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Evaluation of cyclodextrin solubilization of drugs

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Abstract

The most common stoichiometry of drug/cyclodextrin complexes is 1:1, i.e. one drug molecule forms a complex with one cyclodextrin molecule, and the most common method for stoichiometric determination during formulation studies is the phase-solubility method. However, in recent years it has becoming increasingly clear that solubilizing effects of cyclodextrins are frequently due to the formation of multiple inclusion and non-inclusion complexes. The aqueous solubility of 38 different drugs was determined in pure aqueous solution, aqueous buffer solutions and aqueous cyclodextrin solutions, and the apparent stability constant ($K_{1:1}$) of the 1:1 drug/cyclodextrin complexes calculated by the phase-solubility method. For poorly soluble drugs (aqueous solubility <0.1 mM) the intrinsic solubility (S_0) is in general much larger than the intercept of the phase-solubility diagram (S_{int}) resulting in non-linearity of otherwise linear (A_L-type) phase-solubility diagram. This can lead to erroneous $K_{1:1}$-values. A more accurate method for determination of the solubilizing efficiency of cyclodextrins is to determine their complexation efficiency (CE), i.e. the concentration ratio between cyclodextrin in a complex and free cyclodextrin. CE is calculated from the slope of the phase-solubility diagrams, it is independent of both S_0 and S_{int}, and more reliable when the influences of different pharmaceutical excipients on the solubilization are being investigated.

Keywords: Cyclodextrin; Complex; Solubility; Complexation efficiency; Ideal solution

1. Introduction

Aqueous solubility is one of the key determinants in development of new chemical entities as successful drugs. However, new drug development technologies, such as combinatorial chemistry and high throughput screening, are based on the basic principles of medicinal chemistry, teaching that the most reliable method to increase in vitro potency is to add lipophilic moiety at appropriate position of the lead structure. This has led to an increase in the number of lipophilic and poorly soluble molecules being investigated for their therapeutic activity (Lipinski, 2000). Various formulation techniques are applied to compensate for their insolubility and consequent slow dissolution rate. These include formulation of the amorphous solid form, nanoparticles, microemulsions, solid dispersions, melt extrusion, salt formation and formation of water-soluble complexes. By such techniques pharmaceutical formulators

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try to increase the apparent solubility of lipophilic compounds without decreasing their optimized potency.

Cyclodextrins are cyclic oligosaccharides, with hydrophilic outer surface and a somewhat lipophilic central cavity. Cyclodextrins are able to form water-soluble inclusion complexes with many lipophilic poorly soluble compounds (Loftsson and Brewster, 1996; Rajewski and Stella, 1996; Loftsson et al., 2004a). However, cyclodextrins (the hosts) are also known to form non-inclusion complexes (Loftsson et al., 2002, 2004b). Most lipophilic compounds (the guests) form apparent 1:1 guest/host complex although apparent higher order complexes are not uncommon. Cyclodextrins and cyclodextrin complexes have been studied intensively for the past couple of decades and these studies have generated a wealth of information on the structural requirements for complex formation and the forces involved (Bodor and Buchwald, 2002; Katritzky et al., 2004). However, most of these studies have been performed in dilute aqueous solutions under close to ideal conditions, or conditions that can almost never be found in pharmaceutical formulations. Lipophilic drug molecules, as well as drug/cyclodextrin complexes, are known to form aggregates in aqueous solutions, and common pharmaceutical excipients, such as water-soluble polymers and surface-active preservatives, are known to solubilize drugs in aqueous solutions (Loftsson and Másson, 2004; Loftsson et al., 2004b). Still current stoichiometric models treat aqueous formulations as ideal solutions in which dissolved drug and cyclodextrin molecules, and individual complexes, are independent of each other as well as of other excipients. In the present paper, we will investigate some of the discrepancies caused by this over simplification and how they effect the determination of stability constants of drug/cyclodextrin complexes. We will also suggest an alternative constant, the complexation efficiency, for evaluation of drug/cyclodextrin complexes under different conditions.

2. Methods

2.1. Data collection

The drug solubility data was generated in our lab over the past fifteen years during various drug pre-formulation studies (Table 1). Many of the formulation studies resulted in publication (see references in Table 1) but some have remained unpublished. The solubility data, intercepts and phase-solubility profiles were obtained from the original notebooks and reports.

