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REVIEW ARTICLE

A Review on High Performance Capillary Electrophoresis

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ABSTRACT
High performance capillary electrophoresis is a separation technique in which the analysis is separated on the basis of differences in their charge to size ratios. This makes high performance capillary electrophoresis suitable for the analysis of molecules with abroad range of sizes, charge and hydrophobicity, as proteins and peptides. Another attractive feature of high performance capillary electrophoresis is its ability to handle minute sample amounts, with Nano litre injection volumes, which makes it ideal for applications when the sample volumes are limited, as often as the case in analysis of body fluids, single cells and other small volume analysis of bio fluids. Electrophoresis is performed in narrow bore, fused silica capillaries.

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1. Introduction

Liquid Chromatography (HPLC) and Gas Chromatography (GC) the separating force is the difference in affinity of the sample components to a stationary phase, and or difference in boiling point. With both techniques the most important factor is the polarity of a sample component. Along with the uses it also has certain disadvantages like sensitivity issues, sample stacking issues, lack data regarding reliability, and reproducibility of methods, no standardization methods for determining appropriate test conditions for unknown sample. So it has been modified into High Performance Capillary Electrophoresis. The High Performance capillary Electrophoresis instrument combines a unique detection system with power partical tracking and data interrogation algorithms to deliver unrivalled capillary

electrophoresis performance background noise from buffer/ capillary conditios etc is drastically reduced resulting in superb resolution, accuracy and detection levels which would otherwise require the need of expensive distortion techniques. High performance capillary electrophoresis is a separation technique in which the analysis is separated on the basis of differences in their charge to size ratios. This makes high performance capillary electrophoresis suitable for the analysis of molecules with abroad range of sizes, charge and hydrophobicity, as proteins and peptides Separation by electrophoresis is obtained by differential migration of solutes in an electric field moreover, the large surface area-to-volume ratio of the capillary efficiently dissipates the heat that is generated. The use of the high electrical fields results in short analysis times and high efficiency and resolution. Peak efficiency, often in excess of 105 theoretical plates, is due in part to the plug profile of the electro osmotic flow, an electrophoretic phenomenon that generates the bulk flow of solution within the capillary.

Current State and Development

Capillary electrophoresis is a rapidly growing separation technique. One of the greatest advantages is its diverse application range. Originally considered primarily for the analysis of biological macromolecules, it has proved useful for separations of compounds such as amino acids, chiral drugs, vitamins, pesticides, inorganic ions, organic acids, dyes, surfactants, peptides and proteins, carbohydrates, oligonucleotides and DNA restriction fragments, and even whole cells and virus particles. While numerous advances are being made in Capillary electrophoresis, the technique is still in a development and growth stage. The number of publications per year on Capillary electrophoresis has risen from about 90 in 1983, to about 140 in 1987, to more than 300 in 1991. Incumbent with new technology is a lag time between published results generated by researchers developing the technique and the formation of a workable knowledge-base for the user. Scientists should be aware that Capillary electrophoresisis not totally mature, relative to HPLC for example. Both development of the theory and its application to separation problems are still somewhat incomplete.

Characteristics of High Performance Capillary Electrophoresis

- Electrophoresis performed is a narrow-bore fused silica capillaries.
- High voltages and high electric fields are applied across the capillary.
- High resistance of the capillary limits the current generation and internal heating.
- High efficiency and short analysis time.
- Detection performed on the capillary itself (no external detection).
- Simple methods development, automated instrumentation

Principle

In high performance capillary electrophoresis a capillary is filled with a conductive fluid at a certain pH value. This is the buffer solution in which the sample will be separated.

The sample is introduced in the capillary, either by pressure injection or electrokinetic injection.

