LUBRIZOL LIFE SCIENCE

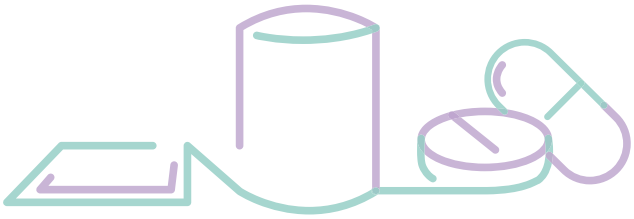
Technical Report

LIPOFER™ microcapsules

Bioavailable and stable source of iron

# TECHNICAL REPORT

## Microencapsulated Minerals



**Competitive advantages**

- Reduces metallic taste
- Controls interactions with other compenents
- Water dispersible
- No digestive tract irritation

Lipofoods™ Nutraceutical Ingredients

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LUBRIZOL | HEALTH

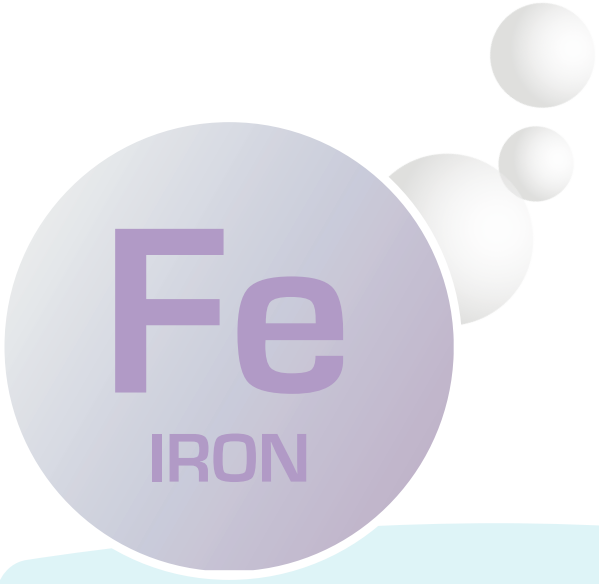
## 1. IRON - AN ESSENTIAL NUTRIENT

From ancient times, man has recognized the special role of iron in health and disease. [1] Iron had early medicinal uses by Egyptians, Hindus, Greeks, and Romans. [2,3] During the 17th century, iron was used to treat chlorosis (green disease), a condition often resulting frzom the iron deficiency.[4] However, it was not until 1932 that the importance of iron was finally settled by the convincing proof that inorganic iron was needed for hemoglobin synthesis. [5] For many years, nutritional interest in iron focused on its role in hemoglobin formation and oxygen transport. [6-8]

### Biochemistry and physiology

In contrast to zinc, iron is an abundant element on earth [2,9] and is a biologically essential component of every living organism. [10,11] **However, despite its geologic abundance, iron is often a growth limiting factor in the environment.** [9] This apparent paradox is due to the fact that in contact with oxygen iron forms oxides, which are highly insoluble, and thus is not readily available for uptake by organisms. [2] In response, various cellular mechanisms have evolved to capture iron from the environment in biologically useful forms.

Examples are siderophores secreted by microbes to capture iron in a highly specific complex [12] or mechanisms to reduce iron from the insoluble ferric iron (Fe+3) to the soluble ferrous form (Fe+2) as in yeasts. [13] Many of the mechanisms found in lower organisms, have analogous counterparts in higher organisms, including humans. In the human body, iron mainly exists in complex forms bound to protein (hemoprotein) as heme compounds (hemoglobin or myoglobin), heme enzymes, or nonheme compounds (flavin-iron enzymes, transferring, and ferritin). [13] **The body requires iron for the synthesis of its oxygen transport proteins**, in particular hemoglobin and myoglobin, and for the formation of heme enzymes and other iron-containing enzymes involved in electron transfer and oxidation-reductions. [3,14] Almost two-thirds of the body iron is found in the hemoglobin present in circulating erythrocytes, 25% is contained in a readily mobilizable iron store, and the remaining 15% is bound to myoglobin in muscle tissue and in a variety of enzymes involved in the oxidative metabolism and many other cell functions. [15]




**Iron is an essential nutrient for the proper growth and maintenance of human body. It is the main component for Haemoglobin in red blood cells (RBC) and muscles that distribute oxygen throughout the body.**

## 2. IRON ABSORPTION AND METABOLISM

**Iron is recycled and thus conserved by the body.** Figure 1 shows a schematic diagram of iron cycle in the body. Iron is delivered to tissues by circulating transferrin, a transporter that captures iron released into the plasma mainly from intestinal enterocytes or reticuloendothelial macrophages.

The binding of iron-laden transferrin to the cell-surface transferrin receptor (TfR) 1 results in endocytosis and uptake of the metal cargo. Internalized iron is transported to mitochondria for the synthesis of heme or iron-sulfur clusters, which are integral parts of several metalloproteins, and excess iron is stored and detoxified in cytosolic ferritin.

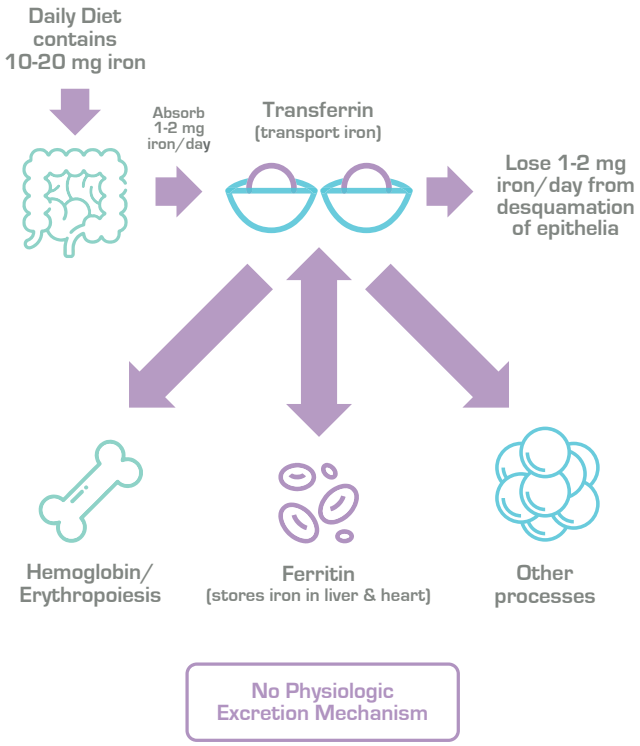



**Figure 1.** Iron is bound and transported in the body via transferrin and stored in ferritin molecules. Once iron is absorbed, there is no physiologic mechanism for excretion of excess iron from the body other than blood loss, that is, pregnancy, menstruation, or other bleeding.

