in different size ranges of granules produced by FHMG SD illustrated good uniformity in both water-soluble and poorly water-soluble formulations, while for the wet granulation process the smaller particles contained more drug than the bigger particles, which indicated that the drug may be transferred from granule to granule during the drying process. The content uniformity of tablets showed similar results. The hardness of tablets produced by the granules from FHMG technique was lower than the tablets made by wet granulation process. The drug-release rates of both water-soluble and poorly water-soluble APIs were increased by using the solid dispersion as a binder for the FHMG process. Furthermore, the lack of drying process in FHMG compared with wet granulation decreased the total processing time considerably.

Conclusions FHMG is a simple and novel granulation process that could be developed to process both water-soluble and poorly water-soluble APIs. The application of solid dispersions in the FHMG process is an effective procedure in pharmaceutical manufacture to improve drug-content uniformity, dissolution rate and hence the bioavailability of drugs. Compared with conventional wet granulation process, FHMG has no drying step, which may be a good way to save process time and energy.

SESSION 3
Analytical Chemistry

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The effect of milling-process parameters on granule and tablet properties

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Objectives The wet granulation process is often used to produce large agglomerates from primary particles. Control of granulation allows optimization of the granule-size distribution, affecting powder flow and the key tablet properties of hardness and dissolution. To produce an optimal uni-modal size distribution and to remove any lumps from the granulation, the milling process is used as a control step. Very little work has been reported in the scientific literature on the importance of the milling process. In this study an experimental investigation was carried out to understand the impact of milling-process conditions on the granule and tablet properties using both laboratory- and pilot-scale equipment. An attempt was made to develop a scale-up relationship between laboratory-scale and pilot-scale cone mills using the geometry of the cone and rotor-tip speeds.

Methods A design of experiment (DoE) approach was used to relate the milling process parameters with the granule and tablet properties. Granule-size distribution was measured using a Malvern Mastersizer (dry powder dispersion) and flow properties were measured using a Ring Shear Tester. Coarse, intermediate and fine granules were used in tablet production to assess their impact on the tablet properties.

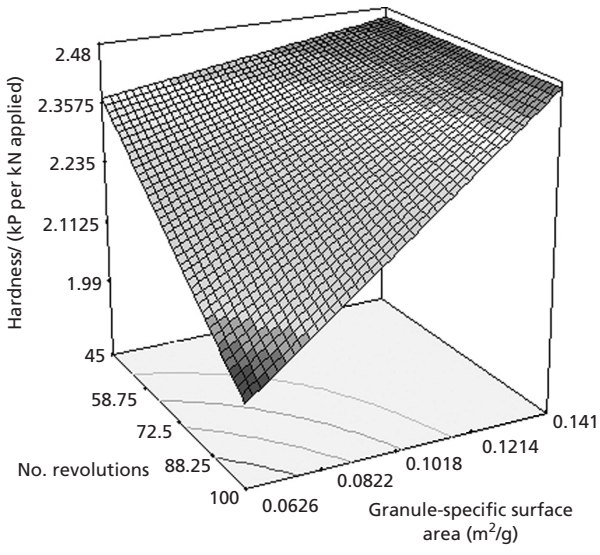

Figure 1 Effect of granule-specific surface area on tablet hardness.

Results The mill speed and screen size were found to have a significant effect on granule properties. Low mill speed and smaller screen size produced granules with minimum fines and good flow properties. A grater-type screen surface (something like a cheese grater, with a bit-shaped surface) generated less fines than a smooth screen surface. Using a mill beater tip-speed calculation, it was possible to produce granules with similar properties at both scales. Three batches of milled granules with various granule-specific surface areas (GSSAs) were compressed to understand the impact of GSSA on tablet hardness. Figure 1 shows tablet hardness as a function of GSSA and blender speed. Coarser granules were sensitive to lubrication and produced softer tablets. However, finer granules had a greater tendency to sticking. This work highlighted the importance of the milling process in producing optimum granules.

Conclusions Optimization of the milling process is important to produce good and consistent granules and tablet properties. The mill speed, screen size and type were found to have significant effect on granule properties. A tip-speed approach was found to be suitable for scaling up the milling process.

