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Preparation and characterisation of chitosan microspheres for antioxidant delivery

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Abstract

Encapsulation of olive leaf extract (OLE) in chitosan micropsheres was carried out by a spray-drying process. The interaction of polyphenolic compounds (PPCs) present in OLE within the polysaccharide matrix was studied using various physico-chemical techniques. Both the placebo and the OLE loaded microspheres were characterised by optical and scanning electron microscopy, particle size distribution analysis, Fourier transform infrared spectroscopy (FTIR) and differential scanning calorimetry (DSC). The particles obtained after spray-drying were found to be spherical and had uniform particle size distribution patterns. FTIR and DSC indicate that there are interactions of polyphenolic compounds in OLE with the chitosan matrix. Further studies on encapsulation of OLE and its activity were analysed after the total hydrolysis of OLE loaded chitosan microspheres. The released polyphenolic compounds in OLE were estimated to confirm the activity of OLE loaded into the microspheres. The loading percent of polyphenolic compounds through encapsulation of OLE achieved was 27%.

Keywords: Microspheres; Chitosan; Polyphenolic compounds; Spraydrying; Encapsulation

1. Introduction

Chitosan is a cationic biopolymer obtained from *N*-deacetylation of chitin, a β-(1-4)-linked *N*-acetyl-D-glycan (Tharanathan & Kittur, 2003). The non-toxic, biodegradable and biocompatible properties of chitosan provide potential for many biotechnological applications (Miles, 1992; Peppas, 1992). The cationic character, along with the presence of reactive functional groups in chitosan, has given it particular possibilities for utilisation in controlled-release technologies (Brannon-Peppas, 1993; Manning & Mathur, 1976). Previous studies have examined pH-sensitive chitosan/gelatin hybrid polymer network microspheres and the release of encapsulated cimetidine has been studied. The drug only delivers in acidic medium, while the release rate can be controlled by the hybrid polymer network composition and the degree of deacetylation of chitosan (Yao, Xu, Yin, Zhao, & Chen, 1996). pH-sensitive chitosan films with or without cross linking by glutaraldehyde were investigated for immobilization of yeast cells. The most appropriate experimental conditions were found to be about 25 °C, at pH 6 for 5 h for immobilization of yeast cells (Oztop, Saraydin, & Cetinus, 2002).

Polyphenolic compounds were found to exhibit highly potent anti-oxidant activity. Incorporation of polyphenolic compounds in various foods as nutraceuticals is a growing area of research. To our knowledge there has been no study on the encapsulation of polyphenolic compounds from olive-leaf extract (OLE) into chitosan microspheres. To date there are no reports of OLE loaded chitosan microspheres prepared by spray-drying and their characterisation to understand the structural interactions of polyphenolic compounds contained in the olive leaf extract with the polysaccharide matrix.

In this study, chitosan microspheres were prepared using a spray-drying technique. Commercially available OLE was encapsulated. Placebo and OLE loaded microspheres were characterised by a number of physico-chemical techniques. Optical microscopy and scanning electron microscopy were carried out to understand the morphological characteristics. Particle size distribution analysis was done. Fourier transform infrared spectroscopy and differential scanning calorimetry were carried out to understand the structural interactions caused by the incorporation of polyphenolic compounds in the microspheres. The loading % of OLE in the chitosan microspheres was also estimated.

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2. Experimental

2.1. Materials

Chitosan was obtained from Sigma-Aldrich and used without any purification. Folin-Ciocalteu's phenol reagent (2.0 N) was obtained from Sigma-Aldrich. All other reagents were used as obtained. OLE was obtained from olive products Australia, 767 Bischoff Rd, Coominya Qld 4311.

2.2. Preparation of chitosan and OLE-loaded chitosan microspheres by spray drying

The placebo and OLE-loaded chitosan microspheres were made using 1% (w/w) of chitosan solution in 5% (v/v) aqueous acetic acid. OLE extract was added to chitosan solution in the ratio of 1.25:1 (w/w) and stirred thoroughly before the spray drying process. Spray drying was done using a DryTech spray-dryer. The inlet and outlet air temperatures were 180 and 74 °C, respectively. The solution flow rate was 2.13 L/h.

