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An investigation into the release of cefuroxime axetil from taste-masked stearic acid microspheres. III. The use of DSC and HSDSC as means of characterising the interaction of the microspheres with buffered media

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Abstract

Stearic acid coated cefuroxime axetil (SACA) microspheres have been studied using differential scanning calorimetry (DSC) and high sensitivity DSC (HSDSC) in order to examine the interaction between the spheres and a range of buffer systems, with a view to further enhance the understanding of the mechanism of drug release developed in earlier studies [Robson et al., 1999, 2000]. DSC studies indicated that after immersion in Sorensens modified phosphate buffer (SMPB) pH 5.9 followed by washing and drying, no change in the thermal properties of the spheres was detected up to 60 min of immersion, with a single endotherm noted at circa 56°C, that corresponded to the melting of the stearic acid used in this study; similar results were obtained for systems immersed in distilled water. After immersion in SMPB pH 7.0 and 8.0 , however, a second peak was noted at approximately 67°C that increased in magnitude relative to the lower temperature endotherm with increasing exposure time to the medium. Spheres that had not been previously washed prior to drying showed complete conversion to the higher temperature endotherm for these two buffers. Systems which had been exposed to a range of pH 7.0 buffers (citrate–phosphate buffer (CPB), phosphate buffer mixed (PBM), boric acid buffer (BAB)) were then examined. Only the CPB systems showed evidence for conversion to the higher melting form. PBM systems to which further sodium had been added were then examined. A maximum conversion was found at 0.05 M sodium, which was in agreement with the maximum in release rate found in a previous study [Robson et al., 2000]. HSDSC was then used to examine systems that were immersed in the buffer. For SMPB, pH 5.9 and distilled water, only the endotherm corresponding to the stearic acid melting was seen. However, for SMPB pH 7.0 and 8.0, three peaks were seen, two corresponding to those seen for the DSC studies and a further lower temperature peak at circa 44°C. Studies on PBM systems to which additional

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sodium had been added showed small levels of conversion to the higher temperature form at higher sodium contents. The data was discussed in terms of the correlation with earlier dissolution studies on the same systems [Robson et al., 1999; 2000].

Keywords: Cefuroxime axetil; Differential scanning calorimetry; Microsphere; Stearic acid; Taste masking

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1. Introduction

Stearic acid microspheres containing cefuroxime axetil (SACA) have been described as a means of taste-masking this antibiotic for administration to children (Robson et al., 1999, 2000). These microspheres are prepared via a spray chilling process and are subsequently formulated as a suspension (Zinnat™) that is reconstituted prior to administration. Previous studies have indicated that this formulation approach meets the dual needs of effective taste-masking and suitable bioavailability (Powell et al., 1991; Gooch et al., 1993; Shalit et al., 1994), although the mechanisms by which the drug was released from the spheres was still not fully understood. Robson et al. (1999) have indicated that the drug is released at a considerably faster rate in an alkali environment, hence it is logical to suggest that release (and hence taste) is delayed until the spheres reach the intestine. However, the mechanism involved in the dissolution process appears to be complex, depending on both pH and composition. Robson et al. (2000) have shown that buffers with the same pH values may yield very different release profiles. These authors have suggested that there are essentially two mechanisms involved. Firstly, the drug may diffuse out of the spheres this process being controlled by a number of parameters such as the ionic composition of the media. Secondly, the spheres may undergo a reaction with the media that leads to loss of integrity of the sphere microstructure. The predominance of these two mechanisms may vary according to the composition of the medium, with the second process generally occurring in a higher pH media.

In this investigation, we described the use of two thermal techniques for the characterisation of the spheres with a view to further clarifying the mechanisms involved. In particular, it is reasonable to suggest that if the reaction that has been suggested to take place between the spheres and the media results in changes to the physical properties of those spheres, these changes will in turn be reflected by the thermal behaviour. Consequently, a thermoanalytical study of the systems after or during exposure to the various dissolution media has been performed. The two techniques in question are differential scanning calorimetry (DSC) and high sensitivity differential scanning calorimetry (HSDSC). The principles of heat flux and power compensation DSC have been described in detail elsewhere (Ford and Timmins, 1989; Coleman and Craig, 1996). HSDSC works on the same principle (although it should be noted that in this study the DSC used is a power compensation instrument while the HSDSC is heat flux). The essential differences between the two approaches are that HSDSC is a more sensitive technique and involves the use of larger volume vessels and slower heating rates (typically, $<2°C$ min^{-1}), rendering the approach particularly suitable for the study of liquid systems such as dilute solutions of biomolecules (Chowdhry and Cole, 1989). However, the slow scanning speeds are also a disadvantage in that experimental time may be increased considerably. For solid state systems that undergo energetically large thermal events, there may be little benefit in using HSDSC, although Wissing et al. (2000) have recently exploited the increased sample pan volume of HSDSC by studying the properties of whole tablets. If, however, the system is in a liquid or semi-solid state and/or the thermal events under study are energetically small, then HSDSC may offer considerable advantages over conventional DSC. In addition to biomolecule systems, the approach has also been used to study systems such as aqueous surfactants, polymers and vesicles (Kaneshina and Yamanaka, 1989; Arunothayanun et al., 2000).

