Comparison of the cohesion-adhesion balance approach to colloidal probe atomic force microscopy and the measurement of Hansen partial solubility parameters by inverse gas chromatography for the prediction of dry powder inhalation performance

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Abstract-

The abilities of the cohesive-adhesive balance approach to atomic force microscopy (AFM) and the measurement of Hansen partial solubility parameters by inverse gas chromatography (IGC) to predict the performance of carrier-based dry powder inhaler (DPI) formulations were compared. Five model drugs (beclometasone dipropionate, budesonide, salbutamol sulphate, terbutaline sulphate and triamcinolone acetonide) and three model carriers (erythritol, α-lactose monohydrate and D-mannitol) were chosen, giving fifteen drug-carrier combinations. Comparison of the AFM and IGC interparticulate adhesion data suggested that they did not produce equivalent results. Comparison of the AFM data with the *in vitro* fine particle delivery of appropriate DPI formulations normalised to account for particle size differences revealed a previously observed pattern for the AFM measurements, with a slightly cohesive AFM CAB ratio being associated with the highest fine particle fraction. However, no consistent relationship between formulation performance and the IGC data was observed. The results as a whole highlight the complexity of the many interacting variables that can affect the behaviour of DPIs and suggest that the prediction of their performance from a single measurement is unlikely to be successful in every case.

KEYWORDS: adhesion; atomic force microscopy; dry powder inhaler (DPI); inverse gas chromatography; materials science; physical characterisation.

1. Introduction

Dry powder inhalers (DPIs) are widely used to deliver drugs to the lungs. Most DPI formulations consist of a mixture of micronised drug and larger “carrier” excipient particles. This is aerosolised as the patient inhales and in order to reach the lungs, drug particles must separate from other particles, as only those <5 μm aerodynamic diameter (i.e. single particles) will reach their target (Timsina et al., 1994). The efficacy of this process determines product efficiency, hence interparticulate adhesion and cohesion are critical to determining performance. It might, therefore, be possible to relate particle-particle interactions within a DPI blend to product performance. Such a system would be highly advantageous during the formulation of DPI products, as it would enable the rapid screening of a range of salt forms or carrier excipients in order to find the combination likely to yield the highest fine particle delivery.

Recently, there have been a number of attempts to develop such predictive techniques. The most promising have utilised colloidal probe atomic force microscopy (AFM) and inverse gas chromatography (IGC), though other techniques have also been investigated (Lohrmann et al., 2007). Using AFM, the force of adhesion between particles can be measured with a sensitivity as high as 10^{-11} N, but as the contact area (to which adhesion is proportional) is unknown, its usefulness has been limited (Bunker et al., 2005). This limitation has been overcome in various ways, including by the cohesion-adhesion balance (CAB) technique, in which the cohesion of a material and its adhesion to a different material are measured using the same particle, giving the same contact area (Begat et al., 2004a). The ratio between cohesion and adhesion can then be calculated, a value independent of contact area. Such CAB ratios have been able to explain the behaviour and predict the performance of a number of types of DPI formulation (Begat et al., 2004b;

Begat et al., 2005; Hooton et al., 2008; Hooton et al., 2006; Jones et al., 2008a; Jones et al., 2008b; Kubavat et al., 2012a, b).

IGC investigates the surface properties of a solid by examining its interaction with organic vapours (Grimsey et al., 2002). The majority of work using IGC to predict DPI performance has focused on the measurement of surface energy at infinite dilution (Alhalaweh et al., 2013; Bernhard and Steckel, 2005; Cline and Dalby, 2002; Jiang et al., 2005; Kumon et al., 2006; Oliveira et al., 2006; Sethuraman and Hickey, 2002; Traini et al., 2008). However, a consistent relationship between surface energy and DPI performance has failed to emerge. A number of studies have considered the dispersive surface energy of either the carrier or drug, finding either a positive (Kumon et al., 2006) or negative (Jiang et al., 2005; Oliveira et al., 2006; Sethuraman and Hickey, 2002; Traini et al., 2008) relationship with formulation performance. In addition, Traini *et al*. found a negative relationship between total carrier surface energy and fine particle delivery (Traini et al., 2008). Other workers, recognising that the interaction between two particles is more complex than simply the surface energy of one of them, have used more complex calculations, either combining surface energy and surface area measurements (with conflicting results) (Bernhard and Steckel, 2005; Sethuraman and Hickey, 2002) or attempting to calculate drug-carrier interaction energy from the components of surface energy (Cline and Dalby, 2002). This latter approach found a positive relationship between drug-carrier interaction energy and fine particle delivery. However, this work was subsequently criticized for the substitution of surface tension with free energy of adsorption during the calculation of the “surface energy interaction”, as well as for the reliability of the method employed for the determination of specific surface area from IGC data (Chow et al., 2004).

Tong *et al*. employed another IGC approach, by measuring the Hansen partial solubility parameters (see Section 2.2.4.1) of salmeterol xinafoate polymorphs and lactose monohydrate, from which the strength of the various adhesive and cohesive interactions within in a formulation could be calculated (Tong et al., 2006). Subsequently, these data were found to relate to the *in vitro* performance of DPI formulations.

All of the studies discussed above make the assumption that adhesion between drug and carrier particles dominates the behaviour of carrier-based DPI formulations. However, this assumption is not universally valid, as drug-only and drug-fine excipient agglomerates are also known to exist within, and influence the performance of, carrier-based DPI formulations, especially for cohesive drugs (Jones and Price, 2006; Xu et al., 2011). This may be an explanation for the inconsistent findings of studies to date.

AFM and IGC each have the potential to develop into useful tools for the prediction of DPI performance, although each has its own strengths and weaknesses. While AFM makes a direct measurement of adhesive forces, due to the time consuming processes involved, it is only possible to do so using a few particles (Weiss et al., 2015). In addition, AFM experiments require a high degree of operator expertise. In practical terms, IGC requires a lower level of operator expertise and can also make measurements across a whole powder surface. It is, however, an indirect measurement of adhesion and does not take account of certain factors (e.g. electrostatic and capillary forces) which can influence interparticulate forces.These are measured by AFM (Bunker et al., 2005). Currently, the relative merits of AFM and IGC for the prediction of DPI performance are unclear, as their capabilities have yet to be directly compared. The aim of this study was, therefore, to compare these two techniques.