### Absorption

**Iron absorption** occurs by the enterocytes by divalent metal transporter 1, a member of the solute carrier group of membrane transport proteins. This **takes place predominantly in the duodenum and upper jejunum.** [16] It is then transferred across the duodenal mucosa into the blood, where it is transported by transferrin to the cells or the bone marrow for erythropoiesis [producing red blood cells (RBCs)]. [14, 16,17,18]

A feedback mechanism exists that enhances iron absorption in people who are iron deficient. The physical state of iron entering the duodenum greatly influences its absorption. At physiological pH, ferrous iron (Fe+2) is rapidly oxidized to the insoluble ferric (Fe+3) form. Gastric acid lowers the pH in the proximal duodenum reducing Fe+3 in the intestinal lumen by ferric reductases, thus allowing the subsequent transport of Fe+2 across the apical membrane of enterocytes. This enhances the solubility and uptake of ferric iron.

Exported iron is scavenged by transferrin, which maintains Fe+3 in a redox-inert state and delivers it into tissues. The total iron content of transferrin (≈3 mg) corresponds to less than 0.1% of body iron, but it is highly dynamic and undergoes more than 10 times daily turnover to sustain erythropoiesis. [19]

**Iron absorption is a complex process that occurs mainly at the duodenal enterocytes; it reflects not only the iron content in the diet but also the bioavailability of iron.**

## 3. IRON INHIBITORS AND ENHANCERS

One of the major difficulties to ensuring adequate absorption is the presence of iron absorption inhibitors in the fortification vehicle itself, or in the accompanying diet. In table 1, some factors that could influence iron absorption are summarized.

### INHIBITORS

#### Phytic acid

The main inhibitory compound is phytic acid (myo-inositol6-phosphate), which is widely present in cereal grains and legume seeds. Phytic acid binds iron strongly in the gastrointestinal tract and can decrease the absorption of even the most bioavailable iron compounds to very low levels. Thus, there are two major technical barriers to overcome when developing an iron-fortified food. The first is the selection of an iron compound that causes no sensory changes but is adequately absorbed; the second is to overcome the inhibitory effect of phytic acid and other food components on iron absorption [20].

**Table 1.** Factors that could influence iron absorption

| **Physical state** (bioavailavility) | **Heme > Fe+2 > Fe +3** |
|---|---|
| **Inhibitors** | phytates, polyphenosis, calcium, some proteins |
| **Competitors; in animal studies** | leas, cobalt, strontium, manganese, zinc. |
| **Facilitators** | ascorbate, citrate, some aminoacids, meat fish, poultry |

### ENHANCERS

#### Ascorbic acid

Ascorbic acid (AA) also referred as vitamin C is the most widely used enhancer of fortification iron. It can increase by several fold the absorption of all fortification iron compounds (and native food iron) that dissolve in the gastric juice and enter the common non-heme iron pool. Ascorbic acid has been demonstrated to be effective in decreasing the negative effects of all major inhibitors of iron absorption including calcium and milk proteins, phytic acid, polyphenols, and soy products) [21].

However, the instability of AA during food processing, storage, and cooking, and the possibility of unwanted sensory changes limits the number of suitable food vehicles for AA, whether used as vitamin fortificant or as an iron enhancer [21,22].

## COMBINATION WITH FOLIC ACID

Data from four trials suggested that women who routinely received daily iron+folic supplementation reached term with higher Hb concentration (MD 12.00 g/L; 95% CI 2.93–21.07). Women who received iron+folic acid supplementation were less likely to have anaemia at term: 8.2% versus 35.5% RR 0.27, 95% CI 0.12–0.56); these results should be interpreted with caution since the heterogeneity between treatment effects was substantial [23].

**Several dietary factors influence iron absorption. For example, ascorbate and citrate increase iron uptake and phytates have a negative effect on iron absorption.**

## 4. HUMAN IRON REQUIREMENTS

During early infancy, iron requirements are met by the little iron contained in the human milk.[58] The need for iron rises markedly 4-6 months after birth and amounts to about 0.7-0.9 mg/day during the remaining part of the first year. [24]

Between 1 and 6 years of age, the body iron content is again doubled.[24] Iron requirements are also very high in adolescents, particularly during the period of growth spurt. Girls usually have their growth spurt before menarche, but growth is not finished at that time. In boys there is a marked increase in hemoglobin mass and concentration during puberty. In this stage, iron requirements increase to a level above the average iron requirements in menstruating women [24] [see Table 2].

