ELSEVIER

Journal of Chromatography A, 919 (2001) 427–436

JOURNAL OF CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

Chiral separations of transition metal complexes using capillary zone electrophoresis

Jamie E. Harris, Nehal Desai, Kathryn E. Seaver, Rhett T. Watson, Noel A.P. Kane-Maguire*, John F. Wheeler*

Department of Chemistry, Furman University, 3300 Poinsett Highway, Greenville, SC 29613-1120, USA

Received 11 May 2000; received in revised form 2 April 2001; accepted 3 April 2001

Abstract

Several buffer additives that may facilitate chiral separation for optically active transition metal (TM) systems are investigated using capillary zone electrophoresis. The TM complexes evaluated exhibit considerable heterogeneity with respect to total complex charge (0 to 4+), ligand type, and identity of the central metal including Ru^{2+}, Ni^{2+}, Cr^{3+}, and Co^{3+}. *threo*-D_s[+]-Isocitrate, potassium antimonyl-*d*-tartrate and dibenzoyl-L-tartrate are identified as the most efficient chiral selectors. Interestingly, TM complexes exhibiting a (3+) total complex charge exhibit a reversal of enantiomer elution order versus all other complexes when separated using the tartrate additives. Operating parameters including pH, temperature, and capillary length are discussed, and chiral separations of complex mixtures are demonstrated.

Keywords: Enantiomer separation; Buffer composition; Transition metals; Metal complexes

1. Introduction

Capillary electrophoresis (CE) has been shown to be an exceptionally effective tool for the separation of enantiomeric substances. Several excellent reviews offer extensive lists of the applications of CE to chiral mixtures, most which are directed toward compounds of pharmaceutical origin due to potential differences in pharmacological activity as a function of the isomeric structure [1–6]. Numerous chiral resolving agents have been investigated as matrix additives (including cyclodextrins, macrocyclic antibiotics, polysaccharides and proteins) that provide stereorecognition for compounds containing asymmetric carbons [7–10].

In recent years there has been a rapid growth in the use of chiral transition metal (TM) complexes for asymmetric synthesis, catalysis, chiral recognition and electron transfer studies. An essential need in each of these applications is knowledge of the enantiomeric purity of the parent and product species. In spite of the widespread use and acceptance of chiral CE for conventional pharmaceuticals, relatively little effort has been directed toward developing suitable resolving agents for TM complexes that display optical activity. Fanali et al. first demonstrated the use of CE for isomeric separation of several ethylenediamine/amino acid complexes of Co^{3+} using sodium (*S*)-(+)-tartrate in the run buffer

*Corresponding authors. Tel.: +1-864-2943-371; fax: +1-864-2943-559.

E-mail addresses: noel.kane-maguire@furman.edu (N.A.P. Kane-Maguire), john.wheeler@furman.edu (J.F. Wheeler).

0021-9673/01/$ – see front matter
PII: S0021-9673(01)00820-2

[11], although separation of the tris-phenanthroline complex of Co^{3+} was not observed. More recently, Elshihabi et al. investigated micellar electrokinetic chromatography (MEKC) as a means of resolving enantiomers of Fe^{2+} based on complexation with tridentate quinoline-type ligands [12]. For many years, the optical activity of TM complexes has been monitored using polarimetry or circular dichroism, from which values for specific rotation or $\Delta\epsilon$ may be obtained, respectively. However, unless these spectroscopic parameters are initially determined using a sample with an *independently* measured optical purity, it is impossible to utilize these values for assessing optical purity. This frequently results in multiple values of the specific rotation or $\Delta\epsilon$ for the same compound being reported in the literature e.g., for Δ-$[Ni(phen)_3]^{2+}$, $\Delta\epsilon_{274} = -550\ M^{-1}\ cm^{-1}$ [13] vs. $-636\ M^{-1}\ cm^{-1}$ [14]), compromising the accuracy of any spectroscopic purity determination based on these values. This problem has been addressed through the use of chiral shift reagents in high field nuclear magnetic resonance (NMR) studies [15]. However such determinations are time-consuming, require expensive instrumentation and solvents, and are restricted to diamagnetic analytes.

Recently, we demonstrated CE to be an effective technique for the separation of an important subset of TM complexes that include the Δ and Λ optical isomer mixtures of $M(\alpha\text{-diimine})_3^{2+}$ species (M= Ru^{2+}, Ni^{2+}, Fe^{2+}; α-diimine=1,10 phenanthroline or 2,2′-bipyridine, see Fig. 1) using potassium antimonyl-*d*-tartrate as the resolving agent [16]. Zone electrophoresis provides a simple, rapid and highly quantitative approach for establishing the enantiomeric purity of these complexes, and when combined with independent analyses using circular dichroism or polarimetry, can be used to provide accurate absolute values for $\Delta\epsilon$ or specific rotation [16,17]. In the present work we examine a broad range of TM complexes including both M^{2+} and M^{3+} systems. The overall charge of the complex associated with the systems under study is intentionally varied between 4+, 3+, 2+, 1+ and neutrality by changing the nature/identity of the associated ligands. We also provide considerable diversity in the selection of possible resolving agents, and examine a host of electrophoretic optimization parameters including separation temperature, buffer pH, capillary length and associated field strength. These efforts are manifested in significantly improved and expanded capabilities for separating chiral TM complexes relative to earlier work, and demonstrate interesting differences in the enantioselectivity observed as a function of the charge of the TM complex mixtures. These results lead us to some preliminary conclusions regarding the most important features of chemical interaction between the analytes and resolving agents, and suggest a rational procedure for efficient method optimization.

