BULETINUL Universităţii Petrol – Gaze din Ploieşti	Vol. LXI No. 1/2009	7 - 12	Seria Tehnică

Microencapsulation of Essential Oils by Using Biocompatible Polymers

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Abstract

In this work, it is proposed the use of biomaterials for essential oils encapsulation. In present, the synthesis and the properties of microcapsules from biocompatible polymers are much studied, which it is proved by the large recent amount of literature. Chitosan (fig.1) is a biomaterial compatible with organisms and causes low pollution.

In this paper, the strategy of chitosan use for essential oils encapsulation was lead on the following directions: to get skills concerning the procedure of encapsulation by using water/oil emulsions in order to obtain microcapsules with uniform shapes and diameters; study of the encapsulation parameters: solvent, temperature, speed stirring; FTIR, UV-VIS, GC-MS analysis of microcapsules, in order to establish new quantitative methods for the released volatile compound.

Keywords: *chitosan, microencapsulation, essential oils*

Introduction

Chitosan is a cationic linear polysaccharide composed essentially of β-(1-4)-D-linked glucosamine units together with some proportion of N-acetylglucosamine units (Figures 1,2). It is generally obtained by extensive deacetylation of chitin, a homopolymer of β-(1-4)-D-linked N-acetyl-D-glucosamine, present in the shells of crustaceans, molluscs, the cell walls of fungi, and the cuticle of insects [1-5].

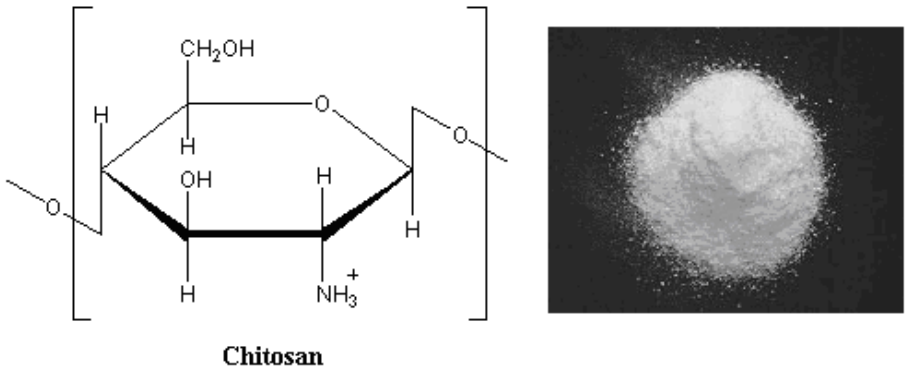


Fig. 1. Structure and presentation of chitosan

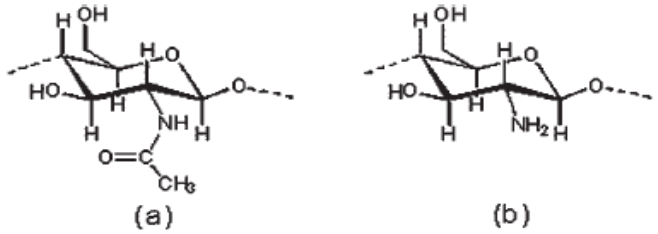


Fig. 2. Structural units of chitosan (a) unit N-acetylglucosamine
(b) unit glucosamine

Chitosan is a chelating polymer very effective for the removal of transition and post-transition metal ions due to the primary amino groups distributed along its chain. Therefore, chitosan has been used as a chromatographic support for the concentration of ions and as a resin for the treatment of residual industrial waters contaminated with toxic metals, like mercury. [6-8]

In solution, chitosan behaves as a cationic polyelectrolyte. Moreover, it has been used in the food industry for the treatment of fruit juices and wines. The antifungal activity of chitosan and its ability to promote metabolic changes in plants allows it to influence favorably on the development of crops, inducing increased germination and greater yields. Chitosan has an excellent film-forming ability. Chitosan films exhibit limited swelling in water [9-10].

