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Applications of Capillary Electrophoresis in Pharmaceutical Drug Analysis

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ABSTRACT

Capillary electrophoresis (CE) is a new analytical technique that has been applied to many fields including biomedical technology, clinical diagnosis, pharmaceutical drug analysis, food science, and environmental analysis. This article reviews the use of CE in the analysis of drugs, with the drugs being categorized by their major mode of pharmacological action. From selected paper, methods are brief described. These descriptions include the mode of CE, the composition of the running buffer, detection methods, sample preparation and running conditions.

Key words: **capillary electrophoresis, capillary zone electrophoresis, micellar electrokinetic chromatography, running conditions, drug analysis.**

INTRODUCTION

Conventional electrophoresis and chromatography have been used as separation tools for many years. However, despite the good separation that these techniques offer, there are also drawbacks such as the laborious multi-stage handling and insufficient long-term reproducibility of the results. Capillary electrophoresis (CE) is a newly developed analytical technique in analytical chemistry. This technique provides a fast, accurate, quantitative analysis for all kinds of molecule, from single ions to macromolecules such as proteins and DNA. Different modes of CE have been developed, depending upon the nature of the analyte. Capillary zone electrophoresis (CZE) is the simplest and most widely used mode of CE. Due to its simplicity, better reproducibility and longer lifetime, CZE is easier to use than some other modes, such as capillary gel electrophoresis (CGE), micellar electrokinetic capillary chromatography (MECC), capillary isoelectric focusing (CIEF), or capillary electrochromatography (CEC). In CZE, electrophoresis takes place in a length of capillary tubing filled with an appropriate buffer solution. A small quantity, typically 1-30 nl, of sample mixture is introduced into one end of the capillary. The capillary is made of fused silica, with silanol groups on the surface. These silanol groups may become ionized in the presence of the electrophoretic medium. The interface between the fused silica tube wall and the electrophoretic buffer consists of three layers: the inner capillary wall is negatively charged, primarily from ionization of the silanol groups. Adjacent to the wall is an immobile layer (the Stern layer or inner Helmholtz plane), and the dif-

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fuse layer of hydrated counter ions (cations). Upon application of the electric field, these cations are attracted to the cathode, inducing a bulk flow of liquid within the capillary. This flow is called electroosmotic flow (EOF). In the meantime, the charged species in the sample mixture migrate toward the electrodes of opposite polarity, which may be against or in the same direction as the EOF. Each species moves through the buffer at a constant speed, determined by the size, shape, and charge of the respective species, as well as the applied voltage. This movement is called electrophoretic movement. Because in general the mobility of the EOF is usually faster than that of the electrophoretic mobility, the net direction of movement of each species is from anode to cathode. When a species of molecule passes through the detector (such as a UV absorption detector, or a potential or amperometric detector) an amplified signal can be recognized.

In spite of the advantage of its simplicity, CZE may not always give a convinsing results, because the CZE separation is based on the charge to mass ratio, so it is not capable of separating neutral hydrophobic molecules. Another mode such as MECC (or MEKC), however, is able to do this. In fact, MECC has been used widely for neutral drugs analyses and on other neutral chemicals. This technique was first demonstrated by Terabe and co-workers [1-2] in 1984. The mechanism is based on solute partitioning between a micellar pseudo-stationary phase and an electroosmotically pumped mobile phase. When surfactant is added to a buffer solution at a concentration above its critical micelle concentration (cmc), leads to the formation of micelles. Micelles consist of aggregates of surfactant molecules which are composed of a hydrophobic hydrocarbon tail and a hydrophilic head formed either by an ionizable or highly polar group. The most commonly used surfactant molecule in MECC is sodium dodecyl sulfate (SDS), which is an anionic surfactant. The micelles formed by this anionic surfactant are negatively charged and are attracted toward the anode. Thus, taking into account the EOF in an uncoated fused silica capillary, these anionic micelles move toward the cathode at a slower rate than the bulk of the liquid because of their attraction towards the anode. Neutral molecules partition in and out of the micelles to different extents depending on the hydrophobicity of each analyte. Consequently the micelles of MECC are often referred to as a "pseudo-stationary phase". Neutral molecules that interact with the micelles to a lesser extent are eluted earlier. Conversely, the hydrophobic neutral molecules that interact with the micelles are eluted later.

