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Original Article

Pretreatment with Ultrasonication Reduces the Size of Azelaic Acid-Chitosan Nanoparticles Prepared by Electrospray

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ABSTRACT

Nowadays, electrospray is becoming a favourable approach for preparing monodispersed nanoparticles. However, this approach is quite recent and requires further works to optimize control over physicochemical properties of its products. This study aimed to determine the possible effects of sonication as a pretreatment to reduce the size of azelaic acid-chitosan particle before using electrospray. The results showed that sonication treatment can produce submicron particles of azelaic acid-chitosan. By diluting the solution and increasing sonication time and amplitude, smaller particles were obtained with the smallest one at 516 nm, sized by dynamic light scattering. The pretreated solution was then electrosprayed to reduce the size of nanoparticles to 80 nm, indicating that sonication may play an important role in reducing the size of electrosprayed nanoparticles. The electrosprayed nanoparticles were nearly monodispersed and almost spherical in shape.
Keywords: Electrospray, Chitosan, Azelaic acid, Ultrasonication

Le prétraitement par ultrasonication réduit la taille des nanoparticules d'acide azélaïque-chitosane préparées par électrospray

Résumé: L'electrospray est aujourd'hui une approche favorable pour la préparation de nanoparticules monodispersées. Cette méthodeest cependant assez récente et nécessite des travaux supplementaires afin demieux contrôler les propriétés physicochimiques des produits traités. Cette étude visait à déterminer les influences possibles d'un prétraitement par sonication sur la taille des particules issues d'une solution de chitosane-acide azélaïque. Les résultats ont montré que le traitement par ultrasons permet de produire des particules submicroniques d'acide azélaïque-chitosane. En diluant la solution et en augmentant le temps et l'amplitude de la sonication, des particules plus petites sontobtenues dont lesplus petites ont été mesurées par diffusion dynamique de la lumière à 516 nm. La solution prétraitée a ensuite été soumise à uneélectropulvérisation afin de diminuer la taille des nanoparticules jusqu'à 80 nm. Ceci montre que la sonication peut jouer un rôle important dans la réduction de la taille des nanoparticules traitées par électrospray. Les nanoparticules issues de l'électrospray étaient plus ou moins monodispersées et de forme sphérique.
Mots-clés: Electrospray; le chitosane; l'acide azélaïque; ultrasonication

INTRODUCTION

Chitosan, a biodegradable polysaccharide, is obtained from deacetylation of chitin (Jayakumar et al., 2007). Chitosan is globally the second largest biopolymer with increasing applications in many industries, such as cosmetics (Jimtaisong and Saewan, 2014), wood (Patel et al., 2013), and biomedicine (Suh and Matthew, 2000). Chitosan and its derivatives in nano- or microparticles have the potential to be employed in drug delivery systems, tissue engineering, and scaffolds (Suh and Matthew, 2000; Kumar et al., 2004; Azra et al., 2012). Effective encapsulation of drug compounds into a biodegradable and non-toxic carrier is an important challenge in designing drug delivery systems (Kumari et al., 2010). Various methods have been established for the preparation of nano- or microparticles and the encapsulation of drugs in the carrier, such as spray drying (Vehring, 2007), single/double emulsion methods (Kumari et al., 2010), and nanoprecipitation (Bilati et al., 2005). However, each method has shown disadvantages such as low encapsulation and loading efficiency, prolonged procedures (Bilati et al.), and heterogeneity in the produced size (Zambaux et al., 1998). Studies demonstrated that electrospray (electro-hydrodynamic atomization) is an effective approach to encapsulate drug into particles when spraying a solution to form tiny droplets using high voltage fields (Arya et al., 2009; Sridhar and Ramakrishna, 2013). This is a simple method that can overcome many of the limitations of other methods (e.g., slow extraction and complex evaporation process) (Yeo et al., 2005) with high drug encapsulation and loading efficiencies (Valo et al., 2009), lack of need to separate particles from the solvent (Lassalle and Ferreira, 2007), and maintaining the biological properties of active components (Braghirolli et al., 2013). In this method, several parameters such as solution concentration, voltage, flow rate, nozzle to collector distance, and method of solution preparation have been reported to influence the size of produced particles (Enayati et al., 2011; Karimi Zarchi et al., 2015). For instance, it has been reported that by decreasing solution concentration and/or spraying flow rate, particle size could be reduced (Zarrabi and Vossoughi, 2009; Zhang and Kawakami, 2010). In addition, when using minimum distance between nozzle and receiver, particles were found to be smaller (Karimi Zarchi et al., 2015). Azelaic acid, a non-toxic dicarboxylic acid, is a natural organic compound, which is found in barley, wheat, and rye (Cornils and Lappe, 2000). It is industrially prepared by the ozonolysis (oxidation) of oleic acid through bacterial degradation of pelargonic acid (Breathnach and Levi-Montalcini, 1995). Topical azelaic acid has been used effectively in the treatment of chloasma and acne (Fitton and Goa, 1991). In our work, the effects of solution concentration, as well as time and amplitude of sonication on the size of nanoparticles in electrospray technique were studied. We first produced solution of azelaic acid-loaded chitosan particles via sonication method. Subsequently, the solution was electrosprayed to allow nanoparticle fabrication, which can be used in biomedical processes. This is the first attempt to investigate the effect of ultrasonication pretreatment in electrospray process.

