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Effect of pH and water-soluble polymers on the aqueous solubility of nimesulide in the absence and presence of β-cyclodextrin derivatives

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Abstract

The aqueous solubility of nimesulide in the absence and presence of β-cyclodextrin (β-CD) and its alkyl derivatives hydroxypropyl-β-CD and methyl-β-CD was studied. We also investigated the effect of water-soluble polymers, hydroxypropylmethyl-cellulose, sodium-carboxymethyl-cellulose, polyvinyl-pyrrolidone and polyethyleneglycol on the solubilization efficacy and complexation ability of cyclodextrins with nimesulide. The solubility of nimesulide in the absence and presence of cyclodextrins and polymers was studied using a phase solubility technique combined with a spectrophotometric method. The study was carried out at 25°C and pH values of 6.0 and 7.0. Conditions in terms of polymer concentration and polymer heating with and without sonication were optimized. Values of the solubility enhancement factor of nimesulide in the presence of each cyclodextrin and in the absence and presence of each polymer were determined and the formation constants, *K*, of the inclusion complexes formed calculated. β-CDs increased the aqueous solubility of nimesulide in the following order: methyl-β-CD > β-CD > hydroxypropyl-β-CD. Addition of hydroxypropylmethyl-cellulose at a concentration of 0.1% (w/v) had the greatest influence on complexation of all three β-CDs with nimesulide, while preheating of the polymer at 70°C under sonication resulted in an additional two-fold increase in the aqueous solubility of the drug. Sodium-carboxymethyl-cellulose, polyvinylpyrrolidone and polyethyleneglycol had minor effects on the aqueous solubility of nimesulide. Thus β-CD, hydroxypropyl-β-CD and methyl-β-CD are proposed as good solubilizing agents for nimesulide in the presence and absence of hydroxypropylmethyl-cellulose in order to enhance its oral bioavailability.

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Introduction

Nimesulide is a non-steroidal anti-inflammatory drug with high anti-inflammatory, analgesic and antipyretic activity, low toxicity, a high therapeutic index and moderate incidence of gastric side-effects (Davis & Brogden 1994), probably attributable to the significant selectivity that nimesulide exhibits for inhibition of cyclooxygenase-2 versus cyclooxygenase-1. Nimesulide is sparingly soluble in water (<0.01 mg mL^{-1}); this poor aqueous solubility and wettability cause difficulties with the pharmaceutical formulation of oral and injectable products, which may result in variable bioavailability. Improving the aqueous solubility of the drug is therefore important.

Cyclodextrins (CDs) are known to form non-covalent inclusion complexes with a large variety of molecules (Szejtli 1982) thus improving drug stability, aqueous solubility, dissolution rate and bioavailability (Duchêne 1987; Loftsson & Brewster 1996; Rajewski & Stella 1996; Stella & Rajewski 1997; Davis & Brewster 2004; Challa et al 2005; Brewster & Loftsson 2007). However, the efficiency of complexation is often not very high and therefore relatively large amounts of CDs must be used to complex small amounts of drugs. Unfortunately, only a limited amount of CD can be used in many drug formulations, such as in aqueous isotonic solutions and tablets; increasing the efficiency of complexation will mean that less CD can be used to achieve the same or even greater solubilizing effect. In addition, since CDs are still relatively expensive, reduction of the amount of CDs in drug formulations will result in lower production costs. Water-soluble polymers such as hydroxypropylmethyl-cellulose (HPMC), polyvinylpyrrolidone (PVP),

carboxymethyl-cellulose (CMC) etc., have been demonstrated to increase complexation of various drug molecules with CDs (Loftsson et al 1996; Loftsson & Masson 2004; Brewster & Loftsson 2007).

The purpose of this study was to investigate the effect of β-CDs, namely β-cyclodextrin (β-CD), its hydrophilic derivative hydroxypropyl-β-cyclodextrin (HP-β-CD) and its lipophilic derivative methyl-β-cyclodextrin (Me-β-CD), on the aqueous solubility of nimesulide in the absence and presence of various water-soluble polymers. Complexation efficiency was mainly evaluated by determining the solubilizing effect of the CDs used. Complexation constants of nimesulide–CD complexes were also determined.

