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ORIGINAL PAPER

Encapsulation of Ascorbyl Palmitate in Zein by Electrospinning Technique

Mohammad Amin Miri[1] · Mohammad B. Habibi Najafi[2] · Jebrail Movaffagh[3] · Behrouz Ghorani[4]

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Abstract

In this study, ascorbyl palmitate was encapsulated in electrospun zein fibers at different loading levels (2.5%w/v, 5%w/v and 10% w/v). HPLC, SEM, FTIR, XRD, DSC and BET characterized the resulting electrospun zein fibers containing ascorbyl palmitate. Results indicated that the diameters of electrospun zein fibers increased with increasing concentration of ascorbyl palmitate. The physical status of ascorbyl palmitate in electrospun zein fibers was determined by X-ray diffraction (XRD), and differential scanning calorimetry (DSC). Fourier transform infrared (FTIR) was used to study the interaction between ascorbyl palmitate, and zein. The results showed that there was hydrogen bonding between protein and ascorbyl palmitate. Pore size was characterized by N_2 adsorption–desorption isotherms. Contact angle results showed that hydrophobicity of electrospun fiber mats was not affected by ascorbyl palmitate. Encapsulation efficiency of ascorbyl palmitate in electrospun zein fibers was about 22.5–65.5% w/v. Release study results indicated that pH and morphology affected the release. This is the first report describing that ascorbyl palmitate was successfully encapsulated in electrospun zein fibers. This product is intended to be used as an active coating for the delivery of ascorbyl palmitate to butter and other food products.

Keywords Ascorbyl palmitate · Zein · Electrospinning · Electro-encapsulation

Introduction

Ascorbyl palmitate (AP), also known as vitamin C palmitate, has been used as a source of vitamin C, and as an antioxidant for foods, pharmaceuticals, and cosmetics [1]. Although ascorbyl palmitate is more stable than vitamin C, low chemical stability limits its utilization. The main problem of ascorbyl palmitate is its oxidation mediated by transition metal ions presented in traces. Besides oxygen, light can also accelerate the oxidative degradation of ascorbyl palmitate. Ascorbyl palmitate is gradually oxidized, and becomes discolored when exposed to light and a high humidity [2].

Encapsulation potentially can protect active molecules from degradation by direct exposure to severe environments such as light, oxygen, chemicals, etc. Various processes have developed for encapsulation and, electrospinning is one of such advances that has received raising awareness and increased number of studies have been undertaken recently [3–7].

Electrospinning is a simple technique to produce fibers from submicron diameters down to the nanometer diameter through an electrically charged jet of polymer solution or polymer melt [8]. In the electrospinning, an electrically charged jet of polymer fluids is created by a high voltage power supply. The hemispherical shape of the pendant droplet at the end of the capillary tip changes into a conical shape with increasing voltage, which is known as the Taylor cone. By applying a critical voltage to the spinneret, the charge

Mohammad B. Habibi Najafi and Jebrail Movaffagh equally contributed.

✉ Mohammad B. Habibi Najafi
habibi@um.ac.ir

✉ Jebrail Movaffagh
movaffaghj@mums.ac.ir

[1] Department of Food Science and Technology, University of Zabol, Zabol, Iran

[2] Department of Food Science and Technology, Ferdowsi University of Mashhad (FUM), P. O. Box 91775-1163, Mashhad, Iran

[3] Department of Pharmaceutics, Faculty of Pharmacy, Targeted Drug Delivery Research Center, Stem Cell Research Center, Mashhad University of Medical Sciences, Mashhad, Iran

[4] Department of Food Nanotechnology, Research Institute of Food Science and Technology, km 12 Mashhad-Quchan Highway, P.O.Box 91895-157-356, Mashhad, Iran

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accumulated in the polymer droplet overcomes its surface tension, and causes the polymer solution to eject from the spinneret tip to the collector. The electrical forces stretch, and thin the jets by substantial ratio, the solvent finally evaporates, and the jet solidifies, resulting in a fibrous mat on the collector [9–11]. Electrospun fibers have outstanding properties such as high surface area to volume ratio, and high porosity. Furthermore, no temperature control is required in the whole process. Electrospinning allows the use of a wide range of food grade, biodegradable, biocompatible polymeric substances as wall materials for encapsulation of bioactive compounds [11]. These properties make electrospun fibers ideal for encapsulation of food ingredients, enzymes and other active compounds such as antioxidants [4, 6, 7, 12].

Zein, the primary storage protein of corn, is one of the most hydrophobic proteins as a consequence of the presence of nonpolar amino acids such as leucine, proline, and alanine. Furthermore, zein is thermal resistant and has excellent oxygen barrier. Apart from biodegradability, and biocompatibility, zein has high elasticity, and film-forming capabilities. These properties make it suitable for different applications like controlled release applications, coatings, halochromic sensors, biodegradable films and plastics [13, 14].

Recently, electro-encapsulation of sensitive compounds through zein fibrous network have been reported for stabilization and improving the bioaccessibility of fish oil [5], gallic acid [6], epigallocatechin gallate [7], β-carotene [12], Barije (*Ferula gummosa Boiss*) essential oil [15], saffron extract [16] and carotenoid extracted from tomato peels [17]. The effective way for encapsulation of sensitive compounds by electrospinning, hydrophobic interaction between the zein and loaded compounds as well as the physical entrapments were manifested by previous researchers.

