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Analysis of inorganic cations as their complexes by capillary electrophoresis

Bi-Feng Liu, Liang-Bin Liu, Jie-Ke Cheng*

Department of Chemistry, Wuhan University, Wuhan 430072, China

Abstract

The development in the analysis of inorganic cations as their complexes by capillary electrophoresis is reviewed. Topics covered are separation strategies, approaches of derivatization, choice of complexing ligands, manipulation of sensitivity and selectivity, and applications to metal speciation analysis with emphasis on separation strategies and choice of complexing reagents. The future development was also predicted.   © 1999 Published by Elsevier Science B.V. All rights reserved.

Keywords: Capillary electrophoresis; Derivatization, electrophoresis; Inorganic cations; Metal complexes

Contents

*Corresponding author.

1. Introduction

Since introduction of using narrow-bore tube (internal diameter less than 100 nm is usual) in electrophoresis by Jorgenson and Lukacs [1] in 1981, capillary electrophoresis (CE) has experienced a rapid progress whether in theory or in practice [2–5]. It brought about a profound revolution in modern separation science, and led to a change of the direction of the advance of separation techniques. In early age of development. CE was mainly employed in the field of analysing biological macromolecules such as proteins, peptides and nucleic acids et al. Currently, the application range of CE is widely exploited. CE increasingly infiltrated into the realm of low-mass-molecule [6,7], and has been recognized as a powerful method for the separation and simultaneous determinations of small molecules and ions, in which, the application of CE to assaying inorganic cations is challengeable, and has been drawn a great attention. Several books [8–10] and reviews [11–15] dealing with principles and practice relevant to this theme have been published. Macka and Haddad [15] presented an excellent review on determination of metal ions by CE. As illustrated in that article, interest in this area has steadily increased.

Although the separation of inorganic cations by CE can be traced even as far back as 1967 [16] when Hjerten pioneered the first separation of bismuth and copper with capillary zone electrophoresis mode using a capillary of a relatively large diameter, it was not so inspiring in its initial development that two main difficulties were encountered. One is the selectivity. It seems very difficult to discriminate these cations that have similar or identical ionic equivalent conductances which directly determine the electrophoretic mobilities of ions, only based on their migration behaviour as their free forms. The other is the sensitivity, because most of metal ions are transparent in UV-Vis region. It was Foret et al. [17] who introduced an indirect UV detection technique that broke the dilemma. The enhancement of selectivity was achieved by incorporating with on-column complexing equilibrium. However, this mode was quite limited because of its low sensitivity, high matrix effect and strict dependency on the co-ion of background electrolyte.

Recently, more and more researches related to CE separation of metal ions as their complexes have been performed due to the advantages of the combination of high performance separation technique and high sensitive derivatization agents. Many researchers begin to focus their interest on exploring and selecting suitable derivatization agents for various inorganic cations, and have made great progress.

This survey is an overview of analysing inorganic cations as their complexes by CE published in journals up to the middle of 1998. The scope of review includes: (i) separation strategies for metal complexes; (ii) derivatization mode; (iii) choice of derivatization agents; (iv) manipulating sensitivity and selectivity; (v) application to speciation analysis,

and (vi) prediction of future development. The emphasis will be placed on the separation strategies and Choice of derivatization agents. Additionally, the CE separations of metal ions based on the on-column complexing equilibrium with weakly complexing agents are not included unless they are taken into account as the examples for comparison, because only little fraction of complexes are formed in the tube. It should be also noted that the work related to the separation in which a metal ion or metal complex is added into the background electrolyte in order to facilitate the separation of other species are not under consideration.

2. Separation strategies for metal complexes

CE separations can be accomplished with various modes, including the popularly employed capillary zone electrophoresis (CZE) [1], micellar electrokinetic capillary chromatography (MECC) [18], capillary isotachophoresis (cITP) [19], capillary gel electrophoresis (CGE) [20], ion-exchange electrokinetic chromatography (IEEC) [21] and capillary isoelectric focusing (cIEF) [22], as well as some newly developed separation strategies that involve affinity capillary electrophoresis (ACE) [23], capillary electrochromatography (CEC) [24], separation on microchip (MC) [25] and using organic solvents as the separation medium – nonaqueous capillary electrophoresis (NACE) [26]. In the applications to analyzing metal complexes, CZE now is the predominant separation mode in CE. And nearly 80% researches on the separations of metal complexes have been achieved under CZE conditions. MECC is also widely employed, but relatively less than CZE. Other strategies are seldom utilized in this field up to date. Consequently, the discussion as follows will be focused on CZE separation mode and MECC mode followed by cITP, cIEF, IEEC, MC and NACE in brief.

2.1. CZE

CZE-based separation is the most widely used operation mode in CE. Its mechanism is based on the differences in the electrophoretic mobility of analytes. The analytes with different electrophoretic

mobility will migrate in separated zones. And the analytes with the same electrophoretic mobility will co-migrate as the same zone in the capillary under the applied electric field. That is the origin for the name *zone*. In 1991, Swaile and Sepaniak [27] proposed the first demonstration of metal complex separation with CZE mode. Three test metal ions like Ca(II), Mg(II) and Zn(II) as their 8-hydroxyquinoline-5-sulfonic acid (HQS) chelates formed by in-situ complexation scheme were separated successfully using laser-induced fluorescence (LIF) detection. Swaile and Sepaniak's work largely stimulated the practice of CZE in metal complexes assaying. From then, the number of applications of CZE to this field greatly increased.

Under CZE conditions, the migration behaviour of the metal complexes in the tube is actually determined by the sum of their intrinsic electrophoretic mobility (μ_{ep}) and electroosmotic mobility (μ_{eo}), due to the action of electroosmotic flow (EOF):

$$\mu_{obs} = \mu_{ep} + \mu_{eo} \tag{1}$$

where μ_{obs} represents the observed electrophoretic mobility of the complexes. Thus, the controlling of EOF plays an important role in CZE separations. On the basis of the discrimination that the direction of μ_{ep} is opposite or identical to that of μ_{eo}, CZE can be divided into two subdirectories: (i) Counter-electroosmotic CZE; (ii) co-electroosmotic CZE.

2.1.1. Counter-electroosmotic CZE

The direction of EOF is consistence with the migration direction of cations provided that no capillary modification procedure has been performed. In most cases, metal complexes are anionic species according to the published papers. So, the direction of μ_{ep} of the complexes is opposite to EOF in general. Neutral complexes can be also viewed as anionic species with zero negative charge. For cationic complexes, things go to the contrary compared to the anionic complexes. Consequently, the following discussion will based on the anionic complexes.

In this investigation, most of CZE separations of inorganic cations as their complexes are operated under counter-electroosmotic CZE conditions. Timerbaev et al. [28] presented an excellent separation of 23 metal-cyclohexane-1,2-diaminetet-
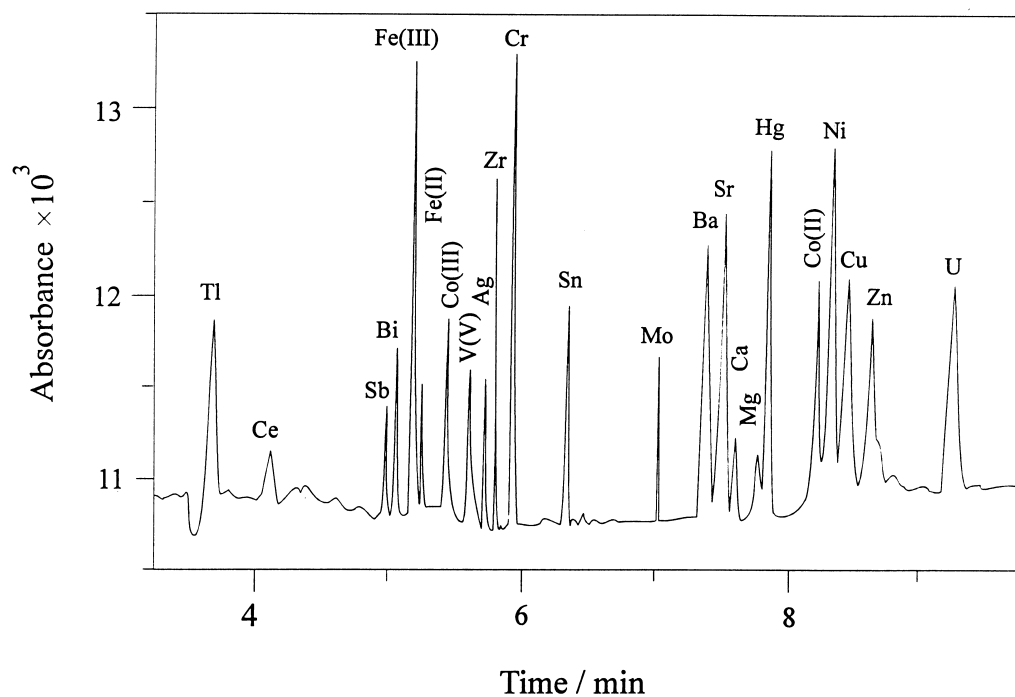
Fig. 1. Separation of metal-CDTA complexes under optimized conditions. Electrolyte: 20 mM sodium borate, 1 mM CDTA and 5% ethylene glycol, voltage: 12.5 kV. Reproduced from Ref. [28] with permission.

raacetic acid (CDTA) complexes within 10 min at a high pH value 9.0 of buffer using μ_{eo} as the driving engine of separation as shown in Fig. 1, in which, some metal ions were scarcely subject to conventional CE procedures, including aluminum(III), antimony(III), bismuth(II), chromium(III), mercury(II), palladium(II), silver(I), tallium(I), tin(IV), uranium(VI), vanadium(IV, V) and zirconium(IV). Liu et al. [29] reported a separation of rare-earth elements from their co-existed metal ions thorium and uranium after complexation with 2-(2-Arsenophenylazo)-1,8-dihydroxyl-7-(4-chloro-2,6-dibromophenylazo)-naphthalene-3,6-disulfonic acid (DBC-As) in acidic buffer of pH3.0. The μ_{ep} of the metal complexes was utilized as the driving force of the separation. Compared with co-electroosmotic CZE, counter-electroosmotic CZE can obtain more ideal resolution between two neighbour peaks as described in the following equation [30]:

$$\Delta t = t_2 - t_1$$
$$= [L_d \cdot L/V]$$
$$\cdot [(\mu_{ep}^1 - \mu_{ep}^2)/[(\mu_{eo} + \mu_{ep}^1)(\mu_{eo} + \mu_{ep}^2)] \qquad (2)$$

where Δt, t, L, L', V are on behalf of the difference of migration time between two neighbour peaks, migration time of metal complexes, the total length of the capillary, the effective length of the capillary, and applied voltage, respectively. The symbol 1 and 2 represent two neighbour peaks. From Eq. (2), it can be obviously concluded that the opposite sign of the μ_{ep} and the μ_{eo} under counter-electroosmotic CZE conditions will be benefit to increasing the value of Δt, and accordingly, improving the separation resolution in case that the influence of efficiency on separation is excluded.

2.1.2. Co-electroosmotic CZE

To accomplish co-electroosmotic CZE system, EOF should be effectively reversed. Many approaches have been utilized, such as derivatization of the capillary wall [31], application of a radial potential to the capillary [32] and addition of long-chain cationic surfactants [33]. Addition of cationic surfactant like cetyltrimethylammonium bromide (CTAB) is a very convenient method to reverse the direction of EOF, and adopted by researchers as the

chief choice. Zhang et al. [34] described the determination of palladium(II) as a chloro complex $PdCl_4^{2-}$ in the presence of Ru(III), Rh(III), Os(III) and Ir(III) chloro complexes by EOF-reversed CZE mode. 0.2 mM CTAB was added to the carrier electrolyte to create a layer of positive charges at the inner capillary wall. The reversal of EOF extremely shortened the analysis time, and the determination was completed in less than 4 min.

While the cationic surfactant additives as the dynamic modifier of the capillary wall are employed for inducing the reversal of EOF, the using of coated capillary can be considered as an alternatively effective methodology for the achievement of a co-electroosmotic CZE system with zero EOF. Jones and Hardy [35] studied a separation and determination of trace inorganic and organomercury species as their dithizone sulphonate(DzS) complexes in a polyacrylamide coated capillary column. In that study, neutral coated capillary was found to be essential to prevent the separation from ruining by a significant EOF.

Thornton and Fritz [36] attempted another approach to accomplish co-electroosmotic CZE conditions without additional modifier. The separation of $AuCl_4^-$ and the chloro complexes of palladium group elements was performed in acidic solution. The pH value of the buffer was controlled from 2.0 to 2.4. With this acidic buffer, the dissociation of silanol group at the surface of fused-silica capillary are completely restrained. So that, EOF was also completely suppressed.

2.2. MECC

Neutral compounds will co-migrate under the classic CZE conditions. Terabe et al. [18] successfully developed a MECC separation mode to resolve this problem. In MECC mode, surfactant with concentration over its critical micellar concentration (CMC) is necessary to be added in the carrier electrolyte to form micelle as the pseudostationary phase. So, there will be two phases in the running buffer: aqueous phase and micellar phase. The principle of MECC mode can be described briefly as the chromatographic partition mechanism between the two phase. Further detailed interpretations can be found in references [37–39] recommended. Although MECC mode is mainly designed for the separation of

neutral compounds, it proves to be very suitable to facilitating the separation of ionic species. Saitoh et al. [40] first probed the applicability of MECC to the separation of metal-complex. 4-(2-pyridylazo)resorcinolato(PAR) was utilized by the authors as the pre-column chelating reagent. As illustrated in Fig. 2, some transition metal ions such as Co(II), Cr(III), Fe(III) and Ni(II) as their PAR chelates were separated in 30 min by using SDS as the micellar phase in the buffer. More recently, a good review [41] given by Haddad et al. on the topic of the separation of metal ions and metal-containing species by MECC. In that review, the separation of inorganic cations as their complexes by MECC were drawn a great attention. Herein we will give a survey from an
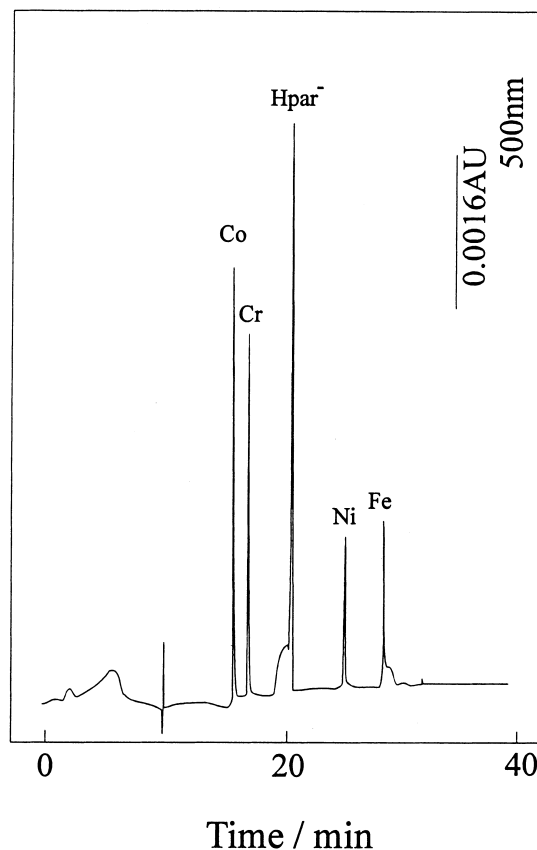
Fig. 2. MECC separation of metal-par chelates. Eluent: [SDS]= 0.02 mol dm^{-3}, [NaB$_4$O$_7$]=0.0125 M, [PAR]=1.0·10^{-4} M; [metal ion]/(10^{-5} M), Co(III) 0.995, Cr(III) 1.23, Fe(II) 1.00, Ni(II) 1.01; [trithanolamine=0.025 mol dm^{-3}(pH=8.8 with HCl), heated at 98°C for 30 min. Reproduced from Ref. [40] with permission.

alternative view angle of using different micelles, because obviously, the employment of micelles is the key for MECC separation mode.

