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ORIGINAL PAPER

MODELING THE CHEMICAL SPECIATION OF IRON RELEASED FROM COMMERCIALLY AVAILABLE ORAL IRON SUPPLEMENTS AND IRON FOOD FORTIFICANTS*

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Abstract

There are several papers presenting results of the studies on alleviating iron deficiency anemia by oral dosage of iron. Most preparations contain iron in the form of salts or chelates of Fe(II), less frequently as Fe(III) compounds. Some foods are fortified with iron by using Fe(II) and Fe(III) compounds. In our study, we aimed to focus on a frequently disregarded aspect of iron bioavailability, i.e. chemical speciation of iron in the gastrointestinal tract. Chemical speciation of iron was predicted using the chemical equilibrium model Visual MINTEQ. Fe speciation calculations were carried out for ferrous bis-glycinate, ferrous sulfate and ferric sodium ethylenediaminetetraacetate. The ionic equilibrium calculations were carried out for a wide range of pH (1÷8), from low values (pH 1÷2) so as to recreate the gastric environment, to pH 5÷8 in order to match the environment in the small intestine. Under assumed gastrointestinal conditions, Fe(II) was the only thermodynamically stable form of dissolved iron. Ferrous sulfate and ferrous bis-glycinate were characterized by very similar speciation of iron in the model gastric juice. The main form of dissolved ferrous iron were aqua-complexes of Fe(II). Poor complexing properties of the glycinate anion became apparent only in a slightly alkaline medium. The presence of phosphate anions limited full solubility of Fe(II) to acidic pH. The speciation prediction of dissolved Fe in NaFeEDTA solutions included virtually only anionic Fe(II)-EDTA complexes. Slight precipitation of Fe occurred only at pH 8. In more complex systems, where other divalent cations forming stable complexes with EDTA (e.g. Zn^{2+} and Ca^{2+}) are present, competitive complexation reactions may lead to an essential change in Fe speciation in neutral and weakly alkaline solutions. The deficit of EDTA available for Fe(II) resulted in precipitation of sparingly soluble Fe compounds.

Keywords: dietary supplements, iron, chemical speciation, chemical equilibrium model.

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INTRODUCTION

Iron deficiency in the human body is most commonly supplemented by the consumption of iron-fortified food products and natural iron-rich mineral water, as well as by means of orally ingested iron preparations. Dietary supplements and iron-fortified foods are also recommended as a preventative measure when iron deficiency has not occurred yet but it is likely to develop (Worwood et al. 1996, Umbreit 2005).

Most preparations contain iron in the form of salts or chelates of iron(II), less frequently as Fe(III) compounds. The commonly used compounds are ferrous sulfate, ferrous succinate, ferrous gluconate, ferrous fumarate, and the chelate ferrous bis-glycinate. In addition to simple iron supplements, complex vitamin-mineral preparations are also available. Some foods are fortified with iron by using Fe(II) and Fe(III) compounds, most commonly as NaFeEDTA (Fidler et al. 2003, Le et al. 2006, Pawlak et al. 2016).

In certain situations, ascorbic acid is added in order to enhance iron absorption (Goddard et al. 2011). Iron supplements are available in uncoated tablets, sugar-coated tablets, effervescent tablets, lozeneges, hard capsules and soft capsules. Additionally, there are also "controlled release tablets" and "sustained release tablets" available on the market. Generally, controlled release formulations are characterized by a lower incidence of gastrointestinal side-effects in comparison to conventional ferrous salt preparations (Santiago 2012). For similar reasons, carbonyl iron, which is a pure form of elemental iron, is gaining popularity among patients with intolerance to orally ingested iron (Pyarelal 2015), who can opt for parenteral preparations that can be administered intravenously or by intramuscular injections (iron sucrose, ferric carboxymaltose and iron(III) hydroxide dextran) (Goddard et al. 2011).

