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**“Development and characterization of bioactive alginate microcapsules with cedarwood essential oil“**

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## Abstract

In this work, sodium alginate microcapsules containing cedarwood essential oil (CWO) for uses in anti-acne tonic were prepared by ionic gelification of alginate with calcium chloride ($CaCl_2$) and subsequent addition of glutaraldehyde to improve the crosslinking degree. Alginate microcapsules with cedarwood essential oil were obtained in an encapsulator with a 600 μm nozzle using different alginate concentrations (1, 3 and 4% w/v) , and different compositions of the coagulation solution with $CaCl_2$ concentrations of 0.1, 0.25 and 0.5 M, and addition of glutaraldehyde at two concentrations: 12.5% and 50 % (0.1 g $L^{-1}$ and 10 g $L^{-1}$ respectively). The effect of alginate concentration was followed by viscosimetry and the influence of the $CaCl_2$ concentration and presence of glutaraldehyde on the microcapsules' shape as well as the total content on encapsulated cedarwood essential oil were evaluated by means of stereoscopic magnifying glass, scanning electron microscopy (SEM) and UV-vis spectrophotometry. Results show that proper shape formation is obtained for an alginate concentration of 3% w/v. With regard to the total encapsulated cedarwood oil, as the $CaCl_2$ concentration in the gelifying-coagulating solution increases the efficiency of the encapsulated oil. Best results in terms of micro-bead shape and total amount of encapsulated oil were obtained for a $CaCl_2$ concentration of 0.5 M without glutaraldehyde with a maximum encapsulation of 177.2 mg per gram of microcapsule.

**Keywords:** Microcapsules; alginate; cedarwood essential oil; anti-acne; coagulation.

## Introduction

Cedarwood oil (CWO) is an essential oil that is generally obtained from *Juniperus* species. For many centuries, the leaves, berries and essential oils from *Juniperus* have been used as cosmetics and for medical purposes [1]. The main chemical compounds contained in cedarwood essential oil are cedrol (13-15%), widdrol (9-12%), 8,14-cedranoxide (7-8%), 14-hydroxy-E-caryophyllene (5-9%), cis-thujopsene (10-11%) and a-cedrene (6-8%) [2]. Some studies have revealed the antifungal, anti-termite and anti-inflammatory properties of cedarwood

oil [3,1]. Cedarwood essential oil is widely used in fragrances as well as flavored soaps, aerosols and sprays, disinfectant agents, cleaning of microscope parts and immersion lens [2].

Microencapsulation is a packing technique in which a material or a mixture of materials in the liquid form, small solid particles or even in the gas state are covered or trapped inside another material, typically a polymer, with the main aim of immobilizing, protecting or maintaining the bioactivity of the encapsulated chemical. Degradation of the polymer entrapment can lead to a controlled release rate of the encapsulated compound [4-6]. In the last years a remarkable increase in the microencapsulation technology has been detected, with a wide variety of applications in different sectors. Among others, the use of microcapsules or micro-beads is generalized in industries such as pharmaceutics, chemical, food, perfumery, textile, agriculture, etc. [4,7-11].

Recently, microcapsules from alginate-based polymers have acquired great relevance as they can be used as controlled delivery systems due to its biodegradability. In addition, alginate is nontoxic, hemocompatible and offers high stability to the encapsulated compound. For these reasons, research has focused on the use of alginate-based microcapsules as drug carrier and delivery systems for the pharmaceutical industry [12] and in bioengineering and biomedicine applications [13-18]. Alginate is a linear non branched polysaccharide obtained from brown marine algae and it mainly consists on linear polymeric chains from α-l-guluronic acid and β-D-mannuronic acid residues joined by 1,4-glycosidic linkages [19,20]. One of the most relevant features of alginate polymers is their ability to form gel structures by reaction with divalent cations such as $Ca^{2+}$ [21]. Typical alginate microcapsules can be obtained by extrusion of the alginate-containing solution in the form of drops over the crosslinking solution with divalent cations such as $Ca^{2+}$, $Sr^{2+}$, $Ba^{2+}$ [22].

