BJPS

Brazilian Journal of
Pharmaceutical Sciences
vol. 50, n. 4, oct./dec., 2014
http://dx.doi.org/10.1590/S1984-82502014000400009

Article

Alginate-pomegranate peels' polyphenols beads: effects of formulation parameters on loading efficiency

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Calcium alginate beads containing pomegranate peels' polyphenol extract were encapsulated by ionic gelation method. The effects of various formulation factors (sodium alginate concentration, calcium chloride concentration, calcium chloride exposure time, gelling bath time maintaining, and extract concentration) on the efficiency of extract loading were investigated. The formulation containing an extract of 1 g pomegranate peels in 100 mL distilled water encapsulated with 3 % of sodium alginate cured in 0.05 M calcium chloride for 20 minutes and kept in a gelling bath for 15 minutes was chosen as the best formula regarding the loading efficiency. These optimized conditions allowed the encapsulation of 43.90% of total extracted polyphenols and 46.34 % of total extracted proanthocyanidins. Microencapsulation of pomegranate peels' extract in calcium alginate beads is a promising technique for pharmaceutical and food supplementation with natural antioxidants.

Uniterms: Pomegranate/polyphenols/microencapsulation. Calcium alginate beads/microencapsulation. Microencapsulation. Polyphenols. Natural antioxidants/microencapsulation.

Pérolas de alginato de cálcio, contendo polifenóis de extrato de casca de romã, foram encapsuladas pelo método de gelificação iônica. Os efeitos de vários fatores de formulação (concentração de alginato de sódio, concentração de cloreto de cálcio, cloreto de cálcio, o tempo de exposição, o tempo de manutenção do banho de gelificação e a concentração do extrato) sobre a eficiência de carga do extrato foram investigados. A formulação que contém 1 g extrato de casca de romã em 100 mL de água destilada, encapsulado com 3% de alginato de sódio curada em 0,05 M de cloreto de cálcio durante 20 minutos e mantido em banho de gelificação por 15 min foi escolhida como a melhor em relação à eficiência de carga. Estas condições otimizadas permitem o encapsulamento de 43,90% do total de polifenóis extraídos e de 46,34% do total de proantocianidinas extraídas. A microencapsulação de extrato de cascas de romã em esferas de alginato de cálcio é uma técnica promissora para a suplementação farmacêutica e de alimentos com antioxidantes naturais.

Unitermos: Romã/polifenóis/microencapsulação. Alginato de cálcio/microencapsulação. Microencapsulação. Polifenóis. Antioxidantes naturais/microencapsulação.

INTRODUCTION

Pomegranate peels (*Punica granatum*) contain a high amount of polyphenols associated with free radical scavenging activity. Epidemiological studies have revealed that consumption of polyphenols correlates with reduced cardio- and cerebro-vascular diseases and cancer mortality (Lansky, Newman, 2007; Shabtay *et al.*, 2008).

Natural polyphenols extracted from pomegranate peels should be protected from the surrounding medium because they are very sensitive to oxygen, light, acid, and alkaline, but relatively less sensitive to heat. Therefore, the administration of phenolic compounds requires the formulation of a finished protecting products able to maintain the structural integrity of the polyphenol until the consumption, mask its taste, and increase its water solubility and bioavailability.

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Among the existing stabilization methods, encapsulation is an interesting means. Conventional microencapsulation as described by Swapan (2006) is a process of surrounding either a liquid droplet or a solid particle core with a defined, solid shell. It is used to either deliver, protect, stabilize or control the release of the core.

There have been a number of recent reviews focusing on the encapsulation of the more widely used polyphenols, discussing their effectiveness, variations, developments and trends (Barras *et al.*, 2009; Das, Ng, 2010; Di Mattia *et al.*, 2009; Dube *et al.*, 2010; Ersus, Yurdagel, 2007; Fang *et al.*, 2006; Hu *et al.*, 2008; Kosaraju, Dath, Lawrence, 2006; Laine *et al.*, 2008; Liang *et al.*, 2011; Lucas-Abellan *et al.*, 2007; Mourtzinos *et al.*, 2007; Shi *et al.*, 2007; Shutava *et al.,* 2009; Tommasini *et al.*, 2005; Zhang, Mou, Du, 2007; Xiong *et al.*, 2006; Yu, Huang, 2010).

