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Anion interactions with (polypyridyl)ruthenium complexes, and their importance in the cation-exchange chromatographic separation of stereoisomers of dinuclear species†

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The separation of the ligand-bridged dinuclear complex cation $[\{(Me_2bpy)_2Ru\}_2(\mu\text{-bpm})]^{4+}$ (Me_2bpy = 4,4′-dimethyl-2,2′-bipyridine; bpm = 2,2′-bipyrimidine) into its two diastereoisomeric forms, *meso* and *rac,* by cation-exchange chromatography has been investigated, using a wide range of organic counter anions (aromatic and aliphatic) in the aqueous eluents. ^1H NMR titration studies not only confirm association between the complex cation and the counter anions in solution, but also reveal differential associations with the two diastereoisomers of $[\{(Me_2bpy)_2Ru\}_2(\mu\text{-bpm})]^{4+}$. Analogous interactions are also evident with mononuclear species, $[Ru(pp)_3]^{2+}$, where pp is a bidentate ligand such as Me_2bpy, 1,10-phenanthroline (phen) or 2,2′-bipyridine (bpy): from ^1H NMR titration studies, the first association with a number of organic anions has a stability constant in the order of 100 $dm^3\ mol^{-1}$ in aqueous solution. This association also affects the emission from the MLCT excited state of the monomers, with up to a 10% enhancement in the luminescence.

Introduction

Octahedral tris(bidentate)-ruthenium(II) and -osmium(II) complexes involving polypyridyl ligands such as 2,2′-bipyridine (bpy) and 1,10-phenanthroline (phen) have been extensively studied, primarily because of their unique combination of chemical inertness, redox properties and photophysical characteristics.[1,2] They have found application within diverse areas of chemistry such as photo-catalysis,[3] molecular recognition,[4,5] and artificial photosynthesis/charge separation schemes,[6–10] and exhibit specific interactions with polynucleotides such as DNA.[11–13] These coordination centers are inherently chiral, and any supramolecular assembly composed of such moieties may exhibit a number of possible stereoisomers, a complication which has until recently received only tacit attention.[14–16]

In our stereochemical investigations of mono- and oligonuclear ruthenium complexes with α,α′-diimine ligands, the cation-exchange support SP Sephadex C-25 has proved remarkably successful in the separation of individual geometric isomers, diastereoisomers and enantiomers of mixtures typically produced in structurally-uncontrolled syntheses.[15–23] This material had been extensively used in the separation of stereoisomers of various octahedral cobalt(III) species,[24–26] 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 propylsulfonate groups.[25] The separation of cationic species is achieved as a result of the differential equilibria between the absorbed cations and the cations in the mobile eluent phase (typically Na^+) with the anionic stationary phase. The lowest-charged cations move faster relative to the other species introduced onto the column, with coulombic forces (charge densities and polarities of the cations) dominating the equilibria leading to the separation.

The initial application of this cation-exchange chromatographic technique to the separation of ruthenium(II) complexes containing polypyridyl ligands utilised aqueous sodium toluene-4-sulfonate solution as eluent.[21] It became apparent that the anion of the eluent was not innocent in the process and associated with the complex cations, effectively reducing their overall charge and causing increased rate of travel down the column.[27] The different geometries of the stereoisomers of the cations and the choice of anion had a profound effect on the strength of these anion interactions, facilitating the separations: the present paper elucidates factors giving rise to these observations.

Unless they occupy a formal coordination site on the metal centre, anions have classically been considered as isolated from the complex in a polar environment, being part of a second-sphere solvent "cage". However, the recent work of Beer *et al.* has shown that a variety of anions can strongly associate with a cationic ruthenium complex containing amide-functionalised polypyridyl ligands.[4,5,28] Such specific interactions rely heavily upon hydrogen bonding, in addition to electrostatic factors which facilitate the association. Photophysical studies of ruthenium polypyridyl complexes with anionic micelles,[29–31] polyelectrolytic anions,[29–35] and DNA[11,12,36–39] have indicated a distinct interaction manifested as an increase in luminescence.

In this paper we illustrate the significant association of anions with both mono- and di-nuclear complexes of ruthenium(II) containing polypyridyl ligands, the connotations of which spread well beyond the chromatographic process.

Experimental

Instrumentation

NMR spectra were recorded on a Varian Unity Inova-500 spectrometer using the solvent as the internal reference. Electronic

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† *Supplementary data available*: NMR titration curves plotted for the sequential addition of sodium 4-toluate to diastereoisomers of $[\{(Me_2bpy)_2Ru\}_2(\mu\text{-bpm})]Cl_4$ and for the sequential addition of $[Ru(Me_2bpy)_3]Cl_2$ to sodium toluene-4-sulfonate; relative ^1H NMR signal perturbations observed on the protons of $[Ru(Me_2bpy)_3]Cl_2$ on the addition of organic anions; relative photoemission of the complex $[Ru(Me_2bpy)_3]Cl_2$ upon the addition of organic sodium salts of organic anions. For direct electronic access see http://www.rsc.org/suppdata/dt/1999/683/, otherwise available from BLDSC (No. SUP 57484, 6 pp.) or the RSC Library. See Instructions for Authors, 1999, Issue 1 (http://www.rsc.org/dalton).

and emission spectra were recorded on Varian CARY 5E and Perkin-Elmer LS 50B spectrophotometers, respectively.

