Poster Session 1 – Pharmaceutical Technology

076
Optimisation of protein and sugar ratio in lyophile development

T. A. Payne-Cook and S. Pygall

Pfizer Global Research and Development, Sandwich, Kent CT13 9NJ, UK. E-mail: toby.payne-cook@pfizer.com

During lyophilisation, proteins are protected by the addition of stabilising excipients, often called lyoprotectants. Sugars in their amorphous state are the most commonly used. To optimise lyophile stability, solutions are frozen to below T_g', preventing collapse during lyophilisation (Franks 1990). During primary drying, the product temperature should be below, but as close to T_g' as possible, as drying time decreases by an approximate factor of two for a 5°C increase in product temperature. The final drug product must also be stored below its collapse temperature (T_c), to prevent deformation of the lyophile "cake" during its shelf life. Our work shows that by varying the ratio of protein and sugar in a formulation, the material properties of the frozen solution and the final cake can be manipulated to shorten the lyophilisation cycle and to improve physical stability. Two proteins were studied: a proprietary highly glycosylated protein with a mean mol. wt. of 48 kDa and bovine serum albumin (BSA). For the glycoprotein, both sucrose and trehalose were studied as lyoprotectants, while only sucrose was investigated with BSA. Isotonic solutions of sucrose and trehalose in 40 mM pH6.5 phosphate buffer were prepared at 75 mg mL^{-1} and 83 mg mL^{-1}, respectively. The glycoprotein and BSA were added to the sugar solutions at concentrations in the range 0–70% mass ratio protein:sugar. Samples were freeze dried using a conservative lyophilisation cycle, with a primary drying stage of 60 h at −40°C/0.1 h Pa. T_c, T_g and T_g' values were measured by differential scanning calorimetry on a TA2910 instrument. T_g' values were obtained at 5°C min^{-1} ramping from −60°C to +30°C. T_c and T_g values were obtained at 10°C min^{-1}, ramping from −30°C to 110°C. T_c was measured on the first heating curve, while T_g was measured during the second heating curve once the heterogeneity and thermal history of the sample was erased. Our data shows that the T_g' values for sucrose in phosphate buffer are −34°C, −30°C, −20°C and −18°C at 0%, 20%, 50% and 70% mass glycoprotein, respectively. The trend is linear with an R^2 value of 0.98. The same trend was observed with sucrose and BSA. Trehalose T_g' increases from −31°C at 0% glycoprotein to −3°C at 40%. The T_c and T_g values also increase for the corresponding lyophiles: the T_c value for sucrose increases from 55°C to 80°C at 0% and 40% mass glycoprotein, respectively, while the residual moisture increases over that range. Normally, moisture acts as a plasticising agent, causing depression of the T_c, yet the expected trend is reversed in our studies. The data suggests that the sugar and protein exist in a mixed amorphous phase and that T_g' values for 100% mass ratio protein can be predicted from the mixture data by linear extrapolation. Most importantly, such significant increases in T_g' can significantly reduce lyophilisation costs by shortening the drying time required. The data also shows that stable sucrose lyophiles can be produced with relatively high T_c values, thus preventing the need for using novel excipients such as trehalose.

Franks, F. (1990) *Cryo Letters* **11**: 93–110

077
Crystallisation better enhances folding reversibility of trypsin than does lyophilisation

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

Drug Delivery Group, School of Pharmacy, University of Bradford, Bradford BD7 1DP, UK. E-mail: r.t.forbes@bradford.ac.uk

An important aspect in the preparation of proteins as pharmaceutical products is stabilisation of the native folded protein conformation, which is required for biological activity. However, it is not enough for this conformation to be stable, but the protein must also be able to find the folding pathway from a denatured, unfolded conformation. Our aims were: to investigate the influences of crystallisation and lyophilisation on folding reversibility of trypsin (a model protein drug) in solution using high sensitivity differential scanning calorimetry (HSDSC) and to determine the integrities of crystallised and lyophilised trypsin solutions in low and high concentrations, employing FT-Raman spectroscopy. HSDSC determined thermodynamic parameters and measured folding reversibility of thermally denatured trypsin in crystallised and lyophilised forms by exploiting two consecutive upscans from 20–90°C at 1°C min^{-1}. After the first upscan, the sample was immediately cooled to 20°C at 0.75°C min^{-1} and the heating cycle was repeated. Transition reversibility was measured as ratio (%) of enthalpy change of second upscan over that of first upscan. FT-Raman spectra of aqueous trypsin solutions (8 and 20% w/v) were the averages of 3500 scans at 4 cm^{-1} resolution. FT-Raman showed minor changes in the spectra of crystallised and lyophilised trypsin at low and high concentrations. We observed that trypsin crystals more readily dissolved in water at high concentration (20% w/v) compared with lyophilised form at the same concentration. After the FT-Raman run, lyophilised samples (8 and 20% w/v) formed a gel, possibly due to aggregate formation. Crystallised samples only formed a gel at 20% w/v, indicating that crystallised trypsin was less aggregated than lyophilised protein in low concentration. Proteins must be able to retain their native state to be biologically active. A change from native folded state to unfolded state can lead to aggregation. Consequently, to complement FT-Raman data, HSDSC was employed to study the conformational performance and folding reversibility of crystallised and lyophilised trypsin. HSDSC plots of crystallised trypsin exhibited an endothermic transition as one single peak at 61.3 ± 0.3°C (Tm_1) for the first heating cycle and also one single peak at 61.5 ± 0.3°C (Tm_2) for the second heating cycle. There was no significant difference ($P < 0.05$) between Tm_1 and Tm_2. The folding reversibility was 76.7 ± 8.8%. For lyophilised trypsin, the HSDSC profiles showed an endothermic transition at 61.7 ± 0.6°C with a shoulder at 54.1°C for the first heating cycle and only a small transition at 61°C for the second heating cycle. The folding reversibility was 27%. In conclusion, trypsin crystals retained a higher folding reversibility compared with lyophilised protein, supporting our earlier findings that lysozyme crystals possessed better folding reversibility than did the spray-dried form. Accordingly, crystallisation shows promise for preparing protein drug delivery systems.

078
Lyophilisation technology in drug dissolution enhancement

R. S. K. Ng, H. N. E. Stevens and A. B. Mullen

Department of Pharmaceutical Sciences, University of Strathclyde, Glasgow, G4 0NR, UK. E-mail: raymong.ng@strath.ac.uk

Lyophilisation was investigated as a means of modifying the solubility and dissolution profile of three model drugs, indomethacin (IND), ibuprofen (IBU) and hydrochlorothiazide (HCTZ), through manipulation of their physical forms. Drug solutions were prepared in a binary co-solvent system consisting of 2-methylpropan-2-ol and water supplemented with additional surfactants or pH modifiers to enhance drug solubility (Table 1). Drug solution equivalent to the desired testing dose was dispensed into lyophilisation vessels and transferred into a −70°C freezer and allowed to solidify. The resultant solidified drug solution was lyophilised in an Edward Modulyo freeze dryer for approximately 12 h before being removed and stored under vacuum in a desiccator until undergoing dissolution assessment. Each batch of experimental drug formulation underwent dissolution testing in accordance with USP II method (1000 mL distilled water at 37 ± 0.5°C, paddle speed 50 rev min^{-1}). Drug release from formulations was monitored by UV spectrophotometry at the following wavelengths: indomethacin (276 nm); ibuprofen (265 nm); hydrochlorothiazide (273 nm). For comparative purposes the dissolution performance of experimental lyophilised formulations were compared with a relevant control or commercial product. Formulation composition, time to maximum release (t_{max}), 'difference factor' (f_1) and 'similarity factor', (f_2) are presented in Table 1 (Pillay & Fassihi 1998). In addition the porosity of select formulations was evaluated by scanning electron microscopy (SEM). The dissolution profiles of each experimental lyophilised formulation were significantly enhanced compared with their respective control or commercial formulation (Table 1). SEM indicated that the formulations had high porosity. This may have contributed to the rapid dissolution profiles observed. Further work is being undertaken to determine the relative contribution of polymorphic/amorphic drug transitions or modifications in formulation porosity to the enhanced dissolution profile of these formulations.

Table 1 Dissolution performance of experimental and commercial formulations of ibuprofen, indomethacin and hydrochlorothiazide

Active ingredient	Formulation	t_{max} (min)	f_1	f_2
IBU	Experimental formulation	4.5	16.2*	32.1*
	Nurofen Meltlets	15		
IND	Experimental formulation	14	10.2	31.2*
	Experimental control	28		
HCTZ	Experimental formulation	20	19.6*	21.0*
	Hydrosaluric	40		

f_1 values ≥ 15 significant at 10% level; f_2 values ≤ 50 significant at 10% level (Costa et al 2003).

