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Review

Separation of metal ions and metal-containing species by micellar electrokinetic capillary chromatography, including utilisation of metal ions in separations of other species

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Abstract

The use of micellar electrokinetic capillary chromatography (MEKC) for the separation of metal ions and metal-containing species is reviewed, together with the use of metal ions as a means to separate other species. Topics covered include the manipulation of separation selectivity through the use of complexation reactions induced by addition of a metal ion to the background electrolyte, enantiomeric separations facilitated through metal–analyte interactions, separation of organometallic species, separation of stable metal complexes in which the entire complex is the analyte and the separation of metal ions as analytes using pre-capillary or on-capillary complexation reactions with a suitable ligand. © 1997 Elsevier Science B.V.

Keywords: Reviews; Background electrolyte composition; Enantiomer separation; Metal cations; Metal complexes; Organometallic compounds

Contents

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1. Introduction

In this review, the separation of metal-containing species by micellar electrokinetic capillary chromatography (MEKC) is discussed. The scope of the review includes the following: (i) separations in which a metal ion or metal complex is added into the background electrolyte (BGE) in order to facilitate separation of other solutes, including enantioselective separations. (ii) Separations in which a metal-containing compound or a metal ion is the analyte. These separations can be subdivided into the following two categories: (a) separation of analytes such as stable metal complexes and organometallic compounds, including enantioselective separations and (b) separation of metal ions as analytes by converting them to complexes of a suitable ligand to facilitate detection and/or to influence the separation selectivity.

MEKC separations are characterised by the presence of surfactant micelles in the BGE, but it should be noted that the critical micellar concentration (CMC) depends strongly on parameters such as temperature and BGE composition [1]. As an example, the CMC for sodium dodecyl sulfate (SDS) in aqueous solution can vary over a range of more than an order of magnitude, according to the conditions used (see Table 1). Consequently, in cases where the CMC has not been determined experimentally, it may not be possible to see from a reported BGE composition whether micelles were present or not. Also, surfactants are often used as BGE additives for purposes other than establishing a micellar solution; e.g., long chain quaternary ammonium compounds are used as EOF modifiers [2] and can be present in the BGE at a concentration above the CMC without any specific recognition that micelles are formed [3]. For these reasons, the border between MEKC and non-micellar CE (capillary zone electrophoresis, CZE) can often be somewhat diffuse, which makes it difficult to decide unequivocally whether a published method falls into the class of MEKC.

The separation selectivity exhibited by metal complexes can be expected to involve not only hydrophobic interactions with the micelles but also polar interactions since compounds incorporating metal atoms can generally be considered to exhibit some polar characteristics. As shown throughout this review, the interaction of metal-containing solutes with the micelle can be either through diffusion into the interior of the micelle or through interaction with the outer surface of the micelle. In the latter case, this interaction may occur either with the charged functional groups of the surfactant or in the case of negatively charged micelles of SDS, with metal cations associated with the negatively charged functional groups of the surfactant. Although the precise mechanism of interaction with the micelle can vary, both interactions lead to an increase in the migration time of the analyte and are often referred to as partitioning into the micelle. Finally, when the metal-containing analytes are charged, the separation selectivity exhibited in MEKC comprises contributions from the electrophoretic migration based on charge (as in CZE) and the interaction of the analyte with the micelle.

2. Separations using a metal ion or metal complex added into the BGE

We consider here those separations which are

Table 1
CMC for SDS at different conditions

Solution composition	CMC (m*M*)	Ref.
Water (at 20°C)	8.2	[4]
5 m*M* borax pH 9.2 (25°C)	5.29	[5]
3 m*M* disodium phosphate	5	[6]
12.5 m*M* phosphate–2.5 m*M* tetraborate buffer (pH 6.8)	5	[7]
50 m*M* phosphate (pH 2.05)	4	[8]
25.0 m*M* phosphate–5.0 m*M* tetraborate buffer (pH 6.8)	2.8	[7]
20 m*M* tetraborate (pH 9.2)	2.6	[7]
0.1 *M* NaCl	0.52	[4]

achieved by the adding a metal ion or a metal complex into the BGE. The basis of separation rests in the establishment of secondary complexation equilibria between the introduced metal ion or metal complex and the analyte(s) to be separated. If the metal complex introduced in the BGE contains a chiral ligand, this introduces enantioselectivity for separation of analytes which can act as ligands towards the metal atom.

2.1. Non-enantioselective separations

Metal ions are well known to adsorb on silica [9]. In CZE and MEKC, carried out in fused-silica capillaries, the addition of metal ions (especially di- and trivalent metals) into the electrolyte usually results in their adsorption onto the capillary wall, leading to a decrease of electroosmotic flow (EOF) [10–17]. Since a decrease in EOF leads to longer migration times in the counter-electroosmotic mode using SDS micelles, this can improve the resolution without changing separation selectivity. However, there are several examples in the non-micellar CZE literature showing dramatic changes of selectivity as a result of adding metal ions into the BGE. In these cases, metal complexes are formed with some of the analytes, leading to separation of previously comigrating species [4,18–23].

There is a growing number of reports which indicate the presence of interactions or adsorption of divalent metal cations onto the negatively charged functional groups of the anionic SDS micelle. These interactions result in an increase of the electrophoretic mobility (i.e., a less negative mobility) of the SDS micelle [13–17]. From a practical viewpoint, there is a potential danger of precipitation of a SDS salt with a particular divalent metal [14], however, Macka et al. have demonstrated for a SDS-based BGE that clouding of the BGE caused by addition of zinc cations can be overcome by an addition of a weakly complexing ligand, such as citrate [15].

Applications of the addition of metal ions to the BGE in MEKC include separation of bases, nucleosides and oligonucleotides [13], oligosacharides [14], metallochromic ligands [15], sulfonamides [16] and polyaminocarboxylic acids [17]. In 1987, Cohen et al. [13] used additions of Cu(II) or Zn(II) to a SDS-based BGE to separate bases, nucleotides and nucleosides. Comparison of separations of a polythimidine mixture without added metal ion and with additions of Mg(II) or Cu(II) to the BGE (Fig. 1) shows a dramatic improvement of the resolution upon the addition of magnesium ions. Another application presented was an improvement in the separation of oligonucleotides in an SDS-based BGE upon addition of Mg(II), Zn(II) or Cu(II), with zinc ions offering the best resolution. In the latter example, the observed decrease of the capacity factor of the micelles when the metal ion was added was considered to be evidence for adsorption of the metal ions on the micelle surface. Complexation of the analytes with the metal ions attached to the surface of the micelle was expected to be a major component of the migration mechanism, as illustrated in Fig. 2.

