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Optimization of Penicillin G Microencapsulation with OSA Starch by Factorial Design

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The purpose of this study was to develop a system for sustained release of penicillin G (PenG) from alginate matrix microbeads for future application in subdermal implants. The beads containing penicillin G were prepared by extrusion method. Initially, different biopolymers were evaluated in combination with alginate. Sodium alginate (3 %,w/v) with different wall materials (maltodextrin, carboxymetilcellulose, pectin and octenyl succinic anhydride (OSA) modified starch at a concentration of 4 % (w/v) was dissolved in distilled water and a pre-calculated quantity of penicillin G was added (10 %, w/v). A 2^{4-1} fractional factorial design was used to investigate the effects of alginate, OSA starch, $CaCl_2$ concentration and times of gelation on retention percentage of penicillin in microspheres. The retention percentage of penicillin was determined in the calcium chloride solution. The best results were obtained with alginate/OSA starch, with entrapment of 51.66 %. Experiments were designed to improve PenG entrapment in microbeads. The retention of PenG in alginate/ OSA starch matrix was optimized using a Central Composite Rotatable Design (2^3 full factorial design). The data indicates that high retention percentage of penicillin G (95.4 %) values was obtained when OSA starch concentration and gelation time were lower. The results obtained in the present study indicate that alginate with OSA starch are wall materials suitable for the microencapsulation of PenG.

1. Introduction

Penicillin is a group of β-lactam antibiotics used in the treatment of bacterial infections caused by susceptible, usually Gram-positive, organisms. Since its discovery in the decade of 20, the penicillin comes being managed through injections. The discomfort associated with the inconvenience of this type of administration device has led patient with rheumatic fever to neglect and even though to give up the therapy (Khoee and Yaghoobian, 2007). For these reason alternative procedures of PenG administration using controlled release systems such as lipids (Santos-Magalhães, 2000) have been proposed.

Biopolymers are chosen predominantly to produce microcapsules because of the advantages of biocompatibility and biodegradability. Beyond that, the biopolymer can be associated to a specific device which could control the drug release. Alginate has been considered one of the most suitable biopolymer for microcapsules production; its composition and sequential structure has a great importance for its function as encapsulation material (Lee et al. 2011). It contains two uronic acids, β -

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(1-4)-linked D-mannuronic acid (M) and α - (1-4) linked L - guluronic acid (G), and is composed of homopolymeric blocks M–M or G–G, and blocks with an alternating sequence of M–G block (Turos et al., 2007). In addition, sodium alginate has a unique property of cross-linking in the presence of multivalent cations, such as calcium ions in aqueous media, which rather complex with G–G sequences in the polymer chain to form the 'egg box junctions'. Alginate forms a reticulated structure in contact with calcium ions and this network can entrap antibiotic (Finotelli et al., 2010).
Other potential biopolymer is Octenyl succinic anhydride (OSA) starch, which is a modified starch developed by the National Starch and Chemical Corporation from the US. This modification consists of adding a lipophylic component (octenyl-succinate) which increases the emulsion stability in the formulations (Finotelli, 2005). Furthermore, it is a polymer of a large application in controlled release systems since it can contribute to obtaining less porous materials. Of this form, OSA starch is widely used for a better entrapment and slow release of many bioactive substances in microcapsules (Bastos et al. 2009). The purpose of this work was to develop a system for sustained release of penicillin G from alginate matrix microbeads for future application in subdermal implants.

2. Materials and methods

2.1 Materials

Sodium alginate was purchased from Keltone LV. Sodium alginate solutions (2 %, w/v) had viscosity at 25 °C and 60 rpm (No. 2 spindle) of 100–300 Pa.s, as determined with LV model of the Brookfield viscosimeter. The ratio of mannuronic acid to guluronicacid residues (M/G) was between 0.4 and 1.9. The Penicillin G and Calcium chloride were obtained from Sigma-Aldrich (Fluka e Science Lab, US) and Vetec, respectively. The biomaterials (maltodextrin, carboxymethylcellulose, octenyl succinic anhydride (OSA) starch and pectin) were purchased from Mor Rex (Corn Products Brasil®), Latinoquímica Argentina®, National Starch® and Sigma-Aldrich, respectively.

