Invited Abstracts

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New regulations for herbal medicinal products: implications for safety, quality and efficacy

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The vast majority of herbal products on the market in the UK are not currently regulated as medicinal products but are exempt from licensing requirements under the Medicines Act 1968. In contrast to licensed medicinal product, there are no specific safeguards on quality and safety. Furthermore, there are no statutory provisions for labelling or information to be provided to the patient. Over the past decade important safety issues associated with the use of herbal products have resulted in regulatory action worldwide in an effort to protect public health. The safety problems emerging with herbal products reflect a growing global market, largely unregulated where many of the safety concerns arise due to lack of effective quality control. Evidence of poor quality and safety standards continues to grow and reports about the use of potentially toxic herbal ingredients and deliberate addition of hazardous substances such as heavy metals and pharmaceuticals are widespread. One emerging area is the evidence of potential drug–herb interactions that may have significant clinical implications. The MHRA has reached a wide measure of agreement with the UK herbal sector that the current arrangements for unlicensed herbal medicinal products do not afford sufficient protection for public health and that there is a need to improve the regulatory position. The European Commission has recognised the difficulties faced by herbal manufacturers in fulfilling the regulatory requirements for marketing authorisations. In an effort to achieve a single market for herbal medicinal products, the Commission has adopted a new Directive that provides a simplified registration procedure for traditional medicinal products. The Directive on Traditional Herbal Medicinal Products (2004/24/EC) was adopted in March 2004 and Member States will have to introduce national simplified schemes by October 2005. Under the Directive, no derogation is made with regard to the quality aspects of the product and manufacture will have to take place in compliance with GMP. Provided that the product is intended for minor conditions that do not require medical supervision and the traditional use of the product is plausible on the basis of long-standing use and experience, evidence of efficacy from clinical trials will not be required. While the Traditional Use Directive may not provide all of the solutions to the problems encountered with herbal medicinal products, it does provide significant advantages over existing regulatory arrangements. It is hoped that the additional safeguards for patients will make an important contribution to protecting public health by ensuring that the patients have access to a wide range of good quality products with comprehensive patient information.

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Galantamine: are plant derived anti-dementia drugs best?

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Prescription drugs for dementia include the cholinesterase inhibitors (ChEI) donepezil, rivastigmine and galantamine, and more recently the glutamate NMDA receptor agonist memantine. Widely used non prescription drugs include *Ginkgo biloba* extracts, and other herbs such as sage and lemon balm are being explored on the basis of their traditional use, bioactivity and clinical efficacy. One reason to expect that traditional herbal remedies might be superior to synthetic chemicals that do not occur naturally is that the safety and efficacy of herbals have been established over centuries or even millennia. There is thus a 'survival of the fittest' (medicine) factor in operation. The alkaloid galanthamine is derived from Amaryllidaceae species such as *Galanthus* and *Narcissus*. There is a limited amount of ethnobotanical information on the application of these species, which have been used in Eastern Europe for conditions such as poliomyelitis (Heinrich et al 2004). In comparisons between the different ChEI, including galantamine, for the treatment of Alzheimer's disease (Ritchie et al 2004) all three drugs had similar cognitive efficacy. However in metanalyses to date the period of treatment has been limited (generally 6 months or less). There is some anecdotal evidence that over longer periods, galantamine may be better tolerated although clinical data is not yet available. In addition allosteric nicotinic receptor modulation, which is part of the action of galantamine but not the other two ChEI, may be relevant in terms of neuroprotection and disease stabilisation or even prevention. The question of whether whole plant extracts or single plant chemicals are superior includes arguments on both sides. The challenge of standardization of plant material in terms of chemicals and bioactivity favours chemical drugs. Poly-pharmacology, including for example in addition to cholinergic also anti-inflammatory and anti-oxidant activities relevant to the treatment of Alzheimer's could favour plant extracts such as sage (Perry et al 2003).

Heinrich, et al (2004) *J. Ethnopharmacol*. **92**: 147–162
Perry, N., et al (2003) *Pharmacol. Biochem. Behav*. **75**: 651–659
Ritchie, C. W., Ames, D., Clayton, T., et al (2004) *Am. J. Geriatr. Psychiatry* **12**: 358–369

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Mother Nature's Pharmacopoeia; the search for pharmacophores from nature

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The use of natural products as sources of drug agents/treatments against a multiplicity of diseases has its roots in Antiquity, with the earliest compilations dating back to the Sumerians approximately 4000 years ago, and actual usage can be traced to graves approximately 50 000 years old where medicinal plant residues have been reported. Even today, over 80% of the world's population uses plant materials as their sole source of medicaments, but these are customarily mixtures of plants parts and each indigenous group tends to have different methods of preparation and/or treatment. This presentation, however, will deal with the use of natural products as sources of single, defined drug entities and not with mixtures. It will briefly cover the earlier plant-derived agents such as aspirin, digitalis, reserpine, etc., and will then move into the current usages of natural products from all sources with the underlying motif of the use of the basic structure from nature as a building block upon which to base modified drug candidates by use of supplemented fermentation, semi-synthesis or basic combinatorial studies but using as the starting molecule, "a (privileged) structure from Nature". It will include an overall analysis of the intellectual sources of the 1301 drugs that were approved, world-wide over the 1981–2002 time frame. Examples will include agents directed against cancer-related targets, the AT1-R inhibitors, anticholestemic agents, ACE inhibitors and molecules that have activity against MRSA and Van^r staphylococci.

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Herbal medicines: a critical evaluation of their economics, safety, and effectiveness

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This presentation will primarily outline how the available data on the economics, safety, and effectiveness of herbal medicines can be evaluated in a critical way. **Economics**: Herbal medicines are big business in Europe. The first part of this presentation will therefore focus on the latest developments in their over-the-counter sales, together with recent figures on their reimbursement in large-scale consuming European countries. It will be argued that herbal prescription medicines with a high level of reimbursement should be submitted to the same rigorous pharmacoeconomic analyses as their synthetic competitors (De Smet et al 2000). **Safety**: The second part will outline that the safety of a herbal remedy is not some kind of static concept, but the dynamic result of various product-related and consumer-related factors. The most important consumer-related factors will be briefly outlined (De Smet 2004). **Effectiveness**: The third and final part of the lecture will emphasize that there is much more to be said about the quality assessment of herbal randomized controlled trials (RCTs) than the mere application of the Jadad method or a similar quality rating instrument. Some of the pertinent questions that should asked by any evaluator of herbal RCTs will be presented together with illustrative examples (De Smet 2002).

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De Smet, P. A. G. M., Bonsel, G., Van der Kuy, A., et al (2000) *Pharmacoeconomics* **18**: 1–7

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Towards Single-Molecule Array based DNA sequencing

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Our approach to genome resequencing relies upon proprietary Single-Molecule Array^{TM} technology. The single-molecule array chips will have up to 10^8 sites per cm^2, each site consisting of a single molecule in a random pattern on the chip surface. Attachment of the DNA is performed such that the molecules are stable, mono-disperse, uniform, universally primed, and allow extension by a polymerase. A step-wise sequencing-by-synthesis biochemistry that uses reversible termination and removable fluorescence has been developed. The optimisation of this sequencing technology to achieve longer read lengths at the single-molecule level is being carried out. By sequencing (e.g., 1.2×10^9 fragments on a 12-cm^2 surface, at 25-base read length) a ten-fold coverage of the haploid human genome is possible. This billion-lane sequencer has the ultimate potential to resequence mammalian genomes for a few thousand dollars in a few days per instrument. Our detection instrument has been designed to allow visualisation of single fluorescent molecules at high signal-to-noise ratios. It can resolve individual features at micron resolution while scanning at high speed across a large chip surface. A number of fluorophores have been evaluated for single molecule detection. The properties of fluorophores at the single molecule level will be discussed, along with data showing the characterisation of four uniquely detectable fluorophores amenable to DNA sequencing on the Single Molecule Array platform.

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A novel ultra-specific approach to detection of DNA sequences: signal-silent dye fragments assembled by their target to give fluorescent exciplexes

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This presentation describes a newly introduced fluorescence method for ultraspecific detection of nucleic acid sequences Douglas & Bichenkova 1999, 2000). The detector is split at a molecular level into two components, which, before a signal can be generated, must be assembled correctly into a particular 3-dimensional arrangement. This assembly process is effected only by the molecular structure of the target itself. The DNA assay system described is designed to have zero or negligible background because the components of the fluorescent detector are silent (with virtually zero emission at the detection wavelength) until assembled at and by their own specific target. The basis of the proposed split-probe exciplex detector system is as follows. To target uniquely a particular stretch of the human genome (10^9 base pairs) would need a long oligo-probe, approximately 18 bases. We use a tandem pair of short oligos, which we call an *Exci*Probe. Two antisense oligos (say 9 mers) with groups A and B appended to their internally directed 5′ and 3′ ends, respectively, are hybridised to their complementary target DNA or RNA. On correctly arranged binding to the correct contiguous target sequence A and B become juxtaposed in space. A and B are chosen to form an *exciplex* of a specific 3D structure. An exciplex is an excited state complex of A* (A in its photoexcited state) with B (in its ground state). The exciplex, stable only for nanoseconds, emits light of longer wavelength (100–150 nm Stokes shifts are common). In their ground states A and B repel each other at the short distances they achieve by their mutual attraction in their exciplex state, so they fly apart on emission ready for re-excitation. Fluorescent signal development can only occur when the A and B units are very close (around 4 Å), a situation enforced by the carefully designed final 3D-correct assembly of the detector. Thus, non-target sequences are silent (e.g. from annealing only of oligo-A or oligo-B to target, or with oligo-A and oligo-B not perfectly co-located on the target). For exciplex emission, distances between A* and B must be about the thickness of a benzene ring, which corresponds to the thickness of a single base pair. *This technique is not related to FRET methods.* FRET operates over much larger distances (usually between about 10 and 50 Å) and thus has poorer resolution. These novel dyes are photostable and show very low background compared with current market dyes. These exciplex fluorophore systems actually undergo a *visible* colour change from pale blue as single-strand probes to green on correct hybridisation. In contrast, other hybridisation dyes currently available only show a change in intensity with very little, if any, shift in emission wavelength on hybridisation. It is possible using this *Exci*Probe approach to pick up single or double mismatches (e.g. SNPs) efficiently.

Douglas, K. T., Bichenkova, E. V. (1999) US patent US 6,475,730 B1, granted 5th Nov., 2002. Filed Dec., 20th, 1999

Douglas, K. T., Bichenkova, E. V. (2000) PCT Int. Appl. WO 99-GB4207 19991220 GB 98-27912 19981219 CAN 133:85107

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The photochemistry of green fluorescent proteins — complexities and opportunities in bioassays

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Green fluorescent proteins (GFP) have produced a revolution in fluorescence light microscopy as probes of proteins within living cells. By manipulation of the sequence in and around the fluorophore, different colour variants have been produced to allow protein–protein interactions to be studied by co-localisation and fluorescent resonance energy transfer (FRET) within the cell. Protein dynamics have also been studied by fluorescence recovery after photo-bleaching (FRAP) and related techniques. GFP is becoming widely used in High Throughput Screening assays to investigate drug action. GFP and its variants also have interesting photochemistry that need to be understood for the optimal use to the above applications. The yellow fluorescent protein (YFP) variants, in particular, show sensitivity to H^+ and Cl^- and also the phenomenon of reversible photobleaching. In the case of the latter, bleaching by 514 nm light can be partially reversed by irradiation at 390 nm. Thus YFP is a potentially photoactivable probe for following the kinetics of cellular events. The nature of reversible photobleached state is not understood, but its properties show that care is required in using YFP in FRET-based assays because the wavelength used to excite the donor will also reactivate bleached YFP. Single molecule fluorescence measurements have shown that YFP blinks on the seconds time scale owing to slow protein conformational changes, which are coupled to ionisation of the fluorophore. The blinking rate therefore encodes the local pH.

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Understanding genomics: the future impact on pharmacy

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The ongoing biotechnological revolution and the completion of the human genome project have fuelled great excitement, optimism and expectation for the delivery of improved healthcare. Recent technological advances in genomics (the study of gene expression and function) and proteomics (the study of proteins and their function) have led to significant advances in understanding the relationship between our genes (genotype) and their role in the development of human disease and our variation in response to therapies (the so-called clinical phenotype). This new knowledge will likely have a dramatic impact on how we deliver healthcare in the future. Pharmacy, as a profession, will be a key player in this new vision of personalised medicine based on genomic ((or commonly termed pharmacogenomic) information (for review pertinent to pharmacy, see Akhtar (2002)). Genomics and proteomics knowledge is set to improve healthcare by increasing our knowledge of the genes or proteins that are, among others, important as diagnostic markers of disease or as potential therapeutic targets for therapy. In addition, genomics information will allow us to identify patients likely to respond to drug treatment and/or identify patients likely to undergo adverse drug reactions. These new developments, when applied broadly, are likely to improve therapeutic outcomes and reduce the costs of healthcare. However, there remain many challenges that we have to overcome if we are to understand fully the contribution of genomics and genetic variation (polymorphisms) to inter-individual differences in drug effects and to translate this new knowledge into clinical practice. These include appreciating the often multiple and complex effects of polymorphisms regulating the same drug's absorption, metabolism, distribution and elimination as well as the social, ethical and legal issues concerning the use or misuse of genetic information, and the possibility of incurring short-term additional costs during the transition to genetically guided decisions about drug therapy. However, in the long run, decreasing the frequency of adverse drug effects and increasing the probability of successful therapy will probably lower the cost of future healthcare. Pharmacogenomics has the potential to facilitate this process by translating knowledge of human genomics into better therapeutics.

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Targeting cell cycle genes for the treatment of cardiovascular disease

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Cardiovascular disease represents a major clinical problem affecting a significant proportion of the world's population and remains the main cause of death in the Western world. The majority of therapies currently available for the treatment of cardiovascular disease do not cure the problem but merely treat the symptoms. Furthermore, many cardioactive drugs have serious side effects and have narrow therapeutic windows that can limit their usefulness in the clinic. Thus, the development of more selective and highly effective therapeutic strategies that could cure specific cardiovascular diseases would be of enormous benefit both to the patient and to those economies where health care systems are responsible for an increasing number of patients. There is increasing evidence suggesting that targeting components of the cell cycle machinery using gene and peptide transfer approaches in cardiovascular cells provides a novel strategy for the treatment of certain cardiovascular diseases, including heart failure, restenosis and bypass graft failure. Those cell cycle molecules that are important for regulating terminal differentiation of cardiac myocytes (e.g. cyclins, cyclin-dependent kinases (CDKs), CDK inhibitors, E2F transcription factors), and whether they can be targeted in these cells using gene transfer to reinitiate division and myocardial repair, will be overviewed. Evidence will be presented to show that targeted overexpression of the cyclin B/CDC2 complex in cardiac myocytes leads to proliferation in these otherwise cell cycle-arrested cells thereby offering hope for regeneration of the damaged myocardium post-infarct (Bicknell & Brooks 2002). Furthermore, studies will be described that have used small peptides that block activation of the transcription factor, E2F, to abrogate the development of hypertrophy in cardiac myocytes since this might prove to be a useful therapeutic approach for preventing the development of heart failure in some patients (Vara et al 2003). Although certain problems are associated with using such approaches (e.g. ensuring sufficient and cell-specific gene delivery), the results of these studies illustrate the possibility of targeting cell cycle genes to improve cardiac function and prognosis for heart failure and for patients with atherosclerosis.

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Glivec: a rationally developed drug based on genomics

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Cancer is the most common genetic disease in the Western world, affecting 1 in 3, and is the second most common cause of death after heart disease. It is not one disease but more than 200 and, although many of these share mechanisms of carcinogenesis across tumour types, different neoplasms have widely differing pathophysiologies and underlying biochemistries. To exploit these differences, molecular biology and genetics have been used to identify and characterise components of the signalling pathways of normal and cancerous cells. From this research, together with epidemiological evidence, the deregulation of protein kinase activity has been shown to play a central role in the pathogenesis of human cancer. In particular, the molecular pathogenesis of chronic myelogenous leukaemia (CML) depends on the formation of the Bcr-Abl tyrosine kinase, a constitutively active fusion product of the Philadelphia chromosome. This abnormal chromosome arises from the reciprocal translocation of the long arms of chromosomes 9 and 22. In CML, the Philadelphia chromosome abnormality, and resultant Bcr-Abl protein tyrosine kinase, is seen in approximately 95% of all patients. Based on these observations, imatinib (Glivec) was developed as a specific inhibitor of the Bcr-Abl protein kinase. Imatinib competes with ATP for its specific binding site in the kinase domain and has been shown to be highly active in the treatment of all stages of CML. In chronic phase CML for example, haematological responses were achieved in 97% of patients and complete cytogenetic responses in 76%. Although the long-term survival benefits are yet to be confirmed in chronic phase, the cytogenetic response suggests a survival advantage. Fifty-five percent of patients in accelerated phase and 14% of blast crisis patients remain alive after 3 years of treatment. Imatinib has also demonstrated activity in the treatment of gastrointestinal stromal tumours (GIST's), which is driven by the deregulated tyrosine kinase activity of the Kit receptor. In a single study of 147 patients with unresectable or metastatic GIST, refractory to conventional chemoradiotherapy, imatinib monotherapy achieved a complete response in 1% of patients, a partial response in 67% and 16% had stable disease at 34 months follow-up. Imatinib is the first specific tyrosine kinase inhibitor introduced for the treatment of cancer. Over an exceptionally short period of time, with more than 12 000 patients enrolled in clinical trials, the observations of the remarkable efficacy and relative safety of imatinib have radically changed the management of patients with CML and GIST. Our understanding of the genetics of cancer has driven the development of imatinib, demonstrating that it is possible to produce a rationally designed anti-cancer drug.

