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Spironolactone-cyclodextrin complexes: phase solubility and ultrafiltration studies

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Summary

Phase-solubility and ultrafiltration techniques, coupled with UV spectrophotometric analyses, have been employed to demonstrate the formation and to determine the stoichiometry of soluble and solid-state complexes in spironolactone : cyclodextrin systems. α-, β- and γ-cyclodextrins (α, β, γ-CDX) all formed complexes with spironolactone in aqueous solution with apparent mole ratios of 1 : 1 for the α- and β-CDX complexes. The γ-CDX system formed more than one stoichiometric form in solution. Apparent stability constants, K, reflecting the spatial compatibility of guest molecules, show the β-CDX : spironolactone soluble complex to be the most stable. Solid-state complexes of mole ratio 3 : 1 and 2 : 1 CDX : spironolactone were formed for the β- and γ-CDX systems, respectively, and these gave 8- and 17-fold corresponding increases in aqueous solubility for spironolactone.

Introduction

Incomplete bioavailability and bioinequivalence of solid oral dosage forms of spironolactone (Sp), a poorly aqueous soluble diuretic, are well recognised (e.g., McInnes et al., 1982). The source of the problem appears to be the unrecognised and/or unavoidable changes in physicochemical properties during formulation and processing, which may involve polymorphic modifications and hence solubility variability (Salole and Al-Sarraji, 1985). One approach to solve this problem is to form drug : adjuvant complexes of improved and controlled aqueous solubility. A particularly useful series of adjuvants are the cyclodextrins.

Cyclodextrins are cyclic and toroid shaped oligosaccharides consisting of 6, 7 and 8 α-(1,4)-linked glucopyranose units for α-, β- and γ-cyclodextrins, respectively. Their ability to form inclusion complexes has been applied in the pharmaceutical field to modify the physicochemical properties of various drug molecules (Lach and Cohen, 1963; Saenger, 1980; Szejtli, 1982).

Spironolactone has been reported to form complexes both in solution and in the solid state with cyclodextrins (Andersen and Bundgaard, 1983; Seo

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et al., 1983); however, the stoichiometry of the solid state complex of Sp and β-CDX is controversial.

As part of wider studies of cyclodextrin complexes and their structure in both liquid and solid states, phase-solubility studies (Higuchi and Connors, 1965; Pitha et al., 1987) and ultra-filtration studies (Jones and Parr, 1987) of the cyclodextrins with spironolactone are presented. The aim of this work is to resolve the stoichiometries of the solution and solid state complexes and to demonstrate the existence and stability of inclusion complexes in solution.

Experimental

Materials

Spironolactone BP (Sp) was obtained from APS Ltd, Cleckheaton, U.K., β-cyclodextrin (β-CDX) (Dexy-Pearl) from Steetley Berk Ltd, Hampshire, U.K. and α- and γ-cyclodextrin (α-CDX and γ-CDX) from Medimpex, London. Methanol was from BDH, Poole, and all water used was double-distilled and deionised. Ultraviolet spectra were recorded using a Hewlett Packard diode array spectrophotometer at 240 nm for Sp.

Ultra-filtration studies were conducted with an Amicon model 8050 filtration cell fitted with Amicon Diaflo ultrafiltration membranes type YM2 (molecular weight cut-off 1000).

Methods

Solubility studies Solubility measurements were carried out by the method of Higuchi and Connors (1965), with excess amounts of Sp, accurately weighed, added to aqueous solutions containing various concentrations of CDX in screw-capped bottles. These were then equilibrated in a shaking water bath at 25°C for 7–12 days. Solutions of Sp with β-CDX were also equilibrated at 37°C. Following equilibration, an aliquot was filtered with a 0.45 μm pore size Millipore membrane filter, diluted with methanol and analysed spectrophotometrically at 240 nm for total Sp against a calibration curve (correlation coefficient = 0.9998). There was no interference from CDX at this wavelength and the systems had been shown to be stable over the period of study.

The equilibrium constants (apparent stability constants), K, are calculated from the initial slope, S, of the graph of concentration of total Sp against concentration of cyclodextrin assuming a stoichiometry of 1 : 1 for the complex

$$K = \frac{S}{S_0(1 - S)}$$

where S_0 is the intercept of the Sp concentration axis.

