Pharmaceutical Research, Vol. 21, No. 8, August 2004 (© 2004) *Research Paper*

Inverse Gas Chromatography: Investigating Whether the Technique Preferentially Probes High Energy Sites for Mixtures of Crystalline and Amorphous Lactose

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Received January 7, 2004; accepted April 9, 2004

Purpose. Inverse gas chromatography (IGC) is rapidly gaining popularity as a method for assessing powder surface energy. It is vital to understand what IGC measures if results are to be useful. This work examines the view that IGC preferentially measures high-energy sites on a powder surface.

Methods. Mixtures of amorphous (high-energy) and crystalline (lower energy) lactose particles were prepared and tested using IGC with nonpolar probes. The surface area of the particles was measured *in situ* in the inverse gas chromatograph.

Results. The results were weighted averages of the surface energy for amorphous and crystalline material until the amorphous content exceeded 15% w/w of the sample, after which the surface energy become equivalent to that of the amorphous form. The amorphous content dominated when the surface area was 40% of the total area. Given that the amorphous particles were much smaller and adhered to the crystalline ones, it is reasonable to conclude that many (most) of the binding sites on the surface of the crystalline particles were masked by the amorphous particles by the time that the amorphous content dominated the surface energy measurements.

Conclusions. IGC does not simply measure the high-energy sites in the packed column, but equally there is a complex process that results in measured data on mixtures not being a weighted mean of the surface energy of the two components.

KEY WORDS: amorphous; inverse gas chromatography; surface area; surface energy.

INTRODUCTION

Inverse gas chromatography (IGC) has been used for the measurement of the surface properties of a wide range of materials, including pharmaceutical powders (1–9). IGC has the advantage of using the powder essentially in the as-received state, whereas contact angle techniques usually require sample preparation, generally through compaction or adhesion. This does, however, give rise to some differences between the two types of techniques, with IGC usually yielding higher values for the dispersive surface energy (5). This has been explained on the basis that IGC is performed in the infinite dilution regime, in which an adsorbed molecule ideally has no other adsorbate molecule within close enough proximity to allow any adsorbate-adsobate interaction. The adsorbed molecules are essentially isolated, and no interactions occur either in the vapor phase, on the surface, or between adsorbed and vapor molecules. It is this lack of interaction or competition for adsorbate sites that leads to the surmise that the highest energy sites of the sample surface will be probed in preference to those of a lower energy. Hence the hypothesis is that IGC will probe the highest energy sites preferentially and so yield a value for the dispersive surface energy that is representative of these sites and not the surface as a whole. In contrast, contact angle studies probe the entire range of surface sites. The energy returned will be an average of the different available sites, and thus is expected to be lower than IGC values. In this study we attempt to investigate this hypothesis.

Physical mixtures of amorphous and crystalline lactose have been used many times as a method of producing samples that contain a defined percentage of amorphous material. In this study we have taken the physical mixtures to observe the change in measured surface energy as a function of amorphous content. If the hypothesis described above is correct (i.e., that IGC preferentially detects high-energy sites), any physical mixture should have a measured surface energy that is approaching that of the higher energy component irrespective of the actual composition of the mix.

MATERIALS AND METHODS

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, Jarfalla, Sweden), as described previously (10). This showed the complete absence of any crystallization event. Previously, it has been shown that amorphous contents of less than 0.1% can be observed using this technique (11); 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, using nonhermetically sealed aluminum pans, with a scan rate of 10 °C/min, using indium to calibrate). These data showed the two peaks associated with α–lactose monohydrate, namely a dehydration occurring at ca. 420 K and a melt at ca. 490 K. The lactose content was assessed by use of gas chromatography (12). This revealed that the β-(beta-) content of the sample was 4.4 ± 0.14%.