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Pharmacogenetics in pharmacy practice

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At the moment there are many classes of drugs that have a low efficacy rate (e.g. those for treating Alzheimer's disease (30%) and cancer (25%)). It is expected in the future that the use of pharmacogenetic information will enable patients to be prescribed the right drug and dosage to improve efficacy, decrease adverse drug reactions and give therapy faster, saving money at the same time. Pharmacists are well placed to play their part in this because of their knowledge, experience and public trust. In addition, the Government wants to see more point of care diagnostic testing and pharmacies are convenient locations for patients. There are a number of pharmacogenetic services that can be performed by pharmacists: advising and counselling, taking samples and conducting tests, interpreting results, prescribing and advising, monitoring and reviewing the treatment prescribed, recording results and educating the public and other healthcare professionals. The Society will be providing practice guidance for the setting up of such services. This will include information on sources of funding, clinical governance issues, choice of equipment, training and advertising. In addition, the British National Formulary will contain relevant pharmacogenetic information on a drug by drug basis. Pharmacogenetic testing is currently carried out in central genetics laboratories using semi-automated equipment and technologies such as gene-chips, real-time polymerase chain reactions or sequencing. However, the development of new technologies and the identification of relevant single nucleotide polymorphs should allow appropriate pharmacogenetic tests to be performed at the point of care such as in a pharmacy. There are few examples of clinical practice being altered practically by pharmacogenetic knowledge. However, there are a number of candidate drugs who's toxicity and pharmacokinetics are known to be genetically controlled via polymorphic drug metabolising enzymes. The most well known examples are warfarin, omeprazole, tricyclic antidepressants, codeine, fluorouracil, mercaptopurine, azathioprine, isoniazid and irinotecan. The Food and Drugs Administration are looking at the possibility of adding pharmacogenetic information to the labels of four of these (warfarin, mercaptopurine, azathioprine and irinotecan) to improve their risk/benefit ratio in clinical use. Last year the UK Government published their white paper on "Our Inheritance, Our Future — Realising the potential of genetics in the NHS" looking broadly across genetics and health. It recognised the important role that pharmacists have to play in pharmacogenetics in the future. As part of this initiative, the Department of Health recently announced the six winning bids for $4 million to fund cutting edge research into pharmacogenetics. These research projects are expected to last one to three years and the results of half of the projects are expected to be used by the NHS or industry within five years of completion. It may be that the widespread clinical use of pharmacogenetic information for the benefit of patients is still some years away.

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Distinct element analysis of the Heckel analysis of bulk powder compression

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Bulk compression of particles is often used to deduce mechanical properties of individual particles and there have been a number of attempts to establish relationships between the two scales of single particle and bulk deformation. A widely used approach, particularly in the pharmaceutical industries, is the Heckel analysis where the applied load and bulk deformation are used to infer the yield stress of individual particles. However it is difficult to analyse this process rigorously and to ascribe any significance to the parameters quantified in the Heckel analysis because the individual particles are not loaded uniformly in the bed. The most appropriate approach for this purpose is the use of the Distinct Element Method to simulate the bulk deformation based on single particle properties. Our analysis shows that there is a critical ratio of Young's modulus to the yield stress of individual particles $(E/\sigma y)_{critical}$, above

which the Heckel analysis does reflect the effect of the yield stress, but below which it in fact reflects the effect of Young's modulus. Heckel's parameter is numerically equal to the yield stress of particles only for a certain value of $E/\sigma y$. For ratios higher than this value, Heckel's parameter can even exceed the yield stress of the individual particles.

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Multi-scale modelling of powder compaction: from granules to tablets

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As a drug delivery technique, tabletting has been known to the pharmaceutical industry for over 150 years, yet only very recently have modern computational approaches, commonly used in drug discovery and development, been applied to achieve a more fundamental understanding the process. In this talk, I will present computational work being undertaken in the recently established Pfizer Institute for Pharmaceutical Materials Science in Cambridge studying the relationship between the size, shape and mechanical properties of the constituent powders and the characteristics of the final compact. Our approach is to use discrete element models (also called granular dynamics) to parameterise appropriate constitutive equations (e.g. Druker-Prager cap model) for the powders, which can then be used in finite element simulations of the actual compaction process. The discrete element models incorporate well-defined elastic moduli and contact forces, including friction to couple the rotational and translational degrees of freedom of the particles (Dutt et al 2004). Currently, we have limited our studies to spheroidal particles, but plan to model frangible granules of arbitrary shape undergoing uniaxial or triaxial compression and simple shear. The finite element simulations were carried out using ABAQUS/Explicit, and we have found that density distributions produced are a close match to those found in real tablets (Wu et al 2004a). Our aim is eventually to link the molecular scale properties of the constituents to the mesoscale powder properties, and ultimately to predict the stress and density distributions in the final compact. At present, we are also carrying out experimental studies of pharmaceutical excipients and using novel imaging techniques, such as X-ray tomography and MRI, to analyse the structure of powders undergoing compaction (Wu et al 2004b).

For further information on the Pfizer Institute for Pharmaceutical Materials Science, please visit http://www.msm.cam.ac.uk/pfizer

Dutt, M., Hancock, B. C., Bentham, A. C., et al (2004) *Comp. Phys. Comm.* Preprint available

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Recent advances in compaction simulation and imaging: characterising the compaction process

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Powder compaction occurs by the action of a stress upon a powder bed. The powder then moves according to the direction of the resulting forces and the constraints imposed by the die wall and punches. These constraints result in varying density distributions that, in turn, affect the final properties of the finished tablet. Some of the key considerations in compressing a powder to form a tablet are: the flow and packing characteristics of the powder; the stress pathway followed during formation; the mechanical properties of the powder itself, including its behaviour under triaxial stress; and the resultant specimen properties, such as density distributions and stored elastic strains. These factors ultimately control tablet performance. Understanding what influences the mechanical properties of a tablet may then help to further the understanding of why problems such as "capping" and "lamination" occur. Enormous advances have been made in recent years in understanding the compaction mechanisms for pharmaceutical powders. A component in this renewed research has been the deployment of compaction simulators in research and development as well as in some pilot plant facilities. A compaction simulator essentially consists of an isolated tablet punch and die set, which is held in a rigid frame. The tooling is moved by hydraulic valves that are under computer control. The computer generates a displacement–time profile to determine the loading and unloading stress pathway for the compaction and ejection process. The punch displacements and the compaction and ejection forces are then measured. In addition the die wall may also be instrumented to give information on radial die wall stresses. In this way the entire compaction cycle can be carefully controlled and monitored. Changing the boundary conditions, such as the geometry of the punch and die set used, will inherently change the direction of the resultant forces, causing changes in the powder movement directions, hence producing differing density distributions. These density distributions can be important in affecting local properties of the material that, in turn, may for example influence the disintegration or dissolution behaviour or subsequent mechanical properties such as friability. These powder movements and density distributions can be characterised in a number of ways, such as by coloured layer analysis and steel shot deflection techniques. Non-destructive methods, such as surface profilimetry or nuclear magnetic resonance imaging (NMRI), can also elucidate structure. Hence, by a number of differing characterisation techniques the compaction process and its resulting impact on tablet properties can be scientifically assessed.

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Nanomedicines: a significant share of the non-generic market by 2010

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The difficulty of discovering and registering new NCE drugs at the end of the 20th century has coincided with the start of sales of biologicals coming from the biotech revolution, a decade or so before. When the growing generic sector is removed, nanomedicines become a significant growth area, an opportunity that has not on the whole been missed by major pharma. Two examples will be illustrated, Mylotarg (gemtuzumab ozogamicin), the first antibody drug conjugate (ADC) to get FDA approval and CDP 870, an antibody polymer conjugate currently in phase III. Mylotarg illustrates many of the issues with drug conjugates (Eaton 2002); its success also rekindled worldwide the antibody magic bullet approaches, popular over a decade before. Much effort is now going into improving what, at the time of Mylotarg, was state of the art technology, with the primary target remaining oncology. The talk will describe many of the barriers that were overcome in bringing together one of the most toxic molecules known and providing a non-immunogenic delivery vehicle targeting the CD33 positive cells (Hamann et al 2002a, b) responsible for acute myeloid leukaemia. Although the concept of targeting goes back to Ehrlich, its lack of demonstration in the clinic has proved to be a major barrier to commercialisation. Much of the work done in academia has lacked the robustness that is required to convert it into a manufacturable product. Solid tumours present problems to most if not all therapeutic approaches; these will have to be understood and overcome if this class of nanomedicine is to be clinically and commercially successful. Antibodies are extremely good targeting and internalising agents giving them an entrée to opportunities that NCEs will not be able to achieve. CDP870 can be described as a polymer therapeutic, it has an effector molecule: the antibody fragment, the other end of the block copolymer being polyethylene glycol. The role of the PEG is to increase the half-life of the fragment, such that a monthly injection in man is possible. The advantages of this type of polymer therapeutic will be discussed.

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Hamann, P. R., Berger, M. S. (2002a) In: Page, M. (ed.) *Tumor targeting in cancer therapy*. Humana Press Inc., Totowa, NJ, pp 239–254

Hamann, P. R., Hinman, L. M., Hollander, I., et al (2002b) *Bioconjug. Chem.* **13**: 47–58

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Applications of stealth liposomes in cancer therapy

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The pharmacokinetics of liposome-encapsulated drugs are controlled by the interplay of two variables: the rate of plasma clearance of the liposome carrier, and the rate of drug leakage from circulating liposomes. Coating of liposomes with polyethylene-glycol (PEG) inhibits the clearance of liposomes by the reticulo-endothelial system (RES), resulting in long-circulating liposomes referred to as Stealth. Bilayer rigidification using high T_m phospholipids coupled with an efficient drug loading methodology reduces the rate of leakage of liposome contents enabling liposomes to retain most of their drug cargo while in circulation. Another critical aspect of these liposome formulations is

their small vesicle size with a mean diameter equal or less than 100 nm. PEGylated liposomal doxorubicin (PLD) is a Stealth liposomal formulation with a distinct pharmacokinetic profile characterized by an extended circulation time, and a reduced volume of distribution (Gabizon et al 2003). Animal studies of tissue distribution of Stealth liposomes indicate preferential accumulation of Stealth liposomes into various implanted mouse human tumours, with an enhancement of liposomal drug tumour levels when compared to free drug. The ability of PEGylated liposomes to extravasate through the leaky vasculature of tumours, as well as their extended circulation time results in enhanced delivery of liposomal drug and/or radiotracers to the tumour site in cancer patients. In malignant effusions, Kaposi's sarcoma skin lesions, and a variety of solid tumours there is evidence of selective tumour uptake detected by various methods. PLD (Doxil, Caelyx) has been approved for clinical use in a variety of neoplastic conditions (Gordon et al 2001; O'Brien et al 2004) owing to its anti-tumour efficacy and unique safety profile with an impressive reduction of cardiotoxicity in comparison with conventional doxorubicin. Following the successful clinical application of PLD, further development of Stealth liposomal formulations to deliver other drugs, such as DNA-topoisomerase I inhibitors, cisplatin, and mitomycin C (MMC) is being pursued. Efforts are also being made at integrating Stealth liposomes with other therapeutic strategies such as radiosensitization, hyperthermia and ligand-specific targeting. Interestingly, efficacy and toxicity can be substantially enhanced or reduced depending on the type of drug tested. It appears that controlling the rate of drug release both in circulation and at the tumour site is critical for optimizing the added value of Stealth technology in tumour drug targeting. The potential added value of Stealth liposomal drug delivery and the opportunities it offers in combination with specific targeting ligands and other therapeutic modalities suggests a broad scope of pharmaceutical applications, which remain largely untapped.

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The design and development of pegfilgrastim (PEG-rmetHuG-CSF)

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Granulocyte colony-stimulating factor (G-CSF) is the primary regulator of the production of neutrophilic leukocytes. These cells are extremely sensitive to the effects of cancer chemotherapy and their absence leaves cancer patients liable to infection. A reliable surrogate of such infections is pyrexia and when detected in the absence of neutrophils is referred to as febrile neutropenia (FN). Filgrastim (r-metHuG-CSF) has been used in over 4 million patients worldwide for the relief of FN and has set a high standard for safety and efficacy. This recombinant protein has to be administered daily to closely mimic natural cytokine exposure. This frequent administration places a considerable burden on patients and health care resources leading in many cases to suboptimal use. A second-generation molecule was sought that would eliminate the need for frequent administration. The routes of clearance of the parent molecule were known to include renal loss and neutrophil-mediated destruction. Since neutrophils are themselves the product of G-CSF action it was hypothesized that a form of G-CSF engineered to evade elimination by the kidney, yet which retained sensitivity to neutrophil-mediated clearance, may present a unique form with a self-regulating feature. PEGylation of recombinant methionyl G-CSF attained by directing the covalent attachment of a 20 kD poly[ethylene glycol] to the N-terminal amino acid residue resulted in the creation of pegfilgrastim. Extensive screening of PEGylated G-CSF candidates suggested this form represented a desirable combination of prolonged retention time in-vivo and minimal loss of in-vitro activity. Preclinically, the profile of pegfilgrastim fulfilled the design criteria that had been prospectively established: a single injection was found to be suitable for chemotherapy cycles of various lengths; the formulation properties and stability of the parent compound were not compromised; the remarkable fidelity for the neutrophililc lineage of filgrastim was retained and in addition the new molecule gained the novel attribute of remaining sensitive to neutrophil-mediated destruction, but evading renal loss. Clinical development of pegfilgrastim progressed without significant issues, the adverse event profile proving to be identical to the parent compound and the benefit of a single treatment per cycle of chemotherapy was found to be broadly applicable. The novel pharmacokinetic profile which was observed in animals was found to extend to humans and suggested pegfilgrastim was capable of adapting to the impact of various chemotherapy treatments (degrees of myelosuppression) and also adapting to the idiosynchratic needs of each individual patient including the applicability of a fixed dose to a arnge of body weights. Post-launch activities continue to explore the limits of neutrophil mediated clearance in settings of mild and harsh myelosuppression and the limits of dose scheduling.

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Enhanced activity and tolerability of poly-l-glutamic acid drug conjugates: XYOTAX^{TM} (CT-2103) and CT-2106

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Macromolecular poly-L-glutamic acid (PG) drug conjugates can enhance the anti-tumour efficacy of cytotoxic compounds by providing prolonged tumour exposure to active drug while minimizing systemic exposure. The enhanced aqueous solubility of such conjugates further improves tolerability by eliminating the need for toxic solubilizing agents. The PG polymer is biodegradable (thus allowing for molecular weight optimization), contains multiple binding sites (giving a reasonable ratio of active compound to polymer), and, perhaps most importantly, creates a highly stable conjugate that preferentially releases the active compound within tumor tissue. XYOTAX^{TM} (CT-2103) is a water soluble, large macromolecule conjugate of paclitaxel and PG. Administered by short infusion without the need for routine premedication, the conjugate undergoes minimal hydrolysis in plasma. XYOTAX^{TM} takes advantage of the enhanced permeability of tumour vasculature and lack of lymphatic drainage to accumulate in tumor tissues. Bypassing MDR mechanisms by entering tumour cells through endocytosis, XYOTAX^{TM} undergoes intracellular metabolism by lysosomal enzymes such as cathepsin B to concentrate free paclitaxel in tumour cells. Preclinical studies demonstrate enhanced efficacy relative to paclitaxel in animal tumour models when administered as a single agent or in conjunction with radiation. Human PK studies demonstrate biphasic elimination of conjugated taxanes with an initial half-life of 7–10 h followed by an elimination phase of >10 days. Free paclitaxel represents only about 3% of the total measured taxanes in circulation. At doses of 175–210 mg m^{-2}, XYOTAX^{TM} has been generally well tolerated, even in patients with extensive prior therapy. Single-agent efficacy has been observed in a variety of tumour types, including lung, ovarian, breast and colorectal cancers. Side effects include short, predominantly grade 3 neutropenia, neuropathy, and rare and generally mild allergic reactions. Unlike with paclitaxel, alopecia and mucositis are rare. XYOTAX^{TM} is currently in phase 3 trials in advanced non-small cell lung cancer as first-line therapy in poor performance status (PS2) patients and as second-line therapy in PS0–2 patients. A second PG polymer drug conjugate, CT-2106, has also been developed. This conjugate links the topoisomerase inhibitor camptothecin through glycine to PG, not only providing enhanced solubility and biodistribution, but also preventing binding of the lactone ring to serum albumin through the 20-S OH group. Human studies have shown that albumin binds camptothecin with high avidity, rendering the compound inactive. Preliminary PK and efficacy analyses from a Phase I trial demonstrate favorable PK and evidence for anti-tumour activity. Reversible marrow suppression is the dose-limiting toxicity. Phase II studies of CT-2106 have been initiated. In summary, emerging data from clinical studies of XYOTAX^{TM} and CT-2106 appear to validate the hypotheses that PG conjugation can enhance the therapeutic effectiveness and tolerability of anticancer drugs.

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A DNA-dendrimer-based viral and non-viral hybrid drug delivery vector

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For gene therapies, viral vectors are by far the most efficient and effective means of DNA delivery, due to highly evolved, and specialized, viral proteins. However, enthusiasm for viral-mediated DNA delivery, unfortunately, has been tempered by growing concerns over the vector safety. Non-viral vectors, on the other hand, have much better safety profiles although they are still generally inferior to most viral vectors. A hybrid system that combines both viral and non-viral advantages may overcome the major barriers to DNA delivery safely and efficiently (Luo & Saltzman 2000; Luo et al 2004). To design such a hybrid system, only those viral components that are responsible for efficient delivery should be included. Non-viral polymers are also needed to serve as scaffolding to physically harness the power of viral components without the entire virions. We believe that DNA themselves are ideal polymers

to serve as delivery vectors. This is because DNA themselves are bona fide polymeric materials that posses many desirable chemical and physical properties. Although much progress has been made recently in DNA computing and DNA nanotechnology, the full utilization of DNA-based materials has not been achieved. This is in part due to the fact that almost all DNA molecules, natural or synthesized, are either in linear or circular forms, which severely restrict their usefulness (Luo 2003). Through nucleic acid engineering, we have created branched, Y-shaped DNA (Y-DNA) as novel building blocks and assembled, for the first time and in a controlled fashion, highly branched, tree-shape DNA dendrimers from Y-DNA (Li et al 2004). They were multivalent and anisotropic, making conjugation of different entities precisely controlled. A variety of entities, including viral peptides, can thus be conjugated separately onto each branch to target specific, individual barriers along the DNA delivery pathway. Indeed we have successfully assembled both in a solution and on a solid-phase a DNA-based, viral-peptide conjugated, anisotropic DNA dendrimer that served as a carrying vector for gene delivery. This viral and non-viral hybrid system successfully condensed plasmid DNA and crossed the cell plasma membrane by viral peptides. They were non-toxic to the cells and stable in the presence of serum. In addition, the gene expression was much higher than controls. We believe that this is the first time that a modular, viral/non-viral hybrid drug-delivery system that is using DNA molecules themselves as a polymeric carrier to delivery other DNA drugs has been realized. These data promise a unique, universal, efficient and specific viral and non-viral hybrid system for multi-drug delivery including gene delivery.