A second series of solubility measurements for the crystalline complexes of Sp with β- and γ-CDX was carried out. In this case, an equilibration time of 24 h was established as being satisfactory for both systems studied.

Preparation of a solid-complex The solid complexes were prepared by mixing appropriate amounts of Sp and CDX (β and γ) in water (i.e., 3.6×10^{-3} M and 13.2×10^{-3} M for Sp and β-CDX, respectively, and 3.6×10^{-3} M and 16.5×10^{-3} M for Sp and γ-CDX, respectively). The calculated mixture was determined by examining the descending portion of the type B_s phase solubility diagrams (Higuchi and Connors, 1965) (see Figs 2a and 3a). The mixtures were stirred for 4 days at room temperature. The complexes which precipitated as microcrystalline powders were filtered, washed with water, dried in a desiccator under vacuum and over silica gel at room temperature for 48 h then stored over silica gel at room temperature prior to use.

Filtration cell A method of detection of inclusion complexation in solution by the filtration cell was reported by Jones et al. (1987). Since the cyclodextrins have relatively high molecular weights (β-CDX = 1135, γ-CDX = 1297) and the guest molecule usually has a lower molecular weight (e.g., Sp = 416.6), a membrane with a known molecular weight cut-off value (e.g., 1000) can be used to separate uncomplexed from complexed guest molecule. In this way, a large percentage of the cyclodextrin and the complex are retained and the guest molecule (uncomplexed) passes freely through the membrane.

The Amicon YM2 ultrafiltration membrane with molecular weight cut-off value 1000 was demonstrated to permit the passage of 76% of the total Sp present in an aqueous solution (30%/h) and 4%/h and 6%/h of β-CDX and γ-CDX, respectively, at room temperature at an operating pressure of 420 kN m^{-2}. The presence of the cyclodextrin was shown not to interfere with the filtration of Sp.

Solutions containing constant concentrations of Sp (molarity) and various concentrations of the cyclodextrins (molarity) were filtered though the membrane in turn. Filtrates collected over a constant time period of 0–1½ h were assayed spectrophotometrically for total Sp filtered, expressed as the percentage of Sp in the filtrate per h. From the graph of Sp against CDX the number of moles of cyclodextrin required to complex with one mole of Sp was determined. In addition, the filtration rate of Sp was measured from solutions prepared from Sp : β-CDX and Sp : γ-CDX solid-state complexes at equivalent initial Sp concentrations.

Results and Discussion

Inclusion complexation in solution

Cyclodextrins have been used extensively as complexing agents to improve the dissolution properties of many drugs. In these complexes, molecules of the drug or portions of the drug are either enclosed in the relatively hydrophobic cavity of the cyclodextrin (torus) or positioned in a channel formed by several molecules of cyclodextrin. Many solid-state cyclodextrin complexes prepared by precipitation are crystalline, and the structures of several have been published (e.g., Saenger, 1980; Szejtli, 1982).

In the present study, the three parent cyclodextrins were used: α-, β-and γ-CDX. Phase-solubility diagrams indicated that all three are capable of complex formation with Sp in solution and for β- and γ-CDX in the solid-state. The solubility of Sp increased linearly as a function of α-CDX concentration (see Fig. 1) and the solubility curve can be classified as type A_L (Higuchi and Connors, 1965). A solid-state complex is not obtained by precipitation from such a system. In contrast,

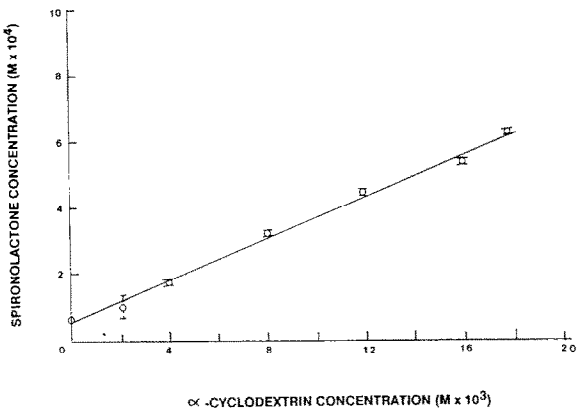

Fig. 1 Phase-solubility diagram of spironolactone with α-cyclodextrin in water at 25°C.

