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Determination of the stoichiometry of 2,4-dichlorophenoxyacetic acid-β-cyclodextrin complexes in solution and in solid state

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Abstract

Chemical analysis, DSC and solubility determinations have been applied to the study of solid inclusion complexes of a pesticide, 2,4-dichlorofenoxyacetic acid (2,4-D) as guest, and β-cyclodextrin (β-CD) as host, in order to produce experimental evidence of the inclusion process and of the stoichiometry of the inclusion compound. Three processing methods have been studied and compared: physical (mechanical) mixing; kneading; and spray-drying. The phase–solubility diagram of complex formation in solution has been also established. The stoichiometric ratio of the complexes was found to be 1 : 1 by solubility and DSC, being confirmed by chemical analysis. Spray-drying was found to be most suitable method for preparing the complexes. © 1998 Elsevier Science B.V.

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1. Introduction

Cyclodextrins (CD) are doughnutshape cyclic oligosaccharides with a central hydrophobic cavity that can enclose one or several molecules, which are held by non-covalent interactions. Among them, β-CD is receiving increasing attention due its low cost and capacity to interact with a wide variety of drug molecules, including pesticides and drugs [1,2]. The molecular encapsulation may result in interesting effects, such as stabilisation of volatile compounds, enhancement of biological effectiveness of solid substances and improved solubility and dissolution.

It is of interest to determine the extent of molecular interaction between host and guest molecules in a quantitative fashion. The knowledge of the stoichiometry and equilibrium constants in solution for inclusion complexes may allow a prediction of further dissolution behaviour.

Different techniques have been used to characterise inclusion complexes in an aqueous solution. Traditionally, the experimental determination and study of phase–solubility diagrams [3,4] and ^{1}H- and ^{13}C-NMR spectroscopies have been widely employed [5,6].

On the other hand, there are also several analytical techniques that can be used in the characterisation applicable for the solid state. In this context, thermoanalytical techniques as DSC, are widely applied to

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confirm the inclusion process. The disappearance of thermal events of guest molecules into the inclusion compound is generally taken as a proof of real inclusion [7–9]. Giordano et al. [10], have employed this technique also to calculate the solid-state stoichiometry of two particular drug-CD binary systems. This application is based on carrying out quantitative DSC studies of mixtures containing excess of drug with respect to their real stoichiometry. Through the measurement of melting enthalpies from DSC curves of a particular drug, a quantitative evaluation of the component in excess should be possible. These results can provide some useful information about what kind of interaction occurs between components on heating, guest molecules and CD in mechanical mixtures, or guest to complex in the inclusion compound, and further to determine the stoichiometry of the inclusion.

In the present work, the application of DSC to determine the stoichiometry of the inclusion complex in solid state is investigated. A commercial organic compound commonly applied as pesticide, 2,4-dicloro-phenoxyacetic acid (2,4-D), widely used for weed control in cereals and other crops, was selected. Three processing methods of preparing binary systems are compared. The results are compared using chemical analysis and phase–solubility determinations.

2. Experimental

2.1. Materials

2,4-D was supplied by Sigma (St. Louis, Missouri, USA) and β-CD by Roquette (Lestrem, France). All other materials were of analytical reagent grade.

2.2. Methods of preparation of binary systems

The preparation of binary systems of 2,4-D with β-CD was performed by mechanical (physical) mixing, kneading and spray-drying, as described below.

2.3. Mechanical (physical) mixing

Physical mixtures were prepared by simple intensive mixing of the two components for 15 min.

2.4. Kneading

2,4-D and β-CD were mixed together in different stoichiometric ratios (1 : 1, 1 : 2, 1 : 3, 2 : 1 and 3 : 1) and kneaded using a porcelain mortar and pestle. During this process, appropriate amounts of ethanol were added as drops to the mixture, in order to maintain a suitable consistency. This process was continued for 45 min, and the paste was dried at 37°C for 48 h using an oven (Selecta, mod. 204). The dried product was gently ground into a fine powder and sieved below 250 μm mesh.

2.5. Spray-drying

Spray-drying was performed using a Büchi 190M miniSpray-Dryer. The molar ratios of 2,4-D and β-CD were 1 : 1, 2 : 1, 3 : 1 and 4 : 1. First, the 2,4-D was dissolved in 400 ml of ethanol (96°) and, separately, the required amount of β-CD in 400 ml of purified water. Next, the solutions were mixed together for 20 min by sonication, to produce a clear solution, which was then spray-dried. The drying conditions were flow rate: 1000 ml/h, inlet temperature: 168°C, outlet temperature: 90°C, airflow rate: 400 Nl/h.

