

A review of the science presentations

Joe Chamberlain, science secretary for the British Pharmaceutical Conference from 1990 to 1999 and a former editor of *The Journal of Pharmacy and Pharmacology*, highlights a selection of the work that will be reported in the science sessions at BPC 2005 next week

Although the British Pharmaceutical Conference theme for 2005 is "A common vision for health: linking science with practice", the nature of the science contributions to the Conference rightly still has a high component of original research rather than being of direct relevance to pharmacy practice.

More than 250 papers will be presented as posters, with a sizeable fraction of these also being presented as short talks. This review, however, does not distinguish between posters and short talks, but groups selected papers according to their classification by the selection panel.

Pharmaceutical analysis

The trend in recent years away from chromatography in analytical research has continued, with researchers concentrating on pushing the limits of spectroscopy without a separation step. Such non-invasive methods make rapid identification of materials or products possible.

Le Roux et al (Molecular Profiles, Nottingham) use Raman microscopy to elucidate the mechanism by which blends of celluloses function as controlled release coatings and carriers. Drug is released through pores in the insoluble ethylcellulose matrix formed by dissolution of the water-soluble hydroxypropyl methylcellulose and consequently the function and release profile of a given formulation coated in this manner depends critically on the distribution, morphology and degree of mixing of the blend. Films of various blends were prepared and sectioned. The chemical nature of circular domains at the sectioned surfaces was elucidated by Raman microscopy which indicated complete phase separation of the two polymers. Ethylcellulose is present as 3–10µm domains dispersed in a hydroxypropyl methylcellulose matrix.

Barrett et al (Queen's University of Belfast) also use Raman microscopy in mechanistic studies. They investigate the formation of temperature controlled Raman microscopy for the imaging of crystallisation and polymorphic transitions in frozen systems containing mannitol. Significantly different spectra were obtained for each of the polymorphs and each of their orientations, allowing assignment of the polymorphic type and orientation. The Raman maps obtained confirm the presence of a boundary region that was rich in mannitol and reveal that the mannitol concentration in this region increased with time.

The School of Pharmacy, University of London, reports further work on the identification of excipients using non-invasive near-infrared (NIR) spectroscopy. **Palmer et al** have constructed an NIR spectral library of excipients and active pharmaceutical



Shin et al have used NIR spectroscopy in identifying counterfeit tablets: **left, authentic Viagra from seven batches; right, counterfeit tablets from 14 batches**

ingredients and use the library to compare five identification methods. Although a procedure described as soft independent modelling of class analogies (SIMCA) was the best individual method, a cascading approach using a combination of the various methods is recommended.

In collaboration with the Korean Food and Drug Administration, **Shin et al** (School of Pharmacy) investigate different authentic batches of Cialis, Levitra and Viagra tablets and counterfeit tablets supplied by the Korean authorities. By including principal

component analysis in the routine, NIR spectroscopy becomes an effective, fast and non-destructive method for identifying counterfeit tablets in this class of drug.

A collaborative effort from Ultrasonic Scientific (Dublin), AstraZeneca (Charnwood), University College Dublin and King's College London extends the applications of high-resolution ultrasonic spectroscopy in pharmaceutical research. **Smyth et al** describe characterisation of microstructural and molecular processes in pharmaceutical systems, including creaming in inhalers, structural transitions in microemulsions, dissolution of respiratory gases in synthetic blood substitutes, and crystallisation of proteins.

Terahertz pulsed spectroscopy (TPS) is a new technique that probes long-range crystalline lattice vibrations and low energy torsion and hydrogen bonding vibrations. It thus has great potential for characterising crystalline materials. **Zeitler et al** (University of Otago, New Zealand) use TPS to monitor for the first time changes in the crystalline structure of both drug and excipients in formulated tablets. Sample tablets were made from carbamazepine polymorph III, alpha-lactose monohydrate and appropriate amounts of talc and magnesium stearate. The tablets were placed into a heatable transmission cell and spectra recorded while heating and cooling the sample. Spectral changes were noted that clearly showed the ability to monitor changes of polymorphic form in pharmaceutical tablets *in situ*.

