Review Article www.ijcps.com

International Journal of Chemical and Pharmaceutical Sciences
2014, June., Vol. 5 (2)

ISSN: 0976-9390
IJCPS

Capillary electrophoresis and its application in pharmaceutical analysis

Ranjith P*, Pavani B, Ajitha A and Umamaheswara Rao V.

Department of Pharmaceutical Analysis and Quality Assurance, CMR College of Pharmacy, Medchal Road, Kandlaykoya, Seethariguda, Hyderabad, Telangana, India.

***Corresponding Author:** E-Mail: rangthkumar@gmail.com

ABSTRACT

Capillary electrophoresis is a physical method of analysis based on the migration, inside a capillary, of charged analytes dissolved in an electrolyte solution, under the influence of a direct-current electric field. Over the past 10 years, capillary electrophoresis (CE) is an analytical tool that has shown great promise in replacing many conventional clinical laboratory methods, especially electrophoresis and high performance liquid chromatography (HPLC). The main attraction of CE was that it was fast, used small amounts of sample and reagents, and was extremely versatile, being able to separate large and small analytes, both neutral and charged. Because of this versatility, numerous methods for clinically relevant analytes have been developed. However, with the exception of the molecular diagnostic and forensic laboratories CE has not had a major impact. A possible reason is that CE is still perceived as requiring above-average technical expertise, precluding its use in a laboratory workforce that is less technically adept. With the introduction of multicapillary instruments that are more automated, less technique-dependent, in addition to the availability of commercial and cost effective test kit methods, CE may yet be accepted as a instrument routinely used in the clinical laboratories. Thus, this review will focus on the areas where CE shows the most potential to have the greatest impact on the clinical laboratory. These include analysis of proteins found in serum, urine, CSF and body fluids, immunosubstraction electrophoresis, hemoglobin variants, lipoproteins, carbohydrate-deficient transferring (CDT), forensic and therapeutic drug screening, and molecular diagnostics. In this review we are highlighting the basic principles and applications of capillary eletrophorosis.

Keywords: Capillary electrophoresis, Carbohydrate-deficient transferring, Immunosubstraction.

1. INTRODUCTION

Electrophoresis is defined as the migration of charged species under the influence of an externally applied electric field. Differences in mobility of the analytes are due to their average charge, size, shape, and on the properties of the electrolyte solution used. Reuss was the first person to carry out separations based on this principle in 1809. During the study of the migration of colloidal clay-particles, he investigated that the liquid adjacent to the negatively charged surface of the wall migrated towards the negative electrode under influence of an externally applied electric field [1]. The theoretical aspects of this electro kinetic phenomenon that Reuss called electro-osmosis were proposed by Kohlrausch in 1897[2]. In their effort to separate diphtheria toxin, globulin, and toxin/antitoxin solutions several researchers carried out electrophoretic separations in so called 'U'-shaped tubes in the late 1800's and early 1900's.

Tiselius greatly contributed to the advancement of the analytical aspects of electrophoresis [3,4]. During his study on the development of free moving boundary electrophoresis, he successfully separated complex protein mixtures based on differences in electrophoretic mobilities. For this reason, Tiselius was awarded the Nobel prize for chemistry in 1948. At that time, the poor resolution observed due to peak broadening caused by Joule heating and in a minor way caused by molecular diffusion was a big challenge concerning electrophoresis. Starch gel, paper, agarose, cellulose acetate or polyacrylamide gels

were used as stabilizers to prevent this peak broadening [5]. Although the above-mentioned stabilizers minimized the problem of convection, they led to other peak broadening phenomena, like eddy diffusion and undesired interactions between analytes and stabilizer. Later Tiselius and Hjertén developed polyacrylamide gel electrophoresis in 3-mm i.d. rotating capillaries and applied this technique in the separation of ribosomes and viruses [6-8]. Hjertén summarized the technique in 1967, in which stabilization was achieved by continuous rotation of the tube about its longitudinal axis and the peak broadening could be avoided [9].