2.2. Determination of drug solubility

The solubility of the drugs in water or aqueous cyclodextrin solutions was determined by a heating method. First, the stability of the drug in the aqueous complexation media was evaluated. Small amount of the drug to be tested was dissolved in aqueous cyclodextrin solution. The solution was then divided into four sealed vials that were heated in an autoclave for one, two, three and four heating cycles, each cycle consisted of heating to 121 °C for 20 min. The drug concentrations in the vials were then determined by a high-performance liquid chromatographic method (HPLC). If the drug degradation was less than 1% during one cycle then the heating method in an autoclave was applied. If the degradation was greater then heating in autoclave was replaced by heating in an ultrasonic bath for 1 h at 60–70 °C. The maximum allowable drug degradation during the solubility studies was under all circumstances 1%. The drug solubility was then determined as follows:

1. Specific amount of cyclodextrin was dissolved in water, aqueous buffer solution or the aqueous formulation vehicle.
2. An excess amount of the drug to be tested was added to the aqueous cyclodextrin solution.
3. The suspension formed was placed in a sealed container and heated in an autoclave (121 °C for 20 min) or sonicated in an ultrasonic bath (at e.g. 70 °C for 1 h). After cooling to ambient temperature the container was opened and a small amount of the solid drug added to the container to promote drug precipitation.
4. After equilibration at ambient temperature (22–23 °C) in a sealed container under constant agitation for 3–7 days, the suspension was filtered through a 0.45 mm membrane filter (discarding approximately the first third of the filtrate) and the solution analyzed by HPLC (after dilution with 70% aqueous methanol solution, if necessary). The time needed to reach equilibrium solubility was determined by

Table 1
The drugs used to generate the data set used in this study and reference to the literature describing the associated preformulation study

Drug	MW	MP	log P[a]	References
Acetazolamide	222.3	260	−0.72	(Loftsson et al., 1994b)
Alfaxalone	332.5	168	2.63	[b]
Alprazolam	308.8	228	3.87	(Loftsson et al., 2001; Loftsson et al., 2003)
Calcipotriol	412.6	167	7.19	(Loftsson and Petersen, 1997)
Carbamazepine	236.3	191	2.25	(Loftsson and Friðriksdóttir, 1998)
Cinnarizine	368.5	–	5.8	[b]
Clotrimazole	344.8	148	6.26	(Loftsson et al., 1994a)
Cyclosporin A	1202.6	150	1	(Loftsson et al., 2003)
Dexamethasone	392.5	270	1.72	(Loftsson et al., 1994c)
Dextromethorphan	271.4	111	3.97	[b]
Diethylstilbestrol	268.4	171	5.64	(Loftsson et al., 2002)
Diflunisal	250.2	212	4.41	(Loftsson et al., 2002)
Digoxin	780.9	240	1.26	[b]
Econazole	381.7	87	5.61	(Loftsson et al., 1994a)
Ergotamine	581.7	213	2.53	[b]
17β-Estradiol	272.4	176	3.94	(Loftsson and Bodor, 1989; Fridriksdóttir et al., 1996)
Finasteride	372.6	254	3.2	[b]
Flunitrazepam	313.3	170	1.91	[b]
Fluoxetine	345.8	138	4.65	[b]
Hydrocortisone	362.5	214	1.62	(Loftsson and Sigurdardottir, 1994; Sigurdardottir and Loftsson, 1995)
Ketoconazole	531.4	146	4.4	[b]
Ketoprofen	254.4	95	3	[b]
Methazolamide	236.3	213	0.33	(Friðriksdóttir et al., 1997)
Miconazole	416.1	182	6.25	(Loftsson et al., 1994a)
Naproxen	230.3	153	3.1	(Loftsson et al., 1993)
Omeprazole	345.4	156	3.4	(Loftsson et al., 1991)
Oxazepam	286.7	206	2.32	(Loftsson et al., 1994a)
Prazepam	324.8	146	3.99	(Loftsson et al., 1996a)
Pregnenolone	316.5	189	3.89	(Brewster et al., 1995)
Progesterone	314.5	127	3.67	(Loftsson et al., 1994a)
Propofol	178.3	19	3.57	[b]
Sulfamethoxazole	253.3	167	0.48	(Loftsson et al., 1996a)
Tamoxifen	371.5	97	6.3	[b]
Tenoxicam	337.4	209	2.4	(Loftsson et al., 1993)
Terfenadine	471.7	148	7.62	[b]
Triamcinolone acetonide	434.5	293	0.96	(Loftsson et al., 1994a)
Triazolam	343.2	234	3.96	(Loftsson et al., 2001)
Triclosan	289.5	56	4.66	(Loftsson et al., 2005)

MW: molecular weight in Dalton, MP: melting point in degrees Celsius; log P: calculated octanol/water partition coefficient.
[a] Calculated log P was calculated on line (http://www.syrres.com/) according to structure.
[b] Unpublished data.

analyzing samples of the equilibrating solution at different time points to establish constant drug solubility.

