RESEARCH ARTICLE

Evaluation of the methylene blue addition in binary polymeric systems composed by poloxamer 407 and Carbopol 934P using quality by design: rheological, textural, and mucoadhesive analysis

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ABSTRACT

This study describes the investigation about the physicochemical behavior of methylene blue (Mb) addition to systems containing poloxamer 407 (Polox), Carbopol 934P (Carb), intended to be locally used by photodynamic therapy. A factorial design 2³ (plus center point) was used to analyze the rheological, mucoadhesive and textural properties of the preparations. Systems containing the lower concentrations of Polox (15 and 17.5%, w/w) exhibited pseudoplastic flow and low degrees of rheopexy. On the other hand, at higher Polox concentration (20%, w/w) the systems display plastic flow and thixotropy. Carb and Mb exhibited a negative influence for the consistency and flow behavior index, due to the interaction between them. For most of the formulations, the increase of Polox and Mb content significantly increased storage modulus, loss modulus and dynamic viscosity. The systems display a sol–gel transition temperature, existing as a liquid at room temperature and gel at 29–37°C. Increasing the temperature and the polymer concentration, the compressional properties of systems significantly increased. The mucoadhesion was noted to all formulations, except to systems composed by 15% (w/w) of Polox. The analyses enabled to understand and predict the performance of formulations and the polymer–Mb interactions, tailoring to the suit systems (Polox/Carb/Mb): 17.5/0.50/0.20 and 20/0.15/0.25.

Introduction

Methylene blue (Mb) is a cationic photosensitizer (PS) from the phenothiazine class, which displays excitation between 600 and 900 nm (therapeutic window) ensuring an efficiently light penetration into biological tissue. Mb is widely used in different fields of medicine, due to its low dark toxicity. Additionally, many studies have shown the efficacy of the aqueous Mb solution associated with photodynamic therapy (PDT) in the treatment of the skin, nails or mucous membrane infections caused by bacteria and fungus. PDT consists in a triad of light, oxygen and PS, to generate reactive oxygen species (ROS) and singlet oxygen (¹O₂). The cytotoxic products generated by PDT trigger a cascade of molecular events that lead to chemical injury of the cells (apoptosis and/or necrosis) and selective tissue destruction.

However, aqueous Mb solution displays many disadvantages, as not remain at the site of application without drain, promoting the generation of Mb dimer and not favoring the formation of ¹O₂. Moreover, the clinical efficacy of PDT is intrinsically dependent on the physicochemical characteristics of the formulation, such as the ease of administration, good retention at/in the target tissue for the desired period of light application, biodegradability, no toxicity, and to be nonirritant. Therefore, it is necessary to find new pharmaceutical dosage forms to provide these properties.

In this context, bioadhesive polymeric systems offer many advantages when compare to the conventional formulations, enabling to promote a uniform and selective distribution of the active agent on the lesion. This pharmaceutical strategy allows an interaction between the polymeric systems and the biological substrate, e.g. mucus-coated tissue and in this case, the system is denominated as mucoadhesive. They are capable of improving on the contact with the administration site and the residence time. Consequently, it is possible to enhance the bioavailability of active agents, decreasing the number of doses as well as the toxicity, and in situ systems cause less irritation/pain during the administration.

In addition, the advance on research and production of environment-sensitive polymers enabled the development of thermoresponsive systems, one of the in situ forming systems and based on the increase of viscosity by the temperature variation, especially on contact with the body temperature. One of the most common polymer classes used for this goal is that of the poloxamers. Poloxamer 407 (Polox) is a nonionic co-polymer composed by ethylene oxide (EO) and propylene oxide (PO) arranged of triblock structure EO–PO–EO, presenting amphiphilic characteristics. Polox can promote the formation and organization of micelles, and consequently, the conversion of the fluid phase (sol) in semi-solid phase (gel) when it reaches the gelation temperature (Tg). However, this characteristic can be modified by the presence of actives and/or additives, which can interfere on the formation of micelles and dewatering of PO units.

