studies on the quench cooled paracetamol showed that low temperature storage (20–50°C below the T_g) can postpone the complete recrystallization of the amorphous form for up to 2 weeks (studies ongoing). The recrystallization behaviour of the amorphous paracetamol prepared by different methods (in the temperature ranges of 50–70°C, 80–90°C and 90–140°C) were studied using HSM (polarised light mode). Differing nucleation, crystal growth and fusion habits were observed corresponding to the transitions between the amorphous and metastable polymorphs of paracetamol. These transitions can also be presented in terms of light density plotted against temperature from the microscopy images. The study has demonstrated that different thermal histories may have a profound effect on the polymorphic form generated from the amorphous state. The investigation has also demonstrated the utility of using HSM and thermal scanning methods in conjunction to study this issue.

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Application of real time characterisation techniques during Zydis product development

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The Zydis dosage form is a fast dispersing tablet designed to disintegrate in less than 10 s and thus dissolves instantly to release the drug when placed on the tongue. The Zydis process involves dispersing the active pharmaceutical ingredient (API) in an aqueous solution of gelatin and mannitol, which is then dispensed into preformed blister pockets. The dispensed aqueous "dispersion" is then frozen and freeze-dried to form a light porous freeze-dried tablet. The API may be dispersed as a suspension or solution and held within the aqueous phase for several hours. Therefore, the ability to monitor API uniformity and potential for morphological changes on line is a significant advantage. Real time particle characterisation is one of several PAT tools being evaluated by Cardinal Health for Zydis product development. This abstract summarised a study on the use of real time analysis to monitor morphological changes, crystal growth, suspension homogeneity and impact of processing conditions, potential physical interactions of excipients and API, for a Zydis "dispersion". For this work, three model Zydis "dispersions" were evaluated using two PAT tools, namely Focus Beam Reflectance Measurement (FBRM) and the Particle Vision and Measurement (PVM) systems. In Example 1, the FBRM tracked the change in the crystal habit of a model drug "A" known to undergo pseudo-polymorphic transformation on prolonged suspension hold and or with an increase in suspension temperature. The data indicated that the particles of the original polymorph dissolved and then transformed into the new pseudo-polymorphic form with new habits. These observations were confirmed by off line microscopy. In Example 2, FBRM and PVM were used to monitor the uniformity of the dispersion and the physical interactions of the API and excipients. The FBRM tracked the particle size and number during the preparation of Zydis suspensions for a model compound "B" with a range of excipients typically used in Zydis formulations. The FBRM was able to quantify the changes to the particle size and particle number as each ingredient was added. The effects of addition of excipients, even in relatively small quantities (e.g. oily flavour, colour pigments, surfactant) were detected. The dispersion and de-aggregation of API particles to reach a stable suspension were also successful detected. The PVM captured images of the dispersion on line, showing clearly the physical interactions of the excipients with each other and with the API. In Example 3, the FBRM tracked the changes that took place as a result of different solid loading of model drug "C", the influence of process conditions on particle dispersion, and stability of the homogenised model suspension while held in an Intermediate Storage Vessel (ISV). In summary, the above examples demonstrated the usefulness of FBRM and PVM in product development by providing real time data for Zydis "dispersion" on events such as changes in crystal growth and habits, physical interactions between the formulation ingredients and the influence on processing conditions on the stability and uniformity of the dispersion.

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Gastro-retentive dosage forms: the characterisation of floating calcium alginate beads

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Gastro-retentive dosage forms have the potential to improve drug bioavailability compared with that from many commercially available immediate release and modified release products. A dosage form, based on freeze-dried calcium alginate beads has been developed and shown to have prolonged gastro-retention in the fed state. The aims of this work were to obtain information regarding the structure, floating ability and changes that occur when the dosage form is placed in aqueous media. Calcium alginate beads were prepared by extruding sodium alginate solution 2% w/w into calcium chloride 0.02 M solution. The precipitated gelled calcium alginate beads were freeze-dried. The formula was modified so that calcium alginate beads containing a model drug, riboflavin, were produced. The characterisation of the calcium alginate beads was divided into five main categories: physical parameters, floating ability, imaging, and release rate of riboflavin from the calcium alginate beads. The weight and diameter of the calcium alginate beads were assessed. The floating ability of the calcium alginate beads was assessed using the resultant weight technique (Timmermans & Moës 1990). The technique considers vertical and gravitational forces exerted by the dosage form, hence providing a quantitative measure of floating ability. The internal and external morphology of the calcium alginate beads was viewed using SEM. Digital photography studied the effect of the aqueous environment on dried calcium alginate beads. Confocal laser scanning microscopy measured the diffusion rate and movement of riboflavin from the calcium alginate beads when the beads were placed in aqueous media. X-ray microanalysis was used to determine the presence of phosphorous and the distribution of riboflavin-5′-phosphate within dried calcium alginate beads. The weight and diameter of the calcium alginate beads varied according to formulation. Calcium alginate beads containing riboflavin were 16.3% larger in diameter, and had a mass 3.2% greater, than placebo calcium alginate beads. The resultant weight technique demonstrated that the calcium alginate beads floated for a time in excess of 12 h, regardless of formulation. SEM showed all calcium alginate beads to be spherical and consist of air filled cavities that enabled floatation. X-ray microanalysis showed the presence of calcium and chlorine, which was expected. The X-ray microanalysis data plot of calcium alginate beads containing riboflavin-5′-phosphate did not show the presence of phosphorus. Only a small part of the riboflavin-5′-phosphate molecule is phosphorous. The absence of phosphorous in the X-ray microanalysis results may be due to insufficient amounts of phosphorous in the calcium alginate bead. Therefore, distribution of riboflavin-5′-phosphate within the calcium alginate beads was not observed. Confocal laser scanning microscopy showed that the movement of riboflavin throughout the bead and rate of riboflavin release from the calcium alginate beads occurred rapidly, (diffusion coefficient $24.70 \times 10^8\ cm^2\ s^{-1}$). Therefore, the drug would not remain in the dosage form long enough to demonstrate prolonged gastro-retention without further formula modifications. Digital photography showed that when calcium alginate beads were placed in aqueous media, air filled cavities remained. The development of a gel barrier, that slows the ingress of the dissolution medium, was also observed. Drug release may therefore occur by erosion and diffusion. The characterisation of calcium alginate beads of different formulations has resulted in obtaining an understanding of the properties of the floating dosage form. The characteristics of the calcium alginate beads make them suitable for further investigation as modified release gastro-retentive dosage form.

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An investigation into the physical properties of a wet granulated formulation at different stages of production

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The prediction of the properties of granules made by wet granulation is one objective the pharmaceutical industry currently works towards, to optimise products and manufacturing processes and ultimately minimise development times. The aim of this research was to study the physical

properties of granules, here by using macro-granules made by a beam formation process from the wet mass, and the final product (i.e. tablets) made from conventional granules of the same wet mass. Wet granulation masses containing microcrystalline cellulose and lactose monohydrate with varying concentrations of a liquid binder, polyvinylpyrrolidone (PVP), dissolved in water (0–6%), were produced using a Glatt high shear mixer granulator. A percentage of the wet mass was used to form model granules in the form of beams as described in a previous study (Pettersson et al 1997). The beams were dried in two ways, in an oven for 1 h at 60°C and at room temperature for 1 week. A notch of defined dimensions was introduced centrally to the beams, which were then subjected to bending stress using a CT5 strength tester with a 3-point bending rig attached. The remaining wet mass was screened and dried and granule size analysis was carried out, retaining the relevant size fractions for tablet production and for further assessment. The results obtained from granules dried at 60°C showed no clear trends between the tablet and beam tensile strengths. Beams containing 3% PVP, which were oven dried, were found to be brittle to an extent where a satisfactory notch could not be introduced into the beams. This study was repeated and the beams produced were left to dry for a week at room temperature. This method of drying resulted in specimens that were easier to handle, and not as brittle. The results of the repeated study showed that a correlation between beam and tablet strength did exist and the tensile strength of the beams and tablets were similar (Table 1). One observation, which occurred in both studies, was that between 3 and 4% PVP the tablet and beam tensile strength increased and then decreased significantly between 4–6%. This was more pronounced in the beam data and was clearly visible when the results were illustrated graphically. This trend was dalso observed in a recent study into the strength and morphology of solid bridges in pharmaceutical powders (Bika et al 2005), where PVP was used as a binder with mannitol. The study has demonstrated that the properties of the model granules and the properties of the tablets are comparable and estimations of the physical properties of the tablets produced from the same formulation could be made from such model specimens.