2.3. Characterisation of chitosan and OLE-loaded chitosan microspheres

The chitosan microspheres prepared by spray drying were subjected to particle-size distribution analysis using the Malvern master sizer. The optical microscopy study was done on an Olympus BH-2 microscope. The SEM study was carried out using a Hitachi S570 SEM at 10 kV. The samples were sprinkled on to conductive glue (Electrodag) on an aluminum SEM stub and sputter coated with gold. The FT-IR was carried out using FTIR Nicolet Avatar 360 (32 scans, Resolution 4.000, Wavenumber range 4000–500 cm^{-1}). The sample was prepared by KBr pellet method. The DSC of the samples was carried out using Perkin–Elmer, Model Pyris Diamond DSC. The scan rate was 10 °C/min.

2.4. Estimation of the polyphenolic compounds

Fifty milligram of placebo and model bioactive loaded chitosan microspheres were incubated with 0.1 N hydrochloric acid for 24 h. The solution was filtered and the amount of encapsulated OLE was estimated by the Folin-Ciocalteu method using a Shimadzu UV 1201V spectrophotometer (Wu, 1920). The amount of encapsulated OLE is expressed as loading percent (in terms of amount of gallic acid in 100 g of microspheres). The reported loading percent represents the Mean ± SD of three independent experiments.

3. Results and discussion

3.1. Preparation of chitosan and OLE-loaded chitosan microspheres by spray drying

Spray drying was used to prepare the placebo and OLE-loaded chitosan microspheres. In these studies, there was no obvious visible interaction as determined by aggregation between OLE and the chitosan matrix at pH 3. OLE was completely in solution before spray-drying. The efficacy of polyphenols as complexing agents derives principally from their relative molecular mass and size and due to the presence of many phenolic groups in the same molecule. The process of complexation between polyphenols and chitosan may or may not be reversible (Popa, Aelenei, Popa, Valentin, & Andrei, 2000). Reversible complexation of polyphenols may be considered as a two-stage process, of which in the first stage, chitosan and polyphenols are at an equilibrium in a soluble complex due to the development of non-covalent binding forces. As equilibrium changes to a second stage, these soluble complexes may aggregate and precipitate from the solution. In our studies, OLE may have been in the form of a soluble complex with the chitosan matrix. This interaction would not have progressed to the second stage, which leads to aggregation of these complexes. However, significant increase in viscosity was observed when higher loading concentrations of OLE in the chitosan matrix were attempted at the same pH. The microspheres prepared by spray drying were found to be in the form of free-flowing powder.

4. Characterisation of the microspheres

4.1. Optical microscopy

Optical microscopy of both the placebo and OLE-loaded particles revealed the spherical geometry. There were a few aggregates formed in both the placebo and OLE loaded microsphere preparations. Further studies using scanning electron microscopy provided a better understanding of the morphological characteristics of these microspheres.

4.2. Scanning electron microscopy

Fig. 1 (a) and (b) show the SEM of placebo and OLE-loaded chitosan microspheres. The SEM of spray-dried placebo chitosan microspheres appeared to have a different morphology when compared to the OLE entrapped chitosan microspheres. The placebo microspheres appeared to have many wrinkles on the surface and there were gaps between the wrinkles. The formation of wrinkles cannot be explained at this stage but similar wrinkled surface morphology was observed in a previous study. (Huang, Chiang, & Yeh, 2003) for spray-dried chitosan microspheres. The OLE-loaded microspheres had a smooth surface morphology except for pores in larger microspheres observed at higher magnification. The same observation was recorded by Huang et al., when chitosan-gelatin and chitosan-pluronic microspheres were prepared (Huang et al., 2003). Based on the above observations, the variation of surface morphology may be attributed to a number of drying parameters such as drying rate and the composition of formulation etc.

