The Use of Inverse Phase Gas Chromatography to Measure the Surface Energy of Crystalline, Amorphous, and Recently Milled Lactose

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Received November 28, 2000; accepted January 22, 2001

Purpose. To assess differences in surface energy due to processing induced disorder and to understand whether the disorder dominated the surfaces of particles.
Methods. Inverse gas chromatography was used to compare the surface energies of crystalline, amorphous, and ball milled lactose.
Results. The milling process made *ca* 1% of the lactose amorphous, however the dispersive contribution to surface energy was 31.2, 37.1, and 41.6 mJ m^{-2} for crystalline, spray dried and milled lactose, respectively. A physical mixture of crystalline (99%) and amorphous (1%) material had a dispersive surface energy of 31.5 mJ m^{-2}.
Conclusion. Milling had made the surface energy similar to that of the amorphous material in a manner that was very different to a physical mixture of the same amorphous content. The milled material will have similar interfacial interactions to the 100% amorphous material.

KEY WORDS: inverse gas chromatography; lactose; amorphous; milling; processing induced disorder; dispersive surface energy.

INTRODUCTION

It is well established that materials can become partially amorphous as a consequence of milling. Materials with a relatively small amorphous content (in the region of a few percent of the total mass) can exhibit significant changes in behavior. These effects are not well documented in the literature, as the data are usually held confidentially by pharmaceutical companies, but the changes in behavior can relate to difficulties in wet granulation of micronised drugs and performance variability in micronised inhalation products. A number of authors have assumed that the reason why small amounts of amorphous content can give rise to a large change in behavior of the material is that the amorphous content may well occupy most of the surface of the material. Elamin *et al.* (1) attempted to show that processing induced disorder was at the surface of particles, by use of sequential solubility determinations on the same sample. Although this work provided an indication that milling disrupted the surface more than the bulk, the data were not conclusive. Other workers (2) have shown that there can be differences in surface energy between micronised and unmicronized material, possibly due to surface disorder. If it is true that milling can make the surface of a particle amorphous, then a sample that has a few percent amorphous content could in fact have a totally amorphous surface. This totally amorphous surface may interact differently to crystalline material during processes such as wet granulation or the formation of inhalation products. To date there has not been a clear demonstration of the effect of small amounts of induced disorder on the surface energies of particles.

In this study a purpose built inverse gas chromatography apparatus has been used to study the surface energies of crystalline, amorphous, and recently milled lactose. Inverse gas chromatography has been used recently as a means of studying the surface energies of a number of pharmaceutical systems (2–8) and offers significant advantages over contact angle methods when comparing the surface nature of particulate samples (5,8)

Measurement of Surface Energies

The retention times for a homologous series of alkane probes were used to calculate the dispersive surface energy of the surface under investigation. The equations for this analysis have been firmly established for some time (15). The measured parameter is the retention time of the probes, t_r. However, the equations deal with the net retention volume, $V_n{}^0$, which eliminates any difference in flow rate and temperature of experiments. These net retention volumes are calculated using equation 1:

$$V_n{}^0 = jF(t_R - t_0)\frac{T}{273.15} \quad (1)$$

where T is the column temperature, j is the James-Martin pressure drop correction factor, F is the exit flow rate measured at 1 atm and 273.15 K, t_R is the retention time of the interacting probe and t_0 is the mobile phase hold up, commonly referred to as the "dead time". The relationship between the net retention time and the free enthalpy of adsorption, ΔG, is given in Eq. 2:

$$\Delta G^0 = RTLnV_n{}^0 + C \quad (2)$$

where R is the gas constant and C is a constant that depends on a chosen reference state. These are further discussed in other texts (16,17). While it is important to understand their meaning, they are not used further in this study. It is also possible to relate ΔG to the energy of adhesion between a probe molecule and a substrate, W_A by equation 3:

$$\Delta G^0 = N_A a W_A \quad (3)$$

where N_A is Avagadro's number and a is the area of surface occupied by the probe molecule. The work of Adhesion can be obtained from the surface energies of the two interacting materials, as shown in equation 4:

$$W_A = 2(\gamma_S \gamma_L{}^D)^{1/2} \quad (4)$$

where $\gamma_S{}^D$ and $\gamma_L{}^D$ are the dispersive components of the surface energy of the substrate and adsorbate.

The dispersive interactions, $\gamma_S{}^D$ are those relating to long range interfacial forces and include London and van der

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Waals interactions. The method used to calculate the dispersive component of the surface energy of the substrate is that used by Schultz *et al.* (18). Combining Eqs. 3 and 4, Eq. 5 is obtained.

$$RTLnV_n{}^0 = 2N_A(\gamma_S{}^D)^{1/2}\, a(\gamma_L{}^D)^{1/2} + C \qquad (5)$$

By plotting a graph of $RTLnV_N{}^0$ versus $a(\gamma_L{}^D)^{1/2}$, which are both properties of the adsorbate, for a homologous series of hydrocarbons, a straight line is obtained. The gradient yields the dispersive surface energy, $\gamma_S{}^D$.