β- and γ-CDX systems showed type B_S solubility curves (Figs 2a and 3a, respectively) with microcrystalline complexes precipitating at higher cyclodextrin concentrations. For the Sp : β-CDX system, a typical type B_S curve was obtained only when the amount of Sp used in the experiment was within a specific concentration range. This was inferred from the work of Andersen and Bundgaard (1983) who obtained a plateau region without a descending portion and no plateau when the amount of Sp used was 4 and 1 mg/ml, respectively, at 20°C. A typical type B_S curve was produced in this study by selecting Sp concentrations of 1.5 and 2 mg/ml for the studies at 25 and 37°C, respectively.

The apparent stability constants, K, were calculated from the initial slope, S, of the phase solubility diagrams and values of K, S and S_0, the intercept, are listed in Table 1. K values, which reflect spatial compatibility of guest molecules for the cyclodextrin cavity and the strength of the interaction, indicate that the β-CDX cavity is the more favourable site of the three CDXs as indicated by the higher slope and K value. This value is reduced at the higher temperature studied, 37°C, but an appreciable interaction is maintained. No precipitation of solid complex was observed for the α-CDX system, and this is reflected in the low values for the slope and K; thus, little penetration of the steroid molecule into the smaller cavity size of α-CDX is indicated. γ-CDX having the largest cavity size might have been expected to produce a

