https://doi.org/10.15255/KUI.2020.056
KUI-20/2021
Original scientific paper
Received August 22, 2020
Accepted October 23, 2020

Optimisation of the Microencapsulation of an Active Ingredient by Crosslinking and the Coating Method to Target Colon Diseases

M. Hammoudi,[a*] *D. Atsamnia*,[b] *K. Otmanine*,[c] *R. Moumen*,[b] and *M. Oumouna*[a]

[a] Biomaterials and Transport Phenomena Laboratory (LBMPT), Yahia Fares University, Department of Chemical Engineering and Environment, Experimental Biology and Pharmacology Team, Médéa, 26 000, Algeria

[b] Biomaterials and Transport Phenomena Laboratory (LBMPT), Équipe Biologie et Pharmacologie exprémentales, Yahia Fares University, Médéa, 26 000, Algeria

[c] Bio-ressources Naturelles Locales LBRN, Chlef University, Faculty of Technology Department of Process Engineering, Chlef, Algeria

Abstract

The aim of this study was to prepare microcapsules based on a natural polymer chitosan solution (high degree of deacetylation (DDA), low molecular weight (*MW*), and low viscosity)/sodium alginate in the presence of a crosslinking agent (glutaraldehyde), in order to encapsulate and vectorise the active principle towards the diseased organ (colon), without being diffused into other levels of the digestive tract, to increase the therapeutic effectiveness of treatment by chemotherapy and to reduce undesirable effects. The method of preparation of the microcapsules obtained from the sodium alginate/chitosan solution/active ingredients system was examined by conventional optical microscopy. In addition, an *in vitro* study was carried out on the active ingredients' release profiles, depending on the pH simulating the gastric and intestinal media for the seven systems proposed. It should be mentioned that, in the basic medium (pH(colon) = 8), the release of the active ingredients is of the utmost importance. Nevertheless, control of this release can be improved by a crosslinking agent and the coating method. The dry [sodium alginate / chitosan solution / active ingredients + crosslinking 2 %] formulation coated with non-crosslinked chitosan (Formulation 7) is the standard formula that meets all the criteria from our earlier work, with a core release rate of 67 %. The PSD was unimodal, with sizes ranging from 750 µm to 900 µm.

Keywords

Microcapsules, colon diseases, alginate, chitosan, vectorisation, coating, crosslinking

1 Introduction

The treatment of colon cancer by chemotherapy is based on drugs that degrade in the stomach, reacting to the mechanism of cell division (slowing down or blocking), whether being healthy or malignant cells, which causes side effects such as allergic reactions, skin disorders, and hand-foot syndrome, hair loss.[1,2,3]

The goal of this this study was to develop another procedure that would allow the drug to degrade and absorb itself only at the level of a diseased organ, such as the colon and tumours or metastasis, to reduce the side effects.

Vectorisation involves transporting an active ingredient in sufficient quantity towards the target to be reached by means of a vector, soluble or insoluble in water, which is inactive from the therapeutic point of view. On the one hand this vectorisation makes it possible to avoid direct administration of the drug into the human body; on the other hand, it allows for improvement of the pharmacokinetic characteristics of the active vector ingredient. It is then the properties of the vector, and not those of the active ingredient, that will determine the fate of the system *in vivo*.[4,5,6] Size and shape have gradually emerged as one of the major factors influencing the properties of smaller drug delivery systems.[7]

Alginate and chitosan as natural polymers have been retained mainly because they are biodegradable, non-toxic, muco-adhesive, and for their good film formation.

They are available in large quantities, and their cost, as well as the cost of their gelling and crosslinking agents is low.[8,9]

Chitosan, a deacetylated derivative of chitin, is a linear b (1-4) copolymer of *N*-acetyl D-glucosamines and D-glucosamines. It is found more rarely in nature: it is present only in the wall of a particular class of mushrooms, *zygomycetes*, and in some insects. Chitin is the most interesting source of chitosan.[10] It is noted that they all have important antioxidant and antimicrobial properties.[11]

The other biopolymer is alginate, a normal linear polysaccharide of brown algae. Alginate is a copolymer of residues β-D-mannuronate and α-L-guluronate,[12] forming homo- and heteropolymer blocks. These biopolymers are in acidic units of different lengths, sequences, and proportions. Alginate salts differ from most other polysaccharides because they have a sol-gel transition if their ionic environment is simply changed.[13,14] Their antibacterial activity

* Corresponding author: Mounir Hammoudi, student
Email: mounir_chimie@hotmail.fr

on *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus*, and *Micrococcus luteus* has been studied.[15]

2 Problematics

Our study aimed to produce a microcapsule vectorised to the large intestine where the pH is basic. Therefore, the following work criteria was proposed.

2.1 Work criteria

Release in weak acidic medium; release in a strong basic medium; release between the two media pH [2–7] is average; degree of swelling in the basic medium.

3 Materials and methods

3.1 Reagents

Sodium alginate was supplied by Fluka; the viscosity of 2 % aqueous solution at 20 °C is greater than 0.3 Pa s.

Chitosan was provided by Fluka, and we deduced the degree of deacetylation (DDA = 78 %).

After examining Table 1, given the different lethal doses 50 (LD50) depending on the concentration of glutaraldehyde in aqueous solutions, we opted for the preparation of an aqueous solution of 1 and 2 % by crosslinking.

The other reagents ($CaCl_2$, *etc.*) were of analytical nature.

Table 1 – Lethal doses 50 (LD50) for different solutions

Aqueous solution / %	50	15	5
LD50 / ml of solution / kg	1.30[(a)]	1.17[(a)]	3.25[(a)]
	0.27[(b)]	0.88[(b)]	1.30[(b)]
	0.20[(c)]	–	0.62[(c)]
	2.54[(d)]	16[(d)]	–

(a): Oral route for a male rat, (b): Oral route for a male mouse, (c): Oral route for a female mouse, (d): Cutaneous route for a male rabbit

3.2 Preparation of microcapsules

The active ingredient was dispersed in a previously prepared solution of sodium alginate (2 %). The suspension thus obtained was drained through a needle of a syringe into a 2 % $CaCl_2$ solution. Table 2 groups together the different formulations of the microcapsules.

▸ Drying:

Drying in open air at room temperature, under atmospheric pressure and shelter from the light.

