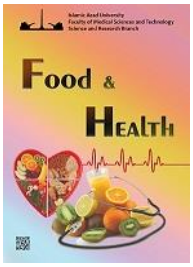

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Comparative evaluation of biodegradable polymeric nanoparticles of casein/poly lactic-co-glycolic acid (PLGA) containing different concentrations of alpha-tocopherol

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ABSTRACT

Biodegradable polymeric nanoparticles have been extensively used as colloidal materials for nanoparticles production designed for various purposes, including drug targeting, enhancement of drug bioavailability and protection of drug bioactivity and stability. In particular, poly (lactide-co-glycolide) (PLGA) as a polyester has been FDA approved for human use. In this research, the biodegradable polymeric nanoparticle of casein/poly lactic-co-glycolic acid (PLGA) containing three concentrations of alpha-tocopherol was prepared. The comparative evaluation of these nanoparticles, including morphology, size, zeta potential, entrapment rate, spectroscopy, thermal resistance, and their release profile was carried out. The comparative results suggested that the sizes of derived nanoparticles were between 150 and 350 nanometers. In addition, there was a significant difference between the nanoparticles size and increase in alpha-tocopherol percentage used in this formulation ($p<0.05$). The accumulated results indicated that the highest entrapment rate belonged to 10 percent of alpha-tocopherol, and higher concentrations decrease the entrapment rate. The using of casein/PLGA can be optimized the characteristics and morphological properties of nanoparticles. The polymeric nanoparticles containing alpha-tocopherol can be used as a biologic preservative to improve drug delivery and consumer health.

1. Introduction

Food fortification or enrichment is the process of adding micronutrients (essential trace elements and vitamins) to food to reduce the number of people with dietary deficiencies within a population (1). In recent years, consumers have more concerns and recommendations to use natural food sources with antioxidant properties rather than synthetic antioxidants, which have been restricted because of their toxicity and carcinogenic effects, although their synthesized types are cheaper and the amount of the natural type is highly limited (2). One of the main natural antioxidants is tocopherols, which are a class of organic chemical compounds mainly found in food products, especially in vegetable oils, and they can extract from the vegetables during the oil extraction. Alpha-tocopherol is a type of vitamin E that known as a fat-soluble antioxidant, but also seem to have many other functions in the body (3). Thus, α- tocopherol is a widely used component in functional food, cosmetic and pharmaceutical industries. However, the design of proper dosage forms is still challenging due to its hydrophobicity and well-known sensitivity to oxygen and light (4). In order to overcome its environmental susceptibility, and improve its solubility in aqueous medium, encapsulation has been recently exploited by several studies (5-7).

The use of nanoparticles besides improving solubility in water also provides controlled release. Polymer nanoparticles (NP) have recently received great attention in the encapsulation and controlled release of bioactive substances (8). Among various polymeric nanoparticles, biodegradable

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synthetic polymers such as poly (lactic acid) (PLA) and poly (glycolic acid) (PGA), as well as poly (lactic-co-glycolide) (PLGA) copolymers may be considered in biomedical applications, i.e., in drug delivery applications, where the drug/biomolecule kinetic release is dramatically influenced by the polymeric degradation rate (9). During degradation, the ester bonds within polymer chains are cleaved due to the hydrolysis reaction in the presence of water. In addition, casein micelles have received much attention in many fields such as food, cosmetics, and medicine. Approximately, caseins can be thought of as block copolymers consisting of blocks with high levels of hydrophobic or hydrophilic amino acid residues (10). Therefore, the objectives of the present research were to produce biodegradable polymeric nanoparticles of casein/poly lactic-co-glycolic acid (PLGA) containing alpha-tocopherol.

