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ORIGINAL PAPER

Application of simultaneous multiple response optimization in the preparation of thermosensitive chitosan/glycerophosphate hydrogels

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Abstract The parameters affecting the sol/gel transition of chitosan-based thermogelling systems, their final properties and structure have been reported. Nevertheless, equations that define this relationship specific for a particular case have not been developed. The aim of this study was to develop these equations for a general formulation without drugs, so they could be used in the preparation of different drug delivery systems. First, a factorial design was built to evaluate the main factors affecting the pH of a gel-forming solution, gelling time at ambient temperature and 37.5 °C and residual mass after exposure to a buffer solution. The analyzed independent factors were: chitosan concentration, β-glycerophosphate concentration, mixing temperature and mixing time. Mixing time was the only factor whose effect on the responses was not significant; the other three factors were considered for the optimization study. Then, a central composite design based on response surface methodology was carried out to develop statistical models which describe the relationship between active independent factors and the studied responses. The optimized gel was prepared and characterized. FTIR experiments showed that hydrogen bonds and electrostatic attraction were the main types of interactions following sol-to-gel transition. The most important finding of this investigation was that the resulting equations could be used to prepare gel-forming solutions with the desired pH, gelling time and residual mass. The significance of these equations was considered in their potential use to design hydrogels intended for controlled drug delivery.

Keywords Chitosan · Thermosensitive hydrogel · Statistical optimization · β-Glycerophosphate · Biopolymer

Abbreviations

CHT	Chitosan
GP	β-Glycerophosphate disodium salt
CCD	Central composite design
D	Desirability function

Introduction

Hydrogels are three-dimensional hydrophilic polymer networks capable of swelling in water and biological fluids, thus retaining large amounts of fluids in the swollen state. The water content in the hydrogel affects permeability, mechanical and surface properties and biocompatibility [1, 2]. According to this definition, films, membranes, spheres and particles made of certain polymers are examples of systems that can behave as hydrogels. In addition, gels obtained from polymer solutions, following the formation of new physicochemical links, behave as hydrogels due to the retention of water inside the polymer network.

Generally, hydrogels can be divided into two categories, namely conventional and smart. The latter are able to change in response to one or various external stimuli, such as pH, temperature, and electric and magnetic fields [3].

Hydrogels can be made from natural and synthetic polymers. Some of the natural polymers commonly used include collagen, hyaluronic acid, gelatin, cellulose, alginate and agarose. Synthetic polymers can be properly

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adjusted by chemical modifications to impart specific properties to the final obtained hydrogels. An important advantage is that the structure and the properties of synthetic polymers are highly reproducible with small batch-to-batch variation during preparation [4]. The most commonly used synthetic polymers are poly(ethylene glycol), poly(lactic-*co*-glycolide), poly(*N*-isopropylacrylamide) and poly(propylene oxide). Chitosan (CHT) is another natural polymer which is also commonly utilized as a support in gel-forming solutions [5–7].

Chitin, the second most abundant natural polysaccharide after cellulose, is a structural component of the exoskeleton of crustaceans and insects and of the cell walls of a variety of fungi. CHT is a biodegradable and biocompatible linear polymer composed of repeating units of glucosamine and *N*-acetyl-*d*-glucosamine, and it is obtained from chitin by partial deacetylation reaction [8, 9].

CHT hydrogels prepared with different methods are of great interest in cell encapsulation [7, 8], drug delivery [10, 11] and tissue engineering and repair [12]. In situ formed hydrogels, in general, can be prepared using ultraviolet irradiation, with thermosensitive properties or pH dependence. Photoreactive groups added to CHT molecules provide them the ability to form an insoluble and adhesive hydrogel after exposure to ultraviolet irradiation [13, 14]. CHT solutions containing a salt of polyol or sugar have the ability to turn into gel after a change in temperature [5, 7, 8]. Moreover, CHT solutions with pH-sensitive properties turn into gel after variations in pH conditions [10].

Since the appearance of CHT hydrogels in research and development, several attempts have been made to improve their properties. Previous works have focused on the preparation and characterization of thermosensitive hydrogels [15, 16], improvement of hydrogel capabilities as controlled drug delivery system of drugs [17–19] and proteins [20] and in vivo studies describing the behavior of the system in contact with normal or abnormal (tumoral) tissue [21–23].

Regarding thermosensitive CHT-based hydrogels, a method for its preparation includes a CHT solution and a salt of a polyol or sugar. Glycerophosphate salts (GP) are the most widely used for this type of hydrogels [5, 17, 24].

