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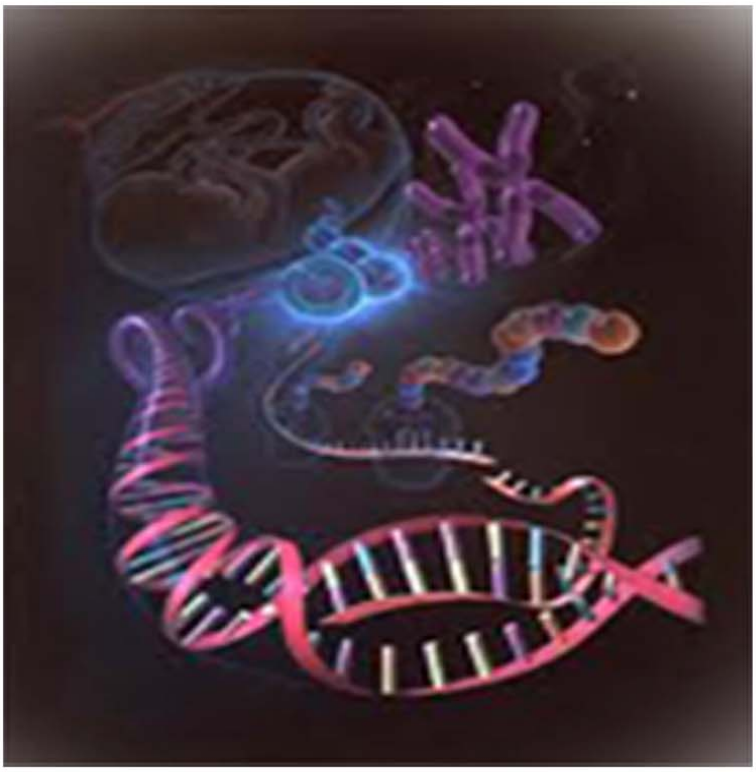

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Research Paper

THE STUDY OF DISPERSION METHOD IN THE FORMATION OF FERRIC SACCHARATE MICROCAPSULES WITH ALGINATE COATING

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About a fourth of the people in the world suffer from anemia. The most major and effective ways of reducing anemia and iron deficiency in the society are iron fortification of food and improving the iron absorption. In this method a certain amount of iron in the form of capsulation is added to the food ingredients which are the main food of the people and are eaten every day. Encapsulation will decrease undesirable changes and iron interaction with the other food ingredients, moreover can usefully affects the bioavailability of iron. The present study describes iron fortification of hydrated and dehydrated food products in which ferric saccharate is as a core and calcium alginate is as a capsule coating. To evaluate the performance range, time and size of the microcapsules, the dispersion method was compared in three ways of extrusion with nozzle, extrusion with needle and emulsion. After studying the experiments and their results, it was observed that microsized capsules can be produced using the extrusion with needle and extrusion with nozzle methods also nanosized capsules can be obtained using the emulsification and spray methods.

Keywords: Capsulation, Ferric saccharate, Alginate, Dispersion

INTRODUCTION

One reason of improving the iron is that it should satisfy some requirements, for example it should has high absorbability (solve in gastrointestinal tract well) and be harmless and stable in the body. When iron compounds (with high absorption power) is added directly to the food ingredients will cause problems such as oxidation, taste change (metal taste) and will bring about the storage time shortening of food gradients, finally result in organoleptic problems creation such as change in taste, color, and so on. Some foods contain inhibitors (phytate) which reduce iron absorption (Monsen, 1988) Modern technology of iron fortification namely encapsulation can be utilized to remove these problems. Encapsulation

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decreases iron interaction with the other food ingredients thus minimize the oreganoleptic changes, increases the beneficial lifetime and stability of products and indirectly prevent fat oxidation also usefully affect the iron bioavailability (Hurrell, 1999).