Biopharmaceutics

Buggins et al (Welsh School of Pharmacy and AstraZeneca, Macclesfield) point out that co-solvents or excipients often used with poorly soluble drugs may have effects on their metabolism and hence may give misleading information on bioavailability. This possibility was investigated by monitoring the effect of common excipients on drug metabolising enzymes in mice. Although dimethylsulphoxide inhibited metabolism by CYP3A, CYP2E1 and mixed function oxidases, at the usual preclinical doses no effects on metabolism of appropriate substrates were apparent. Propylene glycol inhibited metabolism by CYP2E1 but did not significantly affect mixed function oxidases or CYP3A, suggesting a specific effect of propylene glycol. Solutol HS15 did not significantly affect mixed function oxidases or CYP3A, in contrast to its reported ability to inhibit CYP3A *in vitro*. Thus, although excipients can affect the pharmacokinetics of some drugs *in vivo*, excipients that can inhibit metabolism *in vitro* do not necessarily do this *in vivo*.

In recent years pharmacokinetics has begun to fulfil its potential as a useful predic-

BPC science abstracts 2005

The BPC science proceedings abstracts are being published as a supplement to the October issue of *The Journal of Pharmacy and Pharmacology*. Copies of the supplement are made available to those attending the conference science proceedings sessions as well as to *JPP* subscribers. Copies can be purchased for £14.95 from the Pharmaceutical Press, Turpin Distribution, Stratton Business Park, Pegasus Drive, Biggleswade, Bedfordshire SG18 8TQ (tel 01767 604971; fax 01767 601640; e-mail custserv@turpin-distribution.com). The abstracts are also available in the form of PDF files, which can be downloaded via the BPC section of the Royal Pharmaceutical Society's website (www.rpsgb.org/events).

tive tool, particularly in its application to physiological-based modelling. **Rodrigues et al** (University of Manchester and Servier, Courbevoie) demonstrate how the use of known physiological parameters in the rat can accurately predict the kinetics of norfloxacin, including enterohepatic recirculation and active renal tubular excretion. The extension of such methods for novel drug compounds in man is awaited with much interest.

Aminolevulinic acid is used in photodynamic therapy of neoplastic skin lesions. Administration of excess exogenous aminolevulinic acid induces high concentrations of the potent photosensitiser protoporphyrin IX in neoplastic cells. Illumination with light of appropriate wavelength induces protoporphyrin IX excitation and singlet oxygen production. The highly reactive singlet oxygen destroys the target cells selectively. However, topical administration allows aminolevulinic acid to enter the systemic circulation inducing skin photosensitivity and other side effects. **Donnelly et al** (Queen's University of Belfast and the Norwegian Radium Hospital, Oslo) are able to enhance selectivity of protoporphyrin IX accumulation in tumours following topical bioadhesive patch application. The amount of aminolevulinic acid released across normal stratum corneum from a newly developed patch was approximately one-tenth than that from a commercial cream. This latter effect was particularly pronounced in the tumour-bearing mice, such that the entire mouse fluoresced under UV light following application of the cream; the patch led to localisation and may be considered a more efficient delivery system.

Pugh et al (Welsh School of Pharmacy) successfully use discriminant analysis to identify molecules with potential as enhancers of percutaneous absorption. Data available for 73 enhancers of hydrocortisone permeation from propylene glycol across hairless mouse skin were compared with proposed predictors of enhancement. Simple guidelines suggest that high enhancement is associated with carbon chain lengths greater than 12 and between two and five hydrogen-bonding atoms. Discriminant analysis using chain length, octanol/water partition coefficients and molar aqueous solubility correctly assigned 11 of the 12 effective enhancers. Twelve of the 61 ineffective enhancers were incorrectly assigned although three could be considered marginal. The success of this simple approach in identifying potent enhancers suggests that it is sufficiently reliable to identify potential transdermal enhancers for *in vitro* screening.

The Biopharmaceutical Classification Scheme (BCS) classifies drugs according to their *in vitro* solubility and permeability. **Patel et al** (King's College London) give a timely reminder that the solubility parameter of the BCS is determined using standard dissolution compendial media, which do not adequately simulate the *in vivo* condition. Simulated intestinal fluids, devised to improve *in vitro-in vivo* correlation, are examined for their effect on

the classification of model drugs. Only ibuprofen remained within its proposed BCS class when simulated intestinal fluids were used. Paracetamol and allopurinol were reclassified to Class IV and III drugs, respectively.

