Capillary Electrophoresis-Mass Spectrometry for Cancer Metabolomics

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1 Introduction

Metabolomics is an analytical toolbox to profile the whole low-molecular-weight metabolites in a biological system, such as cells, tissues, urine, serum, and plasma. Metabolomic analysis is a promising omics approach to not only investigating the altered metabolic regulation in cancer cells but also identifying biomarkers for early cancer detection and prediction of treatment response in cancer patients. Untargeted metabolomics can be performed to gain a comprehensive metabolite profile of a biological sample. Targeted metabolomics may also be applied to quantitative analysis of preselected metabolites and related metabolic pathway. The goal of a metabolomics study is to obtain an answer to a specific biological or clinical question [1]. While genomic and proteomic analyses may not tell the whole story of what might be happening in a cell, metabolic profiling can give an instantaneous snapshot of the physiology of that cell. To accomplish a comprehensive metabolomic analysis of complex biological samples, there is a high demand for the analytical techniques used in metabolomics due to the large variations in physicochemistry properties and expression levels of metabolites. Currently, advanced analytical techniques, such as NMR spectroscopy, gas chromatography with mass spectrometry (GC-MS), and liquid chromatography with mass spectrometry (LC-MS), have become well-established tools for metabolomics studies [2, 3]. A comprehensive overview of the possibilities of these techniques for metabolomics studies can be found in recent reviews [3, 4]. Despite significant developments in LC column technology and methodology, such as hydrophilic interaction liquid chromatography, the selective and efficient analysis of highly polar and charged metabolites is still challenging.

Capillary electrophoresis (CE) is a powerful separation technique but still underused for complex sample analysis. CE-MS has shown considerable potential for profiling of polar ionogenic compounds in metabolomics. However, relatively speaking, there have been not many papers published on CE-MS-based metabolomic analysis. Hyphenation of CE with MS is generally performed via a sheath-liquid interface. However, the electrophoretic effluent is significantly diluted in this configuration, thereby limiting the utility of this method for highly sensitive metabolomic analysis. Moreover, in this setup the intrinsically low-flow property of CE is not effectively utilized in combination with electrospray ionization (ESI). In this chapter, we will discuss the CE-MS fundamentals, methodologies and interfacing, as well as its applications in cancer metabolomics.

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2 Capillary Electrophoresis with Mass Spectrometry

2.1 Separation Modes of Capillary Electrophoresis

CE is a general term for a range of separation techniques based on different separation principles, including capillary zone electrophoresis (CZE), micellar electrokinetic capillary chromatography (MECC or MEKC), nonaqueous capillary electrophoresis (NACE), capillary gel electrophoresis (CGE), capillary isoelectric focusing (CIEF), capillary electrochromatography (CEC), and capillary isotachophoresis (CITP). Depending on the complexity of the sample and the nature of the present analytes, each of these techniques will provide various advantages for the separation and detection of different substances.

2.1.1 Capillary Zone Electrophoresis

CZE is a main separation mode used with MS because volatile buffers can be employed. It separates the analytes first in CZE based on their charge-to-size ratios and then in the MS on the basis of their mass-to-charge ratio (m/z). Prior to analysis, CE running buffer is flushed through the capillary by pressure. Afterward, the sample is injected and high voltage is applied for the separation (Fig. 1). Since CE separates analytes based on the differences in charge-to-size ratio, relatively small and highly charged analytes have high electrophoretic mobility, whereas relatively large and poorly charged compounds exhibit low electrophoretic mobility. Obviously, neutral compounds will not be separated because their charge-to-size ratio is zero. The CZE-MS method used for global metabolomic profiling in biological samples was demonstrated by Soga et al. [5]. The coupling to MS is important in untargeted metabolomic analysis although it limits the buffer additives to those that can be made volatile [6].

2.1.2 Micellar Electrokinetic Chromatography

The classic CZE method is not suited for the separation of neutral molecules, which migrate toward the detector with the same velocity as the EOF (Fig. 1). MEKC is a commonly used electrophoretic technique developed in the early 1990s that extended the applicability of CE to analysis of neutral analytes. It is based on the differential partitioning of an analyte between the two-phase system: the mobile aqueous phase and micellar pseudostationary phase. In MEKC, surfactants are added to the buffer solution in concentration above their critical micellar concentrations; consequently micelles are formed. These micelles have a nonpolar inside, and a polar (or charged) surface, and undergo electrophoretic migration like any other charged particle under high electric field. Analytes are

