Evaluation of natural polyphenols entrapped in calcium alginate beads prepared by the ionotropic gelation method

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The uses of biopolymers for the encapsulation of polyphenols have gained much more attention due to biocompatibility and biodegradability of these materials. Sodium alginate loaded polyphenols beads were prepared by ionotropic gelation of alginate with calcium chloride, an aqueous solution of chitosan and $CaCl_2$ were used as coagulation fluid also. Natural polyphenols entrapped in alginate beads were extracted from rose hips (*Rosa canina*) with a total phenolic content of 38.9 mg/g Galic acid equivalents. The effects of sodium alginate and chitosan concentration on the encapsulation efficiency were studied. The interactions between polyphenols and biopolymers (sodium alginate, chitosan) were investigated by Fourier Transform Infrared Spectroscopy (FT-IR) and thermal analysis (Differential Scanning Calorimetry - DSC and Thermo Gravimetric Analysis - TGA). In vitro polyphenols release studies in a gastrointestinal system demonstrated that the highest polyphenols content was released in simulated gastric fluid (SGF).

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1. Introduction

The term of biopolymers comprises a variety of materials that are produced both by microorganism, plants animals and synthesized chemically from biological starting materials such as amino acids, sugars, natural fats or oils [1]. One natural polymer, alginate, is a linear copolymer composed of β-D-mannuronic acid and α-L-guluronic acid joined by a 1-4 glycosidic bond. The composition and patterning of the monomers is dependent on the source of the polysaccharide. The most common source of alginate is the cell wall of brown algae. Alginate is known as biocompatible, biodegradable and non-toxic polymer and has shown great promise in biomedical applications due to the reactivity of its carboxylate side groups and its capacity to form spontaneous gelation when exposed to divalent cations such as calcium [2]. Calcium cross-linked alginate hydrogels have been used in many biomedical applications as the entrapment and/or delivery of a variety of proteins [3], drugs [4] and cells [5].

Another biopolymer is the chitosan (CS), a polysaccharide composed of 2-amino-2-deoxy-β-D-glucan combined with glycosidic linkages. Chitosan is obtained from the deacetylation of chitin, a naturally occurring and abundantly available (in marine crustaceans) biocompatible polysaccharide. The primary amine groups render special properties that make CS very useful in pharmaceutical applications [6]. CS has been used for the preparation of nanoparticles, microspheres, hydrogels and films for oral [7], transdermal [8] [9], ophthalmic [10] drug delivery systems.

Phenolic compounds are the most abundant secondary metabolites of plants, with more than 8,000 phenolic structures currently known, ranging from simple molecules such as phenolic acids to highly polymerized substances like tannins. Plant phenolics include phenolic acids (derivatives of benzoic acid and cinnamic acid), flavonoids, tannins (hydrolysable and condensed) and the less common stilbenes and lignans [11]. Various studies have been proven that phenolic compounds have different types of pharmacological properties including anti-oxidative, anti-inflammatory [12]; anti-mutagenic, anti-carcinogenic [13]; anti-diabetic [14], anti-aging effects [15].

These valuable natural compound's uses are substantially limited, only a small proportion of molecules administered orally are absorbed, because of insufficient gastric residence time, low permeability and/or low solubility. Their instability during food processing, distribution or storage, or in the gastrointestinal tract (pH, enzymes, the presence of other nutrients), limit the activity and the potential health benefits of polyphenols. The topical use of natural polyphenols is also delicate because of their important sensitivity to environmental factors, including physical, chemical, and biological conditions. Unfortunately, they oxidize very quickly, leading to the progressive appearance of a brown color with a considerable loss in activity [16].

The aims of this study were to develop polyphenols entrapped in calcium alginate beads and to investigate their encapsulation efficiency and *in vitro* release. The interactions between components of encapsulated rose hips

extract were studied through FT-IR, DSC and TGA analysis. The effect of selected parameters such as polymer: cross linking agent ratio, and polymer: polyphenols ratio (w/w) was also investigated.

2. Experimental

2.1 Materials

Alginic acid sodium salt form brown algae bio-reagent and Chitosan of medium molecular weight, derived from crab shell were purchased from Sigma-Aldrich. Rose hips *(Rosa canina)* were acquired from the local pharmacy and then pulverized and stored in desiccator before extraction and analysis. Ethanol, Folin-Ciocalteau phenol reagent, DPPH (1,1-diphenyl-2-picryl-hydrazyl) were provided by Merck. Gallic acid was also purchased from Sigma-Aldrich. All the other reagents used in the experiments were of analytical grade.

