

SCIENCE PRESENTATIONS

A review of science at Harrogate

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This article highlights a selection of the work that will be reported in the science sessions at the British Pharmaceutical Conference next week

Figure 1: Computer representations showing the similarity of the surface charge distributions of the amino acid lysine (left) and a dimer of cysteine-cysteamine (right). Cystinotic patients are deficient in a functioning cysteine transport protein and accumulate cysteine in lysosomes, causing damage and leading to eventual cell death. Cysteamine can deplete cystinotic cells of accumulated cysteine because the dimer can be excreted from lysosomes and then out of the cells by a lysine transport protein (Tindall et al)

The theme of this year's British Pharmaceutical Conference is "Delivering innovation for patients" and the breadth and novelty of the short science papers presented will underline the ongoing commitment of pharmaceutical scientists to this goal.

Over 220 abstracts will be available, mostly as posters, although five commended papers will also be presented at each of three discussion sessions. Sixteen short papers in pharmaceutical analysis are to be presented at podium sessions, with a prize for the best presentation. Selected highlights in the various categories are presented below. [The full abstracts can be downloaded from the BPC section of the Royal Pharmaceutical Society's website at www.rpsgb.org.uk/events].

MEDICINAL CHEMISTRY

Because of the enormous cost of development and safety testing, a major effort in medicinal chemistry has been directed at using computer modelling to predict both activity and toxicity of new drug candidates. **Lagunin et al** (Institute of Biomedical Chemistry, Moscow) describe an evaluation of the Russian prediction program PASS for the prediction of acute toxicity in rats. Prediction accuracy for the oral route of administration was better than that for intravenous administration. The authors conclude, unsurprisingly, that the moderate accuracy of prediction was due to the use of compounds from different chemical series in the training set and to the relatively small number of compounds included into each class of toxicity.

Tanabe et al (Toyo, Tohoku and Toyama Universities, Japan) refine the use of quantitative structure activity relationships (QSAR) by the application of neural networks to predict carcinogenicity of organic compounds. Prediction accuracy was significantly improved compared with that obtained using statistical methods such as regression analysis.

A less up-beat conclusion is reached by **Dearden and Thomson** (Liverpool John Moores University) in their QSAR study of reversal of multidrug resistance by phenothiazines. Despite all the compounds being of the same general chemical class, good QSARs were not obtained, except by using specific sub-sets, and the authors conclude that it will not be possible to devise a global QSAR for multidrug resistance.

Drug design to alter specific biochemical properties to improve drug action rather than achieve an overall increase in activity may be a more successful approach. Cystinosis is a rare clinical condition in which the cell removal mechanism for cystine is impaired resulting in cystine accumulation within the lysosomes of various cells. **Tindall et al** (University of Sunderland and Tulane University, New Orleans) describe the synthesis of a number of amino acid derivatives of cysteamine which are able to enter the afflicted cells by a natural internalisation process; cysteamine subsequently

released by specific cellular enzymes is the agent which then removes cystine from the lysosomes (see Figure 1).

Cox et al (Aston University) apply molecular modelling techniques and shape changes to understanding the binding of antimycobacterial N¹-benzylidene pyridine-2-carboxamidrazones, rather than aim for increased activity. Postulation that binding of carboxamidrazones was influenced by the distance from the pyridyl nitrogen atom to the furthest extremity of the lipophilic arylidene substituent was supported by a loss of activity when the optimum distance was not achieved.

Similarly, **Casely-Hayford and Searcey** (London School of Pharmacy) study the effect of stereochemistry on biological activity of newly synthesised azinomycin antitumour antibiotics. The analogues contained structural modifications expected to affect DNA-binding and alkylation. However, the findings strongly suggest that analogues of the natural products do not need to crosslink DNA to retain potent anti-tumour activity.

A reminder that the methods of medicinal chemistry are not confined to predicting or modulating the interaction of drug molecules with biological systems is provided by **Ambarkhane et al** (London School of Pharmacy and GlaxoSmithKline); the molecular mobility of molecules in an amorphous state was measured using enthalpy relaxation data obtained by StepScan differential scanning calorimetry. The empirical Kohlrausch-Williams-Watts equation was applied and was shown to differentiate between the molecular mobility and relax-

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ation processes of indomethacin and nifedipine in amorphous states and hence could be of potential use in predicting the relative stability of the amorphous form of different drugs.

