

Transition metal-saccharide chemistry: Synthesis and characterization of some monosaccharide complexes[†]

S P KAIWAR, R P BANDWAR, M S S RAGHAVAN and C P RAO*

Bioinorganic Laboratory, Department of Chemistry, Indian Institute of Technology, Powai, Bombay 400 076, India

Abstract. Synthesis and characterization of mononuclear complexes of Mn(II), Co(II), Ni(II), Cu(II) and Zn(II) with D-fructose and dinuclear complexes of Cr(III) with D-glucose, D-fructose, and L-sorbose are reported. Cyclic voltammetric studies of these complexes suggest their robustness over a wide pH range. Cr(III)-saccharide complexes are found to affect the structure of pSV2 neo DNA irreversibly.

Keywords. First row transition-metal saccharide complexes; syntheses and characterization; absorption, IR, NMR, EPR spectroscopies; magnetic and electrochemical studies; Cr(III)-induced DNA cleavage.

1. Introduction

Saccharides, their oligomers, polymers and various derivatives are widely occurring compounds in the biosphere and have a variety of important roles to play. The polyhydroxy nature of these compounds makes them very interesting in coordination chemistry as ligands and in bioinorganic chemistry in association with metalloenzymes. As a consequence of this, saccharides and their derivatives regulate, to some extent, the behaviour of trace metals in environment and in biological systems. These types of saccharide interactions were first reported in the early nineteenth century when a crystalline adduct of D-glucose (Glu) with sodium chloride was described (Calloud 1825), mainly for the purpose of isolating and purifying D-glucose (Glu) and sucrose (Suc). This finding opened a new class of inorganic biochemistry of saccharides. Later on only few reports appeared in the literature till 1871 regarding these interactions. Rendleman (1966) had reviewed these interactions. Angyal and Davies (1971) reported the relationship between the structure and complex-formation of neutral carbohydrates with non-transition metal cations which generated increased interest in nutritional, medicinal and other aspects of metal-saccharide complexes. Some of these include the use of carbohydrate complexation with metal ions as an analytical tool in separation methods such as chromatography and electrophoresis. Many anti-cancer reagents which have been widely used clinically have saccharide residue(s), e.g. bleomycin, adriamycin and several other antibiotics.

Although it is conceivable that saccharides can form complexes with transition metals, the field of transition-metal saccharides lack detailed investigation. Whereas,

*For correspondence

[†]This paper is dedicated to Prof. C N R Rao, FRS on his 60th birthday

their chemical counterparts, i.e., the metal-alkoxides have been explored to a large extent and the corresponding studies have been the subject of various monographs (Bradley *et al* 1978; Chisholm 1983). The interactions of alkali and alkaline-earth metal ion adducts with saccharides are studied in great detail both in solution and in solid states (Tajmir-Riahi 1988, 1990). The only crystallographically characterised class of transition-metal saccharide complexes are the coordinated amino-glycosides of cobalt(III) and nickel(II) obtained from their amines upon reacting with saccharides (Yano 1988). Nevertheless, the subject has got some attention and few reviews have appeared in recent years (Burger and Nagy 1990; Geraldes and Castro 1990). In our attempts to further revive the chemistry of transition metal ions with these biologically important molecules and to obtain better insights into their interactions, we have taken up systematic and challenging tasks of developing newer methods to synthesize and characterize saccharide complexes of different essential and toxic transition elements of life, such as Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Mo and a non-transition metal, Zn. In the present paper, we report the synthesis and characterization of D-fructose complexes with Mn(II), Co(II), Ni(II), Cu(II) and Zn(II), and D-glucose (Glu), D-fructose (Fru), and L-sorbose (Sor) complexes with Cr(III). Also, the effect of Cr(III)-saccharide complexes on the structure of pSV2 neo plasmid DNA is reported.

2. Experimental

The synthetic procedures that we have developed allow us to categorise the metal ions into different groups depending upon their starting materials. The major group of divalent ions (Mn, Co, Ni, Cu and Zn) utilises the dichloro-dibromo salts with a large tetraethylammonium cation, $[\text{NEt}_4]_2[\text{MCl}_2\text{Br}_2]$. These are prepared by mixing ethanolic solutions of metal(II) dichloride and tetraethyl-ammonium bromide in a 1:2 mole ratio. In order to study chromium(III)-saccharide interactions, the reduction of chromate, Cr(VI) to Cr(III), by saccharides is employed in aqueous solutions followed by the isolation of final Cr(III)-saccharide products.