This paper confirms the feasibility of using electrospinning for functional food applications by encapsulating ascorbyl palmitate in zein. This is the first report on the encapsulation of ascorbyl palmitate as a bioactive compound using food grade polymer by electrospinning for producing nanostructured fiber with different physical properties due to geometric confinement. The objective of the present study was to encapsulate ascorbyl palmitate as a bioactive food compound using electrospinning in food grade polymer.

Experimental

Materials

Ascorbyl palmitate (AP) and zein powder from corn were obtained from Sigma-Aldrich (Madrid, Spain), and used as such without further purification. The acetic acid glacial of 99.7% purity was purchased from molekula (England). Labrasol was provided by Gattefosse (France) as a gift, Methanol (HPLC grade) was purchased from Sigma-Aldrich (Madrid, Spain). Ultra-purified water was obtained from a Milli-QPlus system Millipore.

Electrospinning Process

Production of sub-micron zein solution was achieved by dissolving different amounts of zein powder and different concentrations of ascorbyl palmitate (2.5, 5 and 10%w/v) in acetic acid glacial to obtain 26% w/v zein solutions. The viscosity of zein and ascorbyl palmitate solutions was measured by Brookfield RV DVIII Ultra (Brookfield Co. USA) rotation viscometer. The solutions were electrospun using the electrospinning set equipped with a variable high voltage 0–35 kV power supplier (Fnm Co. Iran). The zein and ascorbyl palmitate solutions were fed into 5 ml plastic syringes and an 18-gauge stainless steel needle spinneret was connected through Teflon tubing to syringes. A syringe pump was used to fed into needle spinneret at 8 ml h^{-1}. Stainless steel needle spinneret attached to the positive electrode of a direct current (DC) power supplier and the applied voltage was 20 kV with a distance between needle and collector of 10 cm. All electrospinning experiments were carried out at 25 °C for 30 min [13].

Morphology

The morphology of the electrospun nanofibers, after coating with the gold-palladium mixture (20 nm) with a sputter coater, was studied using a scanning electron microscope (Model VP-1450; LEO Co, Germany) at an accelerating voltage of 20 kV. Diameters of electrospun fibers were measured with image visualization software Image-J (National Institutes of Health, USA). The average fiber diameters for the samples were determined by measuring about 50 random fibers from the SEM images [13]. Specific surface area (SSA) for release analysis was calculated. To calculate SSA of fibers in a web, SEM images taken in random locations at medium magnification were captured from multiple samples. Based on the fiber diameter distributions, SSA could be evaluated using Eq. (1) [18]:

$$Specific\ surface\ area\ (SSA) = \frac{Total\ surface\ area}{Total\ volume} = \frac{4\sum_i^n (Di\ fi)}{\sum_i^n \left(D_i^2\ fi\right)} \quad (1)$$

D Fiber Diameter, f Frequency of fibers.

Physical Status

X-ray diffraction (XRD) and Differential scanning calorimetry (DSC) tests were performed to study the physical status

of ascorbyl palmitate in electrospun zein fibers. XRD patterns were obtained on XMD-300 diffractometer (Unisantis, Germany) with Cu Kα radiation in the 2θ range of 4–50° at 40 mV and 300 mA. DSC analyses were performed on a DSC88220 differential scanning calorimetry (Mettler Toledo, Switzerland) at a scanning speed of 10 °C min^{-1}. The sealed samples (2 mg) were heated from 25 to 400 °C under nitrogen gas flow [1, 6, 19].

Analysis of Pore Size

Pore size distributions of the zein and AP loaded zein fibers were calculated according to the method of Brunauer–Emmett–Teller (BET) and Barrett-Joyner-Halenda (BJH). The N_2 adsorption–desorption isotherms were measured with a Micromeritics ASAP-2000 automatic analyzer instrument. Before analysis, samples were out gassed for 3 h at 30 °C [20].

Measurement of Contact Angle

The effect of ascorbyl palmitate on the wettability of the electrospun fibers was determined by measuring the contact angle. The contact angle of the fibers was obtained using a commercial contact angle meter (Data physics OCA 15 plus). The mats were attached to a glass slide and 4 µL distilled water was dropped on the samples [20, 21].

Interaction Between AP and Zein

Fourier transform infrared (FTIR) spectroscopy was applied to study the interaction between ascorbyl palmitate and zein. The FTIR spectra were recorded on a Thermo Nicolet AVATRA 370 FTIR spectrophotometer over the wavenumber region of 400–4000 cm^{-1} at a resolution of 4 cm^{-1} [22].

Encapsulation Efficiency of Ascorbyl Palmitate in Electrospun Zein Fibers

Quantification of the ascorbyl palmitate was analyzed using high performance liquid chromatography (HPLC). The percentage of encapsulation efficiency (E.E) was calculated by the following Eq. (2) [23, 24]:

$$\%E.E = \left(\frac{\text{Weight of loaded AP}}{\text{Weight of initial AP}}\right) \times 100 \quad (2)$$

The stationary phase was 250 × 4.6 mm i.d. RP C18 column packed with 5 µm Luna-NH_2 (Phenomenex, Germany). The mobile phase consisted of methanol and distilled water at the ratio of 50:50 (v/v). The flow rate was 1 ml min^{-1}. The UV detector was set at the wavelength of 254 nm and the injection volume was 20 µL [23, 24].

