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Research Article

Supercritical CO_2 Generation of Nanometric Structure from *Ocimum basilicum* Mucilage Prepared for Pharmaceutical Applications

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Abstract. Plant-derived polymers are widely used in the pharmaceutical industry due to their emollient, lack of toxicity, and irritating nature and low cost. In this work, basil seed mucilage was dried using supercritical carbon dioxide phase inversion technique to form a nanometric structure. The obtained polymeric structures were characterized by scanning electron microscopy (SEM), Brunauer–Emmett–Teller (BET) method, and Fourier transform infrared spectroscopy (FTIR) and compared with the oven-derived sample group. It was demonstrated that the product morphology could be controlled by altering the composition of methanol which functioned as the co-solvent in the nonsolvent stream. The most homogeneous product (60-nm mean pore size diameter, 78 m^2/g BET surface area with no agglomeration) was obtained with 2.5% methanol. The FTIR data showed that the presence of hydroxyl and carboxyl groups suggested the bioadhesive property of basil seed mucilage was good and many active pharmaceutical compounds might be loaded to the resultant nanometric structure to enhance drug release. Furthermore, the FTIR analyses indicated that the nature of the final product did not change during the supercritical drying procedure.

KEY WORDS: basil seed gum; drug delivery; FTIR characterization; nanostructure; natural polymer; supercritical phase inversion.

INTRODUCTION

Natural polymers are widely used in the pharmaceutical industry as an emulsifying agent, adjuvant, (1) and adhesive in packaging and are also well suited for cosmetic product development (2). Due to its biocompatibility, gelation ability, water-binding capacity, nonirritating nature, and low cost, natural polymers can be used as contact lenses, linings for artificial hearts, materials for artificial skin, membranes for biosensors, and drug delivery media (3–8). In addition, they possess many functional groups available for chemical modification, which provide an overwhelming variety of products with tailorable chemistries and properties to be obtained (3,9).

Mucilages are plant-derived natural polymers which mainly consists of polysaccharides, proteins, and uranides (10). They are widely used in the pharmaceutical industries as adjuvant, thickeners, suspending agents, binders, and granulating agent and are also used as matrices for sustained and controlled drug delivery (1,2,10–17). Mucilage is a gelatinous substance which when concentrated is called as gum. The main difference between them is that mucilage does not dissolve in water whereas gum dissolves in water and makes hydro-gel. Acacia, tragacanth, gum ghatti, gum karaya, and *Ocimum bascilium* are popular examples of plant mucilages with a demonstrate values in pharmaceutical applications (2,16–19).

O. basilicum L. also known as basil is an herb plant commonly found in Asia, Africa, and Central and South America. Basil seeds have been used in traditional medicine for a long time to treat colic ulcer, dyspepsia, diarrhea, and inflammations, among other ailments (20). Basil seed is black in color and oval in shape with mean dimensions of 3.11± 0.29 mm (length), 1.82±0.26 mm (width), and 1.34±0.19 mm (height) (20). When soaked in water due to the presence of a polysaccharide layer, the outer pericarp of basil seeds swells into mucilage which can be extracted from seeds and dried or concentrated for further applications.

Malviya *et al.* (15) reviewed mucilage applications, as a potent candidate for various pharmaceutical formulations. Jani *et al.* (1) reviewed the developments in natural gums and mucilages for use in the pharmaceutical sciences. Prajapati *et al.* (2) reviewed the pharmaceutical applications of various natural gums, mucilages, and their modified forms for the development of various drug delivery systems. Archana *et al.* characterized mucilage polysaccharide from waste of *Abelmoschus esculentus* for biomedical applications (14). Characterization and *in vitro* drug release studies of *Terminalia catappa* gum were carried out by Meka *et al.* (17). Srinivas *et al.* (12) study focused on *O. basilicum* as disintegrates in the formulation of dispersible tablets.

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The common gel drying techniques have some shortcomings such as time-consuming, organic solvent residuals inside the polymeric network, and limitation in the obtainment and preservation of various levels of porosity. To overcome these limitations, SC-CO_2-assisted gel drying processes have been recently implemented. The SC-CO_2 has been used as an alternative nonsolvent in phase inversion processes to generate polymeric and biopolymeric porous structure (21). The obtained structures possess good interconnectivity and high porosity.

SC-CO_2 rapidly dissolves many organic compounds at moderate temperatures and pressures (for example, at 100 bar and 40°C). The operating conditions of SC-CO_2 processes are readily obtainable, and their low-temperature condition is compatible with thermal stability of polymers. However, at the ordinary temperatures and pressures used in SC-CO_2 processing, it shows a very limited compatibility with polar components, like water. For example, at 40°C and 100 bar, solubility of water in SC-CO_2 is only around 0.5% (22). Therefore, the common supercritical gel drying process is not directly applicable to polymeric hydrogels. To overcome this limitation, CO_2-assisted phase inversion (23) and water substitution SC-CO_2 gel drying process (24,25) have been recently implemented. In both of the mentioned works, water solubility in SC-CO_2 was improved, by utilizing a polar organic co-solvent.

