Application of Capillary Electrophoresis for the Assessment of Enantiomeric Purity of α-Diimine Transition Metal Complexes

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Although polarimetric or circular dichroism measurements are of critical importance for the characterization of optically active molecules, their value as probes of enantiomeric purity is compromised by the necessity for access to data for optically pure reference samples. For transition metal complex systems, this problem has been most successfully addressed via high-field NMR spectroscopic investigations in the presence of chiral shift reagents.[1−3] However, with the increasing availability of modestly priced and user-friendly capillary electrophoresis (CE) instrumentation, an alternative direct CE method for determining enantiomeric purity offers several practical advantages over conventional NMR procedures. For example, less sample is required, no deuterated solvents are employed, and the method is not restricted to diamagnetic analytes. Despite the growing use of CE for chiral separations in the analytical field,[4−6] only a few studies have involved the separation of transition metal complex systems. Fanali et al.[7] achieved isomeric separation of some ethylenediamine/amino acid complexes of Co^{3+} using sodium (*S*)-(+)-tartrate in the buffer, while more recently Bushey and co-workers[8] employed micellar electrokinetic chromatography to resolve enantiomers of several Fe^{2+} complexes containing tridentate quinoline-type ligands. We report here the versatility and convenience of capillary electrophoresis (CE) as an alternative procedure for the determination of enantiomeric purity of a range of transition metal complexes containing α-diimine ligands.

At operating voltages of 10−20 kV, injection of millimolar aqueous solutions of racemic M(α-diimine)$_3^{2+}$ species (M = Ru^{2+}, Ni^{2+}, Fe^{2+}; α-diimine = 1,10-phenanthroline or 2,2′-bipyridine) into a capillary containing 25 mM phosphate buffer (pH 7) and 100 mM potassium antimonyl *d*-tartrate results in effective separations of the respective Λ and Δ optical isomers.[9,10] The electropherogram of racemic Ru(phen)$_3^{2+}$ is shown in Figure 1A.[11] Excellent baseline enantiomeric separation is apparent, with the two peaks yielding identical integrations. The enantiomeric order of migration was established by coinjecting the racemic analyte with a sample of the Δ isomer, which resulted in the selective growth of the peak at longer migration time. We conclude, therefore, that the Δ isomer has the greater interaction with the antimonyl *d*-tartrate anion in the electrophoretic buffer. The corresponding electropherogram of a resolved sample[13] of Δ-(−)$_D$-Ru(phen)$_3^{2+}$ ($\Delta\epsilon_{264} = -600$ M^{-1} cm^{-1}) is provided in Figure 1B. Consistent with the prior spiking experiment, the dominant peak is observed at the longer time. From the relative areas of these two peaks, the enantiomeric purity of our Δ-(−)$_D$-Ru(phen)$_3^{2+}$ sample is assessed to be 98.5%. It is noteworthy that the most widely reported literature circular dichroism value for Δ-(−)$_D$-Ru(phen)$_3^{2+}$ is $\Delta\epsilon_{264} = -540$ M^{-1} cm^{-1},[12,14] which based on our data corresponds to an enantiomeric excess of 89%.

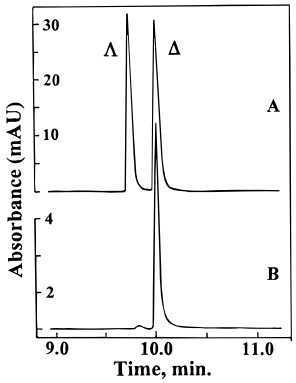

Figure 1. Electropherograms of (A) racemic Ru(phen)$_3^{2+}$ (2.0 mM) and (B) Δ-(−)$_D$-Ru(phen)$_3^{2+}$ (0.3 mM) in 25 mM sodium phosphate, pH 7.0 containing 100 mM potassium antimonyl *d*-tartrate. Electrophoretic field strength was 143 V/cm (50 μm i.d. capillary, 62 cm to detection) using a run temperature of 35 °C. Injection was hydrodynamic for 3 s, and detection was by UV/vis at $\lambda = 450$ nm.

