The Characterisation of Amorphous Material by Inverse Gas Chromatography

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The amorphous state of differently processed lactose samples has been studied by Inverse Gas Chromatography at infinite dilution. The dispersive surface energy was taken as a measure for the energetic state of the samples. Significant differences were found independent of particle size and amorphous content. The observed differences confirm distinctive amorphous states although the experiments allow no conclusion as to whether or not this is due to poly-amorphism. The varying changes in surface energy with amorphous content further suggest that the location and distribution of the amorphous phase within a crystalline matrix also affects the results.

Introduction

The characterisation of amorphous structures in drugs and excipients is of high interest in industrial practise. Various unit operations during formulation and manufacturing can cause an unexpected decrease in crystallinity (sometimes called "amorphisation" or vitrification). This is often associated with a reduced stability and shelf life of a material. Amorphous contributions may also be introduced deliberately as they can cause an increase in bioavailability and dissolution rate [1]. Therefore, many publications are dedicated to the characterisation of amorphous structures and to the determination of the amorphous content. Amongst these characterisation methods are vapour sorption, XRD, microcalorimetry and IR [2]. However, very little attention has been spent on the energetic state of the amorphous material and its form, e.g. are amorphous samples obtained from a spray-drying and a milling process energetically equivalent?

Inverse Gas Chromatography (IGC SEA) is a highly sensitive technique if used at infinite dilution conditions (low concentration of vapour) and is able to detect small differences in energy between similar materials, e.g. batch-to-batch variations [3, 8]. For this reason IGC SEA can be a useful tool to achieve a better understanding of the energetic state of amorphous materials. This paper investigates whether or not IGC SEA surface energy measurements can reveal any significant differences between lactose samples obtained from different processing routes as a function of the amorphous content.

Theory

IGC SEA measurements are typically carried out at infinite dilution. Under these conditions the probe molecules interact only with the high energy sites of the solid surface [4]. Buckton et al [5] have shown amongst others that the amorphous form of a material has a significantly higher energy than the crystalline forms since amorphous sites are reactive "hot-spots". For this reason it can be assumed that IGC SEA measurements at infinite dilution reflect predominately amorphous regions and experiments at higher concentration of probe vapour are required to take lower energy sites into account (finite concentration).

To study the energetic state of an amorphous sample the surface energy of the solid surface is measured. These experiments have been carried out at infinite dilution conditions and the calculations are described in detail in reference [6] and [7]. In short, various vapour probes are injected through the column containing the solid under investigation. The retention time of each probe is measured using a chromatographic detector. The higher the retention time, the stronger the interaction between vapour and solid sample, the higher the surface energy. To obtain the dispersive and specific contribution to the surface energy various non-polar and polar probes are injected.

Method

Several columns (SMS standard column with 3mm ID and 30 cm length) were fully packed with lactose samples of different amorphous contents. Different samples of amorphous lactose were obtained by processing α-lactose-monohydrate (Acros Organics UK, ACS grade, CAS 5989-81-1) via different routes, i.e. milling, spray-drying, freeze-drying and super-critical freeze-drying (SCF). Milling was carried out using a Minigrinder (Micromark, USA) for 10 min. Spray-dried samples were obtained from Imperial College, London while freeze-dried samples were provided by University of Utrecht. A change in amorphous content was performed by an exposure of the sample to a high humidity over different time periods. The amorphous content was measured by dynamic gravimetric vapour sorption (DVS) as an independent method using the method by Mackin et al [8].

All IGC SEA experiments were carried out using an SMS-*iGC* 2000 system. Surface energy measurements have been carried out at 303 K with a series of alkanes (hexane to decane) in duplicate. Helium was used as the carrier gas at 10 ml/min gas flow rate.

Before measurement a pre-treatment was carried out for 5 h at the measurement temperature and flow rate *in-situ*. Methane was used as the tracer molecule (dead-time determination). After pre-treatment probe molecules were injected by a 0.25 ml gas loop at infinite dilution conditions.

Calculations were performed by using the SMS-*iGC* Advanced Software v1.2 .

Results

The first step was to investigate the surface energy at infinite dilution of the samples "as received", meaning regardless of their amorphous content at the start of the experiment. The results are summarised in

Table 1.

Table 1. Dispersive surface energies of different processed lactose samples (measured at infinite dilution, 30 °C).

Lactose sample	Disp. Surface Energy (mJ/m^2)
Crystalline	37.6
Milled	45.2
Freeze-dried	48.1
Spray-dried	43.2

The differences observed between samples are overall significantly higher than the experimental error of <3%. Although the lower surface energy of the crystalline sample was expected (suggesting a pure α-lactose-monohydrate sample) the differences between the amorphous materials are surprisingly clear (maybe with exception of the difference between spray-dried and milled sample which is just outside the error margin). To understand the reason for the observed effects the impact of particle size and amorphous content has been studied in further experiments.