Costa, F. O., et al (2003) *J. Controlled Release* **89**: 199–212
Pillay, V., Fassihi, R. (1998) *J. Controlled Release* **55**: 45–55

079
Verification of a Phase I clinical trial distribution network for cold storage materials

M. A. Elliott and G. W. Halbert

Cancer Research UK Formulation Unit, Department of Pharmaceutical Sciences, University of Strathclyde, 204 George Street, Glasgow, G1 1XW, UK. E-mail: moira.elliott@strath.ac.uk

Article 13 of European Union Directive 2001/20/EC for the implementation of Good Clinical Practice (GCP) in clinical trials will for the first time lay a Good Manufacturing Practice (GMP) legislative obligation on Phase I clinical trial manufacturers and suppliers (stated under Directive 91/356/EEC). The ramifications will be significant throughout the industry, but nowhere greater than in small Phase I/II Units. Voluntary application of appropriate GMP in such Units is probably commonplace. This undoubtedly also goes hand in hand with understandable apprehension of the inspectable standard, specifically in relation to process validation and verification. We report here on one aspect of the GMP/GCP interface looking at the manufacturer's responsibility to distribute medicines at the appropriate temperature until such time as it is received at the clinical centre. The study aimed to verify cold storage shipping arrangements using a representative transport route from the Cancer Research UK Phase I/II Formulation Unit in Glasgow to the Cancer Research UK Drug Development Office in London. Prior to real time transit tests, a laboratory-based study determined the required configuration of frozen gel packs to maintain internal refrigerated conditions (stated as 0–8°C) over a minimum time period of 24 h (representative of overnight transit and delivery). One standard size of insulated shipper with white cardboard outer and one chosen size of gel pack were used. The shippers were packed with boxed dummy product units. Annually calibrated, portable temperature logging devices were placed in three product boxes covering the top, middle and lower internal packing positions. Shipments were made by overnight transit by one courier company. Refrigerated vehicles were not used. On parcel arrival at the London office, the address detail was turned around without opening the parcel, and the same courier company arranged for parcel uplift and re-delivery to the Formulation Unit. Temperature data was downloaded from the logging devices on arrival back in Glasgow. In this way, logging data was obtained for up to 48 h. Thirteen transits were made over a two-year time period to cover seasonal variation in temperature. Refrigerated internal conditions were maintained in autumn (3.9–6.6°C), winter (2.6–5.6°C) and spring (4.1–7.5°C) in shippers configured with 3 ice packs and exposed to a minimum of 4 h pre-chill. The system was modified to 4 ice packs for summer transit (2.6–5.2°C). The study verified the cold chain shipping arrangements in use at the Formulation Unit. The results were repeatable and seasonally robust. The equipment and cold transit arrangements were proven while remaining simplified, cost effective and practically achievable within the staffing structure and budget of a Phase I unit without benefit of a dedicated packaging and distribution section.

080
The effect of polymer molecular weight on polymer-stabilised drug nanoparticle size

D. J. Goodwin, L. G. Martini*, S. J. Holland*, G. S. Leonard* and M. J. Lawrence

Dept. of Pharmacy, King's College London, Franklin-Wilkins Building, Stamford Street, London and *GlaxoSmithKline, New Frontiers Science Park, Third Avenue, Harlow, Essex, UK. E-mail: daniel.goodwin@kcl.ac.uk

Of the new chemical entities identified through combinatorial screening methods, it is estimated that approximately 40% possess poor water-solubility (Lipinski 2002). This poor solubility is manifest in the clinical scenario as low and erratic oral bioavailability and, therefore, sub-optimal performance. A wet-milling process has been developed by GSK, which allows production of drug particles in the submicron or nanometre size range with a concomitant improvement in the bioavailability of the drug. The distinguishing feature of this process is the addition of a "surface modifier", selected from a range of pharmaceutically acceptable polymers or surfactants, which adsorbs at the drug particle surface to prevent aggregation. The resultant aqueous suspension of drug can be used as-is or alternatively processed into various solid dosage forms. As part of an investigation into understanding the nature of the interaction between the stabilising polymer and drug particles, the effect of polymer molecular weight on size reduction has been studied. Aqueous hydroxypropyl cellulose (HPC) solutions were subjected to ultrasonic degradation at 2°C for varying periods up to 24 h. Polymer molecular weights were characterised by determination of intrinsic viscosity at 25°C using capillary viscometry and applying the Mark–Houwink relationship, using the constants K and α determined by Law & Kayes (1983) for HPC. Exposure to ultrasound produced an exponential decrease in HPC molecular weight down to a limiting value of approximately 45 000 g mol^{-1} after 24 h (Table 1). Nabumetone, a non-steroidal anti-inflammatory drug with an aqueous solubility of 0.0047 mg mL^{-1} was subjected to wet-milling in the presence of a 1.5% w/v HPC solution of varying molecular weight (Table 1). The average particle size of the drug was assessed during milling up to a maximum time of 6 h, using a Malvern 2600 series laser diffractometer. The results are summarised in Table 2. Greater than 4 h wet-milling was required to obtain nabumetone particles in the submicron size range using un-degraded HPC. However, nanoparticles were formed after less than 2 h using HPC that had had been exposed to ultrasonic degradation for 24 h. This more rapid reduction in drug particle size using the lower molecular weight HPC could be explained by a greater milling efficiency due to a lower viscosity or the fact that lower molecular weight polymers have a faster ability to diffuse to the newly generated drug particle surfaces. Reduction in processing time has important implications; particularly the economic advantages in that less energy and time are needed to produce nanoparticles.

Table 1 Intrinsic viscosity and molecular weight of ultrasonically degraded HPC

Ultrasonic exposure time (h)	Intrinsic viscosity (dL g^{-1})	HPC molecular weight (10^{-3} g mol^{-1})
0 (Un-degraded)	1.08	110 ± 5
1	0.95	95 ± 5
4	0.82	80 ± 5
8	0.68	65 ± 5
24	0.51	45 ± 5

Table 2 Mean particle size of nabumetone drug particles wet-milled in the presence of various molecular weight HPC solutions: particle size with milling time

HPC molecular Weight (10^{-3} g mol^{-1})	Mean particle size (D[4,3]) after milling time (μm)		
	2 h	4 h	6 h
110 ± 5	2.75	1.08	0.85
95 ± 5	2.14	0.96	0.77
80 ± 5	1.60	0.89	0.75
65 ± 5	1.07	0.79	0.73
45 ± 5	0.94	0.76	0.76

Law, S. L., Kayes, J. B. (1983) *Int. J. Pharm.* **15**: 251–260
Lipinski, C. A. (2002) *Am. Pharm. Rev.* **5**: 82–85

081
Stabilising ability of mannitol, sorbitol and trehalose mixtures on spray dried lysozyme assessed by accelerated rate stability testing

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

Drug Delivery Group, School of Pharmacy, University of Bradford and *Merck Chemicals Ltd., Merck House, Poole, Dorset, UK. E-mail: r.t.forbes@bradford.ac.uk

The biological activity of proteins depends on the three dimensional native state of the protein. The stability of the native state is determined by weak physical interactions and is therefore relatively fragile. To overcome the lack of stability of the native state it is often necessary to produce proteins as solid products. This is possible by dehydrating the protein into an amorphous matrix of suitable stabilising excipients. Excipients that have been reviewed as stabilising agents include sugars (Tanaka et al 1991) and polyhydric alcohols (Gekko 1982). The excipients were present as single excipient/protein mixtures.

Here, the use of mixed excipient/protein systems was investigated to establish if any increase in stability could be achieved by using mixed excipient systems. Single excipients, as well as various mannitol/sorbitol and mannitol/trehalose mixtures, were co-spray dried in a 1:1 w/w ratio with dialysed lysozyme (5% w/v). Spray drying employed a Buchi 190 mini spray dryer. Solutions were atomised through a 0.5-mm nozzle fed by 1-mm tubing. The flow rate of 12–13 mL min^{-1} resulted in inlet temperatures of 135–145°C and outlet temperatures of 60 ± 2°C. Structural analysis of samples was obtained using X-ray powder diffractometry (XRPD) using a Bruker D5000 Diffractometer employing a scanning range of 5–45 degrees of two theta with a step size of 0.05° and step time of 3 s. Fourier transform Raman (FT-Raman) spectra were obtained using an FRA Raman module. The laser operated at 1.064 μm. A laser power of 900 mW with 500 scans at 4 cm^{-1} were used. Thermal analysis was performed using differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA) using Perkin Elmer series 7 systems. DSC utilised heating rates of 10°C min^{-1} scanning from 25 to 200°C while TGA profiles were obtained using a scan rate of 10°C min^{-1} heating from 25 to 275°C. Biological activity assay of lysozyme was determined using the method described by (Shugar 1952). Accelerated stability testing exposed the samples and unprocessed controls to conditions of 40°C and a relative humidity of 75% for 4 weeks. XRPD revealed that spray dried samples containing mannitol/sorbitol mixtures were relatively crystalline before and after exposure. Mannitol/trehalose mixtures were, however, amorphous on production and showed differing degrees of recrystallisation after exposure. FT-Raman showed that spray drying lysozyme alone and exposure to elevated conditions caused shifts in the Amide I and III bands indicating denaturation of the sample. The incorporation of excipients caused a decrease in the degree of denaturation, which varied between samples. DSC revealed that co-spray drying of mixtures reduced the apparent melting point of all of the excipients. TGA analysis indicated that initial mixtures incorporating sorbitol and trehalose had increased water content in the final sample. Biological activity assay results showed both before and after exposure that samples containing trehalose had retained higher activity than those containing either mannitol or sorbitol, or both.