In vitro release tests

▸ Apparatus:

The dissolution tests were carried out in a *Pharmatest PWS300* brand dissolution test apparatus. The device was equipped with 6 cylindrical containers with a hemispherical bottom, one normal capacity of 1000 ml in borosilicate glass, equipped with a stirrer consisting of a rod vertical

Table 2 – Microcapsule formulations

	Formulation	Procedure
1	[sodium alginate + $CaCl_2$ + active ingredients] non-crosslinked	The active ingredient was dispersed in a previously prepared solution of sodium alginate (2 %). The obtained suspension was drained in a solution of $CaCl_2$ of 2 %.
2	[sodium alginate + $CaCl_2$ + active ingredients + crosslinking 2 %]	The same experimental protocol as above was adopted by adding 0.7 ml of 2 % glutaraldehyde (crosslinking agent).
3	[sodium alginate + chitosan solution+ active ingredients + $CaCl_2$ + crosslinking 1 %]	The sodium alginate / active ingredient mixture was stirred for 2 h until a homogeneous solution was obtained. Subsequently, it was drained through a needle of a syringe into a petri dish, previously containing the chitosan solution (2 %), the gelling agent $CaCl_2$ (2 %), and glutaraldehyde (1 %) as crosslinking agent.
4	[sodium alginate + chitosan solution + active ingredients + $CaCl_2$ + crosslinking 2 %]	The concentration of the crosslinking agent was increased to 2 %.
5	[sodium alginate + chitosan solution + active ingredients + $CaCl_2$ + crosslinking 2 %] coated in [chitosan solution + crosslinking 2 %]	The microcapsules [sodium alginate + chitosan solution + active ingredients + $CaCl_2$ + crosslinking 2 %] already prepared, and well dried, were immersed in a 2 % crosslinked chitosan solution.
6	[sodium alginate + chitosan solution + active ingredients + $CaCl_2$ + crosslinking 2 %] wet in chitosan solution	The formed microcapsules (wet) were immersed directly in a non-crosslinked chitosan solution in order to ensure the formation of a new envelope for the membrane of the microcapsules
7	[sodium alginate + chitosan solution + active ingredients + $CaCl_2$ + crosslinking 2 %] dry in chitosan solution	We adopted the same experimental protocol. Microcapsules [sodium alginate + chitosan solution + active ingredients + $CaCl_2$ + crosslinking 2 %] were previously dried for 24 h before immersing in the non-crosslinked chitosan solution

and the lower part of which was fixed to a pallet. Each dissolution tank had several orifices allowing the introduction of a thermometer. The whole of microcapsules was placed in a thermostatted water bath, which maintained the temperature of the dissolution medium.

- Preparation of buffer solution similar to gastrointestinal pH:

Preparation of pH = 2 0.1 M

Prepare 800 ml of distilled water in a suitable container. Add 7.45 g of KCl to the solution. Add 772 mg of HCl to the solution. Adjust solution to final desired pH using HCl or NaOH. Add distilled water until volume is 1 l.

Preparation of pH = 5 0.1 M

Prepare 800 ml of H_2O in a suitable container. Add 1.017 g of K_2HPO_4 to the solution. Add 12.814 g of KH_2PO_4 to the solution. Add H_2O until volume is 1 l.

Preparation of pH = 7 0.1 M

Prepare 800 ml of H_2O in a suitable container. Add 9.343 g of K_2HPO_4 to the solution. Add 6.309 g of KH_2PO_4 to the solution. Add H_2O until volume is 1 l.

Preparation of pH = 8 0.1 M

Prepare 800 ml of H_2O in a suitable container. Add 16.282 g of K_2HPO_4 to the solution. Add 0.888 g of KH_2PO_4 to the solution. Add H_2O until volume is 1 l.

About 40 beads were suspended in the aqueous solutions at different pH = 2, 5, 7, and 8, respectively, simulating the gastric, physiological, and intestinal media. The suspensions were maintained at a temperature of 37 ± 1 °C, and constantly stirred at 100 rpm. The release results were determined by UV-Vis spectrophotometry.

3.4 Study of the physical properties of the different microcapsules

The physical properties of each of the formulations developed were determined by estimating the size of the microcapsules, calculating the encapsulation size, degree of porosity, diffusion coefficient, and degree of inflation. This study also allowed identification of the best formulation for the various microcapsules.

3.4.1 Particle size analysis

The particle size analysis involved 50 microbeads for which the mean diameters, called the central value of the microcapsule, were calculated using an optical microscope. The central value of a microcapsule was calculated from the average diameters of the 50 microbeads.

$$\overline{x} = \frac{(x_1 + x_2 + \ldots + x_n)}{n} = \sum x_i / n \tag{1}$$

3.4.2 Calculation of encapsulation size

Encapsulation size was estimated for the different systems from the LOADING relation, expressed depending on the mass of the encapsulated active ingredients and the mass of dry particles.

$$T = \frac{\text{mass of encapsulated active ingredient}}{\text{mass of dry particles}} \tag{2}$$

3.4.3 Porosity

Porosity, *P,* of the microcapsules was calculated from the determination of the amount of water absorbed by the microcapsules after immersion in the pH = 8 medium.

$$P = \frac{(m_1 - m_0)}{m_1} \cdot 100 \tag{3}$$

where m_0 is the mass of dry microcapsules, and m_1 is the mass of wet microcapsules.

3.5 Chemical structural analysis of SC, Alg, and mixture

Chemical characterisation of the SC, Alg, and mixture was carried out by FT-IR spectroscopy [SHIMADZU 8400 (400–4000 cm^{-1})].

3.6 Microstructure and surface morphology

A scanning electron microscope, Model SEM Quanta 250 from FEI company was used to study the micro-morphology of microcapsules, tested under 12.5–15 kV and 50 mA.

3.7 Statistical parameters

Several statistical parameters can be extracted from the PSD such as mode, the median, mean, and variance or standard deviation.

3.7.1 PSD classification

The number of peaks (modes): the PSD can be unimodal when it has only one peak (one single mode at most) or multimodal when it has several peaks or modes representing a mixture of particles of unimodal distributions.

3.7.2 Mode

The mode corresponds to the peak of the distribution, *i.e.* the size value for which the PSD is maximal.

3.7.3 Median

The median or size D50 is the size for which the cumulative function is equal to 50 %; similarly, sizes D10 and D90 are defined by the size values for which the cumulative function is equal to 10 % and 90 %, respectively.

3.7.4 Standard deviation

The standard deviation measures the width of the distribution around its mean. The standard deviation is the square root of the variance of PSD.

3.7.5 Coefficient of variation (CV)

The ratio between the standard deviation and the mean diameter defines the coefficient of variation (CV).

