JOURNAL OF Applied Polymer SCIENCE

Characterization of Novel Hyaluronic Acid Matrix Systems for Vaginal Administration of Metronidazole

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ABSTRACT: Polymers such as Hyaluronic acid (HA), Polyethylene glycol-400 (PEG-400) and Xanthan Gum (XG) are promising in drug delivery applicationsbecause of their biomedical and pharmaceutical potential applications. In HA 2%-PEG 400 systems, the effect of pH and PEG-400 concentration were evaluated. The viscosity of HA-PEG 400 formulations slightly increased with PEG-400 concentration. Viscoelastic properties and shear thinning character was strongly dependent on pH. Structured systems were obtained at pH 3, with an increase of several orders of magnitude in zero-shear viscosity values. When XG 1% structured system is added on HA (0, 0.5, and 2%) and PEG-400 5%, a sharp increase of viscosity can be observed, obtaining a gel-like behaviour for HA 0.5%-XG 1%-PEG 400 5% formulation. Finally, metronidazole release profiles in HA 2% formulations with different PEG-400 concentrations at pH 4.5 were studied. At least 90% of metronidazole was releasedat 24 h. However, the addition of XG 1% to the HA (0.5 and 2%)-PEG 400 5% systems delayed the drug release. © 2014 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2015**, *132*, 41313.

KEYWORDS: drug delivery systems; hydrophilic polymers; rheology; viscosity and viscoelasticity

Received 1 April 2014; accepted 21 July 2014

DOI: 10.1002/app.41313

INTRODUCTION

Vaginal systems, such as suppositories, gels or vaginal rings, aim to provide not only a localized effect, but through drug absorption, sustained therapeutic levels compared with the traditional oral route.[1]

For topical administration of drugs biodegradable polymers such as Carbopol, Chitosan, Xanthan Gum (XG) or Hyaluronic acid (HA) are commonly used. Moreover, the HA regenerative properties could contribute to restore a normal and functional state of the vaginal tissue in caseit had been damaged during a infection. Therefore, the HA could be considered as an adecuate physiological support for vaginal administration of drugs.

HA is isolated either from animal sources, within the synovial fluid, umbilical cord, skin, and rooster comb, or from bacteria (*Streptococcus zooepidemicus*) through a process of fermentation or direct isolation. It is a linear polyelectrolyte based on β1,4-D-glucuronic acid and β1,3-N-Acetyl-D-glucosamine alternated in the repeated unit. Previous studies have demonstrated as the viscoelasticity of HA in aqueous solutions is pH dependent.[2–5] Biocompatibility, non-immunogenicity, biodegradability and viscoelasticity properties have been proved, so it is an ideal biomaterial for cosmetic, medical and pharmaceutical aplications.[4,6,7] Likewise, HA acts as a space filler, lubricant (at 2%),[8] and osmotic buffer in the native Extracelullar Matrix (ECM). The hydrated HA helps to maintain the viscoelasticity of connective tissues such as the vitreous humor, cartilage and vocal folds.[9]

In order to improve the characteristics of the polymeric matrix, HA was combined with polyethylene glycol 400 (PEG-400). PEG-400 is an excipient commonly used as a humectant agent, antiseptic and plasticizer at concentrations between 5 and 20%.[10,11] Furthermore, PEG 400 reduces the tendency of particles to aggregate by steric stabilization, thereby producing formulations with increased stability during storage and application.[12] PEG-400 is a biocompatible polymer but non-biodegradable (use of low-molar-mass PEGs would be preferable).[12,13] No skin reactions with PEG 400 were seen.[13,14]

Additional polymers to form polymer networks are usually added in order to modulate drug release from the hydrogels.

However, the influence of these substances on the properties of hydrogels requires detailed investigations.