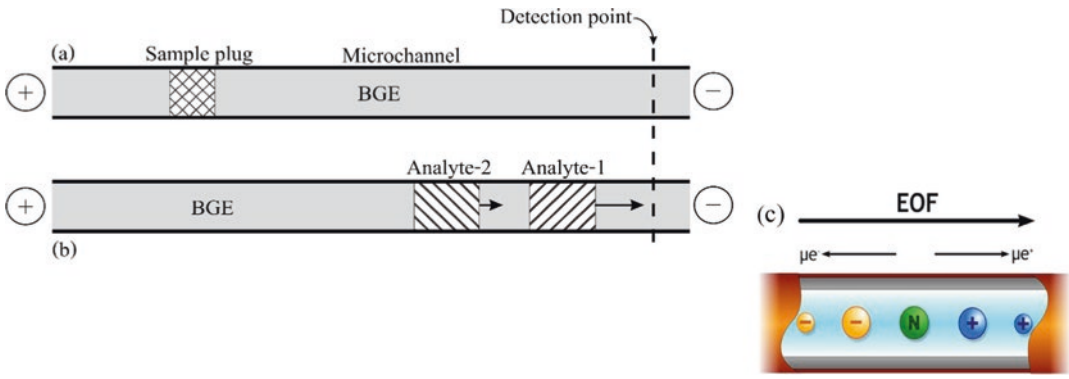

Fig. 1 The principle of capillary zone electrophoresis (CZE). Sample is injected into the separation capillary (**a**) and the analytes are electrophoretically separated by the applied high voltage. The velocity of migration of an analyte in CE also depends on the rate of electroosmotic flow (EOF) of the background electrolytes (BGE) (**c**)

partitioned between the micelles and the buffer solution, dependable on the affinity to the micelles, and separated with the applied high voltage [7].

2.1.3 Nonaqueous Capillary Electrophoresis

With NACE, analytes that are insoluble in water are separated, mainly depending on the use of organic solvents. The viscosity and dielectric constants of organic solvents affect both ion mobility of analytes and the rate of EOF. Recently, NACE was applied as a multiplexed separation platform for analysis of more than 20 nonesterified fatty acids in human serum or plasma samples [8]. Following a simple methyl-tert-butyl ether extraction, seven serum extracts were analyzed directly by multisegment injection-NACE-MS within a single run (<4 min/sample) under negative ion mode detection that incorporates stringent measures for quality control, including batch correction adjustment. Overall, excellent technical variance (RSD = 10%) and mutually agreed results for the measurement of the fatty acids in 50 serum samples were achieved by MSI-NACE-MS and GC/MS within the same laboratory (mean bias = 24%, n = 600).

2.1.4 Capillary Gel Electrophoresis

CGE is carried out with using a gel matrix inside the capillary for size-based separation of biomolecules. Small molecules migrate faster than large molecules under the CGE separation mode. Therefore, CGE is frequently employed for separation of proteins and DNA fragments.

2.1.5 Capillary Isoelectric Focusing

When a pH gradient is formed across the separation capillary, and a high voltage is applied from low pH region (positive) to high pH (negative), analytes would migrate to the pH value that equals their pI value. At lower pH, the analytes are positively charged, whereas at higher pH the analytes are negatively charged. In this separation mode, all the analytes are separated according to their pI. CIEF is commonly used to separate proteins, particularly useful for resolving protein isoforms.

2.1.6 Capillary Electrochromatography

With CEC separation, a capillary is packed with silica-based particles as a stationary phase. When high voltage is applied across the capillary, the buffer starts to migrate due to the present EOF. Similar to HPLC, the analytes are separated based on their interaction with the stationary phase. The difference between HPLC and CEC is that HPLC utilizes a high-pressure pump to mobilize the mobile phase whereas, in CEC, a high voltage is applied to drive the EOF. CEC is capable of separating both neutral and charged molecules. However, CEC has bubble formation issue caused by Joule heating during experiments, which may lead to column dryout and current disruption. Therefore, pressure-assisted CEC (pCEC), with EOF combined with supplemental pressurized flow as its driving force, has been used to overcome this problem. pCEC has been demonstrated for metabolomic profiling of urine samples from lung cancer patients and healthy controls [9].