Although literature data dealing with the interaction of nimesulide with β-CD and HP-β-CD exist (Vavia & Adhage 1999; Nalluri et al 2003), these studies are conducted in distilled water without pH adjustment. In the present work, it has been shown that pH greatly influences the nimesulide–CD interaction, probably shifting ionization equilibrium to the right (i.e. favouring the ionized form of nimesulide). In addition, prolonged equilibration time (7 days or more) has been reported for the nimesulide–CD interaction in distilled water (Vavia & Adhage 1999; Nalluri et al 2003). According to our results, pH adjustment reduced the equilibration time to 24 h or less. The current study therefore focused on the effect of β-CD and its water-soluble derivatives HP-β-CD and Me-β-CD on the solubility of nimesulide under standardized pH and temperature conditions. The effect of various water-soluble polymers on the complexation and solubilization efficiency of the three CDs for nimesulide was also studied.

Materials and Methods

Apparatus

A Perkin Elmer Lambda 6 double-beam UV/VIS spectrophotometer (Illinois, USA) was used for spectrophotometric measurements. A Julabo SW1 (Julabo Labortechnik GmbH, Seelbach, Germany) shaking bath, equipped with an external thermostatic unit (Edmund Buhler GmbH, Tübingen, Germany), was used for solubility experiments, and a thermostatically controlled ultrasonic bath (Strasonic 60, Kerry Ultrasonics Ltd, Hitchin, UK) was used for sonication at constant temperature.

Reagents

All chemicals were of analytical grade. HP-β-CD (molecular weight (MW) 1383) and Me-β-CD (MW 1310) were purchased from Sigma-Aldrich (St Louis, MO, USA). β-CD (MW 1134.9) was purchased from Serva Electrophoresis GmbH (Heidelberg, Germany). Polyethyleneglycol (PG 6000; PEG) was purchased from Clariant GmbH (Heidelberg, Germany). $NaH_2PO_4 \cdot 2H_2O$ and NaOH were purchased from Panreac Quimica (Barcelona, Spain). PVP (Kollidon 30 BASF Aktiengesellschaft) was kindly donated by Roumpoulakis SA (Athens, Greece). CMC sodium salt (NaCMC) was kindly donated by Famar SA (Athens, Greece), while nimesulide was provided by Elpen SA (Athens, Greece). Water purified with the Labconco water pro polishing system (Kansas City, MO, USA) was used in all procedures. Regenerated cellulose syringe filters (Titan 0.45 μm pore size) were purchased from Scientific Resources Inc. (Eatontown, NJ, USA).

Effect of pH on nimesulide spectral characteristics in the absence and presence of β-CD

Aqueous nimesulide solutions and mixed aqueous nimesulide with β-CD solutions (1×10^{-5} M nimesulide, 1×10^{-2} M β-CD) were prepared in 0.1 M phosphate buffer at pH values of 4.5, 5.5, 6.0, 6.5, 7.0 and 8.5; absorption spectra of these solutions were obtained.

Calibration curves

Calibration curves were constructed using nimesulide standard solutions in the concentration range 2×10^{-6} to 1×10^{-4} M prepared in 0.1 M phosphate buffer at pH values of 6.0, 7.0 and 8.5. Absorbance of these solutions at 300 and 390 nm was measured and plotted against nimesulide concentration to obtain calibration curves.

Determination of equilibration time

An excess amount of nimesulide (0.005 g) was added to 0.01 M CD solutions or mixed polymer/CD solutions of known polymer and CD concentration, prepared in 0.1 M phosphate buffer at pH values of 6.0 and 7.0. The resulting suspensions were equilibrated in a thermostatically controlled shaking bath at 25°C for up to 96 h. Samples were withdrawn at 3, 6, 15, 24, 48 and 96 h, diluted appropriately with phosphate buffer and the nimesulide concentration measured spectrometrically.

Solubility studies – effect of CDs and polymers on aqueous solubility of nimesulide

An excess amount of nimesulide (0.005 g) was added to: 0.1 M phosphate buffer (pH 6.0 or 7.0); 0.01 M CD solutions (β-CD, HP-β-CD, Me-β-CD) prepared in 0.1 M phosphate buffer (pH 6.0 or 7.0); 0.1% (w/v) polymer solutions (HPMC K4M, NaCMC, PVP, PEG) prepared in 0.1 M phosphate buffer (pH 6.0); aqueous solutions containing both a polymer (0.1% w/v) and a CD (0.01 M) prepared in 0.1 M phosphate buffer (pH 6.0). Suspensions formed were equilibrated in a thermostatically controlled shaking bath at 25°C for 24 h. When a polymer or mixed polymer/CD solution was used, suspensions were either heated at 70°C for 1 h or sonicated at 70°C for 1 h, and cooled to room temperature before equilibration at 25°C for 24 h. After equilibration, suspensions were filtered through a 0.45 μm membrane filter, diluted appropriately and the nimesulide concentration measured spectrometrically. A significant excess of the drug was always used in these studies; thus, solid drug was always present in the solutions during the entire equilibration period.