For gastric emptying studies it is important that the model meal used is robust and reproducible. **Wilson et al** (University of Strathclyde) investigate the suitability of a commercial product, Clinutren ISO, labelled with technetium-99m. Scintigraphic imaging was performed with the subject in a standing position. A robust and reproducible model of the gastric emptying of this nutrient liquid meal is described which shows similar emptying kinetics in the fasted and the post-prandial conditions.

Chemistry

As in previous years a clutch of papers will be presented by the group at Kingston University, using molecular modelling to aid in the synthesis of novel active compounds or inhibitors, especially with respect to 17 β -hydroxysteroid dehydrogenase. **Soltani-Khankahdani et al** report that compounds which are able to mimic the steroid structure possess inhibitory activity. Such newly synthesised compounds were weak inhibitors of the enzyme, whereas the hydroxy form of the same compounds showed no inhibitory activity.

Chalcones and stilbenes have shown exciting potential as anticancer prodrugs, but their high lipophilicity results in low water solubility and poor pharmacokinetics. **Lohdi et al** (De Montfort University, Leicester) suggest that the functionality essential for bioactivation can be replaced by a nitrogen-containing heterocycle. A prodrug designated DMU-943 was synthesised which is metabolised to a cytotoxic compound by the tumour-specific enzyme CYP1B1 and could prove to be a highly selective anticancer agent.

Gill et al (Aston University) attempt to reduce the side effects associated with the use of andrographolide, a cytotoxic agent against cancer cells. By determining the crystal structure of andrographolide and related compounds, the effect of structure on geometry was investigated. The considerable differences between crystallographically observed conformations and calculated global minima suggest that several low-energy conformations are available, from which crystal packing forces select one for the solid state which may differ from the minimum in solution. Calculations of molecular descriptors did not give strong correlation with activity; substituent-based log P values, unlike activity, increase upon successive acetylation of andrographolide.

Melanoma cells contain high levels of tyrosinase and since this enzyme is virtually absent from other cells, it provides a good target for the *in situ* activation of prodrugs. **Osborn et al** (University of Reading) have synthesised two series of prodrugs for the treatment of melanoma that rely upon novel tyrosinase-mediated bioprocessing to effect

selective drug release. Cytotoxicity studies using a melanotic and an amelanotic cell line have illustrated that the urea-linked prodrugs display enhanced toxicity in the melanotic cell line, as required.

Certain N1-benzylidene-pyridinecarboxamidrazones are known to have antimycobacterial activity and this constitutes most of the published work for these compounds in the antimicrobial area. Most N1-benzylidene-pyridinecarboxamidrazones with antimycobacterial activity contain benzylidene moieties substituted with relatively non-polar functionalities and little by way of hydrogen bond donor functionality. **Rathbone et al** (Aston University) have therefore examined a set of phenolic N1-benzylidene-heteroarylcarboxamidrazones and tested them against both a methicillin-sensitive strain of *Staphylococcus aureus* and a clinical isolate of MRSA. One compound in particular, containing a phenolic hydroxyl as well as two bulky lipophilic alkyl substituents in the benzylidene portion, gave an intriguing and very sharp structure-activity profile. This compound exhibited the most potent activity of the set against Gram-positive bacteria, and high activity was also observed against a panel of seven vancomycin-resistant clinical strains. Any change made to the substitution pattern in this compound or the deletion or modification of substituents resulted in much reduced or completely abolished antimicrobial activity.

Quite removed from the range of papers on directed synthesis and testing of new chemical entities, **Dunn et al** (Pfizer, Sandwich and University of Greenwich) describe the development of new catalysts for N-acylation in industrial processes. A common catalyst is 1-hydroxybenzotriazole hydrate, but this is highly explosive when heated beyond its melting point (around 160C) and the transportation of this catalyst is subject to restriction in Europe. A new catalyst for this type of reaction is 2-hydroxy-5-nitropyridine. It is safe, effective and readily available at a similar price to 1-hydroxybenzotriazole hydrate.

