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Señores

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

DIVISIÓN DE NUEVAS CREACIONES

E. S. D.

Re: Expediente No. 03-059.764

Solicitud de patente titulada: "COMPOSICIONES DE COMPLEJOS DE ESTRÓGENO-CICLODEXTRINA"

Solicitante: SHERING AKTIENGESELLSCHAFT

N. Ref: P2003/000176

RESPUESTA AL OFICIO No. 6794 NOTIFICADO POR FIJACIÓN EN LISTA No. 176, DE FECHA 12 DE JUNIO DE 2009 (REQUERIMIENTO ARTÍCULO 46)

JAMES IAN RAISBECK, mayor de edad y vecino de Bogotá, identificado tal como aparece al pie de mi firma, en mi carácter de apoderado de la sociedad solicitante, me permito manifestar lo siguiente ante el auto emanado por ese Despacho, dentro del plazo legal previsto en el artículo 46 de la Decisión 486:

1. Presento copia completa de los siguientes documentos exigidos por el Examinador en el examen de búsqueda de anterioridades, según lo previsto en el Artículo 46 de la Decisión 486 de la Comisión de la Comunidad Andina:

1) FRIDRIKSDOTTIR, HAFRUN ET AL: "Design and in vivo testing of 17β-estradiol-HPβCD sublingual tablets". PHARMAZIED (1996), 51(1), 39-42. XP 000545921.

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2) AMSTERDAM, NL; LOFTSSON T. ET AL: "The effect of water-soluble polymers on drug-cyclodextrin complexation". INTERNATIONAL JOURNAL OF PHARMACEUTICS, (1994), 110/2, 169-177. XP 001002856

Atentamente,

J. IAN RAISBECK
C.C. No. 79.376.996 de Bogotá
T.P. 135.799 CSJ

Anexo: Artículos mencionados.

1996 Die Pharmazie
51(1996) January, No. 1, Eschborn, DE

p. 39-42 = (4)

Department of Pharmacy[1], Department of Obstetrics and Gynaecology[2] and Department of Clinical Chemistry[3], University of Iceland, and Icelandic Pharmaceuticals[4], Reykjavik, Iceland

Design and *in vivo* testing of 17β-estradiol-HPβCD sublingual tablets

HAFRÚN FRIÐRIKSDÓTTIR[1], T. LOFTSSON[1], J. A. GUÐMUNDSSON[2], G. J. BJARNASON[4], M. KJELD[3] and TANJA THORSTEINSSON[2]

17β-Estradiol is almost insoluble in water. The effect of various cyclodextrins and two different polymers, polyvinylpyrrolidone (PVP) and carboxymethylcellulose (CMC), on the aqueous solubility of 17β-estradiol was investigated. 17β-Estradiol was dissolved in aqueous 50% w/v 2-hydroxypropyl-β-cyclodextrin (HPβCD) solution containing 0.25% (w/v) CMC and the dry 17β-estradiol-HPβCD complex formed by lyophilisation of the solution. Sublingual tablets from [redacted] in a lyophilised HPβCD complex was determined. For reference the dissolution of 17β-estradiol from tablets containing the drug tablets containing physical mixture of 17β-estradiol and HPβCD or tablets containing 17β-estradiol without HPβCD. Sublingual tablets containing 17β-estradiol-HPβCD in the lyophilised complex demonstrated the fastest dissolution profile and those tablets were selected for further studies in humans. Six postmenopausal women received a sublingual tablet containing 17β-estradiol-HPβCD complex equivalent to 100 µg 17β-estradiol. Blood samples were collected over a 12 h period and the 17β-estradiol plasma concentration was determinated. 17β-Estradiol was rapidly absorbed from the sublingual tablets, resulting in a peak 17β-estradiol plasma concentration of 568 ± 97 pmol/l 15 min after administration of the tablets, followed by a biphasic elimination.

Entwicklung und *In-vivo*-Testung von Sublingualtabletten mit 17β-Estradiol-2-hydroxypropyl-β-cyclodextrin-Komplex

17β-Estradiol wurde in Gegenwart von wäßriger 2-Hydroxypropyl-β-cyclodextrin(HPβCD)-Lösung (50%, w/v) und von 0,25% (w/v) Carboxymethylcellulose lyophilisiert. Aus dem getrockneten Komplex wurden durch Direkttablettierung Sublingualtabletten hergestellt und die Liberation von 17-β-Estradiol geprüft. Als Vergleich dienten physikalische Mischungen von 17-β-Estradiol mit HPβCD oder 17-β-Estradiol enthaltende Tabletten. Die Tabletten, die den Komplex enthielten, zeigten die beste Liberation. Bei weiblichen Probanden in der Postmenopause zeigten die so bereiteten Sublingualtabletten eine schnelle Absorption des Wirkstoffs mit Spitzen-17-β-Estradiolkonzentrationen von 568 ± 97 pmol/l 15 min nach Applikation und anschließender biphasiger Elimination.

1. Introduction

The buccal mucosa is an easily accessible and convenient site for drug delivery. It is routinely exposed to food and other "foreign substances" and, thus, it is robust [1, 2]. This makes tablets for sublingual/buccal drug delivery widely acceptable. After administration to the oral cavity, the drug must dissolve in the aqueous saliva, however, the drug molecules must be lipophilic to be able to pass the mucosal barrier into the blood circulation.

Cyclodextrins (CD) are cylindrical shaped hydrophilic oligosaccharides with a lipophilic central cavity [3, 4]. They are capable of forming inclusion complexes with many drugs by taking up a whole drug molecule, or some part of it, into the cavity. The drug-CD complexes can improve the clinical usage of the drugs by increasing their aqueous solubility, rate of dissolution and pharmaceutical availability. Pitha et al. [5] have shown that when testosterone is formulated in a complex with 2-hydroxypropyl-β-cyclodextrin (HPβCD), it is readily absorbed from under the tongue resulting in a rapid increase of testosterone levels in plasma. The complex increases the solubility of the drug in the salvia without effecting its lipophilicity, i.e. its ability to pass the mucosal barrier. Pitha's results have been confirmed by other investigators [6]. In recent years, administration of estrogens as hormone replacement therapy (HRT) during and after the female climacteric has increased [7, 8]. Good clinical effects have been obtained after oral administration of micronized 17β-estradiol. However, due to its first-pass metabolism relatively large oral doses of the drug have to be given in order to obtain physiological plasma concentrations. Also, the first-pass metabolism results in relatively high levels of the metabolite estrone which possesses much weaker estrogen activity than 17β-estradiol [7, 8]. This first-pass effect can be avoided by percutaneous, subcutaneous, intramuscular and intranasal administration. For example, after intranasal administration of 17β-estradiol, the estrone/17β-estradiol ratios were shown to be below unity, whereas, the ratios were above unity after oral administration of 17β-estradiol [9]. Parenteral drug delivery is inconvenient and the intranasal route can cause inflammation of the nasal mucosa. Thus, alternative methods for HRT are being sought.