In this work we have considered an exobacterial gum, XG, which is a heteropolysaccharide with a primary structure consisting of repeated pentasaccharide units formed by two glucose units, two mannose units, and one glucuronic acid unit, in the molar ratio 2.8:2.0:2.0.[15] Xanthan gum is an adhesive and biocompatible polymer, used at concentrations between 0.1% and 1%,[15,16] in pharmaceutical industry.The viscosity of xanthan solutions strongly increases when increasing concentration of the polymer. Likewise, viscoelastic propierties of XG solution has been described for others researchers.[15]

The main objective of this work was the study of HA matrix systems with several polymeric agents (PEG-400 and XG), as potential vehicles for the controlled release of metronidazole, which is used on various types of bacterial and parasitic infections, including bacterial vaginosis and rosacea.[17,18] The effect of pH and PEG concentration in HA-PEG 400 matrix systems and the effect of HA in HA-XG polymeric mixtures on rheological properties. As for healthy women in reproductive age, normal vaginal pH is 3.5 to 4.5 metronidazole release rates were studiedat this pH range. This naturally acidic environment is maintained by the production of lactic acid by the vaginal microflora.[19,20]

EXPERIMENTAL

Formulation of Gels

HA, XG, lactate and PEG-400 were obtained from Guinama (Valencia, Spain). Metronidazole was supplied by Acofarma (Valencia, Spain). Purified water by reverse osmosis (MilliQ®, Millipore Spain) with a resistivity above 18.2 mΩ cm was used.

- HA-PEG 400 matrices: Metronidazole 0.75% (w/w) solutions with differents proportions of PEG-400 (0, 5, 10, and 20%) (w/w) were prepared by dissolving both products in water. Afterwards HA 2% was added.
- HA-XG matrices: Metronidazole 0.75% (w/w) solutions with PEG-400 at 5% (w/w) were prepared by dissolving both products in water. Afterwards XG at 1% (w/w) was added. The matrix system is homogenized and finally, different proportions of HA (0%, 0.5%, and 2%) (w/w) were added.

Formulations were adjusted to the corresponding pH with lactic acid and they were left to stand for 24 h at room temperature for complete hydratation of the polymer and removal of bubbles.

Rheological Tests

Rheological tests were carried out with acontrolled stress rheometer Rheostress 1 (ThermoHaake, Germany) with data acquisition software (Rheowin 4.0.1). Cone plates (2°, 35 mm and 60 mm) and serrated parallel plates (35 mm) were used. All measurements were made in triplicate at 25°C and samples were allowed to rest for at least 600 s prior to analysis. In all cases, the exposed edges of the sample were covered with silicone oil (Dimethicone 350 CP RFE/Ph. Eur.) to prevent evaporation of water during measurement.

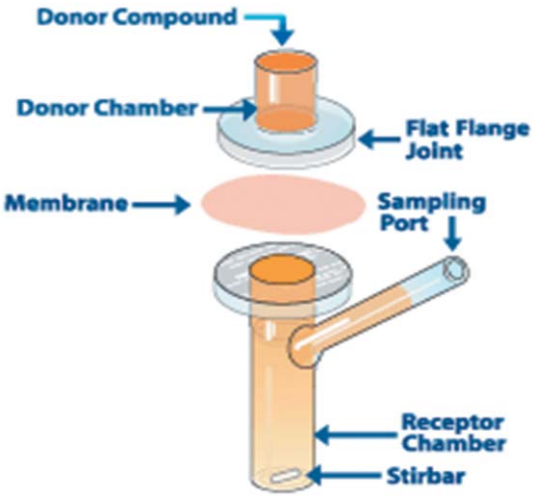

Figure 1. Scheme of a Franz cell type. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Step flow curves in Controlled Shear (CS) mode (30 s each point) were recorded. To determine the linear viscoelastic range (LVR), stress sweeps at a frequency of 1 Hz were performed for all systems studied. The oscillatory rheological parameters used to compare the viscoelastic properties for all the systems were the storage modulus (G′) and the loss modulus (G″). Frequency sweep tests were performed from 0.01 to 10 Hz, at 1 Pa except to HA 2%-GX 1%-PEG 400 5% formulation (carried out 2 Pa). Creep tests were carried out at a constant stress amplitude in LVR. The stress was applied instantantly and maintained for a period of 300 s. After removing stress, compliance was measured also during 300 s.