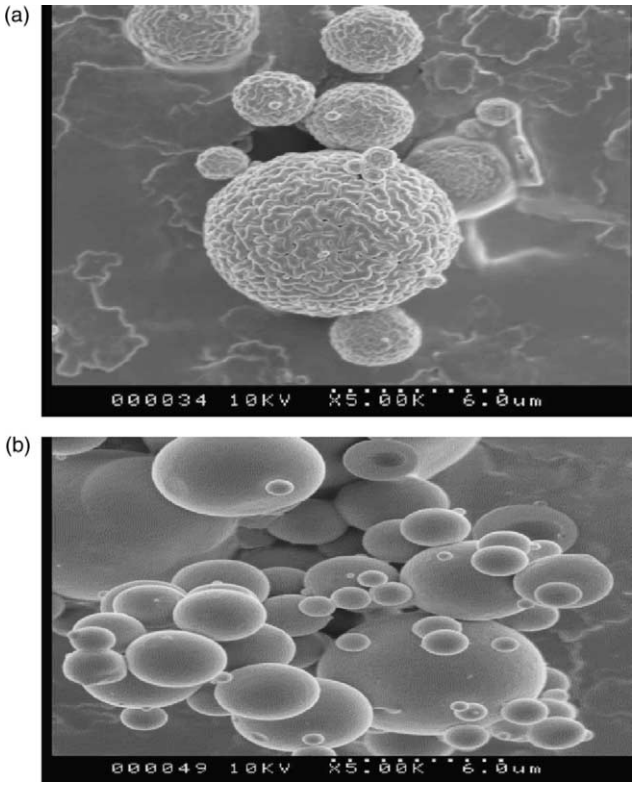

Fig. 1. Scanning electron micrographs of (a) placebo chitosan microspheres and (b) OLE loaded chitosan microspheres.

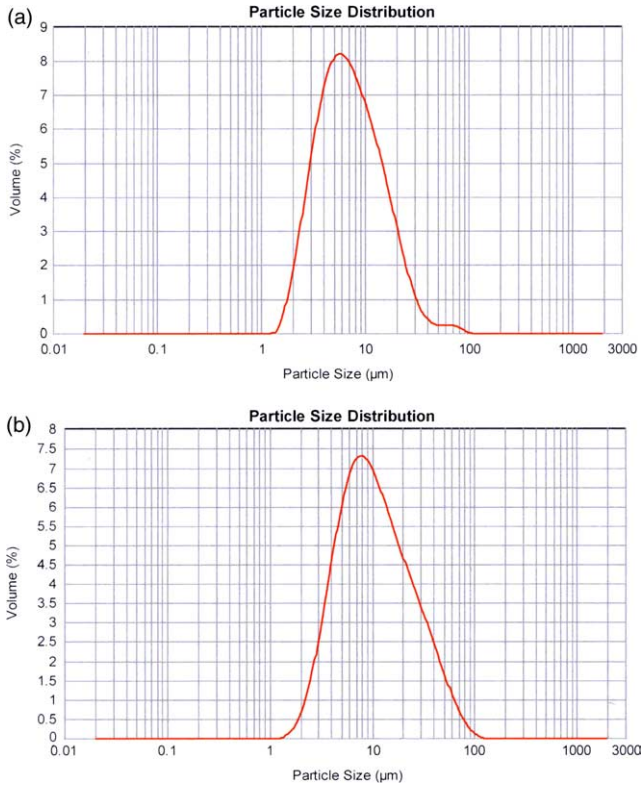

Fig. 2. Particle size distribution of placebo (a) and OLE loaded (b) chitosan microspheres.

4.3. Particle size distribution analysis

Fig. 2 (a) and (b) show the distribution curves for both the placebo and OLE-loaded beads.