In this study, we have attempted to exploit the strengths of both approaches by studying SACA in the solid state after exposure to various dissolution media using conventional DSC. In addition, we describe the use of HSDSC as a means of studying the spheres, while they are suspended in the dissolution media. In this way, it is intended that changes in the thermal properties of the spheres may be correlated to the dissolution data described in earlier studies.

2. Materials and methods

Samples of SACA and all buffer components were supplied and prepared as described in previous studies (Robson et al., 1999, 2000). Similarly, samples for DSC were prepared by exposing the SACA to the dissolution media, washing and drying as described for earlier SEM studies. DSC was performed using a Perkin–Elmer DSC7, incorporating a PE 3700 computer. A nitrogen purge at a flow rate of 30 cm^3 min^{-1} was used to provide an inert gas atmosphere around the immediate vicinity of the sample and reference pans. Indium was used as a calibrant and in all cases a heating rate of 10°C min^{-1} and a sample mass of 4.0 mg were used (crimped aluminium pans). At least three runs were carried out for each sample. All DSC traces were highly reproducible in terms of shape and melting point values, while all enthalpy data had coefficient of variation values $< 5\%$. When clear differentiation from the baseline was not possible, extrapolated onsets were quoted.

High sensitivity differential scanning calorimetry (HSDSC) was performed using a Setaram Micro DSC III. In order to avoid steam condensation in the calorimetric wall, a constant sweeping of dry nitrogen was used. In this study, closed stainless steel 'batch' vessels were used. Vessels were cleaned using acetone and sonication when necessary. Once dry, the bodies and stoppers were weighed to check mass balance (to the nearest mg) and placed into a vessel-holding block. Liquid samples were introduced using a Gilsen pipette. Gloves or pliers were used to handle the vessels at all times. Once filled, the top of the vessel was dried using a tissue as residual water could cause a thermal signal linked to water evaporation. The reference vessel was filled with an equivalent amount of reference liquid (buffer without added spheres). A new seal was placed onto each stopper that was then screwed into the top of the vessel. The instrument was left for 10 min before beginning analysis to ensure baseline stability. In all cases, 4 mg of SACA were mixed with 800 mg buffer and a scanning rate of 1°C min^{-1} was used.

3. Results

3.1. DSC studies

3.1.1. Immersion in Sorensens modified buffer

The melting behaviour of SACA corresponded well with that expected for stearic/palmitic acid (Swern, 1979) and a single endotherm was seen at approximately 56°C with a corresponding enthalpy of 156.1 J g^{-1}. SACA samples that had been exposed to Sorensens modified phosphate buffer (SMPB) (pH 8.0, 7.0 and 5.9), washed and then dried were then examined in order to relate the thermal behaviour to the corresponding dissolution profiles and appearance of the spheres, as assessed by SEM (Robson et al., 1999). In this earlier study, a marked pH dependence was observed, with samples in pH 5.9 buffer and distilled water showing considerably slower release than was observed for the pH 7.0 and 8.0 buffer systems. Similarly, evidence for alterations in the surface integrity of the spheres was observed for the pH 7.0 and 8.0 systems, leading to the conclusion that the spheres were interacting with the buffer in the neutral and alkali systems.

Fig. 1 shows representative DSC traces for microspheres removed at 10, 30 and 60 min intervals from the SMPB pH 5.9 and 7.0 media, washed and dried, with the corresponding quantitative data given in Table 1. The systems suspended in pH 5.9 media (Fig. 1a) and distilled water showed no significant alteration throughout the process and yielded profiles that were effectively identical to those of the untreated SACA. These results mirror those obtained from the earlier SEM studies in that SACA that had been subjected to the same dissolution conditions showed no appreciable surface changes.