3. Results and discussion

The most common type of cyclodextrin complexes is the 1:1 drug/cyclodextrin complex (D/CD) where one drug molecule (D) forms a complex with one cyclodextrin molecule (CD):

$$\mathrm{D} + \mathrm{CD} \overset{K_{1:1}}{\rightleftarrows} \mathrm{D/CD} \quad (1)$$

The value of the stability constant ($K_{1:1}$) is used to compare the affinity of drugs for different cyclodextrins or cyclodextrin derivatives. The total solubility of drug

(S_t) in aqueous cyclodextrin solution will then be:

$$S_t = S_0 + \frac{K_{1:1}S_0}{1 + K_{1:1}S_0}[CD]_t \quad (2)$$

where S_0 is the intrinsic solubility of the drug, i.e. the solubility when no cyclodextrin is present, and $[CD]_t$ is the total concentration of cyclodextrin in the aqueous medium. A plot of S_t versus $[CD]_t$, according to Eq. (2) (i.e. a phase-solubility profile), will give a straight line with a slope ($K_{1:1}S_0/(1 + K_{1:1}S_0)$) less than unity and an intercept (S_{int}) equal to S_0. Then $K_{1:1}$ is calculated from the slope and S_0 (Higuchi and Connors, 1965):

$$K_{1:1} = \frac{\text{Slope}}{S_0(1 - \text{Slope})} \quad (3)$$

The observed value of $K_{1:1}$ is most often between 50 and 2000 M^{-1} with a reported mean values of 129, 490 and 355 M^{-1} for the parent α-, β- and γ-cyclodextrin, respectively (Connors, 1995). However, thus determined $K_{1:1}$ value is strongly affected by accuracy of the intercept. The feasibility of using cyclodextrins in pharmaceutical formulations can be calculated from $K_{1:1}$ and S_0 (Rao and Stella, 2003).

3.1. Intrinsic solubility, intercept and the effects of excipients

According to Eq. (2) the intrinsic solubility should be equal to the intercept but there are numerous exemptions from this. For example, Fig. 1 shows the relative deviation of the intercept (S_{int}) from S_0 as a function of S_0. Ideally S_{int} should be equal to S_0 and all points should fall on the line drawn at the zero value. This is indeed the case for drugs with intrinsic solubility greater than about 1 mM but strong negative deviation is observed at intrinsic solubilities below about 0.1 mM (or approximately 0.03 mg/ml). In this case S_0 is much greater than S_{int}. There is no correlation between the partition of the drug between octanol and water (log P) and the deviation of S_{int} from the S_0 value (correlation coefficient 0.004), or between the slope of the phase-solubility profile and the deviation (correlation coefficient 0.177). It is not clear why the intercept of the phase-solubility diagram is below S_0 but it could be due to the non-ideality of water as a solvent. Usually we treat solvents as homogenous but somewhat random structure of solvent molecules that are more or less independent of each other. We treat aqueous drug

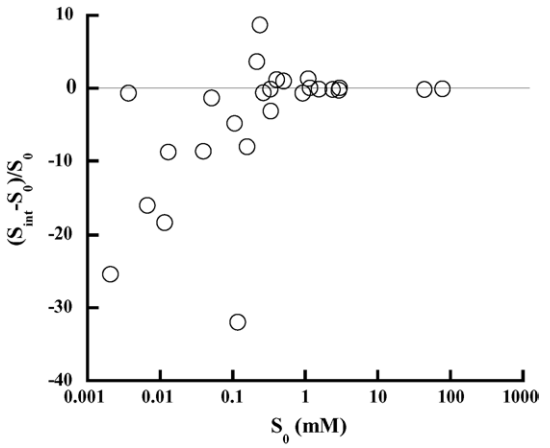

Fig. 1. Plot of the determined intrinsic molar solubility (S_0) vs. the relative deviation of the intercept (S_{int}) obtained from phase-solubility studies from the determined value at ambient temperature. Twenty-six different drugs, molecular weight ranging from 178 to 1202 with mean of 348 Da, melting point ranging from 19 to 293 with a mean of 182 °C.

solutions and perform solubility estimations assuming that water is such an ideal solvent. But in recent years it has become increasingly clear that water is a highly structured solvent with many unique physicochemical properties that have yet to be explained on molecular level (Xantheas, 2000; Schmid, 2001; Kunz et al., 2004). For example, the molecular structure of water allows the water molecules to form a cage around non-polar solute without sacrificing much of their hydrogen bonding capacity. Structured water can close on the solute like an elastic net trapping one or more solute molecules (Schmid, 2001). This physicochemical property of water can be responsible for some of the solubility irregularities observed in pure aqueous solutions.

Common pharmaceutical excipients can also either decrease or increase the apparent intrinsic solubility. For example, polymers commonly used in pharmaceutical formulations are known to form complexes with small molecules in aqueous solutions (Riley et al., 1991; Tomasik and Schilling, 1998). In some cases the polymers form water-soluble complexes but water-insoluble complexes in others (Marcus, 1956; Riley et al., 1991). Table 2 shows the effect of three common water-soluble polymers on the aqueous solubility of poorly soluble drugs. For example, the total solubility of hydrocortisone in 0.25% (w/v) aqueous hydrox-

Table 2
The solubility in pure water or pure aqueous solutions containing 0.25% (w/v) of hydroxypropyl methylcellulose 4000 (HPMC), the sodium salt of carboxymethylcellulose of medium viscosity (CMC) or polyvinylpyrrolidone, MW 40,000 (PVP) at ambient temperature (i.e. 22–23 °C)