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Gene therapy: industrial opportunities and challenges

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Gene therapy is a platform technology for the delivery of the DNA that codes for proteins to be expressed in target tissues and organs resulting in the treatment of disease. The growing understanding of the human genome, proteomics and the relationships between genotype and disease provides a wealth of opportunities for the development of a wide range of therapeutic agents that treat the causes of diseases such as cancer, infection and tissue degeneration rather than the symptoms. It is nearly 15 years since the first gene therapy trial was undertaken and approximately 5000 patients in over 400 studies have been treated subsequently. Success has been limited and no products have been registered for sale with the Regulatory Authorities. There are significant challenges to be overcome and these have been the reasons for the limited demonstration of clinical benefit and the lengthy time for evolution compared for example with biologicals or small molecules. Based on an understanding of the protein(s) involved in a particular disease it is relatively straightforward to identify the DNA coding sequence using conventional molecular biological techniques. The major challenge is to deliver the DNA to the appropriate tissue to enable adequate expression and with sufficient duration to elicit a therapeutic response. Traditionally, non-viral and viral vectors have been utilised in which the DNA is complexed or incorporated to enable cellular and nuclear uptake to express the protein of interest. Each of these approaches has their advantages and disadvantages and presents different challenges. Viral vectors have been the most widely studied class predominantly because of their ability to give significantly greater transfection based on the evolved capability to infect cells and transport their own DNA to cell nuclei. The principal challenges are safety concerns (immunogenicity and insertional mutagenesis), the size of the DNA construct that can be incorporated and Manufacture and Quality Control difficulties. The magnitude of these challenges is virus dependent. There are a range of non-viral delivery vectors based on naked DNA, lipid-DNA complexes, cationic polymeric complexes and the inclusion of components of viral coat proteins to facilitate targeting and uptake. The major disadvantages are inefficient transfection in-vivo and short duration of expression. The greatest challenge is to design systems that can achieve comparable delivery characteristics to viral systems but without the associated safety concerns and manufacturing difficulties. The manufacture of plasmid DNA of high quality can now be undertaken on a large scale and the "formulation" and product development utilise available technologies. The development of all biological products is more complex than for small molecules although following the same basic principles. The challenges for gene therapeutics are associated with identifying the appropriate non-clinical Safety Assessment packages, developing the Chemistry Manufacturing and Control documentation and satisfying both the Regulatory Authorities and Ethics Review Committees.

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Cellular and intracellular barriers for macromolecular drug delivery

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Work on liposome mediated drug delivery over the last two decades has led to the concept that innate biological barriers have a major impact on the targeting and delivery of therapeutic agents. The impermeability of the cell membrane, the viscosity of extracellular matrix, and the active functions of mononuclear phagocytes all limit our ability to successfully target drugs. Many of the lessons learned in liposome research can also be applied to newer therapeutic approaches including antisense and siRNA oligonucleotides as well as viral vectors for gene delivery. These concepts will be illustrated drawing on our recent work on conjugates of cell penetrating peptides and antisense oligonucleotides as well as a novel viral vector for the expression of siRNA.

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Standards for raw materials

H. G. Kristensen

The European Pharmacopoeia Commission

The European Pharmacopoeia is a collection of quality standards presenting the acceptable quality of pharmaceutical substances and finished drug products, and the analytical means to assess their quality. The European Pharmacopoeia is the result of a remarkable international harmonisation work, which took its beginning in 1964. Today, the work on the European Pharmacopoeia is joined by 31 European countries and the EU Commission. In addition, the work is followed by 16 European and non-European observer countries and the WHO. The membership of the EU Commission reflects the close links between the Pharmacopoeia and the European regulatory bodies. According to European directive, the pharmacopoeia standards are mandatory in marketing authorisation dossiers. The standards and texts of the Pharmacopoeia are accordingly designed to meet the needs of regulatory authorities, manufacturers of raw materials and medicinal products, and those engaged in international trade and quality control. The 5th Edition of the European Pharmacopoeia is now available and will become effective by 1st January 2005. With this Edition the coverage of standards on active substances used in two or more countries is almost complete, apart from one manufacturer substance still being protected by a patent. The coverage of excipients used in marketed products is less complete. The 5th Edition presents some important developments within the standardisation of raw materials. The Pharmacopoeia Commission has decided to implement the principles and terminology of the ICH guideline on impurity control of active substances, not only new substances but also the already monographed substances. The decision was taken also because of the global supply to the European market of active substances. The first results were adaptations of the general monograph on substances for pharmaceutical use, and the presentation in the 5th Edition of a general chapter for information on impurity control. The next step is to revise and update a great many monographs to ensure they contain related substances tests and information on specified impurities. The work to make sure that the monographs reflect the purity requirements to marketed products is important also because of the Certification of raw materials. Close collaboration with European regulators is required in this development. The European Pharmacopoeia Commission has decided that in future more attention will be paid to functionality-related characteristics (FRCs) in monographs on excipients. While these characteristics are of importance for the intended use of many excipients, they are essentially a matter for agreement between the excipient supplier and the manufacturer of a medicinal product. For this reason, FRCs are to be dealt with in a specific non-mandatory section of the monograph and the particular use or uses for which a given FRC is relevant are to be specified. The essential aims of the new section are the following: to list the FRCs of importance for the various uses of an excipient, as information for manufacturers and licensing authorities; to indicate suitable methods that are already or will in the near future be included in the general chapters of the European Pharmacopoeia, thus encouraging the use of standardised methods and consequently more easily interpretable data for users; to state for information typical values and tolerances that have been found to be those of satisfactory products. It is assessed that the introduction of FRCs does not conflict with the international harmonisation of excipient monographs.

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Standards for products

D. H. Calam

Chairman, British Pharmacopoeia Commission

Historically, pharmacopoeias have had a dual role of defining formulae for medicinal preparations and of defining the quality of the ingredients used in them. The former role was especially important at a time when medicines were compounded in pharmacies. Today, most medicinal products are manufactured commercially and have to be licensed. Their approval for licensing depends on demonstration of their safety, quality and efficacy to the satisfaction of the appropriate regulatory authority. Quality is based on consistency of manufacture and on adequate analytical control of the product and its characteristics. Modern pharmacopoeias have evolved to reflect this change. Monographs for ingredients, both active substances and excipients, have become much more important while formulae and instructions for compounding have almost disappeared. Reflecting the view of many of its member countries, the European Pharmacopoeia does not have a policy of drafting monographs for specific medicinal products. Instead, it contains general requirements for dosage forms of many kinds that are binding on all preparations of a given type (e.g. tablets, creams). In this way, certain general properties of all formulated products are controlled. By contrast, some national pharmacopoeias in Europe (e.g. the British and Italian) and the USP contain monographs with analytical specifications for individual medicinal products. This presentation will review the development of pharmacopoeial requirements for medicinal products with a particular focus on the British Pharmacopoeia and its place in regulation of medicinal products. It will examine the role and influence of general monographs for dosage forms of the European Pharmacopoeia and the impact of international harmonisation of pharmacopoeial requirements in this sphere. It will also consider the interaction between specifications for active substances and those for dosage forms containing them as well as the impact of other constituents on analysis of dosage forms. Although tests for functionality-related properties have begun to be introduced into some monographs for individual substances, such tests do not usually form part of a pharmacopoeial specification. For products, however, control of properties such as dissolution and disintegration of oral dosage forms and performance tests of inhalation products is critical and the place of these in pharmacopoeial specifications will be examined.

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Global strategies for pharmacopoeias

R. Williams

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History of *United States Pharmacopeia (USP) and National Formulary (NF)*: The United States Pharmacopeial Convention (USP) publishes the *USP* and *NF*. Originally a book of recipes begun in 1820 by practitioners, the modern *USP* contains standards for pharmaceutical manufacturers and compounding professionals. The *NF* was originally published by the American Pharmaceutical Association in 1888, and contained monographs for mixtures widely sold but not recognized by the USP. Convention delegates meet at 5-year intervals (next in March 2005) to elect officers of the Convention, the Board of Trustees and the Council of Experts, and to consider resolutions. The Council of Experts creates content for two compendia, the original *USP* and, with its purchase in 1975, the *NF*. The current *USP 27-NF 22* contains monographs for approximately 4000 prescription and over-the-counter drugs, biologics, allied therapeutic products, and excipients. How Monographs Enter USP and NF: Submission of a Request for Revision that leads to a public monograph is voluntary on the part of manufacturers. With better control of an article's quality, a public monograph has moved beyond a 'one size fits all' approach to one that accounts for different routes of synthesis and different dosage form performance characteristics. Further information is provided in a Guideline (http://www.usp.org/standards/revisionguideline/index.html). Use of USP by Regulators: The original mandatory character of *USP*, to the extent that it existed at all, devolved from practitioners who declared it to be their "official" compendium. Over time, *USP* and *NF* have been adopted and made enforceable, by various bodies, including US federal and state governments and by other national regulatory bodies. USP has no enforcement authority, nor can it make legal requirements unilaterally. Global Use of *USP-NF* and Other Pharmacopoeias: Many countries throughout the world use a pharmacopeia as a means of controlling the quality of a therapeutic article. An important pharmacopeia for all countries is the International Pharmacopeia (IP) maintaining by the World Health Organization (WHO). The IP focuses on medicines contained in WHO's Essential Drug List, which includes only a small fraction of available drug substances and dosage forms. For these and other medicines, manufacturers and regulators can turn to monographs in JP, EP, USP, or to other national pharmacopeias. At a February 2004 meeting of the International Conference of Drug Regulatory Authorities (ICDRA), WHO arranged for a meeting — the first for ICDRA — of pharmacopeias, with several resulting recommendations, including one that WHO should organize an international meeting of pharmacopeias. Harmonization with Other Pharmacopoeias: Harmonization now occurs largely through the Pharmacopeial Discussion Group (PDG), composed of representatives from USP and the European and Japanese Pharmacopeias, with WHO as an observer. PDG meets twice yearly in conjunction with ICH. Harmonization focuses on both General Chapters and excipient monographs. Progress is slow for many reasons, including the need to engage volunteer experts from the three standards-setting bodies, the retrospective impact of harmonization on existing products in a marketplace, constrained resources, and other factors.

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Film coatings based on polymer blends for controlled drug delivery

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Polymeric film coatings are frequently used to control drug release from various types of solid pharmaceutical dosage forms, such as tablets, pellets and capsules. Several natural and synthetic macromolecules have proven to be suitable coating materials, providing different types of drug release behaviour (e.g. zero order kinetics, pulsatile and sigmoidal patterns). However, each polymer has specific physicochemical properties, and it is often difficult to obtain a particular, desired release profile that is adapted to the pharmacokinetic/pharmacodynamic characteristics of the drug. Generally, the coating level is varied to increase or decrease the resulting release rate, and different types and amounts of water-soluble and water-insoluble plasticizers are added, altering the drug permeability through the film coatings. But the variation of these parameters is restricted, because adequate mechanical film properties and processing conditions must be provided. Too low or too high coating levels, as well as extreme film coating flexibility/brittleness, must be avoided. A promising approach to overcome these restrictions is the use of polymer blends for film coating. By simply varying the polymer-polymer blend ratio the coating properties can effectively be altered and broad ranges of drug release patterns can be provided. Blends of enteric and gastro-intestinal-tract-insoluble polymers are of particular interest: in the stomach both types of polymers are insoluble, whereas in the intestine the enteric polymer becomes soluble and might leach out of the films resulting in dynamic changes in the physicochemical properties of the coatings. This type of drug delivery system might for example be used to render the release of basic drugs with distinct pH-dependent solubility (being freely water-soluble at low pH and poorly water-soluble at high pH) pH-independent. The decrease in drug solubility along the gastro-intestinal-tract can be compensated by an increase in drug permeability through the coating material due to partial polymer leaching at high pH. The effects of different formulation and processing parameters (e.g., type of polymer blend, blend ratio and coating technique (aqueous versus organic)) on the resulting drug release kinetics were studied. Furthermore, the physicochemical properties of the coated solid dosage forms and changes thereof upon exposure to different release media were determined using various techniques, such as scanning electron microscopy, differential scanning calorimetry and the puncture test. Based on these experimental results, the underlying drug release mechanisms were elucidated and the observed drug release kinetics could be explained.

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Materials science of film coating formulations

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To produce a film-coated product that exhibits the desired characteristics, the properties of materials used in the film coating process need careful consideration. This is necessary to ensure that the film formation process occurs satisfactorily, the film adheres adequately to the substrate surface and that the desired film properties are maintained after being subjected to handling and storage stresses. These desired properties may include, for example, physical protection of the dosage form or control of the site or rate at which the active component is released. Material properties that require consideration include solution/suspension properties, component solubility, film mechanical properties, film permeability, interaction between the components of the film and interaction between the film and the substrate. The dependence of these properties on the temperature and relative humidity to which the material is

exposed may also be of great importance. This presentation will discuss the material science of film coating formulations and highlight those properties that are of particular importance to the pharmaceutical scientist. Techniques used to assess relevant properties will be detailed and desirable characteristics identified. Data from research work in the area will be used to illustrate the importance of knowing the properties of the materials used in film coating and the potential consequences of using materials with inappropriate properties.

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Science, society and sustainability

H. Kroto

The lecture will explore numerous aspects of science. What science is, how others perceive science and scientists and some of the problems that non-scientists have in understanding the Science, Engineering and Technology (SET) upon which our modern world is so completely and precariously balanced. SET has truly revolutionised our lives. However, our technologies have also catalysed a mindless mass production driven plundering of the Planet's resources, which may be hurtling us towards disaster. We don't need an asteroid. For a 50:50 chance of surviving the next century each segment of society from industrialists, engineers and scientists to farmers and fishermen must now take this matter as the most serious issue the world has ever confronted. As for the science community they must develop as their priority a cultural spirit of sustainability. I see the greatest role in this whole area for Chemistry. I would like to see its whole ethos transformed and actually recognised as the Fundamental Science for Sustainability. Material from the Vega Science Trust (www.vega.org.uk), which makes TV and Internet programmes to improve public awareness and understanding of science and engineering (PAUSE), will be used to illustrate some of the issues.

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The use of increased throughput technologies to aid the rational selection of pharmaceutical salts

J. McCabe

AstraZeneca

This presentation describes the use of increased throughput technologies to screen for salts and polymorphs to aid in the rational selection of an optimum solid form. The use of salt and polymorph screening to find alternative solid forms of pharmaceuticals is an important step in the development of an API. Inappropriate nomination of a solid form can have important financial, regulatory and biopharmaceutical consequences. Formation of a salt provides a means of altering the physiochemical and resultant biological properties of an API without modifying its chemical structure. Traditional methods of salt and polymorph screening involve variation of crystallisation conditions (e.g. solvent, temperature, supersaturation and counterion (for salts)) in sequence. Experiments are usually performed in an iterative manner and can involve significant quantities of material and time. There is an increasing need for this activity to be carried out earlier in discovery when limited compound is available and this has its own associated problems of scale. This presentation will examine small-scale preparation and analysis of pharmaceutical salts and polymorphs. X-ray powder diffraction, optical/Raman microscopy and thermal analysis are used as the primary analytical tools for the identification of new solid forms. Examples will be presented showing the utility of this approach and how it enables the selection of an optimum solid form.

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High speed differential scanning calorimetry (HSDSC) and inverse phase gas chromatography (IGC)

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HSDSC and IGC are two relatively new methods for the characterisation of pharmaceutical materials. IGC conventionally has been used to produce a quantitative assessment of the surface energy of solids, in terms of a dispersive and an electron donor and an electron acceptor contribution. Issues remain with these numbers, both due to the theory used to calculate them (e.g. the use of a surface area of a probe vapour that assumes that the probe molecule is spherical) and also due to variability in instrument performance. Despite these problems, it is possible to find valuable applications for this method. Here we review new approaches to the measurement of glass transition temperatures (Tg) of amorphous solids and especially the ability to study the Tg of the surface of hydrophobic solids at any desired temperature and humidity, by use of analysis of the shape of the retention peak. HSDSC is a new approach in which the sample is scanned at a very fast rate to increase detection sensitivity. In this presentation the value of this approach will be discussed and data generated on different DSC instruments (Perkin Elmer Pyrus and Perkin Elmer Diamond DSC, respectively). The ability to detect Tg and a comparison of Tg values obtained by different methods will be discussed. It is clear that Tg is very much dependent upon method, with conventional scan rates, modulated DSC, HSDSC and IGC all giving different results for certain samples.

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The use of PAT in understanding dosage forms

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If PAT is to be understood within the widest terms as described by the FDA (2003) and, by implication, draft guidance from ICH, then it is a holistic approach to both developing and then manufacturing pharmaceutical dosage forms. PAT, and the scientific principles that underpin it, is used to first understand processes, and the underlying dosage form, and then control them. Within this there are a number of elements that are required including: formulation and process design using appropriate experimental design strategies; process mapping and quality risk analysis; measurements taken during the process as well as measurements to characterize the output of each process step; data mining and modeling of the process. This talk will illustrate how these principles can be applied to better understand raw materials, process intermediates and the final product. Examples of how technology can be used at or on-line to follow-up to contribute to process understanding as well as increase knowledge of the dosage form.