In this work, extracted mucilage from basil seeds was dried using supercritical CO_2 (SC-CO_2) to form a nanoporous structure for pharmaceutical applications such as tissue-replacing applications and drug delivery matrices. The drying procedure was performed using a modified method based on Temtem *et al.* (23) study in which methanol was used as co-solvent to improve water solubility in SC-CO_2. Moreover, scanning electron microscopy (SEM), Brunauer–Emmett–Teller (BET), and Fourier transform infrared spectroscopy (FTIR) analyses were carried out for product characterization.

MATERIALS AND METHODS

Materials

Basil seeds were purchased from a local market. Ethanol and methanol (99.99% purity) were obtained from Merck, and industrial grade carbon dioxide (≥99.9%) was purchased from Zamzam Co. (Isfahan, Iran). All materials were used upon being received.

Experimental Setup

The setup of gel drying process is illustrated in Fig. 1. Carbon dioxide was drawn from the cylinder (1) and pumped with a high-pressure pump (2) (Jasco–Milroyal B, France) equipped with a cooler to liquefy CO_2. High-pressure carbon dioxide was heated in a spiral heat exchanger (3) located in an oven (4) before charging into the 50-mL stainless steel high-pressure vessel (5). Co-solvent was delivered from reservoir (6) to the high-pressure vessel by secondary high-pressure pump (7) (Dionix, GP40, USA) after mixing with CO_2 flow at the entrance of spiral heat exchanger (3). The pressure was controlled by a back-pressure regulator (8) (Tescom, elk river, USA). Pressure and temperature in the high-pressure vessel were monitored using a pressure gauge (PI) and thermocouple (TC) respectively.

Procedures of Mucilage Extraction

The extraction of mucilage from basil seeds was performed using a method modified from Razavi *et al.* study (26). The basil seeds were soaked and swelled in distilled water at 65°C and a water/seed ratio of 50:1 at pH 8 which was adjusted using a 0.2-M aqueous NaOH solution. The mixture was stirred with a rod paddle mixer at 100 rpm until the seeds were completely swelled which was approximate 30-min agitation. The swelled seeds washed with ethyl alcohol/water solution with ethyl alcohol content varied from 10, 30, 60, 70, and 80 to 90%, respectively. Seed solution then passed through an extractor (Pars Khazar 700P, Rasht, Iran) with a rotating rough plate to scrape the mucilage layer off the seed surface. The separated mucilage was passed through a sieve and then centrifuged at 5000 rpm (Himac CR22GII, Hitachi Koki Co. Ltd., Takeda, Japan) for 10 min at ambient condition to remove all likely seed residuals.

In order to remove protein and ash content, after filtration of the crude extract, pure ethyl alcohol was added to the extracted mucilage in the 3:1 ratio and left overnight at 4°C. Finally, crude extract concentrated at 55°C with rotary vacuum evaporator (IKA, RV 10, Deutschland, Germany) to remove the extra water/ethyl alcohol content to form hydrogel. A thick and viscose gel with dried mucilage and water in 1:200 ratios was kept in the refrigerator for further use. Two sets of samples of the obtained gum, hydrogel, were weighted, and each sample was dried in the following manner: The first sample (reference) was dried with air in a laboratory oven at 55°C for 6 h. The second group of samples was dried by SC-CO_2 as described below.

Supercritical Gel Drying Procedure

SC-CO_2 gel drying was performed according to the following procedure: 2 mL of the mucilage solution, as described before, was loaded into a stainless steel cap and then placed inside the high-pressure vessel. The vessel was closed, and pure SC-CO_2 was charged from the top to fill the vessel. When the required pressure and temperature were obtained (220 bar and 55°C), the SC-CO_2 continued to flow at the exact rate of 4 mL/min. Simultaneously, the co-solvent (pure methanol) was introduced into SC-CO_2 using a high-pressure pump at an exact flow rate of 0.1 and 0.2 mL/min to form a nonsolvent solution. Thus, the nonsolvent is a binary mixture of methanol and CO_2, with methanol composition in the range of 2.5 and 5%. The methanol addition mode to the nonsolvent stream is a key parameter to control product morphology (23). Nonsolvent addition lasted 3 h. At the end of this stage, a pure CO_2 flow is passed through the high-pressure vessel, for 30 min, to remove the methanol residue in polymeric structure. The vessel was then depressurized slowly for a time about 20 min to bring the system back to atmospheric pressure.

