Poster Session 3 – Pharmaceutics

183
Predicting process behaviour using physicochemical data: a computational approach

I. J. Hardy and D. A. Adkin

AstraZeneca, Bakewell Road, Loughborough, Leicestershire, LE11 5RH, UK

Formulation scientists are under increasing pressure to produce robust dosage forms, which may be manufactured over a range of scales with minimal time, cost and material. Microformulation approaches have in part addressed this need through the miniaturisation of processing equipment and the use of predictive/correlative techniques. Powder rheometry is one such microformulation technique that measures the flow properties of powders, which can be correlated with the weight uniformity of tablets produced on a rotary tablet press (Hardy & Cook 2002). However, powder rheometry requires approximately 30 g of material, which may not be available during pre-clinical formulation development. In contrast, a physicochemical data set can be generated on a few grams of sample. The following paper describes a preliminary investigation into the use of physicochemical data to predict powder flow and processing behaviour with the aid of computer modelling.

The flow properties of a series of pharmaceutical excipients (Emcompress, Pharmatose 50M, 150M, 450M, DCL11, DCL21, DCL40, Aerosil 130, 200 and 300 and Parteck M300) were measured using a Manumit powder rheometer (200 mL glass vessel, 48 mm diameter blade, 50 mm s^{-1} blade speed, 5° helix angle downward compaction cycle, 178° helix angle upward aeration cycle). The compaction coefficient (indicative of powder flow) was calculated by measuring the area under a force-displacement plot of the compaction cycle. Particle size was measured using a laser diffractometer (Malvern mastersizer, 300 mm lens, Miglyol suspension, polydisperse Fraunhoffer analysis, n = 3). True density was measured using an Accupyc 1330 helium pycnometer (n = 5) and water content was measured using Karl Fischer titration (n = 3). Principal component analysis (PCA) and partial least squares projection to latent structures modelling (PLS) was performed using multivariate analysis software (SIMCA v10.0, Umetrics).

A PCA scatter plot showed that materials formed discrete clusters depending on their compaction coefficient measured by powder rheometry and ultimately by their flow properties. Further analysis as to the source of the clustering behaviour revealed that materials with enhanced flow properties were those with the larger more uniform particle size (Pharmatose 50M and Parteck M300). Increases in true density, reduction in mean particle size and particle size uniformity resulted in a deterioration in powder flow and a distinctive clustering behaviour. PLS modelling showed that the compaction coefficients predicted by the model from the physicochemical data were in close agreement with the observed values measured by powder rheometry.

This study suggests that for a series of excipients, flow properties and ultimately tablet weight uniformity may be predicted from the physicochemical properties of the material using computer modelling. This could offer a potentially powerful tool during pre-clinical formulation development, when drug substance is in scarce supply.

Hardy, I. J., Cook, W. G (2002) AAPS Annual Meeting. Supplement; 4 (4)

184
Effect of punch surface material on the sticking tendencies of model ibuprofen formulations

M. Roberts, J. L. Ford, G. W. Smith*, G. S. MacLeod†, J. T. Fell‡ and P. H. Rowe

School of Pharmacy and Chemistry, Liverpool John Moores University, L3 3AF, UK, *Manesty, Kitling Road, Knowsley, L34 9JS, UK, †FMC BioPolymer, Avenue Mounier 83, 1200 Brussels, Belgium and ‡School of Pharmacy and Pharmaceutical Sciences, University of Manchester, M13 9PL, UK

Coating of tablet press tooling is often used by manufacturers to increase wear and corrosion resistance. Methods such as chromium electroplating and chromium nitride ion bombardment (Schumann & Searle 1992) are also intended to eradicate tablet process problems such as punch sticking. However, minimal data to support these effects has been reported.

The aim of this study was to assess the influence of punch surface material and the tablet formulation on adherence to the upper punch face using a model ibuprofen system.

Two formulations were used: 69.5% ibuprofen BP: (i) 29.5% Lactose DC (Tablettose 80, Meggle, Germany) and (ii) 29.5% microcrystalline cellulose (MCC) (Avicel PH102, FMC). Each contained 0.5% colloidal silica (Aerosil 200, Degussa, Germany) and 0.5% magnesium stearate (BDH, UK). Compaction was performed at 10 kN, 25 kN or 40 kN using an instrumented Manesty (Knowsley, UK) F3 single punch tablet press. Two sets of 12.5 mm flat-faced punches were used with either uncoated steel or hard chrome-plated punch tips. Punch surface quality is influential on sticking (Roberts *et al* 2002). Surface profiles (Taylor Hobson Form Talysurf 120) of steel and chrome-plated upper punch faces indicated similar surface quality. Ra values, the mean of all positive deviations from zero, were 0.05 μm and 0.06 μm, respectively. Each compaction run was 1 min. Following compaction and removal of powder from the punch barrel, the punch face was immersed in 5 mL 96% ethanol; ibuprofen attached to the face was quantified by spectroscopy at 264 nm.

Table 1 Sticking of ibuprofen to the upper punch face

	Steel punch		Chrome punch	
	Lactose	MCC	Lactose	MCC
10 kN	149 ± 43	449 ± 29	1379 ± 136	306 ± 60
25 kN	515 ± 88	636 ± 57	216 ± 32	282 ± 47
40 kN	1031 ± 286	844 ± 120	346 ± 50	481 ± 132

Data are expressed as μg ± s.d., n = 25 for each data set

Sticking with the uncoated steel punch increased with compaction force for both formulations, though the increase in sticking between 10 kN and 40 kN was less pronounced when MCC was used. Sticking was reduced when using the chrome punch for the MCC formulation at all compaction forces and for the lactose formulation at 25 kN and 40 kN. However, sticking to the chrome punch was markedly increased at 10 kN with the lactose formulation. Three-way analysis of variance indicated a strong interaction between punch surface material, compaction force and the main excipient used in the formulation. The cause of sticking appears to be an inter-relationship between the three parameters studied.

Roberts, M., *et al.* (2002) *J. Pharm. Pharmacol.* **54** (Suppl.): S-24
Schumann, S., Searle, G. D. (1992) *Drug. Dev. Ind. Pharm.* **18**: 1037–1061

185
Investigation into the influence of batch variations of non-ionic surfactants and fatty alcohols on the rheology and microstructure of topical formulations

H. Vu Dang, P. Scholes*, J. Dove* and G. M. Eccleston

Department of Pharmaceutical Sciences, Strathclyde Institution of Biomedical Sciences, University of Strathclyde, Glasgow and *Department of Pharmaceutics, 3M Health Care Ltd, Loughborough, Leicestershire, UK

The rheological properties of dermatological creams containing fatty alcohols and non-ionic surfactants are often extremely sensitive to batch variations of the emulsifier components. The ternary phase behaviour of such mixed emulsifiers in water (ternary systems) provides valuable information about the microstructures of semisolid creams prepared with them (Eccleston 1997).

In this work, ternary systems prepared from fatty alcohol (16 g), Tween 60 (4g) and water (180g) were investigated. Two batches of Tween 60 were used of bovine (Batch 1: B1) and non-bovine (Batch 2: B2) origin. Mixed homologue alcohols, cetostearyl, CSA (~ 40% C16, 50% C18) and pure homologue cetyl, C16 (95%

pure) and stearyl, C18 (97% pure) were also used. Rheological tests were performed over 4-month storage at 25°C (Stress Tech Rheometer, RheoLogica, Sweden) with cone and plate geometry (4°, 4 cm). Differential scanning calorimetry, DSC, TC 15 (Mettler, Switzeland) and microscopy (Polyvar, UK) were also used to characterize the ternary systems. Selected data of apparent viscosities η_{app} (calculated at shear rate of 100 s^{-1}), oscillatory parameters G', η', and creep compliances after 90 min, J, are shown in Table 1.

Table 1: Selected data (η_{app}, G', η', J) after 1 week and (in parenthesis) 4 month storage at 25°C

Ternary systems	η_{app} (Pas)	0.01 Hz G' (Pa)	η' (Pas)	J (1/Pa)
				(0.018)
CSA-B1	1.4 (1.5)	756 (686)	8810 (12626)	(0.024)
CSA-B2	0.7 (0.7)	409 (449)	4236 (7613)	—
C16-B1	0.8 (0.2)	415 (102)	3967 (1712)	—
C16-B2	0.4 (0.3)	246 (91)	2514 (946)	—
C18-B2	0.1 (0.1)[a]	—	—	—

[a]1 month storage

All cetostearyl alcohol ternary systems were thicker than corresponding cetyl and stearyl alcohol systems. Each ternary system showed an initial increase in consistency over approximately the first 1–2 weeks of storage (data not shown), followed by slight changes in consistency (cetostearyl alcohol systems) or significant reductions of structure (cetyl alcohol systems) on further storage (Table 1). In contrast, the stearyl alcohol ternary system was mobile immediately after preparation and remained mobile on storage. The initial increase in consistency in all systems is due to hydration of the polyoxyethylene (POE) groups at low temperature to form additional lamellar gel phase on storage. DSC data and microscopy confirm that breakdown (C16) or lack of structure (C18) in the pure alcohol system is due to crystallization of the lamellar gel phase.

The consistencies of all systems made with surfactant Batch 1 was higher than those of systems made with Batch 2. This may be due to variations in the number of POE groups in the two batches. Future work will investigate this.

In conclusion, the manner in which batch variations of non-ionic surfactants and fatty alcohols influence the structure and rheology of ternary system can be explained from their phase behaviour in water. The same effects are seen in full cream formulations containing an oil phase (data not shown), further confirming the value of ternary systems as structural models for oil-in-water emulsions.

Acknowledgement: The authors would like to thank 3M Health Care Limited for offering financial support.

