Invited Abstracts

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Possibilities for pharmacological intervention in the ageing eye

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Pharmacologically active preparations directed towards modulating muscarinic receptor activity in the eye have been used for over 2000 years when extracts from *Atropa belladonna* were first applied to enhance eye appearance through pupillary contraction. The first clinically active drugs targeting a specific eye disease were anticholinesterases (e.g. ecothiophate) applied as eye drops to treat glaucoma in the 1960's. However, cataract was soon detected as a relatively frequent side effect and such drugs are now only used to treat glaucoma as a last resort. As muscarinic agonists have been found to reduce intraocular pressure both by decreasing inflow (through Na-K-ATPase pump inhibitor) and increasing outflow (by muscle contraction), it is likely that treatments will be developed that target specific muscarinic subtypes.

It has recently been shown that the M_1 receptor subtype predominates in the lens and so it is important that this subtype is not targeted so that past cataract side-effects of acetylcholine sparing drugs are avoided.

Form deprived myopia resulting from an increased axial length in the affected eye can be reduced by the application of atropine. An atropine-induced reduction in axial length has been achieved both in a chick model system and in human clinical trials and in the former system atropine has been shown to reduce the production of scleral extracellular proteins.

Muscarinic receptor activation appears to stimulate chick eye growth very early in development and it is interesting that muscarinic receptor expression increases to very high levels in the mammalian retina during early development.

Carbachol stimulates tear fluid production through the activation of muscarinic receptors. Interestingly, at least part of the stimulation occurs via EGF receptors and although the precise signalling mechanisms are not completely understood, it has been shown that calcium mobilisation plays a critical role in both muscarinic and EGF receptor activity.

Muscarinic receptor activation therefore plays diverse roles in the eye and pharmacological intervention based on specific receptor sub-types has potential benefit in a number of ocular problems in the ageing eye. However, potential side effects have also recently been identified.

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Industry perspectives on the biopharmaceutical classification system (BCS)

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Oral drug intake is the totally dominating administration route for pharmaceutical products. Bioequivalence studies, as a reflection of pharmaceutical product quality, is an important issue in the development process for oral products in case of line-extensions, post-approval changes, generics as well as for new chemical entities. The possibility provided by BCS to replace bioequivalence studies by relatively simple in-vitro dissolution test studies could therefore be a significant improvement. The most rewarding benefits are minimisation of drug exposure to large panels of volunteers and in some cases, shortened development time lines. In addition, some reductions of study costs and less risk for in-vivo results outside the acceptance limits due to random effects could be other beneficial effects of BCS.

Presently, the simplified route provided by BCS is only applicable for drugs with high solubility and permeability drugs (class I). These properties are unfortunately often not possible to combine with the desired pharmacological profile of a drug and only a very minor fraction of drugs and drug candidates today belong to class I. It is therefore desirable to extend the type of drugs allowed for this simplified route beyond class I drugs in order to increase the importance of BCS. However, such extensions must clearly be performed without endangering the assurance of appropriate product quality. Additional requirements on the in-vitro dissolution test programme may be one way forward to obtain desirable standards. For example, testing for influence of food and other physiological factors as well as establishment of in-vitro/in-vivo correlations may be considered.

Besides the regulatory applications of BCS, for which it has been originally developed, it is also of significance for other parts of the development process. The main merits of BCS, in a non-regulatory context, are the very clear and simple rules to determine absorption limitations. BCS is therefor today often referred to in strategical development decisions and other issues like selection of new drug candidates, choice of drug delivery system, evaluation of potential for absorption mediated food effects and judgements of likelihood for in-vitro/in-vivo correlation of drug dissolution.

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In-silico methods for absorption prediction

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In recent years, one of the key forces shaping the ever-changing world of drug discovery has been the need to reduce the number of compounds that fail at subsequent hurdles on the way to market. Nowadays, in an effort to decrease the attrition rate of candidate compounds, many companies incorporate ADME (Absorption, Distribution, Metabolism, Elimination) considerations very early in the drug discovery process, embracing a "fail fast, fail cheap" philosophy. Advances in automation technology and experimental techniques, both in-vitro and in-vivo, have enabled the processing of much larger numbers of compounds in absorption and metabolism assays than was traditionally possible.

In addition to the development of experimental assays with greater throughput, there has been considerable effort applied to the conception and validation of computational methods for predicting ADME-related properties, especially intestinal absorption. Compared to experimental approaches, these in-silico methods have the advantage that they do not require compound synthesis. They can therefore be applied to "virtual" compounds permitting the rapid exclusion of likely failures at the "drawing-board" stage. In this presentation, I shall survey the computational methods that have been developed with the aim of predicting intestinal absorption. In addition, some future directions for research in this field will be discussed and the impact of these methods on the drug discovery process, both now and in the future, will be briefly considered.

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Clinical relevance of efflux on absorption – can this be predicted?

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The *MDR1* gene product P-glycoprotein does not only contribute to drug resistance during chemotherapy of tumors but is also an important determinant of drug disposition. It is expressed in healthy tissues with excretory function (apical membrane of enterocytes, canalicular membrane of hepatocytes), in the blood-brain barrier and in peripheral leukocytes. It transports a wide range of structurally unrelated drugs out of cells. Intestinal expression of this transporter determines bioavailability of orally administered P-glycoprotein substrates such as digoxin, cyclosporine and HIV protease inhibitors. Similar to cytochrome P450-mediated drug metabolism (e.g. by CYP3A4), P-glycoprotein function can be induced or inhibited by exogenous factors such as concomitantly administered. The antibiotic rifampin for example induces duodenal P-glycoprotein in humans and the level of intestinal P-glycoprotein expression determines disposition of the orally administered P-glycoprotein substrate digoxin. Increased digoxin plasma concentrations have been observed, when the antiarrhythmic quinidine was co-administered. Using the intestinal colon carcinoma cell line Caco-2, P-glycoprotein knock-out

mice and perfusion catheter studies in humans, it could be shown that the underlying mechanism of this recently recognized type of drug-drug interaction is inhibition of P-glycoprotein-mediated digoxin transport by quinidine. In summary, recognition of the importance of intestinal drug transporters by in-vitro and in-vivo techniques has contributed to a major extent to understanding of inter- and intra-individual variability of drug disposition in humans.

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Modification of compaction properties

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Optimization of the compaction of materials is important for successful production of tablets. High dose drugs, with poor compaction properties, are problematic since these properties may be difficult to overcome by the use of directly compressible excipients. Similarly, the properties of excipients are considerably altered by differences in their water content. The compaction simulator, at Liverpool School of Pharmacy and Chemistry, has been used extensively to assess the compaction of materials. This presentation seeks to review modifications to the compaction properties of hydroxypropylmethylcelullose (HPMC) caused by moisture and of two poorly compressible drugs, paracetamol and ibuprofen induced by crystallization in the presence of HPMC and polyvinylpyrollidone (PVP) respectively.

Increase in moisture content from 0 to 14.9% causes a six-fold increase in the tensile strength of HPMC compacts (Nokodchi et al 1996a). HPMC consolidates by plastic deformation and the increase in moisture content results in elastic recoveries as low as 1.2% (Nokodchi et al 1996a). At compression forces between 5 and 20kN, increase in moisture decreases the plastic energies. This is probably the result of reducing the resistance of particles to flow, enhancing particle deformation and by lubrication effects of moisture (Nokodchi et al 1996b).

Paracetamol is elastic in nature. Incorporating PVP into its crystals by watering out techniques using dilute aqueous solutions of PVP produces agglomerates composed of micro-crystals (Garekani et al 2000a). The incorporation of less than 4.3% PVP results in compacts with improved crushing strengths and a loss of capping at punch speeds up to $250\,mm\,s^{-1}$. Energy analysis from compaction simulation reveals that both elastic and plastic energies increased with increase in compression force and speed. but were less for the agglomerates (Garekani et al 2000b). Improved compression was attributed to a lower elastic energy/plastic energy ratio in the agglomerates. Data suggest a high degree of fragmentation occurs during compression and that in the PVP containing crystals; less compression energy was utilized as elastic energy.

Ibuprofen displays compaction problems because of its elastic nature and low melting point. Crystallization of ibuprofen by watering out, using a dispersion of HPMC K100M, produced crystals with improved compaction (Whelan et al 2000). This was again associated with a decrease in elastic energy and an increase in the plastic/elastic energy ratio, although this was compression force dependent.

The studies indicate that compaction properties are improved when drugs or excipients are processed to contain relatively small quantities of compaction improving materials.

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Understanding agglomerate compaction

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An important strategy to improve the manufacturability of powders is to form agglomerates from the primary particles. Knowledge on the relationship between granular material properties and tablet properties is necessary for agglomerate engineering. This presentation will focus on the compaction properties of agglomerates.

