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THERMOANALYTICAL METHOD FOR STUDYING THE GUEST CONTENT IN CYCLODEXTRIN INCLUSION COMPLEXES

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The interaction of cypermethrin with β-cyclodextrin was investigated using different (coprecipitation, suspension, kneading and 'melting in solution') complexation methods and qualifying the resulted complexes by UV-spectrophotometry, thermal methods (TG, DTG and DSC) and X-ray powder diffraction. The total guest content of complexes can be measured by UV-spectrophotometry in aqueous ethanol solution, while the uncomplexed guest fraction of samples can be determined by DSC based on a previous calibration curve, which was found between the melting enthalpy change of cypermethrin and the guest content of physical mixture samples. The combination of both analytical methods enables the determination of really complexed guest content.

Keywords: *cyclodextrins, cypermethrin, differential scanning calorimetry, inclusion complexation*

Introduction

Natural extracts of the herb *Chrysanthemum roseum* have been used as insecticide for almost 400 years. The active substances of the above herb are called pyrethrins. The synthetic pyrethroids – which are simple chemical analogues of the pyrethrins – were developed in the 1970s. The major advantages of these derivatives are in their increased stability and selective biological activity. The low toxicity for mammals and fast degradation make them a very useful insecticide in the household and agricultural application [1]. The only disadvantage is that the pyrethroids are nearly insoluble in water, and have low light stability, therefore their application is limited.

One of the most effective synthetic pyrethroid is *cypemethrin*, which was synthesized in England in 1974. Cypermethrin similarly to the other pyrethroids possesses very low solubility and UV light instability, especially in alkaline solution. A possible way to improve its properties is the complexation with cyclodextrins.

Cyclodextrins (CDs) are cyclic oligosacharides prepared by enzymic conversion of starch. Organic molecules are able to enter their cavity forming inclusion complexes [2].

In most cases inclusion complex formation with CDs improves stability, wettability and dissolution rate of poorly soluble substances. In consequence of the improved efficacy of the complex the effective dose is lower than that of the pure insecticide. Applying it in complexed form reduces the environmental pollution caused by overdosing [3].

Some pyrethroid/CD complexes have been described in the literature. The complexes showed improved efficacy against insects [4], retarded photodecomposition [5, 6] and reduced contact insecticidal activities [7]. Pyrethroids formulated with methylated CD were reported to be safe to human skin, so a shampoo containing pyrethroids and methylated CDs has been prepared to control the lice in hair [8].

The preparation of solid-state cyclodextrin inclusion complexes can be carried out by simple procedures. Numerous methods exist, the most frequently applied techniques start from solution or suspension of both the guest and CD, the complex isolation is accomplished by removing the solvent by filtration, lyophilization or spray-drying. The complex formation can take place in solid-state by kneading, heating or melting (with a large excess of the active substance component) together the guest with CD in the presence of small amount of water that is essential for inclusion complex production [9, 10]. In case of spherical agglomeration technique the particles are also suspended, but in a non-wetting liquid, and are agitated together with a second wetting liquid (water). The resulting product will be solid spherical pellets which are easily separated from the liquid [11].

It is not guaranteed that the powders obtained by different complexation methods are true, homogeneous inclusion complexes. In many cases the product is a mixture of complex, uncomplexed guest and empty hydrated CD. The total guest content of the

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product can be determined by UV-spectrophotometry, GC or HPLC. Because complexes always have to be dissolved in these methods, we can not get precise information about the ratio of complexed and uncomplexed guest, but with solid-state analytical techniques (IR-spectroscopy, solid-state NMR, X-ray diffraction (XRD) or thermal analysis) [12].

Thermal methods are widely used to characterize CDs and their inclusion complexes. In general, the aim of these examinations is the comparison of thermal behaviour of single components (in this case CD and the active substance) and their inclusion complex. It is considered to be the proof of inclusion complex formation when the melting peak of the guest molecule does not appear in the DSC curve of complex [13–16].

The aim of our work was to compare cypermethrin/CD inclusion complexes prepared by different (coprecipitation, suspension, kneading and 'melting in solution') complexation techniques and investigate them using UV-spectrophotometric and thermal methods to determine the total and uncomplexed guest ratios. Beside DSC technique, justifying the complexation (that is the formation of a new crystalline phase different from the starting materials) can be carried out by X-ray powder diffraction.