The main aim of this work is to obtain alginate-based microcapsules containing cedarwood essential oil (CWO) as potential anti-acne material for uses in cosmetic tonics. Optimization of the microencapsulation process is carried out by varying concentrations and process parameters. The influence of sodium alginate concentration (polymer-based solution),

$CaCl_2$ concentration (gelling solution) and glutaraldehyde (crosslinking agent) on the microcapsule morphology as well as the total encapsulated cedarwood oil is evaluated.

## Experimental

### *Materials*

Alginic acid sodium salt from brown algae (low glucuronic content with low viscosity, 4–12 cPs, 1% in $H_2O$ at 25 °C) and calcium chloride ($CaCl_2$) were supplied by Sigma Aldrich (Sigma Aldrich, Madrid, Spain). Two glutaraldehyde solutions at 12.5% and 50%, supplied by Corquimia Industrial (Corquimia Industrial S.L., Barcelona, Spain) were used as crosslinking agent. Glutaraldehyde was selected to its wide use in the textile industry as crosslinker. These two particular concentrations are typical of crosslinking processes of alginate [23] and were also recommended by the supplier. Cedarwood essential oil (CWO) was supplied by Esencias Martínez Lozano (Esencias Martínez Lozano, S.A., Murcia, Spain). In addition, colorant Conasol 3-C supplied by Proquimac PFC (PROQUIMAC PFC SA, Barcelona, Spain) was used to stain cedarwood thus avoiding a better observation of the encapsulation oil.

### *Cedarwood oil activity against acne*

The benefits of cedarwood oil for acne treatment were tested by measuring its bacterial activity against *Propionibacterium acnes*. The test was conducted with Tryptone soya broth (TSB) and the culture medium was plate count agar (PCA). The contact time with the microorganism was 24 h and the incubation time of plates was 24 h at a temperature of 35 ºC.

### *Preparation of CWO encapsulated by alginate microcapsules*

Alginate solution was prepared with sodium alginate in distilled water at different concentrations: 1, 3 and 4% w/v. The solution mixture was homogenized using an Ultra-turrax T18 homogenizer (IKA, Staufen, Germany) at 5000 rpm for 5 min. Alginate droplets with cedarwood oil were generated with an encapsulator B-390 pro from Büchi (Essen, Germany) equipped with a concentric nozzle system with an inner nozzle diameter of 300 µm and an outer

nozzle diameter of 600 μm. The droplets were generated at a fixed frequency of 400 Hz and an electrode tension of 250 V and were continuously dropped onto a $CaCl_2$ solution at different concentrations (0.5, 0.25 and 0.1 M) and two different glutaraldehyde compositions (0.1 g $L^{-1}$ and 10 g $L^{-1}$). Gelation of alginate microcapsules was maintained for 30 min at a rotating speed of 100 rpm to allow hardening. Table 1 summarizes the main parameters of the microencapsulation process.

**Table 1**

In a first stage, the alginate concentration varied from 1% to 4% w/v to find the optimum concentration in terms of solution viscosity and the spherical shape of the obtained microcapsules, measured by optical microscopy. After this initial stage, the alginate concentration was maintained constant and then, the gelling solution composition was optimized by varying the $CaCl_2$ concentration (0.1M, 0.25M and 0.5M) and glutaraldehyde (0.1 g $L^{-1}$ and 10 g $L^{-1}$).

***Viscosity of the alginate polymer solution***

The easiness of extrusion of the alginate polymer solution through the encapsulator nozzle is directly related to the solution viscosity. For this reason, rotational viscosity of polymer solutions with different alginate concentrations was measured with a Brookfield viscometer DV-I Prime (Brookfield Engineering Laboratories, Inc, Massachusetts, USA) equipped with a RV/HA/HB-2 spindle at a rotating speed of 100 rpm.

