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Received April 8, 2006
Revised May 29, 2006
Accepted June 28, 2006

Review

Optimization of CZE for analysis of phytochemical bioactive compounds

Advantages of CZE such as high efficiency, low cost, short analysis time, and easy implementation result in its wide applications for analysis of phytochemical bioactive compounds (*e.g*. flavonoids, alkaloids, terpenoids, phenolic acid, saponins, anthraquinones and coumarins). However, several aspects, including sample preparation, separation, and detection have significant effects on CZE analysis. Therefore, optimization of these procedures is necessary for development of the method. In this review, sample preparation such as extraction method and preconcentration, separation factors including buffer type, concentration and pH, additives, voltage and temperature, as well as detection, *e.g*. direct and indirect UV detection, LIF and MS were discussed for optimization of CZE analysis on phytochemical bioactive compounds. The optimized strategies were also reviewed.

Keywords: CZE / Phytochemical bioactive compounds / Sample preparation
DOI 10.1002/elps.200600219

1 Introduction

There is a long history of herbal medicine in far Eastern countries. In particular, Chinese people have utilized herbs and plants to treat various diseases for thousands of years. These drugs are complex mixtures, containing usually hundreds of chemical constituents but only a few compounds are responsible for the beneficial and/or hazardous effects. Therefore, efficient and selective methods, including the extraction techniques are required for qualitative and quantitative analysis of the active compounds or quality control. Chromatography and electromigration methods are main techniques applied in this field due to their powerful efficiency of separation combined with sensitive detection [1–13].

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Abbreviations: CCD, central composite design; **FASS**, field-amplified sample stacking; **IL**, ionic liquid; **ITP**, isotachophoresis sample stacking; **PLE**, pressurized liquid extraction

CE is a powerful separation tool, which has rapidly developed and matured since its introduction [14, 15]. It has been widely applied for analysis of pharmaceuticals, including relatively small synthetic drugs [16–19], DNA analogs (antisense DNA drugs) [20–22], naturally produced drugs (traditional medicines) [1, 23–30], and biopharmaceuticals (peptides, proteins, *etc.*) [31–33]. Among various modes of CE such as CZE, MEKC, CGE, CIEF, capillary isotachophoresis (CITP), CEC and non-aqueous CE, CZE is the most frequently used method because it is the simplest and most versatile CE mode (Fig. 1). In addition, CZE, using an uncoated capillary column, is based on differences in the charge-to-mass ratio and analytes migrate into discrete zones at different velocities, which require less maintenance and makes the optimization of conditions easy. Several features of CZE such as high resolution, speed of method development, robustness, simplicity, costs, *etc.*, enable the successful separation of these complex samples.

This review summarizes the optimization of CZE, including sample preparation, separation, and detection for analysis of phytochemical bioactive ingredients in traditional medicines.

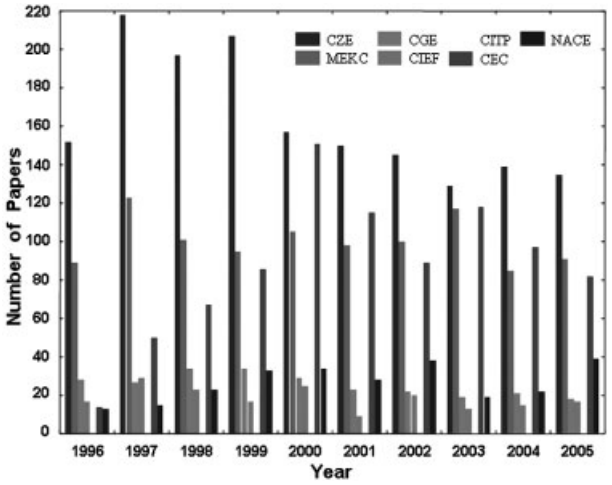

Figure 1. Growth in the number of different CE modes journal articles appearing annually during the last decade based on the data from *ISI Web of Science*.

2 Sample preparation

Sample preparation is the first and usually the most important process, which greatly influences the repeatability and accuracy of the analysis. It is reported that 70–80% of analysis time is spent on sample preparation and more than 60% of analysis error derives from non-standard sample pretreatment. Therefore, a proper sample preparation approach is very important for CZE analysis.

The principal objectives of sample preparation for analysis are isolation of the analytes of interest from as many interfering compounds as possible, dissolution of the analytes in a suitable solvent, and preconcentration if necessary. The ideal approach of sample preparation is to exclude the step altogether or "dilute and shoot" [34]. This approach is sometimes possible when sample levels of targeted analytes are relatively high and the matrix components do not co-elute with the analytes.

2.1 Extraction

Although many of the instrumental analytical techniques have matured and automation is a common place, sample preparation is still considered to be slow, labor-intensive, and even a bottleneck in laboratory processes.