CZE and MECC are the two dominant methods for the analysis of small organic molecules, such as conventional pharmaceutical agents, drugs and physiologically derived metabolites. However, on some occasions, the concentration of the analytes may be under the detection limit with conventional UV/VIS detectors. To overcome this problem, some other detection methods may be applied in combination with CZE or MECC such as mass spectrometric (MS) detection or fluorescence detection. The analytes have to derivatize fluorescence either in the pre- or post-column if they are not normally fluorescent. An alternative method is indirect fluorescence detection.

The use of mass spectrometry for detection provides the unique capability to determine the mass and structure of atoms, and molecules and their fragments in a mixture. The interfacing of CE to MS has been accomplished by two different of approaches: one is "electrospray ionization" (ESI), and the other is "continuous-flow fast atom bombardment" (CF-FAB). In the first attempt at CZE-MS[3] an electrospray ionization interface was used for the connection of the separation capillary to a quadrupole mass spectrometer. Further improvement in the design of the electrospray CZE-MS interface was the introduction of the sheath flow [4] and liquid junction coupling [5-6] to make the electric contact with the outlet of the capillary. In this way the composition of the electrospray liquid could be controlled independently of the CZE buffer. This allowed the use of aqueous and high ionic strength buffers, and independent definition of both the CZE and electrospray field gradients. An alternative design to electro-

spray ionization for CE-MS is based on the continuous-flow fast bombardment interface [7-9]. In this technique, a liquid effluent flows directly onto the probe tip of the mass spectrometer where the bombardment by high energy atoms (e.g., accelerated xenon atoms) induces the desorption of the analyte molecules. To inhibit the freezing of the solvent on the high vacuum probe tip the ionization process involves the flow of a low volatile matrix (e.g., glycerol), which has to be present either directly in the effluent or supplied in the post-column mixer.

Fluorescence detection may increase the sensitivity by at least a thousand times relative to UV/VIS detection [10-17]. Since most analytes are not fluorescent, pre- or post-column derivatization may be required to introduce a fluorophore to the analyte molecules. Recently, a laser monochromatic beam has been widely used to enhance the detection limit. This laser induced fluorescence (LIF) detection method was originally developed because of the low concentration limits of some biologically active peptides, which are found in body fluids and tissues at extremely low concentrations (between 10^{-11} and 10^{-13} M). The first successful use of LIF detection for capillary electrophoresis was accomplished by Gassmann and associates [18], who demonstrated the detection of dansylated amino acids by using the 325 nm line of a He-Cd laser. Another example of an application for CE-LIF is the measurement of nonfluorescent compounds that can be determined by indirect fluorescence. This technique uses a fluorescent buffer and measures the separated zones by a decrease of fluorescence intensity [19-21]. Several approaches improved on detection sensitivity even further by introducing new laser lines and new fluorescent chromophores. For instance, a He-Cd laser (442 nm) was used for the analysis of naphthalene dealdehyde derivatized amino acids and used argon ion laser (488 nm) was used to detect fluorescein isothiocyanate (FITC) derivatized analytes [22-23].

Another mode of CE for increasing sensitivity is "isotachophoresis" (ITP). This technique is carried out in a discontinuous electrolyte system formed by a leading and terminating electrolyte. The leading electrolyte forms the front zone, and the terminating electrolyte forms the rear zone; it is between these zones that the sample substances migrate. During the migration, both the leading and the terminating zones are separated from the sample by phase boundaries. Sample components condense between leading and terminating constituents, producing a steady-state migrating configuration composed of consecutive sample zones. This mode of operation is therefore different from other modes of capillary electrophoresis. In isotachophoresis mode, the resultant electropherogram consists of a series of steps, with each step representing an analyte zone. Unlike in other CE modes, where the amount of sample present can be determined from the area under the peak as in chromatography, quantification in isotachophoresis is mainly based on the measured zone length, which is proportional to the amount of sample present.

