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BIOENCAPSULATED SEABUCKTHORN OIL: CONTROLLED RELEASE RATES IN DIFFERENT SOLVENTS

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Abstract. This work examines the release rates in different solvents (polar and non-polar) of seabuckthorn oil encapsulated in different natural biopolymers (alginate, *k*-carrageenan and chitosan). The sea buckthorn oil was encapsulated using the ionotropically crosslinked gelation technique, and spherical orange beads with diameters ranging from 2 to 3 cm were obtained. The free and encapsulated seabuckthorn oil beads were characterized by Fourier transformed infrared (FTIR) spectroscopy. Determination of release rates of seabuckthorn oil (in hexane and methanol) was monitored by UV-Vis spectroscopy. Our experimental data indicated that release rates are determined by the diffusivity in the biopolymer matrices, and the solvent type, which influences the swelling of the beads.

INTRODUCTION

Controlled release may be defined as a method by which one or more active agents or ingredients are made available at a targeted site and time and at a specific rate (Pothakamury and Barbosa-Canovas, 1995). The advantages of controlled release are: the active ingredients are released at controlled rates over prolonged periods of time, avoiding or reducing the loss of ingredients during processing and cooking, with possible separations of reactive or incompatible components (Dziezak, 1988; Brannon-Peppas, 1993).

Controlled release can be achieved by diffusion, which is controlled by the solubility of the compound in the matrix and the permeability of the compound through the matrix or by degradation (Pothakamury and Barbosa-Canovas, 1995). Can be achieved as well, by swelling when the matrix polymer is placed in a thermodynamically compatible medium and it swells because of a fluid absorption (Gibbs et al., 1999b) and by melting e.g. release lipids, modified lipids or waxes with low melting points (Sparks et al., 1995).

Seabuckthorn oleosomes or extracted oil are rich sources of many bioactive molecules, such as PUFAs, carotenoid pigments, tocopherols, phytosterols (Socaciu, 2003,2007) with many applications in biomedicine and dermocosmetics.

We aim to study the encapsulated forms of sea buckthorn oil in different water soluble biopolymers, the release rates by diffusion and/or by swelling in two different types of solvents, one polar (methanol) and a non-polar one (hexane). The sea buckthorn oil was encapsulated by an ionotropically crosslinked gelation method, a cheap encapsulation method used in the field of bioencapsulation of natural bioactive compounds, cells and enzymes.

MATERIAL AND METHODS

Materials. Sodium alginate (ALG) was purchased from Promova Biopolymer Norway, calcium chloride ($CaCl_2$), chitosan (CHI) (medium molecular weight), natrium tripolyphosphate (NaTPP), acetic acid from Sigma Aldrich, *k*-carrageenan (*k*-CAR) from Danisco, sea buckthorn oil (SBO) was extracted from the fruits of sea buckthorn, which were collected from Cluj county (Transilvania, North of Romania).

Preparation of the beads. A concentration of sodium alginate (2,0% w/v) (ALG) and sodium alginate - *k*-carrageenan (ratio 0.75:0.75) (ALG-k-CAR) were dissolved in de-ionized water. Chitosan (CHI) in different concentrations (1,0% and 1,5% w/v) was dissolved in acetic acid 0,7% in water, all these matrices been used to encapsulate the sea buckthorn oil by ionotropically crosslinked gelation. The emulsions obtained after matrices were homogenized with sea buckthorn oil were dropped using a syringe with a needle (0.4 x 20 mm) into a hardening bath 2% (w/v) solution of $CaCl_2$ in water to obtain the sodium alginate and sodium alginate - *k*-carrageenan complex beads, and 5% (w/v) solution of NaTPP to obtain chitosan beads. After 30 minutes, the beads were separated from the hardening baths by filtration.