For CZE analysis of phytochemical components, different sample extraction methods, including reflux [35–38], Soxhlet extraction [39–45], and ultrasonic extraction [46–58] with different solvents, were used. In addition, some conventional methods such as shaking [59–61], stirring [62–64], marinating [65, 66] and leaching [67]) were also used (Supplementary Table 1). Usually, ultrasonication using running buffer as solvent is easy for the extraction of CZE analysis [49].

Sample buffer [68] or deionized water [51, 56] were also used as solvents for avoiding band broadening induced by extremely high salt concentrations. However, aqueous extracts rich with proteins can contaminate the capillary and affect selectivity, precision and accuracy of analysis. In addition, organic solvents usually have high extraction efficiency for most phytochemicals. Therefore, organic solvents including methanol and ethanol were mainly used for Soxhelt extraction, reflux extraction or ultrasonication of analytes in raw materials. Then organic solvent was removed, and the residue was dissolved with sample buffer or organic solvent to prepare the sample solution for CZE analysis [36, 37, 45, 48, 52, 55, 57, 61, 65]. Actually, methanol extract of anthraquinones was directly diluted with buffer for CZE analysis [69]. Chloroform [36, 65], isopropyl alcohol [61] and 70% ACN [50] were also used for extraction of alkaloids or flavonoids. However, conventional methods suffer from a variety of disadvantages, including long extraction time, relatively large amount of solvent and tedious operating procedures.

Pressurized liquid extraction (PLE) is a new technique, which was developed for sample pretreatment of CZE in recent years [69–72]. It was performed at elevated temperatures and high pressure, which maintain the heated solvent in a liquid state during the extraction process. Therefore, PLE offers many advantages such as short extraction time, little solvent consumption and high extraction efficiency. Generally, the major parameters, which influence the extraction efficiency, are type of solvent, particle size, temperature and static extraction time (Fig. 2). It has been reported that the extraction efficiency of strychnine in *Strychnos nux-vomica* increased about 4-fold when the temperature increased from 80 to 140°C. The solvent required in PLE was approximately six times less and extraction time required was approximately 20-fold faster compared to Soxhlet extraction [72]. The amount of solvent and time required for PLE extraction of glycyrrhizin in *Radix glycyrrhizae* was about half of those required by ultrasonic extraction [70]. Especially, the reproducibility of PLE is generally much better than that of conventional extraction methods [73, 74]. Therefore, PLE is a good alternative method for sample preparation of CZE analysis.

2.2 Preconcentration

Small requirement of sample amounts (nL-level) is one of the most attractive advantages of CZE compared to HPLC. However, just as every coin have two sides, the