2.1.7 Capillary Isotachophoresis

A discontinuous buffer system is used in CITP. The sample is introduced between a zone of fast leading electrolyte (LE) and a zone of slow terminating electrolytes (TE). The analytes of interest have intermediate ionic mobility between LE and TE. Under the high voltage applied, a low electrical field is formed in the LE zone whereas a high electrical field is formed in the TE zone. If an analyte is situated in the TE zone, it will be under a higher electric field, giving it a higher speed. Meanwhile, if an analyte is situated in the LE zone, it would be under a lower electric field and migrate slower; the result is that the analytes are focused at the LE/TE interface. For this reason, CITP is often used to concentrate large-volume injections, and low concentration samples are strongly concentrated into very narrow zones.

It was reported that the integration of transient-isotachophoresis (t-ITP) as an in-capillary preconcentration procedure with sheathless CE-MS resulted in subnanomolar limits of detection for metabolites, and more than 1300 metabolic features were detected in urine.

Compared to the classical CE-MS approaches, the integration of t-ITP combined with the use of a sheathless interface provides up to 2 orders of magnitude sensitivity improvement [10]. It was also reported that the use of t-ITP and pH-mediated stacking, coupled with FT-ICR MS, improved the overall detection of cationic metabolites in bacterium [11].

2.2 CE Interface with MS

ESI is a commonly used ionization mode in CE-MS. ESI enables molecules in the liquid phase to be converted directly into ions in the gas phase. It can be easily adapted for online coupling of MS with CE. CE separates mostly charged compounds and ESI is appropriate for ionization of polar and ionic compounds. The interface to MS is a little more complicated in CE than in HPLC owing to a low flow rate of the effluent from the capillary and incompatible electrolytes used in the running buffer. Most commercial LC-MS systems can be adapted for CE-MS with modification in the interface.

2.2.1 Sheath-Flow Interface

A CE-ESI-MS system has to complete the electrical circuit for analyte separation while simultaneously providing an electrical potential to the spray tip. This is generally accomplished using a sheath-flow or sheathless interface [12, 13]. Sheath-flow interfaces have been popular since the early years of CE-MS applications. In this configuration, the separation capillary is inserted coaxially into a stainless-steel tube with a slightly larger diameter, and a sheath liquid is mixed with the BGE at the capillary outlet. The nebulizing gas is supplied via a third coaxial tube, and this assists with stable spray formation and desolvation [14]. Dovichi's group developed a highly robust electrokinetically pumped sheath-flow nanospray interface for coupling CZE with MS, which has been commercialized by the Agent Technologies (Fig. 2). They demonstrated the system for high-throughput proteomic analysis, and 27,000 peptide and nearly 4400 protein identifications were achieved with single-shot CZE-MS [15–18]. A mixture of an aqueous volatile acid (formic acid or acetic acid) and an organic solvent (methanol, propanol, or acetonitrile) is often used as the sheath liquid. The sheath liquid composition and flow rate and the nebulizing gas flow rate, therefore, need to be optimized to create a stable electrospray and maintain separation efficiency and detection sensitivity [19, 20]. Sarver et al. modified the sheath-flow nanospray interface developed by Dovichi's group [21] in order to perform ESI in negative ionization mode [22]. They obtained stable spray conditions by using 10 mM ammonium acetate in 70% methanol as sheath liquid and coating of the emitter with 3-aminopropyltrimethoxysilane to reverse the EOF in the used glass emitter. However, recent calculations and measurements indicate that the EOF is not the driving force in this type of interface and rather the sheath liquid is important to obtain stable spray conditions [23].

Fang et al. designed a nano-flow sheath liquid interface with an extendable tip, featuring a so-called surface flow [24]. The separation capillary with a 20 μm id is chemically etched to reduce the od to 30 μm and is introduced into a tapered sheath flow capillary made of glass with 35 μm id and 40 μm od (Fig. 3). After optimization, the separation capillary protrudes by approximately 200 μm from the outer capillary and serves as the electrospray emitter. The sheath liquid is driven by a syringe pump and flows on the surface of the separation capillary and mixes with the separation effluent inside the Taylor cone with a volume of 4 pL, under a flow rate of 200 nL/min. Extension of the tip showed improved sensitivity when compared to the assembly with retracted tip. The interface was applied to the analysis of a peptide mixture, showing good repeatability for peak intensity and spray stability. While this interface might offer great sensitivity due to its small dimensions with minimum dead volume and low sheath liquid flows, it might be difficult to manufacture.