The release of iron that has been orally administered occurs in the gastric juice. Iron is also liberated from food during its digestion in the stomach. The released iron is then available for absorption, which occurs predominantly in the duodenal segment of the small intestine, in the intestinal fluid (Zariwala et al. 2013). Both the physical form of the preparation and the chemical form of iron (iron speciation) have influence on the iron uptake and absorption from oral dosage forms (Zariwala et al. 2013). It is generally accepted that only soluble iron can be absorbed, hence it is enough to use a soluble salt of the ferrous form, while in the case of a ferric salt, a sufficient concentration of a complexing agent must be present to keep iron(III) in the soluble phase (Wienk et al. 1999). However, more detailed research information on possible iron species in gastric and intestinal media is difficult to find in literature.

Research on the efficacy of various iron formulations seems to indicate that the knowledge of chemical iron speciation should facilitate following the fate of iron in the gastrointestinal tract as well as contribute to our better

understanding of differences in the absorbability of iron introduced with various iron compounds. The aim of this study was to predict speciation of iron commonly introduced by means of a ferrous sulfate salt, a ferrous bis-glycinate complex and a ferric sodium ethylenediaminetetraacetate. To our knowledge, such an approach has not been presented previously, although the usefulness of modelling ionic equilibria for the description of properties of real aqueous solutions has been discussed by several Polish researchers (Grzybkowski 2006, Baranowski 2007, Świetlik, Malik 2012).

METHODS

The chemical speciation of iron was modelled using Visual Minteq ver. 3.1 software (Gustafsson 2016). The aqueous phase equilibrium composition was obtained by using a simultaneous solution of the nonlinear mass action expressions and linear mass balance relationships. The equilibrium problem was solved (the convergence criterion) when the difference between the input concentration of an individual component and its calculated total concentration (the sum of the concentrations of its species) did not exceed 0.01%.

Fe speciation calculations were carried out for ferrous bis-glycinate, ferrous sulfate and ferric sodium ethylenediaminetetraacetate solutions. The former compound is the most popular chelate used to supplement iron deficiency, while the other one is the most common iron salt used for oral administration, and is also frequently treated as a reference in *in vivo* studies. The ferric compound is often used to fortify food products and as a component of food supplement tablets.

The concentrations of iron(II) = 0.5 mmol dm^{-3}, iron(III) = 0.5 mmol dm^{-3}, sulfate = 0.5 mmol dm^{-3}, EDTA = 0.5 mmol dm^{-3} and glycine = 1.0 mmol dm^{-3} were used in the calculations. In view of the written reports on the negative effects of phosphates in iron(II) absorption (Jackowska et al. 2015), the calculations were also carried out for systems that additionally contained 1 mmol dm^{-3} of phosphate.

The complexing agent of Fe(III) is EDTA, which is a ligand forming stable chelates with practically all metal cations. Therefore, a prediction of the effect of competitive reactions of binding EDTA by bivalent cations on Fe speciation was presented. An upper level of concentrations of $[Ca^{2+}]$ = 1 mmol dm^{-3}, $[Mg^{2+}]$ = 0.5 mmol dm^{-3} and $[Zn^{2+}]$ = 0.02 mmol dm^{-3} in the gastric acid was taken into account (Powell et al. 1992).

All the ionic equilibrium calculations were carried out for a wide range of pH (1÷8), from low values (pH 1÷2) so as to recreate the gastric environment to pH 5÷8 in order to match the environment in the small intestine. In order to obtain a better correlation of speciation analysis results with *in vitro* test results, ionic equilibrium calculations were carried out for pH = 1.2

characteristic for 0.1 M HCl which is used to simulate gastric conditions, and for pH 5.8 which is a characteristic of a phosphate buffer used to simulate intestinal conditions (Zariwala et al. 2013). The temperature assumed in the calculations was t = 37°C. Following the work of McConnell et al. (2008) the ionic strength was fixed at $\mu = 0.15$ mol dm^{-3} (mainly NaCl), and redox potential at Eh = - 100 mV (reducing conditions).

The formation constants of the iron species whose presence was considered in equilibrium calculations can be found in Table 1. Visual MINTEQ uses log K values that reflect the temperature corrections (van't Hoff equation) and activity coefficient corrections (Davies equation).