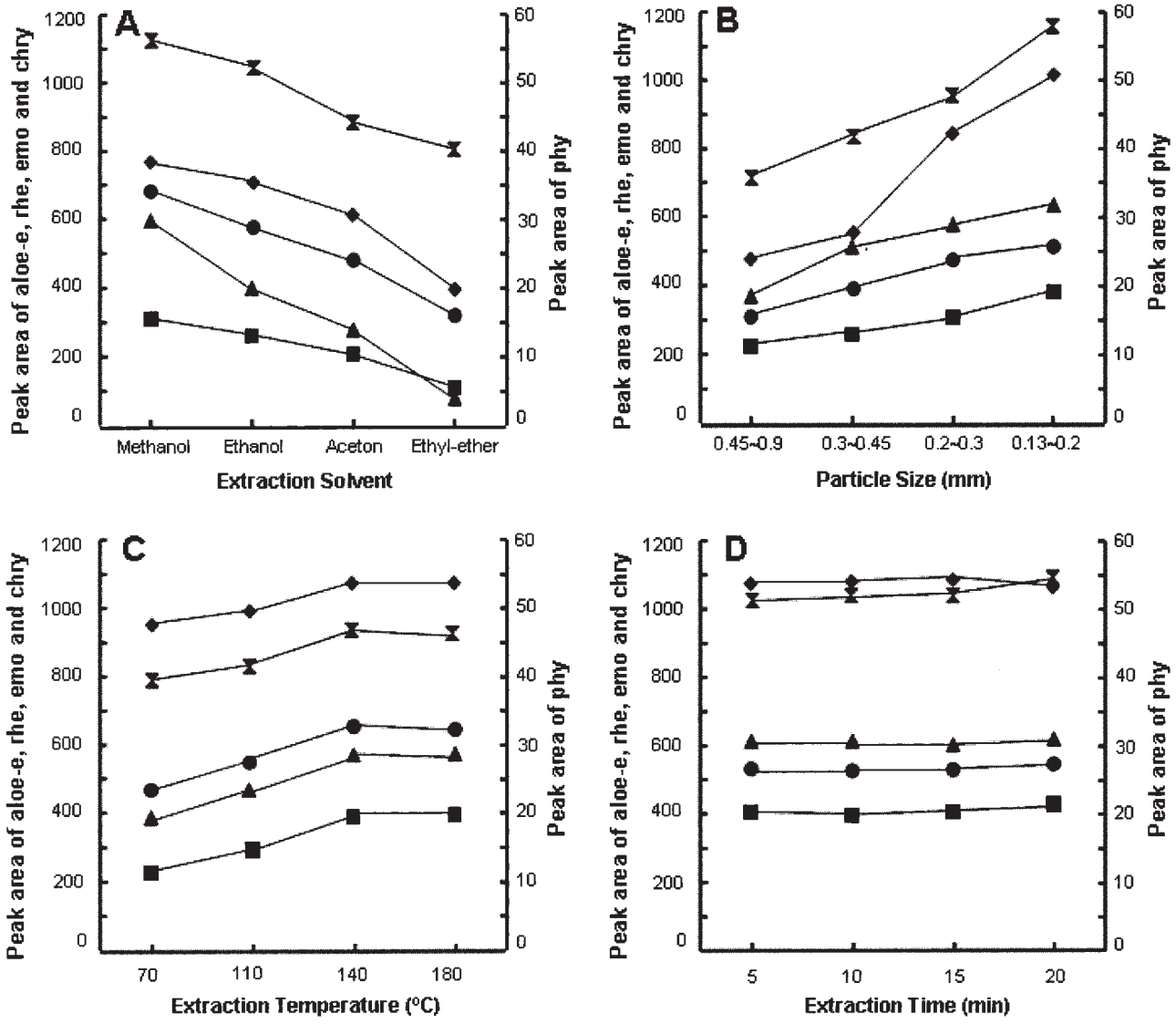

Figure 2. Influence of selected factors including solvent type (A), particle size (B), temperature (C) and static extraction time (D) on the PLE extraction of physcion (phy, ◆), chrysophanol (chry, ⧗), aloe-emodin (aloe-e, ■), emodin (emo, ●), rhein (rhe, ▲) in Rhubarb. Conditions: to determine one of the parameters including temperature and static extraction time, the others were set at the system default value (temperature, 100°C; pressure, 1500 psi; static extraction time, 5 min). Solvent, methanol; particle size, 0.13–0.2 mm.

limited sample volume injectable under standard conditions and a short optical path length of capillary result in high concentration detection limit for CZE. Thus, for trace analysis applications, the amount of analyte injected into the capillary or the detector sensitivity has to be increased. Actually, to increase the amount of analyte injected into the capillary is a good choice, which may be accomplished either by analyte enrichment during a sample preparation or by extended volume injections followed by analyte focusing during the CZE analysis. The former concept, including SPE, solid phase microextraction (SPME), microdialysis and liquid-liquid extraction (LLE) such as electroextraction, supported liquid membrane extraction and liquid-phase microextraction (LPME) has been reviewed [75, 76].

The latter involves sample stacking that may take place when the sample plug is sandwiched between leading and terminating electrolytes (isotachophoresis sample stacking, ITP) [77] or when the sample is of lower conductivity than the running buffer (field-amplified sample stacking, FASS) [78]. As a preconcentration and pre-

separation technique, ITP combined with CZE can dramatically increase the injectable sample volume into the capillary with up to 10^3 orders of magnitude higher than that of CZE [79]. Urbanek *et al*. [80, 81] utilized on-line column coupling configuration (the ITP column with relatively large id and the CZE column connected by a column switching system), which improved the selectivity and efficiency of separation by selective electromigration, to separate and assay several cinnamic acid derivatives and flavonoids in extracts of *Sambucus nigra*, *Crataegus* sp. and *Hypericum perforatum* leaves or flowers. Liu *et al*. [65] applied FASS to the on-column concentration of alkaloids in *Sophora flavescens*. It has been found that water plug at the column inlet is essential for improving the reproducibility and sensitivity in FASS with electroinjection. By applying FASS with the optimal water plug injection time (3 s), the concentration sensitivity was about three to four orders of magnitude higher than in hydrodynamic injection. Sample stacking has been discussed in few reviews [82, 83].

3 Separation

3.1 Optimization of parameters

In CZE, the important question is which electrolyte system (buffer) is needed. The main purposes of a buffer are to provide the transport of electric current and the separation of the analytes, which play an important role in electrophoretic processes. The basic principles of the choice of buffer in CZE have been discussed [84]. Herein, we will discuss the most important aspects of optimization of the parameters.

3.1.1 Buffer pH

The buffer pH is one of the most important parameters for separation of CZE that keep the migration velocity of weak electrolyte components and the velocity of the EOF constant. Generally, for weak anionic and cationic ions the effective mobilities strongly depend on their p*K* values related to the pH of the buffer. However, the substances with zero effective mobilities may still move in the capillary due to the EOF, and this EOF is strongly dependent on the pH of the buffer used. It has been found that the best separation of the nucleosides (adenosine, guanosine and uridine) in *Cordyceps* was achieved at pH 9.5, although the running time was also increased twofold when compared to pH 8.5 (Fig. 3) [68].