Table 1 Tablet and beam tensile strength at varying PVP concentrations

PVP concn (%)	Granule size fraction (μm)	Tablet tensile strength (MPa)	Beam tensile strength (MPa)
0	355	3.44 ± 0.23	3.43 ± 0.66
	500	3.14 ± 0.22	
1	355	2.89 ± 0.24	3.71 ± 0.73
	500	2.74 ± 0.39	
2	355	2.65 ± 0.31	2.50 ± 1.58
	500	2.65 ± 0.13	
3	355	2.76 ± 0.13	2.31 ± 0.99
	500	2.78 ± 0.24	
4	355	2.98 ± 0.28	2.75 ± 1.14
	500	3.19 ± 0.34	
5	355	2.25 ± 0.18	0.83 ± 0.43
	500	2.40 ± 0.27	
6	355	2.36 ± 0.08	1.48 ± 0.71
	500	2.43 ± 0.33	

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Small Angle Neutron Scattering (SANS) from polymer-stabilised drug nanoparticles

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An increasing number of new drugs exhibit extremely poor water-solubility, and hence, low and erratic oral bioavailability. One solution to this problem is to mill the drug in the presence of an aqueous solution of polymeric stabiliser, to produce crystalline drug nanoparticles of less than 400 nm. While this novel technology has been successful in producing stable nanoparticles of a wide range of hydrophobic drugs, the development process has thus far been largely empirical, with very little understanding gained into the fundamental interface science involved in the stabilisation. The aim of this study was to rectify this deficiency and in particular, to determine the amount and conformation of stabilising polymer adsorbed onto the drug nanoparticles with a view to understanding why some stabilisers are more effective than others. Nanoparticles of two poorly water soluble drugs, nabumetone and halofantrine were prepared by milling 4 g of nabumetone or 6 g of halofantrine in the presence of a 1.5% w/v solution of either hydroxypropyl cellulose (HPC) or hydroxypropylmethyl celluloses (HPMC) for nabumetone and HPMC or polyvinylpyrrolidone (PVP) for halofantrine. After removal of the excess polymer, the nanoparticles were re-suspended in either a 31.3 or 33.8 vol% D_2O/H_2O mixture (according to the experimentally determined 'contrast-match' point of nabumetone and halofantrine, respectively). Under these conditions only the scattering from the polymer stabiliser was detected. SANS experiments were performed at the Institute Laue Langevin, France, over the momentum transfer (Q) range 0.007–0.035 $Å^{-1}$. The neutron data were analysed using a "volume fraction profile independent surface Guinier model" (King et al 2000). This allowed Γ, the mass of polymer adsorbed per unit area, also known as the adsorbed amount (in mg m^{-2}) to be determined, as well as s, the 'second moment' of the layer; the distance of the centre-of-mass of the adsorbed polymer layer from the interface (Table 1). There was little change in either the second moment of the polymer layer or the amount of polymer adsorbed onto the nabumetone nanoparticles with molecular weight of HPC and HPMC. Under the present experimental conditions, nabumetone nanoparticles could not be prepared using HPMC of molecular weight greater than 7 kg mol^{-1}. Compared with nabumetone, HPMC adsorbed to the halofantrine nanoparticles to a lesser extent, forming a thinner adsorbed layer and indicating that a different conformation is adopted at the drug nanoparticle surface. Similarly PVP only formed a relatively thin absorbed layer on the halofantrine nanoparticles. To our knowledge these SANS studies are the first that have been performed on polymer-coated drug nanoparticles.

Table 1 Characterisation (using SANS) of polymer layer on nabumetone and halofantrine nanoparticles as a function of polymer molecular weight

Nanoparticle composition	Polymer molecular weight (M_η kg mol^{-1})	σ (Å)	Γ (mg m^2)
Nab-HPC	110	80.4	11.4
	95	76.0	11.6
	80	78.9	11.6
	65	76.5	11.0
	55	78.3	11.0
	45	80.3	11.3
Nab-HPMC	7	76.8	10.5
	5	80.8	10.8
Halo-HPMC	7	42.3	6.9
	5	33.4	7.7
Halo-PVP	46	52.7	1.8
	3	51.6	1.9

Nab, nabumetone; Halo, halofantrine; η, second moment of the adsorbed polymer layer; Γ, adsorbed amount of polymer

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Optimisation of autoclave cycle for a glucose-containing solution for injection

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A glucose-containing solution for intravenous injection is being developed for commercialisation. The proposed formulation is an aqueous solution of an exploratory drug incorporating glucose (5.05% w/v) as a tonicity adjustor. The solution is presented in a 50-mL Type I clear glass vial and is terminally sterilised by autoclaving. Heating of solutions of glucose can result in the formation of 5-hydroxymethylfurfural (5-HMF), a glucose-

related degradation species. For this reason, the sterilisation of this solution for injection by autoclaving represents a challenge. Following formation, 5-HMF concentration increases during storage, making it a critical-to-control product attribute. Based on pharmacopoeial limits of 5-HMF in glucose for infusion and the proposed dosing regimen for the solution for injection, a 5-HMF specification limit of 25 ppm has been set. Autoclave trials were performed with formulation to identify a cycle that produced low levels of 5-HMF. In exploratory studies with a GMP autoclave (Fedegari, chamber size $2\,m^3$), compendial and non-compendial cycles generated different quantities of 5-HMF (Table 1), with high temperature/short time combinations producing less 5-HMF. For manufacture of clinical supplies, a cycle of 121°C/15 min was selected (compendial cycle with acceptable 5-HMF level). Product shelf life was predicted using real time stability data. With a 121°C/15 min cycle, predictions provided a high level of confidence that the concentration of 5-HMF will be lower than the 25 ppm limit at the end of the 2 year shelf life required for the clinical formulation. Additional autoclave trials were conducted in a commercial manufacturing facility in preparation for the manufacturing campaign to support registration stability. Thermocouples (18) were positioned uniformly throughout the autoclave chamber (Fedegari, $3\,m^3$, 3 racks, 11 layers). A full load was autoclaved and two vials beside each thermocouple were analysed for 5-HMF content. An F_0 set point of 25 was investigated initially to increase assurance of sterility level. The resulting initial 5-HMF concentration range was 1.63–3.19 ppm. Due to this high 5-HMF content, the F_0 set point was lowered to 20, producing a lower 5-HMF concentration range of 0.93–2.22 ppm. Based on these findings, this autoclave cycle with F_0 set point of 20 was selected for the registration stability manufacturing campaign. Further studies revealed lower 5-HMF concentrations in the highest layers (8–11) of the autoclave chamber. A stratified sampling plan for 5-HMF was proposed for ICH batches (at least one sample per level in each rack; more samples in lower half of chamber). Sixty-six vials were sampled from each of the three ICH batches and all contained 2 ppm 5-HMF or less, well within the end of shelf life specification. Through optimisation, and validation, of the chosen autoclave cycle, the risk of 5-HMF formation in the glucose-containing solution for injection was understood and controlled.

