EFFECT OF SELECTED MUCOADHESIVE POLYMERS ON PHARMACEUTICAL PROPERTIES OF CHITOSAN FORMULATIONS

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Abstract

The aim of this study was to develop a technical process and composition of mucoadhesive hydrogels containing benzocaine, based on different concentration ratios of the natural polymers chitosan and xanthan gum. For this purpose, lyophilisates of polymeric complexes with the quantitative ratios of 0.5:1, 1:1 and 1:0.5 chitosan to xanthan gum were prepared and subsequently used to prepare hydrogels of various concentrations. The physicochemical properties and pharmaceutical availability of benzocaine were evaluated and diffractograms and Fourier-transform infrared spectra of individual polymers and their polyelectrolyte complexes were compared. The 1:1 formulation exhibited the highest water absorption capacity and the gels showed the highest viscosity and the shortest blurring times. More chitosan increased carrier texture parameters, including hardness, cohesiveness and consistency, whereas more xanthan gum led to the longest gel blurring times and improved carrier stability. The concentration ratio of chitosan to xanthan gum in lyophilisates determined the viscosity, texture, spreadability and blurring time of the gels. Increases in lyophilisate percentage in the gels also affected the physicochemical properties of the carrier. In addition, the proportions of polymers in the mixture did not influence the availability of the drug from the prepared gel; this factor appears to depend more on the lyophilisate content in the carrier. Variations in the ratio of chitosan to xanthan gum in the polymer complex as well as lyophilisate percentage in the gel may impact the properties of the hydrogel and its efficacy as a carrier for therapeutic substances administered to the oral cavity mucosa.

Keywords*: chitosan, xanthan gum, mucoadhesive polymers, hydrogel, benzocaine*

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1. Introduction

Mucosal disorders such as aphthae, erosions or ulcers that occur in the mouth, as well as irritation or mechanical damage to the mucosa caused by braces and dentures, are common causes of discomfort and pain reported by patients. When the lesion is restricted to a small area of the mucous membrane, medical treatments with mucoadhesive properties may present an appropriate solution [1, 2]. Gels or hydrogel films consisting of mucoadhesive polymers can act as carriers for the local administration of therapeutic substances, such as anti-inflammatories, analgesics or topical anaesthetics, to the oral mucosa [3-5].

Two such polymers, chitosan and xanthan gum, are used as components in hydrogel carriers. Their combination in the preparation of such carriers may enhance desirable physicochemical properties for the delivery of therapeutic agents. Chitosan, a polymer of natural origin, is biocompatible, non-toxic, biodegradable and has antimicrobial properties. Xanthan gum is a binding agent and its co-administration with various drug forms may extend the release of active substances [6-8].

The aim of this study was to optimise the preparation and composition of gel carriers based on lyophilised polyelectrolyte complexes of chitosan with xanthan gum. This first involved the preparation of three lyophilised formulations containing different ratios of chitosan to xanthan gum. In addition, hydrogels consisting of 1%, 2% or 3% of the above-mentioned formulations were prepared and analysed as carriers for the topical application of the anaesthetic drug benzocaine. All lyophilisates and hydrogels were subjected to physicochemical tests to select the carrier with the optimal rheological and textural properties and the pharmaceutical availability of benzocaine.

2. Materials and Methods

2.1. Materials

Benzocaine (Fagron, Rotterdam, the Netherlands) was incorporated into natural, highly purified chitosan (high molecular weight, > 75% deacetylated) obtained from Merck (St. Louis, MO, USA). Xanthan gum was provided by CP Kelco (Atlanta, GA, USA). Glacial acetic acid (99.5%-100.5%) was provided by J.T. Baker. Purified water complying with finished product (FP) XI pharmacopoeia monographs was used in the tests.

2.2. Methods

2.2.1. Preparation of Polymeric Combinations of Chitosan and Xanthan Gum

The appropriate quantities of chitosan were dissolved in 1% acetic acid, with stirring at 150 rpm. An aqueous solution of xanthan gum was added dropwise to the chitosan solution while stirring at 150 rpm. The compositions of the chitosan and xanthan gum formulations used to prepare the polymer complexes are shown in Table 1.

The polymer complexes were drained after 24 h, frozen at -30°C and freeze-dried for 72 h at 9°C and 0.5150 mbar pressure. The lyophilised powder was passed through a 0.315-mm sieve to standardise the grain size. Photographs of the formulations in consecutive stages of preparation are shown in Figure 1.

