

Research Article

Studying the Enrichment of Ice Cream with Alginate Nanoparticles Including Fe and Zn Salts

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The aim of this research was developing alginate nanoparticles as a carrier for food enrichment. In this research, Fe/Zn-loaded alginate nanoparticles were prepared and characterized as point size, morphology, FTIR, loading efficacy (LE), and release properties and used in ice cream structure. After this stage, absorption of the salts was measured and sensory and rheological evaluations were taken for samples. Results showed that alginate nanoparticles have average size between 90 and 135 nm. Also, the shape of the nanoparticles is regular and smooth without aggregation phenomena. FTIR certified that Zn/Fe loaded into alginate nanoparticles. Also, loading efficacy of Zn/Fe was 70–85% and release profile of nanoparticles showed a steady state. Alginate nanoparticles could decrease the loss of Fe/Zn in comparison control. Furthermore, these nanoparticles have no side effects on sensory and rheological properties. Hence, this nanoparticle can be suggestive for the enrichment of ice cream and probably other foods.

1. Introduction

One of the most popular aspects of nanotechnology is designing of new vehicle for delivery and transition of materials. Today, researchers achieve new results about probability application of drug delivery systems for food enrichment. Regine et al. studied calcium absorption from fortified ice cream formulations compared with calcium absorption from milk and designed a calcium-fortified ice cream formulation that is lower in fat than regular ice cream and could provide a useful source of additional dietary calcium [1]. Huynh et al. also used solvent-free β -carotene nanoparticles for food fortification [2]. Chan et al. encapsulated herbal aqueous extract through absorption with *ca*-alginate hydrogel beads and found the potential of using absorption process and hydrogel material for the encapsulation of herbal aqueous extract [3]. Goto et al. used soybean ferritin gene for iron fortification of rice seed [4]. There was little investigation in fortification with the nanomaterials field. In recent decades, researchers used polysaccharide polymers such as alginate, chitosan, and dextran due to their biodegradable properties. Alginate has different application in food science such as food additive. Furthermore, alginate was certified by WHO/FDA

expert food committee on food additives at the 39th meeting in 1992 [5]. Alginate is applied to numerous kinds of food, such as ice cream and jelly, for gelling properties. Machado et al. prepared calcium alginate nanoparticles Using water-in-oil (W/O) nanoemulsions [6]. Zhang et al. prepared chitosan/alginate nanoparticles for oral delivery of insulin and found that these nanoparticles can be good vectors showing a good efficiency in oral administration of the protein of peptide drugs [7]. Ahmad et al. used alginate nanoparticles for drug delivery of ATDs (rifampicin, isoniazid, pyrazinamide, and ethambutol) in mice against tuberculosis [8]. Zohri et al. used Nisin-loaded chitosan/alginate nanoparticles as an antimicrobial agent against *Staphylococcus aureus* in raw and pasteurized milk samples and achieved desirable results [9]. It seems that with attention to the alginate in the stabilizing of ice cream, it can be a good candidate for drug delivery in ice cream. Hence, alginate nanoparticles were developed for food fortification and used in ice cream.

2. Method and Materials

2.1. Materials. Dialysis membrane bag Mw 100 kD, Spectrum Laboratories, and zinc chloride salt (ZnCl₂), and ferric salt

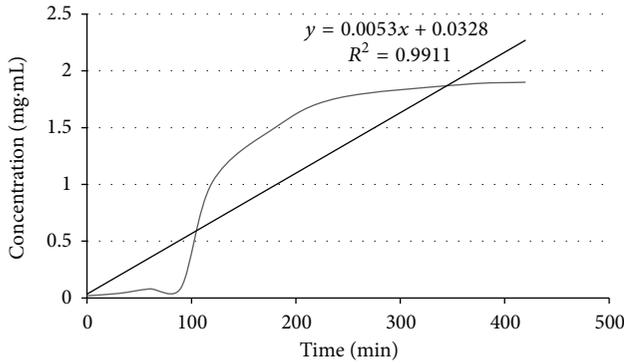


FIGURE 1: Release profile of Fe/Zn from nanoparticles.

(FeCl₃), ultrafilter Amicon cutoff 100 kD, and alginate were purchased from Sigma-Aldrich.