Taverna et al. have used additions of Mg(II) to a SDS-containing BGE to improve separation of oligosacharides released from glycoproteins [14]. They observed a decrease of electrophoretic mobility of the micelles and a reduced negative electroosmotic mobility with increasing Mg(II) concentration in the BGE. They also investigated the influence of the concentration of the sodium phosphate buffer in the BGE, which confirmed the existence of competition between the magnesium and sodium cations for the anionic sites both at the fused-silica capillary wall and on the functional groups of the SDS micelles.

Manipulation of separation selectivity and detection properties for separation of metallochromic ligands through complexation with Zn(II) added to an SDS-based BGE (formate–diethanolamine buffer, pH 9.7, 35 m*M* SDS) has been demonstrated by Macka et al. [15]. Although the zinc ions generally caused poor peak shapes for the metallochromic ligands, probably due to adsorption of the analytes via the sorbed metal ions, the problem was rectified by addition of citrate to the BGE. Under the conditions used, citrate was a weaker ligand than the metallochromic ligands themselves, but nevertheless served to retain good peak shape. This approach proved successful for some ligands (xylenol orange and arsenazo I), but peaks for some other ligands (arsenazo III and chrome azurol S) remained broad.

Silver(I)-mediated separations by MEKC using an SDS-containing BGE have been applied by Wright and Dorsey [16] to the separation of a number of sulfonamides exhibiting selective complexation with Ag(I). Analytes which were previously unresolved

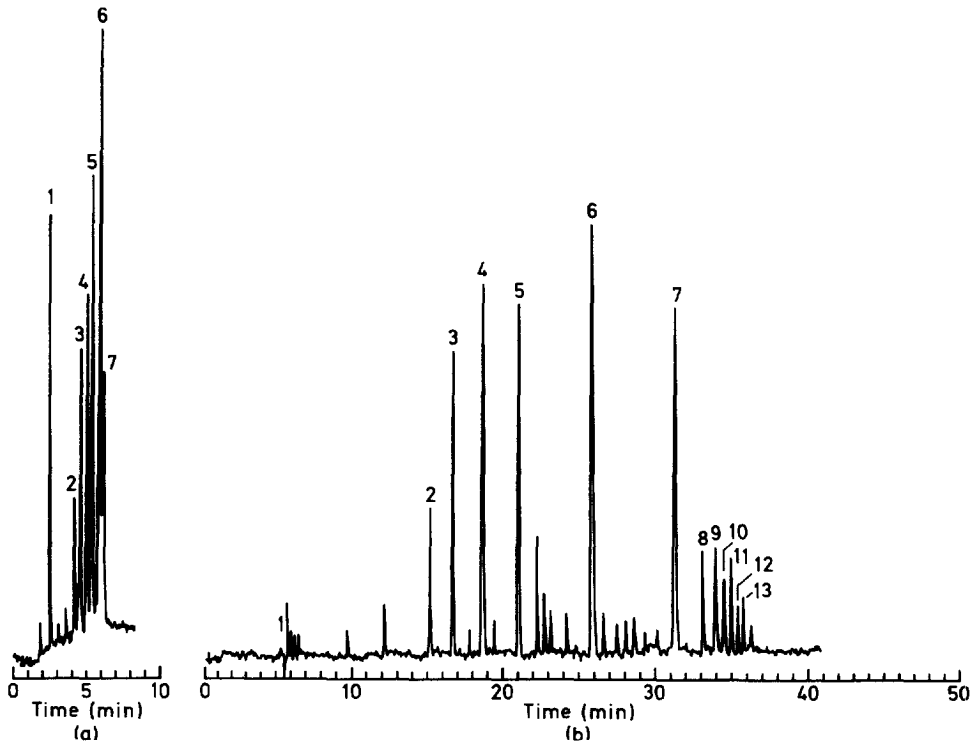

Fig. 1. Separation of a polythimidine (pT) mixture in a SDS BGE (a) without any metal ion addition and (b) with an addition of 5 m*M* Mg(II) to the BGE. Peaks: 1=solvent, 2=$d(pT)_2$, 3=$d(pT)_3$, 4=$d(pT)_4$, 5=$d(pT)_6$, 6=$d(pT)_{10}$, 7=$d(pT)_{12}$–$d(pT)_{18}$, 8=$d(pT)_{13}$, 9=$d(pT)_{14}$, 10=$d(pT)_{15}$, 11=$d(pT)_{16}$, 12=$d(pT)_{17}$, 13=$d(pT)_{18}$. Conditions: capillary, fused-silica; 0.650 m (0.450 m to detector)×50 μm I.D.; temperature, 25°C; BGE, 7 *M* urea, 5 m*M* Tris, 5 m*M* Na_2HPO_4, pH 7; separation voltage, 20 kV (10 μA); detection wavelength, 260 nm. Reproduced with permission from Ref. [13].

without Ag(I) could be separated using this approach. After addition of Ag(I) to the BGE, the migration times for analytes forming a complex with Ag(I) were increased greatly, as was the retention window (t_{mic}/t_0). This was explained by the interactions occurring between the negatively charged SDS micelle and the Ag(I) cation, together with complexation between the neutral analyte and Ag(I).

Separations of several polyaminocarboxylic acids as their stable Cu(II) complexes in both CZE and MEKC have been conducted by Harvey [17]. Both cationic [100 m*M* cetyltrimethylammonium bromide (CTAB)] and anionic (75 m*M* SDS) surfactant-based BGEs were used and their comparison offers an interesting view of the selectivity of these micellar systems. In the SDS system the CuEDTA complex (which carried the highest negative charge) was the most mobile and migrated as the last analyte in this counter-EOF separation. In the CTAB system, and using negative polarity at the injection side (as for classical anion analysis with reversed EOF), the observed migration order was the same as for the SDS system, which indicated that the CuEDTA now had the lowest electrophoretic mobility and therefore the strongest affinity for the micelle (Fig. 3). However, the extent to which the opposite charge of the analyte (−) and the CTAB (+) contributed to this interaction is not clear, since electrophoretic mobilities in the different separation systems were not given.