Drug	MW (Da)	Solubility (mg/ml)			
		Water	HPMC	CMC	PVP
Acetazolamide	222.3	0.64	0.90	0.59	0.94
Carbamazepine	236.3	0.26	0.33	0.18	0.28
Finasteride	372.6	0.04	0.02	0.12	0.05
Hydrocortisone	362.5	0.43	1.40	1.34	0.87
Methazolamide	236.3	0.70	1.12	0.64	0.64
Oxazepam	286.7	0.05	0.27	0.05	0.10
Pregnenolone	316.5	0.03	0.05	0.06	0.05
Sulfamethoxazole	253.3	0.39	0.55	0.62	0.58

ypropyl methylcellulose (HPMC) solution is the sum of the intrinsic solubility (S_0) and the concentration of hydrocortisone/polymer complex ([H_n/HPMC]) where n hydrocortisone (H) molecules form a complex with one HPMC molecule:

$$S_t = S_0 + n\,[\mathrm{H}_n/\mathrm{HPMC}] = 0.43 + 1.0 = 1.43\,\mathrm{mg/ml} \quad (4)$$

In aqueous solution the intrinsic drug solubility is equal to the concentration of free drug (i.e. dissolved unbound drug) in the solution and only the free drug is in equilibrium with drug in the complex. Other common pharmaceutical excipients, such as preservatives and buffer salts, are known to affect the total drug solubility through micellar formations and common ion or salting out effects (Bergström et al., 2004). The conventional methods used to predict drug solubility in aqueous solutions do not, in general, account for these excipient effects.

3.2. Determination of the stability constant

In most cases the main purpose of adding cyclodextrins to pharmaceutical products is to increase the aqueous solubility of poorly soluble drugs. The amount of cyclodextrin needed to obtain desired drug solubility is determined from a phase-solubility profile according to Eq. (2) (Fig. 2). Then the stability constant of the drug/cyclodextrin 1:1 complex is determined from Eq. (3) (Higuchi and Connors, 1965). As mentioned previously poorly soluble drugs frequently show negative

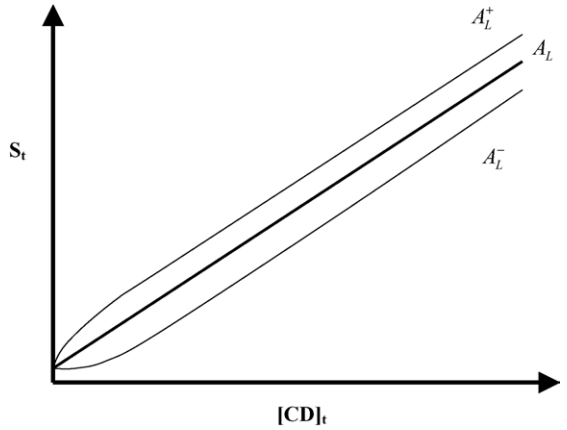

Fig. 2. Linear phase-solubility profiles. A normal profile (A_L), a profile with a positive deviation at low cyclodextrin concentrations (A_L^+), and a profile with a negative deviation at low cyclodextrin concentrations (A_L^-).

intercept deviation, i.e. $S_{int} < S_0$, resulting in A_L^--type profiles in pure aqueous solutions that leads to apparent overestimation of $K_{1:1}$ when determined from the slope and intercept. When drug molecules self-associate to form dimers (D_2) in the aqueous complexation media then only the monomer will be in direct equilibrium with the cyclodextrin complex:

$$\mathrm{D} + \mathrm{D} \overset{K_2}{\rightleftarrows} \mathrm{D}_2 \quad (5)$$

where fraction of free drug monomers in solution will decrease with increasing K_2 and increasing monomer concentration ([D]), the maximum tendency of dimer formation being in a saturated drug solution. Some excipients and impurities can also decrease the amount of free drug molecules that are in equilibrium with the cyclodextrin complex. Thus, self-association of poorly soluble drugs and excipient interactions can in some cases explain the A_L^--type phase-solubility profiles where $S_{int} < S_0$ (Fig. 2). In some cases S_{int} has a negative value resulting in a negative $K_{1:1}$-value which is theoretically impossible. For example, cinnarizine has a sharp A_L^--type phase-solubility profile (Fig. 3) that can be mistaken for A_P-type profile of cinnarizine/cyclodextrin 1:2 complex (Okimoto et al., 1996). However, cinnarizine is a very lipophilic ($\log P_{octanol/water}$ 5.8 (Moffat et al., 2004)) and water-insoluble compound ($S_0 = 1\,\mu g/ml$ at pH 11.9) with large aromatic planar regions, or physicochemical char-