***Morphology and size of the CWO encapsulated alginate microcapsules***

The shape of the wet microcapsules was examined by a digital camera Canon coupled to a stereoscopic magnifying glass Olympus SZX7 (Olympus Corp., Japan). Optical microscopy allowed observation of the encapsulated oil, previously stained with a specific colorant and a warped shape of microcapsules. The average size and spherical shape could not be observed in the wet form. For this reason, microcapsules were previously dried (1 h at 50 ºC) and,

subsequently, observed by scanning electron microscopy (SEM) in a Phenom microscope from FEI Company (Eindhoven, Netherlands). Prior to observation, dry microcapsules were subjected to a coating process with an aurum-palladium alloy for 135 s in a sputter coated EMITECH mod. SC7620 from Quorum Technologies Ltd. (East Sussex, UK)

***Determination of the encapsulated cedarwood essential oil into alginate microcapsules***

The total content of encapsulated cedarwood essential oil (CWO) was determined by weighing a fixed weight of microcapsules (500 mg) and diluting them into 5 g ethanol. After this, the ethanol solution was placed in an ultrasonic bath sonicator for 10 min to allow microcapsule breakage and subsequently subjected to centrifugation in a SCANSPEED mod. 1248 from Labogene ApS (Lynge, Denmark) at a rotating speed of 3000 rpm for 5 min. The supernatant was collected and the total amount of encapsulated cedarwood essential oil was determined by UV-vis spectrophotometry at a wavelength of 326 nm using a Hach Lange DR-3900 spectrophotometer (Düsseldorf, Germany).

The encapsulation efficiency of cedarwood essential oil into alginate-based microcapsules was calculated by considering the total amount of cedarwood used in the encapsulation ($W_{CWO\_T}$) and the remaining cedarwood essential oil contained in the supernatant after centrifugation ($W_{CWO\_S}$) (this stands for the total encapsulated cedarwood essential oil). The encapsulation efficiency (EE%) was calculated by using the following equation.

$$EE\ (\%) = \frac{W_{CWO\_T} - W_{CWO\_S}}{W_{CWO\_T}} \times 100$$

***Cytotoxicity test MMT***

As these microcapsules are intended to be used in cosmetics, it is important to assess their potential cytotoxicity as they are going to be in contact with the skin. For this reason, cytotoxicity of alginate microcapsules was tested *in vitro* with L929 cells (mouse fibroblasts) by the MMT

photocolorimetric assay based on the reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) as indicated in the ISO 10993-5 standard. This standard is designed to determine the response of mammalian cells *in vitro*. A cell culture medium without alginate microcapsules with cedarwood oil was used as the negative control for the viability assay. L929 cells were incubated in a DMEM medium (Dulbecco's modification of Eagle's medium) as recommended by ISO 10993-5 standard as it is a specific medium for mammalian cells culture, with additional fetal bovine serum and L-glutamine (Glutamax). All the tests were carried out in triplicate.

Cells were cultured at a density of approx. $2 \cdot 10^4$ cells per well in plates containing 96 wells for a period of 24 h at a constant temperature of 37 ºC and 5% $CO_2$ in wet atmosphere (higher than 90%) in triplicate. After this, different concentrations of alginate microcapsule with cedarwood oil were added (10 and 500 μg $mL^{-1}$) and the plates were incubated for an additional period of 24 h with the same conditions. These concentrations were selected to cover a wide range of bioactivity at very low and very high concentrations. After incubation, the supernatant was removed and MTT was added; then incubation was kept for 2 h at 37 ºC. The precipitated formazan was dissolved in dimethyl sulfoxide (DMSO) and the absorbance and optical density (OD) was measured at a wavelength of 550 nm in a microplate reader (Multiskan Ascent). Cell survival was determined by comparison of the absorbance values obtained for treated and non-treated cells. The total amount of live cells was then correlated to the generated formazan with the following equation:

$$Cell\ Viability\ (\%) = \frac{OD_{550}\ treated\ sample}{OD_{550}\ negative\ control} \times 100$$

If the cell viability of cultures with alginate microcapsules is lower than values of 70% of the negative control culture, then the material has potential cytotoxicity and if this value is lower than 30%, then the cytotoxic effect is clearly evident.

## Results and Discussion

The anti-acne of cedarwood essential oil has been checked by its activity against *Propionibacterium acnes*. The antimicrobial effect is evident as the microorganism reduction that cedarwood essential oil gives is 99.99%, thus showing its potential in cosmetics.

