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ORIGINAL ARTICLE

Dissolution properties of cypermethrin/cyclodextrin complexes

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Abstract Cypermethrin—a very effective pyrethroid-type insecticide—has been complexed with β-cyclodextrin and peracetylated-β-cyclodextrin with different guest content. Dissolution measurements by reversed phase HPLC method, together with UV-spectrophotometry, differential scanning calorimetry and thermogravimetry were applied to prove the inclusion complex formation and characterize the complexes. With the help of the thermal analysis the really complexed (strongly bound) and surface-bound guests were distinguished. All of the β-cyclodextrin complexes show better dissolution rate than the pure guest. In case of inclusion complexes an oversaturated solution was formed with extremely high concentration of active substance (6–19 mg L^{-1}) during the first couple of minutes then the concentration decreased gradually until it reached the equilibrium solubility value of the complex (2 mg L^{-1}). The cypermethrin/peracetylated-β-cyclodextrin complexes prepared with organic solvent method showed slightly retarded dissolution profile compared to the pure guest. The area under the dissolution curves was introduced for quantitative characterization of the dissolution rate. The release was found to depend on the complexed guest content of the samples. The continuous variation plots used first for this parameter gave information on the stoichiometry of the complexes: 1:2 cypermethrin/β-cyclodextrin and 1:1.25 cypermethrin/peracetylated-β-cyclodextrin.

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Abbreviations

AcβCD	Peracetylated-β-cyclodextrin
β-CD	β-Cyclodextrin
cyp	Cypermethrin
TG	Thermogravimetry
DSC	Differential scanning calorimetry

Introduction

Pyrethroids are common in commercial products such as household or agricultural insecticides and insect repellents. Their major advantage is their selective biological activity for insects; the low toxicity for mammals, effectiveness and fast biodegradation make them more useful compounds than other insecticides [1]. The agricultural application of pyrethroids is limited because they usually are sunlight sensitive compounds, nearly insoluble in water. As solutions of low active substance concentration can be prepared only and the stability of pyrethroids in these solutions is poor, high doses are needed to be spread into the environment to get satisfactory effect. Another disadvantage is that they are toxic to bees as well, because these compounds act through stomach and nervous system of every insect. Pyrethroid/cyclodextrin complexes have been well studied in publications. Inclusion complexation with cyclodextrins may result in increased thermal and photostability of the active substance [2–6] and remarkably enhanced water solubility [6, 7] depending on the type of cyclodextrin derivative. Using cyclodextrin solid formulations with water-controlled (enhanced or retarded)

release of the guest [8], slightly increased effectiveness [9, 10] can be created although inclusion complexes do not show contact activity any more [4, 11], but remain stomach poison for the real plant-eating pests. The enhanced solubility and improved stability make it possible to reduce the environmental pollution caused by overdosing insecticides in fields. The cypermethrin/β-CD complexes were prepared by different complexation methods, and the 'melting in solution' technique was found the most effective complexation process resulting in the highest ratio of included guest [12].

At the solid state, inclusion complex characterization may be provided by thermal analysis, which is a simple technique to investigate CD complexes. Comparing the thermal behaviour of single components, their physical mixture and the inclusion complex candidate, remarkable changes in the thermal properties of the complexes (the characteristic peaks of components disappear as a result of complexation process) indicate the complex formation [13, 14]. Complexation often results in enhanced rate of release of the guest into solution [14]. Cyclodextrins usually increase the apparent solubility of active substances therefore the end of dissolution rate curves of inclusion complexes and physical mixtures shows higher active substance concentration than that of the pure active substance. A very fast initial rate of dissolution can be often observed in case of inclusion complexes: the active substance concentration exceeds the equilibrium solubility (the concentration at the end of the dissolution rate curve), so the supersaturation phenomenon is present within the first minutes then the active substance concentration drops back to the equilibrium solubility [15].