2.2. Enantioselective separations

The separation of enantiomeric species by MEKC utilising formation of a metal complex has been reported. The technique relies on the formation of a

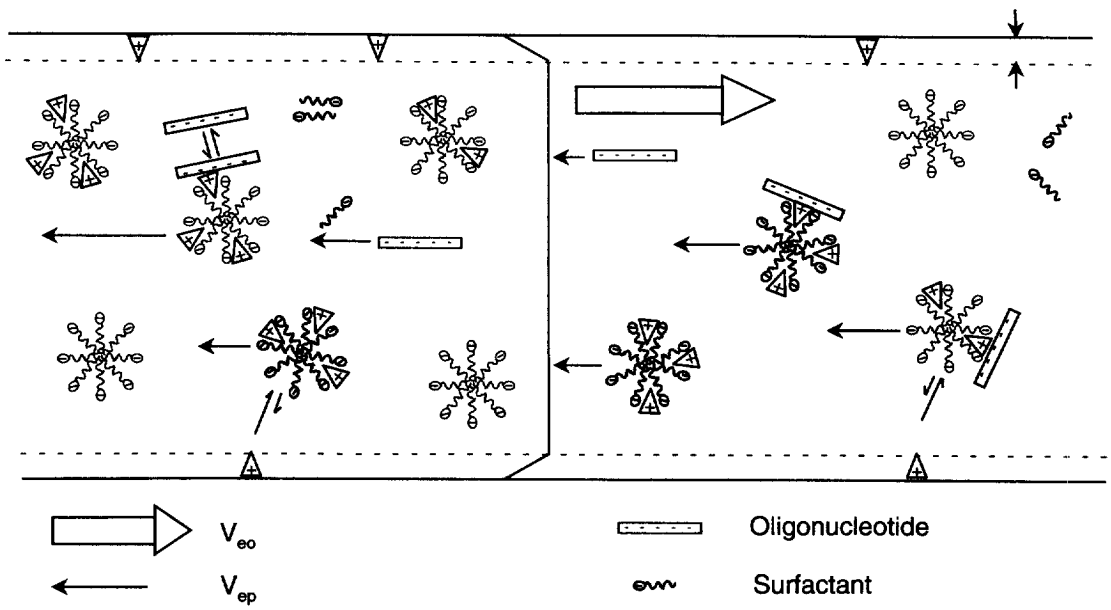

Fig. 2. Schematic illustration of retention mechanism with SDS micelles and metal ions. Reproduced with permission from Ref. [13].

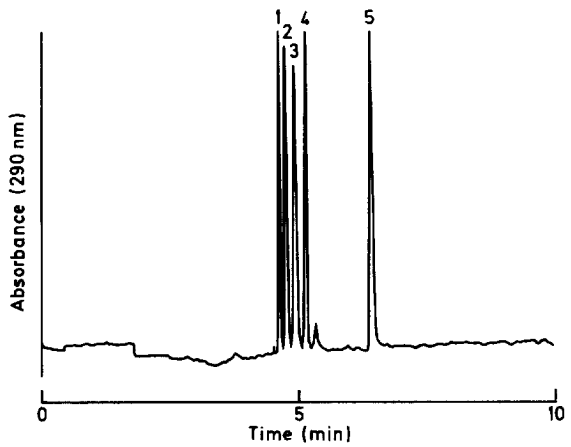

Fig. 3. Separation of copper(II) complexes of polyaminocarboxylic acids by MEKC using CTAB (cetyltrimethylammomium bromide) as surfactant. Peaks: 1=$[Cu(II)ED3A]^-$, 2=$[Cu(II)_2DTPA]^-$, 3=$[Cu(II)HEDTA]^-$, 4=$[Cu(II)NTA]^-$ and 5=$[Cu(II)EDTA]^{-2}$ (ED3A=ethylenediaminetriacetic acid; DTPA=diethylenetriaminepentaacetic acid, HEDTA=hydroxyethylenediaminetriacetic acid, NTA=nitrilotriacetic acid, EDTA=ethylenediaminetetraacetic acid). Conditions: capillary, fused-silica, 0.800 m (0.600 m to detector)×50 μm I.D.; BGE, 100 m*M* CTAB, 10 m*M* $Cu(OAc)_2$, 70 m*M* HOAc, pH 5.5; separation voltage, −20 kV (−37 μA); detection wavelength, 290 nm; hydrodynamic injection. Reproduced with permission from Ref. [17].

diastereomeric ternary complex incorporating the analyte and an enantioselective ligand. The complex formation can be performed either pre-capillary for highly stable complexes (this is referred to as the indirect mode of analysis) or on-capillary (referred to as the direct mode of analysis). Examples of the direct enantiomeric separation of chiral analytes using complexation with a metal ion include the addition of copper(II) and the chiral surfactant N,N-didecyl-L-alanine to the BGE in order to facilitate separation of dansylated amino-acids (Fig. 4) [24–26]. The BGE also contained SDS and the combination of this species with the chiral surfactant resulted in the formation of a mixed micelle. The metal is attached to the chiral chelating surfactant and to the dansylated amino acids. The L and D form of the amino acids will combine with the copper-didecyl-L-alanine–SDS, forming two diastereomeric metal chelates with sufficiently different rates of formation as to yield differences in their migration velocity.

