Journal of Thermal Analysis and Calorimetry, Vol. 94 (2008) 1, 109–118

A DSC AND RAMAN SPECTROSCOPIC STUDY OF MICROSPHERES PREPARED WITH POLAR COSOLVENTS BY DIFFERENT TECHNIQUES

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A study was made of the possibilities of gradually decreasing the concentration of the toxic organic solvent in the process of microsphere preparation. Ammonio methacrylate copolymer-based microspheres were prepared by spray drying or conventional solvent evaporation techniques, and compared. The formulations were designed by varying the preparation methods and the concentrations of four polar cosolvents as independent variables.

DSC was used to study the relationship between the changes in the independent variables and three of the main thermal events of the microspheres. Raman spectroscopy was used to investigate and confirm the possible interactions between drug and copolymer. Appropriate choice of the independent variables led to the molecularly dispersed drug in the polymer matrix. It was demonstrated that only the nature of the preparation method caused significant variations in the structure and thermal behaviour of the microspheres.

Keywords: *cosolvents, DSC, energy-dispersive X-ray fluorescence analysis, microsphere, Raman spectroscopy, spray drying, W/O/W multiple emulsion*

Introduction

The value of microspheres as orally administered controlled-release dosage forms has been evident for years. The $W_1/O/W_2$ multiple emulsion-solvent evaporation technique ensures the formation of lipophilic microspheres of identical size that are uniformly loaded with drug, but nonetheless with a poor encapsulation efficiency (EE) of water-soluble drugs [1]. Ever since microspheres have been formulated via the emulsion method, there has been the problem of the organic solvent. The selection of the organic solvent may determine the microsphere characteristics. The integrity of the microsphere wall is controlled by the migration of the organic solvent between the emulsion phases. The rate of extraction of the cosolvents is limited by their solubility in water, while their evaporation rate depends on the boiling point. The concentration of harmful solvents (Class 2, ICH) [2], such as the commonly used dichloromethane (DCM), should be limited. The use of polar cosolvents with different water miscibilities to prepare microspheres has been investigated, e.g. acetone [3], ethanol [4] and ethyl acetate [5–7]. The present work was designed to evaluate the effects of four polar cosolvents on the thermal characteristics of the microspheres: acetone (Me_2CO), methyl ethyl ketone (MeCOEt), *n*-propanol (*n*PrOH) and *n*-butyl acetate (*n*BuOAc), which were mixed individually with DCM as the organic phase of the multiple emulsion. The cosolvents used were water-miscible (Me_2CO and *n*PrOH) or partially miscible (MeCOEt and *n*BuOAc) at the concentrations used. Their density gap with water is smaller than that of DCM. The characteristic physico-chemical properties of the cosolvents used are listed in Table 1.

The model drug used, diclofenac sodium (DS), is a non-steroidal anti-inflammatory drug (NSAID) with a short plasma half-life (1.1–1.8 h) and C_{max} value (within 1.5–2.5 h), which is unstable in aqueous solution; achievement of sustained release is therefore of great importance. TG, DTG and DSC instruments have been used to study the thermal behaviour of DS [8, 9] and other NSAIDs [10]. The thermal degradation of diclofenac has been investigated [11] and DS has been determined by DSC in tablets, suppositories [12] and a HPMC–β-CD solid complex [13]. The thermodynamics of aqueous DS solutions has also been highlighted [14].

The biocompatible and non-biodegradable polymer component AMC (Eudragit® RS PO, *MW* 150000, Ammonio Methacrylate Copolymer Type B, USP/NF, Ph.Eur./NF.) has been used in the formulation of sustained – release pellets [15], matrix tablets [16] and thermosensitive membranes [17], and as a retardant component in tablet coatings [18] and pellet coatings [19]. AMC is insoluble in the digestive juice, and is slightly permeable to water [20]; the matrix provides a pH-independent slow release [21]. The thermodynamic

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1388–6150/$20.00

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Table 1 Physicochemical properties of the organic solvent and cosolvents used

Used solvents	ICH Class	Boiling point/ °C	Density/ g mL^{-1}	Polarity index	log*P*	Din.visc./ mPa s[a]	Sol. in water/ g 100 mL^{-1}	Saturation[b]
DCM	2	39.5	1.317	3.1	1.511	0.475	1.3	rapid
Me_2CO	3	56.5	0.785	5.1	0.234	0.360	miscible	mixing
MeCOEt	3	79.6	0.800	4.7	0.736	0.415	29.0	–
*n*PrOH	3	97.2	0.807	4.0	0.559	2.072	miscible	mixing
*n*BuOAc	3	125.0	0.882	3.9	1.822	0.730	0.7	rapid

[a]Results from the preliminary measurements (relative densities: water=1.000); [b]Saturation at the maximum cosolvent concentration (mass/mass%) in the W_2 phase

behaviour [22], the fragility index [23] and the degradation of PMMA in different N_2–O_2 atmospheres have been determined using TG/DTA/MS [24, 25]. Thermal investigations (TG and DSC) have been utilized to study PMMA-based microparticles [26]; porous microparticles [27]; spray-dried microcapsules [28]; PMMA–HP–β-CD-based microparticles [29]; PMMA-grafted silica nanocomposites [22]; dry powder-coated pellets [19] and PMMA-plasticizer interactions [30].