The aim of this work was to investigate the dissolution properties of cypermethrin (Fig. 1) inclusion complexes with β-cyclodextrin (β-CD) and peracetylated-β-cyclodextrin (AcβCD). In this paper, we studied the release of active substance from 7 cypermethrin/β-cyclodextrin complexes and 3 cypermethrin/peracetylated-β-cyclodextrin complexes with different guest content. The total guest content of complexes was analysed by UV-spectrophotometry and RP-HPLC, while non-complexed cypermethrin content of samples was determined by differential scanning calorimetry (DSC). With knowledge of water content (measured by thermogravimetry) the complexed guest content of inclusion complex candidates was calculated. We investigated the dissolution rate of cypermethrin, comparing the pure active substance with complexes. The effect of β-cyclodextrin was compared with that of peracetylated-β-cyclodextrin. We have also studied how the release properties of complexes are affected by the cypermethrin content (free and complexed).

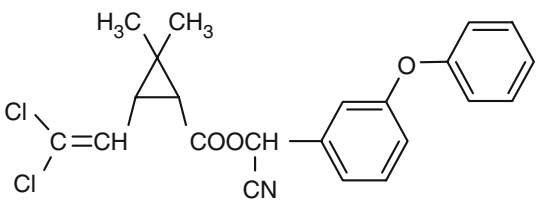

Fig. 1 Chemical structure of cypermethrin

Experimental

Materials

Mixture of enantiomeric isomer pairs of (1R trans S) and (1S trans R) cypermethrin obtained from Chinoin Pharm. Chem. Works (Budapest, Hungary) was used without further purification. The cyclodextrins (β-CD and AcβCD) were from the products of Cyclolab Ltd. (Budapest, Hungary).

HPLC grade acetonitrile for liquid chromatography (Merck, Darmstadt, Germany) and deionised water generated by Milli-QPlus Ultra Pure Water System from Millipore (Billerica, MA, USA) were used for HPLC measurements.

Preparation of the inclusion complexes

"Melting in solution" complexation method with β-CD

The β-CD (0.88 mmol) was dissolved in water (10 mL) at 90 °C, then the cypermethrin (0.10–1.21 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 through a G4 glass filter and dried over P_2O_5 to constant weight.

Complexation in organic solvent with peracetylated-β-CD

The crystalline fully acetylated-β-cyclodextrin (0.25 mmol) was dissolved in acetone (2 mL) in an agate mortar. The crystalline active substance (0.13–0.27 mmol) was dissolved also in 3 mL of acetone, and added to the cyclodextrin solution in the mortar. After stirring, the mortar was put into a drying oven at 40 °C for 2 h. The product was dried in air to constant weight.

Characterization of the inclusion complexes

Thermogravimetry (TG)

A MOM Derivatograph PC was used to obtain TG and DTG curves. Experiments were carried out on ~20 mg of powdered sample in alumina open pan at a heating rate of 5 °C/min, under pure nitrogen flow.

Differential scanning calorimetry (DSC)

The DSC measurements were carried out by a Netzsch DSC 200 Differential Scanning Calorimeter in flowing nitrogen atmosphere, using 10 °C/min heating rate. The sample weight was ~6–7 mg using closed pan with a small pinhole punched in the lid, the crucible material was aluminium.

UV spectrophotometry

Cypermethrin content was analysed at 278 nm with 258 and 298 nm reference wavelengths by a Hewlett Packard 8452A type diode-array UV-VIS spectrophotometer in 50% aqueous ethanol. (Calibration curve: $A = (4.76 \pm 0.03) \times c$ (g L^{-1}), correlation coefficient: $R = 0.9999$, concentration range: 17–230 mg L^{-1})

RP-HPLC

The HPLC experimental arrangement consisted of a Chrompack ISOS isocratic pump equipped with a solvent selection valve, a Rheodyne 7125 manual injector (with 25 μL loop) and with a Chrompack 204 UV/Visible detector (wavelength: 220 nm). Chromspher C18, (100 × 3.0 mm, Chrompack) column and acetonitrile:water = 80:20 eluent (0.4 mL min^{-1} flow rate) were used. (Calibration curve: Area = (14.288 ± 120) × c (mg L^{-1}), correlation coefficient: $R = 0.9989$, concentration range: 0.03–24 mg L^{-1})

Dissolution measurements

The dissolution measurements were carried out under non-sink conditions. 20 mg of cypermethrin or an equivalent quantity of complexes were added to 5 mL of distilled water and stirred continuously at room temperature (22 ± 2 °C). Samples were filtered through 0.45 μm pore size membrane filter (mixed cellulose esters, Millipore Corporation, Bedford) and measured by HPLC. All experiments were carried out in triplicate.

