ELSEVIER

International Journal of Pharmaceutics 108 (1994) 69–75

international journal of pharmaceutics

Interaction of taxol and other anticancer drugs with hydroxypropyl-β-cyclodextrin

Tibor Cserháti *, János Holló

Central Research Institute for Chemistry, Hungarian Academy of Sciences, P.O. Box 17, 1525 Budapest, Hungary

(Received 29 November 1993; Accepted 19 January 1994)

Abstract

The interaction between 23 anticancer drugs and hydroxypropyl-β-cyclodextrin (HPβCD) was studied by reversed-phase charge-transfer thin-layer chromatography and the relative strength of interaction was calculated. HPβCD formed inclusion complexes with 15 compounds, the complex always being more hydrophilic than the uncomplexed drug. The inclusion forming capacity of drugs differed considerably according to their chemical structure. The intensity of interaction significantly increased with increasing hydrophobicity of the guest molecule, demonstrating the preponderant role of hydrophobic interactions in inclusion complex formation.

Key words: Hydroxypropyl-β-cyclodextrin; Anticancer drug; Hydrophobic interaction

1. Introduction

Taxol, a promising anticancer drug, has been isolated from the bark of various *Taxus* species such as *Taxus baccata* L. (Senilh et al., 1984), *T. brevifolia* (Chabner, 1991), *T. cuspidata* (Neto and DiCosma, 1992). Taxol has been successfully used for the treatment of metastatic breast cancer (Holmes et al., 1991) and ovarian carcinomas (Markman, 1991; Marty et al., 1991; Williams et al., 1992). Taxol also exhibits toxic side effects such as anaphylactoid reactions, leukopenia, peripherial neuropathy and oropharyngeal mucositis (Slichenmyer and Von Hoff, 1991). Due to its high hydrophobicity (Kingston, 1991) and the fact that the administration of taxol presents considerable difficulties (Guerite-Voegelein et al., 1992), considerable effort has been devoted to the development of less hydrophobic semisynthetic taxol derivatives with better application parameters (Hanauske et al., 1991; Matthew et al., 1992).

Cyclodextrins (CDs) are cyclic oligosaccharides which have the ability to form inclusion complexes with many organic and inorganic compounds of various chemical structures (Szejtli, 1982, 1988). CDs readily form inclusion complexes with several drugs such as steroids (Chun and Yun, 1993; Loftsson et al., 1993), antimycotic agents (Pedersen et al., 1993), insulin (Watanabe et al., 1992a,b) and anticancer drugs (Distelmans et al., 1991). The formation of an inclusion complex modifies the physicochemical characteristics of the guest molecules, improves the performance

* Corresponding author.

SSDI 0378-5173(94)00029-5

of intravenous formulation (Estes et al., 1990), prolongs the pulmonary absorption of salbutanol (Cabral Marques et al., 1991), sustains the release rate of drugs (Uekama et al., 1990), increases the stability of the guest molecule (Djedainipilard et al., 1993), enhances the peak concentration of drugs in blood (Hostetler et al., 1992) and improves bioavailability (Müller and Albers, 1991).

Charge-transfer reversed-phase thin-layer chromatography (TLC) has frequently been applied to study molecular interactions (Cserháti and Valkó, 1993). This method was used to investigate the formation of an inclusion complex of barbituric acid derivatives with crosslinked water-soluble βCD polymer (Cserháti et al., 1986) and with hydroxypropyl-β-cyclodextrin (Csabai et al., 1993).

The objectives of this work were to study the interaction of taxol and other anticancer 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 formation of inclusion complex.

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 the anticancer drugs are shown in Table 1. The drugs were separately dissolved in methanol at a concentration of 3 mg/ml and 2 μl of the solutions were spotted onto the plates. Water-methanol mixtures were used as eluents, the methanol concentration ranging from 0 to 70 vol.%. As the objective was to study 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 onto the plates. In this way the HPβCD/solute ratio was the same for each compound. Methanol was chosen as the organic solvent miscible with water, since it forms only weak inclusion complexes with β-cyclodextrins (Buvári et al., 1983/1984; Harada and Takahashi, 1984). The application of this wide range of methanol concentrations was motivated by the considerably different hydrophobicities of the anticancer drugs. Hydroxypropyl-β-cyclodextrin (Cyclolab Research and Development Laboratory, Budapest, Hungary) was added to the eluents at concentrations of 0, 10 and 20 mg/ml. Development was 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, by iodine vapour and by phosphomolybdenic acid reagent. Each experiment was run in quadruplicate.