The amphiphilic poly(ethylene glycol stearate) (PEGS) has been applied as a plasticizer in order to make the polymer chains more flexible and enhance the pore-forming process [31]. The presence of plasticizer can cause alterations in the polymer glass transition temperature, fragility, film-forming temperature and drug release [23]. Poly(vinyl alcohol) (PVA) has been used as a protective colloid to protect the W_1/O droplet from coalescence due to the surfactant effect and the increased viscosity of the W_2 phase.

The technique for preparation of the microspheres was the spray drying of a multiple emulsion (SD microspheres). To compare the effects of different preparation techniques, microspheres were also prepared by the conventional solvent evaporation technique (SE microspheres), a process which ensures the 'in-water drying' of the polymer matrix, without heat treatment. The target of this work was to replace harmful organic solvents by more biocompatible solvents. However, the use of polar cosolvents could cause the confluence of the aqueous and organic phases; a good approach, therefore, is to increase the preparation rate by the rapid SD technique, which is a one-step process and can be used to minimize the time necessary for microsphere formation and to ensure uniform product quality. During the process, the multiple emulsion droplets come into a short but appreciable contact with the hot drying air, the intense heat and mass transfer therefore resulting in efficient drying, while the protective envelope of vapour keeps the particle at lower temperature. The thermal treatment of the polymer (e.g. the process of thermal aftertreatment (curing)) above T_g could alter the structure of the polymer due to the internal structural changes (the moving of side-chains, and a shift from a glassy to a more flexible rubbery state) [29]. This could modify the drug release [32], characteristically decrease the release rate [33], and result in a reduction of the microsphere porosity [34].

The primary aim of the present study was to observe the thermal behaviour of AMC-based microspheres, as part of the microsphere product-preparation process optimization strategies, in conformity with PAT (Process Analytical Technology). The quality parameters of the initial ingredients, the physical and model mixtures and the microspheres with in-process testing were measured by DSC and Raman spectroscopy. The preliminary study suggested that the type and increased amounts of polar cosolvents could increase the risk of confluence of the W_1 and W_2 aqueous phases, which could cause marked changes in physical structure and thermal behaviour, with statistically significant relationships between the independent variables and the numerical values of the main thermal events. The secondary purpose was to investigate and confirm the possible interactions between DS and AMC by using Raman spectroscopy.

Experimental

Materials

Diclofenac sodium (DS) (Human Co., Hungary) and Eudragit® RS PO (AMC) (DeGussa Co., Germany) were of pharmacopoeial grade. Dichloromethane (DCM) and the cosolvents (acetone, *n*-propyl alcohol, methyl ethyl ketone and *n*-butyl acetate), the surfactants (sorbitan mono-oleate, HLB=4.3; polyoxyethylene 20 sorbitan mono-oleate, HLB=14.9), poly(ethylene glycol stearate) (PEGS), and poly(vinyl alcohol) PVA (MW=72000) were of analytical grade (Spectrum-3D Co., Hungary).

Preparation of SD and SE microspheres

Four cosolvents in four batches were investigated: Batch A (DCM+Me_2CO), Batch B (DCM+MeCOEt), Batch C (DCM+*n*PrOH) and Batch D (DCM+*n*BuOAc). Four cosolvent concentrations (X_1; 0, 25, 50 and 75 mass/mass%) and two preparation methods (X_2=SD and SE) were selected in every batch as independent variables (Table 2). Other potential variables were kept constant.

SD microspheres: A $W_1/O/W_2$ multiple emulsion was first prepared by a two-step emulsification procedure. An aqueous solution of DS was emulsified in the organic phase (DCM or DCM-cosolvent mixture, AMC, PEGS, and surfactant with HLB=4.3). The resulting W_1/O emulsion was added to the W_2 phase (PVA, and surfactant with HLB=14.9). The multiple emulsion was spray-dried, using a Büchi B-191 Laboratory Spray-dryer (Büchi Co., Flawil, Switzerland) with a standard 0.7 mm nozzle, under continuous stirring, a magnetic stirrer being applied to maintain emulsion homogeneity. The final products were separated in the novel high-performance cyclone. The process was performed under the same conditions, at 700 L h^{-1} air flow, 5 bar pressure and 128 mL h^{-1} pump flow rate. The inlet temperature (140°C) was set above the boiling points of the solvents, according to the preliminary study [35], while the outlet temperature was the result of changing factors and varied in the range 95–108°C. Drug-free SD microspheres with 50% cosolvent concentration were also prepared in each batch.

SE microspheres: The conventional SE technique was carried out at 25±1°C, at normal atmospheric pressure, under continuous stirring. The SE microspheres, prepared with a $W_1/O/W_2$ multiple emulsion, were collected by centrifugation (4000 1 min^{-1}) under cooling, washed three times with bidistilled water, and freeze-dried for 48 h (Christ Alpha 1-2, Christ, Germany). All the SD and SE microspheres were stored under controlled humidity conditions at 4°C.

DS/AMC mixtures (DS/AMC ratio 1:6): Physical mixtures were prepared to identify the DSC events of the microsphere components. A model mixture was prepared as cast film for Raman spectroscopy to gain better-quality spectra: ethanolic solutions of DS and AMC in DCM were mixed, followed by SE by vacuum drying at 60°C for 4 h. In contrast with the physical mixture, the model mixture allows the preparation of a solid solution of DS in the AMC matrix.

