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Design of novel antifungal mucoadhesive films Part I. Pre-formulation studies

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Abstract

In this work, pre-formulation studies concerning the design of novel mucoadhesive films have been carried out. The rationality of the design is based on the utilization of mucoadhesive polymers (carbomer and carboxymethylcellulose), a plasticizer (polyethyleneglycol 400, PEG400) and a surfactant (ascorbyl palmitate, ASC16). In the gel preparation, the casting method using water as a solvent was employed. To provide a better understanding of the structural arrangements produced during the casting process, the changes in morphology (Cryo-TEM) and rheology (viscosity) of the film forming gel were evaluated. When PEG400 was included as a plasticizer, a disorder was produced in the network, reflected in the globular structure adopted by the gel and the consequent decrease in viscosity. The addition of ASC16 improved the solubilization of nystatin and provoked a decrease in gel viscosity. However, as water was removed during casting, ASC16 produced a significant increase in the viscosity at the point in which the polymer concentrations were sufficient to strengthen the inter-polymeric interactions, giving rise to a more rigid tri-dimensional network.

Keywords: Mucoadhesive film; Nystatin; Candidiasis; Ascorbyl palmitate

1. Introduction

Candidiasis is one of the most common pathologies manifested in the oral cavity (Carr et al., 1996), and its treatment requires the long-term administration of antifungal agents. The fungicidal activity of nystatin against *Candida albicans* is well known and its mode of action damages the cell membranes, altering their permeability, causing the death of the fungus (Kerridge, 1986). However, the success of the treatment is dependent of the pharmaceutical dosage form used for its administration in the oral cavity, since it is important to maintain the drug concentration higher than the minimal inhibitory concentration in the salivary fluid, over an extended period of time. Therefore, a mucoadhesive sustained release formulation could be advantageous compared to commonly used conventional pharmaceutical dosage forms, which usually have short residence times at the site of administration. This problem may be resolved using bioadhesive dosage forms, which can improve intraoral administration and reduce the dosage frequency as they are able to produce a sustained release of the antifungal drug while remaining adhered to the mucosa surface. Among novel mucoadhesive drug delivery systems, tablets and films are the most prominent. In previous works, we designed mucoadhesive tablets containing nystatin, in which swellable polymers were responsible for bioadhesion and release modulation (Llabot et al., 2002, 2004). However, buccal films offer advantages over adhesive tablets in terms of flexibility and comfort. Moreover, buccal films are also suitable for protecting wound surfaces, which is important when the affection produces ulcerative lesions. On the other hand, nystatin presents some physical–chemical properties that have to be considered when designing the films; three distinctly different crystal forms have been described which were referred to as types A–C. As nystatin is practically insoluble in water at room temperature and may suffer thermal and autoxidative degradation (Michel, 1977), the low solubility of nystatin is a feature that must be taken into account from a biopharmaceutical and mechanical point of view. In fact, the incorporation of solid particles in the films, even at very small sizes, can produce a decrease in the mechanical strength. Taking into account all these facts, we carried studies into the design of

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novel mucoahdesive films. The rationality of the design was based on the utilization of mucoadhesive polymers (carbomer and carboxymethylcellulose), a plasticizer (polyethyleneglycol 400) and a surfactant (ascorbyl palmitate, ASC16). In their preparation the casting method was employed. In this first part of our research, whose results are reported in this article, we attempted to acquire knowledge related to the structural evolution of the system during casting, the morphology, and the rheological behavior of the precursor gel ("film forming gel").

2. Materials and methods

2.1. Materials

Nystatin USP (Parafarm, Buenos Aires, Argentina), carbomer 934P (Acritamer 934, a gift from RITA Corporation, Woodstock, IL, USA), sodium carboxymethylcellulose viscosity grade: 500–2500 mPa s, (Fluka AG, Buchs SG, Switzerland), polyethyleneglycol 400 (Parafarm, Buenos Aires, Argentina), and ascorbyl palmitate (Sigma, Milwaukee, USA).

2.2. Formulation and preparation of the gels and films

The gels were obtained using Carbomer 934P (CB) and sodium carboxymethylcellulose (NaCMC) (1:1) as mucoadhesive polymers, PEG400 (plasticizer), ASC16 (surfactant) and N (active compound) were also incorporated into the formulations. The composition of the different gels is shown in Table 1.