2.2.2 Sheathless Interface

Sheathless interface benefits from the absence of additional liquid diluting the capillary effluent by

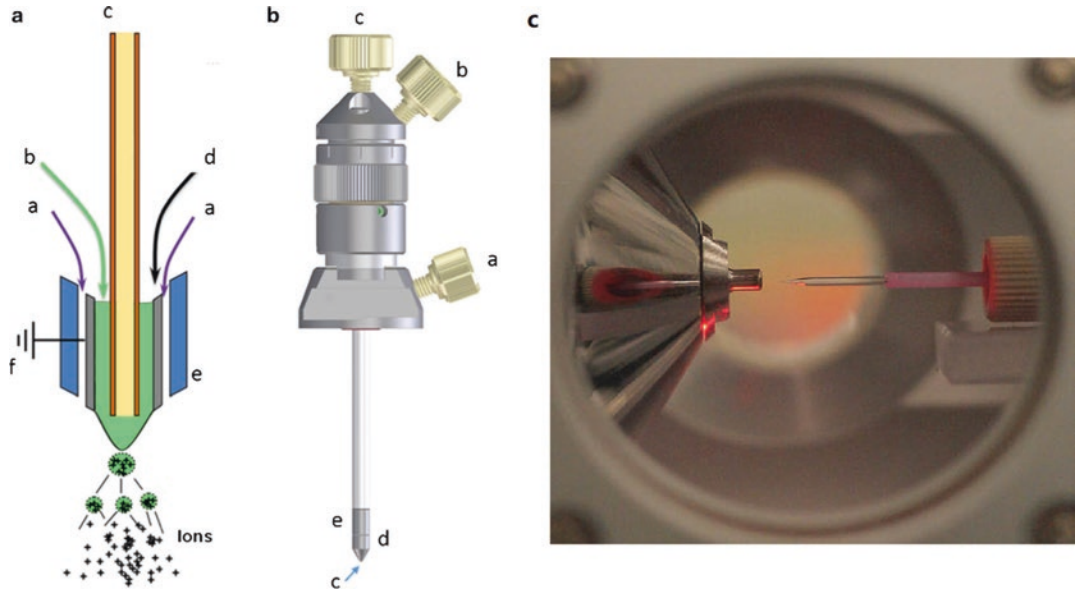

Fig. 2 (**a**) Pictorial representation of Agilent coaxial sheath-liquid CE-MS interface: a, nebulizing gas, b, sheath liquid, c, CE-capillary with BGE, d, stainless steel spray needle 0.4 mm i.d., 0.5 mm o.d., e, outer tube, f, ground connection. (**b**) Engineered sketch of the coaxial sheath-liquid CE-MS interface (graphics courtesy from the Agilent Technologies). (**c**) Electrokinetically pumped sheath-flow nanospray interface developed by Dovichi's group (https://dovichilab.weebly.com/). Reproduced with permission [21]

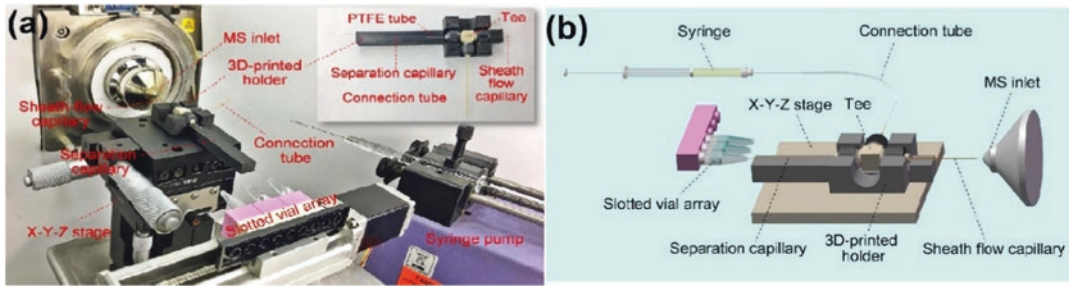

Fig. 3 Image (**a**) and schematic diagram (**b**) of the extendable sheath-flow interface. The inset in (**a**) shows the image of the whole interface. Reproduced with permission [24]

spraying the BGE directly, resulting in high ionization efficiency. However, the lack of supporting liquid can compromise separation and electrospray conditions, since pH value, EOF, capillary coating, organic solvent, or inlet pressure need to be considered to achieve an effective spray. CE-MS with commercial sheathless interfaces have been demonstrated for metabolic profiling of glioblastoma cells and colon and stomach cancer tissues [25, 26].

