

Flow Injection-Capillary Electrophoresis (FI-CE): Recent Advances and Applications

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Abstract: This paper presents a comprehensive review of how recent advances in flow injection-capillary electrophoresis (FI-CE) technology have led to enhanced separation capabilities of a wide range of analytes in such areas as biological, environmental, food, medical and pharmaceutical analysis. Significant developments in design, detection methodology and applications made in the last five years are reported. In addition, future perspectives in FI-CE are considered.

Keywords: Capillary electrophoresis, flow injection, sample introduction, preconcentration, high-throughput screening.

1. INTRODUCTION

Capillary electrophoresis (CE) is a powerful separation tool for examining many types of species including proteins, sugars, cations and anions and DNA [1-10]. CE offers a number of advantages as a separation technique: i) it requires only small quantities of material; ii) it is applicable to water-soluble, high molecular-weight species in aqueous buffer solution, and; iii) various separation modes make it applicable for the analysis of a variety of biological and non-biological species. CE, however, suffers from a number of limitations including discontinuous and biased sample introduction, fouling of the capillary walls and limited amount of sample introduced in the capillary resulting in low concentration limits of detection [11-12]. The majority of these limitations can be reduced or completely eliminated upon coupling CE to a flow injection (FI) front end. This coupling allows enhanced sample throughput and reproducibility as well as the ability to introduce modern preconcentration techniques. In addition, enhanced detection schemes including electrogenerated chemiluminescence (ECL), amperometry and fluorescence using liquid core waveguides can be easily incorporated. Other inherent advantages of coupling FI and CE include high separation efficiency, low reagent consumption, ability to analyze small molecules in complex matrices and overall cost and simplicity. Studies by Kubán *et al.* and Fang *et al.* in 1997 [13-14] pioneered the way for coupling FI with CE for enhanced sample injection and pretreatment. Based on these early studies, as well as others by the Karlberg group [15-16], the majority of the limitations discussed above have been reduced or completely eliminated.

Previous reviews and informative research papers provided systematic studies on early development efforts of FI-CE [12-16, 18-23]. This paper presents a comprehensive review of the advances in FI-CE and related applications

within the past five years. Advances in detection technology are discussed in relation to specific applications within this paper. The first section reviews the principles and concepts of FI-CE. This is followed by a discussion of how recent advances in FI-CE technology have impacted the areas of biological/food, environmental and medical/pharmaceutical analysis. The last section discusses future trends in FI-CE technology. Broader coverage of CE detection methodologies can be found in the comprehensive review by Swinney and Bornhop [17].

2. PRINCIPLES & CONCEPTS

Flow Injection

FI was first introduced in the mid-seventies [24] and continues to be a very useful and versatile tool in modern analytical chemistry [25-38]. FI has many unique features including limited sample/reagent consumption, short analysis time, and on-line separation, preconcentration and physicochemical conversion of analytes into detectable species [24, 39]. FI is based on three principles: i) reproducible timing; ii) reproducible sample injection, and; iii) partial and controlled dispersion of the sample zone. In FI, the length of the manifold tubing remains constant and carrier and reagent flow rates vary little during transport of the sample zone from injection to detection, thus allowing proper sample zone transport and mixing. As the injected zone advances, it broadens forming a dispersed form as it moves downstream and changes from an asymmetrical to a more symmetrical shape (although never becoming completely symmetrical in real systems). This continuum of concentrations can be viewed as being composed of individual elements of fluid, each having a certain concentration (Fig. 1).

Capillary Electrophoresis

CE is a powerful separation technique that has gained widespread use in research laboratories because of its versatility and ease of use. CE separates molecules based on differences in electrophoretic mobilities (μ) under the influence of an applied field. The direction and velocity of

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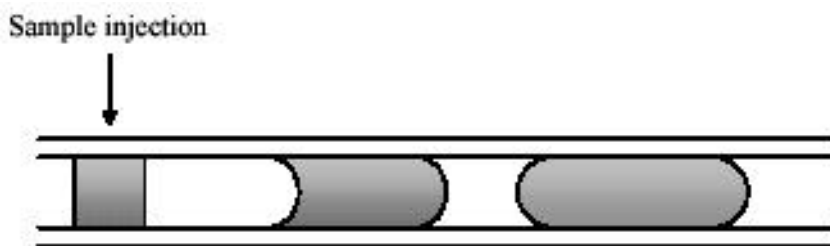


Fig. (1). The advancement of an injected sample zone through a narrow FI tube.

the movement of molecules are determined by the sum of two vector components, the migration and the electroosmotic flow (EOF). Molecules migrate through the capillary column based on their charge-to-mass ratio. In addition, the mobility (μ) of ionic species is directly proportional to the ratio of charge to radius (q/r) and reciprocal to the viscosity (η) of the solution [4]. Thus, small, highly charged ions have high mobilities, whereas large, less charged species have low mobilities. EOF is a bulk hydraulic flow of liquid in the capillary driven by the applied electric field and is a consequence of the surface charge of the capillary. In a capillary column filled with buffer a double layer is formed due to electrostatic forces. This double layer can be described by the zeta (ζ) potential. The EOF results from the movement of the layer of electrolyte ions near the capillary wall under the force of the electric field. The basic CE instrument set-up consists of a high-voltage power supply, two buffer reservoirs, a capillary and a detector (Fig. 2).