Gekko, K. (1982) *J. Biochem.* **91**: 1197–1204
Shugar, D. (1952) *Biochim. Biophys. Acta* **8**: 302–309
Tanaka, K., Tokuji, T., Miyajima, K. (1991) *Chem. Pharm. Bull.* **39**: 1091–1094

082
Mechanical integrity of polymer films used in time-delayed release capsules

L. A. Hodges, H. N. E. Stevens and A. B. Mullen

Department of Pharmaceutical Sciences, University of Strathclyde, Glasgow G4 0NR, UK. E-mail: leeann.hodges@strath.ac.uk

We propose a time-delayed device consisting of an ethylcellulose (EC)-coated hard gelatin capsule with a vegetable oil/drug phase separated from a swelling compartment by a gelatin-based tablet. This study aims to elucidate the effect of polymer concentration on the ethylcellulose coat's tensile properties while also looking at the effects of maintaining either weight ratio of polymer to plasticiser or weight of plasticiser only. The required amounts of EC (Ethocel, Standard 10 grade) and triacetin were added to 50% (v/v) each of acetone and isopropyl alcohol; total volume 150 mL. The mixture was stirred for at least 6 h then poured onto a Teflon-coated plate (31 cm × 31 cm) and the organic solvent allowed to evaporate in a fume cupboard. Once thoroughly dry, the resultant films containing 2–6% (w/v) EC and either 20% (w/w of EC) triacetin or 1.8 g triacetin were carefully peeled off and cut into dumbbell-shaped samples using a sharp scalpel and an American Society for Testing and Materials (ASTM) metal template. Sample thicknesses were measured at five different points. The samples were then secured between two tensile grips on a Texture Analyser (TA-XT2; Stable Micro Systems, Surrey, UK) and the force generated in stretching the films was measured. The data obtained was normalised with respect to mean thickness of each film. Mean and standard deviation (s.d.) for individual data sets were compared and tested for statistical significance using analysis of variance in Minitab v.13 as shown in Tables 1 and 2. Overall, increasing polymer concentration increased tensile stress at break, work done to break film and elastic modulus of film. However increasing polymer concentration in films with constant EC:triacetin weight ratios did not significantly affect the tensile strain at break. Tensile strain at break decreased with increasing polymer concentration in films with constant triacetin weight. This method allows correlation with data obtained from force generation studies (unpublished) as part of a rational approach to the design of a formulation with a pre-determined lag phase dependent on the rupturing of the EC coat by hydration and swelling of a swelling agent.

Table 1 Tensile properties of EC films with constant weight ratio of EC:triacetin (per mm of film thickness)

EC (% w/v)	Tensile stress at break (N mm^{-1})	Tensile strain at break	Work done to break film (Nm)	Elastic modulus of film (N mm^{-2})
2	0.11 (0.02)	0.47 (0.25)	0.03 (0.02)	6.85 (1.11)
3	0.07 (0.03)	0.36 (0.26)	0.02 (0.01)	5.44 (0.69)
4	0.25 (0.03)	0.45 (0.22)	0.06 (0.03)	13.00 (0.73)
5	0.20 (0.05)	0.30 (0.09)	0.05 (0.02)	12.65 (1.65)
6	0.37 (0.07)	0.43 (0.22)	0.22 (0.20)	21.37 (3.51)
P-value	< 0.0005	0.400	< 0.0005	< 0.0005

Data are means (s.d.).

Table 2 Tensile properties of EC films with constant weight of triacetin (per mm of film thickness)

EC (% w/v)	Tensile stress at break (N mm^{-1})	Tensile strain at break	Work done to break film (Nm)	Elastic modulus of film (N mm^{-2})
2	0.11 (0.02)	1.38 (1.06)	0.07 (0.02)	2.99 (0.63)
3	0.09 (0.02)	0.57 (0.46)	0.03 (0.02)	3.98 (1.13)
4	0.10 (0.04)	0.35 (0.14)	0.03 (0.03)	5.39 (1.26)
5	0.16 (0.05)	0.33 (0.14)	0.06 (0.06)	8.85 (1.30)
6	0.37 (0.07)	0.43 (0.22)	0.22 (0.20)	21.37 (3.51)
P-value	< 0.0005	< 0.0005	< 0.0005	< 0.0005

Data are means (s.d.).

083
Guiding the formulation of poorly soluble compounds

S. Branchu, A. P. Plumb, P. G. A. Rogueda, J. A. E. Kraunsoe and W. G. Cook

AstraZeneca R&D Charnwood, Bakewell Road, Loughborough, LE11 5RH, UK

Although a range of solubility-enhancing formulation types exist, there is little published information about how to choose the most suitable formulation types for a given drug. Thus, the objective of this work was to develop a predictive tool to guide formulation type selection for poorly soluble drugs. A data set was built from AstraZeneca proprietary drugs as well as substances and suitable formulations taken from the literature. The properties included pK_a, molecular weight, melting point, 1-octanol/water distribution coefficient, number of hydrogen-bond donor and acceptor groups, dose and dose-to-solubility ratio. That data set was then examined using principal component analysis (PCA). PCA resulted in the identification of latent variables highlighting the presence of structure in the data. Those variables drew on all physicochemical properties. Certain compounds, such as nifedipine, played little part in influencing the principal components. Other compounds, such as chlorzoxazone, made a greater contribution. In addition, a simple case-based reasoning (CBR) procedure was designed and used in an attempt to solve the problem of formulation choice without using explicit rules (Rowe & Roberts 1998). This CBR tool works by nearest neighbour analysis to determine the physicochemical similarity of the test compound to reference compounds in the data set. The CBR-proposed formulation type for the test compound is that of the reference compound with the highest similarity factor. Danazol, amprenavir, saquinavir, ritonavir, tacrolimus, sirolimus, cyclosporine, dipyridamole, naproxen, itraconazole, griseofulvin and cinnarizine were used as test compounds. The CBR procedure identified griseofulvin to be similar to other substances known to be well formulated as solid dispersions. Thus, a solid dispersion was predicted to be suitable for griseofulvin. This is consistent with the way this compound is usually prepared. Itraconazole on the other hand was found to be quite different from the other case-base compounds, which was supported by the PCA results. The CBR method correctly detected some physicochemical similarity, especially within compound series, but neither for itraconazole nor for a number of other compounds was it able to predict successfully the formulation type. The results of this study suggest that empirical approaches combining multivariate projection and case-based reasoning may be applicable in dosage form development.

Rowe, R. C., Roberts, R. J. (1998) *Intelligent software for product formulation.* Taylor & Francis Ltd, London

084
Solution versus solid-state co-crystallisation: sulfathiazole with glucose

P. T. Gavan, N. Blagden, I. M. Grimsey and P. Marshall*

Drug Delivery Group, The School of Pharmacy, University of Bradford, Richmond Road, Bradford, BD7 1DP and *Pfizer Limited, Sandwich, Kent, UK. E-mail: P.T.Gavan@Bradford.ac.uk