The appropriate selection of coating materials, layer (surface and compression), the method of process and storing/transferring process can increase the performance, and selection of proper kind of capsulation can affect considerably the cost and concepts and the range of performance as well (Duxbury and Swientek, 1992).

The bioavailable iron salt, with more preference, is selected from ferrous sulphate and ferric saccharate. Ferrous sulphate has a high absorption power but it's probable interaction with foods leads to the reduction of iron absorbance and change in color and odor of the food ingredients thus more preferably, ferric saccharate is selected as the iron salt (Hurrell, 1992; Jalil and Nixon, 1989; Dominguez *et al.*, 2004). Chelating agent results in the improvement of the iron absorption, it is used as core in this study. The selected chelating agent can contain tartaric acid, malic acid, succinic fumaric acid, citric lactic, oxalic acid or a salt thereof, EDTA, saccharose and more preferably ferric saccharate (Olivares *et al.*, 1997; Hurrell *et al.*, 2004; Chen and Oldewage-theron, 2002).

Coatings are sublayers which protect fortified foods from interaction between iron and absorbance agents in food (Hurrell *et al.*, 2004; Lee *et al.*, 2003; Janda et al., 1995). Alginate is the best option for coating compounds due to its colloid exclusive properties such as stability, suspension, creation a thin layer of film, gel production and emulsion stabilization. Alginate with a high content of guluronic acid blocks is preferable for capsule formation because of their high mechanical stability, high porosity and tolerance to salt and chelating agents (Nicetic *et al.*, 1999). Calcium alginate as edible coating in addition to a significant decrease in microbial load and amount of volatile nitrogen results in the reduction of spoilage rate and increase in the lifetime (Smidsrod and Skjak-Braek, 1990). Calcium alginate is a natural food fiber which it's consumption not only prevents many digestive problems (constipation, bloat, diarrhea) during iron take, but decreases fat in the body as well (De Vos *et al.*, 1997; Gholami, 2010).

In the present study, ferric saccharate iron salt with high bioavailability was selected as the active material of core and calcium alginate as the capsule coating. When a water-soluble iron salt enters in contact with water-soluble alginate salt, cross linking and gel formation of carboxylic group of the alginate takes place with the iron cation (ferric or ferrous). When a core comprising iron alginate is placed in contact with a water solution of calcium salt, a capsule (formed by the core covered with an outer layer comprising calcium alginate) is formed by reaction of the alginate salt with the calcium cations (Silva *et al.*, 2006; De Vos *et al.*, 1997; Annan *et al.*, 2008).

The present invention can produce nano- and microsized capsules and three methods are proposed for its production which it can be used in food and medicinal industries according to its micro or nanosize. This capsulated product can be applied in hydrated and dehydrated food products. Initiative includes formation of iron in form of ferric saccharate capsules with calcium alginate coating. Capsulation was performed by three methods of emulsification with oil, extrusion with needle (stirrer) and extrusion with nozzle (spray).

MATERIALS AND METHODS

Sodium alginate with medium viscosity (Cas No. 9005-38-3) was prepared from the sigma Aldrich (Germany) and calcium chloride ($CaCl_2$) (Cas No. 2380) was bought From the Merck (Germany).

Ferric saccharate iron with average molecular mass of 45200Da (Cas No. 8047-67-4) was parched from a Shanghai Boyle Chemical Company (China) and Tween 80 from Fluka Biochemical, (Germany), and vegetable oil from Varamin Co. (Iran). All other chemicals used in this paper were analytical reagent grade. Ultra pure water from Mili-Q water system was used to prepare the aqueous solutions.

Encapsulation by Extrusion with Needle

At first, the solution of alginate 1.5% was prepared and 0.798 g iron salt was added to it (70:30 ratio of coating to core) and was turned at the high rotation of stirrer at 500 rpm to obtain uniform solution of iron and alginate then this solution was added drop wise using a fine needle (by the smallest needle) to 300 ml of calcium chloride salt 1 M. Upon adding the iron- alginate solution, calcium ion replacement to sodium took place consequently, capsules formed in calcium alginate coating.

