Chromatographic separation of stereoisomers of ligand-bridged diruthenium polypyridyl species

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Cation-exchange chromatographic techniques have been developed to separate stereoisomers of polymetallic complexes, using SP Sephadex C-25 as support. Through the example of the ligand-bridged dinuclear cation 
\[[\text{Ru} \text{dmbpy}]_2 \text{μ-bipyrim}\]^+ (dmbpy = 4,4′-dimethyl-2,2′-bipyridine, bipyrim = 2,2′-bipyridimidine), the isolation and characterisation of the meso and rac diastereoisomers by elution with aqueous sodium toluene-4-sulfonate solution are demonstrated. The effects of variation in salt concentration and temperature on the efficacy of the separation are discussed. The enantiomeric pair of the rac diastereoisomer (ΔΔ and ΔΛ) was subsequently chromatographically resolved on SP Sephadex C-25, relying upon the inherent chirality of the support. Optical resolutions using eluents with chiral anions [aqueous sodium (+)-O,O′-dibenzoyl-d-tartarate and sodium (−)-O,O′-dibenzoyl-l-tartarate] were investigated, with the (−)-enantiomer demonstrating a positive [the (+)-enantomer a negative] synergistic effect in combination with the Sephadex support. Crystals of the meso form were isolated, allowing an X-ray structural determination.

The study of polymetallic ‘supramolecular’ assemblies is a rapidly expanding field of chemistry, largely because of their potential application in materials for such diverse purposes as photochemical molecular devices, and as phototrops of structure and function of polynucleotides such as DNA. In particular, much attention has been focused upon the polypyridyl complexes of ruthenium and osmium as the basis for such assemblies as a result of their favourable photochemical and redox characteristics. When bidentate ligands are involved in such centres, stereoisomerism is inherent in the resultant assemblies, but surprisingly this problem has until recently received only tacit attention.

In general, studies of chirality in octahedral tris(bidentate ligand)ruthenium(II) centres have been limited to mononuclear species, and isolation of enantiomers has relied upon the sometimes inefficient technique of diastereoisomer formation using chiral building blocks for larger assemblies, but this approach requires tedious synthetic procedures where care must be taken to preserve the chiral integrity at each metal centre during every reaction step. Additionally, in the vast majority of the targets studied, crystallisation has proved the most favourable method of diastereoisomeric discrimination. Our aim was to develop general chromatographic techniques for the separation of stereoisomers not only of mononuclear complexes, but also of oligomeric transition-metal assemblies containing polypyridyl ligands. In the latter case the simplest example is that of a dinuclear complex of the type \[[\text{Ru} \text{pp}]_2 \text{μ-L}_4\]^+ (pp is a symmetrical bidentate ligand, \(L_4\) is a bis(bidentate) bridging ligand). There are the possibilities of the meso (ΔΔ) and the rac diastereoisomers, the rac form consisting of an enantiomeric pair (ΔΔ and ΔΛ), as illustrated in Fig. 1.

In these studies, SP Sephadex C-25 was chosen as the cation-exchange support, rather than the more commonly used polystyrene-based resins for which we had previously observed significant absorptions by some complexes containing polypyridyl ligands. This material has been extensively used in the separation of stereoisomers of various octahedral cobalt(III) species, but surprisingly it appears not to have been widely applied to ruthenium(II) complexes containing polypyridyl ligands until our recent studies. SP Sephadex is composed of a cross-linked dextran matrix, functionalised with strongly acidic propanesulfonate groups. The cations to be separated are adsorbed onto the anionic stationary phase, and separation is achieved as a result of differential ion-exchange equilibria involving the components of the mixture and the cations of the eluting electrolyte solution (typically a Na⁺ salt).