Release Analysis

In this research, the effect of morphology and pH on the release of ascorbyl palmitate was studied. To this end, bead-free fibers and fibers with large bead morphology at AP concentration of 10% (w/v) were fabricated, and the release of AP was evaluated at pH values of 3 and 7.2. Fourty mg of loaded fibers were immersed in 20 ml of the aqueous medium prepared by distilled water and Labrasol as an emulsifier. The solution was then placed in shaking incubator at 100 rpm and 37 °C. At the intervals of 15, 30, 45, 60, 90, 120, and 180 minutes, 200µL of the sample was carefully replaced by the same amount of the fresh medium. The amount of the released AP was determined by RP-HPLC [9, 23, 24].

Statistical Analysis

All data were statistically analyzed and expressed as mean values ± standard deviation (SD) of the mean. Statistical analysis was performed using SPSS 16.0 software. The data were analyzed using analysis of variance (ANOVA) at 5% significant level. All experiments were triplicate.

Results and Discussion

Evidence of AP Encapsulation in Electrospun Zein Fibers

The existence of the ascorbyl palmitate in fibers was determined by comparison of standard sample retention time, and test sample retention time in HPLC column (Fig. 1a–d). As it is observed, the retention time for all samples is almost 17.6 min. There is no difference between retention time of standard sample and test samples. Such observation confirmed successful encapsulation of ascorbyl palmitate within the zein fibers.

Effect of Loaded AP on Morphology and Diameter of Electrospun Fibers

The SEM images of the electrospun fibers are shown in Fig. 2a–d. The morphology of the zein and AP loaded fibers has shown bead-free, smooth, uniform and homogenous fibers. Besides beaded and defect free fibers, electrospinning is able to produce other types of fibers such as tubular structures, branched fibers, flat ribbons, ribbons with other shapes and fibers that were split longitudinally from larger fibers [13, 25, 26]. As it is observed in SEM images (Fig. 2), the morphology of the fibers is entirely tubular-like shapes.

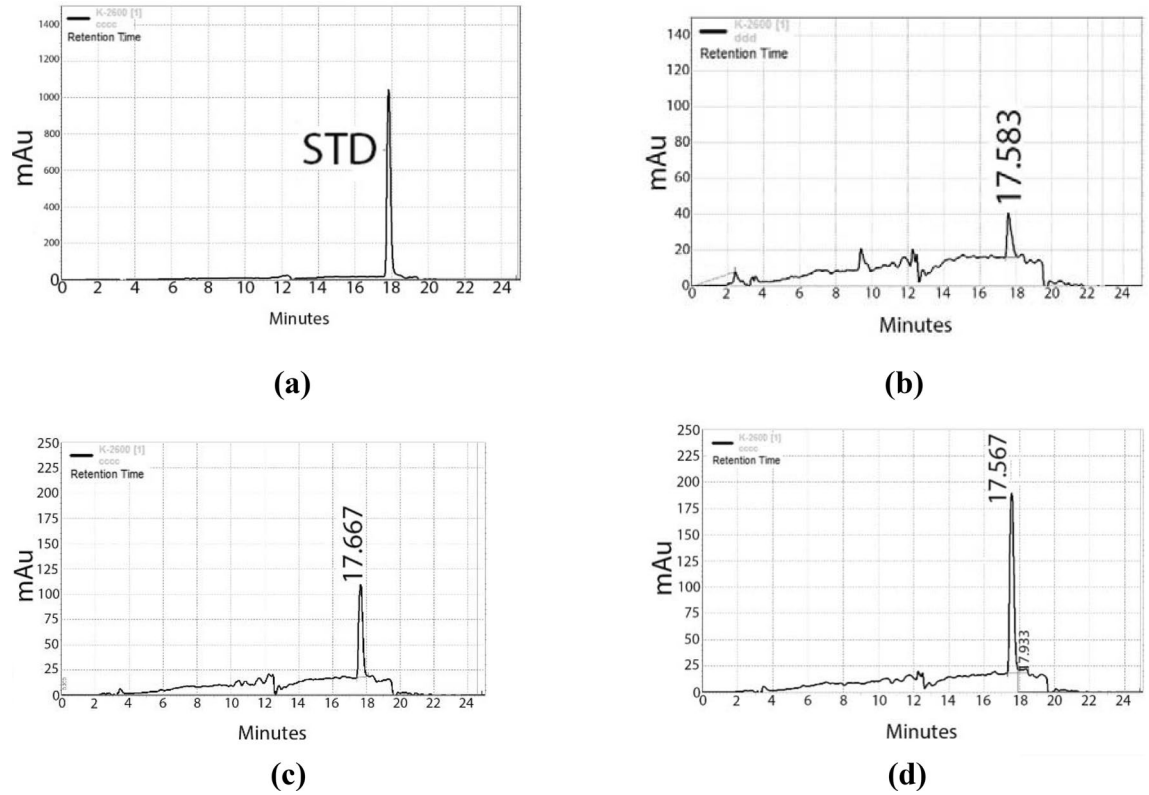

Fig. 1 HPLC chromatograms of **a** standard ascorbyl palmitate (STD) and encapsulated ascorbyl palmitate at levels **b** 2.5% w/v, **c** 5% w/v and **d** 10% w/v

