Journal of New Technology and Materials (JNTM)
J. New Technol. Mater.
Special Issue, Vol. 08, N°03 (2019)93-96

OEB Univ. Publish.Co.

Nanoencapsulation of insulin by alginate/chitosane matrix by ionotropic pre-gelation technique

A. Derbali[a, b*], I. Behlloul[a], N. Faghmous[a, b] And D. Bouzid[a, b]

[a] *Ecole Nationale Polytechnique de Constantine, BP 75, A, Nouvelle ville RP, Constantine, Algérie*
[b]*Laboratoire Génie des procédés pour le développement durable et les produits de santé, Nouvelle Ville Universitaire Ali Mendjeli, Algérie*

*Corresponding author, email: derbaliabir94@hotmail.com

Selected paper of JSSE'18, received in final form: March 11, 2019 ; accepted date: March 23, 2019

Abstract
Insulin is the most effective hypoglycemic agent used to treat the diabetes. Oral administration of insulin is the most comfortable and least restrictive way. However, it encounters the low absorption of insulin by the digestive tract and its degradation by proteolytic enzymes. Several strategies have been developed to insulin protection against enzymatic hydrolysis in the gastro intestinal environment. They also aim to promote absorption and transport of insulin through the intestinal epit helium. In his context, we encapsulated insulin in alginate/chitosan based nanoparticles by the ionotropic gelation method. This technique comprises two steps, which are the cross link of alginate with calcium chloride followed by complexation with chitosan. The nanoparticles obtained are then coated with polyethylene glycol 400. Particle size and zeta potential were characterized, and encapsulated insulin amount was assessed.
Keywords: Insulin, Nanoparticles, Alginate, Chitosan, Ionotropic pre-gelation.

1. Introduction

The oral administration of insulin to treat diabete is now a major challenge for humanity. The different physical and chemical barriers met in the gastrointestinal tract make this mode of delivery ineffective. [1]
Several strategies have been developed to protect insulin, against enzymatic hydrolysis in the gastrointestinal tract, and to promote its absorption and transport through the intestinal epithelium.
Nanoparticles loaded insulin is a new technique that intended to protect it against all possible degradations. It consists of incorporation the bioactive molecule into a nanometric matrix. [2]
In order to encapsulate insulin, many biodegradable polymers have shown potential for use as drug delivery system but the most common are natural polymers, which have the advantage of being stable, bio compatible and biodegradable, [3] the most used are alginate and chitosan. In this context, we encapsulated insulin in nanoparticles by the ionotropic pre-gelation method of alginate crosslink with calcium chloride, followed by complexation between alginate and chitosan.
Nanoparticles size and their surface charge were assessed with Zeta siezer, and the ultra-violet spectroscopy can be used to quantify the insulin loaded into alginate/chitosan matrix. The results obtained are very promising, they allow us to synthesize nanoparticles with a high degree of drug loading and to make sure an appreciable absorption rate and bioavailability of insulin at the epithelium.

2. Materials and methods

Sodium alginate Figure 1, low molecular weight chitosan (40KDA), Figure 2 and calcium chloride were purchased from SIGMA ALDRICH Chemistry. Alginate solution (0.063%) (w/v) was prepared in 100ml of deionized water under magnetic steering. Chitosan (0.07%) (w/v) was dissolved in 1% acetic acid solution. [4] The pH of alginate and chitosan were adjusted to 4.9 and 4.6 respectively with a chloric acid solution (1N) and a concentrated solution of NaOH. [5] The polymers solutions ready above are subjected to magnetic steering for enough time to make sure complete dissolution and good homogenization and followed by filtration with micro pore paper filter (0.22µm) to eliminate undissolved materials.