Sample Injection

In CE, a low volume of sample is loaded into the capillary in order to maintain a high efficiency. Quantitative sample injection can be accomplished by two different modes:

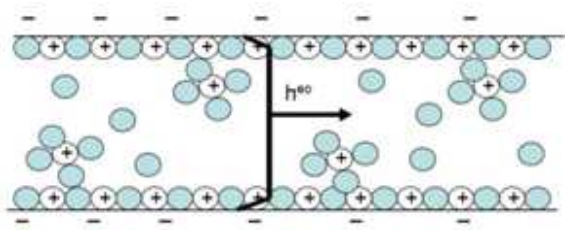
- Hydrodynamic injection: It can be accomplished by application of pressure at the injection end of the capillary (or vacuum at the exit end of the capillary. With hydrodynamic injection mode, the injected quantity of sample is directly related to the injection time and the pressure value
- Electrokinetic injection: It is performed by applying a voltage (some kilovolts) at both ends of the capillary. With this voltage, analyte enters the capillary by migration and by action of the electro osmotic flow. The injected quantity of sample is directly related to the injection time, the voltage value, the electrophoretic mobility of the compound and the electro osmotic mobility.

Transport processes in Capillary electrophoresis

The Transport of chemical compounds inside the capillary is controlled by two phenomena: the electro osmotic flow and the electrophoretic migration.

Electro osmotic flow (EOF)

Under main aqueous conditions (pH > 2.5), silica narrow bore capillary surface possesses an excess of negative charges resulting from the ionization of silanol groups. Counterions (cations, in most cases) ; which build up near the surface to maintain charge balance, form a double-layer and create a potential (zeta potential) difference close to the capillary wall This electro osmotic flow can be modified, inversed or deleted by covalent or dynamic capillary wall modifications using surfactants or neutral or ionized polymers.



Electrophoretic migration

In CE, separation is related to the differential migration of compounds in an applied Electric field. The Electrophoretic Migration velocity is directly related to the electrophoretic mobility. The electrophoretic mobility depends on pH buffer, ionic strength, buffer composition and viscosity.

Basic Theory of High Performance Capillary Electrohporesis

Separation on electrophoresis is based on differential movement of charged species by attraction or repulsion in the electric field. The electrophoretic separation is performed in capillaries made of silica, typically 25-75 micrometres in inner diameter, which are usually fused with a buffer .The use of high electrical fields results in short analysis time and high peak efficiency and resolution. Other properties that characterize capillary electrophoresis are the minimal sample volume requirements and its diversity of application.

2. Instrumentation

The instrumentation needed to perform capillary electrophoresis is relatively simple. The system's main components are a sample vial, source and destination vials, a capillary, electrodes, a high voltage power supply, a detector, and a data output and handling device. The source vial, destination vial and capillary are filled with an electrolyte such as an aqueous buffer solution. In the most common mode of CE, all ions, positive or negative, are pulled through the capillary in the same direction by electro osmotic flow. Capillary electrophoresis was first combined with mass spectrometry by Richard D. Smith and co-workers, and provides extremely high sensitivity for the analysis of very small sample sizes. A process called field-amplified sample stacking (a form of isotachophoresis) results in concentration of analyte in a narrow zone at the boundary between the low-conductivity sample and the higher-conductivity running buffer

Detectors

The high performance capillary electrophoresis system is equipped with a high sensitivity diode array detector. In high performance capillary electrophoresis the capillary itself acts as the detector flow cell. Unlike many detectors in high performance capillary electrophoresis instrumentation, the optical design of the high performance capillary electrophoresis detector has been specifically developed for uv-visible absorbance detection in capillary shape flow cell.

Capillary Modification

Modification of the inner wall in fused silica capillaries can be reasons. Different types of applications require different properties of the separation. For instance analyte-wall interaction can be prevented; in addition, modification of the electro osmotic flow can increase the speed of analysis, improve reproducibility and resolution or increase compatibility with MS detection.