The pharmaceutical industry is continuously investigating methods to alter the physicochemical properties (e.g. solubility, mechanical stability, crystal morphology) of active drugs and their dosage forms. The incorporation of a co-crystallising excipient with a pharmaceutically applicable molecule by non-covalent intermolecular synthesis may modify the bulk material properties, while maintaining the intrinsic activity of the drug molecule. The mechanism of co-crystal formation is far from being understood, and design strategies are currently being researched (Aakeröy et al 2003). Co-crystals may be prepared by evaporation of a heteromeric solution, or by grinding the components together. It is known that co-crystals formed from solution may not form by solid-state grinding, and vice versa, and the addition of small amounts of solvent to the grinding process may facilitate co-crystal formation (Pedireddi et al 1996; Shan et al 2002). Although there have been many studies of co-crystallisation, little has focused on using pharmaceutically acceptable co-crystallising agents. Co-crystallisation of sulfathiazole, a poorly water-soluble antibacterial sulfonamide drug, with glucose, was investigated from solution and solid-state methods. Solution co-crystallisation was attempted by dissolution and recrystallisation of equimolar, 2:1 and 1:2 molar ratios of sulfathiazole and glucose, from a variety of solvents. Mixed solvent co-crystallisation, from tetrahydrofuran-water and nitromethane-methanol, was also attempted, to prevent the least soluble component from precipitating out exclusively. Solid-state co-crystallisation was attempted by milling equimolar amounts of sulfathiazole and glucose together in a Wig-L-Bug grinding mill. The reaction was monitored at 2-min intervals, up to a total milling time of 20 min. The effect of the addition of small amounts of solvent to the solid-state grinding was also examined. The products were characterised structurally using Powder X-Ray Diffractometry (PXRD), and thermally using Differential Scanning Calorimetry (DSC) and Thermogravimetric Analysis (TGA). Glucose was found to be insoluble in toluene, tetrahydrofuran and nitromethane, and sulfathiazole was also insoluble in toluene. No product was formed from mixed solvent co-crystallisation of sulfathiazole with glucose. A sulfathiazole-glucose solid dispersion was formed from methanol, and single component sulfathiazole from water. Potential sulfathiazole-glucose co-crystals were identified as forming from propanol and ethanol solutions. Co-crystal formation and identity could not be confirmed because crystals of suitable quality for structure determination by single-crystal x-ray diffraction were not obtained. Altering the ratio of sulfathiazole:glucose appeared to have no effect on the solution co-crystallisation products from methanol, propanol and water. No product was obtained from the 1:1 sulfathiazole:glucose from ethanol co-crystallisation. Although the 2:1 sulfathiazole:glucose from ethanol potential co-crystals appeared identical to the products formed from propanol, the 1:2 sulfathiazole:glucose from ethanol product seemed unique, based upon their PXRD patterns. The effect of the solvent and the influence of the drug:excipient ratio, on solution co-crystallisation, are therefore not clear. Co-crystallisation by solid-state grinding was not successful, even upon the addition of small amounts of solvent, and simple binary eutectics and a hydrate product were obtained.

Aakeröy, C. B., Beatty, A. M., Helfrich, B. A. et al (2003) *Cryst. Growth Des.* **3**: 159–165

Pedireddi, V. R., Jones, W., Chorlton, A. P. et al (1996) *Chem. Commun.* **8**: 987–988

Shan, N., Toda, F., Jones, W. (2002) *Chem. Commun.* **20**: 2372–2373

085
Non-viral formulation of DNA using N^2, N^3-dioleoyl spermine

O. A. A. Ahmed and I. S. Blagbrough

Department of Pharmacy and Pharmacology, University of Bath, Bath BA2 7AY, UK. E-mail: prsisb@bath.ac.uk

As non-viral DNA-delivery vectors, cationic lipids and polymers have the ability to condense DNA into particles. These can be readily endocytosed by tissue-cultured cell lines and ultimately delivered to the nucleus. Our aims are the design and formulation of a novel non-viral vector capable of efficiently and safely delivering DNA to the nucleus in a variety of cell lines. The characteristics of lipospermines as cationic and lipophilic substances render these compounds promising carriers for DNA through the formation of lipoplexes (Blagbrough et al 2003). In this study, we synthesized and characterized a novel lipospermine in which the tetra-amine spermine (the cationic moiety) and dioleoyl chains (the lipophilic moiety) are linked by amide bonds at the secondary amino groups of spermine to form previously unreported N^2, N^3-dioleoyl spermine (LipoGen). As among the prerequisites for delivery of DNA across intact cytoplasmic membranes are masking the negative charge of the phosphate backbone leading to DNA condensation, we studied the ability of N^2, N^3-dioleoyl spermine to condense calf thymus DNA and plasmid DNA (β-galactosidase, Promega) using an ethidium bromide (EthBr) ($\lambda_{ex} = 260$ nm, $\lambda_{em} = 600$ nm) fluorescence-quenching assay (Gershon et al 1993; Geall & Blagbrough 2000) in comparison with poly-L-lysine (PLL, average molecular weights 9.6 and 27 kDa) and polyethylenimine (PEI, average molecular weights 2 and 60 kDa) as model DNA condensing agents. To quantify the ability of these polyamines in DNA condensation, the binding constants of these polyamines with DNA was calculated. Also, particles of condensed DNA were detected using a UV light scattering assay at $\lambda = 320$ nm. The results indicate the ability of N^2, N^3-dioleoyl spermine to condense DNA by the significant decrease in (intercalated) EthBr fluorescence intensity and the increase in apparent absorption in light scattering experiments. The transfection results revealed high transfection efficiency in both an immortalized cancer cell line (HeLa) and in primary skin cells (FEK4) (in culture) using plasmid DNA encoding for enhanced green fluorescent protein (pEGFP) (Clontech) as the reporter macromolecule with its fluorescent imidazolidinone moiety analysed by FACS. The transfection efficiency and toxicity of N^2, N^3-dioleoyl spermine were studied in comparison with other commercially available liposomal cationic lipids Lipofectin and Lipofectamine. The DNA condensation and gene delivery using N^2, N^3-dioleoyl spermine was achieved at a small ammonium/phosphate (N/P) charge ratio (2.5 +/−) that minimises the toxic effects observed at higher N/P charge ratios. These results obtained with novel N^2, N^3-dioleoyl spermine show it to be efficient in both lipoplex formation and lipofection.

We acknowledge the financial support of the Egyptian Government (studentship to O.A.A.A.). We are grateful to Prof. R. M. Tyrrell for the FEK4 cell line and to Dr C. Pourzand (University of Bath) for help in the cell biology studies and for useful discussions.

Blagbrough, I. S., et al (2003) *Biochem. Soc. Trans.* **31**: 397–406

Geall, A. J., Blagbrough, I. S. (2000) *J. Pharm. Biomed. Anal.* **22**: 849–859

Gershon, H., et al (1993) *Biochemistry* **32**: 7143–7151

086
Development of a coated tablet formulation containing compressed enteric-coated pellets using electrostatic dry powder deposition technology

L. C. Dawson, S. C. Mundy, S. Monajem-Isfahani, A. Dawkins, J. Forster and M. Whiteman

Phoqus Pharmaceuticals Limited, 10 King's Hill Avenue, West Malling, Kent, ME19 4PQ. UK. E-mail: marshall.whiteman@phoqus.com

Traditionally enteric-coated (EC) pellets containing active materials have been filled into hard gelatin capsules. Gelatin, being of bovine origin, can represent a health risk due to the potential risk of transmitting spongiform encephalopathy. The aim of this study was to compress EC pellets of an active into a tablet while maintaining their physical structure and enteric release characteristics. Since a light compression force is needed while manufacturing the tablets to prevent pellet fracture, these tablets are usually not robust. Therefore, a coat was applied to the tablets by electrostatic dry powder deposition technology (EDPDT; Page et al (2002)) to increase the tablet strength while preserving the enteric release characteristics of the pellets to meet USP standards. For this study, commercially available pellets containing 10% w/w omeprazole were chosen as the model (Suppliers: PSA Chemicals and Cornileus Pharmaceticals, India). The integrity of the EC pellets was tested before use according to the method outlined in the USP and was found to be well within the specification limit (i.e. less than 10% w/w of omeprazole dissolved after 2 h in a 0.1 M HCl medium) as analysed by HPLC (Table 1). The PSA batch of pellets was then compressed into a suitable tablet matrix that was capable of protecting the pellets from fracture. Tablets were compressed to 10 mm diameter and 4.2 mm thickness using a single punch press (Manesty F3, UK). The powder blend chosen to suspend the pellets was primarily a lubricated mixture of microcrystalline cellulose and starch. The ratio of pellets to powder and tablet breaking strength were optimised to ensure that pellets remained intact during compression. Integrity of the pellet coat in the tablets was assessed by an adapted USP dissolution method for EC tablets (Farinha et al 2000), performing the acid stage dissolution test. The amount of omeprazole released due to pellet fracture was determined by HPLC analysis. Omeprazole tablets were then externally coated with a water-soluble polymer-based coat using EDPDT and the integrity of the pellets was assessed. The results obtained show that the integrity of omeprazole pellets in uncoated tablets of

similar breaking strength (ca. 2 kp) with a 1:3 and 1:4 ratio of pellets to powder blend was preserved and that these tablets passed USP specifications (Table 1). Coated tablets containing a 1:3 ratio of omeprazole pellets were prepared and were found to be more robust and remain intact without any damage. For these tablets, the water-soluble coat was seen to dissolve away leaving the tablet to disintegrate rapidly. Meanwhile, the integrity of the EC pellets was preserved (5.2% w/v omeprazole release) thus conforming to USP specifications. In conclusion, by using EDPDT, lightly compressed but robust tablets containing enteric-coated omeprazole pellets were prepared without affecting their enteric protection.

Table 1 Omeprazole release from enteric-coated pellets and compressed tablets

Material	Pellets:blend ratio	% w/v omeprazole released
Pellets (PSA)	—	3.0 (5.1)
Pellets (Cornileus)	—	4.2 (1.2)
Uncoated tablet	1:3	0.4 (0.7)
Uncoated tablet	1:4	7.7 (7.6)
Coated tablet	1:3	5.2 (2.5)

Omeprazole release is expressed as mean (s.d.), n = 12.