2. Materials and methods

2.1. Preparing casein/PLGA nanoparticles

The casein/PLGA nanoparticles containing alpha-tocopherol was prepared based on the method described by Narayanan et al. (11) with some modification. Briefly, 55 mg of PLGA and three concentrations of alpha-tocopherol (10, 30 and 40%) were dissolved in 5 ml acetone/dichloromethane binary solvent (2:1 v/v) (oil phase) and 20 ml of deionized water (containing 0.5 % pluronic acid, w/v) was gently added to this solution. Subsequently, the formed suspension is dissolved in 100 ml deionized water and let to evaporate overnight. In order to remove acetone from nanoparticles, the solution was centrifuged at 11,500 rpm at 4 °C for 30 min and spin again with deionized water. In the final stage, particles were dissolved in 30 ml deionized water. Casein/PLGA nanoparticles were obtained by incubating of 2 mg nanoparticles at 0.5 % acetic acid solution for 3 hours at room temperature (11).

2.2. Morphological study of particle by SEM

The morphological properties of nanoparticle samples were determined using scanning electron microscopy (SEM). The samples were spread on a sample holder and dried using a vacuum drier. They were subsequently coated with gold (JFC 1200 fine coater, Japan) and examined by a Scanning Electron Microscopy (12).

2.3. Size distribution studies

The entrapment rate and release of zinc sulfate and magnesium sulfate salts were evaluated by dynamic light scattering (DLS) technique as described by Sourabhan et al. (13). Samples were evaluated before washing as well as after washing after resuspending in phosphate-buffered saline (PBS). The three nanoparticle samples were centrifuged at 15,000 rpm for 10 minutes and put in a phosphate-buffered saline environment in 12-kDa (MWCO) membrane. The phosphate-buffered saline environment was placed into 50-mL glass cylinder containing release media, which was continuously stirred at 300 rpm using a small magnetic stir bar. The release curve was drawn in 0, 30, 60, 90, 120, 180, 300, 360 and 420 seconds for each of the three ratios. The released alpha-tocopherol rate in the phosphate-buffered saline liquid was identified and reported in percentage.

2.4. Zeta potential evaluation

The size and surface zeta potential of the nanoparticles were evaluated using zeta sizer (nano-sizer machine model: ZEN36000, Germany).

2.5. Fourier Transform Infrared Spectroscopy Analysis

FTIR (Thermo Nicolet, Nexus 870 FTIR, USA) was applied for the analysis of three nanoparticles samples, chitosan powder, beta-casein, and alpha-tocopherol powder. At ratio of 2% (w/w), the samples were mixed with dry potassium bromide and the mixture was ground into fine powder using an agate mortar before compressing into the KBr disc under a hydraulic press at 10,000 psi. Each potassium bromide disc was scanned at 4 mm/s at a resolution of 2 cm over a wave number region of 400–4,000 cm-1. The peaks were recorded for the different samples (14).

2.6. Differential scanning calorimetry analysis

The thermal resistance of nanoparticles was analyzed by DSC using DSC-60 system (Shimadzu Co., Japan). The samples were dried in vacuum desiccators, and then the dried powders were crimped in a standard aluminum pan and heated from 20 to 35 °C with a heating rate of 10 °C/min under constant purging of nitrogen. In the meanwhile, the exothermic and endothermic peaks were measured (15).

2.7. Alpha-tocopherol entrapment rate

To measure the alpha-tocopherol entrapment rate, the Amicon ultra filter (100-kDa) was used for each of the three samples and the supernatants were centrifuged at 20,000 RPM in order to separate free polymers. Subsequently, the alpha-tocopherol absorption rate was measured in the lower solution by the atomic absorption spectrometry. By using the following formula, the alpha-tocopherol entrapment rate was calculated:

Entrapment Rate (%) = (Non-entrapped tocopherol (µg))/ (Total tocopherol (µg)-entrapped tocopherol (µg)) ×100.

3. Results and discussion

3.1. Morphological properties of nanoparticles

Results from microscopic micrographs of casein/PLGA nanoparticles containing alpha-tocopherol suggests that the lower densities of alpha-tocopherol show better morphological appearance comparing to higher densities. (Fig. 1). As seen, by the increase in tocopherol density, particle size increased.

However, the surfaces of all three nanoparticles were flat, spherical and homogeneous particles. The results of SEM show some pores on the particle surface at the 40 percent density observed. This phenomenon is due to the imbalance between the ratios of casein and alpha-tocopherol. The using of the proper ratio of nanoparticle components prevents the formation of bridges and agglomeration of particles (16).

