

REVIEW ARTICLE

Challenges and strategies to combat global iron deficiency by food fortification

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Abstract: Iron is an important micronutrient required for healthy living. Therefore, populations dependent primarily on plant-based diets with little or no animal flesh may become deficient in iron. It is found that ~30% of the world population suffers from iron deficiency anemia. Since iron undergoes oxidation which reduces its absorption, formulation of iron supplements and fortificants for treatment of iron deficiency has been studied extensively. Iron fortified food has been introduced in order to overcome the iron deficiency. Chelation of iron with amino acids, EDTA, fumarate, succinate, and gluconate and encapsulating iron by inclusion complexation and liposome encapsulations have improved the bioavailability of iron in the human body. Among these methods, iron delivery via complexation and -encapsulation are common due to their high efficacy and simplicity.

Keywords: iron chelation, iron deficiency, iron encapsulation, iron fortification.

INTRODUCTION

Natural food consumption has undergone a metamorphosis from fresh plucked, home grown to highly preserved and long shelf-life products. Thus, nutrient loss is a concern resulting in food supplementation and fortification. Food supplements in the forms of pills, tablets, capsules, or as liquids in syrup or suspensions are taken in addition to those obtained in the normal diet as concentrated nutrients for nutritional or physiological effect. A fortificant on the other hand, is a nutrient added over the natural level of the nutrient present in the food for the purpose of increasing its nutrient content. Thus, cereals are fortified with iron and vitamins. Iron is used as a food supplement as well as a fortificant in

various food items. Use as a fortificant requires consideration of many factors. The primary factor being sensory problems where unacceptable colour, texture, flavor, or odor may affect the final product. Iron has the likelihood of interactions with components in the food matrix which can affect its stability and make it segregate out of the food matrix (Table 1). In this context, the delivery of iron in the form of supplements and fortificants need careful preparation and processing. Here, we review the different forms of iron delivery methods available along with new developments in delivery techniques.

IMPORTANCE OF IRON AS A NUTRIENT

Iron has the capability of existing at various oxidation states from -2 to +6. However, in biological systems, iron exists mainly in two oxidation states, ferrous state (+2) and ferric state (+3) (Beard, 2001). It is a functional component in many biologically important macromolecules such as hemoglobin, myoglobin, cytochromes, oxygenases, flavoproteins, and redoxins (Vashchenko and MacGillivray, 2013) and is important for cell growth and differentiation. As iron can participate in many electron transfer reactions in the body, it plays a central role in biological processes such as respiration, DNA synthesis, and energy production. Iron can facilitate redox reactions by changing its oxidation state from reduced ferrous state to oxidized ferric state or vice versa (Mackenzie *et al.*, 2008). In addition, this ability to oscillate from one oxidation state to the other allows iron to reversibly bind ligands such as oxygen, nitrogen, and sulphur atoms (Beard, 2001).

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Table 1: Types of iron fortified foods and their iron contents.

Fortified food	Iron source/s	Added iron content	Reference
Curry powder	NaFeEDTA	25 µg Fe/g curry powder	(Ballot <i>et al.</i> , 1989)
Cheddar cheese	Ferric chloride	90 mg Fe/g cheddar cheese	(Zhang and Mahoney, 1989)
	Fe-casein complex	29 mg Fe/g cheddar cheese	
	Ferripolyphosphate-whey protein complex	36 mg Fe/g cheddar cheese	
Yogurt	Ferric chloride	10, 20, and 40 mg Fe/kg yogurt	(Hekmat and Mahon, 1997)
Milk	Ferrous sulfate	1.2 mg Fe/100 ml milk	(Pizarro <i>et al.</i> , 2015)
Sugar	NaFeEDTA	1 g Fe/kg sugar	(Viteri <i>et al.</i> , 1995)
Salt	Ferrous sulfate	1 mg Fe/g salt	(Rao, 1994)
Bread rolls	FeSO ₄ and NaFeEDTA	2.5 mg Fe/50 g wheat flour	(Hurrell <i>et al.</i> , 2000)
Infant cereal	Ferrous succinate	50 mg Fe/100 g rice cereal	(Hurrell <i>et al.</i> , 1989)
	Ferrous saccharate	50 mg Fe/100 g rice cereal	
	Ferrous fumarate	50 mg Fe/100 g rice cereal	
Rice	Ferrous sulfate	3 mg Fe/100 mg rice	(Cook and Reusser, 1983)
Fish sauce	Ferrous sulfate	310-380 mg Fe/l fish sauce	(Walczyk <i>et al.</i> , 2005)
	Ferric ammonium citrate	310-380 mg Fe/l fish sauce	
	Ferrous lactate	310-380 mg Fe/l fish sauce	

Iron containing proteins in the mammalian system carry out functions that are essential for the maintenance of the body. Since iron is an essential constituent in cytochromes that participate in ATP production, it is necessary for efficient energy production in the body. Iron present as a cofactor in heme, is the largest iron reservoir in the body (Johnson and Wessling-Resnick, 2012). Haemoglobin that contains iron is important to transport oxygen throughout the body. The porphyrin prosthetic groups that contain iron as the metal center, reversibly bind the dioxygen ligand and carry the oxygen from the lungs to the tissues. Myoglobin is required for oxygen storage in the body and for the use of oxygen in the muscles (Alaunyte *et al.*, 2014). Iron is a central component in the heme enzymes. Cytochromes A, B, and C contain heme as the active site and carry out electron transfer employing the variable oxidation states of iron. Additionally, iron facilitates transfer of

electrons to molecular oxygen at the end of the respiratory chain (Geissler and Singh, 2011). The catalases and peroxidases which facilitate breakdown of hydrogen peroxide also contain iron in them.