DRUG DELIVERY

More than a quarter of the short papers presented are in the "drug delivery" category. Topics include those related to development of the dosage form itself, including liposomes, microspheres and nanoparticles, and those related to the route or method of delivery such as intravaginal rings, transdermal patches and nasal inserts. The rationale for developing novel drug delivery systems continues to be the need to control rates of delivery of drug to the target and protection of vulnerable molecules against premature degradation. Several papers develop the theme of targeted delivery of DNA and other biologicals.

McNeil *et al* (Aston University and Lipoxen) assess several liposomal/DNA systems for their ability to entrap DNA within the liposome and retain their vaccine payload in the presence of competitive anionic components. The addition of DNA to cationic small unilamellar vesicles led to the formation of complexes, larger than the original empty small unilamellar vesicles with a reduced zeta potential. This decrease in zeta potential reflects neutralisation of the vesicles' cationic surface due to adsorption of the DNA, suggesting that a high proportion of the DNA is entrapped within the vesicles with little surface neutralisation occurring. The data suggest that the dehydration and rehydration of liposomes in the presence of DNA produces structures where the DNA is predominantly entrapped within the liposomes and protected against displacement and degradation in biological milieu.

Davies *et al* (University of Nottingham and AstraZeneca) describe the effect of critical parameters on the properties of microspheres modified with the polycation polyethylenimine in a project to improve the immunogenicity of DNA incorporated into microspheres. The microsphere size was found to have a pronounced effect on both polyethylenimine incorporation and subsequent microsphere DNA loading.

Enhancement of transdermal penetration of estradiol is described by **Essa *et al*** (University of Bradford) using electroporation for enhancing the delivery of the steroid from saturated aqueous solution and ultradeformable liposomes. The work was extended to probe the effect of phosphatidylcholine on repairing skin damage after electroporation. Results suggest that during skin electroporation using liposomes, the phosphatidylcholine molecules released from liposomes at the sites of damage could improve skin repair. The freed phosphatidylcholine monomers would fill up the areas of high skin damage to form liquid crystal microdomains, replacing water molecules.

Malcolm *et al* (Queen's University, Belfast) describe their use of intravaginal rings as a delivery system for the otherwise

poorly bioavailable oxybutynin, and for the local delivery of metronidazole for treatment against *Gardnerella vaginalis*.

The transport across Caco-2 monolayers of polyamidoamine dendrimers conjugated to lauroyl chloride is described by **Jevprasesphant *et al*** (University of Manchester). This modification succeeded in increasing permeation, while also decreasing cytotoxicity to Caco-2 cell monolayers. The route of transepithelial transport, for both dendrimers and dendrimer conjugates, is suggested to be via both paracellular and transcellular pathways.

Effective drug delivery to the lung following inhalation depends upon simultaneous, complicated processes, and direct delivery of therapeutic agents to the lung is desirable. **Somavarapu *et al*** (London School of Pharmacy) have prepared mucoadhesive positively charged poly lactide-co-glycolide microspheres containing rifampicin. The results show that when chitosan is used as the emulsifying agent not only are positively charged microspheres produced but there is enhancement of the loading efficiency of rifampicin.

Singh *et al* (London School of Pharmacy) recognising that poly-ε-caprolactone has some advantages over poly-(lactic-co-glycolic acid) for the preparation of drug delivery systems, suggest that using a copolymer as a nanoparticle vaccine delivery system, the properties and the advantages of both polymers could be exploited. They demonstrated the advantage of co-polymer nanoparticles over both poly-(lactic-co-glycolic acid) nanoparticles and free antigen solution in obtaining higher immune responses.

PHARMACEUTICS

The dominant themes of the pharmaceuticals papers were development of methods for more efficient production of materials to be formulated into products, and the characterisation of the materials and products by a variety of analytical methods including differential scanning calorimetry, near infrared spectroscopy, time-of-flight secondary ion mass spectrometry and scanning electron microscopy. Papers on the prediction of properties of both products and pure materials were also prominent.