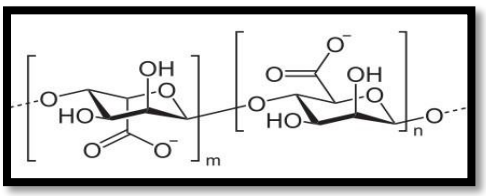

Figure1. Chemical structure of Sodium alginate

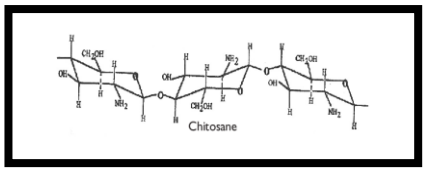

Figure 2. Chemical structure ofChitosan

2.1. Preparation of insulin nanoparticles

Insulin loaded nanoparticles takes place in a three steps procedure based on pre-gelation of alginate by the crosslink agent. In First, an amount of insulin equal to 3.5 mg was added into a beaker containing 58.75 ml of alginate solution, Under a magnetic steering to make sure that it completely dissolved , then 3.7 ml of calcium chloride (18 mM) was drop wised into the beaker under 2000 rpm, Figure 3. The interaction between alginate solution and Ca2+, Figure 4 occurred a pre-gel and forming a homogeneous suspension.

Thus, 12.5 ml of chitosan solution was dropped into the pre-gel for 45 min under steering (2000 rpm), we leave the mixture for 10 minutes before we added 12.5 ml of polyethylene glycol 400 for coating. The addition of chitosan stabilized the alginate and formed a polyelectrolyte complex by sample electrostatic interactions due to existence of opposite charges.

After the complexation between the polyelectrolyte (alginate and chitosan) and Polyethylene glycol coating, we leave the dispersion for 15 minutes under steering to stabilize. [4]

Polyethylene glycol 400 concentrations have been varied from 0.175% (w/v) to 0.35% (w/v) to see the effect of the concentration on the characteristics of nanoparticles.

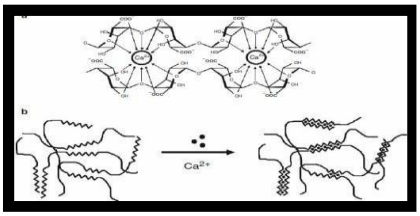

Figure 3. The <<Egg-Box>> model, a: chelation of divalent cations b: formation of junctions between the chains [6]

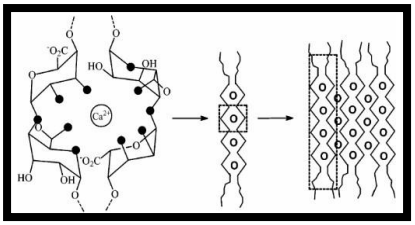

Figure 4. Sodium alginate reticulate [6]

Table 1: The experimental conditions

Experience	Steering speed (rpm)	PEG concentration (w/v)
01	800	0.175
02		0.35
03	2000	0.175
04		0.35

2.2. Nanoparticles characterization

2.2.1. Size

The size and the granulometric distribution of chitosan/alginate nanoparticles were measured by Zeta siezer NANO ZS (100 HORIBA Scientific) granulometer. A sample of 5 ml of the dispersion has been placed into analyzer chamber; collective reading was performed three times at 25°C.

2.2.2. Zetapotential

Measures of surface charge have been realized into capillary electrophoresis cell by granulometer Zetasizer®Nano ZS (100 HORIBA Scientific), at 25°C with angle detection of 173°.

2.2.3. Drugloading

Chitosan/Alginate can be used to deliver proteins and peptidic drugs. They form a polyelectrolyte complex which can retain a large amount of drug. To determinate the amount of encapsulated insulin into nanoparticles, a sample of nanoparticles has been centrifuged at 12000 rpm, for 30 min at room temperature. [5]

Nanoparticles that contain insulin precipitated forming a culot of nanoparticles, while free insulin which is not encapsulated remained in supernatant.

The amount of insulin in the surnageant can be analyzed with two methods: measure with HPLC or measure with UV (280 nm).

Encapsulation rate can be calculated by this formula

$$rate\% = \frac{initial\ insulin - unencapsulated\ insulin}{initial\ insulin} \quad (1)$$

3. Results

Particles size is important in improving how drugs might be absorbed in the intestinal epithelium and in increasing the circulation time of drugs in the body. Oral delivery of insulin by nanometric systems is more effective and moreexact.