Representative electropherograms are shown for paramagnetic Ni(phen)$_3^{2+}$ in Figure 2A (racemic) and Figure 2B (Δ-(−)$_D$-isomer) run under identical conditions. Inspection of Figure 2A reveals, once again, baseline separation of the Λ and Δ enantiomers. From the electropherogram in Figure 2B of a Δ-(−)$_D$-Ni (phen)$_3^{2+}$ sample ($\Delta\epsilon_{274} = -520$ M^{-1} cm^{-1}), it is clear that the Δ-species has the longer migration time (as previously observed for the Ru(phen)$_3^{2+}$ system). Using these data, optically pure Δ-(−)$_D$-Ni(phen)$_3^{2+}$ is calculated to have a

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(10) The CE instrument employed was a SpectraPhoresis 1000 (Thermo Separations Products, Fremont, CA).
(11) At the detection wavelength normally employed (450 nm) the metal to ligand charge transfer absorption band of the complex has a molar absorptivity near 19 000 M^{-1} cm^{-1},[12] providing excellent signal to noise at 1−2 mM analyte concentrations. Even higher sensitivity is achieved if the detection wavelength is shifted to the phenanthroline $\pi-\pi^*$ absorption maximum at 262 nm ($\epsilon = 89\,000$ M^{-1} cm^{-1}).[12]
(12) Mason, S. F.; Peart, B. J. *J. Chem. Soc., Dalton Trans.* **1973**, 949.
(13) Optically active samples of the complexes investigated were isolated via literature procedures: Ru(phen)$_3^{2+}$,[a] Ni(phen)$_3^{2+}$,[b] Fe(phen)$_3^{2+}$,[c] Ru(bpy)$_3^{2+}$,[d] *cis*-Ru(phen)$_2$(py)$_2^{2+}$,[e] cis-Ru(phen)$_2$(CH$_3$CN)$_2^{2+}$.[f] (a) Dwyer, F. P.; Gyarfas, E. C. *J. Proc. R. Soc. N.S.W.* **1949**, *83*, 170. (b) Kauffman, G. B.; Takahashi, L. T. *Inorg. Synth.* **1966**, *8*, 227. (c) VanMeter, F. M.; Neumann, H. M. *J. Am. Chem. Soc.* **1976**, *98*, 1388. (d) Dwyer, F. P.; Gyarfas, E. C. *J. Proc. Roy. S. N.S.W.* **1949**, *83*, 174. (e) Bosnich, B.; Dwyer, F. P. *Aust. J. Chem.* **1966**, *19*, 2229. (f) Watson, R. T.; Jackson, J. L.; Harper, J. D.; Kane-Maguire, K. A.; Kane-Maguire, L. A. P.; Kane-Maguire, N. A. P. *Inorg. Chim. Acta* **1996**, *249*, 5.
(14) It should be pointed out that the authors in ref 12 made no claims as to the degree of optical purity of their samples.

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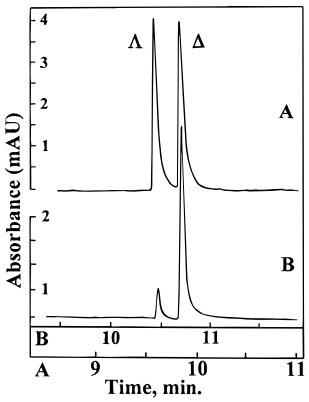

Figure 2. Electropherograms of (A) racemic Ni(phen)$_3^{2+}$ (2.0 mM) and (B) Δ-(−)$_D$-Ni(phen)$_3^{2+}$ (1.0 mM). Detection was at $\lambda = 310$ nm; all other conditions were as in Figure 1.

$\Delta\epsilon_{274}$ value of −650 M^{-1} cm^{-1} (i.e. slightly larger than the value of −636 M^{-1} cm^{-1} recently reported by Rehmann and Barton[15] and considerably higher than a frequently referenced value of −550 M^{-1} cm^{-1}).[12,14] We have also achieved good enantiomeric separations for the complexes Fe(phen)$_3^{2+}$ and Ru(bpy)$_3^{2+}$ (data not shown), with both species showing the same isomeric migration order as that noted for Ru(phen)$_3^{2+}$ and Ni(phen)$_3^{2+}$.