It has been shown that, at relatively high concentrations and when formulated with cyclodextrin, 17β-estradiol is absorbed from the oral cavity [10]. The purpose of this study was to design and evaluate sublingual 17β-estradiol tablets containing only 100 µg of the drug as a 17β-estradiol-HPβCD inclusion complex.

2. Investigations, results and discussion

Various CDs and CD derivatives have been shown to be powerful solubilizers of drugs. One of the most common CD derivative presently used in drug formulations is HPβCD. In this experiment HPβCD was used after studying the solubilising effect of various CD's (at 10% w/v concentration) on 17β-estradiol (Tables 1 and 2). Although both dimethyl-β-cyclodextrin (DMβCD) and the branched cyclodextrin (M/DMβCD) resulted in better solubilization of 17β-estradiol, HPβCD was selected due to its more favourable toxicological profile [4, 11]. The effect of carboxymethylcellulose (CMC) and polyvinylpyrrolidone (PVP) on the solubilising effect of HPβCD on 17β-estradiol, is shown in Table 2 and Fig. 1. The solubili-

Table 1: The effect of 10% (w/v) of various cyclodextrins on the solubility of 17β-estradiol with and without polymer

10% (w/v) Cyclodextrins	No polymer (mg/ml)	0.25% CMC (mg/ml)	0.25% PVP (mg/ml)
HPγCD	2.46	3.01	3.49
HPαCD	0.09	0.09	not determined
M/DMβCD	8.12	8.31	8.0
DMβCD	9.13	10.30	10.31

Hydroxypropyl-γ-cyclodextrin (HPγCD), hydroxypropyl-α-cyclodextrin (HPαCD), maltosyl/dimaltosyl-β-cyclodextrin (M/DMβCD), dimethyl-β-cyclodextrin (DMβCD).

Table 2: The effect of 0.25% (w/v) CMC and 0.25% (w/v) PVP compared to water on the solubilisation of 17β-estradiol in aqueous cyclodextrin solutions

HPβCD (%, w/v)	No polymer, (mg/ml)	0.25% (w/v) CMC (mg/ml)	0.25% (w/v) PVP (mg/ml)
0	0.002	0.172	0.069
2.5	1.324	1.794	2.243
5.0	2.741	3.853	3.100
10.0	4.770	5.340	5.280

sing effect of 10% (w/v) HPβCD on 17β-estradiol was improved by 2–12% when increasing concentration of PVP or CMC (0–0.7%, w/v) was present in the solution (Fig. 1). The optimal concentration of PVP was 0.05% (w/v), but higher concentrations lead to some decrease in 17β-estradiol solubility. The optimal concentration of CMC was about 0.1% (w/v).

The dissolution rate profiles of the lyophilised inclusion complex I (containing 100 µg of 17β-estradiol and 2.5 mg of HPβCD tablet 1), the lyophilised inclusion complex II (containing 100 µg of 17β-estradiol and 4.4 mg of HPβCD, tablet 2) the pure drug (17β-estradiol without HPβCD, tablet 3) and the physical mixture (100 µg 17β-estradiol mixed with 2.5 mg HPβCD tablet 4) are shown in Fig. 2. Composition of the tablets is given in Table 3.

It is evident that complex I, complex II and the physical mixture demonstrate faster dissolution rate than the pure

Table 3: Composition of the tablets

Material	Tablet 1	Tablet 2	Tablet 3	Tablet 4
Complex I	2.6			
Complex II		4.53		
17β-estradiol			0.1	0.1
HPβCD				2.5
Emdex*	52.1	50.2	54.6	52.1
DC-Lactose-II*	23	23	23	23
Polyplastone XL*	6.6	6.6	6.6	6.6
Cab-O-Sil	0.6	0.6	0.6	0.6
Magnesii steras	1.1	1.1	1.1	1.1
Total weight per tablet. (mg)	86.0	86.0	86.0	86.0

Tablets 1, 2, 3 and 4: contained 100 µg 17β-estradiol.
Complex I; one gram powder contained 39 mg of 17β-estradiol. Complex II: one gram powder contained 22 mg of 17β-estradiol. Tablet 3; 17β-estradiol without HPβCD, (pure drug). Tablet 4; Physical mixture of 17β-estradiol and HPβCD (0.1 mg 17β-estradiol mixed with 2.6 mg powder).

drug. After 5 min the amount of 17β-estradiol dissolved was 100% from tablet 1 and 80% from tablet 2, compared to 66% and 42% from the tablet 4 and the tablet 3, respectively.

Generally, the dissolution was much faster from the tablets that contained 17β-estradiol-HPβCD-complex, than from the tablets containing only the pure drug or a physical mixture of the drug and HPβCD. It can also be seen that the fastest dissolution was obtained when just enough HPβCD was used to dissolve the drug. An excess HPβCD decreased the dissolution rate. Because of its dissolution profile, tablet 1 was selected for human testing.

Absorption of 17β-estradiol-HPβCD from tablet 1, given sublingually, produced a supraphysiologic peak of 17β-estradiol concentration in serum, within 15 min after administration (Fig. 3). The mean peak 17β-estradiol concentration was determined to be 568 ± 97 pmol/l (at 15 min). The 17β-estradiol serum concentration versus time profile was fitted to a two compartment model:

$$C_p = Ae^{-at} + Be^{-bt} - Ce^{-kat}$$

where a and b are the distribution and elimination rate constants, and A and B are empirical constants. The

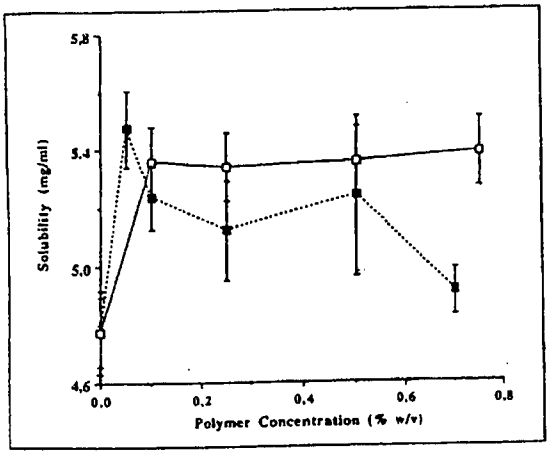

Fig. 1: The effect of increasing concentrations of (■) polyvinylpyrrolidone (PVP) and (□) carboxymethylcellulose sodium (CMC) on the solubility of 17β-estradiol in aqueous 10% (w/v) HPβCD MS 0.6 solution. Each point represents the mean of three measurements and the vertical lines represent the standard error of the mean (S.E.M).