MATERIALS AND METHODS

Materials. Azelaic acid (pharmaceutical grade) was gifted by Sepidaj Pharmaceutical Co. (Tehran, Iran). Chitosan was purchased from Zhengzhou Sigma Chemical Co. (Zhengzhou City, China) (MW=100 kDa and deacetylation degree > 80%). Other chemicals used in the study were analytical grade and acquired from Merck (Darmstadt, Germany).

Preparation of azelaic acid-loaded chitosan solution. The specified amount of chitosan was used in the aqueous solution of azelaic acid (1 g/l) at azelaic acid/chitosan ratio of 1:2. Then, the solution was vigorously stirred for about 60 min until chitosan was completely dissolved. pH of the solution was adjusted at 5.0±0.1. The solutions were then diluted 1:5 or 1:10 with water and sonicated under continuous mode by ultrasonication (UP400S, 24 kHz, Hielscher GmbH) at

400 W, amplitudes of 50% and 100%, for 0, 30, 60, and 120 s.

Electrospray process. The nanoparticles were attained by a single spray nozzle mode (Karimi Zarchi et al., 2015). The obtained solutions from the previous step were loaded into a 1-ml injection syringe and electrosprayed at 1 ml/h flow rate and 6.8 kV voltage by a syringe pump (SP2000, Fannavaran Nano-Meghyas Co., Iran) and a high voltage power supplier (HV35P OC-series, Fannavaran Nano-Meghyas Co., Iran), respectively. A distance of 11 cm was set between the nozzle and collector (i.e., ground electrode). A permanent cone-jet mode was obtained using the settings mentioned above.

Characterization of nano- or microparticles. Size and size distribution of the obtained particles were determined freshly by dynamic light scattering (DLS) using Scatteroscope-I (K-One. Co., Korea). Particle size distribution was calculated using Equation 1:

$$Particle\ size\ distribution = \sqrt{\frac{d75}{d25}}$$

Morphology and particle size of azelaic acid-loaded chitosan nanoparticles were studied by scanning electron microscopy (SEM; Zeiss DSM-960A, Oberkochen, Germany) at a voltage of 15 kV.

RESULTS

Table 1 details the results of DLS experiments using different values for polymer concentration, sonication time, and amplitude. It was noted that increasing the duration of sonication commonly decreases particle size in solutions. Also, enhanced amplitude of sound waves caused a noticeable reduction in particle size. Figure 1 compares the effect of sonication on DLS results from a single sample. To study the effect of solution concentration on particle size, the sonication time was fixed (i.e., 30, 60, and 120 s). Table 1 presents that by reducing the solution concentration, particle size dwindles. According to the results of electrosprayed samples (Figure 2), the chitosan nanoparticle preparations were quasi-monodisperse and almost spherical in shape. To study the impact of sonication pretreatment, three samples (i.e., samples 3 [no pretreatment], 13 [pretreatment for 60 s], and 18 [pretreatment for 120 s] with particle size of 3990,609 and 516 nm, respectively) were electrosprayed. The obtained nanoparticles showed the mean (SD) sizes of 320, 140, and 80 nm, respectively. The results indicated that sonication pretreatment (i.e., reduction in the size of particles to be electrosprayed) remarkably affected the size of generated nanoparticles using electrospray. To summarize, our findings illustrated that sonication of chitosan solution diminished the size of particles, with the minimum size obtained by increasing the time and amplitude of sonication, as well as decreasing the solution concentration. Further, the particle size of electrosprayed chitosan decreased when the solution was pretreated using ultrasonication.

DISCUSSION

The ultrasonication method is applied in a wide range of fields, such as drug delivery (Pitt et al., 2004), degassing, cleaning, homogenizing (Miller et al., 2012), deagglomerating, and dispersing (Hwang et al., 2008). Probe-type ultrasonication commonly shows better efficiency compared to bath-type (Capelo-Martínez, 2009). We used the sonication process for azelaic acid-loaded chitosan particles via ultrasonic-probe apparatus. A DLS technique was then employed to assess the size of the prepared particles in the solution.

