Published in final edited form as:

J Mol Struct. 2008 November 12; 890(1-3): 75–80. doi:10.1016/j.molstruc.2008.02.030.

Enantioseparations of Chiral Ruthenium(II) Polypyridyl Complexes Using HPLC with Macrocyclic Glycopeptide Chiral Stationary Phases (CSPs)

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Abstract

A high performance liquid chromatographic method using macrocyclic glycopeptide chiral stationary phases (CSPs) was used to separate enantiomers of seven ruthenium(II) polypyridyl complexes. Among the five different CSPs, the Chirobiotic T2 was most effective and baseline separated all complexes. All complexes show the same elution order with the δ -enantiomer being retained longer than the Λ -enantiomer. The mobile phase composition, including organic modifier type, organic modifier percent, salt type, and salt concentration, produced significant effects on the enantioresolution.

Keywords

chiral stationary phase; HPLC; macrocyclic glycopeptide; enantioresolution

1. Introduction

The chirality of octahedral transition metal complexes bound by three bidentate ligands has received a considerable amount of attention for several decades. Right- and left-handed configurations of metal complexes are referred to as δ - and Λ -enantiomers, respectively (Fig. 1) [1]. Ru(II) polypyridyl complexes have attracted growing interest due to their robust nature and unique photophysical characteristics[2]. These complexes are widely used as catalysts for asymmetric synthesis [3, 4], and to construct various supramolecular assemblies [5, 6]. Enantiomers of Ru(II) complexes have shown specific interactions with DNA [7-10]. Because many applications require stereochemically pure compounds, there is a great need for preparative methods by which pure single enantiomers can be obtained and analytical methods by which their enantiomeric excess can be determined.

There are different methods available for enantiomeric resolution of Ru complexes. Capillary eletrophoresis (CE) has been successfully applied to separate Ru complex

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enantiomers [11-13]. Enantiomers of sixteen metal complexes, with different metal centers (Ru^{2+} , Ni^{2+} , Cr^{3+} , and Co^{3+}), have been separated by capillary zone electrophoresis (CZE) using tartrate salt as the chiral selector in the running buffer [11]. CZE with double-stranded DNA as the chiral selector was used to separate enantiomers of $[\text{Ru}(\text{phen})_3]^{2+}$ and $[\text{Ru}(\text{bpy})_3]^{2+}$ [12]. However, CE is only an analytical method and not suitable for preparative-scale separations. The chromatographic method using chiral selector as an additive in the mobile phase was also reported with limited success [14, 15]. Due to high efficiency, wide selectivity, and easy operation, HPLC with chiral stationary phases (CSPs) has attracted attention for the enantioseparations of organometallic compounds. Cellulose- and amylose-based CSPs have been used to separate enantiomers of novel chiral metal tetrahedrane-type clusters [16, 17]. Cyclodextrin-based CSPs have been reported to separate enantiomers of metallocene [18] and Ru(II) polypyridyl complexes [19]. Enantiomers of Ni, Co, Cu, and Cr extended metal atom chain complexes have been separated on a Vancomycin CSP [20]. A Teicoplanin CSP was used to separate a series of chiral Ru(II) complexes, including RuL_3^{2+} ($\text{L}=2,2'$ -bipyridine(bpy), 1,10-phenanthroline(phen), and 4,7-diphenyl-1,10-phenanthroline(dpphen)) and some mixed ligand complexes [21]. However, the retention time is unsatisfiably long and the enantioselectivity is not high enough in some cases. Macrocyclic glycopeptides as chiral selectors for HPLC stationary phase were introduced in 1994 [22]. To date, four different macrocyclic glycopeptides have been commercialized to produce CSPs: vancomycin, teicoplanin, teicoplanin aglycone, and ristocetin A. They have somewhat similar structures and consist of an aglycon portion of fused macrocyclic rings that form a characteristic "basket" shape. The unique structure of the macrocyclic glycopeptides and their wide variety of functional groups (e.g. aromatic, hydroxyl, amine, carboxylic acid, and hydrophobic pockets, etc.) give them broad selectivity for a wide variety of compounds [22-27]. In this study, direct high-performance liquid chromatographic methods using the macrocyclic glycopeptide CSPs were developed for the separation of enantiomers of Ru(II) polypyridyl complexes. Baseline resolution was obtained for all seven complexes. Five of them ($[\text{Ru}(\text{phen})_2\text{nitrophen}]^{2+}$, $[\text{Ru}(\text{phen})_2\text{aminophen}]^{2+}$, $[\text{Ru}(\text{phen})_2\text{phendiamine}]^{2+}$, $[\text{Ru}(\text{phen})_2(\text{py})_2]^{2+}$, and $[\text{Ru}(\text{dppz})_3]^{2+}$) have never been separated on the macrocyclic glycopeptide CSPs. The analysis times for $[\text{Ru}(\text{phen})_3]^{2+}$ and $[\text{Ru}(\text{bpy})_3]^{2+}$ obtained in this study were greatly reduced compared to previously reported results using a teicoplanin CSP [21]. In addition, the effects of the mobile phase composition on the separations were investigated.