Table 1

Formation constants for aqueous iron species, t = 25°C, $\mu = 0$

Fe(II) species	Log K	Fe(III) species	Log K
$[FeCl]^{+}$	-0.2	$[FeCl]^{2+}$	1.48
$[Fe(OH)]^{+}$	-9.397	$[Fe(OH)]^{2+}$	-2.02
$[Fe(OH)_2]^{0}$	-20.494	$[Fe(OH)_2]^{+}$	-5.75
$[Fe(OH)_3]^{-}$	-30.991	$[Fe(OH)_3]^{0}$	-15
$[Fe(Gly)]^{+}$	4.31	$[Fe(OH)_4]^{-}$	-22.7
$[Fe(Gly)_2]^{0}$	8.31	$[Fe_2(OH)_2]^{4+}$	-2.894
$[Fe(Gly)_3]^{-}$	9.48	$[Fe_3(OH)_4]^{5+}$	-6.288
$[Fe(SO_4)]^{0}$	2.39	$[Fe(SO_4)]^{+}$	4.25
$[Fe(H_2PO_4)]^{+}$	22.273	$[Fe(SO_4)_2]^{-}$	5.38
$[Fe(HPO_4)]^{0}$	15.975	$[Fe(H_2PO_4)]^{2+}$	23.85
$[Fe(OH)(EDTA)]^{3-}$	6.5	$[Fe(HPO_4)]^{+}$	22.285
$[Fe(EDTA)]^{2-}$	16.01	$[Fe_2(OH)_2(EDTA)_2]^{4-}$	41.676
$[Fe(HEDTA)]^{-}$	19.05	$[Fe(OH)(EDTA)]^{2-}$	19.843
		$[Fe(EDTA)]^{-}$	27.66
		$[Fe(HEDTA)]^{0}$	29.17

RESULTS AND DISCUSSION

The results of the computational analysis of speciation were collected in Table 2. Iron species whose percentage in iron speciation was not lower than 0.1% of the dissolved iron fraction were shown. Iron(II) was the dominant form of dissolved iron under the assumed reducing conditions. The equilibrium concentration of iron(III) was by seven orders of magnitude lower in an acidic medium and by three orders of magnitude lower in a weakly alkaline medium. The predicted Fe(II) speciation in strongly acidic solution (pH 1.2) did not depend on whether Fe was introduced by a ferrous salt, a ferrous chelate or by a ferric chelate. The main form of iron(II) was a divalent iron cation $[Fe(H_2O)_6]^{2+}$ noted as Fe^{2+}, 95.4%÷98.0%. Other inorganic