In addition to the modes described above, inclusion complexes have been used for enhancement of selectivity. The most commonly used inclusion compound in CE is cyclodextrin. Cyclodextrins (CD) are cyclic oligosaccharide molecules that are shaped like a truncated cone and have a cavity which is able to form inclusion complexes with several kinds of chemical compounds. The cavity size and shape are a function of the number of glucopyranose units. The ones most commonly used are α, β and γ-CD with six, seven and eight glucopyranose units, respectively [24]. The complexation mechanism does not involve only a simple partition between the solvent and the cavity of the CD's but also includes hydrogen bonding between the CD's hydroxyl groups and the species of interest, hydrophobic interactions, and solvent effects. The size and shape of the molecules are also important. The effectiveness of using CDs to enhance selectivity depends on the size and geometry of the guest molecule, with respect to the dimensions of the CD cavity. Differences in the stability of the inclusion complexes for series of structurally related solutes and optical isomers provide the

mechanism to improve resolution of both geometrical and optical isomers. This mechanism allows for chiral separations, and has been employed for the resolution of various chiral analytes including amino acids and catecholamine. The use of complex interactions for improvement of the resolution in electrophoresis is not limited to cyclodextrins: other cyclic compounds, such as crown-ethers (e.g.,18-crown-6, which is effective for the resolution of alkali metals in ITP) may be used in CZE as well.

PUBLISHED REVIEWS

Many general review articles of capillary electrophoresis have been published (see for example references [25-26]), as well as specific reviews of the applications of CE in protein analysis [27-30] and in clinical diagnosis [31-33]. This review will focus on drug analysis by CE. The drugs are categorized into nine groups based upon their major pharmacological mode of action.

BRIEF SUMMARIES

I. *Vitamins*

Eleven water-soluble vitamins, pyridoxamine, nicotinamide, pyridoxal, vitamin B_6, vitamin B_{12}, vitamin B_2, vitamin B_2 phosphate, pyridoxamine-5'-phosphate, niacin, vitamin B_1 and pyridoxal-5'-phosphate, were analyzed by using MECC [34]. High pH (pH = 9.0) phosphate-borate buffer (0.02 M) with 0.05 M SDS was used as running buffer. A baseline separation was demonstrated within 20 min. Kenndler et al. [35] used 0.01 M phosphate buffer with a pH of 7.0 to detect the impurities of commercial vitamin B_2, such as riboflavin mono- and diphosphates by fluorescence labeling. Vitamin C, another water soluble vitamin, was analyzed from biological fluids and fruit beverages by Koh et al.[36]. They used 0.1 M tricine buffer, pH 8.8 as running buffer and measured UV absorbance at 254 nm. A stereoisomer, isoascorbic acid, was used as an internal standard to assess the amount of vitamin C in human urine, plasma and fruit juices. The method for analyzing vitamin A, a water-insoluble vitamin, was developed by using laser-excited fluorescence detection [37]. The running electrophoretic buffer was 0.05M Na_2HPO_4, pH 7.8 (pH was adjusted to 7.8 with 1.5 M H_3PO_4). Blood samples were collected and air-dried on a blood collection filter paper. These dried whole blood specimens were pretreated with a solution of 6 M urea in phosphate buffer to dissolve the blood before injecting into the CE column. The detection limit for vitamin A by CE is 3 μg/l. Five retinoids, retinol, retinal, retinoic acid, retinyl acetate and retinyl palmitate, were separated by the MECC method [38]. The running buffer contained phosphate buffer (0.1 M sodium hydrogen-phosphate and 0.1 M disodium hydrogen-phosphate in deionized water) with 3mM Brij 35 and 75 mM sodium deoxycholate. The separation was performed in a 47 cm fused-silica capillary with 50 μm i.d. under a voltage of 20 kV. The five retinoids were separated within 20 minutes. CE may provide a fast and accurate method for vitamin screening tests with a very small amount of sample.