Eccleston, G. M. (1997) *Colloids and Surfaces A* **123–124**: 169–182

186
Reformulation of Magnesium Sulphate Paste

S. Sidhu and T. Ehtezazi

School of Pharmacy, Liverpool John Moores University, Byrom St, Liverpool, UK

Magnesium Sulphate Paste is the most popular over the counter treatment for boils. It reduces the need for antibiotics and surgery when treating boils and carbuncles and is effective in cleaning burns, wounds and ulcers. However, there are a number of problems associated with it, which predispose to its reformulation. On storage the magnesium sulphate crystals settle to from a cake at the bottom of the container, which is not easily redispersed. Consequently the paste must be packed in a jar so that it can be stirred before use, it is usually stirred and applied with a spoon, hence the paste's application is made difficult and messy leading to poor patient compliance. The paste has a low capacity for water, requiring frequent applications to prevent overdilution (Lowthian *et al* 1985). The paste is a coarse suspension which may be rough and abrasive when applied to a sensitive area such a wound or burn. It is not very viscous, often spreading onto healthy skin, damaging skin follicles and predisposing to further boils (BNF 1974).

An ideal preparation would be a stable disperse system or a solution, and so would not settle and could be packed in collapsible tubes, aiding patient compliance. It would be viscous, therefore would not spread onto, and damage, healthy skin which is a serious side-effect of Magnesium Sulphate Paste. It would have a higher capacity than Magnesium Sulphate Paste and so would not need to be frequently re-applied, as this can cause discomfort to the patient and can delay wound healing due to the frequent changing of bandages/dressings.

Magnesium chloride ($4H_2O$), magnesium sulphate (monohydrate), anhydrous sodium acetate, anhydrous sodium sulphate and anhydrous potassium citrate were mixed in different combinations with glycerol, propylene glycol and polyethylene glycol 200. These preparations were placed in a water bath at 32°C and the lid was applied to form a tight seal, producing a high relative humidity environment. The preparations were weighed at intervals to measure the amount of water they absorbed. The two most hygroscopic preparations, glycerol and magnesium chloride/propylene glycol, were selected and thickening agents (aerosil, polyethylene glycol 1500, PVP and carbopol) were added to the preparations and stirred to produce gels. It was found that 4% Carbopol in magnesium chloride/propylene glycol gel was the most hygroscopic gel which also maintained a firm gel structure, even after absorbing 52.31% water. It absorbed 14.81% more water than Magnesium Sulphate Paste and met all the requirements for the ideal preparation.

British National Formulary (1974) The British Medical Association, The Royal Pharmaceutical Society of Great Britain

Lowthian, P., *et al*. (1985) *Lancet* **2**:1186

187
A robust and stable fast disintegrating tablet suitable for packaging in conventional Securitainers

A. L. Page, B. K. Low, A. Leighton, J. Collins, A. Patel and M. Whiteman

Phoqus Pharmaceuticals Limited, 10 Kings Hill Avenue, Kings Hill, West Malling, Kent, ME19 4PQ, UK

Fast disintegrating tablets are generally fragile and require special packaging to maintain tablet integrity and physical stability.

The aim of this study was to assess the ability of electrostatic deposition technology to produce solutions for these fast dissolve issues. The robustness and stability of fast disintegrating tablets (Qdis) packed in desiccated Securitainers and blister packs were also assessed.

Proprietary placebo tablet cores comprising mannitol and a super-disintegrant were prepared by light compression. Tablet faces were coated with a water-soluble polymer coat using electrostatic powder deposition technology (Hogan *et al* 2002). Crushing strength, friability and in-vitro disintegration tests were determined using USP methodology. In-vivo disintegration was measured by holding the tablet on the tongue until it completely disintegrated. Coated placebo tablets were packaged in Securitainers. Stability data were generated up to the one year time point (Table 1).

Table 1 Physical properties of coated tablets in Securitainers

Weeks	0	26	26	52	Target specifications
Storage conditions (°C/%RH)	N/A	25/60	40/75	25/60	—
Crushing strength (Kp)	5.0	4.9	4.4	5.7	>1.5
Friability (% w/w loss)	0.5	0.3	0.4	0.8	< 1.0% w/w weight loss
In-vitro disintegration (s)	27	25	30	30	< 60 seconds
In-vivo disintegration (s)	25	28	27	N/D	< 60 seconds

Coated and uncoated placebo tablets were also blister packed. Tablet robustness was assessed by counting the number of tablets surviving the push-through removal process (Table 2).

Table 2 Robustness testing of coated and uncoated tablets in blister packs

Test	Uncoated	Coated
Crushing strength (Kp)	0.9	3.4
% Survival	62	100
In-vivo disintegration (s)	10	19

Electrostatic dry powder deposition technology can be used to coat friable tablets. Coating improves the robustness while maintaining a short in-vivo disintegration time. Qdis tablets are stable for at least 26 weeks at 40°C/75%RH and 52 weeks at 25°C/60%RH when packed in Securitainers. Furthermore, the coated tablets are sufficiently robust to withstand removal from push-through blister packs.

Hogan, J. E., Reeves, L., Staniforth, J. N. (2002) US Patent 6,406,738 B1, June 18, 2002

188
Distinctive tablet appearances using electrostatic dry powder deposition technology

M. D. Hallett, L. A. Reeves, M. Walton and M. Whiteman

PHOQUS Ltd, 10 Kings Hill Avenue, West Malling, Kent, ME19 4PQ, UK

Tablet appearance is now recognised as an important driver of patient compliance. Electrostatic dry powder deposition technology (EDPDT) allows a variety of distinctive tablet appearances to be created. Specifically, using powders with different electrostatic charge distributions, colour separation can be achieved on a tablet surface and a novel two-colour appearance created (Walton *et al* 2002). This work examines the effect of process variables on the electrostatic separation of coating powders and the quality of the resulting pattern.

A series of placebo test tablets were produced using purpose-designed punches with specific indentation patterns. Indentation patterns were designed to allow the investigation of the effect of depth, shape and area on the coating process.

A 1:1 binary mixture was produced using two, differently coloured, coating powders with a difference in tribocharge values of greater than 15 $\mu C\ g^{-1}$. The electrostatic transfer of this powder mix to the test tablets and to standard biconvex tablets was investigated under a range of electric field conditions. The tablet appearances resulting from the different process conditions were assessed for colour separation and definition.

It was found that an AC component to the electric field was essential for colour separation. DC alone resulted in a mixture of the two colours, with the colour of the lowest charge component predominating.

When an AC component of between 2000 and 4000 V was employed, colour separation was found to take place according to the distance between the tablet surface and the powder source. Hence colour separation occurred between a tablet indent and the tablet surface on the test tablet and at an equivalent distance from the development roller on the curved surface of the biconvex tablet. The high charge powder predominated closest to the source of the powder, while the low charge material predominated furthest away (e.g. in the base of the intagliation). Under the field conditions chosen, colour separation was independent of area but varied with the depth of the intagliation; the greatest separation occurred for depths of 0.4 mm or deeper. The boundary between colour zones was better defined at the vertical edge of the test tablet indent than on the smooth slope of the concave tablet.

The results showed that powder separation using EDPDT is a function of the tribocharge of the coating powders, the electrostatic field conditions and the depth of the intagliation or radius of the tablet surface. Using EDPDT the range of tablet markings can be greatly enhanced. The ability to create a greater range of distinctive tablet appearances could lead to improved safety and patient compliance by alerting patients to the dose and content of a tablet with a strong visual signal.

Walton, M., *et al.* (2002) *AAPS Pharm. Sci.* **4**: Abstract W4133

189
Comparison of the tensile and adhesion properties of different film coating systems

G. S. Macleod and A. M. Twitchell

European Technical Centre, FMC Biopolymer, Brussels, Belgium, 1200 and School of Pharmacy, De Montfort University, Leicester, UK

Tablet film coats must have mechanical properties that prevent film defects such as cracking. Aulton (1995) stated that film coats should be hard and tough without being brittle. Also important is the adhesion between the film and the tablet core with high forces of adhesion being preferable. An optimal coating should have a high tensile stress and tensile strain at break, a high Young's modulus and high tablet-film adhesion. By considering these attributes, the appropriateness of a coating system for a given product can be assessed.

Coating formulations (see Table 1) were prepared at a concentration of 10% w/w (formulations 1 and 2) or 20% w/w (formulation 3). LustreClear LC 103 was supplied by FMC Biopolymer (USA), Pharmacoat 606 by Shin Etsu (Japan), pigments by Roha Caleb (UK), polyethylene glycol (PEG 400) by Vel (Belgium) and Opadry 2 by Colorcon (UK).

Table 1 Coating formulations investigated

	Formulation		
	1	2	3
LustreClear LC 103 (g)	33.3	—	—
Pharmacoat 606 (g)	—	27.0	—
PEG 400 (g)	—	4.0	—
Titanium dioxide (g)	3.9	5.1	—
F D & C Blue No.2 Lake (g)	2.8	3.9	—
Opadry 2 (g)	—	—	40.0
Distilled water to (g)	400	400	200

Cast film samples (120 × 12 mm) were stored at 59% RH and 20°C for two weeks before tensile testing was undertaken using an Instron AMTS 1.3 at a cross-head speed of 12 mm s^{-1}. Ten film samples were tested for each formulation.

Film adhesion was investigated by applying a 3% w/w coat to 11 mm diameter flat bevelled edge waxy tablet cores (15% Sterotex K (Abitec, USA) and 85% Avicel PH 102 (FMC Biopolymer, USA)) compressed on a Manesty (Knowsely, UK) D4 tablet press. The film coats were applied to 1 kg tablet batches using an LDCS3 coating pan (Vector, USA). Coating conditions were the same for all formulations. A Monsanto Tensometer 10 fitted with a 100 N load cell and using a separation speed of 200 mm min^{-1} was used to measure the average force (n = 10) required to remove the different film formulations from the tablets.