Finally, capsules were filtered under vacuum condition and were washed thrice with distilled water (was distilled twice) till the separation of free ions which adhered to the capsules, take place (Janda *et al*., 1995).

Encapsulation by Extrusion with Nozzle

At First, the solution of alginate 1.5% was added to 0.798 g iron salt and was placed at the high rotation of stirrer in order to obtain uniform solution. Next, the uniform solution entered into the spray with atomizer nozzle, and then this uniform solution is sprayed under the high pressure from very narrow nozzles to 300 ml solution of 1 M $CaCl_2$ which is turning on the stirrer. Upon adding the iron alginate solution into the solution of $CaCl_2$ salt, sodium ion replace to calcium. Iron capsules will be formed in fine-seized (micro and nanoseized) in calcium alginate coating, afterwards capsules were washed three times with twice distilled water and were collected and dried (Gibbs *et al*., 1999; Benoit *et al*., 1996).

Encapsulation by Emulsification with Oil

In a solution of 1.5 g of sodium alginate in l00 ml of water, 0.798 gr ferric saccharate (at a coating to core ratio of 70 to 30) was dissolved. The mixture of sodium alginate/ferric saccharate was added drop wise into 500 ml of oil solution (at a water to oil ratio of 1:5) which contains 2.5 g Tween 80 (0/5:100 Tween: oil), was put at 900 rpm of stirrer for 20 min.

After the solution of alginate and iron was added drop wise to the oil phase and was put at 900 rpm for 30 min, calcium chloride salt was slowly added to the solution of oil, alginate and iron, followed by 20 min, the stirrer was turned off and was put at the rest condition for 30 min in order to occur oil/water phases separation. Then the oil and water phase was discarded and capsules were washed three times with distilled water which was distilled twice at centrifuge at 3000 rpm for 15 min, afterwards the capsules were collected, washed and dried (Lee *et al*., 1997; Silva *et al*., 2006; Annan *et al*., 2008).

Morphological Characterization, Size and Surface Charge

The morphological characteristics of micro-spheres were examined by scanning electron micros copy (JSM-5900Lv, JEol, Japan). Microspheres were sputtered with gold and maintained at room temperature for complete dryness before the observation. The particle size distribution was detected by laser diffraction (Nano ZS90, Malvern Instrument, UK; BT-2002 laser particle size Analyzer, Dandong Better size Instruments LTD, China).

Encapsulation Efficiency

The loading efficiency (LE) value was calculated according to the following equation:

$$LE(\%) = \frac{Total\ amount\ of\ Fe - free\ Fe}{Total\ amount\ of\ Fe} \quad ...(1)$$

In Vitro Release Studies

An amount of microspheres equivalent to 10 mg of Fe was placed in glass vials containing 50 ml of artificial gastric fluid (USP XXVI), without enzyme, under magnetic stirring (100 rpm for 2 hours). Microspheres were then transferred into artificial intestinal fluid (USP XXVI), without enzymes. Samples were withdrawn at different time intervals and spectrophotometrically assayed for the Fe concentration as mentioned previously (Huang *et al.*, 1999). Released Fe was calculated accordingly to the following equation:

$$Fe\ release = M_t\ Min \times 100 \quad ...(2)$$

where M_t is the amount of *Fe* at time *t* and Min is the amount of *Fe* in the microspheres at time *t* = 0. All studies were performed in triplicate.

RESULTS AND DISSCUTION

Morphology of Prepared Microcapsules by Extrusion with Needle

Formed capsules by this method at 500 rpm using a stirrer were in microsized. By repeating the experiment at the rotations below than 2000 rpm (300, 500, 750, 1000, 1500), it was found that the stirrer rotation affect more morphology (spherical shape) and surface (Figure 1) distribution of capsules, but capsules size were affected by the output of injector agent of iron alginate solution into the solution of $CaCl_2$ salt. In this method, droplet formation occurs in a controlled manner (Dong *et al.*, 2007).