The ionic gelation process, one of the physico-chemical methods used in microencapsulation, consists of extruding an aqueous solution of polymer through a syringe needle or a nozzle, in which the active material is dissolved or dispersed. Droplets are received in a dispersant phase and are transformed, after reaction, into spherical gel particles, as is the case, for example, with sodium alginate used with a dispersant phase of calcium chloride (Vandamme, Poncelet, Subra-Paternault, 2007).

The encapsulating agent used in this study was sodium alginate. It is an anionic polymer which can be easily cross-linked with calcium chloride, this is because the calcium ions are bound to carboxylate residues of both mannuronic acid and glucournic acid which are components of sodium alginate (Ravindra, Sabitha, 2010). Sodium alginate is a hygroscopic material, although it is stable if stored at relatively low humidity and a cool temperature. Aqueous solutions of sodium alginate are most stable at pH 4–10. Below pH 3, alginic acid is precipitated (Raymond, Paul, Marian, 2009). It is generally regarded as a nontoxic and nonirritant material, it is GRAS listed (Generally Recognised As Safe), and accepted in Europe for use as a food additive. Although excessive oral consumption may be harmful, the World Health Organization (WHO) has not specified an acceptable daily intake for alginic acid and alginate salts as the levels used in food do not represent a hazard to health (Raymond, Paul, Marian, 2009).

Thus the objective of this study is to encapsulate the polyphenol extract in calcium alginate beads to be incorporated as an additive in pharmaceutical or food products.

The effects of various formulation factors (sodium alginate concentration, calcium chloride concentration, calcium chloride exposure time, gelling bath time maintaining, extract concentration, and additives type) on the efficiency of extract loading were investigated.

MATERIAL AND METHODS

Chemical and reagents

Sodium alginate and Folin-Ciocalteu reagent 2 N were obtained from Sigma-Aldrich (Switzerland);calcium chloride, sodium chloride and tri-sodium citrate were obtained from Riedel-de-Haen (Germany); ferric ammonium sulfate (Carl Roth, Germany); sodium carbonate anhydrous, 1-butanol and hydrochloric acid were obtained from Surechem (England).

Sample preparation

Fresh pomegranates were cleaned with water and dried with a cloth. The peels were manually separated, dried for a few days in an open air shade. The dried samples were then powdered in a blender. They were stored at -18 °C until analysis (Zam *et al.*, 2012).

Extraction procedure

2 g of dried and ground peel were placed in a thermostatic water bath shaker with 100 mL of distilled water at 50 °C for 20 minutes. The liquid extract was separated from solids by centrifugation at 2000 rpm for 10 minutes. The supernatant was transferred to a 100 mL flask, and distilled water was added to make the final volume 100 mL (Zam *et al.*, 2012).

Optimization of loading efficiency

The formulation of the calcium alginate beads is based on both the concentration of sodium alginate and the ability of calcium ions to cross link with sodium alginate. The degree of cross linking is dependent on both the concentration of the calcium chloride solution and the time of contact of the beads with this solution. To optimize the parameters affecting the formulation of beads, various factors were evaluated: sodium alginate concentration (1-4.5%), calcium chloride concentration (0.01-0.1 M), calcium chloride exposure time (5-60 minutes), gelling bath time maintaining (5-60 minutes), and extract concentration (0.25-2.5%).

Capsules formulation

Beads were obtained by mixing 10 mL of the active

component with 10 mL of the sodium alginate solution at the best concentration. Once homogenized, 10 mL of calcium chloride solution at the best concentration was added to the alginate solution and was cured for different duration to optimize curing time at 25 °C. The beads formed in this process were maintained in the gelling bath to harden. Then, they were centrifuged at 4,000 rpm and 4 °C for 15 minutes (Anbinder *et al.*, 2011).