Most single electron transfer reactions are facilitated by iron-sulphur complexes present in the enzymes. Aconitase, an enzyme required for the function of the citric acid cycle, ribonucleotide reductase essential for DNA synthesis, phenylalanine hydroxylase, and tyrosine hydroxylase required for melanin synthesis are some examples of biologically important iron-sulphur complexes (Geissler and Singh, 2011). Iron is also found to be an important contributor in immune responses (Mackenzie *et al.*, 2008).

Consequences of iron deficiency

According to World Health Organization (WHO), iron deficiency anemia is the most common micronutrient deficiency throughout the world. Over two billion people in the world have been diagnosed with anemia.

In Sri Lanka, a survey performed in 2009 has shown that 25.2% of children under the age of five, suffer from iron deficiency anemia (Jayatissa *et al.*, 2012). The daily requirement of iron intake depends on the gender and age and the estimated average iron requirement is given in Table 2. Iron deficiency anemia impairs the activity of many enzymes, decreases myoglobin in skeletal muscle, and lowers the level of hemoglobin in the bloodstream (Beard, 2001), resulting in decreased physical activity, lethargy and increased risk of infection due to impaired immune response. Anemia also affects the early physical and cognitive development of infants (Beinner and Lamounier, 2003). The morbidity and mortality of pregnant women at the time of childbirth is shown to increase due to iron deficiency (Hurrell *et al.*, 2000), and therefore, responsible for about 26% of the maternal deaths around the world (Brabin *et al.*, 2001). Low iron content in blood is also linked to inflammatory

bowel diseases, and one third of these patients have been found to suffer from anemia (Gasche, 2004). Further, iron deficiency can cause chronic kidney diseases (Babitt and Lin, 2012) and heart failure (Jankowska *et al.*, 2013). Various measures taken to minimize iron deficiency include intake of iron supplements, fortification of food with iron, and biofortification of crops (Allen, 2002).

Dietary sources and iron metabolism

There are two forms of dietary iron, namely heme iron and non-heme iron. It is found that the relative absorptivity is higher in heme iron than non-heme iron in the human body (Hurrell and Egli, 2010). This distinction is made according to the mechanism of absorption of iron in the body. A certain percentage of iron present in meat, fish, and poultry is heme iron. Plant-based foods such as fruits and vegetables contain only non-heme iron, also known as inorganic iron, in the form of iron salts and chelates (Lim *et al.*, 2013).

Inorganic iron forms about 90% of the total iron absorbed from food (Tandara and Salamunic, 2012). Heme iron is absorbed as a porphyrin complex into the mucosal cells in the small intestine (Lim *et al.*, 2013).

Table 2: Estimated average iron requirement (Source: Institute of Medicine US Panel on Micronutrients).

Age / years	Male	Female	Pregnancy	Lactation
1–3	3.0 mg/day	3.0 mg/day	-	-
4–8	4.1 mg/day	4.1 mg/day	-	-
9–13	5.9 mg/day	5.7 mg/day	-	-
14–18	7.7 mg/day	7.9 mg/day	23.0 mg/day	7.0 mg/day
19–30	6.0 mg/day	8.1 mg/day	22.0 mg/day	6.5 mg/day
31–50	6.0 mg/day	8.1 mg/day	22.0 mg/day	6.5 mg/day
51–70	6.0 mg/day	5.0 mg/day	-	-
> 70	6.0 mg/day	5.0 mg/day	-	-

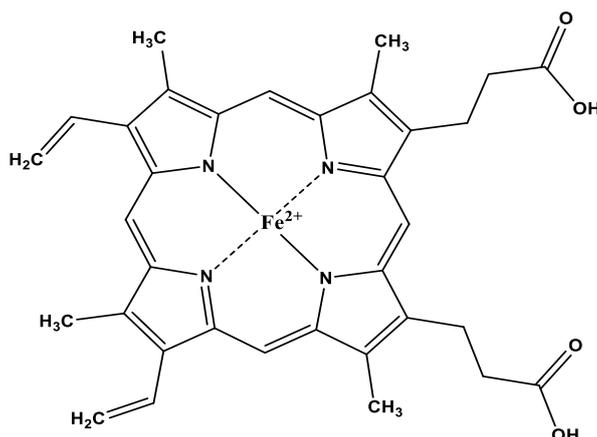


Figure 1: Iron porphyrin ring

Non-heme iron in ferrous state is transported using divalent metal transporter 1 (DMT1), and non-heme ferric iron is converted to ferrous form by duodenal cytochrome reductase (Geissler and Singh, 2011). Then, the ferrous ion enters an exchangeable iron pool in the enterocyte, from where iron will be supplied as required for various functions. A regulated mechanism for excretion of iron is unknown to date and therefore the content of iron in the body is controlled by the absorption at the small intestine (Tandara and Salamunic, 2012). Heme iron remains in the porphyrin complex until it is absorbed by the mucosal cells; the influence of external factors on heme iron absorption is minimal. However, non-heme iron absorption is influenced by factors that enhance or inhibit iron solubility (Osungbade and Oladunjoye, 2012).

Factors affecting iron absorption

Absorption of iron in the intestines can be affected by the presence of several dietary factors associated with the food matrix. Since heme iron is associated with the porphyrin ring, absorption of heme iron is greater than that of non heme iron. Ascorbic acid binds iron and keeps it in the reduced ferrous state and thereby enhances its bioavailability. Similarly compounds such as glycine, citrate, gluconate, and fumarate which can chelate ferrous iron and improve its solubility may increase iron bioavailability. On the other hand, plant components such as polyphenols, phosphates, calcium, and dietary fiber are known to reduce non-heme iron absorption into the body (Chen and Oldewage-Theron, 2002; Hurrell *et al.*, 1999; Roughead *et al.*, 2002) through the formation of insoluble iron salts/complexes.