1.2. Development

On the basis of Giddings' deduction that a very high efficiency must be possible when longitudinal diffusion is the only cause of peak broadening [10], Everaerts *et al.* in 1970 using 200 µm i.d. Teflon® tubes [11] and Virtanen in 1974 using thin glass tubes did the experiments and confirmed this point. They found the stabilizing "wall effect" by using the thin tubes as a separation column in which the small diameter of the column counteracts the convective flow, leading to an increase in efficiency. The increase in efficiency is proportional to the decrease in diameter of the tube due to the increasing surface-to-volume ratio. In 1981 Jorgenson and Lukacs developed an electrophoresis system using open glass capillaries of 75 µm i.d. with an on-column fluorescence detector [12-15]. They applied the system to the separation of the fluorescent dansylated amino acids and the fluorescamine derivatized amino acids in human urine within 25 minutes, which provided the predicted efficiencies (>400000 plates) and illustrated the high efficiency of electrophoresis in open tubes of small diameter.

The possible advantages of performing zone electrophoresis in open tubes of small diameter were summarized:

- Efficient heat transfer within the electrophoresis medium is achieved, leading to minimal temperature gradients.
- Disadvantageous effects of remaining temperature gradients are minimized by solute diffusion back and forth across the tube diameter.
- The medium is stabilized against convective flow by the wall effect.
- After these initial experiments and after the introduction of the first commercially available instruments in 1988, the potential of capillary electrophoresis as a high performance separation technique in analytical (bio) technology, bio analysis and pharmaceutical analysis was soon acknowledged in some early papers [16]. It proved to be a powerful and useful method of analysis, especially when only small amounts of sample are available.

1.3. Principle of capillary electrophoresis

Modern capillary electrophoresis consists of a high-voltage power supply; two buffer reservoirs, a capillary and a detector. This basic set-up can be elaborated upon with enhanced features such as auto samplers, multiple injection devices, Sample/capillary temperature control, programmable power supply, multiple detectors, fraction collection and computer interfacing [17].

1.4. Electrophoretic mobility

The transport of a charged particle in an electric field is very similar to that found for the sedimentation of a particle in a centrifugal field. A molecule with charge q in an electric field E (V cm-1) experiences an electronic force FE: FE = qE (1) Just as in sedimentation, the molecule quickly reaches a velocity, v, and receives a frictional force FF given by: FF = -6πηrv (2) At a steady state during electrophoresis, the two forces are equal but opposite, thus qE = 6πηrv (3). The electrophoretic mobility of the molecule, µ, is the velocity per unit field. µ = v/E (cm2/V-sec) (4) Combination of equations (3) and (4) yield sµ = q / (6πηr) (5) From equation (5), it can be seen that species with small size and higher charge numbers will have high mobilities, while species with large size and lower charge numbers will have low mobilities [18]. Except the above factors, mobility is affected by temperature and electro osmotic flow.

1.5. Electro osmotic flow (EOF)

When a current is applied to the capillaries there is a bulk flow of movement through the system. This is known as electro osmotic flow (**EOF**) and is a result of the surface charge on the capillary wall.

When an aqueous buffer is placed inside a fused silica capillary, its inner surface acquires an excess of negative charges. This is due to the ionisation of the silanol groups (SiOH) that can exist in anionic form (SiO-) and or the absorption of ions from the buffer onto the capillary. EOF becomes significant above pH 4. The negatively charged silanoate groups attract counter ions from the buffer which form an inner layer of tightly held cations, also termed the fixed layer, at the capillary wall. However these cations are not of sufficient density to neutralise all negative charges, so a second outer layer of cations forms

which makes up the diffuse layer. The fixed and diffuse layers make up the diffuse double layer of cations [19]. When an electric field is applied, the outer layer of cations is pulled toward the negatively charged cathode. Since these cations are solvated, they drag the bulk buffer solution with them, thus causing electro osmotic flow. EOF drags all analytes irrespective of charge in one direction. Consequently cations and anions may be simultaneously analysed in one run, which otherwise is not possible in the absence of EOF. EOF makes it possible to analysis analytes with different charge mass ratios within reasonable analysis times. Compared to pumped or laminar flow in HPLC, electro osmotic flow in CE has a flat profile. The advantage of the flat flow profile is that all solutes experience the same velocity component caused by electro osmotic flow regardless of their cross-sectional position in the capillary, and they elute as narrow bands giving narrow peaks of high efficiency [20].