Farinha, A., Bica, A., Martins, J. M., et al (2000) *Drug Dev. Ind. Pharm.* **26**: 785–790
Page, T., Hogan, J. E., Reeves, L. et al (2002) *Patent*: US6406738

087
Evaluation of electrostatic dry powder deposition technology for the precise deposition of active material on solid dosage forms

C. M. Dick, Y. P. Yong, L. A. Reeves and M. Whiteman

Phoqus Pharmaceuticals Limited, 10 Kings Hill Avenue, West Malling, Kent, ME19 4PQ, UK. E-mail: marshall.whiteman@phoqus.com

Electrostatic dry powder deposition technology (EDPDT) can be used to accurately deposit predetermined quantities of powder coating onto solid dosage forms. This study investigates the use of EDPDT to deposit precise amounts of active material within coat formulations onto the surface of solid dosage forms. To demonstrate the high precision of this technology, two studies were performed with anhydrous dicalcium phosphate (DCPA)-based placebo tablets using the EDPDT. In the first study, accurate deposition of a polymethacrylate-based coat on the placebo tablets was carried out. In the second study, accurate deposition of a similar coat containing 2% w/w active material, on the placebo tablets was performed. In both cases the amount of coat deposited was determined by mass difference. In the latter study, the content uniformity of the active was determined by HPLC and the theoretical coat weight subsequently calculated. In the first study, precise deposition of the polymethacrylate based coat was achieved (n = 200, %RSD < 4%). In the second study, HPLC analysis showed that 0.1 mg active was accurately deposited on the surface of the tablets. For mass difference and HPLC analysis, results (n = 10, %RSD < 5%, see Table 1) were all well within USP and Ph. Eur. acceptance criteria. Theoretical coat weights were similar to those obtained by mass difference indicating that the active and coat are deposited on the tablet surface in proportional amounts. High precision deposition of a polymethacrylate based coat onto placebo tablets was achieved using EDPDT. Furthermore, high precision dose loading of a polymethacrylate based coat containing 2% w/w active material onto placebo tablets was successfully achieved. The ability to deposit drugs accurately and reproducibly onto the surface of inactive tablets offers a simple and reliable means of achieving good content uniformity with low-dose actives. It is equally feasible to deposit drug-containing coatings onto cores that contain a second, higher-dose drug or even modified release cores of the same or another drug. The method can therefore simplify the manufacture of a variety of combination products as well as delivering good content uniformity of low-dose drugs.

Table 1 Mass difference and HPLC analysis results from second study

Mass difference		HPLC analysis	
%RSD	Mean weight	%RSD	Mean weight
3.41%	5.16 mg	2.40%	5.67 mg

088
Potential alternatives to lactose in spray-dried powders for pulmonary drug delivery

M. Fahim, R. Harrison, C. McCowen, N. R. Rabbani and P. C. Seville

Inhalation Technology Research Team, Aston Pharmacy School, Aston University, Birmingham, UK. E-mail: p.c.seville@aston.ac.uk

Dry powder inhalers (DPI) are used extensively for the delivery of therapeutic agents in the treatment of local conditions such as asthma and COPD. The pulmonary route is also attracting significant attention for systemic delivery, most notably for proteins and peptides. The majority of DPI systems comprise micronised drug, blended with larger carrier particles to improve powder flow and homogeneity during manufacture and to enhance powder aerosolisation properties (Larhrib et al 1999). Lactose is the most commonly employed carrier, as it displays well-characterised toxicity, broad availability and low cost. However, this traditional approach requires additional steps in the formulation process, including sieving and blending. It has recently been shown that spray-drying lactose-based formulations incorporating leucine as a dispersibility enhancer can successfully generate dry powders with reasonably high fine particle fractions, without the need for large carrier particles (Li et al 2003). While spray-drying offers a viable approach to the production of dry powders for inhalation, the presence of lactose still poses some formulation problems, as lactose is not suitable for use with all drugs, due to the reducing sugar functionality. In this study, we investigate the use of alternative sugars (monosaccharides: glucose, galactose; disaccharides: trehalose, maltose; polysaccharides: dextran T40, dextran 75, raffinose), to generate respirable spray-dried powders, and consider the influence of the sugar on the amount of leucine required for dispersibility enhancement. Aqueous solutions of the sugar and model drug (β-estradiol), plus leucine as a dispersibility enhancer, were spray-dried (Büchi 290 mini spray-dryer) using standard operating conditions to generate appropriately sized dry powders. The spray-drying yield was determined for each powder, and the particle size and morphology determined using laser diffraction and scanning electron microscopy (SEM). Powder aliquots (n = 3) were loaded into gelatin capsules and aerosolised using a Spinhaler DPI into a Twin Stage Impinger (TSI). The emitted dose was determined gravimetrically, and the fraction of drug recovered in the respirable and non-respirable regions of the TSI quantified using HPLC. Particle sizing and SEM indicated particles of all powders were within the respirable range; however, some powders were static and cohesive in nature, and did not display optimal powder flow characteristics. Increasing the leucine content of the glucose-based formulations decreased the spray-drying yield, yet in the galactose formulations, higher yields were seen with greater leucine content. Maltose formulations showed a slight decrease in yield with increased leucine content, and both maltose and trehalose formulations required less leucine to generate a powder with good flow properties. All powders demonstrated high emitted dose, with the majority in excess of 90% capsule contents emitted. The greatest respirable fraction was seen with the dextran T40-based powder, with 77% of total dose detected in the respirable region of the TSI. Recently, alternative sugars have been investigated as potential carrier particles in traditional DPI formulations (Steckel & Bolzen 2004). We have demonstrated that alternative sugars may also be used to generate respirable spray-dried powders, with polysaccharides such as dextran T40 exhibiting particular utility in enhancing the respirable fraction of spray-dried powders.

Larhrib, H., et al (1999) *Int. J. Pharm.* **191**: 1–14
Li, H.-Y., et al (2003) *J. Drug Target*. **11**: 425–432
Steckel, H., Bolzen, N. (2004) *Int. J. Pharm.* **270**: 297–306

089
Influence of leucine concentration and solvent composition on the aerosolisation of spray-dried powders

N. R. Rabbani and P. C. Seville

Inhalation Technology Research Team, Aston Pharmacy School, Aston University, Birmingham, UK. E-mail: p.c.seville@aston.ac.uk

Dry powder inhalers are being developed to deliver systemic agents such as proteins, peptides and hormones to the lung. Spray-drying is widely used in the pharmaceutical industry to prepare respirable powders of therapeutic macromolecules from a liquid formulation; however, the particles generated are prone to aggregation due to inter-particulate cohesion, reducing the dispersibility of the powders (Byron 1986). It has recently been shown that post-mixing an amino acid, leucine, with spray-dried powders effectively eliminates particle aggregation, and improves powder flow properties during inhalation (Lucas et al 1999). However, adding an additional excipient following the spray-drying

process involves an additional processing step, and will inevitably lead to dilution of the spray-dried powder. In this study, we investigate the ability of leucine to enhance the aerosolisation properties of spray-dried β-estradiol, when included in the formulation before spray-drying, and also the influence of solvent composition on the properties of the powders produced. Aqueous ethanol formulations containing β-estradiol and lactose in the presence of leucine were prepared, with a total powder mass of 2% w/v. Ethanol was included as a co-solvent (10–30% v/v). All formulations were spray-dried (Büchi B-290 mini spray-dryer) using standard operating conditions of inlet temperature, spray flow rate, aspirator and pump rate to generate spray-dried lactose-based powders containing 0.22% w/w β-estradiol and 2–12% w/w leucine. The % powder yield was determined, and the particle size and morphology assessed using laser diffraction and scanning electron microscopy. Powder aliquots (n = 3) were loaded into gelatin capsules and aerosolised using a Spinhaler dry powder inhaler into a Twin Stage Impinger. The emitted dose (ED) was determined gravimetrically, and HPLC was used to quantify the fraction of drug recovered in the respirable and non-respirable regions of the TSI, with the respirable fraction (RF) expressed as the percentage of total dose. Particle sizing indicated a unimodal size distribution, with an average size of 2–6 μm, well within the respirable range. Powders consisted of regular spheres, with the exception of the 2% w/w leucine/30% v/v ethanol formulation, which had an irregular morphology. The yield of this powder was also considerably reduced compared to the other formulations. Generally, increasing the proportion of ethanol in the solvent decreased the powder yield, whereas increasing the leucine content of the powders increased the yield. Powder analysis revealed that certain formulations offered improved protection to β-estradiol during the spray-drying process, with some powders containing only 45%, and others containing up to 100%, of nominal dose. The ED was found to be in excess of 90% for all powders, with the exception of the 2% w/w leucine/30% v/v ethanol formulation, which had an ED of 77%. The RF of the powders varied between 25% and 85% of total dose. It has been suggested that leucine may act as a surface active agent at the particle surface (Li et al 2003). We have shown that selection of appropriate leucine and ethanol concentration before spray-drying can generate spray-dried powders with very high respirable fractions, of up to 85% of total dose.