2.3 Capillary Coating

In CE-MS, capillary coatings are used to prevent analyte adsorption and to provide appropriate conditions for CE-MS interfacing. It can enhance the performance and stability of a CE-MS system, producing accurate and reproducible analytical results. Table 1 shows different types of capillary coatings and their advantages/disadvantages. Depending the charge states of the coating

Table 1 Capillary coatings used in CE

Coating type	Advantages	Disadvantages
Covalent	High reusability (in the order of months)	Complex synthesis procedures
	No further additives in the background buffer are required	Lower reproducibility
Dynamic	Higher repeatability and reproducibility	Require the presence of reagents in the background buffer
	Faster and simpler preparation and optimization	Possible compatibility problems with the detection system
		Possible background buffer heating
Semipermanent	Faster and simpler preparation, optimization, and regeneration	Low reusability (in the order of a few runs)
	No further additives in the background buffer are required	

materials, the EOF direction may be modulated, either enhanced, reduced, or neutralized (Fig. 4).

2.4 Sample Preparation in CE-MS-Based Metabolomic Analysis

Preparation of uniform samples for CE-MS-based metabolomic analysis is a critical issue that remains to be addressed. Maruyama et al. presented an easy protocol for extracting aqueous metabolites from cultured adherent cells for metabolomic analysis using CE-MS [27]. Aqueous metabolites from cultured cells are analyzed by culturing and washing cells, treating cells with methanol, extracting metabolites, and removing proteins and macromolecules with spin columns for CE-MS analysis. Representative results using lung cancer cell lines treated with diamide, an oxidative reagent, illustrate the clearly observable metabolic shift of cells under oxidative stress. This protocol would be especially valuable to students and investigators involved in metabolomics research, who are new to harvesting metabolites from cell lines for analysis by CE-MS.

3 Applications of CE-MS in Cancer Metabolomics

Numerous studies have demonstrated that CE-MS is powerful approach for cancer metabolome analysis toward the identification of targets for clinical and therapeutic applications. For instance, metabolomes of colon or stomach cancer tissues obtained from 16 colon or 12 stomach cancer patients were profiled with CE-MS [28]. Quantification of 94 metabolites in colon cancer tissues and 95 metabolites in stomach cancer tissues involved in glycolysis, the pentose phosphate pathway, the TCA and urea cycles, and amino acid and nucleotide metabolisms resulted in the identification of several cancer-specific metabolic traits. Extremely low glucose and high lactate and glycolytic intermediate concentrations were found in both colon and stomach tumor tissues, which indicated enhanced glycolysis and thus confirmed the Warburg effect. Significant accumulation of all amino acids except glutamine in the tumors implied autophagic degradation of proteins and active glutamine breakdown for energy production, i.e., glutaminolysis. In addition, significant organ-specific differences were found in the levels of TCA cycle intermediates, which reflected the dependency of each tissue on aerobic respiration according to oxygen availability. A similar research was accomplished by Chen et al. [29] by using CE-MS for metabolomic analysis of urine sample from colorectal cancer patients and healthy adults. The results indicated that the urine metabolomes of colorectal cancer patients had significant alterations when compared to those of normal controls, and there were also differences in the metabolomes between early stage and advanced colorectal cancer patients. Compared