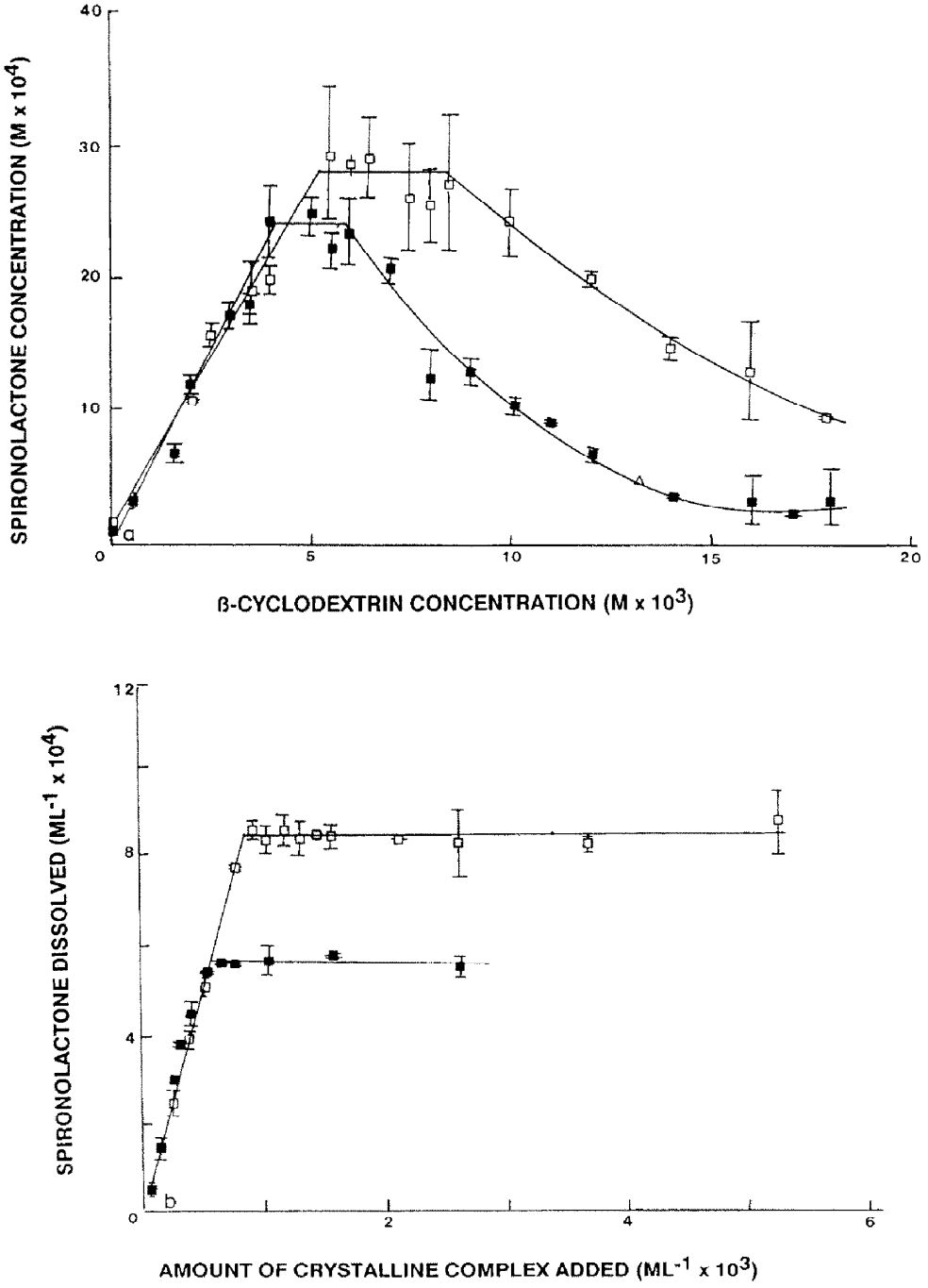

Fig. 2. (a) Phase-solubility diagram of spironolactone with β-cyclodextrin in water at 25°C (■) and 37°C (□). △, point of curve selected for the preparation of the solid-state complex. (b) Phase-solubility diagram of spironolactone : β-cyclodextrin solid-state complex (mole ratio 1 : 3) in water at 25°C (■) and 37°C (□).