Coupling of the two techniques (example set-up presented in Fig. 3) is not without its technical challenges. The FI-CE combined system is based on electrokinetic sample splitting with the bias effect inherent in this type of

introduction unavoidable [14]. Proper calibration measures or use of a non-pressurized sample introduction approach should help minimize such bias. The introduction of air bubbles in the reservoirs and capillary is also of concern in the combined approach. Upon construction, it is important to position the platinum electrode with its end to the right of the separation capillary to avoid interferences from electrolytically generated gas bubbles. The construction of a vertical flow cell has been reported to limit the air which can interfere with the electric current and/or flow conditions [14]. In addition, external high-voltage power sources are used and every effort must be made to effectively ground the instrument through an appropriate earth contact.

3. APPLICATIONS

The coupling of CE with FI has resulted in enhanced separation techniques with advanced sample introduction/pretreatment capabilities of a wide range of analytes. Table 1 gives specific applications and quantitative details in selected FI-CE systems in the last five years. More detailed descriptions are discussed in the sections below.

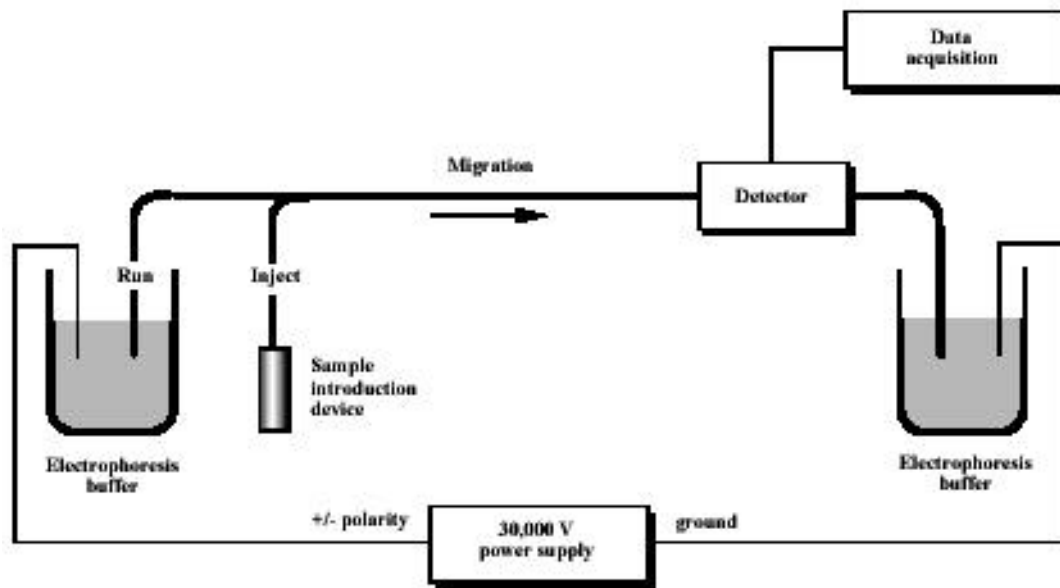


Fig. (2). The basic CE instrument set-up.

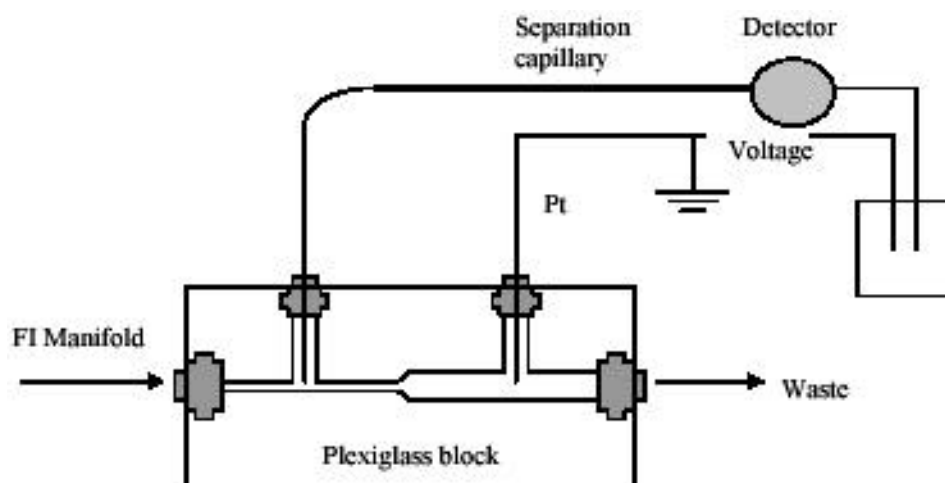


Fig. (3). A basic FI-CE interface set-up.