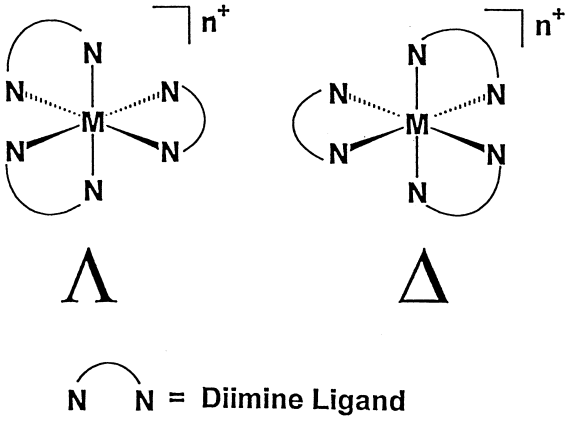


Fig. 1. General structure of chiral $[M(diimine)_3]^{n+}$ (Ru: $n=2$; Cr: $n=3$) transition metal systems.

2. Experimental

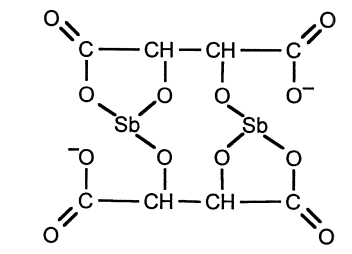
2.1. Materials

Dibenzoyl-L-tartartic acid, antimonyl-*d*-tartrate hydrate, tris(2,2′-bipyridyl) ruthenium(II) chloride hexahydrate, racemic tris(1,10-phenanthroline) ruthenium(II) chloride hydrate and racemic tris(1,10-phenanthroline) iron(II) hexafluorophosphate were obtained commercially and used as received (Aldrich, Milwaukee, WI, USA). *threo*-D_s[+]-Isocitric acid, *O*-phospho-L-serine, *O*-phospho-L-threonine, L-Glu–L-Glu, and L-Asp–L-Asp were used as received (Sigma, St. Louis, MO, USA). Prior to use, dibenzoyl-L-tartaric acid was treated with a stoichiometric quantity of sodium hydroxide and the solution evaporated to dryness to prepare the sodium salt. Optically active isomers of $[Ru(phen)_3]^{2+}$ were

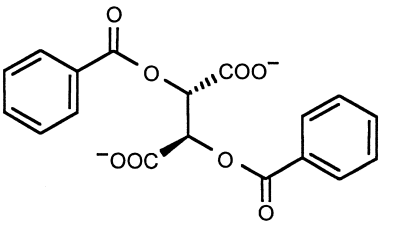
isolated via a slight modification of the literature method [18], as were samples of *cis*-$[Ru(bpy)_2(CN)_2]$ and *cis*-$[Ru(bpy)_2(NO_2)_2]$ [19], where bpy is 2,2′ bipyridine. Racemic and optically active samples of $[Ru(bpy)_3]^{2+}$ [20], $[Ru(bpy)_2(phen)]^{2+}$ and $[Ru(bpy)(phen)_2]^{2+}$ [21], $[Ni(phen)_3]^{2+}$ [22], $[Co(phen)_3]^{3+}$ [23], $[Cr(phen)_3]^{3+}$ [23], $[Cr(bpy)_3]^{3+}$ [24], $[Co(phen)_2(ox)]^{+}$ and $[Cr(phen)_2ox]^{+}$ (where ox= oxalate) [25], *cis*-$[Cr(phen)_2(H_2O)_2]^{3+}$ [26] and the binuclear complex $[(phen)_2Cr(OH)_2Cr(phen)_2]^{4+}$ [26] were prepared via published synthetic routes.

2.2. Methods

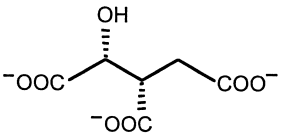
All electropherograms were obtained using either SpectraPhoresis 1000 (Thermo Separations, Fremont, CA, USA) or PACE 5000 (Beckman Instruments, Fullerton, CA, USA) CE instruments equipped with UV–Vis detection. A fused-silica capillary, 50 μm I.D.×363 μm O.D. (Polymicro Technologies, Phoenix, AZ, USA), was cut to desired lengths and activated with 1 *M* NaOH prior to use. Injections were performed hydrodynamically for 5–10 s at a relative pressure of 0.035 bar. All samples were prepared in deionized water (18.3 MΩ cm), and water was briefly rinsed through the capillary between successive injections. In most cases, the cationic TM complexes studied were converted to aqueous solutions of the chloride salt using a Dowex 2-X8 anion-exchange resin prior to sample injection. In order to establish enantiomeric migration order for TM complexes, racemic samples were spiked with optically active samples prior to analysis.



(1)



(2)



(3)

Fig. 2. Chemical structures of resolving agents used for separations. (1) Antimonyl-*d*-tartrate; (2) dibenzoyl-L-tartrate; (3) *threo*-$D_s[+]$-isocitrate.

3. Results and discussion

3.1. Effects of resolving agents

In the preliminary work, we described the separation of $M(\alpha\text{-phen})_3^{2+}$ complexes using potassium antimonyl-*d*-tartrate, $K_2[Sb_2(d\text{-tart})_2]$, at neutral pH demonstrating that chiral discrimination for TM complexes via CE is made possible only through selective association with a chiral resolving agent [16]. Fig. 2 provides the structure for the antimonyl-*d*-tartrate anion. The most important feature of this resolving agent is the presence of the two asymmetric centers on the tartrate backbone which convey its chirality. Since the dianionic complex exhibits a net (2−) charge, we reasoned that electrostatic interaction between the (2+) charged metal complex and the tartrate dimer facilitates the chiral interaction that leads to electrophoretic separation. In fact, antimonyl-*d*-tartrate has been successfully used for many years for isolating *rac*-$[Ru(diimine)_3]^{2+}$ en-