Experimental

Materials

Cypermethrin (α-cyano-3-phenoxybenzyl-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane-carboxylate) obtained from Chinoin Pharm. Chem. Works (Budapest, Hungary) was used without further purification. The chemical structure is shown in Fig. 1.

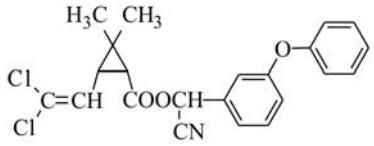

Fig. 1 Chemical structure of cypermethrin

Because of its cyclopropane ring the molecule has two geometrical isomers: *cis*- and *trans*-cypermethrin, which have further 4–4 optical isomers due to the cyclopropane ring and the α-C-atom. Thus the cypermethrin has 8 isomers with different activity. We used the (1R trans S) and (1S trans R) enantiomeric isomer pair in our experiments.

The β-cyclodextrin (β-CD) was obtained from Cyclolab Ltd. (Budapest, Hungary).

Methods

Preparation of cypermethrin–cyclodextrin physical mixtures

Nine physical mixtures were prepared with different cypermethrin content without any solvent at a β-CD/cypermethrin stoichiometric molar ratio of 2/0.3, 2/0.6, 2/0.7, 2/1.0, 2/1.3, 2/1.4, 2/1.6, 2/5.4 and 2/12.7. In due proportions the mixtures of the solid components were kneaded manually for 1 min in an agate mortar with pestle to obtain homogenous powders.

To compare the DSC profiles of complexes and physical mixtures, three other physical mixtures were prepared at the same active substance content than the complexes (at 21.9, 19.1 and 13.7%) using the above-mentioned preparation method.

Preparation methods of the cypermethrin/cyclodextrin complexes

Coprecipitation: The β-CD (0.89 mmol) was dissolved in 50 mL 50% aqueous ethanol at 52°C. Cypermethrin was added (0.60 mmol) in 35 mL 96% ethanol and the solution was stirred for 2 h. The product was filtered on a G4 glass filter and dried in a vacuum desiccator over P_2O_5 to constant mass. 0.92 g of complex was obtained.

Suspension: The β-CD (0.89 mmol) was dissolved in 9 mL water and cypermethrin (0.44 mmol) in 9 mL 96% ethanol was added. The reaction mixture was stirred for 2 h and was filtered on a G4 glass filter. The 0.69 g complex was dried in a vacuum desiccator over P_2O_5 to constant mass.

Kneading: The cypermethrin (0.44 mmol) and β-CD (0.88 mmol) were intensively manually kneaded for 2 h in an agate mortar with dropwise addition of 1 mL water. The 1.17 g of product was dried over P_2O_5.

Melting in solution: The β-CD (0.88 mmol) was dissolved in water (10 mL) at 90°C, then the cypermethrin (0.47 mmol) was added in solid-state and the reaction mixture was stirred for 3 h. The solution was cooled to room temperature. The white precipitate was filtered on a G4 glass filter and dried over P_2O_5 to constant mass (0.97 g).

Analytical methods

The cypermethrin content of samples was analyzed by UV-spectrophotometry at 278 nm with 258 and 298 nm reference wavelengths (Hewlett Packard 8452A type diode-array spectrophotometer) using 50% aqueous ethanolic solutions.

A MOM Derivatograph PC was used to obtain TG and DTG curves. Experiments were carried out

on ~20 mg of powdered sample in aluminum-oxide open pan at a heating rate of 4°C min^{-1}, under pure nitrogen at a flow rate of 40 cm^3 min^{-1}.

The DSC measurements were carried out by a Netzsch DSC 200 Differential Scanning Calorimeter in flowing nitrogen atmosphere (50 cm^3 min^{-1}), using 10°C min^{-1} heating rates. The sample mass was ~6–7 mg using closed pan with a small pinhole punched in the lid, the crucible material was aluminum. The temperature and heat flow response of the calorimeter was calibrated using 6N purity metals.