The association of Polox with bioadhesive polymers (such as carbomers) is very common and can improve the bioadhesive...
features of formulations. This strategy is important when it is necessary to increase the residence time of the system at the site of application. This association was extensively investigated as bio/mucoadhesive platforms for the enhanced delivery of natural or synthetic drugs and to improve both the ease of administration and retention time of formulations at the administration site. In this sense, we have investigated the use of Polox and Carbopol 934P (Carb) as a binary mixture on the development of functional platforms for Mb delivery in PDT. The optimized systems did not show skin permeation and were enabled to produce ROS in a satisfactory amount for PDT. However, the mechanical and mucoadhesive properties of these binary polymeric systems after the addition of Mb were not fully understood. This knowledge is very important to understand the behavior of the systems in the presence of Mb, considering their mechanical and mucoadhesive performance in future in-vivo studies. Therefore, the aim of this work was to investigate the effects of Mb addition in the thermoresponsive bioadhesive systems based on rheological, mucoadhesive and textural characteristics analyzed by the quality of design experiment.

**Experimental section**

**Materials**

Methylene blue (Mb) and mucin (from porcine stomach, type II crude) were purchased from Sigma-Aldrich® (Saint Louis, MO, USA). Poloxamer 407 (Polox) and Carbopol 934P (Carb) were purchased from BASF ( Ludwigshafen, Germany) and from B.F. Goodrich (Brecksville, OH), respectively. Triethanolamine (TEA) was purchased from Synth (Diadema, Brazil) and was used as a neutralizing agent.

**Methods**

**Experimental design**

A full factorial design $2^3$ was employed to determine the influence of Polox ($X_1$), Carb ($X_2$) and Mb ($X_3$) amounts in development of thermoresponsive bioadhesive systems. Each factor is set at one of the two levels, low (−) and high (+), as shown in Table 1. Two center points were added with the aim to detect the curvature of design and errors associated with the isolated effects or the interactions between them.

**Preparation of formulations**

Carb (0.15, 0.20 or 0.25%, w/w) was added to purified water by mechanical stirring until completely dispersed. After this process, Polox (15.0; 17.5 or 20.0%, w/w) was added to and kept in contact with the dispersion for 12 h to ensure hydration of the same. Polox (15.0; 17.5 or 20.0%, w/w) was added to and kept in contact with the dispersion for 12 h to ensure hydration of the same. After the determination of the linear viscoelastic region (LVR), frequency sweep analysis was performed over the frequency range of 0.1–10.0 Hz. The storage modulus ($G'_0$), loss modulus ($G''_0$), dynamic viscosity ($η$), and loss tangent (tan δ) were calculated using RheoWin 4.10.0000 (Haake®) software. Dynamic properties were determined of at least five replicates.

**Continuous shear (flow) rheometry**

The flow rheograms of the systems were obtained at 25.0 and 37.0 °C, using a MARS II rheometer (Thermo-Haake®) and RheoWin 4.10.0000 (Haake®) software. The $T_{sol/gel}$ of the systems was performed in oscillatory rheometry with temperature ramp, and using a cone-plate as described above. Sample of each formulation was carefully applied to the lower plate and the linear viscoelastic region was determined at 5 °C and 60 °C. The temperature sweep analysis was performed over the temperature range from 5.0 to 60.0 °C with a heating rate of 10 °C min $^{-1}$ and frequency of 1.0 Hz. $G'_0$, $G''_0$, $η$ and tan δ were calculated using the RheoWin 4.10.0000 (Haake®) software. The $T_{sol/gel}$ was defined as the point at which $G'_0$ was halfway between the values for the solution and the gel, and was calculated for the systems that had $η$ significantly increased with an increasing of temperature. The $T_{sol/gel}$ was determined in at least five replicates of each system.

**Texture profile analysis**

Texture profile analysis (TPA) of the systems with and in the absence of Mb was accomplished using a TA-XTplus texture analyzer (Stable Micro Systems, Surrey, England). The formulation (16 g) was carefully transferred into McCartney bottles (30 ml), preventing the formation of bubbles. In TPA mode, an analytical probe (10 mm diameter) was compressed twice into each sample at a rate was increased and decreased over a period of 150 s, remaining in the upper limit for 10 s before decreasing. Continuous shear (flow) properties were determined at least in five replicates, and the upward flow curves were modeled using the Ostwald-de-Waele and Herschel-Bulkley equations (Equations (1) and (2))

$$\sigma = k\gamma^n$$

(1)

$$\sigma = \sigma_0 + k\gamma^n$$

(2)

where $\sigma$ is the shear stress (Pa), $k$ is the consistency index ((Pas)$^0$), $\gamma$ is the rate of shear (s$^{-1}$), and $n$ is the flow behavior index (dimensionless).