Fig. 1 shows an optical image of the wet microcapsules. The encapsulated cedarwood essential oil appears blue-stained to observe the efficiency of the encapsulation process. We can clearly see that low concentrations of 1% w/v provide very low viscosity (3160 cP) and the solution is too much fluent so that microcapsules cannot be formed in an appropriate way. On the other hand, an alginate concentration of 4% w/v leads to a thick solution with a viscosity of 14760 cP which leads to a microcapsule aggregation. Best results are obtained for an alginate concentration of 3% w/v with intermediate viscosity values of 9840 cP.

**Fig. 1**

Considering these results, the alginate concentration has been maintained constant at a fixed value of 3% w/v and the effect of the $CaCl_2$ and glutaraldehyde concentration ion the gelling solution has been tested. Fig. 2 shows optical images obtained with a stereoscopic magnifying glass for microcapsules obtained with constant alginate concentration (3% w/v) in the polymer solution and different $CaCl_2$ concentrations in the gelling solution (0.1M, 0.25M and 0.5M) as well as different crosslinking agent (glutaraldehyde), 0.1 g $L^{-1}$ and 10 g $L^{-1}$). As we can see, all microcapsules (in the wet form) show an oval shape in which, both the gelled alginate shell and the encapsulated cedarwood essential oil (CWO) can be detected due to the previous blue-staining of the CWO. The effect of $CaCl_2$ as ionic gelation agent has been widely described in the literature [13,24,25]. In general the shapes are quite homogeneous and slight changes can be detected as a consequence of the $CaCl_2$ and glutaraldehyde concentrations. Higher $CaCl_2$ concentrations lead to more circular capsules. With regard to glutaraldehyde, it has no a clear effect on overall shape of the synthesized microcapsules.

**Fig. 2**

Table 2 shows a summary of the total encapsulated cedarwood essential oil related to 1 g microcapsule in terms of the $CaCl_2$ and glutaraldehyde concentrations in the gelling solution. As we can see the maximum encapsulation is obtained with solutions without glutaraldehyde and, additionally, the encapsulated amount increases with increasing $CaCl_2$ concentration with values of 173.7 and 177.2 mg CWO per gram capsule for 0.25M and 0.5M respectively. Presence of glutaraldehyde leads to more crosslinked structures but the amount of encapsulated cedarwood essential oil is lower. So that, for a $CaCl_2$ concentration of 0.1M, maximum encapsulation efficiency is obtained with gelling solutions containing 10 g $L^{-1}$ glutaraldehyde (12.5% solution) with an encapsulated mass of 175.5 mg, slightly higher that microcapsules obtained with same $CaCl_2$ concentration and low glutaraldehyde concentration (0.1 g $L^{-1}$ glutaraldehyde at 12.5%) leading to an encapsulated mass of 168.4 mg. The use of concentrated solution of glutaraldehyde (50%) leads to an overall decrease in the encapsulated CWO. As it can be observed in Table 2, maximum efficiency of the encapsulation process is achieved for a gelling solution with 0.5M $CaCl_2$ and no crosslinking agent.

**Table 2**

The encapsulation efficiency (EE%) has been determined for the optimized conditions (polymer solution with 3% w/v alginate, gelling solution with 0.5M $CaCl_2$ and no glutaraldehyde) by considering the amount of cedarwood oil used in the process and the total amount that has been trapped or encapsulated inside alginate shells (average values are summarized in Table 3). An average encapsulation efficiency of 65% is obtained with these conditions and this represents an attracting value from industrial point of view.

**Table 3**

Fig. 3 shows a SEM image corresponding to dry microcapsules obtained with the optimum conditions as previously indicated. Wet microcapsules were dried at 50 ºC for 1 h to study the shape and size of the synthesized microcapsules. As we can see, when microcapsules are dry, the shape is clearly spherical with a smooth surface topography. The average diameter is, obviously close to 600 μm as the nozzle diameter is 600 μm.