The dominating compression mechanisms for agglomerates seem to be permanent deformation, densification and attrition (Johansson & Alderborn 1996; Johansson & Alderborn 2001). The degree of changes in physical properties seems to be related primarily to mechanical properties of the materials from which the granules are formed (Nicklasson et al 1999) and to the size (Johansson et al 1998), shape (Johansson & Alderborn 2001) and porosity (Johansson et al 1995) of the granules. The mechanism of compression can thus be controlled by formulation factors and by granulation method. The deformation of granules may also occur in different ways, referred to as different modes of deformation (Tunón & Alderborn 2001), involving changes in the main dimension of a granules or changes in surface shape. Procedures are also developed by which the compression mechanics of agglomerates can be assessed. An interesting example is the calculation of a failure strength of the granules by the Kawakita equation (Nicklasson & Alderborn 2000). The respons of granules to a compressive stress will control the evolution in tablet structure (Nicklasson et al 1999; Johansson et al 1995). The structure will possibly control a number of tablet properties, such as mechanical strength, disintegration and drug dissolution (including drug release from reservoir granules). Other aspects on the tablet forming ability concerns the the effect of the binder on the mechanics of the agglomerates (Nicklasson & Alderborn 2001) and the distribution of binder within the granules (Wikberg & Alderborn 1993).

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Modelling powder compaction

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The preferred drug delivery system today is represented by tablets, which are manufactured using high speed rotary presses where the powder material is compressed in a die between rigid punches. Compression represents one of the most important unit operations because the shape, strength and other important mechanical properties of the tablets are determined during this process. These properties are dictated not only by the characteristics of the powder constituents, but also by the selection of the process parameters, which are essentially imposed by the production machinery.

Numerical modelling is commonly employed as means of optimizing the compaction process in other powder forming industries, such as powder metallurgy and ceramics, where compaction is an important processing step.

We present a review of the current state of analysis of tablet compaction, based on a continuum mechanics approach, which involves solving of equilibrium, compatibility and constitutive equations.

Modelling powder compaction requires knowledge of the following four factors:

1 constitutive behavior of powder under compaction, which relates the strains and stresses during the compaction process, that is the deformation of a volume of powder under a given set of external loads. The details of the contact interaction between neighboring powder particles i.e. the cohesion and interparticulate friction are incorporated in a macroscopic continuum constitutive model.
2 friction behavior between powder and tooling (die and punches)
3 geometry of die and punches (including initial conditions resulting from die filling)
4 loading schedule (sequence of punch motions)

The existing constitutive models and the experimental procedures used for calibration are reviewed with special reference to data generated using equipment which is typically available in an industrial research environment, such as a compaction simulator and die instrumented with radial pressure sensors. A mixture of microcrystalline cellulose lubricated with Magnesium Stearate is used as an example. The elastic and plastic model parameters are expressed using relative density as state variable. The friction coefficient between powder and die wall is determined using the same experimental set-up.

Density variations within tablets are important because they induce local variation of tablet properties, which may affect the dissolution (and possibly the bioavailability) and the mechanical behavior of tablets during post-compaction operations such as coating, packaging, storage and use. We discuss finite element results focusing on density distributions within a tablet after compaction, which are influenced by the combination of the four factors described above. The relative importance of these factors with respect to different design parameters are discussed using parametric studies. The results can be used to aid formulation design, process development, and tablet image and tool design.

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Compaction simulation in tablet development

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Compaction simulators are increasingly used in tablet development. They are single station instrumented tablet presses capable of mimicking the compaction profile of commercial tablet presses. The key advantage of compaction simulation is the ability to measure powder and tablet properties with minimum use of drug substance and significantly reduced timelines during the whole drug development process.

The two most commonly used techniques are the Heckel analysis (Heckel 1961) and the generation of force/hardness profiles.

The main purpose of the Heckel test is to rank different materials/formulations in terms of inherent mechanical properties measured at standard test conditions. Key measurements include the yield stress and speed dependence. Additionally, data can be generated on elastic recovery and radial tensile strength (RTS) at a given force.

In contrast, RTS values over a range of applied forces can be derived from force/hardness (F/H) profiles. These are drug product specific, using commercial tooling and production rates. Such profiles are an ideal tool for screening formulations for robustness prior to large scale experiments.

The two compression techniques can be used in a complementary fashion to guide formulation and process design. As an example, products with suboptimal crushing strength can be identified early in development using F/H profiles. Heckel analysis can then be used to help identify whether the formulation or process parameters are responsible for the low strength.

Table 1 shows the results of Heckel analysis of formulation 1 which has robustness issues. Both the dry blend and the granules of the formulation were tested to elucidate areas for improvement.

Table 1 Results of Heckel analysis of Formulation 1.

Type	Speed (mm s^{-1})	Yield Stress (MPa)	RTS (MPa)
Blend	0.3	63	4
Blend	300	135	1
Granules	0.3	49	1
Granules	300	97	0.4

Historical data shows that robust tablets have an RTS of at least 2 MPa. Table 1 shows that the strength of the granules at both test speeds is well below 2 MPa. The dry blend forms very strong tablets (RTS=4 MPa) at slow speed but the formulation is highly sensitive to speed, and the RTS drops to 1 MPa when compressed at 300 mm s^{-1}. These results prompt towards changes in the formulation, *e.g.* using excipients which are less speed sensitive, prior to optimising the granulation process.

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Mechanisms of B cell ageing

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The elderly are more susceptible to infectious diseases. Mortality and morbidity from infections increases sharply over the age of 65. At the same time, the efficacy of vaccinations in the elderly is decreased. The elderly also have an increased incidence of cancer and inflammatory diseases. All the above indicate an age-related dysregulation of the immune system.

The humoral arm of the immune system is dependent on recognition of pathogens by antibody. The diversity of the antibody response is necessarily large and is, in part, achieved by combinatorial formation of the Immunoglobulin (Ig) genes during B cell development in the bone marrow. Later maturation of antibody affinity and specificity is achieved by the Darwinian process of Ig gene hypermutation and selection after B cell activation. This takes place in the germinal centre of follicles in lymph nodes, spleen, Peyer's patches etc. Finally, antibody function is modified by class switching of the Ig gene to produce different isotypes.

Evidence suggests that the change in the humoral immune response with age is a qualitative rather than a quantitative one; i.e. it is the affinity and specificity of the antibody that changes rather than the quantity of antibody produced. There are a number of possible causes of this failure: 1. A decrease in quantity/diversity of fresh B cells able to cope with new antigen challenge. 2. A failure of B cells to be activated in response to antigen. 3. A defect in the mechanism of hypermutation of immunoglobulin genes. 4. A defect in the mechanisms that select B cells carrying high affinity B cell receptors.

The diversity of the B cell population, and the maturation processes of individual B cells, can be followed by studying the diversity and hypermutation of rearranged immunoglobulin genes. We have studied individual clonal responses within germinal centres of spleen and Peyer's patches in young and old patient groups. Our results indicate that there is no difference in the actual mechanism of hypermutation with age. There are, however, differences that are due either to a change in selection processes, or to a change in the founder cells available for activation.

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Assessing the role of senescent fibroblasts in aged skin pathology

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The purpose of the project is to test the idea that cellular ageing contributes to aged pathology. We have chosen rat skin as our model for investigation since this allows

us to perform our experiments both in-vitro and in-vivo. The aim of the project is to reconstruct dermis using either young or aged (senescent) rat dermal fibroblasts seeded into an acellular fibrous collagen matrix (FDC) derived from either young or aged rat skin. We have performed immunological, biochemical and histological analysis of the reconstituted dermis both in-vitro and following grafting into full thickness loss skin wounds in young rats.

Immunological and histological studies reveal differences in both collagen type I and elastin networks in FDC derived from young donors (3 month) compared with FDC derived from aged donors (2 years).

Both young and aged fibroblasts readily attach to FDC derived from either young or aged donors. However, infiltration assays reveal that young cells repopulate aged FDC more readily than they repopulate young FDC, and that aged cells repopulate FDC (young and old) more readily than young cells. Both young and old fibroblasts secrete higher levels of active matrix metalloproteinase 2 into culture supernatants when cultured with aged FDC than with young FDC. Surprisingly the highest levels of MMP2 activity are found in samples from young cells cultured with aged FDC. This results demonstrates a previously unrecognised response of young cells to an aged environment.

Studies involving grafting young and aged FDC into recipient rats have revealed that grafts are incorporated into full-thickness wounds efficiently regardless of the age of the FDC donor. Re-epithelialisation is initially hyperplastic but after 3 months epithelial coverings of both young and aged FDC grafts are of similar thickness and indistinguishable from host epithlium. We are currently reconstructing skin in-vitro using FDC, dermal fibroblasts and keratinocytes. Histological examination of samples of native young and aged skin show that the density of cells in the aged dermis is reduced by about 35% compared with young dermis. After 1 month in-situ, cell densities in grafts of young and aged FDC are up to 10-fold higher than the adjacent dermis. By 3 months, cell densities in grafts have reduced but are still higher than the adjacent host tissue by 2-3 fold. Interestingly, final cell densities in young and aged FDC are similar suggesting that the age-related decline in cell density *in vivo* is unrelated to age of the matrix but perhaps a function of the age of cells.

Staining frozen sections of retrieved grafts with antibodies reacting with vonWillebrand factor reveals that both young and aged FDC support revascularisation. We are currently quantifying the extent of revascularisation to determine any age-related influence of the matrix.

