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Our Work on Capillary Zone Electrophoresis (CZE) and Micellar Electrokinetic Capillary Chromatography (MECC) under review Since 1989 and proposing a new mode of operation for anions experimentally called Reverse Direction Anion Capillary Electrophoresis

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ABSTRACT

Our work is reviewed on CZE and MECC since 1989.In the experimental part of this manuscript a new mode for operating Capillary Electrophoresis for separation of anions with at using buffer modifiers has been demonstrated. Reverse Direction Anion Capillary Electrophoresis , as the new mode is designated , is performed on two anions , nitrate and nitrite , with similar electrophoretic mobilities at various buffer pH values. Since electroosmotic flow increases as buffer ph is increased, it is shown that resolution is poor at low pH and to high pH.

Key Words: Field Effect Electroosmosis, Metal-Insulator- Electrolyte (MIE)., Field Effect Streaming Potential , Capillary Zone Electrophoresis (CZE) ,

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INTRODUCTION

In 1989 for the first time in University of North Carolina in Chapel Hill U.S.A in Biosensor Technology Conference, we presented the concept of Field Effect Electroosmosis. The proceeding of this conference was published as a book in 1990 by the name of Biosensor Technology [1].

Field Effect Electroosmosis is a novel interfacial phenomenon which is of particular interest of Ghowsi etal. Field Effect Electroosmosis could be demonstrated by combining a Metal Insulator Electrolyte system (MIE) with capillary electroomosis.

Using this Technique a capillary is covered with a metallic coating at its outside surface, and electroosmotic flow is controlled by applying a perpendicular electric field to the flow. Field Effect streaming potential can be defined as converse of Field Effect Electroosmosis. An electric potential is generated across the capillary with the metallic coating and applied electric field perpendicular to the flow, if a liquid is forced through the capillary. The potential application of these effects are examined in the processes which would benefit from a flexible control of electroosmotic flow , such as Capillary Zone Electrophoresis (CZE) in separation science and processes with continous flow electrophoresis where there is a need for elimination of the electroosmotic flow which reduces the effectiveness of these proccsses. These effects are also exploited for the characterization of insulator / electrolyte interfaces.

Based on field Effect Electroosmosis the physical modeling of a Field Effect Electrokinetic Transistor, called a MIEEKFFD (Metal Insulator Electrolyte Electrokinetic Field Effect Device) has been proposed some authors call it Flow (FET), Flow (Field Effect transistor). This device could be considered liquid state equivalent of a conventional solid state Metal Oxide Semiconductor Field Effect Transistor (MOSFET) in electronics.

In a MIEEKFED, the electrolyte flow and ionic current may be controlled, while in a MOSFFT, the electronic current flow may be controlled, in either case, by means of an electric field applied perpendicular to the respective flow. This application of MIEFKFED could be categorized as used of separation based sensor [1]. The MIEFKFFD has great potential for miniaturization, particularly in height of recent advances in the technology of micromachining silicon.

The next reference by us is a conference paper [2]. The conference was High performance Capillary Electrophoresis (HPCE) which was held in Sandiego California (USA). The proceeding was published in Journal of Chromatography in 1991. According to science direct, this paper has been cited one hundred times.

In 1991 American Laboratory Journal which is an Instrumentation Journal invited us to write a paper on this. We published a paper titled Field Effect Electroosmosis: The First Electrokinetic Transistor [3]. In 1995 we published in Russian Journal of Electrochemistry the

detail mathematical modeling of ideal Metal Insulator Electrolyte (MIE) and non ideal (MIE) when chemical equilibriums exit at the interface of insulator / electrolyte [4].

The fifth reference by us is the three dimensional plots of total capacitor, the insulator capacitor and the double layer capacitor as a function of potential and concentration of electrolyte [5].

The next paper published by us [6] is about introducing Field Effect Electroosmosis and three and two Dimensional plots of Zeta potential (the potential in the diffuse layer of the electrolyte) for ideal case of Metal-Insulator-Electrolyte (MIE) structure.

Our contribution is not limited to understanding interfacial phenomena of Insulator/ Electrolyte, but it serves to understand the behavior of Capillary Zone Electrophoresis (CZE) and Micellar Electrokinetic Capillary Chromatography (MECC). Our first work was on MECC.
In CZE ions have different velocities and under electric field they get separated because of different velocities they have.

