

How charge-based separations are applied in pharmaceutical analysis

In a one-day update symposium the technologies and applications of charge-based separations in pharmaceutical analysis were examined by leading experts from industry, academia and the regulatory authorities. **Joseph Chamberlain** reports

Popularity of capillary electrophoresis

The use of capillary electrophoresis (CE) for pharmaceutical analysis has become increasingly popular in recent years said Alex Marsh, of GlaxoSmithKline, Harlow, Essex. The wide range of applications for which its use has proved successful includes assay of drugs, determination of drug-related impurities, chiral separations, small molecule and ion analysis and the analysis of pharmaceutical excipients.

The advantages of CE for pharmaceutical analysis include its speed and low cost, reduction in solvent consumption and disposal, and the possibility of rapid method development. With CE a single set of separation conditions can be applicable for a wide range of analyses. CE instruments can be coupled to a variety of detector types, including mass spectrometers, for special applications and more detailed analysis.

Dr Marsh described applications for the separation of neutral pharmaceuticals, anal-

gesics and nicotine impurities by microemulsion electrokinetic chromatography, a technique which offers optimum selectivity for the widest range of compounds and speeds of analysis of less than 30 seconds. Its extension to water-in-oil microemulsions offers a unique selectivity for analysis of insoluble pharmaceuticals in oil-based formulations.

CE also has a role in the determination of physicochemical characteristics and the determinations of pKa values for naproxen and logP values for cephalexin were described.

An important development has been the use of dynamic capillary coatings to produce repeatable electro-osmotic flows and thus regulate migration times. Future developments expected by Dr Marsh include the use of in-capillary concentrators and loop injectors, the routine application of capillary electrochromatography (CEC), hyphenated techniques such as HPLC-CEC-CE, and in-process instruments.

Linking capillary electrophoresis and mass spectrometry

Linking capillary electrophoresis (CE) to mass spectrometry (MS) combines the high efficiency of CE with the sensitive, widely applicable detection capabilities of MS. Analytes can be distinguished by electrophoretic mobilities and molecular masses and structural information can be obtained from MS analysis. These attributes make it an attractive option for the pharmaceutical industry, said Emma Edwards, of the University of York, but it is still rarely routinely applied, in contrast to LC (liquid chromatography)-MS. This may be because interfacing CE with MS remains technically demanding. As for LC-MS, thermospray and nanospray methods of interfacing are favoured. Special considerations with CE interfacing relate to compatibility of flow rates from CE with the ionisation method, completion of the electrical circuit and the positioning of the electrospray voltage.

Commercial CE-MS interfaces make use of coaxial sheath flow because this approach provides the best compromise of ease of use, robustness, separation efficiency and limits of detection. An ideal system would use a sheathless nanospray interface to take advantage of the excellent limits of detection. However, in practice such interfaces add complexity to the instrumentation, and are generally not robust.

The areas of research for improving the performance of CE-MS include the design of the capillary tip at the interface, the introduction of capillary coating materials which modify the electro-osmotic flow and prevent analyte interactions, and pre-concentration techniques. In a typical application to the analysis of drug metabolites, CE-MS had higher limits of detection compared with LC-MS and was less reproducible. Nevertheless, Ms Edwards concluded that when CE-MS becomes user-friendly and robust, its advantages of rapid method development will prove attractive.

Rapid separations can be achieved with the ion mobility spectrometry technique

The ion mobility spectrometry (IMS) technique is a separation-detection system using gas phase electrophoresis that can be regarded as similar to a time-of-flight mass spectrometer operating at atmospheric pressure. It is quicker to fly than to swim, said Martin Ives, of GlaxoSmithKline, Ware, Hertfordshire, and hence rapid separations, typically less than 30 seconds, can be achieved compared with the more conventional chromatographic methods. The technique was first used for trace environmental pollutants (herbicides and insecticides) and more recently by police and security services for detection of explosives and illicit drugs.

Smith Detection is a large company that specialises in providing instruments for application in all these areas and Mr Ives described experiences with the Ionscan, an IMS instrument that is specifically designed for life science and chemical applications. The instrument comprises an inlet system, an ionisation chamber, a drift region where the electrical voltage is applied and a detection system. For laboratory applications, the inlet

system may be a chromatography effluent or Smith Detection's own high performance injector. Ion mobility is dependent on the size of the molecule, the accelerating voltage gradient, the drift gas employed, the drift tube temperature and the pressure in the drift region. The analytical output is described as a plasmagram, a 3D plot yielding information on the number of segments (multiple scans), the position and intensity of the peaks in the plasmagram, and the fingerprint of the test sample.

For cleaning verification, the technique could be used routinely for samples with a good IMS response. Sample preparation is similar to that for high performance liquid chromatography, but no mobile phase preparation is required, and the system is ready for use in 45 minutes. Disadvantages are the limited linear dynamic range, the limitation to non-thermally labile samples, and variable intermediate precision. Nevertheless, said Mr Ives, developments including improvements to the sample introduction and ionisation steps are likely to overcome these problems.

The symposium was organised by **The Joint Pharmaceutical Analysis Group** and took place at the Royal Pharmaceutical Society's London headquarters on 17 March

Lag time before regulatory acceptance of new analytical techniques

There is a lag time before new analytical techniques, such as capillary electrophoresis, are accepted by regulatory authorities in the submission dossiers for new medicines said Sarah Branch, of the Medicines and Healthcare products Regulatory Agency. Usually new techniques make their first appearance in variations to marketing authorisations because existing methodology for established drugs will provide a ready comparison.

