pharmaceutical formulations containing both anticancer drugs and CM-β-CD their possible interaction has to be taken into consideration. The parameters of Eq. 2 show considerable variations between the drugs, proving that the lipophilicity (R_{M0}), specific hydrophobic surface area (b_1) and their capacity to form inclusion complexes with CM-β-CD (b_2) differ considerably. This finding also suggests that inclusion complex formation may influence differently the biological effect of individual anticancer drugs.

The results of principal component analysis are compiled in Table 3. Five principal components explain the overwhelming majority of variance, signifying that five background variables contain 94% of the information of the 12 variables. Unfortunately, PCA does not define these background variables as concrete physicochemical entities and only indicates the mathematical possibility of their existence. The inclusion forming capacity of anticancer drugs, their measured hydrophobicity and specific hydrophobic surface area have the highest loadings in the second

Table 3
Relationship between the measured and calculated physicochemical parameters of anticancer drugs: results of principal component analysis (for symbols see Section 2)

No of principal component	Eigenvalue	Variance explained (%)	Sum of variance explained (%)
1	5.57	46.38	46.38
2	2.60	21.64	68.02
3	1.30	10.80	78.82
4	1.27	10.55	89.37
5	0.61	5.07	94.44

Principal component loadings

Parameter	No of principal component				
	1	2	3	4	5
π	−0.52	0.25	0.32	0.70	0.03
H-Do	0.10	0.71	−0.22	0.13	−0.63
M-RE	0.89	0.14	−0.01	0.38	0.17
F	0.73	−0.07	0.62	−0.07	0.01
R	−0.90	0.06	0.28	0.31	−0.02
σ	0.67	−0.24	0.53	−0.23	−0.09
E_s	−0.97	−0.15	0.14	−0.14	−0.04
B_1	0.92	0.11	−0.26	0.02	0.00
B_2	0.88	0.23	−0.07	0.38	0.08
R_{M0}	−0.08	0.87	0.41	−0.01	0.09
b_1	−0.35	0.72	−0.31	−0.15	0.40
b_2	0.09	0.76	0.20	−0.53	0.00

principal component whereas the majority of physicochemical parameters have high loadings in other PC components. This finding indicates that the hydrophobicity of drugs exerts a considerable influence on their capacity to form inclusion complexes with CM-β-CD and hydrophobic forces account for the binding of the apolar substructures of drugs to the inner wall of CM-β-CD cavity.

No significant correlation was found between the complex forming capacity of CM-β-CD and HP-β-CD. This result can be tentatively explained by the supposition that the polar substituents on the outer sphere of the CD ring differently influence the sterical availability of the CD cavity. This draws attention to the fact that the effect of various CD derivatives on the pharmacological properties of an anticancer drug may be considerably different and for the preparation of an effective drug-CD formulation as many CD derivatives as possible should be investigated.

Acknowledgments

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References

Betlach, C.A., Gonzalez, M.A., McKiernan, B.C., Neff-Davis, C. and Bodor, N., Oral pharmacokinetics of carbamazepine in dogs from commercial tablets and a cyclodextrin complex. *J. Pharm. Sci.*, 82 (1993) 256–258.

Buvári, A., Szejtli, J. and Barcza, L., Complexes of short-chain alcohols with beta-cyclodextrin. *J. Incl. Phenom.*, 1 (1983/1984) 151–157.

Csabai, K., Cserháti, T. and Szejtli, J., Interaction of some barbituric acid derivatives with hydroxypropyl-β-cyclodextrin. *Int. J. Pharm.*, 91 (1993) 15–22.

Cserháti, T. and Holló, J., Interaction of amino acids with a non-homologous series of anticancer drugs. *Biochem. Mol. Biol. Int.*, 32 (1994a) 201–211.

Cserháti, T. and Holló, J., Interaction of taxol and other anticancer drugs with hydroxypropyl-β-cyclodextrin. *Int. J. Pharm.*, 108 (1994b) 69–75.

