B A R S
Bulletin of Applied and Research Science
ISSN: 1800-556X
Bars journal
Volume 4, Number 2, 2014
Online ISSN 1800-556X
Journal's URL:http://www. barsjournal.net

Preparation of liposomal ferrous sulfate nanocapsules by Reverse-Phase Evaporation Method and nanocapsules structure analysis to apply in the food and medicinal industries

Azadeh Izadyari[*1], Azim Akbarzadeh[2], Ali Vaziri[3], Hosein Attar[3], Abdolhasan Alavi[3]

[1]M.Sc Student, Department of chemical Engineering, Science and Research branch,Islamic Azad university, Tehran, Iran, E-mail : izadyari_a@yahoo.com
[2] Professor, Pilot Biotechnology, Pasteur Institute of Iran, Tehran, Iran, azimakbarzadeh@pasteur.ac.ir
[3] Assistant Prof, Department of chemical Engineering, Science and Research branch, Islamic Azad university, Tehran, Iran, E-mail: a.vaziri@srbiau.ac.ir

Abstract : Iron is a vital metal for doing body essential activities. But reacting this metal with other nutrients or medicines can prevent iron effective absorption in body. One of the best ways for increased iron absorption is the encapsulation. This method minimizes iron interactions and maximizes its absorption. In the research for this objective achievement, Liposome used as nanoparticles cover, due to two-layer phospholipid structure and its similarity with cell membrane, low natural toxicity, biocompatibility and biodegradability; and Polyethylene glycol 2000(PEG_{2000}) due to increasing nanocapsules structure stability. Also Ferrous sulfate selected as nanoparticles core, due to increase in amount of absorption than other ferrous components and effective solubility in the water and the digestive system. Then nanocapsules were prepared by reverse-phase evaporation method and were separated by Refrigerated Centrifuge(30 min, 4˚C, 14000 rpm) and were finally dried with Lyophilized-Edwards high vacuum devices(-20˚C). Physical structure stability, diameter and morphology of nanocapsules were respectively examined by zeta-sizer and Scanning Electron Microscope(SEM). Results showed, all synthetic nanocapsules had the spherical form, diameter about 300 nm and high stability to produce dietary supplementary tablet. In addition, amount of Encapsulation Efficiency (EE) and loading were respectively obtained 75.55% and 7.98%. As a result nanocapsules core will have the most physical stability and structure durability to release medicine in special part of the body. Also, this synthetic product will probably resolve apparent and organoleptic problems which are originated iron in most of the industries especially food and medicinal industries.

Key words: iron deficiency anemia, Ferrous sulfate, liposome, encapsulate

1. Introduction

Nowadays more than half of the population in the world affected by minerals and vitamins deficiency. These compounds, with small doses and supplements form, are playing an important role in persons general health. In the meantime iron as an essential metal is responsible for carrying oxygen and controlling biochemical reactions in the body. Also this metal is one of the important factors for the synthesize DNA, Enzymes, Collagen etc. which is absorbed in the stomach and the beginning of bowel, after reduction and conversion of Fe^{+3} to Fe^{+2}. But reduction process is disrupted by pH increase in the stomach and iron absorption is decreased in the bowel, thereupon iron deficiency anemia occurs. This disease is seen, as important health problem, at all ages and in all countries. For preventing this kind of anemia are used iron compounds such as Ferrous sulfate, Ferrous gluconate, Ferrous fumarate and Ferrous succinate. As respects these compounds, in the dietary supplementary form, can cause side effects (for many person) like stomach discomfort, constipation and the digestive system irritation; food enrichment process was introduced with iron. This method was began to prevent and control side effects of micronutrient

deficiency in 1831. Then Philippines enriched rice with vitamin B_1 in 1947, New Zealand enriched wheat and maize flour with the iron, for decreasing iron deficiency anemia, in 1993 [1] and New Madrid area in Canada enriched flour with vitamin B_1 and B_2 in 1994 [2].