Procedure: the polymers (mixture of CB and NaCMC, also shown in Table 1) were dispersed in water at 60–65 °C, by stirring under vacuum. Incorporation of N, PEG400 and ASC16 was performed by dispersing these compounds in water at 60–65 °C and then pouring this dispersion onto the polymer mixture under slow stirring for 30 min. After that, the product was allowed to cool to reach room temperature giving rise to the gel ("film forming gel"). The gel was dehydrated in an oven (60 °C) until the films were obtained (200 mm thickness). As water was removed, gel samples with different water contents were withdrawn and assayed (Cryo-TEM and viscosity determinations). pH measurement was carried out using a microprocessor bench pH meter HI 9321, sensor HI 2031, Hanna Inst (Padova, Italy).

Table 2
Samples of N subject to different conditions (N was dried until constant weight)

Sample	Dispersion temperature (°C)	Dispersion time (min)	Drying temperature (°C)
N1	65	20	60
N2	25	20	RT
N3	65	20	RT

RT = room temperature.

2.3. Differential scanning calorimetry (DSC)

Differential scanning calorimetry (DSC) experiments were carried out on a DSC 2920 Modulated instrument, (TA-Instruments Modulated-DSC 2920, Universal Analysis-NT software. New Castle, USA), using closed aluminum pans (nonhermetic), in a nitrogen atmosphere (5 °C/min, 25–250 °C).

2.4. X-ray powder diffraction (XRPD) of nystatin

Nystatin was characterized through X-ray powder diffraction using a Rigaku Miniflex diffractometer equipped with the specific software Standard monitoring 3.2 (Texas, USA). The scan range was 1–30° $2\theta/\theta$ with a scan speed of 0.02° $2\theta\,s^{-1}$. N was subjected to different environmental conditions (similar to the procedure for gel attainment) to evaluate possible changes in cristallinity. Briefly, nystatin (5% (w/v)) was dispersed in water at predetermined temperatures ("dispersion temperature") for 20 min ("dispersion time"). These dispersions were dried at selected temperatures ("drying temperature"). Such conditions are summarized in Table 2.

2.5. Morphological evaluation of gels

The microstructure of the samples was examined by freeze-fracture electron microscopy (Cryo-TEM). For the Cryo-TEM, a tiny drop of the sample was placed in a disk and the sample was frozen by plunging it into liquid nitrogen. Fracturing and replication were carried out in a freeze fracture apparatus (BALZERS: BAE 301, Munich, Germany) at −140 °C. Platinum/carbon was deposited at an angle of 45° and this was followed by second deposition of carbon at 90°. The collected replicas were examined with a SIEMENS: ELMISKOP IA electron microscope (Munich, Germany).

Table 1
Composition of film forming gel

Gel[a]	CB[b] (g)	NaCMC[c] (g)	N[d] (g)	PEG400[e] (ml)	ASC16[f] (g)	pH
A	0.25	0.25	–	–	–	3.90
B	0.25	0.25	0.2	–	–	4.03
C	0.25	0.25	0.2	2	–	4.10
D	0.25	0.25	0.2	2	0.1	4.12
E	0.25	0.25	0.2	2	0.2	4.14

[a] Obtained by dispersing the components into the necessary amount of water to complete 100 ml.
[b] CB: carbomer.
[c] NaCMC: sodium carboxymethylcellulose viscosity grade: 500–2500 mPa s.
[d] N: nystatin.
[e] PEG400: polyethyleneglycol 400.
[f] ASC16: ascorbyl palmitate.

2.6. Rheological properties of the gels

Rheological properties of film forming gel were evaluated on samples of gels with different percentages of water, which were obtained by interrupting the casting process at predetermined times. Rheological determinations were performed at 25 °C in a Haake (Karlsrube, Germany) viscometer VT500 equipped with VT500/VT 3.01 software, and a NV sensor. The curve fittings and statistical analyses were performed using a Microcal Origin Version 3.5.