Table 1 Influence of autoclave cycle on 5-HMF formation

Cycle temp.	Cycle time	5-HMF concn
121°C	15 min	1.6 ppm
124°C	4.56 min ($F_0 = 12$)	0.7 ppm
124°C	11.54 min ($F_0 = 25$)	0.7 ppm

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Dextran as a carrier for dry powder inhalation formulations

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Dry powder inhaler (DPI) formulations usually contain a binary blend of coarse carrier particles and micronised drug particles. In this study the effect of different carriers on the in vitro deposition of fluticasone propionate (FP) via the Handihaler at inhalation flow rate of $60\,L\,min^{-1}$ was investigated. α-Lactose monohydrate, recrystallised needle shaped lactose, manitol and dextran (Mw 162 kDa) were sieved and the size fraction of 65–125 μm was retained. Each carrier was mixed with FP in a ratio of 1:10 w/w. A dose of 4.4 ± 0.4 mg of powder formulation containing 400 μg of FP was filled manually into Size 3 gelatine capsule. Fine particle fraction (FPF) of each powder blend was determined by employing the twin stage impinger (cut-off diameter $< 6.4\,\mu$m). Table 1 illustrates recovered dose (RD), emitted dose (ED) and fine particle dose (FPD) of each formulation. Of all formulations used in this investigation, dextran seems to facilitate the aerosolisation of FP compared with all other carriers, as suggested by the highest ED and FPD values. Surface smoothness and lower bulk density of dextran particles compared with lactose particles were attributed to the higher aerosol performance of the dextran particles. Dextran has been shown to have some mucociliary activity and antibacterial properties (Feng et al 1999). Furthermore, a high dose of dextran can be delivered by inhalation ($400\,mg\,mL^{-1}$) without causing lung toxicity (Finlay et al 1999). The amount of dextran used in this investigation (4 mg/dose) was much lower than its lung toxicity limit. Previous work by Larhrib et al (2003) showed the suitability of using needle-shaped lactose as a carrier for hydrophilic drug substances, such as salbutamol sulphate. The deposition profile of FP from needle-shaped lactose was twice that of α-lactose monohydrate, suggesting that needle-shaped lactose can also be used as a carrier to enhance the deposition of hydrophobic drugs such as FP. In conclusion, dextran has been shown to improve the deposition profile of FP from DPIs compared with lactose, mannitol and needle-shaped lactose. Different molecular weights of dextran are commercially available and work is underway to investigate if a relationship between dextran molecular weight and DPIs performance could be established.

Table 1 Deposition of FP from formulations containing different carriers (n = 4)

Carrier	RD (μg)	ED (μg)	FPD (μg)
α-Lactose monohydrate	331.86 ± 13.2	110.24 ± 8.4	32.61 ± 4.3
Needle-shaped lactose	346.16 ± 16.3	176.13 ± 9.5	58.42 ± 3.7
Mannitol	349.59 ± 17.6	140.37 ± 6.4	37.43 ± 5.1
Dextran	340.72 ± 19.6	231.56 ± 11.5	115.87 ± 7.3

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Correlation of near infrared imaging and the dissolution performance of pharmaceutical solid dosage forms

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In the last few years there has been a major emphasis put on the use of more reproducible, rapid and commercially viable techniques for product quality control in the pharmaceutical industry (Hussain 2002). Near infrared spectroscopy (NIRS) fits into these categories. NIRS has generally been applied to solid dosage forms at macro scale providing bulk measurements of samples. It has been employed as an analytical tool in various different situations and has been successfully used to predict such characteristics as dissolution behaviour (Zannikos et al 1991). Pharmaceutical product quality is also known to be dependent on micro scale characteristics within the blends and tablet matrices. Near infrared microscopy (NIRM) is a technique utilised to evaluate the distribution and any interactions of the components present in a tablet matrix. This measurement and statistical quantitation of the ingredient domain distribution allows the generation of chemical images of the sample matrix. As of yet, NIRM has not been utilised for the prediction of product performance, but has been routinely used in a root cause capacity. In this work various Pfizer products were used to measure any differences within the tablet matrix using NIRM in an attempt to correlate the chemical images produced to any changes observed in the dissolution behaviour. Dissolution profiles were monitored using a fibre optic probe method. This would allow a dissolution rate to be obtained, not just an end point value, as this would not be likely to differ between methods. A VK700 dissolution bath with Helma UV probes and a Zeiss UV spectrometer were used to measure the dissolution profiles. At least one example of direct correlation between chemical images and the dissolution behaviour will be shown. A five component system which had known variations in dissolution performance was used in this investigation (Table 1). From the chemical images a variation in the number of magnesium stearate domains was observed, which correlated to the actual dissolution profiles. The blend with fewer magnesium stearate domains had a higher dissolution rate showing that the distribution of hydrophobic material changes the dissolution performance. This was expressed in an equation:

$$f(x) = 3.22 \times 10^{-5} x$$

Where x was the number of magnesium stearate domains in a wafer. The dissolution was predicted to fail when $f(x) \leq 0.0145$. In conclusion, it is possible to utilise NIRM for the prediction of dissolution behaviour from information extracted from the chemical images produced, on a system to system basis.

Table 1

Fail	No. of domains	Pass	No. of domains	Re-work	No. of domains
1	422	1	527	1	548
2	422	2	557	2	619
3	431	3	524	3	648
4	408	4	542	4	660
5	436	5	551	5	676
Mean	423.8	Mean	540.2	Mean	630.2

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On-line monitoring of wet granulation processes using acoustic emission

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Wet granulation is a critical step in the manufacture of many solid dosage forms. Conventional assessments of wet mass properties rely on indirect measurement of motor power or torque. The Process Analytical Technology (PAT) initiative endeavours to introduce technologies that can both monitor and control pharmaceutical manufacturing processes, with the aim of enhancing understanding of the processes and improved quality control of the final product. One such technology is Acoustic Emission monitoring, which passively measures process acoustics using high frequency piezoelectric sensors attached non-invasively to the side of a process vessel. The purpose of the study was to establish whether the GranuMet XP acoustic emission system could be used to monitor a wet granulation process and predict the end-point; and to investigate the robustness of the prediction model when changing process variables such as batch size, liquid dose rate, and impeller and chopper speeds. The work was conducted on a mini-piloting scale high shear granulator (900 mL granulation vessel) using two 650 kHz acoustic emission sensors, which were applied non-invasively to the bottom of the granulation vessel to obtain the acoustic data. A range of off-line solid state tests, such as particle size, bulk density and rheology, were used to characterise both the wet and dried final granule. The prediction model was determined using the process end-point as defined by a Soft Independent Modelling by Class Analogy (SIMCA) package. The model was demonstrated to repeatedly identify a pre-defined process end-point using the same process conditions. Robustness testing was conducted to determine the model's ability to predict further granulation end-points using varied process conditions, such as impeller speed, batch size, and liquid dose rate. The results (Table 1) show that the technique can be used to repeatedly produce granules with consistent physical characteristics, such as particle size distribution and rheology, despite changes in batch size and liquid dose rates. However, the model was affected by changes in impeller speed such that it was unable to identify a process end-point. This is thought to be due to the change in the granule velocity causing a change in the monitored acoustic signal. The study demonstrated that acoustic emission is a viable technology for the monitoring of wet granulation processes and also for predicting the process end-point, and thus help control final product quality.