Characterizations

The structure of the final products was investigated macroscopically and microscopically using scanning electron microscopy (SEM). The samples were sputter-coated with gold

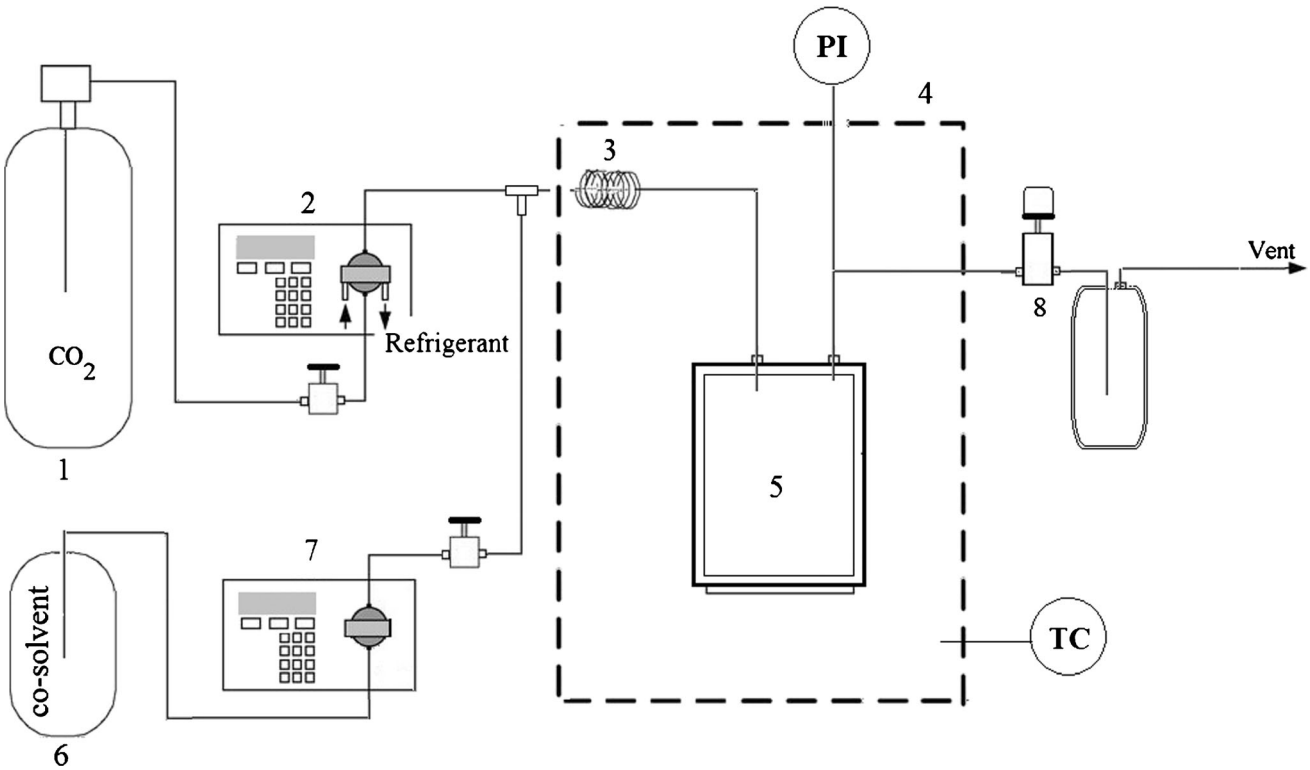

Fig. 1. Schematic diagram of supercritical drying setup: (*1*) CO_2 cylinder, (*2*) CO_2 pump equipped with a cooler, (*3*) heat exchanger, (*4*) oven, (*5*) high-pressure vessel, (*6*) co-solvent reservoir, (*7*) co-solvent pump, and (*8*) back pressure regulator

(SC 7620, Quorum Technologies, UK) at 30 mA for 180 s and analyzed by SEM (DSM 960A, Carl Zeiss AG, Germany) to elaborate the micro and nanostructure and to measure the diameter of structure pores. Surface areas were determined from the isotherm of adsorption of liquid nitrogen using the Brunauer–Emmett–Teller (BET) method (measured on a Quantachrome ChemBET 3000, Florida, USA, which was equipped with a thermal conductivity detector) at degasing temparature of 60°C. FTIR (Tensor 27; Bruker) analysis was used to characterize the chemical structure of basil seed mucilage and identify the effect of drying procedure on the structure of the obtained product. The mean pore diameter of the obtained sample was measured using SEM image analysis (ImageJ software®). Approximately 80 points were selected and measured for each sample and the mean diameter of pores was calculated.

RESULTS AND DISCUSSION

In this work, continuous steady-state supercritical gel drying based on phase inversion method was utilized to generate nanoporous structure from mucilaginous seeds of *O. basilicum* for pharmaceutical applications. Our attention was focused on FTIR characterization and the difference between the obtained structures from common laboratory oven drying and supercritical phase inversion gel drying technique.

Laboratory Oven Drying of Virgin Sample

The reference sample was hydrogel (dried mucilage water, 1/200 *w*/*w*) with no further treatment was put on the stainless steel plate and dried in a laboratory oven at 55°C. As expected, the final product demonstrated a 2-dimensional flat structure (Fig. 2) which SEM image in Fig. 3 shows nonporous structure. The solid sphere formed on plate surface is probably due to gel coagulation and impurities.