2.2.2. Hydrogel Preparation

Gels composed of 1%, 2% or 3% lyophilised polymer complex were prepared by mixing the appropriate amount of lyophilisate with the appropriate quantity of purified water while stirring at 50 rpm. Benzocaine was added to the resulting hydrogels (2 g/100 g), achieving a 2% concentration of the therapeutic substance, as shown in Table 1.

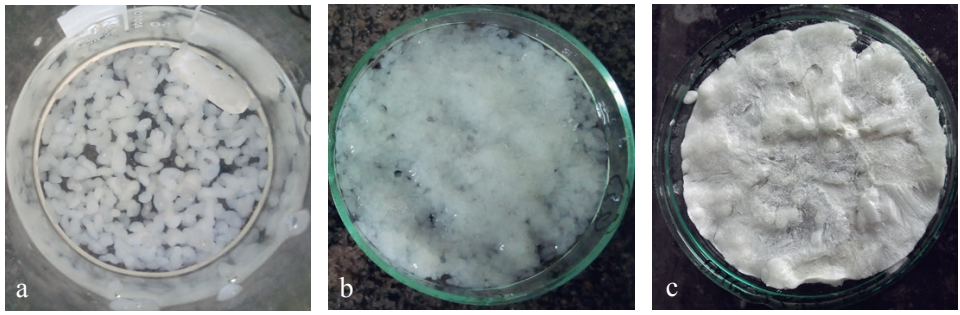

Figure 1. Images of chitosan-xanthan gum hydrogel: (a) the polymer complexes in solution, (b) the polymer complexes after filtration and (c) the polymer complexes after freeze drying

2.2.3. Swelling Ratio of Lyophilised Complexes

To characterise the swelling coefficients of lyophilised complexes, samples (4 mg powder) of each formulation were applied to plates on an area of 1 cm^2. Water (37°C) was added dropwise until a total mass of 100 mg was obtained; the mixture was then left to swell. Excess unabsorbed water was drained off using tissue paper and the sample was weighed again. The average rates of swelling of the lyophilised polymer complexes were calculated based on the formula:

$$W_p = \frac{m_w - m_s}{m_s} \times 100,$$

where W_p is the factor of swelling (%), m_w is the mass of lyophilisate with absorbed water (g) and m_s is the mass of dry lyophilisate (g).

2.2.4. Hydrogel Blurring Time Test

To estimate the blurring time, 1 ml of gel was added to 30 ml of distilled water in a beaker and shaken at 37°C at 100 shakes per min on a thermostat shaker (WB22, Memmert, Germany). The test results are presented in Table 1.

2.2.5. Dynamic Viscosity Test

Dynamic viscosity tests were carried out by means of a rotational viscosimeter (Rheotest 2 Medingen, Ottendorf-Okrilla, Germany) at 37°C using a K-1 cone with a diameter of 36 mm. These measurements were used to calculate the values of shear stress and viscosity. The test results are presented in Table 1.

2.2.6. Consistency Test

Texture profile analysis (TPA) was performed on a TA.XTplus texture analyser (Stable Micro Systems, Godalming, UK) using the Exponent software. The texture of the hydrogel was set by examining the hardness, consistency and density using the back extrusion method. The test parameters were as follows: force of 1 gravity, velocity of 2 mm/s, distance of 20 mm and disc diameter of 35 mm [9].

2.2.7. Pharmaceutical Availability of Benzocaine From Hydrogels

The pharmaceutical availability of benzocaine from hydrogels was investigated based on the paddle dissolution method, using a Vankel VK 7025 dissolution apparatus with

Table 1. Swelling, blurring and viscosity parameters of lyophilised hydrogels determined at 37°C and shear rates of 4860 s^{-1}

Formulation		Swelling coefficient of lyophilisate W_p (average %)	Blurring time T_{av} ± SD (min)	Shear stress (N/m^2)	Viscosity (mPa·s)
Gels at 1% concentration	CH0.25+XG0.5 (0.5:1)	24.42	73.67 ± 2.52	1076.95	22.16
	CH0.5+XG0.5 (1:1)	72.17	8.67 ± 2.08	3060.00	62.96
	CH0.5+XG0.25 (1:0.5)	37.08	68.33 ± 2.52	1700.00	34.98
Gels at 2% concentration	CH0.25+XG0.5 (0.5:1)	24.42	95.00 ± 2.00	1700.00	34.98
	CH0.5+XG0.5 (1:1)	72.17	10.33 ± 1.53	4080.00	83.95
	CH0.5+XG0.25 (1:0.5)	37.08	85.67 ± 2.08	2295.00	47.22
Gels at 3% concentration	CH0.25+XG0.5 (0.5:1)	24.42	132.67 ± 2.52	2210.00	45.47
	CH0.5+XG0.5 (1:1)	72.17	8.67 ± 1.53	7990.00	164.40
	CH0.5+XG0.25 (1:0.5)	37.08	97.67 ± 2.52	2720.00	55.97