antiomers through reactions in which the optically active salts are selectively precipitated as diastereomers (e.g., $[Ru(diimine)_3][Sb_2(d\text{-tart})_2]$ [18]. When identical experimental conditions to those provided in our earlier communication are applied to the racemic *tris*-bipyridine analogs of the M^{2+} metals, only partial resolution is observed using typical CE capillary lengths (i.e., <100 cm effective length), presumably because of the reduced steric bulk of the bipyridine ligand interacting with the tartrate. Further, when $[M(bpy)_3]^{3+}$ or $[M(phen)_3]^{3+}$ systems are investigated using these conditions (e.g., *rac*-$[Co(phen)_3]^{3+}$), no resolution between the Λ and Δ isomers is observed, consistent with the earlier work of Fanali et al. using sodium (*S*)-(+)-tartrate [11]. This led us to select and evaluate a number of additional resolving agent systems, two of which (dibenzoyl-L-tartrate and *threo*-D_s[+]-isocitrate) are shown in Fig. 2.

The criteria used in selecting these agents included the potential to exhibit anionic charge (i.e., to facilitate ion-pair formation), the presence of multiple asymmetric carbons, aqueous solubility, optical transparency in the detection region of interest (typically 300–500 nm) and commercial availability. In total a structurally diverse group of compounds were investigated, including amino acid and dipeptide analogs, isocitrate, and tartrate derivatives. Table 1 provides results obtained from the initial screening studies that utilized *rac*-$[Ru(phen)_3]^{2+}$ and *rac*-$[Co(phen)_3]^{3+}$ as model analytes. Although the *O*-phospho-L-threonine and *O*-phospho-L-serine amino acids did not provide sufficient stereorecognition for chiral separation of either TM complex at any pH investigated (2.5, 7.0 or 10.0), the L-Glu–L-Glu dipeptide did give slight separation for the *rac*-$[Ru(phen)_3]^{2+}$ complex at pH 10.0. Much greater success was achieved using *threo*-D_s[+]-isocitrate as a chiral selector, which gave virtual baseline separation for *rac*-$[Ru(phen)_3]^{2+}$ and partial resolution for the *rac*-$[Co(phen)_3]^{3+}$ complex in borate buffer at pH 10 (Fig. 3). As noted in Fig. 2, isocitrate has two asymmetric carbons with opposite absolute configurations (2*R*,3*S*), and exhibits a net (3−) charge at the pH values where separation is observed. The elution order, established by spiking experiments using the optically pure enantiomer, was found to be Δ followed by Λ for both TM analytes.

Table 1
Chiral separation of $[Ru(phen)_3]^{2+}$ and $[Co(phen)_3]^{3+}$ complexes with various resolving agents

Resolving agent	TM complex	Separation order
Antimonyl-*d*-tartrate	$[Ru(phen)_3]^{2+}$	Λ, Δ[a,b,c]
	$[Co(phen)_3]^{3+}$	Δ, Λ[a]
O-Phospho-L-serine	$[Ru(phen)_3]^{2+}$	Unresolved
	$[Co(phen)_3]^{3+}$	Unresolved
O-Phospho-L-threonine	$[Ru(phen)_3]^{2+}$	Unresolved
	$[Co(phen)_3]^{3+}$	Unresolved
L-Glu–L-Glu	$[Ru(phen)_3]^{2+}$	Λ, Δ[c]
	$[Co(phen)_3]^{3+}$	Unresolved
threo-D_s[+]-Isocitrate	$[Ru(phen)_3]^{2+}$	Δ, Λ[b,c]
	$[Co(phen)_3]^{3+}$	Δ, Λ[c]
Dibenzoyl-L-tartrate	$[Ru(phen)_3]^{2+}$	Λ, Δ[b,c]
	$[Co(phen)_3]^{3+}$	Δ, Λ[b,c]

TM complexes listed as unresolved demonstrate no discernible peak separation (i.e, no observable selectivity) between the Λ and Δ isomers for racemic samples. Experiments conducted in 25 m*M* phosphate buffer, pH 2.5[a], pH 7.0[b], and 25 m*M* borate buffer, pH 10.0[c].

In the absence of any conflicting data, it seems logical to expect that these complexes interact with similar specificity toward the *threo*-D_s[+]-isocitrate, albeit to a different degree, and the results demonstrate that the Λ isomer binds with greater affinity based on its later elution. It should be pointed out that the order of migration is *opposite* to that we previously observed for the $[Ru(phen)_3]^{2+}$ complex using potassium antimonyl-*d*-tartrate as a resolving agent [16], which possesses an (*R*) absolute configuration for both asymmetric carbons of the tartrate backbone.

Additional studies were performed using the sodium salt of dibenzoyl-L-tartaric acid (Fig. 2). As shown in Fig. 4A for *rac*-$[Ru(bpy)_3]^{2+}$ in borate buffer at pH 10, this resolving agent facilitates baseline separation for the M^{2+} complex with the same enantioselectivity observed for the antimonyl-*d*-tartrate, i.e., Λ followed by Δ. The similarity in interaction between these two resolving agents is not surprising since both exhibit *the same absolute configuration* (2*R*,3*R,* see Fig. 2) for the tartrate asymmetric carbons. (Note: It is recognized that the difference in the nomenclature systems for the

