Does the metal influence non-covalent binding of complexes to DNA?

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Electrospray ionisation mass spectrometry, absorption spectrophotometry and circular dichroism spectroscopy were used to investigate the binding of a series of nickel complexes with the general formula $[Ni(phen)_2L]^{2+}$ (L = phen, dpq, dpqC and dppz) to a double stranded DNA hexadecamer. In addition, the binding of the complexes to pUC9 negatively supercoiled plasmid DNA was examined using gel electrophoresis, and their ability to inhibit DNA transcription was measured. Each of the above techniques showed that DNA binding strengthened as the size of the unique ligand L was increased. Comparison of the above results with those obtained previously, and presented here for the first time for the analogous series of ruthenium complexes $[Ru(phen)_2L]^{2+}$, showed that changing the metal ion from nickel to ruthenium consistently resulted in significant increases in DNA binding affinity.

Introduction

There is great interest in understanding the factors that control the binding of metal ions to nucleic acids. This has been fuelled in part by the successful introduction into the clinic of several platinum complexes that exert anticancer activity by forming covalent adducts with the purine bases of DNA.[1,2] In addition, metal complexes that bind non-covalently to DNA have attracted interest for a range of potential applications including use as selective probes of nucleic acid structure,[3] synthetic restriction enzymes[4] or artificial regulators of gene expression.[5] Most studies in this area have focused on octahedral complexes of transition metals such as ruthenium(II) and rhodium(III). This may be attributed partially to the extremely slow rates of substitution reaction of tris chelate complexes of these metals, as well as their interesting and potentially useful redox and photophysical properties. Our understanding of the non-covalent DNA binding chemistry of complexes of these and other metals now has a firm platform to build upon, and has been reviewed recently.[6,7] For example, it has been well established that in order for a mononuclear metal complex to display high binding affinity towards DNA, it must contain at least one ligand such as dppz (dipyrido[3,2-*a*:2′,3′-c]phenazine), which is capable of intercalating strongly with the DNA base stack.[6,8,9] It has also been possible to engineer the structure of metal complexes to display a high degree of selectivity in their DNA binding chemistry. For example, $[Rh(bipy)_2(chrysi)]^{3+}$ (bipy = 2,2′-bipyridine, chrysi = 5,6-chrysenequinone diimine) displays a profound ability to bind to DNA only at regions where base mismatches are present.[10] Despite this, there still remains a great deal to be learnt, particularly about the factors which govern binding specificity towards duplex DNA sequences that are correctly base paired, before it may become possible to tailor-make metal complexes for specific applications.

There have been comparatively few studies of the DNA binding chemistry of tris chelate complexes of first row transition metal ions. This is somewhat surprising, as metal ions such as chromium(III), cobalt(III) and nickel(II) form stable complexes with bidentate ligands, and display spectroscopic and redox properties that can be used to provide information about their DNA binding interactions. In addition, subtle differences in size and shape of complexes of these metals, compared to those of ruthenium(II) and rhodium(III) with the same ligands, may produce significant changes in DNA affinity and selectivity. It is therefore of interest to compare the DNA binding chemistry of related series of complexes such as those of the type $[M(phen)_2L]^{2+}$, where L is either 1,10-phenanthroline (phen) or one of several other bidentate aromatic diimine ligands, and M is different metal ions.

One of the earliest studies of this type was performed by Rehman and Barton, who used ^{1}H NMR spectroscopy to investigate the interactions of $[M(phen)_3]^{x+}$ (M = Ru(II), Rh(III), Co(III), Ni(II) and Cr(III)) with double stranded (ds) 6 *mer* oligonucleotides.[11,12] The nickel(II) complex, like those of the other metals, was found to interact with DNA primarily by two distinct binding modes. One of these was a surface-binding mode that was the predominant means of interaction for the Λ-enantiomer, while the Δ-isomer was shown to interact primarily by intercalation with the major groove. Later work by the same group showed that the racemates of $[Ni(phen)_2(dpq)]^{2+}$, (dpq = dipyrido[3,2-*d*:2′,3′-f]quinoxaline), $[Ni(phen)_2(dppz)]^{2+}$ and $[Ni(phen)_2(phi)]^{2+}$ (phi = phenanthrenequinone) all bind to the major groove of DNA using an intercalative mode, and that the exact binding site was dependent on the base sequence of the DNA molecule.[13] In another study, absorption spectrophotometry was used to show that equilibrium constants for the binding of the complexes $[M(phen)_2(dppz)]^{2+}$ to calf thymus DNA (CT DNA) followed the sequence Ru(II) > Co(III) > Ni(II).[14] Replacement of dppz in the above complexes by either a third phen ligand or phendione resulted in a substantial reduction in binding constant for each metal. It was also found that complexes containing nickel(II) were not able to cleave plasmid DNA upon irradiation, in contrast to analogous complexes containing ruthenium(II) or cobalt(III).[14,15]

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The above studies suggest that varying the metal ion present in metallointercalators can have a significant effect on DNA affinity. Therefore we decided to perform a detailed comparison of the DNA binding properties of two series of metallointercalators featuring identical ligand environments, but containing different metals. In this paper we report the results of DNA binding studies carried out using the four nickel(II) complexes $[Ni(phen)_2(L)]^{2+}$, (L = phen, dpq, dpqC (dipyrido[3,2-*a*:2′,3′-c](6,7,8,9-tetrahydro)phenazine) and dppz). Electrospray ionisation mass spectrometry (ESI-MS), absorption spectrophotometry, circular dichroism (CD) spectroscopy, gel electrophoresis and transcription inhibition assays were used to investigate the interactions of these compounds with dsDNA, and the results are compared to those obtained previously or reported here, from studies performed with the corresponding ruthenium(II) complexes.[16–18]

Experimental

Materials

The structures of the nickel compounds used, which were present as racemic mixtures in all experiments, are shown in Fig. 1. $[Ni(phen)_3]Cl_2$ was prepared by stirring an aqueous solution containing $NiCl_2{\cdot}6H_2O$ (5 mmol) and phenanthroline (15 mmol) for 1 h, and then adding $NaClO_4$ (3 g) to precipitate $[Ni(phen)_3](ClO_4)_2$. The latter compound was converted to the corresponding chloride salt by reaction with a saturated solution of tetrabutylammonium chloride in acetone. $[Ni(phen)_2Cl_2]$ was prepared by the method of Harris and McKenzie,[19] while $[Ni(phen)_2Cl(H_2O)]Cl$ was synthesised using an adaptation of the method of Liu and coworkers.[20] $NiCl_2{\cdot}6H_2O$ (0.005 mol) and phenanthroline (0.010 mol) were homogenised using a mortar-and-pestle forming a pale blue coloured compound, which upon recrystallisation from acetonitrile gave blue crystals after 3 days. The ligands dpq, dpqC and dppz were prepared using reported methods.[21,22] $[Ni(phen)_2(dppz)]^{2+}$ was prepared as follows. Dppz (0.5 mmol) was added to ethanol (25 mL) containing $[Ni(phen)_2Cl(H_2O)]Cl$ (0.5 mmol) and the resulting solution stirred at R.T. for 30 min. The crude $[Ni(phen)_2(dppz)](PF_6)_2$ was isolated by filtration using a sintered glass frit, washed with water and dried. The hexafluorophosphate salt was then converted to the more soluble chloride salt by the same method used for $[Ni(phen)_3](ClO_4)_2$. The above procedures were also used for synthesising $[Ni(phen)_2(dpq)]Cl_2$ and $[Ni(phen)_2(dpqC)]Cl_2$.