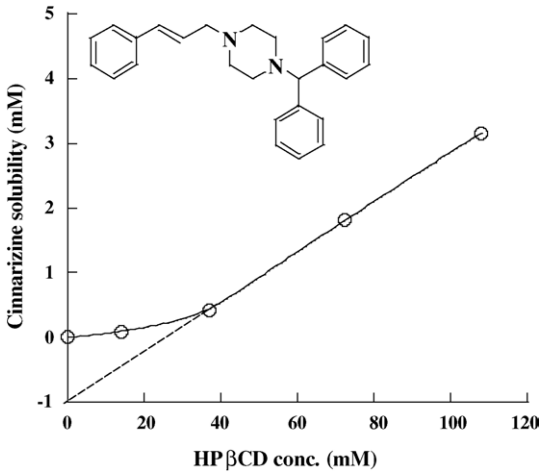

Fig. 3. The phase-solubility profile of cinnarizine in aqueous 2-hydroxypropyl-β-cyclodextrin (HPβCD) solution with molar substitution 0.6 in aqueous 0.01N sodium hydroxide solution (pH 11.9) at ambient temperature. The pK_a of cinnarizine hydrochloride is 7.5 (Roth et al., 1991).

acteristics that promote self-association in aqueous solutions (Yalkowsky, 1999). Self-association of the drug molecules and the drug/cyclodextrin complexes, as well as non-inclusion complexation, can also lead to A_L^--type phase-solubility profiles (Loftsson et al., 2004b). Charged cyclodextrins have been observed to give positive deviation, i.e. $S_{int} > S_0$, resulting in A_L^+-type profiles that leads to underestimation of $K_{1:1}$ when determined from the slope and intercept (Johnson et al., 2004). In this case the A_L^+-type profiles are thought to be composed of one region dominated by ionic interactions between the drug and cyclodextrin and the other dominated by traditional inclusion complex formation.

As mentioned previously polymers can either increase or decrease the amount of free drugs that are in equilibrium with drug/cyclodextrin complexes and the value of $K_{1:1}$ is strongly affected by the value of S_0 used in the calculations. There are three possibilities, i.e. use the true intrinsic drug solubility determined in pure water (S_0), use the intercept (S_{int}) determined by linear regression of the phase-solubility data, or use the determined drug solubility in the aqueous polymer solution when no cyclodextrin is present (see Table 2). Table 3 displays $K_{1:1}$-values (eight drugs with mean S_0 of 0.32 mg/ml) calculated from Eq. (3) by using either S_0 or S_{int}. In general, using S_0 results in significantly larger (on an average about 60% larger) $K_{1:1}$-value than when S_{int} is used, although both positive and negative deviations are observed. Furthermore, the difference between the two $K_{1:1}$-values increases with decreasing S_0. Using drug solubilities obtained in aqueous polymer solutions would give still other $K_{1:1}$-values. Furthermore, various pharmaceutical additives, such

Table 3
The stability constant ($K_{1:1}$) of drug/cyclodextrin 1:1 complex in pure water or pure aqueous solutions containing 0.25% (w/v) of hydroxypropyl methylcellulose 4000 (HPMC), the sodium salt of carboxymethylcellulose of medium viscosity (CMC) or polyvinylpyrrolidone, MW 40,000 (PVP) at ambient temperature (i.e. 22–23 °C)

Drug	S_0 (mg/ml)	Cyclodextrin	$K_{1:1}$ (M^{-1})							
			Water		HPMC		CMC		PVP	
			S_0	S_{int}	S_0	S_{int}	S_0	S_{int}	S_0	S_{int}
Acetazolamide	0.64	HPβCD	85	110	120	160	72	84	95	76
Carbamazepine	0.26	HPβCD	630	270	760	490	650	280	650	440
Finasteride	0.04	RMβCD	7300	–[a]	6800	–[a]	6900	–[a]	7300	–[a]
Hydrocortisone	0.43	HPβCD	1700	1700	–[b]	–[b]	1000	48	1500	260
Hydrocortisone	0.43	RMβCD	1700	–[a]	1400	240	600	150	2100	1800
Methazolamide	0.70	HPβCD	34	32	51	31	44	54	57	56
Oxazepam	0.05	RMβCD	1000	–[a]	480	85	800	–[a]	730	–[a]
Pregnenolone	0.03	HPβCD	1200	–[a]	2800	–[a]	1000	180	2200	2300
Sulfamethoxazole	0.39	HPβCD	360	400	220	310	400	410	780	380

2-Hydroxypropyl-β-cyclodextrin with molar substitution 0.6 (HPβCD); randomly methylated β-cyclodextrin with degree of substitution 1.8 (RMβCD). The stability constant was calculated from Eq. (3) using either the drug solubility determined in pure water (S_0) or the intercept (S_{int}) determined from the phase-solubility diagram (Eq. (2)).

[a] $S_{int} < 0$.

[b] Not determined.

as organic acids and bases, are known to form ternary complexes with drugs and cyclodextrins (Redenti et al., 2000; Redenti et al., 2001; Yamakawa and Nishimura, 2003).