Abbreviations: CH, chitosan; SD, standard deviation; XG, xanthan gum.

a Varian fractional collector (Agilent Technologies, Santa Clara, CA, USA). Gel samples (5 g each) were placed in chambers containing 500 ml of purified water at 37°C and mixed at 100 rpm. Over a period of 150 min, 3-ml samples were collected at 12 time intervals (5, 10, 15, 20, 30, 40, 50, 60, 80, 100, 120 and 150 min). The collected samples were passed through filters with a 10-µm pore size.

Each sample was properly diluted, transferred into a 1-cm cuvette and the absorbance was measured at 284 nm using a V-650 UV-Vis spectrophotometer (JASCO, Pfungstadt, Germany). The concentration of benzocaine in the samples and the average percentage of dissolved benzocaine were calculated by linear regression.

2.2.8. X-Ray Powder Diffraction (XRPD)

XRPD patterns for powders consisting of each pure polymer, polymer mixtures and freeze-dried complexes were recorded on a D2 phaser X-ray diffractometer with a LynxEye detector (Bruker, Billerica, MA, USA), employing CuK∞ radiation at room temperature. The samples were scanned from 5 to 80° (2θ) at a scanning rate of 0.05° per 0.5 s for each step [10].

2.2.9. Fourier-Transform Infrared Spectroscopy (FTIR)

FTIR spectra of individual polymers, polymer mixtures and freeze-dried complexes were obtained using a Spectrum Two FT-IR spectrometer (PerkinElmer, Waltham, MA,

Table 2. Texture parameters and the spread of hydrogels

Formulation		Hardness (g)	Consistency (g)	Density (g·s)	Wheel field (cm^2)
Gels at 1% concentration	CH0.25+XG0.5 (0.5:1)	17.79	55.96	4.04	22.90
	CH0.5+XG0.5 (1:1)	19.39	61.71	4.60	28.63
	CH0.5+XG0.25 (1:0.5)	25.79	69.07	7.20	24.63
Gels at 2% concentration	CH0.25+XG0.5 (0.5:1)	28.05	76.12	7.31	21.24
	CH0.5+XG0.5 (1:1)	32.04.	86.82	6.99	16.62
	CH0.5+XG0.25 (1:0.5)	37.85	92.04	7.12	15.21
Gels at 3% concentration	CH0.25+XG0.5 (0.5:1)	39.22	98.64	11.22	19.63
	CH0.5+XG0.5 (1:1)	41.09	102.43	11.02	13.85
	CH0.5+XG0.25 (1:0.5)	49.57	125.69	15.15	15.21

Abbreviations: CH, chitosan; SD, standard deviation; XG, xanthan gum.

USA). The spectra were collected in the range of 450 to 4000 cm^{-1}, using an attenuated total reflection (ATR) sampling mode [11].

3. Results and Discussion

3.1. Influence of Lyophilisate Formulations on Viscosity Parameters and Textures of Hydrogels

As shown in Table 1, the concentration ratios of chitosan to xanthan gum influenced the swelling rate of the lyophilisates. The highest percentage of absorbed water was 72.12%, observed for the 1:1 chitosan to xanthan gum formulation (CH0.5+XG0.5). The other formulations (CH0.25+XG0.5 and CH0.5+XG0.25) exhibited less lyophilisate swelling.

Regardless of the gel concentration, the longest average blur times were observed in lyophilisate-based carriers with a lower chitosan to xanthan gum ratio. The blurring times for formulation CH0.25+GK0.5 in 1%, 2% and 3% gels were 73.67, 95.00 and 132.67 min, respectively. The shortest blurring time was observed for the carriers containing equal amounts of chitosan and xanthan gum (CH0.5+XG0.5).