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High-performance liquid chromatography method validation for the quantification of tobramycin in urine samples after inhalation using pre-column derivatization with fluorescent 9-fluorenylmethylchloroformate

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Objective A reversed-phase high-performance liquid chromatography (HPLC) method has been developed for determination of tobramycin in urine samples after inhalation of tobramycin. Several previous studies (Essers 1984, Lai and Sheehan 1992, Marples and Oates 1982) used pre-column derivatization with *o*-phthalaldehyde (OPA) but OPA has a poor-stability derivative that results. In this study fluorenylmethylchloroformate (FMOC-CL) was used. Tobramycin was determined following pre-column derivatization with FMOC-CL, which reacts very rapidly with the primary amino groups of tobramycin and other aminoglycosides under mild conditions to form stable derivatives.

Methods Chromatographic separation was carried out on a Phenomenex Luna C_{18} column at ambient temperature using a constant flow rate of 1 mL/minute. The mobile phase was acetonitrile/glacial acetic acid/water (900:2:98, by vol.), and fluorescent detection was at an excitation wavelength of 265 nm and emission wavelength of 320 nm.

Results The assay was linear at seven different concentrations of tobramycin extracted from spiked urine ranging from 0.25 to 3 μg/mL. Tobramycin and neomycin (used as internal standard) were extracted from spiked urine by solid-phase extraction using a carboxypropyl-bonded phase (CBA) weak cation-exchange cartridge and the relative recovery was more than 98% (n = 5). The limits of detection and quantitation in urine were 38 and 115.2 ng/mL, respectively. The intra-day and inter-day precision (in terms of the percentage coefficient of variation) were less than 5.53 and 4.15%, respectively.

Conclusions This assay is simple, precise and accurate and was applied to pharmacokinetic studies to identify the relative lung deposition of tobramycin following inhalation of tobramycin inhaled solution 300 mg per 5 mL (TOBI®) by Pari LC Plus® jet nebulizer.

Essers, L. (1984) *J. Chromatogr. Sci.* **305**: 345–352
Lai, F., Sheehan, T. (1992) *J. Chromatogr.* **609**: 173–179
Marples, J., Oates, M. D. (1982) *J. Antimicrob. Chemother.* **10**: 311–318

122
Evaluation of nuclear quadrupole resonance for use in pharmaceutical analysis

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Objectives Pharmaceutical analysis increasingly emphasizes the use of spectroscopic methods (e.g. Raman and near-infrared) for the direct analysis of solid dosage forms. In this work we report on the use of nuclear quadrupole resonance (NQR) in this field. NQR is a solid-state analysis technique that shows promise in selective and quantitative pharmaceutical analysis (Balchin et al 2005, Perez et al 2005, Latosinska 2007). We have studied NQR signals due to the ^{14}N and ^{35}Cl nuclei in a range of drug substances and drug products including atenolol, sulfapyridine, furosemide and chlorpropamide, without interference from the excipients.

Methods NQR is a radio frequency technique that is related to the more widely known nuclear magnetic resonance (NMR), but is only applicable to nuclei with a

spin of more than 1/2, and to solid samples. NQR does not require a magnet to produce energy-level differences in the nuclei and the samples do not need spinning to obtain good line shapes. NQR is a non-destructive and non-invasive approach and measurements can be made on intact tablets. We have studied ^{14}N, which is present in many pharmaceuticals, and ^{35}Cl, which is less widely applicable but offers greater sensitivity. Advanced data-processing techniques have been used to determine the physical parameters characteristic of the different nuclear environments, and to measure rates of change between the environments.

Results We have evaluated the performance of NQR by evaluating selectivity for different drug substances and different morphological forms of the same drug substance. We have also determined the linearity of response with mass of drug product. The NQR signals for ^{14}N typically occur in the range 0.4–6 MHz with the frequency being sensitive to the local chemical environment. This is a huge spectral frequency range in comparison with NMR and implies high method selectivity. Atenolol, for example, gives very different frequencies for the nitrogen nuclei in the amine and amide functional groups. With ^{35}Cl the frequency range is between 34 and 36 MHz. Polymorphic form also affects the ^{14}N frequencies with different signals obtained for form I and the metastable form II of furosemide. Frequency differences also mean that it is easily possible to distinguish sulfapyridine from its acetone solvate. With furosemide we have also seen small differences in the line width (^{35}Cl and ^{14}N) for the drug substance and the 40 and 500 mg drug products (Lasix tablets). Quantitative analysis has also been carried out by ^{35}Cl NQR and we see good linearity of response with sample mass for both chlorpropamide and furosemide tablets.

Conclusions NQR shows promise as a non-invasive and non-destructive measurement technique for the qualitative and quantitative characterization of solid-state dosage forms at relatively low cost. The selectivity is high and the simplicity of the instrumentation and spectra obtained show significant potential.