2.6. Phase–solubility determinations

The solubility studies were performed according to the method previously proposed by Higuchi and Connors [11]. 2,4-D, in amount that exceeded its solubility (20 mg), was accurately weighed into 50-ml Erlenmeyer flasks, to which 10 ml of water containing various concentrations of β-CD in the range 0.002–0.024 M were added. These flasks were sealed and shaken at 25°C for one week, considered to be sufficient time in order to reach the equilibrium. Next, the samples were filtered with syringe through a 1.2-μm membrane filter (Sartorius cellulose nitrate) and properly washed. A portion of the sample was analysed spectrophotometrically at 284 nm using a Hitachi U-2000 apparatus. Trace amounts of β-CD did not interfere with the assay.

2.7. Chemical analysis

An exactly weighed amount of different powders, previously washed with 2 ml of ethanol (96°), was dissolved in water and the remaining concentration of 2,4-D was determined spectrophotometrically at 284 nm.

2.8. *DSC*

Thermal analysis by DSC was carried out using a Mettler apparatus equipped with a FP85 furnace, FP80 HT temperature control unit and FP89 HT software. Samples of 10 mg were put into aluminium pans. These pans were pierced in order to permit the loss of gases evolved during the heating process. DSC scans were performed in triplicate, under static air atmosphere, at a heating rate of 10°C in the temperature range of 30° to 300°C. Heats of fusion were automatically determined by the software following calibration with Indium (28.4 J/g), using integration of the areas under the DSC endothermic peaks of melting (SD<0.5%).

2.9. *Thermoanalytical procedure for stoichiometric determination*

Let us consider the analytical determination of 2,4-D and β-CD stoichiometry in the solid state from thermoanalytical measurements. Let n_a and n_b be the moles of guest (2,4-D) and host (β-CD) in the starting mixture, respectively, and R the stoichiometric ratio of guest–host interaction (in moles). The free moles of guest after interaction n_a^* will be

$$n_a^* = n_a - n_b R \tag{1}$$

where $n_b R$ represents the interacted moles of guest. Since the total fraction of guest added to the mixture (N) is represented by the equation

$$N = \frac{n_a}{(n_a + n_b)} \tag{2}$$

Consequently,

$$n_a^* = N(n_a + n_b) - n_b R \tag{3}$$

The free mole fraction of the guest (F) can be written as:

$$F = \frac{n_a^*}{(n_a + n_b)} = N - (1 - N)R \tag{4}$$

which can be easily rearranged to

$$F + N(I + R) - R \tag{5}$$

As the total composition of the system (n_a and n_b) is known, by plotting F versus N a straight line with slope $(1+R)$ and intercept at the origin $(-R)$ must be obtained. According to Giordano et al. [10], Eq. (5) bears a physical meaning provided that $n_a/n_b \geq R$ and $F \geq 0$.

3. Results and discussion

3.1. *Phase–solubility diagram*

The data obtained are included in Fig. 1. This phase–solubility diagram can be classified as B_s type, according to the classification of Higuchi and Connors [11].

Considering this curve, it can be seen that, from S_0, the apparent solubility of 2,4-D increases due to the formation of a soluble inclusion complex between