2.2 Preparation of alginate beads containing penicillin G

The beads containing penicillin were prepared in triplicate by extrusion method (dripping method). Initially, different biopolymers were evaluated in combination with alginate. Sodium alginate (3 %,w/v) with different wall materials (maltodextrin, carboxymetilcellulose (CMC), pectin and octenyl succinic anhydride (OSA) starch) at a concentration of 4 % (w/v) was dissolved in distilled water and a pre-calculated quantity of penicillin G was added (10 %, w/v). The solution was stirred thoroughly to ensure complete mixing of drug. The alginate/biopolymers/penicillin mixture was dropped into calcium chloride solution (2 %, w/v) under constant stirring at 300 K. After gelation the beads were maintained for a period of 10 min in the $CaCl_2$ solution. The beads were then removed from the $CaCl_2$ solution and washed several times with Milli-Q water. Beads containing different amounts of penicillin G (10, 12, 14, 16 % w/v) were prepared with the mixture of alginate (4.5 %) and OSA starch (2 %) by the same described procedure.

2.3 Retention percentage of penicillin G entrapment in beads

The retention percentage of penicillin was determined indirectly in the calcium chloride solution by spectrofotometrically (λ = 220 nm, HACH DR/4000V). Preliminary UV studies showed that the presence of dissolved polymers did not interfere with the absorbance of the drug at 220 nm. The retention percentage (%) of entrapment penicillin G was calculated using the following equation:

$$Retention(\%)of\ entrapment = \frac{\frac{mass\ of\ penicillin present in\ beads}{mass\ of\ beads}}{\frac{mass\ of\ penicillin in\ the\ formulation}{mass\ of\ the\ beads}} x100 \quad (1)$$

2.4 Factorial design to optimize penicillin G microencapsulation with OSA starch

Two experimental designs were carried out in order to improve penicillin G microencapsulation with OSA starch. First, a 2^{4-1} fractional *factorial* design was used to investigate the effects of alginate, OSA starch, $CaCl_2$ concentration and time of gelation (Table 1) on retention percentage of penicillin in

microspheres. After that, a Central Composite Rotatable Design (2^3 full factorial design) was used. In this design, a set of 17 experiments, including three replicates at the central point, was performed. The range and the levels of the variables herein investigated are given in Table 2. "STATISTICA" (version 7.0) software was used for regression and graphical analyses of the data obtained.

Table 1: Experimental range and levels of the independent variables used in the 2^{4-1} fractional factorial design

Variable	Level		
	-1	**0**	**+1**
Alginate (% w/v) x_1	1.5	3	4.5
OSA starch (% w/v) x_2	2	4	6
$CaCl_2$ (M) x_3	0.15	0.2	0.45
Gelation time (min) x_4	3	10	17

Table 2: Experimental range and levels of the independent variables used in the 2^3 full factorial design

Variable	Level				
	-1.68	**- 1**	**0**	**+ 1**	**+1.68**
OSA starch (%m/v) z_1	0.32	1	2	3	3.68
$CaCl_2$ (M) z_2	0.35	0.45	0.60	0.75	0.96
Gelation time (min) z_3	8	17	30	43	52

3. Results and Discussion

Initially, different biopolymers were evaluated in combination with alginate. The results reported here show that the retention of penicillin G in the different wall materials comprised between 4.32 % (alginate/maltodextrin) and 51.66 % (alginate/OSA starch) (Figure 1). The best results were obtained with alginate/OSA starch, with entrapment of 51.66 %. This result can be due the favorable intermolecular interactions between the penicillin G and OSA starch, as the hydrophobic interactions, hydrogen-bonded and London dispersion forces.

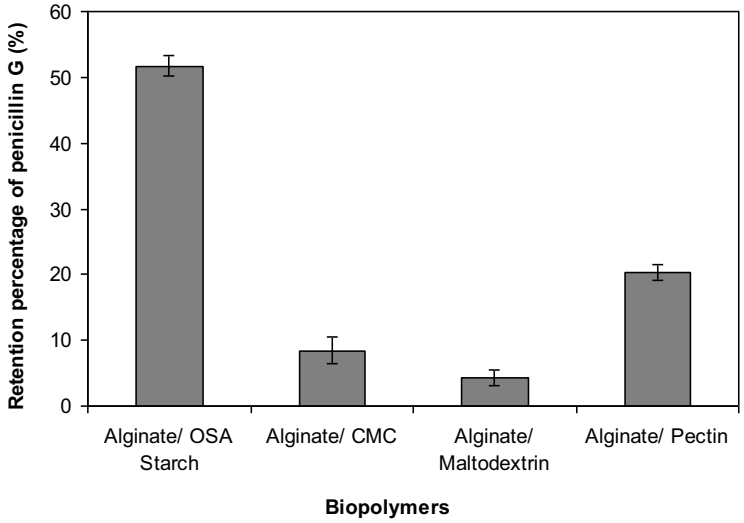

Figure 1. Retention of penicillin G in alginate matrix with different wall materials