Although food enrichment, with micronutrients, was effective method to compensate decrease of various nutrients in the body; but this process caused color and taste changes of food [3]. Therefore new technology of microencapsulation was proposed. Encapsulation includes entrapment of pharmaceutical, enzymes, micronutrients etc. in small capsules and its protection against physical and chemical factors such as moisture, heat and probable reaction [4]. Thus, iron encapsulation would be potentially help to overcome an important and basic challenges in the food enrichment or dietary supplementary tablet production. Also this method could reduce iron interactions with other components and its unwanted changes [5].

Encapsulation is the result of thousands of techniques and thoughts. Components are encapsulated to fall into the trap, in the capsule wall for a specific time, and release in a specific point of the body. First time, encapsulation was examined by Mr L.Schicieher and B.K.Green in 1930 [6] and was used for Pressure- Sensitive covers in 1950 [8-9]. Han and Shahidi proposed a good overview, for components capsulation and their application in food and medicinal industries, in 1993 [10]. Also, Zimmermann used the encapsulated Ferrous Sulfate and Ferric Pyrophosphate salts in cereals, in 2003 and 2004 [11, 12]. Nowadays, Chile and Argentina enriches milk with encapsulated iron and ferrous compounds [12-14].

Various covers are used in encapsulation process to protect nanoparticles core. In the meantime, Liposome is taken into consideration due to low natural toxicity and biocompatibility with body [15]. Those are phospholipid vesicles of two layers which are not eliminated by the immune system due to its similarity to the structure of cell membrane, as a result liposome will be effective for encapsulation process. Actually, the aim of this research is the using encapsulation techniques for ferrous salt (Ferrous sulfate) and producing iron nanocapsules to decrease its interactions in food and supplements tablet and obviation of iron deficiency anemia. Therefore Ferrous sulfate as the nanoparticles core, Liposome as capsules cover and Polyethylene glycol 2000 (PEG_{2000}) for nanoparticles physical stability were used in the structure of nanocapsules. afterward stability, morphology and diameter of liposomal nanocapsules were studied to use in various industries such as food and pharmaceutical industries.

2. Material and Methods

Ferrous sulfate powder was purchased Serva Heidelberg- New York company, Phosphate Buffered Saline (PBS) pH=7.3 provided in Department of Pilot nano-bio technology- Pasteur institute of Iran, Polyethylene glycol 2000, Ethanol (96%), Cholesterol and Lecithin (phosphatidylcholine) were purchased from Merck Darmstadt Germany company. In this research was used Rotary evaporation (RV 10 Digital Rotary Evaporator IKA Company- Germany) to evaporate ethanol, zeta sizer (HSA3000 model- Malvern company) to measure diameter and structure stability of nanoparticles and Scanning Electron Microscope (JEOL JSM-840 -SEM) to examine nanocapsules morphology.

3. Theory

3.1. Samples Preparation

3.1.1. Preparation of empty liposomes:

100 mg Lecithin, 10 mg Cholesterol, 10 mg PEG_{2000}[1] were dissolved in 40 ml Ethanol. The solution was situated on Stirrer for 24 hours. Then, ethanol was evaporated by Rotary Evaporation in 40˚C, 30 min and Reverse-Phase Evaporation (REV) method.

3.1.2. Preparation of liposomes containing ferrous sulfate:

100 mg Lecithin, 10 mg Cholesterol, 10 mg PEG_{2000}, 10 mg Ferrous sulfate were dissolved in 40 ml Ethanol. This solution was situated on Stirrer for 48 hours. In next step, ethanol was evaporated by Rotary evaporation in 40˚C, 40 min and REV[2] method.

[1] Polyethylene glycol 2000

[2] Reverse-Phase Evaporation

Afterward, 15 ml Buffered Saline (PBS) were added to above-mentioned solutions. those were situated on stirrer (15 min) and bath-sonication(10 min), for the better penetration, dispersion and compressibility of particles.

