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DIFFERENTIAL SCANNING CALORIMETRY AS AN ANALYTICAL TOOL IN THE STUDY OF DRUG–CYCLODEXTRIN INTERACTIONS

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Abstract

The interactions of trimethoprim, sulphadiazine and sulphamethoxazole with natural (α-, β-, γ-) and amorphous (RAMEB) or crystalline (DIMEB) methylated β-cyclodextrins were investigated both in aqueous solution (using phase-solubility analysis) and in the solid state (using DSC supported by X-ray analysis). In particular, DSC studies enabled determination of the relative degree of crystallinity of each drug in its physical and ground mixtures with the different cyclodextrins on the basis of the variation of its heat of fusion in comparison with that of the pure drug. In all cases, the host cavity size was a prevalent factor for the inclusion complexation in liquid state. On the contrary, it had a negligible effect on solid-state interactions in terms of drug amorphization. DIMEB and RAMEB exhibited similar performances in aqueous solution, showing that the presence of methyl-groups improved the complexing and solubilizing properties of β-cyclodextrin. However, DSC studies revealed that RAMEB was clearly more active in performing solid-state interactions, i.e. drug amorphization, and as stabilizing agent for the amorphous state brought forth.

Keywords: amorphization, complexation, cyclodextrins, differential scanning calorimetry, sulphadiazine, sulphamethoxazole, trimethoprim

Introduction

Thermal analysis methods, and particularly differential scanning calorimetry (DSC), find a wide and increasing range of applications in the pharmaceutical field, ranging from control of raw materials, to stability, compatibility and preformulation studies for the development of new formulations and drug delivery systems [1–4].

Cyclodextrins are torus-shaped cyclic oligosaccharides currently used as excipients in pharmaceutical technology owing to their ability to include in their hydrophobic central cavity several kinds of drug molecules, thus improving some physicochemical and biopharmaceutical properties [5, 6]. The cyclodextrin complexing power towards a given guest molecule can be strongly influenced by several factors, such as, in particular, the host cavity size [7, 8] and the presence and type of substituents on the ring [9, 10]. Moreover, different performances of non-crystalline and

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crystalline cyclodextrin derivatives in improving the pharmaceutical properties of drugs have been reported [11, 12].

DSC showed to be a very powerful analytical tool in the characterization of solid state interactions between drugs and cyclodextrins [7, 9, 10, 13, 14]. Comparison of the affinities between drugs and cyclodextrins in the solid state with those in liquid state could help shed light on the role of molecular parameters (cavity size, presence of substituents) and amorphous or crystalline nature of the carrier in the host-guest interaction.

Therefore, in the present work we studied the thermal behavior of three different crystalline drugs (trimethoprim, sulphadiazine and sulphamethoxazole) in mixtures with both natural cyclodextrins (α, β, γ) (to assess the role of the host cavity size), and amorphous or crystalline methylated β-cyclodextrin (to evaluate the effect of both the presence of the subtituent and the different solid state). Phase solubility studies in aqueous solutions at different temperatures were also performed, to determine the binding constants of the different complexes and the related thermodynamic parameters. The possible relationships between the solid-state interactions, in terms of drug amorphization, and the host-guest affinity in aqueous solution, in terms of cyclodextrin complexing and solubilizing abilities, were investigated and discussed.

Materials and methods

Materials

Trimethoprim (TMP), sulphadiazine (SDZ) and sulphamethoxazole (SMO), as well as crystalline α-cyclodextrin (αCD) and β-cyclodextrin (βCD) were obtained from Sigma Chemical Co. (St. Louis, Mo, USA). Randomly methylated amorphous β-cyclodextrin with a substitution degree per anhydroglucose unit (DS) of 1.8 (RAMEB) (water content 3.2±0.2% as mass fraction) was kindly donated by Wacker Chemie GmbH (München 70, FRG). Crystalline heptakis-(2,6-di-O-methyl)-β-cyclodextrin (DIMEB) and γ-cyclodextrin (γCD) were purchased from Cyclolab (Budapest, HU).

Preparation of samples

Equimolar physical mixtures of each drug with each cyclodextrin were prepared by tumble mixing 4–5 g of the 75–150 μm sieve granulometric fractions of the respective simple components for 15 min. Equimolar ground mixtures were prepared by manual grinding of the physical mixtures in an agate mortar with a pestle for 15 min.