Results in Table 2 show that the film system having the highest adhesion (Opadry 2) was mechanically weak (low tensile stress and strain at break and low toughness). A typical HPMC-based formulation (formulation 2) was found to be tougher and more extendible but had low adhesion. The LustreClear LC 103 formulation had higher adhesion than the HPMC-based film and was mechanically stronger than the Opadry 2 films.

These film attributes, in combination, are important when optimizing formulations in product development.

Table 2 Mechanical and adhesion properties of various film formulations

Formulation	Stress at break (MPa)	Young's modulus (MPa)	Strain at break (%)	Toughness (MJ m^{-3})	Maximum force of adhesion (N)
1	11.7	1372	1.45	0.120	7.2
2	21.7	1438	5.70	1.052	4.4
3	3.8	1251	0.35	0.014	13.1

Aulton, M. E. (1995) Mechanical properties of film coats. In: Cole, G. C., Hogan, J. E., Aulton, M. E. (eds) *Pharmaceutical coating technology*. Taylor & Francis, London, pp 288–362

190
Characteristics of nitrofurantoin tablets prepared with different disaccharides

S. M. Clow, P. J. Cox, D. L. Munday and M. Shaw

School of Pharmacy, The Robert Gordon University, Schoolhill, Aberdeen, AB10 1FR, UK

Nitrofurantoin tablets containing either lactose monohydrate, trehalose dihydrate or maltose monohydrate were prepared and subjected to a range of tests. The three disaccharide molecules have very similar structures and each anhydrous form has the same molecular weight (342.3). Trehalose has been reported to be more soluble than both lactose and maltose in the temperature range 10–40°C (Lammert *et al* 1998). Lactose and maltose are reducing sugars whereas trehalose is a non-reducing sugar. Lactose is commonly used as a diluent, maltose has been used as a direct compression tablet excipient and trehalose, which has a range of interesting properties, is becoming more widely available. The purpose of this work was to establish if trehalose provided nitrofurantoin tablets with any favourable characteristics.

Preformulation tests (mass flow rate, angle of repose, bulk density and particle size) on the disaccharides showed that the 300 µm grade of lactose (DMV Veghel) was similar to the maltose (Fisons) and trehalose (Sigma) grades available. However, maltose was sieved through a 500-µm mesh sieve and the lower particle size range used to allow a better overall comparison of the disaccharides in subsequent tablet studies.

Compatibility was established by DSC analysis of excipients and drug; λ_{max} and $A^{1\%}_{1cm}$ of nitrofurantoin were determined in phosphate buffer medium (pH 6.0–7.8) and pH 7.4 was selected for dissolution studies.

The tablet formulation (300 mg) contained nitrofurantoin (100 mg), disaccharide (100 mg), magnesium stearate (1.5 mg), sodium starch glycollate (Explotab), PVP and dicalcium phosphate dihydrate (Encompress). The amounts of Explotab, PVP and Encompress were varied to produce suitable tablets by direct compression. The final tablets passed the official tests for uniformity of weight, crushing strength, friability, disintegration and dissolution.

For a constant compression force there was a difference in the hardness of the tablets produced with different disaccharides. For example, average values for the crushing strengths of the tablets in Newtons (N) were: lactose (68 N), trehalose (95 N) and maltose (100 N). When compression forces were changed to normalise tablet hardness, nitrofurantoin tablets containing trehalose as the diluent neither disintegrated nor dissolved as quickly as tablets made with either lactose or maltose. Changing the grade of disaccharide (e.g. lactose BP) and the lubricant (e.g. sodium stearyl fumarate) varied these results. Nevertheless, the overall amount of drug released from tablets containing any of the disaccharides as the diluent after one hour was similar.

Lammert, A. M., Schmidt, S. J., Day, G. A. (1998) *Food Chem.* **61**: 139–144

191
Preparation and characterisation of crystallised and spray dried lactate dehydrogenase (LDH) and trypsin in solid and aqueous states

A. A. Elkordy, R. T. Forbes and B. W. Barry

Drug Delivery Group, School of Pharmacy, University of Bradford, Bradford BD7 1DP, UK

The development of proteins as therapeutic agents is difficult due to their inherent instability, so they are often prepared in a solid form. Hence, crystallisation and spray drying techniques were assessed to determine their effects on protein integrity and activity using LDH, a tetrameric enzyme, and trypsin, a monomeric protein, as model proteins.

Our aims were to characterise unprocessed, crystallised and spray dried proteins in solid and aqueous states, and to determine whether the crystals offered stability advantages as found previously for lysozyme crystals (Elkordy *et al* 2002).

LDH and trypsin were crystallised using a vapour diffusion method, and also spray dried. Unprocessed, crystallised and spray dried proteins were examined in the solid state using polarised microscopy and FT-Raman spectroscopy to investigate crystal shape and secondary structure, respectively, and also in the aqueous state employing high sensitivity differential scanning calorimetry (HSDSC) and enzymatic assay. HSDSC scanned from 20 to 70°C at 1.2°C min^{-1} for LDH and from 20 to 90°C at 1°C min^{-1} for trypsin samples. Enzymatic assay used pyruvate in presence of nicotinamide adenine dinucleotide for LDH assay and N-benzoyl-L-arginine ethyl ester as a substrate for trypsin samples.

Polarised microscopy showed that trypsin crystals were more birefringent than LDH crystals, which in turn were more birefringent than unprocessed trypsin and LDH. Whereas the FT-Raman spectrum of spray dried LDH only exhibited a small shift in a band at 1337–1340 cm^{-1} compared with unprocessed protein, the spectrum for LDH crystals was notably different with respect of peak position and intensity. For trypsin, the crystals maintained the structure with amide I band at 1668 cm^{-1}, while spray dried trypsin showed small drifts ($\sim +1$ cm^{-1}) in amide I band and ~ -2 cm^{-1} in a band at 1341 cm^{-1}. HSDSC data revealed that crystallisation and spray drying did not affect T_m of unprocessed LDH or trypsin in solution and was in agreement with our previous finding for lysozyme. Biological assay indicated that recovered activities from crystallised and spray dried LDH were 100% relative to unprocessed protein. Assay for trypsin showed that recovered activities, relative to unprocessed, were 99.9 and 89.7% for crystallised and spray dried trypsin, respectively.

Thus, crystallisation maintained the secondary structure and activity of trypsin better than spray dried form as indicated previously for lysozyme. However, although LDH crystals revealed a different secondary structure in the solid state as indicated by FT-Raman, after reconstitution crystals retained 100% of biological activity as shown by enzymatic assay. This may be because LDH is a tetrameric or a large molecule. Accordingly, this study supports previous findings (Elkordy *et al* 2002) and confirms once again that protein crystals maintain integrity and activity of proteins.

Elkordy, A. A., Forbes, R. T., Barry, B. W. (2002) *Int. J. Pharm.* **247**: 79–90

192
Spray drying of mannitol as a carrier for dry powder inhalers

K. Gilani, A. Rouholamini Najafabadi, M. A. Barghi* and M. Rafiee-Tehrani

Dept. of Pharmaceutics, School of Pharmacy, Tehran University of Medical Sciences, Tehran and *X-Ray research Laboratory, School of Science, Tehran University, Tehran, Iran

Dry powder inhaler (DPI) formulations are composed of micronized drug particles that are usually blended with coarser carrier particles to form an ordered mixture

(Ganderton 1992). Mannitol is an isomer of sorbitol that can be used as a carrier in DPIs (Tee *et al* 2000). As a preliminary work, four mixtures consisted of disodium cromoglycate and mannitol, prepared from different methods, was formulated in a ratio of 50:50 (Table 1). Forty milligrams of each formulation was filled in size 2 hard gelatin capsules and aerosolized from a Spinhaler. In-vitro deposition analysis was done using Andersen Cascade Impactor operated at 60 L min^{-1} (EP, 2000). Fine particle fractions (FPF), percent of emitted doses (ED%) and dispersibility percents (D%) for all formulations are shown in Table 2.

Table 1 Formulations

Formula	Mannitol	Cromolyn sodium
A	Sieved (63–90 μm)	Commercial
B	Aqueous spray dried	Commercial
C	Ethanolic spray dried	Commercial
D	Ethanolic spray dried	Ethanolic spray dried

Table 2 Deposition data for all formulations

Formulation	FPF	ED%	D%
A	20.6	75.0	27.5
B	16.7	79.87	20.9
C	25.8	73.2	35.2
D	27.3	70.1	39.0

The application of ethanolic spray dried mannitol produced higher FPF and D% than the other types of mannitol. Further investigations are required to confirm the beneficial effects of ethanolic spray dried mannitol as a carrier for DPI formulations. These preliminary results supported the potential application of mannitol as a possible carrier for inhaled drugs.

Ganderton. D. (1992) *J. Biopharm. Sci.* **3**: 101–105
Tee, S. K., *et al.* (2000) *Int. J. Pharm.* **208**: 111–123

193
A novel approach to risk assessment of CIVAS products

M. Taki, P. Tunstell*, P. Forsey* and B. Forbes

Department of Pharmacy, King's College London, 150 Stamford Street, London SE1 9NN and *Guy's and St Thomas' Hospitals NHS Trust, Lambeth Palace Road, SE1 7EH, UK

The Central Intravenous Additive Service (CIVAS) prepares drugs in a ready-to-use form for administration on the hospital ward. The service evolved as a risk management approach to reduce the risk of drug administration errors. However, the service itself introduces different risks, in particular the risk of microbial contamination and drug stability concerns. The Medicines control Agency has recently advocated periodic evaluation of final product for drug content as a quality assurance (QA) exercise. The aim of this study was to develop a method to quantify the relative risk of each CIVAS product prepared at Guy's and St Thomas' Hospitals NHS Trust to allow the systematic introduction of QA testing on the basis of product risk.

