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Note

γCD/HPγCD: Synergistic solubilization

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ABSTRACT

The natural γ-cyclodextrin (γCD) is like linear water-soluble dextrins, and unlike the natural α- and β-cyclodextrins (αCD and βCD), digested by salivary and pancreatic amylase. This gives γCD a very favorable toxicological profile. However, its usage is hampered by its relatively low solubilizing effect and tendency to form turbid solutions. Addition of 2-hydroxypropyl-γ-cyclodextrin (HPγCD) to aqueous γCD solutions increases the complexation efficiency of γCD and reduces the turbidity. For example, 80:20 mixture of γCD and HPγCD is up to 50% more effective solubilizer for dexamethasone and hydrocortisone than expected based on the solubilizing effects of the individual cyclodextrins. Mixing αCD or βCD with their more water-soluble derivatives only resulted in additive effects. Mixing γCD with HPγCD resulted in synergistic effect.

The natural γ-cyclodextrin (γCD), unlike the natural α- and β-cyclodextrins (αCD and βCD), is rapidly and essentially completely digested by salivary and pancreatic amylase (Munro et al., 2004). Studies in humans have shown that γCD is well tolerated and does not cause any gastrointestinal discomfort (Koutsou et al., 1999), which is to be expected since γCD is metabolized just like linear water-soluble dextrins. 2-Hydroxypropyl-γ-cyclodextrin (HPγCD), a water-soluble γCD derivative, is also digested by salivary and pancreatic amylase and therefore believed to possess more favorable toxicological profile than hydroxypropylated αCD and βCD (HPαCD and HPβCD). Although γCD has very favorable toxicological profile its usage is hampered by its limited aqueous solubility and ability to form water-soluble drug complexes. We have observed that the complexation efficiency of γCD can be improved significantly by adding small amount of HPγCD to the aqueous complexation media. Following is a short description of our findings.

Dexamethasone was purchased from Fagron group (Amsterdam, The Netherlands), hydrocortisone from ICN Pharmaceuticals (USA), αCD, βCD, γCD, HPαCD with molar substitution (MS) of 0.6 (MW 1182 Da) and HPγCD MS 0.6 (MW 1576 Da) from Wacker Chemie (Germany), HPβCD MS 0.6 (MW 1400 Da) from Roquette (France), disodium edetate dihydrate (EDTA) from Merck (Germany), benzalkonium chloride from Sigma (USA). Sulfobutylether β-cyclodextrin sodium salt (SBEβCD) MS 0.9 (MW 2163) was kindly donated by Cydex Inc. (USA). All other chemicals used were of analytical reagent grade purity. Milli-Q water was used for the preparation of all solutions. The solubility of the drugs in aqueous cyclodextrin solutions was determined in triplicate by the previously described heating method (Loftsson et al., 2005). The complexation media was either pure water or aqueous eye drop formulation containing 0–20% (w/v) cyclodextrin, benzalkonium chloride (0.02%), EDTA (0.1%) and sufficient sodium chloride to obtain isotonicity. The pH of the eye drop formulation was adjusted to 7.4 with concentrated sodium hydroxide solution. Quantitative determinations were performed on a reversed-phase HPLC component system from Hewlett Packard Series 1100, consisting of G132A binary pump (operated at 1.5 ml/min) with G1379A solvent degasser, G13658 multiple wavelength detector operated at 241 (dexamethasone) and 254 nm (hydrocortisone), G1313A auto sampler, and Phenomenex Luna 5 μm C18 reverse-phase column (150 mm × 4.6 mm). The mobile phase consisted of acetonitrile, tetrahydrofuran and water (33:1:66). The retention time of dexamethasone was 5.1 min and that of hydrocortisone 3.2 min. The phase-solubility diagram was obtained by plotting the total concentration of dissolved cyclodextrin (moles/liter) versus the drug solubility (moles/liter; mean of three determinations). The apparent stability constant of the drug·cyclodextrin complex (D·CD), assuming that one molecule of drug forms a complex with one molecule of cyclodextrin ($K_{1:1}$), was calculated from the slope of the linear phases-solubility profile and the intrinsic drug solubility (S_0) in the complexation media (Higuchi and Connors, 1965):

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

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Table 1
Apparent stability constant ($K_{1:1}$), assuming one to one drug:CD complex formation, and the complexation efficiency (CE) of dexamethasone/cyclodextrin complexes in pure aqueous complexation medium at room temperature (22–23 °C)

