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Investigation to find a suitable reference material for use as an inverse gas chromatography system suitability test

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Abstract

The purpose of this study was to find a suitable material for use as a system suitability test material for inverse gas chromatography (IGC). The purpose of a system check is to measure the variability of the instrument being used rather than the material being analysed. Having such a system check for IGC enables a basic GMP requirement to be met. IGC results can then be used in regulatory submissions to support other already established techniques for characterising pharmaceutical materials. α-Alumina was chosen because it is inorganic, stable at high temperatures, resistant to hydration and is already established as a system check material for surface area determination by nitrogen adsorption. Two columns (1 and 2) packed from the same reference sample jar of α-alumina and analysed under the same conditions yielded dispersive surface energies of 34.8 ± 0.8 and 35.3 ± 0.8 mJ/m^2, respectively, at 15% RH. Column 1 was analysed on a second IGC system, and gave a dispersive surface energy of 34.7 ± 0.2 mJ/m^2. No significant change was evident after 7 months storage under laboratory ambient conditions. Analysis of material from a second reference sample jar of α-alumina produced results not noticeably different to those of the first, yielding a dispersive surface energy of 35.3 ± 0.1 mJ/m^2. A change was seen to occur in the surface properties α-alumina when the humidity was varied, but the change appeared to be consistent across the two columns reported. Based upon the data in this experimental, α-alumina appears to be a suitable material for use as a system suitability test material for IGC.

Keywords: Inverse gas chromatography; System suitability; α-Alumina; Dispersive surface energy

1. Introduction

Changes in the surface properties of a powder can influence the processing and formulation characteristics. The surface energy can be affected by a change in the route of chemical manufacture or by processing (e.g. milling and micronisation), and can impact on the way the drug handles e.g. flow, stability and compactability for tablets. Ultimately the quality and performance of the final product could be affected. Knowledge of the surface energetics of pharmaceutical products is therefore important in the design of pharmaceutical formulations.

Surface free energy (γ) can be regarded as the energy required to produce a unit of surface area. The surface energy of materials will have a non-polar (dispersive) contribution and most will have polar (acid–base) forces. The surface free energy therefore consists of polar (γ^{P}) and dispersive components (γ^{D}). The polar term is often subdivided into an electron donating and electron accepting contribution.

Techniques used to assess surface energy include those which measure contact angle. For powders, sample preparation is a known problem with contact angle measurement. A compact or slide preparation of the analyte is required in order to carry out the sessile drop or Wilhelmy plate methods. The extent of error associated with contact angle measurements has been investigated (Buckton et al., 1995). The techniques, although subject to significant error, provided useful information related to the surface properties of the powder.

Inverse gas chromatography (IGC) offers a methodology which requires minimum sample preparation. The equipment, sample preparation and theory have been described previously by Newell et al. From the fundamental values obtained by IGC i.e. the time required for the probe to pass through the column,

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thermodynamic information can be obtained. These include surface free energy, acid–base properties, solubility parameters, heat of adsorption and the glass transition temperature.

There have been comparisons between IGC and contact angle data, for example the wettability of several pharmaceutical powders has been assessed using contact angle and IGC (Zhang et al., 2002). A drop of water or diiodomethane was placed onto a powder compact and an image taken depicting the contact angle. The contact angle was then fitted to the Young–Laplace equation in order obtain the interfacial tension. In addition to this technique, the dispersive surface energies were also obtained using inverse gas chromatography (IGC). Good correlation between the contact angle and IGC data for hydrophobic powders was reported. Dove et al. (1996) and Ahfat et al. (2000) have also demonstrated similarities between contact angle and IGC data, although the results show that IGC can yield much higher values for surface energy. This is thought to be due to preferential probing of high energy sites with IGC at infinite dilution, compared to an average value of surface energy from contact angle data, as the drop will be in contact with sites of different energy.

Mixtures of crystalline and amorphous lactose have been used to investigate whether IGC preferentially probes higher energy sites (Newell and Buckton, 2004). Scanning electron microscopy (SEM) showed that smaller amorphous particles of lactose adhere to the larger crystalline ones. The surface energy results, derived from IGC data, showed that a substantial part of the surface (40% by surface area) needs to comprise of the higher energy material in order for it to dominate.