Table 1. Applications and quantitative details of advances in selected FI-CE systems in the last five years

Application	Measured Species	Detection method	LOD (unit as reported)	Notes	Reference
Biological/Food Samples	Sucrose, glucose	Amperometric detection	2 μM (sucrose) 1 μM (glucose)	Microchip-based CE system coupled to conventional FI technology.	[23]
Biological/Food Samples	Myo-inositol phosphates	Indirect photometric at 510 nm	11-26 $\mu\text{mol L}^{-1}$	System included a micro-column anionic exchange resin for solid phase extraction of myo-inositol phosphates.	[40]
Biological/Food Samples	Arginine, phenylalanine, glycine	Fluorometric detection	1.3 μM (arginine) 1.9 μM (phenylalanine & glycine)	Miniaturized liquid core waveguide-CE system with FI introduction.	[42]
Biological/Food Samples	Aloperine, sophoridine, matrine, oxymatrine	UV detection at 215 nm	3.28 $\mu\text{g mL}^{-1}$ (ALP) 2.13 $\mu\text{g mL}^{-1}$ (SRI) 2.23 $\mu\text{g mL}^{-1}$ (MT) 3.11 $\mu\text{g mL}^{-1}$ (OMT)	Combination FI with microfluidic capillary electrophoresis.	[43]
Biological/Food Samples	Peptide samples	Amperometric detection	Not reported	On-column polymer-embedded graphite inlet electrode for FI-CE in a poly(dimethylsiloxane) interface.	[44]
Biological/Food Samples	Proline, valine, phenylalanine	Electrogenerated chemiluminescence	1.2 μM (proline) 50 μM (valine) 25 μM (phenylalanine)	System developed on a chip platform to provide easy FI sample introduction and ECL detection.	[45]
Environmental	Phenol pollutants	UV detection at 210 nm	2.12 ng mL^{-1} (PCP) 1.21 ng mL^{-1} (2,4,6-TCP) 1.06 ng mL^{-1} (2-NP) 4.52 ng mL^{-1} (2,4-DMP)	On-line flow stacking in a FI-CE system.	[47]
Environmental	Copper, cobalt	Chemiluminescence detection	1.2 $\times 10^{-8}$ mol dm^{-3} (Co^{2+}) 2.3 $\times 10^{-8}$ mol dm^{-3} (Cu^{2+})	FI-CE with chemiluminescence on a chip platform.	[48]
Environmental	Inorganic ions	Contactless conductivity detection	Range: 20-200 $\mu\text{g L}^{-1}$ for all ions including: Cl^{-} , NO_3^{-} , SO_4^{2-} , HPO_4^{2-} , K^{+} , Ca^{2+} , Na^{+} , Mg^{2+}	On-site determination with an automated FI-CE method.	[49]

(Table 1). Contd.....

Environmental	Nitrate, sulfate	UV detection at 372 nm	124 ppb (nitrate) 77 ppb (sulfate)	Microsequential injection coupled to CE using electrokinetic injection.	[50]
Environmental	Lead, cadmium, cobalt, nickel, zinc	UV-Vis detection at 570 nm	$2.0 \times 10^{-6} \text{ mol dm}^{-3}$ (Pb^{2+}) $8.0 \times 10^{-6} \text{ mol dm}^{-3}$ (Cd^{2+}) $8.0 \times 10^{-6} \text{ mol dm}^{-3}$ (Co^{2+}) $4.0 \times 10^{-6} \text{ mol dm}^{-3}$ (Ni^{2+}) $4.0 \times 10^{-6} \text{ mol dm}^{-3}$ (Zn^{2+})	Separation of heavy metal ions by FI-CE using xylenol orange.	[51]
Environmental	Se(IV), Se(VI)	Contactless conductivity detection	$190 \mu\text{g L}^{-1}$ (Se(IV)) $7.5 \mu\text{g L}^{-1}$ (Se(VI))	FI-CE system with contactless conductivity detection.	[52]
Environmental	Nitrate, nitrite	UV detection	10 ng mL^{-1}	On-line ion-exchange preconcentration.	[53]
Medicinal/ Pharmaceuticals	Artemisinin	UV detection at 292 nm	$5.93 \mu\text{g mL}^{-1}$	On-line conversion of anti-malarial artemisinin using FI-CE. Artemisinin converted to a strongly UV-absorbing compound by treating it with NaOH.	[54]
Medicinal/ Pharmaceuticals	Sulphamethazole Trimethoprim	UV-Visible detection at 254 nm	1 mg L^{-1} 0.5 mg L^{-1}	Miniaturized FI-CE system with ultraviolet photometric detection incorporating a modified falling-drop interface.	[56]
Medicinal/ Pharmaceuticals	Aspirin	UV detection at 214 nm	$1.0 \mu\text{g mL}^{-1}$	Splitting-flow interface used for on-line electrokinetic injection.	[57]
Medicinal/ Pharmaceuticals	Aspirin	UV detection at 254 nm	$3.71 \mu\text{g mL}^{-1}$	Acetylsalicylic acid (ASA) converted to salicylic acid (SA) by on-line alkaline hydrolysis with ASA indirectly quantified by determining the hydrolysis product of SA.	[58]
Medicinal/ Pharmaceuticals	Aspartic acid	UV detection at 214 nm	$0.12 \mu\text{g mL}^{-1}$ (L-Asp) $0.11 \mu\text{g mL}^{-1}$ (D-Asp)	Separation and determination of aspartic acid enantiomers by on-line derivatization with <i>o</i> -phthalaldehyde and mercaptoethanol.	[59]
Medicinal/ Pharmaceuticals	Trimethoprim Sulfamethoxazole	UV detection at 214 nm	$0.31 \mu\text{g mL}^{-1}$ (TMP) $0.70 \mu\text{g mL}^{-1}$ (SMZ)	Microfluidic CE combined with FI. Continuous on-line concentration based on dynamic pH junction.	[60]