Drug delivery

The intestinal barrier is of obvious interest in studying drug delivery. Based on the observation that proinflammatory cytokines are able to affect the protein composition of the tight junction, **Phelps and Duncan** (Welsh School of Pharmacy) attempt to identify polymers that will promote enhanced drug delivery due to their inherent ability to stimulate cytokine release by making the epithelial barriers transiently permeable. Preliminary findings suggest that non-toxic levels of polyacrylic acids and alginates are ineffective, although polyacrylic acid sodium salt shows some promise.

Microgels are dispersions of particles in the size range of 1nm to 1 μ m, which have a high degree of sensitivity to changes in environmental conditions. Thus microgels undergo rapid changes in their physical

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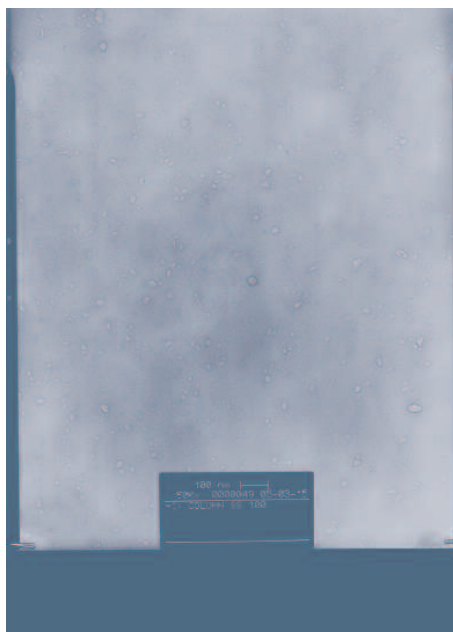
properties, including changes in particle size and surface charge density. **Castro López et al** (University of Greenwich and the School of Pharmacy, University of London) have synthesised new pH- and temperature-sensitive colloidal microgel particles. These materials showed a relationship between the amount of compound entrapped and the solubility and $\log K_{\text{oct/w}}$ of the compounds. The transport rate for the microgels incorporating methyl paraben was one-half to one third that of the saturated solution and one-tenth for propyl paraben; the transport rate for the microgels incorporating salicylamide is the same order of magnitude as that for the corresponding saturated solutions. Transdermal release studies of the saturated colloidal dispersions indicated that pH control of the drug release was marginal.

For improvements in drug delivery to the lung, **Li et al** (Welsh School of Pharmacy) attempt to replace suspensions of micronised drug in propellant with nanoparticles in pressurised metered dose inhalers. Spherical salbutamol sulphate nanoparticles were successfully prepared to a diameter of approximately 10nm. The formulation of the nanoparticles dispersed in hydrofluoroalkane propellant was stable and homogeneous, being either transparent or opaque depending on concentration. *In vitro* deposition demonstrated that the purified nanoparticles are effectively delivered to the pulmonary region, especially the alveolar and lower bronchiolar area.

O'Neill et al (University of Nottingham and AstraZeneca, Charnwood) continue their examination on whether suitably modified microparticles confer steric stabilisation, allowing the possibility of improved targeting of epithelial and dendritic cells in the deep lung. Confocal images at 4C confirmed that microparticles were associated with the outer surface of cells only. However the association observed at 37C was from a combination of particles associated with the outer surface as well as particles internalised by the cells, suggesting some particles are taken up at 37C by an active process.

Low-frequency ultrasound has been found to increase transdermal drug delivery of proteins. In a series of related papers **Dahlan et al** (the School of Pharmacy) investigate factors affecting sonophoresis of proteins through rat skin. With large volumes of coupling medium higher protein permeation was achieved. For the synergistic activity of a surfactant, very low concentrations of sodium lauryl sulphate were as effective as less pharmaceutically accepted concentrations used alone.

Despite much work over several years the use of quantitative structure-permeability relationships (QSPR) may be of limited use for predicting percutaneous absorption according to **Moss et al** (University of Portsmouth and Welsh School of Pharmacy). QSPR analysis suggested that esters and thiol derivatives of captopril would have lower maximal flux values than the parent, decreasing with ester chain length. In practice the esters had flux values for the ethyl ester up to 100 times that



Transmission electron microscopy of salbutamol sulphate nanoparticles, which Li et al have shown can be effectively delivered into the lungs

predicted, peaking for the intermediate chain lengths. These large differences could not be explained by the higher lipophilicity of the esters resulting in higher concentration in the outermost layers, or discrepancies between experimental and calculated solubilities.