The R_M value characterizing the molecular hydrophobicity in reversed-phase TLC was calculated for each drug in every eluent according to:

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

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

To separate the effects of methanol and HPβCD on the hydrophobicity of anticancer 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 is the R_M value for a drug determined at given methanol and HPβCD concentrations, R_{M0} denotes the R_M value extrapolated to zero methanol and HPβCD concentrations, b_1 is the decrease in R_M value caused by a 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 R_M value caused by a 1 mg/ml concentration change of HPβCD in the eluent (related to the relative strength of interaction), and C_1 and C_2 are the concentrations of methanol and HPβCD, respectively. Eq. 2 was applied separately for each anticancer 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 (Cserháti, 1984; Valkó, 1984), linear correlation was calculated between

Table 1
Chemical structure of anticancer drugs

Number	Common name	Chemical composition	Supplier
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 (U.K.)
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 (U.K.)
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-Arzneimetter (Germany)
10	zitazonium	2-[4-(2-chloro-1,2-difenilethynile)fenoxil]-*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-2*H*-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)-1*H*-imidazole-4-carboxamide	Rhone-Poulenc (France)
19	methotrexate	2,4-diamino-10-methylpteroylglutamic acid	Lachema (Czech Republic)
20	myelobromol	1,6-dibrom-1,6-bis(desoxy)-D-mannit	Chinoin (Hungary)
21	zitostop	1,2,5,6-tetramezil-D-mannit	EGIS Pharm. Works (Hungary)
22	elobromol	1,6-dibrom-1,6-bis(desoxy)-D-dulcit	Chinoin (Hungary)
23	taxol	[2*aR*-[2*a*α,4β,4*a*β,6β,9α(*aR**,β*S**)-11α,12α,12aα,12bα]]-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)

the two physicochemical parameters:

$$R_{M0} = A + Bb_1 \quad (3)$$

In order to determine the physicochemical parameters of anticancer drugs that significantly influence their capacity to form complexes, stepwise regression analysis was performed (Mager, 1982). 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. 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, increasing in this manner the reliability of the calculation.

3. Results and discussion

Compounds 4, 9 and 20–22 were near to the front in each eluent system and over the HPβCD front, indicating that these drugs are highly hydrophilic and that their interaction with HPβCD cannot be determined under the experimental conditions employed.

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 cases, an increase in HPβCD concentration also led to a decrease in R_M value, indicating complex (probably inclusion complex) formation. Interaction of the more hydrophilic HPβCD with the anticancer drugs reduces the hydrophobicity of the latter. This finding suggests that the biological properties (adsorption, uptake, half-life, etc.) of drug-HPβCD complexes may be different

Table 2
Parameters of linear correlations between the hydrophobicity (R_M) of anticancer drugs and the methanol (C_1) and hydroxypropyl-β-cyclodextrin concentration (C_2) in the eluent

