

PREPARATION OF FERROUS SULFATE MICROCAPSULES AS A SUSTAINED RELEASE DOSAGE FORMS

FATIMA J. AL_GAWHARI

Department of Pharmaceutics, Faculty of Pharmacy, University of Baghdad, Baghdad, Iraq
Email: thepharmacycollege16@yahoo.com

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ABSTRACT

Objective: The main purpose of this study was to optimize the different methods for the preparation for the preparation sustained release microencapsulated ferrous sulfate as a solid dosage form.

Methods: Ferrous sulfate was prepared as microcapsules using three microencapsulation methods. Complex coacervation, aqueous colloidal polymer dispersions, and solvent removal methods were used to prepare various formulas with different coating agents (acacia, gelatin, sodium alginate and ethylcellulose). The formation and texture characteristics, entrapment efficiency, release profiles, particle size and storage stability of ferrous microcapsules were evaluated in this study.

Results: The encapsulation efficiency and hardening varied considerably among these three preparation methods. Encapsulation of ferrous sulfate by complex coacervation with a coating agent (gelatin and acacia) showed problems in hardening and poor encapsulation efficiency. However, ferrous sulfate when coated by sodium alginate at 1:1 (coat: core) ratio using aqueous colloidal polymer dispersion method showed acceptable encapsulation efficiency ($67\% \pm 0.1$). Moreover, ferrous sulfate/sodium alginate microcapsules hardened successively when dropping into CaCl_2 solution (2% w/v). A same hardening features and values of encapsulation efficiency ($68\% \pm 0.6$) were obtained by solvent removal methods. Especially, after tween 80 and carboxyl methyl cellulose were added to the aqueous phase in the process of coating with ethylcellulose. However, sustained release microcapsules were produced by aqueous colloidal polymer dispersion method. The sustained-release sodium alginate/ferrous sulfate was stable for 30 d in both refrigeration and room temperature.

Conclusion: The aqueous polymer dispersion gave sustain release microcapsules which were uniform, hard and stable during storage at both room temperature and refrigeration.

Keywords: Ferrous sulfate, Microcapsules, Complex coacervation, Aqueous colloidal, Polymer dispersion, Solvent removal

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INTRODUCTION

Ferrous salts are widely used to treat anemia; ferrous sulfate is the favored form of oral salt because of its high bioavailability. However, there are gastrointestinal side effects associated with its intake [1]. Microencapsulation can reduce these side effects by modifying the release of drugs to occur in a specific site of the gastrointestinal tract [2]. In microencapsulation, drugs can be carried inside one or more film coating agents, forming microscopic particles. Microencapsulated drugs hold a new release feature (e. g. Delayed and sustained release) and have the stability by protecting encapsulated drugs from interaction. In addition, taste masking advantage which can be achieved by a coating of drugs inside the core [3, 4].

Therefore, microencapsulation of ferrous sulfate will help to increase the therapeutic efficiency and patient compliance by reducing the gastric side effects. In addition, the frequency of dose can be reduced with the sustained-release formulation.

The aim of the present study is to prepare sustained release microencapsulated ferrous sulfate as a solid dosage form using complex coacervation, aqueous colloidal polymer dispersions, and

solvent removal methods. Then, compare the dissolution profiles of selected formulas with the ferrous sulfate powder, after investigating the best method according to the hardening and productive features as well as microencapsulation efficiency.

MATERIALS AND METHODS

Materials

Ferrous sulfate powder supplied by Samara drug industry (SDI), Iraq; acacia, gelatin, sodium alginate, calcium chloride from AG; Seelze, Hannover, Germany; Ethyl acetate, ethylcellulose, sodium acetate and phenanthroline from BDH chemical Ltd. Pool, (England); Carboxymethylcellulose (CMC) and NaCl from E-Merk. Darmstadt (West Germany) and tween 80 from MERCK-Schuchardt (Germany)

Methods

Preparation of microcapsules

Complex conservation was carried out by preparing 100 ml gelatin and acacia solutions in various strengths at 40 °C as summarized in table 1.

Table 1: Ferrous sulfate microcapsules prepared by complex coacervation method

Formulas numbers	Gelatin (% w/v)	Acacia (% w/v)	Ferrous sulfate (% w/v)	Ratio (Coat: core)
I	1	1	2	1:1
IIII	23	23	46	1:11:1
IV	1	1	1	2:1
V	2	2	2	2:1

Ferrous sulfate was dispersed in the acacia solution and stirred it rapidly for 30 min to form the emulsion. After this, gelatin solution was added to the emulsion in a dropwise manner.