Analogous CE studies carried out on the related *cis*-Ru(α-diimine)$_2$(py)$_2^{2+}$ and *cis*-Ru(α-diimine)$_2$(CH$_3$CN)$_2^{2+}$ complexes employing potassium antimonyl *d*-tartrate as the chiral selector likewise provided effective enantiomer resolution (with the Δ-isomer once again showing the longer capillary migration time). The electropherogram for racemic *cis*-Ru(phen)$_2$(CH$_3$CN)$_2^{2+}$ is presented in the Supporting Information section. The two large peaks of equal area are assigned to the enantiomers of the parent compound, while the two very small peaks at longer migration time are attributed to trace components of the known hydrolysis product, *cis*-Ru(phen)$_2$(H$_2$O)$_2^{2+}$.[16] We have very recently described the isolation of PF_6^- salts of Δ- and Λ-*cis*-Ru(phen)$_2$(CH$_3$CN)$_2^{2+}$ utilizing the antimonyl *d*-tartrate anion as resolving agent,[13f] but were unable to find a suitable chiral shift reagent for NMR spectral studies. Instead, optical purity was determined indirectly by analyte conversion to the well-known Ru(phen)$_3^{2+}$ species. From an analysis of the electropherogram of a Λ-*cis*-[Ru(phen)$_2$(CH$_3$CN)$_2$](PF$_6$)$_2$ sample, the present CE study has provided verification of an estimated $\Delta\epsilon_{264}$ value of +140 for optically pure Λ-*cis*-[Ru(phen)$_2$(CH$_3$CN)$_2$](PF$_6$)$_2$.[13f]

Finally, we note that near-baseline separations of the Λ and Δ isomers of Ru(phen)$_3^{2+}$ and Ru(bpy)$_3^{2+}$ are also effected when calf thymus B-DNA (Sigma) is employed as the chiral medium in 50 mM ammonium acetate buffer (pH 5). A representative electropherogram is shown in Figure 3 for *rac*-Ru(bpy)$_3^{2+}$ in the presence of 1.50 mg/mL of B-DNA as buffer additive. A coinjection of the racemic analyte with Δ-(−)$_D$-Ru(bpy)$_3^{2+}$ established the component with the longer migration time as the Δ-isomer (the same order is also observed when a DNA/phosphate buffer at pH 7 is utilized). It is significant that the small degree of stereopreference for the Δ-isomer is difficult to detect in equilibrium dialysis studies.[17b] We have obtained

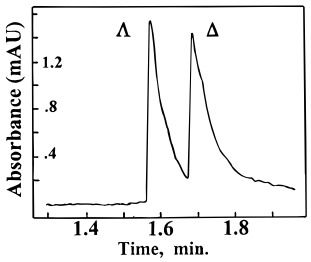

Figure 3. Electropherogram of racemic Ru(bpy)$_3^{2+}$ (0.5 mM) in 50 mM ammonium acetate, pH 5.0, containing 1.50 mg/mL calf-thymus B-DNA. Electrophoretic field strength was 454 V/cm (50 μm i.d. capillary, 25 cm to detection) using a run temperature of 30 °C. Injection was hydrodynamic for 20 s, and detection was at $\lambda = 450$ nm.

similar CE results for Ru(phen)$_3^{2+}$, with the Δ-enantiomer again being detected last.[18] The longer capillary residence times observed for the Ru(phen)$_3^{2+}$ system are consistent with the greater DNA binding equilibrium constant reported for this complex.[17] Our Ru(phen)$_3^{2+}$ results are also in accord with equilibrium dialysis studies by the Barton group and others,[17a,f] which indicate stronger DNA binding by the Δ-isomer. Recent literature data[17d−f] indicate that for both complexes DNA affinity is dominated by electrostatic surface binding. Our observation of a much greater breadth for the Ru(phen)$_3^{2+}$ isomer peaks provides support for the presence of some groove binding in the Ru(phen)$_3^{2+}$ case.[17] We are presently extending our studies to stronger DNA-binding diimine systems such as Ru(phen)$_2$(dppz)$^{2+}$ (dppz = dipyrido[3,2-*a*:2′,3′-*c*]phenazine), where intercalation has been established and a greater enantiomeric stereoselectivity has been observed.[20]

In summary, we have demonstrated that the enantiomeric purity of resolved diimine complexes may be rapidly established on the basis of a single injection and believe this CE method will prove an attractive option for a wide range of optically active inorganic systems. For DNA as the chiral medium, we also anticipate applications in the area of chiral recognition and preferential binding of metal complexes to biological molecules.

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Supporting Information Available: Figure S1, displaying the electropherogram of racemic *cis*-Ru(phen)$_2$(CH$_3$CN)$_2^{2+}$ in the presence of antimonyl *d*-tartrate as chiral additive (1 page). Ordering information is given on any current masthead page.

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