Table 2

Iron speciation in the solutions modeling digestive body fluids

Species	Percentage of precipitated iron fraction and distribution of iron species in dissolved fraction [%]							
	pH 1.2	pH 2	pH 3	pH 4	pH 5	pH 5.8	pH 7	pH 8
$[Fe^{2+}]$ = 0.5 mmol dm^{-3}, [Gly] = 1 mmol dm^{-3}, $[Na^+]$ = 100 mmol dm^{-3}, $[Cl^-]$ = 100 mmol dm^{-3}								
Precipitated Fe	none	none	none	none	none	89.5[a]	100[a]	100[a]
Fe^{2+}	98.0	98.0	98.0	98.0	98.0	97.9	95.4	73.5
$[FeCl]^+$	2.0	2.0	2.0	2.0	2.0	2.0	1.9	1.5
$[Fe(Gly)]^+$						0.1	2.3	16.8
$[Fe(Gly)_2]^0$							0.1	5.1
$[FeOH]^+$							0.4	3.0
$[Fe^{2+}]$ = 0.5 mmol dm^{-3}, $[SO_4^{2-}]$ = 0.5 mmol dm^{-3}, $[Na^+]$ = 100 mmol dm^{-3}, $[Cl^-]$ = 100 mmol dm^{-3}								
Precipitated Fe	none	none	none	none	none	89.4[a]	100[a]	100[a]
Fe^{2+}	97.7	97.3	97.0	96.9	96.9	96.9	96.5	93.2
$[FeCl]^+$	2.0	1.9	1.9	1.9	1.9	1.9	1.9	1.9
$[Fe(SO_4)]^0$	0.3	0.8	1.1	1.1	1.2	1.2	1.2	1.1
$[FeOH]^+$							0.4	3.8
$[Fe^{2+}]$ = 0.5 mmol dm^{-3}, [Gly] = 1 mmol dm^{-3}, $[Na^+]$ = 100 mmol dm^{-3}, $[Cl^-]$ = 100 mmol dm^{-3}, $[PO_4^{3-}]$ = 1 mmol dm^{-3}								
Precipitated Fe	none	none	none	none	40.8[b]	89.3[b]	100[a]	100[a]
Fe^{2+}	95.7	90.1	84.8	83.8	84.9	86.0	74.5	58.9
$[FeCl]^+$	1.9	1.8	1.7	1.7	1.7	1.7	1.5	1.2
$[Fe(H_2PO_4)]^+$	2.4	8.1	13.5	14.5	12.9	9.8	4.7	0.5
$[Fe(HPO_4)]^0$					0.5	2.3	17.1	19.3
$[Fe(Gly)]^+$						0.1	1.8	13.5
$[Fe(Gly)_2]^0$							0.1	4.1
$[FeOH]^+$							0.3	2.4
$[Fe^{2+}]$ = 0.5 mmol dm^{-3}, $[SO_4^{2-}]$ = 0.5 mmol dm^{-3}, $[Na^+]$ = 100 mmol dm^{-3}, $[Cl^-]$ = 100 mmol dm^{-3}, $[PO_4^{3-}]$ = 1 mmol dm^{-3}								
Precipitated Fe	none	none	none	none	40.3[b]	93.9[b]	100[a]	100[a]
Fe^{2+}	95.4	89.4	83.9	83.0	84.0	85.2	75.3	70.9
$[FeCl]^+$	1.9	1.8	1.7	1.7	1.7	1.7	1.5	1.4
$[Fe(SO_4)]^0$	0.3	0.7	1.0	1.0	1.0	1.0	0.9	0.9
$[Fe(H_2PO_4)]^+$	2.4	8.1	13.4	14.4	12.8	9.8	4.7	0.6
$[Fe(HPO_4)]^0$					0.5	2.3	17.3	23.3
$[FeOH]^+$							0.3	2.9
$[Fe^{3+}]$ = 0.5 mmol dm^{-3}, [EDTA] = 0.5 mmol dm^{-3}, $[Na^+]$ = 100 mmol dm^{-3}, $[Cl^-]$ = 100 mmol dm^{-3}								
Precipitated Fe	none	none	none	none	none	none	none	1.2[a]
Fe^{2+}	98.0	94.4	25.2	2.7	0.3	0.1		
$[FeCl]^+$	2.0	1.9	0.5	0.1				
$[Fe(HEDTA)]^-$		3.0	22.8	4.1	0.4	0.1		
$[Fe(EDTA)]^{2-}$		0.7	51.5	93.1	99.2	99.7	98.4	86.1
$[Fe(OH)(EDTA)]^{3-}$						0.1	1.6	13.9

cont. Table 2

$[Fe^{3+}]$ = 0.5 mmol dm^{-3}, [EDTA] = 0.5 mmol dm^{-3}, $[Na^+]$ = 100 mmol dm^{-3}, $[Cl^-]$ = 100 mmol dm^{-3}, $[PO_4^{3-}]$ = 1 mmol dm^{-3}								
Precipitated Fe	none	none	none	none	none	none	none	1.2[a]
Fe^{2+}	95.7	87.0	23.1	2.5	0.3	0.1		
$[FeCl]^+$	1.9	1.7	0.5					
$[Fe(H_2PO_4)]^+$	2.4	7.9	3.9	0.5	0.1			
$[Fe(HEDTA)]^-$		2.8	22.3	4.1	0.4	0.1		
$[Fe(EDTA)]^{2-}$		0.6	50.2	92.9	99.2	99.7	98.4	86.1
$[Fe(OH)(EDTA)]^{3-}$						0.1	1.6	13.9
$[Fe^{3+}]$ = 0.5 mmol dm^{-3}, [EDTA] = 0.5 mmol dm^{-3}, $[Ca^{2+}]$ = 1 mmol dm^{-3}, $[Mg^{2+}]$ = 0.5 mmol dm^{-3}, $[Zn^{2+}]$ = 0.02 mmol dm^{-3}, $[Na^+]$ = 100 mmol dm^{-3}, $[Cl^-]$ = 100 mmol dm^{-3}								
Precipitated Fe	none	none	none	none	none	0.5[a]	79.5[a]	99.9[a]
Fe^{2+}	98.0	94.5	27.0	5.9	4.8	3.7		
$[FeCl]^+$	2.0	1.9	0.5	0.1	0.1	0.1		
$[Fe(HEDTA)]^-$		2.9	22.2	4.0	0.4	0.1		
$[Fe(EDTA)]^{2-}$		0.7	50.2	90.0	94.6	96.0	98.4	86.1
$[Fe(OH)(EDTA)]^{3-}$						0.1	1.6	13.9