Finally, we are using high-resolution FESEM to visualise ultra-structural differences in young and aged FDC. We will then determine whether any changes are observed upon culture with young or aged fibroblasts and/or following grafting into rats.

Our work has provided useful data regarding the interactions between dermal cells and the surrounding matrix and how this relationship may be affecting during organismal ageing.

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Inverse phase gas chromatography

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For many decades scientists have been searching for ways by which to obtain a meaningful assessment of the surface energy of powdered systems. It has been recognised that contact angle methods are not well suited to powdered samples and consequently most workers have looked towards vapour sorption methods as an alternative. Vapour sorption can be studied using gravimetric and calorimetric and chromatographic methods, and in each case the affinity for known vapours provides information about the nature of the solid surface.

In recent years there has been an explosion of interest in inverse phase gas chromatography (IGC) as a means of assessing the surface nature of powders. The basis of the method is to fill a test powder into a column (with sample loads as low as a few hundred mg) and then to monitor the retention behaviour of known vapours, and in so doing to assess the surface energy of the powder. At its best IGC can be used to differentiate between samples that have minor but significant differences in surface ordering and/or various levels of surface contamination.

IGC is a very interesting technique with which to study the amorphous state, allowing measurements of glass transition temperature under defined conditions, such as controlled humidity. Furthermore IGC is a powerful method for the study of changes in the amorphous state, and can be used to demonstrate changes as a function of time elapsed since the formation of amorphous regions in a sample.

Despite the major advantages that IGC has to offer it is essential to remember that the data yield is not always simple to interpret. This is especially true when dealing with polar probes in general and with amorphous solids for which substantial probe absorption can occur. Inevitably for techniques that have recently been introduced to a mass market, there are concerns about inexperience in experimental design and data usage. Without an awareness of these aspects a technique that has enormous value could fall into disrepute.

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Atomic force microscopy and thermal microscopy

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Atomic force microscopy (AFM) is now a well established imaging tool for the characterization of surfaces at the nanoscale. With the provision of new modes of imaging these data can be made to reflect not only surface topography but also surface properties such as friction, adhesion, hydrophobicity and compliance. Scanning thermal microscopy (SThM) is a related probe based technique, but provides images based upon surface thermal conductivity at around the micron scale. SThM hence provides an opportunity to execute calorimetric type measurements normally carried out by DSC with very high spatial resolution and with a very small sample volumes.

I will review how we have applied AFM imaging to provide a unique view of processes at the surfaces of crystalline materials. In particular the ability to acquire data within a range of environments and in real-time will be highlighted. SThM analysis of individual drug and excipient particles will also be highlighted and compared to classical calorimetric approaches.

A number of established methods are used to investigate the adhesive properties of particles, for example centrifuge and electric field detachment techniques. A recent addition to these approaches has been AFM. The ability to measure and map forces between individual particles adhered to AFM tips and a surface has been demonstrated by a number of groups and indeed the AFM has also been used to acquire force measurements relevant to pharmaceuticals applications.

I will show how we have used AFM to rank and quantify the interactions of drug particles with excipients and materials that are incorporated into inhalation delivery devices.

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Investigation of amorphicity and polymorphism in solids using isothermal microcalorimetry and solution calorimetry

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Isothermal microcalorimetry has been extensively used in recent years for the characterization of solids. The pharmaceutical industry, especially, has used this technique to great advantage to investigate a number of issues conventionally examined using other less direct methods. Isothermal microcalorimetry has found its niche through the high level of sensitivity and the versatility of the technique to perform a wide range of experiments for different applications. This paper will give a review of recent work in the field of solid characterization of pharmaceutically relevant materials.

Quantitation of amorphicity is an area where isothermal microcalorimetry has been used. Processing, for example micronisation, can alter the crystalline content of solid samples. Microcalorimetry has been used to quantitate the crystallinity of a sample to levels less than 0.1 percent amorphous content. As with isothermal microcalorimetry, solution calorimetry has also been used to study the amorphous regions of solids and quantitate the level of amorphicity in the sample.

Microcalorimetry and solution calorimetry are excellent tools in the study of polymorphism. It will be shown how microcalorimetry can be used to study the conversion of a metastable polymorph to a stable polymorph. This can be performed at realistic temperatures unlike the more stressed technique of DSC. Solution calorimetry can be used for quantitation of the polymorphic content of a sample. In addition, it is also a very sensitive technique for use in polymorph and solvate identification.

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Adhesion of aqueous-based polymer films to pharmaceutical solid dosage forms

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Polymers are applied to pharmaceutical solids for decorative, protective, and functional purposes. Irrespective of the reasons for coating, the polymeric material must adhere to the solid substrate. Poor adhesion may compromise the mechanical protection the coating provides to the solid, permit an accumulation of moisture at the film-tablet interface, and alter the rate controlling properties of functional polymeric films. The two major forces influencing polymer adhesion include the strength of the interfacial bonds between the polymeric film and the surface of the solid and the internal stresses within the film coating. While various techniques to measure adhesion have been proposed, the most widely accepted procedure is a butt adhesion method where the film coating is removed normal to the surface of the substrate.

Polymeric films are generally applied to solid dosage forms as aqueous-based solutions and dispersions using a spray-atomization technique. The water in the atomized droplets causes dissolution of the outermost surface of the tablet and physical mixing at the film-tablet interface. A method to quantify the thickness of this interfacial area using x-ray photoelectron spectroscopy in combination with intermittent ion sputtering was recently developed. Greater interfacial interaction is thought to be indicative of stronger film-tablet adhesion. In addition, drugs and excipients from the tablet can migrate into the film during the coating process and this migration may affect the mechanical, adhesive, and drug release properties of the polymer.

The physical and chemical properties of the substrate have been shown to influence the strength of the interfacial bonds between the polymer and the solid. A rough surface provides for greater interfacial contact and produces stronger adhesion between the film and substrate. The composition of the solid has been shown to influence polymer adhesion, with more hydrophobic surfaces producing relatively weak interfacial interactions and poor adhesion. The physical properties of gelatin create difficulties in aqueous-based coating of hard and soft gelatin capsules and recent studies have investigated using cellulosic-based hard shell capsules to overcome adhesion problems associated with coating conventional capsules.

Various excipients, such as plasticizers, pigments, and anti-adherents, may be incorporated into polymeric solutions and dispersions to improve the appearance of the film coating or aid in processing. The use of these excipients, however, may influence polymer adhesion. The concentration, morphology, particle size, and surface charge of insoluble excipients have been shown to alter adhesion by affecting both the extent of interfacial interactions and the internal stresses within the film coating. Processing parameters and storage conditions also affect the adhesive properties of polymeric films, presumably by changing the internal stresses in the coating.

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Modelling and simulation of aqueous film coating processes

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The process of aqueous pharmaceutical film coating technology is fundamentally governed by the First Law of Thermodynamics. The first law equation defines the balanced relationships between the input of temperature, humidity, airflow and solution delivery, and the resultant temperature and humidity of the exhaust air. The process in essence is an adiabatic evaporative cooling process. These principles were discussed by Ebey (1987). Understanding the relationships of energy and mass transfers within the process and formulation environment enable increased performance and efficiencies to be gained.

The generation and control of aqueous based droplets are critical to the success of high efficiency, high quality film coating processes. The initial formation of droplets, and evaporation rates during travel, before impact in the coating pan, determine the ability of the droplets to subsequently wet and spread onto, or into, the surfaces of pharmaceutical substrates. The rate of water loss is determined by relative energy and mass transfer effects created by the environmental conditions within the coating pan.

Computational fluid dynamics (CFD) is the science of predicting fluid flow, heat transfer, mass transfer and related phenomena by solving mathematical equations which govern these processes using a numerical process. The physics of droplet vaporisation in the context of spray coating of tablets are discussed in the general texts: White (1999) for fluid mechanics; Chapman (1984) for heat transfer; and Versteeg and Malalasekera (1995) for CFD.

CFD was used to create "near field" and "far field" models to examine the potential effects of thermodynamics and mass flow phenomena and the evaporative rates of droplets. The "near field" model examined predictive effects occurring within the spray zone and immediate surrounding environments, whereas the "far field" model examined related effects of airflow within the internal flow domain of the drum environment. The near field model was bounded by a section of the drum and contained a detailed model of a Schlick 930 flat jet spray nozzle. The tablet bed was modelled using a representative porous medium. The air jet emanating from the nozzle was modelled explicitly while a distribution of water droplets was released into the jet.

A sensitivity study was performed which varied drying air temperature and humidity, spray to bed distance and the non-volatile fraction of the droplets. It was found that air temperature had very little bearing on the evaporation rates while humidity levels were important, with high levels of humidity reducing evaporation rates to a minimum. Complete vaporisation of even the smaller size range of droplets was not achieved under any conditions. However, with a non-volatile fraction present the vaporisation of the smallest droplets was seen.