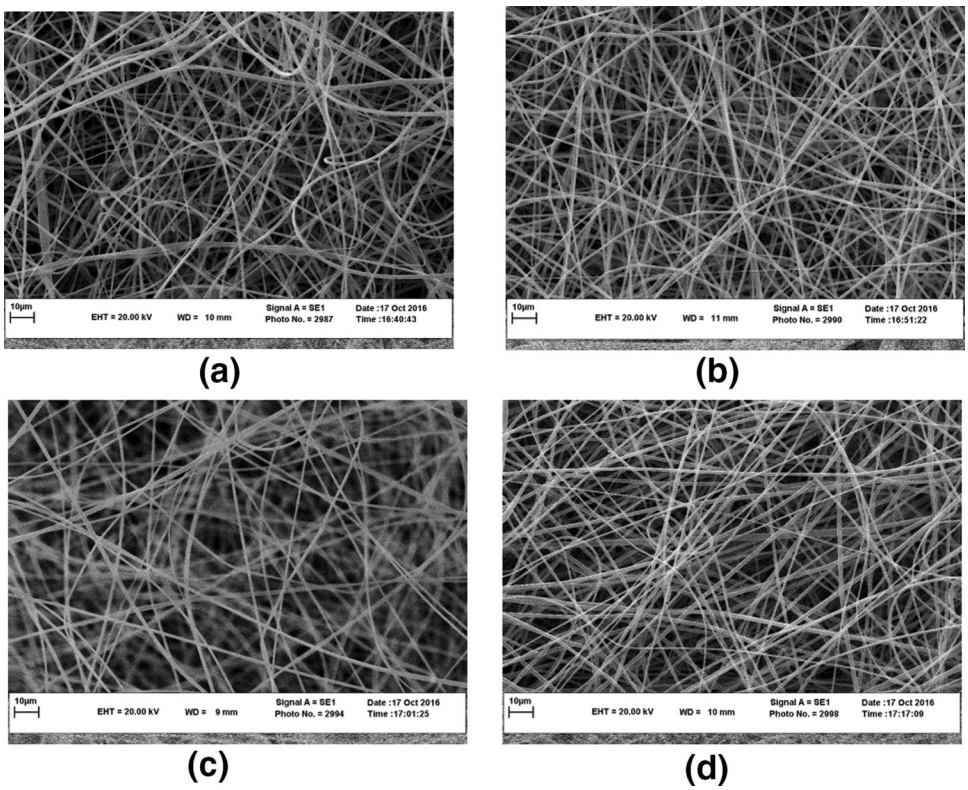

Fig. 2 SEM images of AP loaded electrospun zein fibers at different levels **a** 0%, **b** 2.5% w/v, **c** 5% w/v and **d** 10% w/v

It seems that the round or tubular-shaped fibers are formed when a highly volatile solvent system such as acetic acid is used for electrospinning [13].

The average fiber diameters for AP loaded fibers were ranged from 549 to 750 nm. Results showed that by increasing the AP concentration in the electrospun solution, the average fiber diameter increased (Table 1). Many studies reported that the concentration is the most significant parameter in controlling fiber diameter [8, 13, 25]. By increasing of AP concentration, the viscosity of the solutions increased and thus increased the number of molecular entanglement in the solution, which was reflected in the increase in electrospun fiber diameter [13, 27].

At higher viscosity, there is a great interaction between the solvent and polymer molecules. When a viscous solution is stretched under the electric field, the solvent molecules spread over the entangled polymer molecules, their tendency to come together is reduced and smooth fibers will be created. When the viscosity is increased, the diameter of the fiber also increased [13].

Physical Status of AP in Electrospun Fibers

The DSC and XRD tests were performed to study the physical status of AP in electrospun zein fibers. The DSC thermograms of AP, and the electrospun zein fibers are shown in Fig. 3. The DSC thermograms of pure AP showed a sharp endothermic response corresponding to its melting point of 116.9 °C, which accounts for its crystallinity [24]. The DSC thermograms of zein exhibited a broad endotherm in the range of 40–100 °C with a peak at 70.79 °C. The DSC thermograms of AP loaded zein fibers did not show any melting peak of AP, suggesting that AP had been converted into an amorphous status in the electrospun fibers [6, 14, 19, 24].

The XRD patterns of pure AP, and zein powder are displayed in Fig. 4. The presence of various distinct peaks at diffraction angles 2θ of 5.6°, 15.2° and 20.5° in the XRD pattern of pure AP showed that its physical status was crystalline. The XRD pattern of zein demonstrated a diffuse background pattern with two diffraction halos, indicating an amorphous state. In the XRD patterns of AP loaded zein

Table 1 Viscosity and average electrospun fiber diameter of AP loaded zein solutions

Ascorbyl palmitate content (%,W/V)	Viscosity (m pa s)	Average fiber diameter (nm)
0	635 ± 34^{a}	549 ± 123
2.5	799 ± 38^{b}	700 ± 142
5	842 ± 44^{b}	742 ± 153
10	959 ± 55^{c}	795 ± 190

Means bearing different superscripts are significantly different ($p < 0.05$). Results are presented as mean ± SD ($n = 3$)

