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AN OVERVIEW ON HIGH PERFORMANCE CAPILLARY ELECTROPHORESIS

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ABSTRACT

High performance Capillary electrophoresis (HPCE) is a separation technique in which the analytes are separated on the basis of differences in their charge-to-size ratios. This makes HPCE especially suitable for the analysis of molecules with abroad range of sizes, charge and hydrophobicity, as proteins and peptides. Another attractive feature of HPCE 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 is the case in analysis of body fluids, single cells and other small volume analysis of biofluids. Electrophoresisisperformed innarrow-bore (25- to 75-J.m id), fused silica capillaries. High voltages (10 to 30 kV) and high electricfields (100 to 500 V/cm) are applied across the capillary. In addition, the small injection volume has often been considered as a drawback of the HPCE technique in analysis of complex biological samples, as well as the nature of its injection has represented a reproducibility problem in quantitative chemical analysis.

Keywords: High performance, Capillary electrophoresis, Hydrophobicity, Injection, Electric field.

INTRODUCTION

High performance Capillary electrophoresis (HPCE) is a separation technique in which the analytes are separated on the basis of differences in their charge-to-size ratios. This makes HPCE especially suitable for the analysis of molecules with abroad range of sizes, charge and hydrophobicity, as proteins and peptides. Another attractive feature of HPCE 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 is the case in analysis of body fluids2-4, single cells5,6 and other small volume analysis of biofluids. However, when using HPCE for separation of proteins and peptides their basicity causes adsorption to the capillary inner wall and deteriorates the separation. The adsorption has thus been a problem to address in order to use advantageous properties of the HPCE technique [1].

Characteristics of HPCE

- Electrophoresisis performed in narrow-bore (25- to 75-J.m id), fused silica capillaries
- High voltages (10to 30 kV) and high electric fields (100 to 500 V/cm) are applied across the capillary
- High resistance of the capillary limits current generation and internal heating
- High efficiency (N>105 to 106) and short analysis time
- Detection performed on-capillary (no external detection cell)

Advantages of HPCE

Electrophoresis is a completely different mechanism of separation from chromatography.

1. Non Chromatographic: Provides different information. Should be used with Chromatography.
2. Small sample size required: Provides analysis of samples not possible before.
3. Low Cost. Cost of HPCE and CEC is realized in the purchase and disposal costs of solvents used in Chromatography.
4. Ideal for Compounds that present costly and time

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5. consuming challenges in chromatography (proteins, peptides, nucleic acids, basic drugs, chiral compounds)
6. Easy to develop methods of analysis [2].

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. A sample is introduced in the capillary, either by pressure injection or by electrokinetic injection. A high voltage is generated over the capillary and due to this electric field (up to more then 300 V/cm) the sample components move (migrate) through the capillary at different speeds. Positive components migrate to the negative electrode, negative components migrate to the positive electrode. When you look at the capillary at a certain place with a detector you will first see the fast components pass, and later on the slower components.

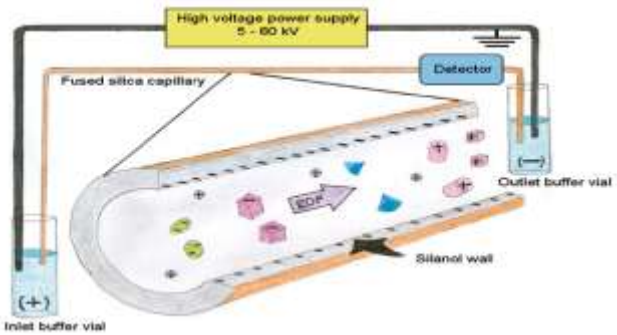
High performance capillary electrophoresis

Basic theory of HPCE

Separation by electrophoresis is based on differential movement of charged species (ions) by attraction or repulsion in an electric field21,30. In HPCE,electrophoretic separation is performed in capillaries made of fused silica (a metal free glass material), typically 25-75 μm in inner diameter, which are usually filled with a buffer. The high electrical resistance of the capillary enables the application of very high electrical fields (100-500 V/cm) with only minimal heat generation. The use of high electrical fields results in short analysis times and high peak efficiency and resolution. Other properties that characterize CE are the minimal sample volume requirements (low nanolitre range) and its diversity of application [3].

HPCE instrumentation

One of the key features of HPCE is the simplicity of the instrumentation. A HPCEsystem consists of a buffer-filled fused silica capillary, where the separation takes place, two buffer reservoirs connected to a high voltage power supply using platinum electrodes and a detector, usually placed closer to the outletbuffer reservoir, connected to a computer for data collection. A basicinstrumental set-up of HPCE.



Schematic picture of the instrumental set-up for capillary electrophoresis

Sample is loaded into the capillary on the opposite side of the detector. Uponapplication of a potential difference over the capillary (usually 15-30 kV) theseparation is started. Ultraviolet (UV) detectors are most commonly used,but numerous other detection techniques can be employed.

Sample Injection

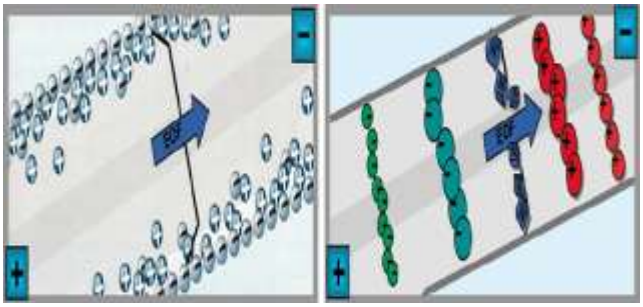
Two injection modes are provided:

- pressure injection, and
- electro kinetic injection.