Materials

Sodium chloride (Ajax; AR) was used without further purification. Sodium toluene-4-sulfonate (Aldrich; 95%) and sodium butyrate (butanoate) (Aldrich) were recrystallised from ethanol. All other sodium salts were prepared by the neutralisation of the corresponding acids (Fluka/Aldrich) with aqueous sodium hydroxide solution until a pH of 8–9 was obtained. The solutions were filtered, and the water was removed under reduced pressure. The resulting white solids were recrystallised from ethanol and dried *in vacuo* prior to use.

The complexes $[Ru(bpy)_3]Cl_2$, $[Ru(Me_2bpy)_3]Cl_2$ (Me_2bpy = 4,4′-dimethyl-2,2′-bipyridine) and $[Ru(phen)_3]Cl_2$ were prepared according to literature procedures.[1] $[\{(Me_2bpy)_2Ru\}_2(\mu\text{-bpm})]Cl_4$ (bpm = 2,2′-bipyrimidine) was prepared according to previously described methods, and the two diastereoisomers (*meso* and *rac*) were isolated using cation-exchange chromatography on SP Sephadex C-25, with aqueous 0.25 mol dm^{-3} sodium toluene-4-sulfonate solution as eluent.[27]

Quantitative column chromatography

A small perspex column (Model K9; Amrad Pharmacia Biotech; dimensions 9 × 600 mm), fitted with an external water-jacket was maintained at a constant temperature of 30 °C by a thermostatted circulating water-bath (Talabo F10). SP Sephadex C-25 was equilibrated in the eluent solution at an electrolyte concentration of 0.25 mol dm^{-3} to overcome problems of contraction of the support on changes of electrolyte concentration, and allowed to settle in the column at a constant eluent flow rate of approximately 0.5 ml min^{-1}, regulated by a Gilson Minipulse 2 peristaltic pump. Once equilibration was achieved, the head of solution was lowered to the level of the top of the chromatographic support and the compound to be separated was introduced (dissolved in the appropriate eluent; 0.25 mg of each isomer in 250 µl), taking extreme care that the surface of the Sephadex was undisturbed during the process. To ensure standardised results, the rate of flow was monitored and the data standardised to give the volume of eluent solution required to move the individual bands by unit length (ml cm^{-1}).

^{1}H NMR titration studies

The ^{1}H NMR titrations on the dinuclear species were performed by the sequential addition of 0.2 equivalents of the sodium salt of the anions contained in 20 µl of D_2O to 2.5×10^{-6} mol of the *meso* or *rac* diastereoisomer of $[\{(Me_2bpy)_2Ru\}_2(\mu\text{-bpm})]Cl_4$ in 0.5 ml of D_2O. The titrations with the mononuclear species $[Ru(Me_2bpy)_3]Cl_2$, $[Ru(bpy)_3]Cl_2$ and $[Ru(phen)_3]Cl_2$ were performed at twice the concentration of complex to provide comparable results. The stability constants were obtained by the application of the program EQNMR,[40] coded in Fortan90 and compiled to execute on Apple Power-Mac systems.[41] However, the parameter refinement routines were based on a constrained quasi-Newton–Rapson procedure which made the optimisation less sensitive to selection of the starting parameters than the original code.

Photophysical titrations

The UV/visible spectra and emission spectra (excitation at 455 nm) were recorded in aqueous solutions at a concentration of 5×10^{-5} mol dm^{-3} for $[\{(Me_2bpy)_2Ru\}_2(\mu\text{-bpm})]Cl_4$ and 1×10^{-4} mol dm^{-3} for $[Ru(Me_2bpy)_3]Cl_2$. The titrations were performed by the sequential addition of the sodium salt of the anion (1 equivalent in 5 µl) to 2.5 ml of the above solutions. Dilution effects were corrected by the subtraction of a blank titration.

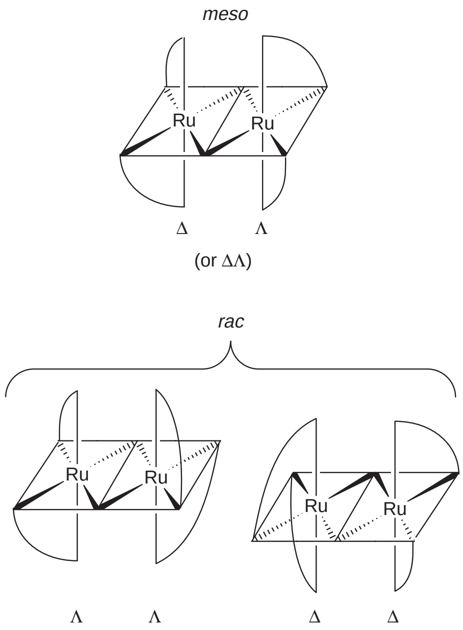


Fig. 1 Schematic view of the isomeric possibilities of the general dinuclear species $[\{M(pp)_2\}_2(\mu\text{-BL})]^{4+}$ {pp is an α,α′-diimine ligand, and BL a di-bidentate bridging ligand}.