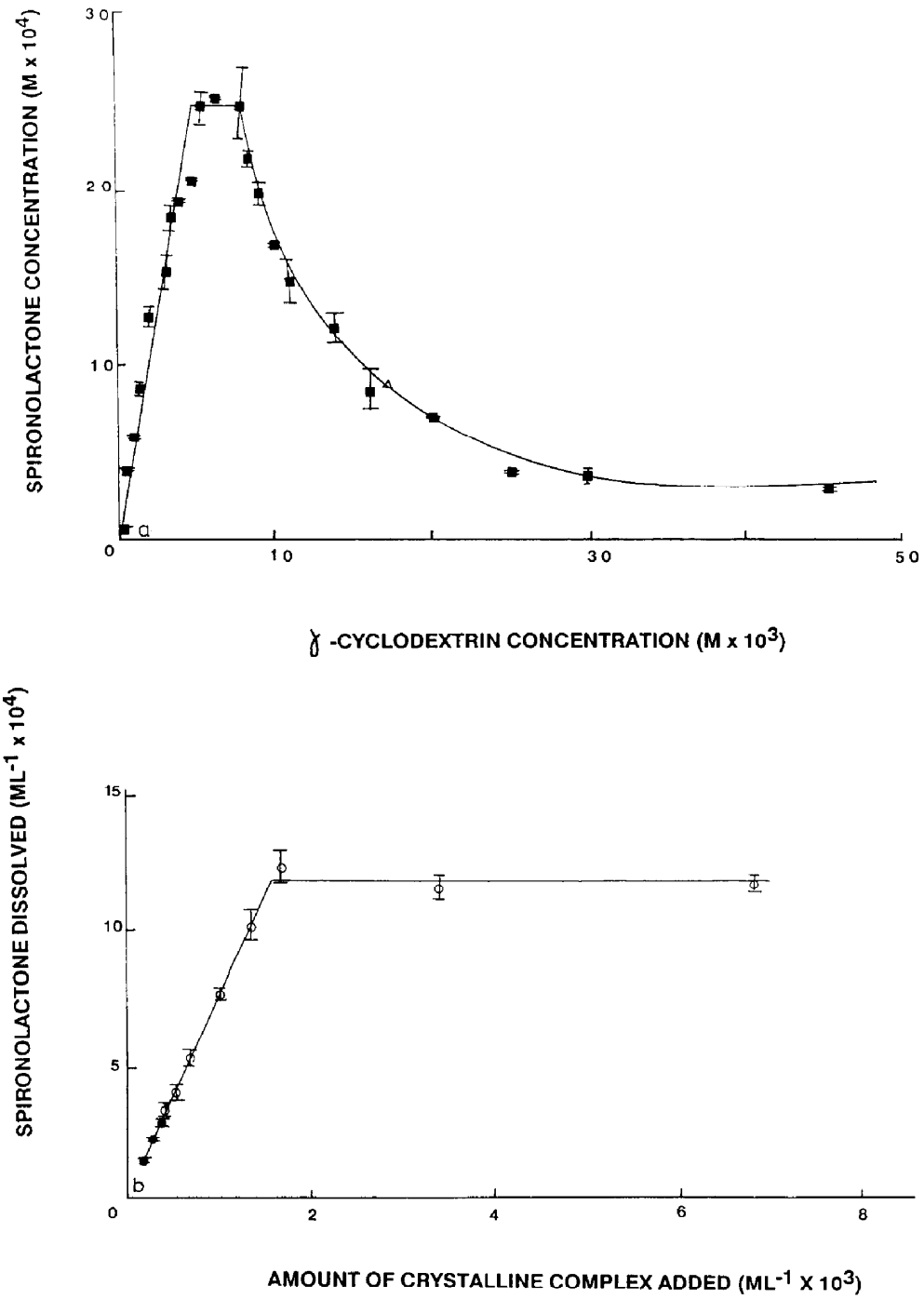

Fig. 3. (a) Phase solubility diagram of spironolactone : γ-cyclodextrin in water at 25 °C. △, point of curve selected for the preparation of the solid-state complex. (b) Phase solubility diagram of spironolactone : γ-cyclodextrin solid-state complex (mole ratio 1 : 2) in

TABLE 1

Apparent stability constants, K, for soluble spironolactone : cyclodextrin complexes

Host cyclodextrin in complex	Intercept (S_0) ($\times 10^{-4}$ M)	Slope (S)	Temp. (°C)	K (M^{-1})
α-CDX	0.566	0.0309	25	564
β-CDX	0.522	0.5653	25	24921
β-CDX	1.128	0.5090	37	9198
γ-CDX	0.897	0.4999	25	11142

complex of greater affinity for the steroid rather than the β-CDX (Uekama et al., 1982). This observation implies that a good, compatible, spatial fit is stabilized by forces that are dependent on bond lengths of intermolecular distances.

Inclusion complexation in the solid state

The microcrystalline complexes were prepared as described in Experimental and subsequently dried at 110°C for 3 h prior to assay. Spectrophotometric analysis confirmed the stoichiometries of the complexes to be 1 : 3 and 1 : 2 for β- and γ-CDX systems, respectively.

The products were demonstrated to be complexes as opposed to mere physical mixtures by the use of high-resolution analytical techniques (Yusuff et al., 1989). Since a clearly identifiable plateau region was obtained in the phase-solubility diagram for the Sp : γ-CDX system, this was also used to estimate the stoichiometry of the solid-state complex at 1 : 2.25 (Sp : γ-CDX). This figure agrees closely with that obtained from the analysis of the solid-state complex. Plateau regions in the Sp : β-CDX phase-solubility diagrams were not as clearly defined so this approach was not adopted for these systems.

The stoichiometries of these solid-state complexes were examined further by the ultra-filtration cell method.

Aqueous solubility of the microcrystalline complex

The solubility diagrams of the β- and γ-CDX solid-state complexes are shown in Figs 2b and 3b.

Dissolution from complex occurred up to the equivalent of 5.52×10^{-4} and 8.40×10^{-4} mole/l of Sp at 25 and 37°C, respectively, for the β-CDX system. Further addition of complex to water had no effect on the limiting values. It can thus be concluded that either the soluble complex formed was composed of a single stoichiometric species at both temperatures or that more than one species was present with identical solubilities.

However, the γ-CDX system gave complete complex dissolution up to the equivalent of 3.29×10^{-4} mole/l of Sp, and incomplete dissolution when further amounts were added (Fig. 3b). The concentration of Sp continued to increase until the addition of an amount of complex equivalent to 1.36×10^{-3} mole/l of Sp, whereby a limiting solubility equivalent to 1.15×10^{-3} mole/l of Sp was established. This phenomenon could result from the presence of more than one stoichiometric species in solution of different aqueous solubilities. On dissolution, the complex reverts to a more soluble complex which accounts for the latter increase in the solubility of Sp in Fig. 3b. It is interesting to note that the aqueous solubility of Sp has been increased by a factor of 8 (25 and 37°C) and 17 (25°C) when complexed with β- and γ-CDX, respectively.