2. Experimental

2.1 Materials

Racemates of seven Ru(II) polypyridyl complexes used in this study are: $[\text{Ru}(\text{phen})_3]\text{Cl}_2$ (**1**) (phen=1,10-phenanthroline), $[\text{Ru}(\text{phen})_2\text{nitrophen}]\text{Cl}_2$ (**2**) (nitrophen=5-nitro-1,10-phenanthroline), $[\text{Ru}(\text{phen})_2\text{aminophen}]\text{Cl}_2$ (**3**) (aminophen=5-amino-1,10-phenanthroline), $[\text{Ru}(\text{phen})_2\text{phendiamine}]\text{Cl}_2$ (**4**) (phendiamine=5,6-diamino-1,10-phenanthroline), $[\text{Ru}(\text{phen})_2(\text{py})_2]\text{Cl}_2$ (**5**) (py=pyridine), $[\text{Ru}(\text{dppz})_3]\text{Cl}_2$ (**6**) (dppz=dipyrido[3,2-a:2',3'-c]phenazine), $[\text{Ru}(\text{bpy})_3]\text{Cl}_2$ (**7**) (bpy=2,2'-bipyridine). At least one single enantiomer (Λ or Δ) for each complex was available for determining elution order. The structure of all complexes is shown in Fig. 2. Chirobiotic T (Teicoplanin), TAG (Teicoplanin aglycone), T2

(Teicoplanin), V (Vancomycin), and R (Ristocetin A) CSPs were obtained from Advanced Separation Technologies (Whippany, NJ, USA). All the columns are 250×46 mm.

Acetonitrile and methanol of HPLC grade were purchased from EMD (Gillbstown, NJ, USA). Water was obtained from Millipore (Billerica, MA). Tetramethylammonium nitrate (TMAN), ammonium trifluoroacetate, ammonium nitrate, and ammonium acetate were purchased from Sigma-Aldrich (St. Louis, MO).

2.2 LC analysis

The chromatographic system is an HP (Agilent Technologies, Palo Alto, CA, USA) 1050 system, which consists of a UV VWD detector, an autosampler, a quaternary pump and Chemstation software. For the LC analysis, the injection volume, the flow rate, and the detection wavelength is 5 μ L, 1 mL/min, and 254 nm, respectively. Separations were carried out at room temperature (~ 22 °C). The mobile phase was degassed by ultrasonication under vacuum for 5 min. Each sample was analyzed in duplicate. The chloride (Cl^-) salts and hexafluorophosphate (PF_6^-) salts of Ru(II) complexes were dissolved in methanol and acetonitrile, respectively. Samples were dissolved at 1.0 mg/ml concentration and subsequently diluted with methanol or acetonitrile for LC injection.