Ascorbic acid

Ascorbic acid has been found to drastically increase iron absorption from meals. According to findings from the research carried out by Hallberg *et al.* in 1987, there is a semi-exponential relationship between the amount of ascorbic acid in the meal and the amount of iron absorbed. Another study was carried out by Derman *et al.* (1977) on 116 Indian women to determine the iron absorption from maize meal porridge containing ascorbic acid. The meals were given with and without tea or coffee and the iron absorption was measured using a radio-iron utilization method. The research revealed that addition of 50-100 mg of ascorbic acid would increase iron absorption by ten-fold when taken without tea, and that using doses of 250-500 mg of ascorbic acid could even overcome the inhibitory effect of tea on iron absorption. Two mechanisms have been suggested to be responsible for the increase in iron absorption in the presence of ascorbic acid. The first mechanism is the formation of soluble iron-ascorbic acid complexes. Absorption of non-heme iron depends on its extent of solubility within the lumen of the upper intestinal tract. Thus, the formation of soluble ascorbic acid iron complexes will lead to an increase in iron absorption. The second mechanism by which ascorbic acid increases iron absorption is reducing iron in ferric to ferrous form. Also, ascorbic acid prevents binding of other ligands in the intestine with iron, which may reduce iron absorption. However, ascorbic acid is unstable under the temperature and humidity conditions used in food processing. Therefore, the use of ascorbic acid in processed food to increase iron absorption may not be fruitful. A study carried out by Lynch and Cook demonstrated that

ascorbic acid is effective only if it is present in the meal. They observed a six-fold increase in iron absorption when 500 mg of ascorbic acid is taken with the meal. However, when the same amount of ascorbic acid was taken 4 to 8 hours prior to the meal, its effect on iron absorption was found to be insignificant (Lynch and Cook, 1980). Human studies using radioisotopes carried out by Lynch and Stoltzfus (2003) concluded that the increase of iron absorption by ascorbic acid depends on the dose of ascorbic acid consumed and that during iron absorption, ascorbic acid needs to be present in the lumen of the upper gastrointestinal tract. They also found that intravenously injected ascorbic acid does not demonstrate an increase in iron absorption. Meat

is also known to increase absorption of non-heme iron to the body.

Phytates

Phytates are powerful chelators which can bind on to metals and form insoluble complexes. Due to its formation of insoluble complexes with iron cations, phytate is considered to be an inhibitor of iron absorption. Cereals and legumes are known to contain phytates and phytic acid. Koreissi-Dembele *et al.* (2013) studied the effect of dephytinization on iron absorption in fonio meals. Incubation at pH 5, 50 °C was found to degrade phytic acid. Iron absorption was shown to increase from 2.6% in the non-dephytinized meal to 8.3% in the dephytinized meal (Koreissi-Dembele *et al.*, 2013).

Table 3: Comparison of oral iron formulations (Source: "Pharmacist's letter/ Prescriber's letter," 2008).

Formulation	Dosage form	Comments
Ferric ammonium citrate	Capsules	Iron is present as a ferric salt and needs reduction to ferrous form in the intestinal lumen for absorption. Less bioavailable than ferrous salts.
Ferrous bisglycinate	Capsules and tablets	An iron-amino acid chelate with high bioavailability.
Ferrous fumarate	Tablets	Efficacy and tolerability similar to ferrous sulfate. Moderately soluble in water. Almost tasteless.
Ferrous gluconate	Tablets	Similar efficacy and tolerability as ferrous sulfate.
Ferrous sulphate	Oral solution, tablets, enteric-coated tablets, and filmcoated tablets	Effective, tolerable, and cheap. Formulation of choice for treatment of iron deficiency anemia.
Heme iron polypeptide	Capsules	More bioavailable than iron salts. Well-tolerated.
Polysaccharide-iron complex	Capsules, solution, and filmcoated tablets	Ferric iron is complexed to hydrolyzed starch. Tasteless and odorless. Bioavailability similar to ferrous sulfate.

IRON DELIVERY METHODS

When dietary intake of iron is insufficient to meet the bodily requirements, iron is taken as a supplement and also iron fortification is carried out on staple food (Beinner and Lamounier, 2003; Mirmiran *et al.*, 2012). Oral iron supplements of widely varying dosages, formulations (quick or prolonged release), and chemical states (ferrous or ferric form) are available (Table 3). A study on the bioavailability of the ferrous and ferric forms has shown that slow-release ferrous sulphate preparations have good bioavailability, efficacy, and acceptable tolerability when compared with ferric iron polymaltose complex preparations (Santiago, 2012).

However, iron can change color of food by forming complexes with compounds such as sulphur compounds, tannins, and polyphenols. Iron is chemically reactive under alkaline conditions, give a metallic taste, cause gastrointestinal discomfort, and give an unpleasant flavor due to fat oxidation (Chen and Oldewage-Theron, 2002; Mehansho, 2006). Also, iron in ferrous state may be easily oxidized into less bioavailable ferric state. Moreover, interaction of non-heme iron with various dietary components in the intestine may reduce the solubility and therefore, lower the bioavailability. To minimize these drawbacks, chelation of iron with biocompatible ligands and encapsulation of iron with various edible coatings have been carried out (Mehansho, 2006). We present some of these methods which have shown promise as effective carriers or delivery agents of ferrous iron.

Chelation

Iron chelation is a technique used to deliver active ingredients that have either low absorption efficiency or are less bioavailable. Chelation is literally the protective enclosure of minerals such as iron and zinc, so that these minerals do not undergo any unnecessary reactions before reaching the target. A set of chelating agents such as EDTA (Ethylenediaminetetraacetate), chitosan, amino acids, gluconate, succinate, fumarate are used in chelating cations that need to be delivered (Flora, 2013). Ferrous sulphate is very soluble and therefore has high bioavailability. However, it is also susceptible to organoleptic changes and interactions with ligands in the intestine to form insoluble

complexes. To minimize these unfavorable reactions, chelation of iron is carried out.