Loading efficiency

The amount of lyophilized extract loaded in beads was estimated, as described by Deladino *et al.* (2007), by dissolving the capsules obtained from 10 mL extract in sodium citrate (10% w/v) during a period of 20 min for alginate capsules in a shaker at 37 °C and 125 rpm. The concentrations of lyophilized extract loaded in the beads were determined by Folin-Ciocalteu and butanol assay method. A blank of sodium citrate was also performed. The percentage of loading efficiency was calculated with the following equation:

$$Loading\ efficiency(\%) = \frac{L}{L_0} * 100$$

where L is the amount of extract determined on the solution of sodium citrate and L_0 is the initial amount of extract dissolved in the alginate solution (Deladino *et al.*, 2007).

Total polyphenol content

The total polyphenol content in the extract was determined by the Folin-Ciocalteu method according to the method described by the International Organization for Standardization (ISO, 2005). A sample of 250 µL of the extract was diluted with distilled water to 10 mL. Aliquots of 1 mL of samples were mixed with 5 mL of 10-fold-diluted Folin-Ciocalteu reagent. After 3 minutes, 4 mL of 7.5% sodium carbonate was added (Zahin, Aqil, Ahmad, 2009). The mixtures were allowed to stand for 30 minutes at 40 °C temperature (water bath) before the absorbance was measured at 734 nm using Jasco V-530 spectrophotometer. The total polyphenol content in the extract was calculated and expressed as gallic acid equivalents (GAE; g/100 g dry mass) using a gallic acid (0-120 mg/L) standard curve (Anesini, Ferraro, Filip, 2008).

Proanthocyanidins content

The proanthocyanidin content in the extract was determined by the Acid Butanol assay according to the method of Porter *et al.* (1986). A sample of 200 µL extract diluted with 300 µL of acetone 70% was pipetted into a 100 x 12 mm test tube. 3.0 mL of butanol–HCL reagent (95:5) and 0.1 mL of 2% ferric acid prepared in HCl 2N were added. The tube was vortexed and then the mouth of the tube was covered with a glass marble and put in the heating block at 97 to 100 °C for 60 minutes. The tube was then allowed to cool and absorbance was recorded at 550 nm. The formula for calculating percentage of condensed tannins as leucoanthocyanidin equivalent is:

$$(absorbance\ 550\ nm * 78.26 * dilution\ factor) \Big/ dry\ matter\ \%$$

RESULTS AND DISCUSSION

Sodium alginate concentration

Different concentrations of sodium alginate were examined namely: 1, 1.5, 2, 2.5, 3, 3.5, 4 and 4.5% (w/v), (calcium chloride 0.05 mol/L as a cross linking agent for 15 minutes and the beads formed in this process were maintained in the gelling bath to harden for 15 minutes).

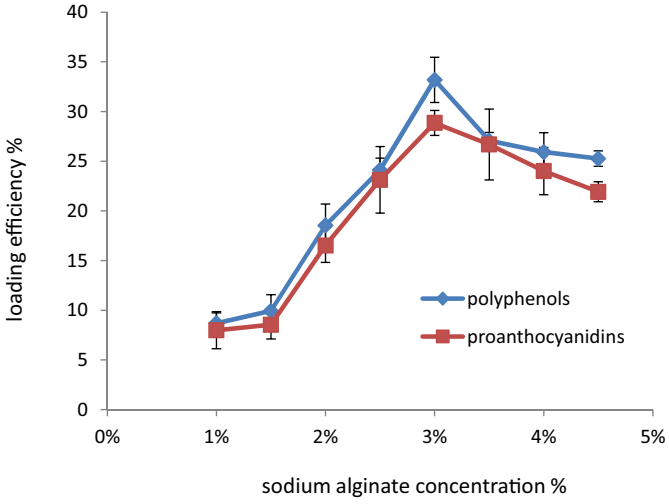

FIGURE 1 - Effect of sodium alginate concentration on total extractable polyphenols and proanthocyanidins loading efficiency. [a] Values are mean ± SD, (n = 3).