Methods

Determination of the encapsulation efficiency (EE)

The amount of DS in the microspheres (mass/mass%) was determined with an energy-dispersive X-ray fluorescence analyser (MiniPal, Philips Analytical, The Netherlands). Pressed microsphere samples were prepared (diameter: 10 mm, height: <1 mm). X-ray tube type: low-powered with side window; anode material: Rh; software-controlled tube setting; tube filters: 5 filters selected by software. He gas purge: to improve the identification of low atomic number elements such as Na. He inlet pressure: 1 bar. Measurement: channel code and compound name: Na; unit: %; line name: KA; measuring time: 600 s; conditions set: 4 kV, 1000 μA. All the measurements were repeated 7 times. DS+PMMA physical powder mixtures with different DS contents were prepared for the calibration, which revealed a linear model (R^2=^{0}.9929, k=0.033). EE (%) was calculated as the ratio of the actual and the theoretical loading of DS.

Thermal analysis

Thermoanalytical measurements were carried out with a Mettler Toledo DSC 821^e instrument (Mettler Toledo,

Table 2 Data on the batches investigated

Batch A (DCM+Me_2CO)			Batch B (DCM+MeCOEt)			Batch C (DCM+*n*PrOH)			Batch D (DCM+*n*BuOAc)		
	X_1	X_2		X_1	X_2		X_1	X_2		X_1	X_2
A0*	50%	SD	B0*	50%	SD	C0*	50%	SD	D0*	50%	SD
A1	0%	SD	B1	0%	SD	C1	0%	SD	D1	0%	SD
A2		SE	B2		SE	C2		SE	D2		SE
A3	25%	SD	B3	25%	SD	C3	25%	SD	D3	25%	SD
A4		SE	B4		SE	C4		SE	D4		SE
A5	50%	SD	B5	50%	SD	C5	50%	SD	D5	50%	SD
A6		SE	B6		SE	C6		SE	D6		SE
A7	75%	SD	B7	75%	SD	C7	75%	SD	D7	75%	SD
A8		SE	B8		SE	C8		SE	D8		SE

X_1: cosolvent concentration (mass/mass%); X_2: type of solvent evaporation technique (SD, spray drying; SE, conventional solvent evaporation); *: drug-free SD microspheres

Table 3 Thermal events and enthalpies (ΔH) of the initial ingredients (1); physical mixtures (2) and microspheres (3) (mean values; n=2)

No.	Appearance	1. event		2. event		3. event	
		T_1/°C	ΔH_1/J g^{-1}	T_2/°C	ΔH_2/J g^{-1}	T_3/°C	ΔH_3/J g^{-1}
1	AMC	66.2 (T_g)	8.6	188.0 (T_m)	9.1	–	–
	PEGS	62.5 (T_m)	214.2	–	–	–	–
	PVA	53.2 (T_g)	8.9	193.6 (T_m)	37.7	322.2 (T_m)	155.1
2	AMC+PEGS	64.0	35.8	187.8	18.5	–	–
	AMC+PGA	66.2	3.0	191.0	14.5	–	–
	AMC+DS	66.3	3.0	187.4	11.3	–	–
	AMC+DS (model mixture)	46.3	2.6	218.7	4.2	–	–
	AMC+PEGS+PVA	66.0	28.1	190.2	20.5	327.5	0.51
	AMC+PEGS+PVA+DS	64.8	19.4	191.3	15.3	–	–
3	SD-microspheres	39.2–43.6	4.4–6.9	179.5–183.6	9.6–13.3	320.7–324.6	11.2–28.9
	SE-microspheres	43.2–46.1	2.7–12.9	181.0–188.5	2.1–10.9	–	–

T_1: peak maximum of first event (PEGS T_m+AMC T_g); T_2: peak maximum of second event (AMC T_m+PVA T_m); T_3: peak maximum of third event (PVA T_m)

Mettler Co., UK). 5.0 mg of the microspheres was placed into an aluminium pan, and heated at a constant rate of 10°C min^{-1} between –5 and +350°C under a dynamic flow of N_2 and Ar. Each sample was analysed in duplicate; measurements were carried out 6 months after the preparation. The curves and the changes in enthalpy (ΔH, J g^{-1}) of the initial materials, the physical mixtures, the model mixture, and the drug-free and drug-containing microspheres were recorded.

Raman spectroscopy

Raman spectra were taken to characterize the solid-state form of the DS in the microspheres, and the possible interactions between DS and AMC. For the characterisation of the DS, the region 1650–1530 cm^{-1} was used, where there were no Raman lines belonging to any other components. Spectra were recorded with a Jobin Yvon LABRam dispersion spectrometer (Jobin Yvon Co., France) with attached an Olympus BX41 microscope. Measurement conditions: the excitation line was 785 nm with 7.5 mW power; measurements were made under a 100× magnification objective. The spectral resolution was 1.2 cm^{-1}, with a 950 g mm^{-1} grating and a 256×1024 pixel CCD detector. The analysis was performed 6 months after microsphere preparation.