**Table 1. Full factorial design $2^3$ (plus two center points) used for the development of the systems.**

| Standard run | Polox (% w/w) ($X_1$) | Carb (% w/w) ($X_2$) | Mb (% w/w) ($X_3$) |
|--------------|----------------------|---------------------|-------------------|
| 1            | 15.0                 | 0.15                | 0.25              |
| 2            | 15.0                 | 0.15                | 0.75              |
| 3            | 20.0                 | 0.15                | 0.25              |
| 4            | 20.0                 | 0.15                | 0.75              |
| 5            | 15.0                 | 0.25                | 0.25              |
| 6            | 15.0                 | 0.25                | 0.75              |
| 7            | 20.0                 | 0.25                | 0.25              |
| 8            | 20.0                 | 0.25                | 0.75              |
| 9            | 17.5                 | 0.20                | 0.25              |
| 10           | 17.5                 | 0.20                | 0.50              |
defined rate (2 mm s\(^{-1}\)) to a depth of 15 mm and allowing a delay period (15 s) between the end of the first and the beginning of the second compression. From the resulting force–distance versus force–time plots, the hardness (force required to attain a given deformation), adhesiveness (work necessary to overcome the attractive forces between the surface of the sample and the surface of the probe), compressibility (work necessary to deform the product during the first compression cycle the probe), cohesiveness (work necessary to become unified the surface of the sample and the surface of the probe) and elasticity (ability the stretch and return to original size and shape) were calculated. At least, five replicate each systems were analyzed at 5.0, 25.0 and 37.0 °C\(^{14,20,25}\).

**In-vitro evaluation of the mucoadhesive strength**

The mucin discs were prepared by compression of crude porcine mucin (300–400 mg). Before the test, the mucin disc was hydrated by mucin solution (5%, w/w) for 30 s and horizontally attached to the lower of the TPA probe using double-sided adhesive tape. At 37.0 °C, each sample was placed under the analytical probe which was then lowered until the mucin disc was in contact with the surface of the sample. A downward force of 0.1 N was applied for 30 s, and the probe was upward at a constant speed of 1.0 mm s\(^{-1}\). The force necessary to remove the mucin disc from the formulation was measured using TA-XTplus texture analyzer (Stable Micro Systems, Surrey, England) in tension mode and was determined as the higher value in the relationship between force and distance. The result was the mean of at least five replicates\(^{14,20,25}\).

**Statistical analysis**

The effects of polymers and Mb concentration on textural properties, consistence index and flow index were statistically evaluated using a design of experiment (DoE). For this, a polynomial model, that correlates the input variables and the response, is further described by Equation (3):

\[
y = b_0 + b_1 x_1 + b_2 x_2 + b_3 x_3 + b_{12} x_1 x_2 + b_{13} x_1 x_3 + b_{23} x_2 x_3\]

where \(y\) is the response, \(b_0\) is the arithmetic mean response; \(b_1 - b_3\) are the estimated coefficients for \(X_1 - X_3\) respectively, and \(b_{12} - b_{23}\) are the estimated coefficients for interaction terms.

The viscoelastic properties (\(G'\), \(G''\), \(\eta'\) and \(\tan \delta\)) at five representative frequencies (0.60, 2.55, 5.04, 7.53 and 10.0 Hz), as well as the effects of the polymer and Mb concentrations on the force required to overcome the mucin-formulation adhesive bond were statistically evaluated by three-way analysis of variance (ANOVA). In all cases of ANOVA analysis, post hoc comparisons of the means of individuals groups were performed using Tukey’s Honestly Significant Different test. The paired Student’s t-test was used to determine if the dynamic viscosity of the formulations increased significantly with rising temperature (gelation). In all tests, \(p < 0.05\) was taken to denote significance, and Statistica 8.0 software (StatSoft, Tulsa, OK) was used throughout\(^{25,26}\).