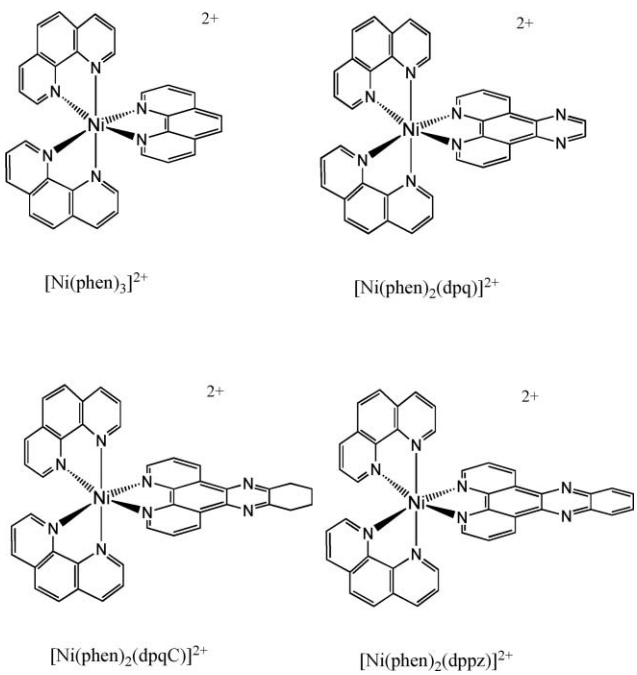

Fig. 1 Structures of the nickel complexes used in this study.

Single stranded oligonucleotides were obtained from Geneworks, South Australia, and purified using procedures previously reported.[23] The concentrations of oligonucleotides were estimated by measurement of UV absorbance at 260 nm using values of ε_{260} for adenine, guanine, cytosine and thymine of 15200, 12010, 7050 and 8400 M^{-1} cm^{-1}, respectively.[24] Stock solutions of individual nickel compounds were prepared by dissolving the appropriate amount of compound in 0.1 M ammonium acetate (NH_4OAc), pH 7.4. Stock solutions containing the double-stranded DNA (dsDNA) molecule D2, shown below, were prepared by heating equimolar quantities of the two single strands (0.4–2.0 mM) in 0.1 M NH_4OAc, pH 7.4, to 20 °C higher than the melting temperature for ~15 min, and then annealing by cooling slowly overnight. Complexes of dsDNA with individual nickel compounds were prepared by annealing samples of DNA, and then adding the required amount of stock nickel solution.

D2 d(CCTCGGCCGGCCGACC/GGTCGGCCGGCCGAGG)

Instrumentation and methods

All mass spectra were obtained using a Waters extended mass range Q-ToF Ultima™ mass spectrometer and conditions previously described.[18] Absorption spectrophotometric titrations were performed using a Shimadzu UV 1700 PharmaSpec spectrophotometer and 1 cm pathlength cells. A spectrum (260–600 nm) was first obtained using 2.5 mL of 10 μM nickel complex. Aliquots of 1.25 mM D2 were then added to the metal solution, which was mixed and allowed to stand at room temperature for 10 min prior to spectral analysis. This process was repeated until there were no further changes in the appearance of the absorption spectrum. Circular dichroism (CD) spectra were obtained using a Jasco J-810 spectropolarimeter and stock solutions consisting of 0.5 mM nickel complex, 20 μM dsDNA and 0.1 M NH_4OAc, pH 7.4. Initially a CD spectrum (200–320 nm) of 20 μM dsDNA in 0.1 M NH_4OAc, pH 7.4 was obtained. After this, the required volumes of stock solution containing both nickel and DNA were added to give final Ni : dsDNA ratios of 1 : 1, 3 : 1, 6 : 1 and 10 : 1. Each solution was allowed to stand at room temperature for 10 min prior to acquisition of another CD spectrum.

Gel electrophoresis experiments were performed using pUC9 negatively supercoiled plasmid DNA and 1% agarose gels together with a TBE (tris–borate–EDTA) running buffer solution. Reaction mixtures (10 μL) containing 0.25 μg of pUC9 together with different amounts of nickel compounds were prepared at 0 °C, and then allowed to incubate at 36 °C for 30 min. Prior to loading samples onto the gel, 2.5 μL of 0.25% bromophenol blue loading buffer and sucrose in water (40% w/v) were added to the reaction mixtures. Gels were obtained at room temperature

by using a Cleaver Scientific Multisub midi horizontal agarose gel electrophoresis system and applying a potential of 30 V for 3.5 h. The resulting gels was soaked in TBE buffer for 24 h, and then stained in ethidium bromide solution (0.5 μg mL^{-1}) for 45 min, after which they were soaked in water for a further 20 min. Gels were visualised under UV light and photographed, using a Bio-Rad Gel Doc™ XR.

In vitro transcription experiments were conducted as described previously,[25,26] using a pGEM express positive control DNA template and RiboMax™ large scale RNA production system with T7 RNA polymerase purchased from Promega. Reaction mixtures containing the DNA template, 5 × T7 transcription buffer (specificed by the provider), 25 mM rNTPs (ATP, UTP, CTP and GTP) and varying amounts of the nickel compounds were mixed together prior to addition of the T7 enzyme mix (specified by the provider). Transcription reactions were allowed to proceed for 1 h at 37 °C. The resulting mixtures were analysed by gel electrophoresis using 1% agarose gels and a TAE (tris–acetate–EDTA) running buffer solution. Gels were run for 2 h at 30 V, and then immediately stained with ethidium bromide.

Results and discussion

Synthesis of nickel complexes

While the synthesis of $[Ni(phen)_3]^{2+}$ proved straightforward, problems were surprisingly encountered during preparation of the complexes $[Ni(phen)_2L]^{2+}$ (L = dpq, dpqC, dppz). Previously the complexes where L = dpq and dppz have been prepared using $[Ni(phen)_2Cl_2]$ as the starting material. Attempts were made to prepare the latter compound using the method of Harris and McKenzie.[19] However, mass spectrometric analysis of samples of $[Ni(phen)_2Cl_2]$ made using this method showed that that they were invariably contaminated with significant amounts of $[Ni(phen)_3]^{2+}$. We therefore decided to investigate other methods for preparing suitable nickel precursor compounds. Liu and co-workers[20] reported the X-ray crystal structure of $[Ni(phen)_2Cl(H_2O)]Cl$ prepared by a procedure which involves initially grinding together $NiCl_2.2H_2O$ and phenanthroline using a mortar and pestle, and recrystallising the resulting powder from acetonitrile. Mass spectral analysis of samples of $[Ni(phen)_2Cl(H_2O)]Cl$ prepared by this procedure showed that they contained far less $[Ni(phen)_3]^{2+}$ and other impurities than $[Ni(phen)_2Cl_2]$ prepared by the method of Harris and McKenzie. It was therefore decided to prepare the $[Ni(phen)_2L]^{2+}$ series using $[Ni(phen)_2Cl(H_2O)]Cl$ instead of $[Ni(phen)_2Cl_2]$ as the starting material.