1.6. Modes of capillary electrophoresis

The origins of the different modes of separation may be attributed to the fact that capillary electrophoresis has developed from a combination of many electrophoresis and chromatographic techniques [21]. In general terms, it can be considered as the electrophoretic separation of a number of substances inside of a narrow tube. The distinct capillary electro separation methods include:

- Capillary Zone Electrophoresis (CZE)
- Capillary Isotachophoresis (CITP)
- Capillary Isoelectric Focusing (CIEF)
- Capillary Gel Electrophoresis (CGE)

1.6.1. Capillary zone electrophoresis (CZE)

CZE is the most commonly used technique in CE. Many compounds can be separated rapidly and easily. The separation in CZE is based on the differences in the electrophoretic mobilities resulting in different velocities of migration of ionic species in the electrophoretic buffer contained in the capillary. Both anions and cations can be separated in the same run. Cations are attracted towards the cathode and their speed is augmented by the electro osmotic flow. Anions, although electrophoretically attracted towards the anode, are swept towards the cathode with the bulk flow of the electrophoretic medium [22].

1.6.2. Capillary Isotachophoresis (CITP)

CITP is performed in a discontinuous buffer system. Sample components condense between leading and terminating constituents, producing a steady-state migrating configuration composed of conservative sample zones. The isotachopherogram obtained contains a series of steps, with each step representing an analyte zone [23]. The quantitation in CITP is mainly based on the measured zone length which is proportional to the amount of analyte present.

1.6.3. Capillary iso electric focusing (CIEF)

CIEF is based on the isoelectric points (pH values) of the substances to be separated. The most common type of sample that utilizes this analytical method is proteins. Under the influence of an applied electric field, charged proteins migrate through the medium with pH gradient until they reside in a region of the pH where they become electrically neutral and therefore stop migrating. Consequently, zones are focused until a steady state condition is reached. After focusing, the zones can be migrated (mobilized) from the capillary by a pressurized flow [24]. Sharp peaks are obtained with good resolution, and a large peak capacity is observed mainly because the whole tube is simultaneously used for focusing.

1.6.4. Capillary gel electrophoresis (CGE)

CGE is based on differences in solute size as analytes migrate through the pores of the gel-filled column. Gels are potentially useful for electrophoretic separations mainly because they permit separation based on 'molecular sieving'. They serve as anti-convective media minimize solute diffusion, which contributes to zone broadening, prevent solute adsorption to the capillary walls and they help to eliminate electroosmosis [25].

1.7. Detection in capillary electrophoresis

Most detectors in capillary elctrophoresis have been adapted from HPLC. Good detector should supply a stable baseline and be responsive to all type of compounds, rugged and not too expensive. Also they must be versatile, provide high sensitivity and low noise level. They may be situated on-column, end-column or post-column. Two types of detectors have been developed for capillary electrophoresis: optical detection and eletrochemical detection

1.8. Optical detection techniques

Optical detection was successfully implemented in commercial state-of-the-art CE instruments, which include UV absorbance and fluorescence detectors. On-column detection is often used, since the light source can be directly focused on to the capillary whilst the electronic transduction of the signal remains galvanically separated from the DC influence of the high-voltage [26].

1.9. UV/Vis

Due to its relatively universal nature; any molecules possessing a chromophore can be detected by UV/Vis; and because of its availability from HPLC work, the UV/Vis absorbance detector is the most commonly used detector in capillary electrophoresis. The advantages of the UV/Vis detector include: non-destructive to the analytes, insensitivity to temperature and gradient changes, low cost, and simplicity. The detector necessitates the presence of an optical window, which is created by removing a small section of the polyimide coating, which renders the capillary fragile and vulnerable to breakage. It should be noticed that not all species of interest possess chromophores, such as most amino acids, sugars and inorganic ions. This problem could be solved by detection in the indirect mode where a chromophore is added into the background electrolyte [27]. This however yields lower sensitivity. Furthermore there are some restrictions in the running buffers due to the optical properties of the buffers themselves.