Results and discussion

Cypermethrin was complexed with β-cyclodextrin using various guest to host ratios to get samples for investigating the effect of the molar ratio on the dissolution properties. As it is shown in Table 1, altogether 10 inclusion complexes (marked with A–J) have been prepared: seven with β-CD (A–G) using 'melting in solution' technique and three with Acβ-CD (H–J) by complexation in organic solvent. The total cypermethrin content of complexes was determined by UV-spectrophotometry and RP-HPLC, respectively. There were no significant differences between the results obtained by the two methods except for complex A, so the averages were calculated. The low active ingredient content of complex A resulted in higher standard deviation.

Thermoanalytical results

The thermoanalytical studies aimed at distinguishing the guest included within the β-CD cavity to those adsorbed on the surface of the product. Table 2; Figs. 2 and 3 summarize the thermoanalytical (TG and DSC) results of the cypermethrin/β-CD complexes.

According to *thermogravimetric* measurements, only cyclodextrins and β-CD complexes have mass loss peak(s) on their DTG curves; pure cypermethrin is stable enough between room temperature and 250 °C, while

Table 1 Guest content of complexes

Complexes	Total cypermethrin content (%)			cyp content on surface (%)	cyp content in complex (%)	Complexed cyp/β-CD molar ratio[a]
	By UV	By HPLC	Average			
β-CD						
A	1.1	2.9	2.0	–	2.0	1:16.2
B	7.9	8.6	8.3	–	8.3	1:3.8
C	11.7	12.5	12.1	–	12.1	1:2.5
D	13.2	13.4	13.3	–	13.3	1:2.2
E	16.9	15.8	16.4	5.4	11.0	1:2.7
F	25.1	23.8	24.5	6.2	18.3	1:1.5
G	31.5	31.4	31.5	10.8	20.7	1:1.2
Acβ-CD						
H	9.2	10.6	9.9	–	9.9	1:1.9
I	14.0	16.3	15.2	–	15.2	1:1.2
J	16.7	18.2	17.4	–	17.4	1:1.0

[a] After deducting water content of complexes

Table 2 Thermoanalytical data of β-CD complexes ([a]Melting peak of the guest)

Complexes	DTG			DSC	
	Temperature range (°C)	T_{peak} (°C)	Δm (%)	T_{peak} (°C)	ΔH (J/g)
A	33–112	81	8.8	99.4	−161.4
B	36–128	72	5.5	97.2	−193.1
C	36–56	52	0.8	92.6	−136.8
	56–127	71	3.8		
D	35–56	53	1.6	69.2	
	56–140	68	4.0	91.3	−241.3
E	37–89	73	1.9	80.8[a]	−3.2
	89–161	101	2.1	99.0	−104.5
F	42–72	60	0.8	70.3	
	72–132	85	1.6	78.5[a]	−4.2
				94.1	−183.2
G	33–55	47	0.8	65.8	
	55–72	61	0.8	76.2[a]	−8.7
	72–121	82	1.6	93.5	−98.9

peracetylated-β-cyclodextrin complexes do not contain any detectable solvent quantity. In case of β-CD a water-loss peak (10.9%) appears in DTG in the 36 and 125 °C temperature range. A smaller portion of solvent content (0.5%) is present in peracetylated-β-cyclodextrin, manifested in a flat peak between 30 and 102 °C in its DTG curve.

Thermogravimetric curves of cypermethrin/β-CD complexes show that they loose their water content in 1–3 stages, presumably reflecting absorbed and bound water of different energies. The higher guest content the complexes have, the more water-loss stages appear on TG-curve: while complexes A and B have only a single water-loss peak, we can see two water-loss stages for complexes C–F and three peaks are present for complex G.