An overview of previous papers shows that some of the experimental factors, which can be modified in gel-forming solutions which affect hydrogel properties, include CHT and GP concentrations [6, 8], solution pH [6, 10], temperature at which CHT and GP are mixed [10, 24] and mixing time, [7] among others.

Optimization of CHT hydrogels for controlled drug release using statistical procedures has been reported [25–27]. Nevertheless, optimization studies to highlight the most significant preparation factors affecting gel properties have not been reported in the form of a statistical equation yet.

In this course of actions, the experimental design and optimization are the skills to examine different types of problems that arise within research, development and production. Screening experiments are performed to determine the experimental variables and interactions that have significant influence on the response. The goal in any optimization is to find out the conditions that produce the desired response. Statistical methods, including response surface methodology and artificial neural networks, are used in developing and optimizing polymeric systems for a wide range of applications [18, 28].

The multi-response problem consists of three stages: data collection, model building and optimization. On the optimization issue, that is, finding the set of input variables that ‘‘optimize’’ all responses simultaneously, several approaches have been proposed, including the desirability function [18, 29]. The desirability function approach transforms an estimated response into a scale-free value, called desirability. It ranges from 0 to 1, and increases as the corresponding response value becomes more desirable. The overall desirability, D, which also varies from 0 to 1, is determined by combining the individual desirability values. By maximizing D, the optimal set of input variables can be obtained. However, the goal of the optimization procedure is not to obtain a desirability value of exactly 1, but rather to obtain a set of response values that meet certain goals, specified by a given practical application. Desirability is only a mathematical method to find the optimum and was used in optimization procedures [28].

This article presents a statistical design carried out to develop models that describe the relationship between selected responses and independent experimental variables related to gel-forming solution preparation. The statistical models could be used to find out the experimental combinations of factors best suited to prepare gels for different potential applications.

Experimental

Materials

CHT was purchased from China Easter Group (China). β-Glycerophosphate disodium salt (GP) was kindly provided by Surfactan S.A. (Argentina). Acetic acid was PA > 99.5 % (Cicarelli, Argentina). The water was of Milli-Q quality. Isotonic phosphate buffer saline (PBS, pH 7.4) was prepared by dissolving 8 g of NaCl, 0.2 g of KCl, 0.2 g of KH_2PO_4 and 1.44 g of $Na_2HPO_4{\cdot}2H_2O$ in 1 L of water. All the salts used to prepare PBS and the potassium bromide were of analytical grades (Anedra, Argentina).

Experimental design, data analysis and desirability function calculations were performed with software Stat-Ease Design-Expert trial Version 7.0.0.

Solution preparation and gel characterization

CHT was dissolved in a 0.15 M acetic acid solution and GP solutions were prepared with water. After the polymer and the salt were completely dissolved, they were mixed (3:1, CHT:GP) at different temperatures and stirred for different lengths of time. The pH value of the final mixture was measured with a pH meter (Altronix TPX-III) before letting the solution turn into a gel at ambient temperature and 37.5 °C. A test tube inverting method was employed to determine the occurrence of solution-to-gel transition. The solution phase was defined as flowing mixture and the gel phase as non-flowing mixture when the test tube was inverted. The gelation point was determined by flow or no-flow criterion after 30 s with the test tube inverted [6]. Gelling time was measured with a chronometer.

For the evaluation of residual mass [15], a portion of the obtained gels was carefully blotted with filter paper to remove the excess of water from the surface and to be weighed. Then it was immersed into a bottle containing PBS. The bottles were maintained at 37.5 °C with agitation using an orbital shaker (100 rpm). At predetermined time points, the gels were removed, gently blotted and reweighed. The residual mass values were established after 7 days of immersion.

Gels were dried at 80 °C for 48 h. Subsequently, they were ground into powder and mixed with potassium bromide and compressed to obtain discs. Discs of CHT and GP were also prepared. The IR spectra were recorded on an FTIR-8001 PC Shimadzu spectrophotometer in the frequency range of 4000–400 cm^{-1} (spectral resolution: 4 cm^{-1}, number of scans: 40).

Rheological measurements were carried out with a Haake RheoStress RS80 rheometer. The elastic modulus (G′) and the viscous modulus (G″) as function of the temperature were determined from the oscillating measurements at a frequency of 1 Hz. The temperature was varied at the rate of 1 °C/min. The acquisition rate was set up at 1 point per 28 s.