The CAB approach to adhesive force measurement by AFM was employed, as the literature contains more comparisons of data from this technique with DPI formulation performance than any other. IGC was employed at infinite dilution to determine the Hansen partial solubility parameters of the study materials because this approach yielded promising results with a limited number of formulations in the first report of its application to DPI systems (Tong et al., 2006) and so it warrants further investigation in a larger study. In addition, the prediction of DPI performance from surface energies measured at infinite dilution by IGC has been widely investigated without consistent success, as discussed briefly above and in greater detail elsewhere (Jones et al., 2012).

It should be noted that IGC analysis at infinite dilution provides information about the most energetic sites on the surface of a powder and so give an incomplete representation of surface properties (Buckton and Gill, 2007; Das et al., 2015; Tong et al., 2005). Therefore, over the last decade a number of groups have developed ever more sophisticated methods which employ finite dilution IGC to map the entire surface energy distribution of a powder (Das et al., 2011a; Smith et al., 2014; Tong et al., 2005; Ylä-Mäihäniemi et al., 2008). Such approaches have proven more effective and reliable than infinite dilution IGC in understanding the effects of pharmaceutical processing and batch-to-batch variability on powder surface properties (Das and Stewart, 2012; Das et al., 2015; Das et al., 2011b). Finite dilution IGC has also been used to calculate the work of cohesion distribution for samples of α-lactose monohydrate, which was related to the deagglomeration of the powders observed by laser diffraction (Das et al., 2012). However, data derived from finite dilution IGC have not yet been applied to the study of the adhesion of a drug to a carrier excipient and their subsequent aerosolisation behaviour, so these approaches were not employed in this study.

2. Materials and Methods

2.1. Materials

Experiments were conducted using five model drugs and three model carrier excipients, allowing the preparation of 15 carrier-based DPI formulations. The model drugs were micronised anhydrous beclometasone dipropionate, micronised budesonide and micronised anhydrous terbutaline sulphate form B (each from AstraZeneca, Macclesfield, UK), micronised salbutamol sulphate (GlaxoSmithKline Research and Development, Ware, UK) and triamcinolone acetonide form I (Sanofi-Aventis, Holmes Chapel, UK). Triamcinolone acetonide was subsequently micronised by one pass through a Trost Gem-T mill (Plastomer Technologies, Newtown, PA, USA) with feed and grind pressures set to 100 psi. Before use, the micronised drugs were passed through a 500 μm stainless steel sieve (Endecotts Limited, London, UK) to remove large agglomerates of particles. Two to four weeks storage under vacuum and over phosphorus pentoxide (0% RH) at ambient temperature were then allowed to elapse.

The model carriers were erythritol (*Eridex*®) and D-mannitol (β-polymorph, *PharmMannidex*®), each supplied by Cargill Excipients (Mechelen, Belgium) and α-lactose monohydrate (*Lactohale*®, Friesland Foods Domo – Pharma, Zwolle, The Netherlands). The as received carriers were sieved to obtain the 63–90 μm size fraction using stainless steel sieves (Endecotts Limited, London, UK) and an AS 200 sieve shaker (Retsch UK Ltd., Leeds, UK) set to an amplitude of 1 mm. Before sieving, the erythritol carrier was milled for 30 min at 200 rpm using a Pulverisetter 5 planetary ball mill (Fritsch GmbH, Idar-Oberstein, Germany), as its initial particle size distribution was almost entirely >90 μm diameter.

The identity, polymorphism and crystallinity of all these materials were confirmed using X-ray powder diffraction before and after processing. Both drugs and excipients were stored under vacuum and over phosphorus pentoxide (0% RH) at ambient temperature prior to analysis.

The following elutants were used in IGC experiments: 1,4-dioxane, acetone, cyclohexane and ethyl acetate (all from Fisher Scientific UK, Loughborough, UK) and acetonitrile and decane (both from VWR, Poole, UK). All elutants were of at least 99% purity. Research grade methane was obtained from BOC (Guildford, UK).

HPLC grade methanol was supplied by Fisher Scientific Ltd (Loughborough, UK) and water was purified by reverse osmosis (PURELAB Option, Elga LabWater, Marlow, UK).

2.2. Methods

2.2.1. Particle size analysis

Particle size analysis of the drug and carrier excipient powders was carried out in the dry state. Samples were dispersed with compressed air at 3 bar through a RODOS dry disperser fed by an ASPIROS micro-dosing unit before sizing with a HELOS laser diffraction sensor (all from Sympatec GmbH, Clausthal-Zellerfeld Germany). The particle size analysis was performed using WINDOX 5 software (Sympatec GmbH, Clausthal-Zellerfeld Germany). Values presented are the mean ± standard deviation of 5 determinations.

2.2.2. True density measurement

Subsequent calculations required the true density of each of the study materials. This was measured using a helium pycnometer (Accupyc 1330 Gas Pycnometer, Micromeritics, Norcross, USA) to determine ten measurements of the volume of a sample of each solid material. Samples were dried at 40ºC for at least 20 hours before analysis.

2.2.3. Quantification of cohesion-adhesion balances by AFM

Drug-carrier CAB ratios were measured with drug particle colloidal probes. They therefore describe the cohesion of drug probes to a substrate crystal of the same drug, relative to the adhesion of the same drug probes to an excipient substrate crystal.

2.2.3.1. Controlled crystallisation of substrate surfaces

The CAB procedure requires the use of extremely smooth single crystals as substrates for cohesion and adhesion measurements. Therefore, such substrates for all the study materials were nucleated and grown on glass cover slips, using techniques described elsewhere (Begat et al., 2004a; Hooton et al., 2006). The surface topography of the resultant crystals was investigated with TappingMode AFM using a Multimode AFM, J-type scanner, Nanoscope IV controller, Nanoscope 5.12b control software (all from Veeco, Cambridge, UK) and a silicon tapping tip (NSG01, NTI-Europe, Apeldoorn, The Netherlands). 10 μm × 10 μm square areas of the crystal surfaces were imaged with a resolution of 512 × 512 pixels and a scan rate of 1 Hz. The roughness of imaged areas was quantified using the mean (R_a) and root mean square (R_q) of the variations in the height of the imaged surface, as calculated by the AFM software. To enable identification

of the Miller index of the dominant growth face of each crystalline substrate, literature data on the unit cell lattice parameters and space group symmetry operators of the study materials (Albertsson et al., 1978; Ceccarelli et al., 1980; Fronczek et al., 2003; Leger et al., 1978; Millard and Myrdal, 2002; Näther and Jeß, 2006; Raghavan et al., 2000; Sengupta and Dattagupta, 1996) were used to model their crystal habits with a 3D crystal simulation program (SHAPE v. 7.0 professional edition, Shape Software, Tennessee, USA).