The pH of the final mixture was adjusted to 4.5 by pH meter (type CG 820 Schott Gerate GMBH, West Germany) with stirring at 250 r. p. m to induce coacervation for 1 hour at 40 °C. The product was cooled

over 3 h and set aside to form sediment. The resulting sediment was filtered to re-suspend in isopropanol and filtered. Then, the filtrate was dried at room temperature [5].

In microencapsulation with aqueous colloidal polymer dispersions, 500 ml of calcium chloride aqueous solution and 100 ml of sodium

alginate aqueous solution were prepared in various strengths at as summarized in table 2. These solutions were prepared as follows: Sodium alginate was slowly added to distilled water with constant stirring on magnetic stirrer followed by mild heating and stirring until the solution became clear. After this, ferrous sulfate was added in quantities which mentioned in table 2.

Table 2: Ferrous sulfate microcapsules prepared by aqueous colloidal polymer dispersions

Formulas numbers	Na. Alginate (% w/v)	CaCl ₂ (% w/v)	Ferrous sulfate (% w/v)	Ratio (Coat: core)
VI	2	1	1	2:1
VII	2	1	2	1:1
VIII	1	1	2	1:2
IX	1	2	2	1:2
X	2	2	1	2:1
XI	2	2	2	1:1

The calcium chloride solution was taken in a beaker and put it on a magnetic stirrer with gentle stirring. Then, the beaker was fixed on a stand and dropped the sodium alginate and ferrous sulfate solution into the calcium chloride solution. The microcapsules particles were formed as drops in calcium chloride solution. The particles were kept in calcium chloride solution for one hour for hardening. After hardening, the particles were sieved through a strainer and washed with distilled water to remove unencapsulated materials. Finally, the collected microencapsulated particles were dried at 60 °C overnight [6].

In solvent removal method, the ferrous sulfate and ethyl cellulose was mixed firstly in ethyl acetate to form an organic phase, which then poured into the aqueous phase, 200 ml cold distilled water (5°C). The mixing of two phases was carried by stirring at 500 r. p. m in the ice bath. The excess solvent was removed by decantation. Finally, the product was dried overnight for 24hours [7].

Surfactant (Tween 80), electrolytes (NaCl) and hydrophilic polymer (carboxymethyl cellulose) were incorporated into the aqueous phase to increase the yield of microcapsules as described in table 3.

Table 3: Ferrous sulfate microcapsules prepared by solvent removal method

Materials and ratio	Formulas numbers				
	XII	XIII	XIV	XV	XVI
Ferrous sulfate (g)	2	3	3	1.5	2
Ethyl cellulose (g)	2	2	2	2	2
Ethyl acetate (ml)	25	25	25	15	15
Tween80 (g)			0.5		0.5
NaCl (g)		1	0.5		0.5
Carboxymethyl cellulose (g)				1	1
Distilled water (ml)	200	200	200	200	200
Ratio (coat: core)	1:1	1:1	1:1	2:1	2:1

Estimation of ferrous sulfate loading in microcapsules

A stock solution of ferrous sulfate of 5 gm/l was prepared and for each standard ferrous sulfate solution, 50 ml volumetric flask was needed. The aliquots of ferrous solution from 1 ml to 8 ml were measured using bulb pipettes and poured in the volumetric flasks. Then, 5 ml of 2% (v/v) hydroxylamine hydrochloride solution, 10 ml of 10% (w/v) sodium acetate solution and 25 ml of 1,10-phenanthroline were added to each flask above. Then the volume was completed to 50 ml total volume by de-ionized water. A blank solution was prepared by placing all of the reagents as described above except the stock ferrous sulfate solution, in a 50 ml volumetric flask and the total volume for flask was then completed to 50 ml with de-ionized water. Reagent blank was analyzed simultaneously with each sample. The absorbance of each sample was measured in a 1-cm glass cuvette, at 510 nm, using spectronic-20 (W-Germany).

Microcapsules samples were prepared by grinding 1 gm of the gained particles with mortar. The resultant powder was transferred into a 50 ml beaker, and 25 ml of HCl (3 molar) was added. The volume of the resultant mixture was reduced by gently boiling under the fume hood to about 15 ml. This solution was left in the hood until cool to the touch. Then filtered into a 100 ml volumetric flask and diluted with de-ionized water.