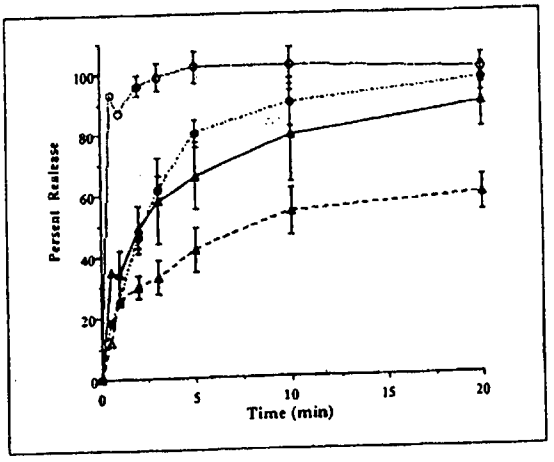

Fig. 2: The dissolution rate profiles of 17β-estradiol from sublingual tablets. Tablets containing pure 17β-estradiol (△), tablets containing physical mixture of 17β-estradiol and HPβCD (▲), tablets containing lyophilised 17β-estradiol-HPβCD inclusion complex with excess of HPβCD, i.e. complex II (●), and tablets containing physical mixture of lyophilised 17β-estradiol-HPβCD inclusion complex without an excess of HPβCD, i.e. complex I (○).

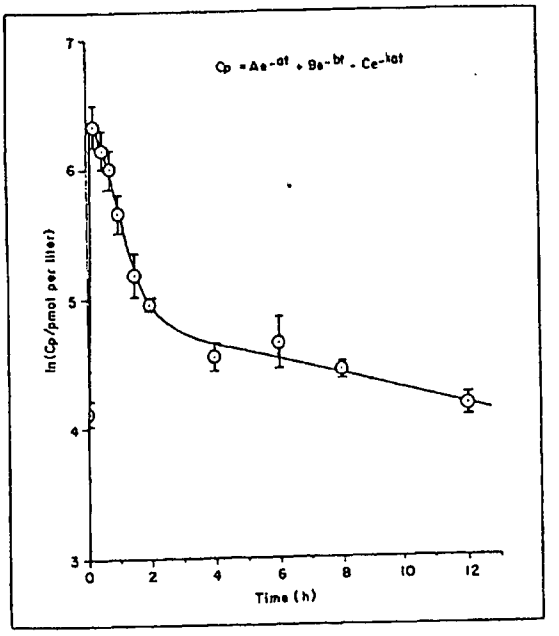

Fig. 3: The 17β-estradiol concentration (ln Cp) versus time, after sublingual administration of 100 µg of 17β-estradiol. Mean values from 6 women; the error bars represent the standard error of the mean (S.E.M). Cp: pmol/l

function of the absorption phase is shown as $-Ce^{-kat}$. The half lives ($t_{1/2}$) of the elimination and the distribution phase are 12.7 h and 0.83 h, respectively. The value of A was determined to be 704 pmol/l, B 131 pmol/l, a $-0.84\ h^{-1}$, and b $-0.05\ h^{-1}$. The half live of the absorption was estimated to be about 7 min, which indicates that about 90% of the dose is absorbed within the first 25 min.

3. Experimental

3.1. Materials

17β-Estradiol, dimethyl-β-cyclodextrin (DMβCD) and maltosyldimaltosyl-β-cyclodextrin (M, DMβCD) were obtained from Pharmatec Inc. (USA), 2-hydroxypropyl-β-cyclodextrin of molar substitution 0.6 (HPβCD), γ-cyclodextrin (γCD), hydroxypropyl-α-cyclodextrin of molar substitution 0.6 (HPαCD) from Wacker Chemie (Germany), carboxymethylcellulose sodium of medium viscosity (CMC) and [illegible] weight 360.000 (PVP) were obtained from Sigma Chemical Co. (USA). Emdex® (dextrans (NF XVI)) from Mendell (U.S.A), DC-lactose-11® (lactose Ph. Eur., 2nd Ed.) from de Melkindustrie Veghel (DMV) (the Nederlands), Polyplastone® (crospovidone NF) from International speciality products, ISP, (USA), Cab-O-Sil® (silica colloidalis ANH., Ph. Eur., 2nd Ed.) from Carbot (USA) and magnesii sterat (Ph. Eur., 2nd Ed.) from Norsk Medicinal Depot (Norway). All other materials and solvents used in this study were of analytical or Ph. Eur., 2nd Ed., grade.

3.2. Solubility studies

An excess amount of 17β-estradiol was added to water, aqueous polymer solution, aqueous CD solution, or aqueous solution containing both a polymer and CD. The suspension formed was sonicated in an ultrasonic bath (Kerry, UK) for 1 h and then heated in an autoclave (M7 Speed Clave from Midmark Corporation, USA), in a sealed container to 120 °C for 20 min. After equilibration at room temperature (22–23 °C) for at least 3d, the suspension was filtered through a 0.45 µm membrane filter (Millex-HV filter units from Millipore, USA), diluted with a mixture of methanol and water (7:3 v/v) and analysed by an HPLC method. Significant excess of the drug was always used in these studies and, thus, solid drug particles were always present in the aqueous cyclodextrin solution during the whole equilibration period.

The phase-solubility of 17β-estradiol in aqueous CD solutions with or without polymer were determined. An excess amount of the drug was added to aqueous solutions containing from 0 to 10% (w/v) CD and from 0 to 0.7% (w/v) polymer. As before the solutions were saturated by sonication and heating. After equilibration at room temperature for 3 d, the suspensions were filtered and analysed by HPLC.

3.3. Preparation of dry complexes

One or two g of 17β-estradiol were dissolved through heating in an autoclave to 120 °C for 20 min in 100 ml of aqueous 50% (w/v) HPβCD solution containing 0.25% (w/v) of CMC. After cooling, equilibration and freezing, the solution was lyophilised in a Snijders Scientific Type 2040 lyophiliser (the Netherlands). Samples of the dry complex powders were then dissolved in 70% (v/v) aqueous methanol and the 17β-estradiol content determined by HPLC. One g of the two lyophilised powders, complex 1 and complex 2, contained 39 and 22 mg of 17β-estradiol, respectively.

[redacted]

The tablets were formed through a direct compression technique. The 17β-estradiol-HPβCD complex, silica colloidalis and magnesium sterate were (manually) screened through 0.3 mm, 0.7 mm and 0.125 mm screens, respectively. The complex and dextrans were blended in a tumble mixer for 15 min. Lactose, [illegible] silica colloidalis were added and the mixture blended further for 10 min. Finally, magnesium sterate was added and blended for 5 min. The tablets without the complex were blended the same way, but 17β-estradiol was first mixed with a HPβCD or dextrans. All tablets were compressed in a stokes model B-2 rotary tablet press (sinjul puncher machine, Stokes-Merrill, INC, USA), using 6 mm punches to hardness of aprox. 40 N. The target weight was 86.0 mg, disintegration time was less than 1 min.