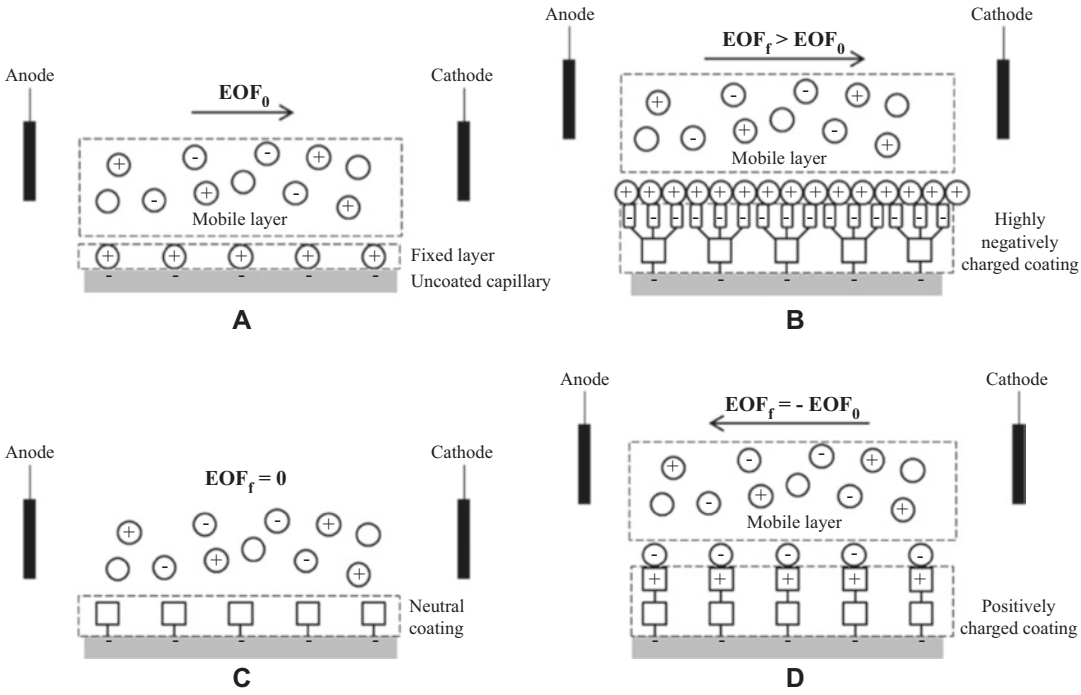

Fig. 4 Schematic representation of the modulation of EOF by capillary coating. (**a**) EOF in an untreated capillary. (**b**) EOF enhancement by formation of a highly negatively charged coating. (**c**) EOF suppression by formation of a neutral coating. (**d**) EOF inversion by formation of a positively charged coating [7]

with the control group, the levels of isoleucine, valine, arginine, lactate acid, and leucine significantly increased, but those of histidine, methionine, serine, aspartic acid, citric acid, succinate, and malic acid significantly decreased in urine samples from colorectal cancer. The levels of isoleucine and valine were lower in the urine samples of patients with advanced colorectal cancer than those in early stage colorectal cancer. Further validation of these urinary markers may lead to a noninvasive method for the early diagnosis of colorectal cancer.

Wu et al. introduced a pCEC method coupled with Q-TOF-MS for metabolomic analysis (Fig. 5) [9]. Three interfaces were compared in this study and a sheathless interface was selected, and the method was applied to lung cancer metabolomic analysis under the optimized conditions. The hyphenated pCEC-Q-TOF-MS system was investigated with mixed standards and pooled urine samples to evaluate its precision, repeatability, linearity, sensitivity, and selectivity. Multivariate data analysis was subsequently performed and used to distinguish lung cancer patients from healthy controls successfully. In a similar approach, Hirayama et al. fabricated a sheathless interface by making a small crack approximately 2 cm from the end of a capillary column and then covering the crack with a dialysis membrane to prevent metabolite loss during separation. CE-MS with the sheathless interface was applied for nontargeted metabolome analysis of human cancer cells and the number of peaks detected was about 2.5 times higher than the standard coaxial sheath liquid interface [30].

The incidence and recurrence rate of bladder cancer is high, especially in developed countries; however, current methods for diagnosis are limited to detecting high-grade tumors using often invasive methods. By using LC-MS and CE-MS, Alberice et al. revealed a total of 27 metabolites that were significantly different among different patient groups, some of which were specific to the stage/grade of cancer or tumor recurrence. The identified

