

Faster and smarter analytical techniques

New techniques that can improve the efficiency of pharmaceutical analysis were discussed at a recent symposium of the Joint Pharmaceutical Analysis Group. Joseph Chamberlain reports

Pharmaceutical analysts have been adopting faster and smarter processes and procedures to deal with increasing commercial and social pressure to discover and develop new medicines while reducing costs and improving productivity. A number of these new techniques were discussed at the JPAG symposium.

Deep Raman spectroscopy Until recently Raman spectroscopy was a surface or near-surface technique, said Pavel Matousek, of Rutherford Appleton Laboratory, but advanced research has transformed it into a deep spectroscopy technique. Two approaches are used: the collected signal may be offset in time or it may be offset in space from the point of laser irradiation. Hence pure Raman spectra at different layers within the sample can be constructed.

Examples of the use of the technique included probing of the active ingredient in pharmaceutical capsules despite a fluorescent shell, study of possible counterfeit tablets contained in glass bottles, the detection of powders in opaque plastic containers, and the non-invasive detection of cocaine in a bottle of rum. Following on from these developments, Dr Matousek described transmission Raman spectroscopy for quantitative analysis of bulk solids without the disadvantages inherent in subsampling methods.

NMR spectroscopy An important parameter affecting the performance of formulations is the extent of polymorphism and pseudo-polymorphism of the ingredients, said Steven Brown, of Warwick University. Nuclear magnetic resonance (NMR) spectrometry is a technique for determining the chemical structure of single molecules in solution by examining the hydrogen atoms. In the solid state, the packing of organic molecules is controlled by weak intermolecular interactions such as hydrogen bonding. Protons are directly involved in hydrogen-bonding interactions and therefore high-field solid-state NMR can be used to determine polymorphism and pseudo-polymorphism. When combined with carbon-13 magnetic resonance spectroscopy, a powerful technique is available to characterise solid-state formulations, said Dr Brown.

Quantitative NMR The further application of NMR in quantitative magnetic resonance imaging of pharmaceutical drug delivery matrices was described by Mick Mantle, of the University of Cambridge. He said that, because magnetic resonance imaging is totally non-invasive, using non-ionising radiation with no need to add tracers, it can be quantitative.

These properties allow the technique to be used to follow the dissolution of capsules. The soluble outer capsule layer dissolves, rapidly releasing many partially soluble pellets. Water diffuses into the pellets, dissolving the drug and releasing it into the surrounding liquid. During this process the evolution of the pore structure can be characterised.

Significant differences can be seen between the drug-loaded and the placebo pellets, with placebo pellets having smaller pores, and a higher tortuosity. Drug-loaded pellets show a large increase in mean pore size with immersion time. These and other NMR data can be used to model the drug release characteristics.

Vibration spectroscopy imaging The use of vibration spectroscopy imaging as a problem-solving tool in pharmaceutical development was described by Don Clark, of Pfizer UK. He said that a chemical image is a photograph with chemical information. Images are constructed with good spatial resolution (microns) and good spectral resolution (1–8cm⁻¹). Information is provided on the identity, polymorphism, size and distribution of components.

Dr Clark explained that a mapping system produces spectra at specific spatial positions, whereas an imaging system produces intensity images at specific wavelengths. Applications of chemical imaging demonstrated that it is useful for increasing understanding and prediction of formulation performance. It is predominantly a technique for tablets, although it may also be promising for inhaled products. Relationships can be established between physical features or defects and chemical structure, content identity, uniformity, homogeneity and stability in the solid state, and of coating properties and effects. It can characterise the tablet interior *in situ*, even for low dosage products, leading to the goal of improved formulation understanding and design.

Terahertz pulsed imaging Terahertz pulsed imaging uses the electromagnetic spectrum between microwaves and infrared. Phil Taday, of TeraView, explained that its versatility allows it to be used in evaluating a wide range of solid dosage forms. It is applicable to different size tablets and not restricted to flat samples. Unlike other technologies, terahertz radiation can penetrate coating layers, allowing the inner content to be imaged. The sample is measured

directly with no dissection or removal of coatings being required.