The average adult stores about 1-3 g of iron in his or her body. A fine balance between dietary uptake and loss maintains this balance. About 1 mg of iron is lost each day through sloughing of cells from skin and mucosal surfaces, including the lining of the gastrointestinal tract.[25] Menstruation increases the average daily iron loss to about 2 mg per day in premenopausal female adults.[26] The augmentation of body mass during neonatal and childhood growth spurts transiently boosts iron requirements.[27]

**A dietary intake of iron is needed to replace iron lost in the stools and urine as well as through the skin.** These basal losses represent approximately 0.9 mg of iron for an adult male and 0.8 mg for an adult female. [28] The iron lost in menstrual blood must be taken into consideration for women of reproductive age [Table 2]

**The highest probability of suffering iron deficiency is found in those parts of a population that have inadequate access to foods rich in absorbable iron during stages of high iron demand. These groups correspond to children, adolescents, and women of reproductive age, during pregnancy. [24-29]**

**Table 2.** Iron requirements of 97.5% of individuals in terms of absorbed irona, by age gropu and sex (World Health Organization, 1989)

| Age/Sex | mg/day[b] |
|---|---|
| 4-12 months | 0.96 |
| 13-24 months | 0.61 |
| 2-5 years | 0.70 |
| 6-11 years | 1.17 |
| 12-16 years (girls | 2.02 |
| 12-16 years (boys) | 1.82 |
| Adult males | |
| Pregnant women | 1.14 |
| First trimester | 0.8 |
| Second and third trimester | 6.3 |
| Lactating women | 1.31 |
| Menstruating women | 2.38 |
| Postmenopausal women | 0.96 |

*Absorbed iron is the fraction that passes from the gastroinstestinal tract into the body for further use. Calculated on the basis of median weight for age. Requirements during pregnancy depend on the woman's iron status prior to pregnancy.

**During pregnancy, there is a significant increase in iron requirement due to the rapid growth of the placenta and the fetus** and the expansion of the globular mass.[29] In contrast, adult men and postmenopausal women are at low risk of iron deficiency and the amount of iron in a normal diet is usually sufficient to cover their physiological requirements. [29]

## 5. IRON DEFICIENCY AND IRON SUPPLEMENTATION

Iron deficiency is defined as a condition in which there are no mobilizable iron stores and in which signs of a compromised supply of iron to tissues, including the erythron, are noted. [30] Iron deficiency can exist with or without anemia. Some functional changes may occur in the absence of anemia, but the most functional deficits appear to occur with the development of anemia.

Even mild and moderate forms of iron deficiency anemia can be associated with functional impairments affecting cognitive development, [31] immunity mechanisms, [32] and work capacity.[33] **Iron deficiency during pregnancy is associated with a variety of adverse outcomes for both mother and infant, including increased risk of sepsis, maternal mortality, perinatal mortality, and low birth weight.** [34] Iron deficiency and anemia also reduce learning ability and are associated with increased rates of morbidity. [34]

**Nutritional iron deficiency arises when physiological requirements cannot be met by iron absorption from the diet.[38]**

### Iron supplementation

The primary causes of iron deficiency include low intake of bioavailable iron, increased iron requirements as a result of rapid growth, pregnancy, menstruation, and excess blood loss caused by pathologic infections, such as hook worm and whipworm causing gastrointestinal blood loss [35-38] and impaired absorption of iron. [39]

**Serum ferritin is a good indicator of body iron stores under most circumstances.** When the concentration of serum ferritin is ≥15 µg/L iron stores are present; higher concentrations reflect the size of the iron store; when the concentration is low (<12 µg/L for <5 years of age and <15 µg/L for >5 years of age) iron stores are depleted. [40]

Iron supplementation may be used as an intervention both to prevent and to treat iron deficiency anaemia and iron deficiency.

However, technical, and practical barriers exist that limit the effectiveness of supplementation due to poor bioavailabilty, interactions that affect organoleptic parameters of formulation as well as side effects that affect compliance, tolerability, and acceptance.

**Iron supplementation is a key strategy to reach target groups that have increased iron needs; however, product development and compliance are challenging, due to the reactive nature of iron. [41]**

## 6. LIPOFER™ microcapsules

Iron deficiency is defined as a condition in which there are no mobilizable iron stores and in which signs of a compromised supply of iron to tissues, including the erythron, are noted.[30] Iron deficiency can exist with or without anemia. Some functional changes may occur in the absence of anemia, but the most functional deficits appear to occur with the development of anemia.

Microencapsulation is currently considered the leading solution for overcoming these limitations via protecting sensitive nutrients and actives throughout processing of fortified foods as well as their shelf life. **LIPOFER™ microcapsules is a microencapsulated source of iron, which is designed to reduce iron's reactivity while improving its bioavailability.**

As shown in figure 2, **LIPOFER™ microcapsules** is a water dispersible micronized source of iron that has been microencapsulated to enhance iron absorption and reduce undesirable organoleptic attributes, thus enabling the enrichment of various types of foods and dietary supplements.

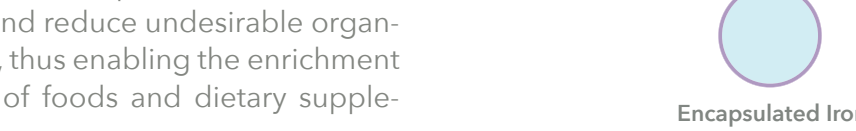



**Figure 2.** Microencapsulation technology of **LIPOFER™ microcapsules**


### Mechanism of absorption

**LIPOFER™ microcapsules** contains lecithin, which is an absorption enhancer that may alter the structural and functional features of cell membranes: fluidity, conformation of protein in mucosal membrane, and cell tight junctions [42]. It may also exchange with the membrane lipids or directly insert into the bilayer of mucosa, thus leading to the increased lipid fluidity of mucosa [42]. Therefore, the emulsifier lecithin can enhance absorption of different ingredients such as iron [43]. Furthermore, micronized iron particles contained in **LIPOFER™ microcapsules** increase surface area, thus enhancing iron absorption and reaching intestinal lumen without any side effects.

**An effect of lecithin on increasing iron absorption in LIPOFER™ microcapsules, probably due to its emulsifying action is likely to occur, thus being responsible for the increase in the solubility of the mineral.**

## 6.1 LIPOFER™ COMPETITIVE ADVANTAGES

Lubrizol has applied its core technological competencies to iron to overcome the issues with traditional iron, by developing a microencapsulated iron source branded as **LIPOFER™ microcapsules**. The competitive advantages are summarized in figure 3.