3. Results and discussion

3.1. Rationality for films design

For the optimization of a mucoadhesive film, either synthetic or natural polymers are normally used, with polyacrylic acid and cellulose derivatives being the most common (Lee et al., 2000). In this work we have selected a mixture of CB/NaCMC (1:1) as mucoadhesive polymers, because in preliminary studies we observed that these polymers at this proportion showed satisfactory "in vitro" mucoadhesion.

It is known that it is necessary to incorporate plasticizers in the films to improve the mechanical properties of the systems. The addition of such substances produces a reduction in film brittleness, and imparts flexibility, thus increasing strength and tear resistance. In preliminary studies we evaluated the efficacy of PEG400 and glycerin (Gly) as plasticizers. However, the incorporation of Gly had a deleterious effect on some properties of the films, since the "in vitro" mucoadhesion was too weak, so we selected PEG400 as a plasticizer in the formulation. Finally, aiming to facilitate the dispersion/solubilization as well as the nystatin release, we incorporated ASC16 as a surfactant, whose aggregation properties depend on molecular weight (alkyl chain length) and temperature (Palma et al., 2002). ASC16 is able to solubilize large amounts of low solubility drugs, and at the same time produce a considerable increase in the stability of the compound in aqueous media (Palma et al., 2003a,b,c). The lack of solid drug particles in the matrix could produce an improvement in the mechanical properties and homogeneity of the films. The casting process to obtain the films involves the dispersion of excipients and drugs in an aqueous vehicle, followed by the controlled evaporation of water. The incorporation of hydrocolloids like CB and NaCMC produces a gel with particular properties. Such properties may vary as water is removed from the system, producing a plastic film at the end of the process. At the first stage of our research, with the aim of gaining a thorough knowledge of the structure involved in the films, we evaluated the properties of the film forming gel during casting and the influence of the different components of the formulation. The composition of the assayed formulations is given in Table 1. The incorporation of nystatin, along with the structural features (morphology) and rheological properties (as water is removed) of the gels were evaluated (see Tables 1, 3 and 4).

3.2. Incorporation of nystatin in gels and films

As previously mentioned, nystatin possesses three different crystalline configurations, types A–C. According to DSC and X-ray powder diffraction (XRPD) determinations, the nystatin used in this work corresponds to type C polymorph. It was described (Michel, 1977) that these polymorphs can be inter-convertible due to changes in environmental conditions (moisture and temperature). Taking into account that the casting process involves the dispersion of polymers, surfactant and drug in aqueous media at a relatively high temperature (>60 °C), we evaluated the possibility that nystatin can suffer crystalline modifications during casting. However, using XRPD (Fig. 1) we observed that nystatin did not change its crystalline structure for different environmental and processing conditions (see Table 2).

In preliminary studies we have assayed the incorporation of nystatin into the gel without the aid of surfactants. The obtained films were not homogeneous even when using very small particles. Also, we observed, as could be expected, that the nystatin release was very slow.

Aiming to solve these two principal drawbacks, we added ASC16 as a surfactant to the system. Nystatin was incorporated into the film forming gel previously solubilized in the supramolecular aggregates that ASC16 forms at temperatures above its critical micellar temperature (CMT, ∼65 °C) (Ambrosi et al., 2004). On cooling, this transparent dispersion became a semisolid liquid-crystal, called "coagel", which is a consequence

Table 3
Composition (%, w/v) and viscosity (η) of gel D as water was removed

Film	% CB[a]	% NaCMC[b]	% ASC16[c]	% N[d]	% PEG400[e]	η[f]
D1	0.575	0.575	0.23	0.46	2.574	257.612
D2	0.636	0.636	0.254	0.509	2.848	267.858
D3	0.671	0.671	–	0.536	3.004	253.614
D4	0.858	0.858	0.345	0.686	3.849	284.906
D5	1.196	1.196	0.477	0.955	5.354	288.104
D6	1.434	1.434	0.575	1.143	6.427	544.374
D7	1.472	1.472	0.589	1.178	6.596	598.634

[a] CB: carbomer.
[b] NaCMC: sodium carboxymethylcellulose viscosity grade: 500–2500 mPa s.
[c] ASC16: ascorbyl palmitate.
[d] N: nystatin.
[e] PEG400: polyethyleneglycol 400.
[f] Viscosity (mPa s^{-1}).