2.2 Extraction of polyphenols from rose hips

Dried rose hips (10 g) were sonicated for 30 minutes at room temperature in ethanol: water (50:50 v/v). The extract was centrifuged at 4000 RPM for 20 minutes. The residue was re-extracted by repeating the procedure mentioned above. The 50% aqueous ethanol combined supernatants were evaporated at 35^{0}C under vacuum to remove solvents. The polyphenols extract was concentrated up to 20 % of solid content was achieved.

2.3 Preparation of microcapsules

Different types of beads (formulation F1-F7) were prepared by the ionotropic gelation method. Sodium alginate (2%; 3% w/v) was dissolved in double-distilled water with agitation. Polyphenolic extract of rose hips was added to the polymer solution at different mass ratio (1:1, 1:3, 1:5, w/w). Then the mixtures were homogenized and added dropwise into $CaCl_2$ /CS-$CaCl_2$ solution at room temperature using a syringe with needle 23G (0.6 x 30 mm) and stirred at 100 RPM. The composition of the microcapsules is presented in **Table 1**.

Table 1. Properties of alginate-chitosan beads

Formulation code	**ALG** (%, w/v)	**$CaCl_2$** (%, w/v)	**ALG/PT** (w/w)	**CS %** (w/v) in 1%
F1	3	3	1:1	-
F2	3	3	3:1	-
F3	3	3	5:1	-
F4	2	1	1:1	-
F5	2	1	1:1	0.25
F6	2	1	1:1	0.5
F7	2	1	1:1	1

The beads obtained were kept for half an hour in the coagulation fluid under stirring. Then the beads were collected, washed with double-distilled water and air-dried.

2.4 Characterization of microcapsules

Fourier Transform Infrared spectroscopy (FT-IR).

Both polymer standards and prepared microcapsules were investigated with FT-IR Spectrum GX Perkin Elmer equipment. The samples were analyzed in KBr by transmission with 64 scans per experiment.

DSC/ TGA. Thermal analysis was performed with DSC 823 and TGA/SDTA 851 Mettler Toledo equipment. For DSC analysis the samples, □ 5 mg (polymer standards, prepared microcapsules) were heated from 25-300 ^{0}C with a heating rate of 10^{0}C/min. In TGA analysis the samples, were heated from 25-600 ^{0}C with a heating rate of 10^{0}C/min.

2.5 Determination of encapsulation efficiency

The polyphenols content in the beads was determined by a digestion method. The polyphenols loaded beads (10 mg) were incubated in 2 mL 0.1 mol/L HCl at room temperature for 24 h. The suspension was then centrifuged at 4000 RPM 30 min. The supernatant was analyzed spectrophotometrically for phenol content [17]. Supernatant of empty beads was taken as blank.

2.6 In vitro release studies

The polyphenols release from beads was studied by incubating 10 mg of beads in 2 mL SGF without pepsin (pH=1.2), at 37 ^{0}C and 100 RPM/min. After 2h, the beads were filtered and transferred to SIF without pancreatin (pH=7.4) incubated at 37 ^{0}C and 100 RPM/min. At fixed intervals of time 1 mL of sample was withdrawn and replaced with the same amount of fresh medium. The total phenolic content in the release medium was measured by the Folin - Ciocalteau method after centrifugation.

3. Results and discussion

3.1 Fourier Transform Infrared spectroscopy

FT-IR spectra with the specific absorption bands are presented in Fig. 1.

By comparing absorbance spectra it can be observed a band near 3440 cm^{-1} which shows the O-H and N-H stretching region. In Fig.1, absorptions associated with ν_a CH_2 and ν_s CH_2 were located at 2930 cm^{-1}- 2850 cm^{-1}. The bands centered near 1630 cm^{-1} corresponding to ν C=O + ν C-N +δ N-H (amide I). Also, some specific calcium alginate bead bands were observed in the symmetric stretching band of the COO^- group centered near 1420 cm^{-1} (ν_s COO^-). The bands located at 1200–870 cm^{-1}

corresponding to the carbohydrate region, respectively, too ν C-O and the δ C-O-H vibrations [18].