Amino acid chelated iron

Use of amino acids to chelate iron causes iron to be absorbed in the jejunum, unlike the non-heme iron which is absorbed in the duodenum. One example of an amino acid chelated iron complex is ferrous bis-glycinate, in which one ferrous cation is chelated with two glycine ligands. Glycine too is capable of protecting iron from binding on to ligands that can reduce iron absorption. Benjamin *et al.* (2000) carried out a study using 10 iron sufficient males to compare the efficiencies of iron absorption from ferrous sulphate, ferrous bis-glycinate, and ferric tris-glycinate from a phytate-rich meal. The subjects were fed whole maize porridge fortified with ^{59}Fe -sulphate on day one and ^{59}Fe bis-glycinate on the second day. Then iron absorption was determined from blood radioactivity. The results showed that iron absorption from ferrous bis-glycinate is four times that of ferrous sulphate. Ferrous bis-glycinate is water soluble, and therefore can easily oxidize from ferrous to ferric state resulting in color and flavor changes (Jiménez-Alvarado *et al.*, 2009; Mehansho, 2006).

EDTA complexes

EDTA ligand has been found to form stable complexes with iron. EDTA is a hexadentate chelate ligand and is capable of forming complexes with many metal cations (Chen and Oldewage-Theron, 2002). Findings from a study carried out by Hurrell *et al.* (2000) demonstrated that the complex NaFeEDTA facilitated higher iron absorption than that from ferrous sulphate and ferrous fumarate. Eighty-four volunteers from ages between 18-40 years were given infant cereals and bread rolls fortified with radio labeled ferrous sulphate or ferrous fumarate and Fe absorption was measured based on erythrocyte enrichment. Then the subjects were fed infant cereals and bread rolls fortified with radio labeled NaFeEDTA. Iron absorption from NaFeEDTA fortified meal was observed to be 1.9-3.9 times greater than that from ferrous sulphate or ferrous fumarate fortified meal. It was also demonstrated that addition of Na_2EDTA can increase iron absorption from cereal fortified with ferrous sulphate.

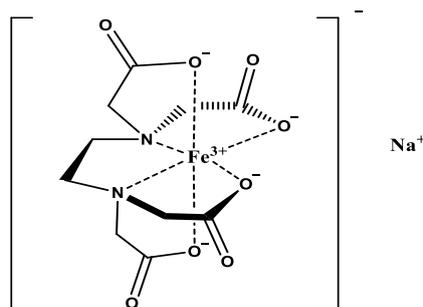


Figure 2: Structure of NaFeEDTA

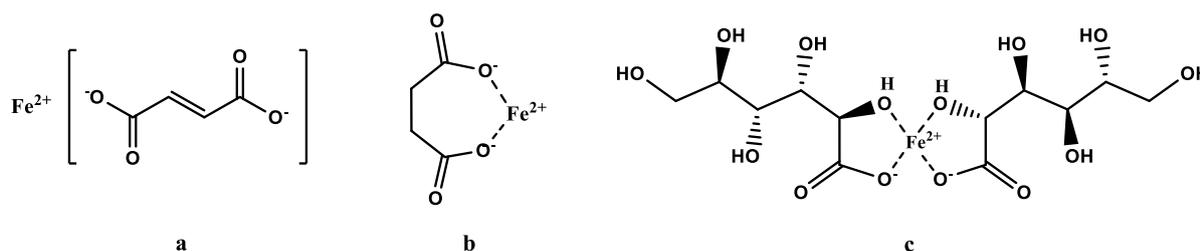


Figure 3: a. Ferrous fumarate b. Ferrous succinate c. Ferrous gluconate

Iron-EDTA complexes are stable and the bioavailability of iron from the complex is not affected by the harsh conditions used in food processing. They are advantageous since they cause fewer organoleptic problems. Presence of EDTA ligand can minimize the effect of phytates on iron absorption. EDTA ligand is not metabolized in the body, and is excreted through the urinary tract. Divalent cations bind EDTA with high affinity and therefore, loss of biologically important divalent cations may occur. This process is named co-chelation (Chen and Oldewage-Theron, 2002). Since, iron-EDTA complexes are about six times more expensive than ferrous sulphate (Le *et al.*, 2006), the use of EDTA as a ligand has its restrictions.

Fumarate, succinate, and gluconate ligands

Unless chelated or protected with a coating, iron in ferrous state is easily oxidized into ferric state. Ferric iron can precipitate proteins and cause gastro intestinal irritation when ingested. However, the precipitation of proteins does not occur when iron is chelated with gluconate or succinate ligands. Therefore, succinate and gluconate complexes of iron are used to deliver iron (Hurrell *et al.*, 1989; Khosroyar and Arastehnodeh, 2007). Ferrous fumarate is commonly used in iron supplements due to its high bioavailability (Chen and Oldewage-Theron, 2002). Also, since fumarate ligand is present in living organisms and is an essential

component in Krebs's cycle, ferrous fumarate shows good biocompatibility and minimal side effects (Kapor *et al.*, 2012).

Encapsulation

Encapsulation involves creating a protective layer around a particular substance. In food and pharmaceutical applications this protective coating can assist controlled release of the entrapped substance of interest. So far, various encapsulation techniques such as polymerization, spray drying, gelation, precipitation, and coacervation with relation to food industry have been reported (Ariyaratna and Karunaratne, 2015; Bastos *et al.*, 2012; Karunaratne *et al.*, 2016; Gadkari and Balaraman, 2015). Encapsulated iron compounds have been used in iron fortification to minimize interactions between the other components in the food vehicle (Chen and Oldewage-Theron, 2002). When choosing the shell material for the capsule, it is important to consider its solubility in gastric juice. Whey protein, chickpea protein, alginate, and hydrogenated oils have been used to encapsulate iron containing compounds (Bezbaruah *et al.*, 2011; Khosroyar and Arastehnodeh, 2007; Martin and Jong, 2012; Mehansho, 2006; Rajakaruna *et al.*, 2015; Zimmermann *et al.*, 2004). Various techniques of encapsulation have been utilized in studies to increase the bioavailability and to solve organoleptic issues related to iron.