Typical applications of IGC have been to try to distinguish difference between batches of the same pharmaceutical raw drug substance which are chemically and structurally identical (Ticehurst et al., 1994). Two batches of a pharmaceutical active (salbutamol sulphate) were found to be equivalent by FT-Raman spectroscopy and X-ray powder diffraction. There was a marked difference in the surface energetics of the two batches, as determined by IGC. The data emphasised the potential of IGC to detect and quantify differences in the surface properties of chemically and structurally equivalent pharmaceutical powders. Ohta and Buckton (2005) have used IGC to show differences in the surface properties of two amorphous samples of the same active.

IGC can certainly be used to increase the understanding in changes of a material as a function of temperature and relative humidity, how it can be used to analyse mixtures and how it can complement other analytical techniques. For example, Newell et al. (2001a,b) have shown the changes in surface energy of amorphous material and how this changes during crystallisation.

Yokoi et al. (2004) have assessed the physicochemical stability of granules and spray dried amorphous cefditoren pivoxil alone and combined with different polymers. IGC data were used to demonstrate that the surface state of the granules was similar to that of spray dried particles. Ohta and Buckton (2004) have related acid–base contributions to surface energy to the tendency for different materials to degrade.

Buckton et al. (2004) have used IGC to study the T_g of the surface (rather than the bulk) of a hydrophobic drug, arguing that water sorption is not even throughout the particle and hence the T_g of the surface will be different to that of the bulk.

All of this work highlights the potential of IGC for acquiring information which would be useful in the development of a new drug substance. The pharmaceutical industry is subject to stringent regulatory control and therefore requires a system suitability check to be performed, prior to analysis, to ensure that the system is working correctly. There is currently no such system check for IGC. The aim of this work is therefore to investigate the potential for alumina to be used as a system suitability check for IGC systems.

Alumina was selected as likely to be a highly appropriate material to be used as a system check-based upon its stability. α-Alumina is used as a system check for surface area equipment (supplied by Micromeritcs Ltd., Bedfordshire). It is inorganic (c.f. drug substance), stable at high temperatures and resistant to hydration.

2. Materials and methods

2.1. Materials

Two different batches of α-alumina (46F-BA106-27 and 46F-BA106-28) were sourced from Micromeritics Ltd. (Bedsfordshire, UK). Each was subsampled from the same bulk by Micromeritics Ltd.

2.2. Equilibrium relative humidity (ERH)

In order to analyse the α-alumina with minimum conditioning of the sample, the ERH was measured at the time of first opening pot 46F-BA106-28 using the AWSprint (TH-500), Novatron UK. The measured value was 23.6% RH at 21.1 °C. This was deemed to be the naturally equilibrated state of the α-alumina (as equilibrated within the original sample pot). Using a psychrometric chart, the water equivalent at 30 °C (temperature being using for analysis by IGC) was found to be 15% RH. All the initial experiments on each column were carried out under these conditions (15% RH 30 °C). Subsequently (as will be described below) the effect of changing the conditions was investigated.

2.3. Inverse gas chromatography

Experiments were performed using an inverse gas chromatograph (Surface Measurement System Ltd.). Approximately 1 g of α-alumina powder was packed into silanised glass columns (Surface Measurement System Ltd.), which were 6 mm o.d., 2 mm i.d. and 300 mm long, by vertical tapping using an IGC column packer (Surface Measurement System Ltd.). Columns were packed for 20 min after the initial filling, monitoring the column visually to check that there were no visible cracks in the powder bed. Both ends of the column were stoppered with 2.5 cm of silanised glass wool (Surface Measurement Systems Ltd.). The columns packed with α-alumina were conditioned at 30 °C and 0, 15, 40 and 60% RH for 10 h. The experimental was carried out at the same temperature and RHs as the conditioning.