The same phenomenon was also found on the separation of rutin and hydrochlorothiazide using CZE [85]. On the contrary, the migration times of the compounds

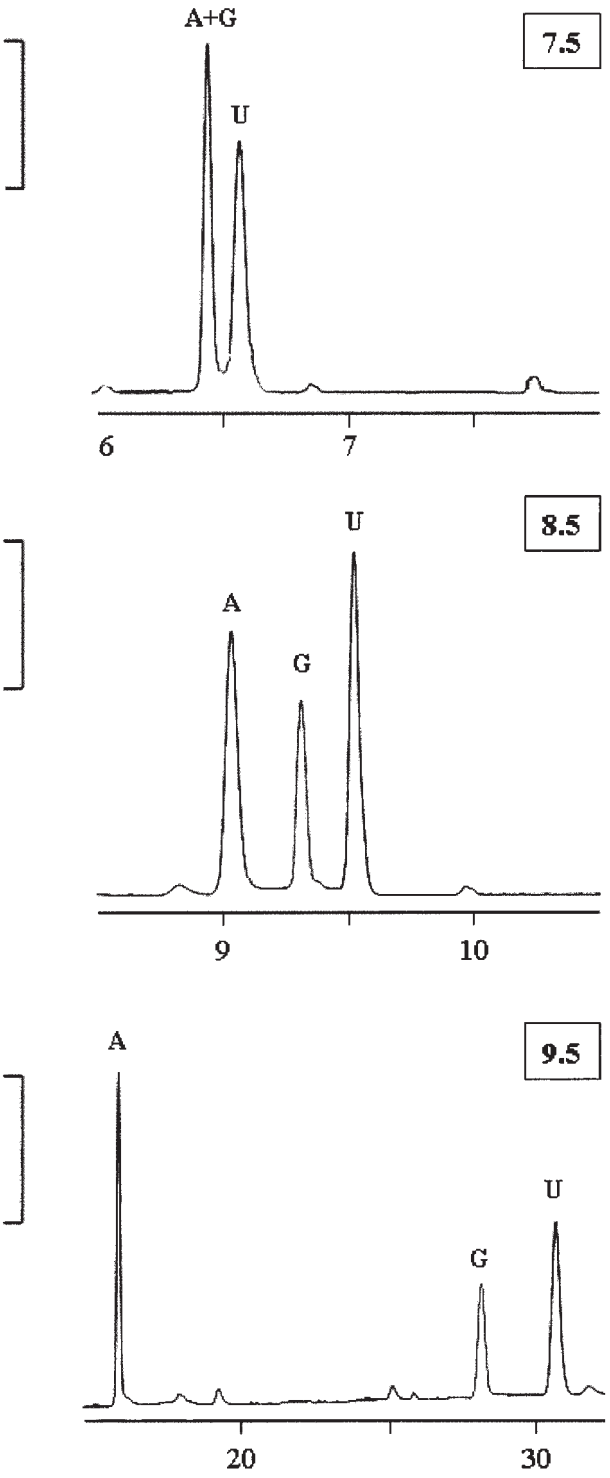

Figure 3. Effect of pH on resolution of adenosine (A), guanosine (G) and uridine (U). Conditions: pressure injection 586 kPa for 6 s, 57 cm × 75 μm column (Beckman untreated fused silica), running buffer 0.2 M boric acid-sodium hydroxide, UV detection at 254 nm. pH at 7.5, 8.5 and 9.5 are shown. The *x*-axis is the migration time in minutes. Bar shows the absorbance of 0.005. Reprinted from [68], with permission.

(saikosaponins a, c and d) decreased with the resolution increased as the buffer pH increased [86]. It is interesting that the variant trend of migration time during CZE of atropine and scopolamine was observed with pH increased from 6 to 9 [87]. Actually, the migration time is dependent on both EOF, which increases with pH augment, and ionization of analytes. It has been found that pH had significant effect on the effective mobility of p-tyrosol and salidroside. The good separation was achieved only at pH 8.93–10.13 [48]. Therefore, optimization of pH is necessary for CZE analysis of phytochemical bioactive compounds to get good resolution with short analysis time.

3.1.2 Buffer type and concentration

A wide variety of buffers (borate, acetate, phosphate, citrate and Tris) can be employed in CZE (Supplementary Table 2). Borate buffers interact strongly with molecules containing polyhydroxyl groups, which imparts a charge on otherwise neutral carbohydrates, so that they will migrate in the electric field. Many different factors must be considered in choosing a good buffer, of which the most important are the ionic strength (conductivity) and the UV absorbance at low wavelengths (190–220 nm). The typical buffer concentration is 30–100 mM as shown in Supplementary Table 2. An increased buffer molarity is, in general, beneficial, as it leeds to enhanced competitive ion pairing of buffer cations with the sample molecules at the capillary wall, thus diminishing sample adsorption. However, these benefits may be outweighed, if the ionic strength exceeds a certain value: increased sample dispersion occurs due to excessive Joule heating [46, 67]. Generally, at equal molarities, oligoprotic buffers like citrate will give substantially higher currents than monoprotic species such as acetate. Therefore, type and concentration of the buffer for CZE should be optimized to obtain a good resolution of the interested compounds in a satisfactory time [40, 42, 43, 48, 58, 63, 87–98].

