Thermodynamic Considerations in Co-ordination. Part XIX.[1] *In vitro* Studies of Complexing Equilibria involved in Oral Iron(II) Therapy

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Formation constants are reported for adding protons and iron(II) ions to ligands currently in use as oral iron preparations (ascorbate, fumarate, and succinate). These constants have been used in computer models of solution equilibria in the small intestine in order to establish that iron(II)-absorption-promoting ligands preferably (*i*) have charge 2−, (*ii*) form uncharged 1 : 1 complexes, and (*iii*) might be low-molecular-weight polymers. These factors suggest a series of additional ligands that conceivably could possess improved absorption-promotion characteristics. Formation constants for these ligand–iron(II) complexes have been determined and the concentration distribution of these complexes over a range of physiological pH values computed, the most promising ligands being galacturonate and malate.

HUMAN beings contain *ca.* 5 g of iron and this quantity remains constant throughout adult life, reflecting a fine balance between that excreted and absorbed. Imbalances can result in iron-deficiency diseases or in hemosiderosis.[2,3] The former condition is usually treated by oral administration of salts of iron(II), these being partly absorbed in the duodenum and jejunum.[4] The extent of this small intestinal absorption is influenced by diet, efficiency of the columnar epithelial absorption–excretion mechanism, and anions associated with the iron(II) ion in the administered dose.

Unfortunately, oral administration of iron(II) salts

[1] Part XVIII, M. D. Walker and D. R. Williams, *J.C.S. Dalton*, 1974, 1186.
[2] M. E. Conrad and W. H. Crosby, *Blood*, 1963, **22**, 406.
[3] S. Granick, *Ann. New York Acad. Med.*, 1954, **30**, 81.
[4] G. W. Bates and P. Saltman, 'An Introduction to Bioinorganic Chemistry,' ed. D. R. Williams, in preparation.

has several disadvantages: (*i*) iron(II) ions are only 5—10% absorbed in normal human beings (iron-deficient patients can absorb up to 29%);[2] (*ii*) unabsorbed iron causes gastrointestinal distress (28% of patients on iron therapy report side effects);[5] and (*iii*) deaths from overdoses of iron(II) sulphate occur occasionally. Thus the upper limit to iron(II) sulphate therapy is the extent of gastrointestinal irritation that the patient can tolerate. A wide variety of ligands (mainly carboxylic acids and carbohydrates) has been used in place of sulphate either to increase the proportion of iron(II) absorbed in the small intestine or to decrease the extent of gastrointestinal irritation (both concepts having the effect of increasing the maximum concentration of oral-iron therapy possible).[6,7]

All these preparations are administered orally as *solids* and this study reports *solution* equilibrium studies on the more promising systems (*i*) to express intestinal iron–ligand equilibria in terms of formation constants, β, and (*ii*) to calculate distribution curves of iron(II) between the range of complexes present in order to seek correlations between the incidence of certain complexes and maximum intestinal absorption or minimal side effects, thus suggesting other absorption-promoting ligands which may have clinical advantages. Most of the ligands prescribed as ethical erythropoietic preparations of iron in the United Kingdom[6] were examined. Protonation pK values and formation constants for their iron(II) complexes were obtained from the literature[8] or, when in doubt, measured at 37 °C and I = 150mM-$NaClO_4$.

EXPERIMENTAL

The following acids were used: L-ascorbic (B.D.H., AnalaR), m.p. 191—193 °C (decomp.) [lit. 192 °C (decomp.)] (Found: C, 41·3; H, 4·6. Calc. for $C_6H_8O_6$: C, 40·9; H, 4·55%); fumaric (B.D.H.), m.p. 297—299 °C (lit. 300·2 °C) (Found: C, 41·45; H, 3·35. Calc. for $C_4H_4O_4$: C, 41·4; H, 3·45%); galacturonic (B.D.H., citrus origin), m.p. 156—157 °C [lit. 156—160 °C (decomp.)] (Found: C, 34·15; H, 5·80. Calc. for $C_6H_{12}O_8$: C, 33·95; H, 5·70%); and succinic (B.D.H., AnalaR), m.p. 187·5 °C (lit. 188 °C) (Found: C, 40·8; H, 5·20. Calc. for $C_4H_6O_4$: C, 40·7; H, 5·10%). Other reagents and potentiometric techniques were as described in refs. 9 and 10. Formation constants were used in the COMPLOT program [11,12] to compute the distribution of complexes present throughout the pH range.