Experimental design and optimization

A factorial design (2^4) at two levels was built to evaluate the main factors affecting the selected responses, i.e., pH of the gel-forming solution, gelling time at ambient temperature and 37.5 °C and residual mass. Two-level factorial designs in f factors require 2^f runs. So, increasing the number of factors greatly increases the number of runs. Due to the different interactions taking place during solution-to-gel transition, the gelation process and/or the final hydrogel network are affected by several parameters: pH of the CHT/GP system, concentration of GP, deacetylation degree, molecular weight and concentration of CHT and acid used for CHT dissolution, among others. In this work, the independent factors analyzed were: CHT concentration (1.67 and 2.67 w/w (%)), GP concentration (25.0 and 35.0 w/w %), mixing temperature (4 and 25 °C) and mixing time (1 and 3 min). The levels were selected based on previous knowledge and experiments. Higher CHT concentration was avoided because the viscosity of the solution was highly increased. With lower CHT and GP concentrations, a hydrogel is not obtained and its consistency is too weak to characterize. For a parameter such as temperature, both selected values can be easily handled and controlled in a laboratory. Higher temperatures are impossible to use due to the fact that they are very close to the temperature of transition. Lower temperatures could form crystals of water or other precipitates. Both the selected mixing times allowed the formation of a homogenous solution. Factors were considered statistically significant if the probability values were smaller than 0.05 and non-significant if the probability values were greater than 0.1.

The factors showing significant effects were then considered for a central composite design (CCD), consisting of 13 experiments, to develop statistical models for each of the studied responses. All gel-forming solutions were prepared following the methodology reported in the previous section. Finally, the desirability function was used to simultaneously optimize the responses.

All experiments were performed in random order to minimize the effects of uncontrolled factors that might introduce a bias on the measurements.

Results and discussion

Screening phase

The factorial design (2^4), built to find out the main factors involved in response evaluations, required a total of 16 experiments. Each of them consisted in the preparation of one gel-forming solution.

The analysis of variance test (ANOVA) was applied to experimental results. ANOVA showed that CHT concentration, GP concentration and mixing temperature were significant factors. These factors were considered for the optimization study in a central composite design. The pH value of the final mixture increased with increasing CHT and GP concentrations, but decreased with higher mixing temperatures. Similar results were reported by Supper et al.'s [7] study, in which raising the GP concentration led to an increase in the pH value of the system, which in turn induced reduction in both CHT protonation and intermolecular electrostatic repulsion, promoting solution-to-gel transition. Furthermore, increasing the polymer concentration allowed faster gelation, due to an increased number of

Table 1 ANOVA results of factorial design

Response	Probability value[a,b]				
	Model	[CHT] (w/w %)	[GP] (w/w %)	Mixing temperature (°C)	Mixing time (min)
pH	0.0037	0.0874 (+)	0.072 (+)	0.0006 (−)	0.6983
Gelling time at 25 °C (min)	0.0002	0.1721	0.0147 (−)	<0.0001 (−)	0.1026
Gelling time at 37.5 °C (s)	0.01	0.2637	0.0015 (−)	0.0024 (−)	0.1801
Residual mass (%)	0.0091	0.0293 (−)	0.0360 (−)	0.2861	0.2555

[a] Significant if $p < 0.05$

[b] Signs between parenthesis indicate the effect on the variable

Table 2 Central composite design

Factors			Responses			
[CHT] (w/w %)	[GP] (w/w %)	Mixing T (°C)	pH	Gelling time at 25 °C (min)	Gelling time at 37.5 °C (s)	Residual mass (%)
1.80	25.0	7.08	6.76	ND	300	64.55
2.53	25.0	21.92	6.72	80	300	41.48
2.53	35.0	7.08	6.9	100	270	39.36
2.17	37.1	14.50	6.94	10	135	58.94
2.17	30.0	25.00	6.75	83	225	62.41
2.17	30.0	14.50	6.85	35	165	52.88
2.17	30.0	14.50	6.84	33	150	44.72
1.67	30.0	14.50	6.84	52	180	57.38
2.17	22.9	14.50	6.86	35	210	55.06
2.67	30.0	14.50	6.87	25	165	46.94
1.80	35.0	21.92	6.83	85	165	41.97
2.17	30.0	4.00	6.77	60	225	91.91
2.17	30.0	14.50	6.88	30	135	48.26

ND not determined

CHT–CHT entanglements [7]. Gelling times were affected by both mixing temperature and GP concentration, showing decreasing values with increasing values of these factors. This result also agrees with those of Supper et al. [7]. In the same way, similar results were obtained for α-D-glucose 1-phosphate (DGP) instead of GP. The addition of DGP into the CHT solution increased the pH and decreased the gelling time [26]. Finally, residual mass values showed a decrease as the CHT and GP concentrations were increased.

Mixing time was not significant ($p > 0.05$) (Table 1). The probability values smaller than 0.05 obtained for the model indicated that it was properly selected.