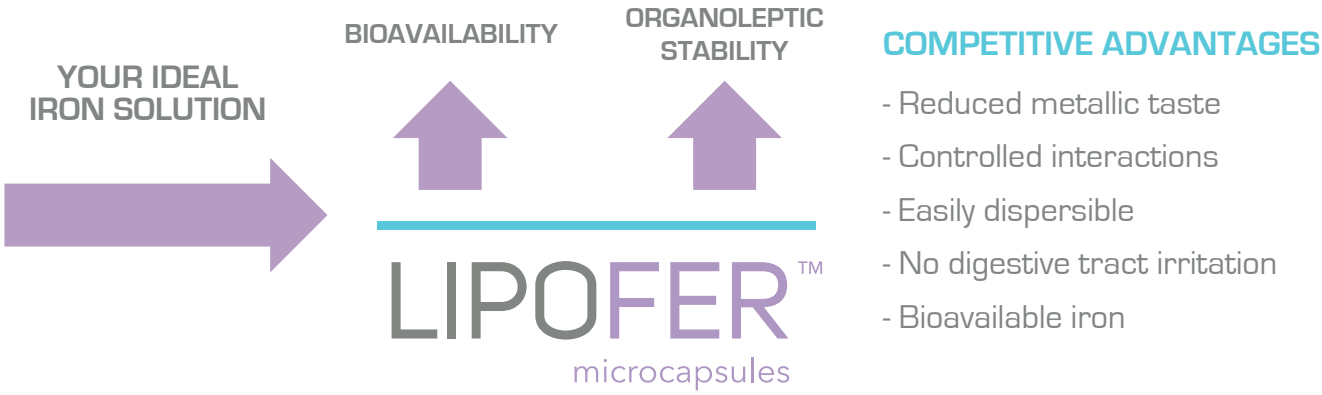



**Figure 3.** LIPOFER™ microcapsules competitive advantages

### Reduced metallic taste

The microencapsulation technology in LIPOFER™ microcapsules allows an easy iron fortification and supplementation by making the iron pyrophosphate more compatible with the food vehicle or minimizing the characteristic unpleasant metallic taste.

### Controlled interactions

One of the major difficulties to ensuring adequate absorption is the presence of iron absorption inhibitors in the fortification vehicle itself, or in the accompanying diet.

LIPOFER™ microcapsules can mitigate some of the interactions with other ingredients during processing and storage, contributing to a stable final product which maintains both its nutritional and sensory attractiveness throughout the entire shelf life while enhancing iron bioavailability.

### Easily dispersible

One of the major difficulties to ensuring adequate absorption is the presence of iron absorption inhibitors in the fortification vehicle itself, or in the accompanying diet.

LIPOFER™ microcapsules is designed as microencapsulated ferric pyrophosphate that is provided as fine powder. It is easily dispersible enabling the addition of various xthis essential nutrient.

### No digestive tract irritation

An important limitation with iron supplementation is low compliance due to gastrointestinal discomfort.

LIPOFER™ microcapsules is mild in the gastrointestinal system, providing no negative consequences like diarrhea, constipation or other digestive disorders that could derive from the intake.

## Bioavailable iron

Several clinical studies have been carried out with **LIPOFER™** microcapsules to assess superior iron absorption.
Some of the efficacy studies done with **LIPOFER™** microcapsules are summarized in figure 4.

### Animal Mode

a) LIPOFER™ absorption vs ferric pyrophosphate and ferrous sulfate in rats

b) LIPOFER™ absorption vs iron fumarate in mice

c) LIPOFER™ assesment on coltis and dysbiosis at adulthood in rats and mice

### Clinical Studies & Applications tests

a) LIPOFER™ efficacy in fortified fruit juice in women.

b) LIPOFER™ bioavailability comparison with iron (III) proteinsuccinylate in women.

c) LIPOFER™ as alternate treatment to intravenous iron in patients with refractory anemia

d) LIPOFER™ comparative of oxidative deterioration of infant formulas during storage.

**Figure 4.** Summary of efficacy tests performed with **LIPOFER™** microcapsules

## ANIMALS

### a) Efficacy of LIPOFER™ microcapsules absorption vs ferric pyrophosphate and ferrous sulfate in rats

Bioavailability of iron in **LIPOFER™** microcapsules compared to other iron forms was demonstrated in a study conducted in the Consejo Superior de Investigaciones Científicas (CSIC) [44]. Four groups of Sprague-Dawley rats weighing 230-250 g were stored in separate cages. Three different iron forms (ferric pyrophosphate, **LIPOFER™** microcapsules and ferrous sulfate) suspended in Carboxymethyl cellulose, were administered orally directly in the esophagus. The iron content of salts administered was equivalent in all cases (10 mg/kg of animal weight) and concentration of iron.

Blood samples were extracted and centrifugated. The concentration of iron in the blood serum was quantified through atomic absorption after 12h. The area under the curve of the iron concentration (mg/dl) values of the different source of iron is presented.

**Results in figure 5 show LIPOFER™ microcapsules is 3.5X more bioavailable than Ferric pyrophosphate and 2.7X than the control.**

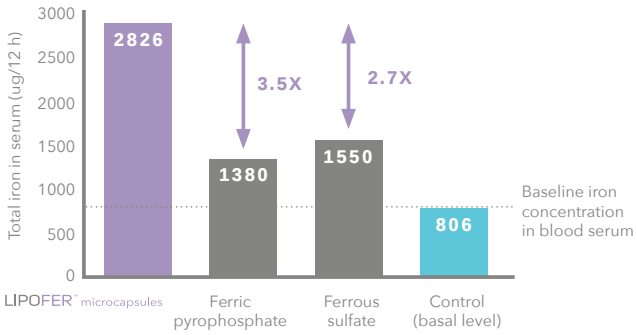



**Figure 5.** Absorption vs ferric pyrophosphate and ferrous sulfate in rats

**A total iron absorption is higher with LIPOFER™ microcapsules, showing to be more bioavailable than other iron sources.**

### b) Efficacy of LIPOFER™ microcapsules vs iron fumarate in mice

A study on three groups of mice was performed to monitor the comparative absorption of iron from iron fumarate, and from **LIPOFER™** microcapsules. Two hours after administration, the blood was collected, and its iron content was further analyzed [45].

