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SOLUTION STABILITY OF IRON-SACCHARIDE COMPLEXES

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Abstract: Several soluble and low molecular weight complexes of mono- and disaccharides with iron were synthesized and found to be stable over a wide range of pH as studied through absorption spectroscopy and electrochemistry. These complexes are expected to act as better candidates for dietary supplementation of nutrient iron.

Introduction

Iron is an essential nutrient for all organisms including humans and must be regulated at certain levels by the organism to carryout its normal physiological functions[1]. This nutrient is generally balanced in humans through diet and administration of appropriate supplements. Various dietary iron supplements available in the market suffer from their poor solubility and absorbability (about 5-15%). The difficulties involved in evaluating the bioutilization of $Fe(OH)_3$ - dextrin complex and other related aspects are discussed in the literature[2]. On the other hand, the parenteral supplements have often exhibited adverse side effects due to metal accumulation. From several nutritional studies it has been understood that the solubility, absorbability and bioavailability of iron is increased when this is supplemented alongwith carbohydrate diets, diets containing ascorbic acid and also milk[3]. Saltman and coworkers have postulated the existence of iron-sugar complexes at neutral pH values and also demonstrated the involvement of these in the transport of iron across biological membranes. Barker and coworkers have reported the redissolvable forms of these complexes[4]. Nutritional utilization of iron-ascorbate and other chelates of iron with phosphoproteins, citric acid and lactobionic acid has been addressed in the literature[5]. All this is meaningful if one envisages the formation of stable complexes between iron and saccharides. In order that an iron compound be a better dietary supplement, it should exhibit better water solubility and stability over a wide range of pH and good transportability. In view of this, we have synthesised several soluble and low molecular weight iron complexes with mono- and disaccharides and studied their stabilities over a wide range of pH using absorption spectroscopy and electrochemistry. As the transition-metal saccharide complexes are interesting in their bonding nature and are expected to play influential roles in catalysis, biology, and medicine, we have recently synthesized these complexes with several first-row transition-metal ions[6].

Experimental

Synthesis: Iron-saccharide compounds reported in this paper were synthesised from methanol solutions using stoichiometric ratios of sodium salts of saccharides (unlike earlier methods in the literature where large excess of saccharides was used in strongly aqueous basic solutions[4]). While sodium salts of monosaccharides were synthesised from 1:2 ratio of saccharide to sodium metal, corresponding sodium salts of disaccharides were synthesised from 1:4 ratio in methanol. To the freshly prepared sodium salts of saccharide *insitu*, was added methanolic solution of anhydrous ferric chloride. The stoichiometric ratio of metal to sodium salts of saccharide employed was 1:3 for monosaccharides and 1:2 for disaccharide cases. A typical procedure for the synthesis of ferric-D-fructose complex is given below.

In about 50 ml of dried and freshly distilled MeOH, D-fructose (2.7 g., 15 mmol) was suspended and to this was added small pieces of metallic sodium (0.69 g., 30 mmol) and stirred until all the sodium was reacted to form disodium salt of fructose. To this salt , anhydrous ferric chloride (0.811 g., 5 mmol) dissolved in 25 ml of MeOH was added with constant stirring. A brownish-yellow precipitate was formed and the mixture was allowed to stir for 4-5 hours for completion of the reaction. The solid was obtained by filtering through frit funnel, washed with MeOH and diethyl ether and dried under vacuum. The solid thus obtained was purified first by dissolving in water-methanol mixture (2:1 v/v) and reprecipitating with absolute ethanol and repeating the same for two times. The solid was further purified by stirring the compound in MeOH, followd by *n*-hexane and filtering the fractions to remove any soluble impurities. This was also performed twice. While methanol fractions showed some colour due to the partial solubility of the compound, upon evaporation, white crystalline saccharide material was also found. A similar procedure was employed in making complexes with other monosaccharides (D-glucose, D-galactose, and L-sorbose) and disaccharides (D-sucrose, D-maltose, and D-lactose).

Spectroscopy: Absorption spectra of iron-saccharide complexes were measured in the pH range 2-11, where the pH of the solution was adjusted by adding appropriate amounts of either HCl or NaOH solutions and were compared with those of ferric chloride. These complexes exhibited similar behaviour immaterial of whether the pH was adjusted from basic to acidic or *vice versa*.