3. MEKC of analytes containing a metal ion

In this section, the separation of analytes containing a metal ion is discussed. Such analytes

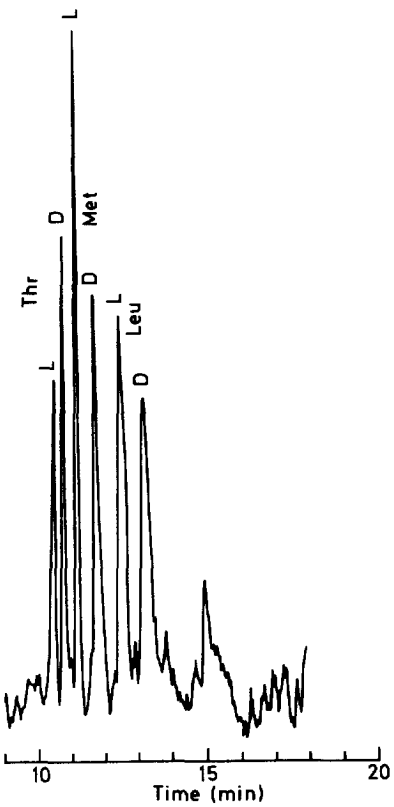

Fig. 4. Resolution of three DL-Dns-amino acids (DL-Dns-Thr, DL-Dns-Met, DL-Dns-Leu) using a mixed chiral micelle. Conditions: capillary, fused-silica, 0.800 m (0.600 m to detector)×75 μm I.D.; temperature, 25°C; BGE, 20 m*M* SDS, 5 m*M* N,N-didecyl-L-Ala, 25 m*M* Cu(II), 10% (v/v) glycerol; separation voltage, 25 kV (20 μA); detection wavelength, 260 nm; electrokinetic injection. Reproduced with permission from Ref. [24].

include organometallic compounds and stable metal complexes.

3.1. Organometallic compounds

Determination of organometallic compounds (or their decomposition products) is a further area showing applications in MEKC. Here the ligand becomes an integral part of a stable organometallic compound and the purpose of the analysis is to separate and determine the organometallic compound as a whole in various matrixes in the same way as other stable organic compounds are determined.

Ng et al. reported the use of MEKC for the determination of several organolead and organoselenium compounds [27]. They observed for a series of CZE experiments (in which no micelles were present) a migration time less than that of the EOF for triethyllead (TEL) and trimethyllead (TML) in an dihydrogenphosphate–borate buffer at pH 6–7. This behaviour was attributed to the partial positive charge of the analytes acquired by the reaction:

$$PbR_3Cl \overset{k}{\leftrightarrow} PbR_3^+ + Cl^- \quad (1)$$

However, at higher pH the TML and TEL migrated after the EOF. When studied in a micellar BGE containing SDS, the migration times increased substantially and their order followed the trend expected from the hydrophobicities of the analytes. Although separation could be achieved in a BGE with SDS, the TEL and phenylselenyl chloride peaks showed tailing. When β-cyclodextrin (β-CD) was added to the BGE the tailing was removed and at the same time separation selectivity could be manipulated by the concentrations of SDS and β-CD. An increase of the neutral β-CD concentration relative to SDS resulted in a decrease of migration times of all solutes. In the final separation (Fig. 5) a BGE containing 50 m*M* SDS, 5 m*M* β-CD in a pH 6.0 buffer was found to give reproducible and efficient (plate numbers >200 000) results. The method has been applied to the determination of the organometallic species in rainwater after preconcentration by extraction into chloroform, giving recoveries from 83% to 104% and limits of detection (LODs) from 8–20 pg (corresponding to 27 ppm to 60 ppm for an injection volume of 0.3 nl).

Li et al. [28] have reported MEKC separations in SDS–phosphate/tetraborate BGEs at pH 7–9 for trimethyltin (Me_3Sn), trimethyllead (Me_3Pb), triethyllead (Et_3Pb), dibutyltin (Bu_2Sn) and tributyltin (Bu_3Sn). The selectivity of the separation was dependent on pH, which could be explained by the partial ionisation of the solutes according to Eq. (1). Liquid–liquid extraction and solid-phase extraction (SPE) using C_{18} membrane discs were used to isolate the analytes from water samples. The recovery of the analytes using SPE was improved greatly when diethyldithiocarbamate (DEDTC) was added to the water samples. This was explained by the formation of a complex between the central metal atom of the organometallic compound and the

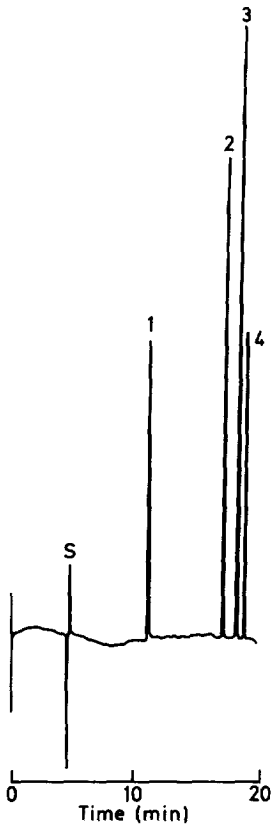

Fig. 5. Typical electropherogram obtained for organolead and organoselenium compounds. Peaks: S = solvent (methanol); 1 = trimethyllead chloride; 2 = triethyllead chloride; 3 = diphenyl selenide; 4 = phenylselenyl chloride. Conditions: capillary, fused-silica, 0.440 m × 50 μm I.D.; temperature, 25°C; BGE, 50 m*M* SDS and 5 m*M* β-cyclodextrin in 25 m*M* phosphate–borate (pH 6.0); separation voltage, 15 kV; detection wavelength, 210 nm; hydrodynamic injection, 20 mm for 4 s. Reproduced with permission from Ref. [27].

DEDTC. The only recovery, which remained low, was for Me_3Sn, which was attributed to the polarity of this compound and its resultant lack of strong interaction with DEDTC. The SPE step achieved preconcentration factors of 1000–40 000 times, resulting in LODs in the low ppb range. However matrix interference was observed for Bu_3Sn.

3.2. Stable metal complexes

In this section we consider the separation of metal complexes which are sufficiently stable that they can be formed and isolated without the necessity to be maintained in the presence of an excess of the ligand. This characteristic enables them to be separated without the requirement for ligand to be present in the BGE.

Hematoporphyrin (HP), protoporphyrin (PP) and their Cu(II) and Zn(II) complexes have been separated using a SDS BGE containing 3-cyclohexylamino-1-propanesulfonic acid (CAPS) buffer at pH 11 [29]. The BGE also contained dimethylformamide (DMF) to improve the solubility of HP and thereby to suppress its sorption to the capillary wall, as well as to improve the reproducibility of the separation. In the optimised BGE (40 m*M* SDS, 20%, v/v DMF), the CMC was determined to be approx. 8 m*M*. The separation of HP, PP and their Cu(II) and Zn(II) complexes was achieved within 24 min.