Phase solubility experiments

An excess amount of the drug (0.005 g) was added to solutions containing 0–0.01 M CD and/or 0–0.5% (w/v) polymer, prepared in 0.1 M phosphate buffer of pH 6.0 or 7.0. When polymer or mixed polymer/CD solutions were used, the suspensions were sonicated at 70°C for 1 h before equilibration at 25°C. After equilibration for 24 h, suspensions were filtered through a 0.45 μm membrane filter, diluted appropriately and the nimesulide concentration measured spectrometrically.

Data analysis

Complexation constants (K) of drug–CD complexes were calculated from the slope of the corresponding phase-solubility diagrams (plots of measured nimesulide solubility, S, expressed as molar concentration vs CD molar concentration) and the drug solubility, S_0, in the absence of CD, according to the following equation (Higuchi & Connors 1965) K = slope / [$S_0 \times (1 - \text{slope})$].

One-way analysis of variance (ANOVA) was used to detect differences between the effects of the various CDs on the aqueous solubility of nimesulide, and the complexation affinity of the three CDs to nimesulide. Two-way ANOVA was used to detect differences between the effects of CD type (β-CD, HP-β-CD and Me-β-CD) on nimesulide solubility in the absence and presence of the various polymers (HPMC, NaCMC, PEG, PVP); the effect of pH on the solubilization ability (effect on aqueous solubility of nimesulide) and complexation affinity (K values) of the three CDs for nimesulide; and the complexation affinity (K values) of the three CDs to nimesulide in the absence and presence of various concentrations of HPMC K4M. Multiple comparison procedure (Tukey's test) was then used to isolate individual differences between the various groups. A significance level of $P < 0.05$ denoted significance in all cases. Statistical analysis was performed using the SigmaStat 2.03 statistical software (SPSS Inc., Chicago, IL, USA).

Results and Discussion

Effect of pH on the spectral characteristics of nimesulide in the absence and presence of β-CD

The effect of pH on the spectral characteristics of nimesulide was studied at six pH values in the range 4.5–8.5 in the absence and presence of β-CD. Results are shown in Figure 1. Nimesulide is a weak acid with a pK_a value of 6.5. In the absence of β-CD at pH 4.5 where the drug was expected to be entirely in its non-ionized form, the absorbance spectrum of nimesulide exhibited a single peak at 300 nm with a slight shoulder (Figure 1A). As pH became more alkaline (pH 5.0–6.0), in addition to the peak at 300 nm a second peak appeared at 390 nm, corresponding to the ionized form of nimesulide. The peak of the ionized form of nimesulide was predominant at pH 6.5 and 7.0. At pH 8.5, where nimesulide exists mainly in its ionized form, a single peak at 390 nm was observed. By contrast, in the presence of β-CD (Figure 1B)

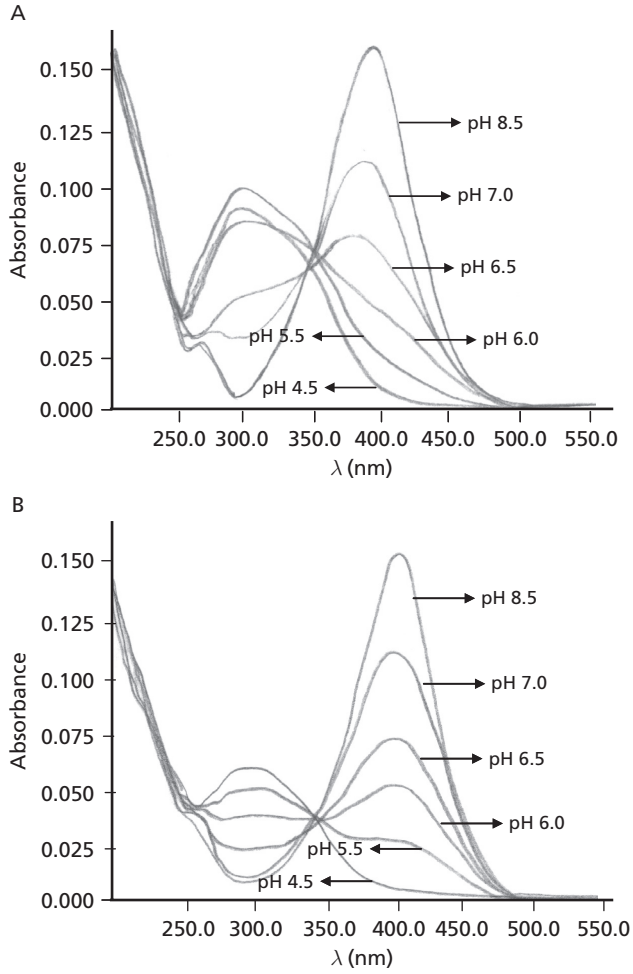

Figure 1 Effect of pH on the absorption spectra of aqueous solution of nimesulide 1×10^{-5} M in the absence (A) and presence (B) of 0.01 M β-CD.