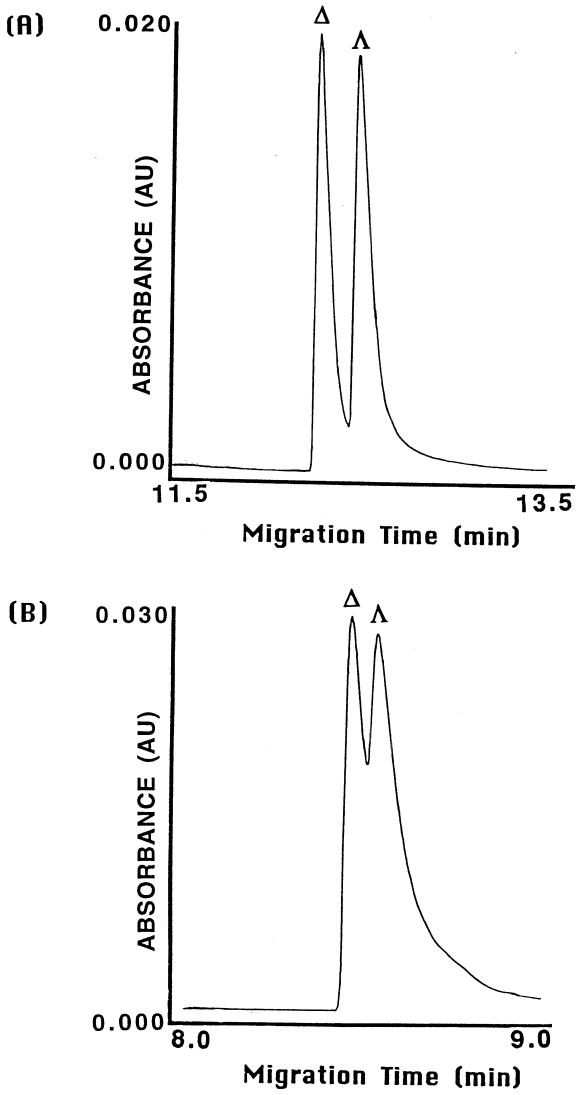


Fig. 3. Electropherograms of (A) 1.0 m*M* *rac*-$[Ru(phen)_3]^{2+}$ and (B) 1.0 m*M* *rac*-$[Co(phen)_3]^{3+}$ using 100 m*M* *threo*-D_s[+]-isocitrate as the chiral resolving agent in 25 m*M* borate, pH 10.0 (57 cm capillary, 50 cm to detection). Conditions: V=350 V/cm, λ=300 nm, T=20°C.

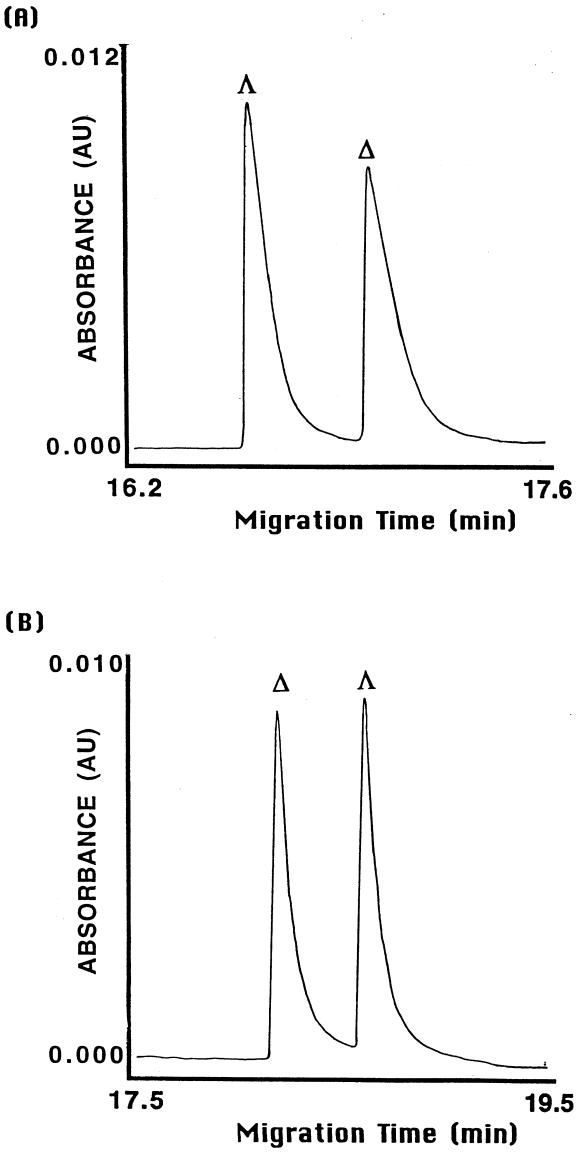


Fig. 4. Electropherograms of (A) 2.0 m*M* *rac*-$[Ru(bpy)_3]^{2+}$ and (B) 0.8 m*M* *rac*-$[Cr(phen)_3]^{3+}$ using 100 m*M* dibenzoyl-L-tartrate as the chiral resolving agent in 25 m*M* borate, pH 10.0 (77 cm capillary, 70 cm to detection). Conditions: (A) V=285 V/cm, λ=450 nm; (B) V=260 V/cm, λ=300 nm, T=20°C.

tartrate additives is inconvenient with respect to their absolute configurations; however, we have chosen to refer to these chiral selectors by their most commonly used names). In contrast to our prior antimonyl-*d*-tartrate studies, when the *rac*-$[Cr(phen)_3]^{3+}$ (Fig. 4B) or *rac*-$[Co(phen)_3]^{3+}$ system is electrophoresed using dibenzoyl-L-tartrate as the resolving agent, baseline resolution is achieved. Furthermore, a reversal in the stereospecificity is observed relative to the M^{2+} complexes with the Δ isomer migrating faster than the Λ isomer. In the earlier work by Fanali et al., this same separation order was observed for the Λ and Δ enantiomers of *rac*-$[Co(en)_3]^{3+}$ (where

en=ethylenediammine) using L-tartrate at pH 5.25 [11].

