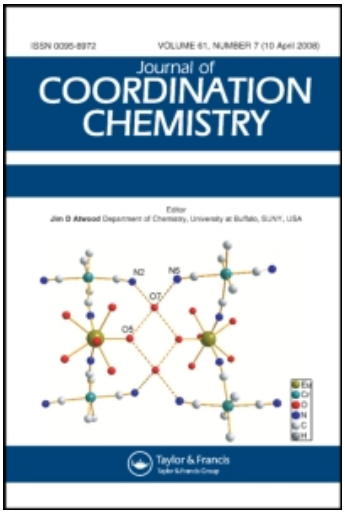

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Spectroscopic and potentiometric studies of the interaction of adenine with trivalent metal ions

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Potentiometric titration was used to determine the potentiometric diagrams of complexes of adenine with Al^{3+}, Cr^{3+}, and Fe^{3+}. IR and Raman spectra of solids show that the interaction of adenine with Cr^{3+} is not as strong as the other metals. Fe^{3+} binds to adenine at low and medium pH values at an Fe^{3+}–adenine ratio of 6, while Al^{3+} binds to adenine at all pH values at an Al^{3+}–adenine ratio of 8. The IR band associated with N-9–H is split into two components at high and low wavenumbers; IR band splitting has been observed for groups with a large dipole moment such as carbonyl and phosphate. To the best of our knowledge, this is the first time to see the splitting of IR bands involving a nitrogen. We also obtained potentiometric titration plots for adenine with Al^{3+}, Cr^{3+}, and Fe^{3+}, independently, in various molar ratios which showed an interaction with adenine, consistent with the IR and Raman findings. Metal–adenine-hydroxo complexes were formed.

Keywords: Adenine; IR and Raman; Potentiometric titrations

1. Introduction

Many functions carried out in biological systems by nucleotides are influenced, or somehow depend on, by the presence of metal ions [1–3]. On the structural level, metals affect DNA and RNA secondary and tertiary structures and conformational states, exerting positive effects as stabilizers or negative effects as destabilizers, acting as mutagens or carcinogens [2, 3]. Study of larger bio-molecules such as DNA and proteins

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are simplified by examining smaller functional groups of which they are composed [1]. Adenine is one of the purines found in DNA and is a fundamental nucleic acid base involved in many nucleosides and nucleotides.

There is confusion in determining the binding of some biologically relevant metal ions to nucleic acid species [4, 5]. It is still unclear whether or not magnesium in Mg-ATP is bound to the heterocyclic purine base. It is difficult to observe metal binding to heterocyclic bases in nucleotide complexes, since phosphate-to-Mg bonding dominates the interaction [4]. Most of the studies have investigated the structural changes in a DNA macromolecule complexed with divalent metal ions [5–8]. Studies involving DNA components with trivalent metal ions are scarce. This project investigates the interaction of trivalent metal ions with adenine at different pH values and metal ion–adenine molar ratios.

The five possible nitrogen binding sites of adenine are the pyrimidines N-1 and N-3, imidazole N-7 and N-9 ring nitrogens, and the exocyclic $-NH_2$ group. The C=C bond between the two cycles and the cycles themselves are also potential binding sites. Studying the interaction of adenine with metal ions may help clarify the role of the N donors in binding.

The accumulation of metal ions such Al^{3+} in nervous tissues is associated with a number of neurological conditions [9–12]. Iron overload has been involved in Wilson's disease patients [13]. Lay *et al*. [14] reported an *in vitro* study on Cr^{3+}-DNA binding *via* reduction of Cr^{6+} to Cr^{3+}, and also suggested that the Cr^{3+}-DNA adducts are mutagenic. Because Cr^{3+} is the final form of chromium within the cell, the interaction of Cr^{3+} with DNA may play a crucial role in the carcinogenic action of Cr^{6+} salts [14–16]. On the basis of spectroscopic data of calf thymus DNA in the presence of Cr^{6+} and Cr^{3+} metal ions, Arakawa *et al*. [15] showed that Cr^{6+} and Cr^{3+} do not induce DNA conformation changes at low or high metal ion concentration; Cr^{3+} induces DNA condensation at high concentration by binding to guanine (N-7) and phosphate. Others have observed a major Cr^{3+}-DNA complexation [17, 18]. Preferential binding of Cr^{3+} to the phosphate backbone leads to only minor 1–2° distortions in DNA duplexes [16].