a single peak without a shoulder was observed at 300 nm at pH 4.5, corresponding to both free non-ionized nimesulide and non-ionized nimesulide complexed into the CD molecule cavity. With increasing pH, the second peak of the ionized nimesulide shifted slightly to the right (at 402 nm) and appeared clearly even at the low pH value of 5.5. This probably reflects a shift of ionization interaction to the right, implying that the presence of β-CD favoured ionization of nimesulide by formation of inclusion complexes with the nimesulide anion. Finally, at pH 8.5, where nimesulide exists only in its ionized form, the absorbance spectrum exhibited only a single peak at 402 nm. The slight shift of this peak to the right compared with the equivalent peak in the absence of CD (390 nm) may be attributed to the formation of nimesulide–β-CD complexes.

Given the observations described above, the interaction of nimesulide with β-CD, HP-β-CD and Me-β-CD in the absence and presence of four water-soluble polymers was also studied under standardized pH conditions, using 0.1 M phosphate buffer at pH 6.0 and 7.0. These particular pH values were selected in order to investigate the complexation efficiency of β-CDs under conditions where either the

non-ionized (pH 6.0) or the ionized (pH 7.0) form of nimesulide was favoured. In addition, results from experiments carried out at pH 6.0 could also be interpreted on the basis of intestinal absorption after oral drug administration, since this pH is close to the physiological pH values of the upper intestine under both fasted (about 6.5) and fed (about 5.5) conditions; pH 7.0 is close to physiological pH (7.4), used for intravenous drug administration.

Calibration curves

Equations describing calibration curves of nimesulide constructed in 0.1 M phosphate buffer were: $y = 5.0 (\pm 0.9) \times 10^{-3} + 5.99 (\pm 0.054) \times 10^{3}\ x$ at pH 6.0; $y = 5.0 (\pm 1.2) \times 10^{-3} + 10.61 (\pm 0.083) \times 10^{3}\ x$ at pH 7.0, and $y = -2.0 (\pm 2.3) \times 10^{-3} + 13.9 (\pm 1.4) \times 10^{3}\ x$ at pH 8.5. The linear response range was $0.25–2.0 \times 10^{-5}$ M at pH 6.0; $1–10 \times 10^{-5}$ M at pH 7.0; and $0.25–10 \times 10^{-5}$ M at pH 8.5; the correlation coefficient, r, ranged from 0.9996 to 0.99992. The limits of detection and quantification were $0.37–0.55 \times 10^{-6}$ M and $1.1–1.7 \times 10^{-6}$ M, respectively. Since nimesulide was fully ionized at pH 8.5, a calibration curve constructed at this pH value was used to quantify the ionized form of the drug.

Equilibration time

Equilibration time was studied over a period of 96 and 48 h at pH 6.0 and 7.0, respectively, in order to obtain the optimum time interval needed to reach equilibrium between drug in solution and in solid state on the one hand, and free drug and drug complexed with β-CD in solution on the other. Equilibrium was reached within 24 h in all cases, although when Me-β-CD was used, equilibrium was reached after 6 h in the thermostatically controlled shaking bath. However, for comparative purposes, all solubility measurement vials were kept in the shaking bath for 24 h.