Sample Preparation

The choice of sample preparation method is crucial in chemical analysis since it is often the most critical and time consuming step in the analytical chain. The isolation of proteins and peptides can be performed using various traditional sample preparation techniques such as homogenization, centrifugation, precipitation. The evolving field of proteomics has created a need for development of new sample preparation.

Various Detection Modes in Capillary Electrophoresis

The Capillary Zone Electrophoresis (CZE) is the most widely used mode due to its simplicity. CZE allows the analysis of ionized or ionizable compounds. Analytes are simply separated according to their charge/hydrodynamic radius ratio and migrate towards anode or cathode according to their charges. Neutral compounds are not separated with this separation mode. The running buffer is extremely important in CZE and is the same in both of separation vials.

Micellar electrokinetic chromatography (MEKC): is one of the most widely used CE modes. This mode can be used for the separation of neutral compounds as well as charged ones. The separation is accomplished by the used of surfactants in the running buffer. At concentration above the critical micelle concentration, micelles are formed in the capillary. During migration, micelles can interact with neutral compounds in a chromatographic manner through both hydrophobic and electrostatic interactions. The more the neutral compounds interact with the micelle the longer is its migration time.

Applications of High Performance Capillary Electrophoresis

Proteomic Analysis:

Proteomics concerns the characterization of the full complement of proteins in a specific organism, tissue or cell type at a given type. In short, proteomics is a field that involves the identification, characterization and quantification of proteins. This challenge can be used to analyze 10 000- 30 000 protein variants expressed in a mammalian cell and characterize all of them at a given time.

Analysis of Proteomics Sample Analysis with High Performance Capillary Electrophoresis

Capillary electrophoresis is able to provide fast and efficient separations of heterogeneous complex samples with low sample volume requirement, is becoming recognized as a suitable alternative among today's bioanalytical approaches.

Pharmaceutical Application

- Identification an quantization of antibodies, their conjugates and complexes. For analyzing biotin in pharmaceutical formulations.
- High performance capillary electrophoresis coupled to mass spectrometry to charecterise the purity and safety of biotechnology drugs.
- Determination of matrine and oxymatrine in sophorasubprostata.
- Direct detection endogenous histamine in rat peritonela mast cells by in-capillary derivatization.
- Analysis of syntheic antidiabetic drugs in aldultered traditional chinese medicines.
- Analysis and confirmation of synthetic anorexics in adultered traditional chinses medicines.
- To analyse carbohydrates especially those in glyco proteins. Fractionation and high performance capillary electrophoretic analysis of phospholipids.
- Determinaion of salbutamol enetiomers.
- A reproducible, simple and sensitive high performance capillary electrophoresis method for simultaneous determination of capreomycin, ofloxacin and pasiniazide in urine.
- Capillary electrophoresis with laser induced fluorescence in clinicsl drug developments.
- Separation and quantitation of azimilide and its putative metabolites by high performance capillary electrophoresis.
- High performance electrophoresis method for determination of ibuprofen enantiomers in human serum and urine.

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4. Conclusion

A good separation strategy before mass spectrometric detection is crucial ia a study involving the understanding of complex mixtures such as biological samples in proteomics profiling. The development of new methods is thus important in order to meet the nalytical challenges represented by the huge dyanamic range of concentrations and the complexity in biological samples. In high performance capillary electrophoresis, a problem that has hampered a reproducible seperation of proteins and peptides has been the protein wall adsorption phenomenon, especially emphasized with increased basicity of their molecules. This thesis also demonstrates the role of sample preparation as a key to improve the possibilities of using capillary electrophoresis- mass spectrometry in the proteomic analysis of high complex human body fluids. Finally, the thesis describes application of capillary electrophoresis in quantitative proteomic analysis with mass spectrometry and how capillary electrophoresis takes advantage of the properties of multiplexing strategies to circumvent the drawbacks of the injection and analyte migration time irreproducibilities.

5. References

[1] J.P. Landers and R.P. Oda. An Introduction to Capillary Electrophoresis. J.P. Landers, Ed. CRC Press, New York, NY, 1997, pp. 1–47.