Nanoparticles were then generated by the electrospray technique from chitosan-azelaic acid dispersion. SEM was then utilized to study the size and morphology of nanoparticles. It can be concluded that solution sonication has a major influence on the size of nanoparticles in solution, as previously reported in other studies (Esmaeilzadeh-Gharedaghi et al., 2012). A study showed that when employing sonication, ampicillin trihydrate-loaded chitosan became smaller with more uniform nanoparticles (Saha et al., 2010).

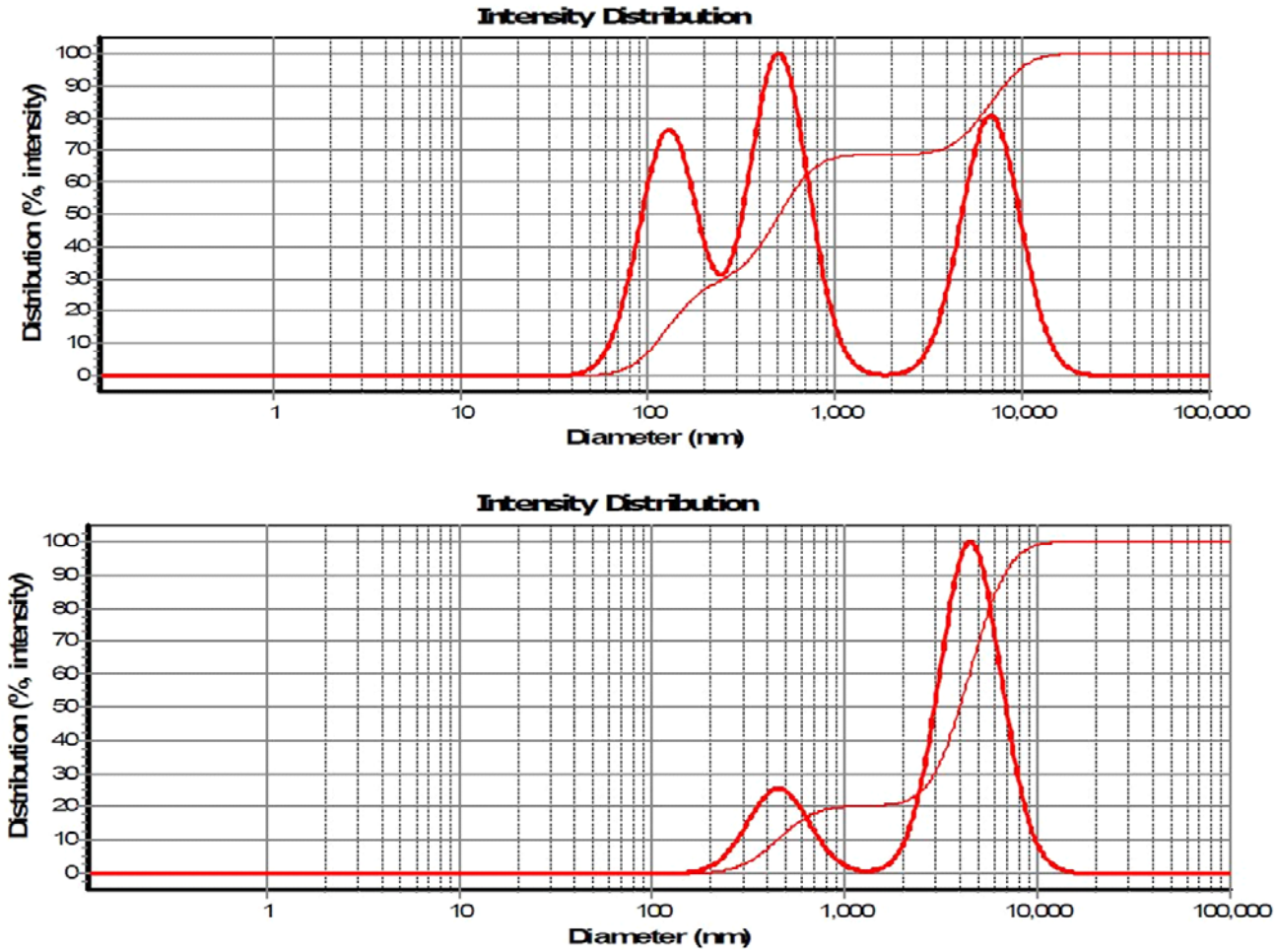

Figure 1. Particle size analysis report of azelaic acid-loaded chitosan, Up: sonicated (sample No. 18, d(50):516 nm) and (B) Down:non-sonicated (sample No. 3, d(50):3990 nm)