Solubility studies

Effect of β-CDs on the aqueous solubility of nimesulide

Aqueous solubility of nimesulide at pH 6.0 and 7.0 at 25°C was determined in either 0.1 M phosphate buffer or 0.010 M solutions of β-CD, HP-β-CD and Me-β-CD prepared in 0.1 M phosphate buffer. One-way ANOVA revealed a significant ($P < 0.001$) increase in the solubility of nimesulide in the presence of β-CD, HP-β-CD and Me-β-CD, at both pH 6.0 and 7.0, in the following order: Me-β-CD > β-CD > HP-β-CD. In addition, two-way ANOVA revealed significant differences ($P < 0.001$) between solubility values observed at pH 6.0 and 7.0, both in the absence and presence of the three CDs. Results are presented in Table 1. The solubility enhancement factor, expressed as the ratio of drug solubility in the presence of CD to that in the absence of CD (S_{CD}/S_0), was calculated for each CD under the experimental conditions used. It is obvious that Me-β-CD was the most efficient of the three CDs used in this study, with a solubility enhancement factor 2–3 times greater than that of β-CD and HP-β-CD. Since nimesulide is a weak acid with a pK_a of 6.5, its solubility is expected to be pH dependent. Indeed, its solubility was 10 times greater at pH 7.0 than at pH 6.0, both in the absence and presence of CDs. However, it is worth noting that enhancement of nimesulide solubility in the presence of CDs is more evident at pH 6.0 than at pH 7.0, probably due to higher saturation solubility of the drug at pH 7.0, where the ionized water-soluble form of nimesulide is favoured.

Phase-solubility experiments of nimesulide

Phase-solubility diagrams of nimesulide in aqueous buffered solutions of β-CD, HP-β-CD and Me-β-CD in the concentration range of 0–0.01 M are shown in Figure 2. In all cases, linear plots of A_L type were observed, with slopes of less than 1, thus enabling calculation of association constants (K), assuming 1:1 complex formation between nimesulide and each of the CDs used. The calculated K values are given in Table 2. Differences between the calculated K values were significant ($P < 0.001$, one-way ANOVA). The complexation affinity of all three CDs increased in the following order: Me-β-CD > β-CD > HP-β-CD, in accordance with the solubilization efficiency of these CDs, as reflected in the values of the solubility enhancement factor (Table 1). Nalluri et al (2003) reported that during formation of inclusion complex of β-CD with nimesulide the nitro-substituted ring of nimesulide molecule is hosted in the CD cavity. Stronger complexation affinity of Me-β-CD to

Table 1 Solubility enhancement of nimesulide in the presence of 0.010 M β-cyclodextrin (β-CD), hydroxypropyl (HP)-β-CD and methyl (Me)-β-CD, with or without addition of the polymer hydroxypropylmethyl-cellulose (HPMC K4M) at 25°C

	Nimesulide solubility $\times 10^{-5}$ (M)					
	pH 7.0	pH 6.0	HPMC K4M concn (% w/v)			
	No polymer	No polymer	0.05	0.1	0.25	0.5
–	4.93 ± 0.25	0.39 ± 0.01	0.40 ± 0.02 (1.0)	0.39 ± 0.01 (1.0)	0.46 ± 0.02 (1.1)	0.42 ± 0.02 (1.0)
β-CD	45.3 ± 1.9 (12.37)	5.92 ± 0.31 (15.14)	11.23 ± 0.6 (31.71)	13.70 ± 0.8 (38.70)	10.70 ± 0.5 (30.05)	11.05 ± 0.7 (31.20)
HP-β-CD	39.7 ± 1.5 (10.85)	4.25 ± 0.3 (12.01)	8.36 ± 0.6 (23.60)	9.70 ± 0.7 (26.94)	7.71 ± 0.6 (21.76)	7.61 ± 0.8 (21.48)
Me-β-CD	107.0 ± 5.7 (29.24)	13.0 ± 0.9 (39.55)	22.4 ± 1.2 (73.04)	26.0 ± 1.4 (77.66)	22.4 ± 1.2 (73.07)	22.3 ± 1.3 (70.14)

Solubility values are given as mean ± s.d. of three or more replicates. Values in parentheses are the solubility enhancement factors, S_{CD}/S_0, where S_0 is the nimesulide solubility value in the absence of CD and S_{CD} is the nimesulide solubility value in the presence of CD or a combination of CD and polymer.