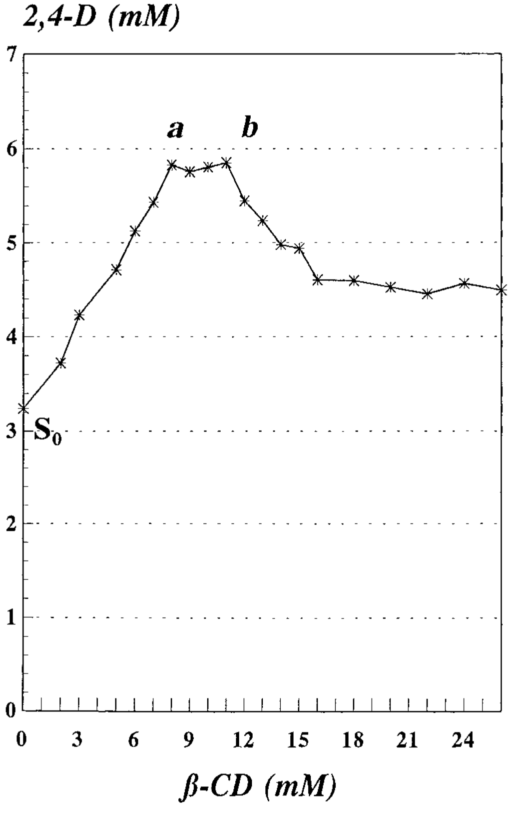

Fig. 1. Phase–solubility diagram of 2,4-D and β-CD binary system obtained in water at 20°C.

2,4-D and β-CD. When the solubility limit of this complex is reached (point a), the ascending linear portion starts levelling-off, and further addition of β-CD results in the precipitation of a microcrystalline complex. The concentration of uncomplexed 2,4-D is maintained constant by dissolution of solid 2,4-D. The complex formation continues in the plateau region and it precipitates from saturated solution as the concentration of β-CD increases. At point b, all solid 2,4-D has been consumed, and further addition of β-CD resulted in depletion of 2,4-D in solution by complex formation and precipitation of an insoluble complex.

The stoichiometric ratio can be easily evaluated from the diagram [11], because the amount of β-CD represented by the plateau (mmol of β-CD in the point b – mmol of β-CD in the point a) is equal to that entering into the complex in this interval, and to the corresponding amount of 2,4-D forming complex (total mmol of 2,4-D added – solubilized mmol of 2,4-D at the plateau). From the results of Fig. 1, it is evidenced that 3 mmol of 2,4-D (9.048–5.960) reacted with 3 mmol of β-CD (11.000–8.000), thus indicating a 1 : 1 molar stoichiometric ratio.

3.2. Chemical analysis

The chemical analysis of the inclusion complex confirmed the stoichiometry calculated obtained from the phase–solubility diagram (Fig. 1). This analysis revealed that the powder obtained after spray-drying (14.7% w/w 2,4-D), and washed with ethanol (13.9% w/w 2,4-D), corresponded to the formation of a complex with a 1 : 1 molar ratio. In contrast, the kneaded (16.3% w/w 2,4-D) and the physical mixture (15.9% w/w 2,4-D) products showed only trace amounts of 2,4-D after washing with ethanol.

It should be noted that the determination carried out on the washed product obtained by spray-drying proved the persistence of 2,4-D, i.e. the existence of a true inclusion compound. However, the product obtained by the kneading method did not yield a true complex, since the ethanol washing practically eliminated the total amount of 2,4-D from the CD.

3.3. Thermoanalytical determinations

From the DSC curves, a melting enthalpy of 30.5 kJ/mol was calculated for pure 2,4-D (peak temperature: 140°C). β-CD showed a broad endotherm in the range of 100–140°C, corresponding to its dehydration process, in accordance with previous thermal data [8]. After heating for 30 min at 90°C, it was noted that this endothermic effect completely disappeared.

In order to allow the accurate evaluation of the endothermic effect of 2,4-D in the kneaded samples, these were previously heated up to 90°C, held at this temperature for 10 min, allowed to cool down to 30°C, and finally rescanned up to 200°C. The same treatment was applied to spray-dried samples, in order to obtain thermal traces from which the fusion enthalpies could be easily calculated.

Using the procedures described in Section 2, mixtures containing an excess of guest (2,4-D) with respect to *R*, and for which the endothermic effect can be reasonably related to the melting of the component in excess, were prepared. DSC runs allowed measurements from peak area of fusion enthalpy of aforementioned excess (*F*), which are associated with the endothermic peak in the melting region of the crystalline guest. Table 1 summarises all these results.

Fig. 2 shows the theoretical straight lines calculated from Eq. (5) for different values of *R* [(a): *R*=(1 : 1); (b): *R*=(1 : 2) and (c): *R*=(1 : 3)]. The points, indicated by open circles and open squares, show the quantities obtained experimentally using the DSC data (Table 1) for kneading and spray-drying methods, respectively. The intercept of the experimental lines

Table 1
Melting enthalpies obtained from DSC measurements

Method	2,4-D (mol)	β-CD (mol)	$-\Delta H_f$ (KJ/mol)	N [a]	F [b]
Kneading	1	3	—	0.25	0.00
	1	2	9.52	0.33	0.10
	1	1	18.12	0.50	0.29
	2	1	47.01	0.66	0.51
	3	1	79.25	0.75	0.64
Spray-drying	1	1	—	0.50	0.00
	2	1	22.73	0.66	0.24
	3	1	54.68	0.75	0.44
	4	1	83.25	0.80	0.54

[a] Total molar fraction.
[b] Free mole fraction.