**Results**

**Continuous shear (flow) rheometry**

Both formulations, with and without Mb, exhibited non-Newtonian flow behavior. The systems with the lowest Polox concentration and/or at 25 °C displayed pseudoplastic behavior and the other systems displayed plastic behavior with various yield values and more pronounced at 37 °C. It was impossible to verify the yield value of formulations containing 20% Polox, and at the higher temperatures, because the initial shear and tension applied to these formulations have changed the polymeric chain conformations.

Moreover, the systems displayed different values for hysteresis area. Preparations containing 15% Polox (at both temperatures), and with 17.5% (at 25 °C) displayed rheopexy. However, the other formulations showed thixotropy.

The influence of Polox, Carb and Mb concentrations on the consistence index and the flow behavior index was evaluated by DoE (Figure 1). It was observed that 99.99 and 99.79% of the variation on the \(k\) at 25 and 37 °C, respectively, are explicated with this model, as well as, that 92.79 and 99.68% for \(n\) at both temperatures. The values of the estimated coefficients for each parameter are described by the equations below:

\[
y = 41.925 + 82.632 X_1 + 7827 X_2 + 3634 X_3 + 6769 X_1 X_2 + 4671 X_1 X_3 + 79,386 X_2 X_3 (k \text{ at } 25 °C)\]

\[
y = 150.555 + 245.139 X_1 + 44,651 X_2 + 20,799 X_1 X_2 - 47.058 X_2 X_3 - 7099 X_3 (k \text{ at } 37 °C)\]

\[
y = 0.645 - 0.431 X_1 - 0.253 X_2 + 0.221 X_3 - 0.225 X_2 X_3 (n \text{ at } 25 °C)\]

\[
y = 0.232 - 0.358 X_1 - 0.074 X_2 - 0.018 X_3 + 0.032 X_1 X_2 + 0.205 X_2 X_3 + 0.037 X_2 X_3 (n \text{ at } 37 °C)\]

For the consistence index, the mains effects and second order interaction showed that the Polox and the \(X_1 X_3\) interaction presented the most important influence on this parameter, at 25 °C. However, when the temperature increases the \(X_1 X_2\) and \(X_2 X_3\) interactions displayed negative influences, and the \(X_2 X_3\) interaction was positive.

On the other hand, the temperature influenced negatively the \(n\). At 25 °C, only the interaction \(X_2 X_3\) and isolated factors interfered on the results, whereas that at higher temperature all interactions display this influence.

**Oscillatory rheometry**

The effects of polymeric concentration and presence of Mb on oscillatory properties (\(G'\), \(G''\) and \(\eta'\)) are presented in Figures 2 and 3. The values of \(G'\), \(G''\), \(\eta'\) and \(\tan \delta\) for all formulations were also reported in Tables S1-S3 (Supplemental data).

The increase of polymer concentration resulted in a significant increase of \(G'\), \(G''\) and \(\eta'\), and the \(\tan \delta\) decreased. Moreover, increasing the temperature from 25 to 37 °C also increased \(G'\) and \(\eta'\), except for formulation 20/0.15/0.75, at high frequency, where higher values were observed at lower temperature. \(G''\) module showed changes related to the poloxamer concentration. For example, systems with 15 or 17.5% showed a considerable increase of \(G''\), while those containing 20% Polox displayed a reduction of \(G''\) at higher frequencies. Exceptions were seen in the presence of 0.75% of Mb, demonstrating that the PS, at elevated concentration, interacts with the system structure.
The influence of Mb concentration was dependent of the temperature and poloxamer concentration. At 25 °C, the systems containing 15% of Polox and 0.25% of Mb showed a reduction of $G'$ and $G''$. On the other hand, an opposite behavior was observed for the same formulation with 0.75% of Mb, and for systems with others Polox concentrations. At the higher temperatures, the addition of 0.25% Mb did not change these parameters, when compared to the systems in the absence of the PS.

Generally all systems showed an increase of $g_0$, similarly to $G'$ for formulations at 25 °C, and to $G''$ at 37 °C, but the systems 20/0.15 did not display significant changes. The increased frequency significantly reduced $g_0$, except for 15/0.15/0.75. The storage modulus also displays an increase in function of frequency, especially at 25 °C. Likewise, the loss modulus ($G''$) displayed different results, which were Polox-concentration dependent. To the lowest concentration of Polox (15%), it was observed an increase in this parameter, whereas for the systems containing 17.5 and 20% it was less perceptible, and in some case did not occur.