For the investigation of particle size dependence, crystalline lactose samples have been sieved and various sieve fractions were collected and the dispersive surface energy measured. The results are displayed in Figure 1.
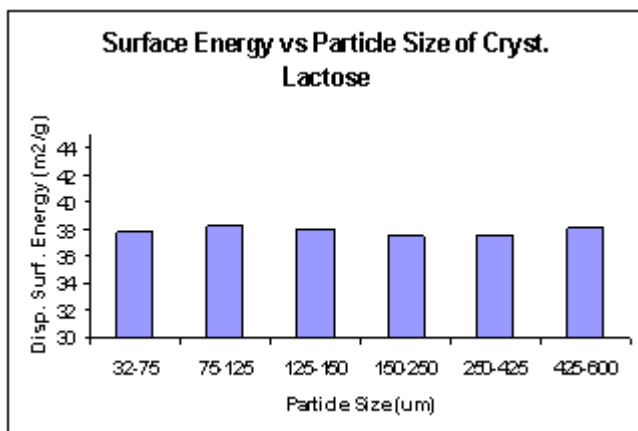
Figure 1. Dispersive surface energy for different sieve fractions of crystalline lactose.

It can be seen that the surface energies of the different samples yield to the same value (within the experimental error margin). This suggests that there is no dependence of the measured results on the particle size. This is not surprising since the surface energy is generally independent of particle size as it is normalised for surface area, i.e. changing the number surface area doesn't change the number of active sites per unit surface. However, in some cases changes in particle size are accompanied by morphological changes, such as a change in preferred crystal face or polymorphic transitions [9].

Another important aspect in this study is the amorphous content of the samples. Figure 2a shows the change in dispersive surface energy with amorphous content. As described above a stepwise reduction in amorphous contents has been performed by exposing the samples to high humidities for different time periods. Amorphous contents have been measured by DVS.
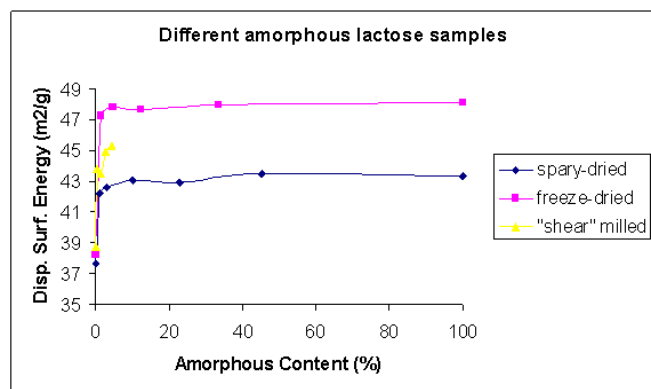

Figure 2a. Dispersive surface energy of differently processed lactose samples as a function of amorphous content. Amorphous contents have been measured by DVS.

The spray- and freeze-dried samples were initially nearly fully amorphous while the milled sample had an amorphous content of less than 5%. This agrees with the observations by Young et al [10] suggesting that the maximum amount of amorphous material produced is relatively small (compared to e.g. spray-drying) although the maximum amount depends a bit on the milling technology. Changes in the graph can only be observed at lower levels of amorphous content. For this reason the region below 10% has been inspected closer in Figure 2b. Above this region the surface energy is nearly constant for the spray- and freeze-dried sample.
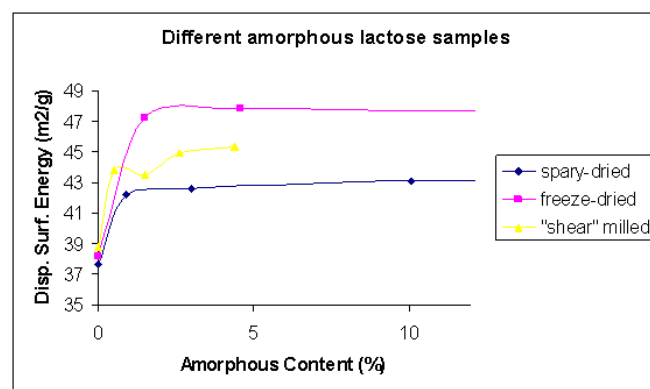

Figure 2b. Dispersive energy of differently processed lactose samples as a function of amorphous content (below 10%).