Ghose [5] found that adenine is coordinated to metal ions through the N-3 and N-7 nitrogens. The N-9–H does not participate in either hydrogen bonding or coordination to metal ions [5]. Giese and McNaughton [19] reported that many reports on the surface-enhanced Raman spectra (SERS) of nucleic acids contradict with regard to the interaction between the base and surface of silver colloids and used Density Function Theory (DFT) calculations to assign the Raman spectra of adenine interacting with silver surface substrates. They assigned the strongest band at $735\,cm^{-1}$ to a NH_2 deformation rather than to the ring breathing mode, in contrast with other reports [7, 20]. Interaction of metal ion preferentially takes place through N-7 and the external NH_2 group, interaction of adenine *via* N-9–H is unlikely [5, 19].

Ghose [5] and Tajmir-Riahi *et al*. [2] suggested that adenine is coordinated to metal ions through N-3 and N-7 nitrogens [2, 5]. Similar discrepancies exist for the interpretation of IR spectra of adenine [7, 19, 20]. A distinct change in the IR or Raman spectrum of a nucleoside upon metal salt addition is a clear indication of complex formation. This work is part of a study on complex formation between biological ligands and metal ions in aqueous solutions. The objective is to determine whether or not complexes are formed, define metal binding sites, and gain new insights into the relative stability of the formed complexes [4, 21–23].

2. Experimental

2.1. *Materials and methods*

Adenine $C_5H_5N_5$ 99% commonly known as vitamin B_4 (formula weight 135.13 g mol^{-1}) was purchased from Aldrich. $AlCl_3 \cdot 6H_2O$, $CrCl_3 \cdot 7H_2O$, and $FeCl_3 \cdot 5H_2O$ were obtained from Fisher and used as received. All solutions including adenine were dissolved in doubly de-ionized (DI) water. Complexes were obtained by mixing the aqueous solutions of the metal and adenine in $1 < R < 12$ molar ratios at pH-values of 2, 7, and 9. Aluminum nitrate nonahydrate, $Al(NO_3)_3 \cdot 9H_2O$, chromium nitrate nonahydrate, $Cr(NO_3)_3 \cdot 9H_2O$, and iron nitrate nonahydrate, $Fe(NO_3)_3 \cdot 9H_2O$, Fisher Scientific 99% were used in collecting the potentiometric titrations. The pH-values of all solutions were adjusted using ~0.1 M NaOH solution and measured using Orion pH electrode model 720 A+ connected to an Orion pH-meter. pH measurements were conducted in neutral gas atmosphere as described elsewhere [24–28]. The ionic strength of all solutions was adjusted to 0.10 M using 1.0 M $NaNO_3$ solution at 25°C ± 1°C [28].

2.2. *Spectroscopic studies (IR and Raman)*

A series of solutions was prepared with a constant adenine concentration and increasing metal ion concentrations. The R value refers to the molar ratio R = [metal]/[adenine]. FTIR measurements were carried out at room temperature on a Perkin Elmer Spectrum equipped with a Diamond attenuated total reflectance (ATR) DuraVision accessory, a DTGS KBr detector and a KBr beam splitter; spectra were recorded between 4000 and 600 cm^{-1} at 4 cm^{-1} resolution and 64 scans were collected. Confocal Raman spectra were acquired with a Horiba Jobin-Yvon HR800 spectrometer using an excitation wavelength of 632.81 nm, a 600 groove grating, and a 10 × microscope objective. Spectra were collected by placing the precipitate on a glass microscope slide and collecting spectra from the top portion of the sample. The spectra presented here represent an average of eight individual spectra, with each spectrum collected over the course of 1 s. The detector was an open electrode CCD and the diameter of the pinhole immediately preceding the detector was 100 microns.

2.3. *System standardization and adenine pK_a values*

A standard phosphoric acid solution (H_3PO_4) was titrated to calibrate the potentiometric titration system before gathering actual potentiometric data for the free adenine, the free metal ions, or the metal ion–adenine reaction system in different molar ratios. Titration of standard H_3PO_4 is presented in the Supplementary materials. When free adenine was titrated without a metal ion, it behaved as H_2L with two protons titrated, from the aromatic nitrogen(s) and the free aliphatic amino group. The pK_a values of these groups are 4.26 and 9.69, respectively [24].

Standardizations of metal ion solutions used in the potentiometric titrations were done by eluting a known volume (typically 1.0 mL) through the Dowex 50 × 8-100 resin packed in a 7 × 1 inch glass column and titrating the eluant with a standard NaOH solution. The stock metal ion concentrations were in the range of 0.05 M.

Typically, seven to nine runs were averaged. These procedures are documented in the literature [25–28].