II. *Antibiotics*

Separation of seven penicillin analogues and nine cephalosporin analogues were demonstrated using MECC by Nishi et al. [39]. They used the same running buffer for separating water-soluble vitamins. The concentration of SDS was from 0.1 to 0.3 M with sodium *N*-lauryl-*N*-methyl-taurate. The detection wavelength was UV 210 or 220 nm. Ampicillin, amoxicillin and its analogue, aspoxicillin, a broad-spectrum semi-synthetic penicillin with an N^4-methyl-D-asparagine residue and an OH group in the benzylpenicillin molecule, was followed in human plasma by MECC [40]. The running buffer was 0.02 M phosphate-borate buffer, pH 8.5 with 0.1 M SDS. Plasma samples without any pretreatment were injected into an untreated fused-silica capillary with UV detection at 210 nm. The running time was about 20 min and the detection limit was 1.3 mg/l. The tetracyclines are a group of broad spectrum antibiotics having similar pharmacological activity. They are

especially effective in treatment of typhus, fever, psittacosis, acute brucellosis, and granuloma inguinale. Six tetracyclines (oxytetracycline, tetracycline, demeclocycline, chlortetracycline, dexycycline, and minocycline) were separated by MECC. The running buffer was ammonium acetate buffer (15 mM) containing SDS (20 mM) or mixed SDS-Brij35 (0.135%, w/v) at pH 6.5. under an applied voltage of 15 kV [41]. Sulfonamides have a broad antimicrobial spectrum and have been used to treat pneumocytis infections in renal transplant and veterinary practice. However, these compounds may be present in animal products such as milk. In addition, there have been reports that sulfamethazine may cause thyroid carcinoma. Many investigators have developed sensitive analytical methods to separate and analyze these compounds. Lin et al. [42] used a phosphate-borate buffer as the background electrolyte with a low concentration of β-cyclodextrin (0.5 mM) at pH 6.85 to separate thirteen sulfonamides very effectively. The separation can be further improved by using a low pH, high concentration citrate buffer (500 mM), with buffer pH having a larger effect on the selectivity and resolution of sulfonamides than buffer concentration [43]. The optimal conditions for separating thirteen sulfonamides were established with a citrate buffer (20 mM) at pH 6.9. The separation was completed within 2.1 min under an electric field strength of 30 kV [44].

III. *Anti-parasitic and Anti-fungal Drugs*

The separation of antimalarial drugs such as quinine and its geometrical isomer quinidine were demonstrated [45]. The running buffer was 0.02 M phosphate buffer, pH 8.3. The separation was based upon the charge difference between quinine and quinidine under the running conditions. The detection wavelength was set at 245 nm and sensitivity reached approximately 1 x 10^{-7} M. An improved separation of quinidine, hydroquinidine and desipramine was demonstrated by adding 15% acetonitrile into the running buffer [46]. Because quinine and its related compounds may induce a series of symptoms collectively called *cinchonism*, which include tinnitus, headache, nausea, abdominal pain, and visual disturbances, and because quinine also causes deafness, limb anomalies, visceral defects, visual alterations and acute hemolysis in fetuses, monitoring serum levels of quinine or its related compounds is often required. A method for analyzing antifungal drugs such as terbinafine and halofuginone has been developed by using phosphate buffer of pH 2.4 with 60% acetonitrile. Krivankova et al. [47] combined CZE with isotachophoresis for analysis of the coccidiocidic drug, halofuginone, in foodstuff concentrates by using a combination of capillary ITP and CZE in column switching mode. High load capacity of ITP and high sensitivity of CZE allowed analysis of up to 25 μl of a sample solution containing 10^{-8} M halofuginone in a capillary of 0.3 mm ID equipped with both conductivity and UV detection. In the first ITP stage, the electrolyte system was composed of 5 mM KOH and MES, pH 5.7, with 0.2% Triton X-100 as the leading electrolyte and 25 mM ε-aminocaproic acid and MES, pH 4.0, with 0.2% Triton X-100 as the terminating electrolyte.