**Fig. 3**

The effect of different concentrations of alginate beads with encapsulated cedarwood oil on cell viability (L929 cells after 24 h incubation) was studied by the MTT assay. Fig. 4 shows cell viability of L929 cells for different concentrations. As one can see, addition of alginate capsules with CWO leads to a slight increase in cell viability with values of about 107% and 111% for alginate concentrations of 10 μg $mL^{-1}$ and 500 μg $mL^{-1}$ respectively. Obviously, these values indicate that alginate beads containing cedarwood oil are not cytotoxic (cell viability remarkably higher than 70%) thus showing that these microcapsules can be used in the cosmetic industry for applications in direct contact with skin.

**Fig. 4**

Fig. 5 shows images of the cell proliferation after an incubation period of 24 h for cultures with different microcapsule concentrations in comparison to the control culture. As it can be observed a slight increase in cell density can be observed for both concentrations so that giving a clear evidence of the absence of cytotoxicity.

**Fig. 5**

## Conclusions

This work has focused on the development of bioactive microcapsules containing cedarwood essential oil (CWO) into an alginate shell obtained by gelation with $CaCl_2$. The study of the viscosity of the polymer solution in terms of sodium alginate concentration revealed a clear effect of the solution viscosity on the ability to form homogeneous-individual microcapsules. Optimum results were obtained with an alginate concentration of 3% w/v. Alginate concentration of 1% w/v gives very low viscosity and microcapsules are not formed in an appropriate way while alginate concentrations of 4% w/v give thick pastes that lead to microcapsule aggregates. Other parameters influencing the encapsulation efficiency and the morphology of the synthesized microcapsules were the concentrations of the gelling agent, $CaCl_2$ and the potential use and concentration of a crosslinker such as glutaraldehyde. For a fixed alginate concentration of 3% w/v, maximum encapsulation efficiency of about 65% (estimated as the ratio between the encapsulated oil and the total oil used) is obtained with a $CaCl_2$ concentration of 0.5M without crosslinker. Glutaraldehyde can contribute to increase hardness and durability of the alginate shell but it has a negative effect on the overall yield of encapsulation. The high encapsulation efficiency (approx. 177 mg cedarwood oil per gram microcapsule) in conjunction with its easy decomposition, its absence of cytotoxicity and the intrinsic antibacterial properties of cedarwood essential oil, make these microcapsules very attractive and useful for the cosmetics industry with potential uses in anti-acne tonics.

## References

1. Tumen I, Suntar I, Eller FJ, Keles H, Akkol EK (2013) Topical Wound-Healing Effects and Phytochemical Composition of Heartwood Essential Oils of Juniperus virginiana L., Juniperus occidentalis Hook., and Juniperus ashei J. Buchholz. Journal of Medicinal Food 16 (1):48-55. doi:10.1089/jmf.2012.2472

2. Tunalier Z, Kirimer N, Baser KHC (2004) A potential new source of cedarwood oil: Juniperus foetidissima Willd. Journal of Essential Oil Research 16 (3):233-235

3. Eller FJ, Clausen CA, Green F, Taylor SL (2010) Critical fluid extraction of Juniperus virginiana L. and bioactivity of extracts against subterranean termites and wood-rot fungi. Industrial Crops and Products 32 (3):481-485. doi:10.1016/j.indcrop.2010.06.018

4. Xiao Z, Liu W, Zhu G, Zhou R, Niu Y (2014) Production and characterization of multinuclear microcapsules encapsulating lavender oil by complex coacervation. Flavour and Fragrance Journal 29 (3):166-172. doi:10.1002/ffj.3192

5. Xiao Z, He L, Zhu G (2014) The preparation and properties of three types of microcapsules of sweet orange oil using alginate and chitosan as wall material. Flavour and Fragrance Journal 29 (6):350-355. doi:10.1002/ffj.3212

6. Sarmah M, Banik N, Hussain A, Ramteke A, Sharma HK, Maji TK (2015) Study on crosslinked gelatin-montmorillonite nanoparticles for controlled drug delivery applications. Journal of Materials Science 50 (22):7303-7313. doi:10.1007/s10853-015-9287-3