A markedly different set of profiles was obtained for the pH 7.0 system (Fig. 1b). After 10 min in SMPB pH 7.0, a second endotherm was seen to occur after the initial melt peak. As SACA underwent longer periods of immersion, the area and peak temperature of this second peak increased, while the same parameters for the first peak diminished. Similar observations were made for SACA, which had been exposed to Sorensens pH 8.0, but on a more pronounced scale. After only 10-min exposure in this medium, the second peak was dominant with respect to the melt peak of SACA (Table 1). It should also be noted that no further thermal event was noted at temperatures up to circa 170°C.

To investigate these observations further, the thermal properties of SACA that had been left suspended in the three media during drying were examined. Previous studies (Robson et al., 1999) had indicated that the surface disintegration effects noted for the pH 7.0 and 8.0 media were considerably enhanced under these drying conditions. The DSC data indicated that the 'conver-

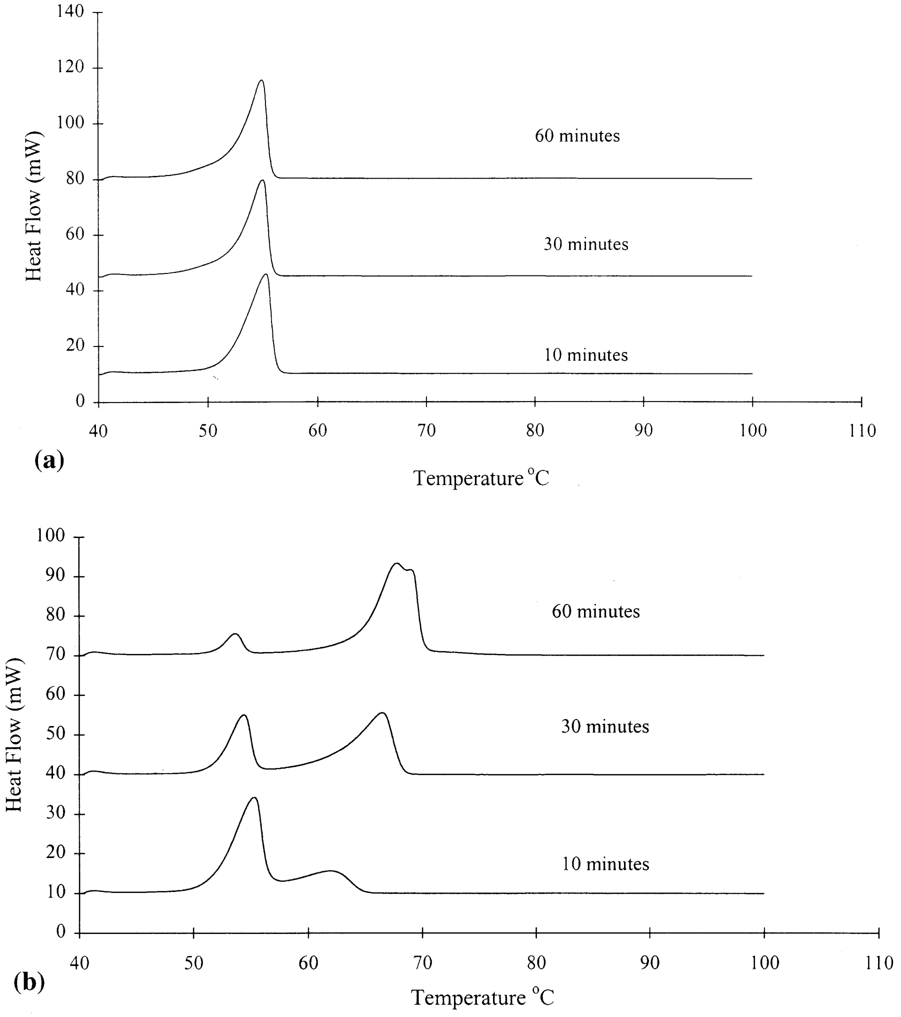

Fig. 1. DSC traces for SACA removed, washed and dried after 10, 30 and 60 min from (a) pH 5.9; and (b) pH 7.0.