Differential scanning calorimetry (DSC)

Temperature and enthalpy values were measured with a Mettler TA4000 apparatus equipped with a DSC 25 cell on 5–8 mg samples (Mettler M3 Microbalance) in pierced Al pans with a perforated lid, at a heating rate of 10 K min^{-1} in the 30–300°C temperature range under static air atmosphere. No oxidation or decomposition phenomena were observed in these conditions before the drug melting process. The relative degree of crystallinity of each drug ($drug_{RDC\%}$) in physical and coground mix-

tures, expressed as percent of the drug mass fraction in the starting sample, was estimated by the ratio between the heat of fusion of the drug calculated in the sample and that of the pure drug, according to the following equation:

$$\text{drug}_{\text{RDC\%}} = \frac{\Delta H_{\text{mix}}}{\Delta H_{\text{pd}}} 100$$

where ΔH_{mix} and ΔH_{pd} are the heats of fusion of each drug calculated in the physical and ground mixtures and in the pure drug sample, respectively [15].

Heat of fusion measurements were carried out in duplicate and the relative deviation of crystallinity data was ±6%. This equation is suitable to evaluate the amorphizing power of a given cyclodextrin toward a given drug [16], but it cannot give information about the actual cyclodextrin inclusion complexation power in the solid state, since it is not able to distinguish if the drug amorphization is a consequence or not of its inclusion into the cyclodextrin cavity.

X-ray powder diffractometry (XRD)

XRD patterns were taken with a Philips PW 1130 diffractometer (CoK_α radiation) at a scan rate of 2° min^{-1} over the 10 to 50° 2θ range.

Phase-solubility analysis

Solubility measurements of each drug were carried out by adding excess amounts of drug (TMP, or SMO or SDZ) to 10 mL of water or aqueous solution of each examined cyclodextrin in different concentration ranges (5 to 15 mmol L^{-1} for βCD, 5 to 25 mmol L^{-1} for αCD and γCD, and 5 to 100 mmol L^{-1} for DIMEB or RAMEB) depending on their different water solubilities. The experiments were performed in sealed glass containers under magnetical stirring at constant temperature (25±0.5, 37±0.5, 45±0.5°C) until equilibrium was reached (3 d). Aliquots were then withdrawn, filtered (pore size 0.45 μm) and spectrophotometrically analyzed for drug concentration (Perkin Elmer spectrophotometer Mod. 552S) respectively at 271 nm (for TMP, ε=6330), 242 nm (for SDZ, ε=14690) and 265 nm (for SMO, ε=4430). Each experiment was performed in triplicate (coefficient of variation CV<5%). The apparent 1:1 binding constants ($K_{1:1}$) of the complexes were calculated from the slopes of the straight lines of the phase-solubility diagrams and the corresponding intercepts, according to the equation proposed by Higuchi and Connors [17]:

$$K_{1:1} = \frac{\text{slope}}{\text{intercept}\,(1-\text{slope})}$$

where the intercept represents the equilibrium solubility of the drug in the absence of cyclodextrin.

Results and discussion

Host–guest interactions in aqueous solution

Phase-solubility analysis showed that the aqueous solubilities of the three drugs linearly increased as a function of carrier concentration with all the examined carriers (Fig. 1). These linear phase diagrams are classified as A_L-type [17] and are considered indicative of the formation of soluble complexes between the substrate (the drug) and the ligand (the cyclodextrin). The 1:1 stability constants ($K_{1:1}$) of the complexes are reported in Table 1. The decrease in $K_{1:1}$ values with increasing temperature indicated the exothermic nature of the inclusion complexation. It is evident that, among the natural cyclodextrins, βCD was the most effective partner for all the three drugs, in terms of both complexing and solubilizing abilities, showing that its cavity size is the most suitable to accommodate the considered guest molecules. The methylated-βCD derivatives showed rather similar complexing and solubilizing properties and were more efficacious than the parent cyclodextrin, not only due to their higher