All the monosaccharides are reacted in the form of their disodium salts generated *in situ* in methanolic solutions except in the case of chromate reaction where simple saccharides are employed. The synthesis of divalent Mn, Co, Ni, Cu and Zn, and Cr(III) saccharide complexes is described here.

2.1 Preparation of Cr(III)-saccharide complexes

A typical method for the preparation of Cr(III)-L-sorbose complex is as follows: A 25 ml aqueous solution of K_2CrO_4 (1.942 g, 10 mmol) was mixed with a 75 ml solution of L-sorbose (10.81 g, 60 mmol) and the reaction mixture was stirred for 8–10 days at 40–45°C after purging with N_2 . The final Cr(III)-L-sorbose product isolated from the completed reaction (as monitored by absorption and EPR measurements; Rao and Kaiwar 1993) was purified by washing in warm MeOH thrice, followed by stirring in *n*-hexane for one day. The filtered product was washed with ether and finally dried in vacuum. Similar Cr(VI) reductions were performed with D-glucose and D-fructose. The product yields obtained after purification were 65–70% based on the chromium content. The elemental compositions are listed in table 1.

Table 1. Elemental percentages * of Cr(III)-saccharide complexes.

Complex	Formula [†]	C	H	Cr	K
Cr-Glu	$K_3[Cr_2(\mu-OH)(D-Glu)_2D-Glu-ox]_2$	28.35 (28.20)	4.23 (4.54)	10.24 (10.54)	11.52 (11.89)
Cr-Fru	$K_4[Cr_2(\mu-OH)_2(D-Fru)_2(D-Fru-ox)]_2$	27.32 (27.48)	3.99 (4.19)	9.87 (10.04)	14.80 (14.84)
Cr-Sor	$K[Cr_2(\mu-OH)(L-Sor-ox)]_2$	24.83 (24.96)	3.62 (3.71)	17.93 (18.00)	6.72 (6.98)

* Values in parentheses are those obtained experimentally; + ox - oxidised form

Table 2. Elemental percentages of metal(II)-D-fructose complexes.

Complex	Composition	C	H	N	Na	Metal
Mn-Fru	$Na[Mn(D-Fru)(OMe)Cl]$ + 0.2(Et ₄ NBr.H ₂ O)	27.96 (27.73)	4.99 (4.41)	0.76 (0.75)	6.23 (5.93)	14.89 (15.23)
Ni-Fru	$Na[Ni(D-Fru)(OMe)Cl]$ + 0.05Et ₄ NBr	26.30 (26.08)	4.44 (3.96)	0.21 (0.19)	6.61 (6.34)	17.38 (17.73)
Cu-Fru	$Na[Cu(D-Fru)(OMe)Cl]$ + 2Na[Cu(OMe)(H ₂ O) ₂ Cl] + 1.2Et ₄ NBr	23.20 (22.87)	5.40 (4.98)	1.75 (1.59)	7.17 (7.29)	19.82 (19.58)
Zn-Fru	$Na[Zn(D-Fru)(OMe)Cl].H_2O$ + 0.1Et ₄ NBr + 0.3NaCl	23.97 (24.11)	4.61 (4.24)	0.36 (0.25)	7.66 (7.19)	16.75 (16.32)

* Values in parentheses are those obtained experimentally

2.2 Preparation of D-fructose complexes of Mn(II), Co(II), Ni(II), Cu(II) and Zn(II)

A typical method for the preparation of the Ni-D-fructose complex is described here. The $[NEt_4]_2[NiCl_2Br_2]$ (1.1 g, 2 mmol) in 15 ml MeCN was added to a pre-reacted mixture of D-fructose (1.082 g, 6 mmol) and sodium (0.279 g, 12.1 mmol) in 75 ml MeOH. A pale green colour developed in the reaction mixture which gradually changed to yellow (12–15 hours) and finally to brownish yellow over a period of two days. The reaction mixture was filtered and the residue collected on a frit was washed with diethyl ether and further dried in vacuum desiccator. This was purified by stirring in MeOH for approximately 2 days, then in MeCN for 2 more days and finally in *n*-hexane for about a day, and then filtered. The purified product was then dried under vacuum. The elemental analysis of this product, Ni-Fru, corresponds to $Na[Ni(D-Fru)(OMe)Cl]$, 0.05NEt₄Br molecular mixtures. Similar reactions with other metal ions using their starting materials were performed and the products obtained in 55–60% yields after purification. The elemental compositions of these complexes are given in table 2.