7. Lopez A, Castro S, Andina MJ, Ures X, Munguia B, Llabot JM, Elder H, Dellacassa E, Palma S, Dominguez L (2014) Insecticidal activity of microencapsulated Schinus molle essential oil. Industrial Crops and Products 53:209-216. doi:10.1016/j.indcrop.2013.12.038

8. Pascual-Villalobos MJ, Lopez MD (2013) New application of guayule resin in controlled release formulations. Industrial Crops and Products 43:44-49. doi:10.1016/j.indcrop.2012.07.001

9. Devi N, Kakati DK (2014) Thermoresponsive submicron-sized core-shell hydrogel particles with encapsulated olive oil. Colloid and Polymer Science 292 (10):2581-2596. doi:10.1007/s00396-014-3309-6

10. Xiao Z, Li W, Zhu G (2015) Effect of wall materials and core oil on the formation and properties of styralyl acetate microcapsules prepared by complex coacervation. Colloid and Polymer Science 293 (5):1339-1348. doi:10.1007/s00396-015-3515-x

11. Thakker SP, Rokhade AP, Abbigerimath SS, Iliger SR, Kulkarni VH, More UA, Aminabhavi TM (2014) Inter-polymer complex microspheres of chitosan and cellulose acetate phthalate for

oral delivery of 5-fluorouracil. Polymer Bulletin 71 (8):2113-2131. doi:10.1007/s00289-014-1176-4

12. Lin N, Huang J, Chang PR, Feng L, Yu J (2011) Effect of polysaccharide nanocrystals on structure, properties, and drug release kinetics of alginate-based microspheres. Colloids and Surfaces B-Biointerfaces 85 (2):270-279. doi:10.1016/j.colsurfb.2011.02.039

13. Cho AR, Chun YG, Kim BK, Park DJ (2014) Preparation of alginate-CaCl2 microspheres as resveratrol carriers. Journal of Materials Science 49 (13):4612-4619. doi:10.1007/s10853-014-8163-x

14. Balcao VM, Santos MG, Martins PR, Chaud MV, Oliveira Junior JMd, Tubino M, Vila MMDC (2014) Development and characterization of a gel formulation integrating microencapsulated nitrofurazone. Current pharmaceutical biotechnology 14 (12):1036-1047

15. Ivanovska TP, Petrusevska-Tozi L, Kostoska MD, Geskovski N, Grozdanov A, Stain C, Stafilov T, Mladenovska K (2012) MICROENCAPSULATION OF LACTOBACILLUS CASEI IN CHITOSAN-Ca-ALGINATE MICROPARTICLES USING SPRAY-DRYING METHOD. Macedonian Journal of Chemistry and Chemical Engineering 31 (1):115-123

16. Chang CP, Dobashi T (2003) Preparation of alginate complex capsules containing eucalyptus essential oil and its controlled release. Colloids and Surfaces B-Biointerfaces 32 (3):257-262. doi:10.1016/j.colsurfb.2003.07.002

17. Khazaeli P, Pardakhty A, Hassanzadeh F (2008) Formulation of Ibuprofen Beads by Ionotropic Gelation. Iranian Journal of Pharmaceutical Research 7 (3):163-170

18. Abbah SA, Lu WW, Chan D, Cheung KMC, Liu WG, Zhao F, Li ZY, Leong JCY, Luk KDK (2006) In vitro evaluation of alginate encapsulated adipose-tissue stromal cells for use as injectable bone graft substitute. Biochemical and Biophysical Research Communications 347 (1):185-191. doi:10.1016/j.bbrc.2006.06.072

19. Lertsutthiwong P, Rojsitthisak P, Nimmannit U (2009) Preparation of turmeric oil-loaded chitosan-alginate biopolymeric nanocapsules. Materials Science & Engineering C-Biomimetic and Supramolecular Systems 29 (3):856-860. doi:10.1016/j.msec.2008.08.004

20. Peretz S, Anghel DF, Vasilescu E, Florea-Spiroiu M, Stoian C, Zgherea G (2015) Synthesis, characterization and adsorption properties of alginate porous beads. Polymer Bulletin 72 (12):3169-3182. doi:10.1007/s00289-015-1459-4

