CAPILLARY ELECTROPHORESIS AND MASS SPECTROMETRY AS BIOANALYTICAL TOOL

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ABSTRACT: - Capillary electrophoresis is a mature separation technology that is effectively coupled with mass spectrometry (CE - MS) and is well - suited for this purpose. CE - MS platforms have attracted considerable interest from the proteomics community as a selection to LC -MS. CE offers advantages over LC, including effective and fast separations paired with low sample consumption. In addition, solvent consumption (waterless or organic) in CE - MS is actually low, making it environmentally profitable. The most common CE - MS approach used in the proteomics field is capillary zone electrophoresis (CZE) coupled with electrospray ionization (ESI). In proteomics exploration, proteins are frequently associated using a well-established most up approach. This strategy is grounded on circular identification of proteins through digestion (enzymatic or chemical) to give peptides that are more analysed by either LC-MS or CE-MS. CE-MS has been used to determine the modesty and the stability of proteins in medicine discovery. These systems grounded on their available type of separation instruments and mass spectrometers.

KEYWORDS: - Mass spectrometry , separation, electrospray ionization, determination , importance.

INTRODUCTION: -

Capillary electrophoresis (CE) is a powerful separation technology that deals with analytes in liquid forms. The most common channel of separation in CE is fused-silica capillary with 360 μm outer diameter and 50–75 μm inner diameter. In a typical CE operation, a small section of

the capillary (usually 1–2 % of capillary length) is filled with the sample. A separation voltage up to 30 kV is then applied at the capillary inlet end, resulting in the analytes being separated by their mobility difference in the background electrolyte (BGE). The mobility of an analyte in CE generally comes from two sides: the electroosmotic force (EOF) and the electrophoretic force (EPF). When an electrical field is applied on the fused-silica capillary inlet end, the ions which are attracted around the slianol groups of the capillary will migrate, pulling the BGE buffer solution toward the outlet end. In addition to the impact of this electroosmotic flow on the mobility of the analytes, the applied electrical field also acts on the analyte ions generating electrophoretic mobility. A classic phenomenon that depicts the combined effect of EOF and EPF is: on a bare fused-silica capillary, when a normal polarity is applied, the analytes migrate toward the outlet end, in the sequence of positively charged, neutral, and negatively charged. The most common CE-MS technique used in the proteomics field is capillary zone electrophoresis (CZE) coupled with electrospray ionization (ESI). Capillary isoelectric focusing (CIEF) is another useful, but less commonly used CE technique. In proteomics research, proteins are often identified using a well-established bottom-up approach. This strategy is based on indirect identification of proteins through digestion (enzymatic or chemical) to provide peptides that are further analysed by either LC-MS or CE-MS.

CAPILLARY ELECTROPHORES COUPLE WITH MASS SPECTROMETRY: -

Since the introduction of CE-MS for analysis of macromolecules, a few soft ionization techniques have been evaluated for CE-MS platforms, but as ESI is the most commonly used technique, for CE-MS applications utilizing ESI. Interfacing CE with ESI requires transferring a voltage to liquid to create the electrospray, which has been done with sheath–liquid or sheath less interfaces. The sheath–liquid interface has proven to be versatile and robust and is currently the more commonly used configuration in CE-MS for proteomics research. This interface uses a coaxial sheath–liquid to produce electrical contact between the CE and ESI source. So, the sheath less configuration is based on the direct application of voltage to the background electrolyte (BGE). The sheath less interface has been widely used in proteomics research to provide ultrasensitive analysis, although it is limited by potential blocking of the pores. It is reported that a successful application of an etched porous-tip sheath less interface for analysis of complex-forming protein standards, a monoclonal antibody, and a ribosomal

proteome of the E. coli in native conditions. This interface enabled characterization of proteins without dilution prior to nano- ESI flow rates (10–20 mL/min). The ultra-low flow rates enabled higher sensitivity from this interface than from sheath–liquid designs. The electric contact between the BGE and ESI source enabled the use of a conductive liquid that is in contact with the metal-coated surface of the ESI emitter. The metal-coating on the ESI emitter was found to be highly sensitive and robust for approximately 100 analyses of a mixture of nine peptide standards. The most common types of sheaths–liquid and sheath less interfaces.

Separation: -

CE separations rely on the electrophoretic mobility of the analytes in an electric field. While many CE-based techniques have been reported for the analysis of proteins and peptides, CZE is the most commonly used in proteomics research. In CZE separation is based on the analyte migration (according to size/charge) in different zones at different velocities. The ionic strength and pH of the BGE drive the solute's electrophoretic mobility. In CZE, band broadening is only caused by longitudinal diffusion. This is particularly attractive for protein analysis because they have small diffusion coefficients. Examples of CZE-MS include the intact mass analysis of biopharmaceutical protein and monoclonal antibodies where complete baseline separation is often achieved. A CZE separation for top-down proteome analysis of E. coli that resulted in high peak capacity (~280, calculated using the average peak width at 50% peak height for the E. coli proteome sample), the value of combining CZE separations with electron dissociation techniques for top- down analysis. In this study, a baseline separation was first obtained for a mixture of four standard proteins and then the CZE strategy was extended to the analysis of a biologically relevant protein mixture derived from the Mycobacterium marinum secretome.