1 ml of this solution was taken into a 50 ml volumetric flask and treated as the standards. The absorbance of these ferrous sulfate microcapsules solutions was measured using spectrophotometer [8]. Encapsulation efficiency was represented by the following equations.

$$\text{Encapsulation efficiency (EE\%)} = \frac{\text{Actual drug loading}}{\text{Theoretical drug loading}} \times 100$$

Release profiles of ferrous sulfate microcapsules (formulas XI, XV and XVI)

The dissolution process was carried out at 37 °C±0.5 °C and 100 RPM using dissolution apparatus (Erweka USP DT6, Germany) according to the United States Pharmacopoeia (USP).

The microcapsules or ferrous sulfate were placed in the basket and the basket was immersed in a vessel containing 750 ml of 0.1N HCl (pH 1.2). Filtered samples of 5 ml were taken for analysis at predetermined intervals (0.25, 0.5, 1, 1.5, 2.0, and 2.5 h) and replaced with the same volume of fresh medium fluid equilibrated at the same temperature. The pH of the dissolution medium was raised to 7.4, and samples were then taken after 3, 4, 6, 8, 12, 14, 16 and 18 h. The samples were diluted and analyzed. All release data were conducted in triplicate and the mean values were plotted versus time with a standard deviation [9, 10].

Determination of storage stability for ferrous sulfate in formula XI

Formula XI was stored at room temperature and refrigerated conditions for 30 d. The stability was assessed by monitoring, color and ferrous sulfate contents every 5 d.

Particle sizes analysis

The particle size of formula XI was measured by optical microscopy. A drop of an aqueous suspension of microcapsules was mounted on a slide and observed under the microscope under 10X magnification. The ocular micrometer was first calibrated with a stage micrometer. The mean particle size was calculated by measuring 100 particles with the help of a calibrated ocular micrometer and using the following equation. Where n is no. of the particle, D is the diameter.

$$\text{Mean particle size} = \frac{\sum nd}{\sum n}$$

Statistical analysis

Data were analyzed by one-way analysis of variance (ANOVA). The mean values obtained for all of the investigated groups were compared with the use of post hoc Scheffe's test. Groups were considered significantly different at $p < 0.05$.

RESULTS AND DISCUSSION

The results of microcapsules production from different methods are presented in table 4. Gelatin/acacia complex coacervation is a simple method of microencapsulation of water soluble drugs and biological agents e. g. peptides and proteins. Although the method is simple, it is not trying to optimize conditions such as a ratio of the coacervation agent (gelatin & acacia) to drug (coat: core ratio) [11]. In this study, the suitable ratio of coacervation agents to the drug was 2:1. Because of with 1:1 ratio, no microcapsules appeared or loose and adherence microcapsules formed. It could be concluded that the obtainment of microcapsules and optimization of hardening depends on the coat: core ratio used in the preparation of microcapsules by complex coacervation [12, 13].

Encapsulation efficiency of gelatin/acacia microcapsules was shown in table 4. Ferrous sulfate was poorly encapsulated by this method. This may be as a result of leaking of ferrous sulfate from the coacervation phase because of its higher solubility in water. No comparative data available in the literature about the encapsulation of ferrous sulfate by gelatin/acacia complex. However, Saravanan and Panduranga (2010) reported 2.92 and 2.65% encapsulation of metronidazole HCl by pectin/gelatin and alginate/gelatin microcapsules which are also considered poor encapsulation [14].

In aqueous colloidal polymer dispersion, sodium alginate is used as an anionic polymer which was cross-linked with CaCl_2 . The gelled ferrous sulfate/sodium alginate particles were formed as a result of the formation of Ca^{++} -carboxylate (mannuronic acid and glucouronic acid) residues bounds. The results indicated that at 1% w/v concentration of calcium chloride, there are no microcapsules were formed. However, ferrous sulfate/sodium alginate (1:1, 1:2 and 2:1) microcapsules were appeared by using of CaCl_2 at concentration 2% w/v. The using of equal portions (1:1) of the coating agent (sodium alginate) and drug (ferrous sulfate) was better to get an acceptable texture of the microcapsules. Because of loose microcapsules were