The X-ray powder diffraction measurements were carried out using front packed powder samples of 1 μm average grain size, on a Bragg–Brentano geometry Siemens D5000 θ–θ diffractometer equipped with a graphite secondary beam monochromator. The data collection was performed using CuK_α (λ=0.154178 nm) radiation scanning the 2–60° 2θ range by 0.02° 2θ stepsize and 5 s counting time/step.

Results and discussion

Thermal characteristics of cypermethrin

The DSC curve of active substance (Fig. 2) shows an endothermic melting peak with onset at 81.0±0.4°C (peak temperature at 84.8±0.4°C), the enthalpy of melting is ΔH_{fus}=95±3 J g^{-1} (from three parallel measurements). Cypermethrin has high heat stability, does not decompose and its evaporation is negligible until 250°C.

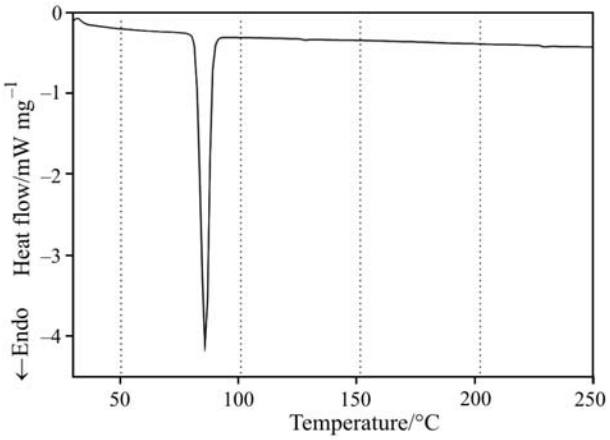

Fig. 2 DSC curve of cypermethrin

Thermal properties of β-CD

The first peak is the water loss on the thermoanalytical curves of β-CD, the temperature range depends on the experimental conditions (open, perforated or sealed lids). After this peak there is no further mass loss detected in TG curve, while small endo or exo or endo–exo effect appears in DSC curve corresponding to a phase transition of β-CD [13, 17].

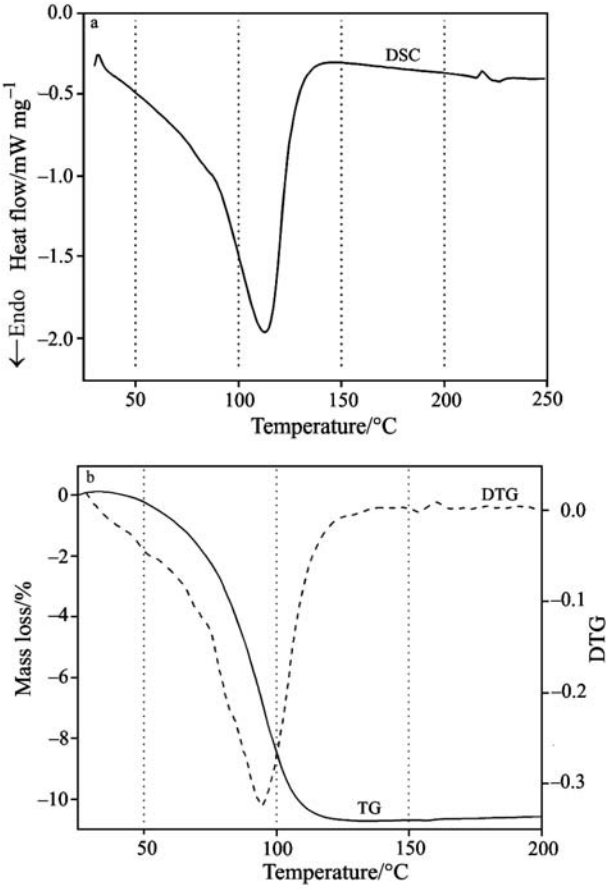

Fig. 3 a – DSC, b – TG (solid line) and DTG (dashed line) curves of β-CD

The TG and DTG curves of pure β-CD are shown in Fig. 3b. Only one stage appears on the curve between 30 and 125°C, the DTG peak minimum temperature is 96°C. This stage of thermal decomposition is related to the dehydration with 10.9% of water mass loss (0.14 mmol).