3. Results and discussions

In developing new methods to study the metal-saccharide interactions, we have prepared a number of complexes of mono-saccharides with metal ions ranging from

Ti to Zn in the first row and a second row element Mo. However, so far we have characterised certain complexes in more detail (Rao *et al* 1990, 1992, 1993; Rao and Kaiwar 1991, 1992; Kaiwar and Rao 1992). This includes mononuclear complexes possessing a single saccharide unit in the coordination sphere of divalent cations of Mn, Co, Ni, Cu and Zn. On the other hand, chromate reduction reactions produced μ -hydroxo bridged dinuclear Cr(III) complexes possessing two saccharide units per metal centre of D-glucose, D-fructose, and L-sorbose complexes. All the complexes are highly soluble in water. Cr(III)-saccharide complexes, on the other hand, are soluble in MeOH, N,N-DMF, DMSO and to a lesser extent in MeCN, in the presence of 18-crown-6 ether. All the compounds need careful handling because of their hygroscopic nature.

3.1 Monosaccharide complex of Cr(III)

All the absorption spectra are characteristic of Cr(III)-oxo bound species but different from aquo and other precursor ones. The diffuse reflectance spectra agree completely with the solution spectra showing that there is no dissociation of the complex units in the solution. The solution pH variation studies in the range 0.5–12 showed only about 10–12 nm blue shift in the ν_1 band position when the medium was made acidic with pH = 0.5, indicating no hydrolysis of the Cr(III) centre in these complexes. This suggests stable complexation in the final Cr(III) products. The CD spectra in the visible region of all the complexes showed $d \rightarrow d$ bands in the same position as in the absorption spectra, the only difference being the opposite sign of rotation in Cr(III)-L-sorbose complex as expected.

The infrared spectra of all the complexes in this series exhibited identical behaviour. The carboxylate group (due to oxidation of the saccharide) stretching vibrations observed in the range 1637–1650 cm^{-1} (strong, ν_{asym}) and 1430–1490 cm^{-1} (weak, ν_{sym}) with a $\Delta\nu \sim 200 \text{ cm}^{-1}$, indicates its weak binding in the complex. The evidence for its formation can also be inferred from ^1H and ^{13}C NMR studies which are described later. The bands observed in the isomer region suggest the dominance of the β -form in the products which is also confirmed by NMR studies.

The solid and aqueous solution X-band EPR spectra of these complexes showed a very broad band near $g \approx 2$, and the data is consistent with the presence of Cr(III) in a distorted octahedral geometry of oxygen environment. The large line widths as compared to the Cr(III)-aquo species may be associated with a more definite *zfs* component arising from geometric distortion at the metal centres (Rao and Kaiwar 1992, 1993). Fe-saccharide complexes also exhibited large line widths for the band around $g \approx 2$ (Nagy *et al* 1986; Rao *et al* 1993).

The ^1H NMR spectra in D_2O are characteristically broad, like those of other paramagnetic complexes. The peak widths are increased about 20–40 times as compared to those in the free ligand spectra. Whereas an aldehydic (< 5%) and a broad carboxylic acid proton resonances are seen around 8.20 ppm and 8.50 ppm respectively in $\text{DMSO}-d_6$ (dissolved by adding 18-crown-6 ether), the 8.50 ppm resonance is masked on deuterium exchange in D_2O as expected.

^{13}C NMR spectra measured in D_2O are slightly broadened on complexation and exhibit the resonances of both α - and β -pyranoside forms of the complexed monosaccharide. Evidence for the presence of a carboxylate group (of the oxidised saccharide) is indicated by two sets of resonances; a downfield set with 2–3 lines in

the region 180–183 ppm due to isomers of C6 oxidised pyranoside and an upfield set with a single line in the range 173.6–174 ppm, indicating an oxidised moiety in the open form. Observation of 173 ppm and 180 ppm resonances of carboxylate with Ca^{2+} ion are generally explained in terms of tight asymmetric and weak symmetric metal carboxylate interactions. The spectra of all the complexes exhibit about five extra lines in the range 64–70 ppm, accountable for the presence of open oxidised saccharide in the coordination sphere.