4 Results and discussion

In vitro diffusion studies

4.1 Effect of crosslinking agent on the release of sodium alginate-based microcapsules

Examination of Table 3a shows that the release rate of the active ingredient is important during the first 3 h in different pH media. Beyond this period, a decrease was observed in the size of the microcapsules until their total disappearance. This phenomenon could be due to the erosion of microcapsules.

The works of *Fabian Nussbaum*[16] on encapsulation by sodium alginate showed that microcapsules based on alginate are characterised by a smooth surface. His work[16] on encapsulation with sodium alginate showed that alginate microcapsules are characterised by a smooth and ridged surface. These striations are due to deep breaks in the microcapsules.

Moreover, *Payet et al.*[17] have also shown that this type of microcapsule is unstable and rapidly degrades in an acidic or basic medium. The results of *Payet* showed that the release of active ingredients for such microcapsules or nanoparticles was impossible, and that alginate degradation occurred in the presence of Na^+ ions as soon as the ratio of Na^+/Ca^{++} was sufficiently high. All these observations corroborate our results.

Bhattarai's research[18] has shown that glutaraldehyde crosslinks with alginate. The crosslinking is between the carbonyl groups of the aldehyde function of glutaraldehyde and the hydroxyl functions of the alginate.

Table 3b suggests that the release rate of the active ingredient was less important than in the case of non-crosslinked microcapsules (Formulation 1). The disappearance of the microcapsules (Formulation 2) occurred only starting from the sixth hour. The addition of the crosslinking agent had strengthened the membrane of the microcapsules produced in this study.

However, it is important to note that the behaviour of active ingredients' release failed to follow the criteria established in this study; namely, a low release in acid and neutral medium and, very important, in basic medium. For this purpose, we combined sodium alginate and chitosan in a new formulation of microcapsules.

Table 3 – (a) *In vitro* release of formulation 1; (b) *in vitro* release of formulation 2

(a)

Formulation 1	Time / h					
	1	2	3	4	5	6
pH = 2	14.9	15.2	25.8	Reduction in the size of the particle until disappearance		
pH = 5	13.7	15.9	23.2			
pH = 7	12.2	16	31.1			
pH = 8	29.8	33.3	38.5			

(b)

Formulation 2	Time / h					
	1	2	3	4	5	6
pH = 2	10.7	11.3	16.1	24.9	35.6	–
pH = 5	10.4	12.0	15.5	22.7	32.2	–
pH = 7	11.4	12.7	14.2	26.4	34.1	–
pH = 8	19.8	26.5	29.2	33.9	36.3	–

4.2 Effect of chitosan and crosslinking concentration on microcapsule release

Adopted were five formulations for the preparation of this type of microcapsule (sodium alginate / chitosan solution / active ingredients) crosslinking by varying the concentration of the crosslinking agent, the coating procedure (monolayer, double layer, coating in dry and wet states).

Uragami et al.[19] showed that, in the presence of sodium alginate and chitosan, glutaraldehyde preferentially crosslinks with chitosan.

The concentration of the crosslinking agent influences the phenomenon of active ingredients release. The low level was observed after the addition of 2 % of crosslinking agent, (Fig. 1b). Microcapsules crosslinked with 2 % glutaraldehyde left their active ingredients diffused in a smaller quantity in acidic media compared to the microcapsules (Formulation 3) crosslinked at 1 %, (Fig. 1a). The increase in crosslinking agent concentration would therefore generate good rigidity of the microcapsules (Formulation 4).

Nevertheless, the values of active ingredient concentrations remain relatively high in the acid and neutral media (pH = 2, 5, and 7), and in order to minimise the release

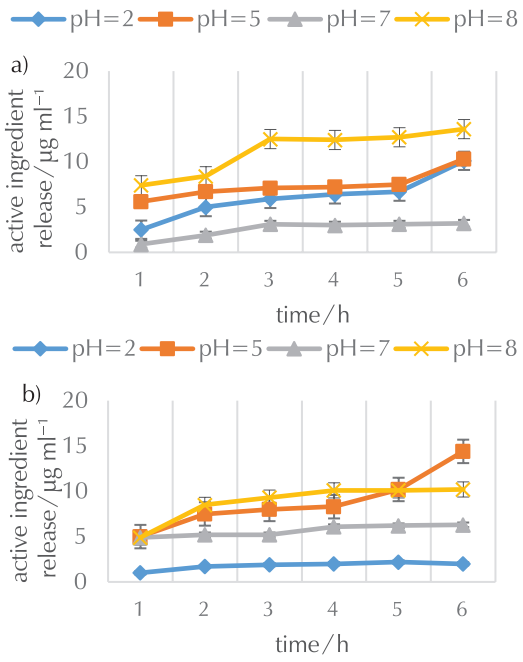

Fig. 1 – a) Release evolution of the microcapsules (F3) as a function of time for the different pH media (1 % crosslinking); b) release evolution of the microcapsules (F4) as a function of time for the different pH media (2 % crosslinking)

rate of active ingredients in the acidic medium, in the next step, the membrane microcapsules were reinforced with a new layer of crosslinked chitosan.

The work of *Y. Song et al.*[20] also confirms the influence of the level of crosslinking of a chitosan membrane on solubility (degradation). This decreases with an increase in concentration of crosslinking agent. In general, when the membranes are crosslinked, the selectivity of the membrane is increased but the release rate is decreased. This confirms our results; the lowest release rate was obtained after adding 2 % of crosslinking agent.

In addition, the 2 % crosslinked microcapsules are stable, resistant, and give higher active ingredients release rates than those crosslinked with 1 % glutaraldehyde whatever the pH of the medium. However, such results do not coincide with our work criteria, and it is mainly for this reason that we prepared another type of microcapsule coated with chitosan that can give rise to better resistance of the microcapsules in acidic medium.

4.3 Influence of coating on release kinetics

In this part, three procedures were followed. In general, the release rate of active ingredient microcapsules coated with crosslinked chitosan (Formulation 5) still remained high in acidic medium. It increased considerably, and stabilised after 3 h in a neutral environment (Fig. 2a). In basic medium, the release rate increased continuously with a medium release rate.

Formulation 6 showed a better release of active ingredients for different pH media compared to the other systems (Fig. 2b). The important result obtained was that in basic medium, the rate of release increased considerably and rapidly upon contact with the medium pH = 8.