3.5. Dissolution studies

The dissolution studies were carried out in the USP XXII described paddle apparatus (Prolabo from Groupe Rhone-Poulenc, France) for dissolution rate determination. The release rate was determined at 37 ± 1 C and 100 rpm by adding tablets containing 100 µg of 17β-estradiol to 250 ml of water. Samples (1.5 ml) were withdrawn at various time intervals, filtrated through 0.45 membrane filters (Millex-HV; Millipore, USA) and analysed by HPLC. The cumulative dilution, cause by replacement of the sample by equivalent volumes of fresh medium was corrected.

3.6. Quantitative determinations

The quantitative determinations of the individual drugs were performed by a reversed-phase HPLC system, consisting of a Milton Roy ConstaMetric 3200 solvent delivery system operated at 1.50 ml/min, a Rheodyne 7125 injector, a Beckman Ultrasphere 5 µ (4.6 × 150 mm) column and a Spectro Monitor 3200 UV/Vis variable wavelength detector operated at 270 nm. The mobile phase consisted of acetonitrile, ethanol and water 154:1:451 and the retention time was 2.4 min.

3.7. In vivo testing in humans

Six postmenopausal women the age of 47–75 years, were recruited for this open study. All the women were inpatients of the Department of Obstetrics and Gynaecology, University Hospital, Landspitali, Reykjavik. The women were in convalescence after gynaecological surgery for benign diseases. They were otherwise healthy and not taking any drugs that could interfere with the metabolism of 17β-estradiol. The study was approved by the local ethical committee and all the women volunteers signed an informed consent form. Before treatment, low endogenous 17β-estradiol production was confirmed by measurement of FSH levels. The women received single doses of 100 µg 17β-estradiol (tablet 1) as sublingual tablets. Blood samples were obtained immediately by venepuncture before the administration of the tablets and then 10 times with decreasing frequency over a period of 12 h for measurement of 17β-estradiol. 17β-estradiol was measured by time-resolved fluoroimmunoassy (DELFIA) with reagents from Wallac, Finland. The detection limit of the assay was better than 50 pmol l.

Acknowledgement: The authors would like to thank Dr. Josef Pitha for numerous helpful suggestions and support throughout this study.

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Improvement of some pharmaceutical properties of drugs by cyclodextrin complexation

5. Theophylline[1]

H. O. Ammar, M. Ghorab, S. A. El-Nahhas, S. M. Omar and M. M. Ghorab

The interaction of theophylline (TPH) with β-cyclodextrin (β-CD) was investigated by spectrophotometry, vapour pressure osmometry and DSC. The results revealed a molecular interaction between TPH and β-CD. The continuous variation method was used to elucidate the stoichiometry of such an interaction by spectrophotometric as well as vapour pressure measurements. Both types of data revealed the formation of two-to-one TPH/β-CD complex. The stability constant of the complex was determined at different temperatures by the vapour pressure osmometric method. The enthalpy and entropy of the interaction were evaluated and the results indicate the liberation of little heat during complexation and the disorder of the guest molecule upon complexation. The effect of β-CD on the solubility of TPH indicates that β-CD exhibits a definite solubilizing effect towards the drug with a typical B_S isotherm. The stability constant of the complex and the amount of drug solubilized in the form of complex reveal that complex-formation is the only factor governing the solubilizing effect of β-CD towards the drug. The dissolution rates of TPH, TPH/β-CD physical mixture as well as the prepared complex were determined according to U.S.P. method and at pH 1.2. In both cases, the dissolution profile of the complex reveals enhanced dissolution properties compared to the drug. The effect of β-CD on the partition properties of TPH reveals decrease in presence of β-CD. The effect of β-CD on the bioavailability of TPH was investigated in human subjects. A clear difference in the biological performance between the drug and the complex was revealed. The pharmacokinetic parameters including C_{max}, t_{max}, C_{min}, $t_{1/2}$, K_e, MRT and AUC revealed that inclusion complexation of theophylline in B-cyclodextrin results in not only an improvement in the bioavailability of the drug, but also to acquired sustained release properties for the drug.

Verbesserung pharmazeutischer Eigenschaften von Arzneistoffen durch Cyclodextrin-Einschluß

5. Theophyllin

Die Interaktion zwischen Theophyllin (TPH) und β-Cyclodextrin (β-CD) wurde mittels Spektrophotometrie, Dampfdruck-osmometrie und Differentialscanningcalorimetrie untersucht. Die Ergebnisse weisen auf eine molekulare Interaktion zwischen beiden Partnern hin, die zu einem 1 : 1-Komplex führt. Stabilitätskonstante, scheinbare Wechselwirkungsenthalpie und Entropie der exothermen Reaktion wurden bestimmt. Die Wechselwirkung mit β-CD führt zu einer Verbesserung der TPH-Löslichkeit durch Solubilisierung. In gleichem Sinne wird auch die Dissolution der Verbindung beeinflußt, wenn sie als β-CD-Komplex eingesetzt wird. Die Bioverfügbarkeit, aus Messungen der Speichelkonzentrationen von THP abgeleitet, wird nicht verbessert. Die Freigabe wird im Sinne eines „sustained release" beeinflußt.

1. Introduction

Theophylline is the drug of choice for sustained release formulations, because it has a relatively short-life of elimination, particularly in children and a rather low therapeutic drug level (8 – 20 mg/l) [1 – 3]. It has a central nervous system stimulant effect which can be minimized by sustained release preparation. Sustained release TPH products are the products of choice for the prophylaxis of chronic asthma symptoms.

The present communication deals with the investigation of the inclusion complexation of TPH in β-CD and the implication of this phenomenon on the solubility, dissolution and bioavailability of the drug.

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The effect of water-soluble polymers on drug-cyclodextrin complexation

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Abstract

In aqueous solutions water-soluble polymers were shown to increase the solubilising effect of cyclodextrins on drugs. For example, the solubilising effect of 2-hydroxypropyl-β-cyclodextrin (HPβCD) with molar substitution of 0.6 was improved by 6–57% (on average 27%) when 0.25% (w/v) carboxymethylcellulose was present in the solution and 12–129% (on average 49%) when 0.25% (w/v) polyvinylpyrrolidone (PVP) was present. Similar results were obtained with other cyclodextrins and other polymers. For PVP and 10% (w/v) HPβCD the PVP concentration for maximum solubilisation appeared to be between 0.05 to 0.25% (w/v). At this low concentration PVP had insignificant effect on the viscosity of the aqueous HPβCD solution. The polymers increased the stability constants of the drug-cyclodextrin complexes. Addition of PVP to the aqueous complexation medium resulted in an increased negative enthalpy change, together with an increased negative entropy change.