Table 1 Showing rheology and mean particle size measurements on final granule in relation to changes in process variables

Liquid dose rate (mL min^{-1})	Impeller speed (rev min^{-1})	Batch size (g)	Rheology (mJ)	Mean particle size (μm)
3	800	120	84.1	210
9	800	120	93.7	322
6	800	120	87.0	263
6	800	120	90.0	217
6	800	120	82.5	224
6	800	100	89.2	212
6	800	140	93.2	187
6	1000	120	101.5	377
6	600	120	79.5	150

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Correlation of permeability through skin and a model PDMS membrane by computational chemistry

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The aim of this study is to compare measurements of membrane permeability for a set of organic compounds through polydimethylsiloxane (PDMS) and excised skin. Permeability measurements through PDMS were obtained from two different experimental techniques with the intent of comparing them with each other and skin respectively. Computational chemistry, in the form of Quantitative Structure-Permeability Relationships (QSPR), has been employed to develop predictive equations relating permeability to physicochemical descriptors. QSPR studies of the acquired permeability data will result in correlation equations between skin and PDMS membranes. Correlations linking each set of membrane permeability data with physicochemical descriptors, both measured and calculated, were also sought as part of the QSPR study. Literature searches were performed to acquire skin and PDMS permeability data. Consistency of the experimental protocol used was sought to minimise data variability. Also, large data sets were chosen to provide the greatest possible compound diversity. A publication by Flynn (1990), in which the permeabilities of 114 chemicals are presented, was selected to provide the permeability data through skin. This data was compiled from several permeability studies using different experimental protocols; however, it offers the largest published collection of skin permeability data and has been well characterised. The PDMS permeability data was selected from a study (Chen 1994) in which the same experimental protocol was applied throughout. This set consists of 256 compounds, mainly small organic molecules and pharmacological actives. A set of 10 compounds common to both literature sets was chosen for the correlation studies. Their permeability through PDMS was determined through a different experimental technique employing a UV diffusion cell. This diffusion cell provides more consistent results combined with greater convenience when compared with the Franz cells employed in the literature for both PDMS and skin. The experimental protocol of Chen was adhered to while determining the flux values (Log J) and apparent permeability coefficients (Log K_P) of the compounds (see Table 1). QSPR equations relating the permeability coefficients to various molecular descriptors through multi linear regression analysis have been determined for each set. The resulting QSPR equations are compared between the three datasets to provide further insights into the correlation between the permeability through model membranes and the permeability through in vitro skin membranes.

Table 1 Flux values (Log J) and apparent permeability coefficients (Log K_P) of the compounds

Compounds	Skin (lit.) Log Kp (cm h^{-1})	PDMS (lit.) Log J (μg cm^{-2} h^{-1})	Log Kp (cm h^{-1})	PDMS (exp.) Log J (μg cm^{-2} h^{-1})	Log Kp (cm h^{-1})
Aniline	−2.65	−0.45	−14.28	1.79	−12.04
Benzylalcohol	−2.22	−0.67	−14.53	1.08	−12.78
Ethylbenzene	−1.15	−0.16	−13.84	−0.17	−13.84
Isoquinoline	−1.78	−0.60	−14.52	1.37	−12.55
Methylnicotinate	−1.59	−0.58	−14.53	1.86	−12.08
Methylparaben	−1.49	−1.14	−15.15	−0.94	−14.94
Phenol	−2.00	−0.41	−14.29	1.93	−11.96
Salicylic acid	−2.20	−0.99	−15.12	0.61	−15.80
Styrene	−0.19	−0.21	−13.92	2.73	−10.98
Toluene	−1.30	−0.10	−13.78	1.87	−11.81

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An investigation into the mechanism of cryoprotection of lactate dehydrogenase by non-ionic surfactants using interfacial rheology

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Non-ionic surfactants are used as cryoprotectants for protein molecules. However, the cryoprotective mechanism is not fully understood. One proposed mechanism is that cryoprotection is afforded by the surfactant preventing adsorption of the protein onto the ice surface and therefore maximum cryoprotection should be obtained at the critical micelle concentration (CMC) of the surfactant and higher concentrations. Hillgren et al (2002) have shown that concentrations below the CMC at the freezing temperature can offer full cryoprotection. Recent surface tension and interfacial rheology studies at the air-water interface have demonstrated that displacement of β-lactoglobulin and β-casein films from the air-water interface by Tween 20 and 60 is not associated with the CMC but instead is a function of surface pressure (Gunning et al 2004). The aim of this investigation was to examine whether the effect observed at the air-water interface may be relevant to the behaviour of proteins at the ice-water interface using lactate dehydrogenase (LDH) as a model protein and the non-ionic surfactants Tween 20, 40 and 80. All materials used in this study were obtained from Sigma. LDH from rabbit muscle was dialysed and filtered before use. 25 μg mL^{-1} LDH solutions were prepared in citrate buffer pH 6.5 and with varying concentrations of the surfactants. Freeze-thaw experiments were carried out on 1-mL samples by cooling in vials to -40°C over 1 h on a freeze-dryer shelf, holding for 1 h and thawing over 3 h. LDH activity was determined (Wroblewski et al 1955). Interfacial rheology was performed using a Camtel CIR100 in normalised resonance mode with a strain of 5 mRad and an oscillation frequency of 3 Hz over a time period of 45 min at the air-water interface with 2.5 μg mL^{-1} LDH solutions in citrate buffer pH 6.5 using a range of concentrations of the surfactants. For both experiments n = 4. All the surfactants were found to protect LDH against freezing increasing the activity recovery in a similar concentration-dependent manner from $52 \pm 5\%$ to $94 \pm 5\%$ for Tween 20 or $93 \pm 2\%$, and $96 \pm 3\%$ for Tween 40 and Tween 80, respectively at a concentration of 30 μM. This indicates that cryoprotection is not related to the CMCs of the surfactants (59 μM, 27 μM and 11 μM for Tween 20, 40 and 80, respectively at room temperature). The nature of the molecules adsorbed to an interface affect interfacial properties, such as elasticity and viscosity, thus the displacement of LDH from the air-water interface by the surfactants was followed using interfacial rheology. The displacement of LDH from the air-water interface by the surfactants occurs in a similar concentration-dependent manner for all three, with complete displacement at a surfactant concentration of 1 μM. A correlation appears to exist between the ability of the surfactants to cryoprotect and to displace proteins from the air-water interface indicating that the two properties are linked. Further work will investigate whether the protein displacement at the air-water interface by the surfactants occurs at the same surface pressure.

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Novel methods to probe surface amorphous states

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Undesirable amorphous material generation during formulation is implicated in a growing number of pharmaceutical problems. Due to the importance of interfacial properties in many drug delivery systems it appears that surface amorphous material is particularly significant. Inhalation devices provide an important example, where amorphous material at the surface of an active or excipient causes alteration of adhesion and cohesion, changing performance (Bérard et al 2002). In this paper, surface sensitive methods are applied to allow detection and imaging of surface amorphous domains. A micron-sized localised surface domain of amorphous Sorbitol is generated using a novel localised heating method. The domain is subsequently investigated using Atomic force Microscopy (AFM) imaging, nanomechanical measurements and Confocal Raman depth profiling. AFM phase and height images reveal nanoscale order variations within both crystalline and amorphous Sorbitol domains. Lamellae organisation within crystalline Sorbitol is apparent, while crystallisation nuclei are visible in the amorphous phase. In addition to qualitative discrimination, AFM nanomechanical measurements are able to quantitatively distinguish the amorphous and crystalline domains through local Young's modulus measurements. For example, at a load of 8 nN the Young's Modulus value are 88 MPa for the crystalline material and 29 MPa for the amorphous domain. A further difference between the amorphous and crystalline domains is revealed by comparing the approach and retract force distance data in the contact region. Curves recorded in the crystalline region show no hysteresis (i.e. approach and retract curves completely overlap). However, the amorphous phase displays an appreciable hysteresis between loading and unloading behaviour. Raman microscopy also distinguishes the amorphous and crystalline Sorbitol. Comparison of the spectrum reveals that the glassy state is characterised by broader peaks and loss of fine spectral structure, features that are typical of a disordered amorphous state (Taylor et al 1998). In particular, the broadening of the peak at 878 cm^{-1} assigned to the C-C-O stretch, provided an intensity independent method of mapping for Sorbitol degree of crystallinity. This is shown to allow mapping of the three-dimensional distribution of the amorphous phase, and is hence complementary to the more surface sensitive AFM measurements. As the confocal plane is lowered into the sample the diameter of the amorphous region reduces, with little contrast visible in the final section, recorded 20 μm below the initial surface image, indicating the depth of the thermally generated domain. Until now, attempts to measure low levels of amorphous content have focused on model systems comprising appropriately blended pure amorphous and pure crystalline particles. However, it is more likely that pharmaceutical processing produces semi-crystalline particles containing both amorphous and crystalline domains. It has been speculated that such particles could respond differently to the blended material when characterised using conventional methods such as isothermal calorimetry (Dilworth et al 2004), highlighting an area where the thee-dimensional capabilities of Raman as demonstrated above, provide valuable information.