Cold-set gelation has been used to entrap iron in the presence of ascorbic acid which can otherwise undergo degradation on heat application. Whey protein has been used to create gel particles by first unfolding the protein structure and then inducing cold-set gelation by addition of Fe^{2+} and ascorbic acid. Particles have then been freeze dried at $-40\text{ }^\circ\text{C}$. Presence of ascorbate has been found to increase the strength of the iron induced cold-set gel. The particles have also displayed protein stability at pH range 2 to 7, indicating high encapsulation efficiency. Also, the presence of ascorbic acid in gel particles has resulted in an increase in *in vitro* iron accessibility from 10 to 80% (Martin and Jong, 2012).

Alginate has been used to encapsulate iron. The carboxylic groups on the alginate molecules and ferric cations can crosslink, creating a capsule with the iron compound at its core, and alginate as the shell. Alginate is insoluble in aqueous media. Therefore, the shell can protect the inner iron compound from interacting with moisture, and reduce organoleptic changes iron may cause. Khosroyar *et al.* (2007) have used coacervation to form ferric saccharate microcapsules using an alginate coating. Here, alginate and ferric saccharate have been mixed at a shell: core ratio of 70:30, and added drop wise to a solution of CaCl_2 . The particles formed have then been washed to remove free ions and dried. The alginate coated ferric saccharate particles have shown an average size of $400\text{ }\mu\text{m}$. Iron release from the capsules under wet or dry conditions at room temperature has been found to be less than 0.04%. When the amount of water in contact with the capsules is increased, a higher release has been observed. Zimmerman *et al.*

(2004) have reported encapsulating iron along with iodine and vitamin A, to be used as a food fortificant. They used hydrogenated palm oil to coat iron and other components employing a single step spray cooling technique. The resultant capsules had a mean particle size of $100\text{ }\mu\text{m}$ and were spherical in shape. On storage for six months, the particles only lost 12-15% of its iron content.

Encapsulation has also been attempted using emulsion formation. Jimenez-Alvarado *et al.* (2009) have reported entrapping ferrous bis-glycinate solution in the inner aqueous phase of water-in-oil-in-water (w/o/w) emulsions. As discussed previously, despite being able to reduce interactions of iron with phytates and polyphenols, ferrous bis-glycinate can cause color changes in food, and is also oxidized easily. Water-in-oil-in-water emulsions are made from dispersing water droplets in large oil droplets, and then dispersing the oil droplets in an aqueous phase. When biopolymers such as proteins are used in the oil phase, chemical interactions through covalent bonding, or physical interactions through electrostatic forces can take place (Dickinson, 2008). Therefore, protein-hydrocolloid systems are increasingly being used in food processing. Chickpea protein coated ferrous fumarate microparticles with high encapsulation efficiency and good release kinetics at pH 2, 4, 6, and 8 have been synthesized by Rajakaruna *et al.* (2015). The isoelectric precipitation technique of adjusting the pH of a homogeneous protein and ferrous fumarate solution to the pI of the protein has been employed to obtain the encapsulate (Rajakaruna *et al.*, 2015).

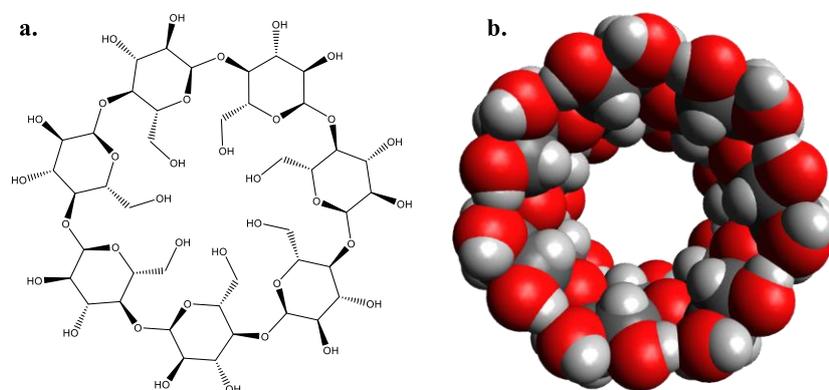


Figure 4: β -cyclodextrin a. Chemical structure b. Van der Waals Sphere structure

Inclusion complexation

β -Cyclodextrin has been used to encapsulate active ingredients by the inclusion complexation technique. β -Cyclodextrin is a cyclic carbohydrate which has a conical cave-like conformation as depicted in figure 4(b). This shape enables β -cyclodextrin to accommodate active ingredients in its central cavity. β -Cyclodextrins contain hydrophilic moieties directed outwards and a lipophilic interior which provides a suitable environment to solubilize organic compounds. The entrapped hydrophobic molecules can easily dissolve in the aqueous media, thereby increasing the stability, solubility, and bioavailability of the hydrophobic molecule. This technique is mainly used to encapsulate organic compounds such as vitamins, essential oils, and some poor water soluble drugs. Compared to other methods, loading efficiency is typically low, but the encapsulation efficiency and the stability of the encapsulated compound against heat is relatively high (Polyakov and Kispert, 2015). Kapor *et al.* (2012) reported formation of ferrous fumarate inclusion complexes using cyclodextrins. To form the inclusion complexes, coprecipitation method was used. A solution of ferrous fumarate and β -cyclodextrin in molar ratio 1:1 was mixed for 72 hours at room temperature, evaporated to reduce the volume and dried in a desiccator over concentrated sulfuric acid. Entrapment of ferrous fumarate in cyclodextrin was shown to increase the solubility of ferrous fumarate (Kapor *et al.*, 2012).

