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Optimization of the Mechanical Properties of Biofilm Based on Alginate - Gellan Plasticized with Glycerol

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Abstract

Objective: In this study, a Box-Behnken design was employed in order to optimize the concentration of sodium alginate (SA), low acyl gellan (LAG) and glycerol (GLY) as a function of tensile strength (TS) and elongation at break (EB) of the biofilms.

Methods: A texturometer Shimadzu model EZ-Test EZ-S was employed to evaluate TS and EB by using constant displacement control with a crosshead speed of 30 mm/min.

Results: The regression model developed herein indicates that the selected variables were statistically determinants ($p < 0.05$) for the studied mechanical properties. The optimal concentration to obtain high TS values (134.38 MPa) were 1.00% (w/v) of SA, 1.00% (w/v) of LAG and 8.00% (v/v) of GLY. However, low values of EB (15.95%) were also obtained using the same concentrations of AS, GBA and GLY.

Conclusions: By increasing the concentration of SA and LAG result in highly resistant biofilms with low flexibility while increasing the glycerol concentration and reducing the concentration of biopolymers yielded the opposite effect. The Box-Behnken design is a useful statistical method for improving the mechanical properties studied depending on the final application of the biofilms.

Keywords: Sodium Alginate, Elongation at break, Low Acyl Gellan, Glycerol, Tensile strength

1 Introduction

The increased production and large application of synthetic materials based films and coatings have grown rapidly during the last decades. This has resulted in serious environmental drawback due to the fact that these materials are non-biodegradable[1]. In order to solve this problem, researchers have focused their attention on biodegradable materials for both food and pharmaceutical applications[2].

Films are formed by natural components that can potentially be used for packaging of several foodstuffs to extend their shelf life by preventing transfer of moisture, flavors or gases across the package, and therefore maintaining their quality. Moreover, biofilms are utilized as carrier of desirable additives such as flavors, nutrients, colorings and antimicrobials[3].

Various polymers have been studied for development of biodegradable films including some polysaccharides as starch, alginate, xanthan, gellan and guar gum. Alginates are polysaccharides produced by brown algae (Laminaria digitata, Laminaria hyperborea, Ascophyllum nodosum y Macrocystis pyrifera) and are widely used in the food industry due to their non-toxic and gelling properties. Within the most important applications of alginate in biotechnology it is the ability to create stable gels through the ionic interaction between two adjacent chains with monovalent or divalent cations, forming junction zones that stabilize the gel structure[4][5].

Gellan gum is an anionic extracellular heteropolysaccharide produced by the bacterium Sphingomonas paucimobilis, and it is available in two forms: high (HAG) and low acyl (LAG). When HAG is exposed to strong alkali treatment at high temperatures, the acyl groups are hydrolyzed and LAG is obtained. These structural differences between HAG and LAG allow great

diversity of its textural properties. Therefore, HAG forms soft, elastic gels; while LAG gum forms strong gels[6][7][8].

The mechanical properties of biofilms are crucial for their adequate selection and application on pharmaceuticals and food matrices. Mechanical properties describe the response of materials to the application of stresses, which may be in compression, tension or shear, and provide insight into the fragility and/or elasticity of the material[9]. Not all the films have the same mechanical characteristics; therefore, it is important to evaluate their mechanical performance considering that for example: fragile films do not perform properly as covering materials and thus should be discarded for the applications described herein.

Design of experiments (DOE) has been frequently applied to optimize different processes because of its various advantages including reduction in the number of experiments, which results in lower consumption of materials and significantly less laboratory work[10][11]. Response surface methodology (RSM) is an effective optimization tool wherein many factors and their interactions can be identified with fewer experimental trials[12]. In addition to that, the response surface can be graphically used to make judgments about the relationship between explanatory and response variables[13].

