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Research Article

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Investigation of Pharmaceutical Nanocapsules of Iron/Alginate Characters via Coacervation Technique

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Abstract Microencapsulation is a process in which solid, liquid or gas components are covered with a septum. The present study has focused on the effect of stirring to produce ferric saccharate capsules with alginate coating applying the coacervation method so that we can obtain the best capsules for fortification of hydrated and dehydrated food products. At first, three methods including stirrer, ultrasonic and sonic bath were compared in order to select the best way of stirring. The experiments results showed that turning was provided by the stirrer method resulted in capsulation with spherical morphology and uniform distribution of surface. In this case the other factors such as the stirrer rotations, alginate concentration and calcium salt concentration were investigated. After studying the various conditions, it is suggested that the best Capsules were formed in alginate 3% at 500 rpm with Concentration of calcium chloride salt 1M and the resulted capsules by this method had a high efficiency and were more stable in hydrated and dehydrated food ingredients network for a long time.

Keywords microcapsules, coacervation, ferric saccharate, alginate, stirrer, ultrasonic, sonic bath

Introduction

One of the reasons of iron deficiency in Third world countries is less iron fortification of food ingredients. Iron encapsulation is one approach of fortification which protect iron against instability and resulting in better processibility (improvement in solubility, dispersion and allegiance), increase of iron life by preventing from decomposition reaction (oxidation), control, stability, timely releasing, safe and proper handling, covering and protection against odor or taste and destructive materials as well [1-4].

Capsules consist of two parts of core and coating in which core contains active compound and coating plays a protective rule for core.

The proper selection of core, coating and method in encapsulation can lead to elimination of many requirements and Cost reduction [5-7].

Therefore, ferric saccharate iron was chosen due to it's high bioavailability and high absorbance power (absorbency) which is in the second group of iron compounds and is less soluble in water but soluble in acidic conditions, with higher absorbance power and the less apparent problems than the first group. On the other hand, ferric saccharate as a chelating agent binds to the iron cation, Fe (II) or Fe (III) and keeps it from precipitating due to a basic pH or to any other compound, which traps and precipitates iron, so improve iron absorption [8-12].

Calcium alginate is a natural fiber extracted from food algae with high nutritional value is water- insoluble with adhesive property which at room temperature and neutral pH forms an even and clear layer around the core to protect iron against it's surrounding environment and release it purposefully in the gastrointestinal tract which resulting in the increase of absorbance power and reduction of the organoleptic problems in food ingredients, more over due to it's insoluble property in aqueous environment can be used in hydrated and dehydrated food fortification [8,13-16].

The method of coacervation was applied in order to produce ferric saccharate capsules with alginate coating.

Water -soluble alginate salt such as sodium carrying carboxylic groups which is able to create complex with metallic polyvalent ions.

When a water- soluble iron salt comes in contact with a water – soluble alginate salt, cross- linking of carboxylic groups of alginate will take place by reaction with the iron cations, such as Fe^{+2} or Fe^{3+} .

When a core comprising iron alginate comes in contact with an aqueous solution of calcium salt, a capsule (formed by the core covered with an outer layer comprising calcium alginate) will form due to the reaction of the alginate salt with the calcium cations. The outer layer, being not soluble in water or in weak acids, avoids the contact of iron with the environment while increasing the mechanical strength of capsules [14, 17].

Whereas contact between iron- alginate core and aqueous solution of Calcium affects the size , morphology and surface distribution of capsules , therefore in this study providing contact in three methods of stirrer, ultrasonic and bath sonic was investigated.

Materials and Methods

Material

Sodium alginate salt with an average viscosity (Cas. No. 9005. 38.3) was prepared from the sigma Aldrich co, Germany, and calcium chloride ($cacl_2$) with average molecular mass of 147.02 (Cas. No. 2380) was bought From the Merck, Germany.

Ferric saccharate iron with an average molecular mass of 45200Da (Cas. No. 8047-67-4) was prepared from shanghai boyle chemical company, chine. All other chemicals used in this paper were agent grade .Ultra pure water from Mili-Q water system was used to prepare the aqueous solutions.

Method

Preparation of micro capsules

Alginate 1.5% is added to 0**.**798g Ferric saccharate iron (at a coating to core ratio of 70 to 30) at the high rotation of stirrer to form uniform solution of alginate iron, then this alginate solution is added to the solution of calcium chloride salt under three conditions of stirrer , ultrasonic and sonic bath. [18,19].