Results and discussion

Thermoanalytical measurements

DSC profiles of initial ingredients

The DS had a characteristic, well-shaped calorimetric profile, revealing endothermic peaks at 285.6 and 290.6°C (T_m) with an enthalpy variation of 18.3 and 37.0 J g^{-1}, respectively, and a single exotherm at 306.8°C (ΔH: 152.8 J g^{-1}), following a decomposition process (Fig. 1, Table 3), in accordance with the literature [10]. The TG curve of the DS demonstrated an 18% mass loss from 300 to 395°C, attributed solely to decomposition.

The form of AMC used was amorphous; as expected, T_g was at 55–60°C [20, 24]. PMMAs generally start to degrade at the side-chain above 150°C, while

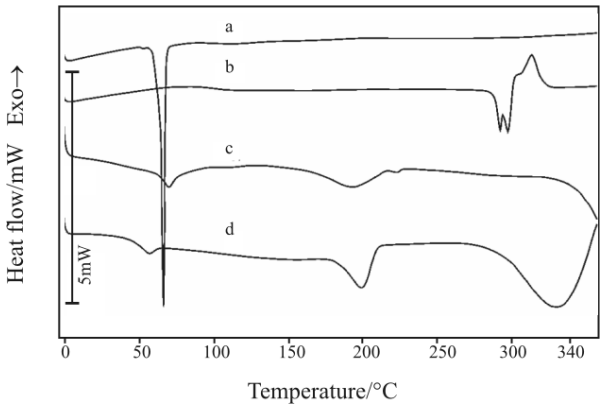

Fig. 1 DSC profiles of a – PEGS; b – DS; c – AMC and d – PVA

depolymerization or other reactions of the main chain start above 180°C [36]. The curves of AMC showed an endothermic peak at 66.2°C (T_g) and a broad endotherm at 188.0°C (T_m), with an enthalpy value of 8.6 and 9.1 J g^{-1}, respectively (Fig. 1, Table 3). There was a small endotherm at 217.5°C, followed by a decomposition process above 320°C. The PEGS had one weak endotherm at 49.5°C (T_g) and a single well-shaped characteristic endothermic peak at 62.5°C (T_m). The PVA exhibited characteristic thermal events: T_g at 53.2°C, a broad T_m at 193.6°C and a wide endotherm at around 322°C.

DSC profiles of physical mixtures and the model mixture

The positions and enthalpies of the AMC T_g and T_m events can be influenced by other components present. Different physical mixtures (AMC+DS, AMC+PEGS, AMC+PVA and their combinations) were therefore prepared and analysed to identify the matrix interference and to assign the endothermic events of the microsphere products. The main endothermic events observed are listed in Table 3. Figure 2 shows the DSC curves of the physical mixtures of AMC with DS, PVA and PEGS separately and in combination.

The quaternary ammonium and ester groups of AMC are capable of interacting with anionic drugs such as DS through hydrogen-bonding, electrostatic and dispersion forces, resulting in a decreased T_g of AMC. For the AMC+DS physical mixture, the endothermic peaks of AMC did not shift; only the characteristic melting of DS was lowered by 15°C (Fig. 2, Table 3) to 270.5°C, due to the formation of a low-melting polymorph of DS. To prepare the molecular dispersion of DS in the polymer matrix, a model mixture (DS/AMC ratio 1:6) was formulated, using the solvent-casting method. In contrast with the physical mixtures, the preparation of the model mixture involved thermal treatment. Thermal treatment of the polymer at a temperature higher than its T_g generally alters its physical structure from a glassy to a rubbery state, and the T_g of AMC therefore disappears [29]. The AMC+DS model mixture exhibited distinct thermal events: a broad and very weak endotherm at around 46.3°C, and an endothermic peak at 218.7°C (T_m of AMC), without the T_m of DS. The DS melted and dispersed in the fused AMC; this should be responsible for the absence of the DS T_m, which implies that DS solubility in AMC was ensured at this DS/AMC ratio, and therefore also in the microsphere products.

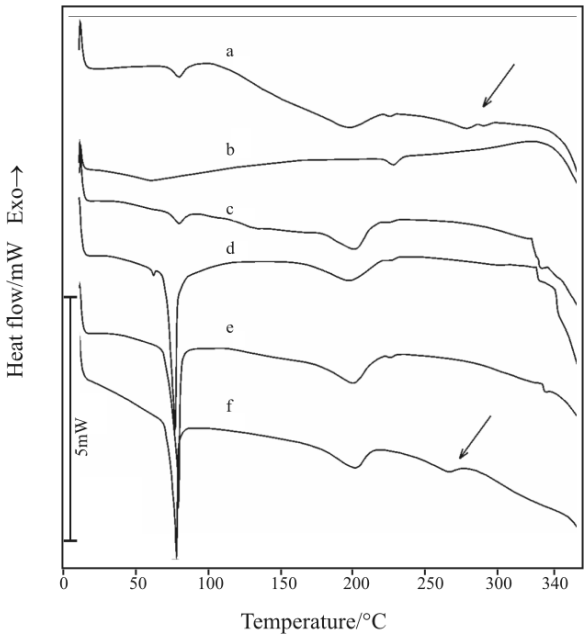

Fig. 2 DSC profiles of physical mixtures: a – AMC+DS; b – AMC+DS – model mixture; c – AMC+PVA; d – AMC+PEGS; e – AMC+PEGS+PVA and f – AMC+PEGS+DS+PVA