The three groups were administered around 200 ml of the test materials orally three times a day. The first group was injected a salt solution, the second ferrous fumarate, and the third **LIPOFER™** microcapsules.

Two hours after administration, the blood was collected, and its iron content was further analyzed by atomic absorption spectrometry (AAS).

**Iron absorption via LIPOFER™ microcapsules is 5X higher than with traditional iron salts as shown in figure 6.**

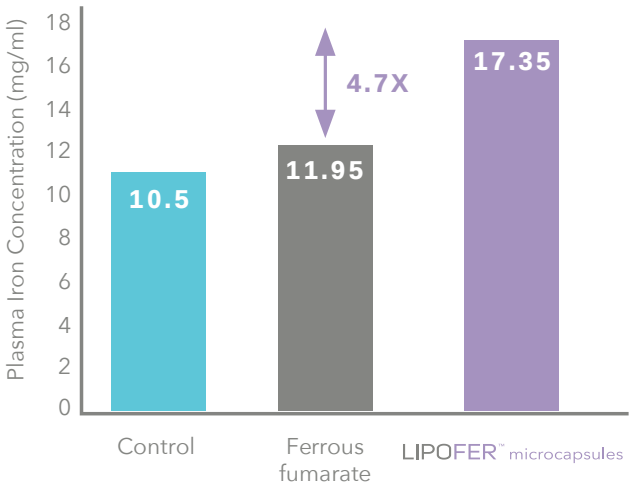



**Figure 6.** Absorption vs iron fumarate in mouse

With **LIPOFER™** microcapsules, a higher increase in the iron absorption is observed.

### c) Juvenile chronic ferric iron ingestion (LIPOFER™ microcapsules) assessment on colitis and dysbiosis at adulthood in rats and mice

In another essay, it was evaluated whether chronic juvenile iron intake leads to colitis and dysbiosis in adulthood in rats and mice. As shown in figure 7, two sets of experiments were designed [46]. In the first group, newly weaned mice were orally administered ferrous iron salt (Fe2 +) or microencapsulated ferric iron (Fe3 +) (**LIPOFER™** microcapsules) for 6 weeks. In the last week of experiments, colitis was induced by trinitrobenzenesulfonic acid (TNBS). In the second group, juvenile rats received microencapsulated ferric iron (**LIPOFER™** microcapsules) for 6 weeks and were also subjected to colitis by TNBS during the last week of experiments.

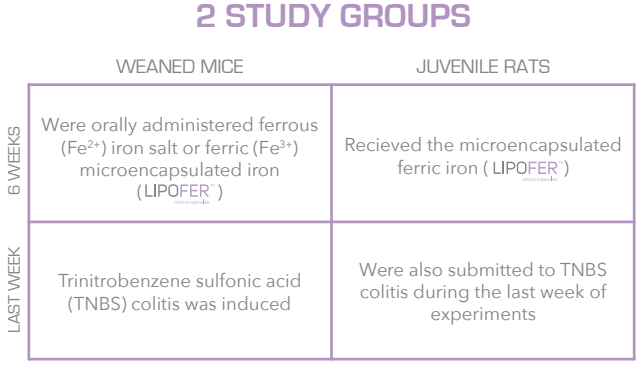



**Figure 7.** Assessment on colitis and dysbiosis at adulthood in rats and mice.

Results show that **LIPOFER™** microcapsules ingestion prevents colitis and dysbiosis at adulthood as assessed by the first interspecies comparison.

**With LIPOFER™ microcapsules there weren´t any negative effects of a repeated administration for 6 weeks (no infflammatory reaction neither microflora proffile modification).**

## HUMANS

### a) Efficacy of LIPOFER™ microcapsules in fortified fruit juice in 130 menstruating women

**The influence of consuming a LIPOFER™ microcapsules-fortified fruit juice on iron status was studied in 130 menstruating women aged 18-35 years with low iron stores (SF40 ng/ml, Hb 110 g/l).**

A randomized, double-blind, placebo-controlled study of 16 weeks of duration was performed [47]. Subjects were randomized into two groups: one group consumed, as a supplement to their usual diet, 500 ml/d of the LIPOFER™ microcapsules-fortified fruit juice (F group, n 64), whereas the other consumed 500 ml/d of the placebo fruit juice (P group, n 66). The fortified juice supplied 18mg Fe/500 ml carton, in the form of microencapsulated LIPOFER™ microcapsules. All juices were fortified with vitamin C. Orange juices provided (per 100 ml) 188 kJ, 0·6 g of protein, 10·5 g of carbohydrate and 19 mg of vitamin C; the Fe-fortified orange juice provided 36mg of Fe, whereas the placebo juice had 0·084 mg. Peach apple juices provided (per 100 ml) 201 kJ, 0·6 g of protein, 11·3 g of carbohydrate and 19mg of vitamin C; the Fe-fortified peach apple juice provided 3·6mg of Fe, whereas the placebo juice had 0·136 mg.

Participants were instructed to alternate between juice flavors (orange juice one day, peach apple juice the next day).