FDA (2003) Draft Guidance Document: PAT — A Framework for Innovative Pharmaceutical Manufacturing and Quality Assurance, U.S. Department of Health and Human Services, Food and Drug Administration, August 2003

ICH Q8 Pharmaceutical Development (draft)

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Molecular simulation of polymorphic phase transformations in crystals

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Pharmaceutical materials (excipients and active substances) often exist in a number of different crystalline forms or polymorphs. By utilizing an appropriate polymorph of a drug substance one can increase the bioavailability, improve the stability of the pharmaceutical, or ease the processing involved. Conversely, an inappropriate polymorph can give rise to problems of instability, where the selected form transforms (on storage or during manufacturing) to a more stable form with drastic consequences (e.g. a significant drop in bioavailability). Consequently, the regulatory authorities require that the polymorphism of any new drug substance is thoroughly investigated and that the stability relationships between the different polymorphs (interconversions) are fully characterized. While we have a good understanding of the many factors (e.g. temperature, pressure and humidity) that can influence phase transitions in crystals, our molecular level understanding is still rudimentary. Experimental methods, including synchrotron-based X-ray diffraction, are currently unable to reveal the molecular level processes taking place in crystal–crystal phase transitions. An alternative approach that is making a significant contribution in this area in providing insight, explaining experimental results and aiding the development of theory is molecular simulation. These studies have the potential to make the molecular mechanisms of phase transitions transparent, thus providing the fundamental understanding and framework for the possibility of controlling crystal-to-crystal phase transitions, and assisting in the rational design and development of materials and processes. Molecular simulations employ methods of statistical mechanics and atom-atom interactions to explore molecular level processes and to calculate thermodynamic quantities of interest. The most commonly employed technique is molecular dynamics (MD) simulations in which the trajectories of the interacting molecules are simulated using Newton's laws of motion. A key impetus to investigating crystal–crystal phase transformations by molecular simulations was the development of the Parrinello-Rahman boundary conditions (Parrinello & Rahman 1981) that enable the system to relieve the internal stress

anisotropically. Although many investigations have been carried out using the Parrinello-Rahman MD method, the applications have largely been restricted to simple systems. My laboratory at King's College London has been investigating the application of this methodology to study phase transitions as a function of temperature and pressure in simple systems such as the alkali halides and more recently organic molecular crystals. We have looked at effects of defects, namely vacancies, on the phase transition pressure as observed in simulations. Real crystals are never absolutely perfect and commonly have a high density of defects. These defects sites are the sites at which the transformations are initiated. The simulations revealed that the hysteresis (P_{trans} forward $- P_{trans}$ reverse transformation) in the phase transition pressures is significantly lower when the vacancy defects were present (Devani & Anwar 1996), which is entirely consistent with experimental observations. Previously simulation studies were invariably carried out on prefect crystals. More recent studies have attempted to investigate displacive-type crystal transformations in the amino acid DL-norleucine (Tuble et al 2004) and a reconstructive-type (where changes in hydrogen bonding are involved) transformation in crystals of resorcinol. For the DL-norleucine system we have also been able to calculate parts of the phase diagram as a function of temperature.

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Lighting up optical imaging: peptide- and antibody-based fluorescent dye conjugates as targeted in-vivo imaging probes

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Biomedical optical imaging is an emerging technology, which created promising opportunities for biomedical research and medical care in the past few years. Major driving forces have been both the instrumental advances in the area of laser and detection techniques and the chemical/biotechnological progress leading to novel fluorescent reporter systems. In particular, fluorescent dyes have demonstrated versatile utility as biocompatible probes for the biomedical optical imaging of diseases at the molecular level. The availability of specific fluorescent compounds reporting molecular characteristics of early diseases in-vivo is crucial for the application of optical imaging as a non-invasive, easy-to-use modality. We have established a cyanine dye platform based on indotricarbocyanines suitable for the conjugation to a desired targeting moiety (peptide, antibody, antibody fragment), which selectively binds to molecular targets in diseased tissues. The synthesis of different linker-modified derivatives and their application for bioconjugation will be illustrated for receptor-targeted peptide vehicles and angiogenesis-specific, bioengineered single chain antibodies (e.g. by employing sulfhydryl linking strategies). The resulting photophysical and in-vitro properties (fluorescence quantum yields, target binding) will be discussed. The potential for near-infrared tumour imaging in animals will be highlighted by different in-vivo models demonstrating that tumours can be detected at different locations and sizes with high contrasts.

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Targeting tumour blood vessels: perfusion MRI in phase I trials

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Selective antiangiogenesis and vascular targeting drugs hold out the promise of improved efficacy and tolerability for anticancer treatments. Early phase 1 drug trials have shown good tolerability for antiangiogenesis agents with biological activity below the maximum tolerated dose. Advanced clinical trials have demonstrated that morphological assessments of tumour response are of limited value in gauging the efficacy of treatment. MRI is a versatile technique that is sensitive to contrast mechanisms that can be affected by antivascular anticancer treatments; this use for MRI has been validated in xenografts and man. Dynamic contrast enhanced MRI (DCE-MRI) using small molecular weight gadolinium chelates enables non-invasive imaging characterization of tissue vascularity. Depending on the technique used, data reflecting tissue perfusion (blood flow, blood volume, mean transit time), microvessel permeability surface area product and extracellular leakage space can be obtained. Insights into these physiological processes can be obtained from inspection of kinetic enhancement curves or by the application of complex compartmental modelling techniques. Combining morphological and kinetic features can increase the accuracy of clinical diagnoses. Potential clinical applications include screening for malignant disease, lesion characterisation, monitoring lesion response to treatment and assessment of residual disease. Newer applications include prognostication, pharmacodynamic assessments of antivascular anticancer drugs and predicting efficacy of treatment. DCE-MRI can serve as pharmacodynamic indicator of biological activity for antivascular cancer drugs, helping to define the biologically active dose. DCE-MRI studies may also predict the efficacy of treatment on the basis of changes observed. If DCE-MRI is to be used for the selection of antivascular drugs that advance into efficacy trials then it will be necessary to develop standardized approaches to measurement and robust analysis approaches with clear accepted endpoints specified prospectively that have biological validity. Such developments will be essential for multicenter trials where it will be necessary to establish effective cross-site standardization of measurements and evaluation.

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Implications of malignant stem cells for the design of cancer therapies

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The continued growth of solid tumours indicates that they contain cells with the stem cell property of indefinite self renewal. Malignant stem cells, as the only cells capable of indefinite proliferation, are ultimately responsible for tumour growth, local recurrence and metastasis. They therefore form the necessary targets of cancer therapy but the size and differential properties of this potentially small sub-population have been uncertain. Cellular heterogeneity is a common feature of malignant cell lines and has usually been attributed to genetic instability and high rates of mutation. However, clonal assays indicate that heterogeneity is also a feature of normal keratinocytes in-vitro and is due to an intrinsic hierarchical stem cell pattern. We have examined cell lines derived from oral, breast and prostate carcinomas by similar clonal assays and shown that: holoclone and paraclone colony morphologies, typical of the stem and amplifying cells of normal epithelia, are consistently regenerated by tumour lines after cloning; the malignant cells forming holoclones differ by being small and rapidly adherent; and immunofluorescent staining, Affymetrix screening and Q-PCR indicate marked differential patterns of gene expression between the holoclone (stem) and paraclone (amplifying) cell populations. Thus both stem and amplifying cell populations can be readily identified in malignant cell lines and, as for normal epithelial stem cells, malignant stem cells can be shown to differ from amplifying cells in many aspects of their behaviour, including motility, growth properties and apoptotic responses. They are therefore also likely to differ in their responses to therapeutic agents. The ability to identify stem cells within malignant cell lines provides methods for analysis of the aspects of their differential responses that may have important implications concerning their therapeutic targeting for elimination.

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Addressing the challenges of bringing cell therapies to the patient

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Adult human cultured keratinocytes have been used successfully in the treatment of patients with extensive full thickness burns injuries since the early 1980s based on a 1975 methodology for the production of thin sheets of integrated keratinocytes. Long-term clinical survival of these cultured epithelial autografts (CEA) strongly suggests that CEA contain keratinocyte stem cells capable of continued proliferation. However, despite early and encouraging clinical success using cultured cells, the next phase of developing this into tissue engineered skin products has so far been disappointing in terms both of clinical uptake and commercial success. In this field, as in no other in tissue engineering, research has gone from a basic, pragmatic approach into over-inflated commercial expectations with a resultant negative impact on clinical progress. There is a need to set more realistic expectations for developing Tissue Engineered skin products which learns from some of the lessons of the last 10 years. The approach within the University of Sheffield, which has led to the spin-out company CellTran Ltd, is to go back to the early methodology of

culturing autologous keratinocytes as sheets of cells and seek to improve on the technology for taking cells from the laboratory to the patient to make the cells easier to use from the point of clinical application. A chemically defined plasma polymerised surface containing 20% carboxylic acid groups was developed which supports the attachment and proliferation of keratinocytes (France et al 1998). (This was launched as the product name Myskin in May 2004). Autologous keratinocytes are initially expanded in the laboratory using conventional methodologies and then transferred to the plasma polymer surface for no more than 2 days before transfer to the patient's wound bed. This allows rapid transfer of cells from the laboratory to the patient for major burns patients and it also gives flexibility of timing for repeated applications in chronic wound patients. Supplying the cells on an easy-to-handle polymer disc (currently 6 cm diameter) obviates the necessity of the surgeon or district nurse handling spray-on cells or fragile sheets of cultured cells. The application of Myskin to the wound bed is also compatible with other on-going treatment regimes for burns therapy and chronic wounds. Our clinical data to date shows accelerated healing of burns injuries following one application of autologous cells and slow steady sustained healing of chronic non-healing wounds with repeated applications of autologous cells (Moustafa et al 2004). The next challenge is to further improve on the culture methodology to avoid the use of bovine serum or murine mouse feeder cells (both routinely used in the initial methodology for expanded keratinocytes) to develop a xenobiotic-free culture. We have established that we can obtain rapid expansion of non-differentiated human keratinocytes grown on irradiated human fibroblasts in the absence of any foetal calf serum (Higham et al 2003). This culture system is now in pre-clinical evaluation.

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Periosteal stem cells in musculoskeletal tissue engineering

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The potential use of periosteum as an autologous cell source for the regeneration of both bone and cartilage tissue will be highlighted. Specific chondrocyte and bone precursor cells important for skeletal development in-vivo and during fracture repair reside within the cambium layer of the periosteum. Periosteum itself can serve as a template for directional evolution of a neo-tissue and inherently contains a source of growth factors. Here we examine the distinct phases of periosteum-derived neo-tissue development, namely cell proliferation followed by differentiation of the cells and active deposition of matrix. In particular, ways to enhance the initial mesenchymal cell proliferation and to achieve control over the final differentiation state of the cells are addressed. The use of raman microspectroscopy to monitor changes in the differentiation state of live stem cells will be discussed and differences in the raman spectra correlated with cellular events during differentiation.

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Stem cell lines for therapy: beyond the "state-of-the-art"

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There are accepted practices and guidelines for sourcing, harvesting, processing and storing tissues and cells taken from patients for transplantation. Similarly there are established guidelines for the evaluation, preparation and control of cell lines used in the manufacture of highly purified biological products from such lines. However, between these clearly defined product areas there exists a rapidly growing range of new therapeutic cell-based products, many involving in-vitro expansion of cell cultures, which can finally fall into different medical product groups but raise common issues for quality and safety not addressed specifically within the established guidelines and regulations for tissues and highly purified products derived from cell lines. This presentation with deal with the implications of this new group of clinical products for the process of sourcing and preparing seed stocks of cell cultures and their use as "starting materials" using the example of the UK Stem Cell Bank as a source of human stem cell lines. It will also review some of the special issues in taking cell culture processes from a rapidly developing research and development background into a pharmaceutical production environment.

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Urine analysis — an MHRA perspective

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Analysis of constituents in urine has been the mainstay of 'scientific' disease diagnosis for thousands of years. That ants were attracted to the sweet taste of urine from diabetics was recognised by the Egyptians; that cloudy urine was a marker for infection was apparent in the middle ages as was the presence of bile as a signal for liver disease. These tests were truly performed at the Point of Care. The accessibility of urine as a fluid for investigation was convenient but that its preparation by the kidney as a fluid free of protein was a great analytical bonus. Laboratory analysis has made urine a prime medium for the diagnosis of secreting tumours of the adrenal cortex and medulla, it has enabled quantitative measurement of glomerular and tubular function and has made urine the first port of call when, in the newborn, suspicion falls on genetic metabolic diseases. Until non-invasive analytical techniques progress from science fantasy to science fiction, urine will remain key to Point of Care testing. The Guildford MHRA Evaluation Centre has twice investigated the ubiquitous urine dipstick, and has recently assessed the merits of stick readers. Those stalwarts of preliminary clinical assessment are not without deficiency. Immunoassay, that exquisitely sensitive and specific method, which literally revolutionised hormone analysis in the late 60s, is now the domain of domestic fertility and pregnancy testing. The spotlight on sports' drug testing has moved to the growing market, drugs of abuse testing, again a competitive protein binding immunoassay which, in its cheap POCT format, challenges the scientist with issues of analytical quality and presents society with ethical questions. The Guildford MHRA Evaluation Centre has reported on 16 of these DOA devices and is currently reporting on 5 devices that measure albumin in urine as an early marker of diabetic complications. The former devices have infiltrated many walks of life and the latter have recently become an essential tool for monitoring all patients with diabetes mellitus. The early Egyptians and the 21st century diabetologists both made urine analysis at the point of care a cornerstone of their clinical practice.

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Pharmacogenetic testing

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Reports of adverse events in response to medications are not new, but now they are appearing in the popular press rather than just being a mater of scientific research buried away in an academic journal. There is now a wealth of academic study linking genetic markers that vary between people, and the individual's response to medication. The opportunity therefore arises for the pharmacist to bring benefit to an increasingly expectant population by offering genetically-based advice on appropriate medication dosage for the individual. Historically such pharmacogenetic testing science has been the preserve of reference laboratories, but now simple test procedures from non-invasive samples, such as a mouth swab, can lead to a DNA test result within a little over 15 min. The process is being designed to work within the non-laboratory environment. While the first applications of such point-of-prescription diagnostics will focus on avoiding adverse reactions, it is expected that in the not too distant future there will be a new generation of medications that will be prescribed on the basis of a efficacy via a pharmacogenetic test. For a number of years a number of drug data sheets have contained in their small print a section relaying additional information and advice noting that the metabolism of the medication is subject to a enzyme that can vary in activity due to genetically predetermined variation. Most importantly some single point changes in the DNA code can inactivate the gene to create a poor metaboliser. Tests to identify single or a small number of genetic changes are suitable for the emerging new point of care technologies. Presently pharmacogenetic tests are not standardly available within the NHS and only undertaken by a limited number of independent testing companies. An important opportunity for pharmacists will be to undertake such tests and to create appropriate patient records for the electronic patient records of the future. The role of the pharmacy may also be empowered by such simple tests to better define the illness to which the prescription is targeted. This may well start with rapid point of care

genetic tests to identify the underlying nature of flu-like symptoms, sore throats etc. before selling unnecessary antibiotics.

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Bioadhesive drug delivery to the upper GI tract

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The oesophagus is a muscular tube, approximately 25 cm long with a diameter of 2 cm, that joins the pharynx to the stomach and serves to move boluses of food, drink or drug formulation from the oral cavity into the stomach. Local diseases that affect the oesophagus include achalasia, dysphagia, reflux disease, oesophageal cancer, Barrett's oesophagus and candidiasis. The impact of these disease states on Western population is not insignificant; oesophageal candidiasis is a common AIDS-defining disease and the incidence of oesophageal cancer is increasing within the UK. In particular, heartburn or acid regurgitation occur at least weekly in 15–20% of the general adult (UK) population (Locke et al 1997). The oesophagus as a site for drug delivery has been much overlooked in comparison with the remainder of the gastro-intestinal tract. The low permeability and transient nature of the oesophagus means that it is unsuitable for delivery of drugs for systemic action. However, oesophageal disorders may be treated using locally acting agents that offer the benefits of reduced dosage and decreased side effects. Historically drug delivery within the oesophagus has been the result of tablets or capsules becoming lodged, leading to localised oesophageal injury. Novel test systems were devised to measure the force of adhesion between ex-vivo oesophageal tissue and a variety of dosage forms, demonstrating that gelatin capsules showed the greatest adhesion followed by film coated tablets then uncoated tablets followed by sugar coated tablets (Swisher 1984). Bioadhesive liquid formulations that adhere to the oesophagus upon swallowing may be utilised to deliver agents directly to the oesophagus. These liquids are either designed as internal bandages to protect against gastric reflux or as vehicles to carry drugs to the site of action. The design of a system targeted to the oesophagus requires a high viscosity to retard transit through this organ and thus leave a coating within which drugs may be retained. Additional strategies include the use of "Smart" systems that form gels at body temperature and those that gel at designated pH values. The high forces involved in a swallow may also be exploited to produce a shear-thickening product that gels in response to this force. The release of drugs from the carrier and their local action must also be examined in the design of a drug delivery device targeted to the oesophagus. Everted ex-vivo oesophageal tubes, ex-vivo oesophageal strips and synthetic substrates have been used in the design of apparatus to measure the adhesion of liquid formulations. Most models include washing to mimic saliva flow, although few account for the presence of peristaltic waves and ingestion of food and drink. Recent work has been performed using ex-vivo test apparatus that examined the retention of liquid formulations, including sodium alginates (Batchelor et al 2002), polyacrylic acid (Smart 2004), Smart Hydrogel, Carbopol and HPMC (Potts 1997) and sucralfate formulations (Antepsin, Gastrogel, Ulcogant) and Carbopol (Dobrozi et al 1999). Comparison of the results suggests that Carbopol exhibits the greatest retention within these in-vitro studies.