Supercritical Gel Drying of Sample

The widespread use of SC-CO_2 to generate porous structure from natural polymers is limited due to the fact that these polymers are only soluble in aqueous solution (pure water or acidified water) which is a solvent with very little affinity/solubility in SC-CO_2. To overcome this limitation, it was necessary to use a co-solvent in the supercritical fluid to increase the mutual affinity between solvent and nonsolvent. Temtem *et al*. have used a similar approach, phase inversion technique, to produce chitosan devices with controlled morphology (23).

In this work, methanol was used as co-solvent. Temtem *et al*. reported that co-solvent addition mode to the nonsolvent stream is a key parameter to control membrane morphology (23). In this work, methanol addition mode with the temperature and pressure being kept constant during the process was examined. In order to maximize the advantages of using supercritical fluid technology, methanol was used with the minimum possible amount. The drying experiments performed with two different methanol compositions of 2.5 and 5% as mentioned before.

Figures 3 and 4 show the SEM images of the porus structures of the final products with methanol composition of 5 and 2.5% in nonsolvent stream, respectively. As shown in Figs. 3 and 4, in both cases, porous structures were obtained. But, the image

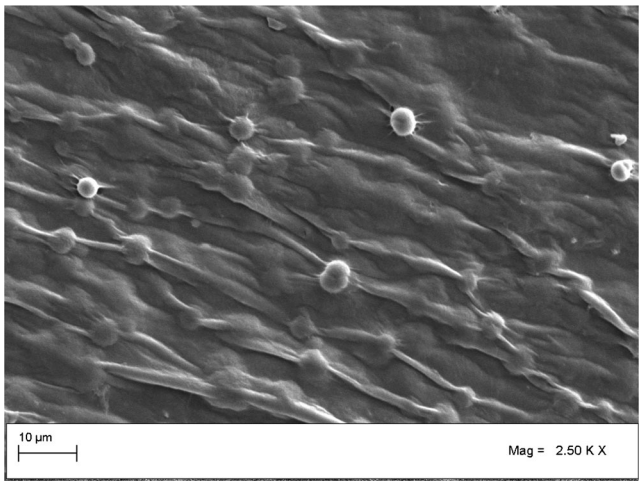

Fig. 2. SEM image of the oven-dried sample at 55°C for 6 h

of 5% methanol in the nonsolvent stream demonstrated some agglomerations formed in the polymeric structure.

Figure 4 shows the uniform nanometric network obtained by SC-CO_2 process with 2.5% methanol in nonsolvent stream. In this case, due to low concentration of methanol in nonsolvent stream, water content of basil seed mucilage solution was removed very slowly; therefore, particle coagulation was avoided. Thus, the nanometric structure of the products obtained by SC-CO_2 process with 2.5% methanol in nonsolvent stream is more homogeneous in comparison to the product obtained from 5% methanol in nonsolvent stream. The mean diameter of the nanometric pores (obtained by 2.5% methanol in nonsolvent stream) was measured to be about 60 nm. Using BET method, the specific surface area of this sample was measured to be 78 m^2/g. The obtained surface area indicated high porosity with good interconnectivity among the pore network in the polymeric structure. The biocompatible structure obtained by this method demonstrated a high level of porosity, with a good interconnectivity among the pore network system. Furthermore, the obtained product has the ability to be loaded with various types of drugs in its structure and used as controlled drug delivery medium.

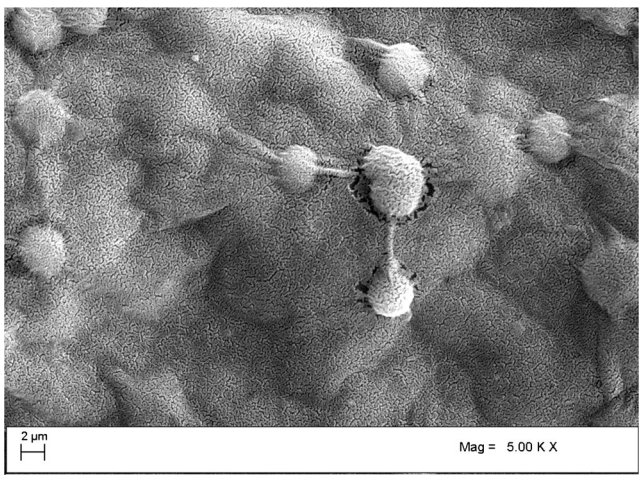

Fig. 3. SEM images of mucilage structure obtained by SC-CO_2 phase inversion drying with methanol composition of 5% in nonsolvent stream