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NMR imaging as a tool to investigate capping propensity in tablets

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Die compaction is a unit operation employed in pharmaceuticals, powder metallurgy, ceramics and other industries. During compaction, complex movement takes place within the powder bed and interactions occur between the powder and tooling (i.e. die wall and punch faces). As a result, density variations are induced in the volume of the compact. The local density distribution in tablets is important because it affects the local material properties, which in turn influence the mechanical response during post-compaction operations, packaging, storage and use. Under certain conditions, in the forming of the compacts, the mechanical integrity and strength can be severely compromised by the development of defects, termed laminations. These may lead to tablet failure during the compression run, known as "capping", a process examined here using Nuclear Magnetic Resonance (NMR) imaging. The main advantage of this method is that porosity cross sections through whole tablets or specific locations could be obtained without mechanically destroying the tested tablet. To image the internal empty volume of the tablets, a filler fluid that gives a good NMR signal is used as a marker. The fluid is introduced into the test samples by vacuum impregnation. In using this technique, we assume adequate penetration of the filler fluid into all the internal volumes of interest, including pore spaces and internal voids. Penetration is assisted by using a fluid of low viscosity and of low interfacial tension with the microscopic surfaces of the sample. The filler fluid approach provides a means to visualize internal volumes that are larger than the spatial resolution of the measurement, which is about 40 μm within the image plane for the measurements presented here. Volumes smaller than the in-plane resolution (specifically, the microscopic volumes that constitute the pore structure) are not resolved. A standard multi-slice spin echo pulse sequence with a short echo time (TE) of about 4 ms was used to minimize signal intensity loss due to diffusion of filler fluid through the interfacial field gradients that exist at the magnetic susceptibility discontinuities of the sample. The work reported here shows how NMR imaging can be used for non-destructively mapping the internal structural weakness of tablets as a function of processing conditions and geometry of die and punches. In most cases, the NMR results were consistent with the observations made during the compression of the tablets (i.e. capping was observed during the

compression run) and the subsequent hardness testing. However, in some cases tablet capping issues during compression and the subsequent hardness testing did not show any unusual trend despite the fact that internal defects could be observed with NMR imaging. This result suggests that hardness data and visual observation during compression alone does not always indicate whether defects are present within the tablet, hence the usefulness of NMR imaging as a means of probing the internal tablet structure. Based on the NMR data generated, recommendations on the compression conditions and tooling were proposed to reduce the capping propensity of the compacts.

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Scintigraphic evaluation of oral formulations in the canine GI tract

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When evaluating a novel oral modified release dosage form, the dog is currently a commonly used model allowing administration of a dose of identical dimensions to the human formulation. However, the literature reports that for such formulations caution should be used in extrapolating results obtained in dogs to man (Davis et al 1993). Gamma scintigraphy in dogs (Iwanaga et al 1998) allows visualisation of the location and behaviour of the dose, which may provide better understanding of the relevance of the results to man. The objective of the current work was to use gamma scintigraphy to assess gastrointestinal transit of two oral formulations in the dog, as a preliminary step to investigating modified release dosage forms. A radiolabelled oral liquid was prepared by mixing 5 MBq of ^{99m}technetium-DTPA with water (2 mL kg^{-1}), and a non-disintegrating plastic capsule (equivalent to a size 1 capsule) was labelled with a small volume of ^{99m}technetium-DTPA (3 MBq), giving a filled weight of approximately 0.68 g. The liquid was administered to four fasted beagle dogs by oral gavage using a nylon catheter, and the capsule on a separate occasion via a pill-dosing device. External markers containing 0.1 MBq ^{99m}technetium were used for positioning. A gamma camera equipped with a low energy collimator was used to obtain images at 2-min intervals until gastric emptying, every 15 min until colon entry, then every 30 min until the end of the study. Posterior imaging was found to provide an optimal combination of animal comfort to minimise stress and transit disturbance, image quality and anatomical definition. All procedures were performed under a UK Home Office Animals (Scientific Procedures) Project Licence, with free access to water allowed during the study, and food allowed when the radioactivity had entered the colon. Regions of interest (ROI) were drawn on the images around the stomach, the caecum and the colon to assess radioactive counts in each area, and the data was then corrected for background activity and radioactive decay. Parameters evaluated for the liquid were time to 50% gastric emptying, small intestine transit time, and time to 50% of the activity entering the colon, and for the capsule time of gastric emptying, small intestine transit time, and time of colon entry (Table 1). The results obtained were in a similar range to those previously described in the literature (Dressman 1986). Significant differences between the formulations were not detected ($P > 0.05$) due to inter-animal variability, although general trends were observed. Gastric emptying of the capsule was slower (7.5 fold) than for the liquid, which was observed to begin almost immediately following dosing. Small intestinal transit time was 1.5 times slower for the capsule, and $T_{50\%}$ colon entry for the liquid was half that of the capsule colon entry time. Gastrointestinal transit times were evaluated in four dogs for a radiolabelled liquid and non-disintegrating capsule, and were found to be in a similar range to literature values, suggesting that the method developed is suitable for further gastrointestinal transit studies of oral dosage forms.

Table 1 Gastrointestinal transit of liquid and capsule

Formulation	Gastric emptying (min)	Small intestinal transit (min)	Colon arrival time (min)
Capsule	70.5 ± 37.4	204.3 ± 95.4	247.8 ± 79.7
Liquid	9.43 ± 5.5	137.7 ± 26.7	147.1 ± 22.4

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Solid-state interactions between ibuprofen and cross-linked poly-vinyl-pyrrolidone

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Solubilization of poorly water-soluble drugs is a challenge within pharmaceutical formulation since many compounds currently within development are BCS Class II compounds (i.e. high permeability but poor solubility). The bioavailability of these drugs is limited by their dissolution rate within the aqueous environment of the gastrointestinal fluids. Various drug:carrier systems have been prepared to improve the dissolution rate of the active pharmaceutical ingredient (API). For example, amorphous frusemide was generated when co-ground with cross-linked polyvinylpyrrolidone (PVP-CL) (Shin et al 1998) and amorphous indomethacin when prepared in a solid dispersion with the same polymer (Fujii et al 2005). We have previously reported that, by dry mixing the two components, an interaction occurred between PVP-CL and a BCS Class II compound ibuprofen (IB) (Rawlinson et al 2004). This resulted in the formation of a relatively stable amorphous fraction of IB upon simple physical mixing – approximately 30% of the original crystalline content. Though some evidence for molecular interactions between APIs and carriers within delivery systems has been described, the mechanisms by which simply mixing an API with a carrier can improve drug dissolution rates remain to be fully elucidated. Here, we discuss possible mechanisms for this specific interaction between IB and PVP-CL, as drawn from spectroscopic analysis. Molecular modes in IB, PVP-CL and in mixes were examined by FT-Raman (Bruker, 4 cm^{-1} resolution, 200 scans) and FT-IR (Matteson Galaxy 6020 series, ATR sampling, 4 cm^{-1} resolution, 1000 scans) spectroscopies. Reduced intensities and peak broadening were observed for characteristic groups, especially the aliphatic moieties of both IB and PVP-CL. This indicates physical restriction of movement caused by interaction between these molecules. The IB (C=O) stretching mode at 1653 cm^{-1} moved to higher wavenumbers in the physical mix and merged with the same mode from PVP-CL, observed at 1670 cm^{-1} in its pure form. Both of these peak shifts can be attributed to changes in environment and bond strength, probably caused by hydrogen bonding between the IB carboxylic acid group and PVP-CL cyclic amide oxygen. The IB quadrant ring stretch modes at 1606 cm^{-1} showed decreased intensity relative to neighbouring peaks, broadening and shifting to a higher wavenumber. A decrease in lattice mode intensities provided further evidence for the partial loss of crystal structure previously established by DSC and PXRD. From these data, the primary mechanism of interaction appears to be via hydrogen bonding. This mechanism been previously been cited for interactions between these two compounds in heat and solvent generated solid dispersions. However, our spectral changes are not as dramatic as would be anticipated for the degree of disorder introduced into the IB in our simple mix. Our data suggests that a secondary mechanism of electrostatic interactions involving the IB benzene ring also operates. Together, these interactions disrupt the crystal lattice of IB allowing the partial conversion of crystalline ibuprofen to an amorphous form, thus generating a dissolution rate advantage over the pure drug.