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Electro-responsive drug release from chitosan hydrogels and microparticles in-vivo

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'Smart' drug delivery vehicles, which release their drug load in a predictable and reproducible manner in response to an internal or external chemical, physical or biological stimulus, may provide optimised drug delivery, for example when mimicking the in-vivo pulsatile release of endogenous chemicals, such as insulin. Electro-responsive drug release from hydrogels is being investigated in many laboratories, including our own and many in-vitro studies have been published (for a review, see Murdan 2003). Meanwhile, there has been only one in-vivo study, showing drops in plasma glucose levels following two pulses of electrical stimulation of a subcutaneously implanted hydrogel containing insulin (Kagatani et al 1997). Our aim was to investigate the in-vivo electrical responsiveness of chitosan hydrogels and microspheres. The latter have the advantage over hydrogels in that they do not need surgical implantation, but can be easily injected. Diclofenac sodium (DFNa) was used as the model drug. Drug-loaded chitosan hydrogels and microspheres were prepared by methods modified from Ramanathan & Block (2001) and from He et al (1999), respectively. In-vitro studies showed that the two formulations released loaded drug in response to an applied electric current (Jahan & Murdan, 2004). The in-vivo studies were conducted on anaesthetised male Wister rats. The gel and the microspheres were hydrated in deionised water for 30 min and 24 h, respectively, before surgical implantation (gel) or subcutaneous injection (microspheres) under the shaved abdominal skin. Pulses of electrical current (0.4 mA, 0.5 mA cm^{-2}) were then applied for 10 min at 0, 30, 60 and 90 min using Ag/AgCl resting ECG electrodes placed on the shaved skin of the rat. The anode was placed on top of the implant while the cathode was placed 2 cm away, still on the shaved abdomen. The experiment was followed for 2 h. Blood samples were taken from the tail vein at time zero and after every electrical stimulus and the plasma was analysed for diclofenac sodium by HPLC. Passive release experiments (control) were conducted in the same way, except that no electric current was applied. We found that under passive conditions, some drug was released from both hydrogel and microspheres, probably due to diffusion along the concentration gradient. Upon electrical stimulation, drug release from both hydrogel and microspheres was increased with respect to passive conditions. This is attributed to drug electrophoresis towards the oppositely charged electrode (gel and microspheres) and electro-induced gel deswelling, with concomitant expulsion of drug from the hydrogel. A pulsatile electro-responsive release of the drug was obtained from the hydrogel, but not from the microspheres formulation. With repeated electric pulses, the extent of drug release from the hydrogel decreased. This could be due to reduced gel responsiveness and deswelling and/or reduced drug content in the hydrogels. We also found that electrical stimulation of microspheres resulted in a burst drug release, followed by a slow and steady release. This profile mirrored that of the control experiment, except that it was twice the extent of passive release. To conclude, we have shown a pulsatile electro-stimulated drug release profile from chitosan hydrogel. A pulsatile release was not shown from microspheres; however, drug release was higher under the influence of an electric current. Further work should be conducted to optimise the electro-responsive drug release in-vivo.

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Prodrug strategies for enhanced percutaneous absorption

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While most drugs are designed primarily for oral administration their activity and stability profiles that may be desirable for this route often make them unsuitable for transdermal delivery. Therefore, we are interested in designing analogues of currently-used drugs for which the sustained steady-state blood plasma level associated with transdermal delivery (and which is usually unattainable orally) would be particularly beneficial. These prodrugs are based on chemical bonds that are readily metabolised to the original, therapeutically active molecule. Mathematical modelling studies (e.g. Potts & Guy 1992; Moss & Cronin 2002) have determined that the main predictors of percutaneous absorption are the molecular weight and lipophilicity of the drug. For absorption from aqueous vehicle these may be modelled as MW and octanol/water partition coefficient (log P):

$$\log kp = 0.74 \log P - 0.0091\ MW - 2.39$$

where kp is the permeability coefficient (cm s^{-1}) (Moss & Cronin 2002). Methods of synthesis, purification and characterisation of the methyl to hexyl captopril-carboxyl ester prodrugs are described elsewhere (Moss et al 2003). Polydimethylsiloxane (Silastic, PDMS) and porcine skin were used as the membranes, and secured in Franz-type cells ($n \geq 3$). Each prodrug was at a concentra-

tion (e.g. 0.02–0.80% w/v) that gave consistent thermodynamic activity. The cells were maintained at $37 \pm 1°C$, sampling the receptor compartment over a 24-h period. Analysis was by UV spectrophotometry and LC-MS with diode array detection, and results were presented as maximum flux (J_{max}) and permeability coefficient (k_p). The permeability coefficient increased with an increase in the carbon number of the carboxyl ester group. For example, the permeability coefficient was approximately 44 times greater for the butyl prodrug than for the parent molecule. However, recorded flux values peaked for the ethyl and propyl prodrugs and dropped thereafter. This latter effect is most likely due to the significant decrease observed in aqueous solubility as the prodrug carbon number increases. The differences observed in k_p and J_{max} as lipophilicity and molecular weight increase would suggest that, at low aqueous solubility, J_{max} provides a more realistic measure of drug transport. Substantial metabolism of the prodrugs was observed, with first-order degradation kinetics being apparent. For example, the methyl ester prodrug was completely converted to its parent drug after 24 h. This study indicates that the prodrugs produced revert back rapidly to the physiologically active parent molecule and suggests that the enhanced percutaneous absorption exhibited by these molecules is complimented by their metabolic performance. The change in physicochemical properties of the prodrugs (i.e. they are oils at room temperature), compared with the parent molecule (a crystalline solid), may be significant in enhancing the permeability more substantially than predicted by QSPR modelling.

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Topical drug delivery strategies for photodynamic therapy

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Photodynamic therapy (PDT) is a medical treatment by which a combination of visible light and a sensitising drug causes the destruction of selected cells. A drug without dark toxicity is introduced into the body and accumulates in rapidly dividing cells. A measured light dose of appropriate wavelength is then used to irradiate the target tissue. This activates the drug and elicits the toxic reaction in the presence of oxygen (Zawislak et al 2002). To date, the applications of PDT have been limited to areas of the body easily accessible to a laser or incoherent light source. Consequently, PDT has been largely investigated as a treatment for tumours and neoplasias of the skin, bladder, mouth and female reproductive tract. Compounds of high molecular weight (> 500 daltons) have impaired ability to cross the *stratum corneum* barrier of the skin. Therefore, despite a few isolated studies pre-formed photosensitisers, which are generally large highly conjugated molecules, are not commonly used in topical PDT. This, coupled with their inherent lack of selectivity, means that 5-aminolevulinic acid (ALA), with a molecular weight of 167.8 daltons, is the most frequently employed agent in modern topical PDT. ALA is a small, water-soluble, prodrug and is a naturally occurring precursor in the biosynthetic pathway of haem. Topical administration of excess exogenous ALA avoids the negative feedback control that haem exerts over its biosynthetic pathway and leads to selective accumulation of the photosensitiser protoporphyrin IX (PpIX) in rapidly dividing cells (Donnelly et al 2003). The rapid development of the field of topical PDT based on ALA and its lipophilic ester derivatives has seen the publication of numerous lab-based and clinical studies. The largely experimental nature of this area has meant that the vast majority of these reports have focussed primarily on the drugs and the outcomes of treatments. Due to the success of these initial investigations, topical PDT has become an established treatment option for a variety of surface lesions. The time has now come for the rational design of dosage forms for the optimised delivery of ALA and its derivatives. This point has often been highlighted as the important next step in the development of topical PDT. The process of rational dosage form design takes into account the physiochemical properties of the drug and dosage form, the nature of the biological barriers to drug delivery, the anatomy of the body site to which the drug must be delivered and the drug release kinetics desired. Purpose-designed dosage forms for topical delivery of ALA or its esters for PDT include creams containing penetration enhancers, pressure sensitive patches and bioadhesive patches (McCarron et al 2003).

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A chemometric approach to the analysis of complex reactions studied by isothermal heat-conduction microcalorimetry

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Isothermal calorimetry is now widely accepted as being a valuable tool for the study of a number of diverse systems ranging from stability of explosives to the quantification of microbial systems. In particular the pharmaceutical industry uses calorimetry for the study of stability and excipient compatibility of individual components and complex formulations. Calorimetry lends itself very well to such studies because of its non-invasive non-destructive nature. It has been previously shown (Willson et al 1995) that calorimetric data, with the aid of appropriate equations, can reveal quantitative information on the kinetic and the thermodynamic (e.g. rate constant, k, enthalpy, H, and order of reaction, n) nature of the system under scrutiny. The direct calculation of equilibrium constants (K, and, by extension, $\Delta_R G$ and $\Delta_R S$) from such data has also been described (Beezer et al 2001). The methods have been developed further to the analysis of solid-state reactions. These analyses, to date, have mainly dealt with simple (single-step) reaction mechanisms. Pharmaceutical formulations generally contain large numbers of ingredients and as a consequence the returned calorimetric data will reflect this complexity. For successful analysis of the thermodynamic and kinetic parameters associated with these systems requires that the data be separated into its component species (which in themselves must reflect a simple reaction mechanism). Successful attempts have been made to derive the equations that describe complex reaction mechanisms but as will be discussed these are unsatisfactory for several reasons. In the only published (Gaisford et al 1999) example of a complex system prior knowledge of the constituent reactions was a pre-requisite. We present here a model-free, chemometric approach to the analysis of such data. It is shown to return target data successfully.

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New safe medicines faster, how, when and by what means: the EUFEPS initiative

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The problem: The pharmaceutical industry faces increasing challenges like spiralling research and development (R&D) expenditure, fewer high-selling products (i.e. "blockbusters"), stagnating or even increased drug development times, falling productivity in terms of new molecules brought to the market and large investments in new technologies which have not yet borne fruit. The situation concerns all of us: the patients who do not get the new innovative medicines fast enough; society, which will have to reimburse the exploding cost of medicines; the industry, which cannot find the capital to fund all the risks of development; the regulatory agencies whose fees are linked to the declining numbers of applications; and the universities who lose students when graduates cannot get jobs in the industry. **The analysis**: Thus it is obvious that all stakeholders see the need for change, but for many reasons coordinated initiatives have been few. In 1999 the European Federation for Pharmaceutical Sciences, EUFEPS, initiated through its Committee on Industrial Relations started to address this complex problem. It became clear that only through the combined effects of academia, regulatory agencies and industry would it be possible to successfully address the problem. As a result of these discussions a position paper "New Safe Medicines Faster" (NSMF) was formulated (Bjerrum 2000). It was followed up by a workshop held in March 2000 in Brussels. The analysis showed that downstream drug development had many bottlenecks but allowed room for methodological and technical improvements. **A solution**: Massive research investments in exploratory and clinical drug development to make this part of the process more efficient, faster and, more importantly, predictable. Major focus areas concern modelling and simulation, biomarker research, clinical assessment and safety science in general. Since this part of the development process is under regulation, extensive validation work is required before methodological and technical advances can be

implemented. Also it became clear that the only independent body large enough to financially support this part of the process was the EU Commission, which explains why it has been the target for EUFEPS' lobbying (Bjerrum 2002). **Activities**: Faithful to the analysis performed and its mission "to serve and advance excellence in the pharmaceutical sciences and innovative drug research", EUFEPS initiated a campaign to raise awareness and support for the NSMF initiative (e.g. through congresses and conferences, workshops and participation in EU activities). The latter included direct contact with the Research Directorate at the Commission, where all reports also have been sent. Further, similar national initiatives (e.g. FIN, NL, DK, AT) have been supported. Also, an NSMF cluster in the pan European industry association EUREKA has been formed (www.NSMF.org). **Outcome**: Support for the pharmaceutical sciences to the value of €300 million in FP6 (2002–2006) on dedicated projects in drug discovery, exploratory and clinical development. A further €1 billion for FP7 has been aired if industry engage heavily in the endeavour! Many highly relevant projects have been launched. **Future**: Taking a 10-year perspective on what really is needed to reverse the current situation, a radical overhaul of drug development and approval based that on the latest (and future) available methodology will be the solution. However, this requires extensive and pre-competitive validation work. The key question being here, who pays? Without doubt, co-ordination is needed. A start could be the formation of a European Technological Drug Development Platform, supported by EU Commission, industry, regulatory agencies and academia. EUFEPS has engaged in the preparative work for such a platform (www.EUFEPS.org). Join forces with us.

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Human microdosing (Phase 0 studies): an approach to smarter drug development

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The expenditure on research and development by the pharmaceutical and biotechnology industry has almost doubled over the past ten years while the number of new drugs being registered worldwide has fallen very significantly. Consequently the price of marketed drugs has increased in order for the industry to recoup their costs. There will be very few patients or healthcare systems that will be able to afford new medicines if this trend continues. Indeed the situation has become so grave that the US FDA has recently published a White Paper entitled 'Innovation or Stagnation' (US FDA 2004) that highlights the Agency's concerns. In particular they are critical of the failure of the pharmaceutical industry to adopt new tools in drug development in contrast to the discovery area. There is general agreement among the informed research community that drug development relies too much on animal and in-vitro models and that these can be poor predictors for man. New approaches are therefore being sought that permit earlier, safe human studies since it is recognised that such studies might prevent drug development failures later. One approach, which is attracting considerable interest, is human microdosing. Microdosing studies (Human Phase 0) involve the administration of trace drug doses to obtain essential metabolism and pharmacokinetic (PK) information at an early stage of drug development. The amount of drug administered in microdose studies is sub-pharmacological and hence the safety testing required is much reduced compared with conventional Phase I studies (European Medicines Agency CPMP/SWP/2599/02/Rev 1). Microdose studies are reliant upon having ultrasensitive analytical methods available. One such technique, known as accelerator mass spectrometry (AMS) and originally developed for radiocarbon dating is now being increasingly used in drug research in a wide range of applications including microdosing (Lappin & Garner 2003). This presentation will describe the AMS technology and how it is being used in microdose studies. PK data will be presented comparing microdoses versus pharmacological doses for a number of drugs and how this information can be used as an aid in candidate selection.

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Appreciating primary clinical efficacy endpoints of neuropharmacological drugs as a guide to directly formulating them into modified release dosage forms

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Clinical and pharmaceutical professionals have consistently sought efficient ways of developing drugs and thereby attempting to streamline the whole process. One such approach is the reason for having these meetings where two pharmaceutical disciplines (i.e., biopharmaceutics (principles governing the development of optimized drug delivery systems) and clinical pharmacology (use of PK-PD)) can be integrated early in drug development to *directly* design controlled- and modified-release dosage forms of new drugs. A drug development paradigm that has evolved in the pharmaceutical industry is reaching 'proof of principle' very quickly (i.e., does the drug work in the patient as expected). This therefore provides for a very early opportunity of understanding the primary relationship between exposure (PK) and response (PD). The major objective of a controlled release dosage form (CRDF) is to achieve a prolonged therapeutic effect while minimizing unwanted side effects due to fluctuating plasma drug levels. They allow for sustained blood levels, which in turn provide for a prolonged and consistent clinical response in the patient. Further, by producing less fluctuation, the CR product produces less unwanted side effects. The two most important requirements for preparing a CRDF are demonstration of safety and efficacy and demonstration of controlled drug release. The advantage may be related to better efficacy and reduced toxicity. The talk blends the best of both sciences, viz., medicine and pharmaceutics, where directness in understanding the primary efficacy measures for various diseases can pinpoint clearly to the early development of CRDF for a given drug. By recognizing early that a drug is showing efficacy (Proof of Principle in early Phase IIa studies), the strategy of drug development could be changed dramatically that the pursuit be towards formulating a CRDF directly, and therefore, not having to pursue the current paradigm of first developing an immediate release dosage form followed by its CR counterpart. Several examples from various neuropharmacologic drug classes, such as anti-epileptics, antispastic agents, antidepressants and anti-Parkinson agents, will be presented. To efficiently pursue development of CRDF every pharmacokinetic study (particularly those with immediate release dosage forms in patients) should incorporate an element of understanding validated primary efficacy measures and, conversely, every efficacy and toxicity trial should incorporate some element of pharmacokinetics. Knowledge so gained and acquired during drug development can be put to efficient, effective, and timely use in developing CRDFs.

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Ultrasound theranostics: antibody-based microbubble conjugates as targeted in-vivo contrast agents and drug delivery system

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One major challenge facing the pharmaceutical industry today is to develop contrast-enhancing agents for molecular imaging. Classic contrast agents primarily document the anatomy. For pathophysiological examinations using differential diagnostic techniques, in other words, characterising the development of a disease, they are only suitable to a limited degree. Molecular imaging selectively tracks down molecules and cell structures to be able to establish proof of diseases at a very early stage — and then to make decisions on a highly individual treatment. The next straightforward vision of medical imaging quite clearly lies in the concept "Find, Fight and Follow." In radiopharmaceuticals we are already pursuing the approach of a triad consisting of early diagnosis, therapy and therapy control. Utilising the nanotechnological concepts of colloid- and interface science imaging on a molecular level can also be achieved via diagnostic ultrasound using tiny gas-filled polymer particles coupled to target-specific ligands (Lindner 2002; Blomley 2004; Hauff et al 2004). Additionally, nanosized polymeric drug carriers for targeting and controlled release have been extensively studied in the past. Here, a nanoparticle or capsule acts like a container for a pharmacologically active agent. Passive and active targeting can be attained by carefully chosen size and surface modification of the carrier. Drug release can be controlled via desorption of surface-bound drugs, diffusion through the particle matrix or the capsule wall or matrix erosion. Moreover, a 'smart' release can be achieved by using smart-polymers (pH or temperature sensitive) or, more interestingly, by applying an external stress to the drug carrier. If the drug carrier is appropriately designed, release can be induced by diagnostic ultrasound (Bekeredjian et al 2003). Building a bridge between therapy and diagnosis opens

the field of "Theranostics". With a "Find, Fight and Follow" strategy, the tissue of interest first can be imaged via target-specific ultrasound contrast particles. In a second step, the same particles, now filled with a pharmacologically active agent, can be used for therapy. Finally, monitoring of treatment effects is possible by sequential imaging. This early approach demonstrates the success of a resolute implementation of nanotechnological concepts in a medical application and will be presented with special emphasis on polymer nanoparticle and microcapsule formation, the control of colloidal structure, surface modification and the resulting in-vitro properties as an "Ultrasound-Theranostic". Investigations with different drugs and targeting sites demonstrate that the approach can serve as a platform technology. In-vivo results will be addressed briefly.