In CZE the neutral molecules do not get separated because they do not have any velocities under electric field. Micelles are added to the solution and CZE is converted to MECC. Micelles are charged and they form a colony, their hydrophobic tale gets the inner of the sphere and the ionic side forms the outside of the sphere. The micelles dissolve the hydrophobic molecules differently. Under the electric field micelles move because they are charged. If hydrophobic molecules like Toluene or Xylene are present in micellar solution, the molecules move under electric field. Because the hydrophobic molecules have different solubility in micelles and micelles move under electric field. The hydrophobic molecules move with the micelles with different velocities under the electric field. In the paper which was published by us in Analytical Chemistry [7]. We defined a novel parameter for the hydrophobic molecule and charged micelle under the electric field.

Since the hydrophobic molecules obtain velocities in the presense of charged micelles and electric field, we proposed the term Effective Velocity and (Effective Mobility) for the hydrophobic molecule in the presence of micelles and electric field. By defining this novel parameter the Effective Velocity and using it two new fundamental equations for Micellar Electrokinetic Capillary Chromatography (MECC) have been derived, which are analogous to the corresponding Capillary Zone Electrophoresis (CZE) eqations for the resolution and the migration time.

This results suggest CZE and MECC are the same in nature since we obtained resolution and migration time for MECC similar to CZE we call these new equations migration time and resolution equation for MECC Electrophoresis Equations for MECC. It is possible to model MECC similar to column chromatography. It is interesting how we model MECC it becomes CZE or column chromatography. If MECC is modeled as column chromatography, the components in the theoretical plate height expression for MECC are compared with the appropriate parameters of the Van Deemter equation in column chromatography. It is interesting to note

that the MECC has dual identities. It could be modeled as Capillary Zone Electrophoresis (CZE) or Column chromatography. In Column Chromatography we have mobile and stationary phase in MECC we have mobile phase and pseudo stationary phase (micelles). It is also shown the optimal ranges of the capacity factors for good resolution and resolution per unit time have been found to be between 2 and 5. These optimal ranges approximate to that for conventional Column Chromatography, even though the physical causes of flow are different, in MECC the flow in a capillary is pumped by an electric field but in Column chromatography the flow is pumped mechanically.

A new model based on Effective Length migrated on a similar to tread mill case for various modes of operation in Capillary Zone Electrophoresis has been constructed. New resolution and number of theoretical plates for Capillary Zone Ellectrophoresis (CZE). For example for separation of anions by CZE, the detector is placed next to the anode. Electrophoretic and Electroosmotic velocity vectors are in opposite divections , but since the electrophorstic velocity is greater than the electroosmotic velocity , anions can be detected at the anode side.

Sine anions are moving in a direction opposite to bulk flow , the effective distance the anion travels is great or than the capillary length. The same scenario happens when a person paddles a canoe upstream. Though a great deal of energy may be expanded in paddling, the shore line distance traveled depends on the downstream velocity. The effective distance the canoe travels is equivalent to the shore line distance plus the distance the stream travels during the time the canoe is in the water by this example we demonstrated, the effective distance the anion travels is greater than the Capillary length. The effective distance is the same parameter as Effective length which was introduced by us for the first time.

This parameter could be used to model (CZE) correctly. In reference 9 a new model based on effective length migrated on a similar to tread mill case for various modes of operation in Capillary Zone Electrophoresis (CZE) was constructed. In present work similar treatment has been applied to (MECC) Micellar Electrokinetic Capillary Chromatography and new equations obtained for the number of theoretical plats and resolution equation [9].

In previous work the equations for migration time and resolution were obtained. These relations were used to obtain optimization using analytical mathematics [7,9]. Optimization of Capillary Zone Electrophoresis (CZE) based on an electroosmotic mobility of ions [10]. A new optimum condition relating electrophoretic mobility of ions and electroosmotic mobility was obtained using new resolution equation proposed in previous work [9] incorporating effective length. Optimization of Micellar Electrokinetic Capillary Chromatography (MECC) was done on the new resolution equation proposed in previous work based on incorporating Effective length [9]. This optimization for Micellar Electrokinetic Capillary Chromatography was done for resolution, R_s, and t_R / R_s^2 which t_R is retention time and R_s is the resolution.