1.10. Fluorescence Detection

A fluorescent detector is used for fluorescent molecules which absorb light at one wavelength and then re-emit it instantaneously at a longer wavelength. Two types of fluorescence detectors have been developed according to the light sources used: lamp10 based and laser induced fluorescence detectors (LIF). In the former, light sources such as deuterium, tungsten or xenon lamps are used for excitation, whilst lasers are used as excitation sources in the latter. Laser induced fluorescence gives rise to even higher sensitivities due to the high intensity of its incident light and the ability to accurately focus light to the small diameter capillaries [28]. Detection limits in the 10-12 M range have been reported. Lasers available include argon ion, helium-cadmium and helium-neon. The LIF detector is expensive and generally limited by the range of excitation wavelengths offered by the laser, there are also possibilities of photo-degradation of the analytes caused by the high light intensity. The detector is also less versatile because many solutes of interest do not exhibit native fluorescence.

1.11. Electrochemical Detection Methods

Electrochemical detection methods in capillary electrophoresis have been reviewed in recent articles. As a universal method, conductometric, amperometric, and potentiometric modes of detection have been successfully coupled to capillary electrophoresis. The positioning of the electrodes, interferences of high electric field, and the materials employed in the fabrication and modification of the electrodes are the main topics concerned. The advantages of the use of electrochemical detection with capillary electrophoresis, regarding to the sensitivity and selectivity, is exemplified with a large number of applications [29]. Especially, the use of electrochemical detection systems in microchip technology is addressed [30].

1.12. Potentiometric Detection

Potentiometric detection can be a powerful alternative in capillary electrophoresis. In this detection mode, a potential developing on an ion-selective electrode or membrane in contact with an analyte ion is measured. Potentiometric detectors can easily be miniaturised without loss of sensitivity because their response is quasi-independent on flow rate. Since potentiometric electrodes respond only to ions with a charge of the correct sign, they are more selective. Miniaturised forms of liquid membrane ion selective electrodes, which are routinely applied in physiological studies, have been used as detectors in CE. They were used for the detection of both cations and anions [31]. In recent works potentiometric detection was carried out with coated-wire liquid membrane electrodes. The detection properties of these electrodes were comparable to those of the micropipette electrodes but they were easier to handle and had a longer lifetime. Reviews on potentiometric detection for capillary electrophoresis are available [32]. The disadvantages of potentiometric detection include the complication of sensor preparation, and handling, fragile micromanipulations and limited lifetime.

1.13. Amperometric detection

Amperometric detection is an important method of detection for CE because it has attractive features including high sensitivity, good selectivity, and low cost. CE with amperometric detection has been established as a powerful analytical technique, especially for the analysis of biological microenvironments such as single cells. Amperometric detection is based on the application of a fixed potential at an electrode. Electro active compounds gain (reduction) or lose (oxidation) electrons to the electrode and the resulting current can be directly correlated with analyte concentration. Amperometric detection requires three electrodes; a working electrode, reference electrode and an auxiliary electrode, which controls the potential difference between the working and reference electrode. A potential is applied across a supporting electrolyte between the working and reference electrode effecting solute oxidation or reduction. Amperometric detection may be carried out in the oxidative or the reductive mode. In the oxidative mode, a

negative potential is applied by the auxiliary electrode. This results in a positive potential difference between the working and reference electrodes. As a result, electrons are transferred to the working electrode. In the reductive mode, the opposite occurs. Reviews on amperometric detection for capillary electrophoresis are available [33].