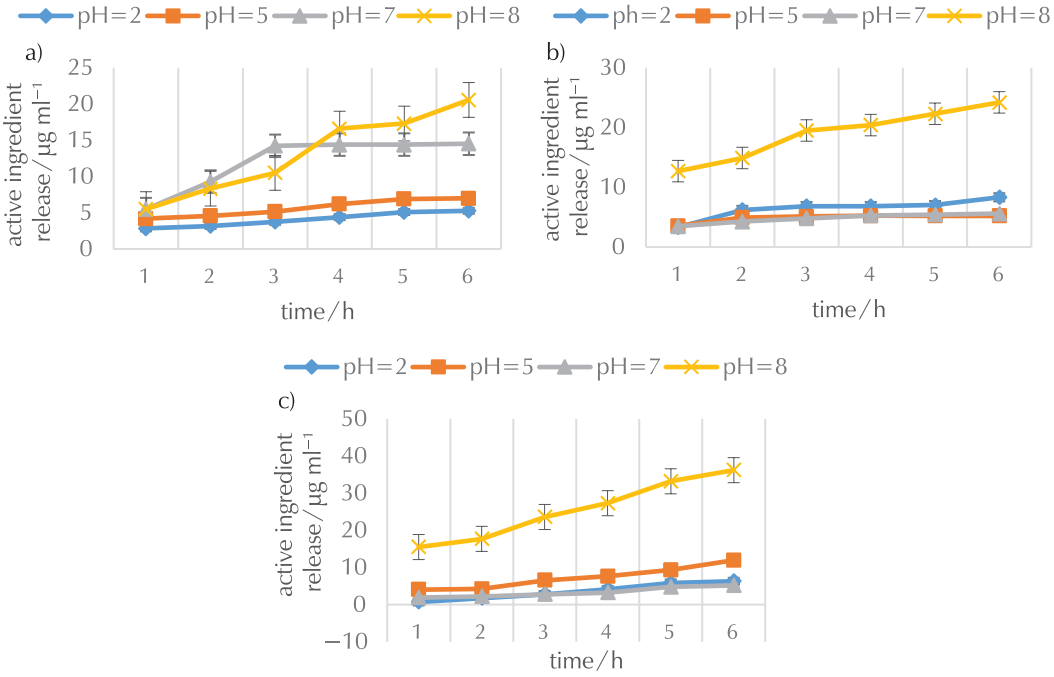

Fig. 2 – a) Evolution of Formulation 5 release as a function of time for different pH media; b) release evolution of wet microcapsules (Formulation 6) in SC as a function of time for different pH media; c) evolution of release of dry microcapsules (Formulation 7) in SC as a function of time for different pH media

This phenomenon can be explained by an inflation of the microcapsules, which was followed by a large and rapid diffusion across the membrane [sodium alginate/crosslinking (2 %)] chitosan solution.

The plots in (Fig. 2c) (Formulation 7), suggest that the decrease in the thickness of the membranes caused an increase in the flux of permeability to the basic medium (34.5 $\mu g\,ml^{-1}$) at the sixth hour better than Formulation 6 (24.2 $\mu g\,ml^{-1}$). Formulation 7 best meets the criteria established in this work. In fact, the release of the active ingredients was slow with a low release rate for acid (0.71 $\mu g\,ml^{-1}$) and neutral (1.9 $\mu g\,ml^{-1}$) medium at the first hour, whereas in basic medium, the microcapsules swelled remarkably, by inducing a faster release and a much larger release rate.

4.4 Study of the physical properties of the different microcapsules

The microcapsules obtained were spherical (reservoir system), with an average diameter equivalent to 800 microns. The study of the release of active ingredients from the different formulations, in pH = 2, 5, 7, and 8 media, showed no deformation or size change of the microcapsules, except in the case of microcapsules coated with chitosan. In basic medium, they showed significant swelling.

In order to estimate the rate of swelling, the different microcapsules were weighed in their dry and wet state (after having immersed them in solution at pH = 8 for 6 h), and their initial and final masses were determined. The results in Table 4 confirm the swelling of dry or wet microcapsules coated with chitosan by increasing the mass of the latter. According to particle size analysis, the average size of the microcapsules was approximately 820 μm and of spherical shape (reservoir system) (Fig. 3), with an encapsulation size estimated at $T = 40$. The calculation of the porosity showed that the dry and wet microcapsules coated with chitosan were the most porous, 69 %, thus generating the highest release rate (results are shown in Table 5).

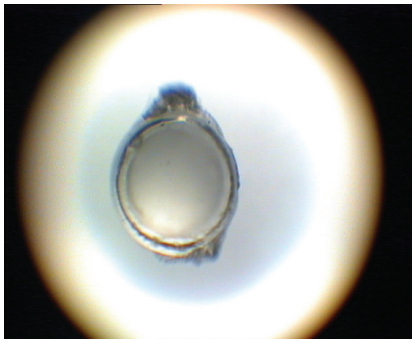

Fig. 3 – Microscopic image of empty microcapsule at 40× magnification

4.5 Chemical structural analysis of SC, Alg, and mixture

The FT-IR spectra of chitosan (SC), sodium alginate (Alg), and their mixture of polyelectrolytes (ACH) are shown in (Fig. 4). The spectrum of (SC) shows a wide and intense band centred at approximately 3409.9 cm^{-1} and corresponds to the stretching vibrations due to the overlap of the OH and NH bonds. The peaks observed at 2881 and 2144.7 cm^{-1} are due to symmetrical or asymmetrical CH_2 stretching vibrations of the pyranose ring. The characteristic absorption bands of chitosan are generally observed between 1654.8 cm^{-1} and 1596.9 cm^{-1}, which corresponds to stretching of C−O (amide I) and bending of N−H (amide II), respectively. The absorption bands at 1080.1 cm^{-1} and 1029.9 cm^{-1} (skeletal vibrations involving C−O stretching) are characteristic of the structure of the polysaccharide. Alginate absorption bands near 1635.5 and 1419.6 cm^{-1} are associated with the asymmetric and symmetrical stretching vibrations of the carboxylate anions, respectively, as others have observed. The spectrum of sodium alginate also shows a strong and wide band at 3444.6 cm^{-1} linked to the O−H stretch, and a weak band of aliphatic C−H stretch at 2927.7 cm^{-1}. Due to its polysaccharide structure, bands of approximately 1245.9 cm^{-1} (C−O stretch), 1038.8 cm^{-1} (C−O−C stretch) were observed. In FT-IR spectra of mixed oppositely charged polysaccharides, we observed changes in the placement and disappearance of certain bands or the appearance of new peaks compared to a single alginate or chitosan. The complexed material has a narrower and more intense band at around 3409.9 cm^{-1}, which is caused by the formation of new hydrogen bonds between the −OH and $-NH_2$ groups of chitosan and the −C=O and −OH groups of the sodium alginate. Bands attributed to movements of the carboxylate salt group were invisible after complexation. This disappearance was due to the lower content of excess alginate and chitosan in all ACH composites.