a – as magnetite, *b* – as vivianite

ligands bonded small amounts of ferrous iron: $[Fe(H_2PO_4)]^+$ – 2.4%, $[FeCl]^+$ – 2.0%, and $[Fe(SO_4)]^0$ – 0.3%. The share of $[FeCl]^+$ was constant in a relatively wide range of pH (1.2÷5.8), whereas the percentage of $[Fe(SO_4)]^0$ had a slightly increasing tendency from 0.3% to 1.2%. Poor complexing properties of the glycinate anion became apparent only in a slightly alkaline medium. At pH 8 glycinate complexes: $[Fe(Gly)]^+$ and $[Fe(Gly)_2]^0$ bonded 21.9% of dissolved Fe(II), whereas inorganic complexes $[FeCl]^+$ and $[Fe(OH)]^+$ bonded only 4.5% of Fe(II). The remaining Fe(II) occurred as Fe^{2+} – 73.5%. In an aqueous solution of ferrous sulfate at pH 8 only 6.8% of dissolved Fe(II) occurred in chemical forms other than Fe^{2+}: $[Fe(SO_4)]^0$ – 1.1%, $[FeCl]^+$ – 1.9%, and $[Fe(OH)]^+$ – 3.8%. It is worth noting that for both ferrous compounds, no precipitation of sparingly soluble iron(II) species was predicted only within the pH range of 1.2÷5, while the presence of phosphate narrowed that pH range to 1.2÷4. The calculations predicted the appearance of the solid phase – *vivianite* $Fe_3(PO_4)_2 \cdot 8H_2O$ at pH 5 and 5.8. In a neutral and a slightly alkaline medium practically all Fe was precipitated as *magnetite* Fe_3O_4. The presence of phosphate also had a visible effect on speciation of dissolved Fe. In the acidic range (pH 1.2÷5.8) for the systems containing ferrous sulfate and ferrous bis-glycinate an initially increasing and then, above pH 4, a decreasing presence of $[Fe(H_2PO_4)]^+$ was predicted. An increasing share of the neutral complex $[Fe(HPO_4)]^0$ was predicted for higher values of pH (5÷8). The formation of phosphate complexes was largely competitive with aqua ions Fe^{2+}.

As expected, results of the computational analysis of Fe speciation for NaFeETDA solutions were different. Under the assumed reducing conditions, practically complete transformation of Fe(III) into Fe(II) occurred. Fe(III)

was only predicted for the solid phase (*magnetite*), which bonded a little over 1% of Fe in a slightly alkaline medium (pH 8). The source of a different speciation of dissolved Fe in this system was the presence of a strong complexing agent – EDTA. The complex Fe(II)-EDTA was not predicted only for pH 1.2. Speciation of Fe(II)-EDTA complexes was practically independent of the presence of ions disturbing the ionic equilibrium in the NaFeETDA solution. The anionic complex $[Fe(EDTA)]^{2-}$ dominated (50.2%÷99.7%) in acidic, neutral and slightly alkaline media. A percentage of the $[Fe(HEDTA)]^{-}$ was lower, max 22.8% at pH 3. At higher pH values Fe(II) was also bound by hydroxochelate $[Fe(OH)(EDTA)]^{3-}$: 1.6% in a neutral medium and 13.9% in a slightly alkaline medium. The presence of phosphate ions only caused binding of several percent of Fe(II) in a dihydrogen phosphate complex, max. 7.9% at pH 2.