Table 1
Peak melting points and enthalpy values for systems immersed in SMPBs and distilled water

Treatment	Time (min)	Peak melting point (°C)	Enthalpy (J g^{-1})
No pre treatment		55.6	156.1
Distilled water	10	56.1	159.6
	60	56.0	159.5
SMPB, pH 5.9	10	55.8	160.9
	60	55.3	156.1
SMPB, pH 7.0	10	55.1	100.4
		61.5	15.8
	60	53.7	13.8
		67.8	144.8
SMPB, pH 8.0	10	53.6	22.2
		69.0	126.0
	60	53.4	1.8
		69.6	137.2
Unwashed SMPB, pH 5.9[a]	10	55.8	149.8
	60	55.9	158.0
Unwashed SMPB, pH 7.0[a]	10	68.8	107.5
	60	69.1	111.2
Unwashed SMPB, pH 8.0[a]	10	69.5	99.5
	60	69.4	100.6

[a] These samples will contain an unspecified quantity of buffer salt, hence the specific enthalpy values are approximate values only.

Table 2
Peak melting points and enthalpy values for systems immersed in pH 7.0 buffers

Treatment	Time (min)	Peak melting point (°C)	Enthalpy (J g^{-1})
CPB, pH 7.0	10	56.6	69.2
		65.4	92.5
	60	54.2	46.4
		64.4	101.6
BAB, pH 7.0	10	55.2	156.1
	60	55.2	154.3
PBM, pH 7.0	10	55.7	152.7
	60	55.1	142.6

sion' from the lower to the higher melting peak was similarly enhanced (Table 1). The pH 7.0 and 8.0 samples showed complete conversion to the higher melting peak at all time intervals, while the pH 5.9 sample showed no such alteration. Once again, therefore, the DSC data mirrors the observations made with SEM.

3.2. Immersions in various pH 7.0 buffers

In a previous study, Robson et al. (2000) indicated that the release of cefuroxime axetil from SACA might vary considerably depending on the buffer used, even if the pH of that buffer was unchanged. More specifically, the authors indicated that SMPB pH 7.0 yielded the most rapid release, followed by boric acid buffer (BAB) and citrate–phosphate buffer (CPB) and finally phosphate buffer mixed (PBM). It was noted that the surface disintegration was only marked for the SMPB and CPB systems, leading the authors to conclude that the predominance of the disintegration and diffusion mechanisms varied between the systems, possibly as a result of differences in ionic strength.

Table 2 gives the corresponding DSC data for these systems. It is interesting to note that there is a clear correlation between the appearance of the higher melting peak and the tendency to exhibit surface disintegration, in that SMPB and CPB show both effects while PBM and BAB show neither. This indicates that the surface disintegration is associated with a fundamental change in the physical and/or chemical structure of the sample, while the diffusion mechanism does not involve this change to any discernible extent.

3.3. Immersion in PBM with different sodium contents

Robson et al. (2000) also investigated the effects of immersion in PBM containing varying amounts of added sodium. The authors found a maximum in the release rate at a sodium content of 0.05 M. While the spheres did exhibit some evidence for surface disintegration at high sodium levels, the effect appeared to be less extensive and more localised into specific regions than was seen for the SMPB and CPB systems and it was difficult to confidently ascribe a rank order with sodium content. Examination of Table 3 indicates that the DSC data shows a similar trend like that seen for the dissolution studies in that a maximum in the appearance of the second peak is seen at 0.05 M sodium. This second peak is either absent or considerably smaller for the 0.01, 0.15 and 1.0 M sodium systems.

Table 3
Peak melting points and enthalpy values for systems immersed in PBM pH 7.0 for 30 min

Sodium molarity (M)	Peak melting point (°C)	Enthalpy (J g^{-1})
0.007[a]	55.7	156.5
0.01	55.2	150.5
0.05	52.9	65.5
	64.4	96.1
0.1	56.7	131.5
	58.9	22.1
0.15	55.0	120.3
	62.1	41.1
0.2	55.0	159.1
1.0	54.8	159.4

[a] No added sodium.

3.4. HSDSC studies

Having established that immersion of the spheres in different buffers may lead to changes in the thermal properties of the SACA, it was of interest to investigate the thermal properties of the spheres while they were still suspended in the dissolution media. This can be achieved using conventional DSC using hermetically sealed pans, although there are numerous practical difficulties associated with this approach. Our preliminary studies indicated that HSDSC was the method of choice for making such measurements for reasons of sensitivity and ease of sample preparation. It should be noted that the reaction between the buffer and the spheres would be taking place during both the equilibration and the heating processes. However, the investigation of the suspended spheres may lead to insights into the mechanism of the interaction between the SACA and the media, as well as providing the possibility of developing a rapid screening method in order to predict the likelihood of such an interaction.