2.2.3.2. AFM colloidal probe preparation

Four colloidal probes of each drug were prepared using the method described in detail elsewhere (Young et al., 2003). In summary, to prepare a probe, a single particle of micronised material was fixed to the apex of a V-shaped tipless cantilever with a nominal spring constant of 0.58 $N.m^{-1}$ (Veeco NanoProbe^{TM}, model number: NP-OW, Veeco Instruments SAS, Dourdan, France) using an epoxy resin glue (*Araldite Precision*, Bostik Ltd, Leicester, UK) and custom built micromanipulation equipment. Once the epoxy resin had cured, the probes were visually inspected using an optical microscope with incident illumination to ensure that a single particle was attached in an appropriate position near the apex of the cantilever and that there was not excess glue present. After adhesive force measurements had been completed, probe integrity was confirmed by scanning electron microscopy.

2.2.3.3. Adhesive and cohesive force measurement

The adhesive or cohesive force between each colloidal probe and the dominant face of a smooth crystal of each relevant material was measured with force-volume mode AFM

using a Multimode AFM, J-type scanner, Nanoscope IV controller and Nanoscope 5.12b control software (all from Veeco, Cambridge, UK). At least 256 (16 × 16) individual force curves were collected over a 10 μm × 10 μm area of the crystal substrate with a z-scan rate of 4.07 Hz and a nominal compressive loading of 11.6 nN. Humidity within the sample area of the AFM head was maintained at 26ºC (±2ºC) and 35% RH (±3%), using a previously described method (Young et al., 2003). The cohesive force between each colloidal probe and the crystalline substrate of the same drug was measured before and after the adhesion measurements, to ensure there had been no changes in the drug particle.

Force-volume data were processed using custom software to extract the force of adhesion/cohesion from each of the force curves. This calculation was performed using the nominal cantilever spring constant of 0.58 $N.m^{-1}$ (rather than the measured spring constant of each individual probe) as the comparison of the cohesion and adhesion of the same probe in the CAB method eliminates the effects of cantilever-to-cantilever spring constant variation (Hooton et al., 2006).

2.2.4. Inverse gas chromatography

2.2.4.1. Calculations

The Hansen partial solubility parameters of the study materials were determined by IGC, following the calculations described by Tong *et al.* (2002). IGC examines the adsorption of gaseous elutants from an inert carrier gas onto the solid sample packed into a glass column. The basic measurement is the retention time, which can be standardised for carrier gas flow rate and column void space in order to calculate the retention volume (V_N)

of each elutant. The specific retention volume (V_G) of each elutant can be calculated by dividing V_N by sample mass. This parameter can be used to calculate the transfer energy of adsorption (ΔE^A) for an elutant interacting with a particular sample:

$$\ln V_G = \frac{\Delta E^A}{RT} + K_G \qquad \text{Equation 1}$$

where R is the gas constant, T is absolute temperature and K_G is a constant. ΔE^A for each elutant can, therefore, be obtained from the gradient of a plot of ln V_G versus 1/T using data obtained at a number of temperatures.

The Hansen partial solubility parameter approach divides the total solubility parameter (δ_T) into three components describing dispersive forces (δ_D), polar interactions (δ_P) and hydrogen bonding (δ_H) (Tong et al., 2002). These are related as described by the following equation (Tong et al., 2002):

$$\delta_T^2 = \delta_D^2 + \delta_P^2 + \delta_H^2 \qquad \text{Equation 2}$$

Elutant ΔE^A is related to the Hansen partial solubility parameters of the solid sample (δ_D^S, δ_P^S and δ_H^S) by the following equation (Tong et al., 2002):

$$\Delta E^A = V(\delta_D^E \delta_D^S + \delta_P^E \delta_P^S + \delta_H^E \delta_H^S) \qquad \text{Equation 3}$$

where V is the molar volume of the elutant and δ_D^E, δ_P^E and δ_H^E are the elutant Hansen partial solubility parameters. Multiple linear regression through the origin using literature

312 values of V, δ_D^E, δ_P^E and δ_H^E can, therefore, be used to obtain δ_D^S, δ_P^S and δ_H^S if ΔE^A is
313 known for a number of elutants.
314
315 Experimental data were initially analysed using IGC Analysis Macros (v1.3 standard
316 edition, Surface Measurement Systems, London, UK) to calculate V_N and Excel 2002
317 (Microsoft, Seattle, USA) was used for the remaining calculations.
318
319 *2.2.4.2. Experimental design*
320
321 There are twenty one volatile compounds with known Hansen partial solubility parameters
322 (Huu-Phuoc et al., 1987a; Huu-Phuoc et al., 1987b; Huu-Phuoc et al., 1986). Of these, five
323 were deemed unsuitable for safety or technical reasons and the alcohols (methanol to
324 butan-1-ol) were excluded when initial data suggested that they may chemisorb to many of
325 the study materials. Twelve potential elutants were therefore available (the “experimental
326 domain”), as listed in Table 1.
327
328 An appropriate choice of experimental elutants was important in order to ensure the
329 validity of the results. The experimental matrix optimisation technique described by Huu-
330 Phuoc *et al.* (Huu-Phuoc et al., 1987a; Huu-Phuoc et al., 1987b; Huu-Phuoc et al., 1986)
331 was therefore employed to rationally select the combination of these potential elutants
332 which would provide the maximum precision and accuracy in the smallest number of
333 experiments. Submatrices containing between 4 and 9 probes were considered, as this
334 was the maximum number of probes that could be loaded into the available IGC
335 instrument. All calculations were performed using Minitab 15 software (Minitab Inc., State
336 College, PA, USA).
337

2.2.4.3. Inverse gas chromatography experiments

IGC experiments were carried out using the commercial iGC system (Surface Measurement Systems, London, UK) with a research grade helium carrier gas flow (BOC, Guildford, UK) of 10 $ml.min^{-1}$ at 0% RH. Elutant vapour injections were carried out using the minimum possible concentration for all samples (2-3%$^{v}/_{v}$ of saturated elutant vapour). Column deadtime was determined by injection of methane (BOC, Guildford, UK). Measurements were carried at 303 K, 313 K, 323 K and 333 K. Each new column was allowed to equilibrate under the IGC conditions for a number of hours before injections commenced. The appropriate equilibration time for each sample was determined by repeated injections of decane onto a freshly prepared column. 60 min equilibration time was allowed following column oven temperature changes and 30 min elapsed between injections.