2.4. *Potentiometric titrations*

In metal–ligand potentiometric titrations, the NaOH solution was always the titrant. The methods used to prepare, standardize, and prevent contamination of the titrant with atmospheric CO_2 have been described elsewhere [24–28]. In a typical titration, 2.0 mL of 0.050 M adenine solution was added to a 100.0 mL volumetric flask. Then 2.0 mL of stock 0.05 M Al^{3+}, or Cr^{3+}, or Fe^{3+} solutions (separately) and 10.0 mL of 1.0 M $NaNO_3$ solution were added before dilution to the mark with DI water. Before each titration, the metal–adenine mixtures were allowed to stir for 20 min to reach equilibrium. The NaOH solution was added in 100 μL increments using an Eppendorf micropipette with continuous stirring. The time intervals between the additions of the NaOH solution were set to 3 min, which was sufficient to get each pH value stabilized and reach complete equilibrium. Titration experiments took about 5–6 h to complete. Each potentiometric titration was performed at least in triplicate.

3. Results and discussion

3.1. *Interaction of Fe^{3+} with adenine*

3.1.1. Fe^{3+}–adenine using FTIR. The IR and Raman spectra of all metal salts used to prepare solutions, free adenine, and metal–adenine complexes of the current study are documented in the "Supplementary material". Table 1 summarizes the wavenumbers and assignments of the main IR bands of free adenine and the metal ion–adenine systems at pH 2 using the assignments proposed by Mathlouthi *et al.* [20]. The spectral changes (intensity and shifting) of the IR peaks associated with the possible binding sites of adenine were monitored. In the 3500–2500 cm^{-1} region, bands at 3282 and 3103 cm^{-1} are assigned to νNH_2 asymmetric and νNH_2 symmetric, respectively. The band at 2970 cm^{-1} is attributed to ν(N-9–H) [20]. In the 1800–600 cm^{-1} region, free adenine includes bands at 1669 cm^{-1} (δNH_2), 1598 cm^{-1} (ν(C=N) or ν(C=C)), 1502 cm^{-1} (δ C-N-9–H), 1449 cm^{-1} (δ imidazole ring), 1250 cm^{-1} (C–NH_2), 910 cm^{-1} (r NH_2), and 866 cm^{-1} (ω N-9–H) [20]. At $R < 8$ for Fe^{3+} (Supplementary material), intensities of the bands cited above exhibit no major intensity change or shift. We conclude that there is no significant interaction between Fe^{3+} and adenine at low concentration.

At a concentration of Fe^{3+} $R = 8$ (Supplementary material), major spectral changes occurred for adenine vibrations at all the possible binding sites. The interaction of adenine with Fe^{3+} is evidenced by the disappearance of peaks associated with NH_2, suggesting that the amino group is involved in metal coordination. A major observation is the splitting of band at 2970 cm^{-1} assigned to ν(N-9–H) into two different bands, 2961 and 2979 cm^{-1}. Concomitantly, the peak at 1598 cm^{-1} splits into two peaks, 1611 and 1575 cm^{-1}. We propose that the assignments of the wavenumbers for the two bands have been swapped, attributed to δ N-9–H in the current study. Splitting of a band

Table 1. Summary of the wavenumbers in (cm^{-1}) and assignments of the main IR bands of solid adenine and its Al^{3+}, Cr^{3+}, and Fe^{3+} complexes at pH 2.

Free adenine	Adenine–Al^{3+}	Adenine–Cr^{3+}	Adenine–Fe^{3+}	Assignments
3282		3282		$\nu(NH_2)$
3101				$\nu(NH_2)$
	2977		2979	ν(N-9–H)
2970		2970		ν(N-9–H)
	2960		2961	
2788	2787		2787	ν(C–H)
2684				ν(C–H)
2593				ν(C–H)
2064		2064		Not assigned
	1948		1947	Not assigned
1893		1893		Not assigned
	1790		1789	Not assigned
1669	1696	1669	1697	$\delta(NH_2)$
1598	1609	1598	1611	ν(C=N), ν(C=C)
1502	1499	1502	1500	δ(C–N-9–H)
1449	1467	1449	1467	(Imidazole ring)
1417	1412	1417	1411	δ(N=CH)
1306	1306	1306	1305	ν(C–N)
1250	1241	1250	1241	$\nu(C–NH_2)$
1154	1185	1154	1186	δ(CH)
1124	1114	1124	1115	δ(C-2–N-1=C-6)
1021	1018	1021	1018	δ(C–N–C)
938	944	938	945	δ(N–C=N)
910	897	910	898	r (NH_2)
866		866		ω(N-9–H)
722	709	722	710	ν(C–C), ν(C–N)
619	617	619	618	δ(N–C–C)

involving a nitrogen has not been reported for this system. The shift and intensity change of the peak at 1449 cm^{-1} assigned to the imidazole ring also indicates involvement of this group in Fe^{3+} coordination. Complexation occurs instantly at a specific R value, and the splitting depends on the nature of the metal ion.