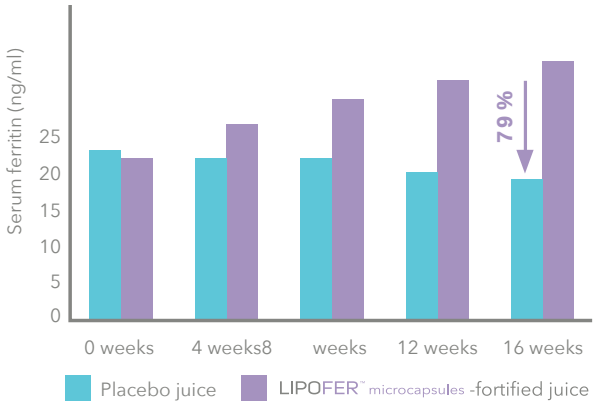



**Figure 8.** Ferritin levels in the LIPOFER™ microcapsules -fortified group vs placebo

For blood analysis, volunteers attended the laboratory facilities at baseline, 4, 8, 12 and 16 weeks. Blood samples were collected by venipuncture after a 12 h fasting period. Serum and plasma were obtained after centrifugation at 1000 g for 15 min and stored at -80°C. At baseline and monthly, dietary intake, body weight and Fe parameters were determined: total erythrocytes, haematocrit, mean corpuscular volume (MCV), red blood cell distribution width (RDW), hemoglobin (Hb) levels, serum Fe, serum ferritin, serum transferrin, transferrin saturation, soluble transferrin receptor (sTfR2) and zinc protoporphyrin (ZnPP) at baseline and monthly.As shown in figure 8, ferritin was higher in the fortified group after 4 weeks and became ~80% higher after 16 weeks (P <0.001). Accordingly, transferrin saturation was significantly higher from week 8 in the F group with respect to the P group.

In figure 9, results showed Haemoglobin concentrations in the F group were significantly higher at week 8 compared to baseline, and at weeks 12 and 16 compared with the P group (P< 0·05).

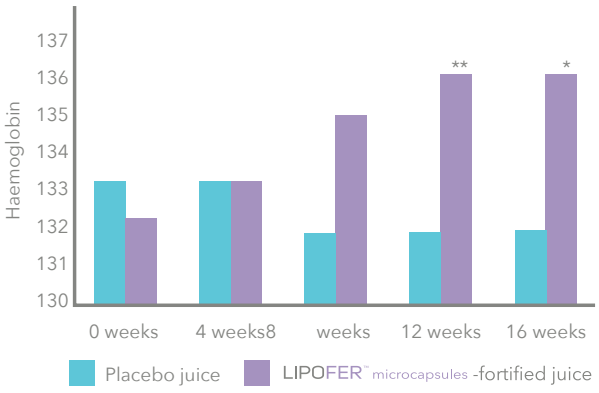



**Figure 9.** Haemoglobin levels in the LIPOFER™ microcapsules group vs placebo

**Daily consumption of LIPOFER™ microcapsules - fortified fruit juice increased iron status in an at-risk population in a short period of time (4 weeks) without side effects.**

**Results show that LIPOFER™ microcapsules fortified fruit juice consumption significantly improved the iron status.**

### b) Efficacy of LIPOFER™ microcapsules bioavailability compared to a drug in humans

The aim of this study was to evaluate the bioavailability of two different iron formulations administered as a single dose. The comparison of the bioavailability of the test formulation 250mg **LIPOFER™ microcapsules** (40 mg elemental iron) with the commercial and well-known pharmaceutical formulation FERPLEX, with 800mg iron III proteinsuccinylate (40mg elemental iron) [48]. As secondary objective, we also report the safety evaluation of two formulations.

The study was conducted as a randomized, open-label (blind for analyst), three periods, crossover, and single-centre study with a washout phase of 7 days. The number of healthy females was 18 and with serum ferritin levels between 9 and 60 ng/ml. The pharmacokinetic properties of **LIPOFER™ microcapsules** and Ferplex iron concentration in blood were measured by evaluating the AUC0-12 (h*ng/ml) (Area Under the time versus serum iron concentration Curve from 0 to 12h after dosing calculated according to the log-linear trapezoidal rule, model-independent approach).

The safety of the **LIPOFER™ microcapsules** was also reported after carefully analysis of adverse events, clinical laboratory evaluation, vital signs, ECG and other observations related to safety.

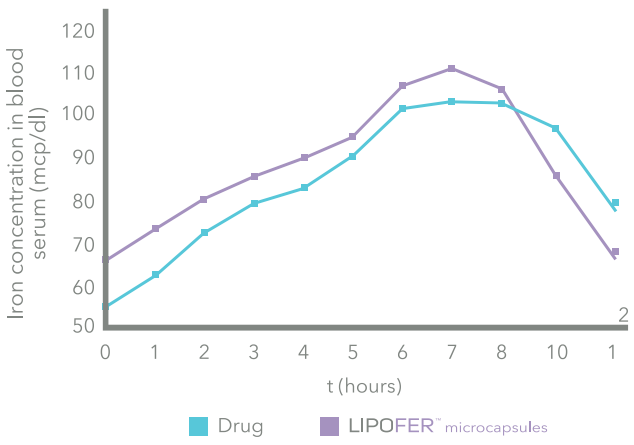



**Figure 10.** **LIPOFER™ microcapsules** bioavailability compared to a drug in humans

**LIPOFER™ microcapsules had a similar absorption profile compared to iron proteinsuccinilate and faster absorption, and it was also well tolerated.**

**LIPOFER™ microcapsules - showed a similar bioavailability to a drug, without side effects at a high concentration.**

### c. Efficacy of LIPOFER™ microcapsules as alternative treatment to intravenous iron in patients with refractory anemia

Randomized study to verify oral iron supplementation treatment in patients affected by refractory anemia is not inferior to intra venous iron support. (n= 24 patients affected by refractory anemia, Hb levels 8.5-11, age= 60-75y, T=12 months). [49]

The group A patients received an iv iron treatment that consisted of sodium ferrigluconate for 1h/day in the day they received alpha erythropoietin 4000UI sc/week + oral supplementation of calcium and vit B12. The group B received an oral iron supplementation that consisted of 2 tablets/day with 14mg Iron (**LIPOFER™ microcapsules**) + alpha erythropoietin 4000UI sc/week + oral supplementation of calcium and vit B12 for twelve months.