Analysing the rheological properties of the gels, the 3% gel containing equal quantities of chitosan and xanthan gum (CH0.5+XG0.5) exhibited the optimal viscosity. The 1% gel composed of CH0.25+XG0.5 showed the lowest values in terms of shear stress and viscosity of all the formulations. It is evident that the concentration ratio of the polymers used has an impact on the rheological properties of the hydrogel. A reduction in either the chitosan or xanthan gum concentration causes a decrease in gel viscosity, whereas

combining them in equal concentrations produces a carrier with the most favourable rheological characteristics, including gel blurring time, swelling coefficient and viscosity, which are closely related parameters that determine the physicochemical properties of the drug.

Based on the above results, it is evident that a high lyophilisate swelling coefficient and low gel viscosity translated into an increase in blurring time. The CH0.5+XG0.5 formulation showed the highest degree of swelling, that is, the best water absorption, and gels prepared using this formulation were quickly blurred due to the loosening of the structure. The CH0.25+XG0.5 formulation exhibited the lowest degree of water absorption and the longest gel blurring time of all the formulations. The texture parameters of the gels were indicative of their potential for therapeutic application. High cohesiveness of the carrier conveys high resistance to damage, while hardness and consistency translate into ease of application of the drug formulae. Based on the results of the texture test (Table 2), it is evident that the values of the tested parameters increased as the percentage of lyophilisate in the gel increased. The carrier with the optimal texture parameters – hardness, cohesiveness and consistency – was the 3% gel prepared from the CH0.5+XG0.25 formulation. A lower proportion of chitosan in the polymer complex led to a reduction in the values of texture parameters, especially hardness and consistency, while an increase in the rubber concentration resulted in reduced cohesion. Among all the gels investigated, the optimal diffusion (with a mean of 28.27 cm^2) was observed for the 1% gel composed of the CH0.5+XG0.5 formulation. This carrier exhibited the best extensibility, a parameter related to the consistency of the gel. This property highlights its potential for application for local oral drug administration. With an increase in chitosan concentration, there was an increase in the swelling of freeze-dried gels, an increase in gel viscosity and a decrease in their blending time. Decreasing the percentage of chitosan in polymer blends resulted in decreasing the values of texture parameters, especially hardness and consistency.

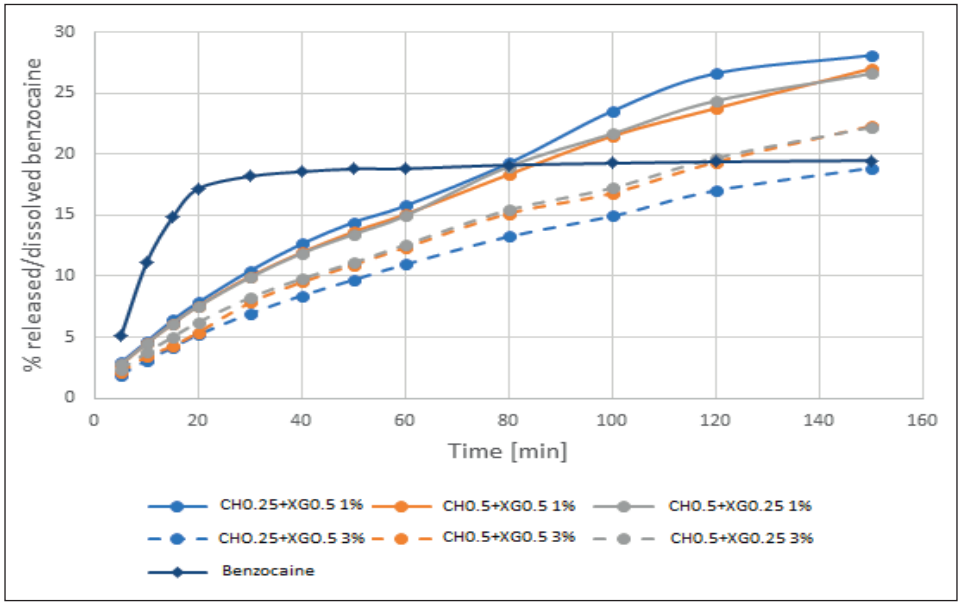

Figure 2. Dissolution and release profiles of benzocaine from 1% and 3% lyophilisate gels with different chitosan (CH) to xanthan gum (XG) ratios

3.2. Evaluation of Benzocaine Release from Gels

The release rate profiles of benzocaine are shown in Figure 2; the dissolution rate of pure benzocaine was used as a reference to compare with the release rate of benzocaine infused in hydrogels. Benzocaine dissolved gradually, in a time-dependent manner, from 5.05% to 19.48% of the tested dose. The pharmaceutical availability of benzocaine released from the carriers depended inversely on the lyophilisate concentration of the gel: an increased lyophilisate content in the carrier resulted in decreased release of benzocaine. Based on benzocaine release curves, after 150 min the 1% gel prepared from the CH0.25+XG0.5 formulation had the highest average percentage of released benzocaine (28.12%).