MATERIALS AND METHODS

Apparatus

Experiments were performed using an inverse gas chromatograph built by Surface Measurement Systems Ltd, comprised of three modules (9) and illustrated in Fig. 1. An integrated flow control system, employing a series of mass flow controllers (MKS Instruments), was used to prepare mixtures of the dry helium carrier gas and the elutant vapor. An automated injection valve was used to inject 250 μL of the elution mixture (appropriate mix of elutant vapor and dry helium) into the reference gas (dry helium) flow, which carried it through the column to the detectors. All injections of elutant vapors are given below in concentrations of % v/v of the saturated elutant vapor. A Hewlett Packard 6890 series gas chromatograph (GC) oven was used to control the solvent temperature. The 6890 GC data acquisition system was used to record data from a thermal conductivity detector (TCD) and flame ionisation detector (FID) plumbed in series at the column outlet. This combination of detectors allowed sensitive analysis of both organic vapor elution and on column relative humidity levels (although RH was not raised above 0% for these experiments). A separate, purpose built column oven was used to control the sample (column) temperature between room temperature and 90°C. The glass columns used were 6 mm OD, 2 and 3 mm ID, and approximately 220 mm long. Columns were treated with a dimethyldichlorosilane solution (DMCS, Repelcote® BDH) and washed thoroughly, to passivate the surface prior to use. DMCS silanised glass wool (Chrompack) was used to hold the powdered samples in place.

The whole system was fully automated by purpose written control software (SMS *i*GC Controller v1.3) and the data analyzed using the SMS *i*GC Analysis macros. Chromatography grade stainless steel tubing of 0.75 mm internal diameter was used throughout to minimize dead volume and wall-solute interactions within the system.

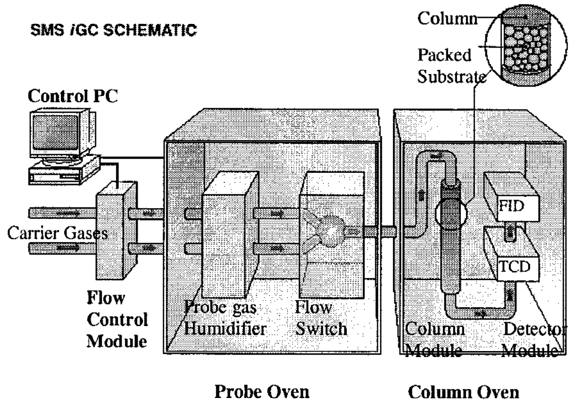

Fig. 1. A schematic diagram of the SMS iGC used in these experiments.

Surface energies were determined by eluting 2% v/v injections of solutes. These were methane (BOC, research grade), for the inert reference and the hydrocarbons heptane (Fisher, HPLC grade), octane (Lancaster, 99%+), nonane (Aldrich, 99%+), and decane (Acros, 99%+). The solutes were held at an oven temperature of 27°C throughout the experiments described. Column temperature and relative humidity were 30°C and 0% RH . The flow rate used was 10 mL min^{-1}, as this gave a good balance between the speed of elution, the shape of the solute peaks (Gaussian), and the pressure drop experienced across the column.

Sample Preparation

Crystalline α-Lactose Monohydrate

The absence of amorphous content in a sample of α-lactose monohydrate (Acros, ACS grade reagent) was confirmed by exposing the material to an environment of 75% RH in a sealed ampoule of an isothermal microcalorimeter (Thermal Activity Monitor, Thermometric), as described previously (10). This showed the complete absence of any crystallization event. Previously (11) it has been shown that amorphous contents of less than 0.1% can be observed using this technique, thus if any amorphous form of lactose had been present an exothermic crystallization event would have been observed.

The material was also investigated using differential scanning calorimetry (Perkin Elmer DSC 7). This data showed the two peaks associated with α-lactose monohydrate, namely a dehydration occurring at *ca* 147°C and a melt at *ca* 217°C. The β-lactose content was assessed by use of gas chromatography (12). This revealed that the β content of the sample was 4.4 ± 0.1%. The same batch of α-lactose monohydrate was used for the experiments that follow.

Amorphous Lactose

The amorphous lactose was prepared in house by spray drying (Buchi spray drier). Conditions were chosen such that the product would be 100% amorphous, as determined previously (13). The amorphous nature of the lactose was confirmed by use of isothermal microcalorimetry, using the method described above (Thermal Activity Monitor, Thermometric), giving an apparent enthalpy of crystallization in keeping with 48 J/g that has been reported in the literature (10). Between preparation and packing the product was stored in a dessicator at 20°C and 0% RH over P_2O_5. Three different batches of spray dried lactose were prepared.