3.1.3 Additives

Besides the buffer characteristics (type, concentration and pH), EOF is also controlled by solvent or additives, which can be employed to change the selectivity of the separation. Buffer additives can alter, among other things, electrophoretic mobilities. In other words, two compounds that have identical mobilities in a simple buffer system may be differentiated with an additive. Various buffer additives can be employed for CZE analysis (Supplementary Table 2), which will be discussed below. Some additives, such as surfactants or CDs, form a heterogeneous environment above the critical concentration that defines MEKC. This topic is not discussed here, as its complexity is beyond the scope of this review and can be found elsewhere [99, 100].

3.1.3.1 Organic additives

The solvent have an important role in modulating EOF. Methanol, ACN, 1-propanol and 2-propanol can be used to improve the selectivity and resolution of CZE by improving both the separation and the peak shape [101, 102], increasing the solubility [48, 64], changing the migration time [50, 91, 103, 104] and/or decreasing the adsorption and diffusion [88] of analytes. Another way of manipulating EOF and reducing analyte interaction with the silica wall is to add amines to the buffer. Triethylamine can dramatically improve the resolution of five alkaloids including matrine, sophocarpine, sophoridine, oxymatrine, and oxysophocarpine in *Sophora* species because the protonated triethylamine can mask the negative charges of $Si\text{-}O^-$ on the inner wall of the fused-silica capillary to reduce the interaction between the inner wall and the analytes [46].

3.1.3.2 Surfactants

Surfactants are also used for controlling EOF of CZE. CTAB (0.5 mM) was added to decrease EOF during analysis of short-chain organic acids in coffee [105]. Tetradecyltrimethylammonium bromide (TTAB) was also used as additive for improving the selectivity and resolution of low-molecular-mass organic acids in plant, *Var splendens* [106].

3.1.3.3 CD

CE is the most dynamically developing branch of chiral selective chromatography [107]. Recently, more than half of all reports dedicated to chiral selective analyses involved CE, where more than two-thirds of chiral separations were made using CD chiral selectors [108]. CD and their derivatives such as α-CD [44, 109], β-CD [52, 53, 110, 111], hydroxypropyl (HP)-β-CD, carboxymethyl (CM)-β-CD [112], sulfated-γ-CD [113], heptakis 2,3,6-tri-*O*-methyl-β-CD (TM)-β-CD [114], heptakis 2,6-di-*O*-methyl (DM)-β-CD [42, 115], sulfated-β-CD [116] and mono-3-phenylcarbamoyl-β-CD [86] are also most frequently used as modifiers for improving selectivity of CZE, or chiral agents for enantiomeric separations of phytochemical bioactive compounds [112, 115, 116].

3.1.3.4 Ionic liquid

Ionic liquid (IL) is currently used to describe a broad class of low-melting semi-organic salts or salt mixtures that have appreciable liquid range. Interests in IL for their potential uses in different chemical processes are increasing [117, 118], because they are environmentally benign and good solvents for both organic and inorganic materials. The applications of IL in CE were focused on their employment as additive, BGE, or coating material in aqueous/nonaqueous CE [119–123]. Yue *et al.* [39] used 1-butyl-3-methyl-imidazolium tetrafluoroborate (1B-3MI-TFB), one of IL, as the additive for CZE separation of three bioactive flavonoids, quercetin, kaempferol and isorhamnetin in the Chinese herbal extract from *Hippophae rhamnoides* and its medicinal preparation (Sindacon Tablet). Baseline separation, high efficiencies and symmetrical peaks of the three flavonoids were obtained. The separation mechanism seems to be the hydrogen-bonding interaction between the H-2 of imidazolium cations of IL and oxygen of hydroxyl in flavonoids because the resolution of three flavonoids was destroyed when the proton at carbon-2 of the imidazolium cation was substituted by a methyl group (Fig. 4C).

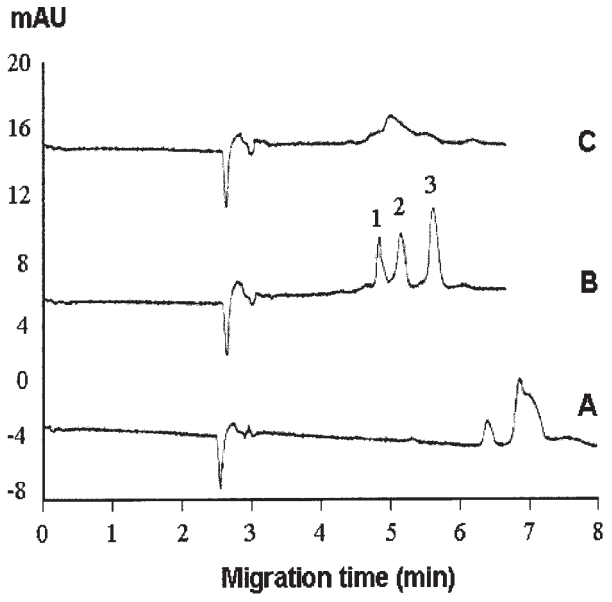

Figure 4. Electropherograms of mixture of flavonoids of isorhamnetin (1), kaempferol (2) and quercetin (3) with and without additives. Electrophoretic conditions: 20 mM borate buffer (A) without IL (ionic liquid) and with 4 mg/mL (B) 1-butyl-3-methyl-imidazolium tetrafluoroborate (1B-3MI-TFB); (3) 1-butyl-2,3-dimethyl-imidazolium tetrafluoroborate (2-methyl-1B-3MI-TFB) at pH 10.00. Voltage, 15 kV; temperature, 25°C; detection, 270 nm. Reprinted from [39], with permission.