The possible shifts towards the shorter wavelengths and the widening of the vibration bands of the functional groups involved, could account for the presence of specific interac-

Table 4 – Variation of swelling of microcapsules in medium pH = 8

Formulation	Initial mass/g	Final mass/g
3	0.0016	0.0018
4	0.0016	0.0017
5	0.0019	0.0022
6	**0.0018**	**0.0051**
7	**0.0017**	**0.0055**

Table 5 – Variation of porosity of microcapsules in medium pH = 8

Formulation	Initial mass/g	Final mass/g	Porosity/%
3	0.0016	0.0018	11.11
4	0.0016	0.0017	5.8
5	0.0019	0.0022	13.63
6	0.0018	0.0051	64.70
7	0.0017	0.0055	69

tions likely to develop. A shift can be seen from the 1654.8 and 1596.9 cm^{-1} (amide I, amide II) and 1635.5 cm^{-1} (alginate carboxylic) peaks.

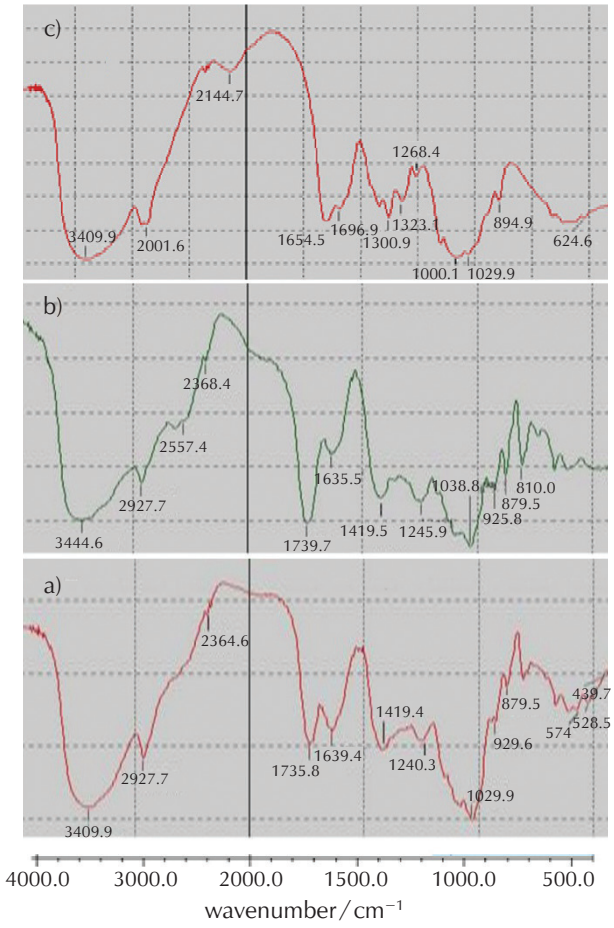

Fig. 4 – FT-IR spectra of chitosan (a), sodium alginate (b), and mixed (Alg/ SC) systems (c)

4.6 Kinetics of diffusion

The diffusion kinetics of microcapsules in different media were studied. For this purpose, calculated the diffusion coefficient was calculated using Fick's law applied to short times from the slope of the plot (m_t/m_∞) depending on $\sqrt{t}$.

The kinetics of diffusion of the formulation microcapsules (Formulation 7) in the different pH media is illustrated in Fig. 5. The values of the diffusion coefficients are summarised in Table 6.

As may be seen from Table 6, a decrease occurred in the values of the diffusion coefficient of active ingredients in acid medium, particularly in the case of dry sodium alginate microcapsules coated with non-crosslinked chitosan. In the pH = 5 medium, the diffusion coefficient for this same formulation was still lower than those determined for the other forms. While in the neutral medium, the values of the diffusion coefficients were practically similar. This would be mainly due to the pH of the interior of the microcapsule, which would be the same as that of the external medium (pH = 7). In basic medium (pH = 8), the diffusion coefficients of the different microcapsules varied between 0.4 and 0.54, thus inducing an extension of the release time of the active ingredients.

Table 6 – Diffusion coefficients apparent at short times of different forms and at different pH

Formulation		pH			
		2	5	7	8
3	Di/r_o^2	0.25	0.54	0.29	0.54
4	Di/r_o^2	0.52	0.35	0.77	0.47
5	Di/r_o^2	0.54	0.6	0.37	0.26
6	Di/r_o^2	0.39	0.68	0.34	0.52
7	Di/r_o^2	0.11	0.33	0.36	0.42

Once again, these results confirmed that Formulation 7 was that which best met the work criteria of this study. In fact, with this formulation, the lowest release of active ingredients was obtained in acid and neutral media, and a majority release in the medium at pH = 8 corresponding to the pH of the colon.

Many parameters, such as pH, degree of acetylation, crystallinity, and porosity influence the final degree of swelling. Studies have shown that a decrease occurs in the crystallinity of non-crosslinked chitosan; therefore, the molecules of the aqueous medium penetrate into the amorphous regions more easily than into crystalline regions. In addition, swellability is greater for strong acetylation degrees.

The microcapsules of the formula dried for 24 h were thinner, which influenced the permeability or even the diffusion of molecules of the aqueous medium penetrating through the pores of the membrane causing the microcapsules to inflate, which was larger for those with a smaller diameter than those with a larger diameter. According to the literature, it has been shown that a decrease in membrane thickness leads to an increase in permeability flow, which

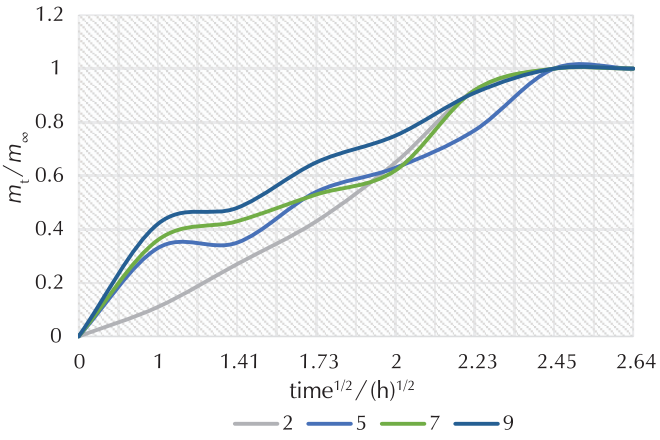

Fig. 5 – Variation of the ratio (m_t/m_∞) according to $\sqrt{t}$ for microcapsules of Formulation 7 dry in SC

confirms the findings of this study. Therefore, the fifth formula was the closest to what this study aimed to produce.