It is obvious that higher active substance content of the complex means less water content, because cyclodextrin inclusion complexes are formed by substitution of included water by the guest molecule, so only absorbed water molecules remain in complexes, water-loss peaks are centred at lower temperature for complexes with higher guest content.

Figure 2 shows the *DSC curves* of cypermethrin, β-CD AcβCD and cypermethrin/β-CD complexes. The free guest itself has a melting peak centred at 85 °C on its DSC-curve, while β-cyclodextrin has a water loss peak (at 113 °C peak minimum) and a phase transition peak (at 220 °C). An endothermic peak appears on DSC curve of peracetylated-β-cyclodextrin, which represents a solid-liquid phase transition of cyclodextrin [16].

While the physical mixture shows a DSC curve with a similar characteristic as the superimposition of the curves

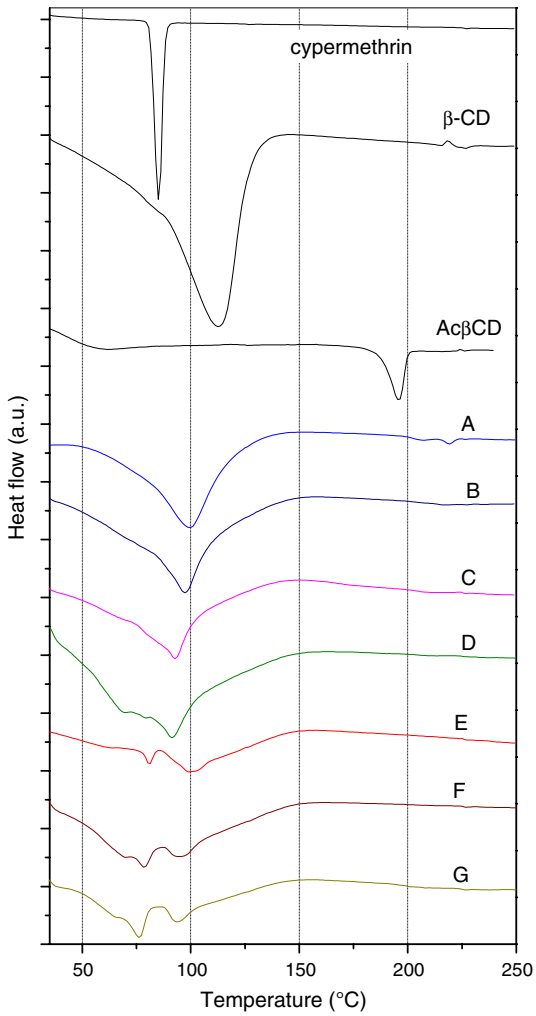

Fig. 2 DSC curves of cypermethrin, β-CD, AcβCD and cypermethrin/β-CD complexes

of pure elements [12, 16], complexes give different characteristics. DSC measurements of β-CD complexes have water loss peak(s) (corresponding to thermogravimetric results). With increasing cypermethrin content in the complex, the melting peak of guest molecule appears on the DSC curve of complexes with higher than 1:2.2 cypermethrin/β-CD molar ratio (E–G). DSC analyses of complexes make it possible to determine the guest content attached onto the surface (free guest content) with a previously reported method based on determination of cypermethrin melting peak enthalpy changes of the samples [12]. It can be observed that the melting point of the free guest shifted to lower temperature. Similar effect has already been reported by other authors [17, 18].

DSC curves of AcβCD complexes do not show any characteristics (not shown): no solvent loss, no melting peak of free guest and no phase transition peak, the latter two indicate a strong interaction between the guest and the AcβCD.