3.1.3.5 Complex-forming selector

Borate buffer of pH 9–11 is widely used for analysis of hydroxy compounds (as relatively weak acids) and in many cases simultaneously the complex-formation ability of B(III) is utilized for manipulating or enhancing the selectivity of CE separation [124–128]. However, polyhydric phenols are readily oxidized by oxygen in alkaline aqueous media, which should be considered during CE assay of such compounds. Possible risk of spontaneous analyte oxidation with oxygen during the sample processing could be reduced by performing the CE separation using a suitable buffer of pH close to 7 containing a complex-forming selector. Molybdate, a novel complex-forming additive, was investigated on the selectivity of separation of polyhydric compounds [129]. As shown in Fig. 5, caffeic acid can be separated with ferulic acid in the presence of molybdate (0.15 mM) due to the formation of Mo(VI)-caffeic acid complex (caffeic acid possesses two aromatic hydroxy groups).

3.1.3.6 Other additives

It has been found that PVP had great influence on separation of flavonolignans [130]. The diastereomers of silybin (SB_A and SB_B) were not separated if no PVP 10 was added to buffer. The higher PVP 10 concentration, the better the resolution of SB_A from SB_B was observed. CZE analysis time is two times shorter than that of HPLC though the PVP 10 addition increases analysis time. Hexadimetrine bromide (HDM), a polimeric alkylammonium salt that efficiently inverts the EOF at very low concentrations, was also used as additive for determining the content of L-ascorbic acid in plants with short analysis time and high reproducibility [131].

3.1.4 Voltage and temperature

Both the electroosmotic and electrophoretic velocities are directly proportional to the field strength, therefore the use of high voltage shortens the time for separation, leading to higher efficiency because diffusion is the most important feature contributing to band broadening. However, if the voltage is too high, more Joule heat is produced, thus reducing the efficiency of separation [35, 42, 48, 54, 55, 85, 88, 94, 111]. With the increase of the separation voltage, the peak shape becomes sharper. However, the peaks of the analytes may overlap when the separation voltage is too high [53, 62, 63, 95, 132]. Experimentally, the optimal voltage is determined by performing runs at increasing voltages until deterioration in resolution is noted.

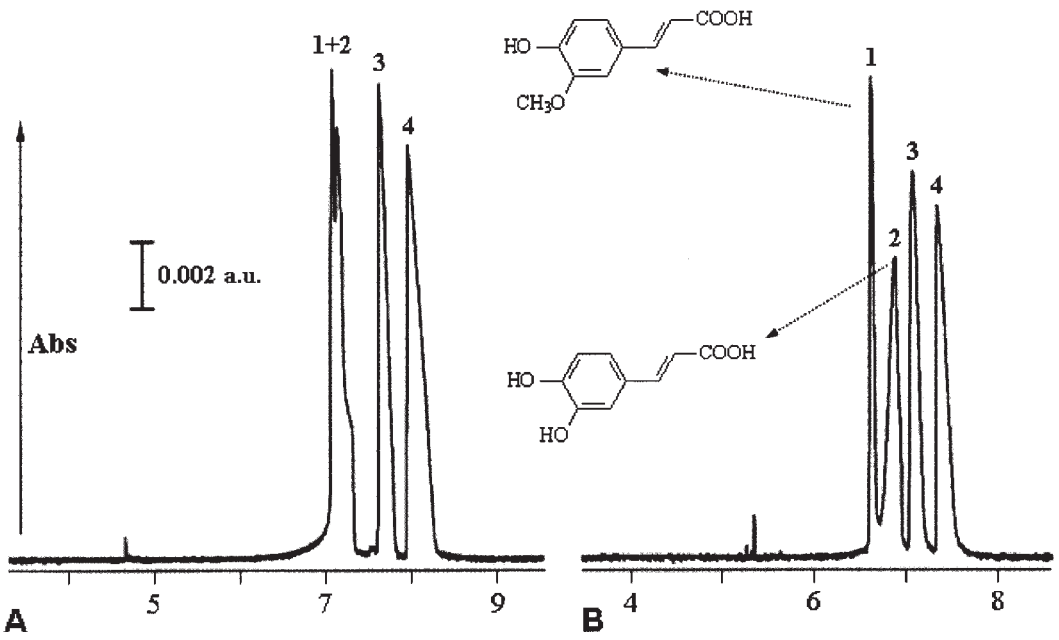

Figure 5. Electropherograms of ferulic acid (1), caffeic acid (2), 3-hydroxycinnamic acid (3) and cinnamic acid (4) with and without additives. Electrophoretic conditions: 25 mM 2-morpholinoethanesulfonic acid (A) without Mo(VI) and (B) with 0.15 mM Mo(VI) at pH 5.4 (adjusted with Tris). Voltage, 25 kV; temperature, 25°C; hydrodynamic sampling at pressure of 50 mbar for 6 s; detection, 280 nm. Reprinted from [129], with permission from Elsevier.