Stirrer

The solution of alginate –iron was added drop wise to 300 ml of calcium chloride salt 1M at 1250 rpm. Upon adding the iron- alginate solution, calcium ion replacement to sodium took place consequently, capsules formed in calcium alginate coating.

After capsulation, they were filtered three times under vacuum condition and were washed three times with water which was distilled twice till all the existing free ions on the capsules were washed away finally they were dried.

Ultrasonic

Alginate- iron solution was added drop wise into the beaker which includes 300 ml of 1M $CaCl_2$ and a sonic probe is located inside it. In this case stirring was provided with sonic, after capsulation they are filtered under vacuum condition and were washed with water which was distilled twice, afterwards they were collected and were dried.

Sonic bath

The alginate- iron solution was added to calcium salt in the sonic bath.

This solution was added drop wise and slowly to the beaker which contained 300ml of $CaCl_2$ 1M and was located inside the sonic bath to form capsules then they were washed and dried.

Morphological characterization, size and surface charge

The morphological characteristics of Micro particles were examined by scanning electron micros copy (JSM-5900Lv, JEol, Japan).

Micro particles 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).
The zeta analyzer (Nano- ZS90, Malvern Instrument; UK) with ultrapure water as solvent (pH=7, 25 °C).
Encapsulation efficiency
The loading efficiency (LE) value was calculated according to the following equations:

$$\text{LE (\%)} = \frac{total\ amount\ of\ Fe - free\ Fe}{total\ amount\ of\ Fe}$$

When capsules were being washed at centrifuge ,every times the existing water on the capsules was threw away and was replaced with new water, by measuring the iron exists in washing water on the capsules using spectrophotometer, the free iron can be calculated.
Capsule Stability
The ability of the capsules to avoid the release of its payload during the storage has been tested in different conditions, close to those that can be found in different media to be supplemented with the capsules. The capsules were stored at room temperature, aqueous solution and solid capsules.
The two different conditions regarding the water content were chosen to simulate the two extreme environments that the capsule are most likely to face: liquid food stuffs or food stuffs with a high content of water, and dry food stuffs or food stuffs with a low content of water.
By weighing about 100mg of capsules and adding 15ml of Distilled water (except in " Solid capsules experiment").
Each sample was kept sealed at room temperature for 0, 0.5, 1 month. After 0, 0.5, 1 month, 15ml of distilled water were added to the "Solid capsules" samples.
All the samples analyzed were then filtered to remove the solid, and the released iron was quantified in the supernatant by spectrophotometer.

Result and Discussion

Effect of the stirring by the stirrer, probe sonic and sonic bath
Stirrer
Upon adding the solution of alginate- iron to calcium salt solution which was turning uniformly on stirrer, ionic exchange took place and this uniform turning caused to form more fine capsules (400 micron) , with spherical morphology and uniform distribution of surface. The SEM results are presented in Figure 1.

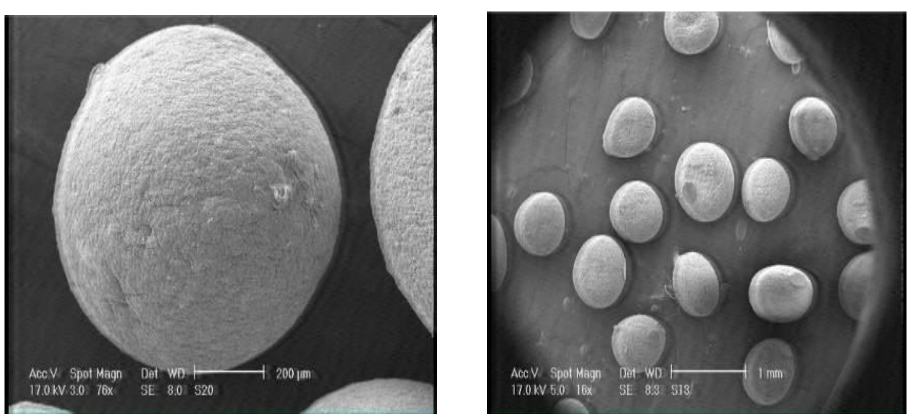

Figure 1: SEM result: Microencapsulation in the condition that stirrer provides contact

Ultrasonic
Since the contact between iron salt- alginate solution and $cacl_2$ salt under ultrasonic condition, is violent with pulse, creates rapid movement and contact and this factor resulting in non- uniform distribution of surface and change in capsules spherical morphology (average size of 450 micron). SEM results presented in Figure 2.