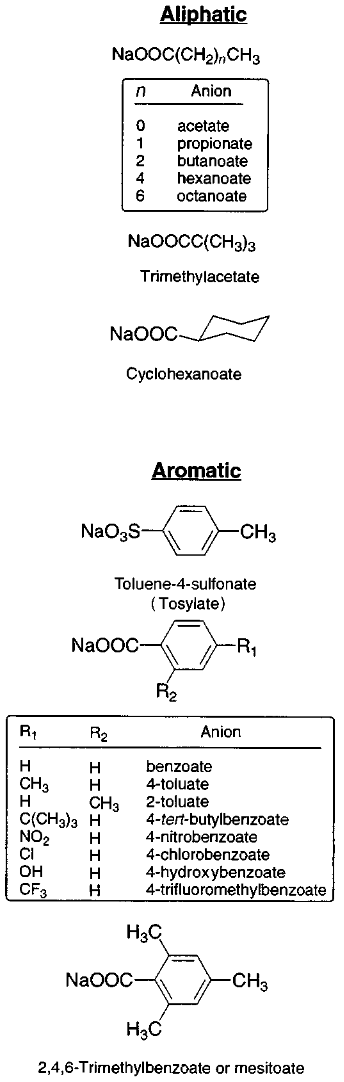
Results

In an achiral synthesis of the ligand-bridged dinuclear species $[\{(Me_2bpy)_2Ru\}_2(\mu\text{-bpm})]^{4+}$, the two diastereoisomers {*meso* ΔΛ (≡ΛΔ) and *rac* (ΔΔ and ΛΛ); Fig. 1} were obtained in approximately equal proportions and were readily separated by cation-exchange chromatography on SP Sephadex C-25 with aqueous sodium toluene-4-sulfonate solution as the eluent.[27] A study of this chromatographic process as a function of eluent concentration and temperature revealed a surprisingly strong association between the cation to be separated and the anion in the eluent.[27] The rate of travel of the *meso* diastereoisomer was considerably faster, indicating its shape led to a preferential interaction of the anion. To elucidate the nature of this interaction, and identify factors controlling its strength, the chromatographic processes were investigated as a function of the variation of the anion in the aqueous eluent (Fig. 2).

Quantitative column chromatography studies

A series of quantitative experiments, similar to those previously described,[27] were undertaken on small columns using a mixture of the two diastereoisomers of the dinuclear cation $[\{(Me_2bpy)_2Ru\}_2(\mu\text{-bpm})]^{4+}$. The relative rates of movement of the two bands down the column of SP Sephadex C-25 cation exchanger were measured using a constant eluent concentration of 0.25 mol dm^{-3}, but varying the organic anion in the solution. The relative rates of passage of the two diastereoisomers down the column with a variety of anions are displayed in Fig. 3, giving the data for two forms (*meso* and *rac*), as well as the average for the combined bands. Several trends are apparent.

Slight movement of the cation down the column (shown by the average movement) was observed with chloride as the eluent anion, although there was very poor separation of the two diastereoisomers. With acetate as the eluent anion no movement was detected, but as the chain length of the aliphatic carboxylate anion was extended the rate of travel down the column dramatically increased (Fig. 3a). Further, the relative difference in rate of movement of the two diastereoisomers (*meso*:*rac*) increased with the chain length of the aliphatic anions (1:0.66 for butanoate compared with 1:0.41 for octanoate). Generally, better separation of the two forms was observed for these simple longer chain aliphatic anions than



n	Anion
0	acetate
1	propionate
2	butanoate
4	hexanoate
6	octanoate

R_1	R_2	Anion
H	H	benzoate
CH_3	H	4-toluate
H	CH_3	2-toluate
$C(CH_3)_3$	H	4-*tert*-butylbenzoate
NO_2	H	4-nitrobenzoate
Cl	H	4-chlorobenzoate
OH	H	4-hydroxybenzoate
CF_3	H	4-trifluoromethylbenzoate

Fig. 2 The organic anions used in these studies.