Cyclodextrin	Ratio[a]	Slope[b]	Correlation coefficient	$K_{1:1}$ (M^{-1})	CE
α-CD	–	0.049	0.999	240	0.05
αCD:HPαCD	80:20	0.051	0.998	250	0.05
αCD:HPαCD	20:80	0.066	0.998	330	0.07
HPαCD	–	0.073	0.999	370	0.08
βCD	–	0.531	0.999	5340	1.13
βCD:HPβCD	80:20	0.476	1.000	4280	0.91
βCD:HPβCD	20:80	0.356	0.999	2610	0.55
HPβCD	–	0.305	1.000	2070	0.44
βCD:SBEβCD	80:20	0.503	1.000	4780	1.01
β-CD:SBEβCD	20:80	0.430	1.000	3550	0.75
SBEβCD	–	0.379	1.000	2880	0.61
γCD	–	0.204	0.904	1210	0.26
γCD:HPγCD	80:20	0.471	0.997	4190	0.89
γCD:HPγCD	20:80	0.594	1.000	6900	1.46
HPγCD	–	0.524	1.000	5190	1.10

The intrinsic solubility (S_0) of dexamethasone in water was determined to be 83 μg/ml (0.2 mM) at room temperature.

[a] The weight-to-weight ratio of dissolved cyclodextrin in the aqueous complexation media (natural cyclodextrin:cyclodextrin derivative).

[b] Slope of the linear phase-solubility diagram.

The complexation efficiency (CE) was calculated from the slope of the linear phases-solubility profile (Loftsson et al., 2005):

$$\mathrm{CE} = \frac{[\mathrm{D}\cdot\mathrm{CD}]}{[\mathrm{CD}]} = K_{1:1} \times S_0 = \frac{\mathrm{Slope}}{1 - \mathrm{Slope}} \quad (2)$$

where [D·CD] is the concentration of dissolved complex and [CD] is the concentration of dissolved free cyclodextrin.

The results are shown in Tables 1 and 2. Mixtures of αCD and HPαCD, βCD and HPβCD, and βCD and SBEβCD did not display synergistic effects. On a molar basis the solubilizing effect of these cyclodextrin combinations was additive and their CEs were close to the weight average or molar average of the two cyclodextrins. Here the weight average is approximately the same as molar average. However, combinations of γCD/HPγCD where 80/20 and 20/80 mixtures displayed CEs those were higher than that of pure γCD and pure HPγCD, respectively (Table 1). For example, the CE of a mixture of 80% (w/w) γCD and 20% (w/w) HPγCD was determined to be 0.89 which is about 110% higher than that of the weight average of

Table 2
Apparent stability constant ($K_{1:1}$), assuming one to one drug:CD complex formation, and the complexation efficiency (CE) of dexamethasone/cyclodextrin complexes in the aqueous eye drop formulation at room temperature (22–23 °C)

Cyclodextrin	Ratio[a]	Slope[b]	Correlation coefficient	$K_{1:1}$ (M^{-1})	CE
Dexamethasone					
γCD	–	0.212	0.886	1320	0.26
γCD/HPγCD	80/20	0.407	0.993	3390	0.69
γCD/HPγCD	60/40	0.650	1.000	9180	1.86
γCD/HPγCD	50/50	0.664	1.000	9770	1.98
γCD/HPγCD	40/60	0.604	1.000	7530	1.53
γCD/HPγCD	20/80	0.557	1.000	6210	1.26
HPγCD	–	0.516	1.000	5260	1.07
Hydrocortisone					
γCD	–	0.238	0.921	240	0.31
γCD/HPγCD	80/20	0.656	1.000	1460	1.90
γCD/HPγCD	20/80	0.617	1.000	1240	1.61
HPγCD	–	0.547	0.999	930	1.21

The intrinsic solubility (S_0) of dexamethasone in the eye drop formulation was determined to be 80 μg/ml (0.2 mM) and that of hydrocortisone to be 470 μg/ml (1.3 mM) at room temperature.

[a] The weight-to-weight ratio of dissolved cyclodextrin in the aqueous complexation media (natural cyclodextrin:cyclodextrin derivative).

[b] Slope of the linear phase-solubility diagram.