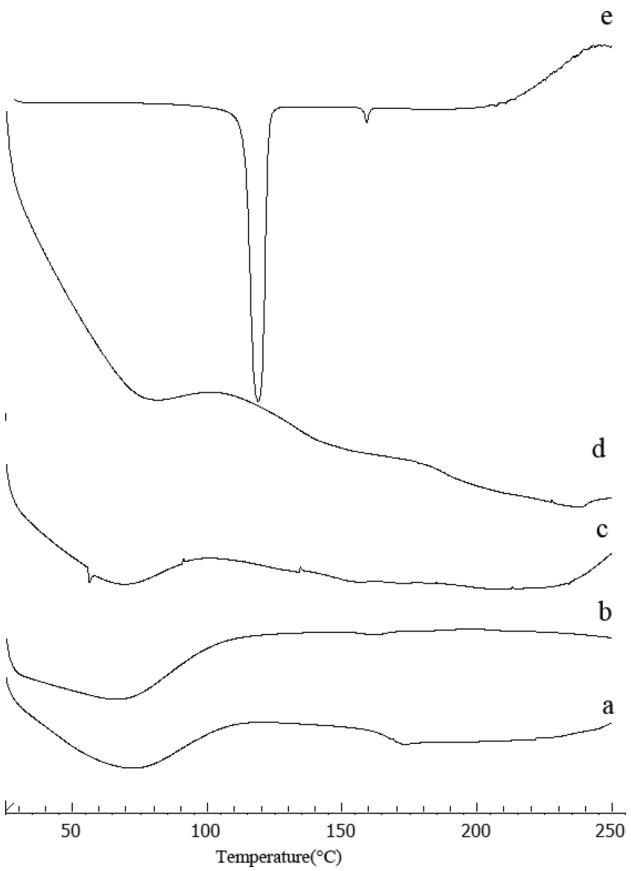

Fig. 3 Differential scanning calorimetry (DSC) thermograms of different samples: **a** zein powder; **b** Ze–AP 2.5%; **c** Ze–AP 5%; **d** Ze–AP 10%nanofibres with 2.5%, 5% and 10% (w/v) ascorbyl palmitate, respectively; **e** ascorbyl palmitate powder

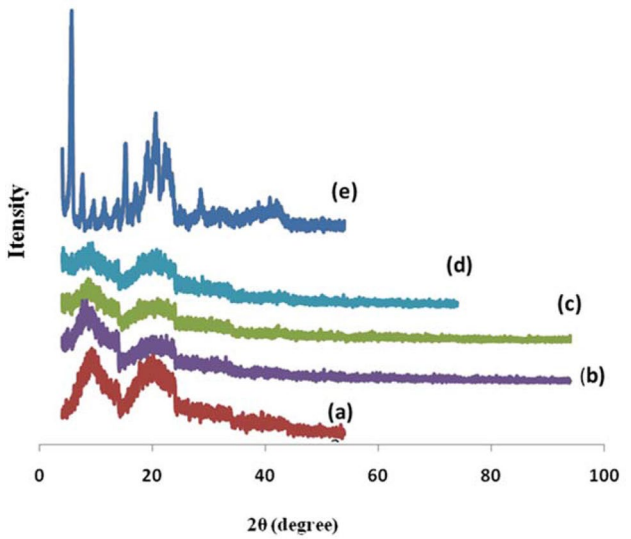

Fig. 4 X-ray diffraction patterns of different samples: **a** zein powder; **b** Ze–AP 2.5%, **c** Ze–AP 5%, **d** Ze–AP 10% nanofibers with 2.5%, 5% and 10% (w/v) ascrbylpalmitate, respectively; **e** ascorbyl palmitate powder

fibers, the characteristic diffraction peaks of AP were absent, suggesting that AP had lost its crystalline structure, and had been converted into an amorphous state. The DSC and XRD results both confirmed that AP was highly distributed in the zein fibers, and was existed as an amorphous material, which is evidence for the successful encapsulation of AP within fiber structure and confirms the HPLC results. The results are comparable and in agreement with other studies [6, 14, 19].

Pore Size Analysis

Figure 5a–d presents the N_2 adsorption/desorption isotherms of regenerated nanofibers. Table 2 presents the pore size and

Table 2 BET test results for analysis of pore size and total volume of pores

Ascorbyl palmitate content (%,W/V)	Pore size (nm)	Total volume (cm^3 g^{-1})
0	11 ± 3.07^{ab}	0.011^{b}
2.5	15.5 ± 3.2^{a}	0.014^{b}
5	16.2 ± 5.01^{a}	0.015^{b}
10	9.3 ± 2.57^{b}	0.023^{a}

Means bearing different superscripts are significantly different ($p < 0.05$). Results are presented as mean ± SD (n = 3)