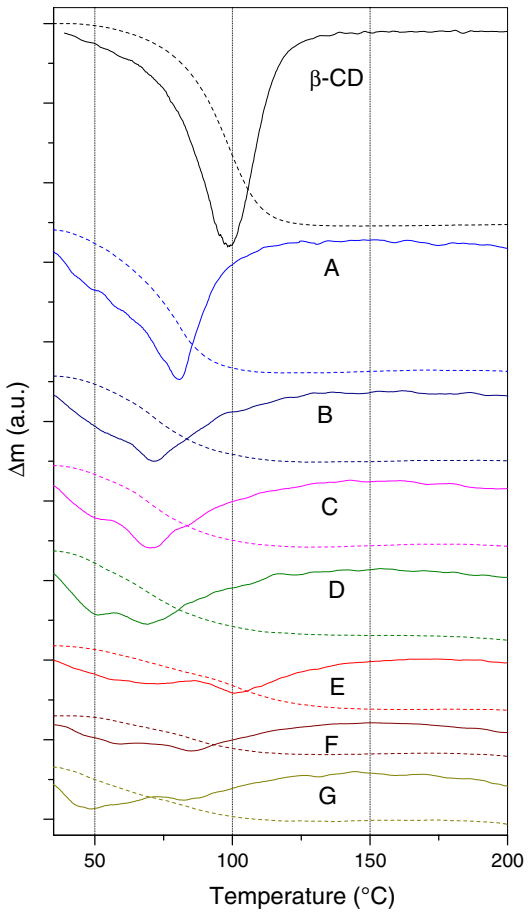

Fig. 3 TG (dashed lines) and DTG curves (solid lines) of *β*-CD and cypermethrin/*β*-CD complexes

Determination of the guest content complexed

The free (non-complexed) guest content values determined by DSC-measurement are listed in Table 1. The differences between the total and free guest content resulted in the assumed complexed cypermethrin content. The molar ratio of the complexed (strongly bound) cypermethrin to cyclodextrin was calculated (last column of Table 1) from the cypermethrin content of the dry material. The solvent (water and acetone) content values measured by thermogravimetry were used for these calculations.

According to our results the *β*-CD complexes possess 2.0–31.5% total active substance content and 8.8–2.4% water content, correspondingly the complexed cypermethrin/*β*-CD molar ratio varies between 1:16.2 and 1:1.2. Four complexes contain only strongly bound guest, which are presumably located inside the cavity (A–D), while complexes with higher total guest content (E–G) have both complexed and free cypermethrin.

The total cypermethrin content of peracetyl-*β*-cyclodextrin complexes (H–J) is 9.9–17.4%. These samples seem to be totally complexed (with 1:1.9, 1:1.2, 1:1.0 complexed cypermethrin/Ac*β*CD molar ratio), because no cypermethrin melting peak was detected on their DSC curves.

Dissolution measurements

The dissolution profiles of complexes and pure cypermethrin have been investigated in distilled water between 0 and 90 min (Figs. 4 and 5). An excessive amount of active substance was added during the test, ensuring cypermethrin saturation of the dissolution medium (non-sink condition). Dissolution rate measurements were characterized by the highest level of cypermethrin concentration of complex and the dissolution time of this maximum value. A new parameter has been introduced: the area under the dissolution curves (until 90 min) to determine quantitatively the changes in the solubilized amount of the active ingredient using the different complexes (Table 3).

The dissolution curve of *pure guest* shows a continuous slight improvement of cypermethin concentration, the area under its curve is 8 ± 1 mg min L^{-1}. In case of *β-CD complexes* a huge increase of cypermethrin concentration can be observed in water during the initial phase (between 0 and 30 min), when supersaturated solution forms (Fig. 4). Dissolution curves of each *β*-CD complex have a peak belonging to a maximum cypermethrin concentration