Biological/Food Samples

Over the past two decades CE has become a must technique in many biological laboratories and, in some cases, is the technique of choice when analyzing small quantities of materials. Because of the versatility afforded by the CE format, it was a natural choice for coupling to other analytical techniques. Mass spectrometry (MS) and nuclear magnetic resonance (NMR) are but two techniques, that when coupled to CE, have yielded critical data on biological species and their physicochemical properties. This examination of biomaterials has continued with the recent coupling of FI and CE.

Simonet *et al.* [40] coupled FI to a commercial CE instrument with indirect photometric detection to examine myo-inositol phosphates in food samples. The FI system served to clean-up and preconcentrate myo-inositol phosphates while the commercial instrument allowed for increased selectivity and programmability. The lower limit of detection for myo-inositol phosphate ranged from 11-26

μM with a coefficient of variation of 3.9-5.0%. The method determined the content of myo-inositol hexakisphosphate in nuts to be 2-3 times higher than that found in legumes.

Kubán *et al.* [41] showed that small inorganic cations in proteinaceous samples could be directly determined using FI-CE. Using a buffer containing 4-aminopyridine (PAP) and cetyltrimethylammonium bromide (CTAB) at pH 4.5 potassium, sodium calcium, magnesium, and lithium could be detected in milk and blood plasma samples. Electropherograms of 20 consecutive direct injections of milk and blood samples are shown in (Fig. 4).

Wang *et al.* [42] described a low cost FI-CE system with fluorometric detection using light emitting diodes (LED). Continuous introduction of 30 μL samples containing fluorescein isothiocyanate (FITC)-labeled amino acids was conducted with a throughput rate of 144 samples/hour and good precision (3.2% RSD). Baseline resolution was achieved for FITC-arginine, phenylalanine, glycine, and FITC in sodium tetraborate buffer (pH 9.5). The limits of

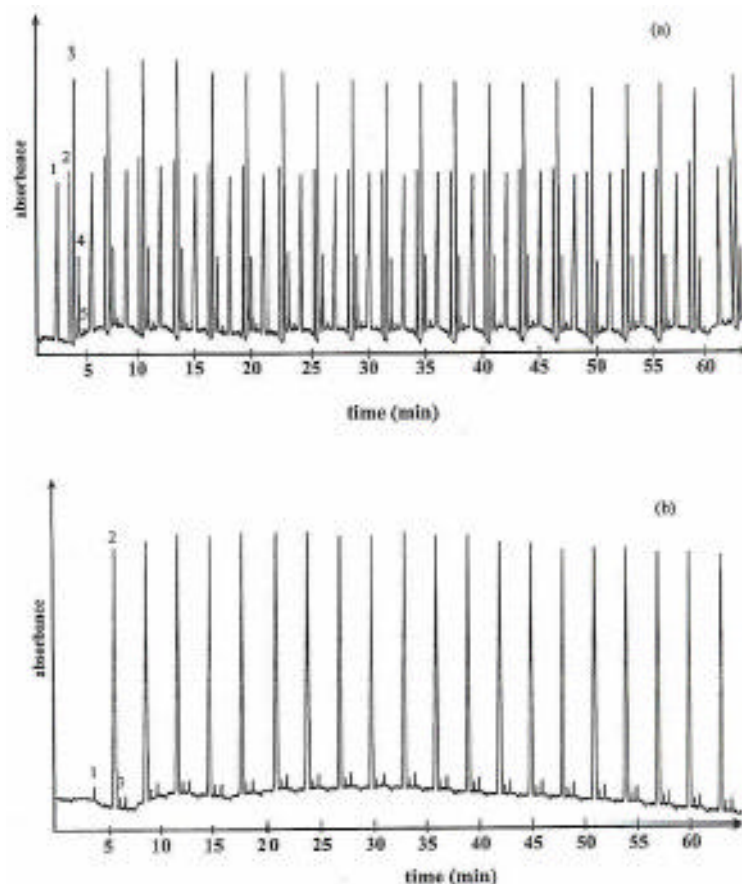


Fig. (4). Electropherograms of 20 consecutive direct injections of milk (a) and blood samples (b) (from reference [41] with permission).

detection of the amino acids was $1.3 \mu\text{M}$ (FITC-arginine) and $1.3 \mu\text{M}$ (FITC-phenylalanine and glycine) corresponding to 1.6 and 2.3 fmol, respectively.

Recently, a rapid and accurate method for separating and determining the bis-alkaloids aloperine (ALP), sophoridine (SRI), matrine (MT), and oxymatrine (OMT) was developed by combining FI and CE [43]. Although the authors called their instrument set-up FI-microfluidic CE, separation of analytes was still conducted in a capillary format and, hence, this system cannot be considered a true “lab-on-a-chip” device. In this work an H-channel structure was produced and a capillary was placed across two vertical side arm tubes placed on either side of the channel. Complete separation of the four bis-alkaloids was readily achieved in 50 mM borate buffer (pH 8.8) at 0.6–1.8 kV.