2.3 Calculations

The retention factor (k') was calculated using the equation $k' = (t_r - t_0)/t_r$, where t_r is the retention time, and t_0 is the dead time which is determined by the peak of the refractive index change due to the sample solvent. Selectivity (α) was calculated by $\alpha = k_2'/k_1'$, where k_1' and k_2' are the retention factors of the first and second eluted enantiomers, respectively. The resolution (R_s) was determined using $R_s = 2 \times (t_{r2} - t_{r1}) / (w_1 + w_2)$, where w is the base peak width.

3. Results and Discussion

3.1 Performance of Chirobiotic CSPs

The Ru(II) complexes in this study contain two or three diimine ligands (phen, bpy or dppz). The simplest of these is $[\text{Ru}(\text{phen})_3]\text{Cl}_2$ (complex **1**), which is used as a model compound. The performance of five Chirobiotic CSPs (T, TAG, T2, V, and R), was tested by analyzing $[\text{Ru}(\text{phen})_3]\text{Cl}_2$ under similar mobile phase compositions (acetonitrile/methanol/water/ NH_4NO_3). The chromatograms obtained using four CSPs are shown in Fig. 3. $[\text{Ru}(\text{phen})_3]\text{Cl}_2$ enantiomers were partially separated on the Chirobiotic R column (Fig. 3). The mobile phase was changed to increase retention and possibly resolution, however, no obvious improvement in the enantioseparation was observed. The analyte was not retained on the Chirobiotic V column, when using the same mobile phase as for the Chirobiotic R. A weaker mobile phase (lower acetonitrile and salt concentration) was used and a broad peak was obtained (Fig. 3).

In contrast to the vancomycin column, the teicoplanin series of CSPs (T, TAG, T2) showed much greater retention for $[\text{Ru}(\text{phen})_3]\text{Cl}_2$ and stronger mobile phases were required for elution. As shown in Figure 3, the Chirobiotic T and T2 columns provided baseline

separations of complex **1**, while the analyte was not eluted on the Chirobiotic TAG column (data not shown). Shorter retention and much greater selectivity were obtained on the Chirobiotic T2 column. The comparison of the performances of five macrocyclic glycopeptide stationary phases revealed that the retention strength order from strongest to weakest was TAG>T> T2>R>V. These five CSPs exhibit distinctly different selectivities toward $[\text{Ru}(\text{phen})_3]\text{Cl}_2$. Of the macrocyclic glycopeptide CSPs tested, the teicoplanin-based CSPs appear to have superior enantioselectivity. The Chirobiotic T2 column is synthesized using a different linkage chemistry and silica gel. It has been reported that T2 exhibits better selectivity than T [28] and has a higher loading capacity. Our results also indicate that the Chirobiotic T2 should offer improved selectivity and resolution for the chiral ruthenium complexes.

3.2 Effect of the mobile phase composition on enantioseparation using teicoplanin (T2) column

The influence of the mobile phase on the enantioseparation of $[\text{Ru}(\text{phen})_3]\text{Cl}_2$ was studied since interactions between stationary phases and analytes can be greatly affected by the mobile phase used. In order to evaluate the effect of organic modifier concentration, the percentage of acetonitrile was decreased from 100% to 0% while maintaining a constant salt concentration (0.08M tetramethylammonium nitrate). Fig. 4 shows the effect of organic modifier concentration on retention, enantioselectivity, and resolution. A typical U-shaped curve (Figure 4a) was obtained when plotting retention vs the acetonitrile percentage. When the acetonitrile percentage is above 70%, increasing the concentration of acetonitrile increases the retention. When acetonitrile is below 70%, the reversed trend is observed. This U-shaped behavior is usually observed on the macrocyclic glycopeptide CSPs [22, 23]. Hydrophobic interactions between the analyte and the stationary phase contribute significantly to retention at higher concentrations of aqueous buffer, while other interactions (e.g. hydrogen bonding) become dominant at higher concentrations of organic modifier [23]. Fig. 4b indicates that the selectivity (α) exhibits a slight increase with reduced acetonitrile concentration. The effect of the organic modifier concentration on resolution is more complex since retention, selectivity, and efficiency all contribute to resolution. In this case study, maximal resolution was obtained at 50%acetonitrile/50% water/0.08M TMAN.