In addition, calculation of diffusion coefficients for different pHs showed that the formula had the smallest coefficient in an acid medium, neutral and suitable for pH = 8 medium, reflected by the low rate of release in different environments where pH varied from (2, 5, and 7), and diffusion in large quantities for pH = 8. Thus, Formulation 7 was the closest to what this study aimed to produce.

4.7 *In vitro* tests of dry microcapsules coated with chitosan in a medium similar to the gastrointestinal tract

Fourty microcapsules of the fifth formulation were placed in a gelatine capsule (in order to preserve their quantity during passage through the digestive tract). The evolution of the microcapsules was then monitored in solutions at pH = 2.7 and 8, simulating the passage of microcapsules in the gastrointestinal environment. The microcapsules were immersed for 2 h (average time) in each pH. The results obtained are grouped in Table 7.

It was found that for acid and neutral media, the release rate remained relatively low, not exceeding 23 %. In the basic medium (corresponding to colon pH), the release rate was 67 %. The microcapsules also underwent a remarkable swelling, causing complete degradation after the sixth hour.

Table 7 – *In vitro* tests of the chosen formula

pH	2	7	8
duration / h	2	2	2
concentration / μg ml^{-1}	2.65	6.65	26.8
release rate / %	6.7	16.5	67

4.8 Statistical parameters

The particle size distribution involved fifty microbeads (formulation F7) for which the diameter of the microcapsule was calculated using an optical microscope.

Particle size distribution (PSD) of the microcapsules of F7 formulation containing active ingredient is shown in Fig. 6. The PSD was unimodal, with sizes ranging from 750 μm to 900 μm. The mean diameter in frequency was 816 μm and D(50) was 800 μm. The current unimodal PSD is considered suitable for the application of microcapsules in the pharmaceutical field.[21]

Table 8 groups the main statistical parameters for the microcapsules of the seventh formulation chosen.

Table 8 – PSD statistical parameters

Median (D50) / μm	D10 / μm	D90 / μm	Standard deviation / μm	Mode / μm	Mean / μm	CV / %
800	750	850	38.39	800	816	4.70

The coefficient of variation was less than 15 %, so the particle size values were homogeneous.

4.9 Microstructure and surface morphology

All the microcapsules (Fig. 7) were of spherical shape and fairly rough surface, with numerous undulations on the outer surface, which increases the surface area. This is a beneficial quality because a higher surface area allows better mass transfer.

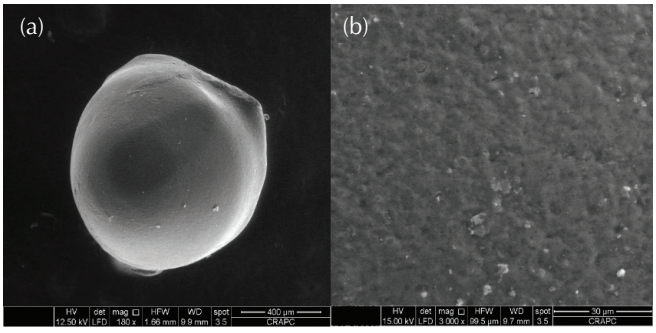

Fig. 7 – (a) Complete image of the microcapsule (formulation F7), and (b) a view of its surface

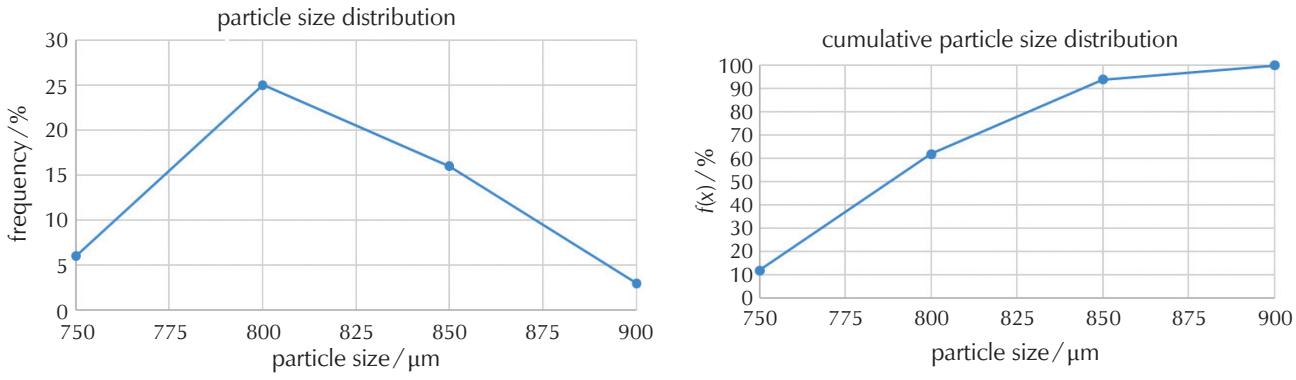

Fig. 6 – Particle size distribution

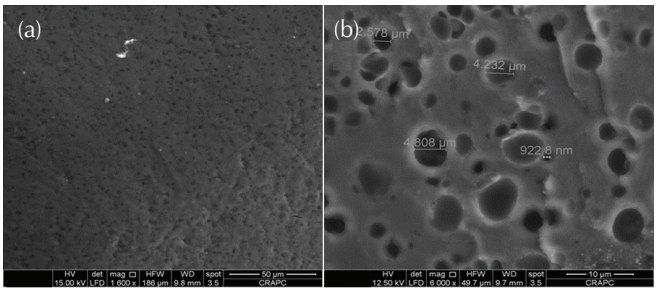

Fig. 8 – SEM images of the microspheres (a), and of the amplified surface of a microsphere (b)

Fig. 8 presents a scanning electron microscopy image of the surface of microcapsules coated with a layer of crosslinked chitosan (Formulation 7) and the amplified surface of a microcapsule. Pores of 900 nm to 5 µm were present on the surface.