A novel electrode design in FI-CE was recently described by Samskog *et al.* [44]. Here, the electrode consisted of a conductive on-column graphite/polyimide coating immobilized onto the CE column inlet. The on-line FI-CE system was coupled to electrospray ionization (ESI)-time of flight (TOF)-MS detection. The authors demonstrated separation of three peptides (methionine-enkephalin, neurotensin, and substance P) in an electrolyte consisting of 50% formic acid/ammonia and 50% acetonitrile. This electrode configuration shows a high mechanical and

electrochemical stability and performance comparable to normal platinum electrodes.

Environmental

Timerbaev *et al.* [46] extensively reviewed the growing acceptance of using CE technology for environmental analysis. This trend has continued over the past five years with major improvements in sample introduction and preconcentration by incorporating FI technology. Kubán *et al.* [47], for example, developed a novel FI-CE on-line flow stacking system for use in the detection of eleven US Environmental Protection Agency priority pollutants. This unique system (Fig. 5) continuously delivered low concentrations of phenols dissolved in distilled water to the capillary by means of a peristaltic pump. This innovative delivery system, one in which the sample temporarily replaced the electrolyte solution forming a water pre-plug, allowed optimized stacking conditions and achieved a 2000-fold preconcentration of phenolic pollutants.

Huang *et al.* [48] enhanced the monitoring capabilities of FI-CE by incorporating chemiluminescence (CL) detection on a chip platform for the determination of Co^{2+} and Cu^{2+} . A falling-drop interface was applied for FI split-flow sample introduction and the CE microchip approach further enhanced sample throughput efforts. The performance of the

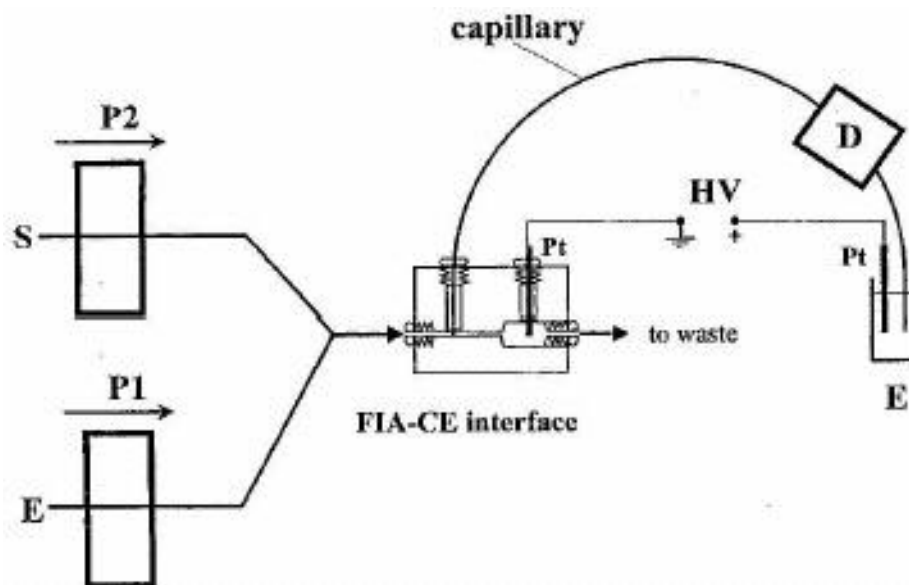


Fig. (5). Schematic of the FI-CE system for sample stacking from a flow. D=Detector, E=Electrolyte, HV=High Voltage Power Supply, P1-P2=Peristaltic Pumps, S=Sample (from reference [47] with permission).

system was studied using the luminal-hydrogen peroxide CL reaction and achieved overall detection limits of 1.25×10^{-8} and $2.3 \times 10^{-6} \text{ mol dm}^{-3}$ for Co^{2+} and Cu^{2+} , respectively.

More recent advances by Kubán *et al.* [49] paved the way for a fully automated field-based FI-CE system for the determination of inorganic ions (e.g., Cl^- , NO_3^- , SO_4^- , K^+ , Ca^{2+} , Na^+ , Mg^{2+}). This system employed dual injection at opposite ends of the separation capillary which allowed concurrent anion and cation determinations at 10 min intervals with detection limits in the range of $20\text{--}200 \mu\text{g L}^{-1}$ for all ions.

Medicinal/Pharmaceuticals

The application of CE to the medical and pharmaceutical industries has burgeoned in recent years with a diversity of applications including the analysis of protein-based pharmaceuticals, drug analysis and design and routine quality control. Coupling with FI has further expanded the medicinal and pharmaceutical applications of CE by providing ultrasensitive, high-throughput analysis capabilities. Chen *et al.* [54], for example, developed an on-line FI conversion method for the determination of the antimalarial agent artemisinin. The coupling of FI allowed rapid analyses (less than 12 min after the conversion of artemisinin to a strongly UV-absorbing compound) with a sampling frequency of 8 h^{-1} . More recent work by Cheng *et al.* [55] led to an improved FI-CE method for artemisinin determination incorporating the use of an orthogonal design in investigating experimental factors.