Amorphous Lactose

The amorphous lactose was prepared in-house by spray-drying (Buchi spray dryer). 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. Between preparation and packing the product was stored in a dessicator at 20°C and 0% RH over P_2O_5. As was shown in previous studies (7,8), there is considerable variation in amorphous lactose batches and in the surface energy measured, thus the same batch of spray-

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dried lactose was used in all the experiments reported here; the surface energy of the stored material remained consistent under these conditions for the duration of this work.

Mixtures

The mixtures of the amorphous and crystalline lactose were prepared by initially sieving both components after storage over P_2O_5. The components were weighed into sample bottles and subsequently mixed for 20 min in a Turbula mixture. The mixtures were then stored over P_2O_5 before the surface energy measurements were undertaken. Mixtures prepared were 1, 5, 10, 12.5, 15, and 20% w/w of amorphous lactose in crystalline lactose. The surface energy of the batch of spray dried lactose was measured, as was the surface energy of the crystalline lactose.

Inverse Gas Chromatography

Experiments were performed in a commercial IGC instrument (Surface Measurement Systems, London, UK). This apparatus has been described in some detail previously (7,8). The experiments were performed with a helium carrier gas (BOC, research grade) with methane (BOC, CP-grade) for the reference injections. The hydrocarbon probes used to determine the dispersive surface energy were heptane (Fisher, HPLC grade), octane (Lancaster, 99%+), nonane (Aldrich, 99%+) and decane (Acros, 99%+). Experimental conditions were 10 ml min^{-1}, 0% RH, and a column temperature of 303 K. Injections were all 3% v/v saturated vapor unless stated otherwise. Each dispersive surface energy measurement is the result of six replicate measurements on a single packed column of material. For the sorption isotherm measurements, a series of injections of a single solute, octane, was performed. These increased in size from 10% v/v to 90% v/v in 10% v/v intervals, while flow, humidity, and temperature conditions were as stated before.

Surface Area Determination

Surface area was measured using the IGC instrument using the experimental conditions described above. A series of injections of a single solute of increasing concentration (0–90% P/Po) was performed, which was intended to cover the finite dilution range. Octane was selected for the probe, as it showed good separation from the methane peak and minimal deviation from a Gaussian peak shape. This indicates that it is not absorbed into the amorphous sample and thus allows comparison of the surface area of the two materials.

Scanning Electron Microscopy

The components and mixtures were examined using a Philips XL 20 SEM system. The samples were gold-coated in an Emitech K550 sputter coater for 3 min at 30 mA. Samples were scanned at a voltage of 10 kV with magnifications of 387× and 773×.

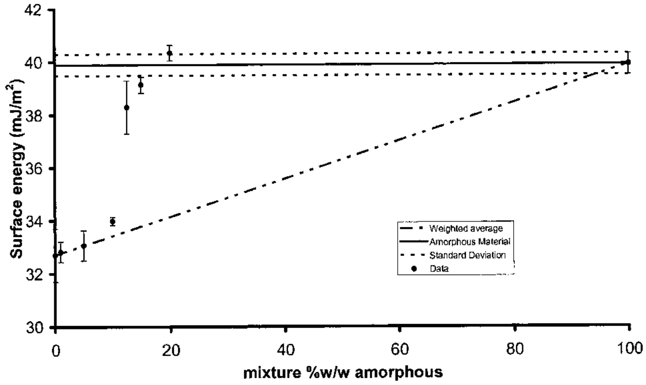

Fig. 1. The surface energy of a range of amorphous and crystalline lactose mixtures as a percentage of amorphous content (w/w).

RESULTS

The dispersive surface energies of the pure amorphous and crystalline samples and the amorphous/crystalline mixtures (%w/w amorphous content) are displayed in Fig. 1. The method used to determine the surface energy values was as described by Schultz *et al.* (14). The surface energy obtained from the pure amorphous material is shown as a horizontal solid line, with the standard deviation of measurement shown as the dotted lines above and below. As can be seen, the surface energy of the mixture approaches that of the pure amorphous material at only 20% w/w amorphous material. It might be expected that the surface energy of the mixture would either approximate to the value obtained from the weighted average of the two constituents of the mixture, or be dominated by the high energy amorphous sites even when the amorphous content is a very low % of the total sample. As can be seen, the surface energies obtained for 1 and 5% w/w amorphous material do fall on the weighted average line, hence they are not dominated by the higher energy sites on the amorphous material. Above 5% amorphous content however, there is a significant deviation from the weighted average.