21. Ichiura H, Konishi T, Morikawa M (2009) Alginate film prepared on polyethylene nonwoven sheet and its function for ellagic acid release in response to sodium ions. Journal of Materials Science 44 (4):992-997. doi:10.1007/s10853-008-3220-y

22. George M, Abraham TE (2006) Polyionic hydrocolloids for the intestinal delivery of protein drugs: Alginate and chitosan - a review. Journal of Controlled Release 114 (1):1-14. doi:10.1016/j.jconrel.2006.04.017

23. Kulkarni AR, Soppimath KS, Aminabhavi TM, Dave AM, Mehta MH (2000) Glutaraldehyde crosslinked sodium alginate beads containing liquid pesticide for soil application. Journal of Controlled Release 63 (1):97-105

24. Pathak TS, Yun J-H, Lee J, Paeng K-J (2010) Effect of calcium ion (cross-linker) concentration on porosity, surface morphology and thermal behavior of calcium alginates prepared from algae (Undaria pinnatifida). Carbohydrate Polymers 81 (3):633-639

25. Tavakol M, Vasheghani-Farahani E, Hashemi-Najafabadi S (2013) The effect of polymer and CaCl2 concentrations on the sulfasalazine release from alginate-N, O-carboxymethyl chitosan beads. Progress in Biomaterials 2 (1):1-8

## Table captions

**Table 1** Microcapsules' formulations and main process conditions of the encapsulation of cedarwood essential oil into alginate microcapsules.

| **Microcapsules' formulations** | |
|---|---|
| **Component** | **Concentration** |
| Encapsulation polymer: alginate | 1, 3, 4 % (w/v) |
| Coagulating agent: $CaCl_2$ | 0.1, 0.25, 0.5 M |
| Crosslinker: glutaraldehyde | 0.1, 10 g $L^{-1}$ |
| **Microencapsulation parameters** | |
| **Variable** | **Value** |
| frequency | 400 Hz |
| electrode tension | 200 V |
| nozzle size | 600 µm |
| stirring speed | 100 rpm |

**Table 2** Values of amount of encapsulated cedarwood essential oil (mg CWO per gram microcapsule) in terms of the concentration of $CaCl_2$ and glutaraldehyde in the gelling solution.

| **$CaCl_2$ concentration** | **Without crosslinking agent** | **With crosslinking agent** | | | |
|---|---|---|---|---|---|
| | | **Glutaraldehyde solution (12.5%)** | | **Glutaraldehyde solution (50%)** | |
| | | **0.1 g $L^{-1}$** | **10 g $L^{-1}$** | **0.1 g $L^{-1}$** | **10 g $L^{-1}$** |
| 0.10M | 160.0 | 168.4 | 175.5 | 141.2 | 141.7 |
| 0.25M | 173.7 | 144.3 | 143.5 | 110.4 | 128.4 |
| 0.50M | 177.2 | 153.4 | 154.7 | 159.3 | 143.2 |

**Table 3** Average values of the weights of total cedarwood oil ($W_{CWO\_T}$) used in the process and encapsulated oil ($W_{CWO\_S}$) trapped inside the alginate shells.

| | |
|---|---|
| **Total weight of cedarwood oil used ($W_{CWO\_T}$), g** | 4.37 |
| **Weight of encapsulated cedarwood oil ($W_{CWO\_S}$), g** | 2.84 |
| **Total weight of microcapsules, g** | 16.02 |

## Figure legends

**Fig. 1** Optical images of the microcapsules obtained with different alginate concentrations in the polymer solution a) 1% w/v, b) 3% w/v and c) 4% w/v

**Fig. 2** Optical images of the microcapsules obtained with a constant alginate concentration of 3% w/v and different CaCl2 and glutaraldehyde concentrations in the gelling solution

**Fig. 3** SEM images of cedarwood alginate microcapsules obtained with optimized conditions

**Fig. 4** Cell viability (L929 cells after 24 h incubating at 37 ºC) of cultures with different concentrations of cedarwood oil encapsulated alginate beads

**Fig. 5** Images of the L929 cells proliferation after 24 h for cultures with different concentrations of cedarwood oil encapsulated alginate beads