Although, as noted earlier, antimonyl-*d*-tartrate does not permit separation of the $[M(phen)_3]^{3+}$ isomers at neutral or basic pH, separation is observed at pH 2.5 (Section 3.3) with the same migration order as observed for dibenzoyl-L-tartrate. In an effort to better elucidate the nature of the unusual difference in chiral specificity for the M^{3+} versus M^{2+} complexes with tartrate containing chiral selectors, we systematically explored chiral separations of an expanded group of metal–ligand complexes as discussed in the following section.

3.2. Effects of analyte charge on stereospecificity

The most obvious difference associated with the reversal in chiral selectivity for the $[Ru(phen)_3]^{2+}$ and $[Co(phen)_3]^{3+}$ complexes separated with tartrate additives is the difference in the total charge of these analytes. Yet we found it interesting that the *same* enantioselectivity (as manifested by migration order) was observed for both complexes using the (3−) charged *threo*-D_s[+]-isocitrate system. In all cases, the $Ru(phen)_3^{2+}$ and $Co(phen)_3^{3+}$ complexes elute significantly faster than an electroosmotic flow (EOF) marker (i.e., acetone), indicating that on average they phorese as cationic complexes. One possible difference in the manner in analyte interaction may be the nature of the ion-pair complex itself. For example, the (2−) tartrate additives have the capacity to form a 1:1 ion-pair with the $[Ru(phen)_3]^{2+}$ isomers (as occurs with tartrate-salt precipitation [18]), but cannot form such a stoichiometrically-matched complex with the $[Co(phen)_3]^{3+}$ isomers. To better ascertain the effect for M^{3+} systems of the identity of the central metal and ligand, *rac*-$[Co(phen)_3]^{3+}$, *rac*-$[Co(bpy)_3]^{3+}$, *rac-cis*-$[Cr(phen)_2(H_2O)_2]^{3+}$, *rac*-$[Cr(phen)_3]^{3+}$, *rac*-$[Cr(bpy)_3]^{3+}$ and their corresponding optically active complexes were assayed with the antimonyl-*d*-tartrate and dibenzoyl-L-tartrate additives as summarized in Table 2. In each case it should be noted that the separation order remains Δ followed by Λ. Interestingly, as the *overall* charge on the metal complex is reduced to (2+), (1+), or neutrality, the migration order mimics that observed for the $[Ru(phen)_3]^{2+}$ system, Λ followed by Δ. Fig. 5 shows the corresponding results obtained for the separation of isomers of *rac*-$[Cr(phen)_2ox]^{+}$ and *rac-cis*-$[Ru(bpy)_2(CN)_2]$, and spiked injections confirm the order of migration to be Λ followed by Δ for these complexes. We have also observed this to be true for $[Ni(phen)_3]^{2+}$ and $[Fe(phen)_3]^{2+}$ using antimonyl-*d*-tartrate as the chiral additive [16]. In addition, analysis of the hydroxy-bridged complex *rac*-$[(phen)_2Cr(OH)_2Cr(phen)_2]^{4+}$ yielded a migration order ΛΛ, ΔΔ as indicated in Table 2. (It should be noted that this bridged complex has been established to form only these two enantiomeric forms [26]). In summary, for all complexes we have evaluated to date with the tartrate additives, only the TM complexes that exhibit a total charge of 3+ demonstrate this unusual reversal in enantioselectivity.

It is particularly noteworthy that neutral complexes (Fig. 5B) can be separated by this electrophoretic approach (see also *cis*-$[Ru(bpy)_2(NO_2)_2]$ in Table 2), demonstrating that significant electrostatic association is not essential for chiral discrimination.

Table 2
Chiral separation of TM complexes in relation to charge using antimonyl-*d*-tartrate (AT) and dibenzoyl-L-tartrate (DT)[a,b]

TM complex	Buffer	Migration order
$[(phen)_2Cr(OH)_2Cr(phen)_2]^{4+}$	DT	ΛΛ, ΔΔ
$[Co(phen)_3]^{3+}$	DT	Δ, Λ
$[Cr(phen)_3]^{3+}$	DT	Δ, Λ
$[Co(bpy)_3]^{3+}$	DT	Δ, Λ
$[Cr(bpy)_3]^{3+}$	DT	Δ, Λ
cis-$[Cr(phen)_2(H_2O)_2]^{3+}$	DT	Δ, Λ
$[Ru(phen)_3]^{2+}$	AT, DT	Λ, Δ
$[Ni(phen)_3]^{2+}$	AT	Λ, Δ
$[Fe(phen)_3]^{2+}$	AT	Λ, Δ
$[Ru(bpy)_2(phen)]^{2+}$	AT, DT	Λ, Δ
$[Ru(bpy)(phen)_2]^{2+}$	AT, DT	Λ, Δ
$[Ru(bpy)_3]^{2+}$	AT, DT	Λ, Δ
$[Co(phen)_2ox]^{+}$	DT	Λ, Δ
$[Cr(phen)_2ox]^{+}$	AT, DT	Λ, Δ
cis-$[Ru(bpy)_2(CN)_2]$	DT	Λ, Δ
cis-$[Ru(bpy)_2(NO_2)_2]$	DT	Λ, Δ

[a] All experiments were conducted using 25 m*M* phosphate buffer, pH 7.0, and 25 m*M* borate buffer, pH 10.0.

[b] Both chiral agents were investigated for all cases except $[Ni(phen)_3]^{2+}$, $[Fe(phen)_3]^{2+}$, *cis*-$[Cr(phen)_2(H_2O)_2]^{3+}$ and $[(phen)_2Cr(OH)_2Cr(phen)_2]^{4+}$; however results are provided only where sufficient separation was observed to permit identification of migration order based on a spiked sample.