IV. *Nonsteroidal Anti-inflammatory Drugs*

Nonsteroidal anti-inflammatory drugs include salicylates (such as aspirin and diflunisal), phenylproprionic acid derivatives (ibuprofen, fenoprofen, flurbiprofen, ketoprofen, naproxen, and oxaprozin), a phenylacetic acid derivative (diclofenac), a pyrrole acetic acid derivative (indomethacin), an anti-rheumatic drug (methotrexate), an anti-analgesic and anti-pyretic drugs (acetaminophen), and drugs for treatment of gout (allopurinol and colchicine). This category of drugs share the same toxic side-effects to corticosteroid with less extend. The major side-effects include inhibition of platelet aggregation, acute renal failure, and gastrointestinal ulceration. CE may provide a fast method for monitoring the drug level to prevent the side-effects.

The separation of acetysalicylic acid, caffeine, ρ-acetamidophenol, salicylamide, and ethoxybenzamide were performed by using MECC. The running buffer was 0.02 M phosphate solution, pH

11, with 0.05 M SDS. The detection wavelength was UV 214 nm [48]. Some other anti-inflammatory drugs such as naproxen, ibuprofen, and tolmetin were analyzed with comparable resolution by using uncoated fused silica capillaries or capillary coated with linear polyacrylamide [49].

V. *Corticosteroids*

Adrenal corticosteroids are among the most widely used drugs in the world. Their use varies from the treatment of mild self-limited conditions to life-threatening problems. Most undesirable effects of corticosteroids are usually proportional to the dose and duration of time over which they are given. These undesirable effects include osteoporosis, Cushing's syndrome, acute pancreatitis, peptic ulcers, and opportunistic infections. Nishi et al. [50] analyzed the lipophilic corticosteroids analogues by MECC with bile salts. The composition of the running buffer was 0.1 M sodium cholate and 0.05 M sodium taurocholate in a 0.02 M phosphate-borate buffer (pH 9.0). Under these running conditions, eight corticosteroid derivatives were separated within 20 min. A further study was conducted by adding cyclodextrins (CDs), which are relatively hydrophilic and not soluble in SDS, into the SDS solution, so that the solute was distributed among three phases: the aqueous buffer, the micelle, and the CD [51]. Thus, by adding 30 mM of β-CD or 15 mM of γ-CD to the SDS buffer solution, a shorter migration time was recorded for the analysis of corticosteroid compounds.

VI. *Drugs that Act on the Central Nervous System*

Psychoactive drugs include the drugs for psychopharmacological therapeutic compounds and drugs of abuse. The latter refers to the administration of a drug or other biologically active substance in order to produce a pharmacological effect unrelated to medical therapy. Psychoactive drugs include CNS depressants (alcohol, benzodiazepines, barbiturates, diazepam, etc), psychostimulants (amphetamine, cocaine, methylphenidate), opioids (heroin, morphine, codeine, methadone), tobacco, cannabinoids, hallucinogens, and caffeine. Psychoactive substance use, abuse, and dependence are of widespread interest in our society, and considerable attention has been paid to this subject in the social sciences.

Barbiturates and its derivatives were separated by MECC [48]. The running buffer was composed of 0.01 M borate buffer, pH 8.5 with 0.05 M SDS, and the detection wavelength was UV 215 nm. Eight barbiturates, barbital, aprobarbital, butabarbital, amobarbital, hexobarbital, pentobarbital, secobarbital, and methohexital, and an anticonvulsant, diphenyldantoin, were separated within 10 min by this method. Thormann et al. [52] used "fast scanning multiwavelength detection" to analyze some other barbiturates such as allobarbital, phenobarbital, thiopental, and isomers of thiopental and pentobarbital in urine and plasma samples.