In reference 11, we specified two Characteristic Equations obtained from the resolution equation of Terabe and Ghowsi [11].These Characteristic Equations are plotted three dimensionally for the first time. Optimum conditions for these Characteristic Equations are discussed from three dimensional, plots.

In reference 12, The Micellar Electrokinetic Capillary Chromatography (MECC) strength of separation is due to Nano Scaled Pseudo Stationary Phase which are micelles. This is the reason that this technique (MECC) could be called Nano Separation Technique. There are four Characteristic Equations obtained from the resolution equation of Terabe and Ghowsi and resolution equation per unit time R_s/t_R [12].These Characteristic Equations are plotted three dimensionally and two dimensionally. Optimum conditions for these Characteristic Equations are extracted from three and two dimensional plots.

In a recent book edited by this author Kiumars Ghowsi, [13] the name of the book is Electrophoresis. The first Chapter of this book contributed by us New looks at Capillary Zone Electrophoresis (CZE) and Micellar Electrokinetic Capillary Chromatography (MECC) and Optimization of (MECC).

There is interesting preface in Electrophoresis [13] which put Electrophoresis in perspective. We present it here. Electrophoresis experiment was first carried out by Tiselius in 1930. In his thesis titled, “The Moving Boundary Method to study the Electrophoresis of proteins “, Tiselius utilized the electric charge carried by the macromolecules to achieve some pioneering separation of blood plasma proteins in free solution on a photographic film.

Electrophoresis is defined as the transport of electrically charded particles in a direct current electric field. Electrophoretic separation is based on differential rates of migration in the bulk of the liquid phase and is not concerned with reactions occurring at the electrodes. In the early days, electrophoresis was carried out either in free solution or in the supporting media such as paper, cellulose, starch, agarose and polyacry lamide gel. In between 1950 to 1970, an enumeration of techniques and instrumentation for Electrophoresis were developed.

Gel electrophoresis has been rarely used for the separation and identification of small charged molecules of molecular weight less that about 1000 Dalton. In addition, the major draw back of gel electrophoresis is lack of complete automation.

To overcome the law efficiency and reduce thermal effects. Hjerten carried out electrophoresis in narrow diameter tubes of 300 µm internal diameter the first time in 1967. This was the birth of open tubular capillary electrophoresis. However, in the following decade, capillary electrophoresis did not draw enough attention from researchers only until 1981 when Jorgenson and Lukacs demonstrated the use of narrow capillaries to produce high efficiency for the separation of dansy and fluorescamine derivatives of amino acids, dipeptides and simple

amines, High performance Capillary Electrophoresis was born and a new era of Capillary Electrophoresis has begun.

After the introduction of commercial CE instrument in late 1988 that allowed the full automation of CE analysis to be possible, more and more research publications and inductrial applications have made Capillary Electrophoresis one of the dominant technologies in the separation field. In 1985 Terabe etal., added a new dimension to Capillary Electrophoresis. They added micelles to the aqueous electrolyte and were able to separate neutral molecules such as benzene and phenol. Using this technique it is possible to separate various drugs which are neutral or even charged. One that can separate enantiomers using this technique is called Micellar Electrokinetic Capillary Chromatography (MECC).

The Electrophoresis book [13] contains few fundamentals on Capillary Electrophoresis and diverse application of Electrophoresis in general. We hope this collection will entertain the readers who are interested in fundamental as well as applications of Electrophoresis in general. In another reference by us [14], a new mode for operating Capillary Electrophoresis for separation of anions without using buffer modifiers has been demonstrated. Reverse Direction Anion Capillary Electrophoresis, as the new mode is designated, is performed on two anions, nitrate and nitrite, with similar electrophoretic mobilities at various buffer pH values. Since electroomotic flow increases as buffer pH is increased, it is shown that resolution is poor at low pH and enhanced at neutral to high pH. Model equations are derived for predicting the resolution and number of theoretical plates for Reverse Direction Anion Capillary Electrophoresis. From these equations, system efficiency (N) and resolution are plotted as a function of electroosmotic mobility to illustrate how performance can be improved by an increase in electroosmotic flow.