Scanning electron micrographs (Figs. 2a–2e) showed (as expected) that the amorphous particles were small spheres (sub 10 μm) (Fig. 2a), and the crystals were large tomahawk particles with a great many small fragments (Fig. 2b). The amorphous particles could not be sized using Malvern light scattering, as it proved difficult to deaggregate in any liquid without giving rise to dissolution or crystallisation of the particles. Given that size was not deemed to be as important as surface area (see below), no further efforts were made to get accurate size distribution data.

The mixtures were prepared by weight and consist of the larger crystalline particles with a selection of smaller particles adhered. At low amorphous content, the adhered particles are predominately crystalline (Fig. 2c), with only the occasional amorphous particle visible. By the time the mixture contains 20% amorphous material by weight, then the amorphous particles are evident (Fig. 2e). They cannot be said to be covering the crystalline surface completely, but there are significant numbers of them. They appear to congregate particularly on the edges of the larger particles and together with clusters of smaller crystalline particles (scanning electron micrographs not shown).

As the particles are of two distinct sizes, it would be beneficial to compare the mixtures by percentage surface area of amorphous material, rather than by mass. In order to do this, it was necessary to determine the surface areas of the two components. It is well understood that assessments of surface area will depend on the probe that is used in the measure-

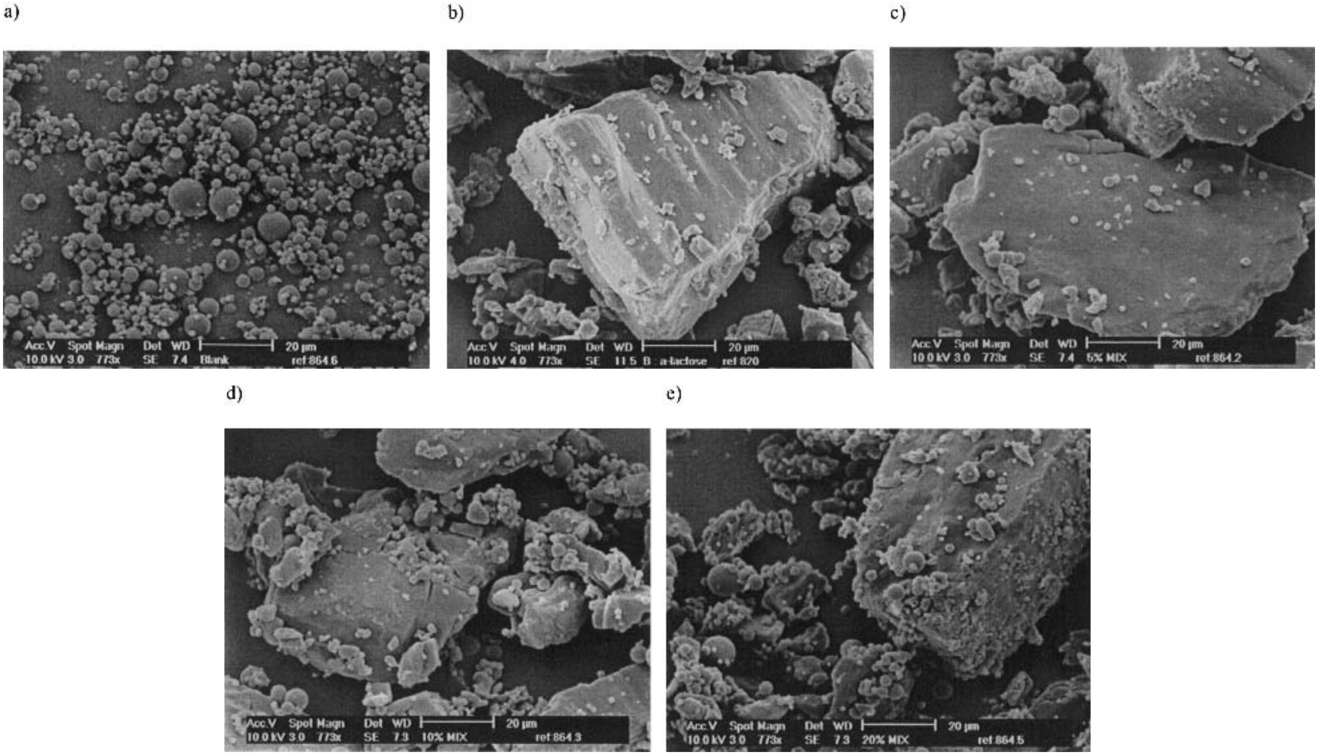

Fig. 2. SEMs of (a) amorphous lactose, (b) crystalline lactose, (c) 5% w/w amorphous mixture, (d) 10% w/w amorphous mixture, and (e) 20% w/w amorphous mixture (all at magnification ×773).