Risk can be defined as the product of the likelihood of an event occurring and the severity of its consequences. Factors affecting the likelihood and severity of the failure of the manufacturing process for each CIVAS product were identified, then a scoring system was developed whereby each product was assigned a score between one (lowest) and five (highest) for the likelihood/severity of each factor. The factors affecting 'severity' were route of administration, clinical indication, and toxicity of the drug/severity of overdosing. The factors affecting 'likelihood' were manufacturing process complexity, frequency/volume of manufacture, product information and the status of the starting material. Using this scoring system, a risk register was constructed for the 15 CIVAS products currently prepared at Guy's and St Thomas' Hospitals NHS Trust (Table 1).

The identification of risk factors pertinent to CIVAS products has allowed the construction of a risk register. The risk register identified dobutamine 500 mg in 50 mL and noradrenaline 8 mg in 50 mL pre-filled syringes as being the highest risk products. The development of HPLC assays for these products to allow QA testing has been prioritised.

Table 1 Risk register for CIVAS products prepared by Guy's and St Thomas' Hospitals NHS Trust

Product	Likelihood (L)	Severity (S)	Risk (L × S)
Dobutamine 500 mg in 50 mL	3	5	15
Noradrenaline 8 mg in 50 mL	3	5	15
Midazolam 50 mg in 50 mL	3	4	12
Fentanyl 2 μg in 1 mL Bupivacaine 0.125%	2	5	10
Erythromycin 200 mg in 100 mL sodium chloride 0.9%	3	3	9
Fentanyl 1.5 mg in 30 mL	2	4	8
Ganciclovir 200 mg in 100 mL	2	4	8
Morphine sulphate 120 mg in 60 mL	2	4	8
Morphine sulphate 50 mg in 50 mL	2	4	8
Desferrioxamine s/c infusions	3	2	6
Alteplase 4 mg in 4 mL	2	3	6
Sodium bicarbonate 1.16% haemofluid	2	3	6
Vancomycin 62.5 mg in 1.25 mL	2	3	6
Colistin 1 MU in 4 mL	2	2	4
Ciprofloxacin 0.2% eye drops	1	2	2

Factors contributing to likelihood and severity of product failure were scored on a scale of one (lowest) to five (highest). These scores are averaged and rounded to the nearest integer

194
Teaching of sterile product formulation and manufacture to pharmacy undergraduates

B. Forbes, A. Hamood and R. Duncombe*

Department of Pharmacy, Kings College London, 150 Stamford Street, London, SE1 9NN and *Pharmacy Department, Broomfield Hospital, Court Road, Chelmsford, Essex CM1 7ET, UK

Pharmaceutical teaching must meet vocational requirements and should equip pharmacy graduates with the abilities and skills that prepare them for pre-registration training. The nature of the manufacturing environment in the NHS has evolved rapidly in recent years with significant regulatory, technological and political developments. Meanwhile the teaching of pharmacy has seen an expansion of the course from three to four years with the consequent reorganisation of many pharmacy programmes. In this climate of change, the need to review the purpose and scope of the teaching of sterile product formulation and manufacture to undergraduate pharmacy students is apparent. The aim of this study was to ascertain the views of NHS technical service managers regarding the skills and attributes required of pharmacy graduates entering pre-registration training.

The views of a cohort of 19 NHS pharmacy managers that had attended a Senior Pharmacy Managers course in 2001 were sought by email questionnaire, yielding 10 responses. There was unanimous agreement by respondents that theoretical knowledge was more valuable than practical skills to new pharmacy graduates. Practical experience in procedures such as aseptic technique was viewed as less important than a fundamental knowledge of pharmaceutical science as practical training provided during the pre-registration year. It was notable that knowledge of both product formulation and methods of sterilisation were regarded as important by 90% of respondents, while training in these subjects did not form any part of the pre-registration year in most institutions. Knowledge of quality assurance, quality control, good manufacturing practice,

microbiology and legislation were similarly regarded as important, but these aspects were generally covered during pre-registration training. In addition to subject specific skills required for sterile manufacture, the promotion of attributes such as initiative and motivation was regarded as an important aspect of pharmaceutical education.

A further questionnaire was designed to evaluate the perceptions of undergraduate students regarding current teaching in sterile product formulation and manufacture at King's College London. Students reported that the course was strong at delivering knowledge, but less effective at generating interest and motivation. Only 3% of 60 students reported that they found the course challenging, leading to the suggestion that the teaching could be improved by making the delivery more challenging, perhaps through increased use of problem-based teaching methods.

Appropriate learning outcomes are vital to the success of teaching programmes. In this study the delivery of knowledge was identified as the most important component for undergraduate programmes in sterile product formulation and manufacture, while motivation and stimulation of students represented the greatest challenge.

195
Miniaturisation of the wet granulation process in tablet formulation: influence of processing on granule compression properties

J. P. Katolik, E. Razzano, A. C. Bentham and R. Docherty

Pfizer PGRD, Sandwich, Kent, CT13 9NJ, UK

Although wet granulation has generally been considered an empirical art, in recent years there have been rapid advancements in the understanding of the fundamental mechanisms that control granule formation and growth (Goldszal *et al* 2001; Ivenson 2002). While the effect of processing conditions on the functional properties of a granulation has been investigated for conventional high shear mixers in numerous studies (Badawy *et al* 2000), little work has been published on the characterisation of laboratory scale granulators, which would be the equipment of choice during formulation design activities in exploratory development.

The purpose of the present study was to optimise the operating conditions of the laboratory scale mixer granulator Mi-Pro (Pro-C-epT, Belgium) by evaluating the effect of key process parameters (impeller speed, binder addition rate and wet massing time post binder addition) on relevant granule properties, including particle size, flowability, friability and compactability. A placebo formulation, containing microcrystalline cellulose and lactose and granulated by the addition of povidone aqueous solution, has been investigated. In order to quantify the effect of the selected process variables and to detect the presence of inter-variable relationships, six granulations (run 0–5) at the scale of 300 g were manufactured according to a range of conditions defined by a factorial experimental design. Relevant functional properties of the resultant granules were analysed and results on particle size parameters (median) and compressibility (radial tensile strength of the compacts) are reported in Table 1. Radial tensile strength measurements were carried out in triplicate by compressing the granules into compacts (Instron 5581) of a constant porosity and by measuring their crushing strength.

Table 1 Granule particle size parameters (median) and compressibility (radial tensile strength)

Granulation	Median (μm)	RTS (MPa)
Run 0	258.5	1.54
Run 1	236.0	2.69
Run 2	213.0	1.70
Run 3	240.0	1.88
Run 4	264.5	1.02
Run 5	254.0	1.55

Results indicated that wet massing time, closely followed by impeller speed, had the greatest impact on the resultant granule properties, while the effect of binder addition time was less evident. Long wet massing time combined with fast impeller speed produced moderately larger, less friable granules, which formed compacts of substantial lower tensile strength. This effect is in line with previous investigations and it is an indication of the impact that the rate of granule consolidation may have on mechanical properties. In conclusion, the effect of key process variables for a laboratory scale granulator has been explored and optimal processing conditions have been identified. In addition, the radial tensile strength measurement of compacts of constant porosity has proven to be a rapid and sensitive test to assess granule compaction properties, with minimal sample size requirements (ca. 1 g).

Badawy, S. I. F., *et al*. (2000) *Int. J. Pharm.* **198**: 51–61
Goldszal, A., *et al.* (2001) *Powder Technol.* **117**: 221–231
Iveson, S. M (2002) *Powder Technol.* **124**: 219–229

196
The discovery of a new lattice transition for theophylline with the use of DVS-NIRS

K. L. Vora, G. Buckton and D. Clapham*

Department of Pharmaceutics, The School of Pharmacy, 29/39 Brunswick Square, London, WC1N 1AX and *GlaxoSmithkline, New Frontiers Park, Harlow, Essex, UK

It has been reported (Lehto & Laine 2000) that anhydrous theophylline converts to the monohydrate form at room temperature and high relative humidities (> 80% RH). At low relative humidities (< 20% RH), the monohydrate is dehydrated to a form which differs from the initial anhydrate (Phadnis & Suryanarayanan 1997). Our previous work using DVS-NIRS has supported the above data from literature. This study shows the emergence of a new and unreported transition during the conversion to the monohydrate from dehydrated theophylline, using the combinatorial techniques of Dynamic Vapour Sorption (DVS) (Surface Management Systems) and Near Infrared Spectroscopy (NIRS) (Foss NIRSystems).

The effect of subjecting anhydrous theophylline (Sigma) to changes in relative humidity on both the mass and the NIR spectra was determined at 25°C. At 95% RH the sample gained approximately 9% mass (equivalent to 1 mole of water), indicating that the monohydrate may have formed. When this presumed hydrate was subjected to 0% RH the mass dropped, again by 9%, suggesting a further change of morphological state (the dehydrate).

The DVS data showed that during rehydration of the dehydrate there was an anomaly between 40% and 50% RH. At this point there was an unexpected loss of mass during hydrate formation. This is a characteristic of an amorphous phase transition, such as one seen during recrystallisation. This occurrence was not seen during the original formation of the monohydrate from the anhydrous sample of theophylline.

Additionally it is well known that under the appropriate conditions or time alone, the dehydrate crystal will revert back to the lattice seen for the anhydrous sample. This is because the anhydrous form is the more stable state for the compound. Therefore it can be postulated that the anomaly may directly be due to the lattice structure of the dehydrate. This is believed to be a result of the expulsion of water during rearrangement of the crystal favouring an anhydrous structural formation.