Optimization procedure

CCD allowed the addition of more levels to the experimental domains and thus provided a better exploration of the design space. The CCD consisted of 13 gel-forming solution tests. The mixing time was 1 min, corresponding to the lower level in the screening experimental study. Table 2 shows the experimental design and the results of CCD.

The models that describe the relationship between the independent factors for every response were developed by backward elimination, while the model coefficients were validated by ANOVA. Table 3 shows the statistical parameters corresponding to the fitted models. The probability values of the selected models were smaller than 0.05 and indicated that the model terms were significant. The probability values of the lack of fit were not significant ($p > 0.05$) and indicated that the proposed models fitted well for a significance level of 95 %. The models can explain more than 90 % ($R^2 > 0.9$) of the variation in the response variable and can be used to navigate the design space. Table 4 shows the probability and coefficient values for response surface reduced models. Irrelevant terms were maintained to fit the hierarchical model. For this reason, hierarchical terms were added after backward elimination regression and were included in the final equation of the model. Model hierarchy maintains the relationships between the main effects

Table 3 ANOVA results of the fitted models

Responses	Model	R^2	Model		Lack of fit	
			p	Conclusion	*p*	Conclusion
pH	Reduced quadratic	0.9194	0.0046	Significant	0.3870	No significant
Gelling time 25 °C (min)	Reduced quadratic	0.9941	0.0029	Significant	0.1264	No significant
Gelling time 37.5 °C (s)	Reduced cubic	0.9680	0.0096	Significant	0.3415	No significant
Residual mass (%)	Reduced cubic	0.9223	0.0042	Significant	0.7195	No significant

p probability value significant if <0.05

Table 4 ANOVA for response surface reduced models

Source	pH		Gelling time at 25 °C (min)		Gelling time at 37.5 °C (s)		Residual mass (%)	
	p	Coefficient estimate	*p*	Coefficient estimate	*p*	Coefficient estimate	*p*	Coefficient estimate
Intercept		6.784		−2207.700		5698.228		−373.116
A	0.3579	−0.133	0.0202	695.349	0.5904	−2599.332	0.006	274.050
B	0.0707	−0.019	0.0248	65.135	0.0432	−173.871	–	–
C	0.6032	0.057	0.0309	77.101	1	−312.661	–	–
AB	0.2954	0.012	0.0080	−14.491	0.1123	83.561	0.0249	−10.815
AC	0.1059	−0.013	0.0010	−19.836	0.3214	148.696	0.0115	−25.503
BC	–	–	0.0011	−1.419	0.0668	9.491	–	–
A^2	–	–	–	–	–	–	–	–
B^2	–	–	0.0384	−0.248	–	–	0.0520	0.138
C^2	0.0011	-1.031×10^{-3}	0.0019	0.331	0.0129	0.564	0.0056	0.110
ABC	–	–	–	–	0.0051	−4.778	0.0019	0.942

A [CHT] (w/w %), *B* [GP] (w/w %), *C* mixing temperature (°C), *p* probability value

Table 5 Optimization parameters

Response	Criteria	Importance	Lower limit	Upper limit	Experimental optimized value
pH	Maximize	3	6.72	6.94	6.92
Gelling time at 25 °C (min)	Maximize	5	10	100	39
Gelling time at 37.5 °C (s)	Minimize	5	135	300	142
Residual mass (%)	Maximize	3	39.36	64.55	63.44

of two-factor interactions and three-factor interactions, etc. Subsequently, to model development, an optimization procedure was carried out. Four responses were simultaneously optimized using the desirability function. To apply the desirability function, researchers have to obtain their right priorities. Its application involves creating a function for each individual response (d_i) and finally obtaining an overall desirability function D that should be maximized. The latter function varies from 0 (totally undesirable value) to 1 (all responses are in a desirable range simultaneously), and can be defined by Eq. 1:

$$D = \left(d_1^{r_1} \cdot d_2^{r_2} \cdot \cdots \cdot d_n^{r_n}\right)^{\frac{1}{\sum r_i}} = \left(\prod_{i=1}^{n} d_i^{r_i}\right)^{\frac{1}{\sum r_i}}, \quad (1)$$

where d_n corresponds to the individual desirability functions for each response being optimized, n is the number of responses, and r is the relative importance of each response. In the desirability function D, to each response it can be assigned an importance relative to the other responses. Importance values (r_i) vary from 1 (the least important) to 5 (the most important) [29]. Table 5 shows the criteria established for optimization of the individual responses, the lower and upper limits and the importance assigned to each response, giving more importance to the gelling time.