ment, hence an area calculated by one method may not be the effective area that is seen for that powder in a different situation. In this case it was possible to measure the surface area with one of the probes that was used to calculate the surface energy, with the same sample packed in the same column for both experiments. Hence there is very high confidence that the surface area that is measured is the relevant surface area that is probed during the surface energy measurements. The chromatogram obtained on crystalline lactose is displayed in Fig. 3. As can be seen, the peaks increase in height with increasing injection size. The time of the peak maxima also shifts to shorter times at the higher injection sizes, which is indicative of the finite dilution regime, in that retention time is dependent on injection concentration, due to the solute molecules interacting in both the gas and adsorbed phase. The adsorption isotherm calculated from this data is displayed in Fig. 4. With these axes, a fit of the line to the BET equation, Eq. 1, is possible.

$$W = \frac{W_m C_B (p/p_0)}{\{[1-(p/p_0)][(1-(p/p_0)) + C_B(p/p_0)]\}} \tag{1}$$

where W is the weight of adsorbate per weight of dry solid, p is the partial pressure of the vapor, and p_0 is the saturation pressure of the liquid at the experimental temperature. The unknowns are W_m, the weight of gas adsorbed as a monolayer on the available surface, and C_B, a constant for each system, reflecting the overall free energy of adsorption. W_m can be translated into an area by knowing the molecular mass and cross sectional area of the solvent molecule.

Fitting Eq. 1 to the isotherms obtained for the amorphous and crystalline materials yields surface areas of 5.48 m^2

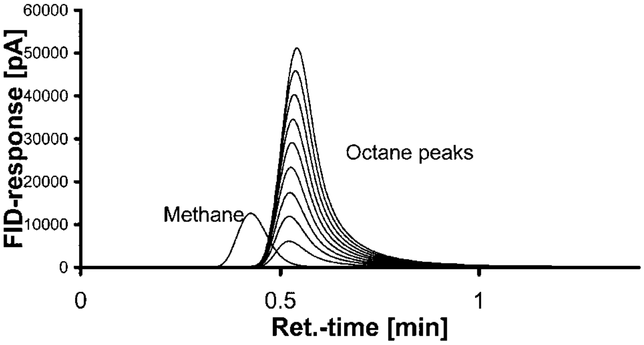

Fig. 3. Chromatograms recorded on crystalline lactose, 10 ml min^{-1}, 0% RH, column temperature 303 K, methane as reference probe, and octane in 10% concentration steps from 10 (smallest peak) to 90 (largest peak) % p/p_0.