Cao *et al.* [56] achieved high separation efficiency ($8 \mu\text{m}$ plate height) and sample throughput (48 h^{-1}) analysis for the determination of sulphamethoxazole and trimethoprim in sulphatrim tablets. In this study, a miniaturized CE system incorporating a modified falling-drop interface was

employed for detection limits of 1 mg L^{-1} and 0.5 mg L^{-1} for sulphamethoxazole and trimethoprim, respectively.

A novel FI-CE system was also developed for the kinetic study of aspirin hydrolysis in aqueous solutions [57]. This system employed a split-flow interface for on-line electrokinetic injection and a knotted reactor for on-line quenching reactions. Overall, the FI-CE system achieved a sampling rate of 20 h^{-1} with a detection limit of $1.0 \mu\text{g mL}^{-1}$.

Cheng *et al.* [58] developed a novel FI-CE system for the determination of aspartic acid (Asp) enantiomers by on-line derivatization with *o*-phthalaldehyde and mercaptoethanol. The enantiomers were converted to UV-absorbing diastereoisomer derivatives, which were reproducibly separated by micellar electrokinetic chromatography (MEKC). Detection limits were 0.11 and $0.12 \mu\text{g mL}^{-1}$ for D-Asp and L-Asp, respectively.

Further applications and advances in miniaturization and automation will continue as technology progresses. We, for example, have recently developed a block FI-CE system incorporating a miniature charge-coupled device (CCD) spectrometer employing $200 \mu\text{m}$ fiber optic cables and an in-house written graphical programming environment for full data acquisition and instrument control. (Fig. 6) shows an electropherogram of four injections of 1 mM vancomycin and nicotinamide adenine dinucleotide reduced form (NADH). This graph shows the applicability of our system for model separations. Continued efforts like this will likely lead to commercially available FI-CE systems with a variety of laboratory and field-based applications.

4. FUTURE TRENDS IN FI-CE

The future of FI-CE in routine analysis looks very promising. Flow techniques such as sequential injection analysis (SIA) and Lab-on-valve (LOV) incorporating

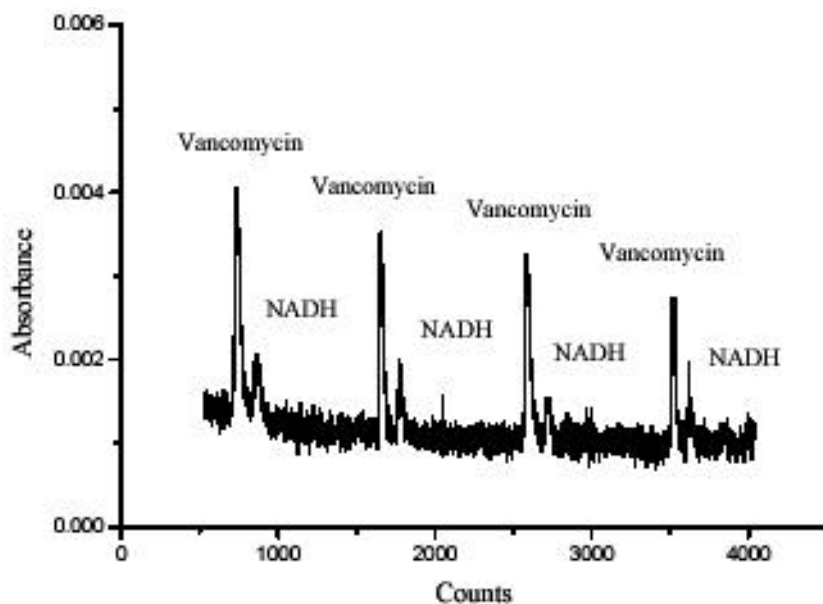


Fig. (6). A representative electropherogram of four direct injections of vancomycin (1 mM) and NADH (1 mM) in 192 mM glycine-25 mM Tris buffer (pH 8.3). The total analysis time in each experiment was 20 min (200 counts equal to one min) at 10 kV using a 36 cm (inlet to detector), 50- μ m I.D. open, uncoated quartz capillary. Flow rate of the combined sample and buffer line was 1.3 mL min⁻¹.

micro-pumping mechanisms and advanced data acquisition and control through graphical programming environments have recently been incorporated with CE [50].

Recent work by Marshall *et al.* [61] has led to further advances in fluid manipulation technology. This approach, termed *Zone Fluidics* (ZF), allows precise manipulation of liquids to accomplish very complex analytical manipulations with relatively simple hardware. ZF principles extend beyond SIA by employing immiscible zones to facilitate zone manipulation in the fluid manifold. This technique will likely have positive impacts on the development of FI-CE instrumentation.

The reliance on minute sample sizes and the feasibility of automation and remote operation of FI-CE-based systems and especially when coupled to microfluidics make it an attractive candidate for drug discovery, environmental monitoring, and biodefense applications. In addition, the need for increased integration and automation of sample preparation and handling, not to mention standardization throughout industry, adds to the attractiveness of FI-CE.