For the pH value of the final mixture, the criterion was to maximize it, to obtain a pH as near as possible to the physiological value. Gelling time at 25 °C was maximized to delay solution-to-gel transition, aiming to obtain proper

handling and administration times. Gelling time at 37.5 °C was minimized to avoid solution leaching outside of the application site. Residual mass was maximized to allow the gel system to remain in the application site as long as possible, enabling the system to work more effectively.

The experimental conditions corresponding to one maximum in the desirability function ($D = 0.761$) were: 2.0 w/w(%) for CHT concentration, 35.0 w/w(%) for GP concentration and 8.12 °C for mixing temperature. Taking into consideration that four responses were simultaneously optimized, the obtained value of desirability was acceptable.

Figure 1 shows a plot of D as a function of a given pair of factors, while keeping the remaining one fixed at its optimal value. Desirability reached values near the optimum when the GP concentration was high. Moreover, desirability values were high for the lowest levels of CHT concentration and mixing temperature.

The theoretical response values corresponding to the selected set of conditions were: a 6.83 value for pH of the final mixture, 66.85 min for gelling time at 25 °C, 135.05 s for gelling time at 37.5 °C, and 65.8 % for residual mass. These theoretical responses were experimentally corroborated by preparing the optimized solution-to-gel mixture according to the conditions corresponding to the maximum in the desirability function mentioned above. Three replicas of the optimized mixture were evaluated.

Table 5 shows the experimental values obtained for every response when the optimized mixture was evaluated. For the response pH value of the final mixture, the experimental value obtained was near the upper limit, so the criterion established for optimization was achieved. The gelling time at 37.5 °C criterion was also attained, since the experimental value was near the lower limit for that response. As stated before, the criterion established for gelling time at 25 °C was to maximize it, to allow convenient handling and administration into the target tissue, delaying the gel formation. However, the experimental values obtained were considerably distant from the expected upper limit; they were substantially higher than the values obtained at 37.5 °C. This difference was significant ($p < 0.05$).

This difference between the theoretical and experimental values of the gelling times at 25 °C could be explained by considering the difficulties that arise in the gelling point determination. The transition to the gel state takes longer at this lower temperature and it is also more difficult to notice. This can lead to a systematic error inherent to the measurement of the gelling time. Other sources of systematic errors could arise from the environmental temperature (ambient temperature approximated to 25 °C), which could interfere with the solution-to-gel transition, and imperfect methods of cutoff test in gelling time. To overcome one of these drawbacks, in future studies a climatic chamber for constant conditions (25 °C) should be utilized.