NIRS data has been obtained to follow the changes that occur during the anomalous period. Anhydrous, hydrate and dehydrate spectra are compared with the patterns at different time points during recrystallisation (30, 40 and 50% RH). The NIRS data suggest that during the 30–50% RH rehydration stages the sample shows a tendency to transform structures towards the anhydrous lattice from that of the dehydrate. A possible explanation is that the water provides the mobility for anhydrous formation (or as near as possible) prior to the emergence of theophylline monohydrate. This would explain the crystallisation behaviour seen at the anomaly during rehydration.

The combination of DVS and NIRS data therefore is valuable in aiding the characterisation of different crystal lattices of a powder, which should aid the understanding of how materials of pharmaceutical relevance may behave under environments they may meet during processing.

We would like to acknowledge Hiden Isochema Ltd for their initial work using the IGASorp.

Lehto, V., Laine, E. (2000) *Pharm. Res.* **17**: 701–706
Phadnis, N. V., Suryanarayanan, R. (1997) *J. Pharm. Sci.* **86**: 1256–1263

197
Physical characterization of spray dried sugar-protein particles

E. Suihko, R. T. Forbes* and D. Apperley†

Department of Pharmaceutics, University of Kuopio, Finland, *Drug Delivery Group, The School of Pharmacy, University of Bradford, UK and †Industrial Research Laboratories, University of Durham, UK

The aim of this study was to evaluate moisture-induced changes in the physical state of co-spray dried trehalose:lysozyme and sucrose:lysozyme formulations. Co-spray dried 1:9, 1:1 and 9:1 w/w trehalose:lysozyme and sucrose:lysozyme powders were stored at 0 % RH or 75 % RH for 5–6 days prior to experiments. Thereafter, molecular mobility and crystallinity of the co-spray dried formulations were determined by using ^{1}H solid-state NMR and X-ray powder diffractometry, respectively. Also enzymatic activity of the lysozyme formulations was measured. Solid-state NMR results showed that increasing sugar content in the co-spray dried formulations stored at 0% RH increased spin-lattice relaxation times (T_1), which are associated with motions of the protein side chains and surface groups, suggesting concomitant decrease in molecular mobility (Table 1.). T_1 values for the 1:9 sugar:lysozyme formulations decreased when they were stored at 75% RH. However, exposure of the 1:1 and 9:1 sugar:protein formulations to moisture led to increase in T_1 values. In addition, T_1 values for these formulations were averages of two T_1 components, whereas for all the other samples the T_1 values were single-component (results not shown). X-ray powder diffraction measurements showed that all the formulations were amorphous after storage at 0% RH. During storage at 75% RH sucrose and trehalose crystallized in the co-spray dried 9:1 and 1:1 sugar:lyzosyme formulations, whereas both the co-spray dried 1:9 sugar:lysozyme formulations remained amorphous. Differences in relative enzymatic activity of lysozyme between the formulations were not significant.

Table 1 Averaged proton (^{1}H) spin-lattice relaxation time constants (T_1)

Material	T_1 (s)	
	0% RH	75% RH
Lysozyme	1.0	n.d.
Sucrose:lysozyme 1:9	1.1	0.6
Sucrose:lysozyme 1:1	2.3	8.8
Sucrose:lysozyme 9:1	5.8	6.7
Sucrose:lysozyme pm 1:1	9.8	n.d.
Trehalose:lysozyme 1:9	1.2	0.7
Trehalose:lysozyme 1:1	1.9	7.5
Trehalose:lysozyme 9:1	4.2	4.5
Trehalose:lysozyme pm 1:1	3.3	n.d.

pm = physical mixture, n.d. = not determined

In conclusion, solid state NMR results indicated strong interaction between sugars and protein, and decreased molecular mobility when sugar content is increased, in the co-spray dried trehalose:lysozyme and sucrose:lysozyme formulations stored at 0% RH (Lam *et al* 2002). X-ray powder diffraction showed that storage at 75% RH induces crystallisation of sugars in the co-spray dried 1:1 and 9:1 sugar:protein formulations. The crystallisation was also shown as an increase in averaged T_1 relaxation times, which actually were averages of two T_1 components, demonstrating that sugar and protein phases were separated in these formulations. The phase separation did not have any significant effect on the enzymatic activity of lysozyme, a result which may not be applicable to other proteins.

Lam, Y.-H., *et al.* (2002) *J. Pharm. Sci.* **91**: 943–951

198
Elevated differential scanning calorimetry heating rates in polymorphism screening studies

W. L. Hulse, R. T. Forbes, M. C. Bonner and J. Pearson*

Drug Delivery Group, School of Pharmacy, University of Bradford, Bradford, W. Yorks and *Merck Chemicals Ltd, Merck House, Poole, Dorset, UK

The rapid identification of polymorphic forms of new drugs is an important objective in preformulation studies. Differential scanning calorimetry (DSC) is typically used to investigate polymorphic relationships. Using a series of polyhydric compounds we investigate the usefulness and limitations of accelerated rate DSC to provide additional information on polymorphic behaviour. Mannitol and sorbitol are isomeric hexahydric alcohols used primarily as diluents in tablet formulations. Mannitol exists in three polymorphic forms and sorbitol in four. Xylitol and trehalose are both polyhydric and are commonly used in pharmaceutical formulations with no true polymorphs of either being reported in the literature.

DSC profiles were obtained using a Perkin Elmer Series 7 DSC thermal analysis system. Samples (1–10 mg) were sealed in standard aluminium pans that were crimped and vented. A standard heating rate of 10°C min^{-1} was employed to gain a reference profile for each compound. Further experiments employed heating rates of 100°C min^{-1}. A further series of experiments employed a heating rate of 150°C min^{-1} for the δ polymorph of mannitol.

At a heating rate of 10°C min^{-1} the thermal profiles of all the polyols exhibit a single melting endotherm, with trehalose also exhibiting dehydration and glass transition endotherms. At the higher heating rate of 100°C min^{-1} the melting points of all the compounds were increased by 12.55–22.17°C.

At the higher heating rate, the thermal profile of the δ mannitol exhibited an incongruent melt peak which was not evident at the standard heating rate. The incongruent melt peak had increased area, height and melting point with increased heating rate (Table 1). The incongruent melt peak was distinct from the main melting endotherm and was not a shoulder.

Table 1 The influence of heating rate on the incongruent melting peak of δ mannitol

Heating Rate ($^\circ$C min^{-1})	Incongruent melt onset (°C)	Incongruent melt peak area (mJ)	Melting endotherm onset (°C)	Melting endotherm area (mJ)
10	Not present	Not present	166.1 ± 0.3	1308.3 ± 24.7
100	159.7 ± 0.3	2.3 ± 0.2	181.6 ± 1.2	1367.5 ± 34.6
150	164.3 ± 0.2	10.3 ± 0.9	188.0 ± 0.5	1355.2 ± 42.0

Means ± s.d., n = 3

Increasing the heating rate of the compounds increased their apparent melting points. At elevated heating rates the incongruent melting peak of δ mannitol increases in size. Additionally, the mannitol phase transition is more resolved than at lower heating rates. Thus, particularly for simple molecules (e.g. non-hydrates), the use of accelerated rate DSC is effective for exploring the presence and interrelationship of polymorphism and could be incorporated into preformulation screening programmes.

199
Co-spray drying of mannitol/sorbitol mixtures

W. L. Hulse, R. T. Forbes, M. C. Bonner and J. Pearson*

Drug Delivery Group, School of Pharmacy, University of Bradford, Bradford, W. Yorks and *Merck Chemicals Ltd, Merck House, Poole, Dorset, UK

Mannitol and sorbitol are polyhydric alcohols commonly used in the pharmaceutical industry as diluents in tablet formulations. Combining the two polyols may achieve a final product with the excellent tabletting properties of sorbitol and the low hygroscopicity of mannitol. Spray drying is a method of combining pharmaceutical

excipients and has been used for the production of solid pharmaceuticals since the 1940s (Corrigan 1995). Both polyols exist in different polymorphic forms, which may interconvert, therefore, any polymorphic transitions that may be induced during spray drying must be investigated.

Spray dried mixtures (Table 1) were produced using a Buchi 190 mini spray dryer. Samples were atomised using a 0.5-mm two fluid nozzle operating under 6 bar pressure. The drying air flow rate was 500 L min^{-1} and the liquid feed rate 13 mL min^{-1}, which created inlet temperatures of 120–150°C and outlet temperatures of 60–70°C.

Table 1 Composition of the spray dried mixtures

Batch	% Mannitol	% Sorbitol
1	100	0
2	0	100
3	90	10
4	80	20
5	70	30
6	60	40

Structural analysis of the spray dried products was performed using X-ray powder diffraction (XRPD) using a Siemens D5000 Diffraktometer, comprising a scintillation counter detector and monochromator with Cu-Kα radiation source (λ = 0.15418 nm). The range used was 2–45° of 2θ with a stepwise scanning mode using a step size of 0.05° and a step time of 3 s. Thermal analysis was performed using differential scanning calorimetry (DSC). Measurements were carried out using a Perkin Elmer series 7 DSC thermal analysis system. Samples (2–10 mg) were placed into sealed aluminium pans with vented lids and heated at 10°C min^{-1} in the range 25–200°C. Water uptake of the samples was determined using Thermogravimetric analysis (TGA). Measurements were performed using a Perkin Elmer series 7 TGA system. Samples (2–10 mg) were loaded onto an open platinum sample pan suspended from a microbalance and heated from 25 to 200°C at 10°C min^{-1}.

Notably, XRPD analysis indicated that batch 4 had a much more diffuse powder pattern than any other batch produced. Batches 3–6 all exhibited peaks corresponding to β mannitol, α and Γ sorbitol with peaks $< 13°$ of 2θ absent. The DSC profile of batch 3 failed to reveal the presence of sorbitol, while profiles of batches 4–6 indicated the presence of Γ sorbitol in increasing amount with batches 5 and 6 having a third endotherm corresponding to α sorbitol. With increasing sorbitol content the melting endotherm of mannitol was broader with a lower onset temperature. TGA revealed that increasing the amount of sorbitol in the mix increased the water content of the mixture.