Results obtained showed that increasing sodium alginate concentration from 1% to 3% elevated loading efficiency from approximately 8 to 33% (Figure 1). The concentration of 3% gave the best loading efficiency for both total extractable polyphenols and proanthocyanidins, with values of 33.19% ± 0.014 and 28.85% ± 0.012, respectively (Figure 1). As reported by El-Kamel *et al.* (2003), this may be attributed to the greater availability

of active calcium-binding sites in the polymeric chains and, consequently, the greater degree of cross linking as the quantity of sodium alginate increased.

Higher concentration of sodium alginate is accompanied by an increase in viscosity and a decrease in loading efficiency. Based on this result, for the next experiment, the concentration of alginate was 3% (w/v), because this concentration is the lowest concentration which can make a good loading efficiency. The concentration of alginate utilized in this study was higher than the one used by Deladino *et al.* (2%, w/v) for the encapsulation of polyphenols extracted from yerba mate.

Calcium chloride concentration

Alginate solution droplets in this experiment are gelled by contact with calcium chloride solution droplets. The concentration of calcium chloride has an important influence on the characteristics of the resulting alginate beads.

Therefore, we examined the effect of the concentration of calcium chloride on alginate bead loading efficiency. The concentration of sodium alginate solution was fixed at 3%, curing time after addition of calcium chloride was 15 min and hardening time in gelling bath was also 15 min. The concentration of calcium chloride varied from 0.01 M to 0.1 M.

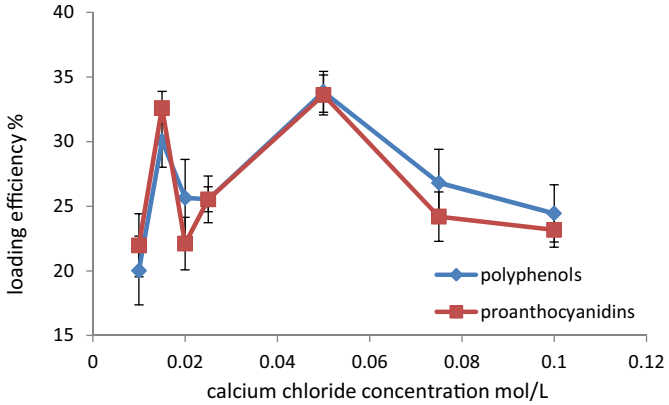

FIGURE 2 - Effect of calcium chloride concentration on total extractable polyphenols and proanthocyanidins loading efficiency. [a] Values are mean ± SD, (n = 3).

Results obtained showed that increasing calcium chloride concentration from 0.01 M to 0.05 M elevated loading efficiency from approximately 20 to 34% (Figure 2). This may be explained by the increase in the gel strength as the calcium ions increased. The concentration of 0.05 M gave the best loading efficiency for both total extractable polyphenols and proanthocyanidins, with values of 33.84% ± 0.007 and 33.61 % ± 0.012, respectively (Figure 2). These results are in agreement with Takka *et al.* (1998) and Mirghani *et al.* (2000).

On the other hand, the loading efficiency was found to decrease at concentrations of more than 0.05 M, this may indicate damage of microcapsules due to possible saturation of calcium binding sites in the glucoronic acid chain, preventing further calcium ion entrapment as reported by Ostberg *et al* (1994) or to osmotic stress as reported by Takayuki *et al.* (2009). Based on these results, we adopted a concentration of 0.05 M in subsequent experiments.

Calcium chloride exposure time

King (1983) suggested that, when calcium and alginate solutions get in contact, a gel is formed immediately at the interface, thus matrix homogeneity depends on the calcium diffusion through the gel network. Continuous exposure of the alginate to the calcium solution will increase the firmness of the gel, as more calcium diffuses into the gel and binds to the G blocks within the alginate structure (Lamkey *et al.*, 2009). Diffusion of calcium chloride towards the beads core, at 5, 10, 15, 20, 25, 30, 45, 60 min, was examined and loading efficiency values were demonstrated in Figure 3.