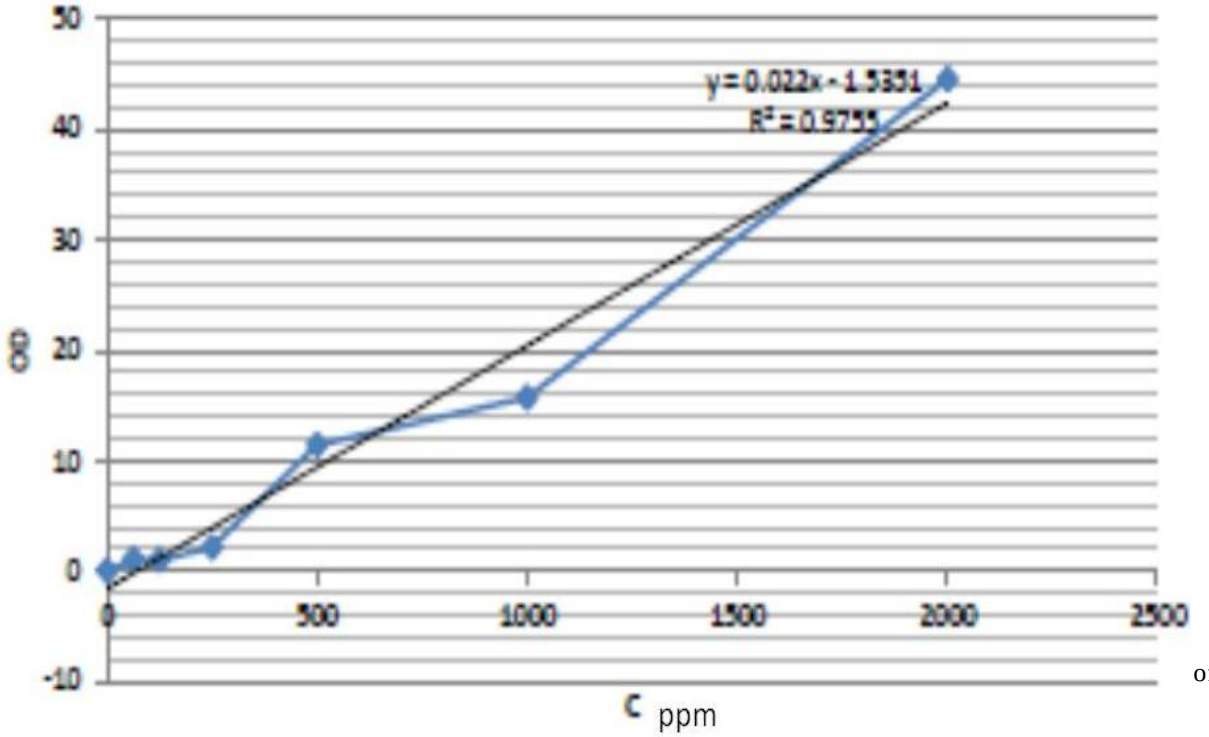
3.2. Separation and absorption determination of nanocapsules:

Ferrous sulfate which was provided according to above method, situated on Refrigerated Centrifuge(GRX-220 model- Tomy) for 30 min, 4˚C and 14000 rpm. Then supernatants amount of absorption was measured by atomic absorption (GBC932AA model, Australia) in 248.3 nm.

4. Results

4.1. Preparation of standard solution and plotting standard curves

40 mg Ferrous sulfate powder were dissolved in 20 ml PBS. The mentioned solution was diluted six times and their absorption were measured by atomic absorption in 248.3 nm and standard curves was plotted by Excel software to calculate the standard equations, Encapsulation Efficiency (%EE) and Loading. Result is shown in figure.1



of

4.2. Zeta Potential (ζ-potential) measurement

For tow solutions (Ferrous sulfate and control), amount of absorption were measured by Spectrophotometer (in 630 nm). Then samples were situated on zeta sizer to determine zeta potential (ζ-potential), nanoparticles diameter, nano-liposomes physical stability and structure durability. results are showed in Table 1 and figure 2.