Materials science

The importance of precisely defining fatty acids used in drug formulations is highlighted by **Qi et al** (University of East Anglia and GlaxoWellcome). Spray-chilled microspheres based on pure stearic acid, pure palmitic acid, and mixed stearic and palmitic acids were prepared and their drug release behaviour investigated. It was noted that the fastest drug release appeared for the mixed stearic and palmitic acids formulations in alkaline media, possibly due to the formation of fatty acid soaps.

Bajwa et al (University of Nottingham, Sheffield Hallam University and Bristol-Myers Squibb, Moreton) seek to elucidate the relationship between the mechanical and molecular properties of hydroxypropyl methylcellulose solutions during the thermally modified sol:gel transition. They use attenuated total reflectance Fourier spectroscopy and oscillatory rheology experiments to show that changes during the transition involved hydrophobic polymer chain interactions.

Although certain general rules can be applied when powders are mixed and compressed into tablets, interactions can be complex and depend on physical properties and manufacturing conditions. **Gibb et al** (AstraZeneca, Alderley Park, and University of Bradford) attempt to understand the consolidation behaviour of powder mixtures by studying the mean deformation pressure of mixtures at high and low compression speeds. Mixtures of Avicel PH200 and Di-Tab

showed a negative deviation from linearity indicating that microcrystalline cellulose dominates the system. In contrast, mixtures of Avicel PH101 and Calipharm D display a linear relationship, although microcrystalline cellulose appears to dominate the properties of the system at higher volume fractions. Computer generated packing simulations support these conclusions.

Stabilisation of proteins is possible by freeze-drying them in a sugar matrix, but temperature and humidity have a strong impact on product performance and storage, as the sugar may go through a glass transition phase, resulting in a loss of thermal stability of the protein. **Thielmann et al** (Surface Measurement Systems Ltd, London) study the impact of bovine serum albumin on the water sorption and glass transition behaviour of trehalose. All samples containing trehalose showed a significant mass loss between 60 and 70 per cent relative humidity. High contents of bovine serum albumin in an amorphous trehalose matrix cause an increase in the humidity at which the glass transition of trehalose occurred.

It is not just the biological activity of the active compound in a formulation that is important but also the physical properties of the material itself. This is exemplified by the report by **Mackin et al** (AstraZeneca, Macclesfield). During the development of a wet granulation process for a development compound, two batches of an immediate release tablet failed the proposed dissolution specification. The subsequent investigation found a correlation between the life-time of the drug substance batch and the dissolution result. To investigate this ageing effect, the surface properties of the drug substance batches were investigated using gravimetric vapour sorption and inverse gas chromatography. The moisture sorption profiles exhibited a high degree of variability. An unusually high moisture uptake for a crystalline compound correlated with the presence of variable levels of sodium chloride on the surface of the drug substance particles. The data for a typical batch of micronised drug substance indicate that micronisation significantly increases the dispersive surface energy in addition to changing the acidic/basic nature of the highest energy sites. The data also show a significant reduction in the dispersive and polar surface energy over time, which is probably due to the recrystallisation of the amorphous material on the surface of the particles.

High-energy milling or micronisation, necessary to produce the small particles for dry powder inhalers, breaks down the surface crystalline structure of the particles introducing an unstable amorphous phase to the powder. **Tang et al** (King's College London and Triton Technology Ltd, Keyworth) have developed powder pocket dynamic mechanical analysis for the detection of amorphous content in salbutamol sulphate. It was possible to determine a limit of detection of 3.6 per cent of amorphous

salbutamol sulphate in otherwise crystalline powders of salbutamol sulphate.

Microbiology

The conference continues to attract a small number of papers in microbiology, most of this year's papers having a bearing on the MRSA problem. **Gallagher et al** (University of Brighton and Welsh School of Pharmacy) suggest that since current antimicrobial agents are gradually being rendered ineffective by resistance developing in target organisms there is an urgent need for alternative antimicrobial approaches and they show that toluidine blue O, a light-activated antimicrobial agent which has been shown to be effective against a wide range of bacteria, is the most successful method of applying the photoactivated dye for the disinfection of silicone biofilms.