Iron liposomes

Liposomes are produced by the association of amphiphilic compounds such as phospholipids into a bilayer structure using various techniques such as sonication, microfluidization, and solvent evaporation. Among these methods, microfluidization is the most promising technique in commercial scale production of liposomes. The encapsulation of food active ingredients in liposomes is still in its infant stages. However, interest in using this technique is becoming more popular. Liposomes are especially suitable for encapsulation of hydrophilic active components such as water soluble vitamins, minerals, and flavours. The encapsulation efficiency of active component depends mainly on the rigidity and the stability of the bilayer structure of liposomes (Matos *et al.*, 2015; Gonnet *et al.*, 2010).

A significant improvement in iron supplementation in animal trials was observed in liposomal encapsulated ferric ammonium citrate (Xu *et al.*, 2014). They conclude that the liposomal iron can be used efficiently with minimal side effects to relieve the iron deficiency anemia caused by excessively exercise. A study that used rotary-evaporated film-ultrasonication method for the preparation of ferric citrate liposomes and heme liposomes demonstrated that this can be used as a supplement with iron fortified food in order to relieve iron deficiency. This study clearly shows that the liposomal iron delivery can significantly improve the iron absorption over direct iron supplements (Yuan *et al.*, 2013a).

Ding *et al.* (2009) synthesized ferrous glycinate nanoliposomes from egg phosphatidylcholine by the reverse phase evaporation technique with high encapsulation efficiency for oral administration. The prepared iron nanoliposomes showed controlled release potential and better stabilization under simulated gastric juice conditions. According to the findings by Yuan *et al.* (2013b), heme liposomes and ferric citrate liposomes demonstrated 119% and 54% increase in serum iron levels when compared to heme and ferric citrate, respectively.

It was found that ferrous sulphate liposomes prepared by reverse phase evaporation method functioned as a strong iron fortifier in fluid milk (Xia and Xu, 2005). The ferrous sulphate liposomes were created with egg phosphatidylcholine using thin-film, thin-film sonication, reverse-phase evaporation, and freeze thawing methods. Out of the four methods, reverse-phase evaporation was seen to create liposomes with the highest encapsulation efficiency (Xia and Xu, 2005).

CONCLUSION

Over the years, the method of iron delivery has evolved from using simple iron salts such as ferrous sulphate to chelates in the form of ferrous fumarate, ferrous bis-glycinate etc. With the advancement of research in carriers for delivery of small molecules, drugs, and nutrients, the use of proteins, alginate, and various hydrogenated oils have become promising candidates for iron delivery. Liposomal iron is no exception in showing that it is a good vehicle for iron delivery. The effect of these carriers on intestinal absorption of iron has indicated that the increase

is probably due to the ability of the carrier to protect iron in the ferrous state. Therefore, for iron supplementation and fortification, the use of an effective carrier may be the method of choice.

REFERENCES

- Alaunyte, I., Stojceska, V., Plunkett, A., and Derbyshire, E. (2014). Dietary iron intervention using a staple food product for improvement of iron status in female runners. *Journal of the International Society of Sports Nutrition*, **11**: 50.
- Allen, L. H. (2002). Forging Effective Strategies to Combat Iron Deficiency: Iron Supplements: Scientific Issues Concerning Efficacy and Implications for Research and Programs. *Journal of Nutrition*, **132**: 813S-819S.
- Ariyaratna, I. R., and Karunaratne, D. N. (2015). Use of chickpea protein for encapsulation of folate to enhance nutritional potency and stability. *Food and Bioprocess Processing*, **95**: 76-82.
- Babitt, J. L., and Lin, H. Y. (2012). Mechanisms of Anemia in CKD. *Journal of the American Society of Nephrology*, **23**, 1631-1634.
- Ballot, D. E., Macphail, A. P., Bothwell, T. H., Gillooly, M., and Mayet, F. G. (1989). Fortification of curry powder with NaFe(III)EDTA in an iron-deficient population: report of a controlled iron-fortification trial. *The American Journal of Clinical Nutrition*, **49**, 162-169.
- Bastos, D. D. S., Gonçalves, M. D. P., Andrade, C. T. D., Araújo, K. G. D. L., and Leão, M. H. M. D. R. (2012). Microencapsulation of cashew apple (*Anacardium occidentale*, L.) juice using a new chitosan-commercial bovine whey protein isolate system in spray drying. *Food and Bioprocess Processing* **90**: 683-692.
- Beard, J. L. (2001). Iron-deficiency anemia: reexamining the nature and magnitude of the public health problem. Summary: implications for research and programs. *The Journal of Nutrition* **131**: 568S-580S.
- Benner, M. A., and Lamounier, J. A. (2003). Recent experience with fortification of foods and beverages with iron for the control of iron-deficiency anemia in Brazilian children. *Food and Nutrition Bulletin*, **24**: 268-274.
- Bezbaruah, A. N., Shanbhogue, S. S., Simsek, S., and Khan, E. (2011). Encapsulation of iron nanoparticles in alginate biopolymer for trichloroethylene remediation. *Journal of Nanoparticle Research* **13**: 6673-6681.
- Bovell-Benjamin, A. C., Viteri, F. E., and Allen, L. H. (2000). Iron absorption from ferrous bisglycinate and ferric trisglycinate in whole maize is regulated by iron status. *American Journal of Clinical Nutrition* **71**: 1563-1569.
- Brabin, B. J., Hakimi, M., and Pelletier, D. (2001). An Analysis of Anemia and Pregnancy-Related Maternal Mortality. *The Journal of Nutrition*, **131**: 604-615.
- Chen, Z., and Oldewage-Theron, W. (2002). Food Fortification to Prevent and Control Iron Deficiency. *African Journal of Food, Agriculture, Nutrition and Development*, **2**: 67-77.
- Cook, J. D., and Reusser, M. E. (1983). Iron fortification: an update. *The American Journal of Clinical Nutrition*, **38**, 648-59.
- Derman, D., Sayers, M., Lynch, S. R., Charlton, R. W., Bothwell, T. H., and Mayet, F. (1977). Iron absorption from a cereal-based meal containing cane sugar fortified with ascorbic acid. *The British Journal of Nutrition*, **38**: 261-269.
- Dickinson, E. (2008). Interfacial structure and stability of food emulsions as affected by protein-polysaccharide interactions. *Soft Matter*, **4**: 932.
- Ding, B., Xia, S., Hayat, K., and Zhang, X. (2009). Preparation and pH Stability of Ferrous Glycinate Liposomes. *Journal of Agricultural and Food Chemistry*, **57**: 2938-2944.
- Flora, S. J. S. (2013). Chelation Therapy. In: Reference Module in Chemistry, Molecular Sciences and Chemical Engineering, *Comprehensive Inorganic Chemistry II*, Pp. 987-1013.
- Gadkari, P. V., and Balaraman, M. (2015). Catechins: Sources, extraction and encapsulation: A review. *Food and Bioprocess Processing*, **93**: 122-138.
- Gasche, C. (2004). Iron, anaemia, and inflammatory bowel diseases. *Gut*, **53**, 1190-1197.
- Geissler, C., and Singh, M. (2011). Iron, meat and health. *Nutrients*, **3**: 283-316.
- Gonnet, M., Lethuaut, L., and Boury, F. (2010). New trends in encapsulation of liposoluble vitamins. *Journal of Controlled Release*, **146**: 276-290.
- Hallberg, L., Brune, M., and Rossander-Hulthén, L. (1987). Is there a physiological role of vitamin C in iron absorption? *Annals of the New York Academy of Sciences* **498**: 324-332.
- Hekmat, S., and Mahon, D. J. M. (1997). Manufacture and Quality of Iron-Fortified Yogurt. *Journal of Dairy Science*, **80** 3114-3122.
- Hurrell, R. F., and Egli, I. (2010). Iron bioavailability and dietary reference values. *American Journal of Clinical Nutrition* **91**: 1461-1467.
- Hurrell, R. F., Furniss, D. E., Burri, J., Whittaker, P., Lynch, S. R., and Cook, J. D. (1989). Iron fortification of infant cereals: A proposal for the use of ferrous fumarate or ferrous succinate. *American Journal of Clinical Nutrition*, **49**: 1274-1282.
- Hurrell, R. F., Reddy, M. B., Burri, J., and Cook, J. D. (2000). An evaluation of EDTA compounds for iron fortification of cereal-based foods. *The British Journal of Nutrition* **84**: 903-910.
-