2.2.1. Using anionic micelles

Among numerous anionic micelles, SDS is the most widely used micelle in MECC mode. In the applications of MECC to metal complexes all the employed anionic micelles are SDS, according to the reported articles.

Timerbaev et al. [42] investigated the migration behaviour of anionic metal complexes in the presence of the anionic micelle SDS in the carrier electrolyte. After compared the separation of metal PAR complexes with the separation of metal ArsenazoIII complexes, it was concluded that SDS-based MECC system was versatile to the separation of negatively charged metal complexes that possessed a hydrophobic nature like PAR complexes. For those highly ionized complexes like ArsenazoIII complexes, the separations were governed by the electrophoresis without chromatographic partition interaction with SDS, due to their highly hydrophilic characteristics. Additionally, the authors presented an important fact that the separation resolution was improved by using ammonium as the cationic component of the carrier electrolyte instead of sodium. It was explained by the formation of ion-pairing which could result in the increasing of hydrophobic characteristics of the complexes and thereby improving partitioning processes.

SDS-based MECC system seems more effective for the separation of neutral complexes in virtue of their good hydrophobic interaction with SDS micelle phase. The separation of metal $\alpha,\beta,\gamma,\delta$-tetrakis(4-carboxypenyl)porphine (TCPP) [43], Acetyl-acetone (acac) [44] and bis(2-hydroxy-ethyl)dithiocarbamate (HEDTC) [45] complexes were achieved using SDS micellar solution. It [45] was reported that the introduction of organic modifier like methol could conveniently used to adjust the partitioning behaviour of the complexes between aqueous phase and micellar pseudostationary phase. In addition, it [44] was found that capacity factors of metal complexes depended little on the free form of SDS but much on the amount of its micellar form in the solution.

For the cationic metal complexes, no separation by SDS-based MECC mode was reported.

2.2.2. Using cationic micelles

Long-chain quaternary ammonium salt is usually used as cationic micelle in MECC mode. Jia et al. [46] studied the determination of palladium in the presence of other platinum group elements as their chloro complexes by MECC. CTAB was utilized to form micellar phase in the bulk solution. The authors compared the results obtained from using three type of micelles: CTAB as the cationic micelle, Dodecylbezone sodium sulfonate (DBC) as the anionic micelle and *p*-octylpolyethylene glycol phenylether(OP) as the non-ionic micelle. The experimental result showed that CTAB was the most suitable surfactant in that investigation, while DBS made peak fronting, and OP did not affect the migration of $PtCl_4^{2-}$ complex. The mechanism of separation can be interpreted by the ion-pairing interaction between cationic micelle and anionic metal complexes.

Timerbaev et al. [47] reported a separation of cationic metal 2,6-diacetylpyridine-bis(N-methyl-enepyridinohydrazone (H$_2$dapmp) complexes by tetradecyl-trimethylammonium bromide (TTAB)-based MECC system as shown in Fig. 3. In the same paper, the authors also proposed the separation of metal-H$_2$dapmp complexes with counter-EOF CZE mode to compare the two separation systems. Because the analytes are cationic species, TTAB was added to the buffer to induce the reversal of EOF for accomplishing the Counter-EOF situation.

2.3. Capillary isotachophoresis

cITP separation mode based on the differences in the μ_{ep} of ionic species is similar to CZE mode. Sample is sandwiched between two different electrolytes named leading electrolyte and terminating electrolyte instead of only one carrier electrolyte employed in CZE. In cITP separation, the zones of analytes migrate in contact with each other at the same velocity as the leading electrolyte to keep the constant of electric current in the capillary. Therefore, the concentrations of sample zones will be adjusted in agreement with the concentration of the leading electrolyte during isotachophoresising by
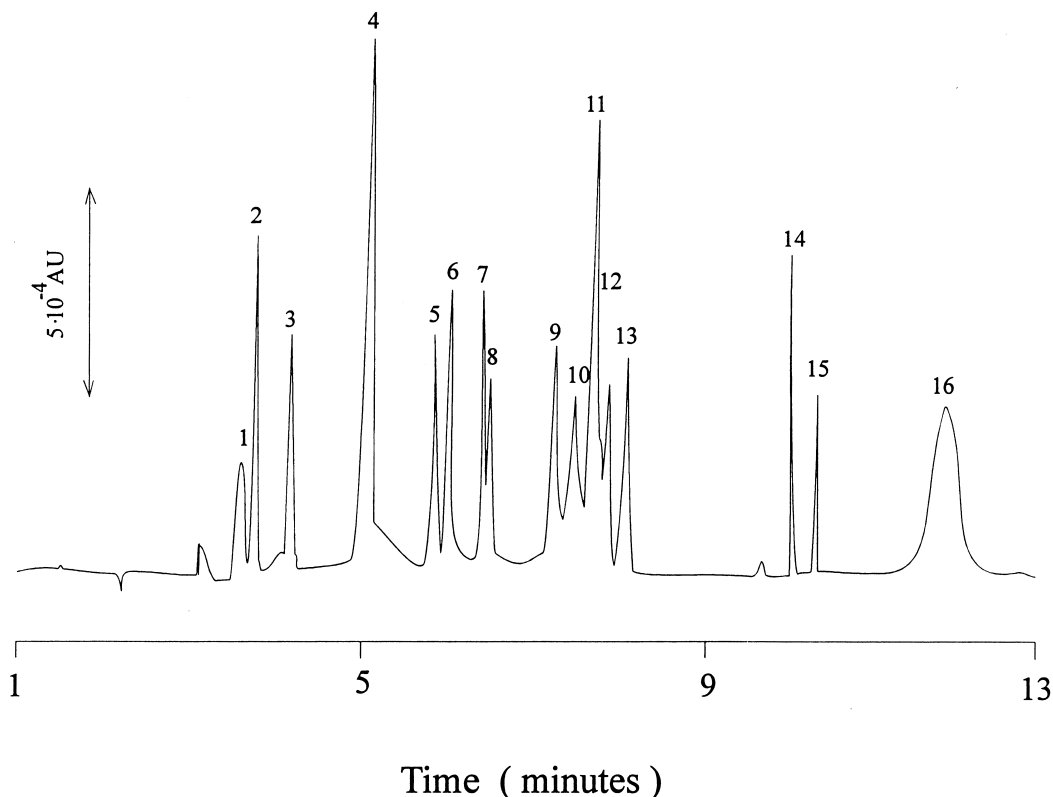
Fig. 3. Separation of 14 metal ions as H_2dapmp complexes. Electrolyte, 10 mM borate buffer(pH 9.0), 75 mM TDTAB and 10 mM SOS; power supply, negative; injection, 10 s. peaks: 1=acetone; 2.4=H_2dapmp; 2=Mo(VI)($1 \cdot 10^{-4}$ M); 5=Sc(III)($8 \cdot 10^{-5}$ M); 6=Fe(III)($4 \cdot 10^{-5}$ M); 7=Y(II)($8 \cdot 10^{-5}$ M); 8=Zn(II)($1 \cdot 10^{-4}$ M); 9=Cd(II)($1 \cdot 10^{-4}$ M); 10=Zr(IV)($8 \cdot 10^{-5}$ M); 11=Co(II)($1.5 \cdot 10^{-4}$ M); 12=U(VI)($1.7 \cdot 10^{-4}$ M); 13=Cu(II)($1.7 \cdot 10^{-4}$ M); 14=Sn(IV)($8 \cdot 10^{-5}$ M); 15=Ta(V)($8 \cdot 10^{-5}$ M); 16=Hg(II)($4 \cdot 10^{-5}$ M). Reproduced from Ref. [47] with permission.

regulating the length of the zones, which are directly proportional to the original sample concentration. Consequently, cITP can be very useful as a powerful concentration method.

In 1985, Hirama and Yoshida [48] described a cITP separation of metal chloro complexes. ClO_4^- and Cl^- were used as the leading and tailing ions, respectively. Under the optimum conditions, Mn(II), Pb(II), Co(II), Zn(II), Cd(II), Hg(II) and Fe(II) were separated. Zelensky et al. [49] report the separation of Mn(II), Pb(II), Zn(II) and Cd(II) as their xylenol orange (XO) complexes by cITP. The chelating reagent was added to the leading electrolye for accomplishing an on-line chelating method. Currently, cITP is employed more as a preconcentration approach than as a separation method and will be discussed in latter section.

2.4. Capillary isoelectric focusing

cIEF operation mode initiated by Hjerten and Zhu [22] in 1985 is established on the differences in the isoelectric point (pI) of the analytes. Now, it is well known for its extremely high resolving power, especially in the application of biomolecules like proteins. In cIEF system, the ampholytes are employed to offer a pH gradient along the capillary when applying an electric field. Species with different isoelectric points (pI values) will move to and be focused on their individual pI, and then be separated from each other. Seldom report concerning analysis of metal ions by cIEF has been published. Michalke and Schramel [50] recently proposed a separation of six inorganic and organic Se species with cIEF. Because the authors utilized an online coupling

inductively coupled plasma spectrometry (ICP-MS) detector, no derivatization procedures were concerned to form metal complex. Scapolan et al. [51] studied in detail the chemical behaviour of uranium in biological medium. By using cIEF, different uranium complexes were separated as a function of their pIs. Very low level of different uranyl complexes were identified in virtue of the utilization of time-resolved laser induced florescence (TRLIF) detection scheme. The author investigated the results obtained in various inorganic chemical and biological systems. The complexation between uranium and human transferrin was also pointed out.

2.5. Ion-exchange elecrokinetic chromatography

IEEC as an auxiliary mode in CE was proposed by Terabe et al. [21] The name IEEC was chosen by the authors mainly for stressing the analogy with the conventional column chromatographic techniques. IEEC is considered a capillary as a chromatographic column with mobile anionic-exchange pseudo-stationary phase. Krokhin et al. [52] reported separations of metal ions as their ethylenediaminetetraacetic acid (EDTA) and 8-amino-2[(2-amino-methylphenoxy)methyl - 6 - methoxyquinoline-N,N,N′,N′-tetraacetic acid(Quin2) complexes and other inorganic anions by IEEC mode using poly-(diallyldimethylammonium chlorid) (PDMAC) as the selectivity controlling additive thanks to its high ion-exchange affinity to inorganic anionic species. In another paper [53], a similar demonstration was also presented. Some transition metal ions like Co(II), Ni(II) and Fe(II) were separated as their PAR chelates from many other anionic species by this mode in 4 min. The authors investigated the effect of polyelectrolyte structure on electrophoreticmobility of analytes, including polybrene, PDMAC and poly-(N-ethyl-4-vinylpyridinium) (PVPyB). It was found that different cationic polymers had different affinities to hydrophobic anions. The association ability of polymer decreased in order as follows: PVPyB>PDMAC>polybrene.

2.6. Separation on microchip

Since 1992 when Manz et al. [54] published their

first research results of separation on MC, MC has obtained a rapid development. It represents a direction of development in electrophoresis as declaimed by Yang et al. [55] that electrophoresis-based method have been moved from the slab through the capillary to the microchip. To date, only one paper directly discussing the separation of metal complexes on MC was reported by Jocobson et al. [56] The HQS complexes of Zn(II), Cd(II) and AL(III) were formed before injections. The employed fused-quartz microchip is fabricated using a series of standard techniques. The separation channel was modified with ployacrylamide for minimizing EOF. Because MC can be more efficient to dissipate the heat generated by the electric current than capillary, higher electric field can be employed for separation. It means higher performance of separation and shorter analysis time. In that work a 870 V/cm electric field was applied in the separation channel, and the separation was accomplished within 15 s as shown in Fig. 4. More recently, Kutter et al. [57] reported a determination of metal cations in MC electrophoresis with LIF detection scheme. Post-column derivatization with HQS of the two model compounds Mg and Ca was carried out on the chip. The complexing regent HQS was added downstream from the injection cross.