The magnetic behaviour of these complexes shows a nonlinear relationship between χ_g and T , indicating a deviation from simple paramagnetic behaviour. A large decrease in the μ_{eff} value on decrease in T from 300 to 80 K (or 10 K), indicates antiferromagnetic coupling of two Cr(III) centres found commonly in the complexes where the Cr(III) centres are bridged by oxo or hydroxo and/or carboxylate group. The decrease is about 60% in the range 300–10 K in the case of the Cr(III)-D-Glu complex, whereas it is only 15–20% in the range 287–80 K in other complexes. The magnetic data were fitted to the theoretical relationship by a nonlinear least-squares procedure (figure 1) which yielded $J = -9.5 \text{ cm}^{-1}$ and $p = 0.185$ showing weak antiferromagnetic coupling of the two Cr(III) centres.

The cyclic voltammograms of the complexes exhibit an irreversible cathodic peak for $\text{Cr(III)} \rightarrow \text{Cr(II)}$ reduction at HMDE in aqueous solutions. The variation in the E_p^c values on going from Cr(III)-L-Sor (-1.14 V) to Cr(III)-D-Fru (-1.46 V) is consistent with the increasing overall negative charge on the complexes giving a 100 mV shift per unit charge increase. E_p^c values were also found to be varying linearly with the pH of the medium, studied in the range 2–12 (figure 2). The slopes (mV/unit pH) of the straight lines obtained for the complexes, in decreasing order, are: Cr(III)-D-Glu [33] > Cr(III)-L-Sor [26] > Cr(III)-D-Fru [12]. These values are expected to indicate the overall sensitivity of the complexes towards pH. Furthermore, none of these complexes were hydrolysed in the pH range studied, hence indicating their robustness.

The final Cr(III)-saccharide products are found to be anionic with the Cr(III) centre, exhibiting a distorted octahedral geometry, and are bound to the oxidised and

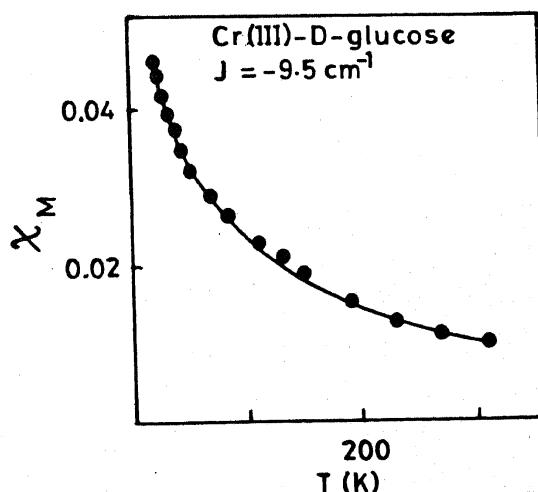


Figure 1. Plot of χ_M vs T Cr(III)-D-glucose complex. Experimental data are shown as points, full lines represent the best fit.