Fig. 2 shows the HSDSC response of the spheres in SMPB (pH 5.9, 7.0 and 8.0) and distilled water. Preliminary studies showed flat baselines for all the media in the absence of the spheres. The distilled water and pH 5.9 media showed a single peak which corresponded well to the melting point of the stearic acid. The pH 7.0 media showed three peaks with maxima circa 44, 53 and 65°C, hence the two higher peaks corresponded to the melting of the stearic acid and the 'new' species, as reported for the DSC studies. It is not clear what the lower temperature endotherm represents. The absence of such a peak using conventional DSC may indicate that it represents a kinetic response rather than equilibrium melting, i.e. the peak may represent the energetics of the conversion process, this reaction having already taken place for the DSC studies.

The effect of sodium addition on the HSDSC response for SACA suspended in PBM was then investigated (Fig. 3). The response was dominated by the melting peak corresponding to the stearic acid (peak II in Fig. 3), with a small peak seen for the circa 65°C response observed using conventional DSC (peak III). In addition, the small peak

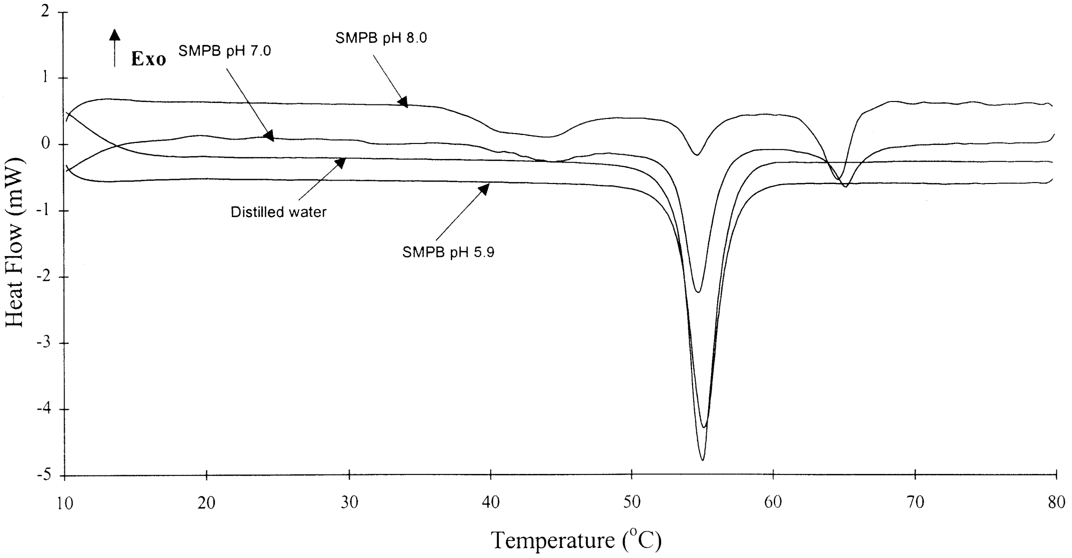

Fig. 2. HSDSC traces for SACA suspended in SMPB (pH 5.9, 7.0 and 8.0) and distilled water.

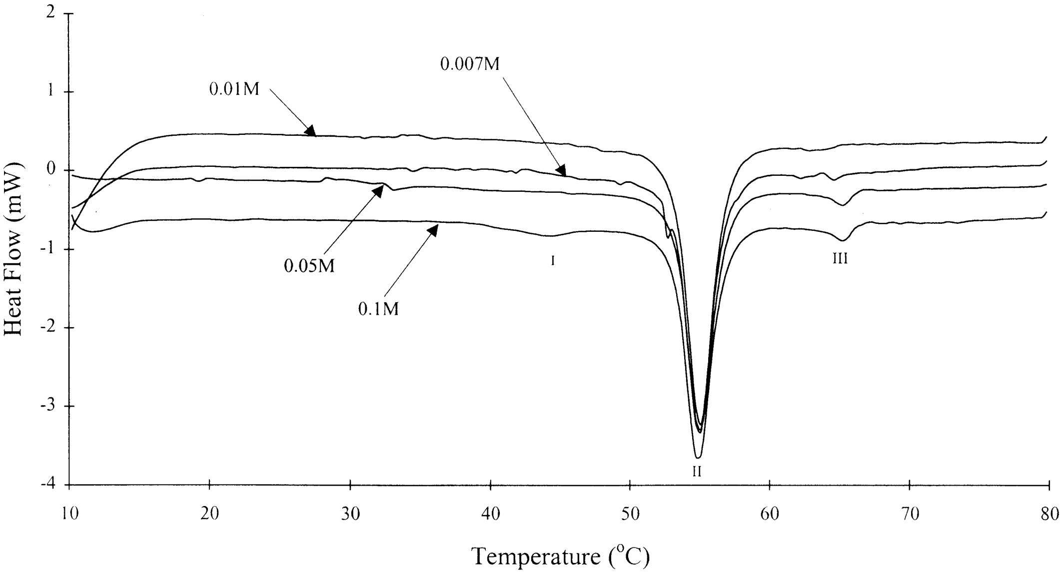

Fig. 3. HSDSC traces for SACA suspended in PBM pH 7.0 with varying sodium concentrations.