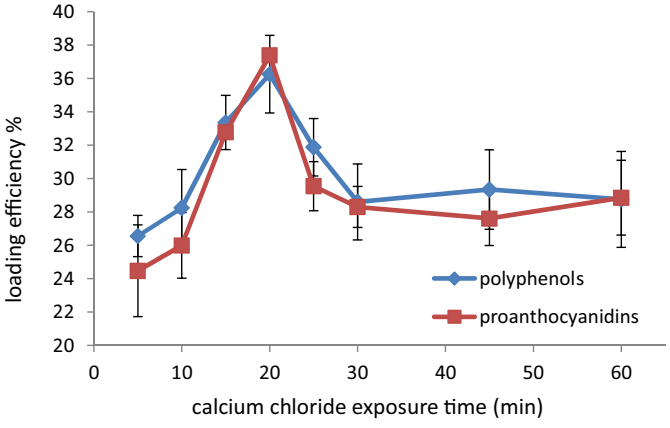

FIGURE 3 - Effect of calcium chloride exposure time on total extractable polyphenols and proanthocyanidins loading efficiency. [a] Values are mean ± SD, (n = 3).

Thus, 20 min in air was enough to get the best loading efficiency for both total extractable polyphenols and proanthocyanidins, with values of 36.25% ± 0.010 and 37.39% ± 0.009, respectively (Figure 3).

Patel *et al.* (2006), showed that the loading efficiency of calcium alginate beads containing metronidazole

decreased with increase in curing time, this could be explained that longer times could cause the shift of the bounded calcium ions by alginate as reported by El-Deladino *et al.* (2007) or may be due to the increased release of polyphenols from the matrix as reported by El-Kamel *et al.* (2003).

Gelling bath time maintaining

The beads formed were maintained in the gelling bath to harden for different times (5-60 min).

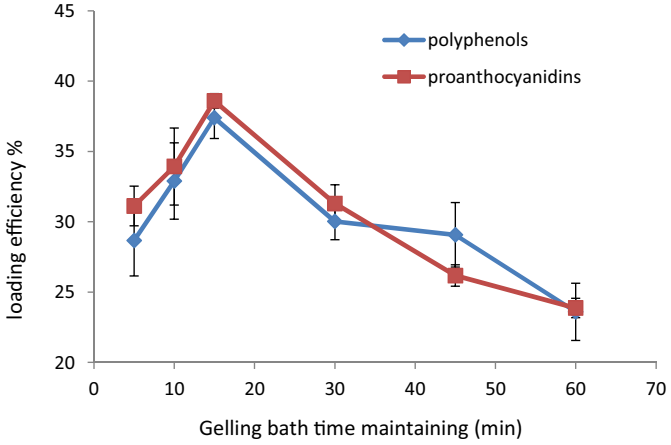

FIGURE 4 - Effect of gelling bath time maintaining on total extractable polyphenols and proanthocyanidins loading efficiency. [a] Values are mean ± SD, (n = 3).

Results obtained showed that increasing maintaining time from 5 min to 15 min elevated loading efficiency from approximately 28 to 37% (Figure 4). This may be explained by the increase in the gel strength as the time increased. The maintaining time of 15 minutes gave the best loading efficiency for both total extractable polyphenols and proanthocyanidins, with values of 37.39% ± 0.007 and 38.59% ± 0.010, respectively (Figure 4).

Results also showed that the loading efficiency of calcium alginate beads decreased with an increase in maintaining time in the gelling bath because longer times could increase the release of polyphenols from the matrix. These results are in agreement with Anbinder *et al.* (2011).

Extract concentration

When the amount of drug is greater, lesser amount of drug diffusion in surrounding aqueous medium was taken place during a definite curing time as mentioned by Jaiswal *et al.* (2009). Trivedi et al (2008) prepared aceclofenac microspheres by emulsion-solvent evaporation, results from this study clearly indicate that encapsulation efficiency is significantly increase as the drug:polymer ratio decreased.