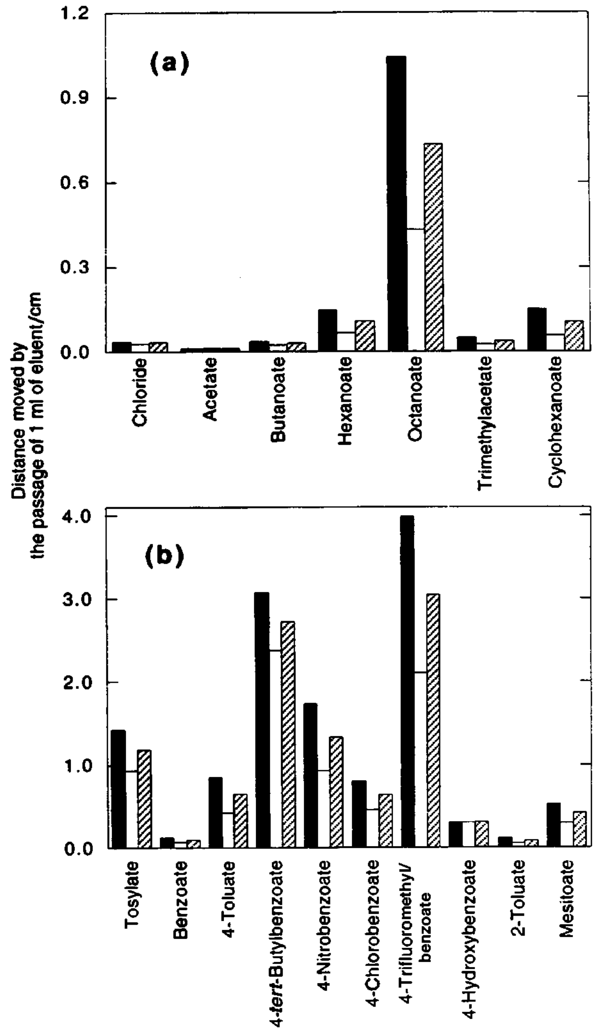


Fig. 3 Plots of relative rates of travel of the diastereoisomers of $[\{(Me_2bpy)_2Ru\}_2(\mu\text{-bpm})]^{4+}$, down a 9 mm (internal diameter) column as a function of (a) aliphatic organic sodium salts, (b) aromatic organic sodium salts as eluent (Concentration: 0.25 mol dm^{-3}, at 30 °C.) {black ≡ *meso*; white ≡ *rac*; hatched ≡ mixture of *meso* + *rac*}.

had previously been observed with sodium toluene-4-sulfonate (1 : 0.66).[27] The rate of travel of the two diastereoisomers down the column was not enhanced by the presence of branched chains (*e.g.* trimethylacetate and cyclohexanoate).

A series of aromatic anions were investigated in an analogous fashion. While it was observed that the behaviour of sodium benzoate closely resembled that of sodium cyclohexanoate, *para*-substituted aromatic carboxylates gave significantly enhanced rates of travel down the column when compared with the aliphatic anions, as illustrated in Fig. 3b. This did not appear to be a function of the difference in electronic character of the appended group, but rather the hydrophobicity (with the possible exception of 4-nitrobenzoate). If the aromatic anion was substituted in the position *ortho* to the carboxylate group (*e.g.* 2-toluate and mesitoate), the rate of travel was significantly decreased: this may arise from the difference in shape between these two anions and the 4-substituted species, but it may also indicate that the hydrogen atoms in the *ortho* position are significant in the interaction. For these two aromatic anions, the relative rates of travel of the two diastereoisomers (*meso* : *rac*) proved to be very similar (1 : 0.46 to 0.56). Notably, no separation was observed for 4-hydroxybenzoate.

Clearly the identity of the anion in the eluent greatly influences the rate of movement of the complex cations by diminution of their effective charges: there must be differences in the association between the tetravalent species $[\{(Me_2bpy)_2Ru\}_2(\mu\text{-bpm})]^{4+}$ and the various anions used in the eluting electrolyte. The association appears greatest for the aromatic anions, which induce a faster rate of travel down the column. The separation of the two diastereoisomers is brought about by differences in the association between the two forms (*meso*/*rac*).

^1H NMR Studies

In recent years, NMR has proved an extremely successful tool in elucidating interactions within host–guest chemistry.[42] In the present case, a series of ^1H NMR titrations were carried out in an attempt to observe perturbations in the signals of the cation (host) as the chloride salt (for which the associations are weak) with the sequential addition of sodium salts of the anions (guest) illustrated in Fig. 2. All of the titrations were carried out in D_2O at 30 °C, in order to maintain the environment as similar as possible to that used in the chromatographic experiments.

The series of anions used with the quantitative columns described above were titrated against the complex $[\{(Me_2bpy)_2Ru\}_2(\mu\text{-bpm})]^{4+}$. Upon the sequential addition of the organic anions as their sodium salts, standard titration curves (such as those provided in SUP 57484 Fig. S1) could be plotted for the small but significant perturbations for the majority of protons on the cation. In most of the curves, the intersection of the initial and final slopes occurred at *ca.* 4 equivalents of the anion, indicating a stoichiometry of 1 : 4 which is consistent with the ratio of the magnitude of the charges of the cation and anion.