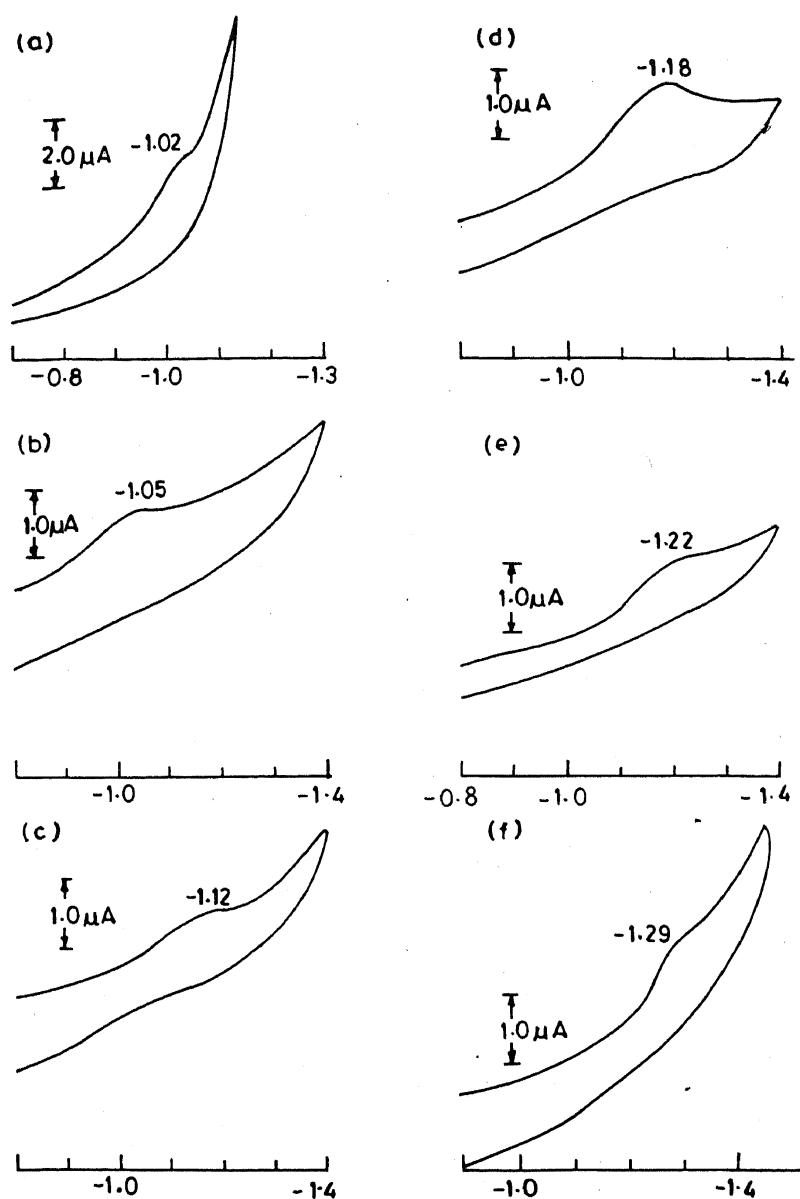


Figure 2. Cyclic voltammogram of Cr(III)-L-Sorbose complex as a function of pH at HMDE (scan speed 100 mV/s). pH = 2.0(a), 4.0(b), 6.0(c), 8.0(d), 10.0(e) and 12.0(f).

neutral saccharide units. The complexes are dimeric and the proposed structures are as follows: $K_3[Cr_2(\mu-OH)(D-Glu)_2(c-D-Glu-ox)(o-D-Glu-ox)]$ for Cr(III)-D-Glu, $K_4[Cr_2(\mu-OH)_2(D-Fru)_2(c-D-Fru-ox)(o-D-Fru-ox)]$ for Cr(III)-D-Fru, and $K[Cr_2(\mu-OH)(c-L-Sor-ox)(o-L-Sor-ox)]$ for Cr(III)-L-Sor, where the short forms *o* – refer to open, *c* – refer to cyclic and *ox* – refer to the oxidised forms of the saccharide.

3.2 Interaction of Cr(III) complexes with pSV2 neo DNA

In order to assess whether the Cr(III) complexes induce any DNA damage, pSV2 neo plasmid DNA was incubated with Cr(III)-saccharide complexes and analysed by agarose gel electrophoresis as this technique readily leads to the observable changes