In reference 15, the effect of injection time on separation in Reverse Direction Micellar Electrokinetic Capillary Chromatography is investigated. In this study, hydrostatic and diffusion injection were studied. The empirical data have been compared with theoretival models for both cases.

EXPERIMENT

Frequently ion analysis in Capillary Electrophoresis involves the separation of cations, Consequently, electrophoretic systems are arranged such that the detector is near the cathode end of the capillary (Fig.1a). As a proposed mode of operation that can make Capillary Electrophoresis useful for detector is positioned near the anode (Fig.1b). Electroomotic flow moves from the anode to the cathode for untreated capillary surfaces. At pH value greater than 2 or 3 the capillary surface acquires a net negative charge , inducing the formation of a compact positively charged layer on the solution side of the interface. Solution bulk moves toward the Cathode as this compact layer of cations is pulled in that direction. In conventional electrophoresis, anions are electrophoretically pulled in the opposite direction to electroosmotic flow, but they may still be detected at the cathode end of the capillary if their electrophoretic velocity is less than their electroosmotic velocity (Fig.1a). If their

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electrophoretic velocity is greater than the electroosmotic velocity , then detection of anions is only possible on the anode side (Fig.1b).

In present study the detector is placed next to the anode (Fig.1b). Electrophoretic and electroosmotic velocity vectors are in opposite directions, but since the electrophoretic velocity is greater than the electroosmotic velocity, anions can be, detected at the anode side. Swparation can be achieved in shorter capillary length. Reducing the capillary length, 1t was surmised, wouid not effect efficiency or resolution since the effective capillary length is much greater that the substantive capillary length.

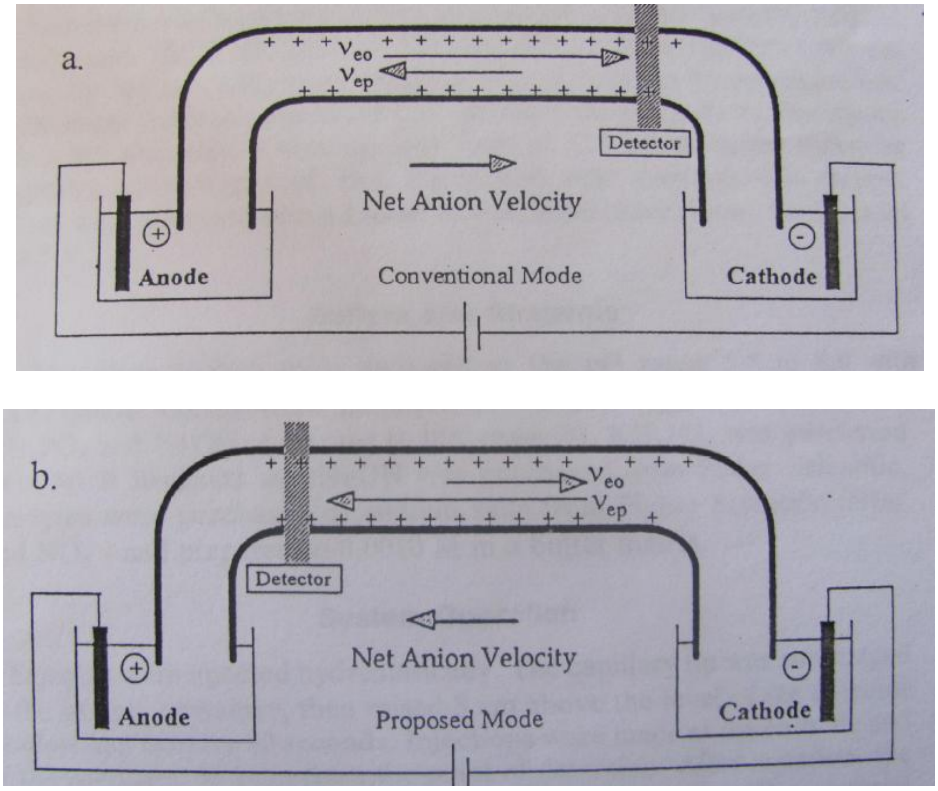

Figure1: Electrophoretic configurations for detection of anions. (a) Conventional Anion Capillary Electrophoresis. (b) Revirse Direction Anion Capillary Electrophoresis without a modifier to coat the capillary surface [14].