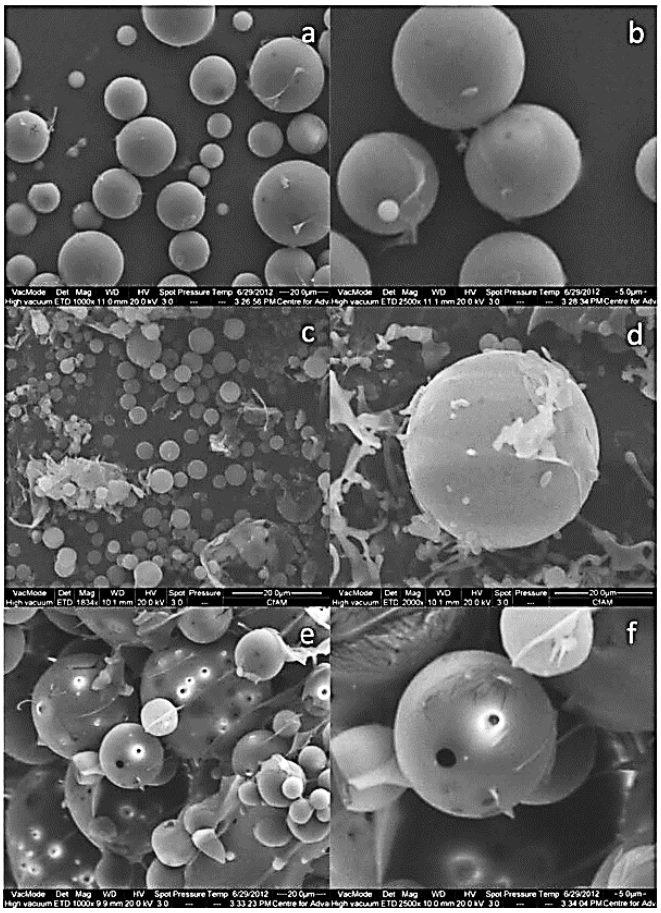

Fig 1. SEM micrographs of casein/ PLGA nanoparticles containing (a & b=10%), (c & d=30%), (e & f=40%) tocopherol.

3.2. Release rate

Fig. 2 presents the release rate of alpha-tocopherol from casein/PLGA nanoparticles. As it could be observed from the figure, the T1 treatment initially has a slower release and after 300 minutes, it releases all of alpha-tocopherols that have accumulated in the nanoparticles. However, the two ratios of T2 and T3 had the capability of releasing 60 to 70 percent of the entrapped alpha-tocopherol for a time of 180 and 90 minutes. Particle size is also one of the factors that influences release. In nanoparticles of smaller dimensions, the surface-to-volume ratio is reduced, hence the release rate increases and vice versa. Nanoparticles with the lowest ratio (T1) had a higher initial release, which was in accordance with Luo et al. (17) results. The second phase of the two-stage release process is called the steady state and continues cumulatively so that the release process is fully executed. One of the main reasons that the T2 and T3 nanoparticles in the second stage are less colorful is due to the presence and accumulation of free alpha-tocopherols on the surface of nanoparticles (17).

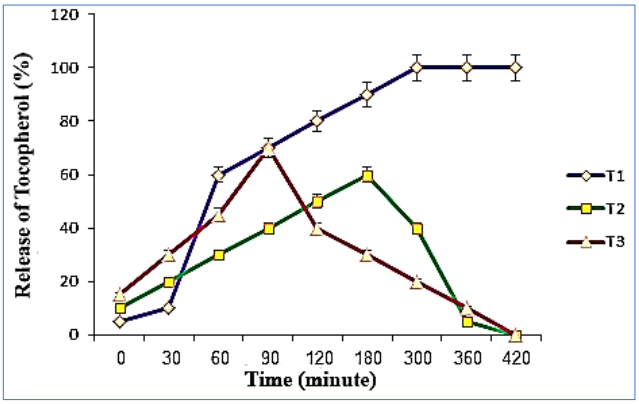

Fig. 2. Release of tocopherol from casein/ PLGA nanoparticles; T1=10%, T2=30%, T3=40%