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Drug delivery evaluation and dosage form development using positron tomography

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A clinical trial shows that asthma patients fail to respond to a new drug formulation, though the active ingredient is highly potent. Why? Does the drug fail to reach the deep lung sites where it needs to be? Does it reach the sites only to be rapidly absorbed and metabolized? Or is it a failed drug, remaining in the lung for hours but failing to produce a response? Can changes in formulation correct the problems? If so, which changes will be successful? Knowledge of the detailed regional deposition and pharmacokinetics of the drug can answer these questions. To have that knowledge during the drug formulation process helps us select good formulations and avoid failed clinical trials. PET imaging of radiolabelled active drug ingredients, such as [C-11] triamcinolone acetonide, [C-11]flunisolide or [F-18]fluticasone propionate, coupled with MRI or CT anatomic imaging, can determine quantitative regional tissue pharmacokinetics in the human lung, nasal passages and sinuses. Initial deposition, measured in micrograms of drug per cc of tissue, and drug absorption rates, wash-out rates, residence times and redistributions are parameters that can be measured. More traditional PET functional measurements (perfusion, metabolism, enzyme and receptor concentrations) can be used in correlation with regional deposition and kinetic measurements to provide information about how drug action couples with drug distribution. Effective use of the PET methods can allow us to produce better drug formulations, eliminate poor drug candidates at an early phase, and in some cases avoid elimination of good drugs that fail due to poor initial formulation. The information obtained can also be used to better understand the action and use of the drugs, to understand unexpected clinical results, to support claims made to regulatory agencies and to improve the quality and impact of the message disseminated for marketing efforts.

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Harvey, Hounsfield and HIF-1: bi-functional PET/CT imaging of drug targets in cardiovascular disease and cancer

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Cardiovascular disease and cancer are the first and second major causes of death in western societies. Hypoxia-inducible factor 1(HIF-1) is a transcription factor that plays a major role in both conditions, limiting ischaemic damage in stroke and myocardial infarction and promoting tumour progression. HIF-1 regulates the expression of more than three dozen genes including those that modulate glucose metabolism, vascular tone and angiogenesis. In 1972, the Nobel Prize winner Sir Godfrey Hounsfield introduced computed tomography (CT). Recently developed imaging systems that combine CT with Positron Emission Tomography (PET) have allowed the anatomical information from CT to be integrated with the physiological information from PET. Furthermore, novel contrast-enhanced CT techniques for perfusion imaging have re-positioned CT as a technique that can also depict vascular physiology, a transition that parallels William Harvey's demonstration of the circulatory nature of blood flow at a time when comprehensive knowledge about the structure of the vascular system had existed for centuries. Thus, by combining CT perfusion imaging with fluorodeoxyglucose (FDG) PET, a bi-functional PET/CT approach offers simultaneous in-vivo assessment of both the vascular and glucose metabolic effects of HIF-1. Examples include the demonstration of increased blood volume and FDG uptake in the ischaemic penumbra surrounding cerebral infarction, flow-metabolism mismatch in ischaemic but viable myocardium and changing flow-metabolism relationships with tumour stage in lung cancer. Regulation of HIF-1 activity and/or its downstream effects are current or potential targets for drug therapy in cardiovascular disease and cancer. Bi-functional PET/CT imaging has the potential to assist the development of such drugs through proof of principle, selection of dose and schedule, provision of early response markers and identification of sub-populations enriched for response.

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Clinical diagnosis to quantitative techniques in early drug development: can we do it?

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A significant problem facing the pharmaceutical industry is that 75% of the expenditure in research and development is on failed candidates. The bottleneck is no longer limited by the generation of targets and chemical ligands but in determining those that will be efficacious in the clinical environment. This needs to be done efficiently and as soon as possible in the drug-development programme. Taking attrition of drug candidates early will add value to the success of the compounds that go through to full development. Robust biomarkers need to be developed to enable this attrition to take place and the identification of new chemical entities (NCE) that have the necessary attributes. For these reasons it is likely that future therapies will include a "treatment package" for individual patients involving single drugs and combinations. To achieve this patients will need to be "characterised" to enable therapies to be optimised for individual needs. This characterisation is likely to include techniques such as metabanomics, genomics, imaging techniques, laboratory biomarkers, etc. In addition combinations of these diagnostic techniques will need to be implemented through multivariate analyses to enable signatures of disease states, both in populations of patients and individuals, to be identified. In this way biomarkers of efficacy and mechanism related to the characterisation of a patient's symptoms would give early signals of a compound's potential success. In parallel with monitoring the efficacy of an NCE it is paramount that we understand its safety profile. This needs to be addressed in a similar way, involving both the elucidation of the patient's profile and that of the NCE. The environment for developing biomarkers for safety and efficacy involves academia (where the principal motivation is diagnosis and treatment), the diagnostic business (here the focus is on the production of diagnostic techniques to aid the clinician's decision-making steps) and the pharmaceutical industry (we need quantitative techniques for safety and efficacy). In broad terms the pharmaceutical industry is engaged in technology development of quantitative biomarkers that is different from other needs in the clinical arena, but there are some synergies with routine clinical practise. (Of course diagnostic procedures are necessary for identifying patient populations and need to be developed in parallel.) This talk will explore the necessary steps we need to take to achieve quantitative biomarkers suitable for drug development. In particular it will concentrate on early clinical development where localised measurements of organ function will be particularly important. The role of imaging techniques and potential "surrogates" will be highlighted.

Secdrive:klww:Bill:general: Clinical diagnosis to quantitative techniques in early drug development

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Phage therapy: bacteriophages as natural, self-replicating and self-limiting antibiotic, a historical perspective

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Bacteriophages are viruses that only infect bacteria. Each has its own narrow range of bacterial hosts. Bacteriophages are ubiquitous in our world — in the oceans, soil and deep sea vents, the water we drink and the food we eat. It is increasingly clear that they are the most abundant living entities on earth — about 10^{31} in total — and play key roles in microbial balance in every ecosystem. Felix d'Herelle discovered phages in 1917 while fighting dysentery in French soldiers, and soon successfully applied them in treating people and

animals around the world. Many entrepreneurs and companies quickly jumped into the field. However, while there were marked successes, the results were inconsistent, probably due to poor understanding of the heterogeneity and ecology of both phage and bacteria, phage inactivation, and frequent lack of appropriate in-vitro testing against the bacteria in question. The approach was largely dropped in the West once broad-spectrum antibiotics became available. The escalating incidence of antibiotic-resistant bacteria, coupled with growing concern about the negative side affects of antibiotics, is leading to widespread renewed public and scientific interest in the possibilities of phage therapy, taking advantage of modern molecular tools and increased knowledge about the biology, ecology and diverse nature of phages. This increased understanding is being coupled with awareness of the extensive prophylactic and clinical experience with phage in the former Soviet Union, involving millions of people. Phage therapy there has reportedly been particularly successful against endemic diarrhoeal disease and against osteomyelitis, diabetic ulcers, gangrene and other localized purulent infections, frequently obviating, for example, foot amputations in diabetic ulcers and osteomyelitis. In such cases, the phage keep multiplying and moving deeper, whereas antibiotics penetrate poorly, leaving major pockets of infection and creating selection for antibiotic resistance with decreasing concentrations. These results fit well with various US experiments from the 1940s that answered fundamental concerns but were lost from view in the refocusing of phage research toward development of molecular biology and the enthusiasm over antibiotics. For example, early animal studies led to widespread conviction that phage are too rapidly cleared by the immune system to be useful. However, those studies involved intravenous injection of healthy animals. René Dubos (1943) elegantly showed that phage *are* seen in the mammalian circulatory system when they enter from a reservoir in other tissues and the animal is carrying a bacterium in which they can multiply. When he injected mice intracerebrally with a lethal dose of *Shigella* and intraperitoneally with appropriate phage, he saw brain levels over 10 million phage/g and blood levels over 10 000 mL^{-1} until the bacteria were destroyed. Such papers and other relevant material are available at www.evergreen.edu/phage. While such reports are tantalizing and encouraging, there are unfortunately few that meet Western criteria for double-blind clinical trials, which are badly needed. Finding funding for such trials, in the West and in Tbilisi, is complicated by the fact that different phages are needed against different bacteria, phage cocktails are used to inhibit the development of resistance, and there are intellectual property complexities.

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Transferring the technologies to the marketplace

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Novolytics

Phage therapy has been around for decades in Eastern countries but only now, since the increase in bacterial resistance to antibiotics, has it generated interest from the Western world. One of the reasons for the lack of companies that provide these products is the shortage of intellectual property. There is a large amount of prior art and so establishing a leading patent position is a formidable task. This has certainly deterred the larger pharmaceutical companies from commercialising this type of product, but the once strong foothold on developing novel antibiotics is fast becoming a serious problem as microbes become increasingly resistant. So what are the barriers to commercialisation of phage therapy? Firstly, the issue of IP, which is crucial for venture capitalists who will want to see that a technology and product are well protected from competitors. This is difficult to achieve given the prior art on the use of phage in therapeutics. It is therefore hard to convince investors of the future protection of the products; in addition to which there is no defined regulatory route to market. Secondly, there is a lack of regulatory approval for such products in the West. What is the best way through the regulatory hurdles and what are they? The FDA has been extremely cautious with Exponential Biotherapies (USA) phage product for VREF and stipulated that they must only use a single phage product in clinical trials. What will happen in the UK and Europe? Novolytics is a phage therapeutics company in the UK that has been in discussion with the MHRA about the use of a phage cocktail as the best type of product to combat MRSA. It is thought that a cocktail approach is best because of the broader host range of the different phage on the cocktail that will kill the majority of relevant host strains. Some phage companies have avoided the complex route to human products altogether and have concentrated on producing phage for use in veterinary applications, sterilisation, or in diagnostics. This talk will highlight the major issues on transferring the technologies to the market place and will attempt to draw attention to ways in which these can be overcome.

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Cannabis as a medicine

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Although cannabis has been used medicinally for many thousand years, the recent discovery of endogenous cannabinoid receptors and their ligands — the endocannabinoids — have prompted great interest in medicinal uses for cannabinoids. Low levels of water solubility and first pass metabolism have stimulated the search for alternative modes of administration, including sublingual, inhaled and suppository formulations. There is also great interest in the "Holy Grail" of cannabinoid formulation — an orally administered compound with low psychotropic effects, but significant therapeutic benefit. Cannabinoids are currently being used to treat nausea, enhance appetite in AIDS and cancer patients, reduce certain involuntary movements, improve neuropathic pain, reduce muscle spasticity, improve bladder control and as neuroprotective agents. Most information has been derived from cannabinoid use in treating multiple sclerosis (MS). The MRC-sponsored Cannabinoids in MS (CAMS) study was designed as a short-term symptom relief study, concentrating on reducing muscle spasticity in patients with relatively stable MS. The results from the main 15-week study demonstrated large patient-perceived benefit, but little objective confirmation of beneficial effects. Results from more prolonged 12-month follow-up have shown significant reduction in muscle spasticity and provide some preliminary evidence for an improvement in disability, which could be consistent with neuroprotection. There is now an urgent need for further well-designed prospective randomised controlled trials to evaluate whether cannabinoids are neuroprotective in MS and other neurodegenerative conditions.

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Biomimetic self-assembling polymeric nanosystems for drug delivery

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Over the last decade there has been increasing interest in the potential use of nanoparticulates to prolong the vascular circulation time of drugs and for the targeting to sites of increased vascular permeability such as sites of inflammation and tumours. These systems need to be engineered to overcome particulate removal from systemic circulation by the mononuclear phagocyte system (MPS) while allowing specific drug release at the site of action. Previous studies have shown that polyethylene oxide polymer coatings may be used to prevent the adsorption of proteins and the adhesion of cells through steric stabilisation of surfaces in aqueous solutions. Although various polyethylene oxide (PEO) block copolymer micelle-based drug delivery systems have previously been investigated, there is growing evidence that some of these systems can elicit an immune response that results in macrophage activation and subsequent scavenging of the micelles (Moghimi & Hunter 2001). Phosphorylcholine-based polymers offer an alternative means of reducing protein adsorption and cell attachment by mimicking the surface of natural phospholipid membrane bilayer, which presents a thermodynamic hydration barrier to surface biofouling (Long et al 2003). Although drug delivery has been evaluated from PC-based coatings, the development of PC-based nanoparticulate self-assembling drug delivery systems has been limited by the inability to synthesise methacrylate-based block copolymers with controlled molecular architectures. However the recent developments in atom transfer radical polymerization (ATRP) has provided a viable route for the synthesis of a wide range of well-defined block copolymers with low polydispersities. We have recently demonstrated that this approach can be used to synthesis pH-responsive PC-based block copolymers (Ma et al 2002). A key aspect of any nanoparticulate drug delivery system is the ability to efficiently load and subsequently maintain the drug in the nanoparticulate whilst in systemic circulation. The use of pH-responsive PC-based block copolymers offers the ability to produce tunable systems, which spontaneously form micelles above a certain critical pH. Block copolymers incorporating poly(amino methacrylates), such as poly (2-(diisopropylamino)ethyl methacrylate), therefore offer the opportunity to engineer nanoparticulate systems that can be tuned to ensure both efficient drug loading and drug release at the site of action. This paper will describe the physicochemical and toxicology characterisation of a series of novel PC-based nanoparticulate drug delivery systems that were developed to engineer a system

stable at physiological pH (pH 7.3), which would releases a loaded drug at sites where local pH is reduced due to inflammation or tumour growth.

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Polymer-mediated intravascular drug delivery: an open and shut case

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The advent of drug-eluting stent (DES) technology has heightened research interests in the area of drug-device combination products. The single digit levels for restenosis that are being realized in clinical practice demonstrate the effectiveness of device-modulated drug therapy for maintaining vessel patency post-stenting. Of those stents that have made it to market, the best use polymer coatings primarily as delivery vehicles for holding and controlling the release of the drugs. Polymers based on phosphorylcholine have been shown to provide a highly flexible biocompatible platform from which a variety of different therapeutic agents can be delivered. These materials are used on commercially available devices (Dexamet coronary stent) and are in clinical trials for DES products from Abbott Vascular Devices and Medtronic Inc. With intravascular drug delivery a very real proposition, attention has turned to other devices that may be used as drug carriers. Embolisation microspheres have been designed as calibrated devices for intravascular use to block a desired blood vessel that may, for instance, feed a tumour. Microspheres based on poly(vinyl alcohol) have been modified to carry the chemotherapeutic agent doxorubicin and have been shown in animal models to deliver high doses of the drug locally to the tumour site (Drug-eluting Beads, DEB). These devices are currently in clinical trials in Asia and Europe for the treatment of primary liver tumours. This presentation describes some of the characteristics of our polymer drug delivery systems and illustrates how the combination devices are effective at either maintaining blood vessels open or shut.

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New polymeric excipients — a challenge for industrial polymer research

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Polymeric excipients are functional polymers that fulfil multiple tasks in the drug delivery process with solid dosage forms. They work as binders, matrix and/or coating for tablets and enable the oral intake of drugs that are on their own not compressible to a tablet. Apart from their role in tablet manufacturing, novel polymeric excipients are being developments to control the drug release and drug stability or even improve the bioavailability of active ingredients. New polymeric excipients can help to improve existing drug formulations and make new therapeutical systems available. Innovations in formulating are becoming more and more an essential part in the development of new pharmaceutical products. Protecting sensitive active ingredients against oxidation or hydrolysis in the gastric juice, controlling release and delivery of drugs has become the major challenge for novel excipient development. Approx. 40% of all new chemical entities synthesized by pharma companies are poorly water-soluble. Making these drugs available for oral dosage forms is the ultimate formulation challenge. The field of synthetic polymers offers a very broad window of opportunities for the development of novel excipients. Advances in polymer research have deepened our insight into correlations between chemical synthesis, production processes, molecular and supramolecular structures and end-use properties. Functional polymer systems can be designed from the molecular to the macroscopic level and thus bulk and surface properties in the solid state, in disperse systems and in formulations can be controlled. Choosing the appropriate monomer composition for the polymer can make the coating of tablets pH-sensitive. Carboxylic group containing polymers become soluble at pH 4.5–6.5 and thus the solid dosage forms pass the acidic environment in the stomach unharmed. Amine-containing polymers give protective and insulating coatings that disintegrate in the acidic environment of gastric juice. Controlling the micro phase structure of a polymer blend of one insoluble polymer (polyvinyl acetate) and a second soluble polymer (polyvinylpyrrolidone) leads to water-insoluble coatings, which can be used for pH-independent sustained drug release. Combining two water-soluble polymer components (polyvinyl alcohol and polyoxyethylene) on a molecular level creates a water-soluble polymer that forms excellent flexible coatings with pH-independent instant release properties. The flexibility of such coatings is due to supramolecular interactions that only work in the solid film. Thus, the aqueous solution of such a polymer can be handled at relatively high concentrations, which is an important factor for the spray coating process of tablets. Hydrophobic supramolecular interactions can be used to design matrix retard systems. Powders of hydrophobically modified water-soluble polymers can be compressed to tablets that show moderate swelling in a aqueous environment and therefore gives rise to a typical diffusion controlled drug release. The molecular architecture of polymers, the polymerisation technology itself or the way a polymer is dissolved in water influences the mesoscopic structure of the polymer solution. These facts make functional polymers attractive candidates as solubilisers for poorly water-soluble drugs.

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Bioactive biomaterials for medical devices and implants

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The use of medical devices for temporary or permanent implantation has become increasingly common and constitutes a major advance in modern medicine. Quality of life is improved immeasurably when medical devices are successfully deployed. Unfortunately, concomitant with this increased use is an increased frequency of complications. In particular, the problem of device-related infection afflicts all medical devices and can cause morbidity and mortality in many instances. To illustrate the extent of this, figures from the USA show that approximately 2 million hospital patients develop nosocomial infections each year with approximately 80% of the 80 000 annual deaths being device-related. At, perhaps, the simplest level, urinary tract infections occur in about 20% of patients with urethral catheters in place for more than 10 days, and in more than 40% of patients after 25 days. The estimated cost of treating nosocomial bloodstream infections in the USA is $1 billion with a persisting mortality of up to 20%. The key to resolving the problem of device-related infection is to prevent bacterial adherence to the device biomaterial and the inevitable formation of antibiotic-resistant microbial biofilm. Silicone (polysiloxane) has gained widespread acceptance as a biomaterial for use in implants and indwelling medical devices. This is due to the low risk of unfavourable biological reactions and the favourable patient comfort associated with its use compared with other biomaterials. However, in common with all device biomaterials, silicone is prone to surface formation of a highly resistant microbial biofilm. Silicone also lacks inherent lubricity and its high coefficient of friction causes pain on device insertion and tissue trauma on removal. An additional problem is platelet adhesion and fibrin blockage of the device lumen. However, significant reductions in these several complications may be realised with the development of novel bioactive silicone materials. Novel highly lubricious condensation-cured silicones can be produced to provide sustained release of an exudate on the biomaterial surface while retaining the mechanical properties of the conventional medical grade silicone. The drug delivery potential of this silicone platform technology is of particular interest as the novel silicones can provide an active entity such as an antimicrobial drug to the device surface. A range of antibiotic and non-antibiotic antimicrobial agents exhibit significant inhibitory, cidal and anti-adherent activity indicating the potential of these bioactive biomaterials for manufacture of medical devices with reduced opportunity for device-related infection. Persistence of antimicrobial activity within the bioactive silicones is also demonstrated in relation to successive microbial challenges.