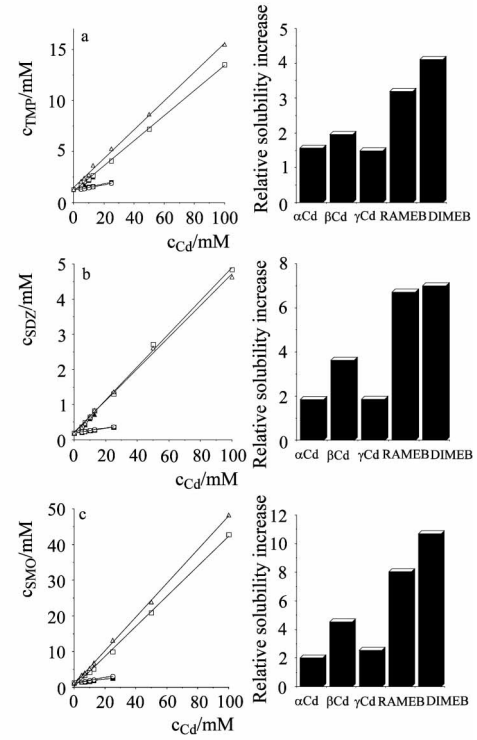

Fig. 1 Phase-solubility diagrams of a – trimethoprim (TMP), b – sulphadiazine (SDZ) or c – sulphamethoxazole (SMO) with ▲ – βCD; ■ – αCD; ○ – γCD; □ – RAMEB; △ – DIMEB in water at 25°C. On the right side the relative increases in aqueous drug solubility are reported in the presence of 25 mmol L^{-1} of the different cyclodextrins or of 15 mmol L^{-1} of native βCD at 25°C

Table 1 Apparent stability constants for the interaction in aqueous solution of sulphamethoxazole (SMO), sulphadiazine (SDZ) and trimethoprim (TMP) with cyclodextrins

CD	Stability constant, $K_{1:1}$/L mol^{-1}								
	SMO			SDZ			TMP		
	25°C	37°C	45°C	25°C	37°C	45°C	25°C	37°C	45°C
αCD	45	35	23	35	31	27	24	19	15
βCD	400	349	231	217	187	174	82	62	48
γCD	70	55	33	36	32	29	20	17	13
RAMEB	591	555	501	252	213	206	110	88	79
DIMEB	725	678	615	240	194	189	129	100	92

water solubility, but also because the substituent methyl groups allow expansion of the hydrophobic region by capping the cavity and thus increase substrate binding via a hydrophobic effect [18]. The complexing ability of the examined CDs towards the three drugs was in the same rank order RAMEB≈DIMEB>βCD>>αCD≈γCD. On the other hand, assuming the stability constant values of the inclusion complexes as indexes of the affinity degree of the drug for the carrier, the rank order observed for each CD was $K_{1:1,\,SMO}>K_{1:1,SDZ}>K_{1:1,TMP}$.

Standard thermodynamic parameters, calculated from the temperature dependency of $K_{1:1}$ values within the 25–45°C temperature range (Table 2) suggested that the complexation process was essentially enthalpy-controlled and that both dipolar or induced dipolar and van der Waals interactions between host and guest molecules are involved in inclusion complexation. A contribution of hydrophobic interactions, which involve the breakdown and displacement of the highly ordered water molecules inside the cyclodextrin cavity and around the apolar guest molecule was also suggested by the positive entropy changes [19] observed for all the complexes with SDZ and for βCD and βCD-derivative complexes with SMO. On the contrary, the unfavorable entropy change observed for all the complexes with TMP and for αCD and γCD complexes with SMO could be mainly attributed to loss of the translational and rotational degree of freedom as a result of the host-guest combination and/or to the smaller disordering of the displaced water molecules released from the drug [20].

Host-guest interactions in the solid state

The results of DSC analyses of SMO, SDZ and TMP and their 1:1 (mol mol^{-1}) physical and coground mixtures with the different CDs are shown, respectively, in Figs 2 and 3 and in Table 3. The drugs' thermal curves were typical of crystalline anhydrous substances and were all characterized by a sharp endotherm, due to drug melting, followed at higher temperatures by endo- or exo-thermic effects due to decomposition phenomena. A rather similar behavior was observed for the three drugs in their blends with the various carriers. In fact, the thermal profiles of all the physical mixtures with natural CDs showed, after the broad endotherm due to the CD dehydration process, the drug melting peak, which appeared substantially unaffected in its shape and area. This indicated that the drug basically maintained its original crystallinity, and, in fact, its amorphization degree in no case exceeded 15%. Some broadening of the drug endotherm was instead observed in blends with DIMEB, and this effect became clearly more marked, and accompanied by a shift to lower temperatures and a strong reduction of intensity in blends with RAMEB, where the loss of drug crystallinity reached as much as 70%. The observed drug–CD solid-state interaction can be attributed to a heating-favored loosening of crystal forces of the drug dispersed within the amorphous carrier phase, as already found for blends of other crystalline drugs with RAMEB [9, 12] or other amorphous CDs [6, 9, 10]. The extent of drug amorphization brought about by natural CDs was positively influenced by the cogrinding treatment, as proved by the sharp reduction in intensity of the drug melting endotherm, up to its disappearance in the coground TMP-βCD (Fig. 3). However, the different cavity sizes of the macrocycles, unlike the important role demon-