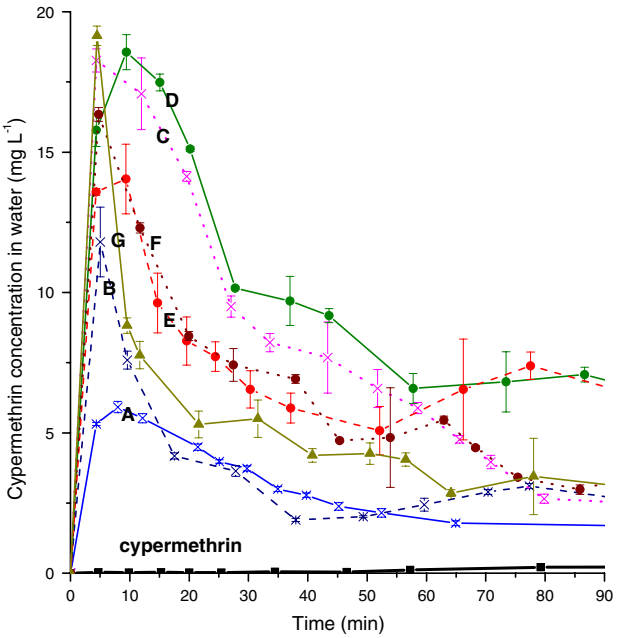

Fig. 4 Dissolution measurements of *β*-CD complexes (compared to the pure guest, solid line, squares) Cypermetrin/*β*-CD complexes: A (with a molar ratio of 1:16.2): solid line, cross symbol; B (1:3.8): dashed line, cross symbol; C (1:2.5): dotted line, cross symbol; D (1:2.2): solid line, circle symbol; E (1:2.7): dashed line, circle symbol; F (1:1.5): dotted line, circle symbol; G (1:1.2): solid line, triangle symbol; pure cypermethrin: thick, solid line, square symbol

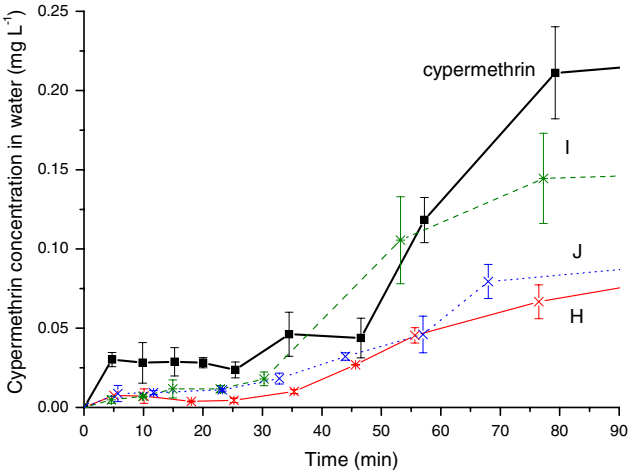

Fig. 5 Dissolution curves of cypermethrin/AcβCD complexes compared to pure cypermethrin (errors calculated from parallel measurements). Cypermethrin/AcβCD complex H (with 1:1.9 molar ratio): solid line, I (with 1:1.2 molar ratio): dashed line, J (with 1:1.0 molar ratio): dotted line; pure cypermethrin: thick, solid line

between 4 and 9 min. The highest active substance concentration (19.1 mg L^{-1}) can be measured in case of complex G with highest complexed guest/β-CD molar ratio (1:1.2), but its dissolution curve shows only a narrow peak with smaller area under the curve (500 mg L^{-1}). On the contrary complexes C and D (1:2.5 and 1:2.2) show similarly high maximum cypermethrin concentration (18.3 and 18.6 mg L^{-1}) with larger area (799 and 920 mg L^{-1}).

After this initial phase the concentration decreased gradually to the equilibrium cypermethrin concentration (3–7 mg L^{-1}), which is higher than the intrinsic solubility of pure guest (0.25 mg L^{-1}). Even the complex with the lowest guest content (2.0% of cypermethrin, with a molar ratio of 1:16.2) shows more effective release property than the pure active substance. The area under its dissolution curve is 281 mg L^{-1}, which is 35 times higher than that of the pure cypermethrin.

On the contrary, *AcβCD complexes* show continuously increasing release of cypermethrin, but lower cypermethrin concentrations and areas under the dissolution curves can be measured in water than for the pure guest (Fig. 5). This slightly retarded dissolution property can be explained with the poor water-solubility of the acetyl-β-cyclodextrin derivative.