Methane (BOC Special Gases) was used for the inert reference material. The non-polar probes used were heptane, octane, nonane and decane (Aldrich), 99.9% minimum purity and were used to calculate the dispersive surface energy. Each probe was injected once per analysis run. The polar probes used were acetone, ethanol and ethyl acetate (sourced from Aldrich and BDH, all >99% purity). The probes were injected at 0.04 P/P_0 (partial pressure of the elutant defined at the column temperature), except acetone which was reduced to 0.02 P/P_0 to reduce the size of the peak. The carrier gas used was helium (BOC Special Gases), and the flow rate was 10 sccm. The IGC system is fully automated, using purpose written control software (SMS iGC Controller Software, v1.6). All results were calculated using the SMS iGC Analysis Macros, v1.2.

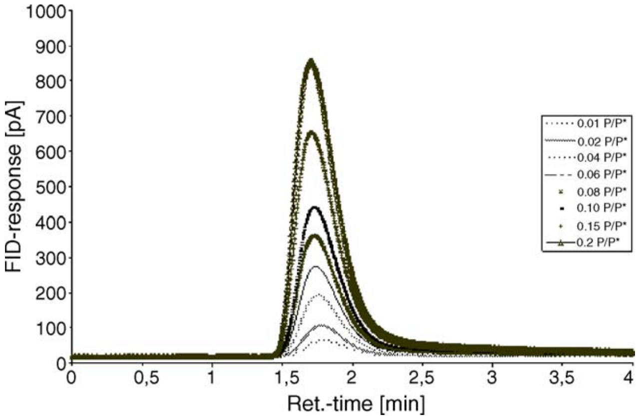

Fig. 2. Eluted nonane peak after injection of different concentrations.

3. Results and discussion

3.1. Peak shape

Peaks with strong tailing factor have been previously observed (Thielmann and Baumgarten, 2000) and were attributed to the microporous structure of alumina, where higher energy was required for desorption out of the micropores than desorption out of the mesopores.

Broad peak shapes were observed during this experimental. An investigation was initiated using nonane to try to reduce the peak-tailing factor. Experiments were carried out varying the flow rate, concentration of elutant, temperature and humidity. In each case the starting method used was the same as detailed in Section 2.3 and then the parameter under investigation varied. The elutant peaks (obtained by plotting the FID response against the relative retention time) for each variable can be found in Figs. 1–4.

An increase in the flow rate, although resulting in an increase in the pressure across the column, had only a slight impact on the tailing factor (Fig. 1). The results obtained by changing the concentration of the elutant (Fig. 2) showed that 0.04 P/P^* is in a suitable region for analysis i.e. a region where the tailing factor is at its lowest and within Henry's Law. Increasing the temperature (Fig. 3(a) and (b)) exhibited peak shapes which appeared to become less Gaussian as the temperature increased until 90 °C, when the tailing factor reduced. Thielmann and Baumgarten (2000) found that complete elution could be achieved by rapid increases to higher temperatures (550–850 °C). The highest temperature which could be achieved on the IGC system used for these analyses was 90 °C.

The peak asymmetry factor and shape appeared to improve with an increase in RH (Fig. 4). This is thought to be caused by the water filling up available pores, affecting the rate of adsorption/desorption of the elutants.

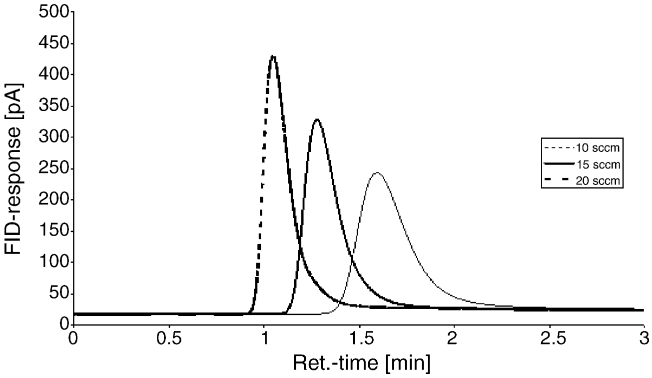

Fig. 1. Nonane eluted at different flow rates in sccm (standard cubic centimeters per minute) of carrier gas (He).