There is increasing pressure for formulation scientists to produce robust dosage forms that can be manufactured over a range of scales with minimal time, cost and material. **Hardy and Adkin** (AstraZeneca, Loughborough) describe the use of physicochemical data to predict powder flow and processing behaviour with the aid of computer modelling. For a series of excipients, flow properties and tablet weight uniformity could be predicted from the physicochemical properties of the material. This could offer a potentially powerful tool during preclinical formulation development, when drug substance is in scarce supply.

Pilot-scale milling trials to determine the most effective and efficient mill often lead to the production of unnecessarily large quantities of bulk active. **Taylor *et al*** (Pfizer,

Sandwich, and University of Greenwich) describe a small-scale technique that could predict the milling properties of bulk powder from single crystals, based on the brittleness index. The brittleness index is sensitive enough to distinguish among materials that were easy, moderate and difficult to mill.

Roberts *et al* (Liverpool John Moores University, Manesty, FMC BioPolymer, Belgium, and University of Manchester) investigate the effect of punch surface material such as uncoated steel or chrome-plating on the sticking tendencies of ibuprofen tablets. Three-way analysis of variance indicates a strong interaction between punch surface material, compaction force and the main excipient used in the formulation. The rapid identification of polymorphic forms of new drugs is an important objective in preformulation studies. **Hulse *et al*** (University of Bradford and Merck Chemicals) use accelerated differential scanning calorimetry as an effective device for exploring the presence and interrelationship of polymorphism, particularly for simple molecules.

Hallett *et al* (Phoqus) stress the importance of the tablet appearance on patient compliance and suggest ways of producing distinctive tablets using electrostatic dry powder deposition technology. The range of tablet markings can be greatly enhanced. The ability to create a greater range of distinctive tablet appearances could lead to improved safety and patient compliance by alerting patients to the dose and content of a tablet with a strong visual signal.

Jordan *et al* (Colorcon and Molecular Profiles) investigate the microscopic structure of the films applied to tablets and the possible migration and partitioning of soluble components into the film during the coating process. They demonstrate that time-of-flight secondary-ion mass spectrometry analysis combined with other complementary analytical tools can have considerable value for the characterisation and understanding of complex heterogeneous modern dosage forms.

Particle size reduction methods to increase dissolution rates for poorly soluble drugs are either not able to produce nano-size ranges (jet milling) or are prohibitively expensive (supercritical fluid particle design). Instead, **Grother** (Cardinal Health) suggests in-line high-pressure particle size reduction as used in the Zydis manufacturing process for drugs delivered by freeze-dried fast-dispersing dosage forms. Product produced in this manner could improve the variable or poor absorption observed with many drugs that exhibit dissolution rate-limited absorption.

The mechanism of the protection of protein drugs during freeze-drying is investigated by **Moran *et al*** (London School of Pharmacy), by study of the near infrared spectra of a model protein, catalase, on exposure to high relative humidity following spray drying with a known stabilising additive, trehalose; spectral comparison suggests an interaction between catalase and trehalose upon spray drying.

MICROBIOLOGY

A small but diverse group of papers appears in the microbiology section. **Behravan *et al*** (Bu-Ali Research Institute and Mashhad University of Medical Sciences, Iran) are searching for new microbiological sources of L-asparaginase enzymes suitable for use against leukaemia. The maximum L-asparaginase activity was found in cultures of *Escherichia coli* PTTC 1330 in a medium containing L-asparagine; however anti-tumour activity of the isolated enzyme needs to be confirmed before scale-up production would be justified.

Field *et al* (University of Calgary, Canada, and Queen's University, Belfast) describe the development of a rapid assay for antimicrobial susceptibility testing, an essential requirement for selecting an appropriate antibiotic in the clinic. A microtitre-based colorimetric method showed promise of providing rapid results to enable appropriate antibiotic therapy to be started more quickly rather than wait for overnight testing.

Similarly, **Moriarty *et al*** (Queen's University Belfast and Belfast City Hospital) developed a colorimetric assay, which correlated well with the conventional plate count method of time kill-analysis, but was less labour intensive and provided results two hours after sampling.