Balchin, E. et al (2005) *Anal. Chem.* **77**: 3925–3930
Latosinska, J. N. (2007) *Expert Opin. Drug Disc.* **2**: 225–248
Perez, S. C. et al (2005) *Int. J. Pharm.* **298**: 143–152

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Novel thermal probe techniques for the physico-chemical characterization of interactions between biological systems

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Objectives As part of a strategy to investigate interactions between materials and living cells, we are developing a number of novel scanning probe microscopy-based techniques. Recently we showed that by using heated and coated tip probes it is possible to successfully measure adhesive forces between the coating material and the surfaces placed in contact with it (Dragnevska et al 2007). In this study we have employed near-field photothermal microspectroscopy (PTMS; Hammiche et al 2004) with the aim of exploring and optimizing its further use and providing further insight into the interactions of the studied biological systems.

Methods *Escherichia coli* cells were chosen for the experiments. These were cultured overnight on agarized Luria broth medium (tryptone 10 g L^{-1}, yeast extract 5 g L^{-1} and NaCl 5 g L^{-1}) on Petri dishes at 37°C. A layer of bacteria was clearly seen. Prior to each photothermal infrared experiment, bacteria were attached to a Wollaston probe tip, simply by touching the cells and then retracting the tip. The set up for the PTMS experiments is based on the integration of an optical interface (Specac, Orpington, UK), a scanning probe microscope (Veeco diCaliber) and a Fourier-transform infrared spectrometer (Bruker Optics, Coventry, UK). Spectra were obtained using a 16 cm^{-1} spectral resolution.

Results Figure 1 shows the typical absorption spectrum of a tip coated with *E. coli* cells. It is clearly seen that the vibrational bands, associated with spectral regions, where significant differences of interest might occur can be identified. These include the amide I (≈1650 cm^{-1}), amide II (≈1540 cm^{-1}), glycoproteins (≈1380 cm^{-1}) and carbohydrates (≈1155 cm^{-1}).

Conclusions PTMS has been successfully applied in the study of *E. coli* cells. Spectra of excellent quality have been generated and regions of interest have been identified. Our ongoing studies are focused on developing PTMS as a method for determining spectral changes in cells treated with pharmaceutically relevant materials.

Dragnevska, Z. et al (2007) *J. Pharm. Pharmacol.* **59** (Suppl.): A-45
Hammiche, A. et al (2004) *J. Microsc.* **213**: 129–134

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Physical and chemical stability of paclitaxel infusions for the application of a dose-banding strategy

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Objectives Dose banding, developed by Plumridge and Sewell (2001), is a chemotherapy dosing method enabling the use of pre-prepared infusion to avoid treatment delays experienced by cancer patients. Adequate physical and chemical stability of drug infusions is a prerequisite for dose banding (normally >14 days is required). The aim of this study was to determine the physical and chemical stability of paclitaxel in 0.9% sodium chloride for dose-banding applications.

Methods Paclitaxel infusions in 0.9% sodium chloride (0.3 and 1.0 mg/mL) were prepared in different containers (Freeflex® polyolefin infusion bags and Ecoflac® low-density polyethylene infusion bottles, n = 3) and stored at 2–8°C. Samples were taken for physical and chemical stability testing at different times up to 35 days. Physical stability was determined by weight loss (%), pH change, visual appearance and sub-visual particulate count (British Pharmacopoeia

Table 1 Physical and chemical stability of paclitaxel infusions over storage up to 35 days

	Paclitaxel (0.3 mg/mL)		Paclitaxel (1.0 mg/mL)	
	Freeflex®	Ecoflac®	Freeflex®	Ecoflac®
Shelf life (days)	20	35	17	31
Weight loss (≤%)	0.02	0.18	0.01	0.20
pH range	3.64–3.73	3.58–3.71	3.42–3.53	3.42–3.49
Visual appearance	Pass	Pass	Pass	Pass
Assay range (remaining %)	98.5–103.6	97.7–104.8	98.8–102.8	95.7–103.3