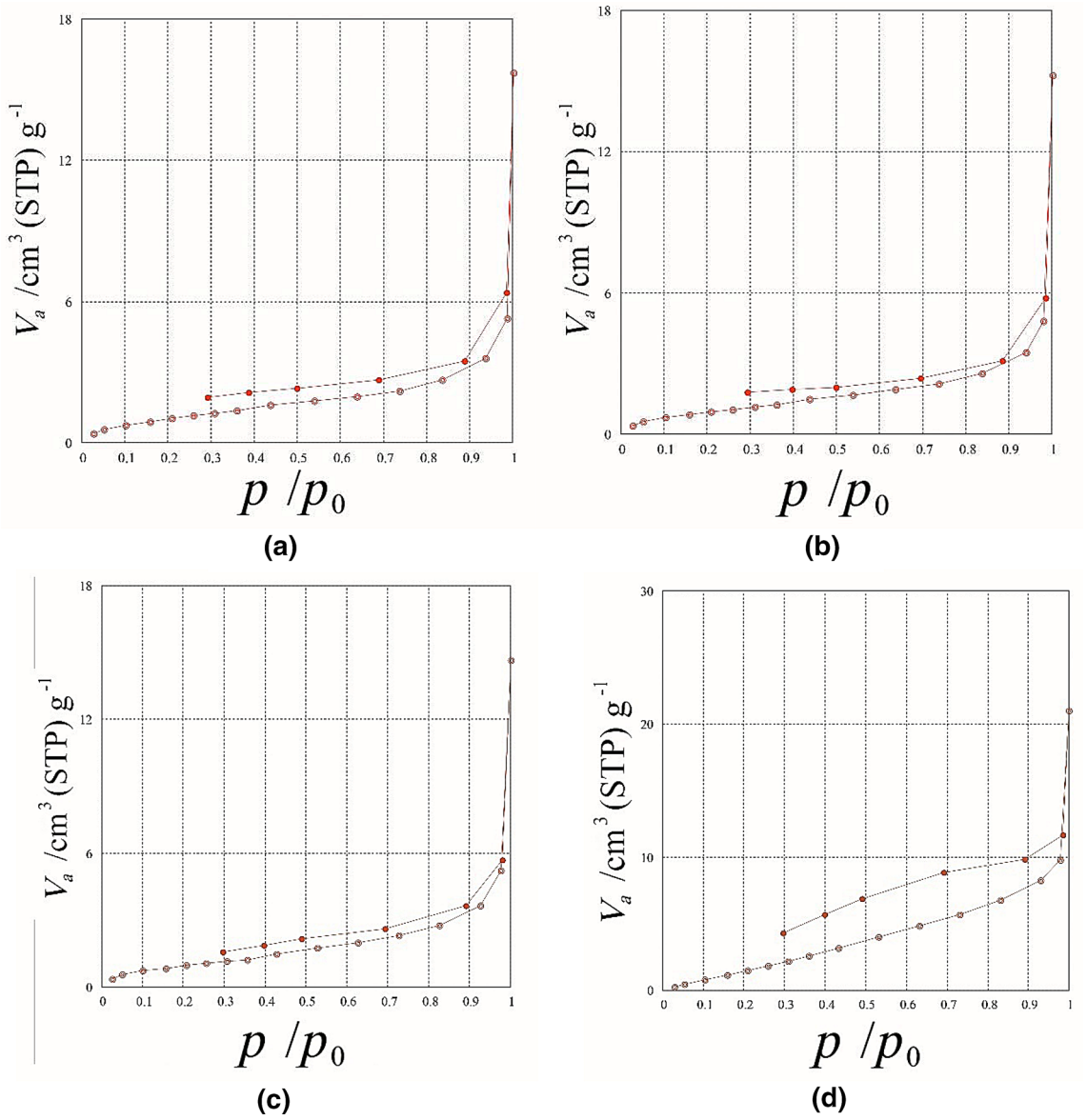

Fig. 5 The adsorption/desorption isotherms: **a** zein fibers and zein fibers loaded with **b** 2.5 w/v %, **c** 5 w/v %, **d** 10 w/v % ascorbyl palmitate

total pore volume of nanofibers. According to the International Union of Pure and Applied Chemistry (IUPAC) classification of adsorption isotherm, type II describes the presence of mesopores (2–50 nm pore diameter) and micropores exhibits a hysteresis loop and a variation point at a lower pressure (P/P_0) in a material [22]. The BJH method is commonly used to determine the volume and size distribution of mesopores (2–50 nm) and small macropores [22]. According to the BJH method, in the electrospun zein, and ascorbyl palmitate loaded zein nonporous nanofiber mostly containing the narrow mesopore size distribution, the pore size is in the range of 9.3–11 nm (Table 2), and the pore volume is 0.011–0.023 $cm^3\ g^{-1}$. This range of pore size is suitable for protecting encapsulant material against environmental conditions.

As ascorbyl palmitate concentration increased from 2.5 to 10% (w/v), electrospun mean fiber diameter also increased from 549 ± 123 to 795 ± 190 nm due to changes in the polymer viscosity (Table 1). Also, the mean pore size increased with increasing fiber diameter for loaded zein fibers and reached to 16.2 ± 5.01 nm at 5% w/v of AP. However, despite the high mean fiber diameter at the concentration of 10% w/v (795 ± 190 nm), the pore size significantly decreased and reached to 9.3 ± 2.57 nm. This behavior might be attributed to the "agglomeration of AP particles" which resulted in decreasing of nitrogen molecules adsorption in pores. The results are comparable and in agreement with Brahatheeswaran et al. [22] and Aliabadi et al. [28].

Based on Fig. 5, the adsorption volume and isotherm graphs for the pure zein fibers and loaded ones at 2.5 and 5 w/v% AP are almost the same and suggesting the presence of ''mesopores'' in the fibrous structures. Moreover, the graphs exhibit type IV adsorption accompanied with almost a type H_3/H_4 hysteresis loop (wedge-shaped or slit-shaped pore sutures). However, the adsorption/desorption isotherms for the zein fibers loaded with 10% w/v AP was changed, thereby a type H_2 hysteresis loop or ''bottle-neck shaped'' pores were observed. These changes in the shape of the pores could be attributed to the 'agglomeration of the AP'' due to high concentration of the loaded AP and therefore decreases in the pore size of the nanofibrous structure up to 9.3 ± 2.57 nm.