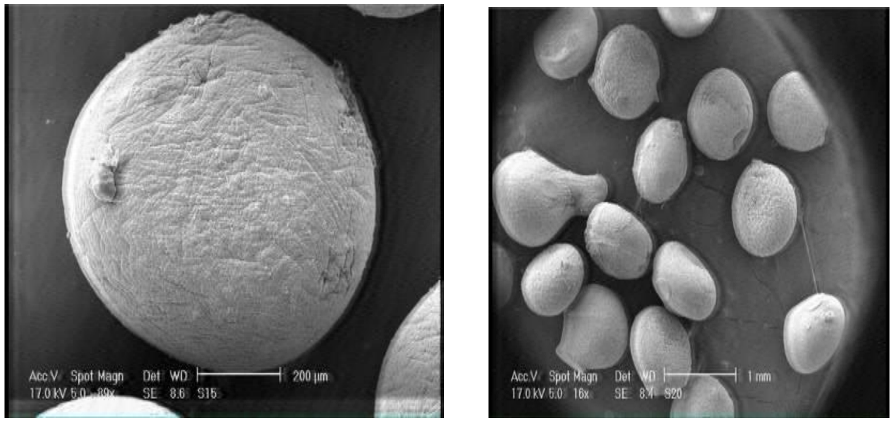

Figure 2: SEM result: Microencapsulation under condition which contact provided by probe sonic

Sonic bath

The solution of calcium salt moves slowly in the sonic bath and this slow stirring leads to form capsules with wider and larger average size (average size of 500 Microns), the SEM result of sonic bath is presented in Figure 3.

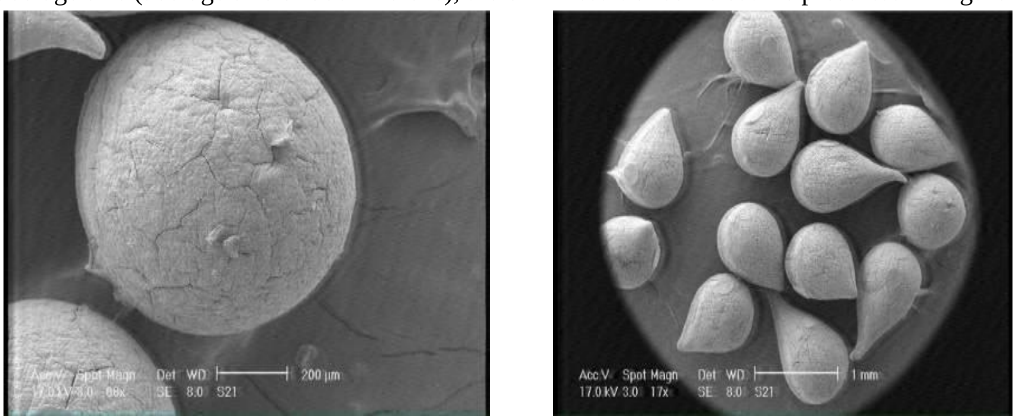

Figure 3: SEM result: Microencapsulation under condition of sonic bath

Determination of the average particles size in three situations is presented in table 1 and Figure 4.

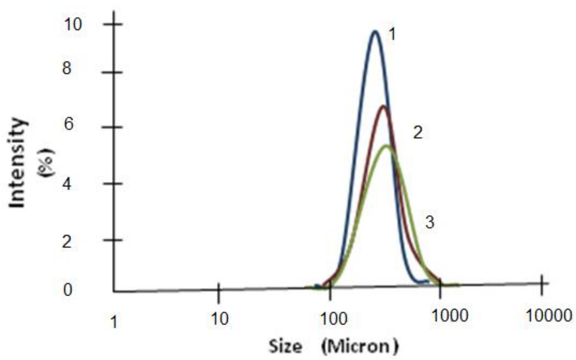

Figure 4: Effect of the agitation by the 1) stirrer, 2) probe sonic and 3) sonic bath on the Particle size distribution of micro capsules

Table1: Effect of the agitation in various situations on the Particles size distribution of micro capsules

Sample	*Mean Diameter (Micron)*	*PDI*	*Zeta potential (mv)*
Stirrer	400	0.2	47.6
Ultrasonic	480	0.28	46.2
Bath sonic	520	0.31	46

In every three methods, efficiency was high and almost constant .But by comparing the morphology and particles size distribution, it was seen that in condition mixing was provided using stirrer, capsules had spherical morphology with more uniform distribution of surface and partly smaller than the other two methods.

The other researches have also utilized the stirrer for turning in coacervation method to obtain better results [19-21].