For Fe^{3+} $R > 8$ (Supplementary material), the interaction shows different features compared to those at $R = 8$, suggesting a different mode of coordination of Fe^{3+} to adenine. Peaks associated with νNH_2 appeared again but shifted to higher wavenumber, suggesting that NH_2 is involved in the bonding [21]. Other peaks not assigned in the literature also appear in our IR spectra of adenine and its metal complexes. At this time we do not have a credible attribution of these new peaks.

To illustrate the effect of pH on the coordination mode of adenine to Fe^{3+}, we recorded the IR spectra of free adenine at pH 2, 7, and 9 (''Supplementary material'', figure 2). Deprotonated amino groups play a role in coordinating metal ions at alkaline pH values. Then we recorded the IR spectra of the Fe^{3+}–adenine complexes ($R = 8$) at different pH values. Figures in the ''Supplementary material'' show the spectra of Fe^{3+}–adenine at pH 2, 7, and 9. Splitting of the band associated with N-9–H is observed at pH 2 and 7. There is no major splitting of the bands at pH 9, suggesting N-9–H is not involved in the interaction with Fe^{3+}.

3.1.2. Fe^{3+}–adenine using Raman spectroscopy. Raman spectra of adenine with its complex of Fe^{3+} in the high wavenumber region are shown in figure 4 of the

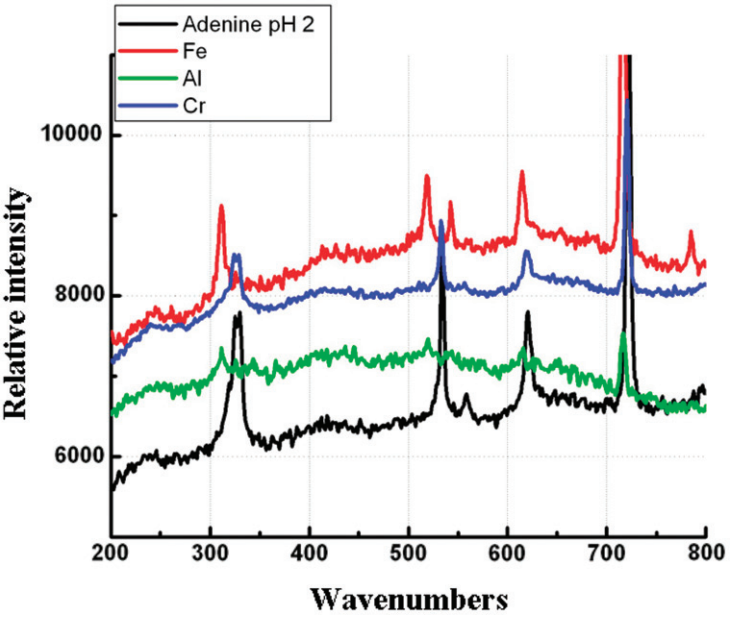


Figure 1. Raman spectra of adenine with Al^{3+}, Cr^{3+}, and Fe^{3+} at pH 2.

Supplementary material. Interaction of Fe^{3+} with adenine is indicated by the disappearance of peak at 1331 cm^{-1} and the very strong intensity increase of the band at 1306 cm^{-1} in the spectrum of the complex. Also, the bands at 720 and 620 cm^{-1} in the Raman spectrum of adenine are shifted to lower wavenumbers 715 and 610 cm^{-1} in the Raman spectrum of the complex, respectively. According to Savoie *et al*. [7], the main spectral indication for the N-9 substitute complex is the absence of a relatively strong band at about 1250 cm^{-1} in the Raman spectrum. Indeed, this band is missing in our Raman spectrum of Fe^{3+}–adenine, suggesting that N-9 is not only H bonded but also substituted through its lone pair. Below 500 cm^{-1}, metal–ligand vibrations occur [21] and assignment of ν(M–N) and ν(M–Cl) have been accomplished by comparing with the reported data [21, 23].

Additional bands observed in the spectra of the complexes (figure 1) in the 600–200 cm^{-1} region are attributed to the coordination of adenine to Fe^{3+}. There are three new bands in the Raman spectrum of the Fe^{3+}–adenine complex. Fe–Cl vibrations are found in Raman spectra of metal salts at 587, 462, 395, 285, and 218 cm^{-1} (figure 6 of the Supplementary material); new bands appeared at 308 and 515 cm^{-1} with the peak at 515 cm^{-1} attributed to ν(Fe–N-6) in agreement with the values reported in the literature [7, 22] and the peak at 308 cm^{-1} attributed to ν(Fe–N) ring [7, 22].