Capillary: -

The proteins have low diffusion coefficients, they should produce very high separation efficiency in CE. However, hydrophobic and ionic interactions between protein and capillary wall surfaces can lead to increased protein retention in the capillary, or to a lesser extent, peak tailing. Coated capillaries can be used to limit protein adsorption on the capillary wall. Although non-permanent coatings such as Ultratrol LN can be used, permanent coatings are preferred, particularly for high-throughput analysis and when interfacing CE with a mass spectrometer. A capillary coating can be achieved using polyacrylamide, aryl pentafluoryl groups, polysaccharides, or polyamines.

MS Interface: -

- Capillary electrophoresis is a separation technique that uses a high electric field to produce electro-osmotic flow for separation of ions.
- Analyte migrates from one end of capillary to others based on their charge, viscosity and size. Higher the electric field, the greater is the mobility.
- The major problem faced when coupling CE to MS arises due to insufficient understanding of fundamental Processes when two techniques are interfaced.
- The separation and detection of the analyte can be improved with a better interface. The most used ionization technique is ESI.
- The three setups commonly used in CE-ESI-MS coupling are:

  a) coaxial sheath liquid

  b) liquid junction,

  c) sheath less or nano spray.

- All these configurations involve closing the high voltage circuit at the outlet of the separation capillary.

INSTRUMENTATION: - CE-MS is the hyphenated technique where CE is connected to the MS with the help of the long capillaries which will increase the analysis time also there is a lack of suitable volatile buffer which has to be compatible with the mass spectrometer.

CE: - 1. Injector

2. Capillary

3. BGE vessel (background electrolyte)

4. High voltage supply

5. Electrode

6. Detector

MS: - 7. MS Interface

8. Analyzer

9. Detector

1. Injector: - Sample is injected into the capillary tube. The most common sample introduction is an electrokinetic and hydrostatic type of injection. With pressure injection, the sample introduction end of the capillary is also placed momentarily into a small cup containing the sample, and a pressure difference is then used to drive the sample solution into the capillary. The pressure difference can end by pressurizing the sample or by elevating the sample end. hydrostatic injections do not discriminate due to ion mobility, but cannot be used in gel-filled capillaries.

2. Capillary: - A buffer filled fused silica capillary that is typically 10 to 100 µm in internal diameter and 40 to 100 cm long extends between two buffer reservoirs that also hold platinum electrodes. The sample is introduced at one end and detection at the other end. The detector should be placed at the right side i.e., near the cathode electrode. A provision is made on the capillary tube to enter the injected sample i.e., 0.5mm detector window. If a provision is absent, then the capillary tube is heated with 96-98% of conc.H_2SO_4 or conc. KOH at 130 °C.

3. BGE (background electrolyte) Vessel: - Buffer plays an important role where the suitable buffer is selected for electro-osmotic and electrophoretic mobility based on analyte behaviour, pH constants. Buffers chosen should be of good quality and should be prepared under optimization concentration Phosphate buffer, ethanoate buffer and borate buffers are the most commonly used buffers in CE. Buffer additives like urea, surfactants, organic & inorganic salts are also used.

4. High Voltage Supply: - High potentials can be applied in CE,with current technology, up to 30 kV can be applied for extremely fast and efficient separations. When the components are migrating at different rates along the length, although separated by the electrophoretic migration, the entire sample is drawn towards cathode by electro endosmosis. Cooling systems are maintained to reduce the temperature or reduce heat from the system.

5. Electrodes: - An electrode in an electrochemical cell is referred to as either an anode or a cathode. The anode is now defined as the electrode at which electrons leave the cell and oxidation occurs and the cathode as the electrode at which electrons enter the cell and reduction occurs. Each electrode may become either the anode or the cathode depending on the direction of current through the cell. A bipolar electrode is an electrode that functions as the anode of one cell and the cathode of another cell.

6. Detectors: - Separation by capillary electrophoresis can be detected by several detection devices. Different detector configurations are possible. Since the amount of each analyte passing the detector is very small, shooting the source lamp along a short section of the capillary increases the path length and, if absorbance is being used, decreases the detection limit. The most commonly used are:

i) UV Absorption

ii) Fluorescence

iii) Conductivity

iv) Potential gradient detector

v) Amperometric detector

vi) Diode array detector

vii) Inductive coupled plasma detector

viii) Refractive index detector

ix) Raman spectroscopy detector

x) Chiral optical activity detector

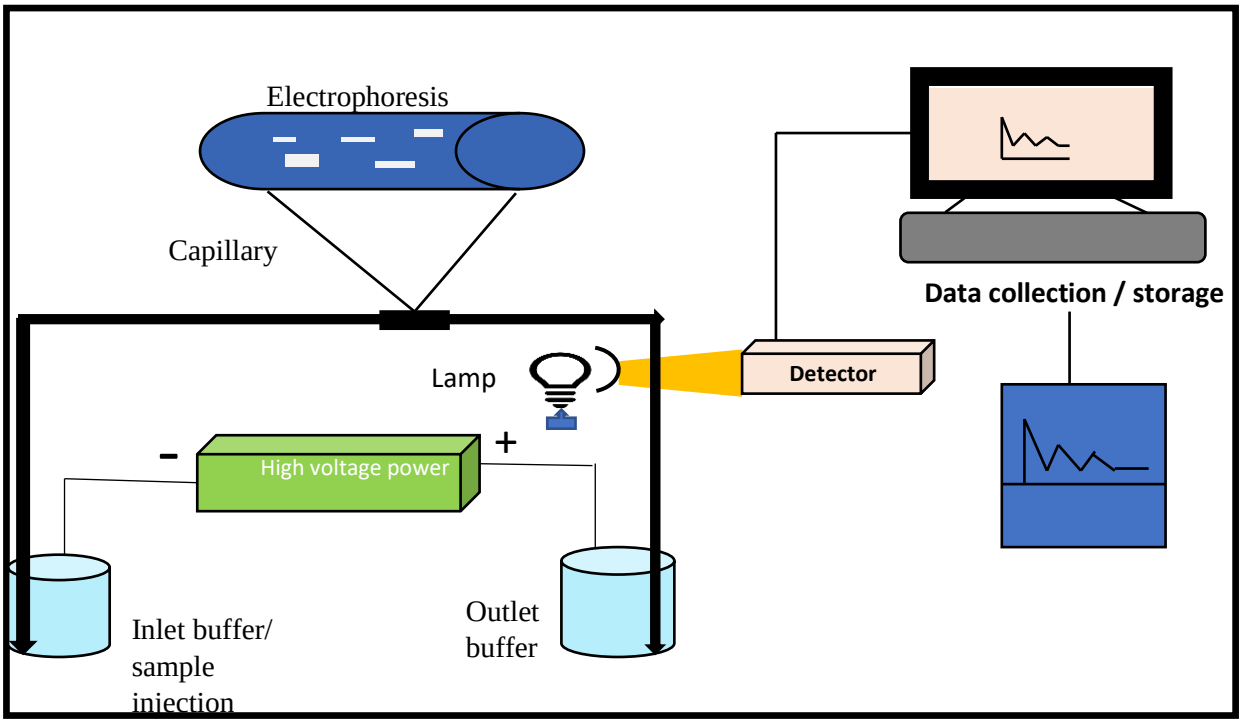
xi) Thermo optical absorbance detector

xii) Atomic absorption.

Electrospray Ionization Interface: - The first CE-MS interface had cathode end of CE capillary terminated within a stainless-steel capillary. Electrical contact was made at that point completing the circuit and initiating the electrospray. This interface system had few drawbacks like mismatch in the flow rates of two systems. Since then, the interface system has been improved to have a continuous flow rate and good Electrical contact. At present, three types of interface systems exist for CE/ESI-MS which are discussed briefly.

Sheath Less Interface: - CE capillary is coupled directly to an electrospray ionization source with a sheath less interface system. The electric contact for ESI is realized by using capillary coated with a conductive metal. Because no sheath liquid is used, the system has high sensitivity, low flow rates and minimum background. However, these interface designs; all

have challenges including low mechanical robustness, poor reproducibility. The latest sheath less interface design features porous ESI emitter through chemical etching.



METHOD DEVELOPMENT: -

- Electrophoresis is done in buffer filled, narrow capillaries.
- Each capillary is about 10-100µm in internal diameter v_{max} =5-30kv.
- When a voltage is applied to the solution, the molecules move through the solution towards the electrode of opposite charge.
- Depending on the charge, the molecules move through at different speeds.
- Thus, separation is achieved.
- A suitable detector is then used to detect the solute as it comes out from the end of the capillary.
- The data obtained are analyzed by a computer
- Finally, the result is represented graphically.

Conclusion: -

CE is a relatively nonperturbing method allowing the structure-dependent electrophoretic separation of monodisperse or polydisperse mixtures of small molecules, macromolecules, polymers and colloids, up to nanoparticle and liposomes, even bacteria and viruses, in various modes of gentle separation techniques using nonaqueous or aqueous buffers with coated or

uncoated capillary columns. The small volume of the analyte needed in CE experiments has made possible the measurement of the cellular contents (native proteins, specific fluorescent-labelled or induced proteins, metabolites), the monitoring of single cells or bacteria communications The coupling of high-resolution separation techniques (CE) to high-precision mass measurements obtained with powerful MS systems is another step toward the understanding of complex mixtures and answering already important questions in cellular biochemistry. The development of CE-MS interfacing should allow for systems that are easy to operate and robust; many authors reached this goal but CE-MS is still in its first steps toward routine analysis when compared to LC-MS.

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