- Hurrell, R. F., Reddy, M., and Cook, J. D. (1999). Inhibition of non-haem iron absorption in man by polyphenolic-containing beverages. *The British Journal of Nutrition*, **81**: 289-295.
- Institute of Medicine (US) Panel on Micronutrients (2001). Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc. National Academies Press (US), Washington (DC), 9, Pp 290-393
- Jankowska, E. A., Haehling, S. V., Anker, S. D., Macdougall, I. C., and Ponikowski, P. (2013). Iron deficiency and heart failure: diagnostic dilemmas and therapeutic perspectives. *European Heart Journal*, **34**, 816-829.
- Jayatissa, R., Gunathilaka, M. M., and Fernando, D. N. (2012). National nutrition and micronutrient survey 2012; Anaemia among children aged 6-59 months and nutritional status of children and adults, 1, Pp 14-15
- Jiménez-Alvarado, R., Beristain, C. I., Medina-Torres, L., Román-Guerrero, A., and Vernon-Carter, E. J. (2009). Ferrous bisglycinate content and release in W1/O/W2 multiple emulsions stabilized by protein-polysaccharide complexes. *Food Hydrocolloids*, **23**: 2425-2433.
- Johnson, E., and Wessling-Resnick, M. (2012). Iron metabolism and the innate immune response to infection. *Microbes and Infection*, **14**: 207-216.
- Kapor, A. J., Nikolić, L. B., Nikolić, V. D., and Stanković, M. Z. (2012). The Synthesis and Characterization of Iron (II) Fumarate and Its Inclusion Complexes with Cyclodextrins. *Advanced Technologies*, **1**: 7-15.
- Karunaratne, D. N., Siriwardhana, D. A. S., Ariyaratna, I. R., Rajakaruna, R. M. P. I., Banu, F. T., and Karunaratne, V. (2017). Nutrient delivery through Nanoencapsulation. In *Nutrient Delivery, Chapter 17*. In: Grumezescu (Eds.), *Nanotechnology in the Agri-Food Industry*. (In press) Elsevier Science Pp. 653–680. ISBN : 978-0-12-804304-2
- Khosroyar, S., and Arastehnodeh, A. (2007). Ferric saccharate microencapsulation with alginate coating using the coacervation method. *Afinidad*, **80**: 121-127.
- Koreissi-Dembele, Y., Fanou-Fogny, N., Moretti, D., Schuth, S., Dossa, R. A. M., Egli, I., Zimmermann, M. B., Brouwer, I. D. (2013). Dephytinisation with Intrinsic Wheat Phytase and Iron Fortification Significantly Increase Iron Absorption from Fonio (*Digitaria exilis*) Meals in West African Women. *PLoS ONE*, **8**: e70613.
- Le, H. T., Brouwer, I. D., Burema, J., Nguyen, K. C., and Kok, F. J. (2006). Efficacy of iron fortification compared to iron supplementation among Vietnamese schoolchildren. *Nutrition Journal*, **5**: 32.
- Lim, K. H. C., Riddell, L. J., Nowson, C. A., Booth, A. O., and Szymlek-Gay, E. A. (2013). Iron and zinc nutrition in the economically-developed world: A review. *Nutrients*, **5**: 3184-3211.
- Lynch, S. R., and Cook, J. D. (1980). Interaction of vitamin c and iron. *Annals of the New York Academy of Sciences*, **589**: 32-44.
- Lynch, S. R., and Stoltzfus, R. J. (2003). Iron and ascorbic Acid: proposed fortification levels and recommended iron compounds. *The Journal of Nutrition*, **133**: 2978-2984.
- Mackenzie, E. L., Iwasaki, K., and Tsuji, Y. (2008). Intracellular iron transport and storage: from molecular mechanisms to health implications. *Antioxidants and Redox Signaling*, **10**: 997-1030.
- Martin, A. H., and Jong, G. A. H. D. (2012). Enhancing the *in vitro* Fe²⁺ bio-accessibility using ascorbate and cold-set whey protein gel particles. *Dairy Science and Technology*, **92**: 133–149.
- Matos, M., Gutiérrez, G., Iglesias, O., Coca, J., and Pazos, C. (2015). Enhancing encapsulation efficiency of food-grade double emulsions containing resveratrol or vitamin B12 by membrane emulsification. *Journal of Food Engineering*, **166**: 212-220.
- Mehansho, H. (2006). Symposium: Food Fortification in Developing Countries The Economics of Food Fortification 1. *The Journal of Nutrition*, **136**: 1068-1071.
- Mirmiran, P., Golzarand, M., Serra-Majem, L., and Azizi, F. (2012). Iron, iodine and vitamin a in the Middle East; a systematic review of deficiency and food fortification. *Iranian Journal of Public Health*, **41**: 8-19.
- Osungbade, K. O., and Oladunjoye, A. O. (2012). Preventive treatments of iron deficiency Anaemia in pregnancy: A review of their effectiveness and implications for health system strengthening. *Journal of Pregnancy*, **2012**: 1-7.
- Pharmacist's letter/ Prescriber's letter. (2008). Comparison of oral iron supplements. *Pharmacist's Letter*, **24**, 1-4.
- Pizarro, F., Olivares, M., Maciero, E., Krasnoff, G., Cócara, N., and Gaitan, D. (2015). Iron Absorption from Two Milk Formulas Fortified with Iron Sulfate Stabilized with Maltodextrin and Citric Acid. *Nutrients*, **7**, 8952-8959.
- Polyakov, N. E., and Kispert, L. D. (2015). Water soluble biocompatible vesicles based on polysaccharides and oligosaccharides inclusion complexes for carotenoid delivery. *Carbohydrate Polymers*, **128**: 207-219.
- Rajakaruna, R. M. P. I., Ariyaratna, I. R., and Karunaratne, D. N. (2015). Development of a protein encased ferrous fumarate microcapsule. *International Research Symposium on Engineering Advancements*, Vol. 5, Pp. 273-276.
- Rao, B. S. N. (1994). Fortification of salt with iron and iodine to control anaemia and goitre: development of a new formula with good stability and bioavailability of iron and iodine. *Food and Nutrition Bulletin*, **15**, 32-39.