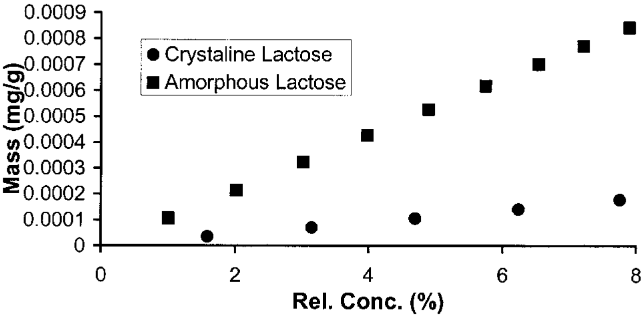

Fig. 4. Adsorption isotherm of crystalline lactose calculated from the chromatogram displayed in Fig. 3 and the equivalent isotherm of amorphous lactose.

g^{-1} for the crystalline material and 16.11 m^2 g^{-1} for the amorphous material. The amorphous lactose has a surface area 2.94 times that of the crystalline material. This ratio can be used for the mixtures produced by weight to be converted into percentage by area. This has been performed and Fig. 1 has been re plotted on these axes as Fig. 5.

DISCUSSION

In this study, the surface energies of a range of mixtures of amorphous and crystalline lactose have been determined. The surface energies of the two components are sufficiently different for the mixtures to contain distinctly different surface energy sites, the higher energy sites occurring on the amorphous material. The data obtained for the 1% and 5% w/w amorphous mixtures seem to contradict the hypothesis that in IGC the solute molecules will predominately access the highest energy sites available to them, as the surface energy of the 1% and 5% w/w mixtures fall very close to the value calculated from the weighed average of the two pure components.

It is only from mixtures above 5% w/w amorphous content that the surface energy substantially departs from this weighted average. In this case, the mixtures between 10% and 15% w/w yield surface energies that are between the energies of the two components. This implies that while more of the higher energy surface sites are being probed, this is not to the exclusion of the lower energy sites. It is not until 20% w/w of the mixture is amorphous that the surface energy measured is within the realms of the amorphous surface energy. This is a higher proportion than would be expected if the assumption that principally the higher energy sites are probed was correct.

It could well be argued that the mixtures were produced by weight, whereas this experiment is probing the surface. The proportion of the surface that is amorphous could be substantially different from the proportion of the mixture as a whole. By measuring the surface areas of the two materials, it has been possible to answer this point. By area, the mixtures appear very similar to the crystalline material until around 15% amorphous content by area. After this value, there is a rapid increase in surface energy with an increase in amorphous content until approaching 40%, where the mixture appears predominately amorphous. This calculation would still dispute the assumption that the probe molecules will always predominately access the highest energy sites available to them.

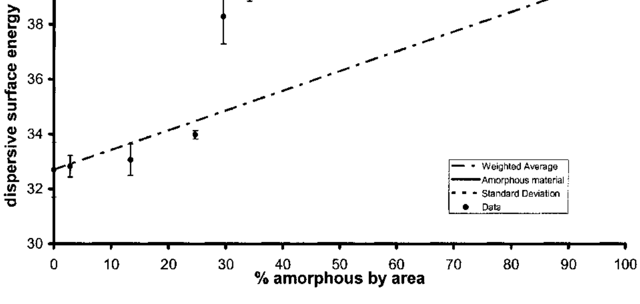

Fig. 5. Displaying surface energy of a range of amorphous and crystalline lactose mixtures as a percentage of amorphous content, measured by surface area.

In conclusion, it has been shown that the highest energy sites do not always dominate the surface area of a system, indeed a substantial part of the surface, 40% by surface area, has to comprise the higher energy material before this material dominates the mixture. Bearing in mind that the small amorphous particles will adsorb to the surface of the large crystalline particles, this 40% of available area may well be magnified as some of the crystalline materials area may be hidden by adsorbed particles, hence explaining the dominant role of the amorphous material. However, SEM pictures show that the surface is not completely covered at this stage, hence there is some element of recording of the amorphous surface energy in preference to the crystalline. This information helps in the future interpretation of IGC data for surfaces (or mixtures) that contain different energy sites.