Parameter	Compound no.				
	1	2	3	4	5
n	26	30	19	23	25
R_{M0}	0.36	1.04	0.93	2.44	2.18
$-b_1$ ($\times 10^{-2}$)	1.75	1.90	0.75	3.20	2.90
s_{b1} ($\times 10^{-3}$)	0.80	0.84	0.99	1.60	2.02
$-b_2$ ($\times 10^{-2}$)	0.97	1.32	2.29	1.42	–
s_{b2} ($\times 10^{-3}$)	1.76	1.86	3.32	2.85	–
b'_1 (%)	79.90	76.07	52.24	80.05	–
b'_2 (%)	20.10	23.93	47.76	19.95	–
$F_{calc.}$	250.24	267.39	52.32	200.66	206.11
r^2	0.9542	0.9503	0.8603	0.9503	0.8957
	Compound no.				
	6	7	8	10	11
n	27	22	29	24	27
R_{M0}	1.96	2.34	1.43	2.15	1.67
$-b_1$ ($\times 10^{-2}$)	2.41	2.86	2.25	2.01	2.12
s_{b1} ($\times 10^{-3}$)	2.52	2.90	3.18	1.56	1.64
$-b_2$ ($\times 10^{-2}$)	1.66	–	–	2.11	2.12
s_{b2} ($\times 10^{-3}$)	5.82	–	–	3.77	3.42
b'_1 (%)	77.08	–	–	69.63	67.64
b'_2 (%)	22.92	–	–	30.37	32.36
$F_{calc.}$	46.86	97.43	50.03	83.83	86.62
r^2	0.7894	0.8227	0.6412	0.8840	0.8739
	Compound no.				
	12	13	15	16	17
n	27	19	30	32	13
R_{M0}	1.58	0.86	1.11	1.18	1.67
$-b_1$ ($\times 10^{-2}$)	1.83	2.26	2.81	2.18	1.47
s_{b1} ($\times 10^{-3}$)	1.41	7.45	2.01	0.98	2.77
$-b_2$ ($\times 10^{-2}$)	2.03	0.91	1.61	1.78	2.59
s_{b2} ($\times 10^{-3}$)	2.94	2.49	4.47	2.16	6.75
b'_1 (%)	65.34	89.25	79.46	72.89	58.11
b'_2 (%)	34.66	10.75	20.54	27.11	41.89
$F_{calc.}$	88.27	467.32	99.06	279.50	22.24
r^2	0.8760	0.9821	0.8762	0.9491	0.8017
	Compound no.				
	18	19	23		
n	30	29	19		
R_{M0}	0.94	0.76	3.50		
$-b_1$ ($\times 10^{-2}$)	2.11	2.26	5.27		
s_{b1} ($\times 10^{-3}$)	1.25	2.56	2.95		
$-b_2$ ($\times 10^{-2}$)	1.66	2.82	1.44		
s_{b2} ($\times 10^{-3}$)	2.50	5.94	4.60		
b'_1 (%)	71.71	64.94	85.08		
b'_2 (%)	28.29	35.06	14.92		
$F_{calc.}$	167.33	46.68	213.07		
r^2	0.9228	0.7757	0.9616		

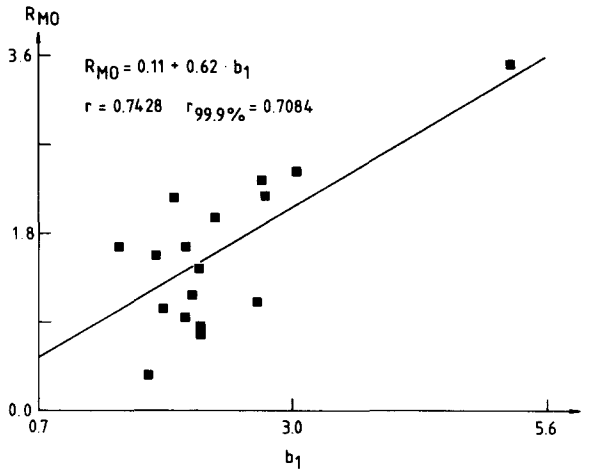

Fig. 1. Relationship between the hydrophobicity (R_{M0}) and specific hydrophobic surface area (b_1) of anticancer drugs.

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 99.9% (see calculated F values). The ratios of variance explained were about 64–98% (see r^2 values). The majority of anticancer drugs interact with

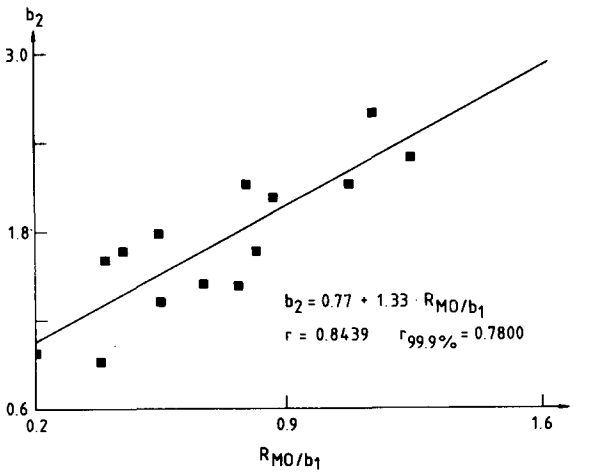

Fig. 2. Relationship between the combined hydrophobicity parameter (R_{M0}/b_1) and complex-forming capacity (b_2) of anticancer drugs.