Key words: Keywords: Complexation; Cyclodextrin; Drug; Polymer; Solubilization

1. Introduction

Cyclodextrins form a group of structurally related oligosaccharides. The important characteristics of the cyclodextrin molecules are their cylindrical shape, somewhat hydrophobic central cavity and hydrophilic outer surface (Szejtli, 1988). Cyclodextrins are capable of forming inclusion complexes with many drugs by taking up a whole drug molecule, or some part of it, into the cavity (Pitha et al., 1986; Duchêne, 1987). No covalent bonds are formed or broken during formation of the complex. The complexes are readily dissociated and free drug molecules are in a rapid equilibrium with the drug molecules bound within the cyclodextrin cavity. This type of molecular encapsulation will affect many of the physicochemical properties of the drugs, such as their solubility and stability. Thus, cyclodextrins are of potential importance as excipients in drug formulations (Loftsson et al., 1991; Duchêne and Wouessidjewe, 1993).

The most common method for preparing drug-cyclodextrin complexes on a laboratory scale is to add an excess of the drug to an aqueous

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cyclodextrin solution. The suspension formed is then agitated for up to 1 week at room temperature. The suspension is then filtered or centrifuged to form a clear drug-cyclodextrin complex solution. For preparation of solid formulations of the drug-cyclodextrin complex, the water is removed from the aqueous drug-cyclodextrin complex solution by evaporation in a rotary evaporator, in a spray dryer or by lyophilisation. Frequently, the efficiency of complexation is not very high and, therefore, relatively large amounts of cyclodextrins must be used to complex small amounts of the drug. Vehicle additives commonly used in drug formulations, additives such as sodium chloride, buffer salts, surfactants, preservatives and organic solvents, reduce the efficiency. For example, in aqueous solutions, low concentrations of ethanol or propylene glycol have been shown to reduce the cyclodextrin complexation of testosterone and ibuprofen by acting as a competing guest molecule (Pitha and Hoshino, 1992; Loftsson et al., 1993). In the same manner, non-ionic surfactants have been shown to reduce the cyclodextrin complexation of diazepam (Kraus et al., 1991) and preservatives to reduce the cyclodextrin complexation of various steroids (Loftsson et al., 1992). However, hydroxypropyl methylcellulose has been demonstrated to increase the complexation of dexamethasone with 2-hydroxypropyl-β-cyclodextrin (Loftsson et al., 1994).

There are several reasons why increased efficiency of cyclodextrin complexation of drugs is desirable. For example, only a limited amount of cyclodextrin can be used in many drug formulations, such as in aqueous isotonic solutions and tablets, and increased efficiency will mean that less cyclodextrin can be used to achieve the same or even greater solubilising effect. Also, since cyclodextrins are still relatively expensive, reduction in the amount of cyclodextrin in drug formulations will result in lower production costs. The purpose of this study was to investigate further the effects of various water-soluble polymers on the cyclodextrin complexation of drugs. The efficiency of complexation was mainly evaluated by determining the solubilising effects of the cyclodextrins, however, the stability constants of the drug-cyclodextrin complexes and their thermodynamics were also determined.

2. Materials and methods

2.1. Materials

The following drugs were obtained from Sigma Chemical Co. (U.S.A.): alprazolam, clotrimazole, econazole, 17β-estradiol, ethoxyzolamide, hydrocortisone, miconazole and progesterone. The following drugs were purchased from Lyfjaverslun rikisins (Iceland): acetazolamide, diazepam, oxazepam, prednisolone, sulfamethoxazole, temazepam and triamcinolone acetonide. Carbamazepine was obtained from Aldrich Chemical Co. (U.S.A.). 2-Hydroxypropyl-β-cyclodextrin [illegible] molar substitution of 0.6 or 0.9 (HPβCD MS 0.6 or 0.9), hydroxyethyl-β-cyclodextrin with molar substitution of 0.6 (HEβCD) and randomly methylated β-cyclodextrin with degree of substitution of 1.8 (RMβCD) were supplied by Wacker-Chemie (Germany). Glucosyl-α-cyclodextrin (glucosyl-αCD), glucosyl-β-cyclodextrin (glucosyl-βCD), maltosyl-α-cyclodextrin (maltosyl-αCD) and maltosyl-β-cyclodextrin (maltosyl-βCD), all monosubstituted, were obtained from Pharmatec (U.S.A.). Carboxymethylcellulose sodium of medium viscosity (CMC), [illegible] (Mol. Wt) 40000 or 360000 and hydroxypropyl methylcellulose of 4000 cP (HPMC) were obtained from Sigma. All other chemicals were commercially available products of special reagent grade.

2.2. Solubility studies

An excess amount of the drug to be tested was added to water, aqueous polymer solution, aqueous cyclodextrin solution, or aqueous solution containing both a polymer and cyclodextrin. The suspension formed was sonicated in an ultrasonic bath (Kerry, U.K.) for 1 h and then heated in an autoclave (M7 Speed Clave from Midmark Corp., U.S.A.), in a sealed container to 120°C for 20 min. After equilibration at room temperature (23°C) for at least 3 days, the suspension was

filtered through a 0.45 μm membrane filter (Millex-HV filter units from Millipore, U.S.A.), diluted with a mixture of methanol and water (7:3 v/v) and analysed by an HPLC method. Significant excess of the drug was always used in these studies and, thus, solid drug particles were always present in the aqueous cyclodextrin solution during the entire equilibration period. The 3 day equilibration was considered sufficient, since further equilibration of selected drug suspensions for up to 10 days did not result in any further drug precipitation.

The phase-solubility diagrams in aqueous cyclodextrin solutions with or without polymer were determined at various temperatures. An excess amount of the drug was added to aqueous solutions containing from 0 to 20% (w/v) cyclodextrin and from 0 to 0.7% (w/v) polymer. The solutions were saturated by sonication and heating. After equilibration at the desired temperature (from 6 to 50°C) for 7 days, the suspensions were filtered and analysed as described above. The stability constants (K_c) of the drug-cyclodextrin complexes were calculated from the slope of the phase-solubility diagrams and the drug solubility in water (S_o):

$$K_c = \text{slope} \times (S_o \times (1 - \text{slope}))^{-1}$$

according to method of Higuchi and Connors (1965). The effect of PVP on the enthalpy (ΔH^o) and the entropy (ΔS^o) of K_c for the drug-cyclodextrin complex was determined according to Martin (1993).

2.3. Quantitative determinations

Quantitative determinations of the individual drugs were performed on a reversed-phase high-performance liquid chromatographic (HPLC) component system consisting of a Milton Roy ConstaMetric 3200 solvent delivery system, a Rheodyne 7125 injector, a Spectro Monitor 3200 UV/Vis variable-wavelength detector and a LiChrosorb RP-18 5 μ (125 × 4 mm) column. For other HPLC conditions, see Table 1.