Donnelly et al (Queen's University Belfast) investigate toluidine blue O in a mucoadhesive patch for photodynamic inactivation of *Candida albicans*. With suitable modifications, the patch may be suitable for delivery of toluidine blue O to the oral cavity for photodynamic antimicrobial chemotherapy of planktonic and biofilm cells of *Candida albicans* causing oral candidosis.

Donnelly et al also report on a study to determine the susceptibility of a clinical MRSA isolate growing planktonically to photodynamic inactivation using a combination of methylene blue and visible light. As the intended lesions for this photodynamic eradication are topical and usually venous in origin, exudation is a problem for effective drug delivery. To overcome this problem, a shear-sensitive PVA-borax gel was evaluated as a potential drug delivery system for methylene blue. Incubation of MRSA biofilms with methylene blue for 30 minutes before irradiation achieved reductions in numbers of viable organisms. Drug release studies demonstrated that PVA-borax gels could accommodate methylene blue and release almost 60 per cent within 15 minutes. In addition, they possessed tensile properties that would make them ideal for both application to, and removal from, exudative lesions.

Vine et al (University of Greenwich) propose biological calorimetry to study the growth and death of microbiological cultures in a unique fashion, distinctly different from traditional analysis. Heat energy is evolved as micro-organisms grow and respire. Calorimetry also offers an opportunity to study the effects of surfactants on polymicrobial cultures or for micro-organisms trapped within complex matrices. Antagonistic or synergistic actions of a combination of two or more surfactants can be assessed, something not easily achieved with traditional techniques.

Pharmaceutical technology

In a manufacturing process, understanding the rheology of the constituent fluids in processes involving piped flow is valuable since this has an impact on both material handling and safety considerations. **Booth** (AstraZeneca, Maccles-

field) investigates the rheology of a viscous gel, formed from a mixture of a drug, polymer and solvent, the precursor to microspheres formed by emulsification. The viscometry data enable the velocity profile of the gel across the pipe diameter to be simulated using a capillary flow model. 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. The models also 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.

Booth also reports on large-scale manufacture of poly(lactide-co-glycolide) microspheres via a continuous microencapsulation process. Particles are formed via rapid solvent removal from an oil-in-water emulsion by quenching into a large flow of water. The viscosity of the gels displays 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. This non-Newtonian rheological response to shear explains why the gels could be emulsified by homogenisation and also why only relatively broad multimodal microsphere particle size distributions are achieved.

A common approach for a rapid, first-in-man formulation is to fill a blend of the active pharmaceutical ingredient and excipients into a hard gelatin capsule, but the density of each powder blend must be known in advance. An alternative approach is assessed by **Fitzpatrick et al** (Merck Sharp & Dohme, Hoddesdon, and University of Nottingham) who use a predictive model to determine the theoretical blend density. The results show that the model was able to predict successfully the blend density for a wide range of materials, particle sizes and shapes.

Many factors can affect the size enlargement process during which granules are produced from fine particles to improve flow properties and content uniformity and to reduce dust. **Dilworth et al** (AstraZeneca, Macclesfield) evaluate the effect of water added, impeller speed and granulation time on the process. All granules undergo an initial period of increasing load required to maintain the increasing linear displacement caused by the probe. At this point, some granules reach a plateau, whereby no further load increase is observed, while others show a sudden drop. The type of profile obtained relates to the different mechanisms of granule compression, and a considerable variation within each batch is observed, reflecting differences in porosity, size and strength between granules. It was

found that granules manufactured with all processing parameters at the lowest level had the lowest average maximum recorded load, and that the average maximum load increased when granulation parameters were at the medium level and again at the highest level.