Previous research has shown that encapsulation of insulin with only alginate and chitosan and for a stirring speed greater than 1600 rpm, particles have tendency to aggregate. In this study, the experiments carried out with a speed of 2000 rpm did not show the phenomenon of

particles aggregation, this is mainly due to the stabilizing nature of polyethylene glycol 400.

Table2: Effect of PEG concentration on particles size and zeta potential

Steering speed (rpm)	PEG Concentration % (w/v)	Particles size (nm)	Zeta potential (mV)
800	0.175	371.87	-35.2
	0.35	197.03	-32.1
2000	0.175	200.2	-30.73
	0.35	100.3	-26.23

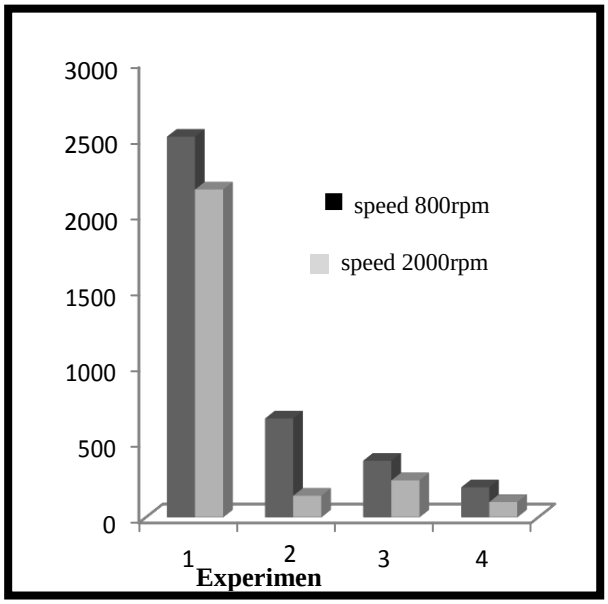

Figure 5. Effect of stirring speed on particles size

The effect of stirring speed was also evaluated when we used two different values of speed, we can see in Table 2 that we found nanometric particles but the particles size obtained with speed 2000 rpm are smaller than those obtained with 800 rpm this can be explained by the shear forces applied to the particles during agitation Figure5.

Polyethylene glycol concentration has an effect on particles size. For a low concentration of polyethylene glycol 400, it has been found that the particles are of a large size; because of the short lengths of hydrophilic chains of the polyethylene glycol 400 which are not sufficient for the complete coverage of the surfaces of the nanoparticles Figure 6. [7]

This situation makes nanoparticles sterically unstable and leads to their aggregation. So the obtaining of larger particles. [7] When density of PEG400 increases to (0.35%) (w/v), nanoparticles size decreases, this is can be explained by the low tendency of nanoparticles to aggregation and complete coverage of nanoparticles surface

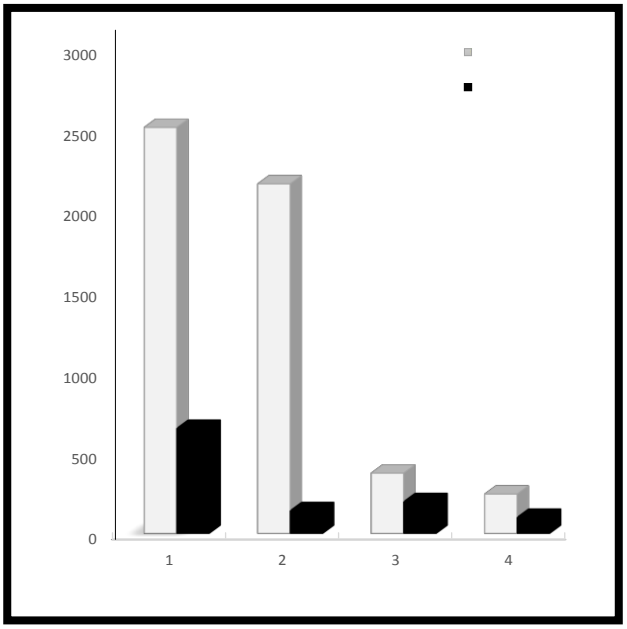

Figure 6. Effect of PEG concentration on particles size

The nanoparticles formed have a high negative charge which makes it possible to ensure their suspended electrostatic stability and their weak aggregation tendencies. [2]