Pranoto et al., 2007 [14] found that the addition of gellan and k-carrageenan modified fish gelatin films by increasing its tensile strength and providing barrier against water vapor, however it made the films slightly darker. Yang and Paulson, 2000[15] also studied the mechanical properties (tensile and puncture) and water vapour permeability of edible gellan films as a function of the glycerol's concentration. They found that the addition of glycerol to gellan flms moderately increased the tensile elongation and puncture deformation, but decreased the tensile strength and elastic modulus of the fims. Likewise, the physicomechanical properties of Ca2+-treated gellan films plasticized with glycerol have been investigated [16][17]. However, most of these studies did not specify the type of gellan gum used (LAG or HAG). Although there have been many studies about gellan gum, there is almost no information concerning the use of SA and LAG mixtures plasticized with glycerol for the production of the film. Thus, this study focuses on using the Box-Behnken design approach to optimize the mechanical properties of binary biofilms formed by LAG, SA and GLY as plasticizer.

2 Materials and methods

2.1 Preparation of the films

A Box-Behnken experimental design with three factors and levels, consisted of SA ($0.50 - 1.00\%w/v$), LAG gum ($0.50 - 1.00\%w/v$) and GLY (8.0–$15\%v/v$).

All factors and their levels were selected from single-factor experiments performed previously. The design matrix consisted of 15 experiments, including three replicates of the central point. Three replicates of the 15-unit design matrix were carried out; each of these replicates represents a block, such that a total of 45 experimental units were performed. The different solutions were prepared separately with deionized water and the corresponding amounts of polymers (Table 1). Subsequently, calcium was added (30 mM) and then the solutions were mixed by using a magnetic stir bar/hot plate magnetic stirrer setup (Thermolyne Nuova II, EEUU) at $90°C$ during 10 min. Samples were then cooled to $35°C$ and the pH was adjusted to 4.0 by adding citric acid ($1\% solution w/v$). Finally, the resulting solutions were poured into petri dishes controlling the volume added, followed by a drying process at $37.5°C$ for three days in an incubator (Memmert IN 160, Germany). All biofilms were conditioned in an environmental chamber at room temperature ($33°C$ and 75% RH) for 24 hours prior to testing[18][19].

Table 1: The Box-Behnken design for response surface analysis

Run no.	**SA % (w/v)**	**GBA % (w/v)**	**GLY % (v/v)**
1	0.50	0.50	11.50
2	1.00	0.50	11.50
3	0.50	1.00	11.50
4	1.00	1.00	11.50
5	0.50	0.75	8.00
6	1.00	0.75	8.00
7	0.50	0.75	15.00
8	1.00	0.75	15.00
9	0.75	0.50	8.00
10	0.75	1.00	8.00
11	0.75	0.50	15.00
12	0.75	1.00	15.00
13	0.75	0.75	11.50
14	0.75	0.75	11.50
15	0.75	0.75	11.50

2.2 Thickness and opacity

The thickness of the biofilms was measured using a digital micrometer (Mitutoyo Japan) with 0.001 mm precision. A total of 8 points were measured for each film. The light transmission of the films was measured by scanning the samples at wavelengths of 200–800 nm using a spectrophotometer ultra-

violet–visible (UV–vis) (Thomson Gold Spectrumlab 54). Three replicates of each film were tested.

Mechanical test Tensile strength (TS) and elongation at break (EB) of the biofilms were measured on a texturometer Shimadzu model EZ-Test EZ-S. Tests were performed at room temperature ($33°C$). Ten samples (15 mm x 50 mm) of each formulation were tested for statistical/uncertainty-reduction purposes. Each test was performed using constant displacement control employing a crosshead speed of 30 mm/min with a load cell of 1 kN. TS and EB measurements were repeated at least eight times for each type of biofilm, and the averages were reported accordingly. TS values were calculated using Equation 1:

$$TS = \frac{F_{max}}{A_t} \tag{1}$$

Where Fmax is the maximum load required to break the biofilms and At is the cross sectional area. EB values were determined using Equation 2:

$$\%EA = \frac{\Delta_l}{l_0} x100 \tag{2}$$

Where Δl is variation in length of the film and lo is the initial length in the biofilm [20].