The effect of co-spray drying mannitol/sorbitol mixtures has been examined and polymorphic behaviour characterised. The anomalous behaviour of batch 4 may have benefits for solid-state formulations.

Corrigan, O. I. (1995) *Thermochim. Acta* **248**: 245–258

200
Predicting bulk powder behaviour from single crystals

L. J. Taylor*†, D. G. Papadopoulos*, P. J. Dunn‡, J. C. Mitchell† and M. J. Snowden†

*Pharmaceutical R&D, PGRD, Sandwich, Kent, CT13 9NJ, †Medway Sciences, University of Greenwich, Medway Campus, Chatham, Kent, ME4 4TB and ‡Chemical R&D, PGRD, Sandwich, Kent, CT13 9NJ, UK

Drug substance is often milled to improve dosage form properties, such as dissolution and homogeneity. Generally, pilot-scale milling trials are run to determine the most effective and efficient mill for each material. However, such trials require relatively large quantities of bulk active. Hence, it would be beneficial to develop a small-scale technique that could predict the milling properties of bulk powder from a small amount of substance.

One small-scale technique that has been reported to give a measure of the fracture behaviour of materials is indentation (Duncan-Hewitt & Weatherly 1989; Prasad *et al* 2001). Indentation allows the measurement of hardness and Young's modulus at the near surface, by the loading and unloading of a sharp indenter into a sample. Measurement of indentation cracking after unloading can be used to calculate the fracture toughness of a material. The ratio of hardness to fracture toughness has been observed to provide a measure of brittleness (Lawn & Marshall 1979). It is hypothesised that materials that fragment extensively during compaction or milling have a high brittleness index. The aim of this study was to investigate that hypothesis.

Nanoindentation experiments were used to measure the mechanical properties and calculate the brittleness index of a number of pharmaceutical actives from a small number of crystals. These results were compared to milling data from pilot and full-scale milling trials. A good correlation between the milling size reduction ratio and brittleness index was observed across the range of materials studied. The brittleness index was sensitive enough to distinguish between materials that were easy, moderate and difficult to mill.

Duncan-Hewitt, W. C., Weatherly, G. C. (1989) *J. Mater. Sci. Lett.* **8**: 1350–1352
Lawn, B. R., Marshall, D. B. (1979) *J. Am. Ceram. Soc.* **62**: 347–350
Prasad, K. V. R., Sheen, D. B., Sherwood, J. N. (2001) *Pharm. Res.* **18**: 867–872

201
Transepithelial transport in and interaction of a lysine-based partial dendrimer (dendron) with Caco-2 monolayers

R. E. S. Rowland, P. W. Taylor and A. T. Florence

Department of Pharmaceutics, School of Pharmacy, University of London, 29/39 Brunswick Square, London, WC1N 1AX, UK

Systemic distribution of partial dendrimers (dendrons) has been shown to occur in female Sprague-Dawley rats after oral administration (Sakthivel *et al* 1999). Further investigation into the processes of uptake could be of benefit. Caco-2 monolayers form a complete and differentiated monolayer after 21 days incubation on polycarbonate membranes, they differentiate, to form tight junctions, microvilli and brush border enzymes and are used here to investigate the effect of a cationic lysine-based dendron (MW 1587, $(C_{14})_3(lysine)_7(NH_2)_8$). The integrity and viability of the monolayers were investigated alongside the permeability of the monolayers to the dendrimer. Permeability of the monolayers to the dendrimers was investigated in the apical to basolateral direction at two different concentrations and in two different types of media. The effect on the viability of the monolayers was tested by using the MTT assay. The integrity of the monolayers was monitored prior to, and during, the permeability study by measuring the transepithelial electrical resistance (TEER).

Results from this study showed that the higher concentrations of the dendron had a detrimental effect on the integrity of the monolayers when incubated in phosphate buffer saline (PBS), but incubation of the dendron with the Caco-2 monolayers in normal growth media (DMEM) had no effect.

The viability of the monolayers was maintained during the incubation period.

Transport of the dendron was higher in PBS than in DMEM (Table 1), perhaps due to the decrease in monolayer integrity. Comparisons of to the transport of the dendron through unseeded polycarbonate membrane were also studied as previous studies in our laboratory have shown that cationic dendrons can also absorb strongly to polycarbonate membranes.

Table 1 Dendron permeability co-efficients (10^6 cm s^{-1})

Concentration	Media	P_{app} (10^6 cm s^{-1})
2 mg mL^{-1}	PBS	10.30 ± 2.6
0.2 mg mL^{-1}	PBS	12.34 ± 0.1
2 mg mL^{-1}	DMEM	8.83 ± 3.4
0.2 mg mL^{-1}	DMEM	13.91 ± 3.4

in Caco-2 monolayers after 2 h

A comparison of the permeability co-efficient of the lysine dendron ranks it equivalent to that of the PANAM[poly(amidoamine)] dendrimers studied in a similar system by El-Sayed *et al* (2002).

These data show that the percentage of the partial dendrimer transported is low (max. 6%) over the time period studied. A tenfold variation in dendron concentration did not statistically affect transport rate, possibly suggesting a receptor mediated pathway. This work forms the baseline for future studies in which internalin molecules will be attached to the dendron. The effects of the media here on P_{app} are relatively modest suggesting there is no aggregation of the dendron, an effect which sometimes complicates interpretation.

The authors would like to thank Drs N. Hussain and T. Sakthivel for their help and advice.

El-Sayed, M., *et al*. (2002) *J. Controlled Release* **81**: 355–365
Sakthivel, T., *et al*. (1999) *Int. J. Pharm*. **183**: 51–55

202
Evaluation of different techniques for preparing indomethacin pellets

T. Toliyat and B. Paknejad

Department of Pharmaceutics, Faculty of Pharmacy, Tehran University of Medical Sciences, P.O. Box 14, Tehran, Iran

The gastrointestinal adverse effects of NSAIDS as a class has led to the search for new forms of delivery to minimize symptoms such as erythema, erosions, ulceration and bleeding. This has led to the development of delayed- release, extended-release or enteric release formulations for these drugs (Elchidana & Deshpande 1999). However, when compared with single unit sustained release tablets, multiunit controlled release dosage forms pass through the gut as if a solution avoiding the vagaries of gastric emptying and different transit rates and release drugs in a more predictable manner. Moreover, a multiunit system spreads in a large area of the absorbing mucosa and prevents exposure to a high drug concentration, when compared to single unit dosage form on chronic dosing (Tamilvanan and Sa 2000).

The aim of this study was to produce indomethacin pellets by using different techniques.

Pan coating, air-suspension technique and matrix-based pelletization were employed and the characters and release pattern from various formulations was then evaluated. Beads composed of indomethacin and lactose were granulated by polyvinylpyrrolidone (PVP) aqueous solution. After drying, the particles with 18/30-mesh size were separated and coated by pan-coating or wurster system. Ethylcellulose dissolved in ethyl alcohol was used for coating of pellets. In matrix pellets, ethylcellulose alcoholic solution was used instead of PVP solution. The prepared pellets were evaluated for appearance, particle size distribution and tapped bulk density (Table 1). Following the assay, the release pattern of each formulation was determined according to US Pharmacopoeia. Release profile from desired formulation with three techniques (Table 2) showed first-order kinetics and passed the USP criteria for extended release indomethacin pellets.

Table 1 Physical characterization of indomethacin pellets

	Tapped bulk density (g cm^{-3})	Mean size (μm)
Air suspension	0.673	684.10
Pan coating	0.612	690.00
Matrix pellets	0.629	722.29

Results showed that all of these techniques could be used successfully for preparation of extended release indomethacin pellets. However, the amount of ethylcellulose required to make a formulation with the desired release profile was 46% less via air suspension (1.37%) than via pan coating (2%). Matrix method requires higher amounts of polymer (5%) than the other methods to produce a similar release profile.

Table 2 Drug release from indomethacin pellets

	% Release					
Time (h)	1	2	4	6	12	24
Air suspension	21.86 ± 1.42	28.57 ± 1.66	40.30 ± 3.61	51.29 ± 0.65	69.25 ± 3.07	86.64 ± 1.43
Pan coating	22.25 ± 0.69	30.49 ± 2.62	41.54 ± 2.53	48.94 ± 3.42	65.01 ± 1.87	84.75 ± 1.61
Matrix pellets	22.07 ± 1.05	29.72 ± 2.01	39.94 ± 2.89	46.87 ± 4.49	64.13 ± 1.16	86.07 ± 1.98
USP criteria	10–25	20–40	35–55	40–60	60–80	⩽ 80

Elchidana, P. A., Deshpande, S. G. (1999) J. *Controlled Release* **59**: 279–285
Tamilvanan, S., Sa, B. (2000*) Int. J. Pharm.* **201**: 187–197

203
Lactose/PEG 4000 co-spray dried fines for dry powder inhalation

L. Schueller, G. Buckton, S. Brocchini, N. Lowther* and D. Singh†

Department of Pharmaceutics, School of Pharmacy, University of London, London WC1N 1AX, UK, *Novartis Pharma UK Limited, Horsham RH12 5AB, UK and †Novartis Pharma AG, Basel CH-4002, Switzerland

Efforts to improve solid formulations for use in DPIs are directed to (1) increase, reproducibly, the effective respirable dose, (2) maintain stability and (3) develop formulations with a wide range of drugs for systemic conditions. One approach is to modify the surface characteristics of lactose carrier particles by blending amorphous fines prior to blending with drug (Al-Hadithi *et al* 2002). Adding crystalline lactose fines did not improve salbutamol sulphate deposition from Pharmatose 325M while amorphous lactose fines followed by partial recrystalisation on the coarse surface had a beneficial effect (Al-Hadithi *et al* 2002).