Because the technique is non-destructive, the whole sample is available at the end of terahertz analysis for further testing. Because no heat is generated, there is no interference with physical or chemical characteristics of the sample.

The technology has now been used extensively for delayed-release products. Terahertz pulsed imaging reveals complex structures within the coating which could affect product performance. The technique allows an increase in understanding of the processes of complex coating structure processes, and preliminary results show that data obtained using terahertz techniques can be correlated with dissolution.

Ion mobility spectrometry Rapid separations by ion mobility spectrometry (IMS), a gas-phase electrophoretic technique, were described by Colin Creaser, of the University of Loughborough. He said that rapid separation of ions occurs as a result of differing mobilities in a buffer gas and it is most commonly operated at atmospheric pressure or in the range 1–5 Torr. Under low field conditions, ion mobility, and hence separation, depends on reduced mass, charge and shape or size. Ion mobility scan times are of the order of 20ms, with 2–5s for accumulation of enough spectra for usefulness. Thus the technique can be considered fast, but it is essentially a low-resolution technique.

For pharmaceutical cleaning verification, analysis of a swab can be completed in 30 to 60 seconds — considerably faster than most high performance liquid chromatography (HPLC) techniques. Combinations of IMS with liquid chromatography (LC-IMS), mass spectrometry (IMS-MS) or both (LC-IMS-MS) are orthogonal separation methods and may be considered smarter but are not fast. Separation of active pharmaceutical ingredients from excipients and impurities could be demonstrated, but slow scan times (greater than one minute) are not compatible with LC peak elution time. Separation of gas-phase ions is on the millisecond timescale and is compatible with a wide range of ionisation techniques.

IMS has traditionally been used in the military and security fields, but has potential for high throughput combined with high selectivity in pharmaceutical analysis, particularly in combination with MS, concluded Professor Creaser.

Ultraperformance HPLC The logical improvements in HPLC for smarter and faster analyses would use smaller particles as

The Joint Pharmaceutical Analysis Group symposium took place at the School of Pharmacy, University of London, on 3 and 4 April

packing material and higher pressures for the mobile phase; that is ultra HPLC or UHPLC. François Lestremou, of Pfizer Europe, considered in depth all the factors affecting a desirable chromatographic outcome, including running the columns at higher temperatures. Orthogonal screening methods — that is, where several independent properties of the analyte are assessed — offer a more consistent and streamlined approach and UHPLC makes it a more rapid approach. However, Dr Lestremou showed the need for balancing parameters using appropriate kinetic plots to visualise the limitations.

Although HPLC with particles as small as 2µm is notoriously unstable, Dr Lestremou considered such systems could be made robust enough for UHPLC to contribute to faster and smarter analyses in pharmaceutical research and development.

DART techniques The acronym DART stands for direct analysis in real time, and thus — unlike other acronyms beloved of analytical chemists — tells us nothing of the technique itself. In fact, it is generally understood to refer to a novel ionisation technique developed by the company JEOL that provides for the rapid ionisation of small molecules, generally under ambient conditions.

Samples exposed to the DART gas stream will rapidly generate ions that are carried by

the gas into the sampling orifice of the mass spectrometer interface. The gas stream can interact with a sample surface and liquids can be sampled by dipping an object (such as a glass rod) into the liquid sample to be measured. Chip Cody, of JEOL, US, described the benefits of these direct sampling techniques in pharmaceutical applications. The procedure enables fast analysis, with little or no sample preparation for many analytes, simple mass spectra for most small molecules, and compatibility with high-resolution MS-MS systems.

However, although the process can distinguish some isomers by fragmentation, it is not a good approach for mixtures of isomers. Some separation by temperature- and time-dependent desorption can be achieved, but separation is certainly not as good as chromatography, and trace-level detection is not comparable with state-of-the-art GC-MS and LC-MS.

DART is currently in use for natural products and herbal medicines, monitoring of synthetic processes, thin layer chromatography plate analysis and toxicological screening. Its usefulness has been demonstrated for counterfeit drug detection and characterisation, and for high-throughput screening. Preliminary investigations show promise for cleaning validation, quality control, and the detection of impurities and degradants. Applications are also feasible in pharmaco-

netic and metabolism studies. For example, for the analysis of drugs in urine, 3µl of unprocessed sample is placed in a melting-point tube and exposed to the source. There is no internal standard and no chromatography and each analysis is completed in 10s.