Ultra-filtration cell

The solubility studies of crystalline complexes showed that the β-CDX system in solution existed as a single stoichiometric species and γ-CDX as multiple stoichiometric forms. An analytical method capable of discriminating the complexed and uncomplexed guest molecule would assist in defining the stoichiometries of these complexes in solution. The ultra-filtration cell method utilises membranes of extremely fine, controlled pore structure and is therefore a selective and sensitive means of separation of relatively low molecular weight compounds.

Fig. 4 shows the results from the filtration of the β- and γ-CDX systems in solution. Data obtained are consistent with the interpretations made from the solubility studies of the crystalline complexes. The decline in the amount filtered up to 1 mole of β-CDX is due to complex formation in solution (see Fig. 4a). Above a 1 : 1 mole ratio, the slope is reduced; in a non-dynamic system a zero gradient would be expected if the complex formed

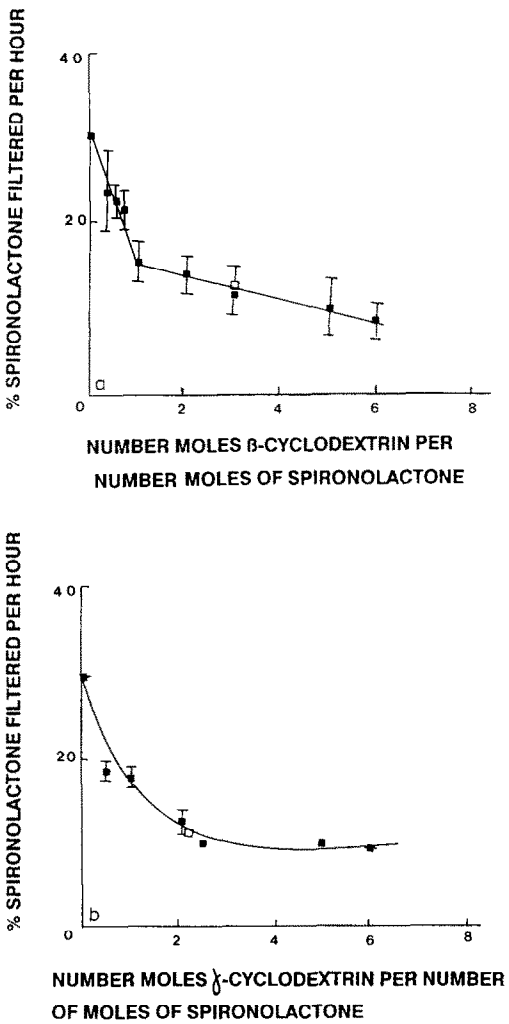

Fig. 4. (a) Fraction of spironolactone filtered (% wt/h) vs number of moles of β-cyclodextrin per number of moles of spironolactone. □, filtration rate for solution formed from solid-state complex. (b) Fraction of spironolactone filtered (% wt/h) vs number of moles of γ-cyclodextrin per number of moles of spironolactone. □, filtration rate for solution formed from solid-state complex.

is of a 1 : 1 mole ratio, whereas complexes with a greater number of moles of CDX would be expected to produce a steeper gradient. This supports the view that in solution a complex of 1 : 1 Sp : β-CDX stoichiometric ratio is formed.

The γ-CDX system is more complicated and appears to consist of more than one stoichiometric form in solution, as proposed above. Above a mole ratio of 1 : 0.5 of Sp : CDX, the slope appears to change continuously until above a mole ratio of approx. of 1 : 2, the curve almost parallels the *X*-axis. At least two species are therefore thought to be present in solution with possibly a 1 : 1 complex at low levels of γ-CDX concentrations and a 1 : 2 mole ratio complex at higher concentrations.

This method, as well as illustrating the existence of complexes in solution, can be utilised to confirm the stoichiometries of the crystalline complexes. The filtration values of solutions formed from the solid-state complexes were compared with those obtained for prepared solutions of known mole ratios. The solution formed from the β-CDX solid-state complex corresponded to a 1 : 3 mole ratio prepared solution and the γ-CDX system to that of a 1 : 2 mole ratio prepared solution.

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