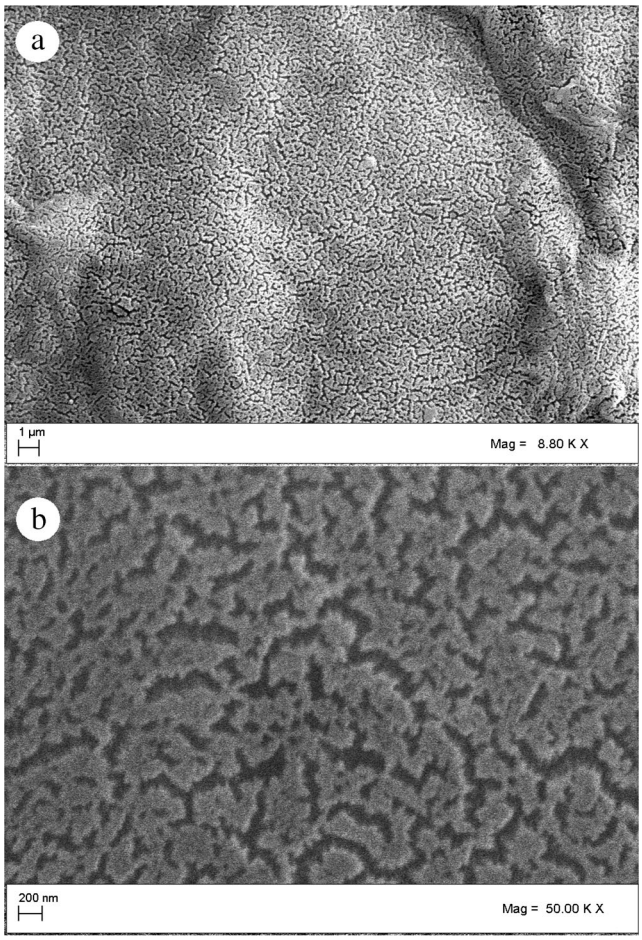

Fig. 4. SEM images of uniform nanometric mucilage structure obtained by SC-CO_2 phase inversion drying with methanol composition of 2.5% in nonsolvent stream; **b** magnified image of **a**

Furthermore, the character of interconnected pores enables many drugs to be loaded in this structure and, thus, controls drug release in a desired manner.

FTIR Characterization of the Products

The FTIR spectra are considered as useful information for partial characterization of seaweeds (27). The mucilage polysaccharide exhibited more or less common constant peaks. In the two similar previous works, the bands of okra mucilage were obtained at 3402.5, 2928.0, 2366.9, 2345.4, 1617.6, 1419.9, 1319.9, 1281.3, 1195.9, 1096.1, and 669.6 cm^{-1} (14) and the bands of *Ficus reticulata* fruit mucilage were detected at 3155.3, 2935.5, 2885.3, 2098.4, 1635.5, 1234.4, 1110.9, 991.3, and 852.5 cm^{-1} (28).

The IR spectrum of oven-dried mucilage sample is shown in Fig. 5. The occurrence of peak at the 2885.4 cm^{-1} signifies C-H asymmetric stretching. The observed characteristic peaks at 3200–3400 cm^{-1} owing to OH stretching of alcohol (or water absorbent) and N-H bond, at 1095.9 cm^{-1} owing to C-O stretching of alcohol and at 2916.1 cm^{-1} due to C-H stretching of alkyl group. The peak at 1589 cm^{-1} was observed in the present study for N-H primary amide and at 883.4 cm^{-1} (C-H aromatic bond) was also detected.