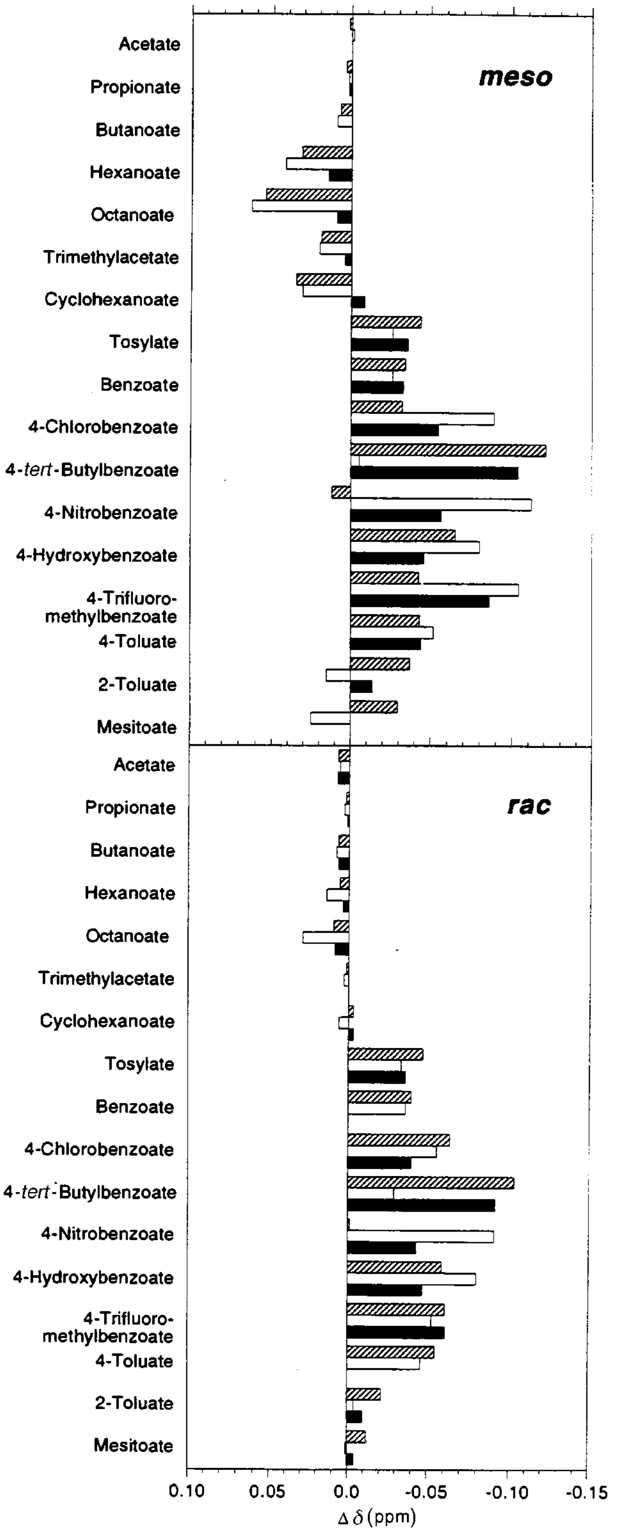
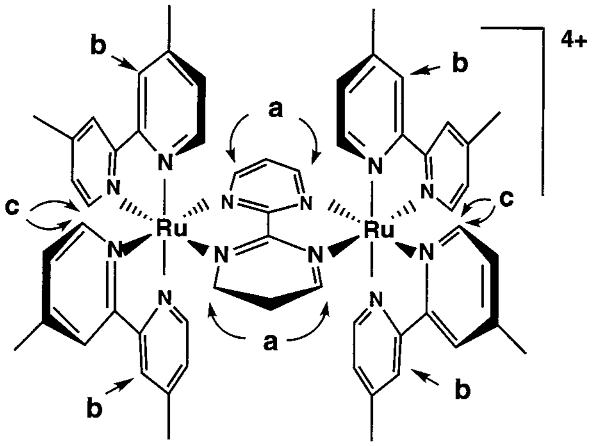


Fig. 4 The relative ^{1}H NMR signal perturbations observed on the protons: bpm-H4/6 (black), Me$_2$bpy-H3 (white) and Me$_2$bpy-H6′ (hatched), respectively, with (i) *meso*-[{(Me$_2$bpy)$_2$Ru}$_2$(μ-bpm)]Cl$_4$, (ii) *rac*-[{(Me$_2$bpy)$_2$Ru}$_2$(μ-bpm)]Cl$_4$ on the addition of 10 equivalents of the organic anions.

Fig. 4 shows the perturbations (Δ ppm) observed after the addition of ten equivalents of the respective anion for three protons {Me$_2$bpy-H6′, Me$_2$bpy-H3 and bpm-H4/6} of both diastereoisomers, selected from three distinct regions of the cation. Several observations can be made from the data. The two diastereoisomers gave essentially similar results, although the magnitude of the observed shifts were significantly less with the *rac* form. While the magnitude of the shift is not necessarily directly related to the strength of an association, it can be assumed that larger shifts are caused by more intimate interactions of the anions with the *meso* than the *rac* diastereoisomer, reflecting differences in structure between the two forms. This was particularly noticeable with the aliphatic anions.



Another very obvious trend is that the aliphatic species all induced down-field shifts in the protons of the dinuclear complex, while the aromatic anions generally gave rise to an up-field shift under the same conditions. The approach of a negative charge has been observed previously to cause down-field shifts in the ^{1}H NMR spectrum of a complex cation, as a result of second sphere interactions,[28] in accordance with the present results for the aliphatic anions. However, the presence of the aromatic anions gave rise to up-field shifts indicating this interaction must have an additional dimension. It is apparent that the aromatic anions occupy a position sufficiently proximate to the protons on the cation that they experience a ring current (anisotropy) from the associated π-electron rich anions, giving rise to the up-field perturbation. Such ring anisotropy implies that the nature of such aromatic associations probably arises from a π-stacking effect.[43]

In both the *meso* and *rac* forms, as the chain length of the aliphatic anions increased greater shifts were observed and by implication there were larger associations. Branched aliphatic species did not improve this effect, and if anything reduced it.