2.3 Response surface methodology and optimization

A three-level-three-factor, Box–Behnken response surface design (BBD) with three central points was applied for studying the simultaneous effects of the defined variables on the optimization of TS and EB. This was accomplished employing the software Minitab version 17. The experimental data obtained for fifteenth experimental runs were fitted to the second-order polynomial model (Equation 3), where $\beta_0, \beta_1 ... \beta_{23}$ represent the regression coefficients, β_0 is the intercept with Y-axes. β_1, β_2 and β_3 are the linear effects of each factor. β_{12} β_{23} β_{13} are the effects from interactions. β_{11} β_{22} β_{33} are the quadratic effects, ϵ is the random error. X_1, X_2 and X_3 represent factors corresponding to SA (0.5 to 1.0), LAG (0.5 to 1.0) and GLY (8 to 15), respectively. Only those statistically significant coefficients ($P \leq 0.05$) were considered in the model; and the remaining coefficients, if considered, yielded much lower R-values, thus, less predictability[21][22].

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{33} X_3^2 + \epsilon \tag{3}$$

3 Results

3.1 Thickness and opacity

Preliminary experiments were conducted in order to determine suitable polymers concentrations to be used in each film-forming solution. Although unplasticized films were also prepared, these films resulted in a brittle-like behaviour and cracked during drying on the casting plates. Thus, the suitable glycerol level was incorporated into the film-forming solutions improving the flexibility of the films. It was established that good films (not to gummy) were obtained. With respect to the biofilms' thickness, these characteristic ranged from 0.07 to 0.08 mm and the transparency values of the films were not significantly ($P < 0.05$) affected by the concentrations of SA, LAG and GLY. As a result, the thickness and the opacity of the films were not considered within Box-Behnken design.

3.2 Surface response methodology (RSM)

Various response surfaces were constructed from the obtained results of TS and EB. Furthermore, the effect of each variable and their interactions were also evaluated. Figures 1A and 1B depict significant influence of both, the SA and LAG concentrations on the TS values. Results revealed an increase on the biofilms strength with increasing concentrations of both LAG and SA. Figures 1C and 1D show a decrease in EB with increasing SA and LAG levels. On the other hand, as the concentration of SA increase and that of GLY decreases results in increased TS values obtaining a maximum strength when the concentrations of SA and GLY are 1.00% and 8.00%, respectively (Figures 2A and 2B).

An increase on the plasticizer content results in a reduction of the biofilm's TS and an increase on EB, maybe due to the reduction of the polymer to polymer interaction, thus, resulting in higher flexibility of the matrix. Figures 2C and 2D show that increasing concentrations of SA yields reduced biofilm's flexibility, however, the GLY content seems to be directly proportional to the elongation.

Figures 3A and 3B reveal that increasing LAG dosage results in TS increase; nevertheless, TS decreases with increasing plasticizer concentration. Figures 3C and 3D indicate that EB decreases with increasing concentrations of LAG conversely, they seem to be directly proportional to the concentration of plasticizer.

The response surface graphs were employed to determine the coefficients use to build model that describes the behavior of each response variable. For each response variable, coefficients were substituted in equation 3; thereby obtaining Equations 5 and 6 for TS and EB respectively..