Chitosan is a good haemostatic agent, but its sulfated derivatives exhibit anticoagulant activity. It is known that chitosan is hipocholesterolemic and hipolipidemic. It has antimicrobial, antiviral and antitumoral activity. The immunoadjuvant activity of chitosan has also been recognized. All these interesting characteristics have led to the development of numerous applications of chitosan and its derivatives in biomedicine, such as: surgical sutures, biodegradable sponges and bandages, matrices (in microspheres, microcapsules, membranes, and compressed tablets) for the delivery of drugs, in orthopedic materials, and dentistry, amongst others [11, 12].

Microencapsulation with polymer matrices has received increasingly growing attention in the last decade, resulting in a great number of applications in industry, agriculture, medicine, pharmacy, and biotechnology. In medicine and pharmacy it has been used for masking unpleasant tastes and odors, the controlled release of drugs, the protection of drugs from aggressive body fluids, such as gastric fluids, the immunoisolation of cells and in immunoassays [13-15].

The techniques employed to microencapsulate with chitosan include, among others, ionotropic gelation, spray drying, emulsion phase separation, simple and complex coacervation, and polymerization of a vinyl monomer in the presence of chitosan. [13] It is well known the preparation method of some cellulose microcapsules by using an emulsion oil/water, followed by the solvent evaporation. The technique of microencapsulation by emulsion phase separation consists of the formation of an emulsion water/oil, with the use of a surfactant [15].

The size and morphology of the particles depend on multiple factors, such as: temperature, stirring rate, amount of gelling agent, concentration of surfactant polymer concentration, the viscosity of the phases, and the configuration of the reaction vessel and agitator, among others. When a drug or other substance is added to the aqueous phase, the loading efficiency is also influenced by many variables, such as: temperature, polymer/drug ratio, solubility of the drug in the phases, and the volume ratio of the phases [16].

In this paper, the strategy of chitosan use for essential oils encapsulation was lead on the following directions: to get skills concerning the procedure of encapsulation by using water/oil emulsions in order to obtain microcapsules with uniform shapes and diameters; optimizing the encapsulation parameters: solvent, temperature, speed stirring.

Experimental

The analyses have been made with high purity chromatographic reagents. High viscosity chitosan was purchased from Fluka. Solvents were purchased from Sigma Aldrich.

The quantitative determination of volatile oil was made by a spectrophotometric method with apparatus: FT-IR Varian 3100, UV-VIS Jasco 550 and a Gas Chromatograph 3800 with FID detector and mass spectrometer Varian 4000.We have used a Factor-Four type capillary Column VF-5 ms (30 m × 0.25 mm ID, DF= 0.25 μm).

Separation of the emulsion was performed with a centrifuge 1-6P Sigma. Microcapsules were broken with an ultrasonicator Sonapuls Ultrasonic HD 2070, H2200 at 50 Hz.

Experiments and Results

For chitosan microcapsule preparation there was used the technique of emulsion phase separation, followed by phases separation by centrifugation.

Preparation of chitosan microcapsules with essential oils: High viscosity chitosan (0.3 g) was dissolved in 20 ml water containing 1% acetic acid (% wt). Polymeric solution was prepared by magnetic stirring for 1 hour, to completely dissolving of chitosan. After its dissolvation, there are added to the polymeric solution: essential oil nerol or *cis*-3,7-dimethyl-2,6-octadien-1-ol (0.4 ml) and a surfactant- linear alkyl benzene sulphonate (0.2 ml) and they are continously stirred for 6 hours, at 30 ^{0}C and stirring rate 4 rpm.

For the two phases separation the obtained mixture in centrifugated at maximum speed (4000 rpm) for 10 min. Microcapsules are separated and washed with ethyl acetate and then dried for 48 hours, in air. After weighting, the yield of microcapsules obtaining was of 75%.

Chitosanic microcapsules were prepared following the above presented method, at different temperatures and stirring rates. The yield of microcapsules obtaining was determined considering microcapsules mass after drying and the initial chitosan quantity and essential oil (nerol). Results are presented in tables 1,2.

Table 1. The yields of chitosan microcapsules obtaining at different temperatures

Sample no.	Temperature (^{0}C)	Yield (%)
1	30	75
2	50	78
3	70	84

Table 2. The yields of chitosan microcapsules obtaining at different stirring rates

Sample no.	Stirring rate (rpm)	Yield (%)
1	4	73
2	5	77
3	6	78

The analysis of chitosan microcapsules was performed both by qualitative methods: IR, UV-VIS spectrophotometryand GC-MS.