Table 2 Thermodynamic parameters for interaction in aqueous solution of sulphamethoxazole (SMO), sulphadiazine (SDZ) and trimethoprim (TMP) with cyclodextrins

CD	$\Delta G_{25°C}$/kJ mol^{-1}			ΔH/kJ mol^{-1}			$\Delta S_{25°C}$/J mol^{-1} K^{-1}		
	SMO	SDZ	TMP	SMO	SDZ	TMP	SMO	SDZ	TMP
αCD	–9	–9	–8	–15	–8	–16	–18	3	–27
βCD	–15	–13	–11	–9	–9	–18	21	13	–23
γCD	–10	–9	–7	–15	–6	–12	–14	8	–15
RAMEB	–16	–14	–12	–4	–11	–14	39	10	–8
DIMEB	–16	–14	–12	–4	–10	–16	40	12	–12

Table 3 DSC data and relative decrease in crystallinity (RDC%) for sulphamethoxazole (SMO), sulphadiazine (SDZ) and trimethoprim (TMP) in 1:1 mol/mol physical and ground mixtures with cyclodextrins

CD	Physical mixtures						Ground mixtures					
	SMO		SDZ		TMP		SMO		SDZ		TMP	
	T_{peak}/°C	RDC/%	T_{peak}/°C	RDC/%	T_{peak}/°C	RDC/%	T_{peak}/°C	RDC/%	T_{peak}/°C	RDC/%	T_{peak}/°C	RDC/%
Native drug	170.0	100	260.6	100	200.1	100	170.0	100	260.6	100	200.1	100
αCD	169.5	90	251.3	93	199.5	92	168.3	26	248.6	28	184.4	22
βCD	169.0	86	250.6	85	198.5	85	168.0	18	246.0	10	–	–
γCD	169.5	95	250.7	90	199.5	95	167.4	23	249.0	32	178.8	10
RAMEB	160.2	32	213.8	43	185.6	64	–	–	–	–	–	–
DIMEB	163.7	83	247.2	85	198.5	80	150.0	37	248.9	46	193.9	68

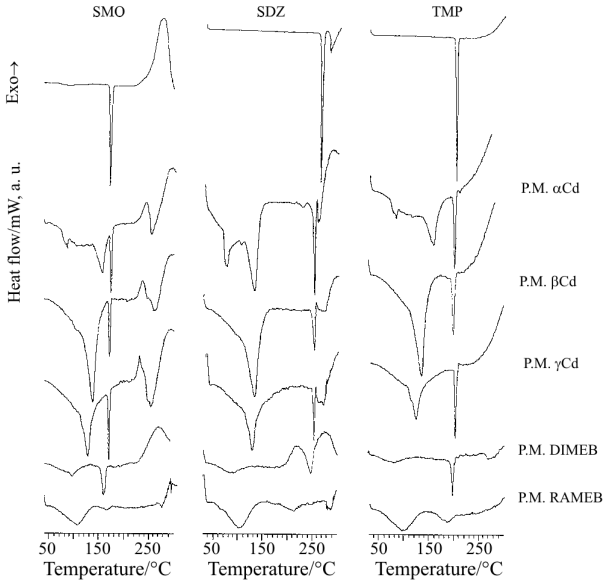

Fig. 2 DSC curves of pure sulphamethoxazole (SMO), sulphadiazine (SDZ) and trimethoprim (TMP) and their equimolar physical mixtures (P.M.) with αCD, βCD, γCD, DIMEB, RAMEB