INSTRUMENTATION

A Bertran power supply (Bertran Associates Inc., Hicksville , New York) running at 30KV was used for the Capillary Electrophoresis system. The current was monitored at the various buffer pH's using an ECG digital multimeter (ECG Multimeter , Taiwan). Fused silica capillaries (61cm long by 363 µmo.d.) were obtained from polymicro Technologies Inc. (Phoenex , Arizona).

An ISCO CV^4 Capillary Electrophoresis absorbance detector was used. Detection was fixed at 214 nm wavelength using the following settings: sensitivity.0.01; rise, 0.8 second ; time

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constant ; 0.36 second. Data were collected with a linear Scientific recorder (Reno, Nevada) set at 5V.

BUFFERS and REAGENTS

Phosphate buffers were prepared in the pH range 5.8 to 8.0 with KH_2PO_4 and NaOH. KH_2PO_4 was purchased from MCB reagents and NaOH was purchased from Fisher Scientific (NO_2^- and NO_3^-) and prepared to 0.0010 M in a buffer matrix.

SYSTEM OPERATION

Samples were injected hydrostatically. The capillary tip was submerged in the sample container, then raised 5 cm above the level of the detector window and held for 30 seconds. Injections were made at the cathode end of the capillary, 30.1 cm from the point of detection. After injection, the capillary tip was wiped clean and placed in buffer solution.
The potential (30KV) was then applied to effectuate the separation.

Before and between each injection , the Capillary was rinsed for from 2 to 5 minutes with buffer solution at the appropriate PH value. A series of electropherograms were recorded for nitrate and nitrite ions at buffer PH values between 5.8 and 8.0. Fig.2 is Reverse Direction Anion Capillary Electropherograms depicting improved resolution of nitrate and nitrite anions as the PH is increased.

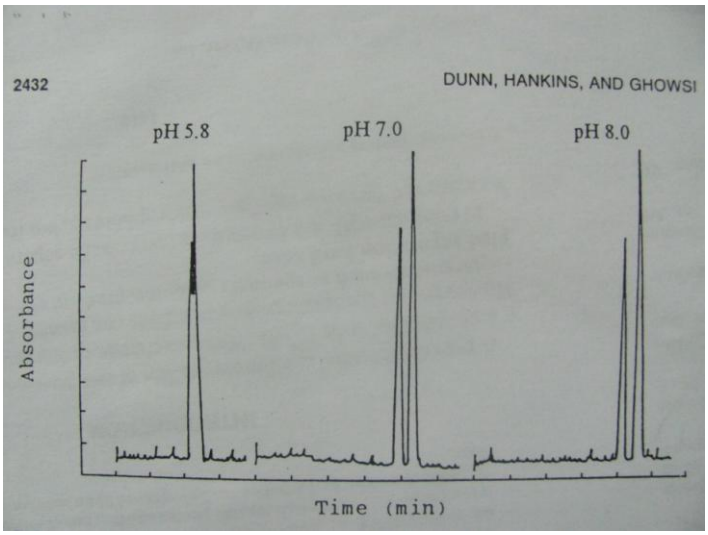

Figure 2: Reverse Direction Anion Capillary Electropherograms depicting improved resolution of nitrate and nitrite anions as the pH is increased

As the PH increases the electroosmotic velocity increases. As Fig.1b demonstrates if the electroosmotic velocity increases since veo is opposite to electrophoretic velocity , the analyte

stay longer under the applied potential and consenquently the electric field. The longer the analyte stays under the applied potential, analyte get separated better. That is why as pH increases Fig.2 the nitrite and nitrate get separated better and consequently the resolution increases as the PH increases Fig.2.

CONCLUSION

In the experimental part, making use of uncomplicated hardware, adequate separation of anions has been demonstrated by utilizing a new mode of electrophoresis base on the principle of sample detection at the anode end of the capillary; but without the use of a buffer modifier to reverse electroosmotic flow.

This proposed mode of operation for separation of anions is named Reversed Direction Anion Capillary Electrophoresis.

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