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Mechanical and dissolution characterisation of gelatin-glycerol films

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Polymeric films for buccal or sub-lingual delivery have been studied mainly for their solubility, permeability and thermal properties, and the aim of this study was to characterise mechanical properties of gelatin-glycerol films and correlate results to dissolution of the films. Gelatin was dissolved in water with heating up to 40°C, before addition of glycerol. The films were cast using a Multicator 411 casting knife on and acrylic plate, dried for ≥48 h at room temperature, conditioned in a 52% relative humidity chamber for ≥72 h and tested using Hounsfield Test Equipment. Tensile strength (TS) and % elongation (E) were produced by Hounsfield QMAT software and elastic modulus (EM) was recorded as a gradient of the initial linear part of stress-strain curve.

Dissolution behaviour was investigated in Sørensen's phosphate buffer pH 5.8, 37°C. Pre-cut films (1 cm^2) were placed within a rigid wire mesh in 15 mL of dissolution medium. The samples were agitated in a shaking water bath and samples were withdrawn pre-determined times. The samples were analysed using microviscometry (Automated MicroViscometer, Anton Paar) with a 1.6-mm capillary tube at 37°C (Esnaashari et al 2005). Dissolution rate constants (k_d) and lag times (t_{lag}) were determined for all samples, and statistical analysis (analysis of variance and Tukey's post-hoc test) was performed using SPSS 12.0.1 for Windows. Plasticizers alter mechanical properties of polymer films due to their interaction with polymer, hence increasing TS and EM with decreasing glycerol content in the formulation (Aulton 1995), which is shown by the results (Table 1). Furthermore % E is expected to increase with increasing plasticizer content, making the film more flexible. Dissolution rates for the films were not different ($P > 0.05$) but formulations 4 and 5 demonstrated significantly increased t_{lag} ($P < 0.05$) compared with the other formulations (Table 2). The swelling rate for formulation 3 was significantly higher than for formulations 1 and 2 (results not shown) indicating increased swelling occurring with reduced glycerol to gelatin. Formulations 1 and 2 are soft but flexible, with low TS and EM values. Addition of plasticizer results in reduced intermolecular forces and softer films (Aulton 1995), which could explain shorter lag times. Additionally, mechanically weaker films require less swelling before breaking and dissolution of the film (Sungthongjeen et al 2004). In conclusion, the mechanical properties of gelatin films can be altered by addition of plasticizer, which affects the dissolution behaviour of the film.

Table 1 Mechanical properties of glycerol-gelatin films

	Ratio[a]	TS (MPa)	E (%)	EM (MPa)
1	5/10	9.98 ± 1.01	53.06 ± 4.73	1.19 ± 0.55
2	5/15	15.81 ± 1.01	18.06 ± 7.78	5.26 ± 1.44
3	1/5	85.52 ± 9.10	5.74 ± 0.69	16.67 ± 5.77
4	1/10	63.42 ± 17.71	2.68 ± 1.28	22.0 ± 2.25
5	1/15	70.62 ± 11.56	3.22 ± 0.65	18.77 ± 1.77

[a]Gelatin/glycerol % in formulation; mean ± s.d., n ≥ 5

Table 2 Dissolution rate constants and lag times for gelatin-glycerol films

Formulation	K_d (min^{-1})	t_{lag} (min)
1	24.89 ± 5.98	0.74 ± 0.21
2	19.81 ± 1.79	0.87 ± 0.57
3	12.44 ± 1.96	no lag
4	20.69 ± 5.15	2.7 ± 0.30
5	19.56 ± 1.84	1.81 ± 0.88

mean ± s.d., n = 3.

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Large scale manufacture of PLGA microspheres via a continuous microencapsulation process

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A manufacturing process capable of yielding kilogram quantities of clinical standard biodegradable poly(lactide-co-glycolide) (PLGA) microspheres has been developed. The process, based on proprietary technology licensed to AstraZeneca from Brookwood Pharmaceuticals, is a continuous emulsification/solvent extraction process whereby particles are formed via rapid solvent removal from an oil-in-water emulsion by quenching into a large flow of water (Tice & Gilley 1995). The challenges in developing this process, for microspheres containing a water-soluble peptide drug (AZD2315), are considered. These include: achieving acceptable drug encapsulation efficiency by limiting drug losses from the microspheres during processing; reducing residual solvent to acceptable levels in the final product; forming and maintaining good particle morphology, particularly after de-watering and drying of the product; and optimizing product yield by limiting processing losses, in particular by maximizing the fraction of product in the target particle size range. A further obstacle to the processing of the PLGA-peptide-system described was the unusual rheological properties of the "dispersed phase" formed from mixtures of drug, polymer and solvent. This combination of materials formed viscous gels that were challenging to pump and emulsify in a controlled manner. Physical characterization techniques, including Modulated Differential Scanning Calorimetry (MTDSC) and Oscillatory Rheometry, were used to probe and address some of the manufacturing issues. Viscometry measurements using Oscillatory Rheometry revealed that the dispersed phase gels were non-Newtonian "power-law" fluids. The viscosity of the gels displayed a high sensitivity to applied shear, with an approximately five orders of magnitude decrease in viscosity at ambient temperature over the shear rate range examined (Table 1). This non-Newtonian rheological response to shear explained why the gels could be emulsified by homogenization and also why only relatively broad multi-modal microsphere particle size distributions were achievable. For one of the microsphere formulations manufactured, MTDSC was used to understand the origin of the poor quality product resulting from interparticle coalescence of the product after collection and drying. The MTDSC data revealed that the glass transition temperature of the product was strongly correlated to residual solvent levels and water, absorbed into the particles during processing. The glass transition temperature fell from approximately 45°C for dry particles containing low levels of residual solvent, to as low as 5°C for damp particles containing a relatively high residual solvent level. The reduction of the glass transition temperature of the product to below the microsphere processing temperature, accompanied by the concomitant increase of the plasticity of the drug/polymer matrix, accounted for the poor morphology of these batches. The strategies used to overcome this problem are discussed. The learning accrued from these and other studies enabled a robust process capable of delivering a clinical quality product to be realized.

Table 1 Typical viscometry data for an AZD2315-loaded polymer gel

Shear rate (s^{-1})	Viscosity (Pa s)
0.002	1.22×10^5
0.0625	6.27×10^3
0.625	8.24×10^2
20	3.38×10^1
625	1.30

Tice, R. T., Gilley, R. M. (1995) US Patent 5,407,609

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Capillary rheometry as an aid to processing a viscous gel in a microsphere manufacturing process