Silanised glass columns of 3 mm or 4 mm internal diameter and 300 mm length (Surface Measurement Systems, London, UK) were packed with powder and tapped for 15 min on a jolting voltameter (Surface Measurement Systems, London, UK) to ensure uniform column packing. Sample mass (0.06-0.15 g for drugs, 2.4-2.6 g for carriers) was chosen to give the maximum possible decane retention time whilst still producing an observable peak. Column ends were plugged with silanised glass wool. Two columns of each material were analysed and each adsorbate was injected into each column three times at each temperature. The ln V_G values derived from each elutant injection into each column were individually included in the regression analysis necessary to calculate the transfer energy of adsorption (ΔE^A) from Equation 1. Columns were weighed before and after analysis, with no changes in mass being recorded.

2.2.5. In vitro *formulation testing*

2.2.5.1. Formulation preparation

Each drug was mixed separately with each carrier in a ratio of 1:67.5 in 4 g batches using a method based on one described previously (Louey and Stewart, 2002). The drug and carrier were geometrically mixed using a microspatula and were then placed in a 15 ml glass test tube with three 10 mm diameter ceramic balls. The tube was then hand-shaken vigorously for 5 min. Following blending, the drug content uniformity of all the formulations was assessed. Formulations were spread evenly over a clean surface and ten samples of 33 ± 1 mg were taken from random positions. Each sample was dissolved in a suitable solvent (see Section 2.2.6) and drug concentration assessed using either a spectrophotometric or spectrofluorimetric assay. The proportion of drug in each sample was calculated and the content uniformity expressed as the relative standard deviation (RSD). Each formulation was then stored in a sealed container with a saturated solution of potassium carbonate (providing a relative humidity of 44% (O'Brien, 1948)) for 24 hours. Formulations were then manually loaded into size 3 gelatin capsules (Capsugel, Bornem, Belgium). Fill mass was 33 ± 1 mg, giving a nominal dose of 482 ± 15 µg drug per capsule. In addition, each of the pure micronised drugs were loaded into capsules with a fill mass of 5 ± 0.1 mg. Following filling, capsules were stored at 44% RH for 24 hours prior to further analysis.

2.2.5.2. Determination of in vitro *fine particle delivery*

The *in vitro* fine particle delivery of the various formulations and micronised drugs was investigated using a twin stage impinger (TSI) (Radleys, Saffron Walden, UK) following the methodology described in the British Pharmacopoeia 2016. 7 and 30 ml of a suitable solvent (see Section 2.2.6) was introduced into stages 1 and 2 of the TSI, respectively. Air flow through the TSI was controlled using a custom built solenoid valve timer and set to 60 $l.min^{-1}$ prior to operation, using a flow meter connected to the mouthpiece of the TSI. Under these conditions, the cut off aerodynamic diameter between the two stages was 6.4 µm. For each test, a *Cyclohaler* (Teva Pharmachemie, Haarlem, The Netherlands) was connected to the TSI using a silicone rubber mouthpiece. The contents of five formulation capsules (or one micronised drug capsule) were then aerosolised sequentially from this inhaler for 5 s each (controlled by the valve timer). The apparatus was then dismantled and the inhaler and capsules, mouthpiece and throat, stage 1 and stage 2 washed out with a suitable solvent (see Section 2.2.6). Each formulation was tested five times in this way.

The drug content of the washings was determined using either a spectrophotometric or spectrofluorimetric assay, from which the amount of drug deposited in each part of the apparatus could then be determined. This enabled calculation of the recovered dose (RD, the total amount of drug recovered), the emitted dose (ED, the amount of drug recovered from all parts of the TSI) and fine particle fraction (FPF, the stage 2 deposition expressed as a percentage of both the RD and ED).

2.2.6. Drug assays

2.2.6.1. Spectrophotometric assay

Beclometasone dipropionate, budesonide and triamcinolone acetonide concentration in solution in 40:60 methanol:water (or pure methanol following the aerosolisation of pure micronised drug) was determined using a Varian Cary 3E UV-visible spectrophotometer. A wavelength of 241 nm was used for beclometasone dipropionate and triamcinolone acetonide, whilst 247 nm was used for budesonide. The UV absorbance of each drug was found to be linear with concentration ($R^2 \geq 0.999$) over the range 0.5-50 $\mu g.ml^{-1}$. The limit of detection of each assay (defined as three times the standard deviation of the absorbance of 40:60 methanol:water (n=10) (Thompson et al., 2002)) was ≤0.18 $\mu g.ml^{-1}$. The presence of erythritol, lactose or mannitol in solution did not affect any of the assays.

2.2.6.2. Spectrofluorimetric assay

Salbutamol sulphate and terbutaline sulphate concentrations in aqueous solution were determined using a Perkin Elmer LS 50 B spectrofluorimeter. The excitation and emission wavelengths were 274 nm and 305 nm for salbutamol sulphate and 280 nm and 308 nm for terbutaline sulphate. The fluorescence intensity of each drug was found to be linear with concentration ($R^2 \geq 0.999$) over the range 0.25-10 $\mu g.ml^{-1}$. The limit of detection of each assay (defined as three times the standard deviation of the fluorescence signal obtained from pure water (n=10) (Thompson et al., 2002)) was ≤0.13 $\mu g.ml^{-1}$. The presence of erythritol, lactose or mannitol in solution did not affect any of the assays.

3. Results and Discussion

3.1. Particle size analysis and true density measurements

The particle size distributions of the study materials and their true densities are summarised in Table 2. There was considerable variation in the size of the drug particles, with d_{50} values ranging from 1.25 ± 0.02 μm (beclometasone dipropionate) to 3.29 ± 0.13 μm (triamcinolone acetonide). Such differences are likely to have an influence on *in vitro* fine particle delivery, but it was not possible to produce powders with a more similar particle size distribution. This reflects the more general difficulty of producing micronised drug powders identical in every property relevant to DPI performance (e.g. size, shape) apart from chemical identity.