Literature methods for preparing the L = dpq and dppz members of the target series required $[Ni(phen)_2Cl_2]$ to be refluxed with 1.5 equivalents of L in ethanol for 1 h, and subsequently stirred at room temperature for a further 4–5 h.[13,15] When this procedure was followed, but with $[Ni(phen)_2Cl(H_2O)]Cl$ instead of $[Ni(phen)_2Cl_2]$ as a reactant, the products obtained were shown by ESI-MS to contain significant amounts of impurities, most notably $[Ni(phen)_3]^{2+}$. Changing the length of time the solution was held under reflux, and recrystallisation of the mixture of compounds obtained after precipitation using ammonium hexafluorophosphate, failed to yield a product with a satisfactory level of purity. For example, Fig. 2a shows the positive ion ESI mass spectrum of a sample of $[Ni(phen)_2(dppz)]^{2+}$ prepared using the

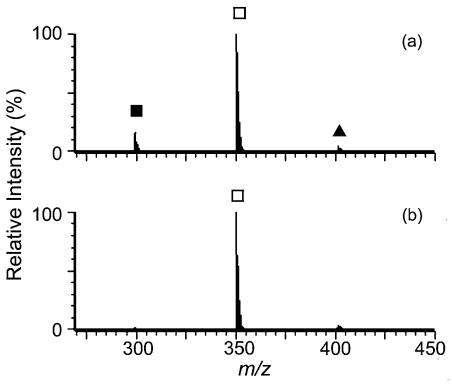

Fig. 2 Positive ion ESI mass spectra of: (a) the product obtained after refluxing $[Ni(phen)_2Cl(H_2O)]Cl$ with 1.5 equiv. dppz for 1 h and subsequently stirring at R.T. for 4 h, and (b) the product obtained after stirring equimolar amounts of $[Ni(phen)_2Cl(H_2O)]Cl$ and dppz at R.T. for 30 min. ■ $[Ni(phen)_3]^{2+}$; □ $[Ni(phen)_2(dppz)]^{2+}$; ▲ $[Ni(phen)(dppz)_2]^{2+}$.

standard literature conditions and $[Ni(phen)_2Cl(H_2O)]Cl$ as the starting nickel complex. The most abundant ions present (at m/z 350.3) are attributable to the desired complex. However, there are also ions of low abundance at m/z 299.3 that indicate a small, but significant amount of $[Ni(phen)_3]^{2+}$ is also present in the product, as well as ions of very low abundance at m/z 405.3 that reveal the presence of trace amounts of $[Ni(phen)(dppz)_2]^{2+}$. Similar results were obtained in reactions between $[Ni(phen)_2Cl(H_2O)]Cl$ and either dpq or dpqC carried out under these conditions. It was therefore decided to see if products with a higher level of purity could be obtained by simply stirring reaction mixtures containing a 1 : 1 ratio of $[Ni(phen)_2Cl(H_2O)]Cl$ and dpq, dpqC or dppz in ethanol at room temperature. Fig. 2b shows the ESI mass spectrum of a typical product obtained from a reaction mixture containing dppz and $[Ni(phen)_2Cl(H_2O)]Cl$ that had been stirred for only 30 min. The spectrum is dominated by ions of high abundance attributable to the desired $[Ni(phen)_2(dppz)]^{2+}$ product, demonstrating that this method does not suffer from the side reactions that occur when similar solutions are heated. This method was therefore also used to prepare the corresponding complexes containing the dpq and dpqC ligands.

Mass spectrometry

Fig. 3 shows the ESI mass spectra of reaction mixtures containing different ratios of $[Ni(phen)_2(dppz)]^{2+}$ and D2. The spectrum of a solution containing D2 alone, (Fig. 3a), contains ions at m/z 1626.4 and 1951.8, which are assigned to $[D2–6H]^{6-}$ and $[D2–5H]^{5-}$, respectively. Addition of 1 equivalent of $[Ni(phen)_2(dppz)]^{2+}$ resulted in the appearance of new ions of low abundance at m/z 1743.2 and 2092.2 (Fig. 3b), which are assigned to $\{D2 + [Ni(phen)_2(dppz)]^{2+} - 8H\}^{6-}$ and $\{D2 + [Ni(phen)_2(dppz)]^{2+} - 7H\}^{5-}$, respectively. Both ions arise from a non-covalent complex consisting of a single intact nickel molecule bound to double stranded D2. The abundances of these ions increased when the Ni : D2 ratio was increased to 3 : 1 (Fig. 3c). This spectrum also contained ions at m/z 1859.9 and 2232.1 arising from non-covalent complexes containing two $[Ni(phen)_2(dppz)]^{2+}$ molecules bound to D2. In addition, ions of low abundance were also detected at m/z 2372.0, which is consistent with assignment to non-covalent

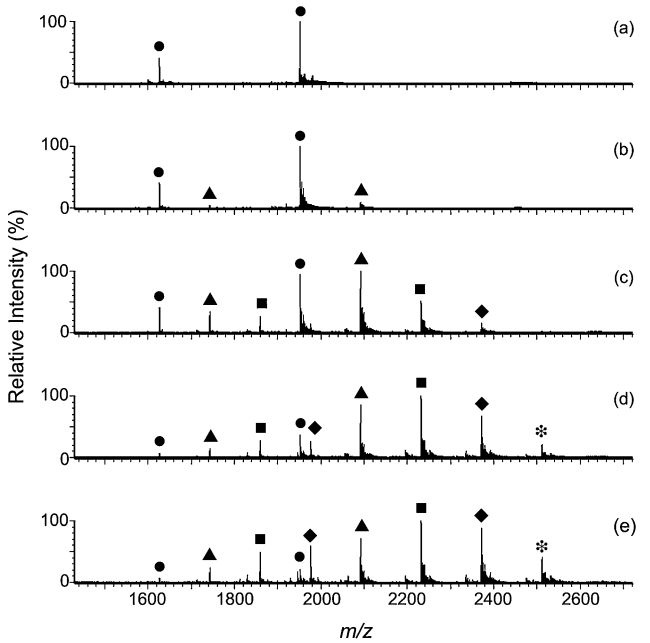

Fig. 3 Negative ion ESI mass spectra of solutions containing different $[Ni(phen)_2(dppz)]^{2+}$:D2 ratios: (a) free D2; (b) Ni : D2 = 1 : 1; (c) Ni : D2 = 3 : 1; (d) Ni : D2 = 6 : 1; (e) Ni : D2 = 10 : 1. The concentration of D2 was 10 μM in each case. ● dsDNA; ▲ dsDNA + $[Ni(phen)_2(dppz)]^{2+}$; ■ dsDNA + 2 $[Ni(phen)_2(dppz)]^{2+}$; ♦ dsDNA + 3 $[Ni(phen)_2(dppz)]^{2+}$; * dsDNA + 4 $[Ni(phen)_2(dppz)]^{2+}$.

complexes containing three $[Ni(phen)_2(dppz)]^{2+}$ molecules bound to D2. Further increasing the Ni : D2 ratio resulted in the appearance of additional ions attributable to DNA molecules containing greater numbers of bound $[Ni(phen)_2(dppz)]^{2+}$ molecules (Fig. 3d and e). At the same time the abundances of ions attributable to free D2 decreased. Similar trends were observed in titration experiments performed with each of the other nickel(II) complexes, and have been noted previously in studies using both ruthenium(II) and platinum(II) complexes.[16–18]