2.7. Nonaqueous capillary electrophoresis

Traditional CE is carried out with water as the separation medium. For hydrophobic analytes, additive-modified CE system is employed. Generally, organic solvent, micelle or cyclodextrin (CD) is added to the buffer for improving the solubility of these analytes and separation resolution. These methodologies cannot solve all the problems, however. Sometimes using nonaqueous medium instead of aqueous medium can be an alternative scheme. Although Walbrohel and Jorgenson [58] pioneered the first separation of NACE in 1984, the organic solvents were employed just as additives of the buffer afterwards. It took until the 1990s before a pure NACE system was further developed. Now NACE has been widely used in the separation of low-molecular-mass compounds due to its advantages such as a wider range of acid/base properties, ion pairing, increased solubility for hydrophobic solutes, reduced adsorption of hydrophobic sub-
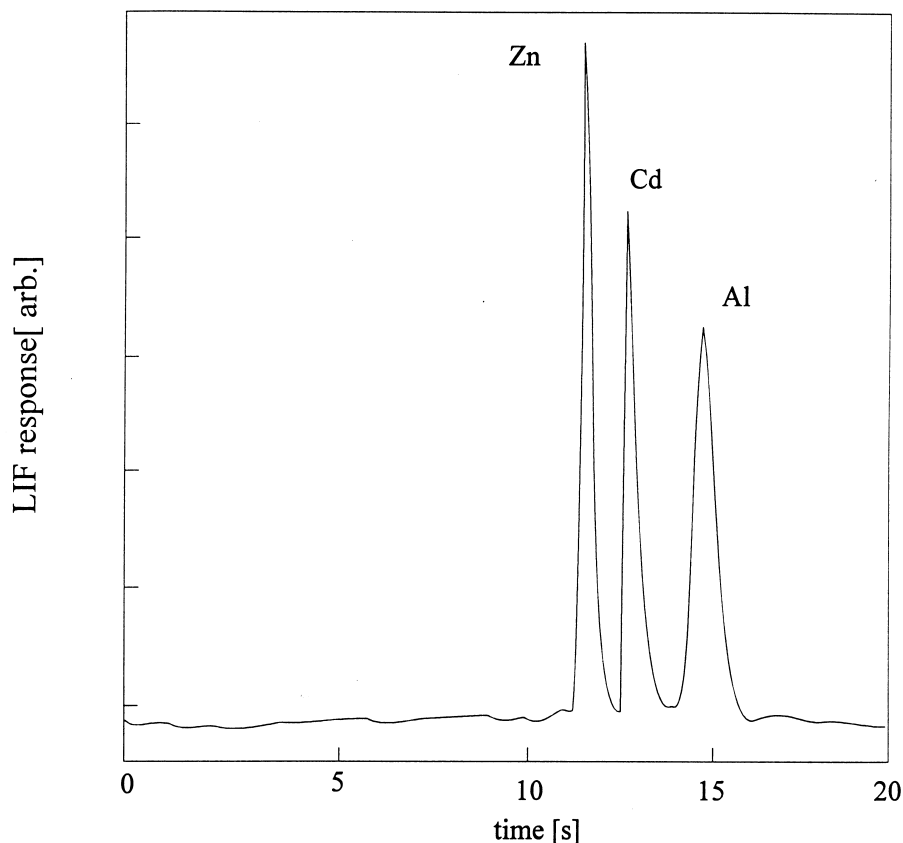
Fig. 4. Electropherogram of Zn, Cd, and Al complexes with HQS: $E_{sep}=870$ V/cm and $L_{sep}=16.5$ mm, The injected concentrations are 8.5(130), 6.7(60), 4.3 ppm (160 μM) for Zn, Cd, and Al, respectively. Reproduced from Ref. [56] with permission.

stances onto the inner capillary surface and reduced Joul-heating et al. For the separation of metal complexes, the application of ligand is essential. Nevertheless many excellent chelating reagents are highly hydrophobic, and their applicabilities in CE are seriously limited (eg. Thiazolyazo-like reagents such as 2-(2-thiazolyazo)-4-methylphenol (TAR), 4-(2-benzothiazolyazo)-4-methylphenol (BTAR) and 2-(5-methyl-2-benzothiazolyazo)-5-diethylamino phenol (MBTAB) which have been widely used in HPLC). Recently, Liu et al. [59] have demonstrated a NACE separation of metal MBTAE chelates. With traditional CZE mode, no peak could be found in the detection window. While employed SDS-based MECC system, the three test metal ions Co(II), Cu(II), Zn(II) as their MBTAE complexes co-migrate with MBTAE because of their highly hydro-

phobic nature. However the employment of NACE resulted in an excellent separation. Three metal complexes and the ligand were separated in 6 min with the theoretical plate numbers over 320 000 as shown in Fig. 5.

3. Approaches to derivatization

There are mainly two approaches for accomplishing the derivatizations of inorganic cations with ligands: (i) in-situ complexation (the name "in-situ complexation", that is, "in-column complexation" as usually called, is chosen to avoid confusion with "in-column complexing equilibria"), (ii) pre-column complexation. The metal complexes are formed before injection.

Fig. 5. Separation of Co, Ni, and Cu as their MBTAE complexes. Conditions: 40 mM NaAc-Hac in nonaqueous medium (EtOH-DMF, 50: 50, v/v), Capillary: 41 cm×75 μm I.D., effective length: 33.5 cm; λ=575 nm; 30 kV; 30°C, Peak identification: 1: Co(II), 2: Ni(II), 3: Cu(II), 4: MBTAE. Reproduced from Ref. [59] with permission.

3.1. In-situ complexation

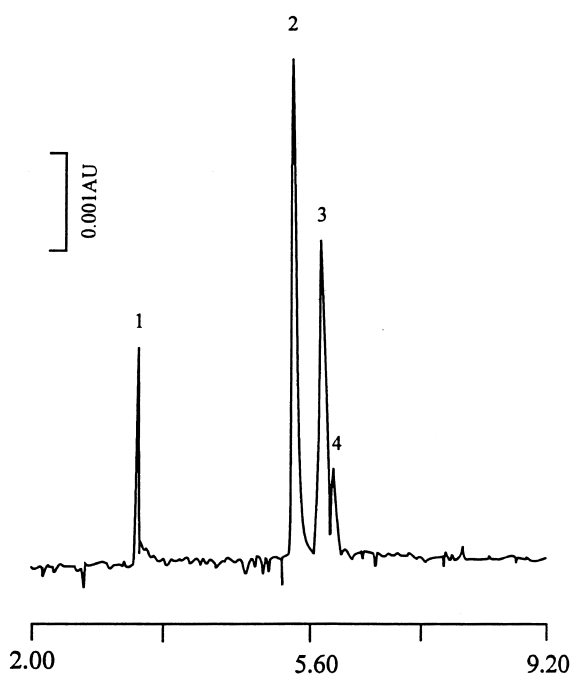In-situ complexation method requires rapid re-actions between metal ions and the ligands. The complexation is usually uncompleted. Swaile and Sepaniak [27] reported an on-column chelation method. The chelating reagent HQS was added to the carrier electrolyte, and the samples containing test metal ions as their free forms were introduced into the capillary directly, so that metal HQS complexes were formed within the tube. This method can be benefit to the enhancement of separation selectivity because of the existence of kinetic differentiation of chelation reactions, at expanse of the loss of separation sensitivity resulted from uncompleted complexation of analytes. Although the laser-based fluorometry was employed for the detection, the detection limits were higher than 2×10^{-6} M Regan et al. [60] tried another good idea to achieve an on-column derivatization using PAR as the chelating

reagent. Before the injection of free metal ions, a specified length of PAR plug with higher concentration was injected into the capillary. Then, the voltage was applied. Because the migration velocity of sample zone was quicker than that of ligand zone under the selected conditions, the two zone would mixed together, and the complexation reaction occurred. At the beginning of the separation, the voltage was kept at a lower level to ensure the adequate complexation of metal ions with the ligand for a period time called pause time by the authors. In this method, the ligand was also added to the buffer at a lower concentration to keep the complexes formed from dissociation while electrophoresising. It proved that the employment of ligand plug could substantially improve detection sensitivity. A detection limit of ca. 1.0×10^{-8} M was achieved if the electrostacking effect occurred. Haumann and Bächmann [61] proposed a new approach to realize the on-column chelation with EDTA. EDTA was injected at cathodic end of capillary while the sample was injected at anionic end. The chelate reaction would occur during the contact time of the two zones in capillary under the controlled electrophoresis conditions. This method required the modification of standard CE instrument, and was limited by more restrict requirements on the speed of complexing reaction and the stability of formed complexes.

3.2. Pre-column complexation

Most researches related to the separation of metal complexes utilized pre-column derivatization mode to achieve the formation of the complexes, due to the distinguish advantage over the on-column derivatization that complete complexation reaction was controlled. In general [28], excessive ligand was added to the solution of metal ions to guarantee the complete complexation of metal ions with ligand. Because there is a dynamic equilibrium among metal ion, ligand and the formed complex. The complexes formed before injection can dissociate during electrophoresising in the capillary in virtue of the shift of the equilibrium. This problem can be easily overcome incorporating addition of the ligand to the running buffer. The added amount of the ligand is agreement with the stability of the complexes. In theory, increasing the concentration of the ligand in

the buffer is in favour of the stability of analyte. Nevertheless, higher concentration will raise the detection background, amplify the analysis noise and the shift of the separation baseline. So that, the concentration of the ligand in the buffer should be controlled within a favourite range.

4. Choice of derivatization ligands

In the area of applications of CE to analysing metal complexes, the selection and utilization of ligand are undoubtedly of the key importance in the research and at the core of the experimental design. A good derivatization reagent should fulfil the requirements as follows: (i) it can be easily synthesized and purified to ensure its purity, (ii) it can form stable, sensitive and single-state complexes with multielements under the controlled conditions, (iii) the formed complex should keep stable during electrophoresising in the capillary, (iv) the maximum absorptive wavelengths of the complexes investigated in an electrophoretic running had better be near to each other, and far away from that of the ligand for the demand of the detection. Over the past decade, much work has been done to exploit suitable derivatization reagents for inorganic cations in CE separations. In this section we will review comprehensively the employment of both organic and inorganic ligands. These separations mentioned in the contexts will not be expounded in detail unless necessary, and just reminded for a complete discussion. For separations belonging to the utilization of the same derivatization reagent, only several examples are presented for conciseness. The overall applications of ligands are given in Table 1.

4.1. Utilization of organic ligands

Many organic colour reagents and organic extraction reagents commonly used in analytical chemistry are utilized as the metal chelating reagents in CE separations such as azo dyestuff, quinoline dyes, porphyrin, Aminopolycarboxylic acids and other types of derivatization reagents.

4.1.1. Azo dyestuff
Pyridylazo dyes. Pyridylazo reagents are a kind of most widely used coordination organic compounds, in which PAR is the distinguish representative. PAR can react rapidly with multielements and form stable chelates in weakly basic solution at room temperature. Because the formed complexes are water-soluble and have hydrophobic nature, both CZE mode [60,62,63] and MECC mode [42] can be conveniently performed for the separation of metal-PAR complexes. Timerbaev et al. [42] reported a separation of 9 metal ions in form of PAR chelates by SDS-based MECC method as shown in Fig. 6, including Cr(III), Fe(III, II), Co(II), Cu(II), Pb(II), Ni(II), Zn(II), and Cd(II) ions. A detection limit of 1×10^{-7} M for Co(II) was achieved. Nearly meanwhile, Regan et al. [60] also reported determinations of Co(II), Fe(II), Zn(II) and Cu(II) in a tablet of vitamin supplement and the pond water as their PAR complexes with CZE method. The detection limits, however, were too high if peak stacking procedures were not performed, due to the poor lamp used in detector.

Some authors studied the applications of other pyridylazo reagents in CE separations of metal complexes. 2-(5′-bromo-2′-pyridylazo)-5-diethyl-aminophenol (5-Br-PADAP) [64,65] by Smyth et al. and 2-(5-nitro-2-pyridylazo)-5-(N-propyl-N-sulfopropylamino)phenol (5-NO$_2$-PAPS) [66] by Motomizu et al. exhibited excellent feasibility in CE. They are superior over PAR in sensitivity of the formed complexes with metal ions. Motomizu et al. [67] proposed a CZE separation of some transition ions like Cd(II), Zn(II), Pb(II), V(VI), Hg(II), Cu(II), Co(II, III), Ni(II) and Fe(II) as their complex forms. Fig. 7 gives the typical electropherogram. 2-(5-bromo-2-pyridylazo) - 5 - (N-propyl-N-sulfopropyl-amino)phenol (5-Br-PAPS) was successfully utilized as the pre-column derivatization reagent. And the detection limit of 5×10^{-8} M for Fe(II) and Co(II, III) was reported. Furthermore, to determine impurities of several nickel salts, the authors lowered the concentration of 5-Br-PAPS in the carrier. Co(II) ion could be determined at a concentration of 1×10^{-8} M as a result of the decreasing of the background noise.

Thiazolyazo dyes. Although thiazolyazo reagents are very popular as the metal derivatization reagents in HPLC due to the advantages that they can form high sensitive metal complexes, be very easily synthesized and purified, they are still seldom uti-

Table 1
Utilization of derivatization ligand

Ligand	Metal ions	Strategy	Buffer components	λ/nm	DL	Application	Ref.
Organic ligand							
Azo dyestuff							
PAR	Co^{II}, Cr^{III}, Ni^{II}, Fe^{III}	MECC	50 mM NaH_2PO_4–12.5 mM $Na_2B_4O_7$, 20 mM SDS, 0.1 mM PAR, pH 9.2	500	$2.3 \cdot 10^{-7}$ M		[40]
	Cr^{II}, Co^{II}, Cu^{II}, Pb^{II}, Ni^{III}, Fe^{III}, Zn^{II}, Cd^{II}	MECC	1 mM ammonium phosphate, 75 mM SDS, 0.1 mM PAR, pH 8.0	254	$1.0 \cdot 10^{-7}$ – $10 \cdot 10^{-7}$ M		[42]
	Co^{II}, Fe^{II}, Ni^{II}, V^{V}	CZE	10 mM NaH_2PO_4–10 mM $Na_2B_4O_7$, 0.1 mM PAR, pH 8.4	500	$1.41 \cdot 10^{-7}$ M		[62]
	Co^{II}, Fe^{II}, Cu^{II}, Zn^{II}	CZE	10 mM TAPS, 0.1 mM PAR, pH 8.4	500	$1.0 \cdot 10^{-8}$ M	vitamin tablet, water	[60]
	Co^{II}, Fe^{II}, Cu^{II}, Zn^{II}	CZE	10 mM TAPS, 0.1 mM PAR, pH 8.40	525	$5 \cdot 10^{-7}$ M	vitamin tablet, water	[63]
	Fe^{II}, Co^{II}, Ni^{II}	IEEC	5 mM K_2CrO_4, 0.05%(w/w) polybrene, pH 8.9	490			[53]
5-Br-PADAP	Co^{II}, Cd^{II}, Ni^{II}, Cu^{II}, Zn^{II}, Pb^{II}	CZE	50 mM NaAc, 0.1 mM PADAP, 20% acetonitrile, pH 6.0	560–585	$5 \cdot 10^{-7}$ – $10 \cdot 10^{-7}$ M	vitamin B_{12}	[64]
	Co^{II}	CZE	50 mM NaAc, $1.0 \cdot 10^{-7}$ M 5-Br-PADAP, 2 mM CTAB, pH 8.0		$5.0 \cdot 10^{-8}$ M		[65]
5-NO_2-PAPS	Cd^{II}, Zn^{II}, Pb^{II}, V^{V}, Cu^{II}, Co^{II}, Ni^{II}, Fe^{II}	CZE	24 mM NaAc, 0.12 mM 5-NO_2-PAPS, pH 4.1	560	$1.0 \cdot 10^{-7}$ M	nickel salts	[66]
5-Br-PAPS	Ag^{I}, Co^{II}, V^{V}, Pd^{II}, Mn^{II}, Hg^{II}, Ni^{II}, Fe^{II}, Cu^{II}, Cd^{II}, Zn^{II}, Pb^{II}, Ti^{IV}	CZE	NaAc, 0.12 mM 5-Br-PAPS, pH 4.9	550	$5.0 \cdot 10^{-8}$ – $1.0 \cdot 10^{-6}$ M	nickel salts	[67]
MBTAE	Co^{II}, Ni^{II}, Cu^{II}	NCE	40 mM NaAc-HAc in EtOH and DMF(50%:50%, v/v) solution, pH 5.0	575	$0.92 \cdot 10^{-7}$ – $4.5 \cdot 10^{-7}$ M		[59]
AAIII	La^{III}, Ce^{III}, U^{VI}, Cu^{II}, Pb^{II}, Co^{V}, Fe^{V}	MECC	10 mM $(NH_4)_2HPO_4$–$NH_4H_2PO_4$, 50 mM SDS, 0.1 mM AAIII, pH 8.0	254			[42]
	Zr^{IV}, Nb^{V}, Ta^{V}	CZE	10 mM $(NH_4)_2HPO_4$–$NH_4H_2PO_4$, 0.1 mM AAIII, pH 8.0	254			[42]
	U^{VI}	CZE	10 mM $HClO_4$–1 mM Na_2HPO_4 –0.6 mM $Na_2B_4O_7$, 10%MeOH, pH 2.7	650	10 ppb	river water	[103]
	U^{VI}, 14 lanthanides^{III}	CZE	15 mM citric–20 mM Tris, 0.025 mM AAIII, pH 4.3	654	5 ppb	river water	[69]
TBDA	La^{III}, Ce^{III}, Pr^{III}	CZE	50 mM citric acid–50 mM Na_2HPO_4, 50% EtOH(v/v), pH 6.0	632.8	$2.2 \cdot 10^{-8}$ M		[70]
	RE^{III}, Th^{IV}, U^{VI}	CZE	30 mM NaAc–HAc, 0.5 mM CTAB, 0.2 mM TBDA, pH 4.3	635	37 ppb		[71]
DBC-As	RE^{III}, Th^{IV}, U^{VI}	CZE	30 mM NaAc–HAc, 0.2 mM DBC-As, pH 3.0	630	6.8 ppb	ore	[29]
SAIII	Ba^{II}, Sr^{II}, Ca^{II}, Mg^{II}	CZE	20 mM MES–10 mM Tris 0.3 mM SAIII, pH 6.2	654	$3.5 \cdot 10^{-7}$ – $3.7 \cdot 10^{-7}$ M	mineral water	[72]