Al-Muti and Podczeck (University of Sunderland) investigate the effect of tablet face curvature and porosity and the type of drug used on the *in vitro* swelling of xanthan gum matrix tablets. Flat round tablets and biconvex round tablets were prepared at three different porosities. Swelling studies were conducted in a hydration medium, with swelling and radial expansion being recorded by a video camera with image analysis software which allowed accurate measurements of the changing tablet dimensions. A significant effect of tablet face curvature, tablet porosity and the type of drug used was seen, in addition to a significant effect caused by the interaction between all three factors. The effect of tablet properties, in terms of face curvature and porosity, on tablet swelling seems to be governed mainly by the type of drug used.

Wet granulation is a critical step in the manufacture of many solid dosage forms but 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. **Gamble** (Bristol-Myers Squibb, Moreton) uses acoustic emission spectroscopy 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 results show that the technique can be used to produce granules with consistent physical characteristics, such as particle size distribution and rheology, despite changes in batch size and liquid dose rates.

Seiler et al (Pfizer, Sandwich, and Johann Wolfgang Goethe-University, Frankfurt, Germany) examine the possibility of preparing controlled-release matrix formulations of theophylline using sucrose-fatty-acid-ester as the matrix-forming agent. Hot-melt extrusion was feasible for all formulations, although process parameter adjustment is necessary to complete the range of drug loadings. Generally, processing of formulations by extrusion/granulation improved critical parameters. The formulations showed a controlled drug release, suggesting sucrose-fatty-acid-esters were suitable for controlled release of theophylline.

Pharmacognosy

The pharmacognosy section of the conference again will provide a guide to the continuing activity in the screening of natural products for medicinal activity, particularly from the King's College London group. Overseas contributors will also be prominent at the conference. **Mehrabani et al** (Kerman,

Iran) investigate essential oils composition and antibacterial activities of the flowering and non-flowering tops of *Stachys acerosa*. The plant materials are subjected to hydro distillation and major essential oil constituents were found to be *cis*-chrysanthenyl acetate and linalool. Twenty-nine compounds of the essential oil of the flowering tops and 37 compounds in the essential oil of the non-flowering tops were identified. Carvacrol, found only in the essential oil of non-flowering tops, showed antibacterial activity.

Lertsatitthanakorn et al (Khon Kaen University, Thailand) isolate several essential oils from Thai herbs. The antioxidant activity of seven essential oils was investigated by a free radical scavenging assay. Of the essential oils tested, all possessed marked activity as hydrogen donors, holy basil oil being the most active with a stronger antioxidant activity than butylated hydroxytoluene, ascorbic acid or alpha-tocopherol.

Van Leo et al (University of Medicine and Pharmacy, Ho Chi Minh City, Vietnam) aim to isolate compounds from the unripe rind of *Citrus reticulata* for use as standards in quality control of traditional medicines. The powder of unripe rind yielded the polymethoxylated flavones tangeretin and nobiletin.

Because of the nature of screening natural product fractions for specific activities, the actual content of the active compounds can be underestimated and several research groups have concentrated on tidying up the isolation procedures. For example, **Aroud and Parkinson** (Dublin City University) sought to maximise the yield of colchicine production and recovery from *Gloriosa superba* root tissue culture in liquid medium by using solid phase extraction. Amberlite resins were enclosed individually in a bag of nylon mesh and incubated with the root culture in liquid medium for four weeks. The mesh bags were removed and extracted with methanol. Amberlite resins greatly enhanced the total colchicine production.

Ahmad et al (Liverpool John Moores University) determine the optimum harvest time of German chamomile (*Matricaria recutita*) by investigating the profile of chemical constituents throughout the growth cycle. Indications are that harvesting could take place earlier in the growth cycle and still yield the major constituents required for biological activity and standardisation. A key finding to support this was the presence of apigenin-7-glucoside only in the budding stage of growth and not in full flower as expected. Because commercial products of the plant are often standardised to apigenin-7-glucoside content this warrants further detailed investigation.

White et al (Welsh School of Pharmacy) investigate Welsh sponges as potential sources of anti-proliferative activity. The growth inhibitory properties of a *Hymeniacidon* sp sponge from south-west Wales has been evaluated in two human cancer lines, MCF7 breast carcinoma and A549 small cell lung cancer. The dried, powdered sponge was extracted with solvents of increasing polarity (dichloromethane, ethyl acetate, methanol)



Chiu and Houghton have demonstrated the anticholinesterase activity of glucosinolates in five common fruits and vegetables

yielding three fractions and an insoluble residue. To determine anti-proliferative activity, stock solutions of each fraction in ethanol were further diluted to suitable concentrations and added to the cell culture medium. The dichloromethane and methanol extracts displayed anti-proliferative activity in both cell lines. In particular, the dichloromethane extract demonstrated extremely potent activity against the A549 cells. Analysis of this fraction by thin-layer chromatography revealed it was a mixture of several distinct compounds.