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Life-cycle management of prescription drugs

P. R. Gellert

AstraZeneca

Today, large Pharma face greater competition for market share, growing costs for both R&D and Sales & Marketing and increasing pressure on product prices. Lifecycle Management Strategies for prescription drugs have therefore grown in importance in recent years as large Pharma seek to maximise the return on their investment to continue to fund the discovery, development and commercialisation of innovative new medicines. Such strategies include defending patent rights, utilising the available regulatory benefits and adding additional product value through new formulations and indications. Lifecycle Management Strategies for prescription drugs will be discussed and illustrated with specific examples from the industry.

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Product life-cycle management in Consumer Healthcare

S. F. Jones

GSK

Consumer Healthcare is a very dynamic, cut-throat business. Patents that could prevent a competitive threat for your product or your positioning are a rarity. The consumer is king, and will determine whether your product gets bought or not – there is no prescribing. Development cycles are short, and there is no shortage of competitors to mimic or undercut your product. Brand development is key, and differentiation from the generics paramount. No brand = no business. Against this backdrop, there are many Consumer Healthcare brands worth many £millions, and have been sold for 50 or even a 100 years. This presentation will discuss a Consumer Healthcare brand from GlaxoSmithKline that has been sold for over 80 years, and has sales well in excess of £100million per annum. The presentation will cover some of the science behind the brand and it's development over the years, as it moves from strength to strength.

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ABC transporter activity in the blood–brain interfaces: consequences for drug delivery to the CNS and possibilities for mitigating their influence

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The blood–brain barrier (BBB) and the blood–cerebrospinal fluid barrier (BCSFB) form a very effective barrier to the free diffusion of many polar solutes into the brain. Essential metabolites that are polar in nature have their brain entry facilitated by specific inwardly-directed transport mechanisms. In general the more lipid-soluble a molecule or drug is, the more readily it will tend to passively partition into brain tissue. However, a very significant number of lipid-soluble molecules, among them many useful therapeutic drugs, have a significantly lower brain permeability than would be predicted from a determination of their lipid solubility. These molecules are substrates for the ABC efflux transporters that are present in the BBB and BCSFB and the activity of these transporters very efficiently removes drug substrates from the CNS, thus limiting their brain uptake. P-glycoprotein (Pgp) was the first of these ABC transporters to be described, followed by the multidrug resistance-associated proteins (MRPs) and more recently breast cancer resistance protein (BCRP). All are expressed in the BBB and BCSFB and combine to reduce the brain penetration of many drugs. This phenomenon of multidrug resistance is a major hurdle when it comes to the delivery of therapeutics to the brain, not to mention the problem of cancer chemotherapy in general. Therefore, the development of strategies for bypassing the influence of these ABC transporters and for the design of effective drugs that are not substrates and the development of inhibitors for the ABC transporters becomes a high imperative for the pharmaceutical industry. The problems are compounded by a poor understanding of the structure–activity relationships between the transporters and their substrates.

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Drug delivery to the CNS using cell targeting peptides

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The blood–brain barrier (BBB) poses a formidable obstacle when attempting to deliver drugs to the brain. As new drugs for neurological disorders are discovered, new delivery techniques will have to be developed in concert to overcome this transport barrier. While researchers have devised many ingenious approaches that avoid, disrupt or exploit the BBB's specialized transport mechanisms, many of these continue to have significant drawbacks. During the last decade, several peptides have been described, such as SynB vectors, penetratin and Tat that allow the intracellular delivery of polar, biologically active compounds in-vitro and in-vivo. These peptides, belonging to various families, are heterogeneous in size (10–18 amino acids) and sequence. However, all these peptides possess multiple positive charges and some of them share common features such as important theoretical hydrophobicity and helical moment (reflecting the peptide amphipathicity), the ability to interact with lipid membrane and to adopt a significant secondary structure upon binding to lipids. The facility with which they cross the membrane into the cytoplasm, even when carrying hydrophilic molecules, has provided a new and powerful tool to deliver drugs across the BBB. SynB vectors have been successfully applied to brain delivery of the anti-cancer agent doxorubicin, the drug-like peptide dalargin and large molecules, such as the protein streptavidin, across the BBB. To conclude, the use of peptide vectors presents a promising avenue for development. Their small size, rapid uptake, ease of drug attachment and versatility in the range of molecules that they can deliver, provide a new approach to develop new drugs for the treatment of CNS diseases.

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Drug delivery to the CNS using nanoparticulate systems

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The blood–brain barrier (BBB) represents an insurmountable obstacle for the delivery of a large number of drugs to the central nervous system (CNS). One of the possibilities to overcome this barrier is drug delivery to the brain using nanoparticles. Drugs that have been transported into the brain and led to a pharmacological effect after intravenous injection using this carrier include the hexapeptide dalargin, the dipeptide kytorphin, loperamide, tubocurarine, doxorubicin and the NMDA receptor antagonists MRZ 2/576 and MRZ 2/596. To achieve a significant transport across the BBB the coating of the nanoparticles with polysorbate 80 (Tween 80) or other polysorbates containing 20 polyoxyethylene units was required. Other surfactants were less successful. After binding to the polysorbate-coated particles, dalargin as well as loperamide exhibited a dose-dependent antinociceptive effect after intravenous injection as determined by the tail-flick and the hot plate test. This effect was accompanied by a Straub reaction and was totally inhibited by pretreatment with naloxone, indicating that it is a central effect and not peripheral analgesia. After brain perfusion of rats with tubocurarine bound to the polysorbate-coated nanoparticles epileptic spikes were observable in the EEC of the rats but not with the controls. The very short anticonvulsive response of the NMDA-receptor antagonist MRZ 2/576 was increased from below 30 to 300 min, and the transport across the BBB of the non-penetrating the NMDA-receptor antagonist MRZ 2/596 was enabled after intravenous injection. Intravenous injection of polysorbate 80-coated nanoparticles loaded with doxorubicin ($5\,mg\,kg^{-1}$) achieved very high brain levels of $6\,\mu g/g$ brain tissue while all the controls, including uncoated nanoparticles and doxorubicin solutions mixed with polysorbate, did not reach the analytical detection limit of $0.1\,\mu g\,g^{-1}$. Moreover, experiments with the extremely aggressive glioblastoma 101/8 transplanted intracranially showed a long term survival for 6 months of up to 40 % of the rats after intravenous injection of the polysorbate 80-coated nanoparticle preparation ($3 \times 1.5\,mg\,kg^{-1}$). The surviving rats were sacrificed after this time and showed total remission by histological investigation. Untreated controls died within 10–20 days; the rats in the doxorubicin control and uncoated doxorubicin nanoparticle groups died between 10 and 50 days. The mechanism of the drug transport across the BBB with the nanoparticles appears to be endocytotic uptake by the brain capillary endothelial cells followed either by release of the drugs in these cells and diffusion into the brain or by transcytosis. After injection of the nanoparticles, apolipoprotein E (apo E) or apo B adsorb on the particle surface and then may promote the interaction with the LDL receptor followed by endocytotic uptake. The nanoparticles thus would mimic the uptake of naturally occurring lipoprotein particles. This hypothesis was supported by the achievement of a antinociceptive effect with dalargin-loaded poly(butyl cyanoacrylate) nanoparticles with adsorbed apo E or with loperamide-loaded albumin nanoparticles with covalently bound apo E.

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Vesicular-mediated drug delivery across the blood brain barrier: what do we know and where do we go?

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Vesicular-mediated transport describes the process of endocytosis, whereby macromolecules or supramolecular assemblies are internalised from the extracellular environment through specialised plasma membrane domains. These domains invaginate to give rise to membrane-enclosed vesicles within the cytoplasm termed endosomes; clathrin-coated pits, non-coated lipid rafts and caveolae are examples of such domains. Through specific and regulated mechanisms these endosomes undergo shuttling through the cell undertaking fusion and fission with each other, which leads to the exchange of internalised cargo between chemically distinct membrane-enclosed vesicles. Endothelial or epithelial cells are

polarised, possessing distinct plasma membrane surfaces. Cargo endocytosed at one membrane surface (e.g. apical membrane), and shuttled through the cell to be released into the extracellular fluid at the opposing surface (e.g. basolateral membrane), is said to have undergone transcytosis. The continuous brain microvascular endothelium is the anatomical basis to the blood-brain barrier (BBB), and possesses clathrin-coated pits and caveolae, but displays only 50% of the vesicle densities found in other continuous microvascular beds such as lung and intestine (Stewart 2000). The exact significance of this to the concept of a more limited endocytic capacity for the BBB remains, however, to be clarified. With respect to receptor-mediated endocytosis, the BBB appears effective in the endocytosis of ligands that include: iron-bound transferin, insulin, low density lipoprotein (LDL) leptin and nerve growth factor. Further, the capacity for adsorptive endocytosis is also clearly recognised and includes the adsorption and vesicular transport of cationised proteins and cationic peptides termed protein transduction domains (Scherrmann 2002), such as HIV TAT peptide, that appear to bind to cell surfaces through membrane glycosaminoglycans. Vector-mediated delivery systems comprising a targeting ligand (e.g. antibody to transferin or insulin receptor), a linker and drug cargo (Pardridge 2002) have displayed significant effectiveness in in-vivo animal models for the transcytotic delivery of proteins and DNA to brain parenchyma. Similarly, the successful exploitation of nanoparticles and protein transduction domains to mediate brain delivery putatively via transcytosis is increasingly evident. It should be anticipated, however, that much can be learned through study of the molecular machinery regulating endocytic and transcytotic events within BBB, and in particular through the mechanisms by which microorganisms and toxins gain entry into the brain. Further, identification of novel internalising ligands may be promoted by exploitation of an increasingly wider range of display technologies now available to investigate protein-protein interactions. Nature has afforded regulated endocytic and transcytotic functions within the BBB; drug delivery scientists will seek to evaluate and exploit these pathways for the safe and effective delivery of biologics and nanoparticulate drug delivery systems.

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The role of transporters in pharmacokinetics and pharmacodynamics of drugs

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The human genome project has revealed that 2–3% of human genes code for transporters. Many of these transporters have been found to determine the pharmacokinetics (PK; absorption, distribution, metabolism and excretion) and pharmacodynamics (PD; efficacy and toxicity) of drugs. These transporters differ in their energy requirements, substrate selectivity, expression across species or human tissues (e.g. intestine vs hepatic), regulation and localization (tissue and/or subcellular). In this presentation, I will cover the major drug transporter families and will illustrate their role in PK and PD of drugs in man with examples from unpublished data, as well as published data. The transporter families covered will include the efflux ABC transporters as well as the influx transporters such as the OATPs, OATs, OCTs and the nucleoside transporters.

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The impact of transporters on oral bioavailability

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The majority (84%) of the 50 most-sold pharmaceutical products in the US and European markets are given orally. Hence, the drug absorption from the intestine and first pass extraction in the gut and the liver is of major interest for drug developers. During the last decade the interplay between membrane transporters and enzymes has been a research focus. Especially the functional characteristics of drug transporters along the intestine and in the liver may provide important knowledge to allow improvements in drug delivery or drug design by targeting. Even if the genomic and postgenomic era in research has published a vast amount of information about membrane transporters, it has also been recognized that no complete understanding has been achieved of their role in drug absorption, hepatic extraction and drug-drug interactions. It is a general belief that the pharmaceutical product development must be more efficient otherwise new biomedical ideas will never be developed to safe and effective treatments, and a better understanding of how drugs are transported across membrane is one important process that requires more in-depth in vivo based studies.

My research group and collaborators are focused on the in vivo function of transporters and enzymes along the intestine and in the liver. We are using advanced, well-established clinical research models (such as GI-intubation techniques, i.e. Loc-I-Gut, Loc-I-Bile-a novel bile sampling tube, Nyberg Capsule) to examine the complex entero-hepatobiliary processing of drug and their metabolites in vivo in humans.

There appear to be a good correlation between in vivo and in vitro intestinal permeability for drugs with passive diffusion as the main transport mechanism, but there is a significant deviation for drugs absorbed through transporters. It is also considered that the interpretation of the importance of efflux carrier on intestinal absorption process is overrated based on results obtained in tissue cell cultures (for instance Caco-2 cells). Many drugs that were initially suggested to undergo significant efflux in vitro were later shown to be completely absorbed in vivo. For instance, based on only Caco-2 cells it was shown that the in vitro permeability of fexofenadine in the absorptive direction increased from $\sim 0.3 \cdot 10^{-6}$ to $1.5 \cdot 10^{-6}$cm/s in the presence of various Pgp-inhibitors (verapamil, ketoconazole and GF 120918) and that low passive diffusion was the main reason for the incomplete and variable intestinal absorption. Interestingly, the human in vivo jejunal permeability ($\sim 0.07 \cdot 10^{-4}$ cm/s) was neither affected by ketoconazole nor verapamil at clinical doses. It shows that the currently applied Caco-2 model is poorly defined to clarify the role of efflux transporter and to make quantitative predictions of drug-drug interactions at the efflux transporter level. We have recently investigated the in vivo intestinal absorption mechanism(s) of fexofenadine in jejunum, ileum and colon in humans with a new intubation approach. The OATP and P-gp inhibitor erythromycin prolonged the t_{max} in the jejunum but not in the ileum or colon. The systemic availability of fexofenadine was highest after the jejunal administration and concomitant administration of erythromycin in the jejunum increased the systemic availability of fexofenadine by 40%.

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Impact of drug transporters on drug disposition and development

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Increasing evidence supports a pivotal role for drug transporters in the absorption, distribution, metabolism and elimination (ADME) of many drugs and new chemical entities (NCEs). As a result, modulation of these processes can have a profound effect on a drug's ADME, potentially resulting in undesirable drug-drug interactions. Drugs that are substrates for these transporters can demonstrate developmentally undesirable pharmacokinetic properties, such as poor bioavailability, an inability to effectively penetrate target organs (e.g. brain), non-linearity due to saturation of the transport processes resulting in unexpected adverse side effects, significant drug–food interactions and variability in drug disposition due to genetic polymorphisms in transporter genes. Understanding the interactions of candidate drugs and NCEs with key drug transporters will aid both in understanding the underlying mechanisms of ADME of and the potential drug-drug interaction liabilities. This level of information provides guidance on potential issues and hurdles that may need to be addressed in the drug development process and future lead optimisation. For drug candidates in certain therapeutic classes, interaction with drug transporters may hinder or even preclude clinical development.

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The psychosocial difficulties encountered by people with skin disorders

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Many people seeking treatment for disfiguring skin conditions experience psychosocial difficulties related to their appearance. A study of 94 out-patients attending a dermatology clinic for treatment of a wide range of skin conditions is reported. Scores on standardised measures of anxiety, depression, social anxiety and quality of life indicated that 26–51% of respondents were experiencing distress at levels significantly greater than normative values. Semi-structured interviews revealed that common difficulties included embarrassment and self-consciousness in social encounters, problems with forming and sustaining relationships, invasive questions and remarks from others. Sixty-seven percent reported that they avoided social situations in which their condition was 'on show'. There was considerable variation in the levels of distress experienced within the sample; however, this variation was not strongly related to the type,

extent, or severity of the condition, or to the time since onset. The current provision of psychosocial care is extremely limited. Clinic staff felt unable to address patients' needs due to time constraints, the lack of an environment conducive to discussions of a personal nature and to a lack of specialist knowledge and training. The majority of patients reported that they would like this kind of support to be provided in the clinic setting.

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Developments in wound healing: a topical approach

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Covering a wound, by whatever means, to effectively duplicate the function of the epidermis, has been understood throughout history as a way of protecting that wound from the potentially harmful external environment. The wound on which this talk will focus is the recalcitrant non-healing or 'chronic' wound (i.e. leg ulceration). In an acute wound, healing is known to take place via a well-defined series of steps with each phase dependent upon the normal progression of a previous phase. If an abnormality occurs in any one phase this may result in abnormal or impaired healing, fibrosis or ulceration. It is generally believed that chronic wounds have resulted from endogenous circumstances, although it is well recognised that exogenous conditions such as bacterial infection or radiation may induce skin lesions. Presently there is no unified biological mechanism available to fully explain chronic wound pathophysiology and until fairly recently the accepted clinical dogma had been that chronic wounds were static and required stimulation, hence major research undertaken to develop topical growth factors. Part of the present problem is that the clinician, nurse and patient are now overwhelmed with a plethora of new products all suggesting potential wound-healing benefits. Discussions will therefore focus on some of the opportunities and pitfalls associated with developing a topical application for chronic wounds, including the use of pharmacological agents, and how not only is choice of delivery vehicle important, but interactions of secondary dressings may also have a role to play. Finally a brief overview of future opportunities will be proposed emphasising that it highly unlikely that a 'silver bullet' approach will provide the answer and that any new advances must consider the financial constraints placed on national health care systems. It is therefore important to obtain a balance between best possible clinical care/practise and affordable quality products that may be beneficial in providing an improved quality of life for individual patients, as well as potentially aiding the healing process.