Table 1 (*continued*)

Ligand	Metal ions	Strategy	Buffer components	λ/nm	DL	Application	Ref.
DHABS	Cu^{II}, Fe^{III}, Co^{II}, Al^{III}, Cr^{III}, V^{V}, U^{VI}	CZE	20 mM NaH_2PO_4, 25 mM TBA, pH 7.0	494	ca. $1.0 \cdot 10^{-7}$ M		[73]
PAA	Cu^{II}, Ni^{II}, Co^{II}	MECC	10 mM NaH_2PO_4–10 mM $Na_2B_4O_7$, 100 mM SDS, pH 8.5	380			[68]
Quinoline dye							
HQS	Ca^{II}, Mg^{II}, Zn^{II}	CZE	10 mM Na_2HPO_4–6 mM $Na_2B_4O_7$, 2.5 mM HQS, pH 8.0	425	$1.0 \cdot 10^{-5}$ M, $-2.0 \cdot 10^{-6}$ M		[27]
	Ni^{II}, Cu^{II}, Zn^{II}, Cd^{II}, Fe^{II} Co^{II}	CZE	10 mM borate, 0.1 mM CTAB, pH 9.2	254	$0.52 \cdot 10^{-7}$ –, $2 \cdot 10^{-6}$ M		[74]
	Mg^{II}, Cu^{II}, Al^{III} Cd^{II}, Fe^{II}, Zn^{II}, Co^{II}, Ni^{II}	CZE/MECC	10 mM borate, 0.1 mM HQS, pH 9.0 /50 mM SDS	254	$4 \cdot 10^{-6}$ –, $5 \cdot 10^{-8}$ M		[75]
	Zn^{II}, Cd^{II}, Al^{III}	MC	60 mM sodium phosphate, 20 mM HQS, pH 6.9		30–57 ppb		[56]
	Ca^{II}, Mg^{II}	CZE	phosphate-borate, 2.5 mM HQS, pH 8			ground water	[76]
Porphyrin							
HP/PP	Cu^{II}, Zn^{II}	MECC	20 mM CAPS–17 mM NaOH in DMF(10:2, v/v), 40 mM SDS, pH 11	405			[77]
TCPP	Mn^{II}, Co^{II}, Zn^{II}, Cu^{II}	MECC	50 mM NaH_2PO_4–12.5 mM $Na_2B_4O_7$, 20 mM SDS, 50 mM imidazole, pH 7.0	422	$8 \cdot 10^{-8}$ M		[78]
Dithiocarbamate							
HEDTC	Cd^{II}, Pb^{II}, Pt^{II}, Co^{II}, Ni^{II}, Bi^{III}, Cr^{III}, Cu^{II}, Hg^{II}	MECC	100 mM borate, 10 mM SDS, 4% MeOH, 0.1 mM HEDTC	254	22–133 ppb		[45]
ED	Cu^{II}	CZE	25 mM phosphate, 35% acetonitrile(v/v), pH 3.5		$1.0 \cdot 10^{-7}$ M		[79]
Aminopolycarboxylic acid							
EDTA	Cu^{II}, Pb^{II}, Cr^{III}, Fe^{III}	CZE	100 mM acetate, 0.1 mM TTAB, pH 5.5	225	$6 \cdot 10^{-6}$ –, $27 \cdot 10^{-6}$ M	waste water	[81]
	Fe^{II}, Fe^{III}	CZE	100 mM borate, pH 9.2	200	$5 \cdot 10^{-6}$ M		[82]
	Ba^{II}, Sr^{II}, Ca^{II}, Mg^{II}	CZE	2 mM bora, 2 mM EDTA, pH 9.2	200	$1.0 \cdot 10^{-5}$ M	water, serum	[80]
	V^{V}, V^{IV}	CZE	50 mM NaAc, 0.5 mM EDTA, pH 4.6	280	0.1, 0.4 ppm	catalyst leachate	[110]
	Cr^{III}, Cr^{VI}	CZE	10 mM phosphate, 0.5 mM CTAB,	254	1.5–5 ppm		[109]
	Pb^{II}, Fe^{III}, Ca^{II}, Co^{II}, Zn^{II} Ni^{II}, Cu^{II}, Cr^{III}	IEEC	30 mM NaAc, 5 mM Na_2SO_4, pH 7.0	240	2.5–		[52]
	Fe^{II}	CZE	25 mM MES, 10 mM BTP, pH 6.6	254	$20 \cdot 10^{-6}$ M, 10 ppb		[104]
	Cu^{II}, Pb^{II}, Ni^{II}, Fe^{II}	CZE	10 mM NaAc, 2 mM TTAB, pH 4.8	242	$0.5 \cdot 10^{-6}$ –, $3 \cdot 10^{-6}$ M		[61]
CDTA	Cu^{II}, Pb^{II}, Mg^{II}, Ca^{II}, Zn^{II}	CZE	300 mM borate, 0.5 mM TTAB, 0.5 mM EDTA, pH 9.0	185	$0.2 \cdot 10^{-6}$ –, $2.5 \cdot 10^{-6}$ M	Mineral water	[83]
	(Ce, Pr, Nd, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb, Lu)^{III}	CZE	20 mM borate, 1 mM CDTA, pH 11.1	214	mid ppb		[85]
	(Ce, Pr, Nd, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb, Lu, Y, Sc)^{III}	CZE	20 mM borate, 1 mM CDTA, pH 11.1	214	mid ppb	nuclear fuel	[84]

Table 1 (continued)

Ligand	Metal ions	Strategy	Buffer components	λ/nm	DL	Application	Ref.
	Tl^I, Ce^{III}, Sb^{III}, Bi^{III}, V^V, $Fe^{II,II}$, $Co^{III,II}$, Ag^I, Zr^{IV}, Cr^{III}, Sn^{IV}, Mo^{VI}, Ba^{II}, Sr^{II}, Ca^I, Mg^{II}, Hg^{II}, Ni^{II}, Cu^{II}, Zn^{II}, U^{VI}	CZE	20 mM borate, 1 mM CDTA, 5% EtOH(v/v), pH 9.0	214	$0.1 \cdot 10^{-6}$ – $4 \cdot 10^{-6}$ M		[28]
	Cr^{VI}, Cr^{III}	CZE	10 mM formate, pH 3.0	214	10, 50 ppb	plating bath rinse water	[107]
	Cr^{VI}, Cr^{III}	CZE	10 mM formate, pH 3.0	214	50 ppb	electroplating	[108]
	$Fe^{II, III}$	CZE	100 mM borate, pH 9.0	185/ 214/ 254	Sub-ppb	pharmaceutical	[105]
	Cr^{III}, Bi^{III}, $Fe^{II, III}$, Ba^{II}, Co^{II}, Cu^{II}, Hg^{II}, Ni^{III}, Zn^{II}	CZE	100 mM borate, 1 mM CDTA, pH 9.0	254	0.06, 0.1 ppm	tap & ground water	[86]
DTPA	Co^{III}, Bi^{III}, Fe^{II}, Cr^{II}, V^{IV}, Pb^{II}, Hg^{II}, Cu^I, Ni^{II}, $V^{IV,V}$	CZE	20 mM Na2HPO4, 5 mM DTPA, pH 8.0/8.5	214	$2-8 \cdot 10^{-6}$ M	electroplating bath	[87]
NTA	Cr^{VI}, Fe^-, Ni^{II}, inorganic and organic Pb, Hg, Se	CZE / CZE / MECC	20 mM Na2HPO4, 5 mM DTPA; 20 mM Na2HPO4, 0.5 mM TTAOH; 40 mM NaH2PO4-Na2B4O7, 5 mM NTA, 40 mM SDS, pH 7.0	214 214 200	0.2 ppb	waste water	[87] [87] [88]
TTHA	inorganic and organic Pb, Hg, Se	CZE	40 mM NaH2PO4-Na2B4O7, 2.5 mM TTHA, 2.0 mM SDS, pH 7.5	200	0.1132– 2.415 ppb	Tap water & seawater	[89]
Quin 2	Fe^{II}, Ca^{II}, Pb^{II}, Zn^{II}, Co^I, Ni^{II}, Cu^{II}	IEEC	5 mM NaSO4, 3 mM NaAc, pH 7.0	240	$5.9 \cdot 10^{-8}$ – $7.1 \cdot 10^{-8}$ M		[52]
Others							
acac	Co^{III}, Cr^{III}, Pb^{II}, Pt^{II}	MECC	20 mM borate, 100 mM SDS, pH 9.2	230	<10 ppb		[90]
DzS	Hg, MeHg, EtHg, PhHg	CZE	10 mM NaAc, 5 ppm DzS, pH 5.0	480	$1.0 \cdot 10^{-6}$ –		[92]
H_2damap	Cd^{II}, Co^{II}, Cu^{II}, Fe^{II}, Hg^{II}, Mo^{VI}, Sc^{III}, U^{VI}, V^V, Y^{III}, Zn^{II}	MECC	10 mM borate, 75 mM CTAB, 10 mM SOS, pH 9.0	254	$44 \cdot 10^{-6}$ M		[47]
Cysteine	Hg, MeHg, EtHg, MeHg	CZE / CZE	100 mM borate, 10% MeOH, pH 3.5; 200 mM borate pH 8.24	200 200	ca. 1 ppm 12 ppb	mussel a certified reference	[111] [112]
phen	Fe^{II}	CZE	50 mM NaAc-HAc, pH 5.0	270	$5 \cdot 10^{-9}$ M	serum	[93]
4,7 Mephen	Fe^{II}, Co^{II}, Ni^{II}, Cu^{II}, Zn^{II}	CZE	20 mM NaAc-HAc, pH 6.0	220	30 ppb	Duralmin	[94]
Citrate	Co^{II}, Ni^{II}, Zn^{II}	CZE	150 mM NaCl,0.5 mM CTAB,	200		milk	[113]
XO	Mn^{II}, Cd^{II}, Zn^{II}, Pb^{II}	CITP	leading electrolyte: 20 mM NH4Ac, 0.4 mM XO, 0.1% (w/v) HEC pH 5.0 terminating electrolyte: 1 mM HNO3, pH 3.0	580	$1 \cdot 10^{-8}$ M		[49]
Inorganic ligand							
CN^-	Au^I, Ag^I, Cu^{II}, Ni^{II}, Fe^{II}	CZE	20 mM Na2HPO4, 36 mM NaCl, 1 mM NaCN, 10/30 µM TTAB, pH 11	204	6-80 ppb	gold mineral &sand	[97]
	Au^I, Ag^I	CZE	10 mM carbonate, pH 9.6	214	2.82, 3.48 ppm	ore	[97]

Table 1 (continued)

Ligand	Metal ions	Strategy	Buffer components	λ/nm	DL	Application	Ref.
	$Fe^{III, II}$	CZE	20 mM phosphate, pH 7	214	$1 \cdot 10^{-5}$ M	waste water & electroplating solution	[98]
	$Fe^{III, II}, Cr^{III}, Co^{III}, Pb^{II}, Ni^{II}, Cu^{II, I}, Ag^{I}$	CZE	20 mM phosphate, 2 mM NaCN, pH 9.4	214	24–335 ppb	enviromental sample	[95]
	$Fe^{III, II}, Ni^{II}, Co^{II}, Pd^{II}, Pt^{II}, Cr^{III}, Au^{I}, Ag^{I}$	CZE	5 mM phosphate–5 mM triethanolamine, 0.8 mM hexamethonium, pH 8.5	214	$3.8 \cdot 10^{-7}$ – $6.6 \cdot 10^{-9}$ M		[99]
	$Fe^{III, II}, Ni^{II}, Co^{II}, Pd^{II}, Pt^{II}, Cr^{III}, Cu^{I}, Au^{I}, Ag^{I}$	CZE	5 mM phosphate–5 mM triethanolamine, 0.8 mM hexamethonium, pH 8.5	214	0.15–2.5 ppm		[100]
Cl^-	$Rh^{III}, Ru^{III}, Os^{III}, Ir^{III}, Pt^{II}$	MECC	50 mM HCl–KCl, 0.15 mM CTAB, pH 3.0	214	0.2 ppm	synthetic sample	[47]
	$Rh^{III}, Ru^{III}, Os^{III}$	CZE	50 mM HCl–KCl, 0.15 mM CTAB,	214	20 ppb		[34]
	$Au^{III}, Pd^{II}, Pt^{IV}$	CZE	100 mM HCl–400 mM NaCl, pH 1	220	2 ppm/ 70 ppb	monitoring	[111]
	$Au^{III}, Ir^{III,III}, Os^{IV}, Ru^{III}, Rh^{III}, Pd^{II, iv}, Pt^{II,IV}$	CZE	4 mM H^+ –25 mM Cl^-, pH 2.4	214			[36]
	$Fe^{II}, Hg^{II}, Cd^{II}, Zn^{II}, Co^{II}, Pb^{II}, Mn^{II}$	CITP	leading ion: ClO_4^- in DMF terminating ion: Cl^- in DMF				[48]