To further evaluate the use of fines to modify the course carrier surface, lactose/PEG 4000 (10:1) co-fines were prepared by spray drying (Chidavanenzi *et al* 2001; Corrigan *et al* 2002). The particles were characterised by SEM, X-Ray Diffraction and Dynamic Vapor Sorption. No amorphous material was detected in the spray-dried fines. The particles produced were rough and covered with needle-shaped crystals. The fines (10% w/w) were first blended with air-jet sieved lactoses (Pharmatose 325M or Foremost Aero 65) and then with micronised salbutamol or beclomethasone dipropionate (BDP) (4% w/w). The carriers were packed into a Clickhaler (Innovata Biomed Ltd). The content uniformity of the blends was assessed prior to determining deposition with a twin Stage Impinger (n = 10 actuations).

Adding the modified PEG-lactose crystalline fines improved salbutamol deposition from both modified lactoses in terms of the fine particle fraction (FPF) however there was decrease in emitted dose (ED) probably due to differences in flowability of the two lactose carriers (Table 1).

Table 1 Deposition pattern for SS

Formulations	ED (mg)	FPF (%)	FPD (mg)
Aero Flo 65	1.27	31.15	0.39
Pharmatose 325M	1.22	24.90	0.30
Aero Flo 65 + Lac/Peg fines	0.69	42.35	0.29
Pharmatose + Lac/Peg fines	1.10	45.80	0.50

Deposition of BDP was also modified (Table 2) with a similar decrease in emitted dose. for the possible reason explained above and an improvement for one of the lactose grade (Pharmatose 325M).

Table 2 Deposition pattern for BDP

Formulations	ED (mg)	FPF (%)	FPD (mg)
Aero Flo 65	0.88	40.64	0.35
Pharmatose 325M	1.11	18.40	0.20
Aero Flo 65 + Lac/Peg fines	0.74	31.62	0.23
Pharmatose + Lac/Peg fines	0.79	30.89	0.24

In conclusion, changes in the surface characteristics of the fines showed mixed effects on drug deposition patterns compared with classical crystalline lactose fines. Further experiments will be undertaken to fully characterise these fines.

Al-Hadithi, D., Buckton, G., Brocchini, S., *et al.* (2002) *Respir. Drug Deliv.* **VII:** 679–681
Chidavanenzi, O. C., Buckton, G., Koosha, F. (2001) *Int. J. Pharm.* **216**: 43–49
Corrigan, D..O., Healy, A..M., Corrigan, O. I. (2002) *Int. J. Pharm.* **235**: 193–205

204
A comparison of two rule induction technologies in generating rules for tablet formulation

Q. Shao, R. C. Rowe and P. York

PROFITS Special Interest Group, School of Pharmacy, University of Bradford, Bradford, West Yorkshire, BD7 1DP, UK

Rule induction is the process of retrieving knowledge from numerical data. Neurofuzzy logic (Rowe & Colbourn 2002) and decision trees (Quinlan 1993) are two well developed rule induction technologies generating knowledge in the form of 'IF (condition 1) AND (condition 2) AND (condition 3) THEN (result)'. The purpose of this study is to investigate and compare neurofuzzy logic and decision trees in generating rules for tablet formulation.

A published database (Bourquin *et al* 1998) relating formulation ingredients (silica aerogel, magnesium stearate, microcrystalline cellulose and sodium carboxymethylcellulose) and process variables (dwell time and compression force) to tablet properties (tensile strength, disintegration time, friability, capping and dissolution at various time intervals) has been systematically studied using both neurofuzzy logic (FormRules, Intelligensys Ltd) and decision trees (C5.0, Rulequest Research). In both cases 177 data records were used for training and 28 randomly chosen data records were used for validation. The analysis of variance correlation coefficient (R^2) and average confidence levels were used to test the predictability of nerofuzzy logic and decision tree models, respectively.

Neurofuzzy logic was successful in modelling tensile strength, disintegration time and dissolution (R^2 in the range 0.55–0.85), but poor for friability and capping (R^2 0.19 and 0.16, respectively). Decision trees, however, were successful in all cases with average confidence levels in the range of 0.61–0.75.

Examples of rules generated by neurofuzzy logic:

1. IF dwell time is high AND magnesium stearate is low AND silica aerogel is low THEN tensile strength is high (0.94*).

IF dwell time is high AND magnesium stearate is high AND silica aerogel is low THEN tensile strength is low (0.63*).

* Confidence levels of the rules computed automatically during the rule generation process.

Examples of rules generated by decision trees:

IF dwell time >35.88 ms AND magnesium stearate $\leqslant$ 1.07% THEN tensile strength is high (52/3†).

IF dwell time >35.88 ms AND magnesium stearate $>1.07\%$ AND silica aerogel $\leqslant$ 1.19% THEN tensile strength is low (9/1†).

†The number of validation records predicted totally/incorrectly.

Neurofuzzy logic was able to deal with numerical data while decision trees required the pre-process of discretizing numerical dependent variables. Both technologies detected similar factors relevant to formulation properties, and were good at generating straightforward and understandable rules.

Bourquin, J., *et al.* (1998) *Eur. J. Pharm. Sci.* **6**: 287–300
Quinlan, J. R. (1993). *C4.5: Programs for machine learning*. Morgan Kaufmann Publishers Inc., San Mateo, USA
Rowe, R. C., Colbourn, E. A. (2002) *Pharm. Tech. Eur.* **14**: 24–27

205
Small scale coating of spherical pellets: inter and intra batch reproducibility

T. Suzuki, R. C. Rowe and P. York

School of Pharmacy, University of Bradford, Richmond Road, Bradford, West Yorkshire, BD7 1DP, UK

There is growing interest in microscaling pharmaceutical formulation and processing operations and developing scale-up rules to reduce drug quantities and time in product development. The purpose of this study is to investigate intra and inter batch reproducibility of a novel mini coater (Caleva Mini Coater/Dryer; Caleva, UK) for pellet coating. Spherical pellets (50% w/w of microcrystalline cellulose (Avicel PH101, FMC, Ireland) and 50% w/w of lactose monohydrate (Pharmatose 100M, DMV, Netherlands) with distilled water 45%w/w of the dry mass) were prepared by extrusion (Alexanderwerk R27, Germany), spheronization (Caleva spheronizer) and drying at 40°C in a hot air oven. They were sieved after drying and the fraction between 1.2 mm and 1.7 mm was used for coating with an aqueous or organic solvent based system. The aqueous coating solution was 5% w/w of HPMC (Pharmacoat 606; Shin-Etsu, Japan) and 5% w/w of a water-soluble red dye. The organic coating solution contained 5% w/w of hypromellose phthalate (HP-55; Shin-Etsu, Japan) and 100 ppm of Sudan III in methanol and dichloromethane 50/50 by weight. Batches of spherical pellets from 1 g to 20 g were coated using a Caleva Mini Coater/Dryer with coating solution flow rates of 10–40 mL h^{-1} (aqueous system) and 10–100 mL h^{-1} (organic system), to spray 1 mL coating solution per 1 g of spherical pellets in all runs. The coating in 10 g scale was repeated three times for investigation of inter-batch reproducibility. Colour differences (ΔE) for coated pellets from a reference state, measured with a colorimeter (Hunter D25A-9, USA) to estimate the film uniformity, increased with quantity of coating solids applied for both solvent systems. Standard deviations of ΔE (see Tables 1 and 2) were small (< 2) in all batches, indicating that the coating achieved was uniform on the pellets in each batch and intra-batch reproducibility was achieved from the 1 g to 20 g scale.

Table 1 Intra-batch reproducibility

Coating System	Loading amount (g)	ΔE(mean $\pm$ s.d.)
Aqueous Solution	1	60.6 $\pm$ 0.5
	2	66.8 $\pm$ 0.6
	5	75.2 $\pm$ 0.5
	10	79.3 $\pm$ 0.7
	20	80.3 $\pm$ 0.5
Organic Solution	1	34.9 $\pm$ 0.7
	2	42.2 $\pm$ 1.2
	5	45.0 $\pm$ 0.9
	10	50.2 $\pm$ 0.9
	20	57.3 $\pm$ 0.6

From analysis of variance for ΔE from repeated experiments at the 10 g scale, a significant difference was not observed at the 95% confidence level for both systems.

Table 2 Inter-batch reproducibility at 10 g scale

Coating System	Batch no.	ΔE (mean ± s.d.)
Aqueous Solution	1	79.3 ± 0.7
	2	78.4 ± 1.5
	3	77.6 ± 0.5
Organic Solution	1	50.2 ± 0.9
	2	50.3 ± 0.8
	3	51.0 ± 0.7

The range from the lower to upper confidence limit was small (< 3) for both systems, thus indicating that inter-batch reproducibility for coating of spherical pellets was also achieved.

206
Selection of a Minicoater system supported by multi-attribute value analysis

D. F. Bain, J. Fraser and A. Maclean

Quintiles, Heriot Watt University Research Park, Edinburgh, EH14 4AP, UK

Multi-attribute value analysis (MAVA) is a structured aid to decision making, which complements and challenges intuition and creates ownership and commitment amongst decision makers (Belton & Vickers 1989). The use of MAVA to select an appropriate small-scale fluid bed coater, following field tests of each, is described. Three minicoaters were identified: Caleva Minicoater (CAL) (Caleva Process Solutions, UK), MP Micro (MPM) (NiroFielder, Switzerland) and Mini AirPro (MAP) (ProCept, Belgium). The MPM and MAP resemble down-scaled pilot coaters also capable of drying and granulation using maximally 50 g of material, whereas the CAL uniquely fluidises the coating bed by a combination of vibration and heated air. This innovation allows coating of remarkably small batches (< 5 g). MAVA requires, variously, identification of alternatives and criteria; model building as a value tree; scoring and weighting (scale of importance) of alternatives and criteria respectively; aggregation of values; cost-benefit analysis and finally sensitivity analysis. These analyses were supported by V.I.S.A software (Strathclyde University GBS).