3.2. Quantification of cohesion-adhesion balances by AFM

Smooth crystals (dominant face R_a and R_q < 1 nm) with a well defined crystal habit were successfully grown. Such smooth surfaces enable reproducible adhesion and cohesion measurements during colloidal probe AFM and so these crystals were used as substrates for the subsequent experiments (Begat et al., 2004a). The habits of these crystals were compared with the appropriate 3D crystal habit simulations to identify the Miller index of their dominant faces (see Table 3). Where a material could crystallise as one of several polymorphs, the probable form obtained was suggested by the crystallisation conditions and the habit of the crystal.

The cohesive forces between four colloidal probes of each drug and the dominant face of the crystalline substrate of the same drug and the adhesive forces between each colloidal probe and the dominant face of the crystalline substrate of each carrier excipient were successfully measured. The distribution of forces obtained for each measurement followed a normal distribution, so were therefore summarised by its mean and standard deviation. The mean cohesive force for each colloidal probe was plotted against its mean adhesive

force to each carrier excipient to produce a CAB graph (Begat et al., 2004a). Linear regression through the origin of each set of CAB data showed a high degree of linearity ($R^2 > 0.91$ in all cases), confirming that the contact area between colloidal probes and substrates remained constant for both adhesive and cohesive measurements (Begat et al., 2004a). Therefore, the gradient of each line of best fit was taken as the AFM CAB ratio (Table 4) (Begat et al., 2004a).

CAB ratios describe the cohesion of the colloidal probe material relative to its adhesion to the crystalline substrate of the second material (Begat et al., 2004a). Therefore, a CAB ratio <1 (known as an adhesive CAB ratio) describes a system where the drug-carrier adhesion is greater than the drug-drug cohesion. The closer a CAB ratio is to zero, the greater was the adhesion compared to cohesion. For example, the beclometasone dipropionate-erythritol CAB ratio was 0.51 ± 0.05, indicating that, all other variables being equal, the cohesiveness of beclometasone dipropionate was 0.51 times smaller than its adhesiveness to erythritol (i.e. the adhesiveness is 1.96 times greater than the cohesiveness). On the other hand, a CAB ratio >1 (known as a cohesive CAB ratio) describes a situation where the drug-drug cohesion is greater than the drug-carrier adhesion. As the relative magnitude of this cohesion increases, so does the CAB ratio. For example, the budesonide-erythritol CAB ratio was 1.38 ± 0.01, indicating that the cohesiveness of budesonide was 1.38 times greater than its adhesiveness to erythritol.

Inspection of Table 4 reveals that the majority of the AFM CAB ratios were adhesive. In contrast to previous studies, where ratios as extreme as 0.22 and 2.39 were observed (Jones et al., 2008b), this set of CAB ratios is reasonably close to 1.00 (i.e. where adhesion and cohesion are equal), with the smallest ratio being 0.51 (beclometasone dipropionate-erythritol) and the largest 1.38 (budesonide-erythritol).

3.3. Inverse gas chromatography

3.3.1. Experimental design

In order to ensure the accuracy of the results, it was important to ensure that there was a low level of multicollinearity between the partial solubility parameters of the twelve potential elutants (Table 1). This was assessed by calculation of their variance inflation factors: $f(\delta_D{}^{E_i}) = 1.376$, $f(\delta_P{}^{E_i}) = 2.140$ and $f(\delta_H{}^{E_i}) = 2.213$. As these values were <4, suggesting little multicollinearity, the experimental matrix was considered satisfactory with regard to accuracy (Huu-Phuoc et al., 1987a; Huu-Phuoc et al., 1987b; Huu-Phuoc et al., 1986; Lewis et al., 1999).

Next, the combination of elutants which would yield the most precise results, whilst still retaining acceptable accuracy, was determined using the matrix optimisation technique. Table 5 shows the results of these calculations for the most precise combinations of between 4 and 9 elutants (the “optimised sets”).

|X'X| is the determinant of the information matrix for each optimised set of elutants containing N compounds. The larger this value, the more precise the experiment. The normalised determinant of the information matrix (**|M|**) is equal to $\mathbf{|X'X|}/N^p$, where p = the number of coefficients in the model equation (in this study, p = 3). **|M|** reflects the quality of information per elutant (D-efficiency) and so should be maximised to ensure an efficient experiment (Huu-Phuoc et al., 1987a; Huu-Phuoc et al., 1987b; Huu-Phuoc et al., 1986). Finally, the variance inflation factors ($f(\delta_D{}^{E_i})$, $f(\delta_P{}^{E_i})$ and $f(\delta_H{}^{E_i})$) were calculated for each

optimised set. As above, these values should be minimised and preferably be less than four to ensure an accurate as well as a precise experiment (Lewis et al., 1999).

Inspection of Table 5 reveals that **|X'X|** increased with each additional elutant added to the optimised set, implying that the use of the set of nine elutants would result in the most precise experiment. However, as the Hansen partial solubility parameters of eight different materials were to be determined by IGC experiments at four temperatures, the use of nine elutants would have resulted in impractically lengthy experiments. Therefore, following the method of Huu-Phuoc *et al.* (Huu-Phuoc et al., 1987a; Huu-Phuoc et al., 1987b; Huu-Phuoc et al., 1986), the values of **|M|** were considered. As Table 5 shows, this was maximised for the optimised set of five elutants, implying that this set would result in the most efficient experiment (highest degree of precision per elutant). In addition, the variance inflation factors for these elutants were amongst the smallest of those calculated and were all <4, implying that this set would result in an accurate experiment (Lewis et al., 1999). It was, therefore, decided to use the optimised set of five elutants described in Table 5 for the measurement of Hansen partial solubility parameters. These were decane, cyclohexane, 1,4-dioxane, acetonitrile and ethyl acetate.

Subsequently, acetonitrile demonstrated an unusual interaction with triamcinolone acetonitrile (highly asymmetrical elution peak with a very small maximum response at ~6 min tailing to at least 30 min), possibly as a result of bulk absorption. The experimental design calculations were, therefore, repeated with acetonitrile excluded from the experimental domain described in Table 1. The results of this process were similar to those described above and resulted in the choice of decane, cyclohexane, 1,4-dioxane, acetone and ethyl acetate for the analysis of triamcinolone acetonide.