RESULTS AND DISCUSSION

Values for logarithms of formation constants for protonating the ligands and for forming iron(II)–ligand complexes are given in Tables 1 and 2; β_{110} values for folate and malonate are omitted primarily because earlier researchers were unable to determine them and so we assume that such species, even if they do exist, constitute insignificant amounts of the iron(II) ion complexed at small intestinal pH values. Some of the formation constants in the Tables refer to conditions other than 37 °C and I = 150mM-(Na)ClO_4 and this introduces a marginal incompatibility into our models. However, the absorption-promotion observations and estimations of side effects are taken from reports by different laboratories using different test systems, and so the β uncertainties are dwarfed by incompatibilities in whole-body counting of radioactive iron in human beings and rats,[2,5] blood-sample counting of radioactive iron in human beings,[5] estimations of oral

TABLE 1

Ligands studied as iron(II) complexes *in vivo*

Ligand	Ascorbate	Citrate [a]	Folate [a]	Fumarate	Gluconate [a]	Succinate
Gastrointestinal absorption promotion	+			+ (?)		+
Side effects w.r.t. $FeSO_4$	increased			decreased	decreased	decreased
log β_{pqr} [b]						
101	10·35 ± 0·05	5·62	8·26	4·39	3·86	5·19 ± 0·01
102	14·31 ± 0·08	9·96		7·41		9·13 ± 0·01
103		12·90				
110	7·09 ± 0·01	4·4		2·78 ± 0·04	*c*	1·42 ± 0·29
210			7·9	4·99		2·92
310				7·19 ± 0·03		
Concn. of species at pH 7 (mM)						
Fe^{2+}	0·13	0·04	0·46	0·39		0·81
$[FeOH]^+$	0·49	0·14	1·70	1·46		2·99
Uncharged complexes	3·38 [d]			1·01 [d]		0·16 [d]
Total Fe^{II} complexed	3·38	3·82		2·15		0·20
Negatively charged complexes		3·82 [d]	1·84 [e]	1·14 [f]		0·04 [e]

[a] Data from ref. 8. [b] Refers to the complexes $[(ligand)_p(metal\ ion)_q(proton)_r]$. [c] Data not available. [d] 1 : 1 Iron (II)–ligand. [e] 1 : 2 Iron(II)–ligand. [f] 1 : 2 and 1 : 3 Iron(II)–ligand.

The following acids were used: L-ascorbic (B.D.H., AnalaR), m.p. 191—193 °C (decomp.) [lit. 192 °C (decomp.)]

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[12] D. D. Perrin and I. G. Sayce, *Talanta*, 1967, **14**, 833.

toxicities in mice and rats, emetic activity in cats, irritant effects on gastric mucosa in rabbits, and hematinic activities in rats.[7]

β Values for each of the ligands whose iron absorption-promotion effects had been investigated *in vivo* {and that of $[FeOH]^+$ (ref. 8)} were used in a COMPLOT [11] model computation of conditions in the intestine at various pH values (total Fe^{2+} and ligand concentrations = 4·00 and 8·00mM, as used in most of the *in vivo* experiments). Examples of the plotter output are shown in Figure 1. Patterns relating absorption promotion, or side effects, to these concentrations were searched for amongst $[Fe^{2+}]$, $[FeOH^+]$, molarities of uncharged iron(II)–ligand complexes, negatively charged iron(II)–ligand complexes, and the total iron(II) complexed. In addition, correlations involving pH values of the peak concentrations of the mono-, bis-, and tris-complexes, molecular weights of the most prevalent species, and $[Fe^{2+}]/[FeOH^+]$ intersection pH values were all researched (see Table 1).

The most important conclusion of this study is that all three absorption-promoting systems [iron(II) ascorbate, fumarate, and succinate] have 1 : 1 neutral complexes present in fairly high concentrations at pH 7 (see Figure 2). Although these were not the major species present at this pH, the concentrations of each of the complexes are buffered (by the presence of the other complexes and the ligand–proton system) and so passage of the 1 : 1 iron(II)–ligand complex into the intestinal lining would merely require the buffering capacity of the intestinal fluid to replenish the concentration of the 1 : 1 complex at the expense of the 2 : 1 complex.

TABLE 2

Ligands studied as iron(II) complexes *in vitro*

Ligand	Aspartate [a]	Galacturonate	Glutamate [a]	Malate [a]	Malonate [a]	Oxalate [a]	Salicylate [a]	Tartrate [a]
log β_{pqr} [b]								
101	9·46	11·42 ± 0·04	9·41	5·11	5·34	2·59	13·0	4·37
102	13·14	14·65 ± 0·08	13·48	8·55	8·19	3·92	15·81	7·39
103			15·78					
110	4·34	9·7 ± 0·6	3·52	2·68	*c*	3·05	6·55	2·24
210	8·5	18·3 ± 0·6			2·22	5·15	11·25	7·09
Concn. of unchanged complexes at pH 7 (mM)	0·40	0·48	0·08	1·57	*c*	1·60	0·02	0·04

[a] Data from ref. 8. [b] Refers to the complexes $[(ligand)_p(metal\ ion)_q(proton)_r]$. [c] Data not available.