These observations show that the phase-solubility method is not a reliable method for determination of $K_{1:1}$ and that the error increases with decreasing drug solubility, especially for drugs with $S_0 < 1$ mg/ml. This is mainly due to the inaccuracy of S_0 determinations of poorly soluble drugs but also due to excipient/complex interactions as well as formation of multicomponent complexes and simultaneous formation of inclusion and non-inclusion complexes. The solubility increase observed in aqueous cyclodextrin containing drug formulations is frequently an additive effect of several different solubilizing processes and complex structural formations. Thus, the $K_{1:1}$-value determined from a phase-solubility diagrams is the observed stability constant that frequently is composed of several different constants describing various drug solubilizing mechanisms that coexist in non-ideal aqueous cyclodextrin solutions.

3.3. The complexation efficiency

For various reasons it is important to use as little cyclodextrin as possible in pharmaceutical preparations and, thus, the solubilizing efficiency of the cyclodextrins in the aqueous vehicle is the important aspect and not the absolute value of $K_{1:1}$. The solubilizing efficiency is determined by either the slope of the phase-solubility profile or the complex to free cyclodextrin concentration ratio, which is referred to as the complexation efficiency (CE) (Loftsson et al., 1999):

$$\mathrm{CE} = S_0 K_{1:1} = \frac{[\mathrm{D/CD}]}{[\mathrm{CD}]} = \frac{\mathrm{Slope}}{1 - \mathrm{Slope}} \quad (6)$$

where [D/CD] is the concentration of dissolved complex, [CD] the concentration of dissolved free cyclodextrin and Slope is the slope of the phase-solubility profile. The slopes and CE of 28 different drugs are listed in Table 4. On an average the CE is only about 0.3, meaning that on an average only about one out of every four cyclodextrin molecules in solution are forming a water-soluble complex with the poorly soluble drug, assuming 1:1 drug/cyclodextrin complex formation. Of the drugs tested diethylstilbestrol has the highest CE, or CE of 2.82 in which case three out of every four cyclodextrin molecules are forming complex with the drug. If CE is 0.1 then 1 out of every 11 cyclodextrin molecules forms a complex with the drug and if CE is 0.01 then only 1 out of every 100 cyclodextrin molecules forms a complex. For solid dosage forms a CE of 0.5 indicates that cyclodextrin formulation of the drug will result in about 13-fold increase in the bulk dose, assuming drug molecular weight of 350 Da and cyclodextrin molecular weight of 1400 Da. Drug dosage of 40 mg will then increase to 520 mg of the drug complex. CE of 0.1 will result in about 40-fold increase in the dosage bulk and CE of 0.01 in about 400-fold increase.

S_0 and S_{int} are strongly affected by common pharmaceutical excipients such as buffer salts, polymers and preservatives, and sometimes S_0 is below the detection limit of the analytical method used for quantitative determination of the drug or S_{int} has negative value. Since the numerical value of CE is only dependent on the slope of the phase-solubility profile less variation is usually observed in the CE values compared to the $K_{1:1}$ values. In Table 3 the value of $K_{1:1}$ is strongly influenced by S_0 and S_{int} but the CE values in Table 4 are independent of S_0 and S_{int}. The CE values in Table 5 show that on an average addition of polymers to the aqueous complexation media has very little effect on the CE. However, there are exceptions. For example, addition of 0.25% (w/v) PVP to the complexation medium increases the CE for sulfamethoxazole from 0.561 to 1.21 increasing the complex to free cyclodextrin molar ratio from 1:3 to about 1:1. Addition of 0.25% (w/v) HPMC to the aqueous complexation media increases the molar ratio from about 1:4 to 1:3 for acetazolamide and from about 1:11 to about 1:8 for methazolamide (Table 5).

3.4. Optimization

In solutions, phase-solubility diagrams must be used for exact determination of the cyclodextrin concentration needed to solubilize the drug. Fig. 4 shows the phase-solubility of acetazolamide in water and aqueous eye drop formulation. Addition of polymer to the aqueous complexation media improves significantly the CE but the highest CE was obtained in the aqueous eye drop formulation. To prevent drug precipitation during storage about 10% excess cyclodex-

Table 4
The solubility of the unionized drug in pure water or aqueous buffer solution, the slope of the phase-solubility diagram of the unionized drug in aqueous 2-hydroxypropyl-β-cyclodextrin (HPβCD) solution or aqueous randomly methylated β-cyclodextrin (RMβCD) solution, the correlation of the linear slope (corr.), the stability constant ($K_{1:1}$) of the drug/cyclodextrin complex calculated according to Eq. (3) using either the intrinsic solubility (S_0) or the intercept (S_{int}), and the complexation efficiency (CE) calculated from the slope according to Eq. (6), at ambient temperature