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Metabolic abnormalities of inflammatory synovium: implications for future therapy

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A major limitation in the treatment of rheumatoid arthritis (RA) is the poor efficacy of currently available drugs (Chikanza et al 1998). Frequently, this results from a lack of selectivity for arthritic tissue that contributes to an unacceptable side-effect

profile. A major requirement exists for the development of new therapeutic strategies for the treatment of arthritic conditions.

The abnormal metabolic profile, found within synovial fluid (SF) of inflamed rheumatoid arthritic joints, implies an impaired vascular supply and/or increased metabolic demand (Naughton et al 1993). Recently, pO_2 profiles within the synovium in inflammatory joint diseases were evaluated (Blake et al 1997). Reduced pO_2 was a characteristic feature of the RA synovium in comparison to both healthy synovium and osteoarthritic synovium. In a more detailed study, an histological assessment of disease severity was performed in tandem with pO_2 determinations on RA knee-joint synovial biopsy specimens (Falchuk et al 1970). Progressively lower SF pO_2 levels correlated with an increase in histological markers of disease severity (cell proliferation, leukocyte infiltration and vascular obstruction) within the synovium.

Hypoxia is associated a wide range of processes that may have pathological consequences in the inflamed rheumatoid joint. These include i) the induction of free radical generating enzymes, ii) modification of activities of free radical generating enzymes, iii) consequent release of redox-active transition metal ions from storage proteins facilitating their mediation of free radical generating processes and iv) elevation of metabolic processes such as glycolysis that may contribute directly to the inflammatory process.

A fuller understanding of the role of hypoxia in the inflamed rheumatoid joint has opened up a number of therapeutic approaches. These include the development of i) novel antioxidants, ii) novel drug delivery systems, iii) enzyme control and iv) hypoxia-based gene therapies. An update on advances in the development of these therapies will be given.

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Near-infrared spectroscopic analysis of phytopharmaceuticals

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Herbal materials have long been vital sources of medicines and in recent years there has been a revival of interest in plant medicines both within the pharmaceutical industry and by the general public. The introduction of the anti-cancer drugs vincristine and vinblastine from the Madagascar periwinkle and taxol derivatives from yew has highlighted the potential of natural products. Health food shops and pharmacies now stock extensive ranges of preparations and extracts claimed to benefit a wide range of conditions eg Echinacea and Hypericum or St John's Wort. A further trend has been the introduction of non-Western styles of alternative medicine into developed countries for example Islamic, Ayurveda and Traditional Chinese Medicine. Particularly in Traditional Chinese Medicine, herbal drugs are often used in very complex multi-component mixtures, including rare and exotic plant materials and which present significant toxicological problems.

Assurance of quality of any herbal material presents a number of interesting challenges to the analyst. Herbal materials are inherently variable and the content of an active principle or of unwanted contaminants can vary according to the sub-species, country or site of growth, climate, season, age, harvesting or storage conditions.

The nature of these problems indicate that a rapid technique for the analytical surveillance of crude herbal products is highly desirable and that is a task for which near-infrared (NIR) spectroscopy is ideally suited as a fast and non-destructive technique.

At the School of Pharmacy a data base of NIR spectra of crude and partially purified herbal pharmaceuticals has been compiled. The objective of the work has been to provide a library of spectra which could be used to test the identity of herbal samples, although much early work has involved the investigation of the optimum combination of chemometric analysis necessary to achieve the highest probability of identification for real samples. One end goal is to make the identification independent of the physical form of the sample, for example of how finely ground it is, while another is to allow discrimination between samples from different closely related species and countries of origin. As part of the investigation of the NIR spectroscopy of solid herbal samples we have distinguished between *Digitalis* samples of different species and measured the spectra of samples of the dried rhizome and roots of Valerian (*Valeriana officinalis*) from different sources and in different physical forms. Moisture content is often another important variable in herbal materials and its determination in these solid samples can often be achieved simultaneously. Not only solid herbal samples are the target in our investigations: a parallel database of essential oils is also under development. The essential oils are being increasingly used in aromatherapy as well as for the treatment of skin conditions and are subject to great variability according to the age of the sample, it's state of oxidation and possible loss of volatile components. NIR spectra can be recorded rapidly from only a very few drops of the oil held in an agricultural reflectance cell.

In this work not only is qualitative identification possible but the principal ingredients can be quantified. NIR has been used for the determination of cineole in eucalyptus oil as an alternative to the official time consuming freezing point BP method. Other examples have included the determination of cinnamaldehyde and eugenol in cinnamon oils, the eugenol content of clove oils, and the citral content of lemon and lemongrass oils at very different concentrations has also been accurately quantified. The adulteration of rosemary oil with eucalyptus has also been studied.

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Innovative approaches to the characterization of plant medicines: the application of nuclear magnetic resonance spectroscopy and proteomics

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The complexity of plant extracts causes severe difficulties for manufacturers and regulators alike. Existing methodologies are invariably reductive and rely on the use of known active molecules (but this belies the increasingly established notion of synergism, or at least, multiple and disparate mechanisms of action) or, even less logically, of marker compounds.

Advances in analytical methodologies and approaches to 'mining' large quantities of data are now beginning to allow comprehensive phenotyping of plant systems by metabolite profiling, using approaches developed originally for medical applications, such as for rapid detection of disease, and the selection of development candidates in drug discovery. A number of analytical techniques are available: all have their advantages and disadvantages. The approach described here involves the use of high field ^{1}H nuclear magnetic resonance spectroscopy to generate chemical fingerprints. The complexity of the resultant data needs to be reduced by chemometric methods such as hierarchical cluster analysis or principal components analysis. Both techniques use all the metabolic data from a given sample to compute an individual metabolic profile and simultaneously compare this profile with those of other samples. The result is a clustering of samples that show multivariate similarity. Such enhanced definition can thus be used to supplement a specification for a plant raw material by means that do not rely on the identification of (essentially random?) components and, moreover, is wholly respectful of the notion of totality. Such a method can also be used to address identity, purity, stability and, above all, consistency, during the production process for botanical drug substances and finished products.

In addition, the use of proteomic analysis to provide a quantifiable measure of total biological activity in relevant human target cells without measuring individual, more or less specific, or marker activities will be described.

A further significant application concerns plant medicines comprising extracts of more than one plant: profiles of consistency can also be generated for such mixtures, so providing a means of enhanced product definition and improved intellectual property. In addition, the approach can also be used to determine which components account for most of the variance between different samples, a technique that has a role in conventional natural products drug discovery. The approach will be illustrated with examples of recent development projects.

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Self-assembled polymers for drug and gene delivery

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Various amphiphilic polymers may be engineered to produce particulate systems for drug and gene delivery applications. Within our laboratories a number of soluble polymers have been covalently converted into amphiphilic molecules by the attachment of hydrophobic and in some cases hydrophilic pendant groups. These amphiphilic polymers based on poly-L-lysine (Brown et al 2000; Wang et al 2000; Wang et al 2001), poly-L-ornithine (Brown et al 2000), glycol chitosan (Dufes et al 2000; Uchegbu et al 2001) and polyethylenimine have been found to assemble into micelles, vesicles and nanoparticles. Higher levels of hydrophobic character give rise to nanoparticles while lower levels of hydrophobic character give rise to polymeric vesicles and micellar systems. We have exploited these various self-assemblies to produce a number of efficient and biocompatible drug and gene delivery systems. Polypropylenimine dendrimers have also been exploited to produce biocompatible gene delivery systems (Zinselmeyer et al 2002).

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Recent applications of pH-sensitive polymers and gels in drug delivery

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The biotechnology field has identified many new biomolecular drugs that act intracellularly, but the effective delivery of these biomacromolecules remains a significant challenge. Such drugs include DNA (in gene therapy), oligonucleotides (ODNs) in antisense therapy, and protein and peptide drugs in immunotoxin therapy and as vaccines. Passive or receptor-mediated endocytosis results in localization of the drug within the endosomal compartment, where the predominant trafficking fate is fusion with lysosomes and subsequent degradation. A variety of viruses and toxins have evolved pH-dependent, fusogenic peptide sequences in their protein coats that are activated in the low pH environment of the endosome to enhance transport of their DNA or RNA cargo into the cytoplasm. Inspired by the principle behind this biological strategy, we have designed a family of novel pH-responsive polymeric carriers that can be incorporated into drug formulations in order to enhance delivery of "fragile" biomolecular drugs out of the endosome and into the cytoplasm, thus avoiding the lysosomal degradation pathway. The first type of polymer is based on poly(alkylacrylic acids) which become hydrophobic and disrupt cell membranes when a sufficient fraction of the carboxyl groups are protonated within the acidic endosomal environment. We have found that poly(propylacrylic acid) (PPAA) is very effective at enhancing transfections in cell culture, even in the presence of serum. PPAA also works to enhance transfections in-vivo in a mouse model. It also enables cytosolic delivery of proteins when it is conjugated to the protein. A second type of polymer is designed with a hydrophobic backbone that is disruptive *per se* to lipid membranes, and to render it soluble as well as to mask it from lysing non-targeted cells, we have grafted hydrophilic PEG groups to it via acid-labile, acetal linkers. Drug molecules and targeting ligands may be linked ionically or chemically at the distal ends of the grafted PEG molecules, or they may be linked directly to the backbone. We have found that these polymer carriers effectively bypass the lysosomal targeting of oligonucleotides (ODNs) that have been internalized through the asialoglycoprotein receptor of hepatocytes. They also have been successful in delivering antisense ODNs and peptides to macrophages in vitro. This work will be described, along with a brief general review of hydrogel DDS.