The separation of platinum(II) anti-tumour drugs and their decomposition products has been investigated by Wenclawiak and Wollmann [30]. The analytes investigated included three neutral compounds, namely carboplatin, lobaplatin and cisplatin, and two cationic aquation products of cisplatin, [*cis*-diammineaquachloroplatinum]$^+$ (ACP^+) and [*cis*-diamminediaquaplatinum]$^{2+}$ (DAP^{2+}). All analytes gave sharp symmetrical peaks but cisplatin migrated close to the EOF peak since it does not contain any organic ligand to facilitate hydrophobic interaction with the micelle. Carboplatin, which is quite polar but contains cyclobutanedicarboxylic acid also showed relatively weak interaction with the micelles, whereas lobaplatin exhibited sufficient interaction to enable both diastereomers to be separated. The two cationic complexes, which might have been expected to migrate towards the cathode at the detector side and appear in the electropherogram before the EOF, in fact migrated after the EOF and appeared in the order ACP^+ followed by DAP^{2+} (Fig. 6). This behaviour was explained by interaction of the cationic species with the anionic SDS micelle, which is in agreement with the behaviour of these complexes in HPLC separations where strong retention on a dynamically coated cation exchanger has been observed [31].

Jia et al. [32] studied the separation of tetrachloroplatinate in an acidic HCl/KCl electrolyte at pH 3.0 using a co-electroosmotic system using CTAB. $PtCl_4^{2-}$ was separated from a mixture containing

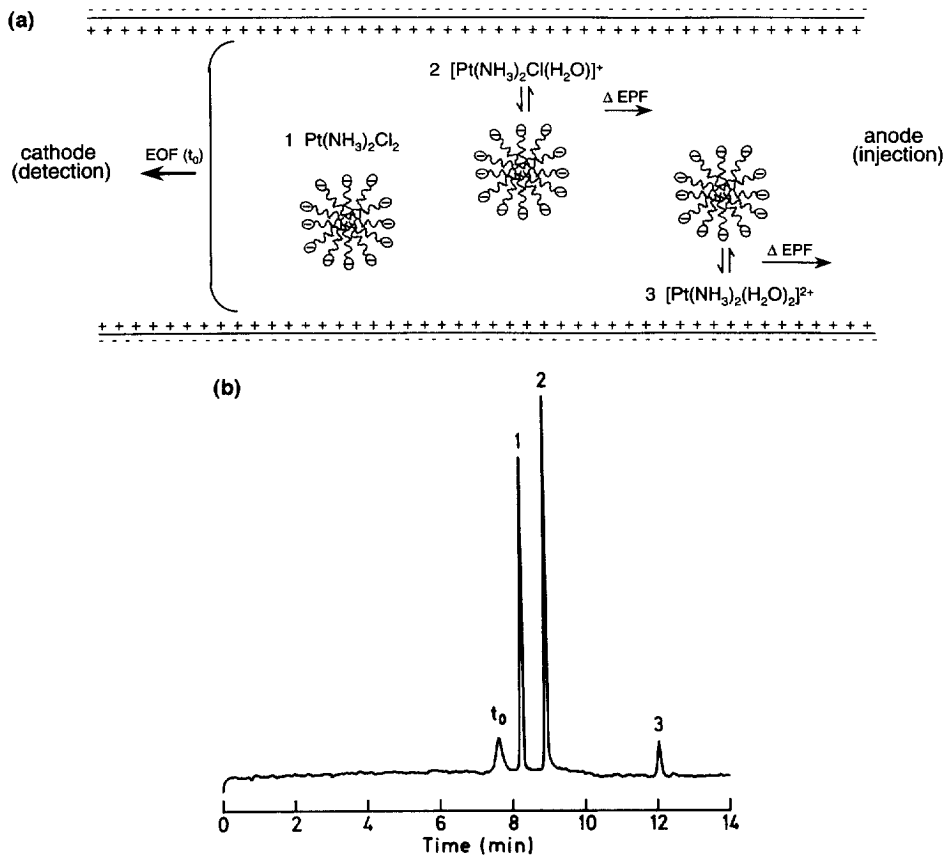

Fig. 6. (a) Schematic representation of the principle of MEKC for cisplatin analysis. The direction of electroosmotic flow (EOF) and electrophoretic flow (EPF) are shown. (b) Electropherogram of a cisplatin solution in water after 2 h at 37°C. Peaks: 1 = cisplatin, 2 = [*cis*-diammineaquachloroplatinum]$^+$, 3 = [*cis*-diamminediaquoplatinum]$^{2+}$. Conditions: capillary, fused-silica, 0.750 m (0.500 m to detector) × 75 μm I.D.; temperature, 25°C; BGE, 100 m*M* SDS in 25 m*M* $Na_2B_4O_7$–50 m*M* $NaHPO_4$ (pH 7.0); separation voltage, 15 kV (50 μA); detection wavelength, 210 nm; hydrodynamic injection, 100 mm for 10 s. Reproduced with permission from Ref. [30].

Rh(III), Ru(III), Os(III) and Ir(III) in less than 4 min.