Encapsulation efficiency of the oil. The encapsulation efficiency (EE%) was calculated taking into consideration the amount of β-carotene contained by SBO, before and after encapsulation. The amount of β-carotene was assayed spectrophotometrically at 454 nm using tetrahydrofuran (THF) as solvent to extract β-carotene from beads after the beads were crushed using a mortal. The following formulae was used:

$$(EE\%) = C_1/(DFxC_2) \text{ x } 100,$$

where: C_1 = β-carotene concentration in beads containing SBO

C_2 = β-carotene concentration in SBO before encapsulation

DF = dilution factor of ß-carotene according to the added encapsulation material (in our case *DF*=1)

FTIR-ATR analysis. The FTIR spectra were obtained with a Fourier transform spectrometer Spectrum One (PerkinElmer), equipped with the universal ATR as an internal reflection accessory which have Composite Zinc Selenide (ZnSe) and Diamond crystals. Each spectrum was registered from 4000 to 650 cm^{-1}. The FTIR spectra were measured for free SBO, ALG, *k*-CAR and CHI (as powders) and blank and SBO-containing beads.

Release rate measurements of the oil from beads. The release of carotenoids (the principal compound of the sea buckthorn oil) from beads was measured spectrophotometrically. The absorption spectra were obtained in a Jasco UV-VIS spectrometer. All measurements were performed with the substances inside a 2 mm long quartz glass cuvette. All spectra were recorded at room temperature and the results are the average of 3 runs. The normalized absorption spectrum of seabuckthorn oil, carotenoids constituents were performed in the range 300-500 nm. Determination of the principal compound of this oil was measured after dissolving 1g oil in 10 mL solvent. Two solvents were used: hexane (nonpolar) and methanol as a polar solvent.

RESULTS AND DISSCUSIONS

Bead sizes. By dropping into the hardening baths the emulsions of ALG, ALG-k-CAR complex and CHI, and SBO into the hardening baths, orange beads with diameters of 2-3 mm and almost spherical shapes were obtained. The concentration of the ALG and CHI influenced the diameter of the beads. Increasing concentrations of the matrices (from 1 to 2%) determined harder beads.

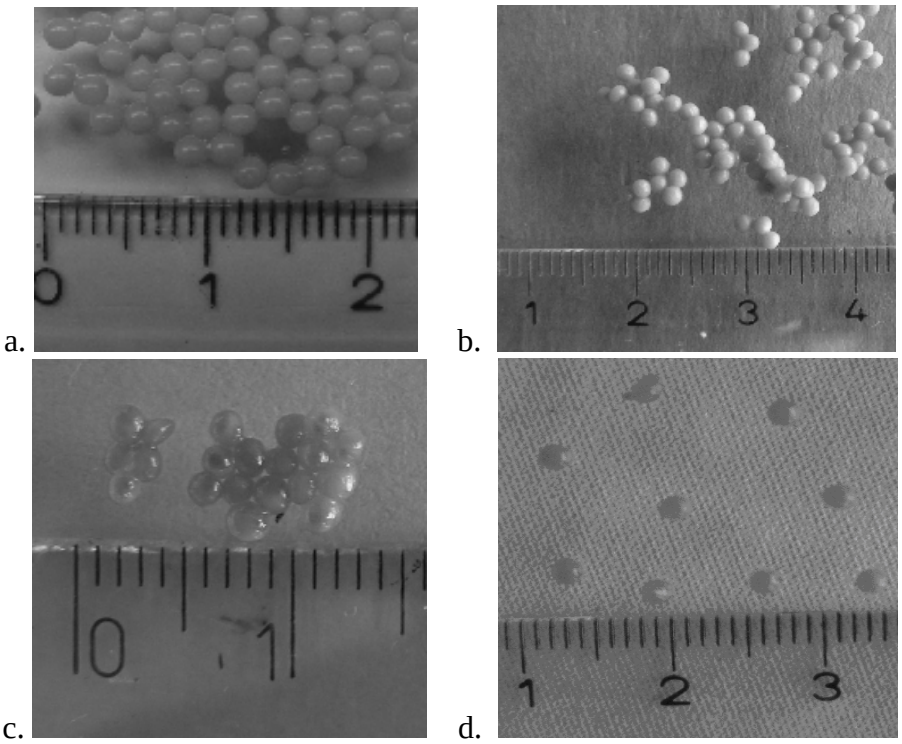

Fig.1 Represents beads containing sea buckthorn oil: a. ALG 2%, b. CHI 1,5%, c. CHI 1% and d. ALG-k-CAR complex (1.5%). The scale is in cm.