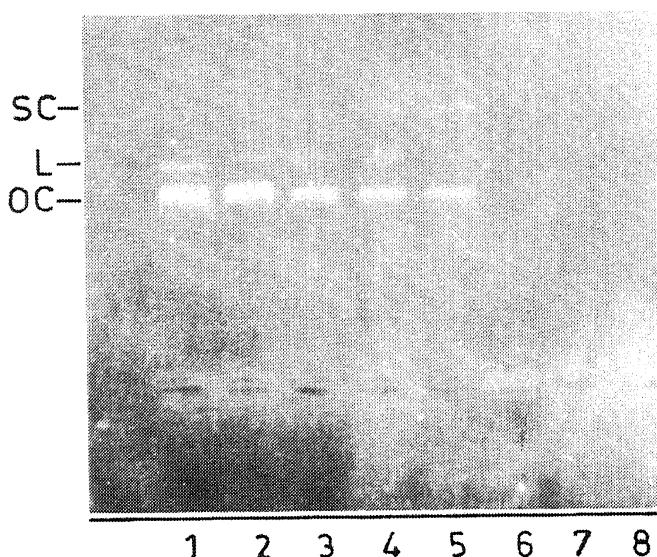


Figure 3. Effects on the electrophoretic mobility of pSV2 neo DNA induced by Cr(III)-compounds. Lanes 1-4: 180 mM, 18 mM, 1.8 mM and 0.18 mM Cr(III)-L-sorbose complex; Lane 5: DNA only; Lanes 6-8: 631 mM, 63.1 mM and 6.31 mM CrCl₃. (SC = super coiled, L = linear and OC = open circular form).

in the electrophoretic mobility of DNA. These are compared with the changes observed with CrCl₃ and other controls.

The pSV2 neo DNA that is used is predominantly in the nicked form (form II), with a small proportion of super coiled form (form I), and this is incubated with the Cr(III)-L-Sor complex. With increase in the concentration of the complex, progressive increase in the amount of the linear form is seen as judged by the intensity of this form (figure 3, lanes 1-4). Further the band corresponding to the nicked form is broadened with increase in the concentration of the complex and complete disappearance of the super coiled form is also noticed. Therefore the gel clearly indicates that in this complex while the nicked form is converted to linear, the super coiled DNA is converted to nicked form. Qualitatively similar trends have been noted with all the Cr(III)-saccharide complexes studied. Controlled experiments carried out with Cr(III)-chloride did not show any bands in the gel (figure 3, lanes 6-8).

3.3 D-fructose complexes of divalent ions of Mn, Co, Ni, Cu & Zn

The use of dichloro-dibromo salts of metal ions gives a viable starting material with four facile halides and a preformed tetrahedral metal center. The aqueous solution absorption spectrum of the Ni-Fru complex (figure 4) is different from its precursor molecules indicating the formation of complex through oxygen atoms. The diffuse reflectance spectrum agrees with the solution spectrum indicating no change in the nature of the complex in solution. The complex formation and the tetrahedral geometry of the metal centre are confirmed in all the cases (except those of Mn and Zn) after comparing appropriately with precursor molecules spectra in the visible region.

Infrared spectrum gives information on the breakage of intermolecular hydrogen bonding (3400 cm^{-1} with $3250 \pm 10\text{ cm}^{-1}$ shoulder) otherwise present in the free

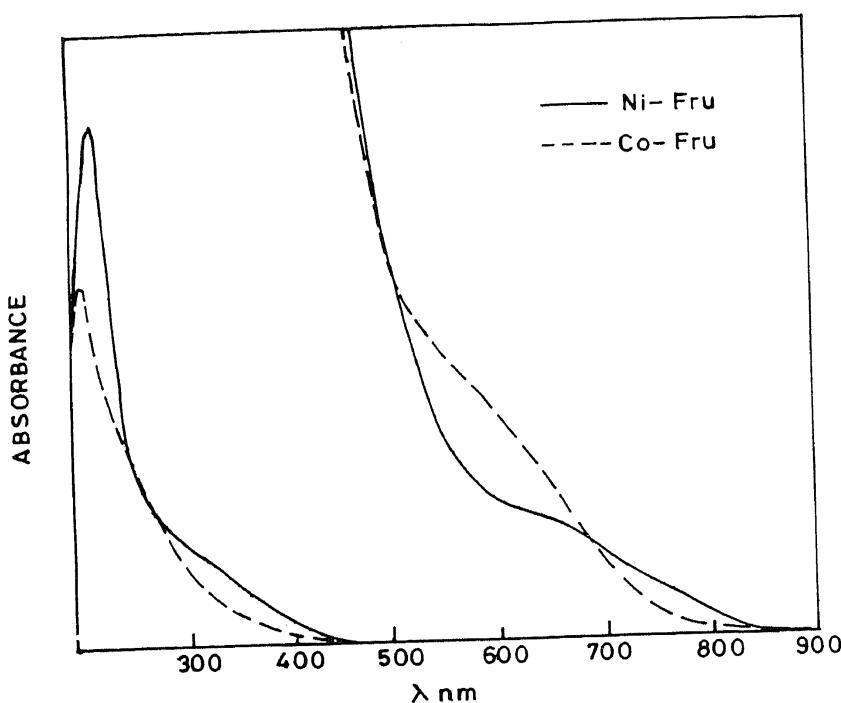


Figure 4. Absorption spectra of D-fructose complexes in H_2O . Pathlengths: 0.5 cm (190–450 nm, 5.0 cm (450–900 nm).