Morphology of Prepared Microcapsules by Extrusion with Nozzle

In this case similar to emulsification method capsules can be produced in nanosized but with less tools and time with uniform surface distribution than emulsification method. In this method by changing the nozzle, microcapsules can be formed in nano- and microsized (Figure 2).

Figure 1: SEM Images of Ferric Saccharate Microcapsules Prepared by Extrusion Method Through Needle

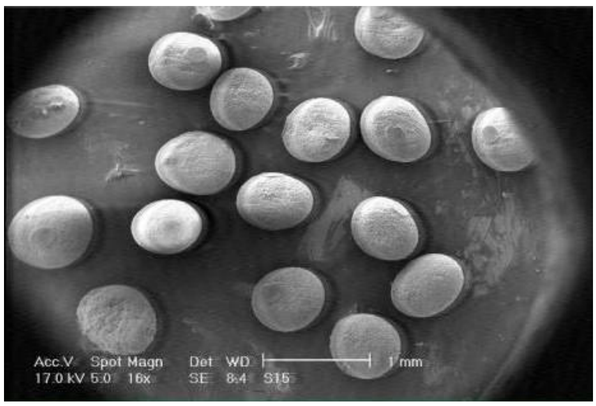
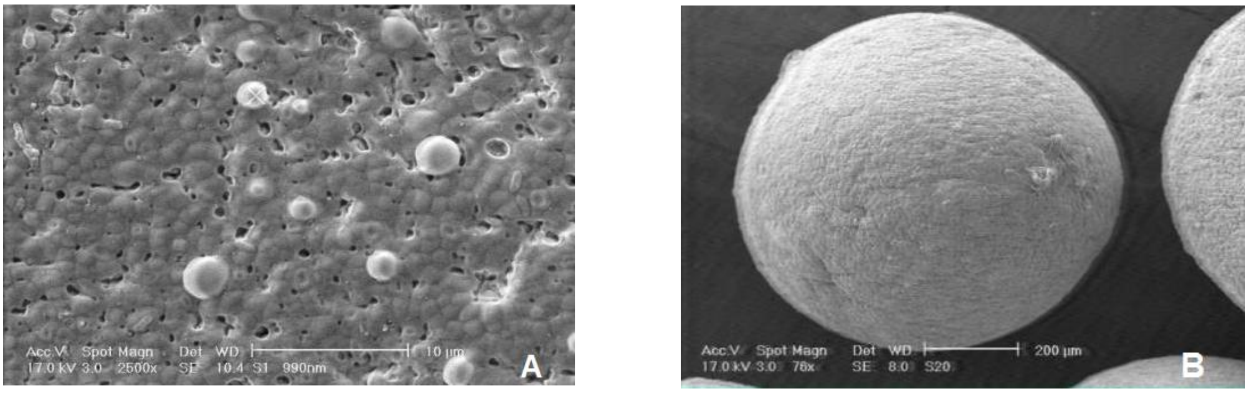


Figure 2: SEM Images of (A) Nanocapsules and (B) Microcapsules Prepared by Extrusion With Spray Nozzle