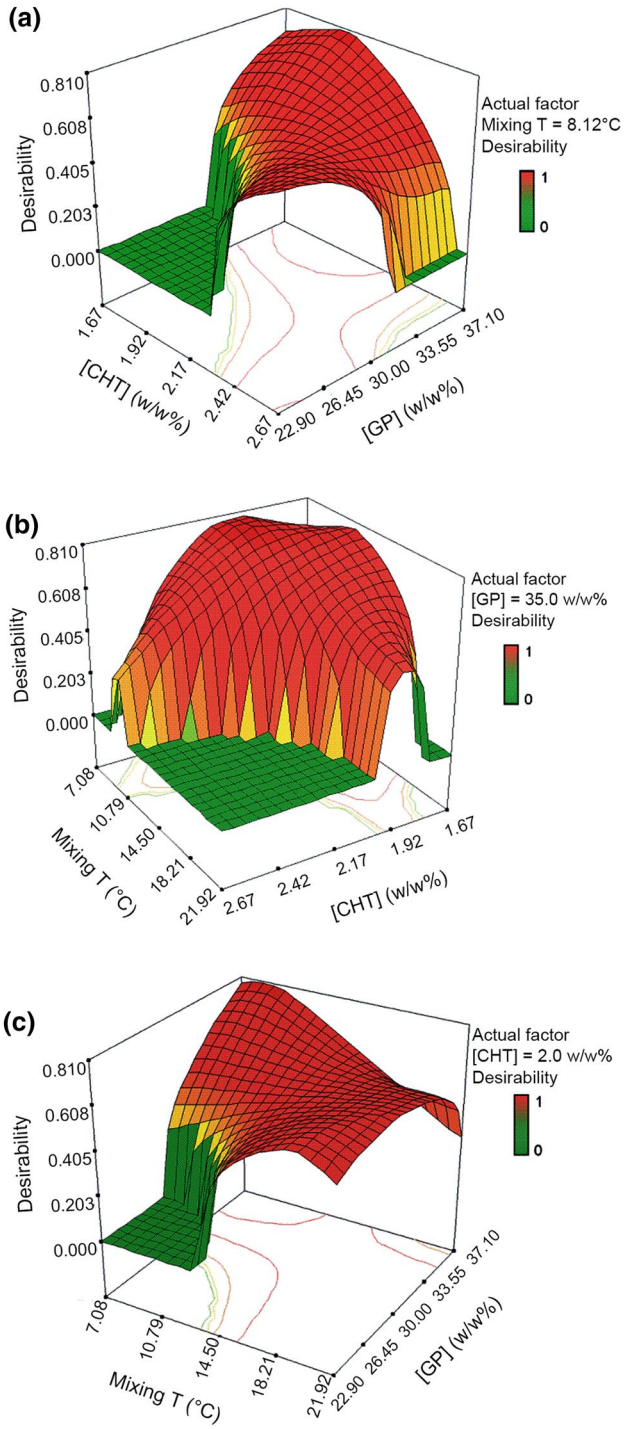

Fig. 1 Response surfaces corresponding to the desirability function for a given pair of factors, while maintaining: **a** mixing temperature, T; **b** β-glycerophosphate disodium salt concentration, [GP]; and **c** concentration of chitosan, [CHT], fixed at its optimal value

Finally, the residual mass criterion of optimization was achieved, since the experimental value was close to the upper limit of the response.

Fig. 2 Chitosan/β-glycerophosphate formulation at room temperature (*top*) and after solution-to-gel transition at 37 °C (*bottom*)

In view of the fact that the criteria established for optimization of the individual responses were almost entirely achieved, the whole optimization process was carried out successfully. The developed models can be used to prepare gel-forming solutions with desired pH, gelling times and residual mass after at least 1 week of exposition to a physiological environment. Figure 2 shows the mixture at room temperature (solution) and at 37.5 °C (gel). Optimization allows the control on the whole process: preparation of gel-forming solutions and in situ gelling. An application of thermosensitive hydrogel would be in the incorporation of anti-cancer drugs for the treatment of solid tumors and prevention of metastasis and tumor re-growth. The drug release process from hydrogel is affected by composition of the CHT/GP solution, pH, temperature as well as the nature of the drug among other factors. The statistical models developed in this work represent an important tool for the mentioned application. Gel-forming solutions to be injected into a tumor or in a tumor resection site after a surgery could be properly formulated based on the optimization process carried out in this work. Researchers can use the statistical models presented in this work to develop platforms for drug delivery, taking into consideration the levels of the significant factors analyzed and if needed making adjustments to the gel-forming solution preparation.

Residual mass values were established after 7 days of immersion. Figure 3 shows the residual mass as a function of time for randomly selected examples of gels from the factorial design (Fig. 3a) and CCD (Fig. 3b). During previous studies and factorial design experiments, it was observed that the curves presented a drop in residual mass up to the first 24 h. Subsequently, mass loss remained stable. However, to ensure a very good estimation of the response during the whole optimization procedure, the cutoff time to establish the response was 7 days. Residual mass versus time plots presented the same shape in all the experiments carried out; therefore the assumption was correct. In addition, these results agree with the findings of Ruel-Gariepy et al. [15]. These authors reported that approximately 40 % of the total weight was lost at the end of the study. Our findings reported that approximately 40 and 50 % of the total weight was lost at the end of the factorial design and CCD experiments, respectively.