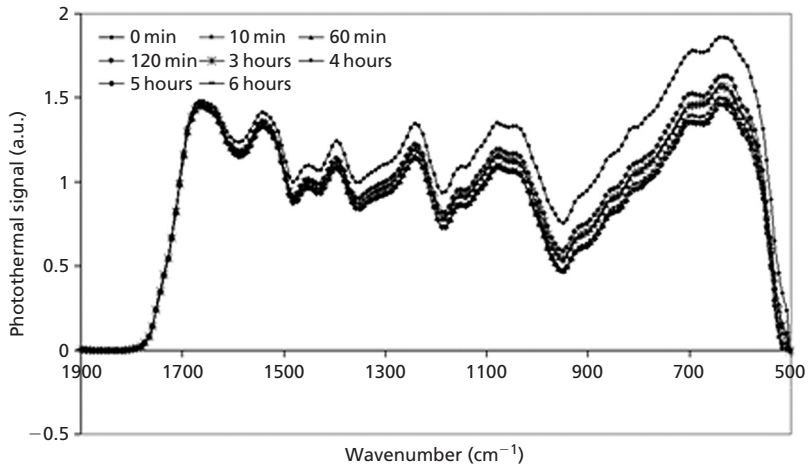

Figure 1 Photothermal spectra of *E. coli* cells. a.u., arbitrary units.

method). Chemical stability was assessed using a validated stability-indicating high-performance liquid chromatography method.

Results The main results from physical and chemical stability testing of paclitaxel infusions during the shelf-life periods are shown in Table 1. The stability of paclitaxel infusions during storage was mainly limited by physical stability due to the precipitation of paclitaxel. This was concentration-dependent and reflects the fact that commercially available paclitaxel concentrates are solubilized with Cremophor EL and ethanol. Sub-visual particulate counts were within the British Pharmacopoeia limits. An adequate stability period ($\geq$17 days) was obtained with both 0.3 and 1.0 mg/mL paclitaxel infusions, which facilitates the dose banding of paclitaxel chemotherapy.

Conclusions Paclitaxel demonstrated robust stability in 0.9% sodium chloride over at least 17 days, which facilitates the feasibility of dose-banding strategy. Further pharmacokinetic studies will be performed by *ex vivo* simulations and clinical studies to evaluate the role of this dose-banding strategy compared with standard BSA-based dosing and flat-fixed dosing.

Plumridge, R., Sewell, G. J. (2001) *Am. J. Health-Syst. Pharm.* **58**: 1760–1764

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Stability of 500 mg/50 mL and 250 mg/50 mL vancomycin B in CIVAS batch-manufactured pre-filled syringes

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Objectives Vancomycin is used as an infusion to treat serious infections in hospitalized patients. Pre-filled syringes for infusion were multi-dispensed by diluting the concentrated vancomycin with diluent in each individual syringe within the aseptic manufacturing unit at Guy's and St. Thomas' Hospitals and assigned 28-day expiry. Following a review of this method of manufacture, improvement in the quality assurance of the product was recommended by production of an aseptically prepared diluted bulk solution that was then packed into 50 mL syringes with no further dilution. The final batch of syringes could be assayed for vancomycin content prior to batch release using a representative sample from the batch. The aim of this study was to establish a stability-indicating assay to validate the new production method and to verify the stability of the vancomycin product.

Methods The high-performance liquid chromatography (HPLC) assay (Figure 1) conformed to International Conference on Harmonisation (ICH) guidelines (ICH 1994) for a stability-indicating assay with regard to specificity, linearity, accuracy and precision. Vancomycin hydrochloride pre-filled syringes (250 mg/50 mL and 500 mg/50 mL) were prepared using the new production method. The pre-filled syringes were evaluated for vancomycin B content (by HPLC assay), pH and clarity. Vancomycin B content was measured for over 63 days under conditions of 2–8°C, 25°C with or without protection from light, and 40°C.

Results No loss of vancomycin B content was measured in syringes stored at 2–8°C over the test period. Syringes stored at 25°C protected from light showed the vancomycin B content to decline at rates of 0.20 and 0.15%/day for 250 mg/50 mL and 500 mg/50 mL, respectively. When exposed to light at 25°C, the rate of vancomycin B decline was 0.23 and 0.17%/day for 250 mg/50 mL and 500 mg/50 mL. Syringes stored at 40°C had the fastest decline in vancomycin B content, at a rate of 1.49 and 1.47%/day for 250 mg/50 mL and 500 mg/50 mL, respectively. Decline in vancomycin concentration was associated with an increase in pH. A white precipitate appeared in the least stable syringes (those stored at 40°C).

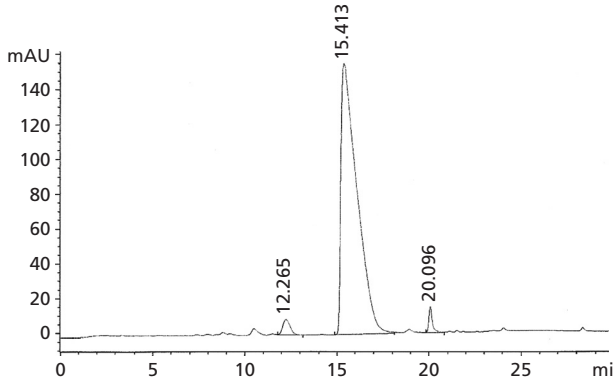

Figure 1 A chromatogram of 2 mg/mL vancomycin B standard. Retention time = 15.4 minutes (vancomycin peak area = 95.4% of area).