Some forensic drugs such as morphine, codeine, heroin, amphetamine, and cocaine and some hallucinogenic drugs such as LSD, psilocin, psilocybin, and cannabidiol, were separated by MECC with 8.5 mM phosphate, 8.5 mM borate, 85 mM SDS and 15% acetonitrile, pH 8.5. The detection wavelength was 210 nm and the separation completed within 40 min [53]. Analysis of illicit drugs in human urine by MECC with on-column fast scanning absorption detection was demonstrated by Wernly et al. [54] using 6 mM sodium borate, 10 mM sodium phosphate with 75 mM SDS, pH 9.1 as running buffer. On-column fast scanning absorption detection may provide sufficient spectral information to identify unknown drugs.

VII. *Drugs that Act on the Sympathetic Nervous System*

This category of drugs includes catecholamines such as epinephrine, norepinephrine, dopamine, and ephedrin, β-adrenoceptor agonists such as isoproterenol, and β-adrenoceptor antagonists such as propranolol. The peripheral autonomic nervous system acts on nearly all organ systems within the body, and controls many important physiological functions. Therefore, those drugs modifying sympathetic or parasympathetic functions have many therapeutic applica-

tions. Three important classes of antihypertensive drugs, α_1-adrenoceptor antagonists, α_2-adrenoceptor antagonists and β-adrenoceptor antagonists, are effective by virtue of their ability to modulate sympathetic neurotransmission. β_2-adrenoceptor agonists represent the primary mode of acute and chronic bronchodilator therapy for asthma and other bronchospastic diseases. Topical β-adrenoceptor antagonists are now the most common approach to therapy of chronic glaucoma. There is increasing interest in α-adrenoceptor antagonists in the treatment of benign prostatic hypertrophy.

Most sympathomimetic drugs have an enantiomer. Thus, for instance, epinephrine shows optical activity and (-)-epinephrine is ten times more potent than its enantiomer [55]. The possibility of using CZE for purity control of this drug was demonstrated by analyzing two different commercial samples. The running electrophoretic buffer was 0.1 M phosphate buffer, at pH 2.5, and supplemented with 20 mM 2,6-di-*O*-methyl-β–cyclodextrin [56]. Finani et al. [57-59] performed a series of studies for separating enantiomers of sympathomimetic drugs by using CD as the chiral agent. They demonstrated that the optical isomers of norephedrine, ephedrine, norepinephrine, epinephrine and isoproterenol were separated in a tris-phosphate solution, pH 2.4, containing 18 mM heptakis (2,6-di-*O*-methyl-β-cyclodextrin) in a coated capillary. They continued to separate a mixture of terbutaline (a selective β_2-receptor agonist used in the treatment of asthma and lung diseases) by phosphate buffer, pH 2.5, containing 5 mM di-OMe-β-CD. In the same study, the separation of enantiomers of propranolol (a β-blocker used in the treatment of *angina pectoris*) was performed with CD with 30% v/v methanol and 4M urea in the running buffer. In a further study they used 0.01 M tris with phosphate buffer, pH 6.4, to separate isoxsuprine, dopamine, norepinepherine, epinephrine and dichloroisoproterenol within 10 min. Lin et al. [60] used CZE to separate β–blocker at low pH (1.8-3.8) with high concentrations of citrate buffer (160-400 mM). Ten β-blocker analogues were separated within 11 min under the 15 kV applied voltage. They also found that the separation of β-blockers could be achieved with a phosphate buffer (70 mM) containing tetradecyl- or hexadecyltrimethylammonium bromides (TTAB or CTAB) when the TTAB or CTAB at a concentration between 15-20 mM and 12-15 mM, respectively, was added into the phosphate buffer and the final pH was adjusted to 7.0. The migration and selectivity of β-blockers were influenced considerably by hydrogen bonding interactions, in addition to hydrophobic interactions in MECC [61].