In Vitro Release Studies

The cumulative amounts, Q, of metronidazole released from HA-PEG 400 were determined using 0.45 μm cellulose-acetate membrane filters (Teknograma, Barcelona, Spain) in Franz type cells (Figure 1) with an available diffusion area of 0.784 cm^2, placed in a heating/stirring device.[21] All experiments were carried out at 37°C. The volume of the receptor compartment was 6 mL and it was filled with distilled water. The HA-PEG 400 (1 g) was placed on the artificial membrane and covered with parafilm to prevent any evaporation. During 24 h at predetermined time intervals, 200 μL samples were taken from the receptor and replaced by the same volume of fresh distilled water to maintain a constant volume. The samples ($n = 6$) were analyzed by HPLC (Perkin-Elmer LC Binary pump) using a C18 column (4.6 mm × 150 mm × 5 μm) and an acetonitrile-water (60:40 v/v) as mobile phase at a flow rate of 1 mL min^{-1}. Ultraviolet detection was made at 320 nm. The calibration curve was linear over a concentration range from 0.015 to 0.75 mg/mL. The limit of quantification was determined as 0.006 mg/mL and the limit of detection as 0.002 mg/mL. The retention was about 1.45 minutes.

RESULTS AND DISCUSSION

Rheological Characterization

Viscoelastic Tests. A predominance of viscous over elastic behaviour was obtained for all HA-PEG 400 matrix systems

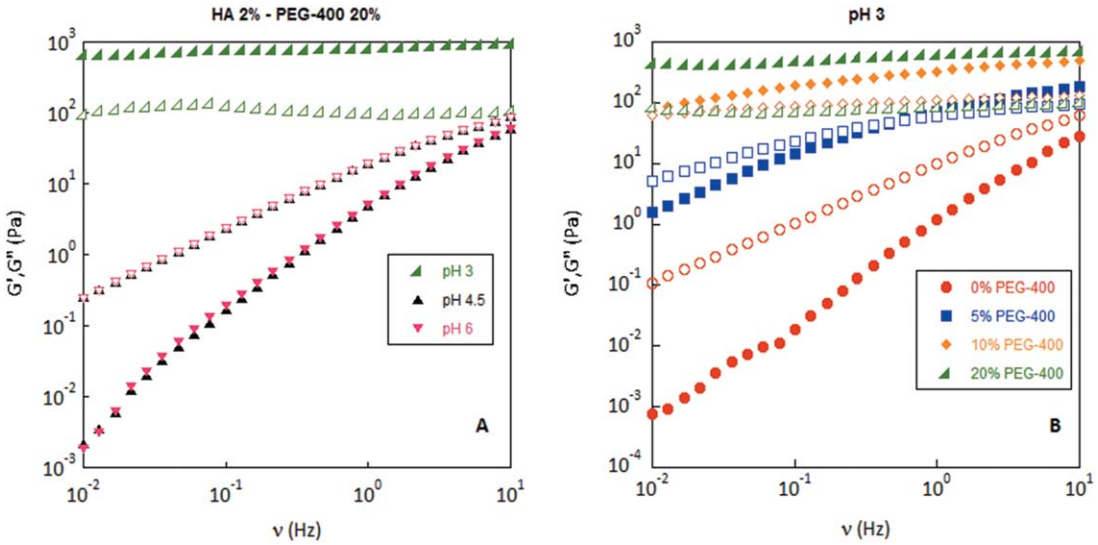

Figure 2. Viscoelastic moduli as a function of frequency. Open symbols G″ and close symbols G′. HA 2%-PEG 400 matrix systems. A: Matrix systems with 20% of PEG-400 at different pH values. B: Matrix systems at pH 3 for all PEG-400 concentrations. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