3.1.3. Fe^{3+}–adenine using potentiometric titrations. The potentiometric titrations of Fe^{3+} (and the other two metal ions) with adenine as a binding ligand have been conducted in 0 : 1, 1 : 1, and 1 : 2 molar ratios. Potentiometric titration is a powerful tool to study metal ion–ligand interactions in aqueous solution [24]. Over the past decade, we studied similar titration systems using the potentiometric technique among other

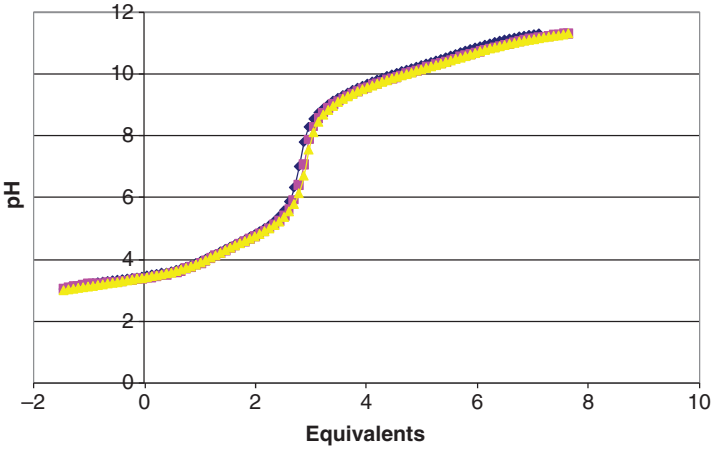


Figure 2. A representative potentiometric titration graph for the Fe^{3+} : adenine system in 1 : 2 molar ratio in triplicate. Summary of all titrations are given in table 2. The equivalents on the *x*-axis are defined as the number of millimoles of titrant per millimole of adenine. If the metal ion is present, the equivalents are defined as the number of millimoles of titrant per millimole of metal ion.

analytical techniques [25–28]. From comparison of titration graphs of free adenine, free M^{3+}, and adenine–metal ion titration systems there is binding of the metal ions to adenine. The shift of the titration plateaus from one equivalent (for free adenine) to three equivalents (for metal–adenine combinations) indicates that there is a reaction taking place, consistent with the significant shifts observed in IR and Raman spectra.

Figure 21 of the "Supplementary material" is the potentiometric titration graph for the free adenine at which five plots were superimposed to show the consistency of the data. The number of equivalents on the *x*-axis is defined as the number of mmoles of titrant per mmole of adenine. Figure 2 is the potentiometric titration graph of the Fe^{3+} : adenine system in 1 : 2 molar ratio in triplicate. Summary of all titrations is given in table 2. We have also measured the effect of the change in pH on the potential response (Supplementary material). Before the end point the potential spanned over +200 mV until the zero potential at the end point. Beyond the end point the potential is spanned over −250 mV.

Figure 22 of the "Supplementary material" is a representative graph of Al^{3+}: adenine titrations in 1 : 2 molar ratio in triplicate. In potentiometric titrations, the presence of a sharp inflection point indicates the formation of a single dominant species, while the position of the inflection point indicates the number of protons released *via* the formation of this dominant species. Table 2 catalogues the exact locations of the inflection points measured for the metal ions with adenine in all molar ratios. All values are reported with the proper value of standard deviation.

3.2. *Interaction of Al^{3+} with adenine*

3.2.1. Al^{3+}–adenine using FTIR. Table 1 summarizes the frequencies and assignments of the main IR bands of free adenine and the Al^{3+}–adenine system at pH 2. Al^{3+} behaves as Fe^{3+} and gives similar shifts. At $R < 6$ for Al^{3+} (Supplementary material),

Table 2. Inflection points for titrations of different (metal ions)$^{3+}$: adenine in different molar ratios at 25°C and $I = 0.1$ M $NaNO_3$.