Drug	Cyclodextrin	Solubility (mg/ml)	Slope	Corr.	$K_{1:1}$ (M^{-1}) using S_0	$K_{1:1}$ (M^{-1}) using S_{int}	CE
Acetazolamide	HPβCD	0.64	0.197	0.995	85	110	0.246
Alfaxalone	HPβCD	0.00	0.553	0.995	–	4100	1.24
Calcipotriol	RMβCD	0.00	0.278	1.000	–	−260	0.385
Carbamazepine	HPβCD	0.26	0.404	0.991	630	270	0.679
Cyclosporine A	HPβCD	0.01	0.004	0.978	660	−44	0.004
Dexamethasone	HPβCD	0.16	0.246	1.000	800	360	0.326
Dextromethorphan	RMβCD	0.09	0.663	0.998	5900	−2800	1.96
Diethylstilbestrol	HPβCD	0.00	0.739	0.997	–	−1300	2.82
Ergotamine	HPβCD	0.00	0.001	0.967	250	700	0.001
Estradiol	HPβCD	0.09	0.243	0.998	970	1100	0.322
Finasteride	RMβCD	0.04	0.458	0.994	7300	−230	0.844
Flunitrazepam	HPβCD	0.00	0.012	0.998	1100	−62	0.012
Hydrocortisone	HPβCD	0.42	0.667	1.000	1700	1700	2.00
Ketoprofen	HPβCD	0.01	0.601	0.997	38000	−5000	1.51
Methazolamide	HPβCD	0.70	0.092	0.999	34	32	0.101
Miconazole	HPβCD	0.09	0.052	0.993	260	55	0.055
Naproxen	HPβCD	0.12	0.282	0.998	780	390	0.393
Omeprazole	HPβCD	0.00	0.004	0.974	69	−230	0.004
Oxazepam	RMβCD	0.05	0.140	0.968	1000	−150	0.163
Prazepam	HPβCD	0.00	0.018	0.995	1400	−180	0.018
Pregnenolone	HPβCD	0.03	0.110	0.999	1200	−310	0.123
Progesterone	HPβCD	0.00	0.240	0.998	150000	−6300	0.315
Propofol	HPβCD	0.16	0.602	1.000	1600	5000	1.51
Sulfamethoxazole	HPβCD	0.39	0.359	0.998	360	400	0.561
Tamoxifen	HPβCD	0.00	0.004	1.000	–	13	0.004
Terfenadine	HPβCD	0.00	0.165	0.988	–	58	0.197
Triamcinolone acetonide	HPβCD	0.11	0.059	1.000	240	640	0.063
Triclosan	HPβCD	0.00	0.391	0.998	–	−88	0.643

The degree of substitution of RMβCD was 1.8 and the molar substitution of HPβCD was in all cases 0.6 except in the case of triamcinolone acetonide where it was 0.9.

Table 5
The complexation efficiency (CE) in water or aqueous 0.25% (w/v) polymer solution at ambient temperature

Drug	Cyclodextrin	CE			
		Water	HPMC	CMC	PVP
Acetazolamide	HPβCD	0.246	0.356	0.209	0.273
Carbamazepine	HPβCD	0.679	0.829	0.709	0.701
Finasteride	RMβCD	0.844	0.789	0.805	0.844
Methazolamide	HPβCD	0.101	0.153	0.130	0.169
Oxazepam	RMβCD	0.163	0.076	0.127	0.115
Pregnenolone	HPβCD	0.123	0.290	0.105	0.231
Sulfamethoxazole	HPβCD	0.561	0.343	0.619	1.21

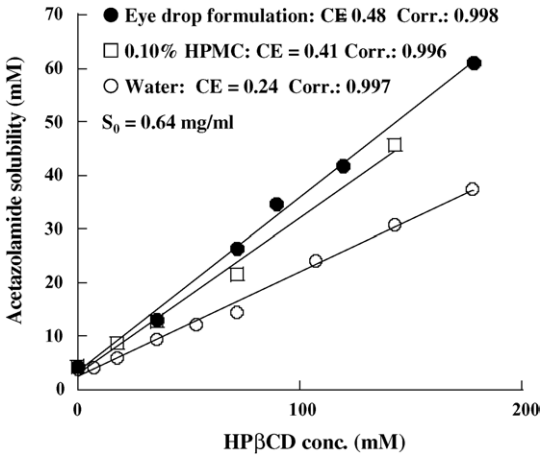

Fig. 4. Phase-solubility profiles of acetazolamide in 2-hydroxypropyl-β-cyclodextrin with molar substitution 0.6 in pure water, aqueous 0.10% (w/v) hydroxypropyl methylcellulose solution and aqueous eye drop formulation containing 0.10% (w/v) benzalkonium chloride, 0.10% (w/v) hydroxypropyl methylcellulose, 0.05% (w/v) sodium edetate and sufficient sodium chloride to make the solution isotonic, at ambient temperature.

trin is used in the final formulation (Loftsson et al., 1996b). Organic solvents frequently reduce the CE. Calcipotriol is a very water-insoluble lipophilic drug that forms a water-soluble complex with randomly methylated β-cyclodextrin with degree of substitution 1.8. Due to its insolubility (i.e. S_0 below the detection limit and $S_{int} < 0$) it is impossible to determine the stability constant ($K_{1:1}$) of the calcipotriol/cyclodextrin complex (Table 4). However, in pure water the CE was determined to be 0.34, 0.25 in 2% (w/v) aqueous glycerol solution and 0.22 in 5% (w/v) aqueous ethanol solution and, thus, the complex to free cyclodextrin molar ratio decreases from 1:3 in pure water to about 1:4 in 5% ethanol solution.