Table 1
Conditions of quantitative drug determination by HPLC

Drug	Mobile phase	Flow rate (ml/min)	Wave-length (nm)	Retention time (min)
Acetazolamide	acetonitrile, acetic acid, water (10:20:88) containing 0.015% 1-octanesulfonate	1.5	263	4.0
Alprazolam	methanol, water (70:30)	1.5	254	2.8
Carbamazepine	acetonitrile, tetrahydrofuran, water (35:1:64)	2.0	278	2.8
Clotrimazole	methanol, 0.01 M aqueous potassium phosphate solution (pH 4.5) (90:10)	1.5	210	2.0
Diazepam	methanol, water (72:25)	1.5	226	4.0
Econazole	methanol, 0.01 M aqueous potassium phosphate solution (pH 4.5) (90:10)	1.5	226	2.0
17β-Estradiol	acetonitrile, ethanol, water (54:1:45)	1.5	285	2.0
Ethoxyzolamide	acetonitrile, water (35:65) containing 0.1% 1-hexanesulfonate	1.0	254	3.2
Hydrocortisone	acetonitrile, tetrahydrofuran, water (30:1:69)	1.5	254	2.6
Miconazole	methanol, 0.01 M aqueous potassium phosphate solution (pH 4.5) (90:10)	1.5	272	2.6
Oxazepam	methanol, tetrahydrofuran, water (55:2:43)	1.5	226	2.8
Prednisolone	acetonitrile, acetic acid, water (17:0.5:82.5)	1.5	242	4.0
Progesterone	acetonitrile, ethanol, water (54:1:45)	1.5	235	5.2
Sulfamethoxazole	acetonitrile, acetic acid, water (30:1:69)	1.5	253	2.4
Temazepam	methanol, water (70:30)	1.5	275	2.8
Triamcinolone acetonide	acetonitrile, water (42:58)	1.5	254	2.8

2.4. Viscosity measurements

The viscosity of the aqueous HPβCD MS 0.6 solutions at room temperature was determined in a Brookfield digital viscometer Model DV-I + (Brookfield, U.S.A.) equipped with a Brookfield UL adapter. HPβCD MS 0.6 and/or polymer was dissolved in water and the solution heated in a sealed container as previously described (120°C for 20 min). After equilibration at room temperature and appropriate adjustments of the viscometer the viscosity of the solutions was determined.

3. Results

[redacted] derivatives [redacted] known to be powerful solubilizers of drugs. The most common cyclodextrin derivatives presently used in drug formulation are the hydroxypropyl derivatives of β-cyclodextrin (Pitha et al., 1986; Loftsson et al. 1991), however, their complexing abilities and, hence, their solubilising effects, are affected by both the average number of hydroxypropyl groups on each glucopyranose repeat unit, i.e., the molar substitution (MS), and the location of the hydroxypropyl groups on the HPβCD molecule (Rao et al., 1991; Loftsson and Baldvinsdóttir, 1992; Loftsson and Jóhannesson, 1994). To a certain limit, HPβCD's complexing ability increases when its MS decreases. For example, in aqueous 10% (w/v) HPβCD solution, the solubility of prednisolone was determined to be 12.7 mg/ml when the MS 0.9 derivative was used compared to 13.6 mg/ml in the case of the MS 0.6 derivative and that of sulfamethoxazole 6.08 mg/ml compared to 10.0 mg/ml. Therefore, we mainly used HPβCD MS 0.6 in our studies. The effect of CMC on the solubilising effect of HPβCD MS 0.6 is shown in Table 2. Solubilities of the drugs in aqueous 10% (w/v) HPβCD solutions (S_{co}) were from 3.6-fold (acetazolamide) to 510-fold [redacted] greater than in water (S_o) but introduction of a small amount of CMC into the solution medium improved the solubility even further. Thus, the solubilising effect of HPβCD MS 0.6 was improved by 6–57% (S_{cp}/S_{co} = 1.06–1.57) when 0.25% CMC was present in the solution, on average the solubilising effect being improved by 27%. An even greater

Table 2
Effect of addition of 0.25% (w/v) CMC to aqueous 10% (w/v) HPβCD MS 0.6 solution on HPβCD's solubilization of various drugs

Drug	S_o (mg/ml) [a]	S_p (mg/ml) [b]	S_{co} (mg/ml) [c]	S_{cp} (mg/ml) [d]	S_{cp}/S_{co} [e]
Acetazolamide	0.70	0.84	2.52	3.11	1.23
Alprazolam	0.07	0.18	1.28	1.55	1.21
Carbamazepine	0.11	0.20	7.00	9.20	1.31
Clotrimazole	0.00	0.00	1.20	1.40	1.17
Diazepam	0.69	0.81	9.14	9.70	1.06
Econazole	0.57	0.60	5.03	7.41	1.47
[redacted]	0.01	0.17	4.78	5.35	1.12
Ethoxyzolamide	0.04	0.07	1.36	1.66	1.22
Hydrocortisone	0.36	1.10	12.2	17.0	1.39
Miconazole	0.04	0.06	1.98	2.50	1.26
Oxazepam	0.03	0.04	0.90	1.42	1.57
Prenisolone	0.38	0.53	13.6	15.3	1.13
Progesterone	0.00	0.00	4.39	6.11	1.39
Sulfamethoxazole	0.36	0.69	10.0	12.6	1.26
Temazepam	0.60	0.65	3.01	3.48	1.16
Triamcinolone acetonide	0.03	0.07	1.84	2.58	1.40

[a] Solubility in pure water. [b] Solubility in aqueous 0.25% (w/v) solution of the polymer. [c] Solubility in aqueous 10% (w/v) cyclodextrin solution. [d] Solubility in aqueous solution containing both 0.25% (w/v) polymer and 10% (w/v) cyclodextrin. [e] Solubility ratio.