Metal-ion-to-adenine molar ratios	Location of the inflection point (equivalents of NaOH)								
	0 : 1	Al^{3+} 1 : 1	Al^{3+} 1 : 2	Fe^{3+} 1 : 1	Fe^{3+} 1 : 2	Cr^{3+} 1 : 1	Cr^{3+} 1 : 2	Cr^{3+} 1 : 3	$(pK_a)^a$ values
	0.960	2.76	3.17	3.13	2.78	3.76	3.76	3.44	pK_{a1} 4.26
	0.960	3.27	2.86	2.96	2.87	3.76	3.44	3.60	
	0.960	3.17	3.07	2.96	2.87	3.76	3.44	3.44	pK_{a2} 9.69
	1.08								
	1.08			–					
Average	1.01	3.07	3.03	3.02	2.84	3.76	3.55	3.49	
Standard deviation, σ	0.07	0.27	0.16	0.10	0.05	0	0.18	0.09	

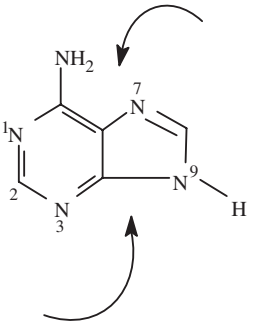
All runs were reported with the proper standard deviation.
[a]pK_a-values were taken from reference [24].

intensities of the bands of free adenine exhibit no major intensity change or shift. For iron at $R = 6$ (Supplementary material), major spectral changes occurred at all possible binding sites. At $R > 6$ for Al^{3+} interaction is shown by similar features to those for iron at R=6, suggesting a similar mode of coordination. Peaks associated with νNH_2 is again shifted towards higher wavenumber, suggesting NH_2 of adenine binds through its lone pair [21].

3.2.2. Al^{3+}–adenine using Raman spectroscopy. The Raman spectra of adenine with Al^{3+} are included in the Supplementary material. Interaction of Al^{3+} with adenine is indicated by the disappearance of peak at 1331 cm^{-1} and the strong intensity increase of the band at 1306 cm^{-1}. Also, bands at 720 and 620 cm^{-1} in the Raman spectrum of adenine shift to 716 and 613 cm^{-1}, respectively. According to Savoie *et al.* [7], the main spectral indication for an N-9 substituted complex is the absence of a strong band at 1250 cm^{-1} in the Raman spectrum. Indeed, this band is missing in our Raman spectrum of Al–adenine, suggesting that N-9 is not only H bonded but also complexed.

Comparison of the Raman spectra of adenine and Al–adenine in the low wavenumber region of figure 1 indicates coordination. The additional bands observed in spectra of the complexes in the 600–200 cm^{-1} region are attributed to the coordination of adenine to Al^{3+}. There are two new bands in the Raman spectrum of the Al–adenine complex at 310 and 518 cm^{-1}. The peak at 518 cm^{-1} may be attributed to ν(Al–N-6), in agreement with the values reported in the literature [7, 22] and the peak at 310 cm^{-1} to ν(Al–N) ring [7, 22].

3.2.3. Al^{3+}–adenine using potentiometric titrations. Figure 22 of the "Supplementary material" is the potentiometric titration graph of Al^{3+} : adenine in 1 : 2 molar ratio in triplicate. The sharpest slopes appeared at 3 equivalents. Table 2 summarizes all titrations with the proper statistical data analysis. Because of the similarities in the behavior of Al^{3+} and Fe^{3+}, we expect similar interaction between adenine and these two metal ions. Scheme 1 shows the most realistic positions of attachments of adenine with Al^{3+} and Fe^{3+}. There were no precipitates for either the Al^{3+}–adenine or the Fe^{3+}–adenine complexes in any molar ratio. By examining table 2 more closely, both metal complexes showed the same maximum slopes at the inflection points for 1 : 1 and 1 : 2 titration systems.



Scheme 1. Proposed sites for the binding of trivalent metal ions to adenine in aqueous solutions at 25°C.

3.3. *Binding of chromium to adenine*

IR spectra of adenine and Cr^{3+}–adenine system are shown in figures 2 and 3 in "Supplementary material". Cr^{3+} does not bind to adenine even at high Cr^{3+} concentration (figure 19 in Supplementary material). Evidence of this comes from the lack of major spectral change (intensity or shift, see table 1) of the prominent adenine vibrations. In addition, a careful comparison of the Raman spectra of adenine and Cr^{3+}–adenine in the low wavenumber region (Supplementary material, figure 6) confirms that Cr^{3+} ions have no significant effect on the structure of adenine. Cr–Cl vibrations are found in Raman spectra of metal salts at 660, 344, 299, 247, and 205 cm^{-1} for Cr–Cl. Gonzalez-Baro *et al*. [29] found Cr–N bands at 448 cm^{-1} and Cr–Cl bands at 337 and 245 cm^{-1} [29]. There is no new band in our Raman spectrum of the Cr^{3+}–adenine complex, and all the Cr–Cl bands have disappeared. Potentiometry generated results that support the conclusion reached from both FTIR and Raman spectra. All the potentiometric titrations for the Cr^{3+}–adenine in different molar ratios indicated similar behavior.