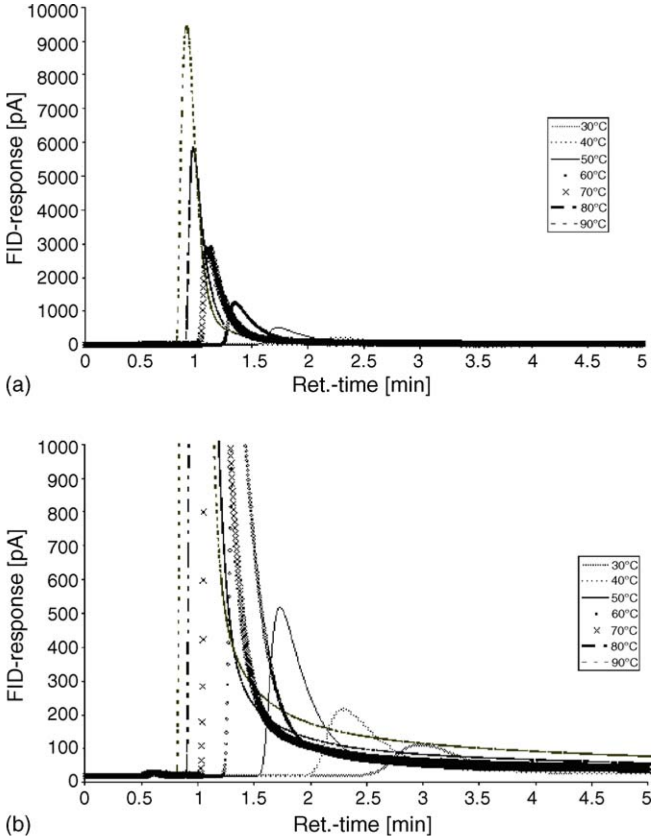

Fig. 3. Nonane eluted at different temperatures.

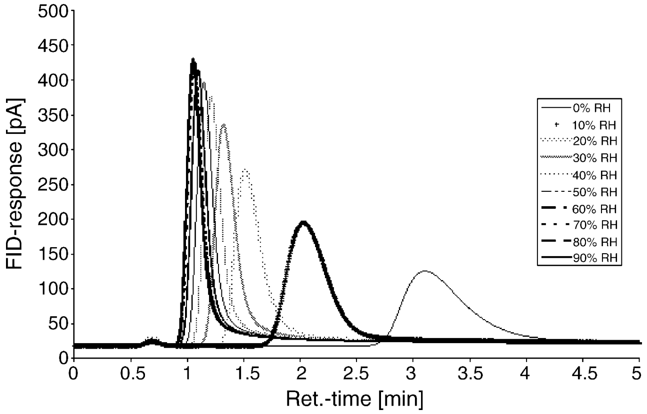

Fig. 4. Nonane eluted at different relative humidities.

3.2. *Analysis of α-alumina at 15% RH*

Two packed columns of sample 46F-BA106-28 gave comparable results for dispersive surface energy and free energies of acetone and ethanol (see Table 1). The data show that there is good agreement obtained for the data for the two different columns. The standard deviations for each column were not noticeably different and the two-tailed *t* values obtained across the two columns were 0.17, <0.01 and 0.02 for the dispersive free energy, and free energies for acetone and ethanol, respectively. This is lower than the value presented in a *t*-table for the 95% confidence level (2.78), demonstrating that there is no significant difference between the two columns packed from a single sample pot of alumina.

3.3. *Analysis of α-alumina at other RH values*

The same columns were analysed at 0, 40 and 60% RH. Given the low initial ERH of the material, analysis was carried out at 0% RH before going to higher RH values of 40 and 60% RH. Ethyl acetate was added to the elutants at 40 and 60% RH. The retention times for acetone, 1,4-dioxane, ethanol and ethyl acetate at 0% RH (and 1,4-dioxane at 15% in addition to 0% RH) were greater than 45 min (i.e. the elutant peak did not return to the baseline during analysis). This data could therefore not be used. During analysis at 40 and 60% RH, the acetone elutant produced a split peak suggesting that some of the acetone is retained on the column for a longer duration, consequently the acetone data have not been included in further analysis.

The data (Table 2) show the dispersive surface energy decreases with an increase in humidity up to 40% RH. The elution of the polar probes was over 45 min at low humidity. There was no noticeable change in the either the dispersive surface energies or the free energies of the polar probes between 40% RH and 60% RH.