For the AMC+PVA physical mixture, common T_g and T_m were seen at 66.2 and 191.0°C, respectively, demonstrating that miscible polymers can exhibit a common, single T_g between the T_gs of the components [37]; the common T_g is indicative of the state of their dispersion. When PEGS was added to AMC, the characteristic sharp T_m of AMC and the T_g of AMC overlapped at 64.0°C; the T_g of AMC did not fall considerably: the difference was approximately 2°C. The total enthalpy might be influenced and increased due to the very sharp enthalpy of PEGS. The addition of DS (alone) to AMC did not change the kinetics of AMC degradation, whereas the addition of PVA or PEGS or both to AMC resulted in an abrupt and decreased thermal degradation temperature (T_D) (>310°C). These phenomena suggested that the latter components exerted a slight destabilizing effect on AMC.

When first PVA and then DS was added to the AMC+PEGS physical mixture, the weak T_g of PEGS (49.5°C), and the second T_m of AMC (217.5°C) disappeared. The addition of DS to the AMC+PEGS or AMC+PVA mixture smoothed over the degradation event (not shown). The sharp endothermic peak (64–66°C) was clearly observed for all the plasticized mixtures. The overlapped T_m of AMC and PVA (191°C) did not shift markedly. The T_m of the low-melting polymorph of DS was clearly visible in the DSC spectrum of the DS-containing AMC+PEGS+DS+PVA physical mixture (250–270°C). Further addition of DS to this physical mixture had no impact as regards altering the temperatures of the endothermic peaks; only the enthalpy of the DS T_m increased (not shown). In the model mixture, DS did not precipitate out in crystalline form, indicating its solid solution form and the applicably higher drug loading level (DS/AMC ratio 1:6).

Influence of the cosolvent type and concentration

The solvent–cosolvent composition and concentration were responsible for controlling the thermoanalytical properties of the microspheres. In consequence of the rapid partitioning of the cosolvents from the organic phase of the W_1/O emulsion, and therefore the increased W_1/O emulsion viscosity and faster emulsion droplet hardening, the drug loading improved [38, 39]. Depending on the solubility of the polar cosolvent in DCM, the rate of polymer precipitation followed by the hardening of the matrix differed. Faster polymer precipitation can lead to uneven-shaped, rather porous microparticles, with free DS crystals on their surface and in the pores, resulting in marked signs of the DS crystals in the DSC record.

The temperatures and enthalpies (ΔH) of the three characteristic endothermic events of the heat-treated SD microspheres were evaluated. Figure 3 shows representative DSC profiles of the SD and SE microspheres. The curves of the batches were identical with each other, apart from minor shifts (Table 4).

The first endothermic event (T_1), formed by the overlapped PEGS T_m and AMC T_g, could be observed in the interval 39.2–47.3°C, with a maximum deviation of 8.1°C, situated 17–25°C before that for the AMC-PEGS+DS+PVA physical mixture (64.8°C). The miscibility/compatibility in the molten state of AMC, PEGS and PVA and therefore the increase in the chain mobility of the AMC molecules and the decrease in the cohesive interactions between the AMC chains were confirmed by the T_g depression. The dissolved state and the plasticizing effect of DS can also increase the mobility of the AMC monomers and weaken the AMC chain segment–segment interactions; as a consequence, the T_g and other thermal events decreased [36]. Similar tendencies were noted for the T_2 event (formed by the common AMC and PVA T_m) observed at 179.5–188.5°C; the effect of the dispersed DS was confirmed by the 2–11°C difference from the AMC-PEGS+DS+PVA physical mixture (191.3°C). The T_3 event (PVA T_m), which could be observed only in the DSC curves of the SD microspheres, was at 320.7-324.6°C. The reason for this was that crystalline PVA was present in all the SD samples, formed from residual PVA during the SD procedure, revealed at around its T_m (322.2°C). The thermograms of the batches did not indicate any sharp thermal event corresponding to the melting of DS crystal domains; this occurred only in the physical mixture. This absence was attributed to the englobing of DS inside the polymer matrix and confirmed the marked decrease in crystallinity and the mainly molecular dispersion.

The residual humidity could exert a plasticizing effect, affecting several parameters, including the matrix permeability and chemical stability of the drug [40]. The elimination of residual water and organic solvents/cosolvents could be ensured by the preparation process; additionally, the AMC used is the least hygroscopic among available the PMMAs, due to the low quaternary ammonium group content (5%). The solvent elimination was proved by the low (1 mass/mass%) mass loss of the product between 42 and 98°C.