Effect of AP on the Wettability of Zein Matrix

The effect of AP on the wettability of zein matrix was investigated by measuring water contact angle. A large water contact angle (θ > 90°) suggests a hydrophobic surface, whilst a small water contact angle (θ < 90°) suggests a hydrophilic surface [29]. According to the results (Table 3), zein has a contact angle of 133° as a consequence of the presence of nonpolar amino acids such as leucine, proline and alanine. Results showed that increasing AP content did not significantly change the contact angle of zein matrix. Panevaet al [30] reported that incorporating ascorbyl palmitate to electrospun PCL fibers did not significantly change the hydrophobic behavior of the fibrous materials.

Table 3 Effect of AP on wettability of zein matrix

Ascorbyl palmitate (%, w/v)	Contact angle
0	133.1 ± 5.02°
2.5	125.6 ± 8.2°
5	114.7 ± 6.1°
10	136.5 ± 1.2°

Results are presented as mean ± SD (n = 3)

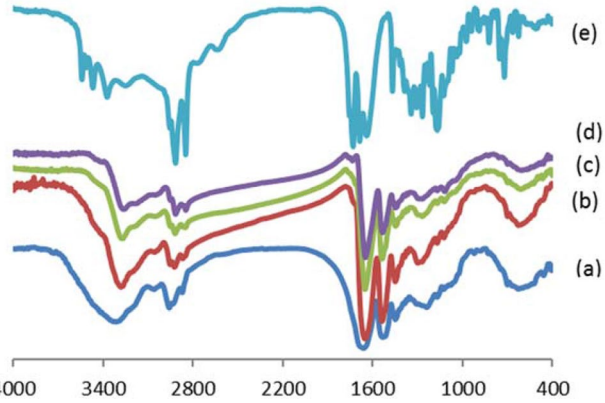

Fig. 6 FTIR spectra of different samples: **a** zein powder; **b** Ze–ASP 2.5%, **c** Ze–ASP 5%, **d** Ze–ASP 10% electrospun fibers with 2.5%, 5% and 10% (w/v) ascorbyl palmitate, respectively; and **e** ascorbyl palmitate powder

Interaction Between AP and Zein Fibers

Figure 6a–e illustrates the results obtained with FTIR for AP, neat zein, and AP loaded electrospun zein fibers. The peak of the zein at 3315 cm^{-1} corresponds to stretching of NH group. The peaks observed at 3060 cm^{-1}, 2959 cm^{-1}, 2937 cm^{-1} and 2873 cm^{-1} indicate CH stretching from functional groups of CH_3 and CH_2. The sharp peaks of zeinat 1660 cm^{-1} and 1532 cm^{-1} present the characteristic vibrational bands of zein known as amid I and amid II. The amid I peak is the evidence of stretching the C=O linkage from peptide groups. The amide II peak refers to stretching of C–N linkage as well as the folding of NH group. In Fig. 6e, the peaks of AP at 1756.40 cm^{-1} and 1731.85 cm^{-1} correspond to stretching of C=O groups. The C=C stretching band of AP is at 1687.83 cm^{-1}. The APOH stretching modes are observed between 1400 and 1200 cm^{-1}. The FTIR spectra of Ze–AP 2.5%w/v, Ze–AP 5%w/v, and Ze–AP 10%w/v electrospun fibers are shown in Fig. 6b–d. The peak of

zein at 3315 cm^{-1} corresponding to the stretching of NH group was observed to shift to 3281 cm^{-1}, 3268 cm^{-1} and 3265 cm^{-1} for Ze–AP 2.5%w/v, Ze–AP5%w/v and Ze–AP 10%w/v electrospun fibers, respectively. By increasing amount of AP in the zein fibers, band shifting was observed for the CH stretch mode of the zein from 2959 to 2949 cm^{-1}. The OH stretching vibration of the hydroxyl groups of AP at 1400–1200 cm^{-1} has disappeared in the spectrum of Ze–AP 2.5%w/v, Ze–AP 5%w/v and Ze–AP 10% w/v electrospun fibers. The zein amide I band at 1660 cm^{-1} was reduced and shifted to 1652.4, 1652.5 and 1649 cm^{-1} for Ze–AP 2.5%w/v, Ze–AP 5%w/v and Ze–AP 10%w/v electrospun fibers, respectively. While, the zein amide II band at 1532 cm^{-1} was increased and shifted to 1538, 1540 and 1535 cm^{-1} for Ze–AP 2.5%w/v, Ze–AP 5%w/v and Ze–AP 10%w/v, respectively. The changes in the zein spectrum demonstrated the interaction between zein and ascorbyl palmitate. AP and zein molecules possess free hydroxyl groups or amino groups that act as potential proton donors, as well as carbonyl groups that act as potential proton receptors for hydrogen bonding.

Encapsulation Efficiency of AP in Electrospunzein Fibers

The encapsulation efficiency of AP in electrospun zein fibers from the blend solution of zein–AP was found to be 22.5 ± 5% for 2.5% w/v AP loaded, 51.1 ± 12% for 5% w/v AP and 65.5 ± 10% for 10%w/v AP loaded, respectively. According to the results, encapsulation efficiency increased significantly ($p < 0.05$) when AP concentration was increased from 2.5 to 10% w/v. The inconsistency from the ideal value of 100% for these samples could be due to the failure of homogenous distribution of AP in the electrospun fibers, which could be influenced by the fabrication techniques [22], and degradation of AP in different steps of production, and storage of loaded electrospun zein fibers, because of its sensitivity to oxygen [23, 31]. Kim et al. [32] loaded ascorbyl pamitate in chitosan nanoparticles and reported the entrapment efficiency was 49%. Yoksan et al. [1] encapsulated ascorbyl palmitate in chitosan nanoparticles by oil-in-water emulsion and ionic gelation processes, and reported the encapsulation efficiency of 77%.