The dispersive surface energy showed greater variability than the free energies of the polar probes (Tables 1 and 2) as demonstrated by the standard deviations shown in Table 1.

3.4. *Analysis of a second sample (46F-106-27) of α-alumina*

A second sample of α-alumina was obtained from Micromeritics Ltd. The results obtained (Table 3) were not noticeably different to those of 46F-106-28.

The two-tailed *t* values obtained across the one column from each sample were 0.12 and <0.01 for the dispersive free energy, and free energies for acetone and ethanol, respectively. This is lower than the value presented in a *t*-table for the 95% confidence level (2.78), demonstrating that there is no significant difference between the two samples of alumina.

3.5. *Stability of α-alumina*

Column 1, prepared using 46F-106-28, having been stored under laboratory ambient conditions with Parafilm at either ends of the column, was reanalysed after approximately 7 months to assess the stability of α-alumina over time. Analysis was performed at 15% RH/30 °C, and compared to results obtained when the column was initially prepared and analysed (see Table 4). A small decrease in the dispersive surface energy was evident; however a two-tailed *t*-test value of 0.28 was calculated for the two sets of data, showing it to be statistically insignificant. (The value presented in a *t*-table for the 95% confidence level is 3.18).

A new column (column 3) was prepared from the bulk of the reference material. The reference material had been stored in its original sealed plastic container under laboratory ambient conditions. Column 3 was analysed using the same conditions as above (Table 5). No change was evident in the dispersive surface energy of the bulk material over time when compared to data generated on earlier samples from the bulk. Using the same two-tailed *t*-test as previous, a *t*-value of 0.71 was obtained across the two columns, showing no significant change in the bulk.

3.6. *Analysis of α-alumina on a second IGC system*

The results discussed above show a method by which a sample can be shown to give reproducible data on the same instrument. It is an important requirement for IGC to know whether the same sample will give rise to the same results when measured on a different IGC instrument.

Table 1
Surface energy results at 15% RH/30 °C for α-alumina, pot 46F-BA106-28

Column	Dispersive surface energy (mJ/m^2)	Free energy for acetone (kJ/mol)	Free energy for ethanol (kJ/mol)
1	34.8, range = 34.2–35.9, S.D. = 0.66	17.2, range = 17.0–17.4, S.D. = 0.15	20.3, range = 20.2–20.5, S.D. = 0.13
2	35.3, range = 34.7–36.2, S.D. = 0.60	17.6, range = 17.4–17.7, S.D. = 0.11	20.5, range = 20.4–20.7, S.D. = 0.13

Table 2
Summary of data for α-alumina (46F-BA106-28), columns 1 and 2, across different relative humidities

	0% RH		15% RH		40% RH		60% RH	
	Column 1	Column 2	Column 1	Column 2	Column 1	Column 2	Column 1	Column 2
Dispersive surface energy (mJ/m^2)	43.8 (42.0–44.6)	45.2 (44.2–46.3)	34.8 (34.2–35.9)	35.3 (34.7–36.2)	30.5 (25.2–33.9)	30.2 (28.9–31.1)	31.0 (29.3–32.7)	29.4 (28.6–29.9)
Free energy for 1,4-dioxane (kJ/mol)	>45 min	>45 min	N/A	N/A	17.3 (15.6–18.7)	18.3 (18.1–18.5)	17.0 (16.7–17.3)	17.1 (17.0–17.2)
Free energy for ethanol (kJ/mol)	>45 min	>45 min	20.3 (20.2–20.5)	20.5 (20.4–20.7)	18.6 (17.5–19.3)	18.7 (18.5–18.9)	18.2 (17.9–18.5)	18.1 (18.0–18.2)
Free energy for ethyl acetate (kJ/mol)	>45 min	>45 min	N/A	20.8 (20.7–21.0)	17.8 (16.7–18.4)	17.8 (17.5–18.0)	17.2 (16.9–17.6)	17.1 (16.9–17.2)

N.B.: The numbers shown in (parentheses) are the range of results obtained over the five runs carried out on each column under the specified conditions.