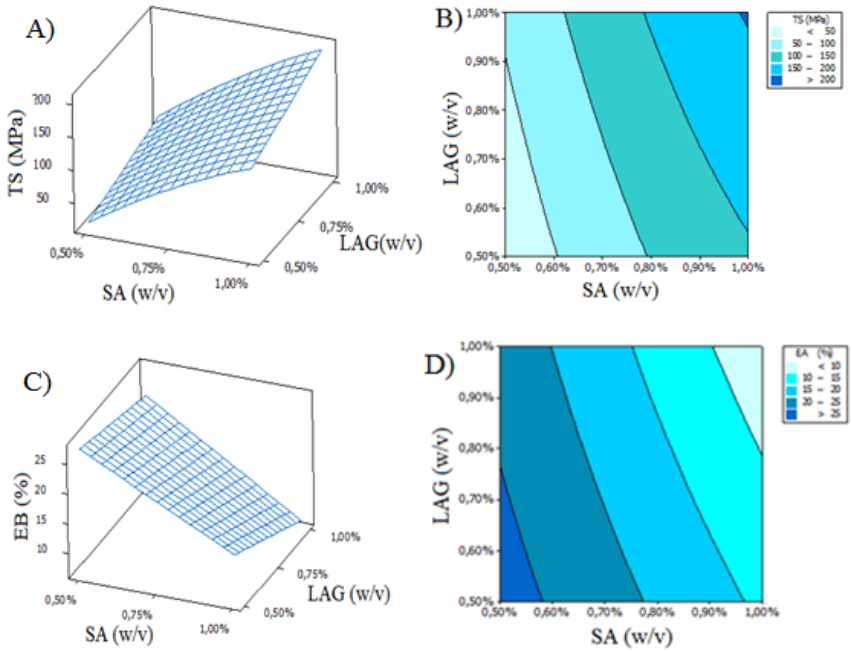

Figure 1: Surface of response and contour plots of TS and EB of SA vs LAG

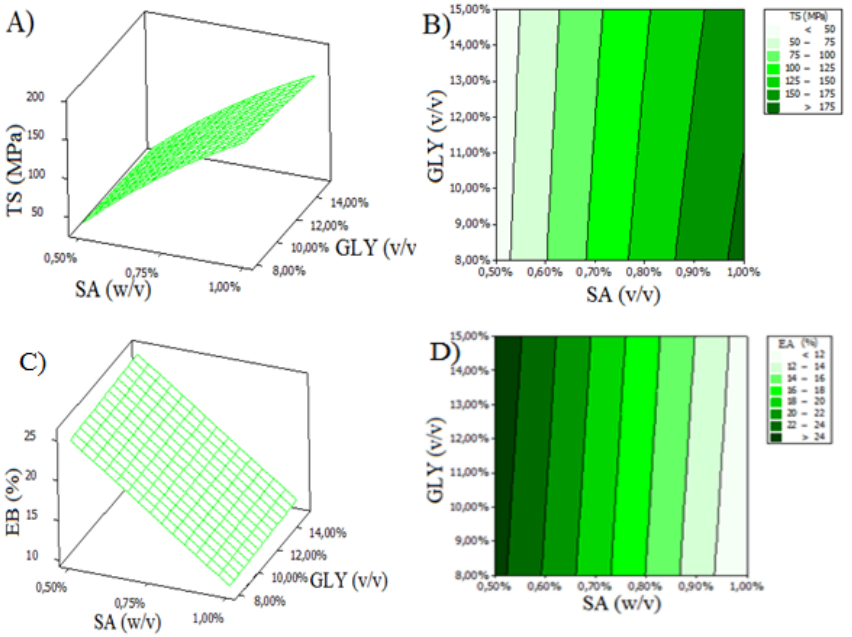

Figure 2: . Surface of response and contour plots of TS and EB of SA vs GLY

$$TS = 47460X_1 + 4471X_2 + 67X_3 + 750160X_1X_2 - 1484737X_1^2 - 29669X_1X_3 - 216 \quad (4)$$

$$R^2 = 0.995 \quad (5)$$

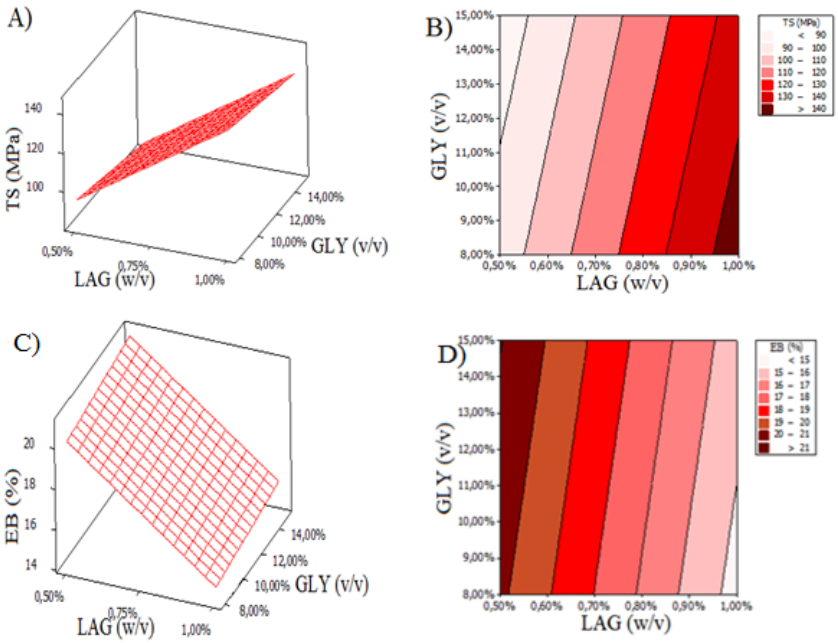

Figure 3: Surface of response and contour plots of TS and EB of LAG vs GLY

$$\begin{aligned} \%EB &= 39 + 12X_3 - 1945 - 146X_2 - 129200X_1X_2 \\ R^2 &= 0.998 \end{aligned} \tag{6}$$