Table 3
Effect of addition of 0.25% (w/v) PVP Mol. Wt 40000 to aqueous 10% (w/v) HPβCD MS 0.6 solution on HPβCD's solubilization of various drugs

Drug	S_0 (mg/ml) [a]	S_p (mg/ml) [b]	S_{co} (mg/ml) [c]	S_{cp} (mg/ml) [d]	S_{cp}/S_{co} [e]
Acetazolamide	0.70	1.05	2.52	3.92	1.56
Carbamazepine	0.11	0.31	7.00	8.50	1.21
Clotrimazole	0.00	0.00	1.20	1.80	1.50
Econazole	0.57	0.64	5.03	5.65	1.12
Ethoxyzolamide	0.04	0.06	1.36	2.72	2.00
Oxazepam	0.03	0.04	0.90	1.14	1.27
[redacted]	0.00	0.00	4.39	5.71	1.30
Sulfamethoxazole	0.36	0.86	10.0	14.8	1.48
Trimethoprim	0.82	1.35	2.83	6.47	2.29

[a] Solubility in pure water. [b] Solubility in aqueous 0.25% (w/v) solution of the polymer. [c] Solubility in aqueous 10% (w/v) cyclodextrin solution. [d] Solubility in aqueous solution containing both 0.25% (w/v) polymer and 10% (w/v) cyclodextrin. [e] Solubility ratio.

effect was obtained when CMC [redacted] PVP Mol. Wt 40000 (Table 3). In this case the solubilising effect was improved from 12 to 129% (S_{cp}/S_{co} = 1.12–2.29), or on average about 49%. Similar results were obtained with other cyclodextrin derivatives and polymers (Table 4). The optimal polymer concentration appeared to be below 1% (w/v). By comparing the solubility of the drugs in water (S_0) with that in aqueous 0.25% solutions of the polymers (S_p) in Tables 2 and 3, it can be seen that the polymers possess a significant solubilising effect themselves. Both the polymers and the cyclodextrins form water-soluble complexes with various drug molecules and can be used to solubilize drugs. However, when polymer and cyclodextrin are mixed together as described above, one achieves a greater extent of solubilization than when the polymer and cy-

Table 4
Effect of polymers on the solubilization of [redacted] in aqueous cyclodextrin solutions

Cyclodextrin	Polymer	S_{co} (mg/ml) [a]	S_{cp} (mg/ml) [b]	S_{cp}/S_{co} [c]
HEβCD	CMC	17.5	26.8	1.53
RMβCD	CMC	18.6	20.1	1.08
RMβCD	[redacted] PVP Mol. Wt 40000	18.6	20.2	1.09
RMβCD	HPMC	18.6	21.8	1.17
Glycosyl-αCD	CMC	2.7	5.4	2.00
Glycosyl-αCD	[redacted] PVP Mol. Wt 40000	2.7	3.6	1.33
Glycosyl-αCD	HPMC	2.7	5.4	2.00
Glycosyl-βCD	CMC	17.0	20.2	1.19
Glycosyl-βCD	PVP Mol. Wt 40000	17.0	22.2	1.31
Maltosyl-αCD	CMC	4.1	6.1	1.49
Maltosyl-βCD	CMC	10.4	18.3	1.76
Maltosyl-βCD	PVP Mol. Wt 40000	10.4	19.5	1.88
Maltosyl-βCD	HPMC	10.4	17.9	1.72

[a] Solubility in aqueous 10% (w/v) cyclodextrin solution. [b] Solubility in aqueous solution containing both 0.25% (w/v) of the given polymer and 10% (w/v) cyclodextrin. [c] Solubility ratio.

clodextrin are used separately. This solubilization enhancement is more than simply additive, it is synergistic.

The optimum amount of the polymer in the aqueous cyclodextrin solutions appeared to be between 0.05 and 0.25% (w/v) polymer concentration, since higher concentrations usually lead to some decrease in drug solubility. Fig. 1 shows the effect of increasing concentration of PVP Mol. Wt 360000 on the solubility of hydrocortisone, 17β-estradiol and triamcinolone acetonide in an aqueous 10% (w/v) HPβCD MS 0.6 solution. The solubility increases sharply with maximum solubility between 0.05 and 0.2% (w/v)

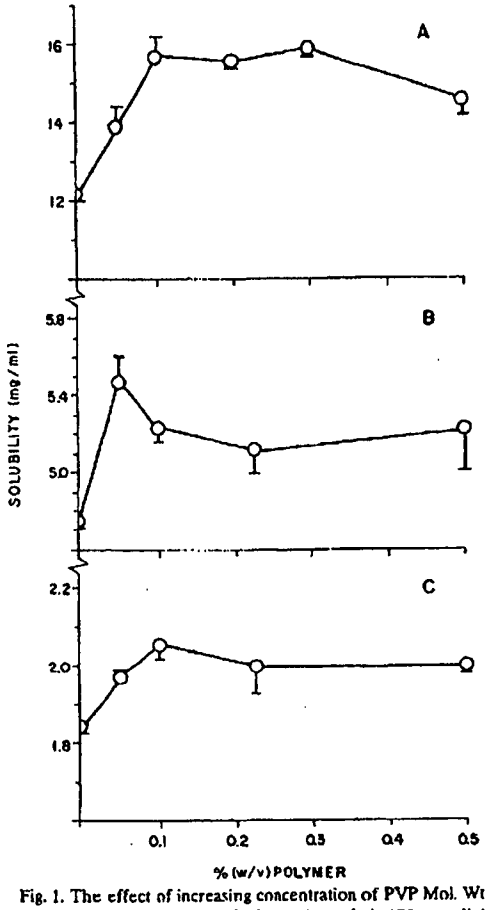

Fig. 1. The effect of increasing concentration of PVP Mol. Wt 360000 on the solubility of hydrocortisone (A), 17β-estradiol (B) and triamcinolone acetonide (C) in an aqueous 10% (w/v) HPβCD MS 0.6 solution at room temperature. Each point represents the mean of three measurments and the vertical lines represent the standard error of the mean.

Table 5
Viscosity of water and aqueous HPβCD MS 0.6, RMβCD DS 1.8, PVP Mol. Wt 360000, CMC and/or HPMC solutions at room temperature (23°C)

Aqueous solution	Viscosity (mPa s)
Pure water	1.00
0.10% (w/v) PVP	1.16
1.00% (w/v) PVP	4.15
1.00% (w/v) CMC	52.9
10% (w/v) HPβCD MS 0.6	1.47
10% (w/v) HPβCD MS 0.6 and 0.05% (w/v) PVP	1.62
10% (w/v) HPβCD MS 0.6 and 0.10% (w/v) PVP	1.72
10% (w/v) HPβCD MS 0.6 and 0.25% (w/v) PVP	2.17
10% (w/v) HPβCD MS 0.6 and 0.05% (w/v) CMC	3.66
10% (w/v) HPβCD MS 0.6 and 0.10% (w/v) CMC	5.40
10% (w/v) HPβCD MS 0.6 and 0.25% (w/v) CMC	10.8
10% (w/v) HPβCD MS 0.6 and 0.50% (w/v) CMC	17.0
10% (w/v) HPβCD MS 0.6 and 0.05% (w/v) HPMC	1.78
10% (w/v) HPβCD MS 0.6 and 0.10% (w/v) HPMC	2.75
10% (w/v) HPβCD MS 0.6 and 0.10% (w/v) HPMC	6.44
25% (w/v) HPβCD MS 0.6	3.07
25% (w/v) HPβCD MS	3.04