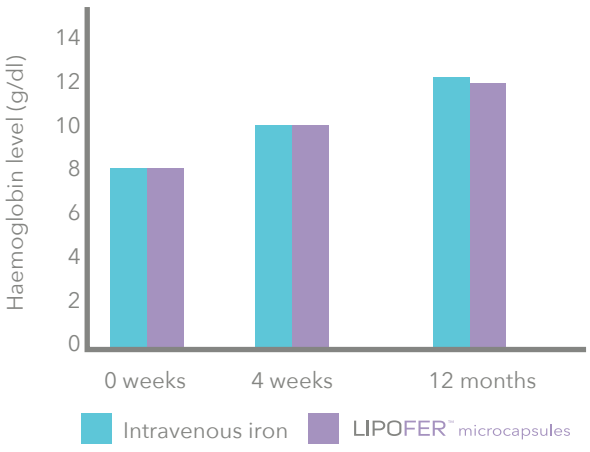



**Figure 11.** **LIPOFER™ microcapsules** oral intake vs intravenous iron support in myelodysplastic patients

**LIPOFER™ microcapsules - support was not inferior to intravenous iron increase after 12 months.**

## APPLICATIONS

### d) Comparative Evaluation of Diagnostic Tools for Oxidative Deterioration of Polyunsaturated Fatty Acid-Enriched Infant Formulas during Storage

Combined analytical study of non-volatile primary products and odor-active secondary products of lipid autoxidation by different analytical techniques with additional aroma profile analysis of 2 base compositions (with/without antioxidants) enriched with LC-PUFA and various of Cu and Fe sources, resulting in total of 18 infant formulas being compared. The mineral sources were: Cu-Lysine complex / Cu-sulphate/ Cu-sulphate encapsulated for cupper and Fe-gluconate / **LIPOFER™** microcapsules / Fe-sulphate encapsulated for iron [50].

The stability of different infant formulas enriched with polyunsaturated fatty acids (PUFAs) to oxidative deterioration was determined. A quantitative study was performed on seven characteristic odor-active secondary oxidation products in the formulations via two-dimensional by 2-dimensional high resolution gas chromatography- mass spectrometry (2D-HRGC-MS/O). Furthermore, photometrical analysis of lipid hydroperoxides (PV) and conjugated dienes and an aroma profile analysis (APA) to reveal presence and intensities of typical odor generated during fatty acid oxidation were carried out. The results can be found in figure 12 and 13.

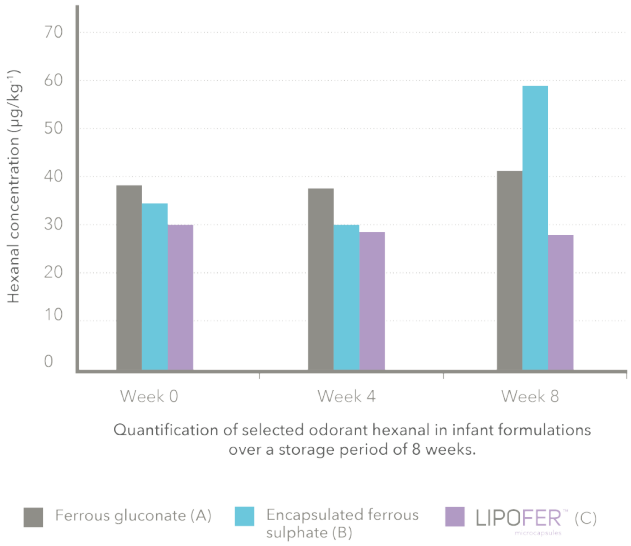



**Figure 12.** Quantification of selected odorant hexanal in infant formulations over a storage period of 8 weeks.

**LIPOFER™ microcapsules formulation was shown to be more stable immediately after production and over storage time with respect to the formulations containing other iron sources.**

**Less secondary lipid oxidation products, such as hexanal detected in the formulas containing LIPOFER™ microcapsules compared to other iron sources.**

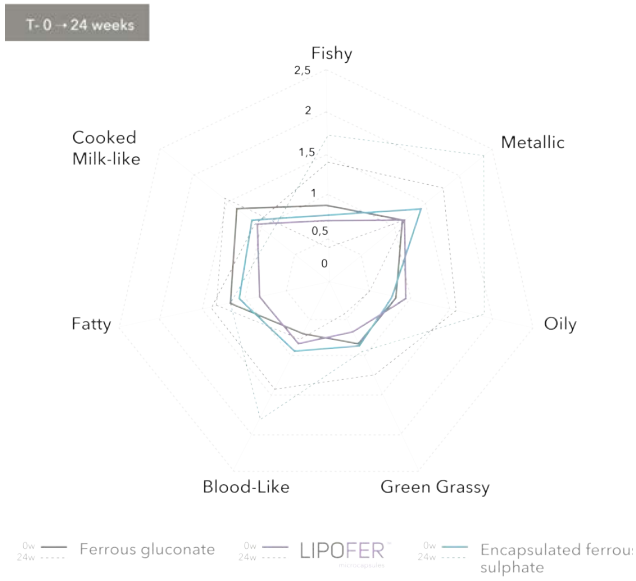



**Figure 13.** Intensity of Odor Quality (Scale 0-3, Mean Values)

**According to the scores obtained via orthonasal evaluation by trained panelists, LIPOFER™ microcapsules formulation remained stable or weakly perceivable over the entire storage period in contrast with other formulations.**

## 6.2 LIPOFER™ APPLICATIONS

### a) Development of LIPOFER™ microcapsules orosoluble sticks

Iron supplementation is one of the most effective recognized ways for the prevention of iron deficiency. However, the development of some formulations can be challenging due to the reactivity of iron, causing difficult product compliance.

**LIPOFER™ microcapsules is a microencapsulated form of iron which permits stable and pleasant tasting products without any side-effects.**

**Powdered formats drive innovation in the dietary supplements market, likely due to their ease of use and portability and because they eliminate the difficulties associated with swallowing large tablets or capsules.**

Orosoluble iron sticks containing **LIPOFER™ microcapsules** with 100% Fe RDA and 20% Vit-C RDA were developed and an accelerated and long-term stability study based on ICH Guidelines (International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use) was performed.