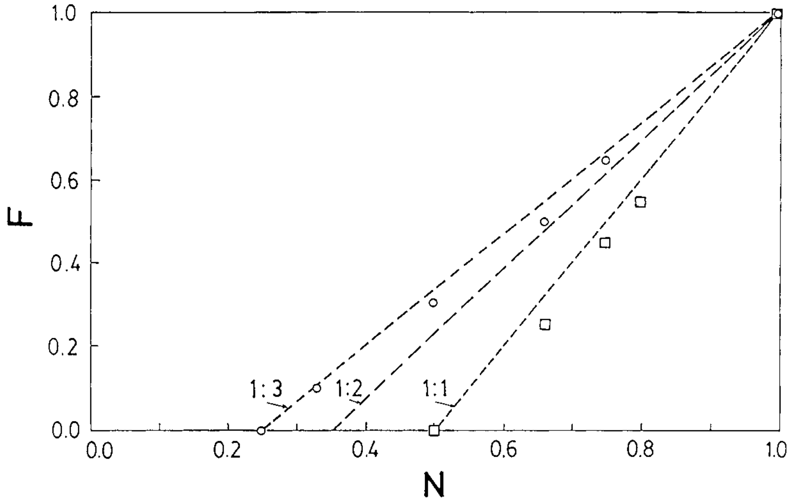

Fig. 2. Plot of free mole fraction of 2,4-D (F) versus total molar fraction of 2,4-D (N) according to experimental and calculated data (dashed lines) provided by Eq. (5) (see the text). Open circles: kneaded samples; open squares: spray-dried samples.

with the composition axis gives the stoichiometry of the inclusion compound.

It is clear that a 1 : 1 molar composition ($R=0.5$) is obtained with the spray-drying method. Then, it is reasonable to assume that this method yield a 1 : 1 2,4-D: β-CD complex, because this stoichiometry would allow maximum contact between the hydrophobic portion of the organic substrate with the apolar cavity of β-CD.

Table 2 displays the stoichiometries of the complexes obtained applying the three analytical methods. Stoichiometries based on the data from the plateau region of phase–solubility diagram (see Fig. 1) were in excellent agreement with those obtained by the chemical analysis and DSC results. However, the stoichiometry obtained using DSC results for the kneaded systems was 1 : 3. Considering the chemical structure of 2,4-D, it is not possible for a molecule to be simultaneously included in three molecules of β-CD, by steric impedance. These results can be interpreted, in accordance with others [12], on the basis of the low yield of complexation obtained by this method, more than on the real stoichiometry of the inclusion complex. Thus, it is deduced that the thermoanalytical method is only adequate when the processing method yields a true inclusion compound.

These results must be taken into account in the processing of this pesticide as commercial formulations using CD and, furthermore, in the establishment of suitable methods of analytical determination of unknown binary compositions in solution and in the solid state.

Table 2
Stoichiometry of 2,4-D and β-CD kneading and spray-drying systems, as deduced by solubility, chemical and thermal analysis determinations

Physical state	Solution	Solid			
		Kneading		Spray-drying	
Methods	Solubility analysis	Chemical analysis	Thermal analysis	Chemical analysis	Thermal analysis
Stoichiometry	1 : 1	0.98 : 1.00	1 : 3	0.85 : 1.00	1 : 1

4. Conclusions

The formation and stoichiometry of an inclusion complex between the pesticide 2,4-D (guest) and β-CD (host) in solution and solid state, have been studied and compared by three processing methods: mechanical (physical) mixing; kneading; and spray-drying. In solution, the phase–solubility diagram has shown the formation of a 1 : 1 molar ratio complex. In the solid state, using DSC, the stoichiometry was the same for the spray-dried complexes. However, the method was not valid for the kneading procedure, because this procedure did not yield a true inclusion compound. These results were corroborated by chemical analysis of the pure solid systems.

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