McMeel *et al* (Queen's University, Belfast) attempted to overcome device-related infection with microbial anti-adherent coatings on the device surface. A series of polymeric films composed of the biodegradable polyester polycaprolactone and the antibiotic rifampicin (see Figure 2) had potential application as microbial anti-adherent coatings for urinary medical devices, the initial anti-adherent properties due to the rifampicin being coupled with the desirable biodegradable and biocompatible properties of polycaprolactone.

ANALYSIS

Most of the analytical papers will be presented orally under the auspices of the Joint Pharmaceutical Analysis Group. Research papers in pharmaceutical analysis include imaginative combinations of spectroscopic and chromatographic methods, and the application of computer methods, with emphasis on characterisation of materials and biological systems rather than measurements of individual components.

Nisar *et al* (London School of Pharmacy and the Innovation Centre, Leicester) applied nano-electrospray ionisation-tandem mass spectrometry for the identification of cytochrome P450s (CYPs) in complex protein mixtures. The combination is a reliable method for the simultaneous identification of multiple CYP isoforms at the level of protein expression found in human liver and hepatocytes.

Millership and Parker (Queen's University, Belfast) describe ratio spectra derivative spectrophotometry for the determination of two drugs together in the same formulation. The method involves record-

Figure 2: *E. coli* seeded agar plates with samples of polycaprolactone impregnated with 1 per cent rifampicin (yellow, left) and 5 per cent rifampicin (red, right). The transparent borders around the materials are the zones of bacterial inhibition (McMeel *et al*)

ing the absorption spectra of mixtures containing both drugs and computer manipulation, with analysis of the resulting curves. The method was found suitable for the determination of the diuretics furosemide and spironolactone in a capsule formulation.

Although the use of near-infrared (NIR) spectroscopy in the pharmaceutical industry has grown rapidly in the past decade, the overall procedure and optimisation of parameters is still perceived as an art rather than a science. **Smith *et al*** (London School of Pharmacy, Cardinal Health and Pfizer) address the issue through the development of the optimal partial least squares regression model for the NIR assay of moisture within a pharmaceutical product. The algorithm was applied successfully, improving the robustness of the calibration model by excluding a variable spectral region not correlated to the analyte of interest.

Elkordy *et al* (University of Bradford) used Fourier transform Raman spectroscopy to detect protein denaturation in the solid state and were able to show that heating of dry proteins above their T_m perturbed the secondary structure with complete loss of biological activity. Thermal stress affected the commercial and spray dried lactate dehydrogenase and trypsin to the same extent. The procedure may be considered a useful method to detect protein structural changes due to thermal stress.

Arnot *et al* (King's College London and Joseph Fourier University, France) investigate the potential of solution calorimetry to characterise dissolution in simulated intestinal fluids by measuring the enthalpy of solution of two solutes, mannitol and propranolol. The concentrations of bile salts and lipid were varied to model the environment present in the fed and fasted intestinal lumen. The study indicated that solution calorimetry has the potential to discriminate both dynamic and thermal characteristics of drug dissolution in complex media.

Ebbens *et al* (Molecular Profiles and the University of Nottingham) demonstrate the use of atomic force microscopy to measure and map the adhesive interaction forces and electrostatic forces in a typical inhaler system between micronised lactose monohydrate, micronised salbutamol sulphate, and polytetrafluoroethylene (PTFE) and a

single salbutamol particle. The adhesion of salbutamol to the substrates is ranked lactose > salbutamol > PTFE and this order of interactions will favour the dispersal of aggregates of salbutamol on to the surface of the lactose carrier. Using force-volume imaging it is possible to map variations in interaction force between particles of interest. This has large potential for the ability to engineer inhalation formulations to provide advanced drug delivery systems.

Madden *et al* (Molecular Profiles and Johnson & Johnson, Belgium) investigate the application of scanning electron microscopy, Raman confocal spectroscopy and scanning thermal microscopy to characterising the differences in itraconazole solid oral capsules. The results correlate with the release and bioavailability data and provide a valuable insight into the microstructure of the formulation in relation to the release profile.