**Table 3.** Formulation of orosoluble stick containing **LIPOFER™ microcapsules**

| Ingredients | % |
|---|---|
| Mannitol | 58.850 |
| Maltodextrin | 25.826 |
| LIPOFER™ DISPERSIBLE | 11.133 |
| Maize starch | 1.800 |
| Ascorbic acid (Vitamin C) | 1.066 |
| Citric acid | 1.000 |
| Flavor | 0.250 |
| Sucralose | 0.075 |


**LIPOFER™ microcapsules enables the formulation of stable and tasty iron-fortified orosolule sticks to consume right away.**

**Simple formulas that can be consumed right away such as orosoluble sticks can be developed with LIPOFER™ microcapsules microcapsules, meeting the consumers' current demand for the on-the-go delivery formats.**

## b) Applications with LIPOFER™ microcapsules

Other possible applications with **LIPOFER™** microcapsules are included in table 4.

**Table 4.** Examples of possible applications with **LIPOFER™** microcapsules

### SYRUP

Most of iron products in the market are not suitable for babies and children and when appropriate they tend to dislike them, or event reject them because of bad taste or side effects.


**LIPOFER™ microcapsules permits to develop organoleptically stable and good-tasting iron drops or syrups without providing side effects.**

### MILK POWDER

UHT milk or milk powder especially for pregnant women is usually fortified with iron and if used as a standard form, interactions may arise.


**LIPOFER™ microcapsules is a microencapsulated form of iron which permits stable and flavored liquid and powder milk for women.**

### CHEWABLE TABLETS

Chewable tablets are designed for use by the children and the older population who may have difficulty swallowing capsules/tablets, or who have digestive complaints.


**With LIPOFER™ microcapsules, organoleptically stable and pleasant tasting chewable tablets without any side-effects can be developed.**

### FORTIFIED FRUIT JUICE

An increasing consumer preference for functional beverages, is driving the innovation for fortified fruit juices.


**LIPOFER™ microcapsules-fortified fruit juices address consumer trends, while meeting demand for nutrient-rich and stable fortified recipes with pleasant taste.**

## 7. PRODUCT HIGHLIGHTS

As shown in figure 14, **LIPOFER™** microcapsules has several technical advantages for formulators and benefits for consumers versus other iron sources.

### Competitive advantages:

- **Water dispersible:** development of powder type products: sticks, sachets, infant formulas, and liquids: drops, syrups, liquid milks
- **Reduced metallic taste:** development of powder and liquid products with significant iron concentration
- **Controlled interactions:** development of more complex formulations

### Benefits for end-consumers:

- **Higher bioavailability:** efficacy demonstrated by clinical studies
- **Lower tract irritation:** purchase repeatability as no side effects
- **Neutral taste:** higher acceptance by the final customers

### Technical information

The composition and different versions of **LIPOFER™** microcapsules can be found in table 5.

**Table 5.** Different versions of **LIPOFER™** microcapsules

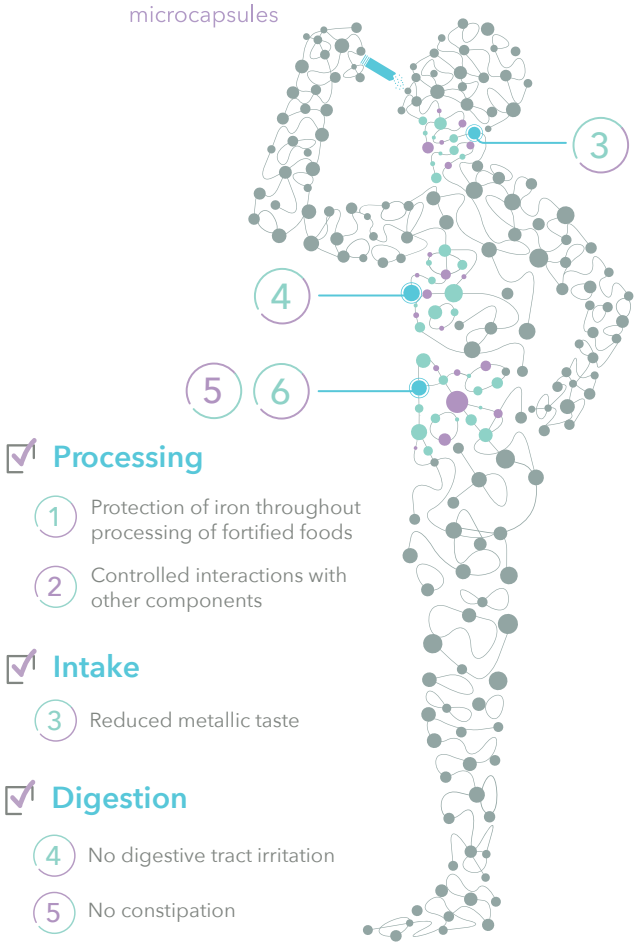
| |
|---|
| LIPOFER microcapsules NA DISPERSIBLE (LI0068) |
| LIPOFER microcapsules NA DISPERSIBLE GRANULATED (LI0075) |
| LIPOFER microcapsules NA DISPERSIBLE PLUS (LI0078) |
| LIPOFER microcapsules NA DISPERSIBLE INFANT (LI0080) |

**Description**
WATER DISPERSABLE coated micronized Iron Pyrophosphate

**Concentration**
8% Elemental Iron

**Certificates**
Halal and Kosher

LIPOFER™ microcapsules




**Processing**

1. Protection of iron throughout processing of fortified foods
2. Controlled interactions with other components

**Intake**

3. Reduced metallic taste

**Digestion**

4. No digestive tract irritation
5. No constipation

**Absorption**

6. Highly bioavailable iron

**LIPOFER™ microcapsules is a water dispersible micronized source of iron that has been micro-encapsulated to enhance iron absorption and reduce undesirable organoleptic attributes, thus enabling the enrichment of various types of foods and dietary supplements.**

Milk powder, dairy products and dietary supplements including drops and syrups.

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