Encapsulation efficiency measured by β-carotene content. Initially, the β-carotene content was 1.212 mg/l SBO, while after oil encapsulation in the ALG 2% beads it was 1.1964 mg/l, showing that the efficiency of β-carotene encapsulation was 98.71%. Encapsulation in the CHI 1.5% resulted in 1.0632 mg β-carotene /l (87.72% efficiency) and in 1% beads it was 0.8184 mg β-carotene /l (68.08% efficiency), while in the ALG-*k*-Car beads it was 0.992 mg/l, showing that the EE% was 81,89%.

FTIR-ATR analysis. The comparative FTIR spectra of SBO, of ALG, *k*-CAR and CHI (as powders) and blank and SBO-containing beads are shown in Fig.2. We described previously (Trif et al., 2007) the most significant FTIR-ATR peaks for SBO and ALG (Fig. 2.A. and B.). This study focuses only on the peak characteristics for CHI, k-CAR, ALG-k-CAR complex and the beads obtained from these matrices with and without SBO.

FTIR spectrum of *k*-CAR powder (Fig. 2.D.) shows various distinct peaks: 3514 cm^{-1} due to polyhydroxy (OH)n group; 2953 cm-1, 2911 cm-1 and 2894 cm-1 due to the C-H stretch; 1474 cm^{-1} and 1400 cm^{-1} due to C-H deformation; 1223 cm^{-1} due to the S=O stretch of sulfate ester salt; 1063 cm-1 C-O stretch of cyclic ethers; 924 cm^{-1} due to the C-O stretch

of polyhydroxy groups attached to carbons (Fatima et al., 2007). The bands at 842 cm^{-1} and 910 cm^{-1} can be attributed to D-galactose-4-sulfate, 3,6-anhydro-D-galactose, glycosidic linkage.

The spectra shown in Fig. 2.E-J confirm the presence of OH and N–H stretching vibration at 3439 cm^{-1} in which the OH stretching vibration are overlapped by N–H stretching which is due to the presence of CHI. The absorption of C=O group of chitosan and sodium alginate is at about 1642 cm^{-1} (i.e. amide I band). The amide II band due to N–H bending appears at 1640, which is overlapped by amide I band (Fig. 2. C.) (Kim et al., 2006). The formation of carboxylate anion of sodium alginate shows another C–O absorption at 1428 cm^{-1}. The N–H bending vibration appears at 832 cm^{-1}.