To date we have successfully achieved the chromatographic separation of a wide variety of stereoisomers of mono-, di- and tri-metallic species using SP Sephadex C-25 as the support with aqueous sodium tolune-4-sulfonate as eluent. Further, chiral resolution of monomeric species such as [Ru(tpmby)phen(ppy)]^2+ and [Ru(tmbpy)ppy]^2+ (where tmbpy = 4,4′,5,5′-tetramethyl-2,2′-bipyridine, phen = 1,10-phenanthroline, ppy = pyridine) have been achieved by using an eluent containing the chiral anion (−)-di-4-toluoyl-l-tartarate. In addition, we have described the separation and isolation of the ΔΔ and ΔΛ forms of the dinuclear complexes \([\text{Ru} \text{dmbpy}]_2 \text{μ-apy}^+]^4+ (\text{dmbpy} = 4,4′-dimethyl-2,2′-bipyridine, apy = 2,2′-azopyridine), as well as the stereoisomers of the dinuclear species \([\text{Ru} \text{pp}]_2 \text{μ-hat}^+]^4+ (\text{ΔΔ}, \text{ΔΛ} and \text{ ΔΛ}) and of the tri-nuclear complexes \([\text{Ru} \text{pp}]_3 \text{μ-hat}^+]^{6+} (\text{ΔΔΔ} and \text{ΔAA}, \text{ΔΔ} and \text{ΔΛΛ}) [\text{ΔΔ}, \text{ΔΛ}, \text{ΔΛ}, \text{ΔΛ} and \text{ ΔΛΛ}] [\text{μopenhagen pants} (2,2′-bipyridine) or phen; hat = 1,4,5,8,9,12-hexaazatriphenylene].

Results and Discussion

Diastereoisomer synthesis, isolation and characterisation

The ligand-bridged dinuclear complex \([\text{Ru} \text{dmbpy}]_2 \text{μ-}
...and in each case the meso isomer (as indicated by $^1$H NMR spectroscopy) was the fastest moving band on the column, despite the differing geometries. The evidence clearly indicates that this method for diastereoisomeric separation is general for a wide variety of dinuclear species of this type.

A sample of the first major fraction (meso) of $\{[\text{Ru}(	ext{dmbpy})_2]_2\mu$-bipyrimidine]$^2$ was repurified by gel permeation chromatography on Sephadex LH20 support (methanol eluent) to remove inorganic impurities. Following removal of the solvent and redissolution in water, oily crystals were grown by slow evaporation from the aqueous solution which were suitable for X-ray analysis. To date, very few crystals of dinuclear ruthenium polypyridyl complexes have been obtained. Ward and co-workers describe an alkoxide-bridged species, where only the rac form was isolated, the meso form being excluded on steric grounds. Two examples of dinuclear species containing unsymmetrical polypyridyl ligands have been reported, where the crystals have preferentially grown in the meso form.

One of the problems in the growth of crystals of such species may well be the existence of a mixture of stereoisomers, and we present here for the first time the selective isolation of one of the various components prior to crystal growth.

The crystals themselves proved to be of poor quality, crumbling easily and being extremely prone to solvent evaporation. However, a data set was obtained and the structure solved, illustrating the connectivity of the complex (Fig. 3). Assuming that the crystal is representative of the bulk solution, this first major band is confirmed as the meso (AA) form. The clefts between the bipyridine rings contained several water molecules, while there are planes of water and chloride anions between the predominantly organic layers of the complex (Fig. 4). These distinct layers are probably the cause of the extreme brittleness and solvation dependence of the material, and another possible reason for the extremely small number of structures of these complexes existing in the literature. The selected bond lengths and angles are given in Table 1. The molecule possesses crystallographic symmetry along the bipyrimidine bridge (the second half of the molecule being generated from the first). The average Ru–N bond lengths (2.04 Å) and N–Ru–N angles correlate with those published for previous structures.

**Effect of eluent concentration on the diastereoisomeric separation**

The variation of eluent concentration by a small amount can have a great influence on the observed separation. To illustrate this, a series of quantitative small-column experiments were undertaken using $\{[\text{Ru}(	ext{dmbpy})_2]_2\mu$-bipyrimidine]$^2$ as the target, with aqueous sodium toluene-4-sulfonate solutions as eluent at concentrations between 0.05 and 1.0 mol dm$^{-3}$; the relative...
played in Fig. 5, giving the data for two forms (given in the Experimental section.)

Column behaviour (in a range of 0.2–2.0 cm although the rate of flow was noted to have little effect on the separation of the two bands did not change, the bands broad-

independent, being between a solid and solute phase.

rates of passage of the two bands down the column of SP Sephadex C-25 were recorded.