The systems with 15%, 17.5% and 20/0.15/0.75 showed tan $\delta$ greater than 1, so they showed characteristics of more viscous preparations. In addition, the other systems presented higher $G'$ to the $G''$ and were considered semisolid or gels.

**Determination of $T_{sol/gel}$**

The results obtained by assessment of the sol–gel transition temperature of the Polox/Carb formulations 15/0.15, 15/0.25, 17.5/0.20, 20/0.15, 20/0.25 were $36.05 \pm 0.05$, $34.08 \pm 0.05$, $32.79 \pm 0.05$, $26.46 \pm 0.04$ and $27.84 \pm 0.07$ °C, respectively. While for the systems containing Mb 15/0.15/0.25, 15/0.25/0.75, 15/0.25/0.75, 17.5/0.20/0.50, 20/0.15/0.25, 20/0.15/0.75, 20/0.25/0.25 and 20/0.25/0.75 the $T_{sol/gel}$ results were $37.38 \pm 0.05$, $36.07 \pm 0.04$, $33.29 \pm 0.05$, $33.29 \pm 0.04$, $31.53 \pm 0.04$ (in both central points), $27.32 \pm 0.04$, $26.87 \pm 0.03$, $29.24 \pm 0.03$ and $26.44 \pm 0.05$ °C, respectively. The lower value of Mb in the system showed the higher $T_{sol/gel}$, when compared to 0.75% Mb. Moreover, the addition of the active generally resulted in an increase of $T_{sol/gel}$.

**Texture profile analysis**

The hardness, compressibility, cohesiveness, elasticity and adhesiveness of systems at three temperatures are displayed in Figures 4–6, which show the significant responses for dependent variables.

Hardness and compressibility analysis showed similar results at the same temperature range. At 25 °C, Polox ($X_1$) has the most important effects ($b_{hard} = +0.224$ and $b_{comp} = +1.174$, for hardness and compressibility respectively). In the same condition, Mb ($X_3$) and the interaction $X_1X_3$ also were directly proportional to the response ($b_{hard} = +0.016$, $b_{comp} = +0.017$, $b_{3comp} = +0.063$ and $b_{3hard} = +0.065$). This means that the increase in the concentration of these substances increases the value of these parameters. On the other hand, Carb ($X_2$) and the $X_1X_2$ interaction displayed to decrease of the results since the value of the estimated coefficient is negative ($b_{2hard} = -0.009$, $b_{2comp} = -0.011$, $b_{2comp} = -0.040$ and $b_{3comp} = -0.150$) and the Carb positive ($b_{2hard} = +0.018$ and $b_{2comp} = +0.083$). For the compressibility at 5 °C, the interaction $X_1X_2$ is the most prominent with $b_{2comp} = +0.004$.

Formulations containing 15 and 17.5% (w/w) of Polox shown adhesiveness only at 37 °C, unlike the formulations with 20% presented this parameter at 25 and 37 °C. Carb was significant only at

![Figure 1](image-url). Response surface graphs for the consistence index (A) and flow behavior index (B) at the temperatures of 25 °C (1) and 37 °C (2).
37 °C ($b_{2adh} = +0.130$), however, the interactions $X_1X_2$ and $X_2X_3$ showed a decrease of adhesiveness, i.e. $b_{12adh} = -0.069$ and $b_{23adh} = -0.053$.

The analysis of cohesiveness and elasticity showed no significant differences with the increase of the temperature. Moreover, at 5 °C a low and negative influence to isolated compounds and interactions to all parameters was verified.

**In vitro mucoadhesive strength**

The force required to promote the detachment of each formulation from mucin disc are presented in Table 2.

