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INVESTIGATION OF THE POLYMORPHISM OF SULFATHIAZOLE
BY A COMBINED DSC-HSM APPROACH

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A combination of differential scanning calorimetry (DSC) and hot-stage microscopy (HSM) has been used to investigate the polymorphism of sulfathiazole. The approach provides a unique insight into the polymorphic transformations and thermal behaviour exhibited by this compound. The results of the experiments show that sulfathiazole tends to crystallize as mixtures of polymorphs, although the literature methods of producing pure polymorph were followed. The use of light intensity profile obtained from the HSM images, as an alternative way to present results of HSM analysis is also introduced here. It was found to correspond very well with the DSC thermogram.

1. Introduction

Crystallizations of active pharmaceutical ingredients (APIs) particularly those that possess multiple polymorphic forms are amongst the most critical, yet least understood pharmaceutical processes. Statistically, about 85% of APIs exhibit polymorphism and 50% have multiple polymorphic forms [KAR06]. The polymorphs of a crystal can exhibit a variety of structures, which have different inter- and intra-molecular interactions. Thus, they have different free energies and consequently different physico-chemical properties and mechanical behaviours [HIL06]. These have an impact on downstream process operations, such as isolation, filtering and drying, and can affect the therapeutic properties of the final product. Therefore, it is desirable for pharmaceutical manufacturers to characterise extensively all known polymorphs of an API. Since all the available characterisation techniques may deliver dubious results, a combination of techniques is used to provide a comprehensive characterisation of the sample. A reliable technique that can quickly differentiate between different polymorphs and determine if a solid contains a pure polymorph or a mixture of polymorphs would be preferable.

One of the most widely used techniques for the investigation of polymorphism is differential scanning calorimetry (DSC). The technique basically involves the application of a heating/cooling signal to a sample and the subsequent measurement of the temperature and energy associated with thermal events including melting and polymorphic transformation [REA07]. The popularity of DSC is due to its simplicity and rapidity of measurement, as well as its requirement for a small sample size. Another technique for the investigation of polymorphism is hot-stage microscopy (HSM), which combines microscopy and thermal analysis, and allows visual observation of the behaviour of a sample through a microscope during heating or cooling [VIT98]. Recent technological advances have expanded the capabilities of HSM, for example with the use of software, which not only captures the image, but also performs an image analysis by computing the total light intensity. The latter may be calculated as the sum of the grey levels in all pixel (pixel value ranges from black = 0 to white = 255). As the image becomes brighter, the light intensity value becomes higher. This provides an alternative way of presenting the results of analysis.

Sulfathiazole has been chosen as the model system in this study. It has four polymorphs that are well characterised and clearly described in the literature ([AND01], [APP99],
Although the polymorphism of sulfathiazole has been extensively and repeatedly investigated by various researchers ([ANW89], [LAG81], [MES71]), it remains difficult to produce a pure polymorph; most of the time, the desired polymorph contains impurities from at least one other form ([APP99], [KOR01]).

In this paper, examples are presented of complementary application of DSC and HSM in the investigation of sulfathiazole polymorphism. The aim of this work is two-fold: (i) to give a contribution to the methods of investigating polymorphism through a combined DSC-HSM approach, and (ii) to gain more understanding on the polymorphism of sulfathiazole.

2. Experimental Section

2.1 Materials. Sulfathiazole was purchased from Sigma-Aldrich with a purity of 98%. The solvents used are deionized water, 1-propanol, acetone and chloroform. Except water, all other solvents were analytical reagent grade purchased from Fisher Scientific.

2.2 Crystallization of the polymorphs. Different polymorphs were generated using techniques available in the literature ([AND01], [ANW89], [APP99]). Form I was prepared by heating 0.5g of sulfathiazole in 50 ml of 1-propanol in a flask on a hot plate to dissolution, followed by natural cooling at 25°C. Crystals of form II were prepared in two batches using two different methods: (i) by boiling to evaporate a saturated aqueous solution of sulfathiazole at 60°C (0.8g in 50 ml water) in a beaker on a hot plate until almost dry, and (ii) by rapid cooling of a saturated aqueous solution of sulfathiazole at 60°C (0.5g in 100 ml water) from 80°C to 4°C at a set rate of 10°C/min in a crystallizer. Form III was prepared by slow cooling of a saturated aqueous solution of sulfathiazole (0.5g in 100 ml water) at a rate of 0.1°C/min from 80°C to 20°C in a crystallizer. Form IV was produced by cooling to 20°C a saturated solution of sulfathiazole in a 50:50 mixture of acetone: chloroform at 50°C (1.3g in 100 ml). All of the crystallized solids were vacuum filtered and immediately dried in a hot air oven at 105°C for 15 minutes. They were then further dried in a vacuum oven at 70°C for 3 hours to remove residual water.