The relative binding affinities of the four different nickel complexes towards D2 was determined to be $[Ni(phen)_3]^{2+}$ < $[Ni(phen)_2(dpq)]^{2+}$ < $[Ni(phen)_2(dpqC)]^{2+}$ < $[Ni(phen)_2(dppz)]^{2+}$, by comparing the spectra shown in Fig. 4, which were obtained using solutions containing a single nickel complex and DNA in a 6 : 1 ratio. The above series is supported in part by the progressive decrease in abundance of ions from unbound D2 that parallels the above sequence. Further evidence is provided by comparing the abundances of ions assigned to non-covalent complexes in the different spectra. For example, when the nickel complex was $[Ni(phen)_2(dpq)]^{2+}$, ions attributable to non-covalent complexes containing one, two and three nickel molecules bound to D2 were observed, with the latter ions (at m/z 2342.0) only of low abundance (Fig. 4b). However, when the nickel complex used was $[Ni(phen)_2(dpqC)]^{2+}$, the abundance of ions at m/z 2374.3 from non-covalent complexes containing three nickel molecules bound to D2 was significantly greater (Fig. 4c), indicating that this nickel complex has a higher binding affinity. Comparison of Fig. 4c and d show that the spectra obtained using $[Ni(phen)_2(dpqC)]^{2+}$ and $[Ni(phen)_2(dppz)]^{2+}$ were very similar in overall appearance. However, the abundance of ions at m/z 2372.0 and 2512.0 in Fig. 4d, assigned to non-covalent complexes

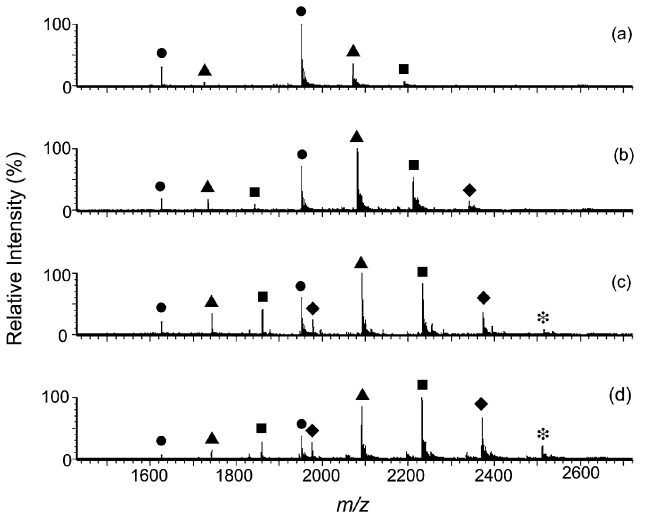

Fig. 4 Negative ion ESI mass spectra of solutions containing a 6 : 1 ratio of nickel complex and duplex D2: (a) $[Ni(phen)_3]^{2+}$; (b) $[Ni(phen)_2(dpq)]^{2+}$; (c) $[Ni(phen)_2(dpqC)]^{2+}$; (d) $[Ni(phen)_2(dppz)]^{2+}$. ● dsDNA; ▲ dsDNA + 1 $[Ni(phen)_2L]^{2+}$; ■ dsDNA + 2 $[Ni(phen)_2L]^{2+}$; ♦ dsDNA + 3 $[Ni(phen)_2L]^{2+}$; * dsDNA + 4 $[Ni(phen)_2L]^{2+}$.

containing three or four molecules of $[Ni(phen)_2(dppz)]^{2+}$ bound to D2, were noticeably greater than that of analogous ions containing $[Ni(phen)_2(dpqC)]^{2+}$ in Fig. 4c. This suggests that the former nickel complex has a slightly greater affinity towards D2 than $[Ni(phen)_2(dpqC)]^{2+}$, and the greatest DNA affinity of the four nickel complexes examined.

It is interesting to note that the order of binding affinities determined here matches that determined by ESI-MS in an earlier study using the four analogous ruthenium(II) complexes.[16,17] This suggests that the relative ability of the ligands (phen, dpq, dpqC, dppz) to enhance overall DNA binding affinity is independent of the metal ion present. A further question that remains is whether the identity of the metal ion has a significant effect on DNA binding. In order to probe this aspect, we compared the ESI mass spectra of solutions containing a 6 : 1 ratio of specific nickel complexes and D2, with those obtained previously using solutions containing the same ratio of the corresponding ruthenium complexes and D2.[17] In the case of the two tris phenanthroline complexes, $[Ni(phen)_3]^{2+}$ and $[Ru(phen)_3]^{2+}$, it proved impossible to determine which has the greater affinity towards D2 owing to relatively low extents of complexation. It also proved impossible to distinguish which of $[Ni(phen)_2(dpq)]^{2+}$ or $[Ru(phen)_2(dpq)]^{2+}$ has the higher affinity towards D2, as spectra of solutions containing these complexes showed ions assigned to unbound D2, and non-covalent complexes containing one and two metal complexes bound to DNA, with comparable abundances.

ESI-MS did, however, provide evidence that $[Ru(phen)_2(dpqC)]^{2+}$ and $[Ru(phen)_2(dppz)]^{2+}$ have significantly greater affinities towards D2 than $[Ni(phen)_2(dpqC)]^{2+}$ and $[Ni(phen)_2(dppz)]^{2+}$. In the case of $[Ru(phen)_2(dpqC)]^{2+}$, our previous ESI-MS study showed that a 6 : 1 solution of metal complex and D2 contained non-covalent DNA complexes containing up to four ruthenium molecules, with those containing two and three ruthenium molecules the most abundant.[17] When the ruthenium complex examined was $[Ru(phen)_2(dppz)]^{2+}$, ESI-MS showed that the most abundant ions in solution attributable to non-covalent complexes

were those containing four ruthenium molecules, with other ions attributable to non-covalent complexes containing five ruthenium molecules also evident.[17] In contrast, Fig. 4c shows that the most abundant ions attributable to non-covalent complexes in solutions containing a 6 : 1 ratio of $[Ni(phen)_2(dpqC)]^{2+}$ and D2 were those containing one and two nickel molecules, while Fig. 4d reveals that the most abundant ions in the corresponding solution containing $[Ni(phen)_2(dppz)]^{2+}$ contained only two nickel molecules. These results therefore suggest that the identity of the metal ion may affect the overall DNA binding affinity of a metal complex, particularly when strongly intercalating ligands such as dpqC or dppz are present.

Circular dichroism spectroscopy

It has been well documented that the non-covalent binding of metal complexes to B-DNA results in perturbation of CD signals due to the latter molecule.[18,27–31] In addition, it is possible for the chiral DNA molecule to induce circular dichroism into both the d–d and charge transfer bands of an achiral metal complex. These spectroscopic changes can be used to qualitatively probe the extent and mechanism of binding of small molecules to DNA, as well as provide quantitative data in the form of binding constants. Fig. 5 illustrates the effect of adding increasing amounts of the four different nickel complexes on the circular dichroism spectrum of D2. The spectrum of a solution containing D2 alone contains a positive CD band centred at 269 nm and a negative CD band at 241 nm. At a Ni : D2 ratio of 10 : 1 all nickel complexes produced shifts to higher energy for the positive CD signal as well as an enhancement of CD ellipticity at 269 nm. The magnitude of these changes are presented in Table 1, together with the corresponding spectroscopic changes observed in our previous CD study involving the corresponding ruthenium(II) complexes and D2.[18] On that occasion it was found that the change in ellipticity of the positive CD signal could be used to provide a relative order of DNA binding affinities that corresponded well with those obtained using several other spectroscopic techniques. Examination of Table 1 shows that the magnitude of the increases in ellipticity at 269 nm increase in the following order: $[Ni(phen)_3]^{2+}$ < $[Ni(phen)_2(dpq)]^{2+}$ < $[Ni(phen)_2(dpqC)]^{2+}$ < $[Ni(phen)_2(dppz)]^{2+}$, which is the order of relative binding affinities derived here using ESI-MS, and similar to that based on binding constants obtained through absorption spectrophotometry (see below). Comparison of the data in Table 1 reveals that the shifts in position and enhancements of ellipticity elicited by the nickel(II) complexes is significantly smaller than what was observed previously with the corresponding ruthenium(II) complexes. This suggests that the nickel(II) complexes generally interact more weakly with D2 than their ruthenium(II) analogues, a proposal that was also put forward earlier on the basis of a comparison of ESI mass spectral data for solutions containing complexes with the dpqC and dppz ligands. While significant changes were observed in the CD spectra between 220 nm and 320 nm only 10 min after mixing of the nickel complexes with D2, no additional features appeared at longer wavelengths. This result also mirrors that obtained using the analogous series of ruthenium complexes.[18]