Separation of enantiomers of tri-azo aromatic complexes of iron by MEKC using bile salt micelles has been reported [33]. The iron complexes, prepared by pre-capillary reaction, were bis{8-[(pyridine-2-methylene)amino] quinoline} iron(II) hexafluorophosphate $[Fe(PMAQ)_2(PF_6)_2]$, bis-{8-[(pyridine-2-methylene)amino] lepidine} iron(II) hexafluorophosphate $[Fe(PMAL)_2(PF_6)_2]$ and bis-[1-(2-pyridinyl) ethylidine]-8-aminoquinoline iron(II) hexafluorophosphate $[Fe(PEAQ)_2(PF_6)_2]$. The complex formed between iron and the pyridine and quinoline moieties in PMAQ has two tridentate ligands in an octahedral geometry about the iron. The ligands are asymmetric with respect to the quinoline and pyridine moieties, and the resulting complex is chiral. MEKC was found to be an ideal tool to investigate the degree of chirality in this complex and those involving similar tri-azo aromatic ligands. As expected, separations in non-micellar systems (phosphate–borate, pH 9.0) did not allow the resolution of the enantiomers or all of the complexes, which comigrated prior to the EOF peak. In phosphate–borate buffer at pH 9.0 and containing 40 m*M* sodium deoxycholate, the doubly charged iron complexes $[Fe(PMAL)^{2+}$, $Fe(PEAQ)^{2+}$ and $Fe(PMAQ)^{2+}]$ were resolved and migrated after the EOF, indicating some degree of partitioning into the micelle. However, the enantiomers of PMAL and PEAQ were not resolved in this system unless

organic solvents were added to the BGE. All of the organic additives studied showed improved resolution and decreased apparent mobility for at least one of the enantiomers when compared to a buffer system employing only the bile salt, with the addition of acetone, dimethylformamide or tetrahydrofurane giving best separation. No mechanism was proposed, but it was suggested that the organic additive altered the micelle to a "better chiral site" for the enantiomers.

4. Separations in which the metal ion is the analyte

This area of application of metal complexes in MEKC concerns methods for the determination of metal ions by converting them to complexes of a suitable, mostly organic, ligand to facilitate detection and/or to influence the selectivity. If one first considers the separation of metal ions by CZE, it can be noted that selectivity for these species is limited because of similar size-to-charge ratios of the metal ions. The addition of a complexing agent is often used to alter selectivity and the metal ions are separated as the corresponding metal chelates or as equilibrium mixtures of the free and complexed metal ion [34,35].

There are two main approaches to the determination of metals by either CZE or MEKC using reaction with a particular ligand: on-capillary complexation and pre-capillary complexation [35]. On-capillary complexation, which is used most commonly in CZE, usually involves the addition of a weak complexing agent to the BGE with subsequent partial complexation of sample cations within the capillary. This results in the formation of metal complexes of differing stability and thus, differing effective mobilities. In CZE, this approach usually employs indirect photometric detection since the fraction of the metal ion existing in the complexed form (that is, the form which is amenable to direct photometric detection) is usually small. On the other hand, pre-capillary complexation, which is the preferred mode for MEKC, enables more complete formation of the metal complex and therefore direct detection is generally used. For pre-capillary complexation the ligand should possess the following properties [36]: (i) the ligand should form complexes with a large range of metals. These complexes should be able to be prepared by simple methods. (ii) The complexes formed should be coordination-saturated. (iii) The ligand should not be too large so that the original properties of the metal are retained. (iv) The donor atoms on the ligand should have low total electronegativity so as to minimise adsorption effects (suitable donor atoms are N, O and S). (v) The complexes formed should have good stability, good detectability and high solubility.

In the pre-capillary complexation approach a strong complexing agent is necessary and this is added to the sample prior to injection. As such, this method can be viewed as a derivatisation of the analyte, so that complete complexation and formation of one stable complex for each analyte (metal ion) is desirable [36]. If the resultant metal complexes formed are charged then CZE may be applied, and if the complexes are neutral they can be expected to partition into micelles so that MEKC can be applied. Advantages of pre-capillary complexation are that the complexes can be detected with direct spectrophotometric detection the elimination of interferences from complex sample matrices may be possible by selective complexation or through the use of an appropriate detection wavelength and problems associated with complexation equilibria, such as bad peak shapes, may often be avoided. Some complexes, which are prone to dissociation during the separation, may be stabilised using a combination of on-capillary and pre-capillary complexation in which the complex is formed prior to injection but a small amount of ligand is also added to the BGE [37]. Examples of separations by MEKC of metal ions as anionic [38–40], cationic [41] and neutral [7,42,43] complexes are discussed in Sections 4.1–4.3. In some cases the method has been restricted to the determination of only a few metal ions with limited applications.

4.1. Anionic complexes

Saitoh et al. [38] have reported the separation of transition metal ions as anionic 4-(2-pyridylazo)resorcinol (PAR) complexes using a SDS micellar BGE. The complexes were formed pre-capillary and separation of Co(III), Cr(III), Fe(III)

and Ni(II) was achieved in 30 min with separation efficiencies greater than 100 000 theoretical plates (Fig. 7). A small amount of PAR (1 m*M*) was added to the BGE in order to suppress dissociation of the less stable Zn(II), Cd(II) and Cu(II) complexes. Other metal ions forming unstable PAR chelates [such as Mn(II) and alkaline earth metal ions] gave no peaks under the conditions employed. The migration behaviour of PAR chelates of Co(II), Cu(II), Pb(II), Ni(II), Fe(II), Zn(II), Cd(II), Cr(III) and Fe(III) has been investigated using micellar solutions of SDS [39]. The complexes were formed pre-capillary and 0.1 m*M* PAR was again added to the BGE, but metals that formed PAR complexes less stable than Cd(II) were not detected. Complexes of La(III), Zr(IV), Sn(IV) and U(VI) exhibited broad, asymmetrical peak shapes and were not included in detailed investigations. The effective mobilities of the PAR complexes were largely unaffected by changes in the surfactant concentration but separation selectivity was influenced strongly by the BGE counter cation. Using a phosphate buffer (pH 8.0), the complexes were resolved when using the ammonium salts but not the sodium salts of the phosphate buffer. This was explained by the authors in terms of ion-pairing between the complexes and the BGE counter cation, with ammonium acting as a stronger ion pairing cation than sodium, thereby allowing more effective partitioning of the ion-paired complexes into the micelles. Separation of the nine PAR complexes was possible within 12 min, with detection limits from 0.1 to 10 m*M* at 500 nm. Complexes of Ni(II) and Fe(II) were not resolved completely and the peak for Cd(II) was very broad compared to the other complexes.