Based on two solubility determination, e.g. in aqueous solutions containing 0 (S_0) and 10% (w/v) cyclodextrin ($S_{HP\beta CD}$), it is possible to estimate the phase-solubility slope and the CE (Table 6). The dosage bulk is estimated from the slope and the drug/cyclodextrin complex:free cyclodextrin molar ration in the solid complex powder from the CE. For example, the solubility of propofol in pure water is 0.16 mg/ml (0.90 mM) but 7.69 mg/ml (43.1 mM) in aqueous 10% (w/v) (71.4 mM) HPβCD solution. Based on these two measurements the slope of the phase-solubility profile is estimated to be 0.591 and the CE to be 1.44. From Eq. (6) the molar ratio of propofol/HPβCD:free HPβCD is estimated to be 1:2, i.e. only one out of every three HPβCD molecules in the complex powder forms a complex with propofol, assuming 1:1 drug/cyclodextrin complex formation. The dosage bulk of the lyophilized complex powder is estimated from S_0 and $S_{HP\beta CD}$ to be 130 mg, which is a 13-fold increase. Through HPβCD complexation propofol could be formulated as sublingual tablet. The feasibility of formulating a given drug as cyclodextrin complex depends on two factors, i.e. the dosage and the CE. Potent drugs with high CE are best suited

Table 6
The oral dosage, the intrinsic solubility in water (S_0), the solubility of the unionized drug in aqueous 10% (w/v) 2-hydroxypropyl-β-cyclodextrin solution ($S_{HP\beta CD}$), both at ambient temperature, the slope of the phase-solubility profile based on the molar drug solubility in pure water and in 10% cyclodextrin solution, the complexation efficiency (CE, see Eq. (6)), the calculated complex to free cyclodextrin molar ratio in the solid complex powder assuming 1:1 drug/cyclodextrin complex formation, and the calculated bulk of the drug dosage as complex

Drug	Dosage[a] (mg)	S_0 (mg/ml)	$S_{HP\beta CD}$ (mg/ml)	Slope	CE	Molar ratio	Dosage bulk (mg)
Alprazolam	0.25	0.07	1.28	0.055	0.058	1:18	20
Clotrimazole	100	0.03	1.21	0.048	0.050	1:21	8500
Digoxin	0.05	0.99	18.2	0.303	0.435	1:3	0.3
Econazole	150	0.37	4.99	0.145	0.170	1:7	3200
Flunitrazepam	1	0.00	0.23	0.010	0.010	1:100	450
Ketoconazole	200	0.01	10.4	0.572	1.34	1:2	1900
Miconazole	1000	0.09	2.46	0.080	0.087	1:12	42000
Oxazepam	10	0.05	2.06	0.098	0.109	1:10	500
Propofol	10	0.16	7.69	0.591	1.44	1:2	130
Terfenadine	60	0.00	7.66	0.227	0.294	1:4	800
Triazolam	0.25	0.03	0.45	0.017	0.017	1:60	60

[a] Approximate dose based on (Anderson et al., 1999).

for cyclodextrin formulations. Due to the dosage bulk only about half of the drugs listed in Table 6 can be formulated as cyclodextrin containing solid dosage forms. It is frequently possible to enhance the CE if the drug/cyclodextrin complex:free cyclodextrin molar ratio is low (1:10 to 1:100) (Loftsson and Másson, 2004), but it can be more difficult to enhance the CE if the molar ratio is high (1:2–1:4).

4. Conclusions

The results show that the phase-solubility method is not a good method for determination of the stability constant of drug/cyclodextrin complexes, especially for that of poorly soluble drugs. Common pharmaceutical excipients such as preservatives, water-soluble polymers and buffer salts, can affect the observed intrinsic solubility and induce formation of higher order complexes. Thus, for poorly soluble drugs the observed $K_{1:1}$-values are most often not the true values for inclusion complex formation. CE can be calculated from the slope of phase-solubility diagrams and it is independent of S_0 or S_{int}, and thus shows less variation than the $K_{1:1}$-values. The CE-values can be used to compare the solubilizing effects of various cyclodextrins, to calculate the drug/cyclodextrin complex:free cyclodextrin molar ration and to study the influence of different pharmaceutical excipients on the solubilization.

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