- Roughead, Z. K., Zito, C. A., and Hunt, J. R. (2002). Initial uptake and absorption of nonheme iron and absorption of heme iron in humans are unaffected by the addition of calcium as cheese to a meal with high iron bioavailability. *American Journal of Clinical Nutrition*, **76**: 419-425.
- Santiago, P. (2012). Ferrous versus Ferric Oral Iron Formulations for the Treatment of Iron Deficiency: A Clinical Overview. *The Scientific World Journal*, **2012**, 1-5.
- Tandara, L., and Salamunic, I. (2012). Iron metabolism: current facts and future directions. *Biochem Med (Zagreb)*, **22**: 311-28.
- Vashchenko, G., and MacGillivray, R. T. A. (2013). Multi-copper oxidases and human iron metabolism. *Nutrients*, **5**: 2289-2313.
- Viteri, F. E., Alvarez, E., Batres, R., Torún, B., Pineda, O., Mejía, L. A., and Sylvi, J. (1995). Fortification of sugar with iron sodium ethylenediaminetetraacetate (FeNaEDTA) improves iron status in semirural Guatemalan populations. *American Journal of Clinical Nutrition*, **61**, 1153-1163.
- Walczyk, T., Tuntipopipat, S., Zeder, C., Sirichakwal, P., Wasantwisut, E., and Hurrell, R. F. (2005). Iron absorption by human subjects from different iron fortification compounds added to Thai fish sauce. *European Journal of Clinical Nutrition*, **59**, 668-674.
- Xia, S., and Xu, S. (2005). Ferrous sulfate liposomes: preparation, stability and application in fluid milk. *Food Research International*, **38**: 289-296.
- Xu, Z., Liu, S., Wang, H., Gao, G., Yu, P., and Chang, Y. (2014). Encapsulation of Iron in Liposomes Significantly Improved the Efficiency of Iron Supplementation in Strenuously Exercised Rats. *Biological Trace Element Research*, **162**: 181-188.
- Yuan, L., Geng, L., Ge, L., Yu, P., Duan, X., Chen, J., and Chang, Y. (2013a). Effect of iron liposomes on anemia of inflammation. *International Journal of Pharmaceutics*, **454**: 82-89.
- Zhang, D., and Mahoney, A. W. (1989). Effect of Iron Fortification on Quality of Cheddar Cheese. *Journal of Dairy Science*, **72**, 322-332.
- Zimmermann, M. B., Wegmueller, R., Zeder, C., Chaouki, N., Biebinger, R., Hurrell, R. F., and Windhab, E. (2004). Triple fortification of salt with microcapsules of iodine, iron, and vitamin A. *American Journal of Clinical Nutrition*, **80**: 1283-1290.
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