Effect of different concentration core/wall ratio

With increasing the core/wall ratio the morphology of micro capsules changed from spherical to irregular and the mean particle size gradually increased and the particle size distribution became wider. (Figure 5, 6). Similar conclusions were also archived by other researchers [14-15, 20-21].

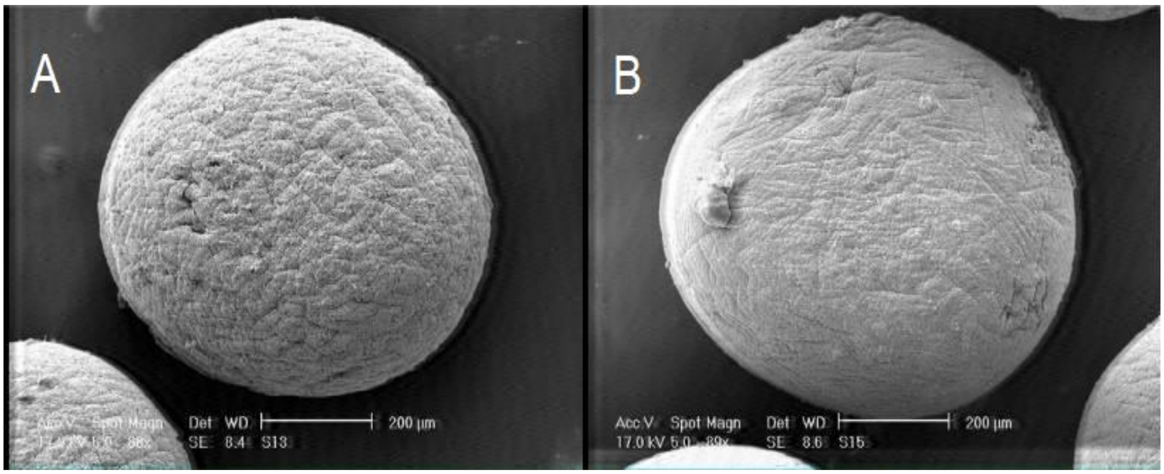

Figure 5: Effect of the increase core/wall ratio on the morphology of micro capsules (Cacl2 1M & stirring speed of 500rpm). A)Alg 1.5% , Fe: 0.798gr (1;2 core/wall) B) Alg 3% ,Fe:0.798gr(1;4core/wall)

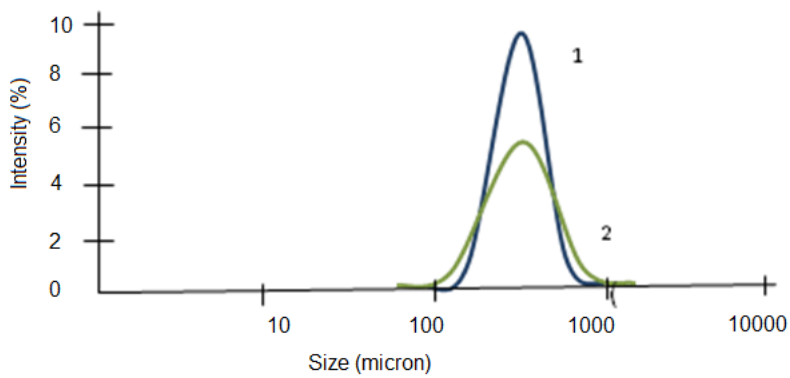

Figure 6: Effect of the increasing core/wall ratio on the particle size distribution of micro capsules: 1)Alg 1/5%, Fe: 0.798gr 2) Alg 3% , Fe: 0.798gr (Cacl$_2$ 1 M & stirring speed of 500rpm)

Effect of the different concentration of $CaCl_2$ on the formation and stability of micro capsules

The result of capsulation showed that in alginate 1.5% at concentrations lower than 0.1M calcium chloride salt by increasing the stirrer rotation, capsules lose their stability and spherical morphology completely and lead them to the linear form, while in alginate 3% at low rotations of stirrer capsules don't have spherical morphology and by enhancing the stirrer rotation, they take spherical morphology and in both percentages of alginate at low salt concentrations, capsules have a less uniform surface and following the increase in concentration of calcium salt at all the rotations form stable and spherical capsules. As the results showed, increasing the concentration of cacl2 brings about the production of better capsules. The test of particles size distribution was performed at two various conditions of alginate. (Table 2)

Table 2: Effect of the different concentration of $CaCl_2$ and Alginate on the particle size distribution of micro capsules (Fe: 0.798gr and stirring speed=500rpm)