1.14. Conductivity Detection

Compared to potentiometric and amperometric detection, conductivity detection does not rely on electrochemical reactions on the surface of the electrode but measures an electrical signal (conductance) between electrodes contacting the solution. In this detection mode, analytes have to be charged in solution for the determination. A conductivity detector cell comprises two inert electrodes across which a high frequency AC signal is applied. Alternating current is used during the detection in order to avoid electrolysis reactions on the surfaces of the electrodes. The signal arises from the difference in conductance between the analyte and the background electrolyte. The higher the conductivity differences between the analyte-molecules and background co-ion, the larger the detector response. Reviews on conductivity detection for capillary electrophoresis can be found in the literature [34].

1.15. Apparatus for Capillary Electrophorosis

An apparatus for capillary electrophoresis is composed of a high-voltage, controllable direct-current power supply and buffer reservoirs, held at the same level, containing the prescribed anodic and cathodic solutions, electrode assemblies (the cathode and the anode), immersed in the buffer reservoirs and connected to the power supply, a separation capillary (usually made of fused-silica) which, when used with some specific types of detectors, has an optical viewing window aligned with the detector. The ends of the capillary are placed in the buffer reservoirs [35]. The capillary is filled with the solution prescribed in the monograph, a suitable injection system and detector able to monitor the amount of substances of interest passing through a segment of the separation capillary at a given time; it is usually based on absorption spectrophotometry (UV and visible) or fluorimetry, but conductometric, amperometric or mass spectrometric detection can be useful for specific applications; indirect detection is an alternative method used to detect non-UV-absorbing and non-fluorescent compounds.A thermostatic system able to maintain a constant temperature inside the capillary is recommended to obtain a good separation reproducibility. A recorder and a suitable integrator or a computer. The definition of the injection process and its automation are critical for precise quantitative analysis. Modes of injection include gravity, pressure or vacuum injection and electrokinetic injection. The amount of each sample component introduced electrokinetically depends on its electrophoretic mobility, leading to possible discrimination using this injection mode [36].

1.16. Applications hemoglobin and its variants

Analysis of hemoglobinopathies and thalassemias is important in the diagnosis and management of the over 600 known congenital hemoglobin (Hb) defects. Analysis requires identification and quantification of the abnormal Hb along with the minor Hb components. Historically, these variants have been characterized by electrophoretic separation using alkaline CAE or AGE combined with citrate agar at acid pH. However, these techniques are being replaced by high performance cation exchange chromatography [37].

1.17. Protine analysis

The first indication that CE could be used in the separation of serum proteins into the classic six bands, e.g. albumin, a-1 globulin, a-2 globulin, h-1 and h-2 globulins, and g globulin, was reported by Jorgenson and Lukas [38]. Although variations in these protein fractions can be correlated with a patient's health status it is usually the changes that occur in h and g fractions that are of the most clinical interest. It is in these fractions that monoclonal proteins are normally found and whose detection is an important part of the laboratory evaluation of patients with lymphoproliferative diseases.

Large monoclonal bands are usually present in patients with multiple myeloma or Waldenstro ¨m's disease; however, lower concentrations may be seen in a variety of other diseases such as light chain disease, leukemia, lymphoma, amyloidosis, or monoclonal gammopathy of undetermined significance (MGUS). Patients that fall into the MGUS category should have annual follow-ups because the chance that these patients will develop myeloma or other lymphoproliferative diseases is increased [39]. Classically, screening serum has been done by either cellulose acetate (CAE) or agarose gel (AGE) electrophoresis. While the reagents for both of these methods are relatively inexpensive, these methods require a significant amount of labour.

1.18. Urine analysis

Urine proteins Increased excretion of protein in urine (proteinuria) is one of the most common abnormalities seen in the clinical

laboratory and can be caused by a number of pathologic conditions affecting the kidney and urinary tract [40]. Characterization of these proteins by electrophoresis can be helpful in determining the cause of increased urinary protein, whether tubular or glomerular. Classically, analysis has been by carried out AGE or CAE, although more recently CE has been successfully used to analyze urine proteins pattern. Cerebrospinal fluid proteins The analysis of cerebrospinal fluid (CSF) proteins can be useful in the diagnosis and management of a variety of neurological diseases including conditions that cause immune responses, destructive brain diseases, or a breakdown in the blood–brain barrier.