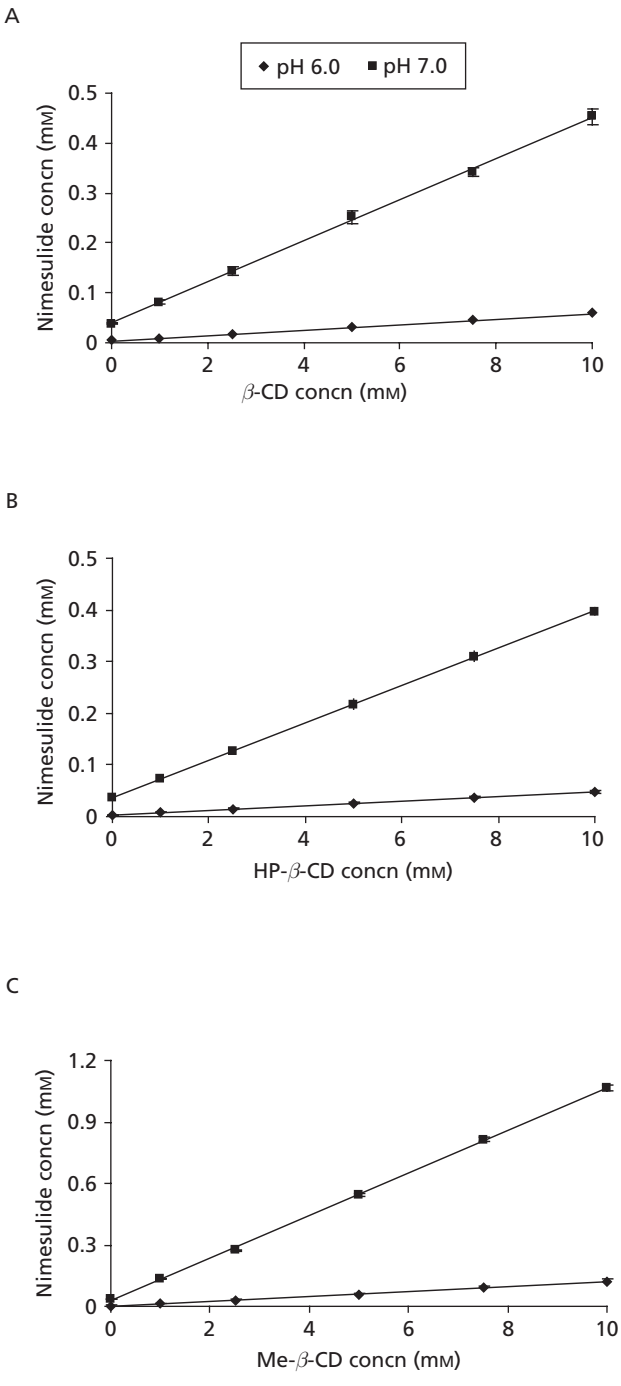

Figure 2 Phase-solubility plots of nimesulide complexed with β-CD (A), HP-β-CD (B) or Me-β-CD (C), at pH 6.0 and 7.0 at 25°C.

nimesulide could probably be attributed to the greater lipophilicity of this CD, which would favour complexation of the non-ionic nitro-substituted ring of nimesulide. By contrast, HP-β-CD creates a more hydrophilic environment compared with the parent β-CD and its lipophilic derivative Me-β-CD. This probably resulted in the decreased complexation affinity of HP-β-CD for the non-ionized form of this drug. In addition, both inclusion and non-inclusion complexation can be considered to add to its solubilization ability. As reported recently (Loftsson et al 2002, 2004), drug–CD complexes can self-associate to form water-soluble aggregates or micelles, which can further contribute to solubilization of drugs through non-inclusion complexation.

Table 2 Complexation constants, *K*, of nimesulide with cyclodextrins (CDs) at 25°C, pH 6.0 and 7.0 and in the presence of hydroxypropylmethyl-cellulose (HPMC K4M) at 25°C and pH 6.0

Experimental conditions	$K(M^{-1})$		
pH	Cyclodextrin		
	β-CD	HP-β-CD	Me-β-CD
6.0	1383 ± 108	1217 ± 90	3641 ± 474
7.0	1004 ± 119	977 ± 66	3438 ± 487
HPMC K4M concn (% w/v)[a]			
0.05	2664 ± 241	2021 ± 185	5846 ± 724
0.10	3090 ± 304	2256 ± 212	6431 ± 610
0.25	2504 ± 234	2221 ± 207	5898 ± 544
0.50	2615 ± 246	2069 ± 196	5465 ± 517

K values are mean ± s.d. of three or more replicates. [a]At pH 6.0, after heating at 70°C under sonication for 1 h prior to equilibration. HP, hyroxypropyl; Me, methyl.

Regarding the effect of pH, complexation affinity of the three CDs to nimesulide was slightly (although not significantly) decreased as pH was increased from 6.0 to 7.0 (Table 2). This was as expected, since CDs are known to be better complexing agents for non-ionized molecules, while the ionized form of nimesulide was predominant at pH 7.0. On the other hand, this behaviour was in agreement with the assumption that the increased solubility of nimesulide in the presence of CDs at pH 7.0 was attributed not only to the complexation mechanism but also to enhanced saturation solubility of the drug at this pH value.