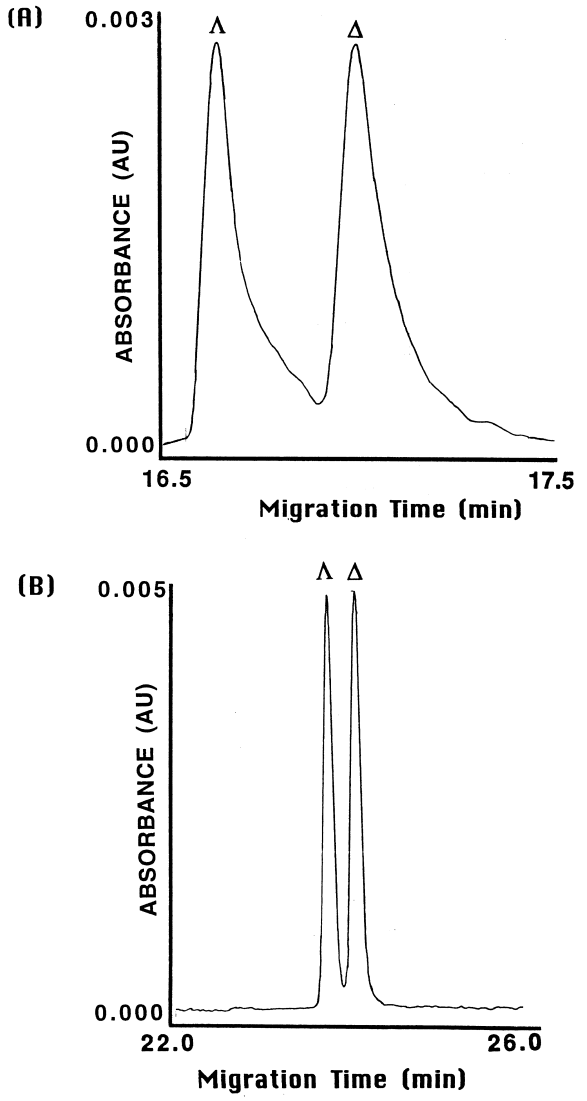


Fig. 5. Electropherogram of (A) 1.0 m*M* *rac*-$[Co(phen)_2ox]^+$ and (B) 2.0 m*M* *rac*-*cis*-$[Ru(bpy)_2(CN)_2]$. Conditions: (A) 50 m*M* dibenzoyl-L-tartrate in 25 m*M* phosphate, pH 7.0, λ=300 nm; (B) 100 m*M* dibenzoyl-L-tartrate in 25 m*M* borate, pH 10.0, λ=450 nm (77 cm capillary, 70 cm to detection, V=260 V/cm, T=20°C).

The peak shapes observed for the neutral complex are more Gaussian with less tailing than for the charged TM complexes, suggesting that the (+)-charge contributes to deleterious wall interaction (which is confirmed by significant tailing in the absence of any resolving agent – data not shown). When a neutral marker is co-injected with neutral TM complexes it elutes ahead of them, indicating that these complexes effectively migrate as anions due to their association with the anionic additive. Additionally, it is useful to note that in certain cases (e.g., *rac*-$[Cr(phen)_2ox]^+$) we have observed that complexation between the analyte and resolving agent may result in the elution of the complex concomitant with the EOF. However, simple adjustment of buffer pH (see below) or reducing the ionic strength of the resolving agent as in Fig. 5A results in a change in either EOF or the average mobility of the TM complex such that this problem can be easily overcome for detection purposes.

3.3. Effects of buffer pH, temperature and capillary length

During the course of this work we noted that longer capillaries typically provide superior separation for TM enantiomers due to increased exposure to the resolving agent. However, these gains can be partially offset by a corresponding loss in separation efficiency associated with lower effective field strengths. One frequently used approach for reducing the rate of EOF in CE is the use of a run buffer of reduced pH. The net effect of the reduced EOF, in this case, is to provide for increased opportunity for analyte/resolving agent interaction without necessitating a compromise in the field strength. As long as the charge on the resolving agent is unaffected by the change in pH, association with the chiral selector is increased (and analysis times lengthened) without an appreciable increase in peak dispersion. Thus, in the case of the antimonyl-*d*-tartrate, isomer resolution at a lower pH is typically improved relative to higher pH buffers to the extent that shorter capillaries (and higher field strengths) can be applied. For example, although excellent resolution was achieved for the $M(phen)_3^{2+}$ systems using the antimonyl-*d*-tartrate at neutral or slightly basic pH, we noted that resolution for the corresponding $M(bpy)_3^{2+}$ species is incomplete at this pH (see above). However, Fig. 6 illustrates the dramatic effect of reduced buffer pH for the separation of a series of four chiral *rac*-$[Ru(diimine)_3]^{2+}$ compounds (including $[Ru(bpy)_3]^{2+}$) into their respective isomers (this figure is discussed further in Section 3.4). While