To ensure consistency in the results, care was taken to pre-equilibrate the column in the desired eluent, overcoming the problems of contraction of the support upon change of the salt concentration. Further, care was taken to monitor the rate of flow by recording the volume of eluent passed and the time. In order to give comparable results from each column, where slight discrepancies in flow rate were observed the data were equilibrated column described above was fitted with an external

librium between the stationary phase and the mobile phase. As a consequence of this the separation of the bands takes a greater length of the column to be achieved. Further, with concentrations over 0.5 mol dm$^{-3}$, the rates of passage of the combined fractions did not increase significantly, implying that there is a maximum possible rate of passage of the compound down the column.

From the observations made, it is apparent that the optimum concentration for efficient separation of the diastereoisomers of [{Ru-(dmbpy)$_2$}($\mu$-bipy)]$^{2+}$ appears to be ca. 0.25 mol dm$^{-3}$.

Effect of temperature on the diastereoisomeric separation

All previous stereoisomeric separations have been carried out at room temperature, and so investigations were made into the temperature dependence of the process, while maintaining the eluent concentration at 0.25 mol dm$^{-3}$. The small pre-equilibrated column described above was fitted with an external water-jacket, and the temperature of the SP Sephadex C-25 controlled using a circulating thermostatted bath.

As illustrated in Fig. 6, the rate of passage of [{Ru-(dmbpy)$_2$}($\mu$-bipy)]$^{2+}$ down the column decreases as the temperature increases. The data indicate a linear relationship, with the relative resolution of the two bands staying approximately constant. At lower temperature greater broadening of the bands was observed, associated with the faster passage through the support and loss of the equilibria.

The association between the SP Sephadex C-25 support and the cationic substrate should be essentially temperature independent, being between a solid and solute phase. Since coulombic forces dominate the rate of passage of species down
there was a clear separation of the two chiral forms of the rac diastereoisomer. These were collected and isolated as the hexafluorophosphate salts, and the resolution into the individual enantiomers confirmed by CD measurements (Fig. 7).

By comparison with the CD enantiomer assignment for stereoselectively synthesized ΔΔ- and ΔΔ-[Ru(bpy)_3]([μ-bipy)m]⁴⁶ by Hua and von Zelewsky,¹² the first band off the column was assigned as the ΔΔ isomer, followed by the ΔΔ form. Importantly, we have achieved for the first time a separation of the two enantiomers using an achiral eluent on the SP Sephadex C-25 cation exchanger. The inference is clearly that the inherent structure of the dextran support itself must provide the chiral environment responsible for this process. The individual units of SP Sephadex are composed of propanesulfonate-functionalised cross-linked d-α-glucopyranoside, and there are five chiral centres in each subunit.

While coulombic forces dominate the cation-exchange chromatographic process, the charge densities or polarities of the cations¹⁴ also exert an influence for species of the same charge. The use of a suitable counter anion in the eluent also has an effect on the second-sphere interactions between cation and anion.¹⁴,¹² Since isomers may differ in their relative interactions with the counter anion, the resultant slight variations in the effective charge and polarity of the species facilitate separation on the column. However, with the chiral resolution observed with an achiral eluent, the effective charge and polarity of the two associated enantiomers must be the same. Hence the separation must be a consequence of the support material itself, where the mechanism has elements of exclusion on the basis of shape.

The significance of this observation is that chiral eluents such as sodium (+)-O,O′-ditoluoyl-d-tartrate and sodium (+)-dibenzoyl-l-tartrate are not always necessary in the separation of simple enantiomeric pairs on the Sephadex support. It is however expected that the efficiency of such resolutions will be synergistically enhanced by the correct choice of chiral eluent.

Chiral resolution of the racemic diastereoisomer with a chiral eluent

Since the support material has such a significant effect on the two enantiomers, it can be assumed that the use of chiral counter anions in the eluent will either oppose or enhance the effect observed above. The rate of passage down the small pre-equilibrated column was therefore investigated to examine the effect of the eluents sodium (+)-dibenzoyl-l-tartrate and (−)-dibenzoyl-l-tartrate (0.154 mol dm⁻³) on the rate of elution of the three individual stereoisomers down the column. The results are displayed in Fig. 8.