The particle-size distribution analysis of placebo chitosan microspheres indicates that the volume distribution of 50% [d (0.5)] of the particles is below 6.8 μm and that of OLE-loaded microspheres is below 9.9 μm. This variation in size may be due to the OLE-encapsulated in the chitosan microspheres. The size distribution was found to be uniform in both the cases except for small amounts of very large particles. This may be explained by aggregation effects as was evident from the optical microscopy study. Aggregate formation was found to be more pronounced in placebo microspheres.

4.4. FTIR spectroscopy

The FTIR spectra of placebo and OLE loaded chitosan microspheres are shown in Figs. 3 and 4. The peaks at 2854 cm^{-1} (ν_s CH_2), 1464 cm^{-1} (δ CH_2), 1182 cm^{-1} (twisting vibration) of CH_2 are sharper in the placebo when compared to the same peaks for the OLE-loaded chitosan microsphere sample. The peaks ranging between 2700–3000 cm^{-1} can be ascribed to the stretching of the $-NH_2$ group with strong overlapping hydroxyl peak between 3000–3600 cm^{-1}. The FTIR of OLE-loaded chitosan microspheres shows a small peak at 1700 cm^{-1}, which was not observed in the placebo chitosan microspheres. This peak was found to overlap with the peaks of amide I and II at 1650 and 1550 cm^{-1} resulting in a broad band ranging from 1700–1550 cm^{-1}. This indicates interaction between the hydroxyl/carboxyl/aldehyde groups of the OLE and the amine functionality of the chitosan molecule. Some of these observations were also reported (Muzzarelli, Tanfani, & Emanuelli, 1984; Jansson-Charrier, Saucedo, Guibal, & Le Cloirec, 1995) for the reactions between chitosan and ascorbic or oxo-2-glutaric acids. Under such conditions a new band corresponding to carbonyl groups appears near 1700–1740 cm^{-1}. The interaction between OLE and chitosan may not be major as evident from the spectra. The chitosan-OLE microspheres show similar absorption peaks to the chitosan

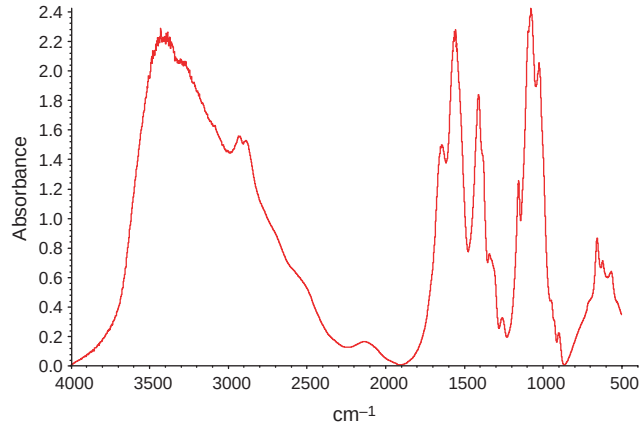

Fig. 3. FT-IR of placebo chitosan microspheres prepared by spray-drying technique.

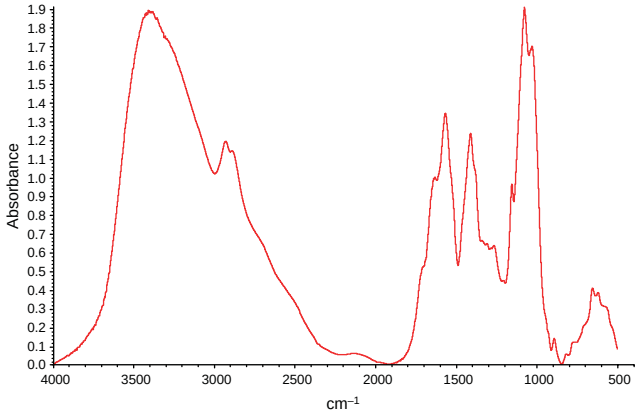

Fig. 4. FT-IR of OLE-loaded chitosan microspheres prepared by spray-drying technique.

molecule. This also indicates that majority of the OLE in the chitosan microsphere is physically encapsulated in the chitosan matrix, although covalent binding of the OLE with the amino group cannot be completely ruled out.