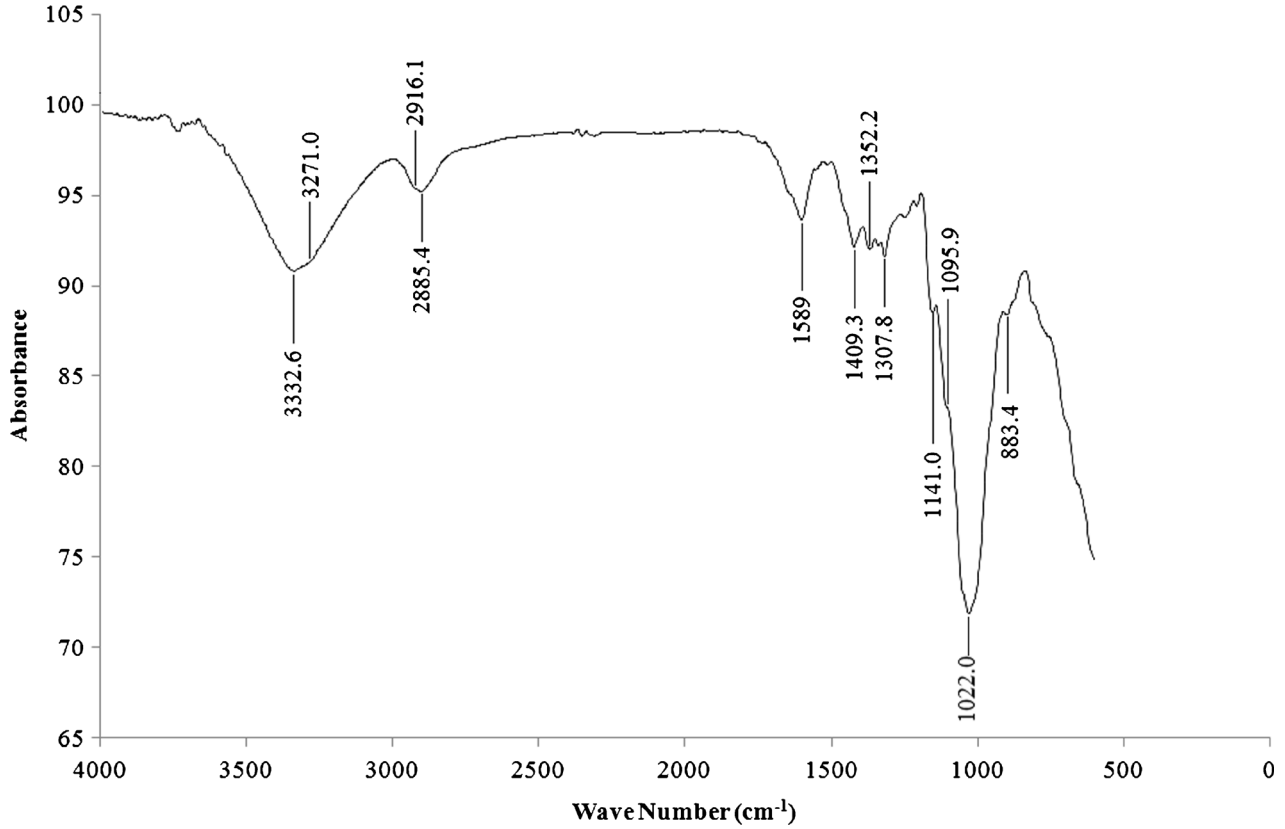

Fig. 5. FTIR spectra of the oven-dried basil seed mucilage

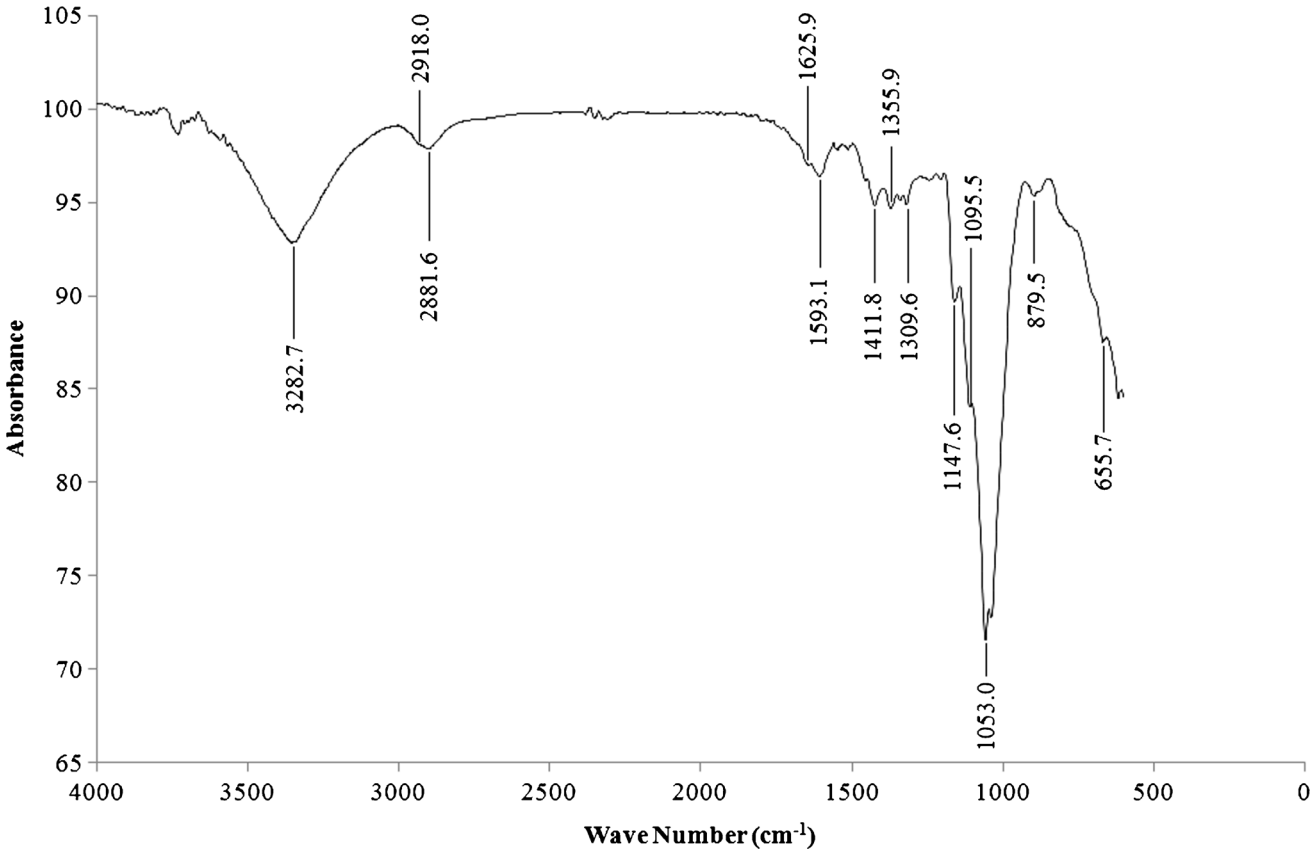

Fig. 6. FTIR spectra of the SC-CO_2 phase inversion-dried basil seed mucilage

Similarly, as shown in Fig. 6, the bands of SC-CO_2-dried mucilage were detected at 3720.5, 3282.7, 2918.0, 2881.6, 1625.9, 1593.1, 1411.8, 1355.9, 1309.6, 1147.6, 1095.5, 1053.0, 879.5, and 655.7 cm^{-1}. The comparison of FTIR spectra of SC-CO_2-dried and oven-dried mucilage indicated that the chemical structure of the final products was not affected by the SC-CO_2 drying procedure.

The results show that except for the usual bands of hydroxyl (3200–3400 cm^{-1}) and amide carbonyl (1589 cm^{-1}) groups, protein (carbonyl stretch 1400–1500 cm^{-1}) and amide deformation (1625 cm^{-1}) are also identified. These results suggested the basil seed mucilage is neither starch nor cellulose, but possess some peptide cross-links and some amino sugars (29).

Furthermore, it is known from literature that polymers with hydrophilic groups, such as carboxyl and hydroxyl groups, can strongly bind with the oligosaccharide chains of the mucous layer (14). It was also noted that carboxyl and hydroxyl groups makes this polymer capable to form hydrogen bond. Thus, basil seed mucilage has good bioadhesive property singly or in combination with polysaccharides like alginate sulfate for biofilm formation and drug delivery systems. Many actives