D-fructose ($\nu_{\text{O}-\text{H}}$: 3240–3460 cm^{-1}). This region is further overlapped by the ν_{OH} of water molecules. The presence of a strong and broad band at $1600 \pm 10 \text{ cm}^{-1}$ indicates bending vibrations of free H_2O present as moisture in these hygroscopic compounds, although a high frequency C=O stretching component of free aldehyde cannot be ruled out as it is quite conspicuous in the PMR spectrum. The structural frequencies in the fingerprint region are merged and grouped together into three broad bands around 1400, 1080 and 800 cm^{-1} respectively on complex formation making the assignments for the anomeric nature difficult. Other complexes in this series exhibit identical behaviour with broad bands in the spectra, while the spectra are very sharp in the case of non-transition metal saccharide adducts (Tajmir-Riahi 1987, 1989).

The PMR spectrum in D_2O shows the paramagnetic transition metal effect on saccharide complexation as seen from merged and broadened peaks in the range 3.5–4.5 ppm and therefore individual assignments are not possible. However the presence of trace amounts of tetraethylammoniumbromide is indicated by the ethyl groups of NEt_4^+ at 1.25 ppm (CH_3) and 3.26 ppm (CH_2), respectively, and the quantitative presence has been estimated based on nitrogen percentages (table 2). The peak at 3.38 ppm can be assigned to methoxy protons indicating its involvement in the complexes. The aldehydic proton resonance at 8.46 ppm suggests possible epimerization (< 5%) of D-fructose on interacting with the transition metal. These characteristics are common in all the complexes. In the case of the Co-D-fructose complex, further purification with hot methanol resulted in the disappearance of the aldehydic peaks.

The cyclic voltammograms of N_2 purged and blanketed solution of the Ni-Fru complex with Me_4NCl as the supporting electrolyte were recorded at 100 mV/s using

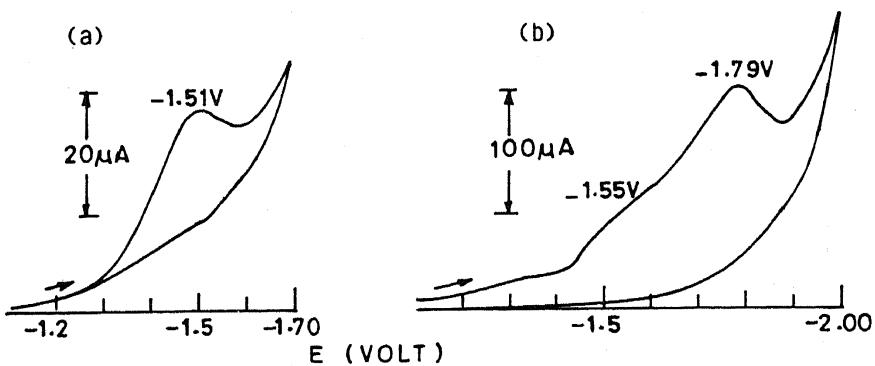


Figure 5. Cyclic voltammograms of Ni-Fru in H_2O at HMDE (scan speed 100 mV/s), $\text{pH} = 2.7$ (a) and 7.5 (b).

Table 3. E_p^c values at HMDE for metal(II)-D-fructose complexes at various pH values.