4. Conclusions

Herein, assignments of the most characteristic IR and Raman [30] bands are proposed for metal–adenine vibrations under ambient conditions. Fe^{3+} binds to adenine at low and medium pH, while Al^{3+} binds to adenine at all pH values. Based on the IR and Raman spectra of solid Cr^{3+}–adenine interaction is negligible at all pH values, even at high metal concentrations, perhaps due to Cr^{3+} being kinetically inert [31].

Many reports regarding adenosine mono-, di-, and tri-phosphate(s), but not adenine, with divalent metal ions [32–41] have been reported until recently [35, 42–44]. Closely examining our data and these reports [32–44] support the claims presented herein [35, 42–44]. Our potentiometric data augmented with both IR and Raman show explicitly the species formed according to equations (1) and (2).

$$[\mathrm{H(adenine)}]^+ + \mathrm{NaOH} \rightarrow (\mathrm{adenine}) + \mathrm{H}^+ \quad (1)$$

$$[\mathrm{H(adenine)}]^+ + \mathrm{M}^{3+} \rightarrow \mathrm{M}[(\mathrm{adenine})(\mathrm{OH})_2]^+ + 3\ \mathrm{H}^+ \quad (2)$$

Free adenine releases one proton and the interaction of metal ions with adenine released three protons. The extra two protons come from the aqua ligands yielding the $M[(adenine)(OH)_2]^+$ complex shown in equation (2). Such hydroxo-complex has been observed in similar recent studies [35, 44]. The locations of the metal binding are clearly shown in scheme 1 due to the fact that the IR band associated with N-9–H was split into two components at high and low wavenumbers.

Highly accurate potentiometric plateaus were collected and refined. The shift and intensity changes of IR and Raman bands associated with the stretching of $–NH_2$, N-1 and N-3 in Al^{3+}–adenine and Fe^{3+}–adenine complexes confirm coordination. Biochemists and physical chemists should work together on the characterization of the interactions between important biological ligands and essential/toxic metal ions, and effects on the conformations of macromolecules such as DNA and proteins. In addition to new potentiometric titrations, we hope that we were able to show that the vibrations for transition metals–adenine bonds in the region of 200–800 are achievable by Raman spectra.