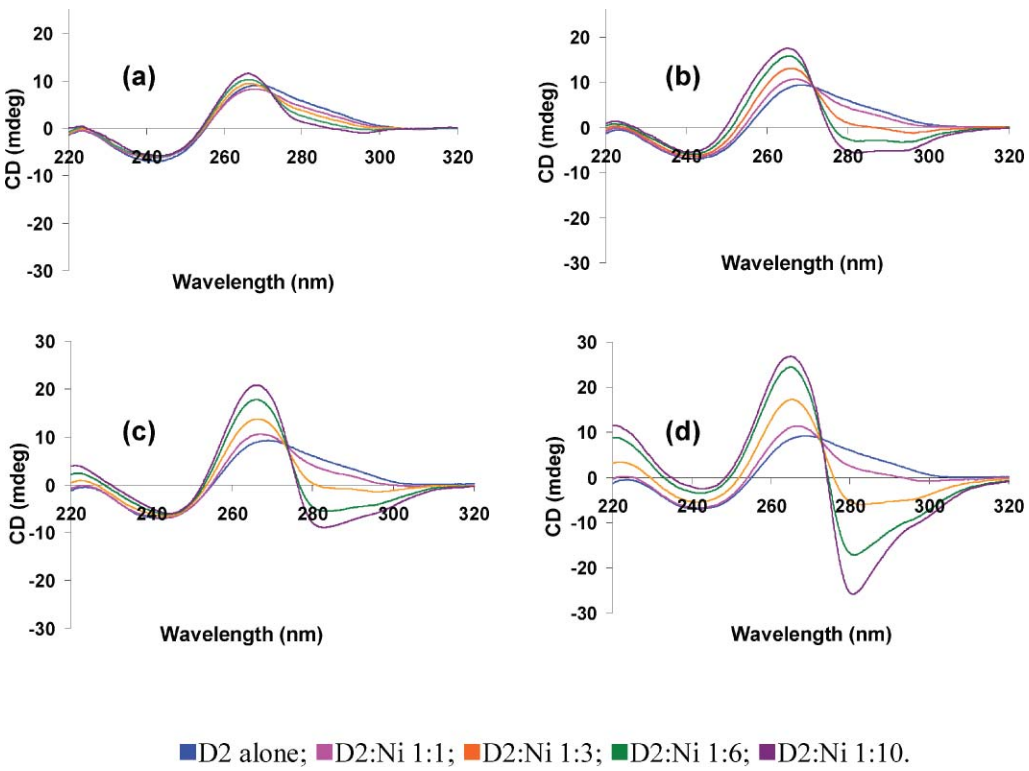

Fig. 5 Circular dichroism spectra recorded over the wavelength range 220–320 nm for solutions containing different ratios of nickel complexes and D2: (a) $[Ni(phen)_3]^{2+}$; (b) $[Ni(phen)_2(dpq)]^{2+}$; (c) $[Ni(phen)_2(dpqC)]^{2+}$ and (d) $[Ni(phen)_2(dppz)]^{2+}$. The concentration of D2 in each solution was 20 μM.

Table 1 Comparison of DNA binding properties of nickel and ruthenium compounds

Compound	$10^4\ K_B$[a]	CD $\Delta\lambda_{max} + \Delta\varepsilon$[b]	$M_{50\%Inh}$[c]/μM	Compound	$10^4\ K_B$[a,d]	CD $\Delta\lambda_{max} + \Delta\varepsilon$[b,d]	$M_{50\%Inh}$[c]/μM
$[Ni(phen)_3]^{2+}$	0.7	−2 (3)	>250	$[Ru(phen)_3]^{2+}$	0.3	−6 (12)	>250
$[Ni(phen)_2(dpq)]^{2+}$	1.8	−4 (9)	>250	$[Ru(phen)_2(dpq)]^{2+}$	1.4	−8 (21)	61
$[Ni(phen)_2(dpqC)]^{2+}$	6.1	−3 (12)	43	$[Ru(phen)_2(dpqC)]^{2+}$	6.1	−7 (25)	18
$[Ni(phen)_2(dppz)]^{2+}$	5.2	−4 (18)	33	$[Ru(phen)_2(dppz)]^{2+}$	6.4	−8 (32)	12

[a] Binding constants ($M(base\ pair)^{-1}$) were determined by absorption spectrophotometric titration. [b] $\Delta\lambda_{max}$ is the shift in nm of the positive DNA CD band at 269 nm. $\Delta\varepsilon$ (the value in parentheses) is the difference between the maximum ellipticity (in °) observed for the positive CD band in the spectrum of a 10 : 1 reaction mixture, and the ellipticity observed at the same wavelength in the spectrum of free D2. [c] $M_{50\%Inh}$ is the concentration of metal complex required for 50% inhibition of DNA transcription. [d] From ref. 18.

Absorption spectrophotometry

The absorption spectra of $[Ni(phen)_2(dpq)]^{2+}$ and $[Ni(phen)_2(dpqC)]^{2+}$ have not been reported previously, and were found to contain a number of absorption bands between 200 and 400 nm attributable to π–π* transitions of the ligands. This includes absorption bands centred at 323 and 338 nm for $[Ni(phen)_2(dpq)]^{2+}$, and at 331 and 347 nm for $[Ni(phen)_2(dpqC)]^{2+}$. Fig. 6 shows the results obtained from a typical absorption spectrophotometric titration involving the incremental addition of D2 to a solution containing $[Ni(phen)_2(dppz)]^{2+}$. For all complexes the addition of DNA was found to result in small (<2 nm) bathochromic shifts for the main absorption bands, as well as significant hypochromism. The latter was analysed initially by measuring the decrease in absorbance for the lowest energy absorption bands of the nickel compounds caused by addition of 10 equivalents of D2. The resulting values of $\Delta Abs_{(\lambda max)}$ varied between 0.02 and 0.32, and increased in the same manner as the orders of relative binding affinity derived from ESI-MS and CD studies, namely $[Ni(phen)_3]^{2+}$ (0.02) < $[Ni(phen)_2(dpq)]^{2+}$ (0.07) < $[Ni(phen)_2(dpqC)]^{2+}$ (0.24) < $[Ni(phen)_2(dppz)]^{2+}$ (0.32). The changes in absorbance of the lowest energy absorption bands were then also analysed using eqn (1) in order to afford an overall DNA binding constant for each nickel complex.