Morphology of Prepared Capsules by Emulsification

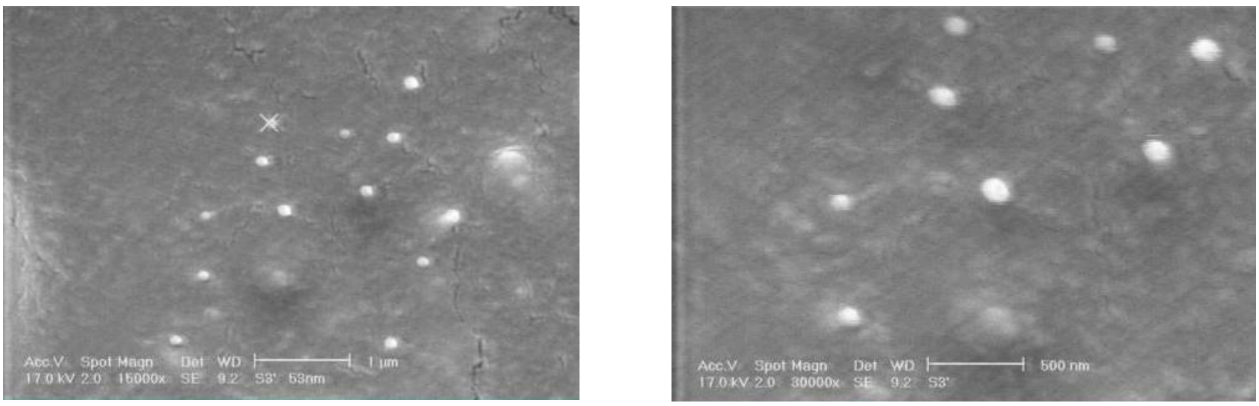
The presence of oil phase in this method, prevent the large particles formation, thus very fine capsules in nanosized (Figure 3) will be produced and would be suitable for high scale production (Groboillot *et al*., 1994). The experiment of emulsification with oil was repeated with and without Tween 80, although had no effect on the particles size but had little effect on morphology and a high percentage of capsules were spherical and a small amount of capsules were oval in shape. Most of the microencapsulation laboratory procedures were reported involved water-in-oil (w/o) emulsion technology (Khalida *et al*., 2000).

Encapsulation Efficiency

In extrusion methods, upon spraying the alginate-iron solution into the calcium salt solution which is located on the mixer, capsules are formed and by washing the capsules, existing free ions on the capsules can be washed. Extrusion method is controlled manually and all the injected droplets turned to capsules, also in the spray method all the solution is sprayed into the calcium chloride solution and capsules are formed. Similar results were obtained by the other researcher in controlled spray which was performed manually (Heinzen, 2002).

As it is seen from comparing the efficiency of

Figure 3: SEM Images of Microcapsules Prepared by Emulsification Method



three methods (Figure 4), both extrusion methods have higher efficiency than the emulsification method. Emulsification method has a lower efficiency in compared to two extrusion methods because in this method, three phases of water, oil and capsules exist at the end of capsules formation. Since produced capsules were very fine, an amount of capsules maybe eliminated during washing by centrifuge.

In Vitro Release Rate

Figure 5 demonstrates the release rate in three methods. As it was observed in the release figure (Silva *et al*., 2006), the release time in emulsification and spray methods is more than coacervation method. As was seen in morphology experiment, the resulting capsules in emulsification method were the finest and the smaller particles size, the more is the release rate.

This outcome is consistent with the research of the relation between particle size and sustained release rate by yamamoto (2002). The reason should be that smaller particle size microcapsules would have larger specific surface area, therefore causes its release rate to be faster than that of larger particle size microcapsules (Hsieh *et al*., 2006).

Figure 4: Encapsulation Efficiencies of Microencapsulation by Coacervation, Spray and Emulsion Methods