Sample	*$CaCl_2$(m)*	*Mean particle size (micron)*	*PDI*	*eta potential (mv)*
Alg 1.5% Speed rotation(rpm 500) Fe 0.798 gr	0.05	412.1	0.499	12.5
Alg 1.5% Speed rotation(rpm 500) Fe 0.798 gr	1	410	0.450	22.5
Alg 3% Speed rotation(rpm 500) Fe 0.798 gr	0.05	405.2	0.393	27.1
Alg 3% Speed rotation(rpm 500) Fe 0.798 gr	1	400	0.34	34

3. Result of the Capsules Stability

The release of iron and calcium from the capsules was used as an indicator of the capsule stability.
The release of iron and calcium are shown in Figure 7:

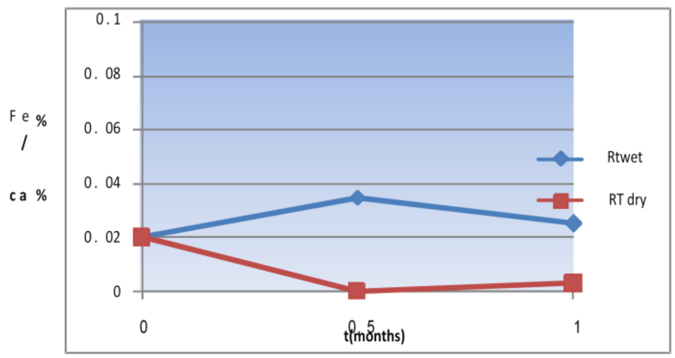

Figure 7: Release (%) of iron and calcium in different conditions of storage (RT: Room temperature in (dry)), (RW: Room temperature in (wet))

The less iron released, the better the ability of the capsules to keep the payload within the capsules, and to protect the environment from being degraded by the iron, and the iron from being captured by the environment.
By comparing the release rate in different conditions it can be seen how the presence of water affect the stability of the capsules as, having more water in contact with the capsules increase the release rate.
The fraction of calcium released into the environment is larger than that of iron, showing that the ability of the capsules to keep the metal inside is much better for the iron than for the calcium. The release of calcium indicates that most probably the capsules get worn out with time, but as calcium is more accessible than iron, calcium is first released into the environment.
The studies of the other researches revealed that in microencapsulation, the presence of outer layer act as a coating protect the core from interaction with the environment and the more aqueous environment , the faster is releasing of capsules than the dry conditions .Since, so far more capsulations was performed in dry conditions. [4, 14, 16, 22-26]
But in the present study, the presence of alginate coating due to it́ s insoluble property in aqueous environment , protect the core for a long time and it́ s storage changes is not much different in dry or wet conditions.

Conclusion

Encapsulation results in the reduction of undesirable sensory changes and decreases iron interaction with the other food ingredients moreover, can strengthen iron within the food network for a long- time but also is able to release it in gastrointestinal tract .Therefore with creating a high bioavailability and having the ability to reduce the organoleptic problems, it can be a good option for fortification of food ingredients.

Iron fortification of dry food products such as cereal flower, spaghetti and biscuits was performed [27].

There have been made a lot of activities for iron fortification of hydrated food products such as coca drinks, spaghetti products and milk powder with the iron from the third group of ferric –pyrophosphate with low absorbance power but without sensational problems [2].

In the present study, capsules contains Ferric saccharate core with high absorbance power with alginate coating which has a high nutritional value and is insoluble in water.

Therefore these capsules can extend the enrichment range of food ingredients and can be used in hydrated and dehydrated food fortification, since they are stable for a long period of time in the food matrix, and are able to release the soluble iron component when they enter the gastrointestinal tract.

Food enrichment using these capsules can greatly reduce the problems of anemia, especially in Third world countries, and also can decrease some digestive problems during iron intake. The present invention can be used for food fortification, more over can be formulated as an oral compound as tablet or syrup together with pharmaceutically acceptable experiments or carriers.

To form these capsules, the coacervation method was applied and contacts were studied in three methods of stirrer, ultrasonic and sonic bath at the stage of forming the calcium alginate coating. The results of experiments showed that the best contact for producing capsules with spherical morphology and uniform surface distribution was provided using stirrer, thus the other significant factors in this method such as the effect of stirrer rotation, alginate concentration, iron calcium chloride salt were studied and it was observed that stable capsules with spherical morphology and uniform surface can be produced at concentration of alginate 3% and calcium salt 1M at 500 rpm.

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