3.3.2. Hansen partial solubility parameters

The Hansen partial solubility parameters of the eight study materials were successfully determined and are shown in Table 6.

Inspection of Table 6 reveals a range of Hansen partial solubility parameters and surface properties. For example, the steroids beclometasone dipropionate and triamcinolone acetonide have similar surfaces in terms of δ_D^S, but very different surfaces in terms δ_P^S and δ_H^S. It is noticeable that terbutaline sulphate had the highest of each of the three Hansen partial solubility parameters when compared to the other study materials. The value of δ_H^S (55.0 ± 32.7 $MPa^{1/2}$) was especially large. This arose from the very long retention time of 1,4-dioxane with this drug (~40 min at 303 K), as this elutant has large δ_D^E and δ_H^E values itself. Visual inspection revealed typical Gaussian peak shape, suggesting that 1,4-dioxane interacted with terbutaline sulphate only via surface physisorption.

Of more concern, however, was the size of the Hansen partial solubility parameter standard deviations relative to the actual values. This is especially noticeable for δ_P^S, where in several cases the standard deviation is larger than the value itself. This is probably a result of the unavoidable use of multiple linear regression during the calculation of Hansen partial solubility parameters. As discussed above, experimental design suggested that the use of a greater number of elutants would have resulted in more precise results, but this was not practical. In addition, similarly imprecise results have been obtained previously despite the use of more elutants. For example, Tong *et al.* report the δ_P^S value of lactose monohydrate to be 11.26 ± 10.36 $MPa^{1/2}$ despite the use of eleven elutants (Tong et al., 2006). Nevertheless, such a level of error could clearly impact on the

usefulness of this technique for detecting small differences between materials and will be borne in mind during subsequent analysis and discussion.

3.3.3. Calculation of particle-particle interactions

Using the Hansen partial solubility parameters reported in Table 6 and true densities reported in Table 2, the relative strength of the interparticulate interactions between the various study materials was calculated, using the methods described by Rowe (Rowe, 1988). These data are presented in Table 7.

To enable comparison with the AFM data, IGC CAB ratios were calculated (Table 8), by dividing the calculated drug cohesive interaction by the relevant drug-carrier interaction.

3.3.4. Comparison of AFM and IGC CAB ratios

From comparison of Tables 4 and 8, it is clear that the AFM and IGC CAB ratios are not similar in absolute terms. However, in order to further investigate the relationship between these two sets of data, a plot of IGC CAB ratio against AFM CAB ratio was produced (Figure 1). This clearly demonstrates that there was no correlation between the two sets of CAB ratios ($R^2 = 0.032$). Overall, these results suggest that the AFM and IGC techniques employed do not produce comparable results. The imprecision of the solubility parameters calculated from the IGC data may have contributed to this negative finding.

In comparing the two sets of CAB ratios shown in Tables 4 and 8, it is clear that for every drug-carrier combination, the IGC CAB ratio was greater (i.e. more cohesive) than the AFM CAB ratio. This may be related to the physicochemical properties of the materials

used for the determination of the drug-drug cohesive interaction by each technique. For the AFM CAB ratios, drug cohesion was defined as being the adhesive force measured between the micronised drug particle forming part of the colloidal probe and the dominant face of the smooth drug crystal. On the other hand, for the IGC CAB ratios, cohesion was defined as being the strength of the interparticulate interaction between two drug particles, which was calculated using Hansen partial solubility parameters relating to micronised material. As highly energetic processes such as air-jet milling are known to produce disorder in crystalline materials and thus increase their surface energy (Buckton, 1997; Newell et al., 2001), the calculated cohesion between two micronised drug particles (as in the IGC method) might be greater than the measured cohesion between a micronised drug particle and the surface of a perfect drug crystal (as in the AFM method). When CAB ratios were subsequently calculated, this would be reflected by the IGC CAB ratios being larger (i.e. more cohesive) than the AFM CAB ratios, as was observed. This phenomenon highlights the differences between AFM and IGC and may have contributed to the lack of correlation between AFM and IGC CAB ratios.

3.4. In vitro *formulation testing*

The relative standard deviation of the drug concentration was <6% for every formulation, confirming them to be sufficiently uniform for further analysis. Visual inspection suggested that drug-only capsules contained a smaller fill volume than carrier-based formulation capsules. The difference in volumes was not as great as the difference in fill masses would suggest, due to the lower bulk density of the micronised drugs. In both cases, less than one quarter of the total volume of the capsule was occupied by powder, so the difference in fill volumes was not expected to effect the aerosolisation processes of the drug-only formulations compared with the carrier-based formulations.

Figure 2 shows the FPF of all the carrier-based formulations tested and its relationship with the appropriate drug-carrier AFM CAB ratio. Figure 3 shows the relationship between FPF and drug-carrier interparticulate interaction strength as calculated from Hansen partial solubility parameters. These figures do not show a clear overall relationship between FPF and the AFM or IGC data. This is unsurprising, because, as discussed, the physicochemical properties of the five drugs used in this study were not identical, especially in terms of particle size (see Table 2), meaning that each will have a different intrinsic level of performance (Jones et al., 2008b). Such differences may be sufficiently great to mask the effect of drug-carrier adhesion on fine particle delivery.

Figure 4 shows the relationship between the FPF and IGC CAB ratio. Given the inconsistent relationship between the AFM and IGC CAB ratios discussed above, it is unsurprising that this figure does not reveal a consistent relationship between FPF and IGC CAB ratio.

3.5. Normalisation of carrier-based fine particle fraction

In the IGC study on which this work is based (Tong et al., 2006) a strong linear correlation (n = 3) was found between the increase in the FPF(RD) of a drug following the addition of a lactose carrier (i.e. carrier-based FPF(RD) minus pure drug FPF(RD)) and the difference between the strengths of the adhesive drug-carrier and cohesive drug-drug interactions calculated from Hansen partial solubility parameters. In order to further investigate this possible predictive relationship, an equivalent plot was prepared for each of the carriers employed during this study (Figure 5). However, as is clear from inspection of this figure, no such linear correlation was observed for any of the carriers. This is still the case if the

data relating to terbutaline sulphate (which was over three times as cohesive as the next drug) are ignored. The explanation for the failure of this previously successful predictive relationship may be the use of a greater range of materials with more similar particle size distributions than those used previously (Tong et al., 2006). It may also be because, with the exception of salbutamol sulphate, the addition of carrier resulted in a decrease in FPF, whereas in the previous study it universally increased FPF. It should also be noted that there were differences in formulation drug concentration, inhaler device and capsule fill mass between this study and the previous work (Tong et al., 2006).