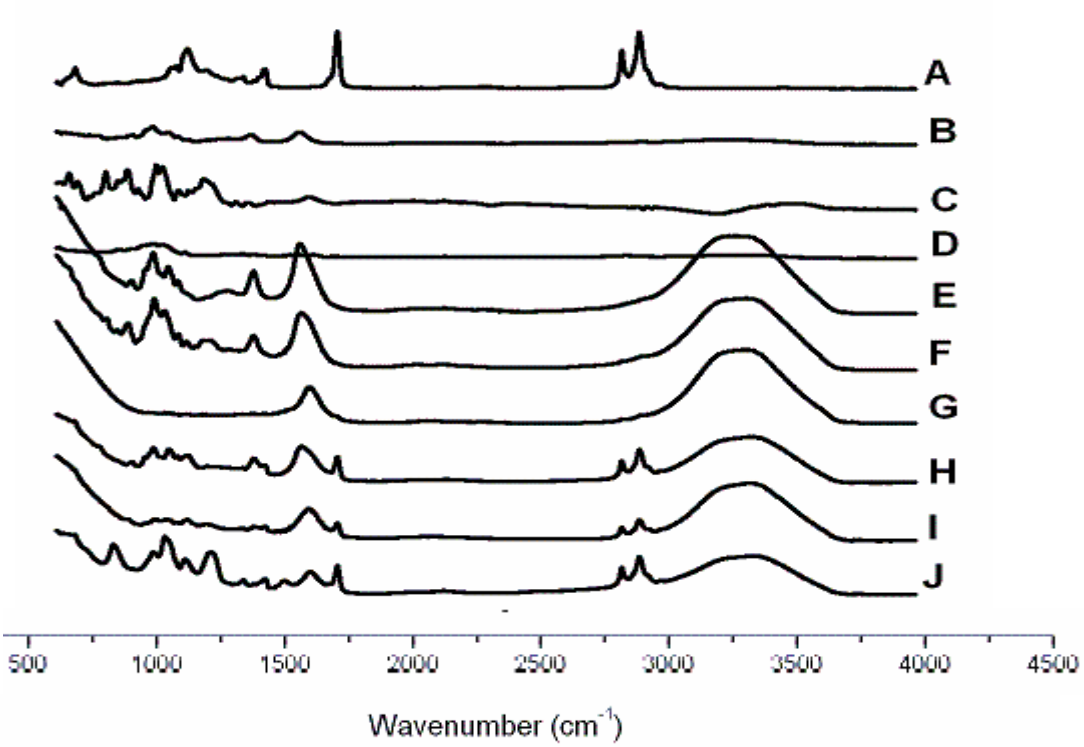

Fig.2 FTIR-ATR spectra for: A. SBO; B. ALG powder; C. CHI powder; D. *k*-CAR powder; E. ALG blank bead; F. ALG- *k*-CAR complex blank bead; G. CHI blank bead; H. ALG bead containing SBO; I. *k*-CAR bead containing SBO; J. CHI bead containing SBO

FTIR spectra of beads containing SBO (Fig. 2.H, I. and J.) show the double peaks specific to SBO (region 2800-2900 cm^{-1}) different matrices which are present also in the free SBO (Fig.2A). This confirms that SBO was encapsulated into the beads.

Release rate measurements of the oil from beads. The release into hexane was complicated by the high surface tension between the aqueous gel particles and the organic solvent, which caused the particles to aggregate and to adhere to the wall of the vessel.

Comparing ALG and ALG-*k*-CAR, in receiving solutions, release rate was faster in methanol as receiving solution for ALG-*k*-CAR and in hexane too. We can say that the complexity and the concentration of the matrix influence the release rate in different receiving solutions.