1.19. Lipoproteins

Atherosclerosis, a chronic disease characterized by the localized accumulation of plaque, is the principal cause of coronary arterial disease leading to the obstruction of arteries. Serum lipoprotein abnormalities, such as increased LDL, decreased HDL, increased Lp (a), etc., have been shown to be a factor in atherosclerotic development. Because of this association, lipoprotein profiles consisting of serum cholesterol, triglycerides, HDL, and LDL (calculated using the Friedewald formula) are routinely run in the main clinical chemistry laboratory [41].

1.20. Serum and urine analysis of drugs

Studies have shown that CE has better resolution, reduced sample preparation, costs less, and is faster than high performance liquid chromatography (HPLC) , although the sensitivity and migration time precision for CE is not good as HPLC. Sensitivity can be increased by using extended light path length capillaries, such as bubble cells or z-cells, where the inner diameter of the capillary is increased only at the detection window [42].

1.21. Molecular diagnostics

Molecular pathology/molecular diagnostics is the newest and most rapidly growing area in laboratory medicine, in which the detection, characterization and/ or quantification of nucleic acids is used to assist in the diagnosis and management of disease. Molecular assays augment classical laboratory medicine by providing additional information that could not be obtained using standard methodologies. Currently, molecular pathology can be separated into six areas: (1) hematology/oncology; (2) solid tumors; (3) genetics; (4) pharmacogenetics; (5) infectious diseases; and (6) identity testing/forensics. Molecular pathology laboratories currently focus on infectious diseases [43].

1.22. Infectious diseases

An important aspect of molecular methods is that they do not require the presence of viable organisms permitting the identification of bacteria, viruses, and fungi that are difficult if not impossible to culture. Identification of infectious agents can also be used for both diagnostic and therapeutic purposes [44] Although the simplest application of molecular techniques is to detect infectious entities in body fluids which are nearly sterile more complex analysis is also done, such as detailed characterization of infectious agents via sequence analysis.

1.23. Genetics

CE has simplified the methodology for performing both simple and complex genetic tests. Numerous inherited genetic diseases, such as Cystic Fibrosis (CF), fragile X, mitochondrial heteroplasmy, spinocerebellar ataxia, and genetic variants of cytochrome P450 that are involved with drug metabolism can be detected using CE. Sequencing and fragment analysis of restriction fragment length polymorphisms (RFLP) and primer extension are commonly used to detect these variants.

1.24. Other

Currently, gene expression profiles using arrays permit pattern identification that may be useful for diagnostic and prognostic purposes. Classically, membrane and silicon arrays identified various patterns but required specialized equipment to perform and interpret the results. More recently, expression arrays formulated with internal expression controls have enabled analysis using standard CE equipment.

2. CONCLUSION

CE is a sensitive and versatile technique and represents an inexpensive and practical method for the determination of many clinically important analytes. Although CE may be maturing in the research genomic, and pharmaceutical arenas, in the clinical laboratory, it still appears to be in its early childhood. Initially, CE was heralded for its speed and low sample volume capabilities, and perhaps more importantly, its ability to be quantitative and to automate, as well as separating compounds that have been difficult to handle by traditional methods. CE has simplified molecular tests by providing a platform that can address all areas of molecular testing. Because of its ease of use and robust nature, it is becoming a standard piece of equipment for every molecular pathology laboratory. In the future, CE will be the

basis of additional molecular techniques, especially in the area of expression arrays.