Table 3
Results from two different pots of α-alumina (46F-106-27 and 46F-106-28) at 15% RH/30 °C

Pot number	Dispersive surface energy (mJ/m^2)	Free energy for acetone (kJ/mol)	Free energy for ethanol (kJ/mol)
46F-106-27	35.3 (35.2–35.4)	17.8 (17.8–17.8)	20.6 (20.6–20.6)
46F-106-28 (column 1)	34.8 (34.2–35.9)	17.2 (17.0–17.4)	20.3 (20.2–20.5)

N.B.: The numbers shown in (parentheses) are the range of results obtained over the five runs carried out on each column under the specified conditions.

Table 4
Comparison of data generated using α-alumina (column 1, 46F-106-28) at time of column preparation and after 7 months (analysed at 15% RH/30 °C)

Run	Column 1, initial		Column 1, after 7 months	
	Dispersive surface energy (mJ/m^2)	Correlation coefficient	Dispersive surface energy (mJ/m^2)	Correlation coefficient
1	34.2	0.9992	34.8	0.9987
2	34.4	0.9992	33.4	0.9993
3	35.9	0.9986	33.8	0.9991
Mean	34.8		34.0	
S.D.	0.93	N/A	0.70	N/A
Coefficient of variance	2.67		2.07	

The dispersive surface energy has been shown to give reliable data across a wide range of conditions (Tables 1 and 2) and consequently this has been used to assess reproducibility of two IGC systems. Column 1 (46F-106-28) was analysed on a second IGC system at 15% RH/30 °C. There was no marked difference between the two systems with regards to the dispersive surface energy (Table 6). A *t*-test was carried out to compare the two data sets. The *t* value obtained across the two instruments was 0.17. This is lower than value presented in a *t*-table for the 95% confidence level (2.78). This demonstrates that there is no significant difference between the two instruments with regards to the analysis of alumina.

Table 5
Comparison of data generated using α-alumina (column 1, 46F-106-28) at time of column preparation and column 3 prepared from the bulk after 7 months (analysed at 15% RH/30 °C)

Run	Column 1, initial		Column 3, after 7 months in the bulk sample	
	Dispersive surface energy (mJ/m^2)	Correlation coefficient	Dispersive surface energy (mJ/m^2)	Correlation coefficient
1	34.2	0.9992	34.2	0.9990
2	34.4	0.9992	35.0	0.9990
3	35.9	0.9986	34.6	0.9999
Mean	34.8		34.6	
S.D.	0.93	N/A	0.41	N/A
Coefficient of variance	2.67		1.17	

Table 6
Comparison of data generated with α-alumina (column 1, 46F-106-28) using two IGC systems (both at 15% RH/30 °C)

Run	System 1		System 2	
	Dispersive surface energy (mJ/m^2)	Correlation coefficient	Dispersive surface energy (mJ/m^2)	Correlation coefficient
1	34.2	0.9992	34.7	0.9988
2	34.4	0.9992	34.8	0.9991
3	35.9	0.9986	34.9	0.9990
4	34.6	0.9994	34.5	0.9993
5	34.8	0.9990	34.7	0.9989
Mean	34.8		34.7	
S.D.	0.67	N/A	0.16	N/A
Coefficient of variance	1.92		0.45	

4. Conclusions

No sizeable change was seen in the dispersive surface energy over time, fulfilling the requirement for a system check material to be stable. The reproducibility of α-alumina as a system check was demonstrated by analysis on a second system. The similarity of the results obtained on two samples supplied by Micromeritics Ltd. again demonstrates the suitability of α-alumina as a system suitability check. All experiments carried out on α-alumina so far have been at infinite dilution.

Sun and Berg (2002) observed that as the water coverage decreases, the oxide surfaces become energetically more heterogeneous. As a result, the surface energy increases and the acid–base interaction potential decreases. Data in this report shows the surface energy increase with a decrease in humidity. The elution times of the polar probes increased to over 45 min with a decrease in humidity.

In summary, a change does occur in the surface properties α-alumina when humidity is varied, but the change is known and therefore does not affect the use of α-alumina as a system check material, provided that the same conditions are consistently used.

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