Conclusions A new batch production process was implemented with a shelf life of 56 days assigned to 250 mg/50 mL and 500 mg/50 mL vancomycin pre-filled syringes when stored at 2–8°C. The HPLC assay is now used as a quality-control test for vancomycin B content prior to batch release.

International Conference on Harmonisation (1994) *Technical requirements for registration of pharmaceuticals for human use*. ICH-Q2A. International Conference on Harmonisation

Chemistry

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Quantitative structure–activity relationship analysis of local anaesthetic activity of a series of 3-aminobenzo[*d*]isothiazole derivatives

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Objectives To test a number of 3-aminobenzo[*d*]isothiazole derivatives as potential local anaesthetics, and to develop a quantitative structure–activity relationship (QSAR) for the local anaesthetic activity of the compounds.

Methods Synthesis of most of the compounds has been reported previously (Geronikaki et al 2003). Local anaesthetic activity of 30 compounds, together with that of lidocaine as a reference compound, was determined in the rat sciatic nerve model using Wistar rats. For the QSAR analysis, descriptors were calculated using TSAR (www.accelrys.com), ADMEWORKS Predictor (www.fqs.pl) and HYBOT (http://software.timtec.net/hybot-plus.htm) software. We used MOBYDIGS (www.talete.mi.it/mobydigs.htm) software to eliminate descriptors with very poor correlation with local anaesthetic activity and those with high pairwise collinearity, leaving a total of 411 descriptors. The MOBYDIGS genetic algorithm procedure was used to select the best descriptors. Cross-validation was carried out using the LOO (leave-one-out) procedure.

Results A six-descriptor QSAR was developed with reasonably good statistics:

$$\log \mathrm{RA}_{100} = -0.961\ \mathrm{FVMX} + 0.103\ \mathrm{ELOW1} - 0.00524\ \mathrm{ECCN} + 28.0\ \mathrm{CARB\text{-}1} - 0.0382\ \mathrm{DPM_Z} + 2.11\ \Sigma\mathrm{Q}^{+} - 7.56$$

$$\mathrm{n} = 31 \quad R^2 = 0.772 \quad Q^2 = 0.648 \quad s = 0.101 \quad F = 13.5$$

where RA_{100} = activity of compound relative to lidocaine activity (= 100), FVMX = maximum free valence value (an indicator of polarity/reactivity), ELOW1 = difference between minimum and maximum electrotopological state values (size/polarity), ECCN = whole molecule eccentric connectivity index (molecular shape), CARB-1 = average charge on carbonyl carbon atoms (polarity), $\mathrm{DPM_Z}$ = dipole moment in Z direction (polarity), $\Sigma\mathrm{Q}^{+}$ = sum of positive charges on atoms (polarity/hydrogen bonding), n = number of compounds in training set, R = multiple correlation coefficient, Q = cross-validated multiple correlation coefficient, s = standard error of estimate and F = Fisher statistic. All P values were <0.02, indicating that each descriptor had a less than 2% chance of having been selected by chance. There have been but few previous QSAR studies of local anaesthetic activity. Recanatini et al (1988) found a weak correlation (r^2 = 0.652) with distribution coefficient for a series of lidocaine derivatives, and Caliendo et al (1996) obtained a reasonable correlation (r^2 = 0.750) with partition coefficient for a series of isobutyramides.

Conclusions The QSAR results are in accord with our previous conclusions (Geronikaki et al 2003) that molecular size/shape, polarity and hydrogen bonding are largely responsible for local anaesthetic activity. Unlike other workers, we did not find that local anaesthetic activity correlated with partition/distribution coefficient, although it should be pointed out that the descriptors that we have found important all contribute to lipophilicity. The QSAR should be useful in the search for more potent 3-aminobenzo[*d*]isothiazole local anaesthetics.

Caliendo, G. et al (1996) *Eur. J. Med. Chem.* **31**: 99–110
Geronikaki, A. et al (2003) *SAR QSAR Environ. Res.* **14**: 485–495
Recanatini, M. et al (1988) *Quant. Struct. Act. Relat.* **7**: 12–18