Table 4
Composition (%, w/v) and viscosity (η) of gel E as water was removed

Film	% CB[a]	% NaCMC[b]	% ASC16[c]	% N[d]	% PEG400[e]	η[f]
E1	0.551	0.551	0.441	0.441	2.468	207
E2	0.611	0.611	0.489	0.489	2.736	205.85
E3	0.69	0.69	0.552	0.552	3.091	212
E4	0.738	0.738	0.592	0.592	3.308	214
E5	0.762	0.762	0.64	0.609	3.413	223.46
E6	0.984	0.984	0.788	0.788	4.407	256.66
E7	1.144	1.144	0.915	0.915	5.126	286.27
E8	1.506	1.506	1.205	1.205	6.747	381.39

[a] CB: carbomer.
[b] NaCMC: sodium carboxymethylcellulose viscosity grade: 500–2500 mPa s.
[c] ASC16: ascorbyl palmitate.
[d] N: nystatin.
[e] PEG400: polyethyleneglycol 400.
[f] Viscosity (mPa s^{-1}).

of the high hydration of lamellar structures (Palma et al., 2002). However, it is necessary to consider that these aggregates will now be formed in an aqueous medium where hydrophilic polymers (CB, NaCMC and PEG400) are present. It is well known that the incorporation of surfactants into a polymeric dispersion can alter the polymer conformation and the viscosity of the dispersion (Barreiro-Iglesias et al., 2001). These alterations depend on the type of polymer and surfactant (ionization), and on the concentration (Alvarez-Lorenzo and Concheiro, 2003; Barreiro-Iglesias et al., 2003a,b). The interaction between both substances can lead to the formation of new aggregates, which could be a consequence of hydrophobic interactions between the non-polar surfactant tail and the polymer backbone, or electrostatic interactions between the polar head of the surfactant and the charged groups of the polymers, or both. In this case CB ($pK_a = 6.10$), NaCMC ($pK_a = 5.8$) and ASC16 ($pK_a = 4.27$) are anionic compounds. At the pH values of the dispersions (see Table 1) CB and NaCMC are practically unionized, while ASC16 is partially ionized. In this way, the hydrophobic interactions are mainly responsible for any possible association.

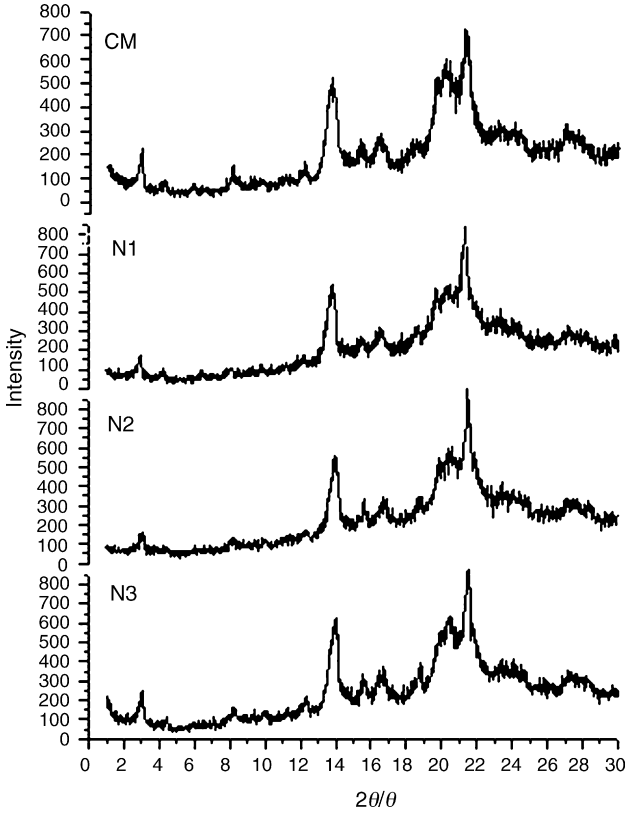

Fig. 1. XRPD of nystatin for different environmental conditions (for details see material and methods Table 2) CM = commercial N.

3.3. Morphological evaluation of gels

Cryo-TEM permits visualization of the conformational structures of the film forming gel as water is removed from the system. In Fig. 2, Cryo-TEM microphotographs of gels for different water proportions are shown.