The electrophoretic mobility and the EOF are related to the viscosity, which is a function of temperature and buffer. As the temperature increases, the viscosity decreases and thus the electrophoretic mobility increases as well. Therefore, the migration time of analytes decreases with increasing temperature [38, 97, 133, 134]. On the other hand, the efficiency of separation is reduced with the increase of temperature [53], partly because of higher Joule heating [94, 106]. The pH of some buffers, such as Tris, is dependent on the temperature. For complex separations such as peptide maps, even small pH shifts can alter the selectivity. Therefore, precise temperature control is important.

3.1.5 Injection mode

Sample can be injected into the capillary mainly by two different modes: pressure injection or electrokinetic injection. Pressure, or hydrodynamic, injection is performed by applying an external force [38, 42, 45, 48, 58, 71, 72, 96, 97, 111, 135, 136] or alternatively by raising the sample vial above the outlet causing siphoning [41, 47, 101, 137]. Especially the former method is often used, as it is not influenced by the conductivity of the sample buffer or the mobility of the analytes to introduce precisely reproducible volumes of sample into the capillary. Electrokinetic injection is simply applied by switching the power source on for a few seconds to cause the sample to flow into the capillary, the advantage of which is that it can be used to concentrate sample in the capillary prior to analysis [62, 63, 67, 85, 106, 132]. The problem with this type of injection is sample discrimination, as the components of the samples with the highest mobility enter the capillary first [138].

Injection is also important during CZE separation because the plug of sample should be kept to a relatively small length (otherwise dispersion will become significant reducing the efficiency of the separation) [139]. It is noted that the voltage influences and the analyte-wall interactions are obvious only in the case of small injection lengths. Therefore, injection time is also an important parameter for optimization of CZE conditions. A long injection time of sample will induce obviously peak broadening of the analytes [62, 63, 67, 132].

3.1.6 Miscellaneous

Some other factors, such as electrode, potential, capillary length and column ID, also have influence on separation of CZE and were optimized in some studies [61–63, 90, 132].

3.2 Optimization methods

For effective optimization of the parameters mentioned above, a systematic approach is required. Many strategies involving univariate design, orthogonal design, uniform design and central composite design (CCD) are used to optimize the parameters for achieving the best resolution and analysis time. Univariate design is the most common and simplest approach applied for optimization of CZE [35, 36, 38, 40–46, 48, 50, 52–55, 58–65, 67, 69–72, 80, 81, 85–97, 101–104, 106, 109–112, 115, 129, 132, 133, 136, 137, 140–148]. However, if the number of factors to be investigated is big or mutual interactions exist among the factors, systematic optimization procedure is needed. Orthogonal design and uniform design, two systematic approaches widely utilized for optimization of other separation methods [149–155], have also been used in CZE analysis. The most remarkable advantage of these two methods is that the number of experiments can be considerably reduced comparing to univariate design. However, the shortcomings of them are also obvious, among of which the most fatal is that the construction of these two techniques is based on a linear model, whereas in most of cases the correlation between the factors and the responses is nonlinear. Furthermore, orthogonal design and uniform design are not applicable to continuous variables. Consequently, the most powerful method is CCD, which has been developed to optimize the parameters of CZE analysis [49, 69, 116, 156]. As shown in Fig. 6, an obvious improvement of CZE separation of nucleosides and their bases from cultured *Cordyceps* was obtained after CCD optimization, especially the peaks of adenine and uracil achieved baseline separation [144].