After the above decision, the effect of total extractable polyphenols concentration in preparation of microcapsules was analyzed. The results showed that when the concentration of polyphenols increased, the loading efficiency decreased at the same amount of polymer in the dispersed (Table I).

Based on these results, the best extract concentration leading to the highest loading efficiency was prepared using 1 g of finely ground pomegranate peels extracted with 100 mL of distilled water.

In conclusion, we optimized the preparation parameters for polyphenols alginate gel beads by ionic gelation method with a view to large-scale production. The optimized concentrations of sodium alginate and calcium chloride in the aqueous phases, curing time and gelling bath maintaining time, extract concentration were 3% (w/v), 0.05 M, 20 min, 15 min and 1 g/100 mL, respectively. The mean loading efficiency was 43.90% ± 0.006 for total extracted polyphenols and 46.34 % ± 0.011 for total extracted proanthocyanidins. Optical microscope was used to investigate the shape of microcapsules prepared by the proposed method. Photos indicate that the microcapsules were covered continuously with sodium alginate coat material (Figure 5).

TABLE I - Effect of extract concentration on total extractable polyphenols and proanthocyanidins loading efficiency

Extract concentration	Loading efficiency Polyphenols [a]	Loading efficiency Proanthocyanidins [a]
0.25 g/100 mL	35.50% ± 0.001	36.58% ± 0.006
0.5 g/100 mL	43.40% ± 0.003	45.49% ± 0.011
1 g/100 mL	43.92% ± 0.006	46.34% ± 0.011
1.5 g/100 mL	37.30% ± 0.015	37.04% ± 0.009
2 g/100 mL	37.39% ± 0.007	38.59% ± 0.010
2.5 g/100 mL	32.89% ± 0.027	31.89% ± 0.009

[a] Values are mean ± SD, (n = 3)

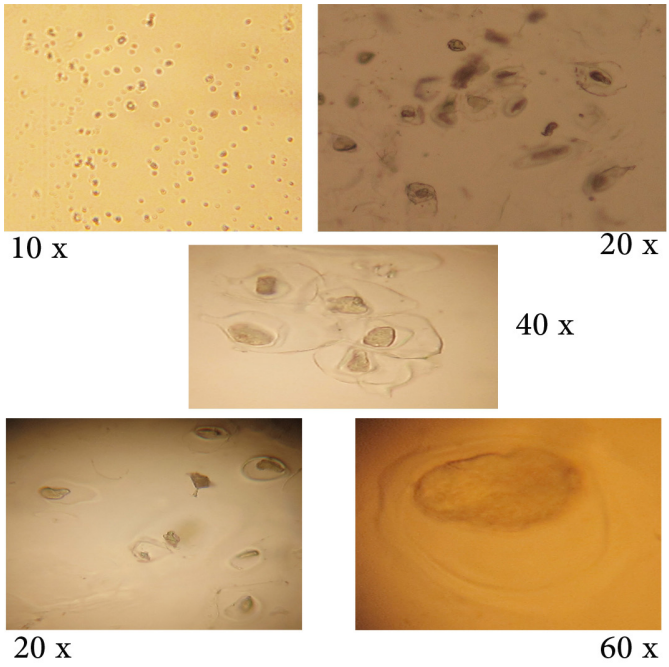

FIGURE 5 – Optical microscope photograph of calcium alginate beads containing pomegranate peels' polyphenol extract.

CONCLUSION

This study shows that calcium alginate microcapsules could be a good carrier for polyphenols. This method of preparation of microcapsules containing pomegranate peels' polyphenol was found to be simple, rapid and reproducible. The protective effect of microencapsulation will help to produce pharmaceutical and food supplements which can improve the physiological efficiency of polyphenols.

ACKNOWLEDGMENT

This work was funded by the Faculty of Pharmacy-University of Aleppo- Syrian Arab Republic. The authors would like to thank D. Jamil Daher and Al-Andalus Language Center for their linguistic assistance.

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Received for publication on 31st January 2013
Accepted for publication on 28th April 2014