Experiments were designed to improve PenG entrapment in microbeads with alginate and OSA starch. The retention of PenG in alginate/OSA starch matrix was optimized using first a 2^{4-1} fractional factorial. The experimental design and the results are presented in Table 3. The data indicates that high

retention percentage of penicillin G (95.4 %) values were obtained when alginate concentrations were high. Figure 2a shows the Pareto chart, with 95 % of confidence level, where is possible to identify that alginate and OSA starch concentration had a significant influence. Figure 2a also depicts that the increase in alginate concentration showed positive effects on penicillin G retention. However, the increase in OSA starch produced a negative effect.

In order to investigate the negative effect of OSA starch, five experiments were carried out with only alginate as wall materials. Different alginate concentrations (3.0; 3.5; 4.0; 4.5 and 5 % w/v) were evaluated. The results show that 4.5 % of alginate was the best condition, reaching 70 % of penicillin G retention (Figure 2b). However, when comparing with the experiment 6 (Table 3) the percentage of penicillin G retention in mixture of alginate and OSA starch was 95.4 %. This result highlights the importance of OSA starch as wall materials for penicillin microencapsulation.

Table 3: Experimental design and results of the 2^{4-1} fractional factorial design.

Nº Exper.	X_1^*	X_2^*	X_3^*	X_4^*	Retention of penicillin G (%)
1	1.5	2	0.15	3	40.17
2	4.5	2	0.15	17	94.97
3	1.5	6	0.15	17	45.0
4	4.5	6	0.15	3	70.12
5	1.5	2	0.45	17	60.32
6	4.5	2	0.45	3	95.34
7	1.5	6	0.45	3	47.55
8	4.5	6	0.45	17	80.68
9	3	4	0.2	10	52.9
10	3	4	0.2	10	52.81
11	3	4	0.2	10	51.34

*The coded variables x_i (i= 1, 2, 3, 4) are defined in Table 1.

With the purpose to analyze the influence of the interaction between alginate, gelation time, OSA starch and calcium chloride concentration in the penicillin G microencapsulation, a second experimental design was carried out with a 2^3 central composite design. The concentration ranges of variables, indicated in Table 2, were calculated according to the results of the previous fractional factorial design. The results for the two-factorial central composite design are present in Table 4.

High penicillin G retention was found at central level conditions (zero level, run n° 15, 16 and 17). The average of retention percentage at zero level was 95.13 %, 1.85 fold higher than the initial penicillin G microencapsulation process (51.66 %).

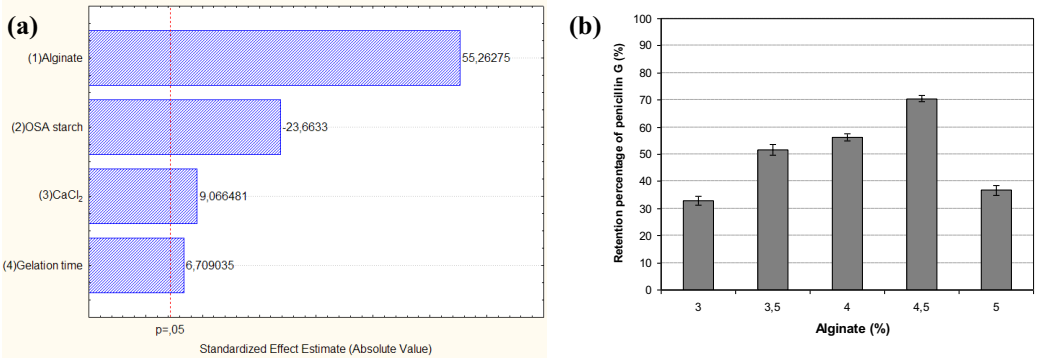

Figure 2. Pareto Chart of standardized effects for retention percentage of penicillin G (a) and Retention percentage of penicillin G in different alginate concentrations (b).