Figure 3a represents the DSC curve of β-CD. The first peak corresponds to the water loss process with –366 J g^{-1} enthalpy change. An endo-exo effect appears at 216–230°C due to the transition of β-CD in the solid-state.

Calibration of melting heat of cypermethrin

On the DSC curve of physical mixtures (measured from 30 to 100°C) the melting peak of cypermethrin appears adding to the water loss peak of β-CD, which causes decreased baseline. The characteristics of physical mixtures (β-CD/cyp molar ratio, cypermethrin content, onset temperature and melting heat of cypermethrin) are summarised in Table 1. The average onset temperature is 81.5±0.2°C.

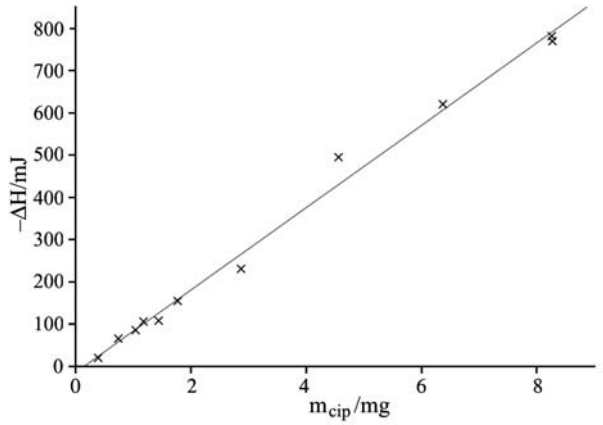

Fig. 4 Calibration curve (slope: (97±3), intercept: (–14±13) and regression coefficient: 0.996)

The linear calibration curve can be seen in Fig. 4, plotting the melting heat of pure active substance and physical mixtures (ΔH_{fus}) as a function of cypermethrin content of samples (m_{cyp}). It is noticeable that the point belonging to the pure cypermethrin lies on the line determined by physical mixtures. The following equation gives the relationship between ΔH_{fus} and m_{cyp}:

$$\Delta H_{fus}\ [mJ]=(-14\pm13)+(97\pm3)m_{cyp}\ [mg]$$

The amount of free active substance of complexes can be determined from the melting heat of cypermethrin on the DSC curve of complexes using the above calibration equation.

When the physical mixtures were kept at 100°C for 30 min, the samples did not show the melting peak of active substance yet denoting that cypermethrin might become complexed only after the melting procedure. This method is also applicable for formation of cyclodextrin inclusion complexes. Analogously, we developed a new complexation method ('melting in solution' technique): the solid cypermethrin was directly added to the hot solution of cyclodextrin in a small amount of water using higher temperature than the melting point of cypermethrin. (The details of this technique are described in experimental section.) The advantage of this method is that there is no need to have a large excess of guest, contrary to other melting procedures [9].

Thermal behaviour of complexes

The resulting solid formulations prepared by four complexation methods were analysed by thermoanalytical methods (TG, DTG and DSC): coprecipitation, suspension, kneading and 'melting in solution' at cypermethrin/β-cyclodextrin stoichometric ratio of 1:2. The thermal properties of complexes are summarised in Table 2.

On the curves of complexes produced by coprecipitation, suspension and kneading methods (Fig. 5, Table 2) three peaks appear: the water loss of β-CD (at 30–120°C), the transition of β-CD (at 210–230°C without mass loss) and the melting peak of cypermethrin (at 82–85°C) verifying that

Table 1 Thermal properties of cypermethrin–β-CD physical mixtures

β-CD/cyp molar ratio of physical mixtures	Cypermethrin content/%	DSC data	
		T_{onset}/°C	ΔH/J g^{-1}
2/0.3	5.1	81.7	–3
2/0.6	9.4	81.3	–8
2/0.7	12.0	81.6	–11
2/1.0	15.3	81.5	–13
2/1.3	18.7	81.4	–14
2/1.4	20.3	81.6	–16
2/1.6	23.1	81.3	–20
2/5.4	49.6	81.4	–54
2/12.7	69.9	81.3	–67

Table 2 Thermal properties of complexes prepared by different complexation methods