Release Analysis

Effect of Morphology on AP Release

The release profile of AP from fibers with different morphologies at different pHs (3 and 7.2) are shown in Fig. 7. At pH 7.2, the maximum AP release values were obtained as 42% and 72% from fibers with large beads and bead-free morphology, respectively. The highest release values

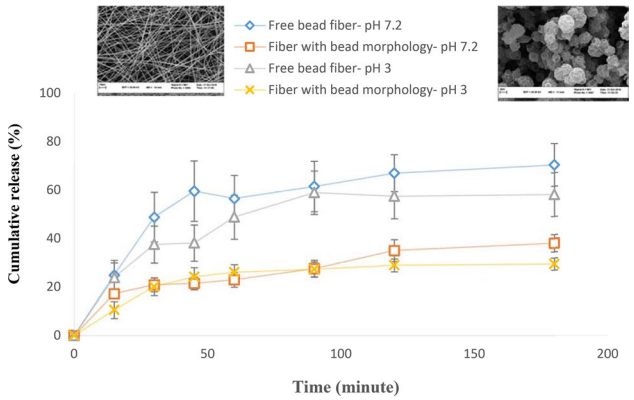

Fig. 7 Effect of morphology and pH on ascorby palmitate release (AP)

of AP at pH = 3 for defected (containing large beads) and bead-free fibers were also measured as 29.5% and 58.2%, respectively. As shown, the amount of released AP from bead-free fibers at both pH conditions (7.2 and 3) were significantly ($P < 0.05$) more than fibers with bead morphology. This clearly confirms that the AP release is dependent on the morphology of fibers.

By comparing the release profile, it is shown that 82.5% of AP release from bead-free fibers occurred during the first 45 min and the rest of AP (18.5%) released during 195 min, whereas, for the fibers containing large beads, only 21.5% of AP released during the first 45 min. It indicates that the rate of release from fibers with bead morphology is lower than bead-free fibers. This behavior could be attributed to the different specific surface area and fiber diameter values in the two studied morphologies.

Bead-free fibers have diameter of 795 nm and specific surface area of 4 μm^{-1}, while fibers with bead morphology have diameter of 2.6 µm and specific surface area of 1.4 μm^{-1}. As mentioned, diameter and specific surface area of bead-free fibers is lower and upper, respectively, than fibers with bead morphology. Compared to fibers with large beads, bead-free electrospun fibers have high surface area when being contacted to the aqueous solution that leads to more release of AP in the environment. Dissolution has a direct relation with the surface area [33]. Therefore, the release of AP from free-bead fibers was more than fibers with bead morphology [34].

Effect of pH on AP Release

The results showed that there was no significant difference between the release of AP at pHs 7.2 and 3. The amount of released AP from bead-free fibers during 180 min at pHs 3 and 7.2 were 58.2% and 70.4%, respectively. However, these values changed to 29.5% at pH 3 and 42% at pH 7.2

for fibers with bead morphology. The release of AP from fibers at pH 7.2 was more than pH 3 which is an indication of higher AP ionization in pH 7.2 and therefore, the solubility and release remarkably increased. To prove this theory, the fraction of non-ionized to ionized AP was calculated by *Henderson-Hasselbalch* (Eq. 3) [33]:

$$pKa - pH = log\frac{fU}{fI} \tag{3}$$

fU Non-ionized drug, fI ionized drug.

Based on the Eq. (3), the fraction of non-ionized to ionized AP at pH 7.2 was 1×10^{-3}, whereas this fraction at pH 3 was calculated as 15.85. Therefore, it can be concluded that solubility and release behavior of AP at pH 7.2 was more than pH 3.

Conclusions

In this study, ascorbyl palmitate was successfully encapsulated in electrospun zein fibers for the first time. Results revealed that the morphology of loaded fibers was bead-free, smooth, uniform and homogenous. The diameter of fibers affected by ascorbyl palmitate, so that increasing ascorbyl palmitate increased diameter of fibers from 549–795 nm. The DSC and XRD results both confirmed that ascorbyl palmitate existed as an amorphous material. The zein and ascorbyl palmitate loaded fibers mostly containing the narrow mesopore size distribution (9.3–11 nm), which is suitable for protecting encapsulant material against environmental conditions. Ascorbyl palmitate did not have a significant effect on the wettability of electrospunzein fibers. According to FTIR spectra, theinteraction between ascorbyl palmitate and zein was hydrogen bonding. Encapsulation efficiency increased from 22.5 to 65.5%significantly ($p < 0.05$) when AP concentration was increased from 2.5 to 10 w/v %. Release study results indicated that pH and morphology affect the release of AP. Concerning the adverse side-effects of synthetic polymers, the food-grade electrospun nanofibers are expected to be produced at industrial scale for various applications such as controlled-release of pharmaceuticals/nutraceutical and particularly active food packaging. This product is intended to be used as an "active coating" for the delivery of AP to butter and other food products. Moreover, since the designed nanofibrous structure increase the stability of AP, they could be applied as an appropriate "carrier" for "fortification" of different food products.

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Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

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