Milled (Partially Amorphous) Lactose

A sample of the crystalline α-lactose monohydrate was subjected to milling in a simple ceramic ball mill for a period of 4 h. The mill and powder were purged with helium to prevent the crystallization of any amorphous content induced by the physical process. The product was stored at 20°C in a dessicator at 0% RH over P_2O_5. Isothermal microcalorimetry was used to confirm the presence of amorphous material within the sample. Although isothermal microcalorimetry is well suited to detection of small amorphous contents, it is not an ideal method for quantification, consequently the amount of amorphous content induced was determined using solution calorimetry. The enthalpy of solution has been shown to vary in a linear manner, as a function of the proportion of amorphous and crystalline lactose in a sample (14). In this study, the enthalpy of solution of the milled material was measured by accurately weighing approximately 270 mg into a thin walled glass ampoule and then storing in a vacuum oven at 50°C to completely dry the sample before commencing analysis, prior to double sealing the ampoule with wax. The ampoule was then temperature equilibrated and subsequently broken into a solution calorimeter (Solcal, Thermometric) containing 100 ml of water at 25°C with a stirring rate of 600 rpm. The measured enthalpy was compared to the data reported previously (14) to calculate the amorphous content. One batch of milled sample was used for the experiments that follow.

Column Packing

The lactose powders were passed through a 385 μm sieve prior to being packed into the glass columns by vertical tapping. Progress was monitored visually while tapping for at least 15 min. Tapping continued until there were no visible cracks, hollows, or channels in the body of the powder. Both ends of the columns were loosely stoppered with silanised glass wool. Columns were subject to a conditioning cycle prior to analysis. This consisted of inserting the column into the IGC and the experimental conditions of column temperature and gas flow were set. The column was left under these conditions for a minimum period of 5 h at 0% RH. The signal traces from the instrument were monitored to ensure that there were no changes still occurring. To ensure that the equilibration process had been completed the TCD trace as well as the retention time and peak shapes of the eluted peaks were monitored at various stages during the experiments. Any changes in these peaks during the course of the experiment would indicate that the substrate was not yet at equilibrium with the applied conditions and further conditioning was carried out. Whenever the same column was investigated under more than one set of experimental conditions, the sample was allowed to re-equilibrate using a similar procedure.

RESULTS

Amorphous Content of the Milled Sample

To assess the amorphous content of the milled lactose sample a solution calorimetry experiment was performed in accordance with that described by Hogan *et al.* (14). A measured enthalpy of solution of 55.2 J g^{-1} was obtained. Using the linear relationship established by Hogan *et al.* (14) between the enthalpy of solution and known amorphous content, it was determined that the amorphous content of the milled sample was 0.7 % ± 0.3% ($n = 2$).

Measured Surface Energy Values

The surface energy of the crystalline sample was found to be 31.2 ± 1.1 mJ m^{-2}. This value was determined from measurements on 5 different columns from the same sample batch, with 3 measurements of surface energy performed on each column (a total of 15 measured values). The difference between the 5 packed columns was statistically insignificant. This confidence led to the reduction in the number of columns packed for each substance under investigation, but the number of replicates was increased.

Three different batches of amorphous (spray dried) lactose were investigated. As would be expected given the fact that amorphous material are in random orientation, a range of surface energies was obtained from the different batches (35.5 to 40.3 mJ m^{-2}). Two columns were packed for each batch and each column was tested 6 times, giving 12 measurements for each batch of spray dried material. Each batch gave self-consistent results, with narrow standard deviations, even when comparing different packed columns. The variability in the data was directly attributable to the differences in surface nature for the different spray dried batches. Taking these three batches as a whole, the mean dispersive surface energy of amorphous (spray dried) lactose was 37.1 ± 2.3 mJ m^{-2}. However, it is very clear that the amorphous state does not define an exact surface nature, consequently amorphous material prepared by different methods could reasonably exhibit a different surface nature.

The dispersive surface energy of milled lactose was determined to be 41.6 ± 1.4 mJ m^{-2}. This comprises a minimum of 4 measurements on each of 3 different columns packed with the same milled sample (12 replicate determinations in total). Once again there was no difference between data generated from different packed columns, hence the number of columns packed was reduced to 3 in this section, but the number of replicates was increased.