BIOPHARMACEUTICS

A feature of the collection of a dozen biopharmaceutics papers was the increased application of design at the molecular level. **Patel and Forbes** (King's College London) investigate the potential of lipid:DNA complexes for delivery to the lung. Because cationic lipids do not have the immunogenicity and carcinogenicity problems of viral vectors they are increasingly favoured as gene delivery vectors. Cationic liposomes were used to complex plasmid DNA to form a lipoplex, which is the delivery unit for DNA transfection. Combining this approach with substitution of pressurised metered-dose inhalers instead of nebulisers, a feasible lipoplex formulation in propellant has been developed.

White and van der Walle (University of Bath) continued the theme of DNA delivery, in particular the problem of overcoming inhibition by the epithelial glycocalyx of the uptake of DNA's particulate vehicles. An ambitious project was initiated to engineer a stalk which projects from the surface of a nanoparticle and has the potential to penetrate the glycocalyx, and hence facilitate endocytosis and internalisation.

In another paper from the same group, **Watson *et al*** studied the optimisation of nanometer-sized agents in the construction

of a polyvalent scaffold for use as a non-viral gene delivery vector. The initial study showed that only a relatively short (5–10 residue) amino acid sequence is needed to complex DNA. In the next step, a synthetic prototype will therefore be furnished with such short polyarginine building blocks and used in mammalian cell transfection studies.

The problem of selectively overcoming the blood-brain barrier for drug delivery is imaginatively tackled by **Omidi et al** (Cardiff University). A comprehensive study was first undertaken to confirm the presence of RNA transcripts for carrier systems recognised to be present within the blood-brain barrier. Several transporters were characterised, including system L, the major Na⁺-independent bidirectional neutral amino acid transporter.

Modulation of this carrier system was achieved suggesting a useful model for the study of carrier-mediated transport processes for solutes entering or exiting the central nervous system.

A more traditional approach to biopharmaceutics is taken by **Strugala et al** (Reckitt Benckiser Healthcare and Norwegian University of Science and Technology, Trondheim) in the investigation of the inhibitory action of alginates on pepsin. The authors conclude that the activity of pepsin can be substantially reduced by aqueous alginate solutions, the extent of inhibition being dependent on the structure of the alginate. There is thus therapeutic potential for alginates, among their other pharmacological activities, to reduce the damaging capacity of the gastric refluxate in patients with gastro-oesophageal reflux.

PHARMACOLOGY

Pharmacology papers of direct pharmaceutical interest include investigations of the mechanisms of drugs used in Alzheimer's disease and non-steroidal anti-inflammatory agents. **Hatzipavlis et al** (University of Bradford), noting that tacrine has both anticholinesterase activity and protease inhibitory activity, compare the ability of some related compounds to inhibit these enzymes in model *in vitro* systems, and determine possible mechanisms of interaction with protease enzymes. They suggest that development of drugs with both antiprotease and anticholinesterase activity provide potentially greater benefits in the treatment of Alzheimer's disease than either activity alone.

At times when epidemiological studies are often used as pointers to the safety of drugs, especially those drugs intended to improve the quality of life, such as hormone replacement therapy, it is important to identify and understand the mechanisms involved in drug action. **McCurrie et al** (University of Bradford) continue their investigation of estradiol's mode of action in relaxing rat aorta and rat portal vein. Their results showed that estradiol action in portal vein is independent of nitric oxide (NO) production while in intact aorta endothelium-derived nitric oxide appears to contribute significantly to relaxation.

Wilson et al (Liverpool John Moores University and University of Brighton) are also concerned with NO, particularly whether NO-prodrugs of ibuprofen could have modified gastrotoxicity associated with non-steroidal anti-inflammatory drugs of this type. Acute effects of ibuprofen and its nitric oxide-releasing prodrug on ulceration, erosion and nitric oxide synthase expression in rat gastric mucosa led the authors to conclude there is some measure of protection afforded by the NO-conjugate.

Clark et al (Liverpool John Moores University and University of Brighton) investigated similar prodrugs of ibuprofen and indomethacin for their effect on rat thymus. It is proposed that if the effects of acute oral treatment with either NO-conjugated or parent forms of ibuprofen or indomethacin are maintained over chronic periods of drug use they may pose a risk to effective immune tolerance.