HPβCD (b_2 values differ significantly from zero), demonstrating that in pharmaceutical formulations containing both anticancer drugs and HPβCD their possible interaction must be taken into consideration. The parameters of Eq. 2 show large variations between the drugs, proving that the lipophilicity (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 formation of an inclusion complex may influence differently the biological effect of individual anticancer drugs. As demonstrated by the calculations, taxol was the most hydrophobic anticancer drug (R_{M0} = 3.50). This finding explains its low solubility in water and in various infusions. Taxol forms an inclusion complex with HPβCD, indicating that the solubility of taxol can be enhanced by HPβCD. The taxol-HPβCD complex may have parameters advantageous to its application. Its elucidation need further investigations.

Significant linear correlation was found between the intercept (hydrophobicity) and slope (specific hydrophobic surface area) values of anticancer drugs (Fig. 1). This finding indicates that from a chromatographic point of view these drugs behave as a homologous series of compounds, although their chemical structures are considerably different.

Significant linear relationship was observed between the complex hydrophobicity index (R_{M0}/b_1) and complex-forming capacity of anticancer drugs (b_2) (Fig. 2). The fact that more hydrophobic drugs form stabler complexes with HPβCD proves that hydrophobic forces are involved in the binding of these drugs to the inner wall of the cyclodextrin cavity.

Acknowledgment

This work was supported by a grant (OTKA T7340) of the Hungarian Academy of Sciences.

References

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

Cabral Marques, H.M., Hadgraft, J., Kellaway, I.Y. and Taylor, G., Studies on cyclodextrin inclusion complexes: III. The pulmonary absorption of β-, DM-β- and HP-β-cyclodextrins in rabbits. *Int. J. Pharm.*, 77 (1991) 297–302.

Chabner, B.A., Taxol. *Principles Pract. Oncol.*, 5 (1991) 1–10.

Chun, I.K. and Yun, D.S., Inclusion complexation of hydrocortisone butyrate with cyclodextrins and dimethyl-β-cyclodextrin in aqueous solution and in solid state. *Int. J. Pharm.*, 96 (1993) 91–104.

Cserháti, T., Determination of lipophilicity of some aniline derivatives by reversed-phase thin-layer chromatography. The effect of organic phase in the eluent. *Chromatographia*, 18 (1984) 318–322.

Cserháti, T. and Valkó, K., *Chromatographic Determination of Molecular Interactions*, CRC Press, FL, 1993, pp. 1–120.

Cserháti, T., Bojarski, J., Fenyvesi, E. and Szejtli, J., Reversed-phase thin-layer chromatography of barbiturates in the presence of soluble β-cyclodextrin polymer. *J. Chromatogr.*, 351 (1986) 356–362.

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

Distelmans, W., Van Ginckel, R., Vanherck, W., Willebrords, R., Wouters, L., De Brabander, M. and Mesens, J., Erbulozole (P.I.N.N.) (R 55 104) encapsulated into cyclodextrins: has it a combined antitumoral and radioprotective potential? *Anticancer Res.*, 11 (1991) 253–256.

Djedainipilard, F., Perly, B., Dupas, S., Miocque, M. and Galons, M., Specific interaction and stabilization between host and guest. Complexation of ellipticine in a nucleobase functionalized cyclodextrin. *Tetrahedron Lett.*, 34 (1993) 1145–1148.

Estes, K.S., Brewster, M.E., Webb, A.I. and Bodor, N., A non-surfactant formulation for alfaxalone based on amorphous cyclodextrin: activity studies in rats and dogs. *Int. J. Pharm.*, 65 (1990) 101–107.

Guerite-Voegelein, F., Guenard, D. and Potier, P., Substances anticancereuses d'origine vegetale. Les poisons du fuseaue: vincaleucoblastine, leurocristine et navelbine; taxol et taxotere. *C.R. Soc. Biol.*, 186 (1992) 433–440.

Hanauske, A.-R., Degen, D., Hilsenbeck, S.G., Bissery, M.C. and Von Hoff, D.D., Effects of taxotere and taxol on in vitro colony formation of freshly explanted human tumor cells. *Anti-Cancer Drugs*, 3 (1991) 121–124.

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

Holmes, F.A., Walters, R.S., Theriault, R.L., Forman, A.D., Newton, L.K., Raber, M.N., Buzdar, A.U., Fye, D.K., and Hortobagyi, G.N., Phase II trial of taxol, an active drug in the treatment of metastatic breast cancer. *J. Natl. Cancer Inst.*, 83 (1991) 1797–1805.