Detector

The HPCE system is equipped with a high-sensitivity diode array detector. In HPCE the capillary itself acts as the detector flow cell. Unlike many detectors in HPCE instrumentation, which have beenadapted from LC detectors, the optical design of the HPCE detector has been specifically developed for UV-visible absorbance detection in capillary-shaped flow cells. In conjunction with the capillary alignment inter-faces the optical design of the detector is optimized for both standard capillaries and for Agilent Extended Light Path capillaries [4].

Fig 3.Development of the electroosmotic flow (A) Hydrated cations accumulating near to negatively charged fused silica surface (Si-O-). (B) Bulk flow towards the cathode upon application of an electric field



Capillary modifications

Modification of the inner wall in fused silica capillaries can be advantageous for various reasons. Different types of applications require different properties of the EOF or the separation. For instance, analyte-wall interactions can be prevented; In addition, modifications of the EOF can increase the speed of analysis, improve reproducibility and resolution or increase compatibility with MS detection. Various strategies, that have been more or less successful, have been introduced to modify capillary walls. Using extremes of pH, which give highly negatively or positively charged capillary wall and repel analytes with the same overall polarity, has perhaps been the most trivial way to minimize analyte-wall interactions.

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 from biological matrices can be performed using various traditional sample preparation techniques such as homogenization, centrifugation, precipitation, solvent extraction, liquid-liquid extraction, solid phase extraction, micro-dialysis and ultracentrifugation. The evolving field of proteomics has created a need for development of new sample preparation methods since it is often important to perform a complete characterization of the natural distribution of analytes in a sample or a set of samples e.g. in clinical proteomics. Global identification of proteins in comparative proteomics studies, where the protein expression of a certain biological system is compared with another or the same system under perturbed conditions, is extremely complex. The interplay between sample components in different physiological processes and their relative abundances related to these processes do not allow for sample preparations where there is risk for sample loss if such differences in the sample composition have to be followed, Common difficulties that also can arise in clinical analysis are limited availability of biological material often in combination with very low concentrations of the molecules that are of interest to analyze.

APPLICATIONS OF HPCE

Proteomic analysis

Proteomics concerns the characterization of the full complement of proteins in a specific organism, tissue or cell type at a given time. In short, proteomics is a field that involves the identification, characterization and quantification of proteins. This challenge can be to analyze 10 000-30 000protein variants expressed in a mammalian cell and characterize all of them at a given time. In an ideal situation the protein characterization would include cellular localization and sequence analysis as well as identification of post-translational modifications and binding partners. This width of the proteomics field brings an enormous number of questions that need to be defined, addressed and answered.

Analysis of proteomic sample analysis with HPCE

Capillary electrophoresis, able to provide fast and efficient separations of heterogeneous complex samples with low sample volume requirements, is becoming recognized as a suitable alternative among today's bioanalytical approaches [5-8].

Pharmaceutical Applications

1. Identification and quantization of antibodies, their conjugates and complexes
2. For analyzing biotin in pharmaceutical formulations—a comparative study.
3. HPCE coupled to mass spectrometry to characterise the purity and safety of biotechnology drugs.
4. Determination of matrine and oxymatrine in *Sophorasubprostata* by HPCE.
5. Direct detection of endogenous histamine in rat peritoneal mast cells by in-capillary derivatization high-performance capillary electrophoresis [9].
6. Analysis of synthetic anti-diabetic drugs in adulterated traditional Chinese medicines by high-performance capillary electrophoresis.
7. Analysis and confirmation of synthetic anorexics in adulterated traditional Chinese medicines by high-performance capillary electrophoresis
8. Application of high-performance capillary electrophoresis to analysis of carbohydrates, especially those in glycoproteins .
9. Fractionation and High Performance Capillary Electrophoretic Analysis of Phospholipids .
10. Determination of salbutamol enantiomers by high-performance capillary electrophoresis.
11. A reproducible, simple and sensitive high-performance capillary electrophoresis method for simultaneous determination of capreomycin, ofloxacin and pasiniazide in urine
12.Capillary electrophoresis with laser-induced fluorescence in clinical drug developments
13. Separation and quantitation of azimilide and its putative metabolites by high performance capillary electrophoresis.
14. High performance capillary electrophoresis method for determination of ibuprofen enantiomers in human serum and urine .
15. Development and validation of a plasma assay for acyclovir using high-performance capillary electrophoresis with sample stacking .
16. Quantitative determination of insulin entrapment efficiency in triblock copolymer nanoparticles by high-performance liquid chromatography [10]

CONCLUSION AND FUTURE ASPECTS

A good separation strategy before mass spectrometric detection is crucial ina study involving the understanding of complex mixtures such as biologicalsamples in proteomics profiling. This is due to that the good separation alsoprovides good conditions to increase the confidence of protein identificationsand to improve the coverage of proteomes..The development of new methods is thus important in order to meet theanalytical challenges represented by the huge dynamic range ofconcentrations and the complexity in biological samples.In HPCE, a problem that has hampered a reproducible separation of proteinsand peptides has been the protein-wall adsorption phenomenon, especiallyemphasized with increased basicity of these molecules.

This thesis also demonstrates the role of the sample preparation as a keyto improve the possibilities of using CE-MS in the proteomic analysis ofhighly complex human body fluids. Finally, the thesis describes applicationof CE in quantitative proteomic analysis with MS and how CE takes advantage of the properties of multiplexing strategies to circumvent thedrawbacks of the injection and analyte migration time irreproducibilities.

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