obtained with 1:2 coats to drug ratio and very hard texture microcapsules were obtained with 2:1 coat to drug ratio. Encapsulation efficiency of microcapsules obtained by this method was shown in table 4. The increase of the coating agents will improve the encapsulation efficiency of the formula X (coat: drug ratio 2:1) in comparison with formula XI (coat: drug ratio 1:1). But this will lead to the formation of very hard texture microcapsules. Aqueous polymer dispersion method reported high encapsulation efficiency in this study and in the previous studies. The average encapsulation efficiency rate of ascorbic acid through this method was 74% in the previous study (6). Encapsulation efficiencies from 80 to 98% were achieved with ibuprofen, theophylline, guaifenesin, and pseudoephedrine HCl when also formulated in gelled particles by dropping sodium alginate solutions containing these drugs into calcium chloride solutions [15].

In solvent removal method, organic solvent like ethyl acetate is preferred because it has a solubility of 8.7% w/w in the aqueous phase at temperatures lower than 25°C and this solubility is enough to form microcapsules [16]. Extracted microcapsules were uniform with no aggregation tendency and they had sufficient hardening [7]. NaCl has electrolyte's repulsion action that prevented aggregation and tween 80 acts as a surfactant. Tween80 was added to the aqueous phase in order to keep the precipitating of ethyl cellulose in uniform particles [7, 17]. The addition of methyl cellulose caused an increasing viscosity of continuous phase that restricts the migration of the ferrous sulfate from the formed microcapsules by decreasing the diffusion of it [18, 19]. Therefore, formulas XV and XVI have a higher encapsulation efficiency than other formulas prepared by the same method.

Microcapsules formula XI, XV, and XVI were used in the dissolution study because they showed the best entrapment efficiency with acceptable texture. Dissolution profiles are shown in fig. 1. There was a wide difference in the profiles of microcapsules and release of ferrous sulfate ($p < 0.05$) because the microcapsules retarded the release of ferrous sulfate at pH 1.2 [20]. Only formula XI showed sustained release at pH 6.8 over a period of 14 hours as shown in fig. 1. The calcium ions and sodium alginate complex leads to a controlled release of ferrous sulfate in the gastrointestinal tract.

Formula XI maintained their ferrous sulfate content throughout the 30 d of storage period under refrigerated conditions. When compared to room temperature stored capsules, which lost 0.5%. Regarding the color, the microcapsules stored at room temperature and refrigerated had no change in color during the storage period of one month.

Table 4: Formation, texture of microcapsules beads and encapsulation efficiency of prepared formulations

Formulas numbers	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV	XVI
Formation of microcapsules	N	N	Yes	Yes	No	N	N	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Texture of microcapsules	o	o	Loose	Hard	Hard	o	o	-	Loose	Very hard	Hard	Hard	Hard	Hard	Hard	Hard
EE%±SD	-	-	13±0.5	15±1.2	40±2.2	-	-	-	45±0.7	78±0.2	67±0.1	52±0.9	57±0.1	60±0.3	65±0.1	68±0.6

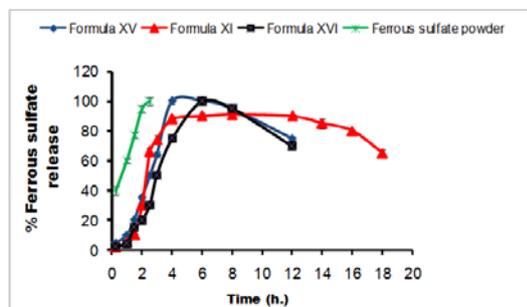


Fig. 1: In vitro release of ferrous sulfate microcapsules

Formulas XI, XV, XVI and pure ferrous sulfate powder dissolution in 0.1 N HCL and phosphate buffer pH 7.4 at 37 ± 0.5 °C and 100 RPM according to the United States Pharmacopoeia (USP). All release data

were conducted in triplicate and the mean. Values were plotted versus time with a standard deviation ($p < 0.05$).



Fig. 2: Sodium alginate/ferrous sulfate (1:1) microcapsules, Formula XI was stored at room temperature. After 30 d there was no change in color, content and particle size ($209 \mu\text{m} \pm 2.5$)

CONCLUSION

The aqueous polymer dispersion and solvent removal methods of preparation were found to be simple and reproducible. The release profiles of producing microcapsules were significantly different in comparison with the pure drug. The aqueous polymer dispersion gave sustain release microcapsules which were uniform, hard and stable during storage at both room temperature and refrigeration.

CONFLICTS OF INTERESTS

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