Table. 1: Amount of Zeta Potential (ζ-potential)

Nanocapsules	Empty liposomes and PEG_{2000} in structure	ferrous sulfate liposomes and PEG_{2000} in structure

ζ-potential	-17.8	-21.098

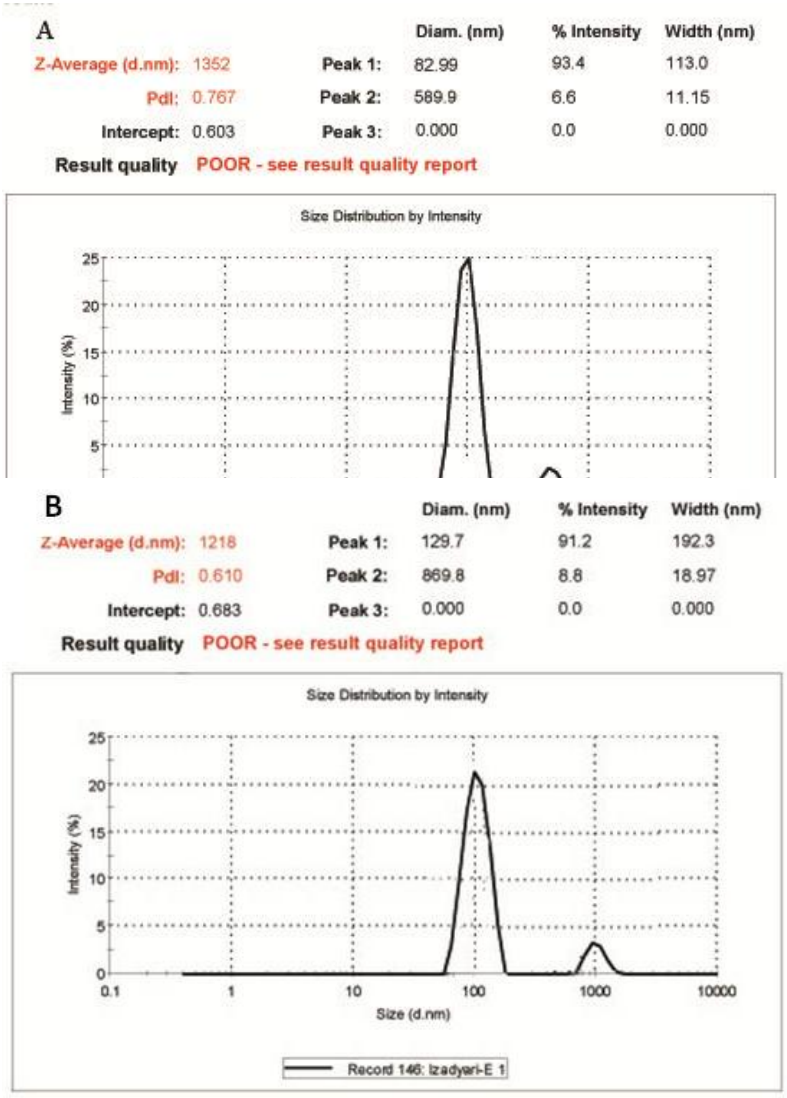
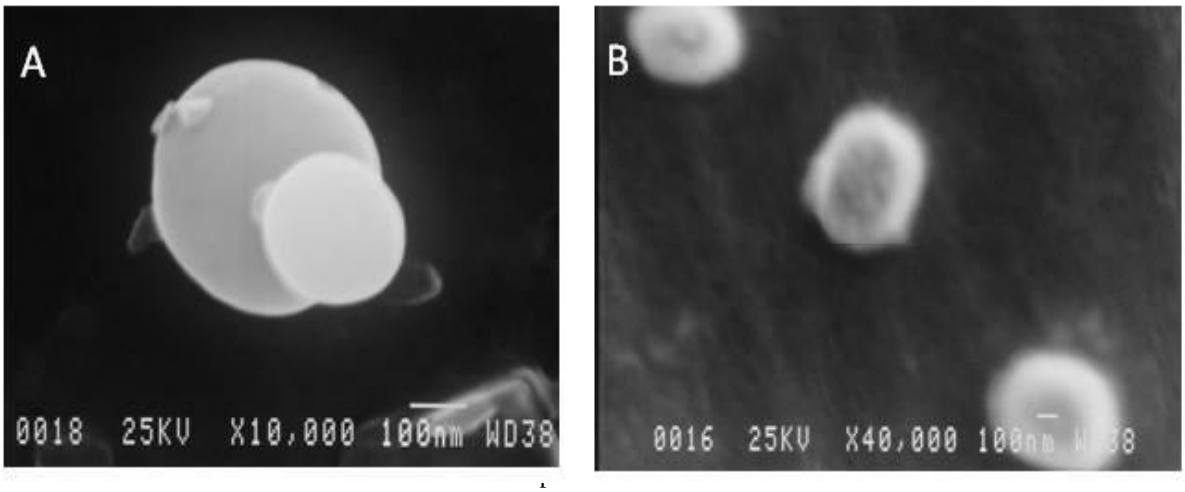