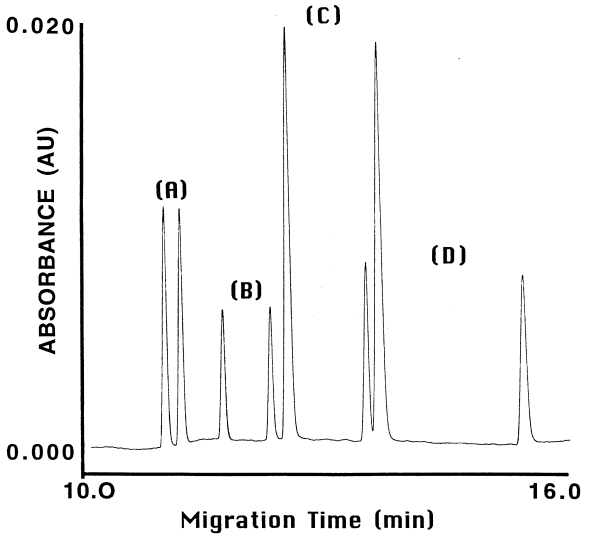


Fig. 6. Electropherogram of (A) 1.0 m*M* *rac*-$[Ru(bpy)_3]^{2+}$; (B) 0.5 m*M* *rac*-$[Ru(bpy)_2(phen)]^{2+}$, (C) 2.0 m*M* *rac*-$[Ru(bpy)(phen)_2]^{2+}$ and (D) 0.5 m*M* *rac*-$[Ru(phen)_3]^{2+}$ using 100 m*M* antimonyl-*d*-tartrate in 25 m*M* phosphate, pH 2.4. Isomers for each solute elute in the order Λ, Δ. Conditions: 77 cm capillary, 70 cm to detection, V=260 V/cm, λ=450 nm, T=35°C.

reduced pH is likewise useful for increasing interaction for the other resolving agents we have considered, for both the dibenzoyl-L-tartrate and *threo*-D_s[+]-isocitrate resolving agents, mildly acidic pH results in protonation of the anionic groups such that chiral interaction is compromised, and in the case of dibenzoyl-L-tartrate inhibits its aqueous solubility at pH<4.0.

In examining the role of temperature in the separation of the $[Ru(diimine)_3]^{2+}$ systems in particular, we have noted an unusual dependence for antimonyl-*d*-tartrate complexation. In the published literature for the preparative separation of the Λ and Δ isomers, it is reported (and we have confirmed) that at 4°C the Λ isomer of $[Ru(bpy)_3]^{2+}$ most effectively ion pairs with antimonyl-*d*-tartrate, resulting in its selective precipitation from aqueous solution [20]. However, in our own labs we have found the *Δ isomer* to selectively precipitate under *ambient* temperature conditions. In the current CE application, this would suggest that improved separations between Λ and Δ isomers might be expected at elevated temperatures, and this is experimentally observed for $[Ru(bpy)_3]^{2+}$ which exhibits superior resolution at temperatures >35°C. Although we have not comprehensively searched for such dependencies with the other TM complexes, resolution is often significantly improved at elevated temperatures with antimonyl-*d*-tartrate in particular, suggesting a similar effect. Indeed in the absence of such an effect (e.g., using *threo*-D_s[+]-isocitrate or dibenzoyl-L-tartrate agents), lower temperatures typically provide superior resolution due to reduced peak dispersion.

3.4. Complex mixtures

As our work with chiral resolution of TM complexes has progressed, we have realized that these additives work exceptionally well with solution mixtures containing different TM metals and/or ligand systems. For example, Fig. 7 shows the

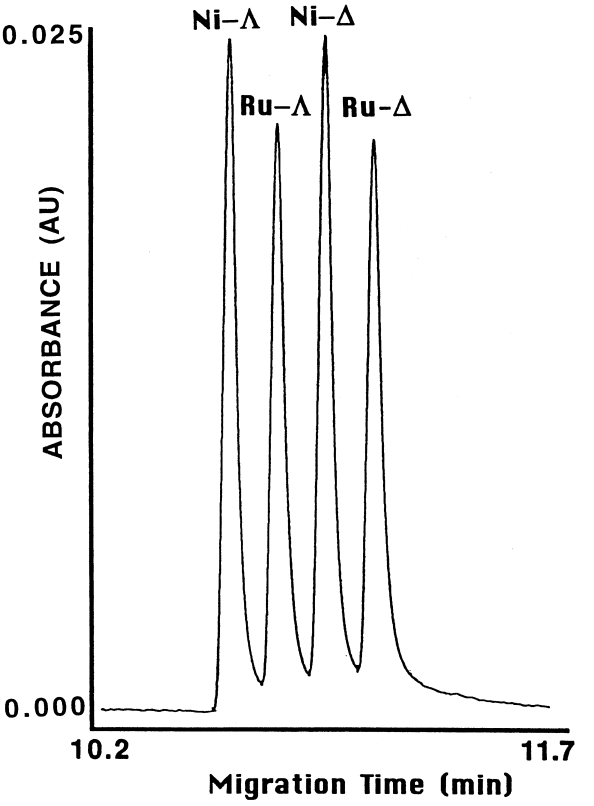


Fig. 7.Electropherogram of 1.0 m*M* *rac*-$[Ru(phen)_3]^{2+}$ and 1.0 m*M* *rac*-$[Ni(phen)_3]^{2+}$ using 100 m*M* antimonyl-*d*-tartrate in 25 m*M* phosphate, pH 7.0. Conditions: 70 cm capillary, 62 cm to detection, V=143 V/cm, λ=310 nm, T=35°C.

separation obtained for a racemic mixture of $[Ru(phen)_3]^{2+}$ and $[Ni(phen)_3]^{2+}$ using antimonyl-*d*-tartrate as the resolving agent at pH 7.0. Baseline separation is observed for this mixed metal system in only ten minutes; we have obtained similar resolution between $[Fe(phen)_3]^{2+}$ and the $[Ni(phen)_3]^{2+}$ or $[Ru(phen)_3]^{2+}$ systems. Likewise, chiral resolution between all isomers for metal systems with different complex charge (e.g., $[Ru(phen)_3]^{2+}$ vs. $[Cr(phen)_3]^{3+}$) is typically straightforward.