The structure of the gels is affected by water content and the addition of PEG400 and ASC16. Also, the solubilization of nystatin is a consequence of the relative proportions of PEG400 and ASC16. As can be seen in Fig. 2A, the gel containing CB and NaCMC (50% (w/v)) presents a "laminar" structure where each lamina is separated from the other by the insertion of free water. This behavior is due to the prevalence of hydrophobic interaction between both polymers, and high crystallinity could be expected, resulting in brittle films. CB is a highly cross-linked resin and the addition of NaCMC also produces physical crosslinks. The partial withdrawal of water (Fig. 2B) shrinks the polymers producing a "fuzz ball" type of gel structure which is characteristic for Carbopol 934P (BFGoodrich Specialty Chemicals, 1994). Fig. 2A and B also shows that nystatin is present as insoluble particles, which were removed from the frozen sample in agreement with the Cryo-TEM technique, giving rise to the formation of dark hollows (pointed out in the figures by arrows heads). When PEG400 is incorporated (gel C, Fig. 2C and D) a notable change in the gel structure is observed, which became more disordered and a globular configuration prevailed. This is a consequence of the plasticizing effect of PEG400, which produces a decrease in the polymer transition temperature and therefore polymeric chains became more flexible. The reduction of water content in gel C (Fig. 2D) did not significantly affect

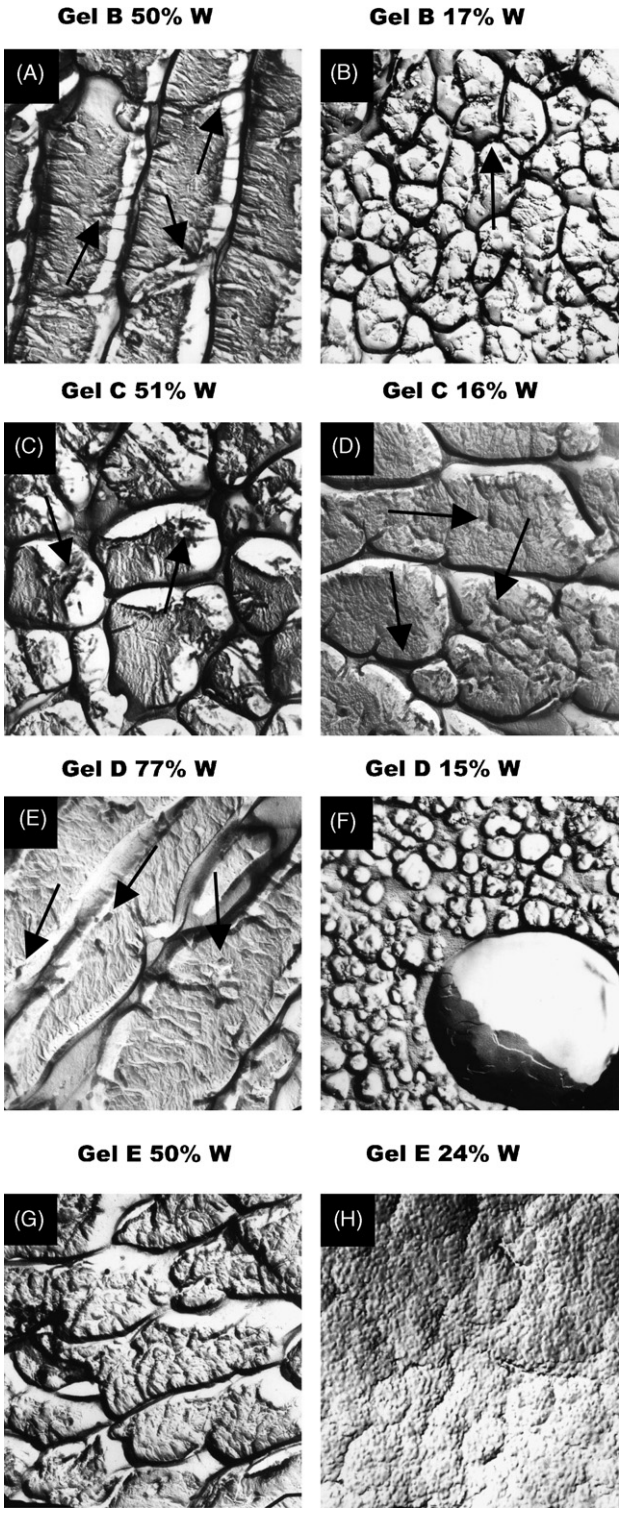

Fig. 2. Cryo-TEM of film forming gel (%W: percentage of water in the gel).