Electrochemistry: The electrochemical behaviour of ferric-D-glucose, ferric-D-maltose and ferric chloride was studied at various pH values using cyclic voltammetry at platinum and glassy carbon electrodes with 0.1M KCl as supporting electrolyte and Ag/AgCl as reference electrode. The solutions were purged with nitrogen gas for 15-30 minutes immediately before the experiment. All the voltammograms were compared with their respective backgrounds.

Results and Discussion

All the iron-saccharide compounds reported in this paper are highly soluble in water and to some extent in MeOH. However, upon the addition of 18-crown-6 ether, the solubility increases even in less polar solvents, *viz.*, DMF, DMSO, and MeOH due to the favourable thermodynamic aspects played as a result of crowning the sodium ions which are actually involved in balancing the anionic charge of the complexes. Compounds prepared by the methods discussed in this paper yields low molecular weight, soluble complexes as deduced from their aqueous solution conductivities, *epr* and magnetic susceptibility studies. For glucose complex, these values are Λ = 140 $ohm^{-1} mol^{-1} m^2$, g = 2.027 & 4.152 and μ_{eff} = 4.85 BM . On the other hand, the compounds prepared from strongly aqueous alkaline media and with large excess of saccharides as reported in the literature[4] often yielded polymeric materials which may be envisaged through the involvement of aquo/hydroxo/oxo groups in bridging the metal species.

As some of the saccharides used in this study are of reducing in nature, one would expect the formation of Fe(II) species to certain extent. As a matter of fact, we found about <2% of Fe(II) species in case of D-fructose, D-galactose, and D-maltose. On the other hand, similar preparations with mono- and disodium ascorbates yielded about 40% of Fe(II) species. Thus the methodology reported in this paper yields mainly Fe(III)-saccharide complexes. As these complexes are anionic and highly basic in aqueous solution, it may be suggested that the saccharides are primarily bound through deprotonated hydroxyl groups (O^-) to result in the formation of very stable complexes. Thus the compounds reported here are rather new though these exhibit some properties which are common with the literature ones.

Absorption spectroscopy: Iron-saccharide complexes, when dissolved in water, showed at least two broad absorption features in the ligand to metal charge transfer region of 250 to 380 nm and two other very weak, and broad d--->d transitions around 460 and 560 nm respectively, highly characteristic of Fe(III) complexes. The charge transfer bands were resolved better in strongly acidic solutions exhibiting their maxima around 265 and 340 nm respectively [Fig. 1a & c]. Thus the absorption spectra were broadly unaltered over 2.5-11 pH range, except that there seem to be a narrowing of the charge transfer bands in highly acidic solutions. The

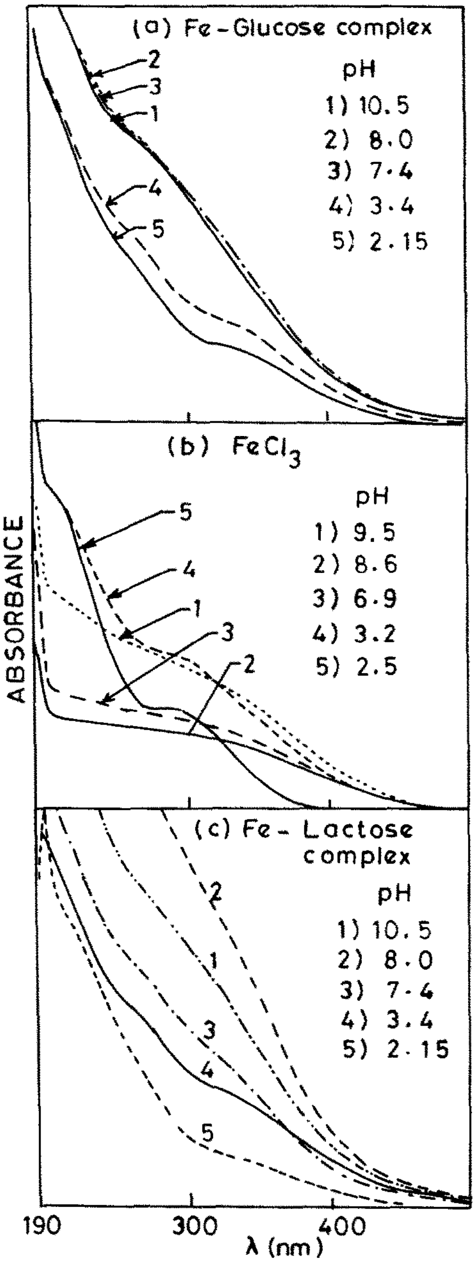

Fig. 1 Absorption spectra of Fe compounds at various pH values.