Cserháti, T. and Valkó, K., *Chromatographic Determination of Molecular Interactions*, CRC Press, Boca Raton, FL, 1993.

Davies, D.M. and Savage, J.R., Cyclodextrin complexes of substituted perbenzoic and benzoic acids and their conjugate bases: free energy relationships show the interaction of polar and steric factors. *J. Chem. Soc. Perkin Trans.*, 2 (1994) 1525–1530.

Fathallah, M., Fotiadu, F. and Jaime, C., Cyclodextrin inclusion complexes. MM2 calculations reproducing bimodal inclusions. *J. Org. Chem.*, 59 (1994) 1288–1293.

Gill, I.J., Illum, L., Farraj, N. and De Ponti, R., Cyclodextrins as protection agents against enhancer damage in nasal delivery systems: I. Assessment of effect by measurement of erythrocyte haemolysis. *Eur. J. Pharm. Sci.*, 1 (1994a) 229–236.

Gill, I.J., Fisher, A.N., Hinchcliffe, M., Whetstone, J., Farraj, N., R. De Ponti, R. and Illum, L., Cyclodextrins as protection agents against enhancer damage in nasal delivery systems: II. Effect on in vivo absorption of insulin and histopathology of nasal membrane. *Eur. J. Pharm. Sci.*, 1 (1994b) 237–248.

Harada, A. and Takahashi, S., Complex formation of cyclodextrins in alcohol solution. *Chem. Lett.*, 12 (1984) 2089–2090.

Hatanaka, H., Komada, F., Mishima, Y. and Okumura, K., Improved bioavailability of para-boronophenylalanine by cyclodextrin complexation. *J. Pharm. Sci.*, 82 (1993) 1054–1057.

Horváth, C., Melander, W. and Molnár, I., Solvophobic interactions in liquid chromatography with nonpolar stationary phases. *J. Chromatogr.*, 125 (1976) 129–156.

Inoue, Y., Liu, Y., Tong, L.-H., Shen, B.-J. and Jin, D.-S., Calorimetric titration of inclusion complexation with modified β-cyclodextrins. Enthalpy-entropy compensation in host-guest complexation: from ionophore to cyclodextrin and cyclophane. *J. Am. Chem. Soc.*, 115 (1993) 10637–10644.

Loftsson, T. and Johannesson, H., The influence of cyclodextrins on the stability of cephalotin and aztreonam in aqueous solutions. *Pharmazie*, 49 (1994) 292–293.

Loftsson, T., Olafsdóttir, B.J., Fridriksdóttir, H. and Jónsdóttir, S., Cyclodextrin complexation of NSAIDs: physicochemical characteristics. *Eur. J. Pharm. Sci.*, 1 (1993) 95–101.

Mardia, K.V., Kent, J.T. and Bibby, J.M., *Multivavariate Analysis*, Academic Press, London, 1979, pp. 213–254.

Ramusino, M.C. and Pichini, S., A ^{1}H NMR study of inclusion complex formation between β-cyclodextrin and monohydroxypyridines. *Carbohydr. Res.*, 259 (1994) 13–19.

Sammon, J.W., Jr, A nonlinear mapping for data structure analysis. *IEEE Trans. Comput.*, C18 (1969) 401–407.

Shao, Z., Li, Y., Chermak, T. and Mitra, A.K., Cyclodextrins as mucosal absorption promoters of insulin: II. Effects of β-cyclodextrin derivatives on α-chymotryptic degradation and enteral absorption of insulin in rats. *Pharm. Res.*, 11 (1994) 1174–1179.

Verhoef, J.C., Schipper, N.G.M., Romeijn, S.G. and Merkus, F.W.H.M., The potential of cyclodextrins as absorption enhancers in nasal delivery of peptide drugs. *J. Controlled Release*, 29 (1994) 351–360.