Pharmacognosy research is not limited to deriving active constituents from esoteric plants and animals. **Chiu and Houghton** (King's College London) follow up the received wisdom that common fruits and vegetables contain cholinesterase inhibitors by studying pesticide-free orange, radish, apple, broccoli and potato. All five sources showed significant activity with broccoli being the most promising. The activity appeared to be due to glucosinolates, with the authors suggesting this is the first time anticholinesterase activity has been reported for glucosinolates. Disappointingly, however, they suggest that consumption of broccoli would not have any beneficial effect on Alzheimer's disease.

Pharmacology

Three groups address different aspect of the pharmacology of nitric oxide. **Goyal et al** (Jan Nayak Ch Devi Lal College of Pharmacy, Sirsa, India) investigate S-nitrosothiols, biological metabolites which represent a more stable form of nitric oxide for storage or transport, as vasodilatory drugs and showed that S-nitroso-mercaptobenzimidazole and S-nitroso-mercaptobenzothiazole produced a dose-dependent relaxation of the muscle of rat tracheal chains.

Ingram et al (University of Brighton and University of Portsmouth) synthesise a nitric

oxide-releasing prodrug, captopril methyl ester nitric oxide, and show a relaxation effect similar to that of isosorbide dinitrate.

Jones et al (Liverpool John Moores University) look at the relative abundance of nitric cells in the thymus of three substrains of the BioBreeding rat, an animal model of human insulin-dependent diabetes mellitus — a diabetes-resistant non-diabetic substrain, a diabetes-prone pre-diabetic substrain and a diabetic substrain. They show significant difference between compared groups and identify an association between the diabetic-prone genotype and deficiency in nitric cell abundance, and suggest that the diabetic phenotype exacerbates this deficiency.

Airley et al (Liverpool John Moores University) noting that the level of the facilitative glucose transporter, Glut-1, expression in individual tumours may influence the activity of tyrosine kinase inhibitors, test the hypothesis using methyl-thiazol-tetrazolium and clonogenic cell survival assays following exposures to quercetin and imatinib in the human colon carcinoma HT-29 cell line. Although cells were significantly less sensitive to quercetin after exposure to anoxia alone, overexpression of Glut-1 increased sensitivity in both normoxic and anoxic conditions. Imatinib showed greater toxicity, but this was not influenced by the level of Glut-1 expression or the level of oxygenation. It is suggested that knowledge of structurally related ATP-binding, which may partially exert toxicity through Glut-1 inhibition, may assist in the design of novel anticancer agents inhibiting Glut-1.

Loneragan et al from the same group test the hypothesis that the induction of Glut-1 expression in hypoxia may serve as a feedback mechanism by the transcription factor HIF-1 (hypoxia-inducible factor 1) activity is decreased upon reoxygenation, by determining the effect of Glut-1 overexpression upon ascorbate-mediated toxicity in tumour cell lines. Although the precise mechanism of ascorbate toxicity is unknown in these experiments, the data suggests that Glut-1 expression leads to increased accumulation of ascorbate by the cell, which may in turn serve a critical role in HIF-1-dependent survival pathways.

McCurrie et al (University of Bradford) continue to investigate the problems of interaction of estrogens and progestogens in the pharmacology of smooth muscle relaxation, using rat intestine. Estradiol and progesterone cause similar concentration-related relaxation of intestinal smooth muscle and the addition of progesterone does not significantly change oestrogen-induced relaxation in this preparation and therefore is unlikely to antagonise the beneficial blood vessel-relaxant effects of the estrogens in hormone replacement therapy. However, there are differences between vascular and intestinal muscle and the synthetic progestins present in the wide range of hormone replacement therapy preparations available could affect vascular relaxation produced by estrogens, since the properties of these progestins differ considerably.