2.2 Thermal analysis of polymorphs. Differential scanning calorimetry (DSC) – The thermal behaviour of the polymorphs was examined using a TA Instruments Q10. About 8 mg of sample was weigh into an aluminium pan and sealed hermetically. Analysis was carried out by heating the sample from 100 to 250°C at heating rates of 2, 10, 20 and 40°C/min under constant purging of nitrogen at 40 mL/min. An empty aluminium pan was used as reference in all the runs.

Hot-stage microscopy – To complement the DSC studies, the thermal behaviour of the polymorphs was visually examined using a Mettler Toledo FP90 hot-stage system and a Leica DMLM microscope at a heating rate of 10°C/min. The light intensity of the image in each captured frames was measured and recorded using Studio Capture version 2.1 (Studio86Designs).

3. Results and Discussion

3.1 Crystallization of the polymorphs. The microphotographs of the obtained crystals are shown in Figure 1. Based on the morphology, some of them appear to be in agreement with those of the pure polymorphs reported in the literature ([BLA98] and [ANW89]). According to literature, form I is a rod- or needle-like, form II is a cuboid, form III is a truncated hexagon and form IV is a plate-like hexagon.
3.2 Thermal analysis of polymorphs.

3.2.1 Form I. The DSC thermograms of form I at various heating rates are presented in Figure 2. Two major endotherm peaks were obtained by all runs. Generally, the first peak was significantly smaller than the second one. The onset of the first peak was observed between 121°C to 134°C, with the respective peak maxima between 125°C to 142°C, depending on the heating rate. The onset of the second peak was observed at almost the same temperature of 200°C for all the runs, but the peak maxima varied from 202.5°C to 207°C, with increasing heating rates. The movement of the onset peak and the peak maximum to higher temperatures as the heating rate increases is due to the effect of “thermal lag” within the system. The effect comes about as the result of the individual or combination of the thermal lag between the DSC’s furnace and the bottom of the sample pan, the lag between the bottom of the sample pan and the sample, and the lag throughout the sample [LEV07]. Therefore, although the use of higher heating rate will reduce the experimental time, but the thermal lag may affect the accuracy of the results. It can also be observed that the baseline of the curves is increasingly offset with an increase in the heating rate. The height and width of the peak are also increased, which consequently increased the detection limit, but reduced the resolution. In order to compromise with all these effects, a heating rate of 10°C/min was chosen for the subsequent thermal analysis experiments.
Figure 3 shows the DSC thermogram and the HSM light intensity of form I, while Figure 4 shows the snapshots of the crystals during HSM analysis. Both experiments, DSC and HSM, were conducted at a heating rate of 10°C/min. Based on the DSC thermogram in Figure 3, it can be seen that there are three major peaks. The peak with a maximum at 203.4°C corresponds to the melting peak of form I, while the peak at a maximum of 198.8°C corresponds to the melting peak of form II. Form I and form II are enantiotropically related, in which form I is stable from its melting point of 202°C down to 116.5°C, while form II is a stable form below 116.5°C [URA02]. Some crystals of form I may have transformed into form II at room temperature during storage and when they were heated in the DSC experiment, some of them transformed back into form I. The formation of the peak at the maximum of 131°C may correspond to the polymorphic transformation from form II to form I. The thermal lag effect as mentioned earlier may have contributed to the difference between the obtained transition temperature and that from the literature. These events, which were implied by the results of the DSC analysis, were verified by the HSM analysis. HSM shows no melting at the expected polymorphic transformation peak, but it shows an optical property change, as can be observed by the difference between the highlighted crystals in (a) and (b) in Figure 4. The change reduced the brightness and thus the light intensity of the crystals as indicated in Figure 3 by the reduction in the HSM light intensity from 131°C onwards. Melting of form II was indicated by the small increase in the value of the light intensity, while melting of form I was shown by the continuous increase in the value of the light intensity as all the crystals have melted. Figure 4 shows the melting of form II and form I in (c) and (d), respectively.

3.2.2 Form II. Two batches of form II crystals were prepared using two different methods: (a) by boiling to evaporate, and (b) by rapid cooling. Figure 5 shows the profiles of the DSC thermogram and the HSM light intensity of the respective crystals obtained.