The drug-free SD microspheres displayed a similar behaviour to that of the drug-containing SD microspheres; nonetheless, the plots revealed pronounced shifts in the characteristic T_1 and T_2 events, while the difference was negligible for T_3 (Table 4). The T_1 event appeared at around 50.2–55.8°C, indicating that the process of melting of PEGS and AMC tend to

Table 4 Summary of thermal events, enthalpies (ΔH) and EE values of the microsphere products

Microspheres		1. event		2. event		3. event		EE/%
Batch	Prep.	T_1/°C	ΔH_1/J g^{-1}	T_2/°C	ΔH_2/J g^{-1}	T_3/°C	ΔH_3/J g^{-1}	
A0	SD	54.5	9.1	189.6	9.4	334.5	14.8	–
A1, 3, 5, 7	SD	40.9–41.6	5.1–6.9	179.5–181.3	12.3–12.5	320.7–322.6	22.8–28.9	14.6–32.8
A2, 4, 6, 8	SE	43.2–47.3	6.8–7.7	181.0–187.0	2.1–10.9	–	–	20.5–70.3
B0	SD	54.1	5.7	190.1	15.1	323.2	23.2	–
B1, 3, 5, 7	SD	39.2–43.1	5.6–6.6	180.3–181.5	12.0–12.6	322.1–323.9	21.3–28.6	14.6–31.7
B2, 4, 6, 8	SE	43.9–46.4	5.9–9.9	185.1–187.8	6.8–10.9	–	–	21.0–39.6
C0	SD	55.8	9.1	188.6	12.5	325.7	20.6	–
C1, 3, 5, 7	SD	39.7–43.6	5.7–6.6	179.9–183.6	9.6–12.6	321.8–324.5	11.2–28.6	14.6–15.3
C2, 4, 6, 8	SE	44.2–46.4	7.4–12.9	183.0–188.5	7.7–10.9	–	–	20.9–55.8
D0	SD	50.2	4.0	185.8	12.9	323.8	29.2	–
D1, 3, 5, 7	SD	39.6–43.6	4.4–6.6	179.9–183.5	11.6–13.3	321.8–324.6	14.3–28.6	14.6–27.1
D2, 4, 6, 8	SE	44.5–46.1	2.7–11.8	185.0–187.0	5.6–10.9	–	–	19.2–39.6

T_1: peak maximum of first event (PEGS T_g+AMC T_m); T_2: peak maximum of second event (AMC T_m+PVA T_m); T_3: peak maximum of third event (PVA T_m)

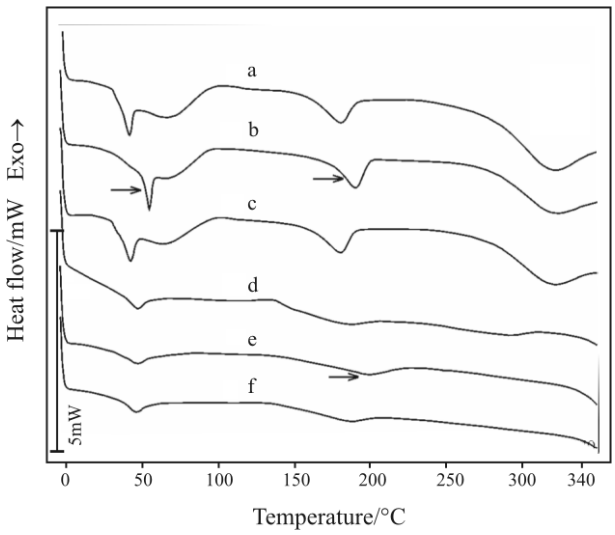

Fig. 3 Typical DSC profiles of the microspheres: a – batch A1 (100% DCM, SD); b – batch C0 (50% DCM, SD, drug-free); c – batch C5 (50% DCM, SD); d – batch A2 (100% DCM, SE); e – without batch number (50% DCM, SE, drug-free); f – batch C6 (50% DCM, SE)

overlap. Thermal events were present at 185.8–190.1°C (T_2) and 323.2–334.5°C (T_3) (Fig. 3), due to the T_m of AMC-PVA and PVA, respectively. In comparison with the drug-containing SD microspheres, the T_1 and T_2 events moved towards higher temperature, with a difference of 7–16 and 2–11°C, respectively.

The effects of the dependent and independent variables on the thermal events were not significant, though the EE values correlated well with the independent variables in several cases (results not shown). The EE values clearly demonstrated that a considerable proportion of the entrapped DS diffused off the multiple emulsion before microsphere hardening, particularly when Me_2CO and *n*PrOH were used as water-miscible cosolvents. The noteworthy differences between the physical mixtures and the microspheres furnished evidence of the formation of a DS solid solution in the matrix when these preparation methods were applied, but the usage of polar cosolvents had less effect on the thermal behaviour of the microspheres; only the presence of DS was of decisive importance.

Influence of the preparation method

To observe the differences in thermal characteristics relative to the SD microspheres, SE microspheres were prepared by the conventional SE technique, using the same cosolvents. It was clear that the SE technique ensured higher EE values (Table 4).

All the SE microspheres displayed an analogous trend, with broad and weak endothermic peaks and frequently lower ΔH values; representative DSC profiles are given in Fig. 3. Comparison of the microspheres prepared by the different techniques revealed that the T_1 and T_2 events of the SE microspheres began at around the temperature where the SD thermal events ended.