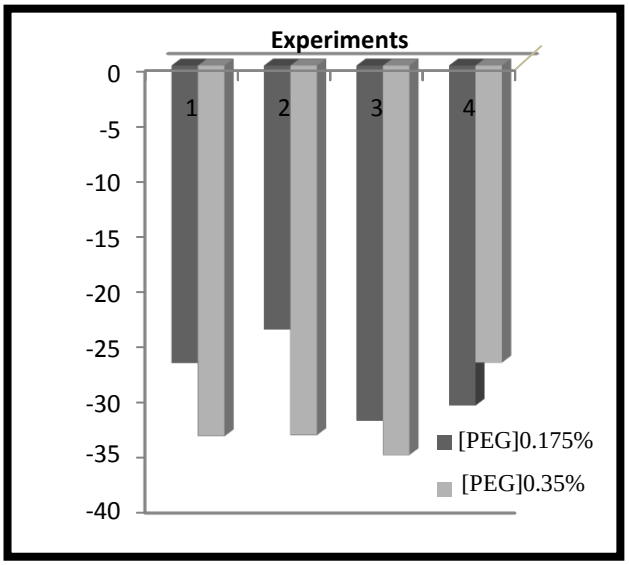

Figure 7. Effect of PEG concentration on Zeta potentiel

The effect of the polyethylene glycol on potential zeta is shown in Table 1. The increase of polyethylene glycol concentration from 0.175% (w/v) to 0.35 %(w/v) decreased the negative charges of the obtained nanoparticles Figure7.

The experiments made it possible to get nanoparticles with an encapsulation rate of approximately 85 %. The increase in the polyethylene glycol concentration from 0.175% (w/v) to 0.35% (w/v) leads to a decrease in encapsulation rate from 87.89 % to 82.52 %. Polyethylene glycol plays the role of a barrier that prevents the combination of insulin to the nanoparticles

4. Conclusion

Insulin loaded nanoparticles were successfully obtained by pre-gelation of reticulate alginate followed by complexation between polyelectrolytes, the interaction between them made a complex system which can be used to deliver insulin orally. Zeta siezer was used to characterize particles size and potential zeta; the results have shown that we get nanometric particles with a high encapsulation rate about 85%. The experiments also show the effect of stirring speed and polyethylene glycol concentration on particles size and zeta potential of insulin loaded nanoparticles

Reference

[1] P. Guhmann, Délivrance Orale D'insuline Par Double Encapsulation : Développement Et Evaluation De L'Efficacité Et De La Sécurité Des Systèmes Entériques Et Nanoparticulaire, Doctoral Thesis, 2013, Doctoral School of Life Sciences and Health, University of Strasbourg, France

[2] Callet, Administration Orale d'Insuline Par Double Encapsulation : développement du système nanoparticulaire par coacervation complexe insuline /chitosane, Doctoral Thesis, 2010, University of Strasbourg, France

[3] T. A. Sonia, C.P. Sharma, An Overview Of Natural Polymers for Oral Insulin Delivery, Journal of Drug Discovery Today, Elsevier, India (2012) 784-792

[4] B.W. Camile, A. J. Ronald, R. J. Neufeld, F. Veiga, Acta Biomaterialia (Ed.7), Collloidal Carrier Integration Biomaterials for Oral Insulin Delivery: Infuence of Component Formulation on Physicochemical and Biological Parameters, vol.5, Elsevier, Portugal, Canada, (2009) 2475-2484

[5] N. Faghmous, Mise au Point de Nanoparticules à Base d'Insuline et Caractérisation Physicochimique et Biopharmaceutique, Magisterialmemory 2011,University of Science and Technology, Houari Boumediene, Algeria

[6] D. Prisca , Système Intégré pour L'Encapsulation Monocouche des Cellules, Doctoral Thesis, 2012, University of Grenoble, France

[7] P. Colombo, R. Bettini, G. Massimo, P. Gatelani, P. Santi, N. A. Pepasse , Drug Diffusion Front Mouvmentis Important in Drug Release Control From Swellable Matrix Tablets, Journal of Pharmaceutical Sciences, (1955) 991-997