Figure. 2: (A) is showing diameter of control sample (empty liposomes) and (C) is showing diameter of ferrous sulfate sample (ferrous sulfate liposomes)

4.3. Determination of nanocapsules morphology

Nanocapsules which were ejected from centrifuge, were dried by Lyophilized-Edwards high vacuum devices(P.2.T.S model) in -20˚C and their morphology were examined by Scanning Electron Microscopy (SEM). Results of this study are showed in figure 3.



4.4. method of Encapsulation Efficiency and Loading measurement:

Encapsulation Efficiency and Loading were calculated according to the following equations.

$$\%\text{Encapsulation Efficiency} = \frac{C_{fe,\,total} - C_{fe,\,encapsulate}}{C_{fe,\,total}} \times 100 \quad (1)$$

$$\%\ \text{Loading} = \frac{W_{fe,\,capsule}}{W_{material,\,total}} \times 100 \quad (2)$$

Actually, amount of encapsulation efficiency is iron encapsulated in liposomes to total iron in liposomes and amount of loading is iron entrapped to total components.
Based on these two equations, amount of Encapsulation Efficiency (%EE) and loading were respectively calculated 75.55% and 7.98%

5. Discussion

Iron is a vital micronutrient for the body and its deficiency could cause iron deficiency anemia. The main reasons of this disease are attributed to use insufficient of iron (poor utilization of iron), its inadequate absorption, or both causes [16]. So far, the different methods have been proposed to overcome iron deficiency diseases such as diet modification, using dietary supplements, foods enrichment (adding micronutrients to foods). Also many researches have been done for encapsulation of iron salts and their bioavailability, in different countries. Nowadays micro-encapsulation is used in the food industry (to maintain flavor, foods durability) and the pharmaceutical industry (to release medicine in special part of the body). Since the prevalence of iron deficiency anemia is high, in developing countries and in Iran, in this study was used encapsulation method to create nano-sized capsules (for enrichment of food and medicines) and their application in supplement tablet for batter absorption of iron.
To achieve this aim, the liposome (above method) was used as the nanoparticles cover, for the first time. Because liposomes as carriers of nutrients, have an important advantage which is compatible with the body (due to the similarity of liposome two-layered structure with the cell membrane) and Ferrous sulfate selected as nanoparticles core, due to increase in amount of absorption than other ferrous components and effective solubility in the water and the digestive system.