at circa 45°C reported above was seen for the 0.1 M sodium systems (peak I). It is therefore interesting to note that the HSDSC studies do not, in this case, reflect the conventional DSC response. This may be an indication of the less marked conversion seen for the systems containing added sodium. If the kinetics of the conversion reaction are such that it does not occur extensively within the timescale of the experimental run, then the approach may not be sufficiently sensitive to detect the change to the higher melting peak with confidence. That aside, the HSDSC method does provide a rapid and experimentally simple screening method which also allows observation of what may be a kinetic process associated with the change in physical integrity of the spheres.

4. Discussion

The thermal studies have clearly indicated that the immersion of SACA in certain buffers causes a fundamental change in the physical integrity of the spheres, as demonstrated by the appearance of a discrete peak at approximately 67°C and, in the case of the HSDSC studies, a lower temperature peak at circa 44°C. The interrelationship between the surface effects, the DSC data and the release profiles may be highlighted as follows. The DSC data may be assumed to indicate whether the system in question is undergoing a specific interaction with the buffer or not. On that basis, one may state that the SMPB pH 7.0 and 8.0 systems, the CPB systems and the 0.05 M PBM systems are undergoing such a reaction. There is therefore a correlation between the DSC data with both the dissolution rate and the surface morphology data in that, in general, the above systems showed rapid release profiles and changes in surface morphology (Robson et al., 1999, 2000). There are two apparent contradictions to this analysis. Firstly, the CPB systems showed extensive surface disintegration but did not exhibit as rapid a release profile as might be expected; this might be due to the high ionic strength of the medium inhibiting release despite the surface effects. Secondly, it was not possible to discern a clear trend between added sodium concentration and surface changes for the PBM systems, although alterations were noted. This may be a reflection of the sensitivity of the visual method for detecting such changes in physical integrity. Indeed, it should be emphasised that the excellent correlation between the DSC and release data for these systems strongly supports the suggestion of the release being a function of sodium-dependent changes in physical integrity. Overall, therefore, there does appear to be a good but not complete correlation between the surface changes seen using SEM, the dissolution behaviour and the conversion to the higher melting form seen using DSC. This lends support to the hypothesis that certain buffers will react with the stearic acid to form a new species, this transformation leading to alterations in the physical integrity of the spheres, in turn enhancing the drug release process.

This then leads to the question of the nature of the change incurred. The obvious interpretation would be a conversion to sodium stearate. However, the DSC data does not fully support this hypothesis, as sodium stearate undergoes a series of thermal events > 100°C (Small, 1986). No such events were seen in this case, hence the mechanism is unlikely to involve simple conversion. However, this observation does not in itself exclude the involvement of sodium stearate formation, as it is possible that a low melting form of the material may have been generated. In particular, the sodium stearate and stearic acid may be forming a eutectic or some other interactive species such as an acid soap. Finally, it should be borne in mind that binary mixes almost inevitably show different melting behaviour to the components alone (Lloyd et al., 1997), with significant lowering of the higher melting point seen as a result of the presence of the molten second component. Overall, on the basis of these studies, one can therefore state with some confidence that a new species is being formed on exposure to certain buffers. One can also suggest that the new species is not simply sodium stearate that but may be a binary form of this material with stearic acid. These issues will be addressed in a subsequent communication.

5. Conclusions

The study has indicated that immersion of SACA into various buffers may result in profound changes to the melting behaviour of the spheres, with a correlation found between the appearance of a second endotherm at circa 67°C, the surface integrity of the spheres and the drug release rate. The data presented here therefore support the hypothesis suggested by Robson et al. (1999, 2000) that drug release may occur via changes in the physical integrity of the spheres as a result of an interaction with the surrounding media. It may now be concluded that this interaction involves the generation of a new physical or chemical species, the identity of which has yet to be identified. In addition, the study has highlighted the use of HSDSC as a means of monitoring the thermal behaviour of the spheres while they are still immersed in the dissolution media.

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