Separations can also be affected by the nature of the organic modifiers. Acetonitrile and methanol are commonly used as organic modifiers with the Chirobiotic columns. Methanol/water/0.08M TMAN was tested as the mobile phase for comparison with acetonitrile. K_1' , α , and R_s of complex **1** are 11.974, 1.684, 2.5, respectively, when using 70% methanol/30% water/0.08M TMAN as the mobile phase. Compared with acetonitrile (data shown in Table 2, the optimized results of **1**, $[\text{Ru}(\text{phen})_3]\text{Cl}_2$), higher selectivity and longer retention time were observed using methanol as organic modifier. Methanol has a lower elution strength than acetonitrile because it is more polar, however the reason for the beneficial effect of methanol on enantioselectivity is unknown. It has been reported that methanol produces higher enantioselectivity on the teicoplanin-based CSPs [23, 27]. In order to combine the good efficiency of acetonitrile and the high enantioselectivity of methanol, optimization can be carried out using the two organic modifiers simultaneously. This dual modifier system proved to be successful and necessary for the separation of $[\text{Ru}(\text{dppz})_3]\text{Cl}_2$

(complex **6**). It can not be baseline-separated using acetonitrile-aqueous or methanol-aqueous mobile phases, but baseline resolution was obtained using 60% acetonitrile/20% methanol/20% water/0.08 M TMAN.

In our study, salt additive was necessary for the elution of ruthenium(II) complexes. Different salts (ammonium nitrate, tetramethylammonium nitrate, ammonium trifluoroacetate, and ammonium acetate) were used as the additive in 70% acetonitrile/30% water at identical concentrations (0.08 M), since the minimum retention was observed with this mobile phase composition. The empirical results (Table 1) reveal that different salts do affect retention and selectivity. Since tetramethylammonium nitrate (TMAN) as an additive produced the highest selectivity and resolution, it was applied to optimize separations of the other Ru(II) complexes. Furthermore, an increase in salt concentration was found to decrease retention and resolution (Fig. 5). This may be due to increased competition of salt for the interaction sites of the stationary phase (e.g. ionic interaction sites) [31, 32].

3.3 Enantioseparation of Ru(II) complexes

Based on its high enantioselectivity for complex **1**, the separation and optimization of all seven Ru(II) polypyridyl compounds were carried out on the Chirobiotic T2 column. Various mobile phases composed of acetonitrile/water/TMAN were tested. If resolution was not adequate, a small amount of methanol was added in order to improve selectivity. Baseline enantioseparation of six of seven compounds was achieved with only acetonitrile as the organic modifier, while complex **6** was only baseline separated using acetonitrile/methanol/water/TMAN. The chromatographic results at optimal separation conditions are shown in Table 2. For Ru(II) polypyridyl complexes, the Chirobiotic T2 CSP provided good enantioselectivity (in the range of 1.149-1.465) and all seven compounds were baseline separated (R_s 1.5). Selected chromatograms are shown in Fig. 6. The separation results demonstrate that this method is applicable for the accurate determination of enantiomeric excess (ee).

The enantiomeric elution order indicated in Table 2 was established by injecting a single enantiomer under the same experimental conditions. The elution order of Ru(II) polypyridyl complexes was very consistent: the Δ -enantiomer eluted first. This indicates that the Δ -enantiomer shows greater affinity for the chiral stationary phase. Interestingly, a study with cyclodextrin-based CSP's showed the opposite elution order for these complexes [19]. The fact that all seven complexes show the same elution order could be explained by their similar ligands, which may interact with the stationary phase in similar ways. The consistency of elution order using the teicoplanin CSP, while not absolute proof of the absolute configuration of the Ru polypyridyl complexes, provides some circumstantial evidence at the point in time.