Sodium (−)-O,O′-dibenzoyl-l-tartrate behaved in a similar fashion to sodium toluene-4-sulfonate as an eluent, in that the meso-ΔΔ forms moved the fastest, with the rac-ΔΔ next and the rac-LLL slowest on the column. The chiral resolution of the rac form was however achieved in a much shorter distance, demonstrating a positive synergistic effect between the chiral eluent and the Sephadex support. On the other hand, with sodium (+)-O,O′-dibenzoyl-d-tartrate as eluent, the order of travel of the two enantiomers was reversed with the ΔΔ form travelling faster than the ΔΔ. Similar behaviour has also been observed using simple monomers such as [Ru(bpy)]³⁺ and [Ru(phen)]³⁺ and is currently undergoing intense study to try to rationalise this observation.²⁴

von Zelewsky and co-workers¹⁵ have shown by X-ray crystallography that there is a specific association between Δ- [Ru(bpy)_3]([py])²⁺ and (−)-O,O′-dibenzoyl-l-tartrate, and between Δ-[Ru(bpy)_3]([py])²⁺ and (+)-O,O′-dibenzoyl-d-tartrate. While in this case the possible π-stacking interactions between the aromatic benzoyl groups and the ligand pyridyl groups cause selective crystallisation, the same type of associations appear to dictate the order of travel down the column.

Chiral resolution of the racemic diastereoisomer with an achiral eluent

Previously, we have established that the ΔΔ and ΔΔ enantiomers of the complex [Ru(dmbpy)_2]([μ-apy)]⁴¹ can be separated chromatographically using the chiral eluent sodium (−)-O,O′-di-4-toluoyl-l-tartrate.²² However, during our studies on the [(Ru(dmbpy)_2]([μ-bipy)m]⁴⁶ system, the observation was made that there was considerable spreading of the slower-moving (rac) band on the column during the separation of the meso and rac diastereoisomers using sodium toluene-4-sulfonate as the eluent. To investigate whether chiral resolution could be achieved using this achiral salt, the slower-moving band from the diastereoisomer separation was reintroduced onto the top of the SP Sephadex C-25 column, and allowed to recycle several times down the length of the column (1 m). After it had travelled an effective column length (ECL) of ca. 2.5 m
A comparison of the use of sodium (−)-O,OO′-dibenzoyl-tartrate with sodium (+)-O,OO′-dibenzoyl-tartrate as eluent indicates that there is much slower passage down the column for the latter eluent (1.25 times slower for the meso diastereoisomer). Additionally, the distance required to achieve chiral resolution between the enantiomeric pairs is larger with (+)-O,OO′-dibenzoyl-tartrate. The implication is that the effect of the chirality of (+)-O,OO′-dibenzoyl-tartrate opposes that of the Sephadex itself, slowing the rate of travel down the column and hindering the chiral resolution. Accordingly, care must be observed in the choice of chiral eluent to use this synergistic behaviour to enhance the separation.

Conclusion
In order to characterise simple ligand-bridged dinuclear polypyridyl species by standard techniques such as NMR spectroscopy the isolation of the individual diastereoisomers must first be achieved. Further, the isolation of stereochemically pure samples is likely to facilitate the growth of crystals appropriate for X-ray structural analysis. Such separations of diastereoisomers has been achieved using SP Sephadex C-25 cation exchanger and elution with sodium toluene-4-sulfonate. The variable-temperature experiments clearly indicate that there must be a significant degree of association of the eluent anion and cation which affects the interaction of the cation with the support. For the first time, chiral resolution of the racemic form has been achieved with the use of this achiral counter anion, rather than by using a chiral auxiliary. The respective bands from the column have been identified by a combination of a simple resolution of enantiomers.

Experimental
Instrumentation
The NMR spectra were recorded on a Varian Unity Inova-500 spectrometer using the solvent as the internal reference. CD spectra in acetoni triole solution using a Jobin Yvon spectrophotometer (λmax/nm) and high-resolution mass spectra on a BRUKER BioApex 47e ICR spectrometer with an electrospray ionisation source, using solutions ca. 2 µg cm−2 in methanol. Microanalyses were carried out on a Carlo Erba EA 1108 CHNS analyser. For the column chromatography studies, preparative columns C16/100 and C26/100 were from Pharmacia Biotech. The semiquantitative column K9 (Pharmacia Biotech) was fitted with an external water-jacket, temperature regulated with a Talabo F10 circularly thermostatted water-bath. Column flow rates were regulated with a Gilson mini bus 2 peristaltic pump.