IGC seems ideally suited to the study of complex powder interactions, especially to understand the behavior of powder mixtures.

ACKNOWLEDGMENTS

H. N. is grateful to Astra Zeneca, Glaxo SmithKline, Pfizer Central Research, and Roche for financial support.

REFERENCES

1. M. D. Ticehurst, R. C. Rowe, and P. York. Determination of the surface properties of two batches of salbutamol sulphate by inverse gas chromatography. *Int. J. Pharm.* **111**:241–249 (1994).
2. M. D. Ticehurst, P. York, R. C. Rowe, and S. K. Dwivedi. Characterisation of the surface properties of α-lactose monohydrate with inverse gas chromatography, used to detect batch variation. *Int. J. Pharm.* **141**:93–99 (1996).
3. P. York, M. D. Ticehurst, J. C. Osborn, R. J. Roberts, and R. C. Rowe. Characterisation of the surface energetics of milled dl-propranolol hydrochloride using inverse gas chromatography and molecular modelling. *Int. J. Pharm.* **174**:179–186 (1998).
4. I. M. Grimsey, M. Sunkersett, J. C. Osborn, P. York, and R. C. Rowe. Interpretation of differences in the surface energies of two optical forms of mannitol by inverse gas chromatography and molecular modelling. *Int. J. Pharm.* **191**:43–50 (1999).
5. J. W. Dove, G. Buckton, and C. Doherty. A comparison of two contact angle measurement methods and inverse gas chromatography to assess the surface energies of theophylline and caffeine. *Int. J. Pharm.* **138**:199–206 (1996).
6. N. M. Ahfat, G. Buckton, R. Burrows, and M. D. Ticehurst. An exploration of inter relationships between contact angle, inverse phase gas chromatography and triboelectric charging. *Eur. J. Pharm. Sci.* **9**:271–276 (2000).
7. H. E. Newell, G. Buckton, D. A. Butler, F. Thielmann, and D. R. Williams. The use of inverse phase gas chromatography to measure the surface energy of crystalline, amorphous and recently milled lactose. *Pharm. Res.* **18**:662–666 (2001).
8. H. E. Newell, G. Buckton, D. A. Butler, F. Thielmann, and D. R. Williams. The use of inverse phase gas chromatography to study the change of surface energy of amorphous lactose as a function of relative humidity and the processes of collapse and crystallisation. *Int. J. Pharm.* **217**:45–56 (2001).
9. O. Planinsek and G. Buckton. Inverse gas chromatography: Considerations about appropriate use for amorphous and crystalline powders. *J. Pharm. Sci.* **92**:1286–1294 (2003).

10. L-E. Briggner, G.Buckton, K.Bystrom and P.Darcy. The use of isothermal microcalorimetry in the study of changes in crystallinity induced during the processing of powders. *Int. J. Pharm.* **105**: 125–135 (1994).
11. G. Buckton, P. Darcy, and A. J. Mackellar. The use of isothermal microcalorimetry in the study of small degrees of amorphous content of powders. *Int. J. Pharm.* **117**:253–256 (1995).
12. S. K. Dwivedi and A. G. Mitchell. Gas chromatographic analysis of anomeric composition of lactose. *J. Pharm. Sci.* **78**:1055–1056 (1989).
13. O. C. Chidavaenzi, G. Buckton, F. Koosha, and R. Pathak. The use of thermal techniques to assess the impact of feed concentrations on the amorphous content and polymorphic forms present in spray dried lactose. *Int. J. Pharm.* **159**:67–74 (1997).
14. J. Schultz, L. Lavielle, and C. Martin. The role of the interface in carbon fibre-epoxy composites. *J. Adhesion* **23**:45–60 (1987).