Table 6
Effect of PVP Mol. Wt 360000 on the stability constant (K_c) of the hydrocortisone-HPβCD MS 0.6 complex at room temperature (23°C)

PVP concentration (% w/v)	Slope	Corr.	K_c (l/mol)
0	0.502	0.988	1010
0.01	0.528	0.972	1120
0.025	0.532	0.994	1140
0.05	0.544	0.977	1190
0.1	0.591	0.999	1450
0.15	0.577	0.999	1390
0.2	0.548	0.999	1290
0.5	0.544	0.998	1190
0.7	0.543	0.999	1190

Table 7
Effect of PVP Mol. Wt 360000 on ΔH° and ΔS° for the stability constant (K_c) of various drug-cyclodextrin complexes

	PVP concentration (% w/v)	ΔH° (kJ mol^{-1} K^{-1})	ΔS° (J mol^{-1} K^{-1})
Acetazolamide-HPβCD MS 0.6 complex (K_c = 86.2 M^{-1} at 0% PVP and 20°C)	0.00	−18.4	−26.0
	0.10	−25.8	−49.6
	0.25	−24.8	−46.2
	0.50	−25.8	−49.9
Hydrocortisone-HPαCD MS 0.6 complex (K_c = 112.0 M^{-1} at 0% PVP and 20°C)	0.00	−32.1	−70.2
	0.10	−39.3	−94.5
	0.25	−48.4	−124.2
	0.50	−35.7	−81.9
Hydrocortisone-HPβCD MS 0.6 complex (K_c = 2100 M^{-1} at 0% PVP and 20°C)	0.00	−20.4	−6.2
	0.10	−41.0	−68.6
	0.25	−36.5	−56.4
	0.50	−38.8	−64.9
17β-Estradiol-HPβCD MS 0.6 complex (K_c = 52880 M^{-1} at 0% PVP and 20°C)	0.00	−71.1	−151
	0.10	−75.3	−166
	0.25	−89.5	−213
	0.50	−81.2	−185

PVP Mol. Wt 360000 and then decreases slowly (Fig. 1). The viscosity of the aqueous 10% (w/v) HPβCD MS 0.6 solutions remained essentially constant, being 1.47 mPa s when no PVP Mol. Wt 360000 was present in the solution but 2.17 mPa s when the PVP concentration was 0.25% (w/v). Addition of CMC or HPMC resulted in somewhat larger viscosity increase but the HPβCD solutions were never viscous (Table 5).

The effect of increasing concentration of PVP Mol. Wt 360000 on the stability constant (K_c) of the hydrocortisone-HPβCD MS 0.6 complex was determined at room temperature. As expected from Fig. 1, initially the value of K_c did increase with increasing PVP concentration, reached its maximum at 0.1% PVP and then decreased slowly upon further addition of PVP. Also, addition of PVP Mol. Wt 360000 to the aqueous complexation medium resulted in more negative standard free energy changes (Table 7).

4. Discussion

Water-soluble polymers have been used in pharmaceutical formulations for many years and although the polymers are usually considered to be chemically inert, they are known to form complexes with small molecules in aqueous solutions. About 40 years ago Takeru Higuchi and coworkers (Riley et al., 1991) investigated interactions of various drugs with a number of water-soluble polymers, i.e., polyethylene glycols, polypropylene glycols, PVP and CMC. In aqueous solutions, they frequently observed a marked increase in drug solubility due to the formation of water-soluble drug-polymer complexes, however, phase separation was sometimes observed which was characterised in pharmaceutical systems as rather embarrassing incompatibilities. In our study, the addition of a very small amount (0.25% w/v) of PVP, CMC or HPMC resulted in a significant increase in the aqueous solubility of most of the drugs tested. The solubility enhancement of both CMC and PVP was on average about 1.7-fold (Tables 2 and 3). HPMC was also shown to increase the aqueous solubility of various drugs. The polymers mainly interact with drug molecules via electrostatic bonds, i.e., ion-to-ion (in the case of CMC), ion-to-dipole and dipole-to-dipole bonds, but other types of forces, such as van der Waals forces and hydrogen bridges, frequently participate in complex formation (Rácz, 1989). In aqueous solutions, the poly-

mer molecules also tend to form electrostatic bonds between themselves and at high concentrations heat-reversible gels.

Monomeric cyclodextrins are powerful drug solubilizers which do not form gels at high concentrations. Even aqueous 25% (w/v) HPβCD and RMβCD solutions have low viscosity (Table 5). In aqueous solutions, water-soluble polymers increased the solubilising effect of cyclodextrins. Our results show that the polymers increased the efficiency of the complexation by increasing K_c. In the case of hydrocortisone, addition of 0.1% (w/v) PVP Mol. Wt 360000 results in about 45% increase in the value of K_c (Table 6).

The driving force for drug-cyclodextrin complex formation is thought to be the release of enthalpy-rich water molecules from the cyclodextrin cavity. The water molecules located inside the cavity cannot satisfy their hydrogen bonding potentials and therefore exist in a higher energy state compared to those in the aqueous cyclodextrin solution (Bergeron, 1984, García Sánchez et al., 1990). These enthalpy-rich water molecules are readily replaced by suitable guest molecules which are less polar than water. The expulsion of the water molecules from the cyclodextrin cavity results in a negative enthalpy (Table 7). The large negative $\Delta H°$ value outweighs the unfavourable negative $\Delta S°$ value. However, a variety of other physicochemical phenomena have been proposed as driving forces for cyclodextrin complexation, such as the release of strain in the cyclodextrin ring, hydrophobic effect, hydrogen bonding, and van der Waals and London dispersion forces. Addition of PVP to the aqueous complexation medium results in an increased negative enthalpy change, together with an increased negative entropy change (Table 7). Again the unfavourable $\Delta S°$ changes were outweighed by the large $\Delta H°$ values and the net results were more negative $\Delta G°$ values. Thus, the compexation was enhanced (i.e., K_c was increased) upon addition of PVP to the complexation medium. This is consistent with the general observation that the values of $\Delta H°$ and $\Delta S°$ become more negative as K_c for molecular complexation increases (Andrews and Keefer, 1964). The stronger binding between the complexing agent and the drug is reflected in a more negative $\Delta H°$ value and a greater structural restraint in a more negative $\Delta S°$ value. PVP complexation of hydrophobic drugs is usually characterised by a small positive (or negative) $\Delta H°$ value and a positive $\Delta S°$ value (Martin, 1993). The binding can be considered as a kind of hydrophobic interaction with the partial destruction of the iceberg-like structure surrounding the molecules and, thus, an increase in entropy. The fact that both $\Delta H°$ and $\Delta S°$ become more negative in the presence of PVP supports the previous observation that the effect of PVP on the cyclodextrin complexation is not a simple additive effect.

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