Materials
The compounds 4,4′-dimethyl-2,2′-bipyridine (Aldrich), 2,2′-bipyridimine (Lancaster), 1,10-phenanthroline, 2,2′-bipyridine and sodium toluene-4-sulfonate (Aldrich) and ruthenium tri-chloride hydrate (Strem) were used as received without further purification. Aqueous sodium (−)-O,O′-dibenzoyl-tartrate and sodium (+)-O,O′-dibenzoyl-tartrate solutions were prepared by the addition of sodium hydroxide solution to the corresponding acids (Fluka), until a pH of 8–9 was obtained. SP Sephadex C-25 and Sephadex LH20 in anhydrous form were from Pharmacia Biotech, Amberlite IRA 400 from Aldrich. The precursors [Ru-L2Cl2] (L = dmbpy, phen or bpy) were prepared according to the literature method.24 Laboratory-grade solvents were used unless otherwise specified.

Complex syntheses

\[ [\text{Ru(dmbpy)}]_{2}^{2+} (\mu-\text{bipyrm}) \] (PF6)_4 are diastereoisomers which are likely to facilitate the growth of crystals appropriate for X-ray structural analysis. Such separations of diastereoisomers has been achieved using SP Sephadex C-25 cation exchanger and elution with sodium toluene-4-sulfonate.

Resolution of the racemic form
Using a similar method to that described above, [Ru(dmbpy)]_{2}^{2+} (\mu-\text{bipyrm})Cl4 (ca. 50 mg) was introduced onto a column (dimensions 16 × 1000 mm). To increase the effective length of the column, the Sephadex had equilibrated to the eluent a plunger was lowered onto the surface of the support and the system allowed to recycle. After the third passage down the column definite resolution had been achieved, and the two individual bands were collected and isolated as the hexahalourourphosphate salts. Band 2, ΔΔ complex: CD λmax/ nm (CD2CN) 256 (Δλ/dm3 mol−1 cm−1) +65.4), 278 (−110.8), 300 (0.4), 318 (−32.5), 392 (+28.2) and 623 (−4.3), Band 3, ΔΔ complex: CD λmax/ nm (CD2CN) 256 (Δλ/dm3 mol−1 cm−1) +53.2), 278 (−109.1), 300 (7.1), 318 (−32.5), 392 (−22.6) and 607 (+4.6).
The complexes \([\text{Ru(dmbpy)}_2\mu\text{-bipym}])^\text{2+}, \[\text{Ru(phen)}_2\mu\text{-bipym}]\text{PF}_6^\text{−}\) and \([\text{Ru(bpy)}_2\mu\text{-bipym}]\text{PF}_6^\text{−}\) were prepared in an analogous fashion, and gave characterisations in accordance with the literature.\(^2\)

Quantitative column techniques

A small Persepx column (dimensions 9 × 600 mm), fitted with an insulated water-jacket connected to a circulating thermostat, was set to a constant temperature (30 °C for all measurements, unless otherwise stated). SP Sephadex C-25 was equilibrated in the eluent solution aqueous 0.1–1.0 mol dm\(^{-3}\) sodium toluene-4-sulfonate for the variable-concentration data and 0.25 mol dm\(^{-3}\) for the temperature-dependent measurements; 0.154 mol dm\(^{-3}\) in the case of the investigations using sodium (+ or −) O.O′-dibenzyol-(D or L)-tartrate solutions, and allowed to settle in the column at a constant eluent flow rate of approximately 0.5 cm\(^3\) min\(^{-1}\). Once equilibration was obtained, the head of salt solution was reduced on the top of the support, and the compound to be separated was carefully introduced, dissolved in the eluent (0.25 mg of each isomer in 250 μl), so as not to disturb the Sephadex surface. With the sodium (+ or −) O.O′-dibenzyol-(D or L)-tartrate eluents, each isomer/enantiomer was added separately, since separation down the column was not always possible. The rate of flow through the column was carefully monitored as was the rate of travel of the individual bands. To ensure standardised results, the data are given in the volume of solvent required to move the individual bands by unit length (cm \(^3\) cm\(^{-1}\)).

Crystallography

A sample of the first major fraction (meso) of \([\text{Ru(dmbpy)}_2\mu\text{-bipym}])^\text{2+}\) was repurified by chromatography, using Sephadex LH20 as support with methanol as eluent to remove any excess of inorganic impurities. Following removal of the solvent, and redissolution of the residue in water, slow evaporation of the solution realised oily crystals which were suitable for X-ray determination.

A unique room-temperature diffractometer data set (Enraf-Nomonius CAD-4 diffractometer; Suitable for X-ray determination.)

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