studied at pH>3, as loss moduli (G″) were greater than storage moduli (G′). As an example of this behaviour, the oscillatory tests for the HA 2%-PEG 400 20% matrix system (with pH values between 3 and 6) have been plotted in Figure 2(A), with the values of viscoelastic moduli as a function of frequency. However, a gel-like behaviour was obtained at pH 3 for HA 2%-PEG 400 10% (Figure 2(B)] and 20% formulations [Figure 2(A,B)] with storage moduli greater than loss moduli. This gel-like behaviour of HA-PEG 400 matrix systems could be due to an isoelectric point located around pH = 3 for HA. Both the decrease of the carboxylic group dissociation and the protonation of the —NH— HA groups favour the H-bond formation. The protonation of the —NH— HA groups gives a positive net charge able to interact with the negative charge of few —COOH.[4,5] Also, Figure 2(B) shows as HA 2% matrix systems without PEG-400 at pH 3 did not exhibit a gel-like behaviour. Addition of PEG-400 semmed to have a reinforcement effect on the matrix, as rheological behaviour indicated the existence of structured systems when concentration of PEG-400 is increased [Figure 2(B)] as moduli are less dependent on frequency and $G' > G''$.[22] Furthermore, both moduli were strongly dependent with frequency for the highest pH values [Figure 2(A)].

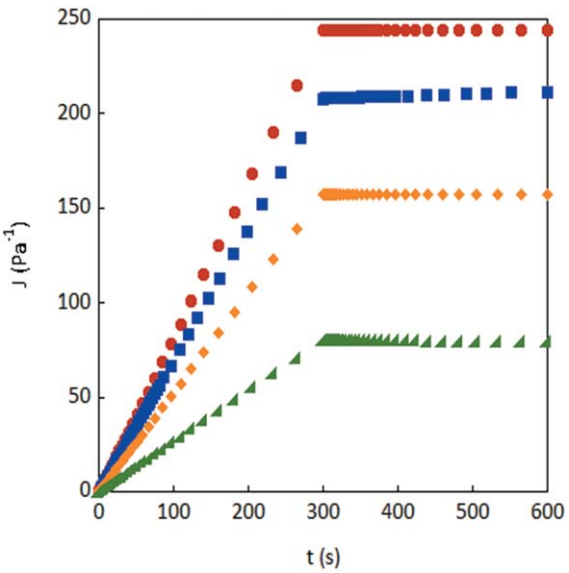

Figure 3. Compliance versus time in creep and recovery tests for HA 2%-PEG 400 matrix systems at pH 4.5. % PEG-400. ● 0; ■ 5; ♦ 10; ◢ 20. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Figures 2 and 3 show the values of compliance $J=\gamma/\sigma$, as a function of time, for the creep and recovery tests corresponding to the HA-PEG 400 and HA-XG-PEG 400 5% matrix systems at vaginal pH (around 4.5), respectively.

In Figure 3(A) predominance of viscous behaviour of formulations with HA-PEG 400 matrix systems at pH 4.5 was observed, because creep test exhibited a linear strain being unlimited as long as the stress is applied. When the stress was removed the deformation was fully maintained. This behaviour is indicative of non structured systems, as it was previously deduced from oscillatory tests. Moreover, the maximum strain decreased in a linear relationship when increasing PEG-400 concentration.

In Figure 4 a gel-like behaviour can be observed in formulations HA 0%-PEG 400 5%-XG 1%, and HA 0.5%-PEG 400 5%-XG 1% at pH 4.5. Creep test exhibited a non linear strain and a partial recovery was observed when the stress was removed. The recovery was greater when decreasing HA concentration. However, a non structured system can be observed in HA 2%-PEG 400 5%-XG 1% as creep test exhibited a linear strain. Moreover, maximum strain was higher when increasing HA concentration.