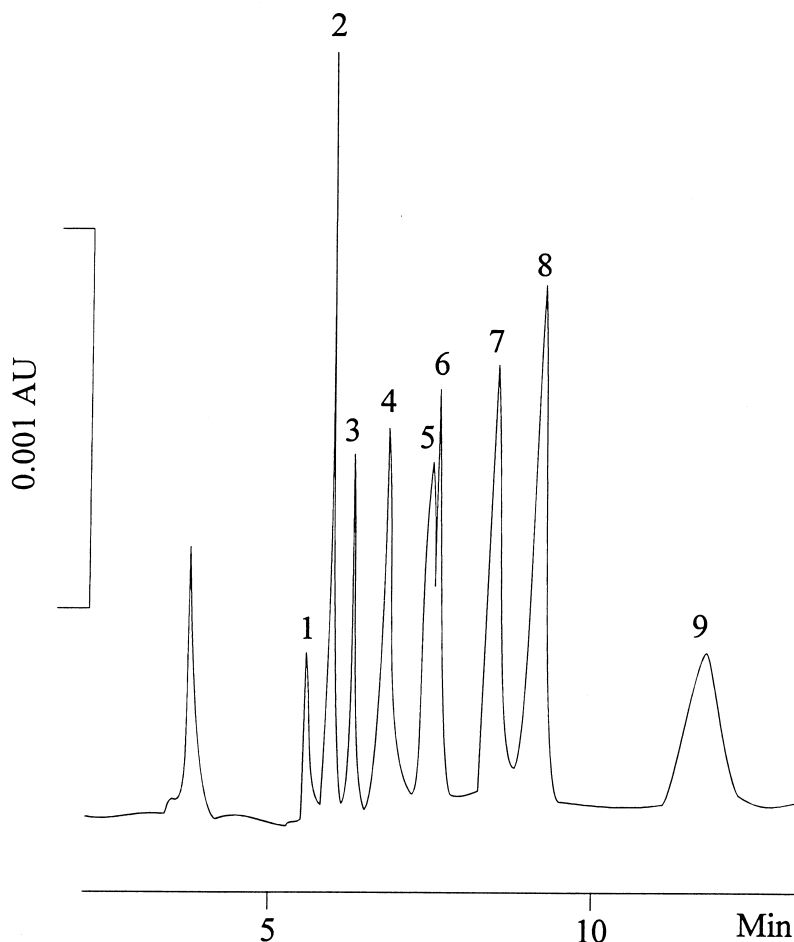Fig. 6. Micellar CE separation of metal chelates of PAR. Capillary, 50 cm (effective length 42 cm)×75 μm I.D.; carrier electrolyte, 10 mM ammonium phosphate buffer containing 75 mM SDS and $1 \cdot 10^{-4}$M PAR(pH 8.0); voltage, 15 kV; injection, 30 s hydrostatic); detection, 254 nm. Metals(mol/l): $1 = Cr(III)(2.4 \cdot 10^{-4})$; $2 = Co(II)(6 \cdot 10^{-5})$; $3 = Cu(II)(8 \cdot 10^{-5})$; $4 = Pb(II)(8 \cdot 10^{-5})$; $5 = Ni(II)(8 \cdot 10^{-5})$; $6 = Fe(II)(8 \cdot 10^{-5})$; $7 = Zn(II)(1.6 \cdot 10^{-4})$; $8 = Fe(III)(8 \cdot 10^{-5})$; $Cd(II)(2.4 \cdot 10^{-4})$. The first migrating peak belongs to acetone. Reproduced from Ref. [42] with permission.

lized in CE separation. An important factor is the solubility of the complexes in aqueous medium. The formed metal complexes of thiazolyazo reagents are highly hydrophobic and nearly insoluble in water. T. Saitoh et al. [68] ever reported a determination of partition constant of Fe(II)-TAR complex into SDS micelle with some other thiaolyazo dye by MECC system. But no electropherogram was presented. Liu et al. [59] demonstrated an excellent separation of metal ions as MBTAE chelates by NACE as mentioned in previous section. Except the two papers, no other research literature has been published.

Bisazochromotropic acid. Bisazochromotropic acid reagents can complex with various metal ions, especially high-valence metal ions such as lanthanoids, thorium, uranium et al. Because the formed complex is stable and highly hydrophilic, CZE mode is usually chosen for accomplishing the separation. Macka et al. [69] conducted a separation of uranium(VI) and lanthanides as their AAIII complexes by CZE system. An on-capillary complexation method was performed due to the believes in better shape of lanthanides peaks compare to precapillary complexation. Meanwhile the authors also
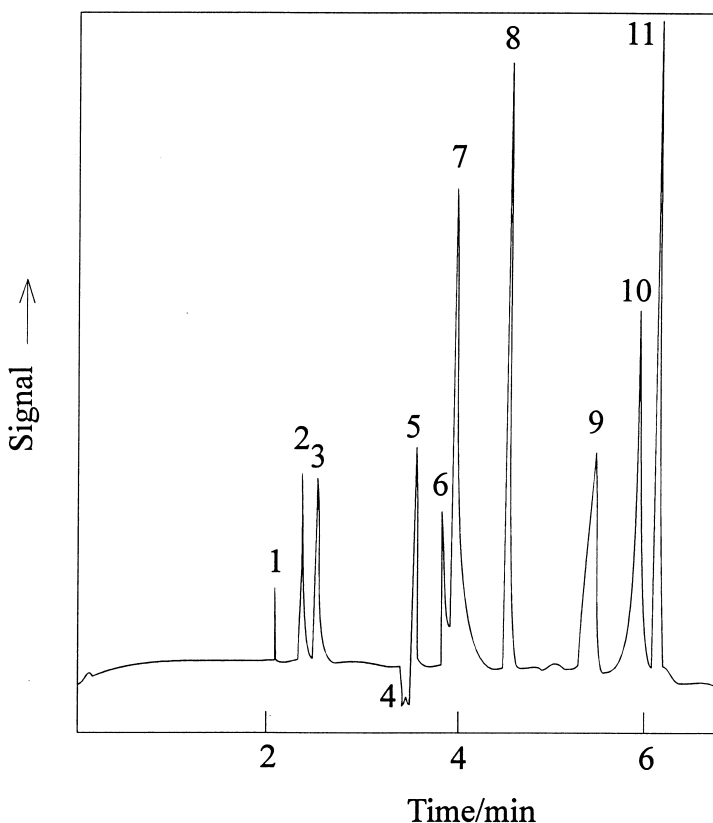
Fig. 7. Electropherogram for nine metal ions at pH 4.9. Carrier, $1.2 \cdot 10^{-4}$ M 5-Br-PAPS + acetate buffer; Sample, metal ions + $.2 \cdot 10^{-4}$ M 5-Br-PAPS. 1, $2 \cdot 10^{-5}$ M Cd^{2+}; 2, $1 \cdot 10^{-5}$ M Zn^{2+}; 3, $2 \cdot 10^{-5}$ M Pb^{2+}; 4, water dip; 5, $1 \cdot 10^{-5}$ M V^{IV}; 6, $1 \cdot 10^{-5}$ M Hg^{2+}; 7, $1 \cdot 10^{-5}$ M Cu^{2+}; 8, $3 \cdot 10^{-6}$ M Co^{2+}; 9, 5-Br-PAPS; 10, $3 \cdot 10^{-6}$ M Ni^{2+}; 11, $3 \cdot 10^{-6}$ M Fe^{2+}. Reproduced from Ref. [67] with permission.

pointed out that addition of a second ligand competing with AAIII for the metal ions was critical to achieve good peak shape. Under the optimised conditions uranium(VI) and 14 lanthanides as their AAIII complexes were separated in 7 min with no resolution of Gd, Eu and Sm, as well as Yb and Lu. Timerbaev et al. [42] have ever studied the separation of metal ArsenazoIII(AAIII) complexes with MECC mode. However, the obtained result was not encouraging.

Polyhalogenated bisazochromotropic acid derivatives are more favorable for chelation due to the very high sensitivity of the formed metal complexes. The molar absorptive coefficient (ε) of cerium-TBDA complex for example reaches up to 1.33×10^5 M^{-1} cm^{-1} which is 2–3 times as high as AAIII. Additionally, there is a large shift of maximum absorptive wavelength(usually $\Delta\lambda > 100$ nm) between these

colour reagents and the formed metal complexes. So that, excessive ligand added to the buffer for keeping stability of the formed complexes can only cause little detection background. Hu et al. [70] proposed the separation of some lanthanoid elements La(III), Ce(III), Pr(III) as their 2-[(2-arsenophenyl)-azo]-1,8-dihydroxy-7-[(2, 4, 6 - tribromophenyl)azo] - naphthalene-3,6-disulfonic acid (TBDA) complexes with CZE. Owing to the employment of photointerference detection scheme instead of UV detection, a very low detection limit of 2.1×10^{-8} M was achieved. Liu et al. accomplished two determinations of rare-earth elements(III) from their co-existing elements in an ore sample like thorium(IV) and uranium(VI), as their TBDA [71] and DBC-As [29] complexes, using co-EOF and counter-EOF CZE system, respectively.

Other azo dyes. SulfonazoIII(SAIII) is a derivative of AAIII in which the -AsO$_3$H$_2$ groups have been

replaced by -SO$_3$H group, and is one of the most sensitive metallochromic reagent for barium with $\varepsilon = 1.73 \times 10^4$ M^{-1} cm^{-1} at 638 nm. Macka et al. [72] conducted the determination of barium(II) and strontium(II) in presence of 100 000 folds disparate levels of calcium(II) and magnesium using SAIII as the chelating reagent as shown in Fig. 8. The detection limits of 3.5×10^{-7} M and 4.7×10^{-7} M for Ba(II) and Sr(II) were reported, respectively, with a linear range from 1.25 μM to 80 μM. The analysis results of a mineral water sample were compared with those obtained by ICP-MS.

Iki et al. [73] used 2,2′-dihydroxyazobene-5,5′-disulphonate(DHABS) as a pre-column derivatization reagent to examine the CZE separation of its complexes with Al(III), Co(III), Cr(III) and Fe(III) ions, in which, Cr(III) chelate formed extremely slow and could not be judged by the authors whether or not quantitative formation was occurred. Because the formed complexes are equally and highly charged (identical-5 charges), ion-association additives were added to the running buffer to facilitate the separation resolution.

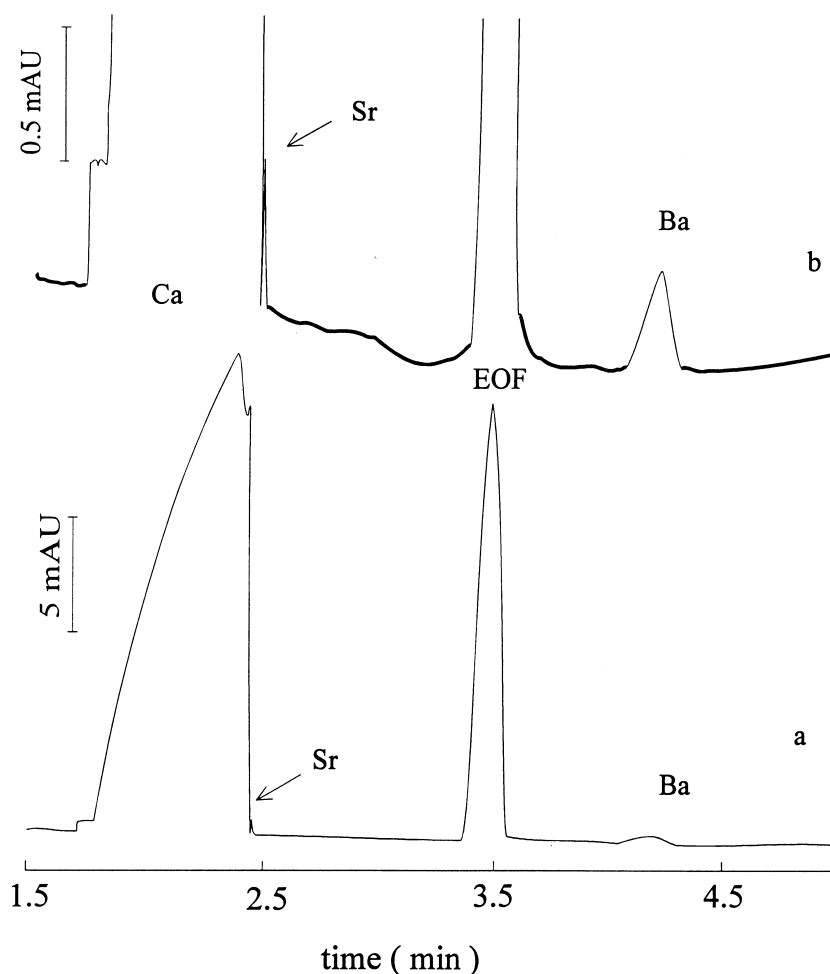A separation of Co(II), Ni(II) and Cu(II) as their

Fig. 8. Electropherogram of (a) a standard of 5μM strontium(II) and barium(II) in 40 mM Ca(NO$_3$)$_2$ and (b) the same electropherogram scaled to show the barium(II) and strontium(II) peaks. The injected solution contained an addition of 20 mM HSNa. BEG: 0.3 mM SAIII in 20 mM MES and 10 mM Tris (pH 6.2), separation voltage: +30 kV(15 μA), temperature: 25°C, detection: LED 654 nm. injection, hydrostatic (30 s). Reproduced from Ref. [72] with permission.

2-pyridylaldehyde-4-amino benzoylhydrazone (PAA) chelates by SDS-based MECC mode was carried out by T. Saitoh et al. [68]. The peaks of Cu(II) and Ni(II) were not completely resolved due to a very small differences in partition constant into SDS micelle. In the same paper, the authors also presented a determination of partition constants of 2,2′-dihydroxyazobenzene (DHAB) complexes with Fe(III) and Co(III) into SDS micelle by MECC.

4.1.2. Quinoline dyes

Of quinoline reagents, HQ and HQS are both good ligands for chelation with metal ions. Actually, however, HQ is not reported to be used in CE separations. On the contrary, HQS is dazzlingly brilliant to be widely utilized, either for UV detector [74,75] or for fluorescence detector [27,56,76] in CE. Timerbaev et al. [74,75] developed a CZE method for the separation and determination of some transition metals using HQS chelating system before an investigation of various separation mode. Borate buffer of pH 9.2 was selected as the separation medium and a detection limit of 5×10^{-8} M for Cu(II) was achieved. The method established was successfully applied to determine the content of zinc in tap water.