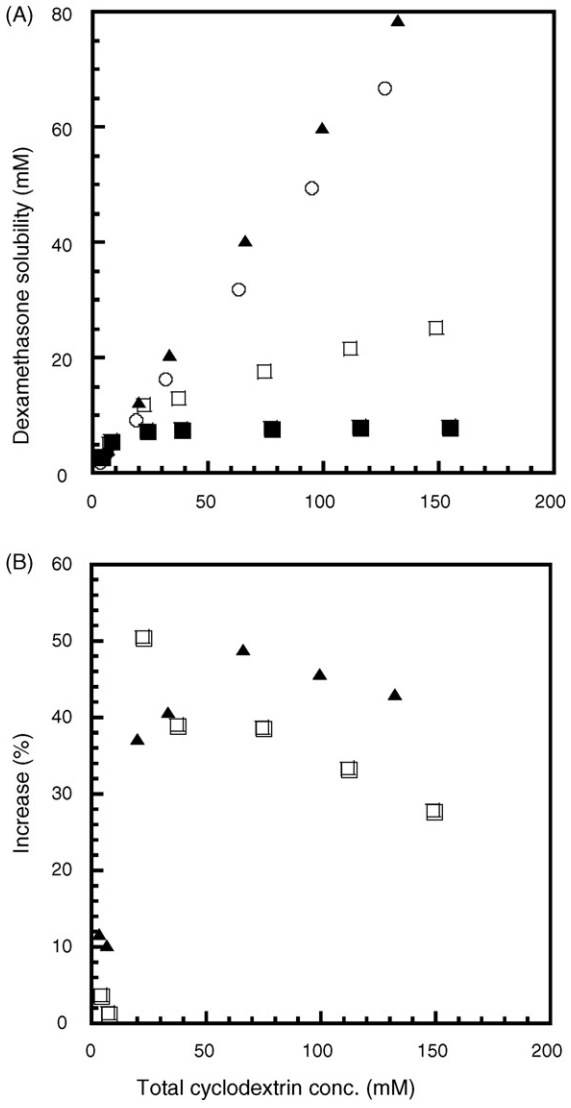

Fig. 1. Phase-solubility profiles of dexamethasone in aqueous eye drop formulation containing mixtures of γCD and HPγCD (A), and the percent increase in dexamethasone solubility above the additive effect of the two cyclodextrins (B). The γCD:HPγCD ratio: 100:0 (■), 80:20 (□), 20:80 (▲), 0:100 (○).

γCD and HPγCD (i.e. 0.8 × 0.26 + 0.2 × 1.10 = 0.43) and 3.4-fold that of pure γCD. The CE of CD in eye drop formulation is somewhat lower than in pure water due to the presence of additives in the complexation medium; however, the significantly improved solubility by the mixing of γCD/HPγCD was not impeded. For example, the CE of a mixture of 80% (w/w) γCD and 20% (w/w) HPγCD was determined to be 0.69 which is about 65% higher than that of the weight average of γCD and HPγCD (i.e. 0.8 × 0.26 + 0.2 × 1.07 = 0.42) and 2.7-fold that of pure γCD (CE 0.26). Highest CE was obtained when the mixture consisted of 50–60% (w/w) of γCD (Table 2). Similar results were observed when dexamethasone was replaced by hydrocortisone where an 80/20 mixture resulted in almost 4-fold larger enhancement in the CE than expected based on an additive effect. The observed solubilization enhancement in water was about 50% (Fig. 1) or in other words the synergistic effect of the γ-cyclodextrins resulted in up to 50% greater solubilization than could be expected based on their additive effect (Fig. 1B). The mean stability constants ($K_{1:1}$) of αCD complexes has been determined to be 130 ± 8 M^{-1}, that of βCD to be 490 ± 8 M^{-1} and that of γCD to be 350 ± 9 M^{-1} (population mean ± standard deviation; Connors,

1995; Brewster and Loftsson, 2007). Thus the stability constants of βCD are about 40% larger than those of γCD. Although the stability constants of drug/βCD complexes and the complexation efficiency of βCD are larger than those of drug/αCD and drug/γCD complexes the usage of βCD is hampered by its low aqueous solubility. Addition of HPγCD to γCD formulations can make the relatively water-soluble γCD just as effective or even better complexing agent and solubilizer than βCD. In conclusion, addition of relatively small amounts of HPγCD to the natural γCD makes it a better solubilizer for lipophilic water-insoluble steroids than the natural βCD, which in turn is a much better solubilizer than αCD.

It is not clear how mixing the parent natural γCD with its more water-soluble derivative HPγCD enhances the CE but it might be related to the intrinsic properties of this cyclodextrin. γCD, and not αCD and βCD, does form turbid solutions due to self-aggregation of γCD (Szente et al., 1998). Filtration or centrifugation of γCD solutions does not prevent the solutions to become again turbid during storage. Addition of HPγCD to aqueous γCD solutions appears to reduce turbidity of the solution.

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