Charge-based separations are now making an appearance in the regulatory submissions. The method is particularly powerful for chiral assays. The relevant guidelines for chiral drug products relate to identity, degradation and assay. A stereospecific test is generally not required where racemisation is insignificant

during manufacture or storage; otherwise a chiral assay or enantiomeric impurity test is required. For degradation products, control of the opposite enantiomer is required if racemisation occurs during manufacture or storage. If racemisation is insignificant, an achiral assay is sufficient.

CE has been used in regulatory dossiers on drug development for stability-indicating assays even if it is not the final analytical method for routine control. It has also been used as an orthogonal technique for validation of the specificity of the routine method, as justification for the absence of a test for inorganic impurities in the final specifications, and as confirmation of a racemate to justify the omission of stereochemical tests in the final specifications.

Charge based separations are more applicable for biological substances, where a variety of electrophoretic techniques has been used. CE has been used for the control of protein degradation and by-products, determination of free proteins and free polysaccharides, and for confirmation of structural integrity. The main issues with CE in marketing authorisation applications involve translation from use in development studies to use in the control of marketed product. The developed methods tend to be more instrument-specific and there is a need for cross-validation with the method to be used for routine control. Instrumentation or techniques are not well established and there is still a problem of low sensitivity. There is also often a lack of proper validation data in the dossier, Dr Branch noted.

Chiral analysis

There are several good reasons for using capillary electrophoresis in chiral analysis said Melissa Hanna-Brown, of King's College London. It is comparable to gas and high performance liquid chromatography in terms of peak efficiency but is cheaper to establish and maintain. Most impressive was the speed of analysis possible, eg, complete amino acid analysis in 3.5 seconds.

Chiral selectors, the special requirement for chiral analysis, should be stereoselective, soluble and chemically stable in the system, and efficient with respect to rapid complexation kinetics. Such selectors include crown ethers, surfactants, ligand exchange complexes and macrocyclic antibiotics. Cyclodextrins are also extensively used. The ring size is determined by the number of glucose residues (giving control over the exclusion characteristics) and interaction with the solute can be modified by ring substitution.

For method development, the strategy should be to consult the extensive literature for the most appropriate cyclodextrin and consider the appropriate pH for the analyte. This strategy can be successful, with often only two or three experimental runs being required to arrive at an appropriate system. Dr Hanna-Brown reviewed an extensive list of selectors and native and modified cyclodextrins used in combination for the analysis of racemates of compounds such as flurbiprofen, chlorpheniramine and brompheniramine and their metabolites.

Other strategies in method development will include intelligent experimental design, knowledge-based systems, and artificial neural networks. The huge scope for development of large and diverse libraries, increases in chip speed and use of multiple arrays, extended combinations of selectors, the use of biological matrices, and the application of integrated sample preparation suggests capillary electrophoresis will be a powerful technique.

CE gradually appearing in EU monographs

John Miller, of the European Directorate for the Quality of Medicines, Strasbourg, reviewed capillary electrophoresis from a European Pharmacopoeia perspective. So far, he said, CE had not found great favour in quality control work and the technique was only gradually appearing in European monographs. CE encompasses free solution CE, capillary gel electrophoresis, capillary isoelectric focusing and micellar electrokinetic chromatography. Suitable systems for analysis would address the apparent number of theoretical plates, resolution, the symmetry factor and the signal-to-noise ratio, factors similar to those for chromatographic systems.

Examples which have been considered for inclusion in European monographs include the analysis of erythropoietin in concentrated solution for the isoform content, a micellar electrokinetic chromatographic procedure for levocabastine and CE for establishing the

enantiometric purity of ephedrine and methyl dopa. One problem is that not all these procedures are accepted by the group of experts as the equipment required may be deemed too sophisticated.

Professor Miller described an international collaborative trial to compare isoelectric focusing and CE for the control of impurities of rDNA-somatropin. Fourteen laboratories took part and the CE procedure has been accepted and will be published later this year.

CE may provide excellent selectivity, concluded Professor Miller, but is often insufficiently sensitive to control impurities in pharmaceutical substances at the levels required. It can be good for the control of an unwanted optical isomer, but there may be a sensitivity issue. It is most useful for biological substances which are not covered by the International Committee on Harmonisation impurity guidelines or the EP.

A pressing need for more rapid methods

Within the industry there is a pressing need for methods that allow the rapid analysis of large numbers of complex samples with ever-decreasing sample amounts, said Norman Smith, of King's College London. Only micro techniques can possibly address this problem. Apart from the well understood increase in sensitivity that micro techniques provide, they are also ideally suited to coupling to a mass spectrometer, which is important considering the demands for sample identification.

To convert conventional high performance liquid chromatography to a microtechnique requires the production of tiny particles as packing materials. The use of micro methods, however, can limit the sample size and hence overall sensitivity; this can be overcome by concentrating the sample.

Using such strategies and available microparticles for packed and capillary columns, Professor Smith demonstrated that fast, efficient analysis of complex mixtures can be achieved using short columns or capillaries packed with 2 μ m packing materials, or long capillaries packed with 3 μ m materials, where the combination of narrow capillaries and small diameter phases give rise to highly efficient separations. However, these require the use of very high pressure pumping systems.

For the future, monolithic materials have the potential to provide rapid, highly efficient separations. Their large pore sizes give rise to high through flow, while micropores supply the adsorption sites for separation. They also are inert and can therefore be used over a wide pH range, concluded Professor Smith.