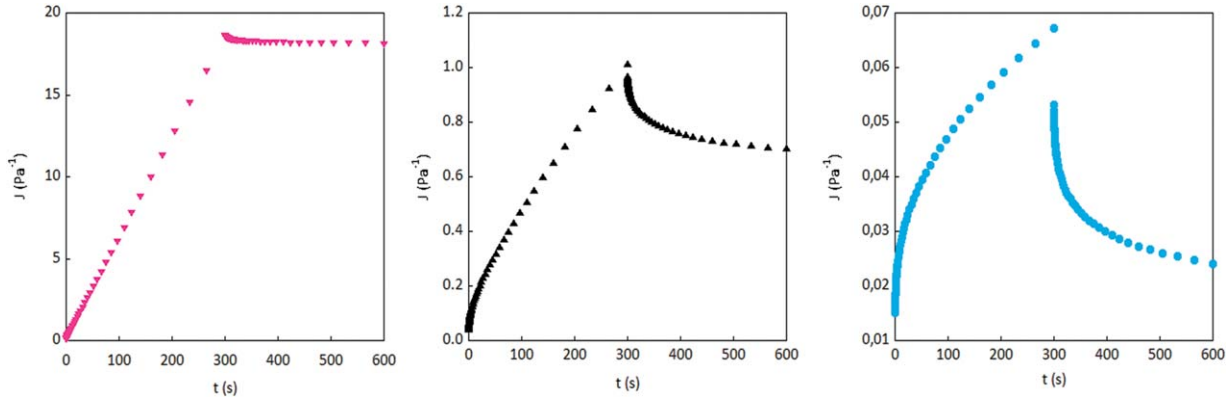

Figure 4. Compliance versus time in creep and recovery tests for HA-XG matrix systems at pH 4.5. HA and XG matrix systems concentration with PEG-400 at 5%. ▼ HA 2%-XG 1%; ▲ HA 0.5%-XG 1%; ● HA 0%-XG 1%. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Flow Curves. All the formulations studied had a shear thinning behaviour (Figure 5), so flow curves were fitted to Carreau model:

$$\eta = \frac{\eta_0}{\left(1+\left(\frac{\dot{\gamma}}{\dot{\gamma}_c}\right)^2\right)^c} \quad (1)$$

where η_0, is zero-shear viscosity, $\dot{\gamma}_c$, is the critical shear rate, and c is the shear thinning index. Parameters obtained through Carreau model for HA-PEG 400 matrix systems for all pH's and HA-XG-PEG 400 mixtures are shown in Tables I and II, respectively. When analyzing pH dependence on HA-PEG 400 matrix systems, it is interesting to point out that in formulations with pH>3.5 a similar behaviour is observed, with zero-shear viscosities between 1.85 and 8.45 Pa s (Table I). However, in formulations with pH 3, zero-shear viscosity was some orders of magnitude greater than those for systems with higher pHs (Table I), what is in agreement with thegel-like behaviour previously described. Moreover, a higher viscosity values when increasing PEG-400 concentration on HA 2% formulations can be observed [Figure 5(A)], obtaining a linear relationship of zero shear viscosity with PEG-400 concentration.Therefore, as the changes on the rheological properties should be proportional, the minimum PEG-400 concentration studied (5%) was selected for HA-XG polymeric mixtures. On the other hand, slopes of the curves were very similar for all formulations while critical shear rates decreased slighty when increasing PEG-400 concentration.