can be released through such bio adhesives, as steroids, anti-inflammatory agents, pH-sensitive peptides, and small proteins such as insulin, and local treatments to alleviate pain in the buccal cavity (14). From the IR data, it is concluded that those which are rich in protein can form strong gels when lignin is bonded with Ca^{2+} ions, Ba^{2+}, and Sr^{2+} (30) by cross-linking, similar to guar and okra gums.

CONCLUSION

The present work presents application of the supercritical modified gel drying method for producing drug delivery materials from basil seeds mucilage. SC-CO_2 phase inversion technique for hydrogel drying successfully generated nanometric structure from mucilaginous seeds of *O. basilicum*. The most homogeneous product (60-nm mean pore size diameter, 78 m^2/g BET surface area with no agglomeration) was obtained with 2.5% methanol in nonsolvent stream. It was shown that the obtained structure *via* SC-CO_2 phase inversion is more uniform and porous than the sample *via* oven-dried process. It was also demonstrated that the product morphology can be controlled by the composition of the co-solvent in the nonsolvent stream. The results of IR data show that basil seeds mucilage has good bioadhesive property and many drug actives can be released through such bio adhesives, as steroids, anti-inflammatory agents, pH-sensitive peptides, and small proteins such as insulin, and local treatments to alleviate pain in the buccal cavity.