4.1.3. Porphyrin

Macrocyclic porphyrin reagents should be a kind of ideal derivatization reagent due to their high sensitivity, high stability and quite close maximum absorptive wavelengths of their complexes with various metal ions, regardless of some disadvantages. Unfortunately, their synthesized yields are very low, usually less than 10%, and difficult to be purified. Furthermore, the reaction conditions are harsh. Consequently, their applications in CE are largely limited.

Saitoh et al. [77] described an application of TCPP as the pre-column labelling reagent for the separation of Mn(II), Co(II), Zn(II) and Cu(II) ions by MECC mode. Because of the high inertness of TCPP chelate, free ligand was not necessary in the buffer. It could be a reason taking the responsibility for the low detection limits except their high molar absorptive coefficients(eg. $\varepsilon = 6.28 \times 10^5$ M^{-1} cm^{-1} for

Zn(II)-TCPP at 422 nm). A concentration of 8×10^{-8} M for Zn(II) was detected.

Kiyohara et al. [78] illustrated the separation of two typical bioporphyrin haematophoryrin IX (HP) and protoporphyrin IX(PP) complexes with Cu(II) and Zn(II) by MECC. A mixture of a micellar solution of SDS at pH 11 and dimethylformamide (DMF)(10:2, v/v) which proved to be necessary to ensure the reproducibility of the separation and the solubility of the analytes, was employed for optimizing the separation condition.

4.1.4. Dithiocarbamate

The employment of dithiocarbamate reagents in this area is very late. Hilder et al. [45] newly developed a separation of 9 metal ions as their HEDTC complexes by SDS-based MECC system. As shown in Fig. 9, Cd(II), Pb(II), Pt(II), Co(II), Ni(II), Bi(II), Cr(III), Cu(II) and Hg(II) were resolved within 8 min. The detection limits were in the range from 22 ppb to 133 ppb. Tsukagoshi and co-workers [79] demonstrated the separation and determination of Cu(II)-emetine dithiocarbamate(ED) complex, and will be discussed in Section 5.2 for details.

4.1.5. Aminopolycarboxylic acids

A tremendous amount of papers related to aminopolycarboxylic acids as the derivatization reagents of inorganic cations in CE have been published in the past decade. Aminopolycarboxylic acids such as EDTA [80–83], CDTA [28,84–86], DTPA [87] NTA [88] and TTHA [89] attract so much interest, because they can form very stable and water-soluble complexes with so many metal ions. However, it should be noted that the sensitivity of the formed complex is usually very low. The molar absorptive coefficient is at 10^3 M^{-1} cm^{-1} level. Some preconcentration procedures should be employed if they were used for determining trace metal ions.

EDTA. In 1992, Motomizu et al. [80] investigated a separation of Mg(II), Ca(II), Sr(II) and Ba(II) ions as their EDTA chelates by CZE. Another alkaline-earth metal ion Be(II) was not detected. With this method, metal ions in waste water and serum were determined. Baraj et al. [81] discussed the simultaneous determination of Cr(III), Fe(III), Cu(II) and
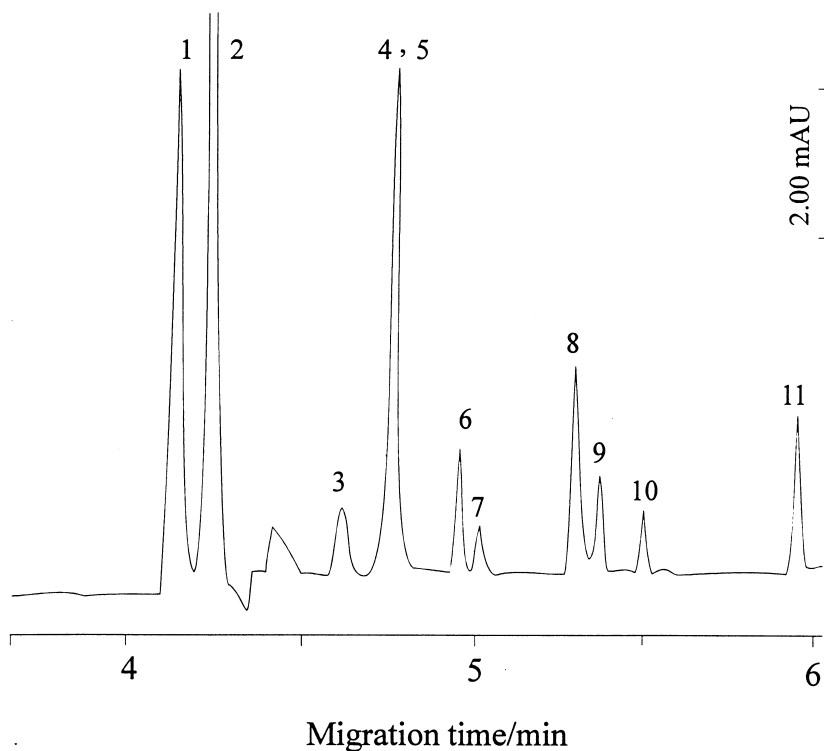
Fig. 9. Separation of metal-HEDTC complexes using methanol added to the BEG. Electrolyte, 100 mM borate buffer (pH 9.2), 10 mM SDS and 0.1 mM HEDTC, 4% (v/v) methol, with detection at 254 nm. Peak identities: $1=EOF$, $2=$oxidation product, $3=Cd^{2+}$, $4=Pt^{2+}$, $5=Pb^{2+}$, $6=Co^{2+}$, $7=Bi^{3+}$, $8=Ni^{2+}$, $9=Cr^{3+}$, $10=Cu^{2+}$, $11=Hg^{2+}$, $12=$HEDTC. Each metal ion was of concentration 10 μM. Reproduced from Ref. [45] with permission.

Pb(II) using EDTA By counter-EOF CZE. TTAB was chosen to induce the reversal of EOF. The detection limits achieved in this separation were in the range 6–27 μM. Schaeffer et al. [82] examined a novel method for the direct determination of Fe(II, III) and total iron. EDTA and o-phenanthroline(phen) were simultaneously utilized to complex Fe(III) and Fe(II) selectively and to achieve the separation of iron under its two oxidation states by CZE. To further obtain the total amount of iron, Fe(II) was oxidized into Fe(III), and a single complexing reagent EDTA was utilized.

CDTA. Compared with other aminopolycarboxylic acids, CDTA seems more useful to chelate with metal ions for CE separation. Timerbaev and co-workers [28,84–86] systematically studied the application of CDTA to separating various metal ions. In 1994, they found CDTA was most satisfactory after investigating the migration behaviour of lanthanoids as their four types of aminopolycarboxylic acids

complexes. In that separation 15 rare-earth elements were completely discriminated including Ce, Pr, Y, Nd, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb, Lu, and Sc. The method was further evaluated by applying it to two types of model samples from the nuclear power industry. Although the waste did not contain any transuranium elements which are inevitable in real-world samples and usually co-migrate with lanthanoids, no interferes were found in the presence of a 200-fold excess of uranium. Another similar report [85] concerning 13 lanthanoids metal-CDTA complexes was also presented for achieving the theoretical estimation of CZE behaviour of metal complexes using multivariate regression analysis.

DTPA. After examining several aminopolycarboxylic acids such as CDTA, EDTA and DTPA in detail, DTPA was chosen by Padarauskas and Schwedt [87] as the pre-column chelating reagent to achieve the CZE separation of Co(III, II), Bi(III), Fe(III), Cr(III), V(IV), Pb(II), Hg(II), Cu(II)and Ni(II) in a

single electrophoretic running with a detection limit range from 2×10^{-6} M to 8×10^{-6} M. The authors also investigated the simultaneous determination of V(IV, V) in weakly acidic buffer of pH 4.0, and Cr(III, VI) in weakly basic buffer (pH 8.0) under co-EOF situation induced by TTAOH surfactant.

NTA. More recently, Liu and Lee [88] reported a simultaneous analysis of inorganic and organic lead, mercury and selenium as their NTA complexes by SDS-based MECC. A theoretical model was also presented for validating the migration behaviour of metal complexes with the consideration of pH value of carrier electrolyte, SDS and NTA in the buffer. Detection limit down to 0.2 ng/ml were obtained by amplified field sample injection method.

TTHA. Triethylenetetraminehexaacetic acid (TTHA) is an aminopolycarboxylic acid with six carboxylic groups. It was utilized in titration analysis as a good mask agent originally. Recently, Liu and Lee [89] presented an excellent demonstration dealing with the separation of inorganic and organic lead, mercury and selenium as their TTHA complexes by CZE mode with on line complexation approach. After carefully discussed the influence of pH value of the buffer, the concentration of TTHA and SDS in the background electrolyte, a model was effectively established for describing the migration behaviour of the metal complexes. The applicability of the method was evaluated on seawater samples.

Quin2. Quin2 is an aromatic homolog of poly-aminocarboxylate. Compared with other amino-polycarboxylic acids, it can form more sensitive complexes with metal ions. Krokhin et al. [52] described the employment of Quin2 as the pre-column tagged agent for Fe(II), Ca(II), Pb(II), Zn(II), Co(II), Ni(II) and Cu(II). The separation of meal chelates was performed under IEEC conditions. A detection limit of 5.9×10^{-7} M was achieved, which was nearly 40-fold lower than that obtained by the employment of EDTA under the same separation conditions.

4.1.6. Other organic chelating reagents

β-Diketonate. The mobility of metal β-di-ketonate(acac) complexes in MECC system were studied by K. Saitoh et al. [90] Co(III), Cr(III), Pb(II) and Pt(II) complexes were separated success-fully in 30 min. A linear log–log relationship

established between the distribution coefficient and the partition coefficient of the complex between SDS and water was effectively used for prediction of migration behaviour of other metal complexes. Kat-suta and Nakatani [91] described a MECC method to evaluate the distribute constants of Nd(III), Sm(III), Tb(III) and Tm(III) between SDS micelles and water. No electropherogram was given by the au-thors.

Dithizone sulfonate. Dithizone can form intensive-ly coloured and stable complexes with a wide range of metal ions including heavy elements. But its complexes are uncharged and together with highly hydrophobic nature which makes them unsuitable for CZE separation. And no report of employment of dithizone was found. Jones and Hardy [35] utilized home-made chelating reagent dithizone sul-fonate(DzS) which can form water-soluble complex-es with metal ions for accomplishing the separation of trace inorganic and organomercury species. The authors measured methylmercury [92] in fish and crab meat.

H_2dapmp. H_2dapmp is also considered as a good chelating reagent regardless of the modest molar absorptivity of its complexes. It can react with a wide range of metal ions and formed very stable and water-soluble complexes. Because the formed com-plexes are cationic species, the electrophoretic be-haviour is not as described as usual. Timerbaev et al. [47] proposed the utilization of H_2dapmp in a CE separation of 11 metal species. Both CZE and MECC system were investigated. The later mode proved to be more effective for resolving these metal chelates, by using cationic surfactant CTAB as the micellar phase in the buffer.

Phen and its derivatives. In 1996, Ma and co-workers [93] conducted a CZE method for determi-nation of Fe(II) as its phen complex. The author selected 270 nm as the detection wavelength instead of 508 nm as usual. Although at this detection wavelength the detection background was very high, the sensitivity of the formed complex increased much higher than the background. A detection limit of 5×10^{-9} M was obtained, which is up to now the highest sensitivity obtained by derivatization pro-cedure in CE separation, and can even compete with that achieved usually by LIF detection scheme. Yokoyama et al. [94] report a group of separations of

five transition metal ions like Fe(II), Co(II), Ni(II), Cu(II) and Zn(II) after complexation with phen and phen derivatives such as 5-NO$_2$phen, 5-Clphen, 5-Mephen, 4-Mephen, 4,7-Mephen and 5,6-Mephen etc. 4,7-dimethylphen was found to be the best derivatization reagent among the phen derivatives for CE separation of metal ions, and was selected to the determination of Cu(II) in duralmin alloy.

4.2. Utilization of inorganic ligands

While utilization of organic ligands occupies the predominant status in CE separation of metal complexes, the use of inorganic ligand also plays an important role in this field, like cyanide(CN$^-$) and chloro(Cl$^-$), even though the sensitivity was worse than that achieved by the employment of organic ligand.

CN$^-$. Buchberger et al. [95] proposed a CZE separation of metal ions after conversion to their cyanide complexes. Fe(III, II), Ni(II), Cr(III, II), Ag(I), Co(III), Hg(II), Cu(I) Cd(II), Zn(II) and Pd(II) were effectively separated within 30 min. Other authors [96–100] also reported many similar separations of metallocyanide by CE. Because cyanide is wide spread used in hydrometallurgical industry, it is versatile to being utilized as the ligand in analysing metal ions in ores [96,97], industrial waste water [95] and monitoring environment pollution [92] by CE. Nevertheless, on the other hand the toxic nature of cyanide seriously limited its application in CE.

Cl$^-$. The employment of chloride ion as the complexation ligand of metal ions in CE can be dated to 1985. In that year Yoshia et al. [48] demonstrated a separation of seven metal-chloro complexes by cITP in a nonaqueous system. Zhang et al. [34] found that chloro ligand could be very useful for the separation and determination of palladium group elements in acidic environment, due to their belief in the advantage that chloro complexes of palladium group metals are the basic of the qualitative and quantitative analysis of these metals and they are usually dissolved in HCl–HNO$_3$ solution. The separation of palladium group elements as their chloro complexes were accomplished with both CZE mode and CTAB-based MECC mode. Baraj et al. [101] also expressed similar idea to other noble

metal ions like Au(III). Thornton and Fritz [36] performed a separation of the chloro complexes of osmium(IV), platinum(IV), rhodium(III), palladium(II), gold(III) and ruthenium(III) as illustrated in Fig. 10. Acidic solution of pH 2.4 was chosen for effectively compressing the EOF. It proved that molecule H$_2$O as the second ligand took part in the complexation with metal ions in place of chloro in part.

5. Manipulation of sensitivity

There is no doubt that the success of CE can essentially contribute to the employment of narrow-bore fused silica tube by scanning the development of electrophoresis. Because of large periphery to cross-sectional area ratio, much higher electric field is allowed for accomplishing high performance and rapid separation with less danger of excessive Joul heating compared with classic electrophoresis techniques. As another consequence of narrow-bore capillary, however, the detection sensitivity is quite damaged by the limitation of short optical length available if a popular UV detector is equipped for on-line detection. Consequently, the manipulation of the detection sensitivity becomes challengeable in CE. To analysis metal ions as their complexes, many methodologies have been wielded to achieve higher sensitivity, and will be discussed in this section.