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Progress and problems in the computational prediction of polymorphs

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The unexpected appearance of a new polymorph in production can be a disaster for the pharmaceutical industry. It is very difficult to establish experimentally whether all possible polymorphs of a product are known, let alone how to control which polymorph is formed. Hence a computational method to predict all likely polymorphs of a molecular product would be a considerable help in pharmaceutical development.

In the past decade there has been considerable progress in developing computational methods of crystal structure prediction. These methods seek to predict the complete crystal structure (space group, cell dimensions and atomic coordinates) from the chemical diagram, so could be applied prior to synthesis of the molecule. Most of these methods are based on seeking the most thermodynamically stable crystal structure, by seeking for the global minimum in the lattice energy. From this assumption, it follows that any local minima that are energetically competitive are possible polymorphs. This approach has been moderately successful at predicting the known structures of a range of simple organic molecules (Beyer et al 2001a), with some successful prediction even under blind test conditions (Lommerse et al 2000). Unfortunately it is relatively rare to have a correct prediction where the known crystal structure is be found significantly more stable than other possibilities, and therefore there will not be any other polymorphs. The more common result is that more energetically feasible structures are found than are known, or probable, polymorphs.

This thermodynamic criterion is clearly necessary but does not take into account the kinetic effects that make some of the energetically feasible structures unlikely to be observed. Preliminary work (Beyer et al 2001b) suggests that considering the mechanical stability and relative growth rates of the hypothetical crystal structures can eliminate some as unlikely to be observed. However we need to incorporate a better understanding of the causes of polymorphism, such as the effect of solvents on nucleation rates, into the computer prediction models, to correct this tendency to predict more polymorphs than are likely to be found.

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Characterization and the stability assessment of pharmaceutical hydrates

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Hydrates are molecular complexes that incorporate water molecules, usually stoichiometrically, in their crystal lattice. The use of the automated moisture sorption apparatus enabled the rapid evaluation of the physical stability of pharmaceutical hydrates. For example, the transition water vapor pressure of amoxicillin trihydrate was determined to be 10.5 Torr at 68°C. X-ray powder diffractometry, thermoanalytical techniques (differential scanning calorimetry, thermogravimetric analysis) and spectroscopic techniques are routinely used for the characterization of pharmaceutical hydrates. The use of a differential scanning calorimeter (DSC) at elevated pressures resulted in the separation of dehydration and vaporization events, and enabled the determination of the enthalpy of dehydration. At high pressures, the water liberated on dehydration was not immediately removed, and its presence influenced the solid-state of the anhydrous phase formed. Variable temperature X-ray powder diffractometry at elevated pressures permitted *in situ* study of dehydration and this confirmed the DSC results. Hydration and dehydration reactions are routinely encountered during pharmaceutical processing. Aqueous wet granulation of anhydrous theophylline resulted in the formation of theophylline monohydrate, which transformed to a mixture of metastable and stable anhydrous phases during the drying of the granules. Using XRD, all three phases were simultaneously quantified during the various stages of granulation. Such phase transitions during pharmaceutical processing can have a profound impact on product performance.

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Drying foods and protecting structures

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Drying provides the benefit of long term microbial stability on foods and food components, but has the disadvantage of irreversibly damaging some cellular structures. Furthermore, these powders and particulates are extremely sensitive to moisture which complicates their subsequent use. In most cases, we are handling materials which are of heterogeneous structure and composition and it is this complexity that we are trying to protect, construct and use.

Nonetheless, systematic approaches to material science, and observations of naturally desiccating systems are providing the basis for design principles.

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Stem cell technologies: problems and possibilities

Paul Kemp

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Stem Cells are specialised cells that have the capacity to both renew themselves or to give rise to different types of differentiated cells. This ability to differentiate down a variety of cell lineages is called multipotency and several different types of Stem Cells have been found in many locations in both embryonic and adult tissues. Not all stem cells have the same properties and they differ in part by possessing a greater or lesser amount of multipotency. There is a particular class of stem cells residing in the embryo and foetus that can develop into any of the more than 200 cell types in body that are called Pluripotent Stem Cells. And one particular type of Pluripotent Stem Cell is the now famous Embryonic Stem Cell which is part of the inner cell mass of a 4 to 5 day embryo.

Stem Cells have already had clinical application for many years and it is the haematopoietic stem cell that is the main functional component of a bone marrow transplant. In recent months there have been many discussions in the lay press about the anticipated abilities of stem cells from various sources to replace cells and tissues in patients suffering from a variety of diseases. This has in turn led to intense political debate around the world and a great deal of public interest.

It is important however to understand the great many technical challenges that will have to be overcome before stem cells fulfil their medical potential and even when these are solved, there are additional economic hurdles to address before they can be shown to have a commercial future.

This talk will provide an overview of the Stem Cell and review what is so far understood about them and attempt to clarify some of the misconceptions that are common in this area.

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Tailoring ligands to high-order DNA structures

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Three- and four-stranded DNA structures are important targets in drug design. Antigene strategies for protein down-regulation require adjuvant ligands to stabilise triplex structures; four-stranded DNA is implicated in the abnormal genomic DNA of Werner's, Bloom's and Fragile-X syndromes, besides its role in the regulation of telomerase. Furthermore, there is evidence that sequences in the promoter regions of some genes (e.g. the c-*myc* oncogene) are able to form tetraplex structures and that tetraplex-stabilising ligands can be used to control gene expression. Ligands with precisely-defined structure selectivity therefore have a range of potential therapeutic applications. Furthermore, the need to avoid undesirable cytotoxic effects dictates that any competing affinity for duplex structures must be eliminated in the course of design.

Pyrimidines bearing *para*-substituted (ω-aminoalkyl)aryl substituents have been found to exhibit intriguing binding preferences for DNA secondary structures. Whilst biphenyl ring systems cannot adopt planar conformations, the analogous phenylpyrimidine has a steric energy minimum around the planar conformation; in consequence ligands built on a diarylpyrimidine skeleton behave, in part, as DNA intercalators. Two series of ligands have been investigated: one with the ω-aminoalkyl side chain attached through a thioether, the other linked by an amide. Although the thioethers bind modestly to duplex DNA, model building predicted the threading distance of the putative planar portion of the amide series to be a closer match to the larger cross-sections of tetraplex DNAs than to either duplex or triplex.

A novel and versatile synthesis (Scheme) has been developed that utilises a Suzuki cross-coupling reaction to afford this class of compounds in greatly improved yields and structural diversity. Biophysical techniques were subsequently employed to discern an SAR for the binding preferences of these ligands for nucleic acid secondary structures.

Ligands based on the thioethers showed very selective stabilisation of polydA•[polydT]$_2$ triplex DNA ($\Delta T_M \leqslant 25\,^{\circ}C$) with no effect on polydA•polydT polydT duplex melting; minor structural alteration to the amide inverted this selectivity but also indicated a much weaker association. Competition equilibrium dialysis confirmed that, generally, neither family of ligands had a strong affinity for duplex nucleic acid structures, irrespective of sequence or backbone; the preference of the thioethers for triplex and the amides for tetraplex DNA was quite remarkable. Further discernment of the effects of the side chain on the fine details of binding was also possible: in both series, variation of simple diaklyamino groups only affected binding strength (e.g. $X = NMe_2 > X = NEt_2$); however, more significant structural alteration was also able to modulate the pattern of binding preferences.

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Molecular modelling and drug design: some puzzles and solutions

C. A. Laughton

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I will present some of our recent work applying various molecular modelling methods to problems, or at least puzzles, in drug design and development.
Firstly I will describe how a range of modelling methods, from protein structure prediction to quantum mechanics, have been applied to help us understand the mechanism of action of a new class of antitumour molecules. The 2-(4-aminophenyl)benzothiazoles are a novel series of selective antitumour agents discovered by the Nottingham Group and under advanced preclinical development both in Nottingham and the National Cancer Institute, exhibiting potent (sub-nanomolar IC_{50}) in vitro and in vivo activity only in certain human breast, ovarian, renal, colon and lung cancer cell lines. The compounds are actually prodrugs, being activated through metabolism by P4501A1 to as yet unidentified products which exert their antitumour effect via DNA alkylation. However, for certain members of the series this pathway instead leads to inactivation, via 6-hydroxylation. By building a homology model for P4501A1 and performing docking studies, we have been able to show that the pathway followed by members of this series – activating or deactivating – is not governed by different binding modes. However, through quantum mechanical calculations on their chemical reactivity we have been able to show that the metabolic pathway probably progresses via a common nitrenium ion intermediate. Examination of their Frontier Molecular Orbitals is a very reliable guide as to whether the molecule will ultimately produce an active, or deactivating, metabolite and is being used predictively now to guide drug development.
Secondly I will describe how another modelling method, molecular dynamics, has enabled us to explain puzzling experimental data regarding drug-DNA interactions and the role of DNA flexibility. Fuelled by developments in genomics, there is much current interest in the development of drugs that will exert their effect directly on DNA, selectively interfering with the expression of certain genes. However the 'rules' governing the design of molecules that can 'read' the genetic code is still poorly understood. We have been investigating a small model system for drug-DNA recognition that shows co-operativity – the DNA sequence binds two molecules of the drug (Hoechst 33258) in preference to just one. Yet NMR studies show that the two drug molecules bind to two separate sites that are not particularly close to each other and that the binding does not noticeably alter the structure of the DNA. Through extended molecular dynamics simulations we have been able to calculate thermodynamic parameters for the recognition process and show that the recognition of these molecules for the DNA is driven by entropy, not enthalpy. This explains why the origin of the co-operativity is effectively 'invisible', but also provides a warning regarding how difficult rational drug design may be in this area.