the arrangement and the interaction of the polymers. In both cases, nystatin remained insoluble in the system, indicating that the addition of PEG400 is of little practical use in drug solubilization. In this context, it was expected that the film obtained from gel C would have good physical and mechanical properties, although solid nystatin particles were still present. In the formulation of gel D, 0.1 g of ASC16 (see Table 1) was added and the respective microphotographs are depicted in Fig. 2E and F. Unfortunately, in the assay we could not obtain any picture from a gel containing about 50% of water in order to compare it with the preceding samples. Correspondingly, in Fig. 2E a relatively ordered configuration is observed, and it can be postulated that this is due to the large amount of water that is still present in the gel. When the solvent was removed, the system had a tendency towards the formation of a globular structure. The presence of a surfactant (ASC16) produced the formation of small globules, with vesicular structures also observed (Fig. 2F). Furthermore, ASC16 increased the solubilization of nystatin and consequently only a few particles could be observed in the frozen gels (dark hollows).

Finally, an increment in ASC16 (gel E) accentuated this effect on gel structures. From Fig. 2G and H, it can be observed that the size of globular units substantially decreased and solid nystatin is practically unobservable, indicating that the drug could be solubilized in the system. This observation was corroborated through XRPD of the films (data not shown). The structure of the gel was very compact when the water amount decreased (Fig. 2H). The respective film obtained from gel E possessed similar properties to that of gel D, although an increase in drug release could be expected.

3.4. Rheological properties of the gels

Even though the analysis of the different properties of the film forming gel may contribute to understanding the structure of the film, the collected data could also permit evaluation of the behavior of the gels as mucoadhesive pharmaceutical dosage forms. It is widely known that this kind of system has been studied as a potential dosage form in local and transmucosal drug delivery systems. Consequently, in the context of this work, we have analyzed these results with the hope of getting information about film structures. Nevertheless, further studies will need to be carried out regarding mucoadhesive gel formulations.

The rheological changes that the film forming gel can suffer as the water content is reduced are a consequence of the rearrangement of the polymeric chains and its interaction with PEG400 and ASC16, which could be reflected in the flow curves and variation in viscosity (η) of the systems. The flow curves of different gels were determined and the results are shown in Fig. 3.

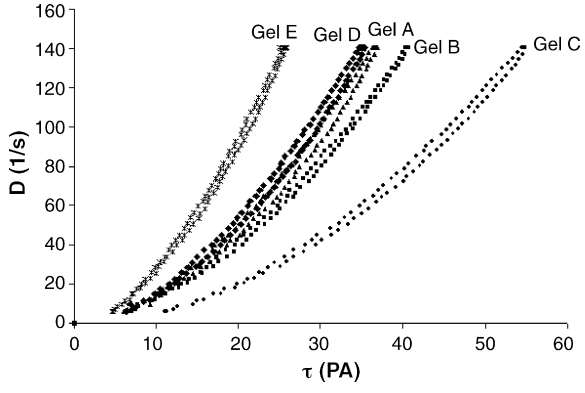

Fig. 3. Flow curves of film forming gel.