PHARMACOGNOSY

Papers to be presented in the pharmacognosy section involve natural products from West Africa, Iran, Venezuela, Malaysia, Korea, Italy, India, Kenya, Panama, China and even the British Isles, tested for a wide variety of applications (cytotoxicity, antidiabetic activity, Alzheimer's disease, antiprotozoal activity, antimutagenicity and psoriasis).

The pharmacognosists of King's College London have been particularly active. **Ren et al** tackle the isolation of novel inhibitors of acetylcholinesterase from the dried root of *Salvia miltiorrhiza* (Danshen). Isolated compounds were identified as the diterpenes dihydrotanshinone, cryptotanshinone, tanshinone I and tanshinone IIA not previously identified as acetylcholinesterase inhibitors.

Ali et al use an α -amylase inhibition *in vitro* model to screen for possible antidiabetic agents in Malaysian plants; a hexane extract of *Phyllanthus amarus* was further fractionated using bioassay guided techniques and yielded a fraction with high inhibitory activity.

Bawden et al used similar procedures to investigate α -amylase inhibitors in plants of Indian origin and isolated partially purified extracts of *Murraya koenigii* Spreng. (Rutaceae) and *Cyperus rotundus* L. (Cyperaceae) with significant α -amylase inhibitory activity.

Anao et al investigated extracts of medicinal plants used in malaria-endemic areas of Nigeria, Zimbabwe and Argentina and found previously unreported antiprotozoal activity for *Sclerocarya birrea*, *Ipomea involu-crata* and *Chromolaena odorata* and antitrypanosomal activity for *Buddleia globosa*.

Houghton et al, in collaboration with colleagues in Korea, examined seven herbal medicines used in Korea to treat memory-related disorders and found promising anticholinesterase activity in methanolic extracts of *Acorus calamus* root and *Epimedium koreanum* herb.

The Anti-Inflammatory Natural Products from Plants (AINP) project utilises the

potential of plant drugs and natural products specifically to inhibit activated nuclear factor- κ B. **Bremner et al** (London School of Pharmacy), on behalf of a consortium which draws members from at least seven European countries, review progress in two separate papers. The primary screening group have now screened over a thousand extracts representing 225 species from 75 families. Overall, 3.1 per cent of the tested extracts have NF- κ B inhibitory activity without associated toxicity.

Satyanarayan et al (Gulbarga University, India, and University of Sunderland) have been screening extracts from the seeds of *Momordica charantia* (Cucurbitaceae) for anti-fertility activity. A surprising discovery has been the isolation of phenolphthalein and its confirmation as an antiandrogen, although this well-known laboratory agent has not previously been reported as a natural product.

DRUG METABOLISM

Drug transporters are carrier proteins involved in translocation of chemical substances across the biological membranes in the body. **Sharma et al** (Johnson & Johnson, Belgium) study the *in vitro* biliary transport by hepatic transporters, using dog hepatocytes, seeded on to collagen-coated 12-well plates, overlaid with gelled collagen, and cultured up to 15 days. It was observed that taurocholate excretion was considerably higher (59-fold) in these sandwich cultures compared with primary hepatocyte cultures and it is concluded that sandwich cultures of dog hepatocytes represent a good model for biliary excretion of drugs. Such *in vitro* drug metabolism studies make use of fresh tissue and cell fractions from laboratory animals; however, there is greater relevance to drug discovery in man when human tissues are available.

The UK Human Tissue Bank receives donations of human liver from surgical resections and non-transplantable livers from cadaveric multi organ donors and these tissues can be used for the isolation of the functional cells (hepatocytes) which are subsequently used for drug metabolism, toxicity studies, bioartificial liver and cell transplantation research. **Lloyd et al** (Leicester General Hospital and De Montfort University) described the effect of patient, operative and processing variables on the quality of isolated human hepatocytes. Hepatocyte yield was reduced by decreasing body mass index, and visual assessment of the success of tissue digestion gave a good indicator of increased yield. Hepatocyte viability was also improved with decreasing patient age, increased liver steatosis and the nature of the cannulation method employed. The authors argue that, given the precious nature of human tissue, all donations should be considered for the isolation of human hepatocytes for research. Easily accessible good quality human hepatocytes are now routinely available to researchers in the UK. However the tissue bank is seeking improvement to this service with active research into cryopreservation.