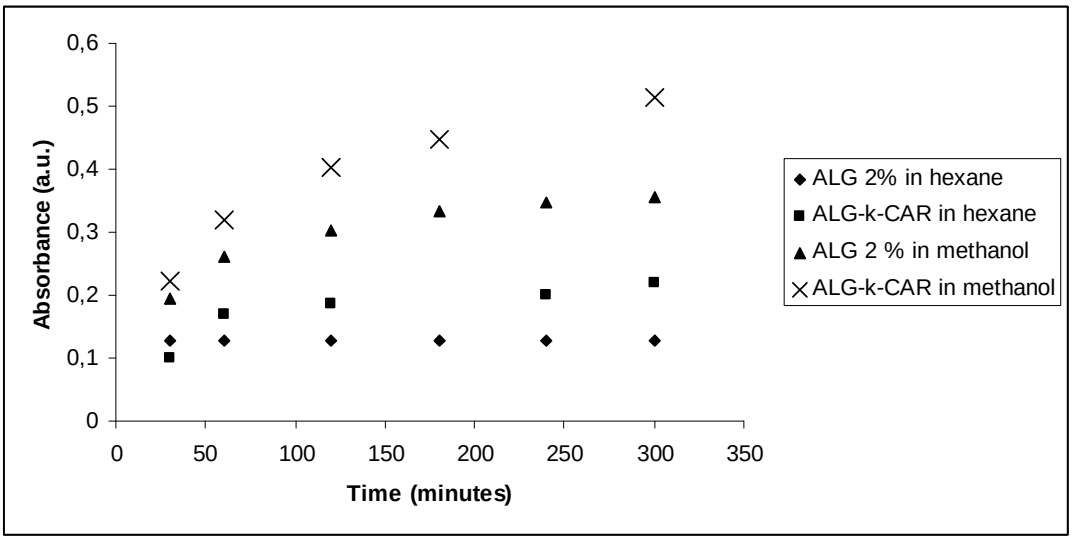

Fig. 3 Graphic representation of the absorbance values (a.u.) corresponding to the oil release in time from alginate 2% and alginate-carrageenan complex fresh beads into methanol and hexane

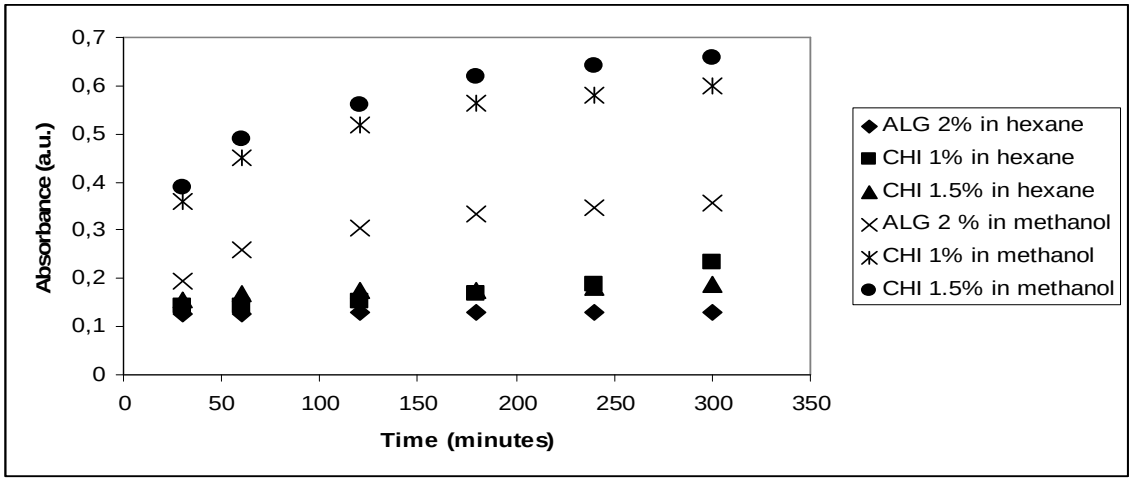

Fig. 4 Graphic representation of the absorbance values (a.u.) of oil release in time from alginate 2% and different chitosan concentrations fresh beads into methanol and hexane

The release was faster in methanol as receiving solution, and as a good matrix was found to be CHI in 1.5% concentration followed by 1% concentration, and in hexane CHI 1%. The CHI was found to be the best matrix for release of the SBO in both solvents used. Comparing the release rates in both solvents from CHI and ALG-*k*-CAR complex beads the best release of SBO was from CHI beads.

The best matrix for a high SBO release into methanol was found for CHI in concentration 1%, and into hexane for the ALG-*k*-CAR complex.

CONCLUSIONS

We succeeded to obtain different beads, which encapsulate seabuckthorn oil in chitosan, alginate and alginate-carrageenan complex matrices, by a ionotropically cross linking gelation mechanism. The best encapsulation efficiency 98.71% was obtained using alginate 2%, which suggests that alginate in this concentration is one of the best matices for seabuckthon oil

encapsulation. Release rates of oil from the beads showed that the alginate, alginate-carrageenan complex and chitosan are suitable microencapsulation matrices for oils.

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