The DSC profiles of the drug-free and drug-containing SE microspheres were also identical, except for the T_2 event of the drug-free microspheres, which was shifted from 180–190 to 200°C, indicating the absence of the plasticizing effect of DS. When the SE technique was used, the characteristic T_3 event (PVA T_m) was not observed. The reason for this is that the SE technique allows the elimination of residual PVA in the course of the preparation process. Furthermore, reduced enthalpy (ΔH) values were obtained with the SE technique because there was more time for the englobing of the PEGS and the formation of the polymer matrix structure. These curves did not exhibit any thermal event corresponding to DS melting. It was found that neither the concentrations nor the types of the cosolvents changed the temperatures of the thermal events or the enthalpies significantly; coherence of the independent variables and the EE values could be observed (data not shown). In spite of the longer preparation time, the SE microspheres had a higher DS content.

For the SE microspheres, the characteristic endothermic event due to residual moisture did not appear, because freeze drying (for 48 h) led to effective drying of the SE microspheres, in accordance with the literature [40]. In spite of the efficacy of the SD procedure, the SD microspheres contained traces of absorbed moisture, indicating that the duration of SD might be too short to eliminate the total residual moisture. The endothermic event relating to residual moisture elimination appeared in the temperature range 55–80°C, which was not considered.

Raman spectroscopy

Raman spectroscopy was employed to study the possible physicochemical interactions between DS and AMC. The spectra of AMC (Fig. 4g), DS (Fig. 4f), the model mixture with a DS/AMC ratio of 1:6 (Fig. 4e), the drug-free (Fig. 4d) and drug-containing SE microspheres (Fig. 4c), and the drug-free (Fig. 4b) and drug-containing SD microspheres (Fig. 4a) in the spectral range 1800–650 cm^{-1} are presented in Fig. 4. In the measurements, the spectra in Figs 4a–f were adjusted for the selected and characteristic band of AMC (1452 cm^{-1}) (spectrum Fig. 4g), to observe the principal differences between the model mixture, the thermally treated SD microspheres (drug-free and drug-containing) and the thermally non-treated SE microspheres (drug-free and drug-containing), and the specific changes in the characteristic bands of DS in the products.

The spectra demonstrated that DS and AMC had characteristic bands, and that the model mixture yielded the sum of their individual spectra. The drug-free and

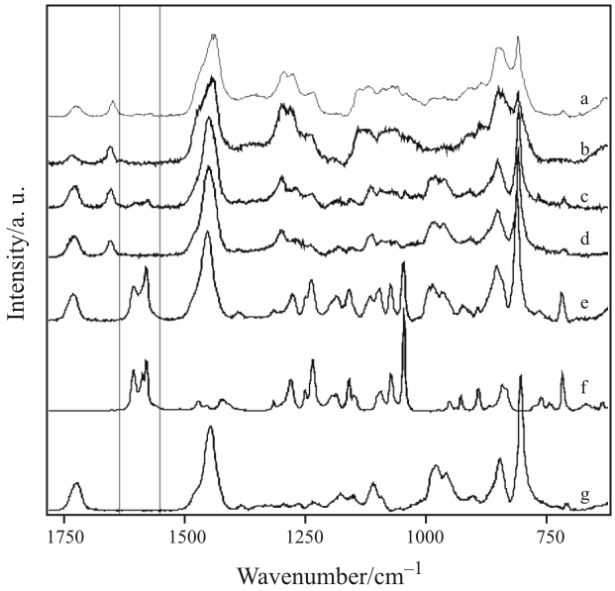

Fig. 4 Raman spectra of a – drug-containing and b – drug-free SD microspheres; c – drug-containing and d – drug-free SE microspheres; the model mixture with a DS/AMC ratio of e – 1:6; f – DS and g – AMC in the spectral region 1800–625 cm^{-1}

drug-containing SD and SE microspheres gave spectra with similar structures, containing broad bands. The study focused on the Raman bands of the DS and AMC molecular vibrations in selected spectral ranges where other ingredients did not exert any disturbing effect.

The spectrum of the model mixture (Fig. 4e) could be regarded as the superposition of the spectra of DS and AMC. As compared with the model mixture, the corresponding Raman bands of the SD and SE microspheres were unchanged (811 cm^{-1}), or were broader (854, 1452, 1736 cm^{-1}), indicating mutual interactions of these functional groups. The overlapped bands in the interval 1005–930 cm^{-1} were present in the spectra of the model mixture, the drug-containing and drug-free SE microspheres and the drug-containing SD microspheres, but not in that of the drug-free SD microspheres. Broadening was seen, whereas there was no dramatic shift in the band of the carbonyl group of the trimethyl-ammonioethyl methacrylate segment of AMC (1736 cm^{-1}), which is responsible for control of the swelling and water permeability of the AMC matrix [41]. However similarly to the bands at 1005–930 cm^{-1}, the intensity decreased significantly. In the spectra of the drug-free and the drug-containing SD microspheres, no difference was observed in the positions of the absorption bands. The shape of the band at 1452 cm^{-1} altered only in the case of the SD microspheres, the reason being the disturbing effect of PEGS; the absence of this alteration from the bands of the SE microspheres might indicate a lower amount of PEGS in the product. During the longer time of preparation of the SE microspheres, the amphiphilic PEGS could leach out from the W_1/O emulsion, which would explain its lower amount. Incidentally, PEGS, used as a plasticizer in low amount, could cause a more rigid microparticle structure, and therefore a higher drug content and lower drug release rate. The presence of the high HLB value surfactant resulted in the band at 1650 cm^{-1}.