At a first glance, the size of the observed shifts for the aromatic anions correlates with the respective rates of passage through cation-exchange support. A comparison of cyclohexanoate and benzoate reveals that in the latter the shifts have inverted, and increased considerably in magnitude. Thus while the column data imply a similar behaviour of these two anions, the observed shift perturbations imply a differing mechanism of association. The *para*-substituted benzoates each produced significant shifts (up to 0.15 ppm with 4-*tert*-butylbenzoate and 4-trifluoromethylbenzoate) on the majority of the terminal ligand protons of both diastereoisomers. Surprisingly, the size of the shifts does not correlate to the electronic character of the substituted groups, again implying the effects are not a consequence of the basicity of the carboxylate. By placing the substituents at the *ortho*-position to the carboxylate (2-toluate and mesitoate) the shift is greatly reduced or even turned down-field, indicating that the aromatic interaction is being blocked. As discussed above, this observation may arise from differences in the shapes of the respective anions, or it implies that the H2/6 protons on the aromatic anion play an important role in the association, in which case it emphasises that the association probably relies on an edge-to-face π-stacking.[43]

The protons on the bridge (bpm) show somewhat different behaviour to those on the terminal ligands. The shifts can vary greatly, from extremely large with 4-trifluoromethylbenzoate to an extremely small down-field shift with 4-nitrobenzoate. Hence it is postulated that this *para*-substituent on the aromatic anion

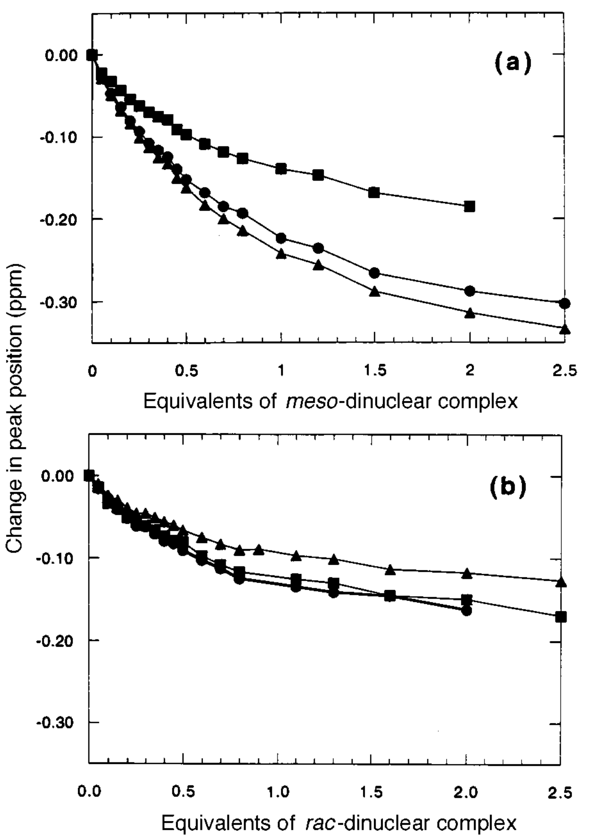


Fig. 5 ^{1}H NMR titration curves plotted for the sequential addition of (a) *meso*-[{$(Me_2bpy)_2Ru$}$_2$(μ-bpm)]Cl_4, (b) *rac*-[{$(Me_2bpy)_2Ru$}$_2$(μ-bpm)]Cl_4 to sodium toluene-4-sulfonate [H-Me (▲), H2/6 (■) and H3/5 (●)].

lies over (or below) the bridge. These differences are more pronounced for the *meso* than for the *rac* form, implying the geometry over the bridge is more favourable to the association in the *meso* isomer.

To reinforce the observations, the cation was titrated against sodium toluene-4-sulfonate to examine whether the anion also experienced similar perturbations in the proton behaviour (Fig. 5). The change in peak positions of the anion were larger than those observed in the cation, and the titration curves ($\Delta\delta$ *vs.* equivalents of complex added) indicated a 4:1 stoichiometry. The shifts were all in an up-field direction indicating the anion is not only being brought into an electron-rich area (shielding environment), but also that the aromatic system of the anion experiences the ring currents of the polypyridyl ligands. There are some pronounced differences in the behaviour of the protons with the two different diastereoisomers (Fig. 5). While the H2/6 protons of the toluene-4-sulfonate anion do not experience much difference for the two diastereoisomeric forms, the H3/5 and H-Me protons both show greater perturbations with the *meso* form.

While the difference in structure of the two diastereoisomers must contribute to their respective differences in behaviour, the complexes both interacted in a 1:4 ratio with the anions. It is unlikely that such associations would only involve the region between the two metal centres (in the vicinity of the bridge), so that the interactions must also involve the terminal ligands. If this were the case, similar patterns should be observed with mononuclear polypyridyl complexes of ruthenium. Accordingly, a similar series of titrations were made with the complex $[Ru(Me_2bpy)_3]^{2+}$ in order to judge the strength of its associations with the same anions. The titration curves obtained indicated a 2:1 stoichiometry. Additionally, a titration of the cation against the anion indicated very similar-sized shifts to those observed for the dinuclear isomers, described above (SUP 57484 Fig. S2).