3. REFERENCES

1. Janini GM and Issaq HJ. The buffer in capillary zone electrophoresis, in Capillary Electrophoresis Technology, (Guzman, N.A., ed.), **Marcel Dekker, Inc., New York,** 1993; 119–160.
2. Cifuentes A and Poppe H. Rectangular capillary electrophoresis: some theoretical considerations. **Chromatographia,** 1994; 39: 391–404.
3. Virtanen R and Kivalo P. Quantitative high-voltage zone electrophoresis method. **Suomen Kemistilehti B**, 1969; 42: 182.
4. Jorgensen JW and Lukacs KD. Zone electrophoresis is open-tubular glass capillaries. **Anal. Chem.**, 1981; 53, 1298–1302.
5. Lukacs KD and Jorgensen JW. Capillary zone electrophoresis: effect of physical parameters on separation efficiency and quantitation **J. High Resolut. Chromatogr. Chromatogr. Commun.** 1985; 8: 407.
6. Kuhn R and Hoffstetter-Kuhn S. Capillary Electrophoresis Principle and Practice. **Springer Verlag, Berlin,** 1993; 153.
7. Lambert WJ and Middleton DL pH hysteresis effect with silica capillaries in capillary zone electrophoresis. **Anal. Chem.** 1990; 62: 1585–1587.
8. Cobb KA., Dolnik V and Novotny M. Electrophoretic separations of proteins in capillaries with hydrodynamically stable surface structures. **Anal. Chem.** 1990; 61: 2478–2483.
9. Hjerten S. High performance electrophoresis: elimination of electroendosmosis and solute absorption. **J. Chromatogr.** 1988; 347, 191–198.
10. Bruin GM, Chang JP, Kuhlmann RH, Zegers K, Kraak JC and Poppe H. Capillary zone electrophoretic separations of proteins in polyethylene glycol-modified capillaries. **J. Chromatogr.** 1992; 471: 429–436.
11. McCormick RM. Capillary zone electrophoretic separation of peptides and proteins using low pH buffers in modified silica capillary. **Anal. Chem.** 1988; 60: 2322–2328.
12. Novotny MV, Cobb KA and Liu J. Recent advances in capillary electrophoresis of proteins, peptides, and amino acids. **Electrophoresis,** 1990; 11: 735– 749.
13. Wiktorowicz JE and Colburn JC. Separation of proteins via charge reversal in capillary electrophoresis. Electrophoresis, 1990; 11: 769–773.
14. Emmer A, Jansson M, and Roeraade J. Improved capillary zone electrophoretic separation of basic proteins, using a fluro surfactant buffer additive. **J. Chromatogr.** 1991; 547: 544–550.
15. VanOrman BB, Liversidge GG and McIntire GL. Effects of buffer composition on electroosmotic flow in capillary electrophoresis. **J. Microcol. Septo.** 1991; 2: 176.
16. Josic D, Zeilinger K, Reutter W, Bottcher A and Schmitz G. High performance capillary electrophoresis of hydrophobic membrane proteins. **J. Chromatogr.** 1990; 516: 89–98.
17. Lehmann R, Voelter W and Liebich HM. Capillary electrophoresis in clinical chemistry. **J Chromatogr B,** 1997; 697:3– 35.
18. Shihabi ZK and Friedberg MA. Analysis of small molecules for clinical diagnosis by capillary electrophoresis. **Electrophoresis** 1997; 18: 1724– 32.
19. Von Heeren F and Thormann W. Capillary electrophoresis in clinical and forensic analysis. **Electrophoresis,** 1997; 18: 2415–26.
20. ThormannW, Aebi Y, Lanz M and Caslavska J. Capillary electrophoresis in clinical toxicology. **Forensic Sci Int**., 1998; 92: 157–83.
21. Perrett D. Capillary electrophoresis in clinical chemistry. Ann Clin Biochem 1999; 36:133–50.
22. Altria KD. Overview of capillary electrophoresis and capillary electro chromatography. **J Chromatogr A**., 1999; 856: 443– 63.
23. Maurer HH. Systematic toxicological analysis procedures for acidic drugs and/or metabolites relevant to clinical and forensic toxicology and/or doping control. **J Chromatogr B**, 1999; 733:3– 25.
24. Thormann W, Wey AB, Lurie IS, Gerber H, Byland C and Malik N. Capillary electrophoresis in clinical and forensic analysis: recent advances and breakthrough to routine applications. **Electrophoresis**, 1999; 20: 3203- 36.
25. Blessum C, Jeppsson JO, Aguzzi F, Bernon H and Bienvenu J. Capillary electrophoresis:

principles and practice in the clinical laboratory. **Ann Biol Clin**., 1999; 57:643–57.