Finally, Tong *et al.* calculated the Hansen partial solubility parameters of their carrier material (α-lactose monohydrate) from IGC data obtained at a humidity of 50% RH. However, the Hansen partial solubility parameters of their drugs were calculated using IGC data obtained at a humidity of 0% RH, as comparison of IGC parameters obtained at 40°C and either 0% RH or 50% RH found no significantly differences. In this study, all IGC experiments were carried out at 0% RH. 50% RH is more akin to the environmental conditions encountered during the preparation and testing of DPI formulations. However, the presence of water molecules within the IGC column also results in an increased number of mechanisms by which elutant molecules might interact with the powder surface, making the interpretation of such results much more complex (Buckton and Gill, 2007).

The principle of the above analysis by Tong *et al.* (examining the relationship between the increase in the FPF(RD) following the addition of a carrier and the difference between the strengths of the adhesive and cohesive interactions) is to normalise formulation FPF to account for differences in the intrinsic *in vitro* performance of the model drugs that might otherwise confound the theoretical relationship between drug-carrier adhesion and fine particle delivery. A significant factor determining the intrinsic *in vitro* performance of a

model drug is the proportion of particles with an aerodynamic diameter <6.4 μm (the cut-off diameter between stages 1 and 2 of the TSI). Therefore, another approach to normalise formulation FPF is to calculate the proportion of particles of each drug with an aerodynamic diameter <6.4 μm and then use this figure to adjust the actual FPF obtained. The proportion of particles with an aerodynamic diameter (d_{ae}) <6.4 μm can be estimated from laser diffraction particle size distributions using the following equation (Shekunov et al., 2007):

$$d_{ae} = d_v \sqrt{\frac{\rho}{\chi}} \qquad \text{Equation 4}$$

where d_v is volume equivalent diameter (from the laser diffraction particle size data), ρ is particle density and χ is the dynamic shape factor. Using the density values from Table 2 and making the assumption that the particles were spherical (i.e. that χ = 1), values of d_v equivalent to a d_{ae} of 6.4 μm were calculated for each drug. Using the values of d_v calculated in this way, the laser diffraction particle size distributions of the model drugs were analysed to determine the percentage (by volume) of each drug contained in particles with a d_{ae} <6.4 μm. Finally, based on the calculation of Tong *et al.* (2006) (carrier-based FPF(RD) - pure drug FPF(RD)), the subsequent calculation was used to estimate the aerosolisation efficiency of drug particles with a d_{ae} <6.4 μm:

Normalised FPF(RD) = carrier-based formulation FPF(RD) - % of drug particles with d_{ae} <6.4 μm

Equation 5

A plot of normalised FPF(RD) versus AFM CAB ratio was prepared (Figure 6).

Having normalised for differing intrinsic drug particle size distributions (one of the major confounding variables that may mask the effect of drug-carrier adhesion on the performance of DPI formulations) in this way, Figure 6 follows the pattern seen in previous studies (Hooton et al., 2008; Hooton et al., 2006; Jones et al., 2008a), where the performance of the carrier-based formulations was optimised when the AFM CAB ratio was slightly cohesive. (The only one of these previous studies which made comparisons between formulations containing different drugs used micronised powders with much more similar particle size distributions than those used in this investigation (Jones et al., 2008a)). However, there was no consistent relationship between FPF normalised in this way and either the IGC CAB ratios or the strength of the drug-carrier adhesive interaction.

3.6. Possible reasons for the discrepancies between AFM and IGC results

The results presented above suggest that the AFM and IGC techniques employed in this study were not measuring the same phenomenon and that they cannot be used interchangeably. This may be attributable to the use of materials with different physical properties for the AFM and IGC experiments (discussed above) or to the imprecision in the Hansen partial solubility parameters calculated from the IGC data. As discussed, the use of a greater number of elutants may have increased the precision of the IGC results, but in a study of this size, this would have been impractical. An alternative explanation for the absence of a strong relationship between the AFM and IGC CAB ratios lies in the consideration of what each technique measures. AFM makes a direct measurement of the total adhesive force acting between the colloidal probe and the substrate. This may be made up of van der Waals', capillary and electrostatic forces (Podczeck, 1998). On the other hand, IGC measures the properties of the surfaces involved in the interaction and then seeks to calculate the strength of the resultant adhesion. Therefore, unlike AFM, IGC

takes no account of capillary or electrostatic forces, although these are likely to be present in DPI formulations (Zeng et al., 2001).

It was a requirement of the calculations used in this study that the IGC experiments were carried out at infinite dilution, suggesting that the solubility parameters obtained would have been biased towards the highest energy sites on the powder surfaces (Buckton and Gill, 2007). Such sites may not have been representative of all the areas involved in particle-particle adhesion, resulting in further discrepancies between the AFM and IGC data. Further developments of the previously discussed finite dilution IGC techniques to enable the calculation of drug-carrier work of adhesion distributions might overcome this limitation. In addition, the differing particle size distributions (Table 2) of the various drugs and excipients suggest that they had different specific surface areas, but the same elutant vapour injection concentrations were used for all samples. This might have resulted in different degrees of surface coverage for different materials, introducing a further source of variability into the IGC results (Gamble et al., 2012).