$$[DNA]/(\varepsilon_A - \varepsilon_F) = [DNA]/(\varepsilon_B - \varepsilon_F) + 1/K_b(\varepsilon_B - \varepsilon_F) \quad (1)$$

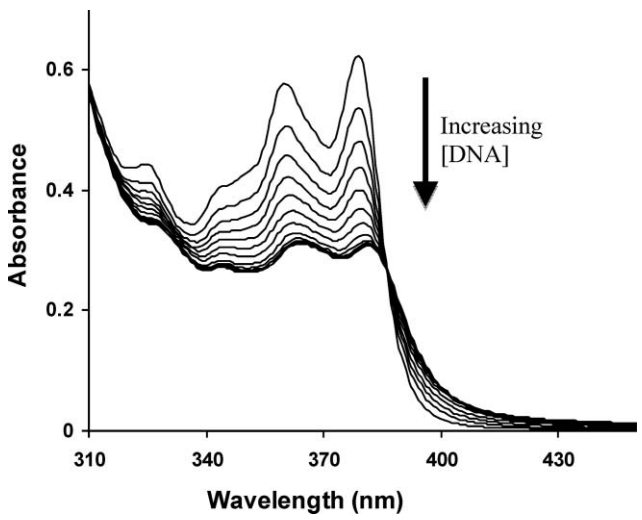

Fig. 6 Visible absorption spectra of $[Ni(phen)_2(dppz)]^{2+}$ (10 μM) in the presence of increasing (0–40 μL) volumes of D2 (1.25 mM).

In this equation ε_A, ε_F and ε_B correspond to A_{obsd}/[Ni], the extinction coefficient for the free nickel complex, and the extinction coefficient for the nickel complex when fully bound to DNA, respectively. The above equation was initially developed by Binesi and Hildebrand to determine equilibrium constants for the binding of iodine to aromatic hydrocarbons,[32] and was later used by Meehan and co-workers who examined the binding of polycyclic aromatic hydrocarbons to CT-DNA.[33,34] Since then its use has been adopted by many others in order to afford a convenient estimate of the overall strength of binding of metallointercalators to DNA.[14,15,31,35–45]

Table 1 shows the binding constants derived after analysis of the results of absorption titrations using eqn (1). As expected, the binding constant for $[Ni(phen)_3]^{2+}$ was the lowest measured, with that determined for $[Ni(phen)_2(dpq)]^{2+}$ approximately two and a half times greater. The binding constants for both $[Ni(phen)_2(dpqC)]^{2+}$ and $[Ni(phen)_2(dppz)]^{2+}$ were larger than that for $[Ni(phen)_2(dpq)]^{2+}$, again as expected in view of the larger intercalating ligands present in the former two complexes. One surprising result, however, was that the binding constant for $[Ni(phen)_2(dpqC)]^{2+}$ was slightly larger than that for $[Ni(phen)_2(dppz)]^{2+}$. Binding constants of $(9 \pm 2) \times 10^5\ M^{-1}$ and $1.5 \times 10^5\ M^{-1}$ have been reported previously for $[Ni(phen)_2(dppz)]^{2+}$ with CT-DNA.[14,15] Both values are significantly larger than that reported here ($5.2 \times 10^4\ M^{-1}$). While this may be attributed partially to the different DNA used in our study, it should be noted that other metal complexes containing the dppz ligand have been reported to have even larger binding constants.[8,14] Therefore it appears that our binding constant for $[Ni(phen)_2(dppz)]^{2+}$ may be anomalously low. In this regard the results here parallel those obtained in our previous study of the analogous ruthenium complexes, where binding constants determined by the absorption titration method for both $[Ru(phen)_2(dppz)]^{2+}$ and $[Ru(bipy)_2(dppz)]^{2+}$ were found to be 1–2 orders of magnitude smaller than literature values.[18] This was attributed to the relatively high concentration of salt (100 mM ammonium acetate) present in the titration mixtures in order to closely mimic the conditions of ESI experiments. The use of solutions with an identical ionic strength in the current study may also account for the relatively low binding constant for $[Ni(phen)_2(dppz)]^{2+}$. Comparison of the binding constants for the nickel complexes with those obtained previously for their ruthenium analogues suggests that changing the metal ion has little effect on overall binding affinity. This conclusion contrasts with what was found using both ESI-MS and CD spectroscopy, although both the spread and magnitude of binding constants obtained by the absorption titration method

may have been significantly affected by the use of solutions with relatively high ionic strengths.

Gel electrophoresis

The binding of organic or inorganic molecules can cause unwinding of negatively supercoiled DNA, resulting in lengthening and changes to its shape that also reduces its electrophoretic mobility by an amount that reflects both the nature and extent of binding. In the case of metal complexes that bind non-covalently to DNA, many researchers have used gel electrophoresis to provide evidence for or against an intercalative mode of interaction,[15,35,46–49] as well as to determine whether the complexes cleave DNA upon irradiation.[14,37,50–52] In one of the former types of studies, $[Ni(phen)_2(dppz)]^{2+}$ was reported to alter the mobility of pBR322 plasmid DNA, while $[Ni(phen)_3]^{2+}$ had no effect.[15] However, previously there has not been a systematic study of the effect of binding of each of the four nickel complexes shown in Fig. 1 on the electrophoretic mobility of plasmid DNA, nor has there been a detailed comparison of the effects on DNA mobility of binding by related series of complexes containing different metal ions.

Fig. 7 shows the gel electropherograms obtained after allowing the nickel complexes to interact with pUC9 plasmid DNA for 30 min. Addition of increasing amounts of both $[Ni(phen)_2(dppz)]^{2+}$ and $[Ni(phen)_2(dpqC)]^{2+}$ significantly retarded the mobility of the closed, negatively supercoiled form of the plasmid, whereas $[Ni(phen)_2(dpq)]^{2+}$ and $[Ni(phen)_3]^{2+}$ had little effect. This suggests that the former nickel complexes interact to a greater extent with the DNA. In the case of $[Ni(phen)_2(dppz)]^{2+}$, the closed, supercoiled form of the plasmid was found to co-migrate with the open, circular form when the nickel : nucleotide ratio was 0.06 : 1. Since co-migration did not occur on addition of $[Ni(phen)_2(dpqC)]^{2+}$ until a nickel : nucleotide ratio of 0.08 : 1 was used, these results lead to the same conclusion as that reached using ESI-MS and CD spectroscopy, which was that $[Ni(phen)_2(dppz)]^{2+}$ has the highest DNA binding affinity of all four nickel complexes. At $[Ni(phen)_2(dppz)]^{2+}$: nucleotide ratios greater than 0.06 : 1 the mobility of the closed supercoiled form of pUC9 increased to a small extent, owing to the formation of positive supercoils after the plasmid had been fully unwound.