The effect of the counteranions of the Ru(II) complexes on the separation was investigated. Six analytes with the same chiral cation ($[\text{Ru}(\text{phen})_3]^{2+}$) and different anions (Cl^- , Br^- , F^- , BF_4^- , PF_6^- , and CF_3SO_3^-) were injected on the T2 CSP using the same mobile phase (70% acetonitrile/30% water/0.08M TMAN). The various anions showed no effect on the retention time, selectivity, and efficiency. It is likely that the mobile-phase nitrate anion

exchanges rapidly with the initial counter-ions, and this new ion-pair undergoes the chiral recognition process.

4. Conclusion

This study has demonstrated that the teicoplanin-based CSPs are the best macrocyclic glycopeptide columns for the enantioseparation of Ru(II) polypyridyl complexes. The newly developed Chirobiotic T2 column works more successfully than the Chirobiotic T. Enantioseparation was optimized by variation of the mobile phase composition. As a result, baseline separation was achieved for all seven complexes. This method was highly suitable for monitoring enantiomeric excess. In all cases tested, the Λ -enantiomer eluted first.

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Fig. 1.
Mirror image relationship of Ru(II) trisdiimine enantiomers

Fig. 2.
Structure of Ru(II) polypyridyl complexes

Fig. 3. Separation of $[\text{Ru}(\text{phen})_3]\text{Cl}_2$ (complex **1**) on different Chirobiotic CSPs. The mobile phase composition is: (a) 40% methanol/40% acetonitrile/20% water/0.08M NH_4NO_3 ; (b) 60% methanol/20% acetonitrile/20% water/0.04M NH_4NO_3 ; (c) 20% methanol/60% acetonitrile/20% water/0.08M NH_4NO_3 ; (d) 20% methanol/60% acetonitrile/20% water/0.08M NH_4NO_3 .

Fig. 4. Effect of acetonitrile concentration on (a) retention factor, and (b) selectivity and resolution. The analyte and CSP are [Ru(phen)₃]Cl₂ (1), the Chirobiotic T2, respectively. The mobile phase is acetonitrile/water/0.08M tetramethylammonium nitrate.

Fig. 5. Effect of the salt concentration in the mobile phase on separation. The analyte and CSP are [Ru(phen)₃]Cl₂ (1), the Chirobiotic T2, respectively.

Fig. 6. Selected chromatograms in the optimal mobile phase on Chirobiotic T2 column. The mobile phase compositions of each complex are shown in Table 2.

Table 1

Effect of salt type in the mobile phase on enantioseparation

Salt type	k_1'	α	R_s
NH_4NO_3	0.9666	1.417	1.7
$\text{N}(\text{CH}_3)_4\text{NO}_3$	1.813	1.477	2.5
$\text{NH}_4\text{COOCF}_3$	0.6197	1.444	1.5
$\text{NH}_4\text{COOCH}_3$	6.542	1.392	2.0

Note: $[\text{Ru}(\text{phen})_3]\text{Cl}_2$ (complex **1**) is separated on Chirobiotic T2 column. The mobile phase is 70%acetonitrile/30% water/0.08M salt.

Table 2

Optimized separation of seven complexes on Chirobiotic T2 CSP

Number	Name	k_1'	α	R_s	Elution order	Mobile phase
1	[Ru(phen) ₃]Cl ₂	1.813	1.477	2.5	Λ,	A
2	[Ru(phen) ₂ nitrophen]Cl ₂	3.009	1.266	1.6	Λ,	B
3	[Ru(phen) ₂ aminophen]Cl ₂	2.356	1.426	2.2	Λ,	A
4	[Ru(phen) ₂ phendiamine]Cl ₂	3.260	1.434	2.4	Λ,	A
5	[Ru(phen) ₂ py ₂]Cl ₂	2.358	1.216	1.5	Λ,	B
6	[Ru(dppz) ₃]Cl ₂	7.968	1.234	1.5	Λ,	C
7	[Ru(bpy) ₃]Cl ₂	3.334	1.160	1.5	Λ,	D

Note: the mobile phase compositions are:

A: 70% acetonitrile/30% water/0.08M TMAN

B: 60% acetonitrile/40% water/0.08M TMAN

C: 60% acetonitrile/20% methanol/20% water/0.08M TMAN

D: 50% acetonitrile/50% water/0.08M TMAN