**Discussion**

The optimization of polymeric systems is extremely important to ensure the best combination in terms of improved administration performance and the residence time of systems on the application site. In this context, it is very important to understand and predict the behavior of the active agent together the polymeric components of formulation, considering the best physicochemical stability of formulation and its components, active agent availability, and the system performance. Moreover, the effectiveness of PDT is directly related to the production of ROS and $^{1}\text{O}_2$ by the photosensitizer. The utilization of the systems composed by Polox and Carb resulted in slightly alkaline pH. The pH for Mb is extremely important for the production of ROS. In the alkaline pH, the production of $^{1}\text{O}_2$ is five times higher when compared with the same systems at acid environment $^{12}$. The continuous shear rheology can help to understand the flow behavior of the systems, and was dependent of polymeric and Mb concentration as well as the temperature. The results of continuous shear analysis demonstrated that flow behavior of systems containing 15 and 17.5% (w/w) of poloxamer were pseudoplastic. However, in presence of 20% (w/w) Polox, with the continuous shear promoted by the test, the carboxylic groups of Carb were exposed and, consequently, more susceptible interaction with Mb resulting in structural alteration of the preparations, making impossible to check the yield value.

The increase of temperature only influenced the conversion of rheopexy to thixotropy for the systems containing 17.5% Polox. The results for the other systems containing Mb are in disagreement with previous studies of the platforms in the absence of
Mb, and may be due to the presence of Mb. These nonlinear answers are due to structural alteration generated by shear stresses, since without external action, the systems are composed by long chain molecules in a relaxed state, but under a shear stress these chains disentangled and line up direction of shear. Consequently, the solvent is released, and apparent viscosity decreased. This property is important to expulsion from packaging material and to skin application.

For the consistence index and flow behavior index, it was possible to verify a negative influence by X1X3 and X2X3 interaction (Equations (4)–(7)). This occurs because the charge of Mb interferes in the structure of the formulations, especially in the presence of a largest concentration of Carb. In this case, we could infer that the difference of Carbopol and Mb charges promotes the complexation between them and resulting in a slight disorganization of the systems, being more pronounced at the highest concentrations of these substances. Furthermore, the increase of temperature increases the value of k.

Viscoelastic properties provide important information about the structural nature of the formulation and rheological response to sub destructive forces. These properties directly influence the performance of the semisolid systems, for example in terms of primary physicochemical properties (e.g., drug release/diffusion) and mucoadhesion. Following the removal of initial shear stress employed during the application of the formulation, the product will undergo rheological recovery and the viscoelastic properties will predominate. In addition, within the biological environment, formulations will be exposed to oscillatory stresses with different frequencies that will be insufficient to cause deformation. Therefore, it is pertinent to examine the effects of such stresses on the viscoelastic properties of the formulations using a wide range of frequency in order to mimic the in vivo conditions.

The increase of polymer concentration and temperature resulted in significantly increase of G', G'' and η'. This behavior is related with the increase of polymer entanglement and the strong association between the polymeric molecules by non-covalent interactions (e.g., ionic interaction and hydrogen bond) which results in an increase on deformation resistance. Furthermore, the increase in temperature provided a greater number of entanglement and aggregates between polymeric chains, besides the Polox molecules aggregate in the form of micelles by dehydration of PO blocks hydrophobic.

Gels present higher G' to G'' in all frequency range, i.e. in low frequency the gel has sufficient time to occur the formation and breaks of entanglement of polymeric chains during oscillation predominating the viscous characteristics, but with the increase occurs

Figure 3. Response surface graphs at 37 °C displaying the influence of poloxamer 407 and Carbopol 934P, poloxamer 407 and methylene blue and Carbopol 934P and methylene blue on storage modulus (Pa), loss modulus (Pa) and dynamic viscosity (Pa s) at frequency of 10.00 Hz.
a distortion of this entanglement, and the gel acquires elastic properties\textsuperscript{30}. The sol/gel transition temperature is characterized by a drastic change of viscosity. The range is considered suitable for thermoresponsive systems between 25 and 37°C. Below 25°C, the formulations are susceptible to form gel at room temperature, hindering the manufacturing, handling and administration. On the other hand, temperatures above the corporeal temperature the gel not be formatted, preventing the permanency on the skin\textsuperscript{20,31}. Therefore, it is very important to characterize the $T_{\text{sol/gel}}$ of the systems for the understanding of the effects of Mb presence. In this context, the difference observed for $T_{\text{sol/gel}}$ value of formulations containing 0.25 and 0.75% (w/w) of Mb may be due to competition between Mb and Polox by the water of the medium, resulting in displacement of balance between the number of micelles and the number of the unimers of poloxamer, decreasing the micelle volume fraction. For the occurrence of gel formation is necessary that micelle fraction >0.53\textsuperscript{32}. Moreover, it has been reported that addition of Carbopol in systems with poloxamer reduces the $T_{\text{sol/gel}}$ especially in the presence of other substances\textsuperscript{33}.