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Antiviral drug discovery

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In this presentation we will review the discovery of antiviral nucleosides such as AZT and d4T(**1**) and describe the discovery in our laboratory of a new method for enhancing the potency of such agents based on phosphoramidate pro-drugs such as the phenyl methoxyalaninyl phosphoramidate (**2**). These achieve the intracellular delivery of the bio-active phosphate forms and by-pass the rate limiting dependence on nucleoside kinase – mediated activation.
We will also describe the recent discovery in our lab of new, exquisitely potent and selective inhibitors of Varicella Zoster Virus (VZV) by unusual bicyclic furano pyrimidine deoxy nucleosides such as (**3**). These agents inhibit VZV at 1nM and are non-toxic at 200 μM.
We will describe the extension of these technologies to other viral diseases and to non viral disease such as cancer.

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Gamma irradiation and parenterals

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Sterilization of healthcare products using ionizing radiation is a critical activity and is deemed a special process as verification of sterilization is not practical as a post processing activity.
Therefore, validation of the process is vital to ensure effective sterilization. Validation can be divided into two activities:

1 Dosimetric validation
2 Microbiological validation

Dosimetric validation is the activity that takes place in the sterilization plant and is also known as dose mapping. This involves the placement of dosimeters throughout the product to determine the minimum and maximum dose positions. This ensures the minimum and maximum dose specification selected by the manufacturer can be achieved.
Microbiological validation is the dose setting exercise performed to ensure that the selected minimum dose will achieve a sterility assurance level (SAL) of at least 10^{-6}. This is the minimum level required for healthcare products sterilized within Europe to enable them to be labelled sterile and is referenced in the European standard EN556.
This presentation will discuss the requirements for both dosimetric and microbiological validation and will also cover routine control of sterilization using ionizing radiation.

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Lyophilisation: formulation and process design

Larry Staines

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Formulation of a successful lyophile assures a physical and chemical stable product with physical strength to withstand the rigours of distribution, manufactured in an efficient and reproducible manner.
Formulation will require the evaluation of bulking agents to assure physical strength, cryoprotectants to maintain molecular integrity of the active moiety and buffers for the stability of pre and post constituted lyophile. Extensive optimisation by thermal evaluation to evaluate eutectic (T_{eu}) or collapse (T_c) temperatures will ensure robust characteristics capable of reproducible and successful processing.
Successful lyophilisation is achieved by an understanding of each component of the cycle from loading to backfill/stoppering.
The design of the manufacturing process requires consideration of the issues of variable scale, in particular freezing rate and thermal fluctuation in smaller plant. Rate of freezing can have a significant effect apon the nature of the frozen crystal lattice. This can, in turn impact skin formation and rate of sublimation, which may be overcome by thermal conditioning as part of the freezing process.
Primary drying should be designed with full knowledge of significant endotherms encountered during temperature conditioning of the frozen plug. Factors such as product depth and chamber pressure control will impact the design, length and efficiency of sublimation.

End point control is essential to ensure adequate but not excessive drying, which can have implications for product stability and structural integrity of the finished product.

Freeze drying from formulations containing inflammable solvents requires special consideration of plant and process design.

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Advances in pre-filled syringe technology

Peter Gassmann

Vetter Group, Germany

Prefilled injection systems enable various convenient, safe and cost-effecive ways to administer parenteral drugs. More and more proteins and other drugs come to the market each year. The focus is therefore to ensure aseptic filling of these high-end products.

The presentation outlines how a company can develop, validate and utilize new technologies in barrier systems to aseptically fill both liquid and lyophilized products. Examples will be given for the the processing of syringes with in situ lyophilization, filling of proteins, monoclonal antibodies, as well as novel systems developed to enhance parenteral drug delivery.

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How the industry makes inhalers

Andrew R Clark

Senior fellow & Chief Scientist, Inhale Therapeutic Systems, San Carlos, CA, USA

"Pharmaceutical inhalers" have been used to treat respiratory diseases for many centuries. Early therapies involved the use of vapors from aromatic plants, balsams, myhrr, sulfur, etc. However, around the turn of the 19th century these early therapies developed into liquid nebulizers, where solutions of an active compound are atomized to produce fine droplets that can penetrate into the airways and deposit in the lungs. In the 1920s adrenaline was introduced as a nebulizer solution, in the1930s nebulized porcine insulin was used in experimental studies in diabetes and in the 1940s, pulmonary delivery of the recently discovered penicillin was investigated. By 1950, steroids were introduced for the treatment of asthma and nebulizers were used extensively. However, nebulizers have their limitations and in 1956 the nebulizer was joined on the market by the more convenient pressured metered dose inhaler (pMDI). Invented at Riker laboratories in Santa Barbara this dosage form uses compressed propellants to produce an aerosol from either a solution or a suspension of drug. As a result, formulation development is a little more challenging than a nebulizer solution. However, over the past 5 decades, helped by the advances in molecule design, the pMDI has risen to become the main stay of asthma treatment around the globe. As with nebulizer technologies, though, the pMDI also has it's limitations and in the late 1950s the dry powder inhaler (DPI) was added to the mix. The DPI uses powder formulation techniques to facilitate the generation of an effective aerosol directly from dry powder drug particles.

Over the past decades many device and formulation technologies have been added to this basic list. For example add-on devices for pMDIs, which help patients coordinate and reduce unwanted oropharyngeal deposition have become popular. Multidose and powered DPI devices have been developed and nebulizer machines with higher efficiencies and reduced dosing time are now available.

Particle manufacture for pMDIs and DPIs has also advanced dramatically with spray drying and super critical fluid precipitation beginning to replace the established solvent crystallization and miconization techniques. On the nebulizer front the old glass ampoule technology has been replaced with the aseptic form-fill-seal technology, which produces plastic ampoules.

With the advent of these modern technologies development, product manufacture and quality control have become more complex. The need to understand how a patient uses an inhaler and the facilitation of realistic laboratory testing has become of paramount importance. Research and quality control scientists alike need to test inhalers in a realistic fashion in order to obtain meaningful results. Measurement of the delivered (emitted) dose and the aerodynamic size distribution of the delivered dose are the two key in-vitro assays currently described in the world's pharmacopoeias. In-vivo assays have also developed well beyond simple efficacy measures with gamma scintigraphy and pharmacokinetics being used extensively to investigate product performance.

All of these sophisticated technologies and test methods have aided the development of ever more efficient and patient friendly inhalers. A trend that will probably continue into the 21st century as the industry continues to "make inhalers".

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Industry experiences of the HFA transition

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The Montreal protocol is a legally binding international agreement, signed in 1987, which obliges signatory countries to reduce and eventually eliminate production and consumption of ozone depleting substances including CFCs. The phaseout date for developed countries was 1996, however an essential use exemption has allowed continued use of CFCs in pressurised metered dose inhalers (pMDIs). Various national transition strategies towards non-CFC pMDI products are in place, the completion of which will eventually end this exemption.

In the early 1990s HFA 134a and HFA 227 were identified as potential replacements for CFC propellants in pMDIs. Toxicological safety was demonstrated by two international consortia: IPACT I (HFA 134a) and IPACT II (HFA 227). The physicochemical properties of these propellants are such that surfactants used in CFC formulations are not soluble without an adjuvent. Also, poor valve function occurs with traditional 'CFC' metering valves and elastomers. Replacement of CFC products has therefore presented a major technical challenge to the pharmaceutical industry. Overlaid upon this has been a trend towards more stringent regulatory requirements.

In 1999 it was estimated that 1400 scientists at 90 laboratories in 19 countries were working on replacement products and that industry had spent approximately $1 billion on the transition (IPAC data). Prior to launch of the first HFA pMDI in the UK in 1995 approximately two CFC products came to market each year. Since 1995 there have been 7 HFA products launched in the UK. CFC pMDIs continue to be manufactured however the amount of CFC propellants licensed by the European Commission decreases each year.

Companies have generated a considerable amount of Intellectual Property during the HFA transition. Formulation patents include the use of Povidone and PEG (Fisons/Aventis), HFA 134a and ethanol (3M), and excipient free (GSK). In general the HFA replacement products launched have targeted equivalent performance to the CFC original, however the formulation of the HFA product QVAR? (3M) is such that lung deposition is improved, which has enabled the total dose of steroid delivered to the patient to be reduced.