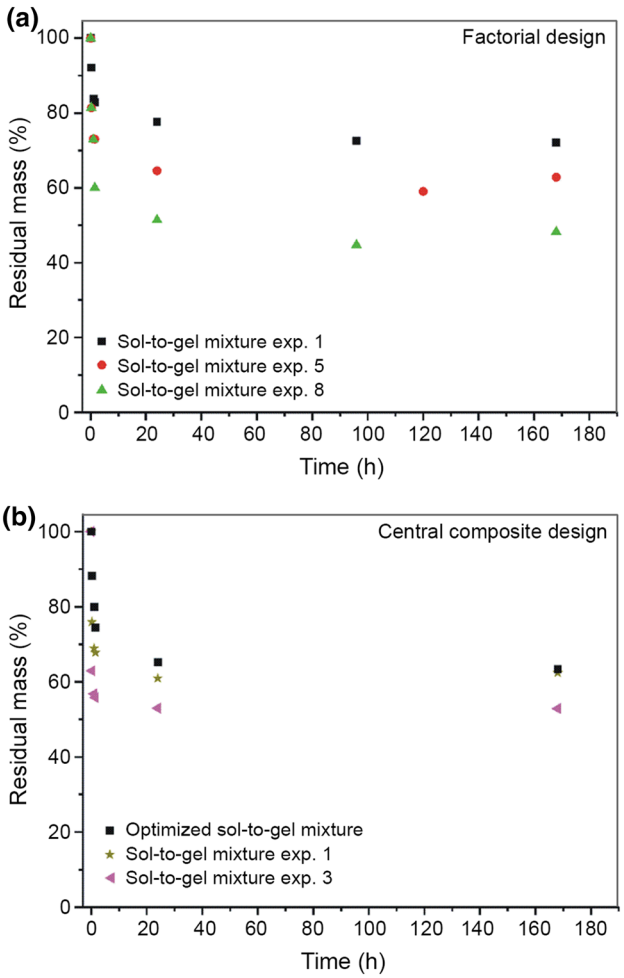

Fig. 3 Residual mass as a function of time for random selected examples of gels from factorial design (**a**) and central composite design (**b**)

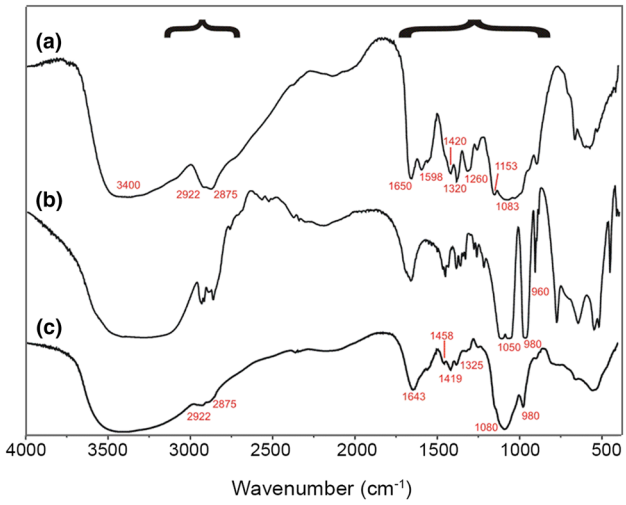

Fig. 4 IR spectra of chitosan (**a**), β-glycerophosphate (**b**) and optimized gel (**c**)

Gel characterization

Figure 4 shows the IR spectra of CHT, GP and the optimized gel. The regions where the changes in the spectra were notable are indicated with a bracket. The spectrum of CHT presents a strong and broad band centered at about 3400 cm^{-1} as a result of the overlap of the –OH and –NH stretching vibrations. These groups can be involved in the formation of inter- and/or intramolecular hydrogen bonds, which play an important role in the solution-to-gel transition. The CHT spectrum also exhibits the distinctive absorption bands at 1650 cm^{-1} (C=O stretching vibration in amide I), 1598 cm^{-1} ($–NH_2$ bending in non-acetylated 2-aminoglucose primary amine) and 1560 cm^{-1} (–NH bending in amide group, amide II vibration). Absorption bands at 1153 cm^{-1} (anti-symmetrical stretching of the C–O–C bridge), 1083 and 1031 cm^{-1} (skeletal vibrations involving the C–O stretching) are characteristics of the CHT structure. The bands at 2922, 2875, 1420, 1320 and 1260 cm^{-1} belong to symmetrical and anti-symmetrical –CH and –OH vibrations of the carbohydrate ring [30]. Analyzing the spectrum of the optimized gel, two bands of CHT at 2922 and 2875 cm^{-1} appear, but almost bound in a minor shoulder in comparison with the CHT spectrum. The peak strength of C=O, –OH and –NH stretching bands changed after the solution-to-gel transition. This is an indication of the occurrence of hydrogen bonds between C = O of CHT and –OH of GP, and –NH of CHT and –OH of GP. These results agree with those of Zhou et al. [6]. In addition, the C=O stretching peak displayed a slight shift toward a lower wavenumber and constituted one peak with those corresponding to NH bending. This indicates electrostatic attraction between the protonated amino groups of CHT and the phosphate groups of GP [31]. Other types of interactions apparently involved in the heat-induced gelation process are: hydrophobic interactions and hydrogen bonding between the CHT molecules. The negative charges of GP neutralize the electrostatic repulsion between the polymer chains and then increase the hydrogen bonding interactions between the CHT molecules [7]. The peaks at 1420, 1320 and 1260 cm^{-1} corresponding to –OH and –CH bending and C–O stretching of the polymer molecule were shifted to higher wavenumber in the optimized gel [31]. In addition, there were significant changes in the spectral shape from 900 to 1250 cm^{-1}. In this region, characteristic peaks of GP emerged. The band at 1050 cm^{-1} indicates aliphatic P–O–C stretching, the band at 980 cm^{-1} is characteristic of the $–PO_4^{2-}$ group, whereas the band at 960 cm^{-1} may indicate the presence of the $–HPO_4^{-}$ group. In the spectrum of the optimized gel, two characteristic bands for GP appear in this region at 1080 and 980 cm^{-1} [31]. Therefore, changes can be attributed to the formation of bonds between CHT and GP during solution-to-gel transition. Figure 5 shows a schematic representation of the expected sol-to-gel transition.