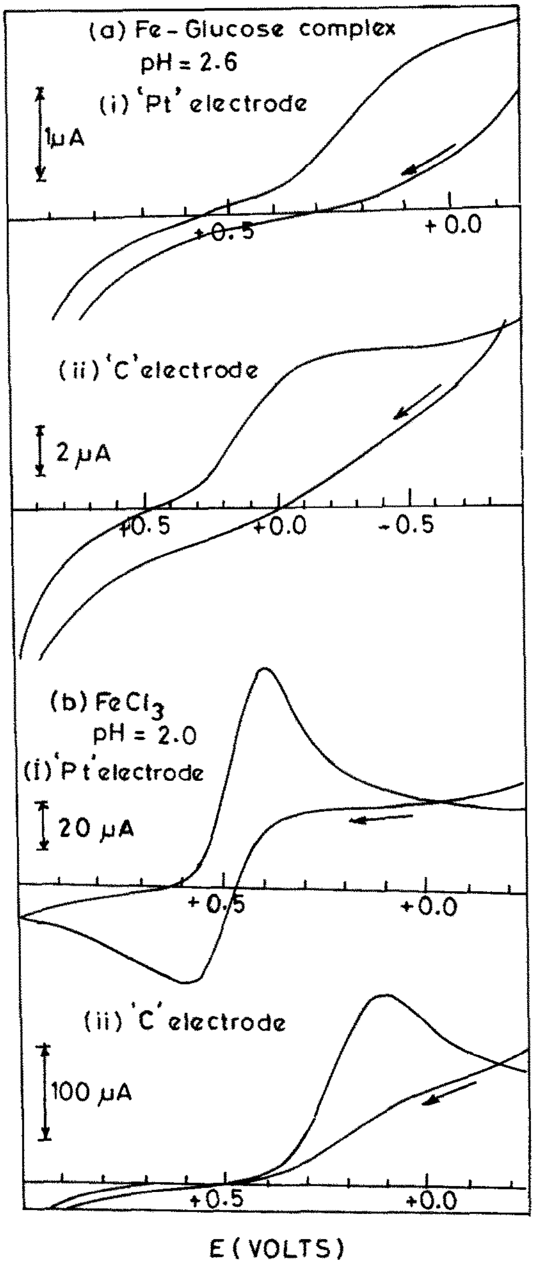

Fig. 2 Cyclic voltammograms of Fe compounds at Pt and C electrodes

situation with $FeCl_3$ is altogether different as the metal ion gets precipitated out in this pH range. The extent of precipitation is dependent upon the pH value, while it is large in the vicinity of neutral pH, there is significant solubility at other pH values[7] [Fig. 1b]. Thus the solution behaviour was found to be same both for mono- and disaccharide complexes of iron. While the ferric chloride solutions were precipitated out beyond pH 2.5, the saccharide complexes stayed in solution for at least several weeks without any significant degradation, an important indication for the stability of the latter ones.

Electrochemistry: At Pt electrode, while ferric chloride showed a reversible voltammogram [E_{pa} = 0.59V, E_{pc} = 0.40V], the Fe-D-glucose complex exhibited only one broad cathodic peak with its maximum lying in the range +0.05V to -0.05V in strongly acidic solutions (pH < 3) [Fig. 2. b(i) & a(i)]. As the pH of the solution was gradually increased, the Fe^{3+} from ferric chloride started precipitating out beyond pH 2.5 while the complex was found to be stable to a very high pH (>11.0). In this process, the cathodic reduction of Fe^{3+}---> Fe^{2+}shifted to more negative potentials and became unobservable at high pH values in the potential range studied for this complex. Similar behaviour in Fe^{3+}---> Fe^{2+} potentials was reported in the literature regarding iron-ascorbate complexes[5a]. Our studies indicate that in highly basic solutions the overall charge on the complex becomes more negative due to the deprotonation of several free OH groups, as a result of which the Fe^{3+} binds more strongly to the saccharide and hence becomes less susceptible for reduction. This was further supported by the fact that the Fe^{3+} is not precipitated in solution even at high pH.

