Methods of chemical analysis

Introduction

Electrophoresis is a technique used for separating molecules through migration on a support medium while under the influence of an electrical charge. It was first used as a separation technique by Tiselius in 1937 . He described the separation of proteins which were placed in a buffer in a tube and an electric field was applied. The importance of this work was recognized and earned him the Nobel Prize for Chemistry in 1947. Initially the technique was carried out on a stationary flat solid phase (slab gel technique). This technique was slow and cumbersome and required long analysis times and had low efficiencies (Anastos, 2007). In the 1960's the technique was greatly enhanced by carrying out the separations in narrow bore tubes (columns). Hjerten describes the separation of many different types of molecules, inorganic ions, nucleotides, proteins and viruses in tubes only a few millimeters thick. The technique was further refined by Jorgenson and Lukacs (1981) in the 1980's and resulted in the separations taking place in narrow bore tubes. This pioneering work led to the development of a technique known as capillary electrophoresis (CE).

Capillary Electrophoresis is used in a wide variety of analytical applications. Tavares (2003) describes it use and versatility in areas such as clinical, forensic, cosmetological, environmental, nutritional and pharmaceutical samples. The first use of CE for forensic analysis was demonstrated by Weinburger and Lurie in 1991. They describe the use of CE in the analysis of illicit drugs in synthetic mixtures.

Since then CE is widely used as a separation technique for a variety of samples for example, drugs, amino acids vitamins, organic acids and

inorganic ions. It has also developed as a technique which is used widely in forensic science laboratories, areas include, analysis of drug seizures; gun shot residues, explosive residues, ink analysis and toxicology,

2 Forensic Analysis Of Samples

Traditionally forensic samples were analysed using instruments available in an analytical chemistry laboratory. The main instruments used were based (and in some cases still are) on chromatographic techniques. Gas Chromatography and High Performance Liquid Chromatography HPLC are the two major type of analytical techniques conventionally used for forensic samples. Currently in forensic science laboratories there is a preference/trend to use CE techniques over the traditional chromatographic technique.

Why Is This So?

There are many reasons for this changeover. Chromatographic techniques usually require pre-treatment of the sample which can be very time consuming. Also some compounds are thermally unstable and are not suitable to GC analysis.

On the other hand the separations that can be achieved by CE are highly efficient, have a rapid analysis time and can be applied to neutral and non-neutral species.

Other advantages of CE are that it requires low sample and low solvent amounts, has low limits of detection and is a relatively simple, inexpensive technique to carry out.

3 Types Of Capillary Electrophoresis

The term CE really describes a number of related techniques in which separations are carried out in narrow bore capillaries under the influence of an electric field. Other techniques include;

Capillary Zone Electrophoresis – CZE

Micellar Electrokinetic Chromatography – MEKC

Capillary Gel Electrophoresis – CGE

It is beyond the scope of this essay to describe each of these in detail. It should be noted that they all operate on the principle of separation of molecules in narrow bore capillaries with an applied electric field.

4 Applications Of CE In Forensic Science

Drug analysis

CE is widely used in the analysis of drug seizures. All the different types of CE have been used in this type of analysis. There are numerous scientific papers published in this area . In 2005 Anastos reviewed the use of CE in forensic drug analysis for the years 2001-2005. This review showed how popular CE had become as a routine method of forensic analysis. There are many studies showing the advantages of CE over traditional chromatographic techniques of drug analysis. Anastos (2005 B) described a CE technique for the separation of caffeine, paracetamol, morphine, codeine, heroin, and acetylcodeine. The mixture was resolved in less that 1. 5 minutes and the results obtained were in agreement with a validated GC method.

Di Pietra (2001) developed a CZE technique for the analysis of amphetamines and compared the results obtained with HPLC analysis. The conclusion was that the CE method provided greater peak symmetry and shorter run times than the HPLC method. Luric (2003) used CZE and HPLC for the determination of opium alkaloids in opium gum and opium latex. It was found that the CZE method gave better resolution that the HPLC method, with significantly faster analysis times – 12 min v 29 min.

Aturki (2009) describes the use of a relatively new CE technique capillary electrochromatograohy CEC to analyse 10 different drugs of abuse. This new technique combines the advantages of the high speed and efficiency of CE with the high selectivity and increased sampling loading of HPLC. This study concluded that this method offers many advantages over the traditional HPLC, these include fewer costs, minimal consumption of solvents and samples and shorter analysis times.

Toxicology

Capillary electrophoresis is also used to analyse different biological samples for the presence of drugs. Blood and urine samples can be successfully tested for a variety of drugs using CE. The advantages of CE over traditional methods of analyzing biological samples are similar to the analysis of drug seizures;

– shorter run times, small sample requirements, and lower limits of detection.

The analysis of amphetamines in blood using a CZE method is described by Boatto (2002). A detection limit of between 10 and 30 ng/ml was recorded and a run time of 7 minutes.

Alnajjar (2004) describe the analysis of urine for the presence of heroin using a CZE method.

The determination of creatinine in urine samples using CZE is outlined in the research paper published by Liotta (2009). The results indicate that the assay described meets the strict requirements of forensic analysis and meets the need of a simplicity, rapidity and low cost required by routine toxicological screening..