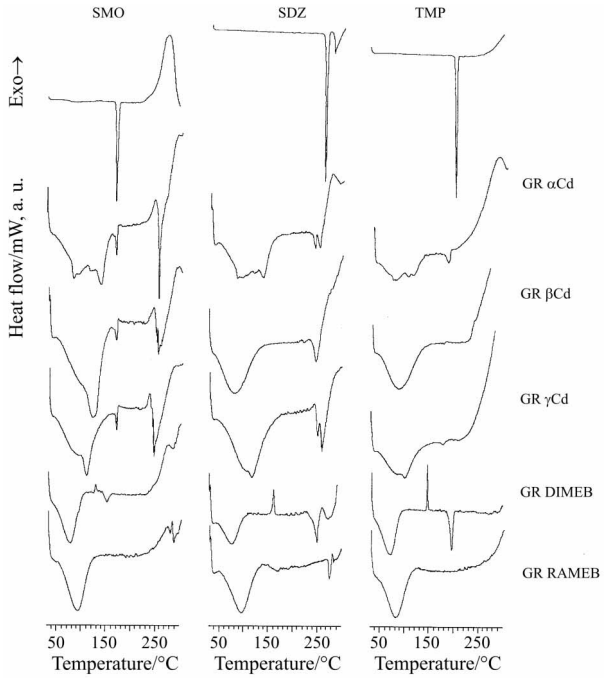

Fig. 3 DSC curves of pure sulphamethoxazole (SMO), sulphadiazine (SDZ) and trimethoprim (TMP) and their equimolar ground mixtures (GR) with αCD, βCD, γCD, DIMEB, RAMEB

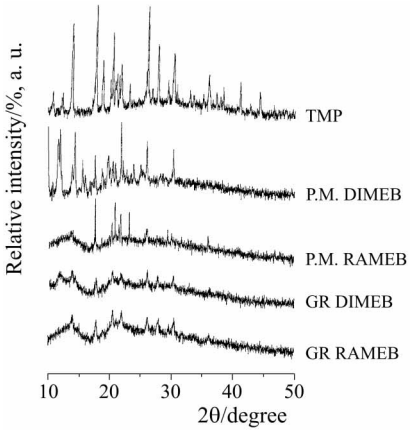

Fig. 4 Powder X-ray diffraction patterns of pure trimethoprim (TMP) and its equimolar physical mixtures (P.M.) and ground mixtures (GR) with DIMEB and RAMEB

strated in drug–CD interactions in aqueous solution, seemed to not be directly involved in the amorphization process of the drugs. In fact, as shown in Table 3, the differences in loss of crystallinity of the drugs were rather small, even though βCD was always slightly more effective than αCD or γCD. An important role in the solid state interaction was, on the contrary, played by the presence of substituents on the CD moiety and by its amorphous or crystalline nature. Complete disappearance of the drug melting peak, an index of total drug amorphization, was obtained in all ground systems with the amorphous RAMEB, which therefore confirmed, also in the solid state, its higher ability in performing drug–CD interaction in comparison with the parent cyclodextrin, as already found in phase-solubility studies. An unexpected behavior was instead observed in the thermal curves of ground mixtures with DIMEB where, in all cases, after the initial broad endotherm due to the CD dehydration process, a sharp exotherm appeared, due to the recrystallization of the drug, amorphized during the cogrinding process, followed by the melting peak of recrystallized drug. X-ray diffraction analysis of physical and ground mixtures with RAMEB and DIMEB revealed that (as shown for example in Fig. 4 for systems with TMP) after the grinding treatment, an analogous drug amorphization degree was obtained with both the βCD derivatives. However, evidently, the amorphous state achieved in the system with the crystalline derivative was clearly less stable than that obtained in the presence of the amorphous carrier, since the heating supplied during the DSC scan was enough to cause the drug recrystallization. DSC curves of drug–RAMEB ground systems recorded after 6 months storage at room temperature were unchanged with respect to those of freshly prepared samples, confirming the stability of the achieved drug amorphization.

Conclusions

Analogies were observed for the interactions of TMP, SMO and SDZ with the different CDs in both aqueous solution and solid state, where the same rank orders of affin-

ity were observed in terms, respectively, of cyclodextrin complexing and solubilizing ability (DIMEB≈RAMEB>βCD>>αCD≈γCD) and of drug amorphizing power (RAMEB>>βCD>DIMEB>αCD≈γCD).

The host cavity size was a determinant factor for the drug–CD interactions in aqueous solution, strongly influencing the carrier complexing and solubilizing properties. On the contrary, it played a secondary role regarding the interaction process in the solid-state, i.e. drug amorphization.

The presence of methyl groups on the βCD ring markedly improved both the complexing and solubilizing capacities (as shown from phase-solubility studies) as well as the amorphizing power (as shown from DSC studies) of the carrier. Interestingly, DSC analysis made it possible to demonstrate the lower stability of the drug amorphous state obtained in ground systems with DIMEB with respect to RAMEB.

Therefore, despite the analogous performance shown by the methylated βCD derivatives in aqueous solution, the highest amorphizing efficacy of RAMEB pointed out by DSC studies makes this derivative the carrier of choice for all the examined drugs. It should be stressed that the availability of amorphous pharmaceuticals which are adequately stable in the conditions of pharmaceutical processing make it possible to overcome the technological problems linked to the intrinsic thermodynamic instability of the amorphous state, which represents the major handicap for practical uses.

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