Table 1 Stability constants obtained using EQNMR[40,41] from ^{1}H NMR titration data for the mononuclear tris(α,α′-diimine)-ruthenium(II) complexes

Complex	Anion (introduced as Na^+ salt)	K_{stab1} (±50%)/ $dm^3\ mol^{-1}$	K_{stab2} (±50%)/ $dm^3\ mol^{-1}$
$[Ru(Me_2bpy)_3]Cl_2$	Octanoate	250	4
	Toluene-4-sulfonate (tosylate)	120	6
	Benzoate	125	10
	4-Chlorobenzoate	55	31
	4-*tert*-Butylbenzoate	75	14
	4-Nitrobenzoate	64	14
	4-Hydroxybenzoate	130	7
	4-Trifluoromethyl-benzoate	16	15
	4-Toluate	75	14
$[Ru(phen)_3]Cl_2$	Benzoate	160	9

Generally the shifts observed on the addition of five equivalents of the anions to the complex $[Ru(Me_2bpy)_3]^{2+}$ revealed a similar magnitude and pattern to the addition of ten equivalents to the *rac* diastereoisomer of [{$(Me_2bpy)_2Ru$}$_2$(μ-bpm)]$^{4+}$, showing that they both behave in a similar manner (SUP 57484 Fig. S3). From the titration data obtained by the ^{1}H NMR titrations, the program EQNMR[40,41] was used to estimate the stability constants for the association of the first (k_{stab1}) and then the second (k_{stab2}) anion with the mononuclear target. Not all of the anions provided sufficiently large shifts on the sequential addition of the anion to the complex cation, but using data for several of the aromatic anions and the octanoate ion it was possible to determine the stability constants (Table 1). The errors in the values are approximately ±50%, but the majority of the values for the first association constant (k_{stab1}) fell into the range of 50 to 130 $dm^3\ mol^{-1}$ (with the exception of 4-trifluoromethylbenzoate, which indicated anomalous behaviour). While these values are small they are nevertheless significant, especially in aqueous solution where the polarity of the solvent effectively negates the electrostatic attractions. As expected, the second stability constants are an order of magnitude smaller. Such data would have proved invaluable in the case of the dinuclear species, but the number of independent variables proved to be too extensive to allow the EQNMR software to provide meaningful answers.

Similar behaviour was observed with the mononuclear complexes $[Ru(bpy)_3]^{2+}$ and $[Ru(phen)_3]^{2+}$ as for the complex $[Ru(Me_2bpy)_3]^{2+}$ upon the sequential addition of both sodium octanoate and sodium benzoate. The perturbations observed typically were not as large as those observed with $[Ru(Me_2bpy)_3]^{2+}$ (Fig. 6), however it provided strong evidence that this effect is not simply limited to complexes containing Me_2bpy.

It is noted that recent structural studies of the complex Λ-(+)-$[Ru(phen)_3]L\cdot 8H_2O$ {L = (+)-*O*,*O*′-di-4-toluoyl-D-tartrate} showed the $[Ru(phen)_3]^{2+}$ cations formed layers which alternated with layers containing water molecules.[44] For L = (+)-*O*,*O*′-di-4-toluoyl-D-tartrate the anions were positioned such that the tartrate backbone (containing the two carboxylate functionalities) resided in the water layer (hydrophilic), while the toluoyl rings penetrated into a virtually hydrophobic region in the complex cations where they underwent π-stacking with the phenanthroline rings. While this observation was made in the solid state, the interaction is analogous to the present proposal for the mode of association of similar anions and complexes in solution.

Absorption/emission spectra

Studies of the photophysical properties of the mononuclear and dinuclear targets in the presence of the various anions were

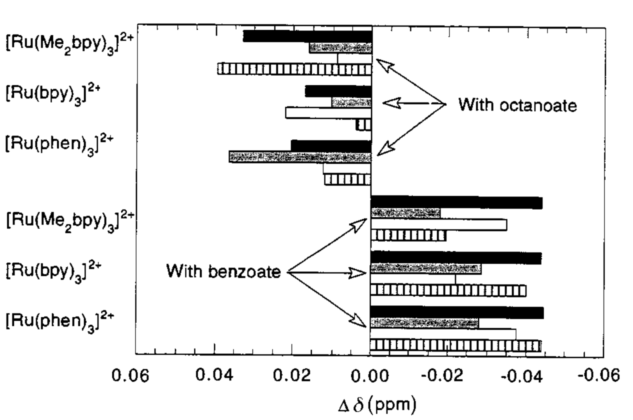


Fig. 6 The relative ^{1}H NMR signal perturbations observed with $[Ru(Me_2bpy)_3]Cl_2$, $[Ru(bpy)_3]Cl_2$ and $[Ru(phen)_3]Cl_2$ on the addition of 5 equivalents of the organic anions octanoate and benzoate: H3 (Me_2bpy and bpy) and H5/6 (phen) {black}, H-Me (Me_2bpy), H4 (bpy) and H4/7 (phen) {grey}; H5 (Me_2bpy and bpy) and H3/8 (phen) {white}; H6 (Me_2bpy and bpy) and H2/9 (phen) {hatched}.

carried out in aqueous solution to investigate whether the associations could be detected by other methods. Unfortunately, there was no indication of any form of interaction in the UV/visible absorption spectra of the ruthenium complexes even up to the addition of a 100-fold excess of the aliphatic or aromatic anions. The studies in the latter case were somewhat hindered by the absorption of the anions themselves which prevented an examination of the sensitive π–π^* transition at 280 nm.