Quantitative NMR Andy Phillips, of Astra-Zeneca, described the use of quantitative NMR for productivity improvements in the areas of impurity determination, drug assay, solubility measurement, potential genotoxic impurities detection, stereochemical issues and characterisation of polymorphs. Using NMR, quantitative analysis is possible, with little method development, one experiment yielding many results. For determination of small amounts of impurities, the inherent low sensitivity of NMR can be enhanced by combination with preparative liquid chromatography. Advanced techniques such as two-dimensional NMR and decoupled spectra can improve the specificity of such methods.

For compounds containing fluorine atoms, fluorine-19 magnetic resonance spectroscopy is ideal for the analysis of formulations because excipients will not interfere and sensitivity is on a par with proton magnetic resonance.

In the future NMR could be used more as magnet technology significantly improves and complete automation becomes possible. It will often be quicker than HPLC, said Dr Phillips.

Benefits of automation and LIMS

Although pharmaceutical manufacture has become highly mechanised and automated over the past 25 years, automation of analytical chemistry has not progressed much beyond sample analysis, said Robert Cripwell, of GlaxoSmithKline. Most systems are built after the product to be tested already has a manual test so that the automation must attempt to replicate the existing manual sample preparation.

“We should look at what we are trying to achieve with the manual test and define the test with the automation in mind,” said Dr Cripwell. For example, for inhaled products the way the device is held, the way it is fired and the way the dose is captured for the test must be considered. Within GSK the testing portfolio for new inhaled products has been re-defined so that all testing uses the same core elements.

Speaking on laboratory information management systems (LIMS), Bob McDowall, of McDowall Consulting, said that the overwhelming increase in data from automated and computerised systems in the pharmaceutical analysis laboratory had added to the importance and implementation of electronic aids such as LIMS and electronic laboratory notebooks. In their implementation — from sample in to report out — there was a need to review working practices and avoid such things as parallel systems.

The basic operating principles of the LIMS environment are that the data must be captured at the point of origin, there should be no transcription checks, and the destination of the data must be known. Critical for an electronic laboratory is the effect of increase in network traffic, which is often overlooked, and IT departments need to be consulted.

Dr McDowall argued that, in implementing a LIMS, the laboratory procedure should be changed to fit the application. Implementation will be faster, there is no customisation or rewriting of software, validation will be faster, and the overall cost of the system will be reduced.

Finally, Dr McDowall emphasised that the people involved in the implementation, from senior management to users, should not be forgotten. Their involvement will ensure success but their non-involvement will ensure failure, he concluded.

Impact of new techniques on regulatory submissions

After the technical presentations, a review of the symposium as it might be seen by a regulator was provided by Brian Clark, of the University of Bradford, who is an academic adviser to the Medicines and Healthcare products Regulatory Agency.

Professor Clark said that analysis underpins the bringing of drugs from discovery to the market, and many of the methods discussed at the symposium showed that the analytical community is alive to its part in the process.

As to how faster and smarter analyses are likely to be seen by the MHRA in the near future, it would be difficult to speculate. For example, process analytical technology (PAT) submissions or resubmissions have still to be seen by the MHRA and the European Medicines Agency. On the other hand, near infrared data in submissions are now accepted without question, and the routine use of solid-state NMR is under discussion.

Professor Clark said that some of the the methods described at the symposium would find an outlet in improving formulations and in the development of an active ingredient. Techniques such as spatial offset and transmission Raman spectroscopy and terahertz spectroscopy have clear pharmaceutical applications. The question is whether these methods will be carried forward to regulatory submissions. The licensing authorities will accept new methods as well as the traditional methods provided they have been clearly validated and are reproducible and robust.

Professor Clark added that the regulators and their committee members keep up to date by attendance at meetings such as JPAG symposia and visits to industry to look at new processes and procedures. Many of the procedures described during the symposium were either already in use in the industry or had great potential for the future, and thus the meeting had clearly lived up to its billing of “faster and smarter analysis”.