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Capillary rheometry (Barnes et al 1989) is a technique for probing the rheological properties of fluids in pipes and, as such, it represents an optimal approach to studying the relationship between fluid viscosity, volume flow rate and pipe geometry in processes involving piped flow. In a manufacturing process, understanding the rheology of the constituent fluids is valuable since this impacts on material handling and "processability", as well as safety considerations. In this study, the rheology of a viscous gel, formed from a mixture of a drug, polymer and solvent, was characterized by capillary rheometry. This material, which is the precursor to microspheres formed by emulsification, is analogous to those described in an accompanying poster – "Large Scale Manufacture of PLGA Microspheres Via a Continuous Microencapsulation Process". The experiments involved extruding gels, formed in situ in the rheometer barrel, through a length of capillary, which had an inner diameter and length identical to the pipe used to deliver the gel into the emulsification stage of the microsphere manufacturing process. "Shear sweeps" were performed by measuring the steady-state pumping pressure as a function of volume flow rate of the gel, enabling the viscosity versus shear rate profiles to be calculated. Consistent with other oscillatory rheological measurements, the viscosity/shear rate profiles were characteristic of a non-Newtonian, shear-thinning "power-law" fluid. From the raw pressure/flow rate data, a linear relationship was

inferred between log(viscosity) and log(shear rate), from which values of approximately 0.4 and 200 Pa.s$^{0.4}$ were calculated for the "power-law index" and "consistency" parameters, respectively. The viscometry data enabled the velocity profile of the gel across the pipe diameter to be simulated using a capillary flow model for a power-law fluid. The model predicts that the gel flow becomes increasingly "plug-like" as flow rate decreases and the pipe diameter increases, such that the gel only experiences significant shearing at the pipe walls. The delivery of essentially unthinned, semi-solid gel into the emulsification process to form microspheres may explain the observation of broad multimodal particle size distributions of the final product. A model was also derived from the experimental data to relate pump pressure to the flow rate and the dimensions of the pipe through which the gel flows. The model predicts that the gel pumping pressure is very sensitive to the inner diameter of the pipe, with pressure proportional to approximately 1/diameter^2. Simulations predict that a pump pressure of approximately 230 psi is required to achieve a flow rate of 120 mL min^{-1} in a 3-mm diameter, 40-cm long pipe, rising to in excess of 10 000 psi for a 0.5-mm diameter pipe. This far exceeds safe working limits. These models show that reducing the pipe diameter is the most effective way of introducing shear into the gel, but that there is a penalty to pay in respect of the high pumping pressures to achieve acceptable volume flow rates. Since pump pressures must in practice be kept to within safe limits, this imposes constraints on the geometry of pipes that can be employed to transport such a fluid.

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Further investigations into fine excipient particles in carrier-based dry powder inhalation formulations using atomic force microscopy

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Carrier-based dry powder inhalation formulations typically consist of a blend of coarse lactose and micronised drug. A small amount of fine excipient may also be incorporated and this has been shown to increase the fine particle fraction (FPF) of various drugs (Islam et al 2004). The newly developed cohesive-adhesive balance (CAB) approach to adhesive force measurement by colloid probe atomic force microscopy (AFM) (Begat et al 2004) was recently applied to the study of this area, suggesting that there might be a relationship between the magnitude of the adhesion of the drug to the fine excipient and formulation performance (Jones et al 2004). To further investigate this phenomenon, this study applies the same techniques to the study of a different drug. Drug B and four excipients (erythritol, lactose, mannitol and trehalose) were used in this investigation. Three AFM colloid probes of drug B were prepared and atomically smooth crystals of each material grown. The force of cohesion between each colloid probe and a crystal of drug B and the forces of adhesion to a crystal of each excipient were measured using force-volume AFM. By plotting the mean cohesive force of each drug probe against its mean adhesive force to each excipient, the ratio between cohesion and adhesion (CAB ratio) could be calculated (Begat et al 2004) (Table 1). These data show that drug B is more cohesive than it is adhesive to erythritol, lactose and trehalose, but it is more adhesive to mannitol than cohesive. This is in contrast to the work of Jones et al (2004), where the drug used was more adhesive to all the excipients than it was cohesive. Binary and ternary formulations were produced containing 2.5% drug B and a coarse lactose carrier. Ternary formulations also contained 10% micronised excipient (erythritol, lactose, mannitol or trehalose). Formulations were tested by aerosolisation into an Anderson Cascade Impactor with the amount of drug deposited on each stage analysed by HPLC. The FPFs obtained from each test of each formulation are shown in Table 2. The FPF of the binary formulation appears to be less than that of any of the ternary formulations, in accordance with other research in this field (Islam et al 2004), while the FPF of the ternary formulation containing mannitol appears to be larger than any other. Despite the CAB ratios between drug B and erythritol, lactose and trehalose varying approximately two-fold, no large differences were seen in the FPFs of these formulations. This suggests that when a drug is more cohesive than it is adhesive to the fine excipient, the precise magnitude of its adhesion to the excipient does not influence formulation performance. The formulation containing mannitol appeared to give the greatest FPF and mannitol was also the only excipient to which drug B was more adhesive than cohesive, suggesting that adhesion of the drug to the fine excipient to form agglomerates may be important for the optimisation of formulation performance.

Table 1 CAB ratios between drug B and each excipient

Erythritol	Lactose	Mannitol	Trehalose
1.1248	2.3856	0.6488	1.3746

Table 2 Fine particle fractions (% of emitted dose) obtained from each test of each formulation (n = 2)

Binary	Erythritol fines	Lactose fines	Mannitol fines	Trehalose fines
14.6	23.8	26.0	37.2	26.6
16.0	24.2	25.2	34.6	24.4

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Evaluation of ethanolic extract of *Asteracantha longifolia* on cyclophosphamide induced anaemia in albino rats

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From ancient times, medicinal plants are believed to be useful in strengthening the haematopoietic and immune system (Chopra 1986). Our objective was to investigate ethanolic extract of aerial part of *Asteracantha longifolia* (AL) on haematological parameters, as well as the suppressive effect of cyclophosphamide-induced toxicity. An animal study was performed with due permission from institutional animal ethical committee (Registration No. 397/01/ab/CPCSEA). Anaemic animal model was used to evaluate haematopoietic activity. Haematological study was performed using five groups of albino rats. Group I acted as control, group II was cyclophosphamide control (3 mg kg^{-1}, i.p.), group III was treated with ethanolic extract alone (500 mg kg^{-1}, i.p.), group IV and V were given cyclophosphamide (3 mg kg^{-1}) and ethanolic extract at a dose of 250 and 500 mg kg^{-1} body weight intraperitoneally, respectively. Anaemia was produced by cyclophosphamide (3 mg kg^{-1}) given intraperitoneally for 7 days. On the seventh day blood samples were collected and observed by autoanalyser (Table 1). After seven days, cyclophosphamide was withdrawn from group IV and V and then, group III was treated with ethanolic extract alone at a dose of 500 mg kg^{-1} intraperitoneally, group IV and V were treated with ethanolic extract at a dose of 250 mg kg^{-1} and 500 mg kg^{-1} intraperitoneally, respectively, for the next 15 days and blood was collected on the 22nd day and evaluated for haematological parameters (Table 2) (Ziauddin et al 1996). Ethanolic extract was found to significantly ($^{a,a1,a3}P < 0.0001$, $^{a2}P < 0.05$) improve erythrocyte and haemoglobin count on the 22nd day. This effect may be due to the presence of iron in extract (1050 μg in 50 mL) estimated quantitatively by spectrophotometric method. Suppressive effect of cyclophosphamide-induced toxicity was studied in three groups of albino rats and used to determine the effect of *A. longifolia* on bone marrow cellularity. Group I was treated with cyclophosphamide (25 mg kg^{-1}), group II was treated with cyclophosphamide and *A. longifolia* (20 mg dose per rat; i.p.) and group III was treated with *A. longifolia* alone. Three albino rats from each group were sacrificed on the fifteenth and nineteenth day of treatment and the bone marrow was collected from the femur into medium containing 2% FCS. The number of bone marrow cells were counted using a haemacytometer and expressed as total live cells/femur. Bone marrow preparation was stained with Harris Haematoxylin to determine the α-esterase activity. Treatment with ethanolic extract along with cyclophosphamide was found to significantly ($P < 0.001$) increase the bone marrow cellularity (12. 9×10^6) as compared with the group treated with cyclophosphamide alone (7.7×10^6). Administration of *A. longifolia* increased ($P < 0.001$) the number of α-esterase positive cells (1140/4000) in the bone marrow of cyclophosphamide-treated rats, compared with those treated with cyclophosphamide alone (660/4000). Significance of results was determined with INSTAT 2.1. We conclude that *A. longifolia* exhibited potent haematopoietic and stem cell proliferation activity (Davis et al 1998).