The analysis of heroin metabolites in human urine is described by Jong. (2009) using the MEKC method. The separation was achieved in less than 10 minutes and the results were confirmed using liquid chromatography/mass spectrometry.

A simple rapid low cost MEKC method of analyzing anabolic steroids in urine is presented by Zhang (2009). This method was validated by GC-MS. This new technique is less expensive and a much quicker analytical technique that GC-MS for this type of analysis.

Aturki (2009) also demonstrated the advantages of CE over traditional chromatographic methods of drug analysis in biological samples. The results obtained for the simultaneous analysis of 10 illicit drugs in human urine demonstrate several advantages, these include fewer costs, minimal consumption of solvents and samples and shorter analysis times.

CE has also been used for the analysis of human hair samples for the presence of drugs. Tavares (2003) analysed hair samples for the presence of opiates and metabolites. A recent study by Zhang (2009 online) illustrates how CE can be used in the determination of cobalt in a single human hair sample. Traditional spectroscopic techniques are used for hair analysis. They are rarely used in the analysis of single hair samples due to their insufficient insensitivity and large sample requirements. The CE method described overcomes these limitations. As a result the determination of trace elements in hair samples can lead to useful forensic information. This includes living location, the working environment of the person, and perhaps their dietary habits which could narrow the search for the suspect(s).

Gun Shot Residues

Gun shot residues are produced when a firearm is discharged and can be deposited onto a surface after discharge. These residues are typically divided into organic and inorganic components. The methods of analysis of both differ, the organic residue have traditionally been analysed using chromatographic techniques, GC-MS, LC-MS and HPLC. The inorganic components are analysed using non chromatographic techniques (SEM-EDX).

These tradition techniques tend to be slow and require expensive equipment. The need for fast, cheap alternatives has seen Capillary Electrophoresis being used to analyse both the organic and inorganic components of gun shot residues. Morales (2004) developed a capillary electrophoresis method to analyze simultaneously 11 organic and 10 inorganic components of gunshot residues. This technique is suitable as a cheaper and possibly more specific method comparing to traditional

techniques. The results obtained were compared with electrothermal atomic absorption spectroscopy, an established technique for gunshot residue analysis. Good agreement between both techniques for lead was found. MacCrehan (1998) describe how the MEKC technique can be applied to the analysis of gunshot residues from tape lifts and swabs. A number of other studies (Northrop 2001, MacCrehan 2003, and Cascio 2004) all describe the suitability of using MEKC in the characterization of organic gunpowder components.

Explosives

Since the mid 1990's CE is routinely used technique for the analysis of the organic and inorganic components of explosive. The reasons for its rapid rise in popularity are similar to its advantages in the previous mentioned forensic samples. This rapid rise can be seen in the review of the use of CE in explosive analysis published by McCord and Bender (1998).

Tagliaro (2001) describes a CE method for nitrite and nitrate determination to be used as a screening tool for investigating the residues of firearm discharge. The use of CE allowed the rapid determination of nitrite and nitrate, which are major inorganic components of gunshot residues. The method is simpler, cheaper, and faster than the modern approaches (AA, ICP-MS, and SEM) to gunshot residue analysis

A further development of Capillary Electrophoresis, CE microchip, has led to the possibility of a portable analytical instrument for field analysis of explosive residues. Two studies (Lu, 2002, Vladislav 2000) have demonstrated the viability of using this microchip technology to analyse

explosive residues and have shown the advantages it offers over traditional more cumbersome laboratory based techniques.

Inks analysis

The characterization of inks is important in forensic science because it can allow the forensic scientist determine the authenticity of a questioned document. Traditionally inks were analysed by chromatographic techniques, initially paper and subsequently thin layer chromatography.

HPLC has replaced these as the separation technique most commonly for the analysis of inks. GC tends to have limited use in ink analysis as most components are non-volatile.

CE has gained popularity in the analysis of inks due to its advantages over HPLC. These include, small samples required (nanolitres), this can a big advantage over HPLC as the damage to the document in question will be minimized.

The first report of the use of CE for ink analysis was documented in 1991 by Fanalli. Different black red and water soluble fibre tipped pen inks were quantitatively distinguished using CE.

A study by Tsutsumi (1996) compared the use of HPLC and CE for the discrimination between red and blue pen inks. CE was found to be more suitable for analyzing inks that contain ionic dyestuffs. Fakhari (2006) examined the use of CE for the separation of ink samples from 6 fibre tip and 2 ball point blue or black pens and showed that a unique migration time for the main dye component in seven of the eight pens could be obtained.

A number of studies report the use of MEKC to the analysis of inks from different pens (Vogt 1999 and Mania 2002). Both studies indicate that the rapid analysis time and the small sample requirement show the advantage of this technique over traditional chromatographic techniques.

5 Conclusion

Capillary electrophoresis is widely used in forensic laboratories for the routing analysis of a variety of samples typically found at a crime scene. It offers many advantages over chromatographic techniques for the analysis of these samples. These advantaged include, quicker analysis time, the requirement of less ample and solvents, lower limits of detection and less expensive equipment. These advantages will ensure that analysis of forensic samples using CE techniques will continue to grow and develop in the coming years.

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