Effect of polymers on the aqueous solubility of nimesulide in the absence and presence of β*-CDs*

In the absence of β-CD, HP-β-CD and Me-β-CD, the four polymers had no effect on the aqueous solubility of nimesulide (Table 3), confirmed statistically by two-way ANOVA using Tukey's test for all pair-wise multiple comparisons. The presence of CDs had significant effects on nimesulide solubility ($P < 0.001$), which depended on both CD type and polymer type. More specifically, in the presence of β-CD, HP-β-CD and Me-β-CD, addition of 0.1% (w/v) HPMC K4M increased the nimesulide solubility by 140%, 115% and 53% respectively, at 25°C and pH 6.0 (Tables 1 and 3). Activation of polymer (HPMC K4M) by heating at 70°C under sonication for 1 h was required before solubility experiments to achieve the greatest enhancement of nimesulide solubility in the presence of all three CDs. Heating at 70°C under sonication for 1 h was recently described by Loftsson (1998) as a polymer activation procedure for water-soluble polymers such as HPMC K4M used with CDs as drug-solubility enhancement agents. However, the exact mechanism of activation is not known. The solubilization efficacy of β-CD, HP-β-CD and Me-β-CD

Table 3 Effect of polymer presence (0.1% w/v) and polymer activation by heating at 70°C under sonication on the aqueous solubility enhancement of nimesulide by 0.010 M β-cyclodextrin (β-CD), hydroxypropyl (HP)-β-CD and methyl (Me)-β-CD (all 0.01 M) at 25°C

Polymer present	Polymer activation	Nimesulide solubility $\times 10^{-5}$ (M)			
		Cyclodextrin			
		No CD	β-CD	HP-β-CD	Me-β-CD
–	–	–	5.92 ± 0.31	4.25 ± 0.3	13.0 ± 0.9
	70°C	–	6.60 ± 0.53	–	–
	70°C + SC	0.45 ± 0.02	6.46 ± 0.71	–	–
HPMC K4M	–	0.39 ± 0.03	7.71 ± 0.96	7.84 ± 0.23	15.6 ± 1.17
	70°C	0.38 ± 0.02	7.84 ± 0.61	7.33 ± 0.51	18.26 ± 0.81
	70°C + SC	0.39 ± 0.01	13.70 ± 0.8	9.7 ± 0.7	26.0 ± 1.4
PVP	–	0.44 ± 0.02	6.75 ± 0.35	–	–
	70°C	0.51 ± 0.05	7.23 ± 0.37	5.35 ± 0.05	16.63 ± 0.32
	70°C + SC	0.48 ± 0.03	6.81 ± 0.35	5.43 ± 0.05	14.96 ± 0.27
PEG	–	0.46 ± 0.01	6.23 ± 0.31	–	–
	70°C	0.47 ± 0.02	6.51 ± 0.41	4.76 ± 0.08	12.47 ± 0.24
	70°C + SC	0.48 ± 0.04	6.19 ± 0.36	4.55 ± 0.05	13.75 ± 0.14
NaCMC	–	0.43 ± 0.04	6.52 ± 0.52	–	–
	70°C	0.44 ± 0.03	6.67 ± 0.81	4.72 ± 0.07	12.69 ± 0.35
	70°C + SC	0.44 ± 0.07	6.32 ± 0.39	4.41 ± 0.04	12.77 ± 0.25

Nimesulide solubility values are given as mean ± s.d. of three or more replicates.
HPMC K4M, hydroxypropylmethyl-cellulose; NaCMC, sodium carboxymethyl-cellulose; PEG, polyethyleneglycol; PVP, polyvinylpyrrolidone; +SC, with sonication.

was at least doubled in the presence of 0.1% HPMC K4M compared with that in the absence of polymer. This was reflected in the corresponding values of the calculated solubility enhancement factor S_{CD}/S_0 (Table 1).

The presence of NaCMC and PEG (0.1% w/v) had no significant additional influence ($0.151 < P < 0.998$) on the aqueous solubility of nimesulide in the presence of β-CD, HP-β-CD or Me-β-CD under the pH and temperature conditions studied (Table 3). This observation is in agreement with recently reported data (Dutet et al 2007), where PEG 6000 did not improve the dissolution rate of nimesulide when added with β-CDs to solid dispersions of the drug. This is probably due to the lower aqueous solubility of the polymer compared with that of the nimesulide inclusion complexes of β-CDs. By contrast, the presence of 0.1% (w/v) PVP resulted in a slight but significant ($P < 0.001$) additional increase in nimesulide solubility in the presence of Me-β-CD. However, PVP had no additional influence on the aqueous solubility of nimesulide in the presence of β-CD or HP-β-CD ($0.056 < P < 0.766$).