The characteristic emission behaviour of the mononuclear species gave more satisfactory evidence. A strong quenching effect was observed for the addition of sodium 4-nitrobenzoate to a 10^{-4} mol dm^{-3} solution of $[Ru(Me_2bpy)_3]Cl_2$ in (non-deaerated) water at room temperature. It is known that nitrated aromatic species are capable of accepting an electron from an excited state species, indicated here by the quenching of the emission,[45] although no indication of an association was observed. The presence of the majority of other organic anions listed in Fig. 2 produced no effective change in the emission. However, on the sequential addition of the anions 4-*tert*-butylbenzoate, 4-trifluoromethylbenzoate, 4-toluate or octanoate, there was a small increase (5–10%) in the emission from the initial value upon the addition of 50 equivalents of the anion (SUP 57484 Fig. S4). All of these particular anions demonstrated good associations with both the dinuclear complex on the chromatography column and in the ^{1}H NMR studies (see above), and are thus assumed to be the most likely to cause a change in the emission behaviour. This increase can be rationalised in terms of the displacement of a layer of solvent molecules surrounding the excited state cation by a protective layer of the anions, held there by the association described above. This would render the excited state complex less susceptible to solvent collision and the resultant radiationless decay, and hence an increase in the emission is observed. Similar behaviour has been observed by the inclusion of complexes such as $[Ru(phen)_3]^{2+}$ and $[Ru(bpy)_3]^{2+}$ in polyelectrolytes,[32,35] micelles[29–31] and DNA.[38] While the change in environment is considerably greater upon the addition of these large polyanionic species, the same effect can be brought about by considerably smaller anions, presumably by discrete associations.

Discussion

We have had particular success with the separation on the cation-exchange support SP Sephadex C-25 of stereoisomers of polynuclear complexes of ruthenium containing bidentate α,α'-diimine ligands. The present work provides an understanding of the mechanism of the separation process, which relies heavily on a "host–guest" association of an organic anion of the eluent with the complex cation.

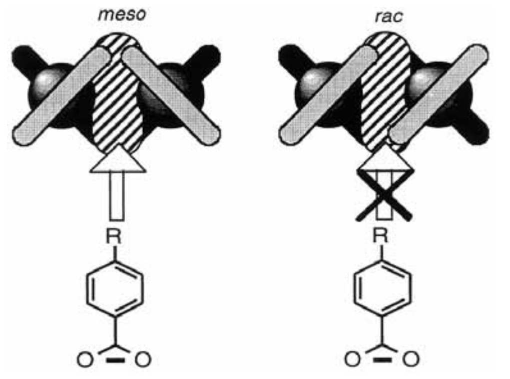


Fig. 7 Schematic illustration of the possible difference in association of the *meso* and *rac* forms of the dinuclear species.

A variety of anions have been used to explore the nature of these interactions, and these studies indicate that there are several factors involved. While there is inevitably a certain electrostatic attraction between the cation and the anion, it would not be exected to be large or specific in a highly polar solvent such as water. Ruthenium(II) complexes containing polypyridyl ligands exist as rigid structures with well-defined clefts between the ligands which provide hydrophobic cavities into which the organic entities present in the bulk polar solvent environment may insert. The resultant specific associations cause changes in the ^{1}H NMR spectra of both the complex (host) and anion (guest), and give rise to the behaviour of the complex cations on the cation exhange chromatographic support. While the use of aromatic anions such as 4-toluate are driven by such hydrophobicity, with the polar carboxylate grouping probably pointing away from the cation, there are additional possibilities arising from the formation of energetically favourable edge-to-face and face-to-face π-stacking interactions or aromatic anions with the pyridyl rings.[43] By the inclusion of *para*-substituted hydrophobic substituents to the aromatic rings of the anions, the binding is enhanced. However *ortho*-functionalised groups inhibit the association, reducing possibilities of the aromatic π-stacking.

From our studies, it is clear that the relative shapes of the two diastereoisomers of the complex $[\{(Me_2bpy)_2Ru\}_2(\mu\text{-bpm})]^{4+}$ give rise to the chromatographic separation by virtue of differential associations with certain anions. The structural features of the cation determine the access by the aliphatic or aromatic anions for either hydrophobic binding or π-stacking interactions, as demonstrated both in the NMR studies and by the rate of passage of the diastereoisomers down the cation exchange column.

The *meso* form has a cleft above and below the bridge, formed by the orthogonal orientation of the terminal ligands, as indicated in Fig. 1. From simple molecular models, this cleft is of an appropriate size to accept an aromatic moiety. In the *rac* form the terminal ligands lie parallel to each other above and below the plane of the bridging ligand, and do not provide such a well-defined cleft, and consequently do not allow as good an association with the organic anions (Fig. 7).

The implications of such "host–guest" associations are of much wider significance than the chromatographic separations. It has been known for several decades that polypyridyl complexes of ruthenium(II) interact with DNA,[46] although there remains conjecture regarding the actual mode of bonding; *i.e.* whether the complexes intercalate and form a π-stacking interaction into the aromatic base pairs of the right-handed helix, or rely upon hydrophobicity to the surface of the helix, or the attraction is merely electrostatic.[11,12,37,38,47]

From the present studies, it is apparent that both hydrophobic and π-stacking interactions are possible between anions and complexes of this type, and the two types of association are of a similar order of magnitude for the simple complex $[Ru(Me_2bpy)_3]^{2+}$. However, the ^1H NMR signal perturbations are in opposite directions for the two interactions, a characteristic which could prove invaluable in the investigation of the actual mode of binding of similar complexes with the larger biological anions.

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