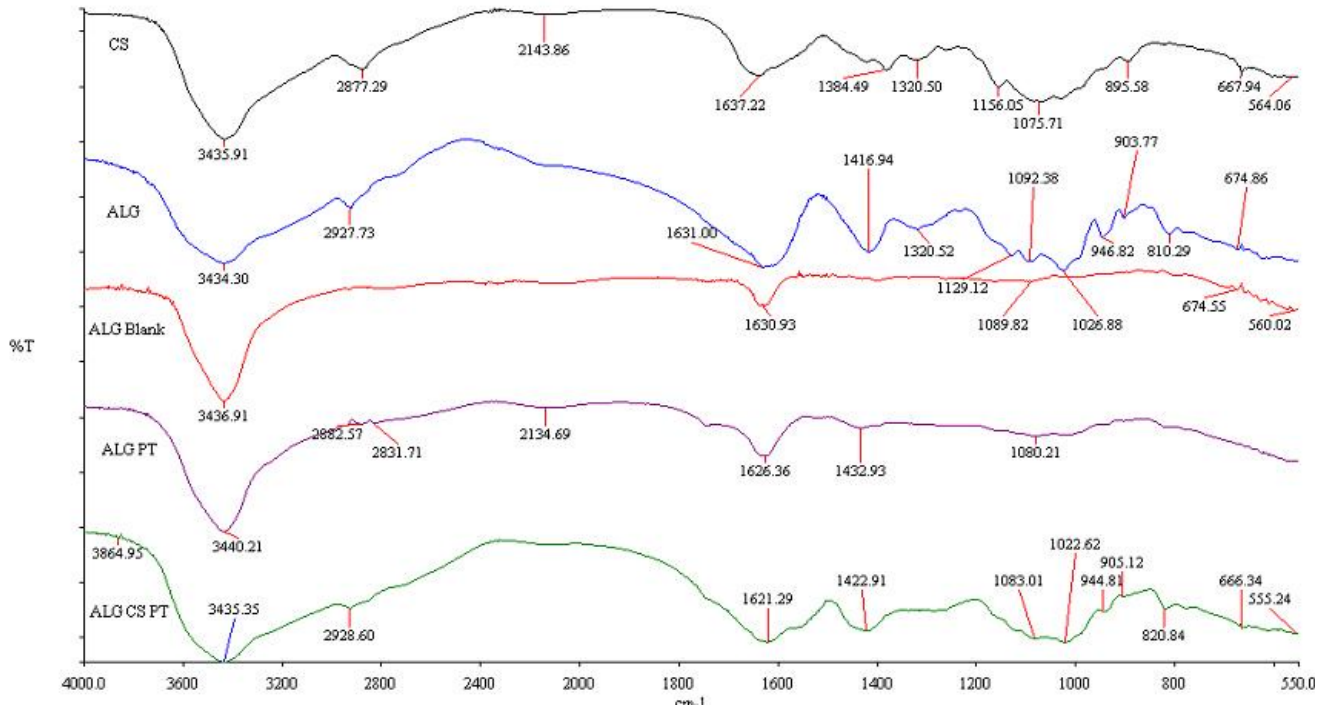


Fig. 1.FT-IR spectra: CS= chitosan; ALG=sodium alginate; ALG Blank = control microcapsule of calcium alginate; ALGPT = calcium alginate microcapsules loaded with polyphenolic extract of rose hips; ALGCSPT =alginate-chitosan microcapsules with polyphenolic extract of rose hips

Thus, in the alginate-chitosan microcapsules loaded with polyphenolic extract of rose hips can be seen that some bands are downshifting, respectively, from 3440 cm^{-1} to 3435 cm^{-1}, from 1626 cm^{-1} to 1621 cm^{-1} and from 1433 cm^{-1} to 1422 cm^{-1}, explained by the interaction with chitosan.