26. Issaq HJ. A decade of capillary electrophoresis. **Electrophoresis**, 2000; 21:1921– 39.
27. Jenkins MA. Clinical applications of capillary electrophoresis. Status at the new millennium. **Mol Biotechnol**, 2000; 15: 201–9.
28. Jin LL, Ferrance J and Landers JP. Miniaturized electrophoresis: an evolving role in laboratory medicine. **BioTech**., 2001; 31: 1332– 53.
29. Palfrey SM. Clinical applications of capillary electrophoresis. Totowa, **NJ: Humana Press**, 1999.
30. Jenkins MA, Kulinskaya E, Martin HD and Guerin MD. Evaluation of serum protein separation by capillary electrophoresis: prospective analysis of 1000 specimens. **J Chromatogr B**, 1995; 672: 241– 51.
31. Bossuyt X, Bogaerts A, Schiettekatte G and Blanckaert N. Detection and classification of paraproteins by capillary immunofixation/subtraction. Clin Chem 1998; 44:760– 4.
32. Bossuyt X, Schiettekatte G, Bogaerts A, Blanckaert N. Serum protein electrophoresis by CZE 2000 clinical capillary electrophoresis system. **Clin Chem**., 1998; 44:749–59.
33. Wong MS and Aw TC. A preliminary evaluation of serum proteins by capillary electrophoresis. **J Capillary Electrophor**., 1998; 5:217–21.
34. Clark R, Katzmann JA, Wiegert E, Namyst-Goldberg C, Sanders L and Oda RP. Rapid capillary electrophoretic analysis of human serum proteins: qualitative comparison with high-throughput agarose gel electrophoresis. **J Chromatogr A,** 1996; 744:205–13.
35. Gonzalez-Sagrado M, Lopez-Hernandez S, Martin-Gil FJ, Tasende J, Banuelos MC and Fernandez-Garcia N. Alpha1- antitrypsin deficiencies masked by a clinical capillary electrophoresis system (CZE 2000). **Clin Biochem**, 2000; 33: 79– 80.
36. Elghetany MT and Davey FR. Erythrocytic disorders. In: Henry JB, editor. Clinical diagnosis and management by laboratory methods. **Philadelphia: Saunders;** 2001; 560–71.
37. Jorgenson JW and Lukacs KD. Zone electrophoresis in open tubular glass capillaries. Anal Chem 1981; 53: 1298–302.
38. Plevak MF. Long-term study of prognosis in monoclonal gammopathy of undetermined significance. **N Engl J Med**., 2002; 346(8):564–9.
39. Johnson AM, Rohlfs EM and Silverman LW. Proteins. In: Burtis CA, Ashwood ER, editors. Tietz fundamentals of clinical chemistry. **Philadelphia: Saunders;** 2001; 325–51.
40. Zabzdyr JL and Lillard SJ. Measurement of single-cell gene expression using capillary electrophoresis. **Anal Chem**., 2001; 73:5771–5.
41. Avins AL and Browner WS. Improving the prediction of coronary heart disease to aid in the management of high cholesterol levels: what a difference a decade makes. **JAMA J Am Med Assoc**., 1998; 279: 445– 9.
42. Shihabi ZK and Oles KS. Felbamate measured in serum by two methods: HPLC and capillary electrophoresis method. **Clin Chem** 1994; 40:1904– 8.
43. Wynia GS, Windhorst PC, Post PC and Maris FA. Development and validation of a capillary electrophoresis method within pharmaceutical quality control environment and comparison with high -performance liquid chromatography. **J Chromatogr A**, 1997; 773: 339– 50.
44. Arakawa H, Watanabe K, Kashiwazaki H and Maeda M. Detection of two variant Vero toxin genes in Escherichia coli by capillary electrophoresis. **Biomed Chromatogr**., 2002; 16(1):41– 6.