6. Conclusion

In this study is used phosphate buffer to dissolve the compounds and polyethylene glycol 2000 (instead of Tween80) and cholesterol for the physical stability and structure durability of nanocapsules. Nano-liposomal carriers which were produced in this research, had optimal diameter (about 300nm), stability structure and amount of encapsulation

efficiency (%EE) and loading respectively 75.55% and 7.98%. Thus, these will be effective in food enrichment or production of pharmaceutical supplements.

Acknowledgement

We are grateful Dr. Ebrahimi and Dr. Khosroyar for theirs valuable guidance.

Reference

[1] Layrisse,M., Cháves, JF., Méndez-Castellano,H., Bosch,V., Tropper,E., Bastardo,B., Gonzalez,E. Early response to the effect of iron fortification in the Venezuelan population. Am. J. Clin. Nutr., 64; 972-3, 1996.
[2] Allen,L., Benoist,B., Dary,O., Hurrell,RF. Guidelines on Food Fortification with Micronutrients. World Health Organization and Food and Agriculture Organization of the United Nations., ISBN 92 4 159401 2, 2006.
[3] Diosady,LL., Alberti,J.O , Venkatesh Mannar, MG. Microencapsulation for iodine stability in salt fortified with ferrous fumarat and potassium iodide, Food Research International 35; 635-642, 2002.
[4] Gonnet,M., Lethuaut,L. & Boury,F. New trends in encapsulation of liposoluble vitamins, Journal of Controlled Release, 146; 278, 280-281, 286, 2010.
[5] Nedovic,V., Kalusevic,A., Manojlovic,V., Levic,S. & Bugarski,B. An overview of encapsulation technologies for food application. Procedia FoodScience, 1; 1806-1815, 2011.
[6] Zhongxiang Fang and Bhesh Bhandari. Encapsulation Techniques for Food Ingredient Systems, Food Materials Science and Engineering, Ferst Edition by Bhesh Bhandari and Yrjo H. Roos. 320- 325, 2012.
[7] Ronald J., Versic. Coacervation for flavor encapsulation. Ronald T. Dodge Company. Flavor Encapsulation., 14:126–131, 1988
[8] LuzziL,A. Microencapsulation.J.pharm.Sci ., 59 ; 1357-1376, 1970.
[9] Benita,S. Microencapsulation Mathod and Industrial Application, Marcel Dekker Inc., N.Y., USA, 756, 2006.
[10] Schooyen,PMM., Meer,VD. and Kruif,D. Microencapsulation : Its application in nutrition. Proceedings of the Nutrition Society; 60; 475-479, 2001.
[11] Zimmermann,MB., Zeder,C. , Chaouki,N., Saad,A., Torresani,T., Hurrell,RF. Dual fortification of salt with iodine and microencapsulated iron: arandomized, double blind, controlled trial in Moroccan schoolchildren.Am .J .Clin .Nutr .,77;425–32, 2003.
[12] Zimmermann, MB., Wegmueller, R., Zeder, C., et al. Dual fortification ofsalt with iodine and micronized ferric pyrophosphate: a randomized,double blind, controlled trial. Am .J .Clin. Nutr, 80; 952–9, 2004.
[13] Rita WM , MichealB,Zimmerman , Diego M , Myrtha A, Wolfgang L,Hurrell RF. Particle size reduction and encapsulation effect the bioavailability of ferric pyrophosphate in rats. AM.J.Sci.Nutr. ,134; 3301-3304, 2004

[14] Chunming, C. ,Hertrampf.E. ,Olivares,M., Pizarro, F. & Walter, YT. Iron fortification of milk in Chile. Food, Nutrition and Agriculture, no. 32, 2003.
[15] Marsanasc,M., Marquez,A. Wagner,J., Alonso,S., Chiaaramoni,N. Liposomes as vehiches for vitamins E and C : An alternative to fortify orange juice and offer vitamin C protection after heat treatment. Food Research International, 44; 3039-3046, 2011.
[16] Gaucheron, F. Iron fortification in dairy industry. Trends in Food Science and Technology, 11; 403–409, 2000.