The diverse behaviour of the four water-soluble polymers used in this study can probably be attributed to the different types of CD–polymer complexes formed, due to differences in the molecular structures. More specifically, linear polymers such as PEG are reported to form polymer–CD inclusion complexes in which many CD molecules are threaded onto the linear polymer (Harada 1997; Loftsson & Masson 2004; Brewster & Loftsson 2007). Such a complex formation between polymer and CD molecule probably reduces the complexation affinity of the CD and its ability to solubilize drugs through complexation. On the other hand, 'bulky' polymers such as PVP and HPMC, which are able to form hydrogen bonds, are reported to create non-inclusion complexes with CDs. In these cases, CD molecules form hydrogen bonds with hydroxyl groups (or other groups) on the polymers (Loftsson & Masson 2004; Brewster & Loftsson 2007). This interaction ultimately leads to formation of inclusion complexes with drug molecules. Therefore, ternary complexes between drug, CD and polymer can be formed, enhancing solubilization and complexation abilities of CDs. Formation of ternary complexes has been recently reported by Valero et al (2004, 2007) during the interaction of naproxen with HP-β-CD and nabumetone with β-CD in the presence of PVP. In the latter case, a drug:$(\beta\text{-CD})_2$:PVP ternary complex was identified, where the drug was wrapped at both ends by two β-CD molecules, the polymer acting as a bridge between the two β-CD molecules binding the nabumetone molecule (Valero et al 2007).

In the present study, only HPMC was found to significantly improve, not only on a statistical basis but also on a physiological basis, the solubilization efficacy of the three CDs used in this study. The fact that addition of PVP does not significantly affect the solubilization efficacy of CDs probably reflects the low polymer concentration (0.1% w/v) used in this study. However, these concentrations were kept constant to maintain uniform conditions for all four polymers studied. Moreover, according to Loftsson (1998), when a water-soluble polymer is used to enhance the solubilization efficacy of a CD, the effect of the polymer is synergistic rather than additive. Usually, solubility increases upon addition of the polymer and reaches a maximum at a polymer concentration between 0.1 and 0.2% w/v, but it can then decline upon addition of further polymer; although for some drugs maximum CD solubilization was observed at a polymer concentration as high as 1% w/v (the usual optimum concentration range is 0.1–0.2% w/v). Depending on the drug under investigation, the optimum polymer concentration is rarely as high as 1% w/v.

Effect of HPMC K4M concentration on the solubilization and complexation efficacy of β-CDs – phase-solubility experiments

Based on the results above, only HPMC K4M was found to significantly affect the solubilization efficacy of CDs. Accordingly, the effect of its concentration on the solubilization efficacy of all three CDs was investigated further (Table 1). In addition, phase-solubility experiments were conducted in the entire range of HPMC K4M concentrations studied in order to determine the effect of the polymer on the complexation affinity of each CD to nimesulide. The solubility enhancement factors of each CD are shown in Table 1. The order of solubilization efficacy of CDs in the presence of HPMC K4M at a concentration range of 0.0–0.5% (w/v) remained the same as in the absence of this polymer (i.e. Me-β-CD > β-CD > HP-β-CD). Statistical evaluation of minesulide solubility results (Table 1) by two-way ANOVA revealed significant differences caused by the use of various CDs in the presence of a variable concentration of HPMC K4M. In all cases, the highest values were achieved when HPMC K4M was present at a concentration of 0.1% w/v ($P \leq 0.001$).

Values for the complexation constant, K, were calculated from the corresponding linear phase-solubility diagrams and are presented in Table 2. Complexation affinity of the three CDs also increases in the presence of HPMC K4M ($P < 0.001$) and there is a slight but significant interaction between the CD type and HPMC content ($P = 0.028$). In all cases, the difference was greater when HPMC was present at a concentration of 0.1% (w/v) ($0.029 < P < 0.035$).

Conclusions

Solubility of nimesulide was found to be greatly affected by CDs in the order: Me-β-CD > β-CD > HP-β-CD; solubilization and complexation ability of the three CDs was further increased in the presence of the water-soluble polymer HPMC K4M. Overall, β-CDs (β-CD, HP-β-CD and Me-β-CD) can be proposed as good solubilizing agents for nimesulide both in the presence and absence of HPMC K4M in order to enhance its oral bioavailability. Taking into account the low toxicity of HP-β-CD, its nimesulide inclusion complex could be also considered for intravenous administration.

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