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References

[1] S. Kalyanaraman, V. Krishnakumar, K. Ganesan. *Spectrochim. Acta, Part A*, **66**, 1340 (2007).
[2] H.A. Tajmir-Riahi, M. Langlais, R. Savoie. *Nucl. Acids Res.*, **16**, 751 (1988).
[3] M. de la Fuente, A. Hernanz, R. Navarro. *J. Biol. Inorg. Chem.*, **9**, 973 (2004).
[4] L.G. Marzilli, B. de Castro, J.P. Caradonna, R.C. Stewart, C.P. Van Vuuren. *J. Am. Chem. Soc.*, **102**, 916 (1980).
[5] R. Ghose. *Synth. React. Inorg. Met.-Org. Chem.*, **22**, 379 (1992).
[6] S.V. Kornilova, P. Miskovsky, A. Tomkova, L.E. Kapinos, E.V. Hackl, V.V. Andrushchenko, D.N. Grigoriev, Yu.P. Blagoi. *J. Mol. Struct.*, **408/409**, 219 (1997).
[7] R. Savoie, J.J. Jutier, L. Prizant, A. Beauchamp. *Spectrochim. Acta, Part A*, **38**, 561 (1982).
[8] M . de la Fuente, J.M. Gavira-Villejo, A. Hernanz, R. Navarro. *J. Mol. Struct.*, **565**, 265 (2001).
[9] R.W. Hutchinson, A.G. Cox, C.W. Mcleod, P.S. Marshall, A. Harper, E.L. Dawson, D.R. Howlett. *Anal. Biochem.*, **346**, 225 (2005).
[10] A. Campbell, S.C. Bondy. *Cell. Mol. Biol.*, **46**, 721 (2000).
[11] S. Desroches, S. Dayde, G. Berthon. *J. Inorg. Biochem.*, **81**, 301 (2000).
[12] C. Exley. *J. Inorg. Biochem.*, **76**, 133 (1999).
[13] M. Bruehlmeier, K.L. Leenders, P. Vontobel, C. Calonder, A. Antonini, A. Weindl. *J. Nucl. Med.*, **41**, 781 (2000).
[14] A. Levina, P.A. Lay, N.E. Dixon. *Chem. Res. Toxicol.*, **14**, 946 (2001).
[15] H. Arakawa, R. Ahmad, M. Naoui, H.A. Tajmir-Riahi. *J. Biol. Chem.*, **275**, 10150 (2000).
[16] K. Slnikow, A. Zhitkovitch. *Chem. Res. Toxicol.*, **21**, 28 (2008).
[17] L.C. Bridgewater, F.C. Manning, S.R. Patierno. *Carcigenosis*, **15**, 2421 (1994).
[18] N.A. Floro, K.E. Wetterhahn. *Biochem. Biophys. Res. Commun.*, **124**, 106 (1984).
[19] B. Giese, D. McNaughton. *J. Phys. Chem. B*, **106**, 101 (2002).
[20] M. Mathlouthi, A. Seuvre, J.L. Koenig. *Carbohydr. Res.*, **131**, 1 (1984).
[21] N. Kazuo. *Infrared and Raman Spectra of Inorganic and Coordination Compounds*, 5th Edn, Wiley & Sons, New York, NY (1977).
[22] A. Torreggiani, A.D. Esposti, M. Tamba, G. Marconi, G. Fini. *J. Raman Spectrosc.*, **37**, 291 (2006).
[23] K. Golcuk, A. Altun, M. Kumru. *Spectrochim. Acta, Part A*, **59**, 1841 (2003).
[24] A.E. Martell, R.M. Smith, R.J. Motekaitis. *Critical Stability Constants Database, Version 6.0*, NIST, Texas A&M University, College Station, TX, USA (2001).
[25] Y.Z. Hamada, N. Bayakly, D. George, T. Greer. *Synth. React. Inorg., Meta.-Org., Nano-Met. Chem.*, **38**, 664 (2008).
[26] Y.Z. Hamada, C. Roger. *J. Coord. Chem.*, **60**, 2149 (2007).
[27] Y.Z. Hamada, N. Bayakly, A. Peipho, B. Carlson. *Synth. React. Inorg., Meta.-Org., Nano-Met. Chem.*, **36**, 469 (2006).
[28] Y.Z. Hamada, W.R. Harris. *Inorg. Chim. Acta*, **359**, 1135 (2006).
[29] A.C. Gonzalez-Baro, R. Pis-Diez, O.E. Piro, B.S. Parajon-Costa. *Polyhedron*, **27**, 502 (2008).
[30] J.D. Ingle Jr., S.R. Crouch. *Spectrochemical Analysis*, Prentice Hall, Englewood Cliffs, NJ (1988).
[31] B. Douglas, D. McDaniel, J. Alexander. *Concepts and Models of Inorganic Chemistry*, 3rd Edn, John Wiley & Sons, Inc, New York (1994).
[32] A. Gasowska, L. Lomozik. *J. Coord. Chem.*, **52**, 375 (2001).
[33] L. Qian, Z. Sun, J. Gao, B. Movassagh, L. Morales, K.B. Mertes. *J. Coord. Chem.*, **33**, 155 (1991).
[34] C.M. Mikulski, R. Minutella, N. De Franco, G. Borges Jr., A. Renn, N.M. Karayannis. *J. Coord. Chem.*, **20**, 39 (1989).
[35] L. Alderighi, S. Dominguez, P. Gans, S. Midollini, A. Sabatini, A. Vacca. *J. Coord. Chem.*, **62**, 14 (2009).
[36] M. Strasak, Z. Durcova. *J. Coord. Chem.*, **22**, 99 (1990).
[37] M.S. Mohan, M.M.T. Khan. *J. Coord. Chem.*, **8**, 207 (1979).
[38] R.B. Jarzebowska, L. Lomozik. *J. Coord. Chem.*, **60**, 2567 (2007).

[39] A.A. Al-Najjar, M.M.A. Mohamed, M.M. Shoukry. *J. Coord. Chem.*, **59**, 193 (2006).
[40] M.M.A. Mohamed, M.R. Shehata, M.M. Shoukry. *J. Coord. Chem.*, **53**, 125 (2001).
[41] Q. Li, P. Yang, E. Hua, C. Tian. *J. Coord. Chem.*, **40**, 227 (1996).
[42] B.P. Operschall, E.M. Bianchi, R. Griesser, H. Sigel. *J. Coord. Chem.*, **62**, 23 (2009).
[43] G. Arrambide, D. Gambino. *J. Coord. Chem.*, **62**, 63 (2009).
[44] Y.Z. Hamada, H. Holyfield, K. Rosli, T. Burkey. *J. Coord. Chem.*, **62**, 721 (2009).