Using a standard theophylline bead product, the assessment of coating performance permitted scores to be assigned on the Performance branch. Whereas set-up with the MAP took longer, coating performance was superior and temperature stability greater. Resultantly, the equipment demonstrated improved repeatability based on coating efficiency when compared with the other coaters, where static was a persistent problem. This assessment also allowed a rational scoring of other attributes across the performance branch.

The Performance branch of the Value Tree included criteria families of design, software, customer service and process. Weights are shown in Table 1. Deemed most important to the Decision Team (DT), Process (Wgt = 0.6) was broken down into sub-criteria of ascending importance: adaptability, robustness, time to process and manufacturability, the latter representing coating, drying and granulation.

Table1 Decision Tree and high level weighting (Wgt)

Criteria	Wgt	Criteria	Wgt	Criteria	Wgt
Cost	0.21	Running	0.5		
		Capital	0.5		
Performance	0.79	Software	0.1		
		Design	0.1	Portability	0.4
				Build	0.1
				Accessories	0.5
		Customer service	0.2		
		Process	0.6	Time	0.28
				Robustness	0.17
				Manufacturability	0.4
				Adaptability	0.13

Upon scoring and weighting, initial normalised values for overall benefits were 12, 66, 80 for the CAL, MPM and MAP, respectively. Because the CAL was dominated across all attributes (with the exception of minimum batch size) by the other coaters, this option was disregarded from later sensitivity analyses. The result confirmed DT intuition. However, upon examination of the cost (capital & running) vs performance (benefits) plots, the MPM was shown to be superior as this equipment's costs are lower. Further examination of the Performance Profiles for the three coaters across the higher level criteria, showed the overall superiority of the MAP, but the MPM scored more highly on Design. This pattern indicated sensitivity of the overall scores to weights for these attributes, providing a focus for sensitivity analysis. The weight sensitivity graph for Design vs overall score demonstrated that the importance of this factor (weight) would have to be elevated to 0.5 for the overall score to favour the MPM, a factor which would only enhance the cost-performance of this apparatus. The DT could not conceive this elevation, however.

The selection made was based more on the technical and business requirements of the selecting organisation, not on the capabilities of apparatus in question. However, the single model provided a considered, justifiable and explainable basis for equipment selection for scrutiny elsewhere.

Belton, V., Vickers, S. P. (1989) *Economic and mathematical systems*. Volume 335, Springer Verlage, Heidelberg, Germany, pp 287–304

207
Miniaturisation of extrusion-spheronisation: an evaluation of scalability

J. Fraser, S. McDonald, J. R. Bloor* and D. F. Bain

Quintiles, Heriot Watt University Research Park, Edinburgh, EH14 4AP and *Alizyme, Granta Park, Great Abington, Cambridge, CB1 6GX, UK

Miniaturised solid dose processing equipment, capable of making key measurements on minimal (often < 10 g) quantities of product can greatly improve understanding of formulation performance before committing larger quantities of active to larger scale processing. Extrusion-spheronisation is a multi-stage process with many interacting formulation and process factors. Accordingly, optimisation and scale-up costs are invariably high. This report describes the utility of miniaturised equipment for extrusion-spheronisation for early identification of critical process parameters and their optimum levels, around which scale-up can centre.

Wet massing of a test formulation was performed in a small planetary mixer to which serial additions of water were made (30–60% dry weight) and a sample (10 g) withdrawn for torque measurement during extrusion using an adapter to the Mixer Torque Rheometer (MTR) (Caleva Process Solution, UK). This extrudate was subsequently spheronised using a Microspheroniser, the speed of which was increased to match typical peripheral velocities of pilot scale equipment. Spheronisiation time was fixed at 7 min. Using pellet morphology as a surrogate marker for the suitability of the wet mass, these observations were related to mean torque readings during extrusion. At low levels of water (30–40%), rounded cylinders were formed due to inadequate plasticity. Fines were also present. As water content increased (40–50%), intermediate shapes were seen. At higher levels, embryonic beads fuse during the process to form agglomerates and bead size is positively skewed. As water content increased, torque readings reduced to form a plateau at 50–55% water, at which level, spherical beads resulted, providing an optimum and target for mass plasticity during scale-up studies.

Table 1 Predicted and actual spheronisation yields

Speed (rev min^{-1})	Time (min)	Yield (0.85–1.4 mm) (%)		
		Miniature	Pilot	
		Actual	Predicted	Actual
800	5	95.1	75.4	80.6
600	7.5	98.9	91.9	95.7
880	7.5	86.0	86.9	77.2
320	7.5	80.0	74.1	81.3
600	4.0	48.2	59.0	37.5
600	11	58.2	57.2	39.1
400	5	78.2	69.2	75.5
400	10	75.2	74.2	73.2
800	10	70.6	70.1	72.3
800	5	74.2	80.9	73.7
600	7.5	85.0	91.9	82.8

Having established a value approximating to the optimum for water content, spheronisation parameters were examined. Because two factors, time and speed of spheronisation, affect bead characteristics but these interact, a Central Composite design (Design Expert 6, Stat-Ease, USA) was used to achieve maximal coverage of the experimental space and hence allow statistical modelling of the yield response (Table 1). Response surface predicted an optimum at 7.5 min and 750 rev min^{-1} (adjusted rev min^{-1} to pilot equipment), which gave an exceptionally high yield (97.2%) at pilot scale and provided the confidence to execute the remaining pilot batches. In general, actual values were lower at pilot scale than predicted, but the response surface was equivalent in shape and importantly, the predicted and actual optimum yield coincided.

Miniaturised equipment for extrusion-spheronisation can identify critical and optimum process parameters during feasibility studies and provide focus to later expensive and time-consuming process development and optimisation work.

208
Thermal transitions of trehalose

A. Khalid, S. Brocchini and G. Buckton

Department of Pharmaceutics, School of Pharmacy, University of London, 29–39 Brunswick Square, London, WC1N 1AX, UK

It is not clear how trehalose stabilises proteins. Mechanisms that include (1) water-replacement and (2) vitrification have been proposed. Interfacial phenomena during freeze-drying may also contribute to protein stabilisation in the presence of trehalose. The formation of a dihydrate crystal protects the remaining amorphous trehalose from the added water (Crowe *et al* 1998), so conversion of this material between amorphous and crystalline forms may be a key property in its ability to stabilise proteins. Different pathways for thermally induced conversions of the amorphous and crystalline forms of this dissacharide have been observed (Sussich *et al* 2002). The different metastable morphologies of trehalose may be a key property that influences protein stabilisation.

Conventional DSC (10°C min^{-1}) and HyperDSC (100°C min^{-1}) (Trademark of Perkin-Elmer) have been used in a comparative study to determine thermal variations of trehalose dihydrate (T_h). Heating rate and sample size may provide conditions where the relative rates of water diffusion can influence molecular rearrangement to provide an opportunity to observe different morphologies. The experiments were carried out in non-hermetically sealed pans and the transitions are summarised in Table 1.

It can be seen that for 1 and 5 mg at both scan rates there was a dehydration at just below 100°C with an enthalpy of ca. 150 J g^{-1}, however the subsequent transitions varied depending upon sample mass and scan rate. For 1 mg samples there was a very small transition (1.5 J g^{-1} at 10°C min^{-1} and 17.0 J g^{-1} at 100°C min^{-1}) at ca. 130°C. For the 5 mg samples there was a large transition at 114–126°C depending upon scan rate. Transitions in this range have previously been described as being due to the α-anhydrate or the γ-form melt (Sussich *et al* 2002).

On further heating the samples scanned at 10°C min^{-1} display a large crystallisation event, whereas those scanned at 100°C min^{-1} show either a very small, or no crystallisation. Finally at ca. 210°C there is the melt of the β-anhydrous form for which the enthalpy is lower for the 1 mg samples. These data indicate that all samples dehydrate in a similar fashion, but the 5 mg samples convert to the α-anhydrous form and then predominantly proceed through a solid transition (little or no crystallisation) to the β-anhydrous form, which then melts. The small samples (1 mg) dehydrate to yield an amorphous material with only modest amount of the α-form, which then by solid transition and some crystallisation produce a smaller amount of β-form than is seen with the large sample masses.

Table 1 Thermal transitions of trehalose in non-hermetically sealed pans

Sample size, Scan rate	Onset temperature (°C) (s.d.); Enthalpy (J g^{-1}) (s.d.)			
1 mg,	97.8 (0.1);	127.0 (0.7);	182.8 (2.7);	211.2 (0.2);
10°C min^{-1}	144.0 (9.1)	1.5 (0.9)	−38.2 (20.0)	73.2 (14.9)
5 mg,	96.5 (1.3);	113.6 (0.9);	196.5 (1.1);	209.6 (0.2);
10°C min^{-1}	159.1 (9.7)	125.4 (9.8)	−8.9 (4.7)	110.7 (4.7)
1 mg,	99.6 (0.7);	130.0 (2.3);	168.7 (3.0);	210.7 (0.3);
100°C min^{-1}	156.5(10.5)	17.0 (6.9)	−0.9 (0.7)	95.9 (3.4)
5 mg,	99.3 (0.4);	126.2 (0.9);		208.6 (0.8);
100°C min^{-1}	149.3 (5.1)	174.2 (37.9)		125.8 (2.9)

These data show that the conversions between the different forms of the trehalose are complex and are affected by scan rate and sample mass. The conclusions here differ from those reported previously (Sussich *et al* 2002).

Crowe, J. H., *et al.* (1998) *Annu. Rev. Physiol.* **60**: 73–103
Sussich, F., *et al.* (2002) *Thermochim. Acta* **391**: 137–150