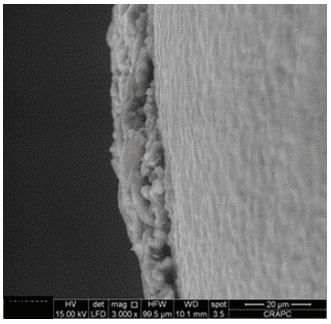

Fig. 9 – Surface of the crosslinked chitosan shell material

The enlarged SEM of the microcapsules (formulation F7) (Fig. 9) shows the surface of the crosslinked chitosan shell material. The reaction of the crosslinked chitosan at the interface of the microcapsules formed the shell of the capsule, and the surface of the microcapsules gradually became coarse and covered with granular deposits as the reaction proceeded. The rough surface resulted from the deposition and SC.

The synthesised microcapsules were analysed by SEM. Fig. 10 shows that the microcapsule was composed of a rough outer surface and a second wall of the microcapsule [sodium alginate + chitosan solution + active ingredients + $CaCl_2$ + crosslinking 2 %] with a relatively porous. Cross-section of the capsule wall revealed that the thickness of the outer shell was approximately 13 µm. The structure of the envelope of the synthesised microcapsules was in good agreement with those described in other publications.

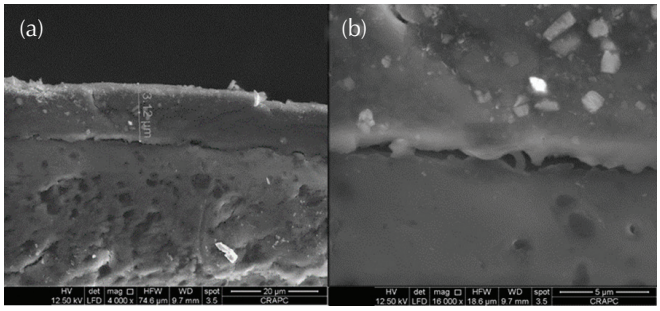

Fig. 10 – (a) Cross-section of the capsule wall, (b) space between the layers

5 Conclusion

This study, concerning the variation of the release rate of the active ingredients as a function of the pH, time, and diffusion coefficient calculation of formulas in different pH media, shows that the crosslinking of microcapsule matrices of chitosan-alginate systems with glutaraldehyde controls the release of active ingredients depending on the desired medium (pH). This control was improved by changing the microporous morphological structure of the microcapsules from the coating. The dry [sodium alginate / chitosan solution / active ingredients + crosslinking 2 %] formulation coated with non-crosslinked chitosan, Formulation 7, was the standard formula to target colon diseases, which met all the work criteria of this study, with a core release rate of 67 %. According to the SEM analysis and the parameters obtained from PSD, the structure of the envelope of the synthesised microcapsules is in good agreement with those described in other publications.

ACKNOWLEDGEMENTS

The authors thank the staff of the Pharmaceutical Company (SAIDAL) of Médéa (Algeria), and the Laboratory of National Institute of Medical Equipment Maintenance of Médéa. We are extremely grateful to Boualem BENAYAD.

References
Literatura

1. *P. Gao, X.-Z. Huang, Y.-X. Song, J.-X. Sun, X.-W. Chen, Y. Sun, Y.-M. Jiang, Z.-N. Wang,* Impact of timing of adjuvant chemotherapy on survival in stage III colon cancer: A population-based study, BMC Cancer **18** (2018) 234, doi: https://doi.org/10.1186/s12885-018-4138-7.
2. *A Lambert, T Conroy*, Standards de chimiothérapie, perspectives et thérapies ciblées dans l'adénocarcinome du pancréas, Oncologie **17** (11-12) (2015) 519–527, doi: https://doi.org/10.1007/s10269-015-2562-8.
3. *A. Chan, A. Bauwens, S. Pontre, S. Jackson, F. McGlone, T. Ernenwein, J. Chih, C. Reid,* Efficacy of scalp cooling in reducing alopecia in early breast cancer patients receiving contemporary chemotherapy regimens, Breast J. **41** (2018) 127–132, doi: https://doi.org/10.1016/j.breast.2018.07.006.
4. *T. Watanabe, K. Muro, Y. Ajioka, Y. Hashiguchi, Y. Ito, Y. Saito, T. Hamaguchi, H. Ishida, M. Ishiguro, S. Ishihara, Y. Kanemitsu, H. Kawano, Y. Kinugasa, N. Kokudo, K. Murofushi, T. Nakajima, S. Oka, Y. Sakai, A. Tsuji, K. Uehara, H. Ueno, K. Yamazaki, M. Yoshida, T. Yoshino, N. Boku, T. Fujimori, M. Itabashi, N. Koinuma, T. Morita, G. Nishimura, Y. Sakata, Y. Shimada, K. Takahashi, S. Tanaka, O. Tsuruta, T. Yamaguchi, N. Yamaguchi, T. Tanaka, K. Kotake, K. Sugihara,* Japanese Society for Cancer of the Colon and Rectum (JSCCR) guidelines for the treatment of colorectal cancer 2016, Int. J. Clin. Oncol. **23** (1) (2018) 1–34, doi: https://doi.org/10.1007/s10147-017-1101-6.
5. *L. Casadaban, G. Rauscher, M. Aklilu, D. Villenes, S. Freels, A. V. Maker,* Adjuvant Chemotherapy is Associated with Improved Survival in Patients with Stage II Colon Cancer, Cancer **122** (21) 3277–3287, doi: https://doi.org/10.1002/cncr.30181.
6. *J. Poutou, M. Bunuales, M. Gonzalez-Aparicio, B. German, I. Zugasti, R. Hernandez-Alcoceba,* Adaptation of vectors and drug-inducible systems for controlled expression of transgenes in the tumor microenvironment, J. Control. Rel. **268** (2017) 247–258, doi: https://doi.org/10.1016/j.jconrel.2017.10.032.
7. *S. C. Larnaudie, J. Sanchis, T.-H. Nguyen, R. Peltier, S. Catrouillet, J. C. Brendel, C. J. H. Porter, K. A. Jolliff, S. Perrier,* Cyclic peptide-poly(HPMA) nanotubes as drug delivery vectors: *in vitro* assessment, pharmacokinetics and biodistribution, Biomater. **178** (2018) 570–582, doi: https://doi.org/10.1016/j.biomaterials.2018.03.047.
8. *J. P. Benoît, J. Richard, M.-C. Venier-Julienne*, Microencapsulation, Techniques de l'Ingénieur J2210 (2013) 1–22, url: https://www.techniques-ingenieur.fr/base-documentaire/procedes-chimie-bio-agro-th2/cosmetiques-procedes-de-formulation-42634210/microencapsulation-j2210/.
9. *N. Sorasitthiyanukarn, C. Muangnoi, P. R. Na Bhuket, P. Rojsitthisak, P. Rojsitthisak,* Chitosan/alginate nanoparticles as a promising approach for oral delivery of curcumin diglutaric acid for cancer treatment, Mater. Sci. Eng. C **93** (1) (2018) 178–190, doi: https://doi.org/10.1016/j.msec.2018.07.069.
10. *M. N.V. R. Kumar*, A review of chitin and chitosan applications, React. Funct. Polym. **46** (1) (2000) 1–27, doi: https://doi.org/10.1016/S1381-5148(00)00038-9.
11. *J. Hafsa, M. A. Smach, B. Charfeddine, K. Limem, H. Majdoub, S. Rouatbi,* Antioxidant and antimicrobial proprieties of chitin and chitosan extracted from *Parapenaeus Longirostris* shrimp shell waste, Ann. Pharm. Fr. **74** (1) (2016) 27–33, doi: https://doi.org/10.1016/j.pharma.2015.07.005.
12. *W. Paul, C. P. Sharma*, Chitosan, a drug carrier for the 21st century: A review, STP Pharma Sci. **10** (1) (2000) 5–22.
13. *C. M. Silva, A. J. Ribeiro, M. Figueiredo, D. Ferreira, F. Veiga*, Microencapsulation of hemoglobin in chitosan-coated alginate microspheres prepared by emulsification/internal gelation, The AAPS Journal **7** (4) (2005) E903–E913, doi: https://doi.org/10.1208/aapsj070488.
14. *N. Fiola, J. Pochb, I. Villaescusaa*, Chromium (VI) uptake by grape stalks wastes encapsulated in calcium alginate beads: Equilibrium and kinetics studies, Chem. Spec. Bioavailab. **16** (1-2) (2004) 25–33, doi: https://doi.org/10.3184/095422904782775153.
15. *J. Liu, J. Xiao, F. Li, Y. Shi, D. Li, Q. Huang,* Chitosan-sodium alginate nanoparticle as a delivery system for ε-polylysine: Preparation, characterization and antimicrobial activity, Food Control **91** (2018) 302–310, doi: https://doi.org/10.1016/j.foodcont.2018.04.020.
16. *F. Nussbaum*, Development of a biosorption facility on a pilot scale, Diploma of High School of Engineering, University of Applied Sciences Western Switzerland, 2008, url: https://doc.rero.ch/record/12828/files/Nussbaum_5782579_TD.pdf.
17. *L. Payet, A. Ponton, F. Agnely, P. Colimart, J. L. Grossiord*, Rheological characterization of alginate and chitosan gelification: Effect of temperature, Rheology **2** (2002) 46–51.
18. *N. Bhattarai, M. Zhang*, Controlled synthesis and structural stability of alginate-based nanofibers, Nanotechnol. **18** (45) (2007), doi: https://doi.org/10.1088/0957-4484/18/45/455601.
19. *V. R. Sinha, A. K. Singla, S. Wadhawan,R. Kaushik, R. Kumria, K. Bansal, S. Dhawan,* Chitosan microspheres as a potential carrier for drugs, Int. J. Pharm. **274** (1-2) (2004) 1–33, doi: https://doi.org/10.1016/j.ijpharm.2003.12.026.
20. *A. J. Varma, S. V. Deshpande, J. F. Kennedy*, Metal complexation by chitosan and its derivatives: A review, Carbohydr. Polym. **55** (1) (2004) 77–93, doi: https://doi.org/10.1016/j.carbpol.2003.08.005.
21. *T. Rosenbaum, L. Tan, J. Engstrom*, Advantages of Utilizing Population Balance Modeling of Crystallization Processes for Particle Size Distribution Prediction of an Active Pharmaceutical Ingredient, Processes **7** (2019) 355, doi: https://doi.org/10.3390/pr7060355.