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REFERENCES

1. Jani GK, Shah DP, Prajapati VD, Jain VC. Gums and mucilages: versatile excipients for pharmaceutical formulations. Asian J Pharm Sci. 2009;4(5):309–23.
2. Prajapati VD, Jani GK, Moradiya NG, Randeria NP. Pharmaceutical applications of various natural gums, mucilages and their modified forms. Carbohydr Polym. 2013;92(2):1685–99.
3. Gomes M, Azevedo H, Malafaya P, Silva S, Oliveira J, Silva G, *et al*. 16 natural polymers in tissue engineering applications. In: Ebnesajjad S, editor. Handbook of biopolymers and biodegradable plastics. Boston: William Andrew Publishing; 2013. p. 385–425.
4. Dang JM, Leong KW. Natural polymers for gene delivery and tissue engineering. Adv Drug Deliv Rev. 2006;58(4):487–99.
5. Sell SA, Wolfe PS, Garg K, McCool JM, Rodriguez IA, Bowlin GL. The use of natural polymers in tissue engineering: a focus on electrospun extracellular matrix analogues. Polymers. 2010;2(4):522–53.
6. Liu Z, Jiao Y, Wang Y, Zhou C, Zhang Z. Polysaccharides-based nanoparticles as drug delivery systems. Adv Drug Deliv Rev. 2008;60(15):1650–62.
7. Jagur-Grodzinski J. Polymeric gels and hydrogels for biomedical and pharmaceutical applications. Polym Adv Technol. 2010;21(1):27–47.
8. Simões S, Figueiras A, Veiga F. Modular hydrogels for drug delivery. J Biomed Nanotechnol. 2012;3(2):185–99.
9. Kurita K. Controlled functionalization of the polysaccharide chitin. Prog Polym Sci. 2001;26(9):1921–71.
10. Shah V, Patel R. Studies on mucilage from Hibuscus rosasinensis linn. as oral disintegrant. Int J Appl Pharm. 2010;2(1):18–21.
11. Sheth N, Shah N, Shah N. Extraction of mucilage from ocimum americanum linn and its role as disintegrant in tablets formulation. JGPT. 2010;2(12):26–31.
12. Srinivas K, Prakash K, Kiran H, Prasad P, Rao M. Study of *Ocimum basilicum* and *Plantago ovate* as disintegrants in the formulation of dispersible tablets. Indian J Pharm Sci. 2003;65(2):180–3.
13. Patel D, Prajapati D, Patel N. Seed mucilage from *Ocimum americanum* linn. as disintegrant in tablets: separation and evaluation. Indian J Pharm Sci. 2007;69(3):431.
14. Archana G, Sabina K, Babuskin S, Radhakrishnan K, Fayidh MA, Babu P, *et al*. Preparation and characterization of mucilage polysaccharide for biomedical applications. Carbohydr Polym. 2013;98(1):89–94.
15. Malviya R, Srivastava P, Kulkarni G. Applications of mucilages in drug delivery—a review. Adv Biol Res. 2011;5(1):1–7.
16. Malviya R. Extraction characterization and evaluation of selected mucilage as pharmaceutical excipient. Polim Med. 2011;41(3):39–44.
17. Meka VS, Nali SR, Songa AS, Kolapalli VRM. Characterization and in vitro drug release studies of a natural polysaccharide *Terminalia catappa* gum (badam gum). AAPS PharmSciTech. 2012;13(4):1451–64.
18. Das A, Wadhwa S, Srivastava A. Cross-linked guar gum hydrogel discs for colon-specific delivery of ibuprofen: formulation and in vitro evaluation. Drug Deliv. 2006;13(2):139–42.
19. Manikoth R, Kanungo I, Fathima NN, Rao JR. Dielectric behaviour and pore size distribution of collagen–guar gum composites: effect of guar gum. Carbohydr Polym. 2012;88(2):628–37.
20. Hosseini-Parvar S, Matia-Merino L, Goh K, Razavi S, Mortazavi S. Steady shear flow behavior of gum extracted from *Ocimum basilicum* L. seed: effect of concentration and temperature. J Food Eng. 2010;101(3):236–43.
21. Reverchon E, Adami R, Cardea S, Porta GD. Supercritical fluids processing of polymers for pharmaceutical and medical applications. J Supercrit Fluids. 2009;47(3):484–92.
22. Sabirzyanov A, Akhunov A, Gumerov F. Solubility of water in supercritical carbon dioxide. High Temp. 2002;40(2):203–6.
23. Temtem M, Silva L, Andrade PZ, dos Santos F, da Silva CL, Cabral J, *et al*. Supercritical CO_2 generating chitosan devices with controlled morphology. Potential application for drug delivery and mesenchymal stem cell culture. J Supercrit Fluids. 2009;48(3):269–77.
24. Cardea S, Pisanti P, Reverchon E. Generation of chitosan nanoporous structures for tissue engineering applications using a supercritical fluid assisted process. J Supercrit Fluids. 2010;54(3):290–5.
25. Reverchon E, Cardea S, Rapuano C. A new supercritical fluid-based process to produce scaffolds for tissue replacement. J Supercrit Fluids. 2008;45(3):365–73.

26. Razavi S, Mortazavi SA, Matia-Merino L, Hosseini-Parvar SH, Motamedzadegan A, Khanipour E. Optimisation study of gum extraction from Basil seeds (*Ocimum basilicum* L.). Int J Food Sci Technol. 2009;44(9):1755–62.
27. Madhaiyan B, Rajasulochana N. Chemical and spectral characterization of cell wall polysaccharide of *Gracilaria edulis* (Gmelin) Silva. 2012.
28. Ahad HA, Sreeramulu J, Bindu VH, Ramyasree P, Padmaja BS, Sravanthi M. Isolation and physicochemical characterization of *Ficus reticulata* fruit mucilage. Int J Green Pharm. 2011;5(2):131–4.
29. Mishra A, Clark JH, Pal S. Modification of Okra mucilage with acrylamide: synthesis, characterization and swelling behavior. Carbohydr Polym. 2008;72(4):608–15.
30. Zhang Z, Wang F, Wang X, Liu X, Hou Y, Zhang Q. Extraction of the polysaccharides from five algae and their potential antioxidant activity in vitro. Carbohydr Polym. 2010;82(1):118–21.