Byron, P. R. (1986) *Drug Dev. Ind. Pharm.* **12**: 993–1015
Lucas, P., et al (1999) *Pharm. Res.* **16**: 1643–1647
Li, H.-Y. et al (2003) *J. Drug Target.* **11**: 425–432

090
The use of amino acids as formulation excipients in lactose-based spray-dried powders

N. R. Rabbani and P. C. Seville

Inhalation Technology Research Team, Aston Pharmacy School, Aston University, Birmingham B4 7ET UK. E-mail: p.c.seville@aston.ac.uk

Spray drying has been used for many decades for the production of dry powder aerosols and offers potential for systemic delivery of therapeutic macromolecules; however, inter-particulate cohesion results in low respirable fractions (Li et al 2003). Spray dried l-leucine has demonstrated anti-adhesive properties in tablet formulation (Rotthauser et al 1998). Amino acids have also been found to show surfactant characteristics, with micelle growth being controlled by side chain length (Miyagishi et al 2002). In this study, we explored the viability of using amino acids as formulation excipients in lactose-based spray-dried powders to enhance their flowability. Aqueous solutions of lactose and amino acid (leucine, norvaline, isoleucine, norleucine, tert-leucine, valine, tryptophan, alanine or glycine) with a total powder mass of 2% w/v were spray dried (Büchi B-290 mini spray-dryer), to generate powders comprising 10% w/w amino acid. Before spray drying, viscosity and surface tension of all the formulations were measured using an AMVn automated microviscometer (Anton Paar) and a torsion balance, respectively. The percentage yield was ascertained and laser diffraction was used to determine the size of the powder particles. Amorphous content and morphology of the particles were assessed using DSC and SEM, respectively. Powder tap density was calculated using a tamping voltameter. Powders were loaded into gelatin capsules (n = 3) and aerosolised using a Spinhaler dry powder inhaler into an Andersen-type cascade impactor. DSC revealed the presence of amorphous material in all powders with the exception of 10% w/w tryptophan. Particle sizing (median particle size range 3.4–8.9 μm) showed unimodal size distributions for leucine and norvaline powders while the remainder displayed multimodal distributions. All powders other than those containing alanine and glycine consisted of spherical particles. Surface tension measurements indicated that addition of lactose to water increased the surface tension from 69 mN m^{-1} to 78 mN m^{-1}. Inclusion of amino acids lowered the surface tension; however, there was no apparent correlation between the spray-dried powder characteristics and surface tension. An inverse relation was found between the viscosity of the formulations and the size of the particles being produced. Incorporation of appropriate amino acids resulted in higher viscosity formulations, which when spray-dried produced finer particles with low tap density, resulting in higher emitted dose (Table 1).

Table 1 Powder characteristics

Amino acid	Yield (%)	Emitted dose % (s.d.)	Kinematic viscosity (mm^2 s^{-1})	Surface tension (mN m^{-1})	Tap density (g cm^{-3})
Leu	50	94 (0.1)	1.073	55	0.35
Nva	50	92 (0.1)	1.070	60	0.38
Isl	40	82 (0.1)	1.068	65	0.42
Nle	45	80 (0.1)	1.068	64	0.44
t-Leu	35	75 (0.1)	1.067	61	0.45
Val	20	25 (0.1)	1.050	58	*
Trp	10	20 (0.6)	1.060	61	*
Ala	5	5 (0.1)	1.050	62	*
Gly	5	5 (0.2)	1.064	56	*

*Insufficient powder generated for tap density studies.

Li, H.-Y., et al (2003) *J. Drug Target.* **11**: 425–432
Miyagishi, S., et al (2002) *Colloid Surf. A-Physicochem. Eng. Asp.* **197**: 125–132
Rotthauser, B., et al (1998) *Eur. J. Pharm. Biopharm.* **46**: 85–94

091
Use of a powder rheometer to characterize wet granule properties during product development

V. L. Wright, T. Ashfield and A. B. Dennis

Bristol-Myers Squibb, Pharmaceutical Research Institute, Reeds Lane, Moreton, Merseyside, CH46 1QW, UK. E-mail: andrew.dennis@bms.com

For many solid dosage forms, the process of wet granulation is a critical step. Conventional assessments of wet mass properties rely on indirect measurement of motor power or torque, dependent on batch size and machine type (Horsthuis et al 1993). This work reports the use of a simple, rapid, off-line measurement using a sensitive powder rheometer (Freeman Technologies) to measure total energy values (E) of wet granules. An investigation was undertaken using a new drug product to explore if a relationship between E and tablet hardness, H (a known final product characteristic critically dependent on the granulation), could be measured adequately, and to determine if any such relationship was independent of batch size. The chosen granulation contained a freely water-soluble drug (49% w/w) and sodium CMC (5% w/w), responsive to level and extent of binder (water) addition. Dried granules were subsequently milled and mixed with HPMC (36% w/w), microcrystalline cellulose (10% w/w) and magnesium stearate (0.1% w/w) before compression on a Kilian T100 rotary tablet press at fixed speed. Granulation batches were manufactured in a high shear mixer with either a 4 or 25 L bowl, using three different levels of water addition. Relative impeller tip speed and water addition times were maintained constant. For all batches the final blend operation was undertaken at the same scale (740 g). Wet mass samples from each batch were determined using two rheometer conditioning methods, non-compressed (NC) and compressed (C) to determine E and compared with the mean maximum tablet hardness (H). For the NC method, wet mass energy increased as maximum tablet hardness increased (Table1). Importantly, results from each granulation batch size formed a continuous trend. However, for method (C), the apparent sensitivity to weak granule structure was less, and correlation not as strong between E and H, in this method sample compression may have obscured differences in granule properties. The NC method was able to predict a critical final product characteristic (compressibility as indicated by maximum tablet hardness) independent of process scale. The importance of method development and selection was also highlighted. The technique has promising applications for monitoring product quality during scale up, technology transfer and routine production.

Table 1 Relationship between extent of granulation and wet mass rheology (total energy value) and maximum tablet hardness

Granulation scale	Granulation water level (% w/w)	Mean total energy (mJ, n = 2)		Maximum hardness (Scu, n = 5)
		NC method	C method	
4 L	13.3	63.6	146.8	32.4
	18.3	157.0	207.5	54.7
	21.3	332.3	333.3	61.6
25 L	13.3	66.6	107.9	31.8
	18.3	126.8	160.0	46.1
	21.3	239.5	232.8	55.5

Horsthuis, G. J. B., Van Laarhoven, J. A. H., Van Rooij, R. C. B. M., et al (1993) *Int. J. Pharm.* **92**: 143–150

092
Method for screening DPI powder blend formulations using Sympatec laser diffraction

D. J. Derbyshire and H. McGlynn

Aventis, Respiratory Technology, London Road, Holmes Chapel, Cheshire, CW4 8BE, UK. E-mail: Darren.Derbyshire@aventis.com; Hayley.McGlynn@aventis.com

The purpose of this study was to establish if laser diffraction can be used to screen early Dry Powder Inhaler (DPI) powder blend formulations to gain knowledge of their predicted fine particle mass without the need for DPI manufacture and Andersen Cascade Impactor (ACI) analysis. The Sympatec laser diffractometer (HELOS H1115) in conjunction with the RODOS dry powder dispersion unit and VIBRI vibrating powder feeder was used to measure the particle size distribution of blended formulations, and the variation with changing feed pressure. DPI's are currently used for the delivery of drug to the lung for the treatment of asthma. Initial DPI formulation studies are focused on identifying the optimum excipients and their concentrations by examining their influence on product performance and in particular on fine particle mass. One current method for evaluating the performance of inhalation delivery systems is the Andersen Cascade Impactor (ACI). This is used to determine the fine particle mass that is potentially respirable (defined as the mass of particles with a mass mean aerodynamic diameter $< 5.8\,\mu$m) and penetrate below the main conducting airways to reach the central and peripheral lung regions. However, this method of determining the particle size and fine particle mass is very resource intensive, as the drug substance collected on each stage of the impactor is quantified separately. Previous studies have shown that a correlation between the particle size distribution of the DPI product using laser diffraction and Andersen cascade impaction can be established (Derbyshire et al 2003). This poster describes the work undertaken to show that the Sympatec laser diffractometer, in conjunction with the RODOS dry dispersion unit and VIBRI vibrating powder feeder, provides a less resource intensive method for determining particle size distribution of powder blend formulations prior to manufacture of DPI product. In the first instance a correlation between the fine particle mass by ACI and the particle size result, at various feed pressures, of the powder blend obtained by laser diffraction was first established by analysing blends of different drug concentrations. Having defined a correlation with an $R^2 > 0.9$, several powder blend formulations were screened using the Sympatec laser diffractrometer at the optimum feed pressure and the optimum formulations were selected for further development. This was found to be less resource intensive than manufacture and assessment of DPI product using ACI.