CONCLUSIONS

Advances in, for example, molecular biology and combinatorial chemistry techniques have hastened the need for high-throughput techniques that can effectively screen many thousands of compounds in a very short period of time. In addition, since many of these compounds are available in only limited quantities, lower limits of detection are a must in any new analytical technique. In only the past five years FI-CE has demonstrated the promise of becoming *the* technique of choice to examine large numbers of compounds in a high-throughput fashion. With the drive for miniaturization and low sample volume requirements the

next generation of FI-CE devices will most likely utilize microfluidic and "lab-on-a-chip" platforms. This statement is borne out by some of the very recent articles detailing the coupling of FI-CE to microfluidic-like systems. The fabrication of interfaces between the FI and CE components and the microfluidic platform is currently a focus of researchers including the authors herein and will no doubt be a major area of study for years to come.

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REFERENCES

- [1] Clohs, L.; McErlane, K.M. *J. Pharm. Biomed. Analysis*, **2001**, *24*, 545.
- [2] Guzman, N.A. *Anal. Bioanal. Chem.*, **2004**, *378*, 37.
- [3] Simal-Gándara, J. *Crit. Rev. Anal. Chem.*, **2004**, *34*, 85.
- [4] Landers, J.P. *Handbook of Capillary Electrophoresis*, CRC Press LLC: Boca Raton, FL, **1997**.
- [5] Beale, S.C. *Anal. Chem.*, **1998**, *70*, 279R.
- [6] Novotny, M.V.; Hong, M.; Cassely, A.; Mechref, A. *J. Chromatogr. A*, **2001**, *752*, 207.
- [7] Villareal, V.; Kaddis, J.; Azad, M.; Zurita, C.; Silva, I.; Hernandez, L.; Rudolph, M.; Moran, J.; Gomez, F.A. *Anal. Bioanal. Chem.* **2003**, *376*, 822.
- [8] Flurer, C.L. *Electrophoresis*, **2001**, *22*, 4249.
- [9] Anderson, D. J.; Guo, B.; Xu, Y.; Ng, L.M.; Kricka, L.J.; Shogerboe, K.J.; Hage, D.S.; Schoeff, L.; Wang, J.; Sokoll, L.J.; Chan, D.W.; Ward, K.M.; Davis, K.M. *Anal. Chem.*, **1997**, *69*, 165R.