The perceived advantages and disadvantages of pMDIs compared to other portable devices such as dry powder inhalers and small volume nebulisers are well documented. It is clear that there is no 'universally ideal device' and that each will have its own market segment and continue to coexist for the foreseeable future. Concerns have been raised over the future environmental impact of HFAs however they have significantly lower global warming potentials than CFC propellants. It is estimated that the contribution to climate change of HFAs from pMDIs in the year 2010 will be no more than 0.02% of all greenhouse gas emissions (IPAC data). The industry has lobbied strongly for the Parties to the Kyoto Protocol on climate change to provide protection for this critical medical use.

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Device trends

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Devices for the administration of medication into the respiratory tract date from time immemorial. The use of smokes and fumes of very fine particle size employed from early times revealed an intuitive grasp of particle mechanics that has since progressed to mathematical precision as understanding of the behaviour of airborne particulates advanced in recent times. Consensus of the key factors involved in the entry and deposition of particles in the respiratory tract was reached in the middle of the 20th century with the work of the USA based "Task Group on Lung Dynamics", coinciding unsurprisingly with the beginnings of modern inhaler device development. The legendary development by Thiels of the pressurised inhaler in the 1950's, followed by the first substantive breath actuated system, a dry powder inhaler by Altounyan in the 1960's, are milestones. The trickle of novel inhalers and nebulisers initiated by these devices has grown to a veritable flood of awesome devices in the past decade that show few signs of abating.

Trends in device development up to the late 1980's were in the main unremarkable, influenced by a perceived need to enhance topical treatment of local airway disease. By 1990 around 300 million standard pressurised inhalers and perhaps 10 million dry powder devices were in use annually. Then suddenly with the immediacy of 9/11, the leisurely world of device development changed. Wholly unanticipated factors emerged to stimulate exciting projects. Growing concerns of destruction of the Earth's ozone layer by CFC propellants attracted a plethora of new entrants to the inhaler field, generated a myriad of new non-CFC inhaler designs and an explosion in the device patent literature. A hitherto uncooperative international industry driven by committed global regulators responded to the threat to patient treatment - and their markets - with ozone-friendly propellants. And stimulated discovery. A principal cause for concern, asthma, inexplicably showed rising incidence in many countries, adding further pressure. Technologies in other fields, particularly electronics, were opening up new opportunities to create magical portable devices with the promise to overcome the surprisingly difficult task of achieving consistent drug administration into the lungs of a curiously variable patient population. And a new vision. Crick and Watson's brilliant elucidation of the structure of DNA founded the modern biotechnology industry. A consequent need for delivery systems for sophisticated protein and peptide structures created a whole new challenge for systemic drug administration via the respiratory tract - but where were the devices? Whilst man's ability and technology to create exciting and novel answers to the problems has never been in serious doubt, a powerful inhibition has emerged in recent years. There is a limit to the price that beleaguered health authorities are able to pay for innovation.

The past teaches that it is not simply new technology that drives trends in device development. Cleverest, most elegant, most inventive is not enough. The inhalers of the future as in the past are being forged by a complex interaction of forces that includes prevalent disease, new drug characteristics, changing economics, and the ever-present unexpected. The market's own particular form of Darwinism will dictate what devices emerge to serve the patient's need.

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Why drugs that are effective in animal models then fail in the clinic: an industry perspective

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To date, every neuroprotective agent that has been shown to be efficacious in animal models of acute ischaemic stroke has failed in a subsequent clinical trial. There have been diverse reasons for failure, including poor clinical trial design, but primarily, most failures have been due to adverse events or lack of efficacy. This problem was recently addressed at an academic/industrial roundtable meeting that discussed guidelines for drug selection that would result in an increased likelihood of subsequent clinical success. The group has published the criteria it considers necessary for a compound to meet if it is to be progressed to clinical evaluation. Requirements suggested include dose-response criteria (including minimum and maximum dose efficacy); therapeutic time window evaluation (that is, the time after the stroke at which the drug can be given and still have a beneficial effect); functional behavioural testing, in addition to histological evidence for neuroprotection; use of appropriate animal stroke models (permanent occlusion as well as reperfusion models) and reproducibility of observations in external (independent) laboratories. The presentation will review these criteria and show, using examples of previous clinical failures, why the guidelines appear logical in the light of these past failures. It is now generally agreed that plasma levels required for neuroprotection in animals must be matched in patients. However, until recently, there has been little attempt to match exactly the therapeutic time window in animals with the time of drug administration in patients. Furthermore, since most patients reperfuse slowly, if at all, after a stroke it is also logical to demand efficacy in a permanent occlusion model. Clinical evaluation of stroke patients and evidence of improvement relies on neurological tests, therefore functional motor tests should be performed in at least one animal model, rather than relying on histology alone as evidence for a neuroprotective action of a compound. Finally, since we model large strokes in animals, patient selection should similarly concentrate on patients with neurological evidence of a large stroke.

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Adaptative tolerance to ischaemia: a novel strategy for the development of neuroprotective agents

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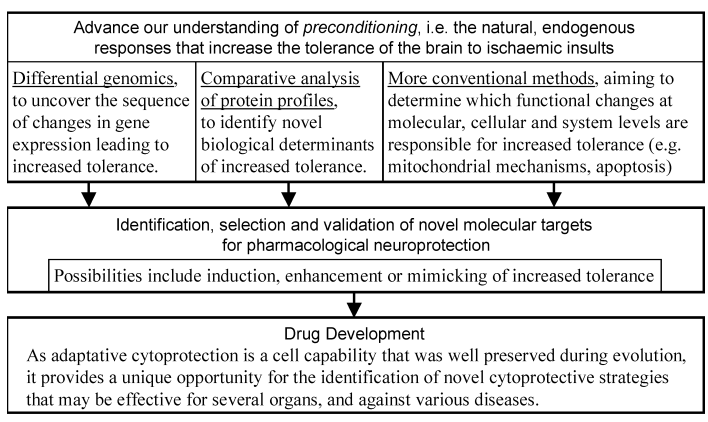
During the last two decades, a tremendous effort has been made to develop drugs designed to correct single, specific biochemical abnormalities associated with cerebral ischaemia. So far, the output from this research strategy has been very disappointing in term of drug development, possibly because this approach has overlooked the very complex and multifactorial origin of ischaemic damage. In contrast, much less resources have been directed towards the inherent capability of most living cells to enhance their tolerance to cytotoxic stress. This fundamental biological process, often referred to preconditioning, adaptative cytoprotection, or increased tolerance (e.g. to ischaemia), provides a radically different scientific rationale and strategy for the discovery of neuroprotective agents, which is illustrated in the diagram below.



This rationale is receiving increasing attention with the discovery that delayed increased tolerance to insults could also be achieved with some drugs (Blondeau et al 2002). In our laboratory, we have developed a method of preconditioning that is especially suitable for genomics and proteomics, as whole cerebral hemicortices of rats of mice can be uniformly subjected to a controlled stimulus (i.e. repetitive cortical spreading depression (Godukhin & Obrenovitch)).[2] The effectiveness of this method of preconditioning was confirmed in a mouse model of stroke, and differential gene expression analysis is being carried out at several time points after preconditioning as part of the activities of a European research programme (StrokeGene).[3] We have also found that the intermediate filament protein nestin was upregulated 24-h post-preconditioning,[4] a feature that may provide the basis for an important, complementary experimental strategy, i.e. the generation of mice with conditional, disrupted gene expression for the study of preconditioning, by coupling the expression of the targeted mutated genes to that of nestin. Finally, we have discovered that the expression of several ionotropic receptor protein subtypes was differently altered by preconditioning, and some of these changes point to novel molecular targets for neuroprotection (Chazot P, Godukhin OV, Obrenovitch TP; unpublished data, March 2002).

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Cytokine gene polymorphisms: gateways to immunotherapy of autoimmune diseases?

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Cytokines are small, secretory proteins that induce, amplify and modify the course of immune responses. Most cytokines can be classified into one of 2 distinct groups, i.e. TH1-cytokines (e.g. interferon-γ, tumor necrosis factor-β and interleukin-2) or TH2-cytokines (e.g. interleukin-4, -5 and –10). TH1 cytokines are typically associated with cell-mediated immune reactions, while TH2 cytokines play a role in strong antibody and allergic responses.

Chronic inflammatory diseases, such as multiple sclerosis (MS) and rheumatoid arthritis (RA), are characterized by excessive production of endogenous TH1 cytokines bringing about a state of sustained immune activation, inflammation and accompanying tissue destruction. A well-documented feature of such diseases is that these occur with a 'gender bias' effect, i.e. men tend to develop MS and RA 2 to 3 times less often than women. Though it is thought that hormonal factors may contribute to this bias, no robust link has ever been demonstrated.

DNA polymorphisms within or close to cytokine genes can affect transcription regulation, and hence production level of these cytokines. Such cytokine gene polymorphisms may predispose their carriers, or protect them from, specific chronic inflammatory conditions. In addition, these polymorphisms may alter disease course from aggressive to benign.