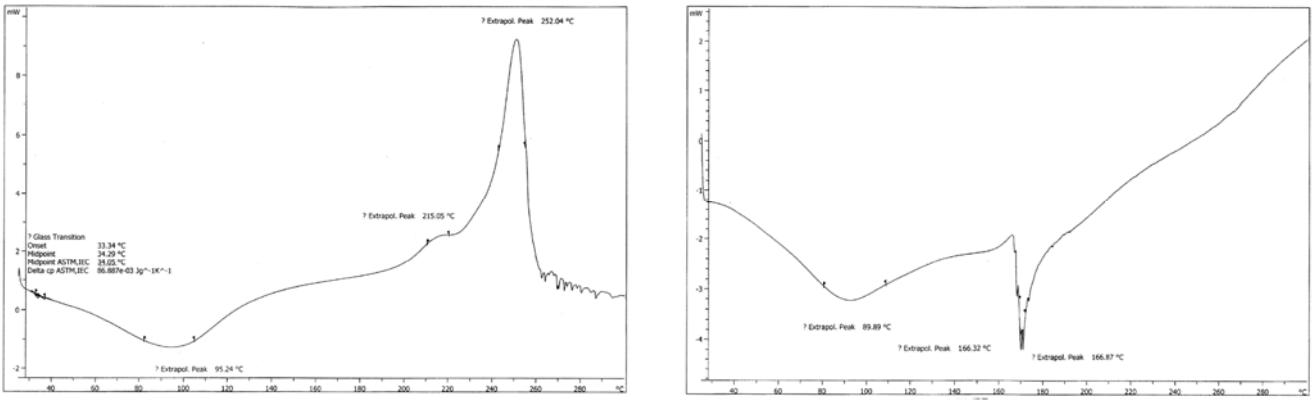
3.2 Thermal analysis

Differential scanning calorimetry thermograms of pure compounds and polyphenols-loaded sodium alginate microbeads were observed by DSC analysis. Alginic acid presents initially a dehydration process followed by decomposition (Fig. 2 A).

Calcium alginate microcapsules with polyphenolic extract of rose hips showed two endothermic peaks around 89 ^0C and 166 ^0C the peaks been shifted to lower temperatures. The degradation exotherm peak of sodium alginate at 252˚C was absent in calcium alginate F4 microcapsules this could be ascribed to interaction between alginate and polyphenols.

Table 2. DSC/TGA analysis of pure compounds and prepared microcapsules.

Compound	Attribution	TGA	DSC	
		Mass loss %	pic/^{0}C	
ALG	DEH		95.24	(endo)
	DEC		252.04	(exo)
F1	DEH		103.84	(endo)
	DEC		173.04	(endo)
F2	DEH		122.40	(endo)
	DEC		168.29	(endo)
F3	DEH		124.72	(endo)
	DEC		170.18	(endo)
F4	DEH	49.30	89.89	(endo)
	DEC		166.32; 66.87	(endo)
CS	DEH		88.28	(endo)
	DEC		223.80	(endo)
F4 blank	DEH		99.30	(endo)
	DEC		193.63;195.52	(endo)
			250.20	(exo)
F5	DEH	47.23	55.54	(endo)
			108.61	(endo)
	DEC		176.92	(endo)
F6	DEH	48.36	74.17	(endo)
	DEC		224.34	(exo)
	DEC		268.91	(exo)
F7	DEH	46.91	77.37	(endo)
	DEC		193.38	(exo)
	DEC		269.25	(exo)
DEH- dehydration; DEC - decomposition				



(A) (B)

Fig. 2. DSC analysis of sodium alginate (A) and F4 microcapsules (B).

It can be observed that dehydration temperature increased from F1 to F3 formulation (*Table 2*). Also, it can be seen that the microcapsules F4 dehydrate and decompose more easily than F1, with decreased concentrations of alginate and calcium chloride. Also in F5-F7 microcapsules can be seen that the dehydration temperature increases from 55.5^0C to 77.4^0C with increasing CS concentration of coagulation fluid from 0.25% - 1%.

The DSC endothermic peak around 269 ºC, which was absent from either the chitosan or alginate thermogram, could be ascribed to the formation of an ionic pair between the carboxylate group ($-COO^-$) of alginate and the ammonium group ($-NH_3^+$) of chitosan [19].

In the one-stage procedure, a complex coacervate membrane is formed at the interface between the alginate and chitosan solutions when the alginate solution is dropped directly into a calcium chloride–chitosan solution.

If the beads are made by a one-stage procedure, only low amounts of chitosan are bound. This is probably because the chitosan only binds onto the surface, creating a membrane with such small pores that further diffusion of chitosan into the beads and subsequent binding onto the gel network is restricted [20].

Mass loss determined from TGA analysis, with values ranging from 46.9% (F7) - 49.3% (F4) can be correlated with the encapsulation efficiency of polyphenolic extract.

3.3 Encapsulation efficiency

Changes in the concentrations of sodium alginate, calcium chloride, and chitosan had a significant effect on encapsulation polyphenolic extract.

Encapsulation efficiency is expressed as a percentage of total polyphenols in the extract that are encapsulated in alginate / chitosan beads.

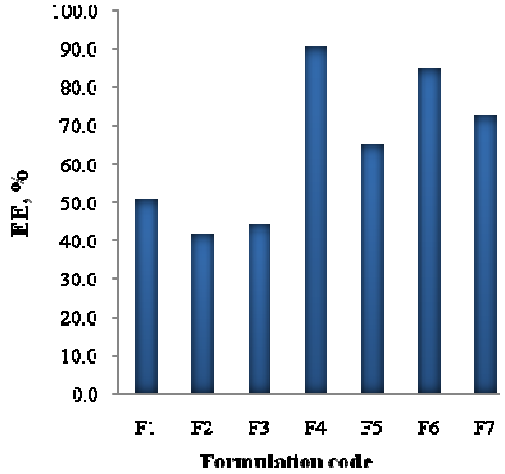


Fig. 3. Encapsulation efficiency of microcapsules.