In the spectrum of DS, four spectral regions were of particular interest. The broadening of the band at 719 cm^{-1} was indicative of a decrease in the vibrational relaxation time. The intense bands at 1049, 1075 and 1097 cm^{-1} were observed as weak spikes in the spectrum of the SD microspheres. The spectral region 1300–1220 cm^{-1} showed the absence of the bands at 1254 cm^{-1} for the model mixture, and the absence of bands at 1170–1120 cm^{-1} for the SD microspheres. However, these findings can not be interpreted with certainly as the spectra of the microspheres, since the spectra were seriously affected by the other ingredients.

The fingerprint region of DS between 1700 and 1550 cm^{-1} was selected for closer investigation; it was necessary to assume that other ingredients did not interfere in this region. The characteristic DS bands were at 1582, 1590 and 1608 cm^{-1} (Fig. 4f). These typical bands were also detected in the spectra of the model mixture (DS/AMC ratio 1:6; Fig. 4e) and the drug-containing microspheres (Figs 4a and c), but not in that of the drug-free microspheres (Figs 4b and d). There was no dramatic shift in the band of DS at 1581 cm^{-1}; the absence of the band at 1590 cm^{-1} in the spectrum of the model mixture and especially in that of the SD microspheres suggested the possibility of weak interactions.

Comparison of the DSC profiles and Raman spectra of the model mixture and the SD microspheres allows the assumation that, if DS is dispersed in the model mixture, then the DS in much lower concentration in the SD microspheres must be present in a similarly dispersed state, in accordance with the literature (DS in β-cyclodextrin) [42]. The distribution of the drug inside the microparticles is an important factor, because the drug can crystallize during the preparation, resulting in a decreased solubility rate and a polymorphic form [43]. The Raman measurements did not prove the existence of strong intermolecular interactions between DS and AMC, which could be responsible for the additional retaining effect on DS, altering the drug release rate. The measurements revealed that the preparation methods used did not significantly influence the structure of DS, but the small shifts, the absence of particular bands, and the changes in the relative intensities of the microsphere bands with respect to the Raman bands of the initial DS and AMC did not permit the exclusion of the possibility of weak DS-AMC interactions.

Conclusions

In this work the effects on the thermal behaviour of microspheres of the type and amount of four polar cosolvents and the preparation methods were investigated. Formulation design was performed to determine the significance of differences in the main DSC events of the microspheres. The results were evaluated in order to characterize the state of the englobed SD and the state in the microsphere products, using thermal analysis and Raman spectroscopy. We established the preliminar finding that, the more polar the cosolvent used, the higher risk of confluence of the aqueous phases, resulting in the formation of different physical structures and therefore characteristic thermal behaviour.

The thermal behaviour of microspheres is often predetermined by the design concept in the early phase of microsphere product investigations. Thermal analysis, among other methods, is a useful tool with which to identify the matrix interference through the thermal behaviour of the initial ingredients, physical mixtures, and microsphere products, and hence to confirm the possible interactions.

It was found that the polar cosolvents used can serve as effective ingredients, replacing DCM in 25–75% mass/mass% concentration to prepare AMC-based DS-containing microspheres. Irrespective of their type, even at high concentration (75 mass/mass%) the cosolvents revealed only minor structural changes and differences in DSC events, while the microspheres varied only in their EE values. The ranges of the main thermal events did not change significantly: major differences were observed only between the SE and SD microspheres and the drug-free and drug-containing microspheres, due to the different methods of preparation and the presence of DS, respectively. The analyses demonstrated the dispersed state of the DS in the microspheres, without free DS crystals. The small changes in the temperatures of the DSC events, the absence of the characteristic T_m of crystalline DS and the non-appearance of any unexpected thermal events confirmed the stability and the uniformity of the microspheres. Raman measurements indicated that the structure of AMC did not alter appreciably during the preparation process, and also revealed weak interactions between AMC and the dispersed DS, without sufficient strength to exert an additional retaining effect on DS from dissolution. The results confirmed that both SE and SD preparation techniques can be used for microsphere production, in spite of the thermal treatment nature of the SD technique. These results are important from the aspect of the further refinement of microsphere formulation.

Abbreviations

AMC	ammonio methacrylate copolymer
DCM	dichloromethane
DS	diclofenac sodium
DS/AMC	drug/polymer ratio
EE	encapsulation efficiency
Me_2CO	acetone
MeCOEt	methyl ethyl ketone
*n*BuOAc	*n*-butyl acetate
*n*PrOH	*n*-propanol
PEGS	poly(ethylene glycol stearate)
PMMA	poly(methyl methacrylate)
PVA	poly(vinyl alcohol)
SD	spray drying (technique)
SE	solvent evaporation (technique)
$W_1/O/W_2$	water-in-oil-in-water emulsion

Acknowledgements

The authors thank Mme F. Perraud, Dr. C. Coralie (Jobin Yvon Co., France) and Professor Gy. Marosi (Budapest University of Technology and Economics) for their kind help with the Raman measurements. This work was supported by the Hungarian National Fund, OTKA (project number: T046908).

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Received: November 19, 2007
Accepted: January 22, 2008
OnlineFirst: August 15, 2008

DOI: 10.1007/s10973-007-8881-2