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Multidisciplinary approach to the development of skin products

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The term 'skin products' encompasses an extremely wide variety of commercially available formulations and includes everything from cosmetics, such as lipstick, to transdermal patches for the prevention of chronic pain. In the cause of brevity this discussion will focus on pharmaceutical products only. By its very nature, the plethora and breadth of applications for such products means that a multidisciplinary approach to their development is vital. Indeed, if 'development' is not taken at its more exact pharmaceutical definition (activity related to moving products through clinical trials and registration), and is extended to include research activity also, the multidisciplinary nature of the approach required becomes quite staggering. The professions involved in this area of research and development range from particle physicists to dermatologists. The techniques used reflect the nature of the professions involved and range from neutron and X-ray diffraction to the clinical assessment of both local (skin) and systemic disease. In short, it could be argued that there are very few professions of a scientific bent that are not involved in the development of skin products. Given the rather exotic and esoteric nature of some of the research techniques available it would be easy to forget that much of the work conducted involves the more conventional pharmaceutical practices of formulation development and testing. Hence, there will always be a place for pharmacists and pharmaceutical scientists in the product development process but they will need to be increasingly skilled in the art of communicating with scientists from numerous other disciplines. The remit of this presentation is, therefore, not only to impress upon the audience the multidisciplinary nature of the different approaches used, but also the need for good communication between the various groups of professionals involved. Like any value chain associated with any product, it is not always necessary for all participants to be aware and understand the activity of all others. However, there are certain links in the development chain that require an intimate exchange of knowledge and understanding if products are to achieve their maximum potential in satisfying a clinical need. In its broadest sense, this is perhaps most markedly demonstrated by the need for the research arm of a pharmaceutical company to be firmly tempered in its efforts by the commercial division (and vice versa). This requirement can be partially achieved by careful and rigorous portfolio design, management and review with input from across an organisation. Ultimately a broad-based profession requires that all voices are represented in the decision making process if it is to deliver the products that patients deserve.

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Bioactive polysaccharides from Malian medicinal plants

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Mali has a long and still living tradition for the use of medicinal plants for treatment of different types of illnesses. One of these are wounds of different types, both external and internal, the latter often defined as gastric ulcer. As part of the collaboration program between Department of Pharmacognosy, UiO and the Department of Traditional Medicine, Bamako, Mali (DMT), a survey has been conducted to record plants used for wound healing in different parts of Mali. So far the survey has been performed in the south west part, including Bamako, the area north of Bamako, the Kangaba region and in Dogonland, north east Mali. It was observed that many of the plants used for wound healing were rich in polysaccharides, and a screening for effect on the complement system of polysaccharides isolated from the plants investigated showed a high activity for many of the polysaccharides investigated. This study identified plants containing polysaccharides that were further studied for their structure, effect in various bioassays, and structure activity studies were also performed. The results reported in the talk will be based on studies on the different bioactive pectins isolated from the following plants: *Vernonia kotschyana, Cochlospermum tinctorium, Glinus oppositifolius, Biophytum petersianum, Trichilia emetica* and *Entada africana*. *V. kotschyana* and *C. tinctorium* are both used against gastric ulcer and are important ingredients in products sold on the market in Mali for the treatment of gastric ulcer; the former is also registered as an Improved Traditional Medicine, ITM, called Gastrocedal by the government in Mali. All plants contain different types of pectic polymers. The water extracts have been fractionated by anion exchange chromatography and further purified by gel filtration. The MW has mainly been determined by gel filtration, but for some products by SEC-MALLS. The monomeric composition has been determined by GC, linkage analysis performed by GC-MS on partly methylated, partly acetylated alditol obtained after methylation of the polymers followed by hydrolysis, reduction and acetylation. The polymers are degraded by different enzymatic methods and the structure of the obtained fractions determined. The bioactivity of the products obtained are determined in the complement system as well as in other systems involved in the immune system. The results form the basis for the structure activity proposed.

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Research strategy in the search for bioactive polypeptides from plants

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The appearance of biologically active metabolites in nature is determined by ecological needs and biosynthetic possibilities. Strategies and methodology for isolation of polypeptides from plant biomass have recently received attention for several reasons. Firstly, plants containing unique pharmacologically active polypeptides have been discovered through natural product-based drug discovery programmes. Secondly, plants have been found, like animals, to make use of peptides as signalling substances. Finally, genetically transformed plants can be used as an alternative method for production of high value, recombinant polypeptides. Our major aim is to develop strategies for identification of novel molecules of natural origin based on evolutionary structure–activity optimisation, to generate lead compounds in the area of inflammation and cancer. Recently, we have focused on two groups of polypeptides, thionins and cyclotides, with the objective of exploring the structural and functional diversity of polypeptides that are found in plants. Fractionation protocols have been developed to adress major challenges encountered when dealing with plant material and to be able to isolate highly purified polypeptide fractions.

Bioassay-guided fractionation using multitarget functional assays has been used for isolation of pure bioactive polypeptides, with an emphasis on cytotoxic and anti-inflammatory effects. Expanding knowledge of similarities in structure and function in plants and animals of these type of polypeptides promises opportunities in the search for novel compounds with biomedical and biotechnological potential.

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Heparins and other sulphated polysaccharides: more than just anticoagulants

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Sulphated polysaccharides (SP) are an important subset of complex polysaccharides that represent the third major class of biopolymers, along with polypeptides and polynucleinic acids. Natural SP can be subdivided into the glycosaminoglycans (GAG) of vertebrates, which are mainly located on the surface of cells or in the extracellular matrix (ECM), and the SP of marine origin. The latter include the SP present in the cell walls of algae (e.g. carrageenans, agar and fucoidans) and those occurring in other marine organisms like sea cucumber, sea urchin, corals, mussels and snails. The only SP that has become important in medicine so far is the glycosaminoglycan heparin. Due to its anticoagulant activity, heparin has been the drug of choice in the prevention and treatment of thromboembolic disorders for more than sixty years. For a long time, however, it was just empirically used and little was known about its structure, biosynthesis and functions. Only with the development of effective methods of chemical analysis and biomedicine during the last two decades, did knowledge about heparin rapidly increase and in addition, the important physiological functions of the GAG in the body were recognised. Due to their special physicochemical properties, the endogenous GAG, as well as other SP, interact with a multitude of biomolecules: enzymes (e.g. heparanase, hyaluronidase, elastase, matrix metallo proteinases), serpins (e.g. AT, HCII, TFPI) adhesion proteins (e.g. P- and L-selectin), growth factors (e.g. β-FGF), chemokines (e.g. C5a, MCP-1), ECM components (e.g. laminin, fibronectin), cell receptors, lipoproteins, nucleoproteins and even viral proteins. Accordingly, both heparin and other SP exhibit not only anticoagulant activity, but have been shown to display a wide range of other biological activity. Among others, antiatherosclerotic, antiproliferative, antiadhesive, antiangiogenic, antimetastatic, anti-inflammatory, anticomplementary and antiviral effects have been described. A crucial point is that all these effects of SP are not just due to unspecific electrostatic binding to the target molecules, but are strongly dependent on the individual structure of the SP. The most prominent example is the specific pentasaccharide sequence of heparin, which is required for the binding of heparin to antithrombin and thus for its anticoagulant activity. All these findings lead to the conclusion that SP may represent a promising substance class for the development of new therapies for a variety of pathophysiological conditions. Their widespread occurrence in nature creates the option to utilise renewable primary products of non-animal origin. A major task will be to identify the structural elements within a SP required for a specific activity. By starting from naturally occurring SP and additional application of synthetic, semisynthetic and biotechnology-based approaches, it might be possible to obtain new drugs based on sulphated carbohydrates. The synthetic heparin pentasaccharide fondaparinux, which has been approved as an antithrombotic drug, and the biotechnologically produced derivatives of the *E. coli* polysaccharide K5, which is currently under clinical investigation, represent the first proofs of concept.

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Colloidal medicines for parenteral delivery: past, present and future

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Since publication of "The World of the Neglected Dimensions" in 1906 by Wolfgang Ostwald there has been a revolution in the field of colloid science and particle nanoengineering. A catalogue of colloidal entities, ranging from a few to hundreds of nanometers, with remarkable characteristics has now been engineered. Such entities encompass polymeric micelles, dendrimers, liposomes, polymeric nanospheres, ceramic nanoparticles and polyplexes. Therapeutic and diagnostic agents have been encapsulated or incorporated in these nanocarriers to enhance effectiveness, decrease side effects and overcome solubility issues. Some of these systems are designed in such a way that they can be activated by changes in the environmental pH, by chemical stimuli, by applying a rapidly oscillating magnetic field or even by application of external heat, thus turning them into multifunctional systems or offering precise control over drug-release profiles. In some cases, a combination of two or more technologies has yielded better results. Furthermore, by controlling the size, morphology and surface characteristics, colloidal nanoparticles can be targeted precisely to designated locations following intravenous and subcutaneous routes of administration. Targets have included mononuclear phagocytes, dendritic cells, endothelial cells and cancers (tumour cells as well as tumour neovasculature). However, for the full potential of colloidal nanocarriers in targeted drug delivery to these sites to be realized, particles have to get "smarter". Pertinent to realizing this potential is a clear understanding of both physicochemical and physiological processes. These form the basis of complex interactions inherent to the fingerprint of a nanovehicle and its microenvironment; examples include interaction with the biological milieu, such as opsonization, and other barriers en-route, be it anatomical, biochemical, etc., and exploitation of opportunities offered by disease states. Inherently, nanocarrier design and targeting strategies may vary in relation to the type, developmental stage and location of the disease. Such approaches can collectively turn promising molecular discoveries into benefits for patients. In addition to these, recent evidence is also drawing attention towards potential pitfalls or side effects associated with different nanosystems, but investigation in this avenue of research is scant. For example, what is the ultimate fate of nanocarrier constituents in the body? Can these constituents or their degradation products exert immunological and pharmacological activity? Can polymeric nanosystems (polyplexes) used in gene transfer interfere with cellular machinaries or induce genetic alterations? Care must also be taken when translating results observed in animal models because there are distinct intra- and inter-species variation. Nevertheless, nanoengineering and nanoscience approaches to formulation have already begun to change the scale and methods of parenteral drug delivery; it is also making a significant impact on global pharmaceutical planning and sales (market intelligence and life-cycle management). First generations of small unilamellar vesicles with encapsulated doxorubicin are already in the market for management and treatment of AIDS-related Kaposi's sarcoma, refractory ovarian cancer and metastatic breast cancer. A second generation of anti-cancer nanosystems are in late-phase clinical trials, and approval is being sought from the regulatory bodies for a number of advanced diagnostic nanocolloids for detection of lymph node metastases, as well as vascular pathologies (arthritis and atherosclerotic plaques) by magnetic resonance imaging.

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Oxidation-responsive colloids

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Inflammation is a natural response of an organism to aggression or damage. This reaction becomes pathological when it is directed towards the organism itself, possibly taking place in the absence of a dangerous agent. Most inflammatory reactions are characterized by the secretion of oxidizing substances, such as hydrogen peroxide, by phagocytic cells, which use them both as signalling and cytotoxic agents. We aim to use the resulting oxidizing extracellular environment as a signal for the release of anti-inflammatory drugs. We have designed two classes of colloidal objects that exhibit oxidation-sensitive behaviour, in the form of polymeric vesicles and nanoparticles, respectively. Polymer vesicles are obtained by self-assembly in water of amphiphilic block copolymers of poly(ethylene glycol) (PEG) and poly(propylene sulphide) (PPS), while nanoparticles are composed by cross-linked poly(propylene sulphide) displaying a PEGylated surface. Both materials are therefore mostly composed of an organic polysulfide (PPS), which is a hydrophobic, amorphous polymer containing sulphur (II) atoms in the main chain; their oxidation converts the material into a hydrophilic polysulfoxide or polysulfone, causing a phase transition or a swelling of the colloid and finally its solubilization. We aim therefore to use these colloids to incorporate anti-inflammatory drugs and release them in response to the presence of oxidizing species. Specifically, polymer vesicles are suitable for the incorporation of water-soluble drugs, while nanoparticles are better suited for hydrophobic drugs. In this communication we discuss the details of the responsiveness to oxidation and the different characteristics of the release mechanism.

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Nanoparticle formulations of poorly soluble drugs

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A significant proportion of drugs on the market are poorly soluble in water and it is expected that this will be even more pronounced in the future. Formulations of poorly water-soluble compounds offers a challenge to the formulation scientist, from the early discovery phase through the development to the launch of the pharmaceutical product. Liquid formulations of poorly soluble compounds can be for example aqueous pH-shifted solutions, provided the molecules are ionizable, mixtures of water and organic cosolvents, or by solubilization in cyclodextrin or micellar systems. With the exception of the pH-shifted aqueous solutions, significant amounts of additives are often needed to increase the solubility into a practical range, which may induce unwanted side effects. An interesting alternative to these formulations is aqueous nanosuspensions with typical particle sizes of the order of 100 nm, and such suspensions can contain crystalline or amorphous particles. In the presentation the preparation of crystalline as well as amorphous nanosuspensions will be described as well as the characterization of such systems. The latter will focus on stabilizer adsorption, Ostwald ripening and the determination of bulk concentration in such systems.

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Scintillating pharmaceutics

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Do you know the "average" healthy 70 kg man for whom we have optimised our drugs and formulations? Considering the diversity that encompasses the human race, height, weight, temperament, enzymatic capacity, etc., it is not surprising that it is rare to find a "one pill fits all" solution. To add further complexity, medicines are designed for sick people who, by definition, have disordered physiological processes, so how does this affect the intricate interplay between drug and its formulation? Technically sophisticated advanced drug delivery is a rapidly growing field. It currently accounts for 6% of the global pharmaceutical market and this figure is estimated to grow to 20% by 2005. The global market for drug delivery technologies in 1998 reached US$28 billion and is expected to grow to US$78 billion by 2005. It is no accident that the rapid growth in sustained release and other sophisticated oral dose forms coincided with the development of pharmacoscintigraphy. New delivery systems are utilised in line extensions, but also increasingly where the target pharmacokinetic profile will not be reached using conventional formulations due to the compound's physicochemical properties. The technique of gamma scintigraphy has been used for studying the subtle interplay between physiology and pharmaceutics for 25 years. Before adding scintigraphy to pharmacokinetic studies very little was known about the in-vivo behaviour of dose forms, and it was assumed to be integral with the absorption phase of the pharmacokinetic profile. This was adequate for simple tablets, but the added complexity of sophisticated formulations designed to modulate the pharmacokinetic profile of compounds could not be understood without a detailed knowledge of their in-vivo behaviour. In fact some devices rely on successful interaction with physiological processes (e.g. those designed to be retained in the stomach for delivering acid-soluble drugs) or prolonging transit and hence absorption through sustained release devices. When adding complex drug delivery technologies to already complex physiological systems, it is hardly surprising that standard pharmacokinetic studies can often produce more questions than answers. The primary strength of the technique is that delivery system behaviour can be acquired simultaneously with blood levels in individual patients. This frequently reveals that a significant part of the inter-subject variability in plasma levels can be ascribed to variations in dose form behaviour, rather than pharmacokinetic differences as was previously assumed. Surprising results have been obtained from "immediate" release formulations dosed to fasted subjects. Quite frequently the units have travelled through the gastrointestinal tract as a bolus due to the small volume of fluid available for dissolution, and this has resulted in reduced absorption. The technique can also give a valuable insight into variable pharmacokinetic data as phenomena such as oesophageal adhesion or erratic gastric emptying can be identified. The main challenge for performing combined scintigraphic–pharamacokinetic studies is having confidence in the radiolabelling and this aspect should not be underestimated as it can be consuming in both time and money. One confidence has been gained with this aspect then the data obtained from scintigraphy studies can be very powerful.

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The development of UV-calorimetry for pharmaceutical stability assessment

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Isothermal Calorimetry (IC) is ideally suited for the stability assessment of pharmaceuticals for a number of reasons: it is indifferent to physical form or sample heterogeneity; modern instruments are extremely sensitive; and the sample property it measures, heat, is a universal indicator of chemical and physical change. However, calorimetric data can often be complex, the observed response representing the sum of the power-time signals for all the processes that are occurring in the sample, and can be difficult to analyse quantitatively. A further problem is that calorimetric data do not contain any direct molecular information. In this work the development of a new instrument is reported, in which a UV spectroscopic probe is incorporated into a calorimetric ampoule. The calorimeter records the total power change in a liquid sample over time while the spectroscopic data allow the change in concentration of selected reactants or degradants to be followed; knowledge of the enthalpy of reaction for each step allows reconstruction of the power-time responses for individual reaction steps from the spectroscopic data and consequent deconvolution of the measured calorimetric data. The construction of a similar instrument has been previously documented (Johansson & Wadsö 1999) but the UV probe in that case was used only to measure bacterial growth as the transmitted signal became obfuscated; the use of such a system for chemical interpretation of data has not been reported and offers great potential for pharmaceutical stability assessment. The UV-calorimeter was housed in a TAM (Thermometric AB, Järfälla, Sweden) and consisted of a standard glass ampoule (20 mL) with a stainless steel lid through which was mounted a custom-designed stainless steel spectroscopic immersion probe (Astranet Systems Ltd, Cambridge, UK). The probe, with a 2 mm path length, contained two fibre-optic cables, one routing light into the vessel from a deuterium light source and one routing transmitted light to an external diode-array UV spectrometer (EPC2000, Astranet Systems Ltd). Experiments were conducted at 37°C; calorimetric data were collected every 10 s using Digitam 4.1 (Thermometric Ltd) and spectroscopic data were collected every 10 min using Spectrawiz (Astranet Systems Ltd). Data analysis was performed using Origin (Microcal Software Inc., USA) and the chemometric package Insight (Diknow Ltd, UK). The performance of the instrument was tested by studying the Maillard reaction in solution, a well known pharmaceutical degradation reaction between carbohydrates and amines. In this case a solution of toluidine hydrochloride (0.06 M) and lactose (0.6 M) was prepared in NaOH solution (0.06 M). The mechanism of Maillard degradation is complex and depends on many factors, including the chemical structures of the reactants, temperature and pH, and the system was observed to give very convoluted power-time data that were not open to conventional kinetic analysis. However, analysis of the spectroscopic data using the chemometic software suggested the likelihood of there being three reaction steps. A similar analysis of the calorimetric data using the chemometric software then allowed successful deconvolution of the overall data into their component parts; each step could then be analysed by fitting to kinetic models to recover reaction rate constants.

Johansson, P., Wadsö, W. (1999) *Thermochim. Acta* **342**: 19–29