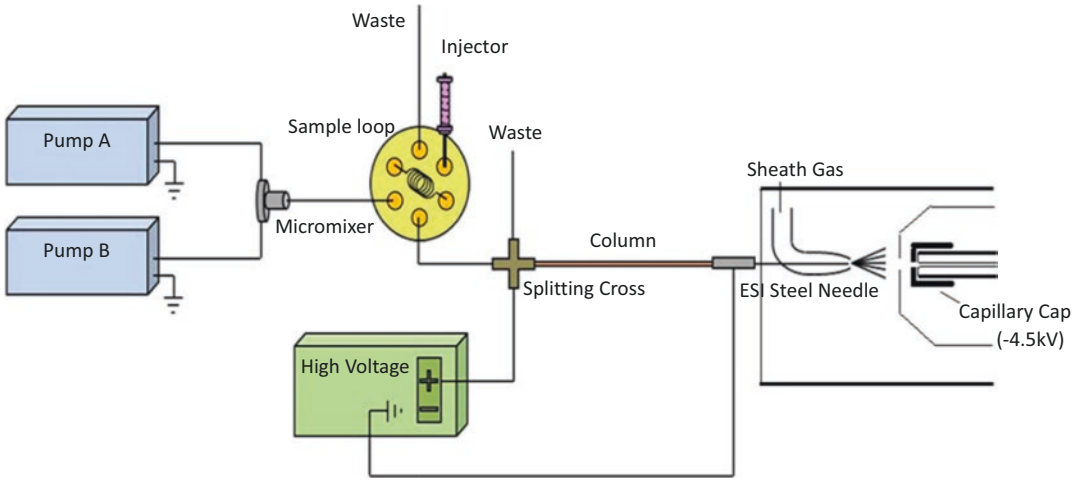

Fig. 5 Schematic overview of pCEC coupled to QTOF-MS. Chromatographic separation was performed on a reversed-phase column of 25 cm (25 cm was packed) × 150 μm id packed with 5 μm C18 particles using a TriSep-2100 pCEC system. (Reproduced from [9] with permission)

potential biomarkers were betaine, cysteine, histidine, tyrosine, carnosine, decanoylcarnitine, and uric acid, where the former four were associated with high risk and the latter three with low risk. Potential biomarkers associated with recurrence were Nε, Nε, Nε-trimethyllysine, N-acetyltryptophan, dopaquinone, leucine, and hypoxanthine, where the former two coincided with high risk and the latter three with low risk [31].

In general, metabolomic analyses lead to the detection of the total amount of all covered metabolites. This is currently a major limitation with respect to metabolites showing high turnover rates, but no changes in their concentration. A stable isotope tracing CE-MS metabolomic approach was developed by Zeng et al., to cover both polar metabolites and isotopologues in a nontargeted way [32]. An in-house developed software enables high-throughput processing of complex multidimensional data. The method was used for analyzing [U-^{13}C]-glucose exposed prostate cancer and non-cancer cells. This CE-MS-based analytical methodology complements polar metabolite profiles through isotopologue labeling patterns, thereby improving not only the metabolomic coverage but also the understanding of metabolism.

Schönemeier et al. reported possible biomarkers in saliva to distinguish between pancreatic cancer and chronic pancreatitis [33]. Salivary samples were collected from patients with pancreatic cancer (PC, n = 39), those with chronic pancreatitis (CP, n = 14), and healthy controls (C, n = 26). Polyamines, such as spermine, N1-acetylspermidine, and N1-acetylspermine, showed a significant difference between patients with PC and healthy controls, and the combination of four metabolites including N1-acetylspermidine showed high accuracy in discriminating PC from the other two groups. These data show the potential of using salivary biomarkers for screening test of PC.

A sheathless CE-MS method has been developed to anionic metabolomic profiling of glioblastoma cells [26]. The BGE, i.e., 10% acetic acid (pH 2.2), previously used for cationic metabolic profiling was assessed for anionic metabolic profiling by using MS detection in negative ion mode. For test compounds, RSDs for migration times and peak areas were below 2 and 11%, respectively, and plate numbers ranged from 60,000 to 40,0000. Critical metabolites with low or no retention

on reversed-phase LC were efficiently separated and analyzed by the sheathless CE-MS method. An injection volume of only circa 20 nL resulted in LODs between 10 and 200 nM (corresponding to an amount of 0.4–4 fmol), which was an at least tenfold improvement as compared to LODs obtained by conventional CE-MS approaches for these analytes. The method can also be used for cationic metabolic profiling studies by only switching the MS detection and separation voltage polarity (Table 2).