In more complex systems where other divalent cations forming stable complexes with EDTA (e.g. Ca^{2+} and Mg^{2+}) are present, competitive complexation reactions may lead to an essential change in Fe(II) speciation in neutral and weakly alkaline solutions. The deficit of EDTA available for Fe(II) resulted in precipitation of a sparingly soluble iron compound - *magnetite*. At the assumed concentrations of divalent metals, Fe precipitation (79.5%) was expected in the neutral medium, whereas at pH = 8 practically all iron occurred as the solid phase (99.9%). Complementary calculations have shown that the presence of divalent cations does not affect speciation of iron introduced with ferrous bis-glycinate and ferrous sulfate.

According to the present speciation model, iron should occur almost entirely as Fe^{2+} in the gastric acid of patients who have been administered ferrous sulfate or ferrous bis-glycinate. Differences may appear in the intestinal fluid. Fe^{2+} would still be the dominant form of Fe(II) in the duodenum section, but complete precipitation of iron and thus a significant decrease in its absorption can be expected at the jejunum stage. The presence of phosphate ions may result in limiting full solubility of Fe(II) exclusively to the gastric tract. In this situation, even the predicted nearly 20% share of dissolved glycine chelates at pH 8 would not have any effect on the efficacy of iron absorption.

Under analogous conditions, Fe(III) supplemented by means of NaFeETDA undergoes reduction to Fe(II), and the resulting anionic chelates: $[Fe(HEDTA)]^{-}$, $[Fe(EDTA)]^{2-}$ and $[Fe(OH)(EDTA)]^{3-}$ stabilize dissolved Fe(II) in an almost entire physiological range of pH. This pattern of iron speciation may change in the presence of divalent cations competitively bound by EDTA. As a result, Fe(II) may undergo partial or complete precipitation in a further section of the small intestine (pH 7÷8).

Our results of speciation analysis of iron(II) correspond well with the general view that different chemical forms of iron ingested lost their differences during their passage through the gastrointestinal tract (Orozco et al. 2012). The fact that for a large part of the physiological range of pH (1.2÷5),

Fe^{2+} was the main Fe species of ferrous salts and moderately stable complexes is in good accordance with the latest reports on similar effectiveness of ferrous sulfate with ferrous bis-glycinate (Duque et al. 2014), ferrous fumarate with ferrous bis-glycinate (Patil et al. 2013) and ferrous sulfate with ferrous fumarate (Pyarelal 2015). Whereas the predicted limitation of complete solubility of Fe(II) to the pH range of (1.2÷4) caused by the presence of phosphate ions is in conformity with reports on the negative effects of phosphates in iron(II) absorption (Wienk et al. 1999).

In general, one should not expect a very good compatibility of *in vivo* test results with the results of iron speciation analysis obtained from considerably more simplified systems. In this case, prediction of iron speciation should rather be treated as an indication of stable iron-species under given conditions, e.g. at a given pH and Eh. In future, the availability of reliable data, particularly for organic components of the gastric juice and intestinal fluid (concentration, stability constant) will facilitate prediction of iron speciation also in more complex biochemical systems.

CONCLUSIONS

1. Only divalent iron is a thermodynamically stable form of dissolved iron in the gastrointestinal fluid.

2. Ferrous iron administered as a commercial formulation of salts e.g. ferrous sulfate, and moderately stable complexes e.g. ferrous bis-glycinate should occur only in the form of cationic species, mainly as aqua complexes Fe^{2+}. The ionic equilibrium model predicts that a difference in dissolved iron(II) speciation may appear in a slightly alkaline medium favouring the formation of glycinate chelates of iron(II).

3. Anionic chelate $[Fe(EDTA)]^{2-}$ is the predicted main iron species originating from dissolution of oral formulations containing ferric sodium ethylenediaminetetraacetate.

4. The presence of phosphate anions in the gastric medium may limit the occurrence of dissolved ferrous species to acidic pH, whereas competitive binding of EDTA by calcium and magnesium cations may limit the occurrence of dissolved ferrous species in neutral and slightly alkaline media.

5. Computational speciation analysis can serve as a useful tool in studying factors that may affect subsequent iron absorption, especially for comparison studies on the efficiency of orally administered iron preparations.

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