As a result of the high R^2 predictive values obtained (> 95%) in Equations 5 and 6, they can be considered validated models of the behavior of TS and EB versus factors studied within the experimental region (eg, SA, LAG and GLY dosage), where X_1, X_2 and X_3 represent the concentrations of SA, LAG gum and GLY respectively.

Observing the coefficients of Equation 5, it can be concluded that the coefficients of SA and LAG have a positive effect on the TS films values, and this effect is increased when these components are combined. However, when GLY is included, the opposite effect is observed (decreasing the TS). Conversely, Equation 6 shows that only GLY concentrations have a positive effect on EB, while SA and LAG concentrations and their interactions have a negative effect, resulting in biofilms with less flexibility.

Optimization process The maximum values of TS and EB obtained (data not shown) were considered along with the predictive models described above (Equation 5 and 6) to determinate the optimum combination of factors that provide biofilms with highest strength and flexibility values. Moreover, the resulting function is to be employed for simultaneous optimization of every

response. This function allows for a combination of independent variables that simultaneously enhance each respond in the design.

Tensile strength can be optimized to reach values up to 134.3845 MPa having maximum concentrations of SA (1.00%w/v) and LAG (1.00%w/v) but less GLY levels (8.0%v/v). Conversely, using lowest concentrations of SA (0.50%w/v) and LAG (0.50%w/v), and maximum GLY levels (15.0%v/v) results in maximized EB at 15.95% (see Figure 4). Finally, employing concentrations SA, LAG, and GLY of 0.91% (w/v), 0.50% (w/v) and 8.0% (v/v), respectively, yielded biofilms with maximum values of TS (138.5 MPa) and EB (16.1%) simultaneously (data not shown).

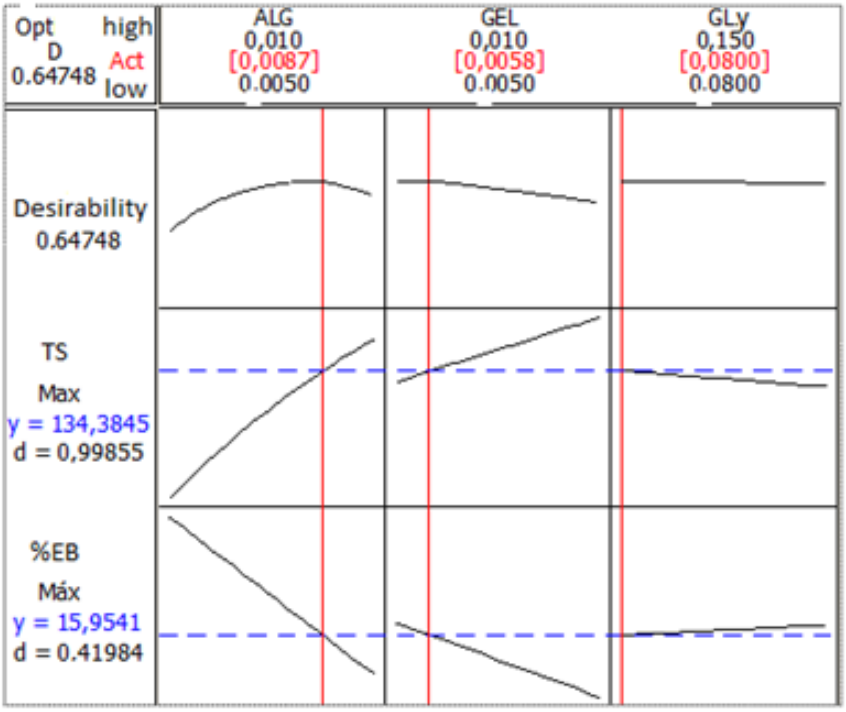

Figure 4: Optimization of TS and EB values of biofilms based on alginate, low acyl gellan and glycerol