Table 2 A partial list of CE-MS applications in cancer metabolomics

Analytes	Sample matrix	BGE	Sample pretreatment	MS analyzer	Refs.
Cationic metabolites	Urines from patients diagnosed of lung cancer	Binary solvents of A (0.1% FA in 2% ACN, v/v) and B (1% FA in ACN-methanol-water 49:49:2, v/v/v) were used in gradient elution	Mixed into chlorophenylalanine, vortexed for 1 min, and then centrifuged	TOF	[9]
Cationic metabolites	Colorectal cancer cells	10% acetic acid (pH 2.2)	Homogenization, centrifugation	TOF	[34]
Cationic metabolites	Colon cancer cells	1 M formic acid (pH 1.8)	Ultrafiltration, methanol precipitation, and two SPE procedures evaluated	TOF	[35]
Cationic metabolites	Colon cancer cells	1 M formic acid (pH 1.8)	Methanol purification; cytosolic fraction centrifugated with 3 kDa filter	TOF	[36]
Cationic and anionic metabolites	Colon and stomach cancer tissues	For cation, 1 mol/L formic acid; for anion, cationic-polymer-coated SMILE(+) capillary (Nacalai Tesque) filled with 50 mmol/L ammonium acetate solution (pH 8.5)		TOF	[28]
Cationic metabolites	Urine from patients diagnosed of urothelial bladder cancer	0.8 ml L − 1 formic acid (pH 1.9) and 10% methanol (v/v)	Diluted with Milli-Q water (1/5 v/v), centrifuged, and transferred to vials for analysis	TOF	[31]
Cationic and anionic metabolites	Prostate cancer and non-cancer cells	For cation, 1 mol/L formic acid; for anion, ammonium acetate solution (50 mmol/L; pH 8.5)	Centrifugally filtered	TOF	[32]
Anionic metabolites	Glioblastoma cell line extracts	10% acetic acid (pH 2.2)	Ultrafiltration	TOF	[26]
Cationic and anionic metabolites	Saliva of pancreatic cancer	For cation, 1 mol/L formic acid; for anion, ammonium acetate solution (50 mmol/L; pH 8.5)	Centrifuged, add 2 mM of methionine sulfone, 2-[N-morpholino]-ethanesulfonic acid (MES), D-Camphol-10-sulfonic acid, sodium salt, 3-aminopyrrolidine, and trimesate	TOF	[33]
Cationic and anionic metabolites	Saliva and tissue of oral cancer	For cation, 1 mol/L formic acid; for anion, ammonium acetate solution (50 mmol/L; pH 8.5)	Saliva: the same as tissue. Homogenized, centrifuged, and filtered	TOF	[37]

4 Conclusion and Perspective

Over the past decade, the applicability of CE-MS for targeted and nontargeted cancer metabolomics studies was well demonstrated. CE-MS represents a high efficiency microscale separation and identification platform for profiling of polar/ionic metabolites that is ideal for volume-restricted biological specimens with minimal sample workup. Compared to other analytical techniques employed for metabolomics studies, the application of CE-MS in this field remains rather limited, which might be due to the issues with concentration sensitivity and reproducibility However, significant progress has been made in the development of robust interfaces for coupling CE with MS over the past decade. In this context, the sheathless porous tip sprayer and the flow-through microvial interface offered new perspectives for highly sensitive metabolic profiling of biological samples [38–42], as both interfaces allowed to perform CE-MS analyses at low flow rate conditions. Moreover, the use of in-capillary preconcentration techniques, such as dynamic pH junction and transient isotachophoresis, could improve the concentration sensitivity of CE-MS for metabolomic analysis as they allowed the injection of large sample volumes. Chromatographic preconcentration techniques, using SPE, can also be used for improving the concentration sensitivity.

In order to increase the applicability of CE-MS for (clinical) metabolomics studies, the utility of CE-MS needs to be demonstrated for the analysis of large cohorts of samples. In this regard, reproducibility of migration times and peak areas is of utmost importance for a reliable comparison of metabolic profiles and to observe small changes in large sample cohorts. A promising approach to achieve reproducible CE-MS methods for metabolomic profiling of body fluids is the use of non-covalently coated capillaries. Small-volume sample consumption is another important aspect of CE-MS. Scaling down LC-MS approaches is still a challenging process, while CE, on the other hand, is a "nanoscale technique" by its nature. Therefore, the analysis of volume-limited samples remains to be an important advantage of CE-MS in the field of metabolomics. In fact, CE-MS has great potential for metabolomic analysis of individual cells [43]. To improve the sensitivity of CE-MS for single cell metabolomic analysis, an efficient ionization emitter, named as a "nanoCESI" emitter, was recently demonstrated, which had a thin-walled (~10 μm) and tapered (5–10 μm) end. The thin conductive wall enabled sheathless ionization and minimized the flow rate of ionizing sample, and the tapered end efficiently ionized analytes via ESI, providing up to 3.5-fold increase in sensitivity compared with a conventional sheathless emitter. CE-MS with such nanoCESI emitter achieved a limit of detection of 170 pM (850 zmol). Meanwhile, a sample enrichment method, large-volume dual preconcentration by isotachophoresis and stacking (LDIS), was combined with nanoCESI to achieve up to 800-fold increase of sensitivity in total when compared to normal sheathless CE-MS. By using this method for metabolome analyses of single HeLa cells, 20 amino acids were successfully quantified and 40 metabolites were identified with quadrupole-time-of-flight MS [44]. Such CE-MS-based system may have great potential to study the heterogeneity of cancer cell metabolism and cancer microenvironment.

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