Table 1 Correlation of fine particle mass and particle size results at a range of feed pressures

Feed pressure on Sympatec (bar)	R^2 value for correlation of particle size by laser diffraction and fpm by ACI
0.5	0.9456
1.0	0.9508
2.0	0.9380
3.0	0.9308

Derbyshire, D. J., Davies, P. A., Shaikh, T., et al (2003) Drug delivery to the lungs X1V, Dec 11–12, pp 144–147

093
Solution calorimetry as a method for quantifying drug-excipient interactions

S. A. Osmundsen, H. N. E. Stevens, P. G. Royall* and A. B. Mullen

Department of Pharmaceutical Sciences, University of Strathclyde, Glasgow, Scotland and *Department of Pharmacy, Kings's College London, Franklin-Wilkins Building, 150 Stamford Street, London, SE1 9NN, UK. E-mail: sigrunn.osmundsen@strath.ac.uk

Previous work has demonstrated that in-vitro dissolution of hydrochlorothiazide (HCT) formulations granulated with dicalcium phosphate (DCP) had a significantly slower and incomplete drug release compared with HCT granulated with lactose (Osmundsen et al 2003), which was suggestive of drug–excipient (hydrophobic) interactions between HCT and DCP. It was anticipated that solution calorimetry might have allowed the extent of this interaction to be quantified by measuring the enthalpy of solution, ΔH_f, for HCT-DCP granules (1:2 weight ratio) and comparing it with relevant controls. Known weights of test sample were added into a solution calorimeter glass ampoule. The calorimeter glass vessel was filled with 100 mL ethanol. Ethanol was used as a dissolution medium since it permitted instant solubility of formulation HCT. The solution calorimeter water bath temperature was maintained at 25°C with the stirrer speed operated at $500\,\text{rev}\,\text{min}^{-1}$. Formulations were analysed in triplicate. After solution calorimetry experiments were complete, the resultant concentration of HCT in the ethanol solution was calculated by UV spectroscopy. An increase in ΔH_f was observed when comparing the HCT powder with the HCT-DCP granules (Table 1). The dry blend of HCT and DCP was not significantly different ($P > 0.05$) from HCT powder alone suggesting no interaction resulted from a dry blend. The calorimetry results were paralleled by UV spectroscopic studies which indicated comparable final solution concentrations of HCT in both samples. However the concentration of HCT in ethanol was reduced in HCT-DCP granules and was associated with a decreased ΔH_f. The solution calorimeter data correlates with previous dissolution experiments. However the differences in enthalpy values were very modest when performed in ethanol solutions and is likely to be larger in aqueous systems where the aqueous solubility of HCT is approximately 21 times less than ethanol (Dreppler 1981) and hydrophobic interactions between HCT and DCP will predominate. Further research into the contribution of particle size and granule moisture content on ΔH_f are being investigated. However, the use of solution calorimetry may prove a useful screening technique for studying drug–excipient interactions particularly if the results can be translated to a physiological environment.

Table 1 Enthalpy of solutions for HCT and DCP Formulations

Formulation	Mean ΔH_f (J/g HCT) (± s.d.)
HCT powder	29.4113 ± 1.1407
HCT-DCP granules	27.2513 ± 0.7164
HCT-DCP dry blend	30.7873 ± 0.6492

Dreppler, H. P. (1981) In: Florey, K. (ed.) *Analytical profiles of drug substances and excipients*. Academic Press, New York, pp 181–197

Osmundsen, S. A., et al (2003) *Proc. Int. Symp. Controlled Release Bioact. Mater*. 526

094
Solid-state interactions between ibuprofen and cross-linked poly-vinyl-pyrrolidone within a physical mix

C. F. Rawlinson, A. C. Williams and P. Timmins*

Drug Delivery Group, School of Pharmacy, University of Bradford, BD7 1DP and *Bristol Myers Squibb, Reeds Lane, Moreton, Wirral, CH46 1QW, UK. E-mail: c.f.rawlinson@bradford.ac.uk

Many compounds currently under development within the pharmaceutical industry are Biopharmaceutical Classification System (BCS) class II compounds (i.e. low solubility, high permeability) (Amidon et al 1995). As a consequence of increased lipophilicity due to highly specific receptor targeting, the dissolution characteristics of these drugs are compromised. This situation has

driven the development of strategies to maximise bioavailability by influencing the dissolution process of such compounds. Stabilisation of amorphous drug species is an example of such a strategy (Craig 2002; Lu 2002). Here we report on studies investigating the formation of an amorphous species of ibuprofen (IB) upon combination with cross-linked poly-vinyl-pyrrolidone (PVP-CL) using simple mixing. Triplicate samples from physical mixes of IB and PVP-CL were analysed using a Perkin Elmer Series 7 power compensated Differential Scanning Calorimeter (DSC). Samples (2–5 mg) were heated under dry nitrogen purge, at 10°C min^{-1}, from 5 to 85°C. They were also analysed using a Siemens D5000 X-ray diffractometer (PXRD); CuK_{α} radiation ($\lambda = 1.5406$ Å) over 2–73°, with a step size of 0.05°(2Θ) and count time of 3 s was used at 40 mV, 30 mA with sample rotation of 30 rev min^{-1}. Molecular modes in IB, PVP-CL and in mixes were examined using FT-C Bruker, 4 cm^{-1} resolution, 200s cans and "conventional" (Renishaw, 785 and 633 nm lasers, 10 s accumulations) Raman spectroscopies. IB displayed its characteristic melting peak with a maximum at 77°C, as well as a small exothermic peak at about 11°C. The physical mixes showed a decrease in enthalpy of this peak that indicated disordering of ≈30% of the IB. PXRD showed broadening and diminution of intensity of the characteristic IB peaks. This is consistent with some of the crystalline IB disordering. PVP-CL did not show any peaks within the higher temperature ranges of the DSC trace. However, exothermic events were observed at low temperatures, notably two overlapping exothermic peaks with maxima around 6 and 12°C. This region was followed immediately by a small endothermic peak. Concomitant with a reduction in the enthalpy of IB melting peak, the physical mixes displayed an area of exothermic activity at low temperatures. This was similar to the activity attributed to PVP-CL, but varied in enthalpy. PXRD could not be used to identify any of these changes, as PVP-CL is X-ray amorphous. The low temperature exothermic activity is probably due to the PVP-CL polymer chain forming intramolecular hydrogen bonds. The formation of amorphous ibuprofen may be facilitated by interaction with the PVP-CL polymer chain. It follows that the presence of these IB molecules will prevent some intra molecular bonding of PVP-CL. The location of IB along the PVP-CL chain would alter the intramolecular bonding, accounting for the varying proportional enthalpies of the two exothermic peaks. Raman spectroscopy showed disorder was introduced into the IB in the mixed samples, evidenced by bond broadening phenomenon. In conclusion, the combination of IB and PVP-CL by simple mixing to facilitate intimate contact, is causing the partial disordering of IB. Modifications of thermal events attributed to intramolecular bonding of PVP-CL provide supporting evidence for molecular interaction between the two compounds.

Amidon, G. L., Lennernas, H., Shah, V. P., et al (1995) *Pharm. Res.* **12**: 413–420

Craig, D. Q. M. (2002) *Int. J. Pharm.* **231**: 131–144

Lu, M. (2002) PhD Thesis, University of Bradford, Bradford

095
Novel amphiphilic polyethylenimine (PEIs) for oral delivery of poorly soluble drugs

W. P. Cheng and I. F. Uchegbu

Department of Pharmaceutical Sciences, University of Strathclyde, 27 Taylor Street, Glasgow G4 0NR, UK. E-mail: Woei.cheng@strath.ac.uk

The oral delivery of hydrophobic drugs is still a challenge as drugs that do not dissolve appreciably in the aqueous environment of the gastrointestinal tract suffer from poor bioavailability. We have previously reported that polyethylenimine (PEI) amphiphiles are able to solubilise the hydrophobic immune suppressant cyclosporine (CsA, MW = 1202 Da) (Cheng & Uchegbu 2003). CsA has an intrinsic aqueous solubility of 23 $\mu g\,mL^{-1}$ and PEI amphiphiles increase the aqueous solubility 87 fold and promote the absorption of CsA via the oral route, producing similar blood levels to that obtained with the commercial microemulsion formulation Neoral (Cheng & Uchegbu 2003). The mechanism by which these oil free PEI formulations promote the oral absorption of this hydrophobic drug is currently unknown and we thus set out to examine whether the inhibition of the intestinal P-glycoprotein efflux pump or the opening of paracellular transport pathways was involved in the absorption enhancement observed with these solubilising PEI amphiphiles. Nine PEI amphiphiles were synthesised and characterised (Table 1) using techniques, previously reported (Cheng & Uchegbu 2003) and their ability to reverse the P-glycoprotein efflux pump evaluated by using a doxorubicin MTT assay on a cell line expressing the P-glycoprotein pump — the A2780 AD cell line. Doxorubicin (dox) has an IC50 in the resistant A2780 AD cell line that is 190 times that shown in the sensitive A2780 cell line, the latter of which is largely devoid of the P-glycoprotein efflux pump. Additionally the cytotoxicity of these PEI amphiphiles against Caco-2 cells was studied using the MTT assay and the effect of these amphiphiles on the transepithelial resistance of a Caco-2 cell monolayer evaluated. The high (25 kDa) and medium (10 kDa) molecular weight amphiphiles were less cytotoxic than their parent molecules (Table 1), whereas with the low molecular weight amphiphile (1.8 kDa), amphiphilicity had very little effect on cytotoxicity. These data (Table 1) indicate that increasing the number of amine groups increases the cytotoxicity of the polyamines and that amine substitution (presumably conversion to quaternary ammonium non protonable amines) reduced the cytotoxicity of the long chain polyamines. Up to a level of 2 $\mu g\,mL^{-1}$ (10–13% of the Caco-2 IC50 values), none of the polymers had any effect on the integrity of the paracellular junctions in the Caco-2 cell monolayer or on the P-glycoprotein pump (Table 1). It appears that, at biocompatible concentrations, the PEI amphiphiles do not act by either a modulation of the P-glycoprotein pump or by altering the paracellular junctions.