Benzocaine was released according to first-order kinetics. Comparing the profiles of benzocaine release from gels with the outcomes of solubility tests, it becomes apparent that infusion in 3% gels decreased the rate of benzocaine release, while infusion in 1% gels seemed to improve the solubility of the drug. Prolonging therapeutic substance release from the gel is a desirable feature of mucoadhesive carriers. This property enhances the therapeutic effect of the drug by extending the duration of its contact with the pathologically affected area on the oral cavity mucosa.

3.3. FT-IR Spectra of Chitosan, Xanthan Gum and Their Complexes

FT-IR spectra for chitosan, xanthan gum and complexes of both these agents are shown in Figure 3. The FT-IR spectrum of chitosan shows a wide absorption band at 3300 cm^{-1}, which illustrates the stretching vibrations between N-H and O-H. An absorption band at 2900 cm^{-1} corresponds to the tensile vibrations of the C-H bonds in the pyranose ring. A peak visible at 1650 cm^{-1} results from the C=O bond vibrations in the amide group, and a peak at 1580 cm^{-1} shows the N-H bond vibrations in the amide group. An intensive band at 1150 cm^{-1} can be associated with glycosidic bonds present in the molecule (lithium). The xanthan gum spectrum is characterised by a wide absorption band

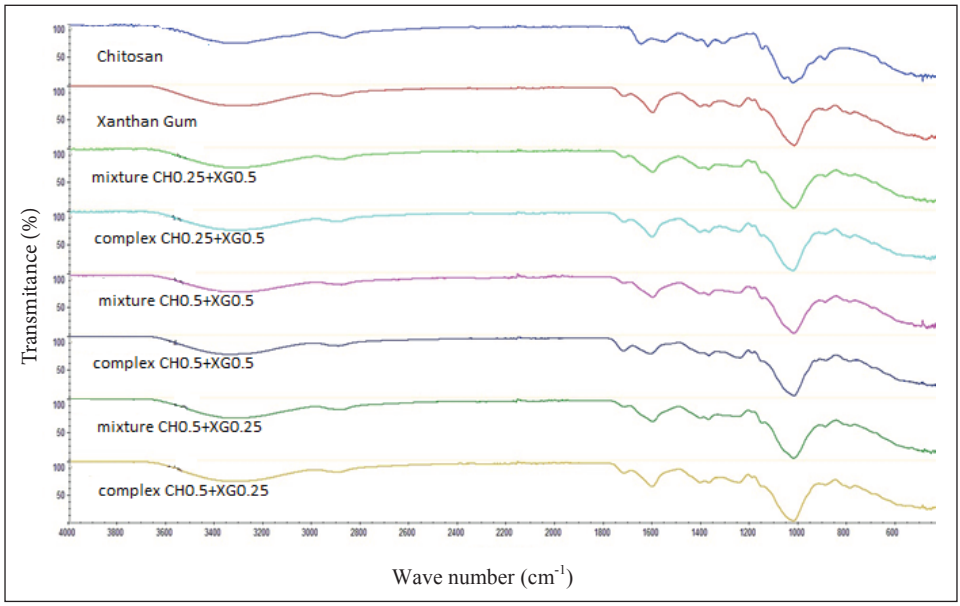

Figure 3. Fourier-transform infraredspectra of mixtures and complexes of chitosan (CH) with xanthan gum (XG)

at 3300 cm^{-1} corresponding to the stretching vibrations of bonds in the hydroxyl group. A peak at 2900 cm^{-1} represents vibrations of C-H bonds, a band at 1700 cm^{-1} corresponds to the vibrations of C=O bonds of ester and carboxylic groups, while a peak visible at 1600 cm^{-1} represents the vibrations C=O of the enol (β-diketone) group. A clear band at 1050 cm^{-1} is an effect of C-O (lithium) bond vibrations.

The analysis of FT-IR spectra of chitosan-xanthan gum complexes and their lyophilisates did not reveal additional peaks that could have indicated interactions which result in new structural properties.