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

Hostetler, J.S., Hanson, L.H. and Stevens, D.A., Effect of cyclodextrin on the pharmacology of antifungal oral azoles. *Antimicrob. Agents Chemother.*, 36 (1992) 477–480.

Kingston, D.G.I., (1991) The chemistry of taxol. *Pharmacol. Ther.*, 52 (1991) 1–34.

Loftsson, T., Baldvinsdottir, J. and Sigurgardottir, A.M., The effect of cyclodextrins on the solubility and stability of medroxyprogesterone acetate and megestrol acetate in aqueous solution. *Int. J. Pharm.*, 98 (1993) 225–230.

Mager, H., *Moderne Regressionsanalyse*, Salle, Sauerlander, Frankfurt am Main, 1982, pp. 135–157.

Markman, M., Taxol: an important new drug in the management of epithelial ovarian cancer. *Yale J. Biol. Med.*, 64 (1991) 583–590.

Marty, M., Extra, J.M., Culine, S. and Rousseau, F., Taxol et adenocarcinomes ovariens. *Path. Biol.*, 39 (1991) 834–835.

Mathew, A.E., Mejillano, M.R., Nath, J.P., Himes, R.H. and Stella, V.J., Synthesis and evaluation of some water-soluble prodrugs and derivatives of taxol with antitumor activity. *J. Med. Chem.*, 35 (1992) 145–151.

Müller, B.V. and Albers, E., Effect of hydrotropic substances on the complexation of sparingly soluble drugs with cyclodextrin derivatives and the influence of cyclodextrin complexation on the pharmacokinetics of the drugs. *J. Pharm. Sci.*, 80 (1991) 599–604.

Neto, A.G.F. and DiCosmo, F., Distribution and amounts of taxol in different shoot parts of *Taxus cuspidata*. *Planta Med.*, 58 (1992) 464–466.

Pedersen, M., Edestein, M., Nielsen, V.F., Sarpellini, A., Skytte, S. and Slot, C., Formation and antimycotic effect of cyclodextrin inclusion complexes of econazole and miconazole. *Int. J. Pharm.*, 90 (1993) 247–254.

Senilh, V., Blechert, S., Colin, M., Guenard, D., Picot, F. and Potier, P., Mise en èvidence de noveaux analogues du taxol extraits de *Taxus baccata*. *J. Natural Prod.*, 47 (1984) 134–139.

Slichenmyer, M. and Von Hoff, D.D., Taxol: a new and effective anticancer drug. *Anti-Cancer Drugs*, 2 (1991) 519–530.

Szejtli, J., *Cyclodextrins and Their Inclusion Complexes*, Akadèmiai Kiadó, Budapest, 1982.

Szejtli, J., *Cyclodextrin Technology*, Kluwer, Dordrecht, 1988.

Uekama, K., Matsubara, K., Abe, K., Horiuchi, Y., Hirayama, F. and Suzuki, N., Design and in vitro evaluation of slow release dosage form of pyretamide: utility of β-cyclodextrin-cellulose derivative combination as a modified-release drug carrier. *J. Pharm. Sci.*, 79 (1990) 244–248.

Valkó, K., General approach for the estimation of octanol/water partition coefficient by reversed-phase high performance liquid chromatography. *J. Liq. Chromatogr.*, 7 (1984) 1405–1424.

Watanabe, Y., Matsumoto, Y., Kawamoto, K., Yazawa, S. and Matsumoto, M., Absorption enhancement of polypeptide drugs by cyclodextrins: 2. Enhancing effect of cyclodextrins on nasal absorption of insulin and its duration in rabbits. *Chem. Pharm. Bull.*, 40 (1992b) 3100–3104.

Watanabe, Y., Matsumoto, Y., Seki, M., Takase, M. and

Matsumoto, M., Absorption enhancement of polypeptide drugs by cyclodextrins: 1. Enhanced rectal absorption of insulin from hollow-type suppositories containing insulin and cyclodextrins in rabbits. *Chem. Pharm. Bull.*, 40 (1992a) 3042–3047.

Williams, S., Mutch, D.G., Xu, L. and Collins, J.L., Divergent effects of taxol on tumor necrosis factor-α-mediated cytolysis of ovarian carcinoma cells. *Am. J. Obstet. Gynecol.*, 167 (1992) 1870–1876.