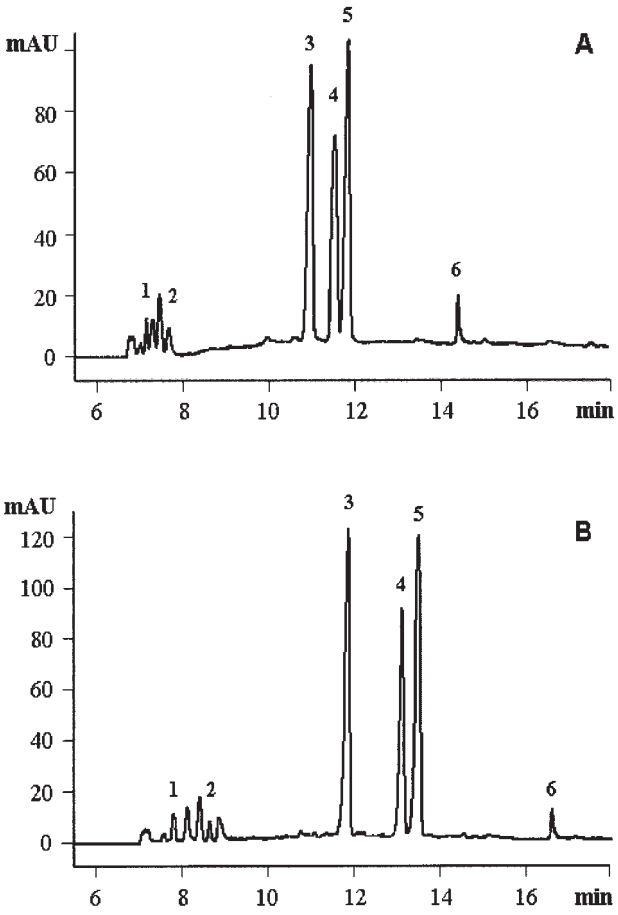

Figure 6. Electropherograms of cultured *Cordyceps* before and after CCD optimization. Electrophoretic conditions: pressure injection 50 mbar for 10 s, 56 cm × 75 μm ID capillary (48 cm effective length, Agilent fused-silica), voltage 20 kV at temperature 20°C, detected at 254 nm. (A) Running buffer 0.5 M boric acid–sodium hydroxide (pH 8.6) without ACN as organic modifier. (B) Running buffer 0.5 M boric acid–sodium hydroxide (pH 8.6) with 12.2°C ACN as organic modifier. (1) Adenine, (2) uracil, (3) adenosine, (4) guanosine, (5) uridine, and (6) inosine. Reprinted from [144], with permission from Elsevier.

4 Detection

To date, the majority of commercially available CE instruments employ UV–Vis absorbance detectors because of their simplicity and versatility. Therefore, direct UV detection is widely used for determination of phytochemical bioactive components [35–43, 45, 47–61, 64–66, 69–72, 80, 81, 86, 89–97, 101–104, 109–112, 115, 116, 129–131, 133–137, 140, 141, 146–148, 156, 157]. It is also used for detection of UV-transparent components after derivatization [158, 159]. However, in the context of routine work, the drawbacks of derivatization techniques are widely recognized (dependence of various experimental parameters, incompleteness of derivatization reactions, analyte degradation, prolonged analysis time, additional cost for derivatization system and reagents). Therefore, in some cases, indirect UV detection is employed for CZE [106, 160]. In the indirect mode, the detector monitors a suitable ionic component of the buffer and substitution of this component by ionic analytes, and the buffer with UV-absorbing properties is used. Unfortunately, the small detection volume of CE often results in a poor concentration detection sensitivity. Therefore, the development of techniques that can improve detection has been a principal area of CE research. Up to date, electrochemical (EC) [62, 63, 67, 85, 132, 161–168], LIF [22, 29, 31, 169], chemiluminescence (CL) [170–175], electrochemiluminescence (ECL) [176, 177] and MS [11, 89, 145, 178–186] detections have been successfully applied for CZE analysis.

Increased detector sensitivity may be accomplished by utilizing LIF detection, which typically provides an enhancement of the S/N by a factor of 3–6 using extended light path or extremely high mass sensitivity for LIF detection [83]. The LOD of LIF for aristolochic acid I (AA-I) and aristolochic acid II (AA-II) in some Chinese herbal samples were 8.2 nM and 5.4 nM, respectively [29]. Unfortunately, currently, direct LIF detection is only applicable for some analytes, as the number of wavelengths available with the commercial LIF detectors is limited.

MS detector, which reveals unambiguous information on an analyte's molecular weight and offers structural information, is helpful to identify the intricate mixtures in herbs or plants. However, there are still some technical problems needing to be resolved in the on-line combination of CE and MS, in which the most critical is probably the interface technique. Among the different types of CE-MS interfaces involving sheath liquid, sheathless, liquid-junction and direct electrode, sheath liquid flow system is the most commonly used mode [179]. As the CE flow is not high enough to maintain stable ionization within the MS source, an extra flow of liquid must be added to the CE eluant to obtain gas phase ions of the solutes. It has been found that sheath liquid composition and sheath liquid flow rate had significant influence on MS response [181, 183, 184]. Buffer is a key for successful coupling CZE and MS. Volatile BGE of 40–100 mM ammonium acetate or ammonium formate in aqueous or organic solution have been proven well suitable for CE-MS [145, 180, 184–186].

5 Conclusions

Several aspects including sample preparation, separation, and detection have significant effects on CZE analysis. Therefore, optimization of these procedures is necessary for the method development. The strategies involving univariate design, orthogonal design, uniform design and CCD can be used as the optimization methods, among them CCD being the most powerful approach.

We are grateful to Macao Science and Technology Development Fund (077/2005/A) and Research Committee, University of Macau (RG086/04–05S) for supporting the research to S. P. Li.

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