Figure 3a illustrates the Pareto chart for the estimated effects in absolute values for retention percentage of penicillin G. It is possible to observe that gelation time and OSA starch concentration had significantly influenced the dependent variables. On the other hand, calcium chloride concentration did not significantly influence the penicillin G retention in the range studied. These results are in accordance with the literature (Finotelli, 2010). Figure 3a also depicts that the increase in gelation time and OSA starch showed negative effects on penicillin G microencapsulation.

Table 4: Experimental design and results of the 2^3 central composite design.

Nº Exper.	Z_1^*	Z_2^*	Z_3^*	Retention of penicillin G (%)
1	1	0.45	17	89.21
2	3	0.45	17	79.36
3	1	0.75	17	90.11
4	3	0.75	17	86.74
5	1	0.45	43	80.22
6	3	0.45	43	65.41
7	1	0.75	43	70.12
8	3	0.75	43	73.49
9	0.32	0.6	30	88.40
10	3.68	0.6	30	80.13
11	2	0.35	30	93.54
12	2	0.96	30	94.32
13	2	0.6	8	86.54
14	2	0.6	52	70.44
15	2	0.6	30	93.4
16	2	0.6	30	95.6
17	2	0.6	30	96.41

* The coded variables z_i (i= 1, 2, 3) are defined in Table 2.

According to the response values obtained from the designed experiments (Table 4), a second – order regression equation was calculated for the response surface as follows.

$$\text{Retention of penicillin G}(\%) = 94.65 - 2.81z_1 - 5.14{z_1}^2 - 6{,}08z_3 - 7.17{z_3}^2 + 3.08z_1z_2 \quad (2)$$

The model was checked and it was found to be adequate as expressed by the coefficient of determination (R^2), which was calculated to be 0.98 for retention percentage of penicillin G. The variance analysis of the quadratic model for retention shows that the model is highly significant, as is evident from the fisher F test, where the calculated F values, F = 303.2, are greater than the tabular F values, $F_{0.05;\,4;\,6}$ = 4.5.The validation of the mathematical model was performed using the mean values achieved in the central points. The experimental maximum of penicillin G retention perfectly agreed with predicted maximum. The difference between the model prediction and the experimental data was less than 0.5 %.

Beads containing different penicillin contents had been prepared in order to understand the effect of antibiotic concentration on encapsulation efficiency. Figure 3b shows the relation between the penicillin G retention and penicillin initial concentration in beads. Increasing the penicillin content from 10 to 16% of beads, the entrapment efficiency decreases from 94.19 % to 44.41 %. However, the total amount of entrapped penicillin in the beads increased almost linearly with the antibiotic concentration.

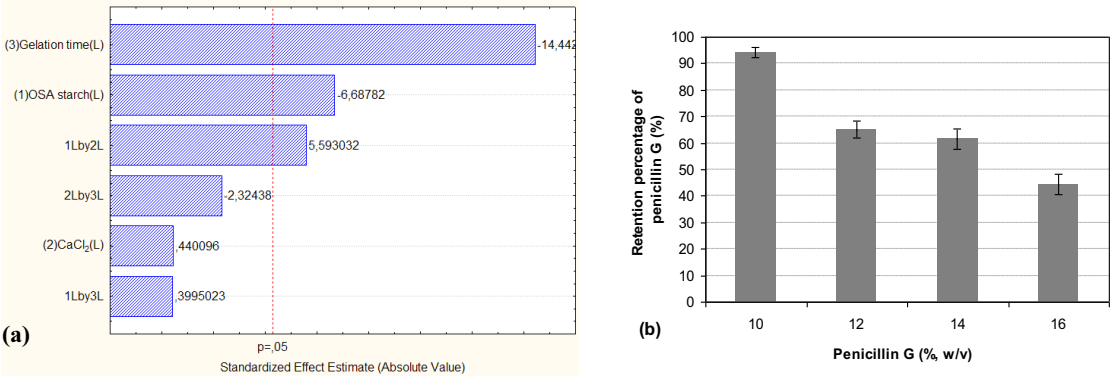

Figure 3. Pareto Chart of standardized effects for retention percentage of penicillin G (a) and The retention percentage of penicillin entrapment in alginate/OSA starch matrix as a function of penicillin content (b).

4. Conclusions

In conclusion, we have developed a delivery system for penicillin G based on natural polymer. The simplicity of preparation, the high drug encapsulation/loading, probably will improve the pharmacokinetic as well as high therapeutic efficacy encourage further evaluation of the formulation in higher animal model. Our results indicate that alginate and OSA starch are wall materials suitable for the microencapsulation of PenG.

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