Temperature range/°C	Coprecipitation				Suspension				Kneading				Melting in solution			
	TG		DSC		TG		DSC		TG		DSC		TG		DSC	
	T_m	Δm	T_m	Δm	T_m	Δm	T_m	Δm	T_m	Δm	T_m	Δm	T_m	Δm	T_m	Δm
30–120	90	6.6	85 97	–17 –216	86	7.4	82 99	–14 –164	87	10.1	82 103	–9 –296	53 68	1.6 4.0	69 91	–241
210–230	–	–	224	–12	–	–	219	–3	–	–	223	–5	–	–	–	–

T_m – peak maximum temperature/°C, Δm – mass loss/%, ΔH – enthalpy/J g^{-1}

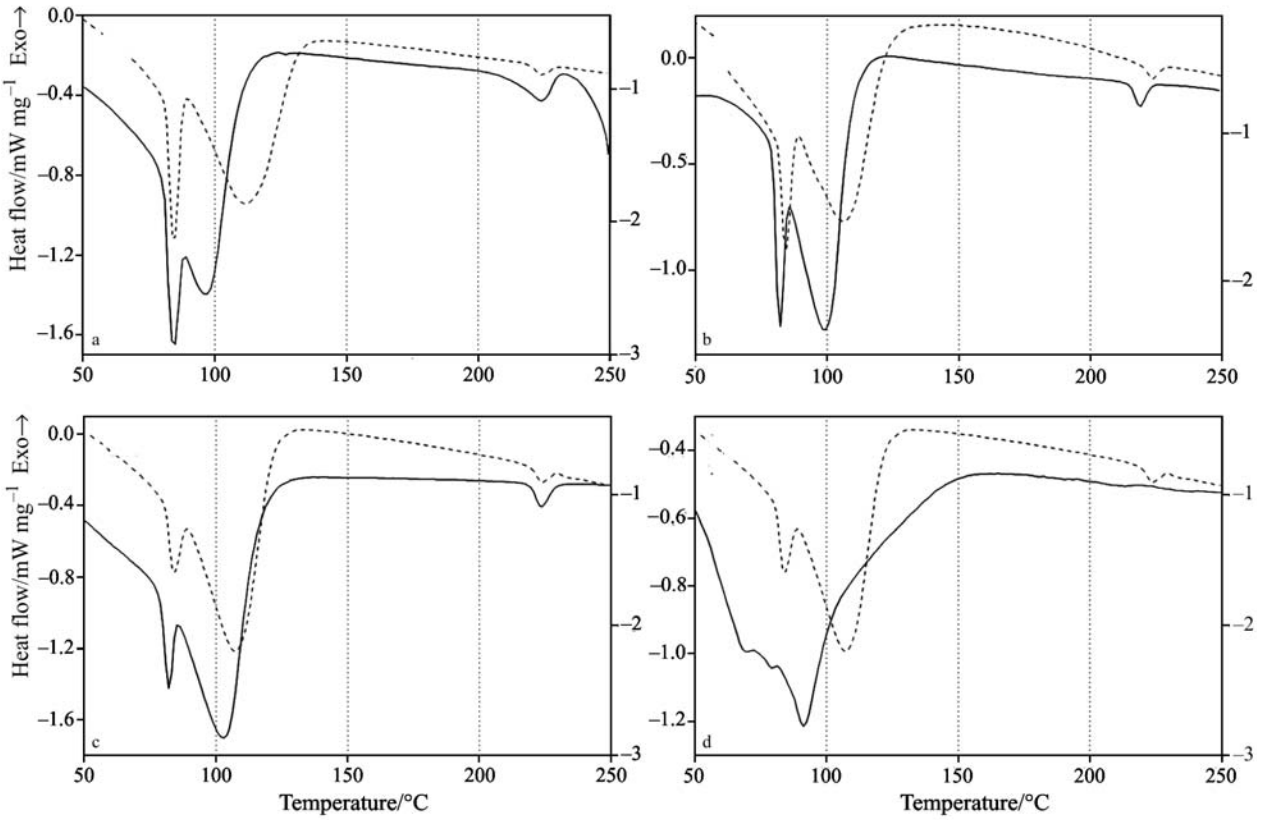

Fig. 5 DSC curves of complexes; a – by coprecipitation, b – by suspension, c – by kneading, d) by 'melting in solution' method; --- – cyp-β-CD physical mixtures, — – cyp/β-CD complexes

cypermethrin exists both in complexed form and as an uncomplexed guest in the samples. The complex prepared by melting does not show the individual melting peak of cypermethrin, this fact might indicate that the entire amount of the pyrethroid is molecularly entrapped into the CD.