Only at very low levels of amorphous content (<5%), values start to decrease until they all reach roughly the dispersive surface energy value of crystalline lactose. The obtained results were not surprising since measurements were carried out at infinite dilution. At these conditions only the high energy sites, namely the amorphous regions, interact with the probe molecules. Only at very low amorphous contents do the lower energetic crystalline sites start to impact significantly on the overall interaction with the vapour probes and therefore on the surface energy.

For the milled samples, a similar trend was observed. The results suggest that the differences reported in Table 1 are not simply an amorphous content effect as they have been obtained at higher percentages where the surface energy changes little with the amount of amorphous material. These kinds of differences have been

observed in other studies, too [11] and it has been speculated whether they are related to poly-amorphism (multiple discrete energy states) as illustrated in Figure 3.
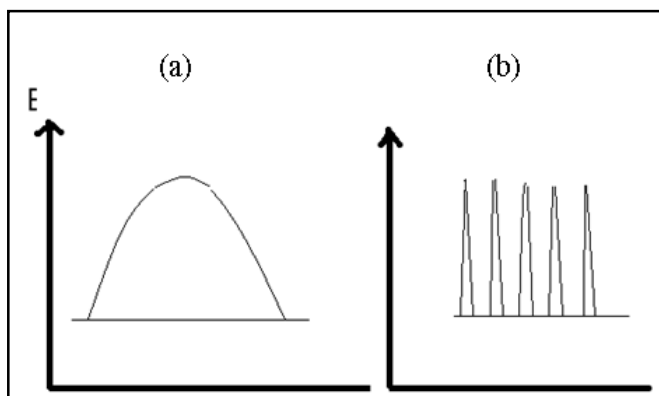

Figure 3. Illustration of poly-amorphism; (a) continuous distribution of energy and (b) multiple discrete energetic states.

From the results in this study this question can still not be answered satisfactorily. However, there are indications that the changes in surface energy at low amorphous contents might be related to the location and distribution of the amorphous form within a crystalline matrix. For example it is well known that the amorphous regions in milled materials are located on the surface of the particles, while for spray-dried samples the amorphous form is more or less evenly distributed throughout the particle [12].

Results were also compared with work published in [13]. This study investigated changes in the dispersive surface energy with changes in the amorphous content of *physical mixtures*. Different physical mixtures were obtained by mixing 100 % amorphous (from spray-drying) and 100 % crystalline lactose in different ratios. The results obtained by the authors are presented in Figure 4. Due to the differences in column silanisation (passivation) absolute surface energies are shifted to lower values and were therefore not comparable. However, the differences in surface energy were in the same order as for the spray-dried lactose investigated in this paper.
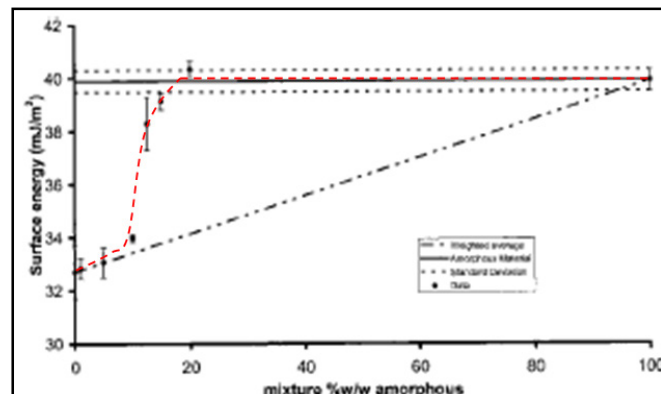

Figure 4. *Dispersive surface energy of a physical mixture (100% crystalline/100% amorphous, spray-dried) as a function of the content of amorphous lactose in the mixture (after [14]).*

When the change in surface energy as a function of amorphous content is considered it becomes obvious that the physical mixture behaves very differently at amorphous contents lower than 20 % (compared to the "real" amorphous spray-dried sample). This suggests that the location and distribution of amorphous regions in physical mixtures is very different in comparison to "real" amorphous systems and this affects the observed surface energy, similar to the effects described for a milled material in comparison to a spray-dried sample. This is also an interesting result in terms of calibration issues as it shows that physical mixtures and "real" amorphous system are energetically very different below 20 %. Having this in mind, it is not surprising that different techniques applied for amorphous content determination can give extremely different absolute results, particularly in the range of low amorphous contents.

Conclusion

Dispersive, acid-base and total surface energies as well as van Oss acid and base numbers have been determined on different minerals by IGC SEA. IGC SEA was shown to be a fast and accurate method for the characterisation of mineral surfaces in terms of their dispersive and acid-base properties. The results can also be used to predict affinities to bituminous components.

Acknowledgement:

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