Complex	pH	E_p^c (V)	Complex	pH	E_p^c (V)
Mn-Fru	4.0	-1.52	Ni-Fru	2.7	-1.51
	6.0	-1.56		3.3	-1.60
	8.3	-1.60		7.5	-1.55, -1.79
Co-Fru	2.3	-1.47, -1.71	Zn-Fru	2.5	—, -1.50
	3.3	-1.50, -1.70		3.8	-1.03, -1.50
	7.4	—, -1.73		4.5	-1.07, -1.54
				7.0	-1.13, -1.56

a hanging mercury drop electrode (HMDE) and an Ag/AgCl reference electrode (figure 5). These studies conducted at various pH values, ranging from acidic to weakly basic conditions, exhibit cathodic reduction peaks for $\text{M}^{2+} \rightarrow \text{M}^+$ only, whose potentials E_p^c shifts to more negative values on increasing the pH (table 3). This shift can be attributed to a stronger binding of the metal with the saccharide due to deprotonation of several free hydroxyl groups. However at pH 7.5 (figure 5(ii)), the splitting of the wave occurs possibly due to the formation of some weakly bound metal complex species allowing the reduction, $\text{M}^{2+} \rightarrow \text{M}^+$ to occur at lower E_p^c values. At higher pH values, the formation of metal hydroxides made it difficult to study electrochemical behaviour. All the D-fructose complexes of these divalent metal ions showed similar behaviour. Similar studies with iron-monosaccharide complexes at platinum and glassy carbon electrodes have shown them to be stable over the pH range 2–12 (Rao *et al.* 1992, 1993).

The complexes of divalent metal ions with D-fructose characterized invariably possess the tetrahedral formula $\text{Na}[\text{M}(\text{D-Fru})(\text{OMe})\text{Cl}]$ as deduced based on various spectroscopic studies and elemental analysis.

4. Conclusions

Our extensive work on transition-metal saccharides has led to some deeper understanding of their interactions. The synthetic procedures convincingly demonstrate complex formation, which is also supported by various analytical and spectroscopic

techniques. pH variation studies clearly indicate their robustness over a wide range of pH; therefore these complexes are useful in the context of environmental detoxification of certain toxic metals. DNA cleaving studies on the Cr(III)-saccharide complexes may be useful in understanding the role played by these molecules in biological aspects of chromate reduction.

Acknowledgements

We thank Prof S Mitra, Tata Institute of Fundamental Research, Bombay and Dr P Chaudhuri, Ruhr University, Germany for magnetic measurements. Council of Scientific & Industrial Research and Department of Science & Technology, New Delhi are acknowledged for financial support and the Regional Sophisticated Instrumentation Centre, Bombay for spectral measurements. SPK thanks the CSIR for a fellowship.

References

- Angyal S J and Davies K P 1971 *Chem. Commun.* 500
Bradley D C, Mehrotra R C and Gaur D P (eds) 1978 *Metals alkoxides*
Burger K and Nagy L 1990 *Biocoord. Chem.* (ed.) K Burger, and references cited therein
Calloud F 1825 *J. Pharm.* **11** 562
Chisholm M H 1983 *ACS Symp. Ser.* **221** 243
Geraldes C F G C and Castro M M C A 1990 *NATO ASI Ser. G* **23** 105, and references cited therein
Kaiwar S P and Rao C P 1992 *Carbohydr. Res.* **237** 203
Nagy L, Burger K, Kurti J, Mostafa M A, Korecz L and Kiricsi I 1986 *Inorg. Chim. Acta* **124** 55
Rao C P, Geetha K and Bandwar R P 1992 *BioMed. Chem. Lett.* **2** 997
Rao C P, Geetha K and Raghavan M S S 1993 *Biometals* **6** (in press)
Rao C P and Kaiwar S P 1991 *Inorg. Chim. Acta* **186** 11
Rao C P and Kaiwar S P 1992 *Carbohydr. Res.* **237** 195
Rao C P and Kaiwar S P 1993 *Carbohydr. Res.* **244** 15
Rao C P, Sarkar P S, Kaiwar S P and Vasudevan S 1990 *Proc. Indian Acad. Sci. (Chem. Sci.)* **102** 219
Rendleman J A Jr 1966 *Adv. Carbohydr. Chem.* **21** 209
Tajmir-Riahi H A 1987 *Inorg. Chem. Acta* **135** 67
Tajmir-Riahi H A 1988 *Carbohydr. Res.* **183** 35
Tajmir-Riahi H A 1989 *Carbohydr. Res.* **190** 29
Tajmir-Riahi H A 1990 *J. Inorg. Biochem.* **39** 33
Yano S 1988 *Coord. Chem. Rev.* **92** 113, and references cited therein