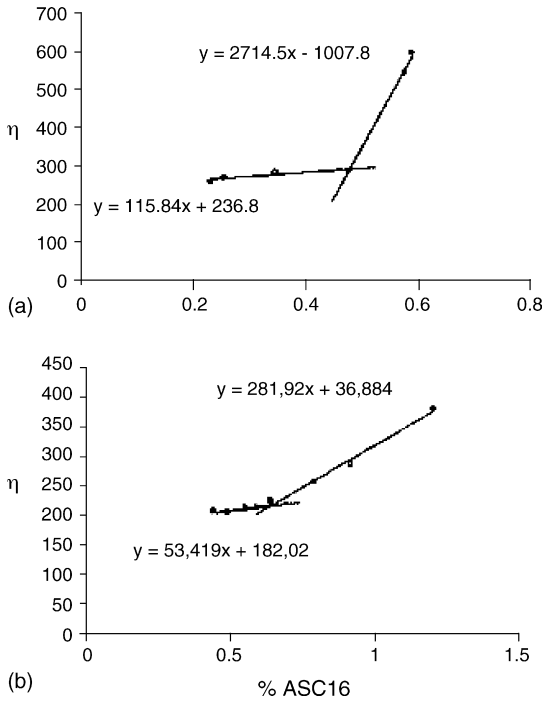

Fig. 4. Variation of (a) gel D and (b) gel E viscosities as a function of ASC16 concentration.

All gels showed a characteristic pseudoplastic flow. The incorporation of nystatin (gel B) caused an increase in viscosity compared to the gel composed by the polymers alone (gel A). An expected very significant increase in the viscosity was observed when PEG400 (gel C) was added to the formulation, which may be attributed to the plasticizing effect of this polymer. This effect is related to the ability of the plasticizer to weaken polymeric intermolecular attractions, thus allowing polymer chains to move more readily, which improves the flexibility of the polymer. Consequently, the higher relaxation and mobility of the chains provoke greater entanglement and increase the gel viscosity.

On the other hand, incorporation of ASC16 (gels D and E) caused a notable decrease in the viscosity. This effect is dependent on the ASC16 concentration, being more pronounced as the concentration of the surfactant is increased. In these cases, where the polymer concentration is relatively low (CB + NaCMC = 0.5%), the viscosity decreases because the surfactant facilitates the intra-polymeric interactions (mainly hydrophobic) resulting in gel shrinkage. However, this behavior was not observed when the concentration of polymers, PEG400 and ASC16 was raised in the gel. This situation corresponds to the gels obtained after partial removal of water. The viscosities of these gels as the water was removed were determined and the results are summarized in Tables 2 and 3. The η versus %ASC16 plots were obtained and the results are shown in Fig. 4a and b.

In both gels (D and E) the viscosity remains practically constant until the water content reaches a critical value, from which point η starts to increase in a pronounced way. This fact is not observed for gels A and B, while in the case of gel D it is observed at water concentrations lower than 45% (Table 3: data#5, CB + NaCMC = 2.4%, ASC16 = 0.477%) and for gel E at concentrations lower than 59% (Table 4: data#5, CB + NaCMC = 1.5%, ASC16 = 0.64%). These observations could be explained by considering the influence of the surfactant on intra and inter-polymeric interactions (Barreiro-Iglesias et al., 2001, 2003a,b).

During the first stages of water removal, the polymers and ASC16 concentrations remain sufficiently low to permit the intra-polymer interactions to prevail. In this environment the amount of ASC16 is still not enough to promote inter-polymeric interactions. At the moment at which the surfactant reaches the critical concentration, an increase in hydrophobic interactions between different polymeric chains is facilitated by ASC16, producing the formation of a more rigid tri-dimensional network, and a related significant increase in the viscosity. It is important to note that the %W for gel D (45%) is lower that for gel E (59%), which is due to the larger amount of ASC16 incorporated in this case. This means that the presence of the surfactant is mainly responsible for this behavior, since the ratio %ASC16/%W at the critical point is constant for both gels (0.011). In this way, the polymer concentration determines the value of the gel viscosity, while the concentration of ASC16 and water determine the viscosity changes as long as water is removed from the system.

4. Conclusions

The properties of the film forming gel were evaluated with the objective of inferring the structure of the mucoadhesive film obtained from the casting process. PEG400 included as a plasticizer produced disorder in the network, which is reflected in the globular structure adopted by the gel, and the consequent decrease in viscosity. The addition of ASC16 improved the solubilization of nystatin and provoked a decrease in the gel viscosity. However, as water was removed during casting, ASC16 produced a significant rise in viscosity to the value at which the polymer concentrations were sufficient to strengthen the inter-polymeric interactions, producing a more rigid tri-dimensional network. From these results, the proposed formulation could permit mucoadhesive films to be obtained that possess adequate physical–mechanical properties with solubilized nystatin.

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