2.2. Preparation of Fe/Zn-Loaded Alginate Nanoparticles. Alginate nanoparticles were prepared using a desolvation method. Alginate powder was added to distilled water. Ethanol solution was added continuously or intermittently into 1% alginate solution at pH 7 under stirring at 700 rpm at room temperature until the solution became just turbid. In continuous addition method ethanol was added continuously in the solution with rate addition about 1.0 to 2.0 mL per min, and for intermittent method, 2 mL of ethanol was added for every 5 min interval. After this 5 mgr of zinc salt and Fe⁺³ salt, solutions continuously were added to the solution and then stirred for 10 min and nanoparticles centrifuged at 11000 rpm for 10 min and separated.

2.3. Nanoparticle Characterization

2.3.1. Release Profile and Loading Efficacy Determination of Nanoparticles. Release studies for Fe/Zn salts were done at 0, 15, 30, 60, 90, and 120 min and thermograms of release were drawn at different times by dialysis membrane bag methods (Figure 1) [9]. For this purpose, the 1000 KD (MWCO) membrane was selected as the MWCO being sufficiently large to allow the passage of Fe/Zn salts. For the experiment, 5 mL of a 1 mg/mL solution of phosphate-buffered saline (PBS) at pH 7.4 (release media) was poured into the inner tube of the dialyzer. The dialyzer tube was placed into a 50 mL glass cylinder containing release media, which was continually stirred at 300 rpm using a small magnetic stir bar to prevent the formation of an unstirred water layer at the membrane/outer solution interface. Diffusion to the outer solution at 37°C was assessed by sampling the contents of the outer solution at periodic intervals of 0, 15, 30, 60, and 120 min [8, 10]. For loading studies, samples were centrifuged in Amicon Ultra-15 (Ultracel-100k Millipore Co., USA) with a molecular weight cutoff to remove free molecules of polymer as well. The alginate nanoparticles were prepared separately to be used as blank solution in subsequent atomic absorption analysis. Samples were centrifuged in Amicon Ultra-15 (Ultracel-100 K) for 20 min at 5000 rpm, and then the absorbance of the solutions in the tubes was measured.

The amount of iron in nanoparticle samples was determined on the basis of Tautkus et al., and the loading efficacy was evaluated as follows (Tautkus et al. 2003, [5]):

$$LE (\%) = \frac{\text{Fe}}{\text{Zn}} \text{ salts Total} - \frac{\text{Fe}}{\text{Zn}} \frac{\text{supernatant}}{\text{Fe/Zn}} \text{ salts Total} \quad (1)$$

2.3.2. Size Disruption of Nanoparticles. For this purpose, Zetasizer 3000 HS (Malvern Instruments, UK) was used. Nanoparticles were diluted and measured by the device. Mean and standard deviations of the free nanoparticles and Fe/Zn-loaded nanoparticles were measured and presented for comparison.

2.3.3. Morphology of Nanoparticles. The nanoparticles morphology such as shape and incidence of aggregation phenomena was studied by SEM (scanning electron microscopy, JSM-6610, 30 kv). For this purpose, the samples of the nanoparticle suspensions (5–10 ll) were mounted on metal stubs, plating coated under vacuum, and then observed.

2.3.4. FTIR Evaluation. FTIR spectra were obtained using 8400S (Shimadzu Co., Japan) FTIR spectrometer. Samples were dried in a vacuum desiccator, mixed with micronized KBr powder, and compressed into discs using a manual tablet press [11].

2.4. Ice Cream Making Producing. For ice cream making, first all of the consequents of mixture include milk, sugar, cream, dried milk, and flavoring agent (Vanilla) calculated by Pierson square method and weighted. Then, milk and cream are mixed well for 5 min with electrical mixer. This mixture was poured in steel bottle and heated till 40°C slowly. The other materials added to ice cream after this stage. The mixture hashed and then set at 80°C for 25 seconds and stored at refrigerator for 4 hour. After this, Fe/Zn-loaded nanoparticles added to ice cream. There were three-trial sample and one blank sample for experimental analysis.

3. Ice Cream Analysis

3.1. Fe/Zn Absorbance Amount. Seven people (women and men with 20–30 ages) were studied and the enrichment ice that was used cream varied from 10 mg Fe to 14 mg Zn at the morning before food eating. The amount of Fe/Zn calculated at urine samples and the average of the results were reported.