Figure 4. Images of sulfathiazole crystals of form I during HSM analysis taken at (a) 100.0°C; (b) 142.6°C; (c) 198.8°C; and (d) 203.9°C.

Figure 5. DSC thermograms and HSM light intensity of sulfathiazole crystals of form II prepared by (a) boiling to evaporate; and (b) rapid cooling.
Figure 6 shows the snapshots of the crystals during HSM analysis of form II crystals prepared by rapid cooling (corresponds to Figure 5(b)). Based on Figure 5, the DSC thermograms for both batches show the presence of three major endotherm peaks. The first peak at a maximum between 161°C and 168°C was not a melting peak as verified by the HSM analysis. The peak was contributed by the change in the optical properties of the crystals, as shown by the difference in the appearance of the highlighted crystals between (a) and (b) in Figure 6. The phenomenon was indicated by the continuous reduction of the HSM light intensity value after 161°C for the batch prepared by evaporation and 168°C for the batch prepared by rapid cooling. The second peak at a maximum of 197°C on the DSC thermograms corresponds to a melting peak of form II, while the third peak at a maximum of 203°C corresponds to a melting peak of form I. The melting of form II and form I were verified by HSM analysis as shown in Figure 6 (d) and (e) and the subsequent increase in the HSM light intensity value from the corresponding melting temperatures in Figure 5. The HSM analysis was able to detect melting of form III as shown in Figure 6(c) and as indicated in Figure 5(b) by a small increase in the HSM light intensity value at that temperature. This melting of form III was not detected by the DSC. According to a report [LAG81], complete melting of form II at its melting temperature can only be observed if the crystals were purely form II. If only the slightest amount of form I present, form I will crystallise during the melting process of form II [LAG81]. This indicates that the crystals obtained by both methods are actually a mixture of form I, form II and a trace amount of form III.

### 3.2.3 Form III

The obtained DSC thermogram and the HSM light intensity for the sulfathiazole crystals of form III are presented in Figure 7. Three endotherm peaks were shown by the DSC thermogram. The first peak was formed at a maximum of 122°C, the second at 163°C and the third at 204°C. The third peak corresponds to the melting peak of form I. The formation of the second peak is in agreement with the observation by the previous researchers [ANW89], who deduced that at low heating rates, form III transforms into form I in the temperature range of 150 to 170°C without showing any melting (of form III at 175°C). Therefore the peak may correspond to the polymorphic transformation of form III to form I. The formation of the first peak may correspond to the polymorphic transformation of form II to form I as observed in section 3.2.1. This indicates that form II crystals may be present initially together with form III crystals. In general the HSM light intensity in this case shows the expected trend caused by the change in optical properties of the crystals after the polymorphic transformations and melting of form I towards the end of the experiment; the fluctuations in light intensity in Figure 7 are due to the movement of the crystals during heating. This indicates a limitation of this technique in that it can be very sensitive to noise and strongly dependent on the sampling spot.
3.2.4 Form IV. The results of the DSC and HSM analyses of the crystals obtained using the procedure for producing form IV of sulfathiazole are presented in Figure 8. The DSC thermogram indicates the presence of two endotherm peaks. The peak maximum of the first peak occurs at 143.5°C whereas the second peak occurs at 203°C. The trend of the thermogram is in accordance with the result obtained by previous researchers ([ANW89], [KOR01]), in which the first peak was said to be due to the transformation of form IV into form I, whereas the second peak indicates the melting of form I. The HSM light intensity confirmed that the first peak was not a melting peak since from 141°C onwards, its value is continuously reduced until it stabilised from 150°C onwards. A slow decrease of light intensity value is shown from 180°C until suddenly the value increased very fast starting from a temperature that corresponds to the melting of form I. The results show that only form IV was initially present in the sample, which in turn indicates the successful production of pure crystals of this form.

4. Conclusions

The crystallization of sulfathiazole polymorphs was investigated by a combined approach using DSC and HSM. The approach was able to provide a useful insight into the thermal behaviour of the polymorphic system. The use of the HSM light intensity as an alternative presentation of the results was found to be useful in describing and verifying thermal events. The results of the experiments showed that, although the methods to produce pure polymorphs were used; most of the time sulfathiazole crystallized as mixtures of polymorphs.

5. Abbreviations

DSC – Differential scanning calorimetry
HSM – Hot-stage microscopy

6. References


**Acknowledgements**

Financial support provided by the Engineering and Physical Sciences Research Council (EPSRC), U.K., (grant EP/E022294/1) is gratefully acknowledged. One of the authors (MRAB) is grateful to the Malaysian Ministry of Higher Education for a scholarship.