[2] Hunt DF. Personal Commentary on Proteomics. Journal of proteome research, 1, 2002, 15-19.

[3] P.D. Grossman, J.C. Colburn eds., "Capillary Electrophoresis – Theory and Practice", Academic Press.

[4] Lapainis T, Sweedler JV. Contributions of capillary electrophoresis to neuroscience. Journal of chromatography a, 2008,1184,144-158.

[5] R. Kuhn and S. Hoffstetter-Kuhn. Capillary Electrophoresis: Principles and Practice. Springer-Verlag, New York, NY, 1993.

[6] S. Terabe, Koji Otsuka, Kunimichi Ichikawa, Akihiro Tsuchiya, Teiichi Ando. "Electrokinetic separations with micellar solutions and open tubular capillaries", anal. chem.1984,51,131.

[7] R.B. Bird, W.E. Stewart, E.N. Lightfoot. Transport Phenomena", John Wiley & Sons Inc., 2nd edition, 2001.

[8] Senk P, Kozak L, Foret F.Capillaru Electrophoresis and mass Spectrometry for screening of Metabolic Disorders in Newborns. Electrophoresis,2004,25,1447-1456.

[9] M.M Dittmann."Capillary electrochro-matography - a high-efficiency micro-separation technique". J. Chromatogram. A. 1996, 744, 63.

[10] N.W. Smith, M.B. Evans. "The analysis of pharma-ceutical compounds using electrochromatography". chromatographia. 1995, 38, 649.

[11] L.A. Colón, Glamarie Burgos, Todd D. Maloney,José M. Cintrón, Ramón L. Rodríguez. "Recentprogress in CEC", Electrophoresis. 2000, 21, 3965.

[12] Nobuo Tanaka, Hisashi Nagayama, Hiroshi Kobay-ashi, Tohru Ikegami, Ken Hosoya, Norio Ishizuka,Hiroyoshi Minakuchi, Kazuki Nakanishi, Karin Ca-brera, Dieter Lubda."Monolithic silica columns forHPLC, micro-HPLC and CEC". J. High Resol. Chro-matogr.2000, 23,111.

[13] F. Svec, T.B. Tennikova, Z. Deyl. "Monolithic materials: Preparation, properties and applications", Anal. Bio. Chem., 2004, 8,379.

[14] Guttman, A.S. Cohen, D.N. Heiger, B.L. Karger. "Analytical and micropreparative ultrahigh resolutionof oligonucleotides by polyacrylamide gel high performance capillary electrophoresis", Anal.Chem., 1990, 62, 137-146

[15] F. Kilar, S. Hjertén. Fast and high-resolution human serum transferrin by high-performance isoelectric focusing in capillaries, Electrophoresis, 1989, 10, 23-29.

[16] J. Wu, J. Pawliszyn. Capillary isoelectric focusing with a universal concentration gradient images system using a charge-coupled photodiode array". Anal. Chem., 1992,64,2934.

[17] J. Wu, A. Watson. Automated sample introduction for an imaged capillary isoelectric focusing instrument via high-performance liquid chromatography sampling devices". J. Chromatogram. B, 1998, 714, 113-118.

[18] F. Kohlrausch. Über Konzentrationen – Verschie-bungen durch Electrolyse im Inneren von Lösungenund Lösungsgemischen". Annalen der Physik undChemie, 1897, 62, 14.

[19] Th. P.E.M Verheggen, A.C. Schoots, F.M. Everaerts. "Feasibility of capillary zone electrophoresis with suppression of electroendosmotic flow in completely closed systems". J. Chromatogr., 1990, 503, 245-255.

[20] X. Huang, M.J. Gordon, R.N. Zare. "Bias in quanti-tative capillary zone electrophoresis caused byelectrokinetic sample injection". Anal. Chem., 1988, 60, 375-377.