A much more frequently encountered analytical problem is the mixture of several different ligand combinations of a common central metal produced as a result of competing substitution pathways during TM syntheses or as a result of ligand lability in solution. For example, we previously demonstrated that *cis*-$[Ru(phen)_2(CH_3CN)_2]^{2+}$ could be separated into its respective Λ and Δ isomers using antimonyl-*d*-tartrate, and that as H_2O replaces the labile acetonitrile groups in aqueous solution, the appearance of two new peaks in the electropherogram signify the presence of the corresponding Λ and Δ isomers of *cis*-$[Ru(phen)_2(H_2O)_2]^{2+}$ [16]. As noted in Section 3.3, Fig. 6 provides the electropherograms obtained for a mixture containing four different chiral *rac*-$[Ru(diimine)_3]^{2+}$ complexes with antimonyl-*d*-tartrate. Remarkably, baseline separation of the Λ and Δ isomers is achieved for all four members of the series, $[Ru(bpy)_3]^{2+}$, $[Ru(bpy)_2(phen)]^{2+}$, $[Ru(bpy)(phen)_2]^{2+}$ and $[Ru(phen)_3]^{2+}$. In contrast, in the absence of the chiral resolving agent, only a single peak is observed in the zone electrophoresis of these four compounds as a result of their virtually identical hydrated radii. Thus, this application effectively illustrates the applicability of this buffer system not only to chiral separations, but also to ''real'' synthetic mixtures containing diverse ligand substitutions with similar net mobilities.

4. Conclusions

Capillary zone electrophoresis has been shown to be an exceptionally powerful and versatile tool for the separation of optically active TM complexes using a variety of readily available chiral resolving agents added to the electrophoretic run buffer. The relationship between charge of the resolving agent and the charge of the TM complex appears to play the most significant role in determining migration order irrespective of the identity of the central metal. Unlike much larger resolving agents used for separations of chiral organic molecules, small, highly charged organic salts containing multiple asymmetric carbons and possessing high aqueous solubility would appear to work most effectively as chiral resolving agents for TM complexes. This approach has been shown to be generally applicable to a large variety of TM systems, and can be used to assess product purity for a species generated as a result of new synthetic pathways, and establish enantiomeric purity based on the ratios of the Λ and Δ isomers. We anticipate additional future applications of CE to chiral TM systems, including studies on the kinetics and mechanisms of racemization and ligand exchange reactions in aqueous solution.

Acknowledgements

The authors wish to gratefully acknowledge financial support of this work provided by the Duke Endowment, Research Corporation and the National Science Foundation REU program. We also thank Mr. Jeff Glaeser and Ms. Olga Mytykh for their assistance in the preparation and analysis of the *cis*-$[Cr(phen)_2(H_2O)_2]^{3+}$ and $[(phen)_2Cr(OH)_2Cr(phen)_2]^{4+}$ complexes.

References

[1] R.L. St. Claire III, Anal. Chem. 68 (1996) 569R.
[2] M.L. Marina, A.L. Crego, J. Liq. Chromatogr. Rel. Technol. 20 (1997) 1337.
[3] R. Vespalec, P. Bocek, Electrophoresis 18 (1997) 853.
[4] A. Guttman, S. Brunet, N. Cooke, LC–GC 14 (1997) 32.
[5] S.C. Beale, Anal. Chem. 70 (1998) 279.
[6] B. Chankvetadze, Trends Anal. Chem. 18 (1999) 485.
[7] R.L. Williams, Gy. Vigh, J. Chromatogr. A 730 (1996) 273.
[8] H. Wan, L.G. Blomberg, Electrophoresis 18 (1997) 943.
[9] H. Nishi, J. Chromatogr. A 735 (1996) 345.
[10] Y. Tanaka, S. Terabe, Chromatographia 44 (1997) 119.
[11] S. Fanali, L. Ossicini, F. Foret, P. Bocek, J. Microcol. Sep. 1 (1989) 190.
[12] S. Elshihabi, J.A. Burke, M.M. Bushey, J. Microcol. Sep. 7 (1995) 199.

[13] S.F. Mason, B.J. Peart, J. Chem Soc., Dalton Trans. (1973) 949.
[14] J.P. Rehmmann, J.K. Barton, Biochemistry 29 (1990) 1701.
[15] D. Parker, Chem. Rev. 91 (1991) 1441.
[16] C.M. Shelton, K.E. Seaver, J.F. Wheeler, N.A.P. Kane-Maguire, Inorg. Chem. 36 (1997) 1532.
[17] B.J. Herbert, D.L. Chalk, N.A.P. Kane-Maguire, J.F. Wheeler, in preparation.
[18] F.P. Dwyer, E.C. Gyarfas, J. Proc. R. Soc. N.S.W. 83 (1949) 170.
[19] R.T. Watson, J.L. Jackson, J.D. Harper, K.A. Kane-Maguire, L.A.P. Kane-Maguire, N.A.P. Kane-Maguire, Inorg. Chim. Acta 249 (1996) 5.
[20] F.P. Dwyer, E.C. Gyarfas, J. Proc. R. Soc. N.S.W. 83 (1949) 174.
[21] B. Bosnich, Inorg. Chem. 7 (1968) 2379.
[22] G.B. Kauffman, L.T. Takahashi, Inorg. Synth. 8 (1966) 227.
[23] C.S. Lee, E.M. Gorton, H.M. Neumann, H.R. Hunt, Inorg. Chem. 5 (1966) 1397.
[24] N.A.P. Kane-Maguire, J.S. Hallock, Inorg. Chim. Acta 35 (1979) L309.
[25] J.A. Broomhead, M. Dwyer, N.A.P. Kane-Maguire, Inorg. Chem. 7 (1968) 1388.
[26] M.P. Hancock, J. Josephsen, C.E. Schaffer, Acta Chem. Scand. A 30 (1976) 79.