Table 1 Polyethylenimine amphiphiles

Polymer	MW (k Da)	% Cetyl group	% Quarternary methyl groups	Caco-2 cell IC50 ($\mu g\,mL^{-1}$)	Magnitude of change in dox IC50 in A2780 AD cell line (polymer concn = 2 $\mu g\,mL^{-1}$)
PEI25	25	—	—	3.7	
Q125	25	5.0 ± 0.35 (n = 2)	62.2 ± 2.60 (n = 4)	15.2	1.1
Q225	25	5.0 ± 0.35 (n = 2)	79.6 ± 2.37 (n = 4)	12.7	1.7
PEI10	10	—	—	4.5	
Q110	10	5.6	62.1 ± 3.0 (n = 3)	21.9	1.5
Q210	10	5.6	88	24.3	0.81
PEI1.8	1.8	—	—	12.4	
C1.8	1.8	6.4	—	6.1	1.5
Q21.8	1.8	6.4	109	13.8	5.3

Cheng, W. P., Uchegbu, I. F. (2003) *Proceedings of Winter Symposium & 11th International Symposium on Recent Advances in Drug Delivery Systems in Salt Lake City, Utah*

096
The effect of increasing hydrophilicity of the counterion of the salts of two non-steroidal anti-inflammatory drugs, ibuprofen and etodolac

S. David, B. R. Conway, P. Timmins* and W. J. Irwin

Medicines Research Unit, Aston University, Birmingham, West Midlands, B4 7ET and *Bristol Myers Squibb, Reeds Lane, Moreton, Wirral, Merseyside, CH46 1QW, UK. E-mail: davids1@aston.ac.uk

Ibuprofen and etodolac are carboxylic acids with limited aqueous solubility. A range of amine counterions of increasing hydrophilicity were chosen to form salts with the drugs to improve their water solubility; these were tert-butylamine (tert) 2-amino-2-methylpropylamine (AMP1), 2-amino-2-methylpropan-1,3-diol (AMP2) and tromethamine (tris). The salts were made by dissolving 0.01 mol of ibuprofen or etodolac in acetonitrile (40 mL). Tris or AMP2 (0.01 mol) were dissolved in a warmed solution of methanol (40 mL) and 0.01 mol AMP1 and tert were dissolved in acetonitrile (40 mL). The amine and drug solutions were mixed together and placed in a freezer for 48 h. The resultant precipitate was filtered and dried overnight under vacuum at 40°C. The salts were analysed by NMR to confirm a 1:1 drug:amine ratio and their melting points were determined by DSC. The aqueous solubility was measured by adding excess solid to 15 mL double distilled water with continuous stirring over 48 h. The resultant suspensions were filtered, diluted and analysed by HPLC. Surface tension was measured with a Du Noy Ring Tensiometer using aqueous solutions of 1, 10 and 100 mg mL^{-1}. The salts were all confirmed to be of a 1:1 molar ratio and DSC suggested a relationship between melting point and solubility (Table 1). All salts increased the aqueous solubility of ibuprofen and etodolac by over 70 fold. However, increase in hydrophilicity of the counterion is not a controlling factor in increasing water solubility, as the tris salts have solubilities similar to AMP1 salts whereas salts AMP2 have solubilities exceeding 100 mg mL^{-1} and showed surfactant properties. It was found that these materials lower the surface tension of water from 72.5 mN m^{-1} (Yuan & Herold 2001) to 32.9 mN m^{-1} for etodolac AMP2 and 47.5 mN m^{-1} for ibuprofen AMP2. The addition of one hydroxyl group to tert counterion results in an increase in solubility of 300- (etodolac AMP1) and

1900 fold (ibuprofen AMP1). When two hydroxyl groups are added, the ion pair acts as a surfactant, the hydroxyl as the hydrophilic moiety and the drug as the hydrocarbon chain. As ibuprofen and etodolac have rigid plane structures they are expected to stack in planes and not form micelles (Attwood 1995). However, when the amine has three hydroxyl groups, no surfactant behaviour is observed and there is a reduction in solubility. This indicates that hydrophilicity is inhibited either by steric hindrance or by an unidentified mechanism. Examination of the crystal habit may help to explain these results and the surfactant activity of these salts could be further exploited in selecting an appropriate salt from for an immediate-release formulation.

Table 1 Physico-chemical properties of ibuprofen and etodolac and their salts

	Mp (°C)	Aq. sol. ($mg\,mL^{-1}$)
Ibuprofen (Ibu)	76–79	0.071
Ibu tert	185–190	5.020
Ibu AMP1	138–140	135.0
Ibu AMP2	114–118	> 100
Ibu Tris	160–164	8.97
Etodolac (Eto)	151–158	0.222
Eto Tert	178–185	15.691
Eto AMP1	164–167	> 100
Eto Tris	159–161	101.028

Attwood, D. (1995) *Adv. Colloid Interface Sci.* **55**: 177–303
Yuan, Z., Herold, K. E (2001) *Appl. Thermal Eng.* **21**: 881–897

097
Influence of particle size on mechanical properties of pharmaceutical crystals

L. J. Taylor*, D. G. Papadopoulos, J. C. Mitchell* and M. J. Snowden

Pharmaceutical R&D, Pfizer Global Research and Development, Sandwich, Kent, CT13 9NJ and *Medway Sciences, University of Greenwich at Medway, Chatham Maritime, Kent, ME4 4TB, UK. E-mail: Lisa_Taylor@Sandwich.Pfizer.com

Milling of pharmaceutical materials is often employed (to reduce particle size as a method) for the improvement of the properties of both drug substance and dosage form. A major problem in the (comminution) milling of drug compounds is the selection of the correct mill type required for individual materials. A method employing nanoindentation has been used to measure the breakage propensity of materials (brittleness) from commercially manufactured single crystals. This measure of brittleness was found to correlate to bulk milling performance and provide a technique to predict milling behaviour (Taylor et al 2004). The main disadvantage for this technique is related to the required particle size of the test samples. Successful nanoindentation studies have a minimum particle size requirement (crystals 100×50 micron). However, pharmaceutical crystals can often be below this limit meaning that a compound cannot be studied. The ability to have crystals grown to a suitable size would be advantageous. It has been reported in the literature that particle size influences the magnitude of hardness, Young's modulus and fracture toughness, (Rowe & Roberts 1995). However, the majority of mechanical property measurements are carried out using compacts. Formation of compacts involves the deformation (both elastic and plastic), as well as fracture, of the drug substance. Therefore, the change in mechanical property values may not be a function of particle size per se, but rather a change in the compaction process as a result of different sized crystals. The milling behaviour of materials will be dependent on the crystal properties rather than those of compacted materials. Single crystal measurements using nanoindentation allow us to see clearly which mechanical properties are influenced by particle size. A study was carried out to investigate the influence of crystal size on hardness, Young's modulus and fracture toughness on a pharmaceutical compound. Indentation of the smallest size fraction was not possible as the crystals underwent complete fracture. The results show that while fracture toughness (K_c) and brittleness (BI) are affected by particle size, both hardness (H) and Young's modulus (E) are independent of size.

Table 1 Influence of particle size on mechanical properties

Particle size (μm)	H (GPa)	E (GPa)	K_c ($MPa\,m^{1/2}$)	BI ($km^{-1/2}$)
< 100	—	—	—	—
250–300	0.55	13.9	0.008	66
> 500	0.55	14.7	0.005	104

Rowe, R. C., Roberts, R. J. (1995) *Adv. Pharm. Sci.* **7**: 1–62
Taylor, L. J., Papadopoulos, D. G., Dunn, P. J., et al (2004) *OPRD* In press