3.3. Nanoparticles size and zeta potential

Zeta potential is the potential difference between the dispersion medium (e.g., polymer) and the layer of fluid attached to the dispersed particle (e.g., solid filler) (18). Due to the presence of ionic features and also membrane constituents (such as proteins, lipids, and sugars) and distribution of load on their membrane surface, cells have an electric charge on the membrane surface (19). As can be seen in Table 1, the highest zeta potential of the nanoparticles belongs to the T1 treatment and the minimum was related to the T3 treatment. Also, the difference between nanoparticles size in T1, T2, and T3 were significant ($p<0.05$).

Harnsilawat et al. (20) reported that the pH can affect the properties of sodium alginate (NaA), β-lactoglobulin (β-Lg) and their mixtures in aqueous solutions. They concluded that at pH 5, β-Lg and sodium alginate formed fairly soluble complexes due to electrostatic attraction between the anionic polysaccharide and cationic patches on the surface of the protein. They also suggested that it should be available in adequate amounts of polysaccharides to prevent self-accumulation of small amounts of sodium caseinate. Zambrano-Zaragoza (21) studied the impact of nano carriers containing alpha-tocopherol and xanthan gum on longevity and browning index in red delicious apples and they reached the zeta potential average of 44-57 millivolt and the average particle size of 170-240 nm.

Table 1. The size and zeta potential of nanoparticles.

Nanoparticles Type	Nanoparticles Size	Zeta Potential
T1 (10%)	150±0/05 nm [a]	-34±0/15 mv [a]
T2 (30%)	200±0/09 nm [b]	-22±0/13 mv [b]
T3 (40%)	350±0/03 nm [c]	-19±0/1 1mv [c]

**Lowercase letters within the same column with different superscript are significantly different ($p<0.05$).*

3.4. Particles bonds properties using FTIR machine

The main objective in analyzing the IR spectrometry in this research is identifying chemical groups of casein and PLGA samples and subsequently identifying the complex formation between them. Fig. 3 shows the formation of the polymer/protein binding in the core of nanoparticles to form encapsulating materials. Presence of bonds in 870 and 1760 cm-1 is due to the stretching of C-O-C and C=O links that generally formed the peaks of PLGA (22).

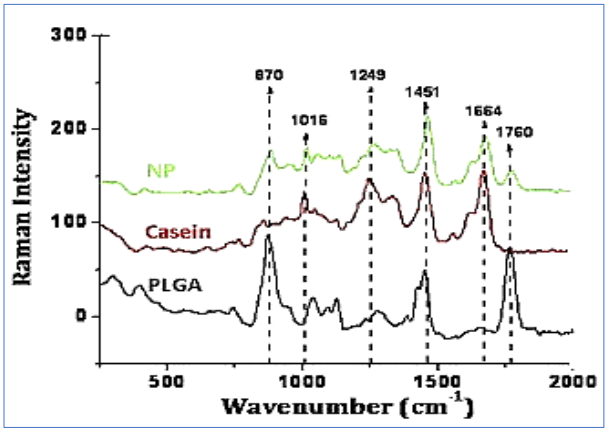

Fig. 3. Results of FTIR spectrometry of casein, PLGA and casein/PLGA nanoparticles containing alpha-tocopherol.

The amide bonds of casein at the core of the system (Amid I, II and III) in 1664, 1451 and 1248 cm-1 have been changed slowly to 1677, 1463 and 1267 cm-1, respectively. In addition, a peak at 1016 cm-1 expresses a P-O-C bond of phosphoprotein which seems to be at the core of the nanoparticles. The 1451 cm-1 peak in the core of the nanoparticles is the internal fluctuation of the CH2 bond of alpha-tocopherol structure, which expresses the coupling of alpha-tocopherol to the casein core and entrapment of it into the matrix structure of the nanoparticles. The 754 cm-1 peak presents the OH groups in the structure of alpha-tocopherol. The spectrum of casein and PLGA nanoparticle containing alpha-tocopherol creates peaks of 1240 and 1764 cm-1, suggesting that alpha-tocopherol is trapped in casein/PLGA nanoparticles. The results of this research are in accordance with the findings of Pool et al. (23). They approved peak changes of carbonyl and carboxyl groups in PLGA and quercetin and attributed them to the structure of nanoparticles.