5.1. Choice of ligand

The influence of ligand selected for complexation with metal ions is evidently direct and effective on detection sensitivity. First, ligand with higher molar absorptivity can achieve better detection limit. It is agreement with spectrophotometry measurement method as described by Beer law. To determinate alkaline-earth elements, for example, SAIII [71] is more favourable as the chelating reagent compared with EDTA [80]. The difference of detection limits achieved between the two ligands reaches up to nearly two orders of magnitude. Likewise [52], for sensitive detection of Fe(II) and Ni(II), Quin2 is superior over EDTA. Subsequently, the stability of the complex should be also taken into account as an important factor. It determines the concentration of
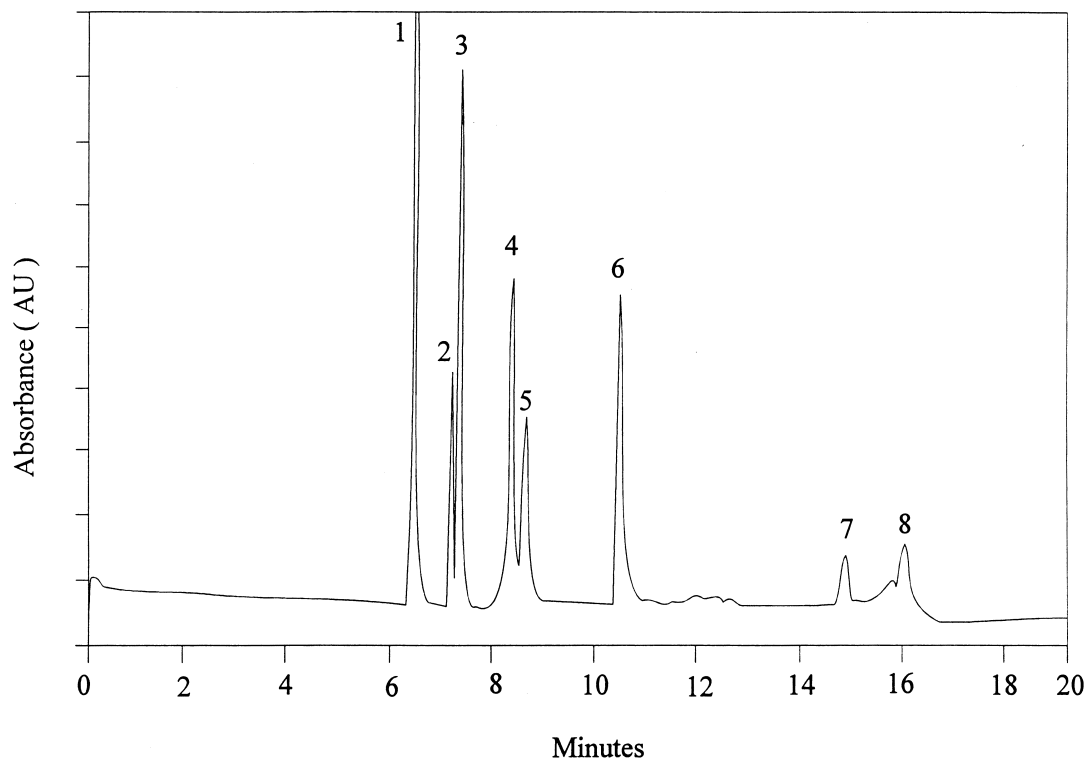
Fig. 10. Electropherogram showing the separation of the chloro complexes of osmium(IV), platinum(IV), rhodium(III), palladium(II), gold(III) and ruthenium at 10 μg ml^{-1} each. Peaks: (1) contaminant, (2) Os(IV)(OsCl$_6^{2}$), (3) Pt(IV)(PtCl$_6^{2-}$), (4) Rh(III)(RhCl$_6^{3-}$), (5) Pd(II)(PdCl$_4^{2-}$), (6) Au(III)(AuCl$_4^{-}$), (7) Pt(IV)[PtCl$_5$(H$_2$O)]$^{-}$ and (8) Ru(III)[RuCl$_3$OH(H$_2$O)2]$^{-}$. Conditions: fused-silica capillary, 60 cm(52.5 cm to detector)×75 μm I.D.; carrier solution, 4 mM H^{+}−25 mM Cl^{-}; applied voltage, −10 kV; UV detection at 214 nm; sampling time, 30 s. Reproduced from Ref. [36] with permission.

ligand added to the carrier. Lower concentration of the ligand in the buffer means lower detection background and higher ratio of signal to noise. Finally, the selection of detection wavelength should be paid more attention to. Xu et al.'s work [93] gave

Table 2
Detection limits (mol/l) of metal ions as PAR complexes

Metal	Detection wavelength (nm)	
ion	254[a]	500[b]
Co(II)	$8 \cdot 10^{-7}$	$1 \cdot 10^{-7}$
Cu(II)	$2 \cdot 10^{-6}$	$6 \cdot 10^{-7}$
Fe(III)	$4 \cdot 10^{-6}$	$9 \cdot 10^{-7}$
Ni(II)	$3 \cdot 10^{-6}$	$1 \cdot 10^{-6}$
Zn(II)	$2.4 \cdot 10^{-5}$	$8 \cdot 10^{-7}$

[a], Quanta 4000; injection time, 5 s (ca. 8 nl).
[b], Model 270 A; evaluated in a 8 nl injection volume.
Reproduced from Ref. [42] with permission.

a powerful evidence while determining Fe(II) ion as its phen complex by CE. At 508 nm as used in usual, a detection limit of 3.0×10^{-6} M was achieved. However, with option of 270 nm in place of 508 nm, the detection limits could be lowered to 5×10^{-9} M(600-fold lower). Timerbaev et al. [42] compared the results of detection limits achieved at two different wavelengths as shown in Table 2 when they performed a CE separation of metal-PAR complexes. Based on Table 2, it could be concluded that right choice of detection wavelength could result in re-markable improvement of detection sensitivity.

5.2. Utilization of alternative detection scheme

Fluorescence detection is versatile to CE in an on-column configuration, especially LIF, which is much preferred due to its high sensitivity. Because

inorganic cations are seldom intrinsically fluorescent, derivatization procedure should be carried out. However, few chelating reagents can form metal complexes with fluorescence. As a result, the application of LIF detection scheme is limited in several reported separations of metal-HQS chelates [27,56,76].

Chemiluminescence (CL) is also considered as a highly sensitive detection method. Tsukagoshi et al. [79] demonstrated a CE separation of copper(II),as its ED complex coupling a CL detector. Tris(2,2′-bipyridine)ruthenium(II) ion $(Ru(bpy)_3^{2+})$ electrogenerated CL system was utilized and was expected to be useful for analysing transition metal ions. However, the obtained result was not as exciting as achieved by Flow injection analysis (FIA) system. A modification of the detection cell geometry, flow rate of the CL reagent needed further improvement.

Hu et al. [70] proposed a photothermal interference spectrometry (PIS) detection scheme to accomplish the detection of some lanthanoids as their TBDA complexes. This crossed-beam thermoptical detection provide a concentration limit of 2.2×10^{-8} M for La(III)-TBDA complex.

Seibel and Faubel [102] newly introduced laser induced thermal lens spectroscopy as a sensitive on-column detection scheme into CE for determination of Fe(II)-phen complex. A limit of detection of 3.6×10^{-8} M was accomplished with a signal-to-noise ratio of 7:1.

5.3. Preconcentration by on-line sample stacking

Sample stacking is wide spread used as an easily operated on-line preconcentration method for improving detection sensitivity. As a presupposition, the ionic strength of sample should be lower than that of carrier electrolyte. It causes a non-uniform distribution of electric field across the whole capillary while the high voltage is applied. Obviously, the electric field in sample zone is higher than in the running buffer due to its higher resistance resulted from its lower ionic strength. As a result, ionic species migrating with higher velocity in the sample zone will be slowed down at the boundary between sample zone and buffer. Thus, the sample segment is compressed to be a narrow zone and be concentrated before real electrophoresis.

As performed in usual, a large volume of sample dissolved in pure water or diluted carrier electrolyte is injected hydrostatically into the capillary. Colburn et al. [103] reported a sample stacking approach to determine low ppb levels of uranyl cations as its AAIII form by CE. Because U(VI)-AAIII complex has an electrophoretic velocity greater in magnitude and opposite in direction than the velocity of EOF under selected CE conditions, the sample solvent is backed out into the buffer container as the complex stacks at the boundary between the sample and the running buffer. Although increasing the length of injected sample plug will result in the increasing of preconcentration factor in theory, the factual injection length should be controlled within a effective range due to the existence of laminar flow, which originate from the different local EOF value resulted from the non-uniform distribution of the electric field in the capillary. In that experiment, the authors optimized the injection plug, and a length of 7.8 cm was chosen as the optimum introduction volume. For cationic metal complexes, the direction of EOF should be reversed and a polarity switching should be performed to achieve such electrostacking procedure as described by Smyth et al. [64]. In this situation, the sample solvent is removed by applying a positive voltage. Initially, the electric current is low and resistance is high because of the presence of sample solvent. The current was allowed to increase until it reaches a level, usually 95% of the highest value to avoid the loss of sample components, and then, a polarity switching is performed to start electrophoresis.

The sample stacking method can also be utilized with amplified field sample injection. Because the matrix differences between sample and running buffer is desired in ionic strength, ions experience a higher electric field in sample than in running buffer, thus, they will stack up into a smaller volume at the inlet of the capillary. Liu et al. [88] performed sample stacking by electrokinetic injection in a determination of some elements in organic and inorganic species as their NTA complexes. A 40–600-fold on-line enrichment was achieved. In another paper [89] the authors achieved up to 1500-fold on-line enrichment and down to sub-nanogram-per-milliliter detection limits when analysing metal-TTHA complexes. It was also used by Aguilar et al.

[96] to determine the metal cyanide complexes in gold processing solution with the detection limit of mid-ppb level. Regan et al. [60] described an in-situ complexation with PAR following trace enrichment by sample stacking. The metal ions as their free forms in sample were injected by amplified field sample injection method, then derivate with PAR in a specified plug in the capillary. The detection limit was down to 1.0×10^{-8} M which could fulfil the requirement of the analysis of trace metal ions in a pound water.

5.4. Preconcentration by on-line coupling of capillary isotachophoresis

Currently, cITP is utilized mainly as a high-performance preconcentrator and couples with other separation techniques as described in Section 2.3. To analyse metal ions as their complexes by CE, such preconcentration is necessary for the demand of the detection sensitivity. Blatny et al. [104] illustrated an on-line coupling of cITP and CZE method to determine iron as its conversion form of Fe(III)-EDTA complex in water at the μg/l level. 10 mM HCl+20 mM L-histidine+0.1% hydroxypropylmethyl cellulose(pH 6.0), 5 mM MES and 25 mM MES+10 mM bis-tris-propane(pH 6.6) were severed as leading, tailing and background electrolytes, respectively. And a detection limit of 10 μg/l for Fe(III) was accomplished. Compared with the result without cITP preconcentration procedure by other authors, the detection limit was improved by a factor of about 50-fold.

5.5. Preconcentration by supported liquid membranes

Over the past few years, supported liquid membranes(SLMs) have gained great attention as a powerful sample preparation and preconcentration procedure, due to the easy operation, low consumption of reagent and higher efficiency of extraction. Because a diffusive transport of analytes across the liquid membrane employed in SLMs from a relatively large volume of sample into a much smaller volume of acceptor solution, it can be utilized as an effective preconcentration method. Recently, it was Kuban et al. [99] who proposed the first application

of the combination of SLMs and CE. The authors discussed several factors to optimize SLM system, such as the concentration of the ion paring reagent in the organic phase, the reagent in the acceptor phase and the flow-rate of the sample solution. Determination of metallo-cyanides by CE after concentration on SLM was performed with an enrichment factors ranging from 50 to 600 folds for cyanide complexes of Fe(II, III), Ni(II), Co(II), Pd(II), Pt(II), Cr(III), Au(I), and Ag(I). the detection limits can reach up to 6.6×10^{-9} M.

6. Methodologies on improving selectivity

To resolve metal cations by CE, the formation of complexes by pre-column or on-column presents an access to enlarge the differences in their elecrophoretic mobility. Nevertheless, it can be usually unequal to all the problems in separation selectivity. Therefore, many further methodologies are performed to achieve the improvement of the selectivity while separation metal ions as their complexes. Massart and co-workers [105] reviewed the method development for the separation of small ions by CE. System and sample variable and buffer electrolyte were under their consideration for the optimization of the separation conditions. Recently, Timerbaev [13] also discussed the selectivity control in CE of metal species. In this section, only several major methodologies on the enhancement of selectivity of metal complexes are surveyed briefly.

6.1. Variation of buffer pH value

Buffer pH has much influence on the separation selectivity of metal complexes. It controls the improvement of resolution of complexes through three approaches. (i) Adjusting the behavior of EOF. Buffer pH value predominantly determines the value of EOF if no modification procedures on EOF are performed. So that, variation of buffer pH can be a very convenient method to control EOF and separation resolution [85] as described by Eq. (2) given in Section 2.1.1. (ii) Controlling the acid/base dissociation equilibria of complexes. On one hand, variation of buffer pH can result in the change of charge density of complex [85], which is directly

proportional to its μ_{ep} as it derived from the following equation:

$$\mu_{ep} = I_e/(6\pi\eta) \qquad (3)$$

where I_e and η are charge density of complex and the viscosity of the medium. On the other hand, different metal ions as their complexes have different response to the variation of buffer pH due to their different acid/base dissociation constants [40]. (iii) Changing the existing state of complex. For example, it was reported by Zhang et al. [34] that the $PdCl_4^{2-}$ complex slightly hydrolyses with increasing pH. The hydrolysate might be $PdH_2OCl_3^-$ or $PdOHCl_3^{2-}$ etc. Timerbaev et al. [84] also reported similar phenomenon while separate lanthaloids as their CDTA complexes. It can be viewed as the formation of binary ligands complexation. OH^- acted as the second ligand to participate the coordination of metal ions.

6.2. Solvent effect

Organic solvent is often added to the running buffer for improving the selectivity of neutral complexes. It can ameliorate the solubility of hydrophobic complexes [77], reduce the adsorption onto the capillary wall [77], regulate the distribution of complexes between aqueous phase and micellar phase [45], adjust the viscosity of the separation medium [28], accordingly accomplish the improvement of selectivity.

6.3. Micellar interaction

Micellar interaction functions in CE is based on two mechanisms: partition and ion-association. It is determined by the charge or the polarity of micelle and metal complex which mechanism on earth participates in the improvement of selectivity while separate metal chelates. For separation systems that micelle and complex own charges with opposite symbol, the two mechanisms function at the same time. And if employed micelle and complex are with identical charge symbol, only partition mechanism can be responsible for the enhancement of resolution.