A similar pattern of results was obtained from gel electrophoresis studies using the four analogous ruthenium complexes (Fig. 8), indicating that their order of relative DNA binding affinities was $[Ru(phen)_3]^{2+} \sim [Ru(phen)_2(dpq)]^{2+} < [Ru(phen)_2(dpqC)]^{2+} < [Ru(phen)_2(dppz)]^{2+}$. For both this and the nickel series the tris(phen) and dpq-containing complexes had the lowest DNA affinity, as none significantly changed the mobility of the closed, negatively supercoiled form of the plasmid. This lack of mobility prevents any meaningful comparison of the relative DNA binding affinities of these complexes. However, Fig. 8 shows that co-migration of bands due to the closed, supercoiled and open, circular forms of the plasmid occurred when the ratio of ruthenium to nucleotide was 0.05 : 1 in the case of $[Ru(phen)_2(dppz)]^{2+}$, and between 0.06 : 1 and 0.07 : 1 for $[Ru(phen)_2(dpqC)]^{2+}$. Both ratios are lower than those at which co-migration occurred with the analogous nickel complexes, suggesting that the ruthenium complexes containing dppz and dpqC ligands have higher DNA binding affinities than their nickel analogues. This conclusion is consistent with those deduced from ESI-MS and CD spectroscopic studies.

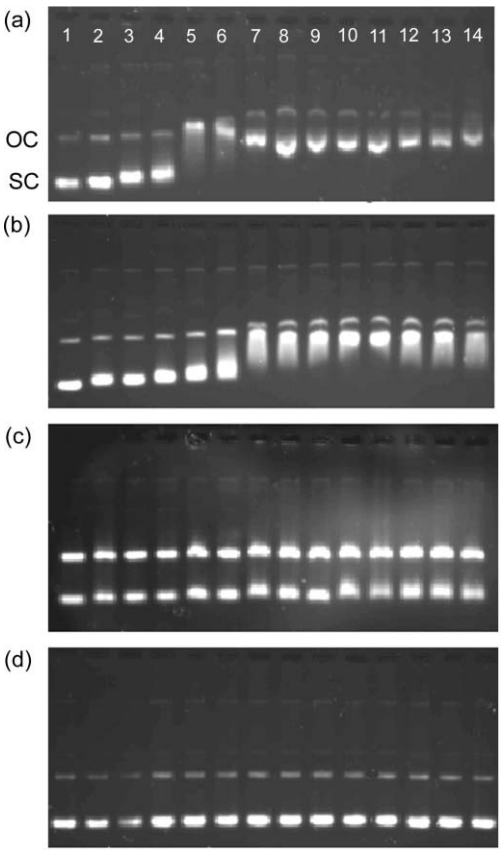

Fig. 7 Gel electropherograms of the products obtained from reaction of pUC9 negatively supercoiled plasmid DNA with varying amounts of nickel complexes for 30 min at 36 °C, pH 7.0: (a) $[Ni(phen)_2(dppz)]^{2+}$; (b) $[Ni(phen)_2(dpqC)]^{2+}$; (c) $[Ni(phen)_2(dpq)]^{2+}$; (d) $[Ni(phen)_3]^{2+}$. The ratio of nickel to nucleotide in lanes 1–14 are: 0 : 1; 0.02 : 1; 0.04 : 1; 0.05 : 1; 0.06 : 1; 0.07 : 1; 0.08 : 1; 0.1 : 1; 0.2 : 1; 0.3 : 1; 0.4 : 1; 0.5 : 1; 0.7 : 1; 0.9 : 1; 1 : 1 and 1.5 : 1. OC = open circular form of DNA, SC = closed supercoiled form of DNA.

Inhibition of transcription

A variety of small organic molecules and metal complexes are known to function as anticancer agents as a result of their ability to interfere with DNA transcription.[53–55] Inhibition of transcription can occur because compounds bind to RNA polymerase or the dsDNA template. In the latter case, the binding event stabilises the duplex structure and inhibits DNA strand separation, a key event in transcription. Turro and co-workers showed that ruthenium and rhodium complexes containing phenanthroline and quinone diimine ligands are able to inhibit transcription to varying degrees as a result of their ability to bind to dsDNA.[25,26] The magnitude of transcription inhibition was conveniently monitored by using reagents readily available in kit form, and was found to correlate with the degree of duplex stabilisation revealed by increases in DNA melting temperature. In view of the chemical similarities between the metal complexes examined as part of the current study, and those studied by Turro and co-workers, it was decided to examine whether the transcription inhibition assay would

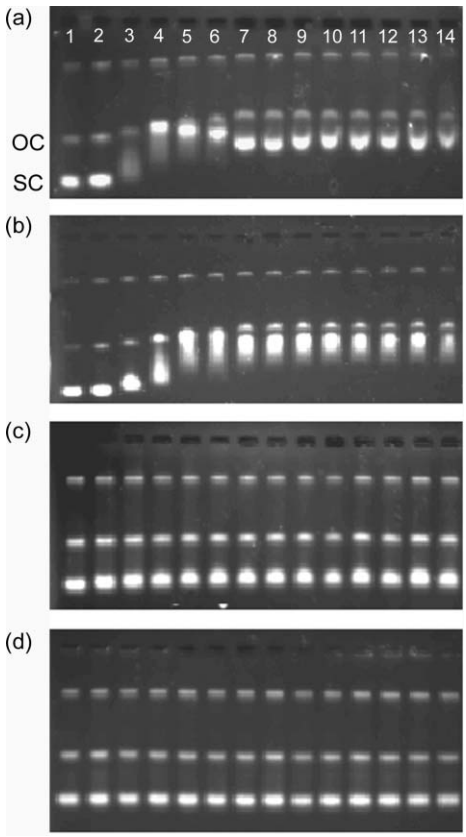

Fig. 8 Gel electropherograms of the products obtained from reaction of pUC9 negatively supercoiled plasmid DNA with varying amounts of ruthenium complexes for 30 min at 36 °C, pH 7.0: (a) $[Ru(phen)_2(dppz)]^{2+}$; (b) $[Ru(phen)_2(dpqC)]^{2+}$; (c) $[Ru(phen)_2(dpq)]^{2+}$; (d) $[Ru(phen)_3]^{2+}$. The ratio of ruthenium to nucleotide in lanes 1–14 are: 0 : 1; 0.02 : 1; 0.04 : 1; 0.05 : 1; 0.06 : 1; 0.07 : 1; 0.08 : 1; 0.1 : 1; 0.2 : 1; 0.3 : 1; 0.4 : 1; 0.5 : 1; 0.7 : 1; 0.9 : 1; 1 : 1 and 1.5 : 1. OC = open circular form of DNA, SC = closed supercoiled form of DNA.

also reveal the systematic differences in binding affinity between ruthenium and nickel complexes noted above.

Fig. 9 shows the effect of increasing the concentration of the nickel complexes on the amount of transcribed mRNA. Two bands attributable to mRNA are present at low concentrations of all nickel complexes due to the formation of two mRNA molecules with different lengths. Increasing the concentration of all nickel complexes resulted in smaller amounts of mRNA being produced, however the effects were most marked in the case of $[Ni(phen)_2(dppz)]^{2+}$ and $[Ni(phen)_2(dpqC)]^{2+}$. In the case of the former compound, no bands attributable to mRNA were detected when its concentration was increased to 30 μM, while a higher concentration (50 μM) of $[Ni(phen)_2(dpqC)]^{2+}$ was required to completely arrest DNA transcription. Although the results shown in Fig. 9 also demonstrate that $[Ni(phen)_2(dpq)]^{2+}$ and $[Ni(phen)_3]^{2+}$ inhibit DNA transcription, the extent to which this occurred was significantly less than with the previous two compounds. Subsequent experiments using higher concentrations of these nickel complexes showed that $[Ni(phen)_2(dpq)]^{2+}$ and $[Ni(phen)_3]^{2+}$ did not totally arrest DNA transcription when present at 250 μM concentration. Since the mechanism of transcription inhibition most likely involves binding of the metal complexes to DNA, the results support the following order of relative