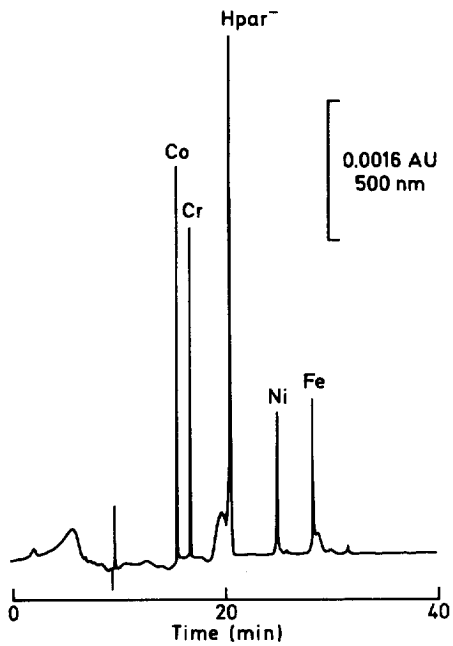

Fig. 7. MEKC separation of metal–PAR [(4-2-pyridylazo)resorcinolato] chelates. Complexation conditions; [PAR]=1 m*M*; [metal ion]/(0.01 m*M*) Co(II) 0.995, Cr(III) 1.23, Fe(III) 1.00, Ni(II) 1.01; [triethanolamine]=25 m*M* (pH 8.8 with HCl), heated at 98°C for 30 min. Separation conditions: capillary, fused-silica, 0.850m (0.700 m to detector)×50 μm I.D.; temperature, 25°C; BGE, [SDS]=0.02 m*M*, [NaH_2PO_4]=50 m*M*, [$Na_2B_4O_7$]=12.5 m*M*, [PAR]=0.1 m*M*; separation voltage, 16.5 kV (25 μA); detection wavelength, 500 nm; hydrostatic injection. Reproduced with permission from Ref. [38].

The separation of metal ions in the form of 8-hydroxyquinoline-5-sulfonic acid (HQS) complexes has been studied by Timerbaev et al. [40]. Complexes of Mn(II), Cu(II), Cd(II), Fe(II), Zn(II), Co(II), Ni(II) and Al(III) were formed pre-capillary and separated in just over 6 min using CZE. The addition of SDS to the BGE as a micellar phase did not affect the electrophoretic mobilities of the complexes, indicating insignificant partitioning of the anionic complexes into the anionic SDS micelles.

4.2. Cationic complexes

The only separation of cationic complexes by MEKC has been reported by Timerbaev et al. in 1994 [41]. Using CZE, 2,6-diacetlypyridinebis(N-methylenepyridinohydrazone) (H_2dapmp) complexes of Cd(II), Fe(III), Zn(II) and Cu(II) were separated. With the addition of tetradecyltrimethylammonium bromide as a micellar phase, separation of Cd(II), Co(II), Cu(II), Fe(III), Hg(II), Mo(VI), Sc(III),V(V), U(VI), Y(III) and Zn(II) was achieved within 13 min (Fig. 8). Whilst separation efficiency was considerably lower than for typical CE separations (<25 000 theoretical plates), the detection limits achieved (mid ppb range) and the range of metals analysed demonstrated the analytical potential of this method.

4.3. Neutral complexes

Separation of Mn(III), Co(III), Zn(II) and Cu(II)

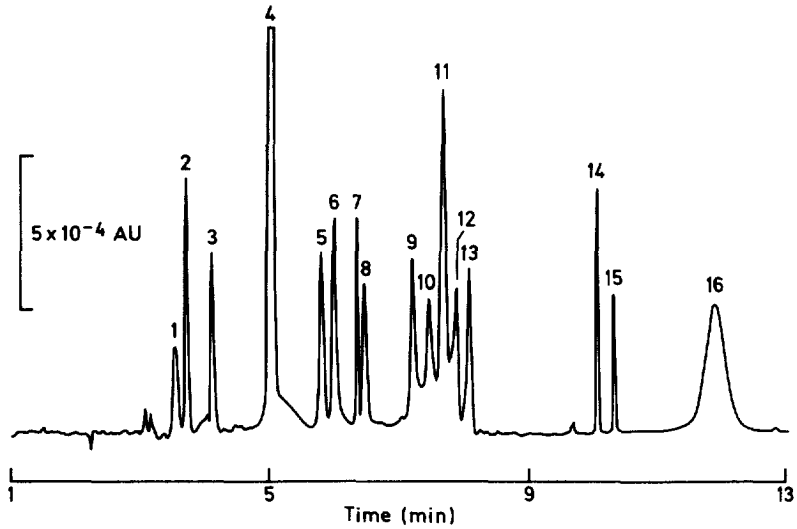

Fig. 8. Separation of fourteen metal ions as H_2dapmp (2,6-diacetylpyridine bis(N-methylenepyridiniohydrazone) complexes. Peaks: 1=acetone; 2,4=H_2dapmp; 3=Mo(VI) (0.1 m*M*); 5=Sc(III) (0.08 m*M*); 6=Fe(III) (0.04 m*M*); 7=Y(III) (0.08 m*M*); 8=Zn(II) (0.1 m*M*); 9=Cd(II) (0.1 m*M*); 10=Zr(IV) (0.08 m*M*); 11=Co(II) (0.15 m*M*); 12=U(VI) (0.17 m*M*); 13=Cu(II) (0.17 m*M*); 14=Sn(IV) (0.08 m*M*); 15=Ta(V) (0.08 m*M*); 16=Hg(II) (0.04 m*M*). Separation conditions: capillary, fused-silica, 0.500 m×75 μm I.D.; BGE, 10 m*M* borate buffer (pH 9.0), 75 m*M* tetradecyltrimethylammonium bromide and 10 m*M* SOS (sodium *n*-octanesulfonate); separation voltage, 15 kV; detection wavelength, 254 nm; injection, hydrostatic for 10 s at 100 mm. Reproduced with permission from Ref. [41].

as neutral chelates of α,β,γ,δ-tetrakis(4-carboxypenyl)porphine has been reported using MEKC [42]. The complexes were formed pre-capillary and SDS was used as the micellar phase. Although the method was limited to these four metals, concentration detection limits for Mn(III), Co(III) and Zn(II) were approximately three hundred times lower than for HPLC due to far superior separation efficiencies. Quantitative determination of Cu(II) was problematic due to lower stability of the complex and resultant poor peak shape.