It can be seen that good encapsulation efficiencies were obtained from a ratio alginate / polyphenolic extract 1:1 and $CaCl_2$ 1%, this proportion being maintained for the following investigations. F4 microcapsules (**Fig.** *3*), showed the highest encapsulation efficiency (90.7%), were prepared with 2% (w / v) sodium alginate and 1% (w / v) $CaCl_2$. Addition of 0.25-1% CS in coagulation fluid (F5-F7) determined an improvement of encapsulation efficiency compared with F1-F3 formulations. This is probably due to more firmness in the alginate-chitosan complex during gelation caused by increased ionic interactions between the carboxylate groups in the alginate and the protonated amine groups in the chitosan. As a result less polyphenol is lost during gelation. In the presence of more chitosan, the process will go faster. Moreover, a denser membrane will be formed because the greater number of alginate-chitosan ionic linkages [21].

3.4 In vitro release studies

In vitro polyphenols released of prepared beads was carried out both in simulated gastric fluid (SGF) and simulated intestinal fluid (SIF).

It can be seen that the total polyphenols release rate in SGF was between 40.7% (F1) - 93.6% (F2) and in SIF was between 3.7 - 15.4%, the highest content of polyphenols was released in SGF (**Fig.** *4*). The release rate (RR) of polyphenols from microcapsules is influenced by the concentration of alginate, this phenomenon is in agreement with the previous study where it is reported that the release rate was quicker for beads prepared in low concentration of alginate but slower for beads prepared in high concentrations [22]. The release rate of F1 formulation with 3% (w /v) sodium alginate was 40.7% in SGF but in F4 beads it increased to 93.6% by reducing the alginate concentration to 2% (w /v).

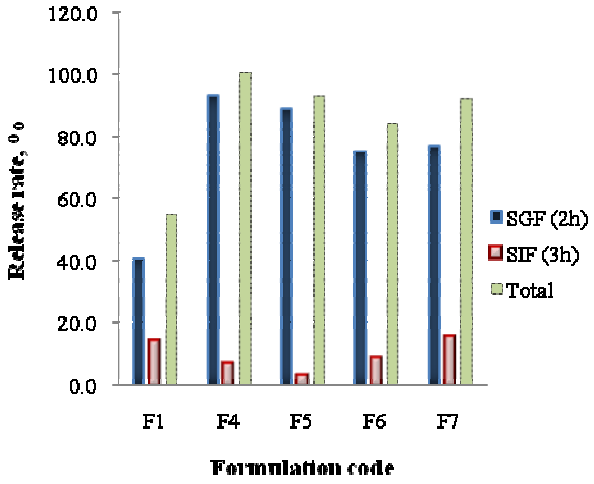


Fig. 4. Polyphenols release rates of beads in SGF and SIF fluids.

By comparing the release rate of polyphenols from microcapsules F5-F7 in SIF can be seen that with the addition of CS (0.25-1%, w / v) in coagulation fluid slightly increased the phenol content released in SIF from 3.7 - 15.4%. This effect can be attributed to the greater stability of polyphenols-chitosan interactions compared to the polyphenols-alginate. The lower rate of release

coincides with the delay in the erosion of the beads. The delayed erosion and concomitantly the more sustained release of polyphenols from chitosan reinforced alginate beads probably reflect the strengthening of the beads by ionic interaction of chitosan ($NH3^+$) with alginate (COO^-) ions [21].

4. Conclusions

Calcium alginate beads were prepared for the entrapment and controlled release of polyphenols. The interactions between alginate/chitosan /polyphenols were studied by DSC/TGA and FT-IR. The best encapsulation efficiency (90.7 %) was obtained for 2% (w / v) sodium alginate and 1% (w / v) $CaCl_2$ with a ratio alginate / phenolic extract 1:1. For microcapsules prepared by adding chitosan in coagulation fluid the best encapsulation efficiency (85.2%) was obtained with 0.5% CS (w / v).

Weak interactions between polyphenols and calcium alginate have allowed most of the polyphenols to be released in SGF. With the addition of CS in the coagulation fluid is observed a slight increase of polyphenols released in SIF.

Further studies must be done in the preparation of the alginate-chitosan beads to prevent the erosion of the polymer matrix and to achieve a more controlled release of polyphenols.

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