In donepezil-loaded chitosan nanoparticles, reduced particle size after using sonication (down to 116.8 nm) has also been reported (Raja Azalea et al., 2012). In this case, sonication helps break intra- and intermolecular bonds to reduce particle size (Azra et al., 2012). Furthermore, we found that higher amplitude values lead to smaller particle sizes. In a similar study, when using sonication, smaller chitosan particles were collected (amplitude > 55) (Esmaeilzadeh Gharehdaghi et al., 2014). Another work on chitosan nanoparticles and molecules showed that when applying an amplitude of 80 for 10 min, a smaller chitosan could be obtained compared with using the sonication amplitude of 40 for 10 min (Tang et al., 2003). Additionally, it has been shown that by decreasing chitosan concentration, particle size decreases, as well. For instance, by diluting the chitosan solution from 2 mg/ml to 0.1 mg/ml, a reduction in the size of particles from ~ 800 nm to ~ 100 nm is expected (Esmaeilzadeh-Gharedaghi et al., 2012). The primary objective of this study was to evaluate the size of electrosprayed nanoparticles when pretreated with sonication. Simultaneously, the effect of particle size of chitosan dispersion (i.e., before the elctrospray process) may be determined based on the size of electrosprayed nanoparticles. Further studies are required to investigate the possible effects that may be

observed in encapsulation efficiency and drug loading of nanoparticles. Globally, sonicated samples generated smaller electrosprayed nanoparticles. The results showed that azelaic acid-loaded chitosan nanoparticles with the mean size of approximately 100 nm can be fabricated by using electrospray on the samples pretreated with ultrasound. To the best of our knowledge, no work has so far used the combination of both methods.

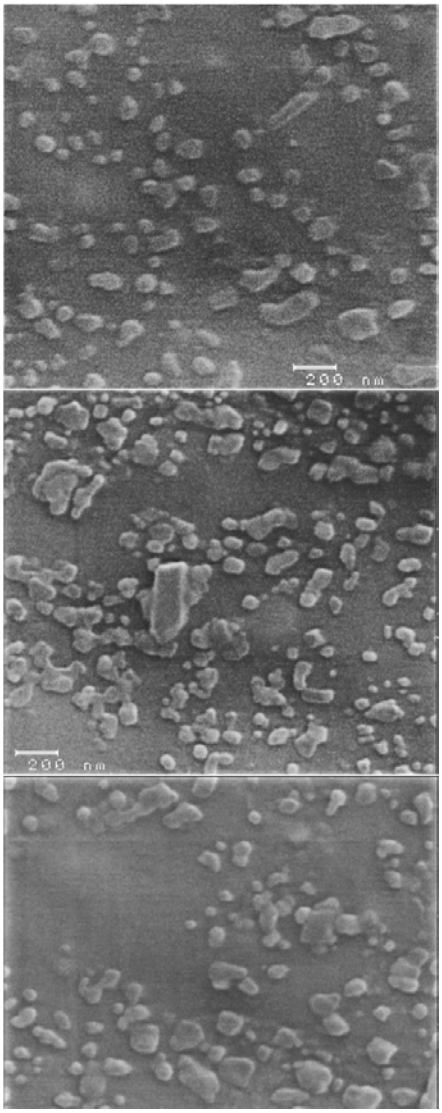

Figure 2. Scanning electron microscope micrograph of azelaic acid-loaded chitosan, Up: sonicated 120 s (sample No. 18), Middle: sonicated 60 s (sample No. 13) and Down: non-sonicated (sample No. 3).

The optimal conditions to collect smaller nanoparticles were obtained with pretreated ultrasonication and appraised as 120 s, 1:10, and 100% for sonication duration, dilution, and amplitude, respectively.

Table 1 .The size of particles obtained in dynamic light scattering

Sample No.	Dilution	Sonication time (s)	Amplitude (%)	Measured size, d_{50} (nm)	Particle size distribution
1	No dilution	-	-	6920	2.81
2	1:5	-	-	5480	3.46
3	1:10	-	-	3990	1.44
4	1:5	30	50	4690	3.49
5	1:10	30	50	3960	2.85
6	No dilution	30	100	6740	1.10
7	1:5	30	100	3350	1.42
8	1:10	30	100	2540	2.48
9	1:5	60	50	4570	3.30
10	1:10	60	50	3760	2.78
11	No dilution	60	100	6460	4.66
12	1:5	60	100	3630	3.41
13	1:10	60	100	609	3.69
14	1:5	120	50	3680	2.96
15	1:10	120	50	3220	2.96
16	No dilution	120	100	1440	2.22
17	1:5	120	100	766	3.08
18	1:10	120	100	516	5.20

Ethics

I hereby declare all ethical standards have been respected in preparation of the submitted article.

Conflict of Interest

The authors declare that they have no conflict of interest.

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