The absorption peaks from IR spectrum of nerol, at 3359 cm^{-1} are assigned to the stretching vibration of O-H group. The bands at 1457 cm^{-1} correspond to C=C stretching vibrations. The bands at 1021cm^{-1} correspond to C-O stretching vibrations from alcohols.

Isolation of the essential oil: Determination of nerol quantity from chitosan microcapsules was performed by FT-IR spectroscopy (by reflexion on ATR with diamond crystal).

Calibration curve setting: There were prepared 4 reference solutions of different concentrations: 0,0111mg/ml, 0,02226 mg/ml, 0,1113 mg/ml and 1,113 mg/ml nerol in methanol.

It was chosen from IR spectrum of nerol the vibration frequency characteristic to a quantitative determination of nerol: $\nu = 1021 cm^{-1}$. (fig.3,4)

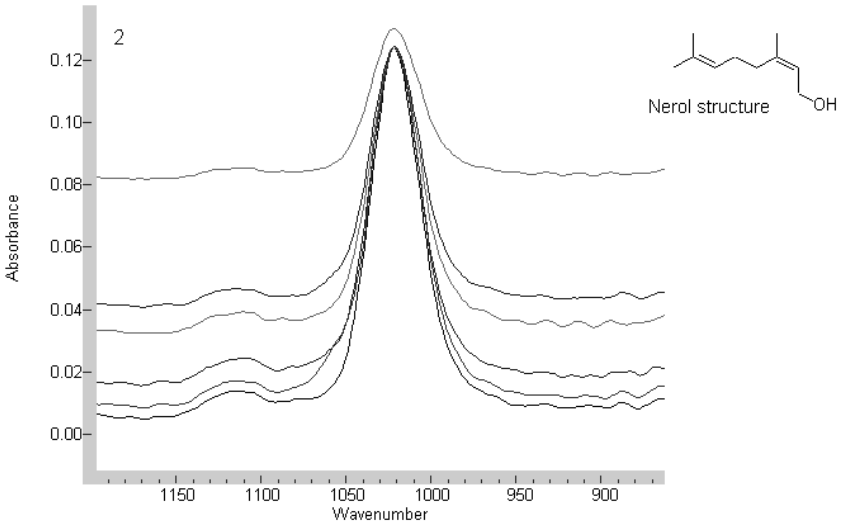


Fig. 3. Characteristic band for nerol

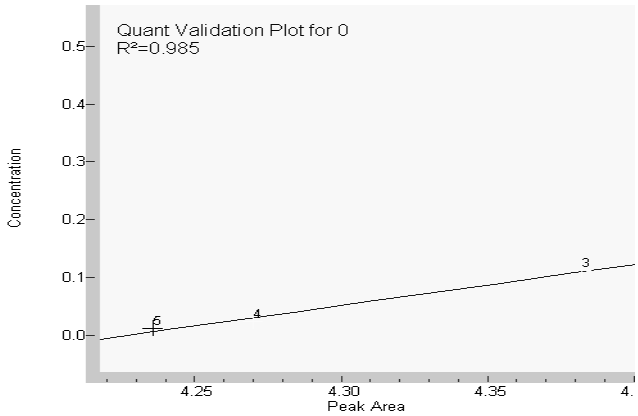
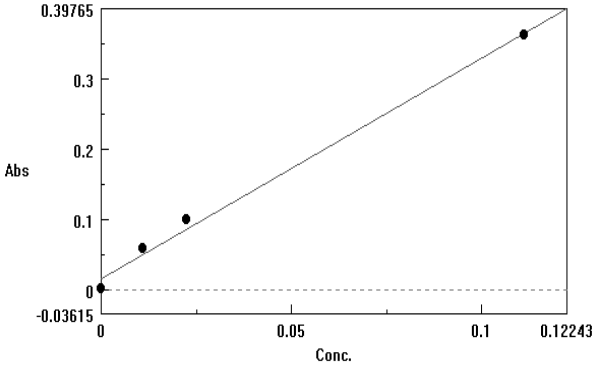