Acetylacetone (acac) has been used as a complexing agent for the determination of Co(III), Rh(III), Cr(III) and Pt(II) by MEKC [43]. These neutral complexes were sufficiently stable to migrate through the capillary without addition of ligand to the BGE. From the capacity factors the distribution coefficient of each metal between the SDS micelles and the aqueous buffer was calculated and was found to correlate well with the partition coefficient for a dodecane–water system. The mobilities of the complexes were predicted and agreed well with experimental values. This work demonstrated that if the resolution of the metal complexes is governed by differential distribution between the micelle and aqueous phase, prediction of such separations maybe possible from liquid–liquid partition data.

Recently Hilder et al. have demonstrated the separation by MEKC of transitional metal ions as complexes with bis(2-hydroxyethyl)dithiocarbamate (HEDTC) [44,45]. Pre-capillary complexation was shown to be superior to on-capillary complexation in terms of the number of metals which could be separated and the peak shapes. Study of the specific complexation conditions required showed that for most metals a molar excess of the HEDTC of at least 25 times over the total metal concentration was required. Under such conditions, Cd(II), Pb(II), Pt(II), Co(II), Ni(II), Bi(III), Cr(II), Cu(II) Hg(II) and Ag(I) could be analysed, while Zn(II), Mn(II), Fe(II), Fe(III), As(III), Te(IV), As(V) and Mo(VI) gave no response and Au(III), Pd(II) and Se(IV) complexes were insoluble in the BGE. The BGE composition (buffer type and concentration, pH, ionic strength, HEDTC concentration and organic modifier type and concentration) was investigated with respect to separation selectivity. The concentration of methanol, as the preferred organic modi-

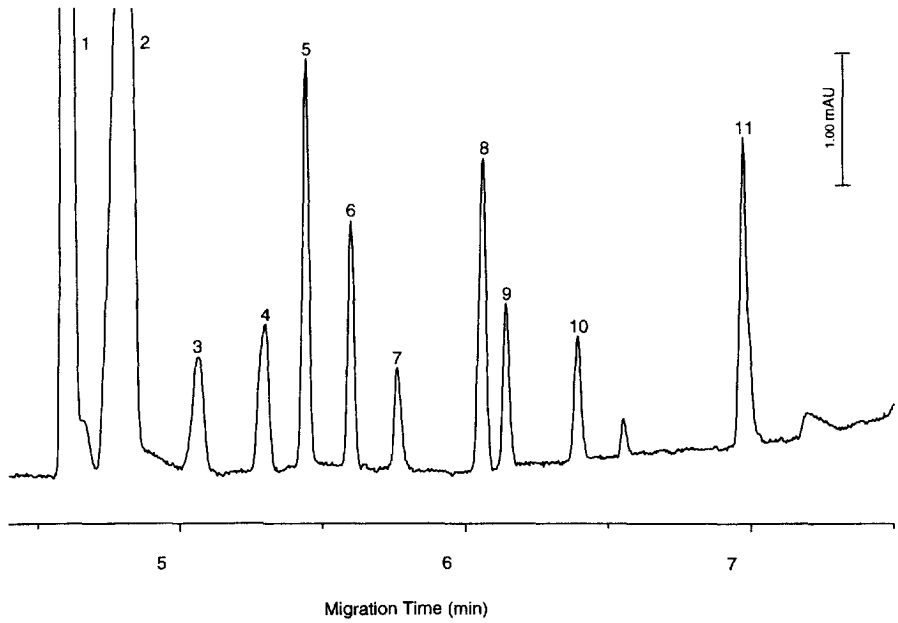

Fig. 9. Separation of HEDTC [bis(2-hydroxyethyl) dithiocarbamate] complexes. Peaks: 1=EOF, 2=oxidation product, 3=Cd(II), 4=Pb(II), 5=Pt(II), 6=Co(II), 7=Ni(II), 8=Bi(III), 9=Cr(III), 10=Cu(II), 11=Hg(II). Separation conditions: capillary, fused-silica, 0.600 m (0.520 m to detector)×75 μm I.D.; BGE, 60 m*M* MOPS (morpholinepropanesufonic acid), 30 m*M* Tris, 10 m*M* SDS, 0.1 m*M* HEDTC, (pH 7.2); separation voltage, 20 kV (27 μA); detection wavelength, 254 nm; injection, hydrostatic injection for 10 s at 100 mm; sample 10 μ*M* each metal, 30 μ*M* HEDTC. Reproduced with permission from Ref. [45].

fier, was optimised using an interpretative optimisation method. Two BGEs were suitable for final analytical methods, namely 100 m*M* lithium borate, 10 m*M* SDS, 0.1 m*M* HETDC, 4% (v/v) methanol, pH 9.2, or 60 m*M* 3-(morpholino)propane sulfonic acid (MOPS), 30 m*M* tris(hydroxymethyl)-aminomethane (Tris), 10 m*M* SDS and 0.1 m*M* HEDTC, pH 7.2. The ten metal complexes were baseline separated with the second of the above BGEs in under 7 min (Fig. 9). Separation efficiencies were up to 500 000 theoretical plates, reproducibility was typically <3% R.S.D. and linearity extended across two orders of magnitude of concentration. For detection at 254 nm, concentration detection limits were in the range 9 to 177 ppb, corresponding to mass detection limits at sub femtomole levels.

5. Conclusions

MEKC is applicable to the analytical separation tasks covered by the review, namely separations in which a metal ion or metal complex is added into the background electrolyte, or separations in which the analytes are metal-containing compounds (e.g., stable metal complexes, organometallic compounds and enantiomers) or metal ions (i.e., after conversion to complexes of a suitable ligand to facilitate detection and/or to influence the selectivity). However, due to the limited number of publications in the reviewed area up to date, it is difficult to evaluate precisely whether MEKC is being applied to its full analytical potential. Compared to HPLC as a well established separation technique generally applicable in the same areas, MEKC has the clear advantage of considerably higher separation efficiencies and its separation selectivity may be different, thus making MEKC a suitable complementary technique to RP-HPLC.

The separation mechanisms in MEKC applicable to the reviewed area are dependent on the type of analytes. In case of lipophilic species, hydrophobic interactions dominate the separation process. However, for polar or charged species, interactions with

the functional groups on the outside of the micelle have been shown to be the prime separation mechanism, similar to that occurring in ion-exchange HPLC.

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