Wallingford and Ewing [62] used an electrochemical detector for analyzing catecholamine from a single cell. The running buffer contained boric acid, which reacted with catechols to form a complex. The separation was based upon the net charge changed after complex formation. An extremely small volume of approximately 430 pl of a mixture of biogenic amines and carboxylic acid metabolites was analyzed in a fused silica capillary of 12.7 μm ID with electrochemical detection [63]. Ewing et al. [64] analyzed a cytoplasmic sample from a single giant dopamine neuron of the pond snail, *Planorbis coneus*. Chien et al. [65] quantified the amount of dopamine released from a single giant dopamine neuron after being stimulated with ethanol. They measured the concentration of dopamine (which ranged from 1.4×10^{-4} - 4.7×10^{-5} M) by CE with electrochemical detector.

Serotonin (5-hydroxytryptamine) is a neurotransmitter in serotoninergic neurons in the central nervous system. These neurons are clustered in a few discrete sites in the brain, with the Raphe nuclei in the mesenecephalon. The neurons are involved in pain perception, behavior, and regulation of such autonomic functions as temperature, blood pressure, and respiration. An important function of the brain serotonin system is in regulation of neuroendocrine functions such as adrenocorticotropic hormone, growth hormone, prolactin, and thyroid-stimulating hormone release. However, the largest pool of serotonin is found in enterochromaffin cells, even though the physiological role of serotonin is still unknown. The mobility of serotonin and dopamine are very

close, so the resolution is very poor. For improving the resolution, Wallingford et al. added 20% 2-propanol to the running buffer [66]. Alternatively, a borate phosphate buffer can be used because serotonin does not form a complex with borate and is therefore retained compared to the catechols including dopamine that are present in the sample [66].

VIII. *Antiepilepsy Drugs*

Antiepilepsy drugs usually have a narrow therapeutic dosage. For example, the therapeutical blood concentrations of carbamazepine range from 6 to 12 μg/ml. This narrow therapeutical range is because of the drug's tolerance and serious adverse effect, so that frequent drug monitoring is required during the treatment. Conventional methods for routine clinical monitoring are either immunological assay or HPLC. However, these methods are prone to problems such as cross-reactive interference and often require a high level of analytical skill and high-cost procedures. MECC is an attractive tool for this kind of drug analysis because it is capable of separating hydrophobic neutral molecules with high separation efficiency, it is easy to operate and has low running costs. Lee et al. [67] used MECC to separate six antiepileptic drugs simultaneously. These drugs were ethosuccimide, phenytoin, primidone, phenobarbital, carbamazepine and valproic acid. The running buffer contained 25 mM phosphate buffer (pH 8.0) with 50 mM SDS. A multi-wavelength detection with a photodiode-array detector was used for peak identification. Shihabi et al. [68] analyzed felbamate, a new antiepileptic drug, in serum by pretreating sampled with acetonitrile to precipitate proteins. The deproteinized solution was then monitored for felbamate by MECC. The running time for this assay was less than 5 min.