As discussed above, both techniques employed in this study have different limitations. AFM makes a direct measurement of adhesion, but employs semi-idealised materials (Weiss et al., 2015), whereas IGC makes use of the actual materials employed in the DPI formulation but relies on the calculation of interparticulate adhesion from measured surface properties. The data discussed here suggest that after normalisation to account for particle size differences, the measurement of AFM CAB ratios is more successful at predicting carrier-based DPI performance than the measurement of solubility parameters using IGC. Therefore, the limitations of AFM seem to be more acceptable than those of the IGC technique employed here. IGC remains, however, a valuable technique when considering other aspects of DPI formulation, including the effects of batch processing

750 techniques on powder surface properties and batch-to-batch variability (Jones et al.,
751 2012).
752
753 *3.7 Relationship to similar studies*
754
755 Unlike some previous studies in this area, this paper has attempted to relate the
756 interaction between various surface properties of drug and carrier particles to DPI
757 formulation performance, rather than relying on the measurement of only one surface
758 property of one type of particle. If an appropriate method can be found, such an approach
759 might be expected to yield more useful predictive relationships, as it takes greater account
760 of the complex nature of interparticulate interactions. In contrast to this account, the one
761 previous major study that attempted to take all these factors into account found a positive
762 relationship between interaction energy and formulation fine particle fraction (Cline and
763 Dalby, 2002). Without a direct comparison, it is impossible to fully account for these
764 differences, but it is noteworthy that previously a number of drugs and carriers were
765 combined in an incomplete and unsystematic way and aerosolised under differing
766 conditions (Cline and Dalby, 2002), whereas this study investigated the aerosolisation of
767 all possible combinations of a number of drugs and carriers under uniform conditions.
768
769 Previous IGC studies have almost invariably found either a positive or negative linear
770 relationship between some calculation of the potential of two surfaces to interact and
771 formulation performance (Bernhard and Steckel, 2005; Cline and Dalby, 2002; Jiang et al.,
772 2005; Kumon et al., 2006; Oliveira et al., 2006; Sethuraman and Hickey, 2002; Traini et al.,
773 2008), whereas the data presented here suggest that there might be an optimum level of
774 drug-carrier adhesion. It is possible this may have arisen from previous studies not

considering material combinations with a wide enough range of drug-carrier interactions, resulting in the examination of data exclusively above or below the optimum point.

The optimisation of fine particle delivery when the AFM CAB ratio was slightly cohesive may relate to the state of the formulations. With adhesive CAB ratios, drug-carrier interactive units might dominate the formulation, so reduced drug-carrier adhesion results in increased fine particle delivery. However, with cohesive CAB ratios, drug agglomerates might also be present, producing more complex aerosolisation mechanisms (Xu et al., 2011) and a different relationship between drug-carrier adhesion and fine particle delivery.

4. Conclusions

There are several studies that describe a consistent relationship between drug-carrier AFM CAB ratio and the *in vitro* performance of carrier-based DPI formulations (Hooton et al., 2008; Hooton et al., 2006; Jones et al., 2008a). After normalisation for differing drug particle size distributions, the results of this study were consistent with these previous findings. However, to date there has only been one study suggesting a relationship between drug-carrier interactions calculated from Hansen partial solubility parameters derived from IGC measurements and formulation performance (Tong et al., 2006), which was not replicated in this study. Therefore, of the two approaches described in this study, the measurement of AFM CAB ratios proved to be more successful at predicting carrier-based DPI performance, but even this technique was not without its limitations.

This study as a whole serves to demonstrate the complexity of the many interacting variables that can affect the performance of DPIs and suggests that the prediction of the fine particle delivery of such formulations from a single type of measurement (e.g. drug-

carrier adhesion) will not be successful in every case (Alhalaweh et al., 2013; Hickey et al., 2007; Jones et al., 2012). Data normalisation to control for confounding variables such as particle size can be useful, but it is currently not possible to control for every possible influence on DPI behaviour. Finally, the observation of an optimum point in the relationship between drug-carrier adhesion data and fine particle delivery, both in this study and others (Hooton et al., 2008; Hooton et al., 2006; Jones et al., 2008a), suggests that it is overly simplistic to expect a linear relationship between these variables, as the best performance from these complex systems is likely to be obtained as a result of achieving the optimum balance between various opposing factors.

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Captions

Table 1: Molar volume (V) and Hansen partial solubility parameters (δ_D^E, δ_P^E and δ_H^E) of the various potential elutants forming the domain from which the experimental elutants were selected (Huu-Phuoc et al., 1987a; Huu-Phuoc et al., 1987b; Huu-Phuoc et al., 1986).

Table 2: Summary particle size statistics (n = 5) and true densities (n = 10) of the study materials.

Table 3: Probable polymorph and dominant face Miller index of extremely smooth crystal substrates used for colloidal probe AFM adhesion and cohesion measurements.

Table 4: AFM CAB ratios ± SD and respective coefficients of determination (R^2) for each drug-carrier interaction.

Table 5: Results of matrix optimisation calculations used to determine the best combination of elutants to use for measurement of Hansen partial solubility parameters by IGC.

Table 6: Hansen partial solubility parameters determined for the eight study materials and the correlation coefficient of the multiple linear regression analysis used to obtain these data.

Table 7: Relative strength of interparticulate interactions between each model drug and either itself (cohesion) or each of the model carrier excipients, as calculated from their Hansen partial solubility parameters (MPa).

Table 8: IGC CAB ratios for each drug-carrier interaction, as calculated from Hansen partial solubility parameters.

Figure 1: Comparison of IGC and AFM CAB ratios. × - beclometasone dipropionate; ★ - budesonide; △ - salbutamol sulphate; ☐ - terbutaline sulphate; ● - triamcinolone acetonide.

Figure 2: The relationship between FPF (based on both ED and RD) and AFM CAB ratio. × - beclometasone dipropionate; ★ - budesonide; △ - salbutamol sulphate; ☐ - terbutaline sulphate; ● - triamcinolone acetonide.

Figure 3: The relationship between FPF (based on both ED and RD) and drug-carrier interparticulate interaction strength as calculated from Hansen partial solubility parameters. × - beclometasone dipropionate; ★ - budesonide; △ - salbutamol sulphate; ☐ - terbutaline sulphate; ● - triamcinolone acetonide.

Figure 4: The relationship between FPF (based on both ED and RD) and IGC CAB ratio. × - beclometasone dipropionate; ★ - budesonide; △ - salbutamol sulphate; ☐ - terbutaline sulphate; ● - triamcinolone acetonide.

Figure 5: Relationship between increase in FPF(RD) following addition of each carrier and the difference in strength between the adhesive drug-carrier and cohesive drug-drug interactions calculated from Hansen partial solubility parameters.

Figure 6: The relationship between the difference between carrier-based formulation FPF(RD) and the proportion of drug particles with a d_{ae} <6.4 μm and AFM CAB ratio.