3.2. Sensory Evaluation. Ice cream samples were organoleptically evaluated for texture, flavour, body/texture, and overall acceptability. For sensory evaluation, 15 trained panelist were selected and samples included ice cream as blank and ice cream including nanoparticles in three replications were evaluated with a 5-point hedonic test.

3.3. Rheological Properties. For this purpose, Yield stress assay was done for ice cream, and it was used from Bohlin

Viscometer with thermal circulator. Bob spindle opted on the basis viscosity of mixture. The mixture after ageing time was poured in C30 cup and its temperature reached 10°C and yield stress was between 14.2 and 501.7 (Figure 4).

3.4. Statistical Analysis. Statistical analysis was conducted using Spss (version 16) to determine the means and standard deviation of the rheological data and sensory data. An ANOVA was conducted to determine differences between means.

4. Results and Discussion

4.1. Release Profile and Loading Efficacy Determination of Nanoparticles Evaluation. There are numerous possible applications of alginate polymer drug delivery systems using both encapsulation with matrices and encapsulation with alginate surfactant oil in water emulsions as delivery agents. As a designed carrier for protein or peptides delivery, the alginate-chitosan microspheres have to experience the gastrointestinal tract when they are orally administrated. Hence, alginate nanoparticle has benefit for oral delivery [7]. As seen in the profile, nanoparticles first release at initial time that named burst release. These phenomena occur due to swelling properties and alginate nanoparticles; this result was in agreement with Gazori and Zohri results [9, 10, 12]. After this nanoparticle reaches a steady-state stage and this situation continues till all of Zn/Fe are released. This type of release profile is suitable due to gradual absorption for oral delivery of encapsulates [7]. Also loading study showed that nanoparticles have 70–85% from Fe/Zn salts.

4.2. Size Disruption of Nanoparticles Evaluations. It can be observed that the mean size of nanoparticles is 98 nm and the mean of PDI (polydisparsity index) of the nanoparticles was 0.1 PDI and is related to homogeneity of the particles. It showed that these nanoparticles have good stability and can be good candidates for drug delivery.

4.3. Morphology of Nanoparticles. SEM was used for certification of alginate nanoparticles and provided morphological information on the Fe/Zn-loaded alginate nanoparticles. Spherical, distinct, regular, and smooth shapes were seen for the nanoparticles through the SEM images (Figure 2). The particle size range of the nanoparticles was 90–200 nm.

4.4. FTIR Evaluation. The FTIR spectra of alginate powder and nanoparticles are shown in Figure 3. The spectra showed a band at approximately 3757 cm^{-1} , 2855 cm^{-1} , and 1739 cm^{-1} which are assigned to Amide I, Amide II, and Amide III, respectively. The most prominent FT Raman band centered at 880 cm^{-1} is mainly due to the O/H deformation mode, while the band at 1400 cm^{-1} is ascribed to deformation of the CH₂ groups. The C/O/C and C/OH stretching modes give rise to several close-lying bands in the spectral regions of 1250/1290 and 1000/1025 cm^{-1} , respectively, but in nanoparticles spectra two additional band in 3390 cm^{-1} and 1636 cm^{-1} were

TABLE 1: Sensory properties of ice cream with Zn/Fe-loaded nanoparticles.

Variables correlated with sensory properties	A	B	A × B
Flavor	70.08**	154.14**	16.05**
Aroma	613.00**	904.00**	121.00**
Oral texture	99.90**	61.18**	7.18**

Means ± standard errors of the measured parameters are presented. The common superscripts in each column is indicative of statistical insignificance that is $P > 0.05$.

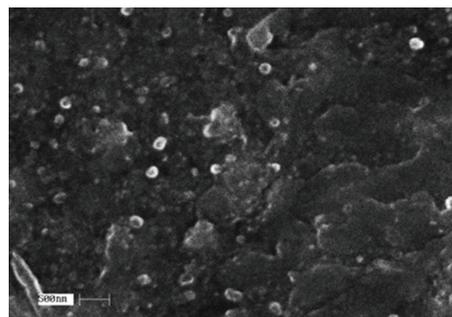


FIGURE 2: Scanning electron microscope image of Zn/Fe-loaded nanoparticles.

observed that this changes related to loading of Fe/Zn functional groups in alginate egg structure and appearance of new bands, Zohri et al.'s result was in agreement with this also [10].