4.5. Differential scanning calorimetry

The DSC curves for both the chitosan and OLE loaded chitosan microspheres is shown in Fig. 5. The DSC of placebo chitosan microspheres shows an exothermic peak at 150 °C, which may correspond to the initiation of polysaccharide chain scission. In the OLE loaded chitosan microspheres, this exothermic transition shifted to a lower temperature (ca 125 °C). A second exothermic thermal transition at 260 °C in the chitosan microspheres also shifted to a lower temperature in OLE loaded microspheres. Thermal transitions around 239.9 and 280 °C are ascribed to a complex process including dehydration of the saccharide rings, depolymerisation of the acetylated and deacetylated units and the galactose group of the polymer (Can, Qineng, Ya, Yao, & Jian, 2004). These shifts in thermal transitions indicate the encapsulation and interaction of PPC in OLE with the chitosan matrix in the chitosan microspheres. A small glass transition in placebo chitosan microspheres was found at 86.5 °C while in OLE-encapsulated microspheres it was at 84 °C. The observed shift

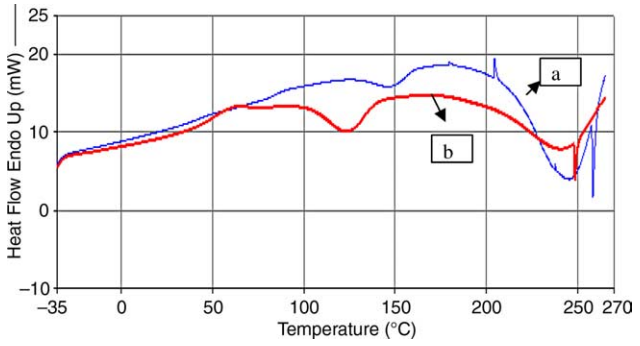

Fig. 5. DSC of placebo (a) and OLE loaded (b) chitosan microspheres prepared by spray-drying technique.

in the glass transition of the OLE-loaded microspheres also indicates an interaction of OLE with the chitosan matrix albeit to a minor extent. These minimal interactions between chitosan and OLE support the encapsulation of polyphenolic compounds such as OLE in chitosan based matrices using the spray-drying technique.

4.6. Estimation of entrapped polyphenolic compounds in chitosan microspheres

The polyphenolic compounds encapsulated in chitosan microspheres were analysed by the direct method of hydrolysing the microspheres in hydrochloric acid. The released polyphenolic compounds were estimated by the Folin chicolteau method. The loading percent in terms of gallic acid equivalents obtained from the analysis was 27%. These results also confirm that the encapsulation of OLE by spray-drying process has not lead to inactivation of the polyphenolic compounds. The loading percent of polyphenolic compounds obtained was found to be much higher by the spray-drying process when compared to the extrusion process applied in our earlier studies of encapsulation of OLE (unpublished results). The PPCs that were covalently linked to the chitosan matrix may not be available for estimation even after hydrolysis of the chitosan matrix. These studies indicate that the PPCs that lead to structural interaction with the chitosan matrix may be available after bacterial degradation of chitosan in the lower gut of humans.

5. Conclusion

Encapsulation of OLE in the chitosan microspheres was achieved successfully by a spray-drying process. These studies demonstrate that there are minor interactions between polyphenolic compounds in OLE and the polysaccharide matrix. The loading percent of PPC in chitosan microspheres was found to be 27%. The activity of the PPC encapsulated through OLE in the chitosan matrix was found to be retained even after the spray-drying process. Further, these preliminary studies to understand the interactions of PPC with the polysaccharide matrix, during the process of encapsulation, form a stepping stone for the many future investigations in this emerging area of research on targeted delivery of PPCs in humans.

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