The concentrations of sodium alginate and low acyl gellans are directly proportional to tensile strength under the conditions studied. However, these polymeric concentrations result in reduced elongation at break. The development of predictive models provided optimal values to produce biofilms based on sodium alginate, low acyl gellan and glycerol, with maximum strength and flexibility. It is estimated that maximum concentrations of sodium alginate (1.00%w/v), low acyl gellan (1.00%w/v) and low glycerol content (8.0%w/v), produce films with maximum values of resistance (134.3845MPa). Whereas, minimum values of SA, LAG and high content of GLY produce films with highest elongation at break percentage 15.95%.

4 Discussion

4.1 Determination of tensile strength of biofilms

The tensile strength values obtained in this paper are in disagreement with those published by some authors[23] who reported TS values ranging from 31.3 to 15.8 MPa in film based on polyhydroxyalkanoates plasticized with different types and concentrations of plasticizers. These authors also stated that a decrease of TS depends of both, the concentration as well as the type of plasticizer employed. The TS results of the biofilms studied herein align very well with results obtained in other studies [24][25]. In general, the results presented herein indicate that TS values are directly proportional to the polymeric concentrations (SA and LAG). Most likely, these high TS values are caused by the presence of hydrogen bonding and cross-linked between each helix of the polymeric matrix which is facilitated by the calcium ions [6][16].

The correlation between GLY concentration and the mechanical properties is in accordance with other studies with alginate-polyvinyl alcohol blend films[26], and cross-linked alginate-pectin composite biofilms[27][28]. Pongjanyakul and Puttipipatkhachorn (2007)[29] already described the reduction of TS with increasing amount of GLY; however, they reported measured values higher than those presented in this study. The same type of behavior was observed by others works [30][31][32] who found that the addition of plasticizers cause a reduction in TS values. A possible explanation for this phenomenon is due to the low molecular weight of glycerol which favors the reduction of intermolecular and intramolecular hydrogen bonding in the polymer network.

4.2 Determination of Elongation at break of biofilms

The determinations of EB values on biofilms play a key role during the design of optimum biofilms for food and pharmaceuticals applications. By increasing the chain mobility of the base polymer, a suitable plasticizer increases the EB. This effect was slightly found in most of the tested samples. Consequently, an increase in GLY does not result in further increase of the EB. Similar behavior was stated by some authors [23] in biofilms prepared with polyhydroxyalkanoates containing GLY and propylene glycol. This might be caused by the low molecular weight of plasticizerd used which results in a saturation of polyhydroxyalkanoates at small concentrations.

The EB values of the SA and LAG biofilms are higher than values obtained in other studies [24][25]. This leads to the conclusion that the preparation process of the films (e.g. SA/LAG ratio, dry matter content, composition, handling, drying parameters) has an effect on the final properties of the biofilms.

4.3 Surface response methodology (SRM)

SRM plays a very critical role in efficiently exploring the optimal values of explanatory variables. As a function of two factors, three dimensional response surfaces and their corresponding contour plots are more helpful in understanding both the main and the interaction effects of these two factors, maintaining all other factors fixed [33]. There is a marked influence of both, the SA and LAG ratio on the TS values. Most likely, there are many active sites to which cross-linking agent binds (calcium ions) promoting a cross-linking between the polymeric chains of SA; thus, increasing the biofilms strength [34][35].

It is noteworthy that as the concentration of SA increase and GLY decrease resulted in increased TS, yielding a maximum strength when the concentrations of SA and GLY are 1.00% and 8.00%, respectively. Nevertheless, the calcium excess may encourage competition for anionic groups of polymers destroying some of the bonds because of repulsive forces [34][36]. This could explain the TS reduction of films when lowest concentrations of polymers were employed. Most of the biofilms obtained in this study can be used in packaging of solid food, however, some of them because of their limited elongation at break values could require the protection of a secondary packaging.

Conflict of interests. The authors declare that there is no conflict of interests regarding the publication of this paper.

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