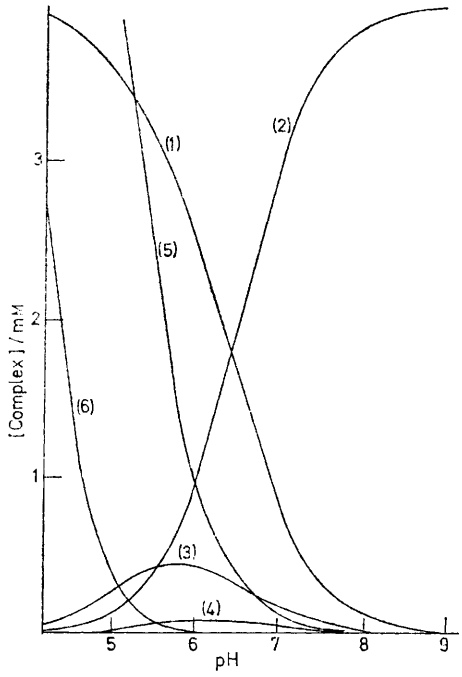

FIGURE 1 Complex concentration as a function of pH in the iron(II)–succinate system: (1), Fe^{2+}; (2), $[FeOH]^+$; (3), [Fe(succinate)]; (4), $[Fe(succinate)_2]^{2-}$; (5), Hsuccinate$^-$; and (6), H_2succinate

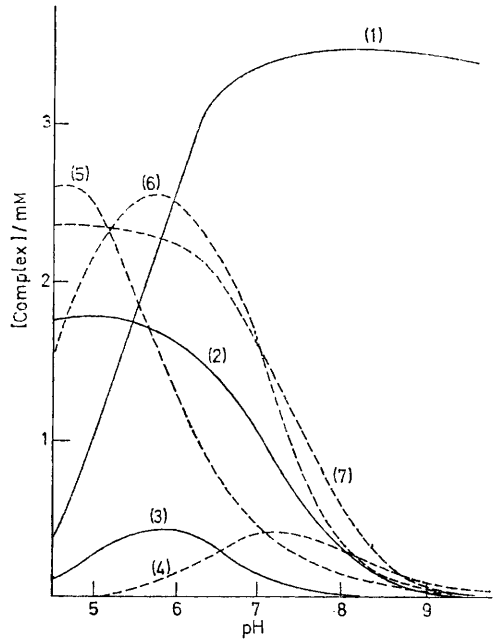

FIGURE 2 Concentrations of 1 : 1 neutral iron(II) complexes in systems whose absorption-promotion characteristics have (——), and have not (— — —), been investigated *in vivo*: (1), ascorbate; (2), fumarate; (3), succinate; (4), aspartate; (5), galacturonate; (6), malate; and (7), oxalate

These computer-model studies suggest the following factors for improving oral-iron preparations. Iron(II) complexes of other 2— carboxylic acid anions might be

tested in animal experiments. Table 2 lists β values obtained from the literature [8] for a range of such ligands, computer models suggesting that aspartate, galacturonate, and malate form the desired high concentration of neutral 1 : 1 complex at pH 7. (The [iron(II) oxalate] figure is also high but need not involve animal tests because of the toxicity of the parent acid.[13]) Lakatos *et al.* have suggested using polymers in the form of humic acids to increase metal absorption.[14] Terato *et al.* have promoted iron absorption using polymers having a molecular weight of less than 10 000.[15] Provided that the iron(II)–polymer complex satisfies the uncharged complex at pH 7 criterion we envisage such polymer systems having additional advantages in that polymers tend to become adsorbed on to the intestinal wall (thus encouraging the first stage of their absorption), and, further, as compared to 1 : 1 iron(II)–ligand complexes in Tables 1 and 2 in which four of the metal-ion bonding positions are aquated (and so vulnerable to hydrolysis), iron(II)–polymer complexes are expected to be less aquated and consequently more resistant to hydrolysis at pH 7. A naturally occurring polymer that appears to satisfy these criteria is pectic acid (polygalacturonic acid, $M \simeq 3\,000$). It was encouraging to find that the monomeric unit, galacturonate, complexes with iron(II) ions according to our neutral complex criterion. Clearly many more *in vivo* and *in vitro* studies are necessary to test the concepts discussed in this paragraph.

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