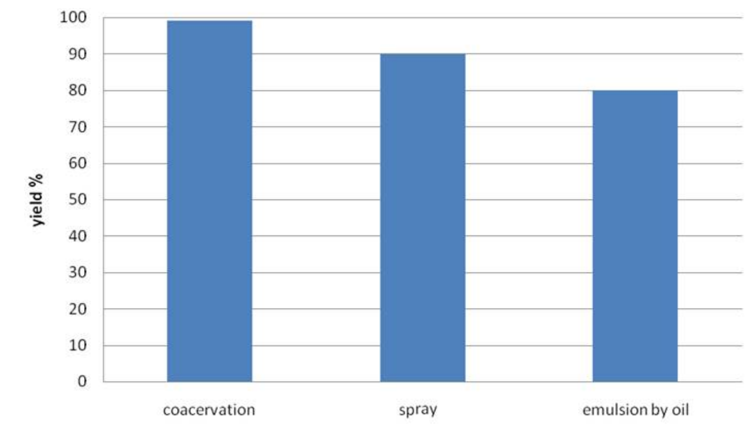
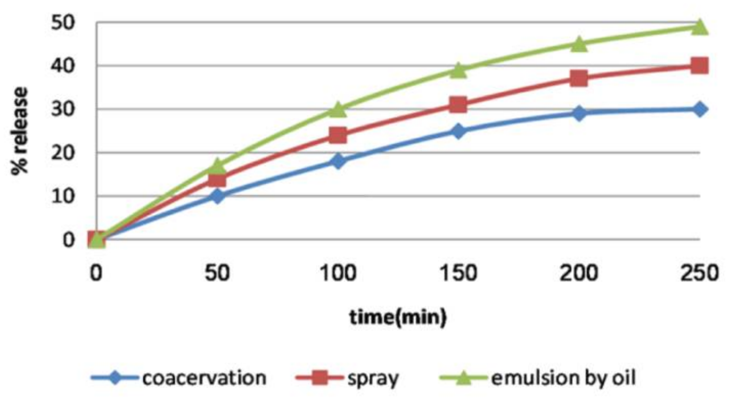


Figure 5: Release Rate of Fe *In Vitro* Comparistion Between Prepared Microcapsules by Coacervation, Spray, and Emulsion Methods



DISCUSSION

Iron is one of the vital micronutrients in human food and its deficiency may cause anemia and iron deficiency. The main reason of anemia is insufficient take or inadequate iron absorption or combination of both of them. About a fourth of the people in the world suffer from this disease. According to the published statistics, about 20 to 50% of pregnant women and 50% of total women afflicted with anemia. So far, three ways has proposed to solve iron deficiency: Dietary modification and diversification, using the supplement, and Food fortification (micronutrient addition to the processed foods). The present case is the best and most practical method in long-term to compensate the nutrient product deficiencies. There have been made a lot of researches in the field of iron salts microcapsulation and their bioavailability especially in dry foods.

Since the prevalence of iron deficiency anemia in the third world countries is more than the other countries, we attempted to solve this problem by capsule production in nano- and microsized for fortification of hydrated and dehydrated compounds and medicine take.

Thereupon, in this study it was tried to produce capsules with ferric saccharate as a core and calcium alginate as a coating. Calcium alginate is a water-insoluble nutrient and useful for body, which solve in gastroestinal tract and release ferric saccharate iron (which is a chelating agent and part of the second group of iron compounds with high absorption power). Finally can provide the main goal of encapsulation namely increase the absorption power and bioavailability and decrease the organoleptic problems.

Capsules were formed using three methods of spray, emulsification with oil, and coacervation. In the coacervation method (by needle), we were able to produce microcapsules. One of the effective factors in this method was stirrer rotation, according to the results, increase in stirrer rotation lead to form capsules with spherical morphology and uniform distribution of surface.

Capsules size affected by the outer of injector, as the finer the outer, the finer are capsules. In emulsification method, capsules were produced in nano- and microsized. In the method of spray by changing the nozzle can produce nano- and microsized capsules.

CONCLUSION

Iron is one of the vital micronutrients in human food and its deficiency may cause anemia and iron deficiency. The main reason of anemia is insufficient take or inadequate iron absorption or combination of both of them. About a fourth of thepeople in the world suffer from this disease. According to the published statistics, about 20 to 50 % of pregnant women and 50 % of total women afflicted with anemia. Sofar, three ways has proposed to solve iron deficiency: Dietary modification and diversification, Using the supplement, and Food fortification (micronutrient addition to the processed foods).The present case is the best and most practical method in long term to compensate the nutrient product deficiencies. There have been made a lot of researches in the field of iron salts microencapsulation and their bioavailability especially in dry foods.

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