Fig. 4. FT-IR calibration curve for nerol

There were weighed samples of chitosan microcapsules (0.03 g), it was added methanol (20 ml) and then they were broken at ultrasonicator. The resulted mixture was filtered and from the filtrate there were extracted samples of 10 ml volume and they were diluted to 50 ml with methanol. It was drawn IR spectrum for each sample and from the calibration curve it was determined nerol concentration. For comparison, nerol content was also determined by UV-VIS spectroscopy. It was registered the UV-VIS spectrum and it was chosen the wavelength $\lambda = 238$ nm. There were used the same nerol concentrations for the calibration curve. (fig.5)



R^2=0.997222

Fig. 5. UV-VIS calibration curve for nerol

Nerol content was then determined by GC-MS, using the same reference samples and there was drawn the calibration curve (fig. 6). It was observed that nerol concentration values for these samples were very closed to those determined by FT-IR and UV-VIS.

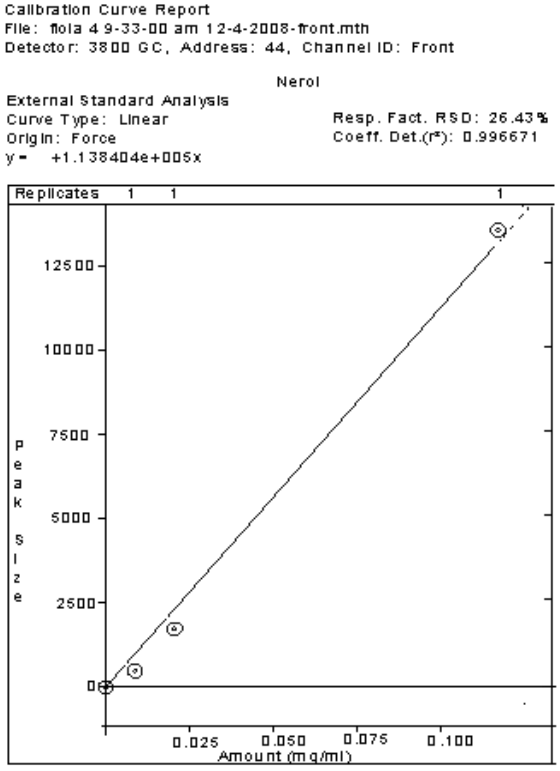


Fig. 6. GC-MS calibration curve for nerol

In table 3 there are presented nerol concentrations encapsulated by chitosan.

Table 3. Nerol concentrations determined by FT-IR , UV-VIS, GC-MS

Encapsulation matrix		**c (g/ml), IR**	**c (g/ml), UV-VIS**	**c(g/ml), GC-MS**
Chitosan	Sample 1	0.022	0.020	0.022
	Sample 2	0.024	0.023	0.0235
	Sample 3	0.023	0.024	0.023

Conclusion

There were prepared chitosan microcapsules by emulsion phase separation technique, at different temperatures and sitirring rates and it was observed that the reaction yield increases with temperature increasing and stirring rate.

The essential oil quantitative determination from microcapsules matrix was performed by FT-IR, UV-VIS, GC-MS spectroscopy and it certifies the obtained experimental results.

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Microîncapsulări de uleiuri esențiale utilizând polimeri biocompatibili

Rezumat

În această lucrare se propune utilizarea biomaterialelor pentru încapsulări de uleiuri esențiale. În prezent, sinteza şi proprietățile microcapsulelor din polimeri biocompatibili este intens studiată, fapt dovedit de literatura recentă. Chitosanul (fig.1) este un biomaterial compatibil cu organismele şi nu cauzează poluare.

În această lucrare, strategia utilizării chitosanului pentru încapsulări de uleiuri esențiale a fost direcționată către: obținerea de abilități pentru încapsulări, folosind emulsii apă/ulei pentru a obține microcapsule cu formă şi diametru uniforme; studiul parametrilor de încapsulare: solvent, temperatură, viteză de agitare; analiza FTIR, UV-VIS, GC-MS a microcapsulelor, pentru stabilirea unor noi metode cantitative pentru determinarea compusului volatil eliberat.