6.4. Ion paring effect

Ion pairing can be used as effective approach to improve separation. Iki et al. [73] systematically investigated the effect of ion paring agents on the migration behaviour of metal complexes in free solution CE. Six types of ion pairing agents were investigated for optimizing the separation conditions. Iki et al.'s work proved the important value of the employment of ion pairing effect for the separation of the metal chelates. Additionally, ion-pairing agent can be utilized in MECC system to regulate the partition process between the micelle and the bulk solution of hydrophobic or weakly hydrophobic metal complexes [42].

7. Applications to speciation analysis

Based on the derivatization method, CE has been successfully applied to the separation and simultaneous determination of inorganic cations in a wide range of fields, as reviewed in previous sections, such as industry, agriculture, environment, pharmaceutical, clinic, geology and nuclear technique etc. Recently, it has been increasingly recognized that the physical and chemical properties of a certain metal species depend critically on its oxidation state and binding forms with organic binding partners. Consequently, a comprehensive understanding of speciation of metal ions is of special interest and importance in researches of biology, chemistry, physics and environment etc. More recently, Dabek-Zlotorzynska et al. [14] reviewed the state-of-the-art of CE for metal speciation analysis. In that review, a further investigation of strengths and limitation of CE and comparison with conventional chromatographic techniques with respect to speciation studies were described.

Fe(II)/Fe(III). Iron, as one of most common elements in the earth crust and an active centre serving in the biosphere has attracted much interest in CE separation. Schaeffer et al. [82] reported the determination of Fe(II, III) with CZE system. EDTA and phen were added to the sample solution for accomplishing the pre-column derivatization with

Fe(III) and Fe(II), respectively. Pozdniakova et al. [106] also decribed a simultaneous determination of Fe(II, III) after selectively complexed with phen and CDTA. The detection limits achieved were 60 ppb for Fe(II) and 100 ppb for Fe(III). This method established was applied for the determination of iron species in tap water and ground water. Aguilar et al. [97] separated Fe(II) and Fe(III) as their cyanide complexes in 5 min. The results of separation were applied to the determination of iron cyanide complexes as impurities in samples from zinc electroplating processes. Other researches conducted the determination of the two iron species by CE together with many other metal ions based on derivatization with varied ligands such as PAR [42], 5-Br-PAPS [67] and CN^- [99,100] etc.

Cr(III)/Cr(VI). Cr(III) and Cr(VI) are oppositely charged in form of Cr^{3+} and CrO_4^-. To accomplish the separation and the determination by CE in only one run, usually, Cr(III) is converted into its anionic complex. Timerbaev and co-workers [107,108] proposed the separation of speciation of chromium ions by CZE. Cr(III) existed as a formed of CDTA complex by adding excessive CDTA ligand (20:1) in the sample solution. With the borate buffer of pH 9.0, the separations were quite unsatisfactory with either negative or positive power supply due to the large velocity of EOF. For rendering the analysis time in a reasonable range, acidic buffer of pH 3.0 was chosen by the authors to suppress the EOF. Although a rapid separation of Cr(III) and Cr(VI) was achieved in 5 min, and the method was further applied to simultaneous determination of the two states of oxidation of chromium in electroplating sample and pharmaceutical sample, the electrophoretic peaks were asymmetry stemmed from a great difference in the mobility values between analyte and buffer electrolyte. It could be overcome by adding cationic surfactant CTAB in the running buffer as reported by Jung et al. [109] while separating CrO_4^- and Cr(III)-EDTA complex. The addition of CTAB reversed the direction of EOF and resulted in a short analysis time and better peak shape with high pH value buffer. Padarauskas and Schwedt [87] presented a determination of Cr(III) as its DTPA complex and Cr(VI) in waster water obtained from electroplating plant. TTAOH was employed in the

carrier to induce the reversal of EOF, which was converted from TTAB. The presence of bromide ion could reduce the detection sensitivity.

V(IV)/V(V). Jen et al. [110] separated vanadium(IV, V) as their EDTA complexes by CE. Hexadecyltrimethylammonium bromide (HTMAB) was used as a modifier of capillary column to decrease the velocity of EOF. The method was applied to determination of waste catalyst-leachate sample. Padarauskas and Schwedt [87] reported a similar separation of vanadium species after complexation with DTPA. Although the analysis time was found to be 26min and band broadening occurred, common inorganic anions such as NO_2^-, NO_3^-, Br^-, CrO_4^{2-} and MoO_4^{2-} showing higher mobility and most other metal chelates showing lower mobility did not interfere in the determination of V(IV) and V(V).

Organometallic compounds. Organomercury is one of the most dangerous chemical species in environment owing to its toxicity to living organisms. Medina et al. [111] studies a CZE method to determine methylmercury, ethylmercury, phenylmercury and inorganic mercury which were extracted from marine samples by conventional Westoo procedure thus giving their cysteine complexes. Results were compared with those obtained by gas chromatography(GC) commonly accepted procedure. For improving the detection limit, Carro-Dias et al. [112] recently reported a CE separation of methylmercury with sample stacking. In that paper a detection limit of 12 ppb for methylmercury was achieved. The method was evaluated by different reference materials with a certified methylmercury content. Jones and Hardy [35] demonstrated the separation and determination of trace inorganic and organomercury species as their DzS complexes. Capillary column was coated with polyacrylamide to eliminate the EOF. As shown in Fig. 11, four mercury species and the ligand were separated in 7 min with detection limits in the low ppb range. Other foreign metal ions like Ag, Au, Cd, Co and Pd gave distorted peaks close to inorganic mercury but well away from the organomercury peaks. The authors determined methylmercury in fish and crab meat after extraction as DzS complexes by the established method [92]. A classical Westoo extraction procedure was utilized
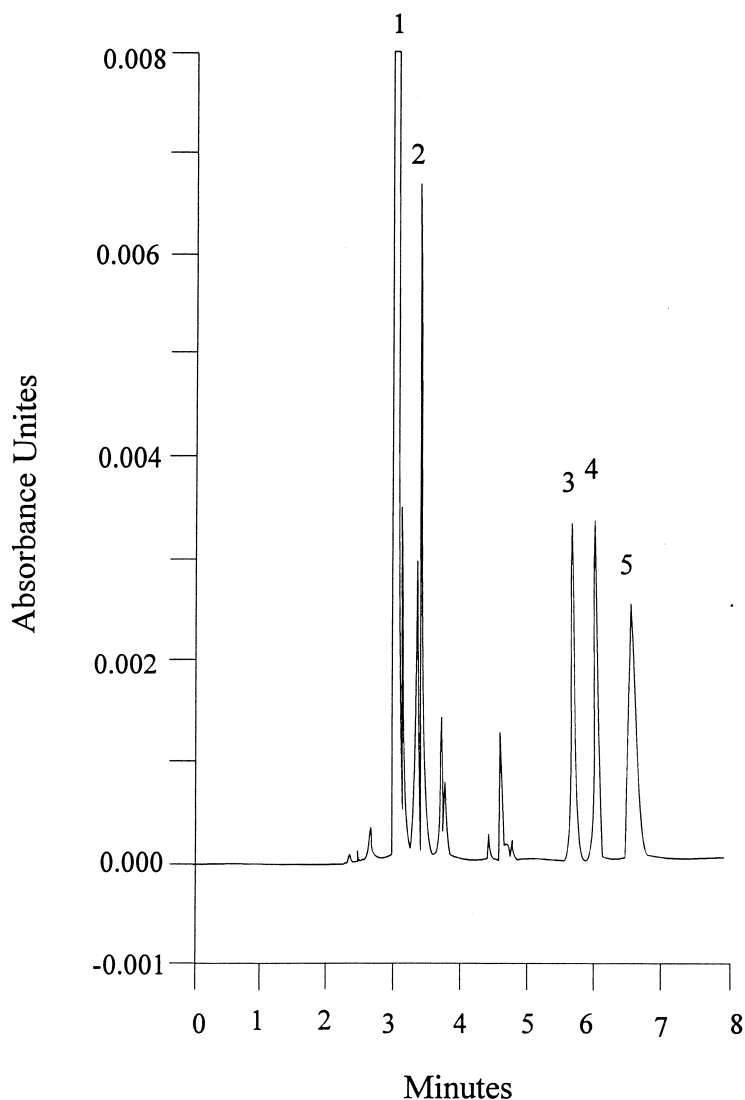
Fig. 11. Separation of inorganic and organomercury species. Separation conditions: buffer = 10 mM sodium acetate, pH 5, containing 5 mg l^{-1} DzS. Voltage + −25 kV; Detection = 480 nm; Hydrostatic injection for 30 s with a 100 mm height difference. Capillary, fused-silica, internally coated with polyacrylamide, 60 cm effective length 55 cm × 100 μm I.D. 1 = DzS; 2 = Hg, 1 mgl^{-1}; 3 = MeHg, 1 mgl^{-1}; 4 = EtHg, 1 mgl^{-1}; 5 = PhHg, 1 mgl^{-1}. Reproduced from Ref. [92] with permission.

before CE separation. Liu and Lee [88,89] proposed simultaneous analysis of inorganic and organic lead, mercury and selenium as their NTA and TTHA complexes by MECC mode and CZE mode. SDS proved to be useful not only for improvement of selectivity also for enhancement of sensitivity.

Theobald and Dunenann [113] described the anal-

ysis of metal ions and their organic binding partners in biological system like cow's milk and human milk by CZE. Sodium chloride was utilized as the carrier electrolyte because of its low UV absorption and its weak complexing ability instead of phosphate and Tris buffer. The samples were defatted by centrifugation and ultrafiltrated. Thus, the results of metal

species with citrite and glutamate were obtained in presence of only low molecular weight substance.

8. Future prediction

Although analysis of metal ions as their complexes by CE has been rapidly developed over the past decade, contrary to other applications of CE, there is still a long way to run to make it a matured and routine method. It remains large virgin land and developing space either in theory or in application. Application to metal species analysis and accomplishing simultaneous separation of inorganic cations and anions in a single electrophoresis run will be the hopeful and challengeable developing direction where the strength of this method is. Additionally, it also suggests based on the recent literature that further development in CE on separation metal complexes is as described as follows:

1. Synthesize and screen versatile chelating reagents for the demand of derivatization with metal ions interest.
2. Couple with on-line or off-line preconcentration approaches.
3. CEC as a newly developed CE or HPLC strategy is expected to be a more reasonable choice due to the hybrid combination of advantages of CE and HPLC.
4. Broaden its applications to various factual samples.

9. Conclusion

CE has proved to be a powerful technique for the analysis of inorganic cations by conversion into their organic and inorganic complexes. Much work has been done focusing on the development of separation strategies, choice of suitable derivatization reagent, manipulation of sensitivity and selectivity and application to the analysis of various real-world samples. Although this method just makes its initial step of progress and has not been fully exploited compared with other application fields of CE and HPLC separations concerning metal complexes, the blueprint constructed by literatures exhibits high potential

of derivatization-based CE method in application to the analysis of trace metal ions, due to its high separation efficiency and resolution, low time, sample and reagent consumption, as well as other important advantages like the immunity of the interferes from complicated sample matrix and the improved detection sensitivity etc. stemmed from the combination of CE with the derivatization method.

10. Abbreviations

AAIII	Arsenazo III
acac	Acetylacetone
ACE	Affinity capillary electrophoresis
5-Br-PADAP	2-(5′-Bromo-2′-pyridylazo)-5-diethylaminophenol
5-Br-PAPS	2-(5-Bromo-2-pyridylazo)-5-(N-propyl-N-sulfopropylamino)-phenol
BTAR	4-(2-Benzothiazolyazo)resorcinol
BTP	Bis-tri-propane
CDTA	Cyclohexane-1,2-diaminetetraacetic acid
CD	Cyclodextrin
CE	Capillary electrophoresis
CEC	Capillary electrochromatography
CGE	Capillary gel electrophoresis
cIEF	Capillary isoelectric focusing
cITP	Capillary isotachophoresis
CL	Chemiluminescence
CMC	Critical micellar concentration
CTAB	Cetyltrimethylammonium bromide
CZE	Capillary zone electrophoresis
DBC-AS	2-(2-Arsenophenylazo)-1,8-dihydroxyl-7-(4-chloro-2,6-dibromophenylazo)-naphthalene-3,6-disulfonic acid
DBC	Dodecylbzone sodium sulfonate
DHABS	2,2′-Dihydroxyazobene-5,5′-disulphonate
DMF	Dimethyl formamide
DTPA	Diethylenetriaminepentaacetic acid
DzS	Dithizone sulphonate
ED	Emetine dithiocarbamate
EDTA	Ethylenediaminetetraacetic acid
EOF	Electroosmotic flow
FIA	Flow injection analysis

H₂damap	2,6-Diacetylpyridine-bis(N-methylenepyridinohydrazone
HEC	Hydroxyethylcellulose
HEDTC	Bis(2-hydroxyethyl)dithiocarbamate
HP	Haematophoryrin IX
HPLC	High-performance liquid chromatography
HQS	8-Hydroxyquinoline-5-sulfonic acid
HTMAB	Hexadecyltrimethylammonium bromide
ICP-MS	Inductively coupled plasma
IEEC	Ion-exchange electroknetic chromatography
LIF	Laser-induced fluorescence
MBTAE	2-(5-Methyl-2-benzothiazolyazo)-5-diethylamino phenol
MC	Microchip
MECC	Micellar electrokinetic capillary chromatography
NACE	Nonaqueous capillary electrophoresis
5-NO₂-PAPS	2-(5-Nitro-2-pyridylazo)-5-(N-propyl-N-sulfopropylamino)-phenol
NTA	Nitrilotriacetic acid
OP	*p*-Octylpolyethylene glycol phenylether
PAA	Benzoylhydrazone
PDMAC	Poly(diallyldimethylammonium chlorid
phen	*o*-henanthroline
PP	Protoporphyrin IX
PVPyB	Poly-N-ethyl-4-vinylpyridinium
Quin2	8-Amino-2[(2-aminomethylphenoxy)methyl-6-methoxyquinoline-N,N,N′,N′-tetraacetic acid
RP-HPLC	Reversed-phase high-performance liquid chromatography
SAIII	Sulfonazo III
SDS	Sodium dodecyl sulfate
SLM	Supported liquid membrane
SOS	*n*-Octanesulfonate
TAR	4-(2-Thiazolyazo)resorcinol
TBA	Tetrabuthylamine
TBDA	2-[(2-Arsenophenyl)-azo]-1,8-dihydroxy-7-[(2,4,6-tribromophenyl)azo]-naphthalene-3,6-disulfonic acid
TCPP	α, β, γ,δ-Tetrakis(4-carboxypenyl)porphine
TRLIF	Time-resolved laser induced florescence
TTAB	Tetradecyl-trimethylammonium bromid
TTAOH	tetradecyl-trimethylammonium hydroxide
TTHA	Triethylenetetraminehexaacetic acid
XO	Xylenol orange

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