DISCUSSION

The difference between the measured dispersive surface energies of the crystalline and the amorphous forms of lactose is substantial and well outside the spread of data for the different batches and different packings of columns. The amorphous values measured are in excess of 4 standard deviations distant from the crystalline data obtained. Consequently, it can be confirmed that amorphous lactose has a surface energy that is very different to that of crystalline α-lactose monohydrate. It is not surprising that the amorphous form gives a higher surface energy than the crystalline form, as the amorphous form is thermodynamically unstable and thus in a higher energy state. The range of surface energies obtained for the amorphous form is also not unexpected, the amorphous state contains disordered molecules and it is perfectly possible to envisage a range of orientations of the lactose molecules on the surface of each amorphous particle. It is indicative of the fact the amorphous state, in contrast to the crystalline state, is by its very denotation not well-defined. Each batch of spray dried material could reasonably have a different surface orientation of molecules and hence a slightly different surface energy while still remaining amorphous.

The dispersive surface energies obtained for the amorphous and milled lactose are similar, however the amorphous material has a higher surface energy than that recorded for the spray dried samples. This indicates that the orientation of the molecules in the milled samples is different to that observed for the spray dried batches. It would also be expected that amorphous material made by other techniques, such as quenching (if possible for the selected material) or freeze drying, could be different to that obtained by milling or by spray drying. A different surface energy does not mean that one surface is more or less amorphous, but does reflect different orientations and spacing between the surface molecules, giving rise to the different energy states.

The surface energy data indicate that the milled sample has a surface that is highly amorphous, yet the amount of amorphous content induced during the milling process is only of the order of 1% of the total sample mass. The surface energy values obtained for both the amorphous and milled samples of lactose are also very similar to values previously reported in the literature for α-lactose monohydrate of 40 to 44 mJ m^{-2} (4). From the published data, we believe that the samples analyzed in these previous papers may well have contained small but measurable proportions of amorphous lactose on the surface.

In the current study, the instrument was operated at infinite dilution. Infinite dilution was proved by injections of different concentrations of probe vapor and ensuring that the retention time did not vary with concentration. It could be argued that the use of infinite dilution of the probe on the powder column surface could result in the data being distorted by a few higher energy sites in the column. Consequently, it could be possible that the milled sample contained some amorphous particles mixed with mostly crystalline particles, but the measured response was dominated by the amorphous particles. To test this a physical mixture of 1% amorphous and 99% crystalline particles was prepared. The physical mixture was mixed in a Turbula mixer for 30 minutes to ensure homogenisation. This mixture was packed into two different columns and the dispersive surface energy ($n = 4$ on each column, thus 8 replicate determinations, NB it was felt unnecessary to pack more than 2 columns as the data were consistently showing that column packing did not alter the measured surface energy) was found to be 31.5 ± 0.4 mJ m^{-2}. The dispersive surface energies for each sample are shown in Table I to aid comparison. The surface energy for the mixed sample is very close to that of the crystalline sample (31.2 mJ m^{-2}) and distinctly different to the values recorded for the amorphous material (37.1 mJ m^{-2}, batches ranging from 35.5–40.3 mJ m^{-2}) and the milled sample (41.6 mJ m^{-2}). The possibility remains that the surface energy obtained for the mixture is a weighted average of the two components present. The weighted average value for 99% crystalline and 1% amorphous has been calculated to be 31.3 mJ m^{-2}, this is extremely close to the value obtained experimentally for the 1% amorphous physical mix, especially given that there is always some uncertainty about the composition of a physical mix. Thus, a mixture containing 1% amorphous material has a surface energy that is dominated by the 99% crystalline material, whereas the milled sample that has *ca* 1% amorphous content has a surface energy similar to the wholly amorphous spray dried material. This provides strong evidence that the milled material does indeed have an amorphous surface and a crystalline bulk.

Table I. The Dispersive Surface Energy Of Lactose Determined Using IGC (Mean ± SD)

Sample	Dispersive surface energy/mJ m^{-2}
Crystalline	31.2 ± 1.1
Spray dried (completely amorphous)	37.1 ± 2.3
Milled (0.7% amorphous)	41.6 ± 1.4
Physical mixture 99% crystalline 1% amorphous	31.5 ± 0.4

CONCLUSION

There is a substantial difference between the surface energy of amorphous and crystalline α-lactose monohydrate. Ball milling of α-lactose monohydrate has induced the formation of around 1 % amorphous material within the sample. Although this forms only a tiny fraction of the bulk sample the data presented here indicate that the amorphous form is present on the powder surface and this is likely to be significant with respect to the interaction of the milled powder with other phases in formulations. The use of IGC allows this surface amorphous material to be detected. Given that the difference in performance between crystalline and partially amorphous materials is often related to their interfacial interactions (wet granulation and inhalation suspensions or dry powder mixing), it had been assumed that the surface nature of the partially amorphous form must be different from that of the crystalline. It is shown here that materials with small amounts of amorphous content can have very different surface energies to the crystalline form.

ACKNOWLEDGMENTS

AstraZeneca (Charnwood), Pfizer Central Research, Roche Discovery Welwyn and SmithKline Beecham are acknowledged for financial support for HEN and consortium funding for this project.

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