The mechanical (textural) properties were evaluated at three temperatures considering the need to storage at low temperature (5°C), in which the system remains as a fluid. The other two conditions are associated to the room temperature (25°C) and the body temperature (37°C). These properties are responses of the systems to the external mechanical influences and demonstrate the ability of the formulations to develop reversible and irreversible

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**Figure 4.** Response surface graphs at 5°C display the influence of poloxamer 407 and Carbopol 934P, poloxamer 407 and methylene blue, and Carbopol 934P and methylene blue on compressibility (N mm) and hardness (N).
deformation. The hardness is intimately related to applicability of the systems at the application site, and the compressibility is related to the system removal of packaging material and spreadability on the application site, since it measures the systems deformation by compression. The adhesiveness results are in agreement with those obtained by Bruschi et al.\textsuperscript{20}, reinforce the existence of interaction between the polymers, and suggest a possible mucoadhesion providing a better retention in mucocutaneous lesions. However, the Carb only display significantly results at 37°C, contrary to what expected, since it is a polymer with bioadhesive characteristics. In addition, the presence of Mb impaired the adhesive properties of this polymer.

Cohesiveness shows the effect of multiple shearing stresses on the structural properties of the formulation\textsuperscript{34}. Comparing these results for all parameters, with the same systems without active, it was possible to see a proportional decrease in these values with the increase of Mb concentration. This reduction observed in the presence of PS occurs by disorganization of the systems, caused by the high affinity of Mb by water\textsuperscript{6} that possibly alters the interaction between Polox and Carb. At the same time, the carboxyl groups present on the Carb are transformed into carboxylate groups. These groups allow the formation of ion pairs between Carbopol and Mb, reducing the interaction between polymers weakening the systems.

It is important to consider the mucoadhesive properties of formulations because they could be utilized in cavity and not only in the skin. This characteristic provides higher contact between bioadhesive material and the application site, resulting in increased availability of the drug\textsuperscript{13}. In PDT, the increase of the residence time of systems in combination with light irradiation can promote the formation de ROS and resulting in the required cytotoxicity lesions\textsuperscript{35}.

For the systems 15% (w/w) Polox with Mb and the system Polox/Carb 15/0.15 (in the absence of Mb) the strength of cohesiveness generated by bonds between the polymers were lower than the semisolid-mucin adhesive bond, so the direct determination of mucoadhesion could not be possible\textsuperscript{36}. The increase of polymer concentration conduced to a significant increase in this parameter in systems without Mb. On the other hand, the presence of Mb reduced these values. The complexion between Mb and Carb can reduce the number of available polymeric chains to produce the swelling, resulting in lower unwinding of these chains and, consequently, the interpenetration in the glycoproteic mucus chains\textsuperscript{37}.

Figure 5. Response surface graphs at 25°C display the influence of poloxamer 407 and Carbopol 934P, poloxamer 407 and methylene blue, and Carbopol 934P and methylene blue on compressibility (N mm), hardness (N) and adhesiveness (N mm).
Conclusions

Binary polymeric systems containing poloxamer 407, Carbopol 934P and methylene blue were prepared and analyzed by quality by design in terms of rheological, textural, and mucoadhesive properties. The polymeric and methylene blue concentration, as well as the temperature, influenced the flow, oscillatory, compressional, and mucoadhesive characteristics of the systems. In this way, the formulations displayed different sol-gel transition temperatures. Moreover, the combination of the highest polymer concentrations with the lowest amounts of methylene blue provided the formation of higher viscous systems, which ensured higher compressive properties and the higher interaction with mucin. The analysis revealed that the formulations 17.5/0.50/0.20 and 20/0.15/0.25 displayed good characteristics to be administered and retained on the application site. Therefore, this investigation protocol helped to understand and predict the performance of these systems and the interactions between the polymeric components of the formulation with methylene blue, tailoring to the suit system for pharmaceutical utilization and local administration.

Disclosure statement

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