3.5. Thermal resistance of nanoparticles

The information about the structure of bioactive materials in nanoparticles was assessed through DSC. Analyzing the casein, alpha-tocopherol, PLGA, and three types of nanoparticles are presented in Fig. 4. The curve shows the two endothermic transfers at 137 and 305 °C which is related to dehydration and melting of alpha-tocopherol. The peaks of 175 and 215°C are related to PLGA melting points and forming new bonds. In the PLGA sample, the endothermic peak is observed at 47 that related to melting point and structure change of alpha-tocopherol. The peak of 52 in the curve is related to the structural changes of phospholipid groups in casein. Peak transfer results in derived nanoparticles approve the breakage of old bonds and the formation of new bonds that also have a higher thermal resistance. The results for endothermic points of T1, T2, and T3 nanoparticles are reported to be the same, showing that these nanoparticles are formed with certain proportions of materials and the presence of higher levels of them does not affect the accumulation (23).

Trombino et al. (7) have shown that the stability of alpha-tocopherol and beta-carotene in solid/liquid nanoparticles based on mono-sterile ferrules is higher than of free alpha-tocopherol and beta-carotene samples.

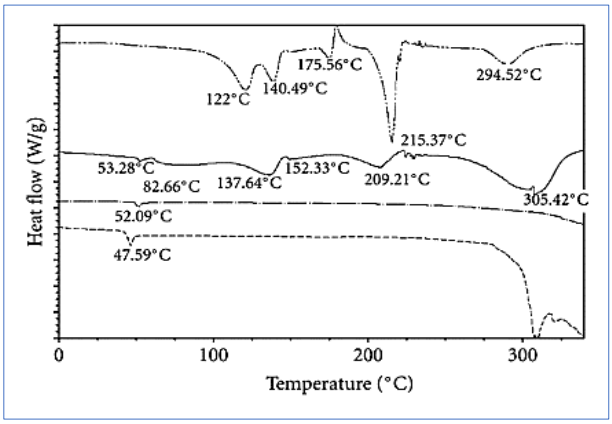

Fig. 4. DSC heat-thermogram results of treatments, casein, and PLGA.

3.6. Alpha-tocopherol accumulation rate

Table 2 presents the entrapment rate of alpha-tocopherol accumulated in casein/PLGA nanoparticles. As it could be observed, the highest rate of entrapment was related to the treatment with 10% alpha-tocopherol and the lowest rate was associated with treatment with 40% alpha-tocopherol that had an accumulation of about 65%. Also, the treatment with 30% alpha-tocopherol had 75 percent accumulation rate. There is a significant difference between T1 treatment and the two other treatments ($p<0.05$). The reasons for the decrease in accumulation rate are increasing in the level of alpha-tocopherol, the presence of free alpha-tocopherol in the vicinity of nanoparticles, the formation of environmental zeta potential and the imbalance in the electrostatic balance for the formation of the bonds (11).

Table 2. Alpha-tocopherol accumulation rate in casein/PLGA nanoparticles.

Nanoparticles Type	Accumulation rate (%)
T1 (10%)	100% [a]
T2 (30%)	75% [b]
T3 (40%)	65% [b]

*Lowercase letters within the same column with different superscript are significantly different ($p<0.05$).

4. Conclusion

This study looked at the possibility of using polymeric nanoparticles containing alpha-tocopherol to produce biologic preservatives to improve health and focus on consumer health. The optimization of nanoparticles characteristics and the study of morphological properties, size, zeta potential, the bond properties and their calorimetry were carried out. The smallest size of nanoparticles was related to the sample with the lowest alpha-tocopherol density, which was increased by increasing the percentage of alpha-tocopherol percentage. Despite the decrease in the accumulation rate, due to the imbalance in electrostatic balance and surface accumulation of alpha-tocopherol.

Conflict of interest

The authors declare no conflicts of interest.

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