The elastic modulus (G') and the viscous modulus (G''), as a function of temperature, were determined (Fig. 6). Upon heating between 5 and 50 °C, a sharp increase of the elastic modulus (G') near 37 °C was observed. This increase indicated the solution-to-gel transition.

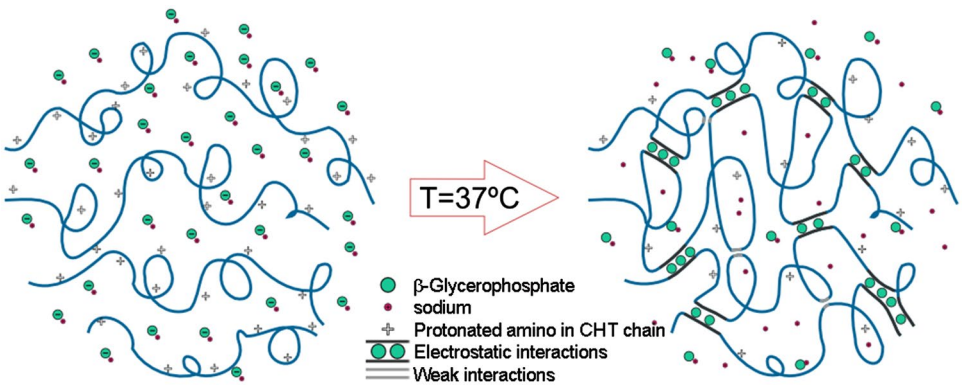

Fig. 5 Schematic representation of expected sol-to-gel transition

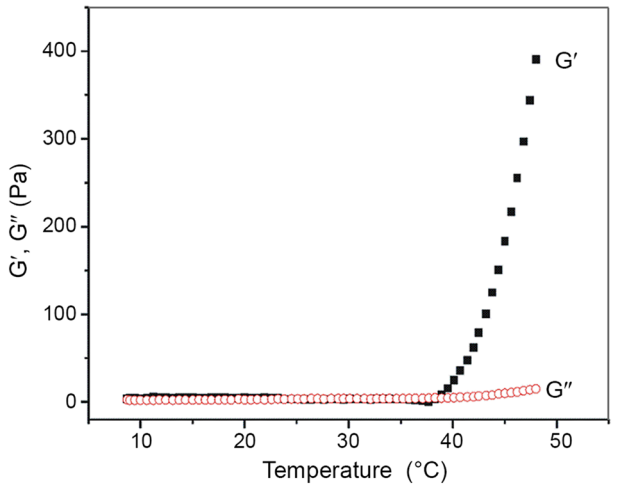

Fig. 6 Elastic modulus (G') and viscous modulus (G'') as a function of temperature for the optimized gel

Similar results were reported by Chenite et al. [16]. According to these authors, the strength of the gel can be appreciated by the magnitude of G'. In the current case, it was in the range of hPa (0–400 Pa). Also, the great difference between G' and G'' indicates a strong gel [16].

Conclusion

An optimization was achieved in the preparation of thermosensitive CHT gel. Firstly, a factorial design was built to evaluate the main factors affecting the pH of the gel-forming solution, gelling time points at ambient temperature and at 37.5 °C and the residual mass. The study of independent factors included CHT concentration, GP concentration, mixing temperature and mixing time. ANOVA showed that CHT concentration, GP concentration and mixing temperature were significant factors. These factors were considered for an optimization study in a central composite design to develop statistical models for each of the responses under study. Finally, a desirability function was used to simultaneously optimize the responses. The resulting equations were used to prepare gel-forming solutions with favorable pH, gelling time, and residual mass as was demonstrated in this work. The importance of these equations lies in their potential use for the design of hydrogels applied in controlled drug delivery. In addition, the FTIR spectrum of the system after sol-to-gel transition presented characteristic peaks for CHT and GP. This result suggested interactions between the polymer molecules and between the polymer and the salt. Hydrogen bonds and electrostatic attraction were the type of interactions observed. Gel-forming solutions to be injected into a tumor or in a tumor resection site after a surgery could be properly formulated thanks to the optimization process carried out in this work.

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