Table 1 Effect of *A. longifolia* extract (Ethanolic) in Cyclophosphamide-treated albino rats (after 7 days)

Group	I	II	III	IV	V
Mean RBCs ($10^6/\mu L$)	7.58 ± 0.75	3.95 ± 0.95	7.65 ± 0.21^{a}	6.20 ± 0.26^{a}	6.40 ± 0.34^{a}
Mean Hb (g/dL)	14.60 ± 0.90	8.50 ± 0.52	14.90 ± 0.20^{a1}	9.20 ± 1.36^{a1}	11.4 ± 1.85^{a1}
Mean WBCs ($10^3/\mu L$)	8.10 ± 0.25	3.80 ± 0.35	7.90 ± 0.66^{a2}	5.50 ± 1.24^{a2}	6.90 ± 0.93^{a2}
Mean HCT (%)	48.50 ± 0.86	34.28 ± 1.62	47.12 ± 2.53^{a3}	38.60 ± 2.63^{a3}	41.20 ± 3.76^{a3}

$n = 6$ albino rats per group, tabular value represents mean ± s.e.m.
$P > 0.1$ (comparison of all Groups of I with each of II).
$^{a}P < 0.005$; $^{a1,a2}P < 0.01$; $^{a3}P < 0.05$ (comparison of II with III, IV and V)

Table 2 Recovery period observations after withdrawal of cyclophosphamide (on 22nd day)

Group	I	II	III	IV	V
Mean RBCs ($10^6/\mu L$)	7.58 ± 0.75	5.85 ± 0.45	8.00 ± 0.13^{a}	6.42 ± 0.10^{a}	7.78 ± 0.12^{a}
Mean Hb (gm/dL)	14.60 ± 0.90	9.00 ± 0.53	15.10 ± 0.39^{a1}	12.90 ± 0.23^{a1}	13.40 ± 0.47^{a1}
Mean WBCs ($10^3/\mu L$)	8.10 ± 0.25	6.20 ± 0.33	8.00 ± 0.42^{2}	6.90 ± 0.53^{a2}	7.5 ± 0.33^{a2}
Mean HCT (%)	48.50 ± 0.86	38.90 ± 1.45	48.60 ± 1.10^{a3}	44.50 ± 1.22^{a3}	47.80 ± 1.14^{a3}

$n = 6$ albino rats per group, tabular value represents mean ± s.e.m.
$P > 0.1$ (comparison of all Groups of I with each of II).
$^{a,a1,a3}P < 0.0001$, $^{a2}P < 0.05$ (comparison of II with III, IV and V)

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Pulmonary delivery of spray-dried and crystallised lysozyme

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Maintaining protein stability during formulation is critical. Consequently, the formulation and delivery of proteins present challenges due to their inherent instability. Innovation in biotechnology has resulted in the development of proteins as drugs and several macromolecules are in development for inhalation delivery. Our aims were to assess the effects of spray drying and crystallisation of lysozyme, a model protein, on inhalation performance of the protein in a dry powder form and to investigate the feasibility of using Clickhalers (a dry powder inhaler) as a device for protein pulmonary delivery. Lysozyme was spray-dried and crystallised using different methods to control the protein crystal size. The particle morphology and size distribution of spray-dried particles and crystals were determined using scanning electron microscopy (SEM) and Zetasizer, respectively. The fine particle deposition of lysozyme, from the spray-dried and crystallised forms, was investigated using in vitro lung (Andersen Cascade Impactor) at a flow rate of $57.3\,L\,min^{-1}$. The method of Bradford (1976) was exploited to assay the amount of lysozyme deposited on each stage of the cascade. The particle morphology of dried powder varied from that of crystals. For crystals, SEM revealed tetragonal crystal shape without aggregates. For spray-dried protein, the particles were of a spherical shape with a smooth surface texture as indicated by SEM. The average particle size distributions were 3.35 and 1.36 μm, as determined by Zetasizer, for crystals and spray-dried lysozyme, respectively. The impaction of protein particles on stages of Andersen Cascade Impactor demonstrated that spray-dried lysozyme powder was retained on the mouth piece of the Clickhaler in the concentration of 19.9%, as determined by Bradford method, with a fine particle depositions of about 37.9% (%wt. of particles $< 5\,\mu$m in the aerosol cloud) and 20.5% (%wt of particles $< 3\,\mu$m). For lysozyme crystals, there was no retention of crystals on the mouthpiece of the inhaler. The fine particle depositions were 18% for the protein crystals $< 5\,\mu$m and 12.6% for the protein particles $< 3\,\mu$m in the aerosol cloud. Although the fine particle deposition for crystals was lower than that for spray-dried protein, the spray-dried protein tended to accumulate on the mouthpiece of the dry powder inhaler and this may lead to blockage of the inhaler and accordingly affect the subsequent dosage. Moreover, absorbing moisture by the spray-dried protein can result in microbial growth. For crystals, there was no device retention. Also, the amount of protein crystals recovered, using the Andersen Cascade Impactor, was higher compared to the spray-dried form (83.5% vs 57.4%). This low mass balance may be explained on the basis that some lysozyme denatured on recovery from the impactor, particularly since the protein was used without any additives. Loss was higher in spray-dried protein than in crystals. Our earlier findings (Elkordy et al 2002, 2004) demonstrated that protein crystals better maintained stability, conformational integrity and biological activity. In conclusion, crystallisation shows promise for pulmonary delivery of proteins. It is feasible to use Clickhalers as a pulmonary device for protein inhalation delivery.

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Poster Session 3 – Biopharmaceutics

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The importance of bio-relevant media in the classifying drugs according to the Biopharmaceutical Classification Scheme (BCS)

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To accurately predict the in vivo performance of a drug from in vitro results, it is critical that the in vivo environment encountered by an orally administrated drug is mirrored in vitro. The Biopharmaceutical Classification Scheme (BCS) classifies drugs according to their in vitro solubility and permeability. Four classes exist, of which Class II (low solubility, high permeability) and Class IV drugs (low solubility, low permeability) are dissolution rate limited. However, the solubility parameter of the BCS has been constructed using standard dissolution compendial media, which do not adequately simulate the in vivo condition. Simulated intestinal fluids (SIFs) have thus been devised (Dressman et al 1998), which hopefully bridges some of the gap in achieving in vitro/in vivo correlation. The composition of these media is largely that of bile salt, lecithin and pH, mimicking both the fasted and fed states of digestion. The aim of this study was to examine if the use of SIFs altered the classification of, in the first instance, Class II drugs (ibuprofen), and then was further extended to other BCS class drugs. Phosphate buffer (pH 7.2), fasted state simulated intestinal fluids (FaSSIFs; pH 6.5) and fed state simulated intestinal fluids (FeSSIFs; pH 5.0) were used as dissolution media. Dissolution testing using the paddle method (BP dissolution apparatus II, stirrer speed $50\,rev\,min^{-1}$ and 25°C temperature) was carried out. Paracetamol (Class I) and allopurinol (Class IV) were also investigated. In brief, a drug tablet was added to a dissolution vessel ($n = 3$) and 5-mL samples of media removed, with replacement of 5 mL fresh media, at specific time points over a period of 2 h. A dissolution profile was constructed with percent drug dissolved versus time (min). It was found that the dissolution profiles of the drugs tested varied in the different dissolution media. In general, drug solubility was enhanced upon changing from phosphate buffer to the SIFs, the result most likely being due to the effects of solubilisation and wetting. Examination of ibuprofen solubility showed a pH dependency, which will impact on its dissolution behaviour in the various media tested. A comparison between tablet coating of ibuprofen (film coated and sugar coated) revealed a faster dissolution for film coated compared with sugar coated tablets. For the other drugs, no pH dependency on drug dissolution was observed. Only ibuprofen remained within its proposed BCS class when SIFs were used (based on dissolution and solubility results).