The complexes were examined by UV-spectrophotometry to determine the total cypermethrin content of samples. The uncomplexed fraction (adsorbed onto the surface) of active substances were measured by thermal analysis (DSC, TG and DTG) using the above-mentioned calibration equation. Their difference results in the assumed complexed cypermethrin content.

In Table 3 the results show that the active substance is principally attached onto surface in the case of complexes prepared by well-known complexation methods (coprecipitation, suspension and kneading methods) and only to a small extent (0.7–2.3%) is complexed. However, the 'melting in solution' technique was found to be the most effective complexation method because all cypermethrin seemed to be complexed.

Results of X-ray powder diffraction measurements

The complex prepared by 'melting is solution' method was analysed by X-ray powder diffraction in order to confirm the complexation. X-ray powder pattern of the physical mixture at the same molar ratio as that used in complexation reaction, as well as those of the pure β-CD and cypermethrin were corroborated with the

Table 3 Comparison of complexation methods

Complexation methods	Cypermethrin content/%		
	total*	on the surface	in the complex
coprecipitation	1.9	20.1	1.8
suspension	18.5	17.8	0.7
kneading	14.3	12.0	2.3
melting in solution	13.3	–	13.3

*measured by UV spectrophotometry

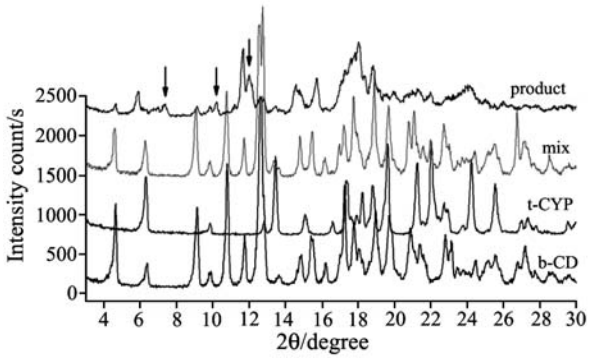

Fig. 6 XPD patterns of β-CD ('*b*-CD'), cypermethrin ('*t*-CYP'), the physical mixture ('mix'), and that of the reaction product of 'melting in solution' complexation method ('product')

observed X-ray powder pattern of the product of complexation reaction. The pattern of the reaction product labelled 'product' on Fig. 6 shows distinct differences (some of them marked by arrows) from the patterns of β-CD ('*b*-CD'), cypermethrin ('*t*-CYP') and that of the physical mixture ('mix') in both the characteristic d_{hkl}-values and the corresponding scattered intensities. This difference is a clear indication of the formation of a new crystalline phase different from the three above. An obvious interpretation of the presence of a new phase is that it represents a cyclodextrin–cypermethrin complex compound.

Conclusions

Cypermethrin/β-CD inclusion complexes were prepared by different complexation methods, which are common from the literature (coprecipitation, suspension, kneading methods) and developed in our laboratory ('melting in solution' technique).

Physical mixtures were created at different composition, and using DSC measurements a linear relationship was found between the melting enthalpy change of cypermethrin and the cypermethrin content of samples. This calibration curve is utilisable to measure the amount of uncomplexed active substance in inclusion complexes. The total active substance content can be determined by UV-spectrophotometry, the assumed complexed guest ratio can be calculated in view of the total guest content and the amount of uncomplexed substance using both of the above mentioned analytical methods.

The new 'melting in solution' technique developed in our laboratory found to be the most effective complexation method, because according to the results of DSC and X-ray diffraction measurements the cypermethrin content of this sample seems to be complexed. This new technique has some more advantages in environmental respect: its material requirement is lower, does not need organic solvent to prepare solution of water insoluble cypermethrin and using water solution there is no loss of active substance in the filtrate.

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