IX. *Anti-cancer Drugs*

Conventional anti-cancer drugs such as methotrexate are highly toxic. For minimizing the toxic effect during the treatment, a simple, rapid and sensitive method for separation and quantification has been developed [69]. The method used high-voltage CZE combined with laser-induced fluorescence detection. The detection limit for methotrexate was as low as 5 x 10^{-10}M. The running buffer contained 5 mM 2-(N-morpholino) ethanesulfonic acid, 5 mM tris and 1 mM sodium chloride. The pH of the solution was adjusted to 6.7 with 1 M sodium hydroxide. The sample had to be treated either by deproteinization with TCA or by being pressed through a Sep-pak C_{18} cartridge. The pretreated sample was oxidized with potassium permanganate to form 2,4-diaminopteridine-6 carboxylic acid, a highly fluorescent compound. The 325-nm radiation (17 mW) of a Model 4050B He-Cd laser was directed into the capillary to excite the sample. The fluorescence emission from the exit end of the 1000-μm fiber was passed through a monochromator or sodium nitrite-saturated solution filter to minimize background radiation, and then passed through a 450-nm interference filter to maximize the collected fluorescence signal. The quantitative results showed a good correlation with a conventional enzyme-multiplied immunoassay technique. An assay for the anti-leukemic cytosine-β-D-arabinoside (ara-C) was demonstrated by using CZE [70]. Samples were prepared by solid-phase extraction, using Bond-Elute 3-ml C_{18} cartridges. The cartridge was first washed with three column volumes of acetonitrile and then two volumes of water. Samples (200 μl) of plasma were added to the cartridge and allowed to soak into the packing material. The cartridge was then washed with 1.2 ml of water, sucked dry, and then ara-C was extracted with 1 ml of acetonitrile. The organic eluent was evaporated at room temperature under a stream of nitrogen. The solid residue was then reconstituted in 100 μl of water. The electrophoretic separation was performed using citrate buffers (10-40 mM) with pH 2.5. The detection wavelength was at 280 nm and running time was less than 10 min.

In the last decade, recombinant lymphokines or cytokines have become one of the most important agents for cancer treatment. CE has been examined as an alternative method for the determination of a recombinant cytokine in a pharma-

ceutical dosage form. Compared with conventional methods such as radioimmunoassay, enzyme-linked immunosorbent assay, bioassay, polyacrylamide gel electrophoresis and HPLC, CE provided a rapid and high resolution method with minimum sample volume requirements for a recombinant lymphokines and cytokines assay. Guzman et al. [71] used a running buffer containing 0.05 M sodium tetraborate with 0.025 M lithium chloride to analyze formulation mixtures of recombinant leukocyte A interferon and recombinant interleukin-1 α. The detection wavelength was 210 nm and the running time was less than 30 min. Anticancer drugs can also be extracted from plant. The sioflavones are a group of plant extract which have anti-cancer activity. They specifically inhibit tyrosine kinase and DNA topoisomerase. Analysis of these compounds from plant extracts is difficult, often requiring a prior derivatization of the compounds before analysis. Shihabi et al. [72], however, managed to use a simple CE method to separate five different isoflavones as well as coumestrol within 10 min. The running buffer was 0.2 M boric acid, pH 8.6, and detection wavelength was 254 nm. A good correlation was found between CE and HPLC. However, CE was faster, did not require solvent gradient and column equilibration, and did not consume organic solvents for elution. In addition, the capillaries for CE are much less expensive than the column for HPLC.

CONCLUSION

Conventional methods for pharmaceutical drug analysis such as radioimmuno-assay, HPLC, polyacrylamide gel electrophoresis, bioassay and enzyme-linked immunosorbent assay (ELISA) still suffer from certain limitations. Some limitations include the complexity and time-consuming nature, the high degree of variability, the low separation efficiency and/or lack of sensitivity of some of these methods. Capillary electrophoresis can often provide a faster, simpler and more accurate analysis than conventional methods. The application of CE to drug analysis will become much more common in the future.

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毛細管電泳在藥物分析之應用

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摘 要

毛細管電泳是一種新的分析技術，由於它的準確性高、解析度高及分析速度快及易於自動化操作，目前已漸應用於生物醫學研究、臨床診斷、藥物分析、食品分析及環境污染之監控。本文專就藥物分析上之應用，依藥物之藥理作用爲分類將目前毛細管電泳在藥物分析之應用上共計較爲重要之文獻共72篇，作一綜合之歸納，以幫助讀者在方法的取捨上作爲參攷。

關鍵詞：毛細管電泳，毛細管區域電泳，微胞電動力層析法，電泳之條件，藥物分析。