3.4. Diffractograms of Chitosan, Xanthan Gum and Their Combinations

The diffractograms of chitosan and xanthan gum and their combinations are shown in Figure 4. Diffraction peaks for chitosan at angle 2θ can be detected at 9-10° and 19-20°. For xanthan gum, a single, broad, low intensity peak is visible at 18-20°. The diffractograms observed for polymer complexes and their lyophilisates did not show additional peaks that could indicate changes in the polymer structure and confirm the amorphous nature of both individual polymers and polymer complexes.

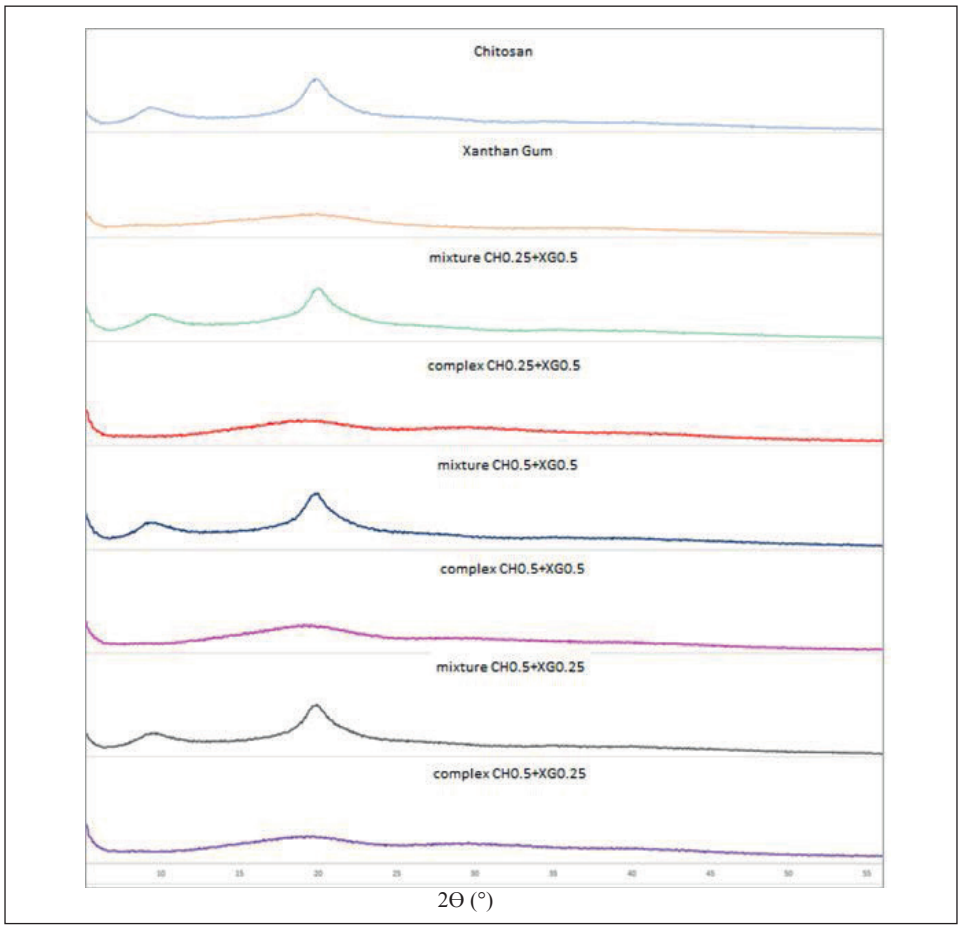

Figure 4. X-ray diffraction spectra of mixtures and complexes of chitosan (CH) with xanthan gum (XG)

4. Conclusions

In conclusion, lyophilisate prepared with a 1:1 ratio of chitosan and xanthan gum exhibited the highest water absorption capacity and hydrogels prepared from this formulation exhibited the highest viscosity. The texture parameters (hardness, consistency and cohesiveness) of carriers increased as the chitosan concentration increased in the formulation and with an increase in lyophilisate percentage in the gel. Similarly, the blurring time of hydrogels depended on the concentration ratios of polymers in the formulation and the percentage of lyophilisate in the gel. Finally, the pharmaceutical availability of benzocaine decreased as the lyophilisate content in the gel increased. The carrier that slowed the drug release to the greatest extent was the 3% gel made of CH0.25+GK0.5, whose benzocaine release rate was lower than its dissolution rate throughout the study. Prolongation of the release time of the drug substance from the gels is a desirable feature of mucoadhesive carriers, allowing an increase in the therapeutic effect of the drug by increasing its contact time with the pathologically altered site on the oral mucosa.

5. Acknowledgements

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6. Literature

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