We have collected evidence that polymorphisms within the IFN-γ gene are associated with susceptibility to multiple sclerosis. Especially, we found that a single allele of a microsatellite polymorphism located within the first intron of this gene is underrepresented in men, but not women with MS. This observation was confirmed in 3 ethnically distinct populations, Sardinian, Northern Irish and American, attesting to a wider relevance. We identified several novel single nucleotide polymorphisms (SNPs) in the IFN-γ gene, some of which showed a similar gender association. We performed a linkage disequilibrium analysis that revealed that this gender bias trait maps to a 100-kb interval surrounding IFN-γ gene. Thus, our findings suggest that common polymorphisms in the IFN-γ gene contribute to the relative protection of men from MS.

This study fits into a rapidly evolving concept of genomics-integrated approaches to drug development. The information we have collected may reinforce understanding of the genetic/functional mechanisms that cause gender bias in autoimmune diseases, and such information may be of importance both in future diagnostic genetic screening and as a means to define patient strata that are likely to benefit from cytokine-based immunotherapy.

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Nicotine: from pariah to panacea

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Nicotinic receptors in the CNS were originally recognised as the mediators of the psychomotor stimulant and rewarding properties of nicotine. Thus nicotine acts through such receptors to produce the addiction that characterises tobacco use. Nicotinic receptors in the brain are now known to comprise a family of pentameric ligand gated ion channel receptors that normally exert a modulatory influence on brain function. For example, presynaptic nicotinic receptors can enhance the release of a variety of neurotransmitters, while the high calcium permeability of some subtypes of nicotinic receptor enables them to interface with calcium-dependent cell signalling pathways. These mechanisms make nicotinic receptors attractive drug targets for moderating brain activity. Hence, they are candidates for therapeutic intervention in a diverse range of neurological disorders, including Alzheimer's and Parkinson's diseases.

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Sleep dysfunction: novel sleep modulating hormones and their cellular actions

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Sleep dysfunction may reflect acute anxiety but is a frequent complication of a variety of psychiatric or neurological diseases. Normal circadian sleep and dreaming occurs in well-defined physiological phases (in terms of the EEG profiles). Ageing is negatively correlated with sleep, which should be stressed when elderly patients present with insomnia in the clinic or pharmacy. Cellular circuits involved in sleep are reasonably well characterised but the molecular signalling mechanisms remain enigmatic. The hypothalamus is an important regulator of sleep and recently a new class of peptidergic ligands has been characterised here which regulate arousal, metabolism and satiety. The hypocretins (or orexins) stimulate firing in pontine nuclei (crucial for arousal), increase metabolic rate and psychomotor activity and stimulate feeding behaviours. Two peptides (28-33mers, derived from a common precursor) have been described which activate one of two G-protein linked receptor isoforms (Hcrtr 1&2). Hypocretin cells are innervated by axons from supra-chiasmatic nucleus (a key regulator of circadian rhythm) and project to other areas of hypothalamus, pontine nuclei, basal forebrain, pre-optic area, thalamic nuclei and spinal cord. Disruption of Hcrtr expression in transgenic animals can mimic the symptoms of narcolepsy and a significant body of evidence exists to suggest that human narcolepsy reflects auto-immune attack of these receptors in susceptible patient families.

The drive to sleep increases with sleep deprivation and several humoral factors have been implicated (e.g. a variety of peptides, cytokines and adenosine). In 1995, a hypnogenic lipid fatty acid amide was detected in the CSF of sleep deprived cats. Synthetic *cis*-Oleamide enhances total sleep time without overt changes in sleep architecture (most clinically useful drugs depress paradoxical sleep). Like many hypnotics in clinical medicine (e.g. barbiturates and benzodiazepines) oleamide is a stereoselective modulator of the widespread $GABA_A$ receptor (expressed at 1/3 of all CNS synapses) at inhibitory neurochemical junctions. Oleamide enhances GABA currents and the duration of inhibitory synaptic potentials but these actions are insensitive to the selective benzodiazepine antagonist flumazenil. Oleamide can suppress the frequency of inter-ictal seizures in a 4-AP model for epilepsy in CA3 of rat hippocampus (see also associated poster). Oleamide concurrently targets nerve terminals to depress transmitter release secondary to a state-dependent blockade of voltage-gated sodium channels. Some of the *in vivo* effects of

oleamide respond to CB1 antagonist but this may reflect the fact that both anandamide and oleamide are cleaved by FAAH enzymes. Unpublished data demonstrate that oleamide does not antagonise the orexin receptors described above. We can find no evidence in the CA1 subfield of hippocampal slices, or in recombinant fluorescence assays, that oleamide is a high affinity modulator of metabotropic 5HT receptors. Brain permeant modulators of these novel physiological signalling pathways hold much promise for the treatment of sleep disorders in the future.

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Prion-related problems associated with the sterilisation of medical devices

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The unconventional agents that cause transmissible spongiform encephalopathies (TSEs) such as bovine spongiform encephalopathy (BSE), scrapie in sheep, and Creutzfeldt-Jakob disease in humans, are known to be relatively resistant to a wide variety of inactivation procedures that are effective with conventional micro-organisms (Kimberlin et al 1983; Brown et al 1986; Taylor et al, 1994; Taylor 1999a; Taylor, 1999b, Taylor, 2000). It is anticipated that the agent that causes variant CJD will share this property (because it is the BSE agent) but this has not yet been formally demonstrated. Even some inactivation procedures that were previously considered to be completely effective, are now known to provide a substantial degree of, but not complete, inactivation, Such procedures include exposure to 1M sodium hydroxide for an hour at room temperature, gravity-displacement autoclaving at 132°C for an hour, or porous-load autoclaving at 134-138°C for 18-60 minutes (Taylor et al, 1999a). Nevertheless, the recommended use of sodium hypochlorite solutions containing at least 20,000 ppm of available chlorine still appears to be an effective method although it is not a particularly user- or product-friendly procedure (Kimberlin et al 1983; Taylor et al 1994). Despite the doubts about the efficiency of achieving complete inactivation by either sodium hydroxide exposure or autoclaving, a number of studies have indicated that complete inactivation can be achieved by combining these procedures consecutively or simultaneously, even at an autoclaving temperature of 121°C (Taylor 2000). In addition, an indication that these conditions provide a good degree of "overkill" has been provided by studies in which the 301V strain of mouse-passaged BSE agent was completely inactivated after boiling in 1M sodium hydroxide for only one minute (Taylor et al 1999a). The 301V agent is known to replicate to relatively high titres in mouse-brain, and is the most thermostable mouse-passaged agent that has yet been identified (Taylor et al 1999b).

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Is there a need for biocide rotation? An examination of evidence for and against

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Biocides (collectively disinfectants, antiseptics and preservatives) are used for a wide range of applications in the hospital, home and pharmaceutical manufacturing industries, to name but a few. They can be used to effectively decontaminate surfaces and reduce bacterial counts.

The practice of alternating biocides has caused some uncertainty as questions over the value of implementing such a programme have arisen. Current Medicines Control Agency (MCA) guidance for the manufacture of sterile medicinal products, in the pharmaceutical industry state: 'Where disinfectants are used, more than one type should be employed' (MCA 1997). No information is provided on the type of agents to be used nor the frequency of rotation.

In hospital pharmacy aseptic units (HPAUs) where pharmaceuticals, dressings and instruments are prepared and sterilised, this guidance is not always implemented. Approximately 65% of HPAUs surveyed employed a biocide rotation policy (Murtough et al 2000).

The scientific evidence to support biocide rotation is scant, partly due to the difficulty in designing laboratory experiments, which mimic the use of biocides in a rotation policy.

The majority of microorganisms can be eradicated by "in-use" concentrations of biocides, but some types of micro-organisms are inherently resistant to these agents. Spores and mycobacteria are particularly difficult to destroy due to their highly impermeable barriers, which limit the uptake of biocides. The types of microorganisms usually found in aseptic manufacturing units are most likely to be Gram-positive cocci with occasional Gram-negative rods. These bacteria are more susceptible (with a few exceptions such as *Proteus* spp.) to the action of biocides and therefore if biocides are employed correctly, they should be eliminated.

In the laboratory, some types of microorganisms (particularly Gram-negative rods) have been adapted to survive exposure to increased biocide concentrations. This has been achieved in two ways, *viz.* i. 'step-wise training' where cells are grown in or on media containing increasing concentrations of biocide or; ii. repeated exposure to a 'residual' biocide concentration.

Using these methods, researchers have been able to increase the insusceptibility of certain bacteria to biocides. However, the final biocide concentrations that the bacteria can survive in these experiments are still well below the in-use concentrations of these agents. Furthermore increased insusceptibility can be lost upon withdrawal of the biocide.

Biocide rotation remains a controversial subject. To ensure uniformity, the MCA needs to clarify its guidance. Particular attention should be paid to the types of agents which are to be used and the period of biocide rotation. The guidance should be backed with sound scientific results, which will give confidence to the end-user when implementing the guidance.

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