It is interesting to note that the highest area under the dissolution curve does not belong to the highest cypermethrin content. To investigate this relationship, Fig. 6 shows the area under the dissolution curve of complexes as a function of complexed cypermethrin/CD molar ratio. Maximum curves can be seen in both graphs: for β-CD complexes the maximum value is at 0.5, which refers to 1:2 molar ratio (upper curve), while for AcβCD complexes the maximum is located at 0.8, corresponding to 1:1.25

Table 3 Dissolution parameters of complexes: dissolution peak characteristics (dissolution time of peak, peak value) and area under the dissolution curve (0–90 min)

Complexes	Dissolution peak		Area[a] (mg L^{-1})
	Dissolution time of peak (min)	Maximum value (mg L^{-1})	
β-CD			
A	8	5.9	281 ± 9
B	5	11.8	324 ± 15
C	4	18.3	799 ± 27
D	9	18.6	920 ± 29
E	9	14.0	636 ± 56
F	5	16.3	686 ± 11
G	4	19.1	500 ± 22
AcβCD			
H	–	0.08	3 ± 1
I	–	0.18	7 ± 2
J	–	0.09	4 ± 1

[a] Standard deviation values were calculated from three parallel measurements

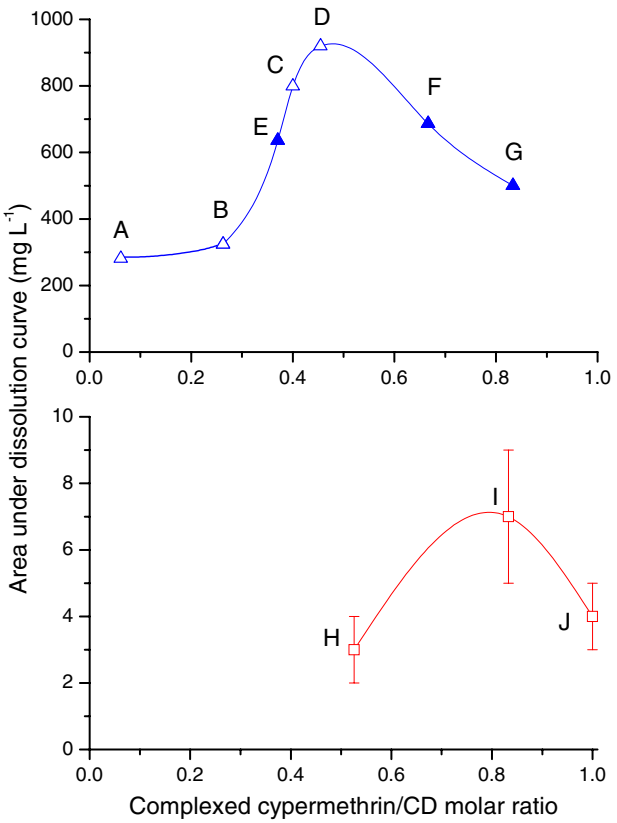

Fig. 6 Area under the dissolution curve as a function of complexed cypermethrin/CD molar ratio Upper graph: cypermethrin/β-CD complexes with totally complexed guest (A–D, empty triangle symbol) and those containing also free guest (E–G, full triangle symbol); lower graph: cypermethrin/AcβCD complexes with totally complexed guest (H–J, empty square symbol)

complexed cypermethrin/CD molar ratio (lower curve). On the upper curve sample E (which contains both complexed and non-complexed guest) clearly fits together with samples containing only complexed guests, and is located between samples B and C. These results suggest that the non-complexed active substance content of complex products does not have any influence on area under the dissolution curve. We think that this phenomenon can be general in case of poorly soluble guests: their contribution to the dissolution should be negligible.

According to the knowledge of the authors the maximum curves on area under the dissolution curve vs. guest/host molar ratio is new. It resembles to the classical Job's plot used for determination of the molar ratio of inclusion complexes based on spectral data [19, 20]. We can assume that the stoichiometry of the complexes can be calculated from these plots in Fig. 6, too.

Conclusion

Inclusion complexes of cypermethrin with β-CD and peracetylated-β-cyclodextrin have been prepared and analysed by different analytical methods (UV-spectrophotometry, RP-HPLC, thermoanalytical techniques and dissolution measurements).

Based on our results solid cypermethrin formulations with controlled release property can be produced using cyclodextrins. Both enhanced (with β-CD) and slightly retarded (with peracetylated-β-cyclodextrin) dissolution rate can be obtained.

The novel parameter characterizing the dissolution behaviour of cypermethrin from the complexes (area under the dissolution curve) gave the opportunity to get preliminary information on the stoichiometry of the complexes.

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