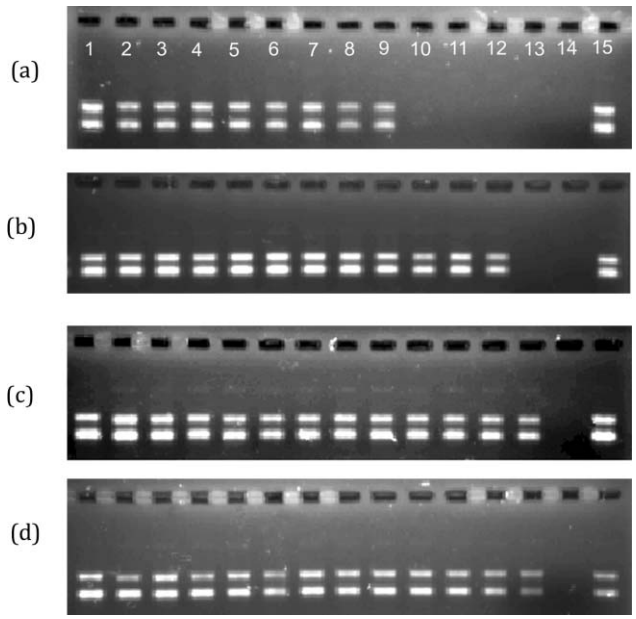

Fig. 9 Ethidium bromide stained agarose gel (1%) of transcribed mRNA in the presence of increasing concentrations of: (a) $[Ni(phen)_2(dppz)]^{2+}$; (b) $[Ni(phen)_2(dpqC)]^{2+}$; (c) $[Ni(phen)_2(dpq)]^{2+}$; (d) $[Ni(phen)_3]^{2+}$. Both sets of bands imaged in each of the gels are due to mRNA. Lanes 1–15 correspond to: [Ni] = 0 μM, 2 μM, 4 μM, 6 μM, 8 μM, 10 μM, 15 μM, 20 μM, 25 μM, 30 μM, 35 μM, 40 μM, 50 μM, Blank, 0 μM.

DNA binding affinity: $[Ni(phen)_3]^{2+}$ ~ $[Ni(phen)_2(dpq)]^{2+}$ < $[Ni(phen)_2(dpqC)]^{2+}$ < $[Ni(phen)_2(dppz)]^{2+}$, which is similar to binding affinity series revealed by most other techniques used here. Experiments performed with the analogous series of four ruthenium complexes produced similar results to those shown in Fig. 9, with the exception that $[Ru(phen)_2(dpq)]^{2+}$ was able to produce 100% inhibition of DNA transcription when present at 75 μM.

In order to compare the effect on DNA transcription of complexes containing the same set of ligands, but different metal ions, the intensities of bands in the gel electropherograms were integrated and plotted as a function of metal ion concentration. The resulting curves were then used to determine values of $M_{50\%Inh}$, the concentration that resulted in 50% inhibition of DNA transcription. These values are presented in Table 1, and show that in most instances the ruthenium complexes are more effective at inhibiting DNA transcription than their nickel analogues. The only exception to this trend was for the two tris phenanthroline complexes, neither of which produced 50% inhibition even when present at the highest concentration examined (250 μM).

Conclusion

The results of ESI-MS and CD studies revealed the following order of increasing DNA binding affinity: $[Ni(phen)_3]^{2+}$ < $[Ni(phen)_2(dpq)]^{2+}$ < $[Ni(phen)_2(dpqC)]^{2+}$ < $[Ni(phen)_2(dppz)]^{2+}$. This sequence is similar to the order of binding affinity derived from absorption titration experiments involving D2, and is supported by the results of gel electrophoresis studies and transcription inhibition assays. Overall these results therefore provide a further demonstration of the potential of ESI-MS for analysis of binding interactions between metallointercalators

and DNA, first revealed by studies involving the analogous ruthenium(II) complexes[16,17] and, more recently by experiments with square planar platinum(II) complexes.[18] In addition, ESI-MS proved to be a valuable tool for developing an improved method for the preparation of nickel(II) complexes.

Each of the techniques used support the conclusion that nickel(II) complexes generally interact more weakly with DNA than the corresponding ruthenium(II) complexes. This was most evident in the case of complexes containing dpqC and dppz ligands owing to their greater overall binding strengths. However, circular dichroism experiments and transcription inhibition assays also supported this conclusion in the case of the two complexes containing dpq ligands. One possible explanation for these observations is that ruthenium and nickel complexes with identical ligand environments show systematic differences in metal–ligand bond distances, and therefore overall size. A survey of the crystallographic literature shows that the Ru–N(phen) bond distances in $[Ru(phen)_3](PF_6)_2$ (av. 2.063(4) Å),[56] $[Ru(phen)_2(bipy)]Cl_2.6H_2O$ (2.073(9)–2.087(10) Å)[57] and $[Ru(phen)_2(dpq)](PF_6)_2$ (2.065(6)–2.073(6) Å)[22] are slightly shorter than the Ni–N(phen) bond distances in compounds such as $[Ni(phen)_3](NO_3)_2$.thiourea monohydrate (2.0701(15)–2.1060(16) Å),[58] $[Ni(phen)_3](ClO_4)_2.0.5H_2O$ (2.078(8)–2.103(7) Å[59] and $[Ni(phen)_2Cl(H_2O)]Cl$ (2.092(3)–2.116(3) Å).[20] Unfortunately no crystallographic data exist for any of the other five complexes examined here. However, if the above data are representative of Ru–N(phen) and Ni–N(phen) bond distances, then it appears that the ruthenium complexes might be slightly smaller in size than their nickel analogues. This may enable the former complexes to participate in slightly stronger electrostatic interactions with the negatively charged DNA molecule. Alternatively, the smaller ruthenium molecules may fit more readily into the major or minor groove of DNA, and insert their dpq, dpqC and dppz ligands into the DNA base stack to a greater extent. In a very recent study,[60] Ramakrishnan and Palaniandavar considered the effects of overall size and coordination geometry on the DNA binding of related series of zinc(II) and copper(II) complexes. These workers found the complex $[Cu(5,6\text{-dmp})_3](ClO_4)_2$ (5,6-dmp = 5,6-dimethyl-1,10-phenanthroline) binds more tightly to CT DNA than its zinc analogue $[Zn(5,6\text{-dmp})_3](ClO_4)_2$. This was attributed as being possibly due to the smaller size of the former complex, which would favour stronger electrostatic interactions, as well as its more flexible coordination geometry.

An alternative explanation for the consistently stronger DNA binding exhibited by ruthenium complexes in the present study, is that they bind to different DNA base sequences and/or interact using slightly different binding modes than the analogous nickel complexes. There has already been significant work in this area, primarily on the interactions of ruthenium complexes with CT-DNA. These studies have led to considerable debate as to the exact means by which complexes such as $[Ru(phen)_2(dppz)]^{2+}$ bind to DNA.[13,61–64] However, in the case of nickel complexes it has also been shown that a slight change in DNA base sequence could alter the exact binding location of the metal complex.[13] These studies all serve to highlight the fact that more detailed structural data, especially information from X-ray studies on crystals containing the above metal complexes bound to the same DNA molecule, is required before it may be possible to assign the differences in binding affinity observed here between nickel and ruthenium complexes to variations in binding mode, or slight differences in size or shape of the metal complexes themselves.

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