SAŽETAK

Optimizacija mikrokapsulacije aktivnog sastojka umrežavanjem i metodom premazivanja za liječenje bolesti debelog crijeva

Mounir Hammoudi,[a*] *Djamel Atsamnia*,[b] *Khaled Otmanine*,[c] *Riadh Moumen*[b] i *Mustapha Oumouna*[a]

Cilj ove studije bio je pripremiti mikrokapsule na bazi prirodne polimerne otopine kitozana (visokog stupnja deacetiliranja (DDA), niske molekulske mase (*MW*) i niske viskoznosti)/natrijeva alginata u prisutnosti umreženog agensa (glutaraldehida), za inkapsuliranje i vektoriziranje aktivnog sastojka prema bolesnom organu (debelom crijevu), bez difuzije u druge razine probavnog trakta, kako bi se povećala terapijska učinkovitost liječenja kemoterapijom i smanjili neželjeni učinci. Metoda pripreme mikrokapsula dobivenih iz sustava natrijeva alginata/otopine kitozana/aktivnih sastojaka ispitana je uobičajenom optičkom mikroskopijom. Uz to, provedeno je istraživanje *in vitro* na profilima oslobađanja aktivnih sastojaka, ovisno o pH koji simulira želučani i crijevni medij za sedam predloženih sustava. Treba napomenuti da je u osnovnom mediju (pH(debelog crijeva) = 8) oslobađanje aktivnih sastojaka od najveće važnosti. Ipak, kontrola tog ispuštanja može se poboljšati sredstvom za umrežavanje i metodom premazivanja. Suha formulacija [otopina natrijeva alginata/kitozana/aktivnih sastojaka + umreženog 2 %] presvučena neumreženim kitozanom (formulacija 7) standardna je formula koja udovoljava svim kriterijima iz našeg ranijeg rada s brzinom otpuštanja jezgre od 67 %. PSD je bio unimodalan s veličinama koje su se kretale od 750 μm do 900 μm.

Ključne riječi
Mikrokapsule, bolesti debelog crijeva, alginat, kitozan, vektorizacija, oblaganje, umrežavanje

[a] *Biomaterials and Transport Phenomena Laboratory (LBMPT), Yahia Fares University, Department of Chemical Engineering and Environment, Experimental Biology and Pharmacology Team, Médéa, 26 000, Alžir*
[b] *Biomaterials and Transport Phenomena Laboratory (LBMPT), Équipe Biologie et Pharmacologie exprémentales, Yahia Fares University, Médéa, 26 000, Alžir*
[c] *Bio-ressources Naturelles Locales LBRN, Chlef University, Faculty of Technology Department of Process Engineering, Chlef, Alžir*

Izvorni znanstveni rad
Prispjelo 22. kolovoza 2020.
Prihvaćeno 23. listopada 2020.