4.5. Fe/Zn Absorbance Amount. Results of urine assay showed that more than 90% of Zn/Fe was absorbed. This is because chelating properties of alginate structure can be loaded minerals and it can be prevent its lost. Generally, the aim of drug delivery systems is to prevent interaction between encapsulate and the other ingredients and also deliver proposed material to the appropriate place at a suitable time [13].

4.6. Sensory Evaluation. Table 1 showed that there were any significant difference between control samples and ice cream with Fe/Zn-loaded nanoparticles. One of the reasons for this result can be related to the size of nanoparticles. The average size of the nanoparticles is between 90 and 135 nm. This size is smaller than to be tasted by tasting buddies. There were not any differences between samples but there was significant relation between characteristic of ice cream at 1% and 5% levels [13, 14]. Zohri et al. certified that alginate can play debittering role and improve the sensory properties of UF feta cheese [15].

4.7. Rheological Properties Evaluation. The changes in viscosity and yield stress against shear rating in ice cream samples that include nanoparticles were similar to control samples and it decreased with increase of shear rate. This occurs due to the accumulation of molecules in low shear rates, and this causes increasing in viscosity. When shear rate increases, the molecules set one side and this causes an increasing in friction

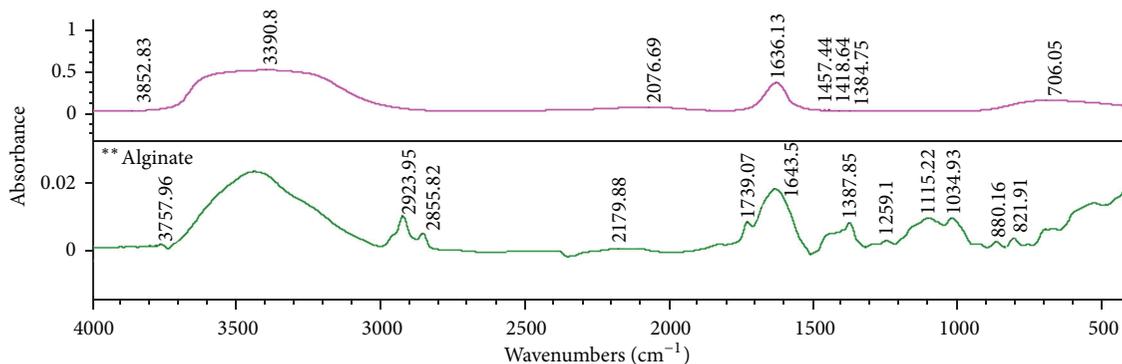


FIGURE 3: FTIR spectra of the alginate powder and nanoparticles.

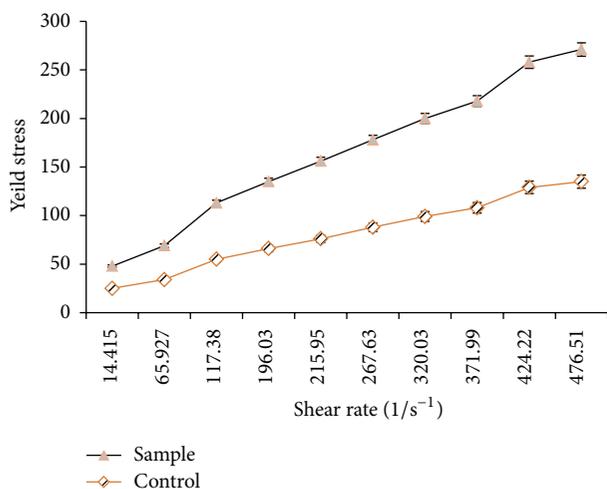


FIGURE 4: Rheological properties of control sample and Fe/Zn-loaded nanoparticles.

and the viscosity decreases. The other reason it can be related to the size of nanoparticles and dispersing of nanoparticles in ice cream structures. Hence, these nanoparticles have no rules in rheological properties [16]. This result was in agreement with Mandar et al.'s [13].

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