- [10] Thormann, W.; Molteni, S.; Caslavská, J.; Schmutz, A. *Electrophoresis*, **1994**, *15*, 3.
- [11] Guzman, N.A. *LC-GC*, **1999**, *17*, 16.
- [12] Fang, Z.L.; Chen, H.W.; Fang, Q.; Pu, Q.S. *Anal. Sci.*, **2000**, *16*, 197.
- [13] Kubán, P.; Engström, A.; Olsen, J.C.; Thorsén, G.; Tryzell, R.; Karlberg, B. *Anal. Chim. Acta*, **1997**, *337*, 117.
- [14] Fang, Z.L.; Liu, Z.S.; Shen, Q. *Anal. Chim. Acta*, **1997**, *346*, 135.
- [15] Kubán, P.; Karlberg, P. *Talanta*, **1998**, *45*, 477.
- [16] Kubán, P.; Tennberg, K.; Tryzell, R.; Karlberg, B. *J. Chromatogr.*, **1998**, *808*, 219.
- [17] Swinney, K.; Bornhop, D. *Crit. Rev. Anal. Chem.*, **2000**, *30*, 1.
- [18] Morishita, F. *J. Flow Inj. Analysis*, **1999**, *16*, 163.
- [19] Valcárcel, M.; Ríos, A.; Arce, L. *Crit. Rev. Anal. Chem.*, **1998**, *28*, 63.
- [20] Karlberg, B.; Kubán, P. *J. Flow Inj. Analysis*, **2000**, *17*, 5.
- [21] Miranda, C.E.S.; Carrilho, E.; Gervasio, A.P. *Gine, M.F. Química Nova*, **2002**, *25*, 412.
- [22] Dantan, N.; Frenzel, W.; Kuppers, S. *Chromatographia*, **2001**, *54*, 187.
- [23] Fang, Z.L.; Fang, Q. *Fresenius J. Anal. Chem.*, **2001**, *370*, 978.
- [24] Ruzicka, J.; Hansen, E.H. *Anal. Chim. Acta*, **1975**, *78*, 145.
- [25] Hanrahan, G.; Gledhill, M.; Fletcher, P.J.; Worsfold, P.J. *Anal. Chim. Acta*, **2001**, *440*, 55.
- [26] Dacaronnet, A.; Cheregi, M.; Calatayud, J.M.; Garcia Mateo, J.V.; Aboul, E.H. *Crit. Rev. Anal. Chem.*, **2001**, *31*, 191.
- [27] Wang, J.; Hansen, E.H. *Trends Anal. Chem.*, **2003**, *22*, 836.
- [28] Lyddy-Meaney, A.J.; Ellis, P.; Worsfold, P.J.; Butler, E.C.V.; McKelvie, I.D. *Talanta*, **2002**, *58*, 1043.
- [29] Shpigun, L.K. *J. Anal. Chem.*, **2003**, *58*, 658.
- [30] Oshima, M.; Wei, Y.; Yamamoto, M.; Tanaka, H.; Takayanagi, T.; Motomizu, M. *Anal. Sci.*, **2001**, *17*, 1285.
- [31] Barriada, J.L.; Truscott, J.B.; Achterberg, E.P. *J. Autom. Methods Manage. Chem.* **2003**, *25*, 93.
- [32] Nyman, J.; Ivaska, A. *Anal. Chim. Acta*, **1995**, *308*, 286.
- [33] Willumsen, B.; Christian, G.D.; Ruzicka, J. *Anal. Chem.*, **1997**, *69*, 3482.
- [34] Nan, J.; Jiang, Y.; Yan, X.P. *J. Anal. At. Spectrom.*, **2003**, *18*, 946.
- [35] Lipe, L.L.; Purinton, S.M.; Mederios, E.; Harrell, C.C.; Efta, C.; Murray, M.; Wood, M.; Portier, R.B.; Chalk, S. *J. Anal. Chim. Acta*, **2002**, *455*, 287.
- [36] Jeanty, G.; Wojciechowska, A.; Marty, J.L.; Trojanowicz, M. *Anal. Bioanal. Chem.*, **2002**, *373*, 691.
- [37] Wang, S-L.; Huang, X-J.; Fang, Z-L.; Dasgupta, P.K. *Anal. Chem.*, **2001**, *73*, 4545.
- [38] Teshima, N.; Nobuta, T.; Sakai, T. *Anal. Chim. Acta*, **2001**, *438*, 21.
- [39] Ruzicka, J.; Hansen, E.H. *Anal. Chim. Acta*, **1986**, *179*, 1.
- [40] Simonet, B.M.; Ríos, A.; Grases, F.; Valcárcel, M. *Electrophoresis*, **2003**, *24*, 2092.
- [41] Kubán, P.; Oldhoff, O.; Karlberg, B. *J. Chromatogr. A*, **1999**, *857*, 321.
- [42] Wang, S.L.; Huang, X.L.; Fang, Z.L. *Anal. Chem.*, **2001**, *73*, 4545.
- [43] Cheng, Y.; Chen, H.; Li, Y.; Chen, X.; Hu, X. *Talanta*, **2004**, *63*, 491.
- [44] Samskog, J.; Bergström, S.K.; Jönsson, M.; Klett, O.; Wetterhall, M.; Markides, K.E. *Electrophoresis*, **2003**, *24*, 1723.
- [45] Huang, X.J.; Wang, S.L.; Fang, Z.L. *Anal. Chim. Acta*, **2002**, *456*, 167.
- [46] Timerbaev, A.R.; Dabek-Zlotorzynska, E.; van den Hoop, M.A.G.T. *Analyst*, **1999**, *124*, 811.
- [47] Kubán, P.; Berg, M.; García, C.; Karlberg, B. *J. Chromatogr. A*, **2001**, *912*, 163.
- [48] Huang, X.J.; Pu, Q.S.; Fang, Z.L. *Analyst*, **2001**, *126*, 281.
- [49] Kubán, P.; Reinhardt, M.; Müller, B.; Hauser, P.C. *J. Environ. Monit.*, **2004**, *6*, 169.
- [50] Wu, C.H.; Scampavia, L.; Ruzicka, J. *Analyst*, **2002**, *127*, 898.
- [51] Chung, Y.; Motomizu, S. *Anal. Sci.*, **2001**, *17*, 411.
- [52] Kubán, P.; Kubán, P.; Kubán, V. *Anal. Bioanal. Chem.*, **2004**, *378*, 378.
- [53] Arce, L.; Kubán, P.; Ríos, A.; Valcárcel, M.; Karlberg, B. *Anal. Chim. Acta*, **1999**, *390*, 39.
- [54] Chen, H.L.; Wang, K.T.; Pu, Q.S.; Chen, X.G.; Hu, Z.D. *Electrophoresis*, **2002**, *23*, 2865.
- [55] Cheng, Y.Q.; Chen, H.L.; Fan, L.Y.; Chen, X.G.; Hu, Z.D. *Anal. Chim. Acta*, **2004**, *525*, 239.
- [56] Cao, X.D.; Fang, Q.; Fang, Z.L. *Anal. Chim. Acta*, **2004**, *513*, 473.
- [57] Cai, Z.; Chen, H. *J. Liq. Chromatogr. Relat. Technol.*, **2003**, *26*, 1695.
- [58] Chen, H.L.; Fan, L.Y.; Hu, Z.D.; Zhao, Z.F.; Hooper, M. *J. Sep. Sci.*, **2003**, *26*, 863.
- [59] Cheng, Y.; Fan, L.; Chen, H.; Chen, X.; Hu, Z. *J. Chrom. A*, **2005**, *1072*, 259.
- [60] Fan, L.; Liu, L.; Chen, H.; Chen, X.; Hu, Z. *J. Chrom. A*, **2005**, *1062*, 133.
- [61] Marshall, G.; Wolcott, D.; Olsen, D. *Anal. Chim. Acta*, **2003**, *499*, 29.