Similar irreversible behaviour has also been observed at glassy carbon elctrode, where the cathodic reduction peaks were obtained for both $FeCl_3$ (+0.11V) and Fe-D-glucose complex (-0.10V to -0.30V) [Fig. 2 b(ii) & a(ii)]. These potentials are more negative than those observed at Pt electrode as expected based on the catalytic properties of these electrode surfaces. A behaviour similar to Fe-D-glucose complex was observed in case of disaccharide complex, Fe-D-maltose complex. The electrochemical behaviour of iron-saccharide complexes is entirely different from that of simple saccharides[8].

Conclusions

The present study clearly indicates that these low molecular weight, water soluble iron-saccharide complexes are very stable both in acidic and basic conditions and do not give insoluble precipitates at high pH values even over several weeks. This behaviour is distinctly different from that observed with ferric chloride. The demonstrated stability and solubility can make these complexes as potential dietary supplements of iron with better absorbability and bioavailability. However, further biological experiments will provide better insight into the latter aspects.

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References

1. Forth, W.; Rummel, W. *Physiol. Rev.* **1973**, 53, 724; Angyal, S.J. *Tetrahedron* **1974**, 30, 1965; Bothwell, T.H.; Charlton, R.W.; Cook, J.D.; Finch, C.A. *Iron Metabolism in Man*; Black Scientific Publications: Oxford, **1979**.

2. Forth, W.; Schneider, W. *Med. Klin.* *(Munich)* **1985**, 80, 697 [Chem. Abstr., Vol. 104, No. 108272w].

3. Hallberg, L. *Ann. Rev. Nutr.* **1981**, 1, 123; Kies. C. (ed.), *Nutritional Bioavailability of Iron, ACS Symposium Series* 203, Amer. Chem. Soc.:Washington, D.C., **1982**; Lynch, S.R.; Cook, J.D. *Annals of the New York Academy of Science* **1980**, 355, 32; Morck, T.A.; Cook, J.D. *Cerial Foods World* **1981**, 26, 667; Nadeau, D.B; Clydesdale, F.M. *J. Food Sci.* **1991**, 56, 145.

4. Charley, P.J.; Sarkar, B.; Stitt, C.F.; Saltman, P. *Biochem. Biophys. Acta* **1963**, 69, 313; Saltman, P. *J. Chem. Edn.* **1965**, 42, 682; Barker, S.J.; Somers, P.J.; Stevenson, J. *Carbohydr. Res.* **1974**, 36, 331.

5. Gorman, J.E.; Clydesdale, F.M. *J. Food Sci.* **1983**, 48, 1217; Galdi, M.; Valencia, M.E. *J. Food. Sci.* **1988**, 53, 1844.

6. Rao, C.P.; Sarkar, P.S.; Kaiwar, S.P.; Vasudevan, S. *Proc. Ind. Acad. Sci. (Chem. Sci.).* **1990**, 102, 219; Rao, C.P.; Kaiwar, S.P. *Inorg. Chim. Acta* **1991**, 186, 11; Kaiwar, S.P.; Rao, C.P. Carbohyd. Res. **1992**; Rao, C.P.; Kaiwar, S.P., Carbohyd. Res. **1992**.

7. Bard, A.J.; Parsons, R.; Jordan, J. (eds.), *Standard potentials in aqueous solutions*, Marcel Dekker (N.Y.), **1985**, Chap. 14, p391.

8. Larew, L.A.; Johnson, D.C.; *J. Electroanal. Chem.* **1989**, 262, 167; Wolfson Jr., S.K.; Chan, L.T.; Krupper, M.A.; Yao, S.J. *Biomed. Biochim. Acta* **1989**, 48, 919.