Figure 5(B) shows how HA 0%-XG 1%-PEG 400 5% formulation reached zero shear viscosity values of 4000 Pa s (Table II) far greater than in XG 0%-HA 2%-PEG 400 5% formulation (1.35 Pa s). However, when XG 1% was added to the HA 2%-PEG 400 5% system a zero shear viscosity of 27.9 Pa s was

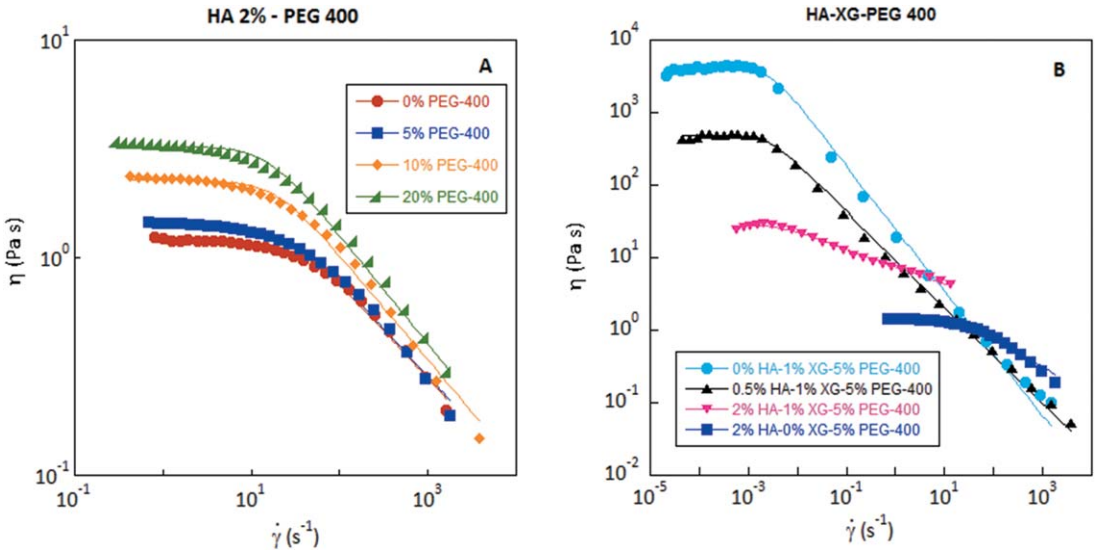

Figure 5. Viscosity versus shear rate in flow curves for matrix systems at pH 4.5. A: HA 2%-PEG 400 matrix systems for all PEG 400 concentrations. B: HA-XG-PEG 400 polimeric mixtures. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Table I. Zero-Shear Viscosity Values Obtained when Fitting Flow Curves to Carreau Model [eq. (1)] for HA-PEG 400 Matrix Systems at all pH's

	η_0 (Pa s)[a,b]			
	% PEG-400			
pH	0	5	10	20
3	1.22 ± 0.01	93.4 ± 3.4	2200 ± 20	68000 ± 700
3.5	1.18 ± 0.01	1.37 ± 0.01	1.82 ± 0.03	8.45 ± 0.09
4.5	1.12 ± 0.04	1.35 ± 0.07	2.3 ± 0.3	3.25 ± 0.12
6	1.67 ± 0.05	2.07 ± 0.04	2.47 ± 0.01	3.7 ± 0.2

[a] Parameter values shown correspond to mean data ± standard deviation.
[b] The correlation coefficient was higher than 0.99 in all cases.

obtained (Table II). Moreover, in Table II an exponential decrease of zero-shear viscosity when increasing HA concentration 0.1 and 2% can be observed.

A decrease of several orders of magnitude was observed when incorporating HA to the XG-PEG 400 systems. On the other hand, shear thinning behaviour was dependent on HA-XG proportion, as a linear decrease in c was obtained when decreasing HA concentration (Table II). Moreover, HA-PEG 400 systems at pH 4.5 had a Newtonian behaviour up to shear critical rates between 15 and 25 s^{-1}, while shear critical rates were much lower (closer to rest, i. e., about 10^{-2} s^{-1}) in HA-XG-PEG 400 mixtures. This could be attributed to the existence of interactions between HA and XG polymers.

Drug Release Studies. Drug releas estudies were performed both in HA-PEG 400 and HA-XG matrix systems at pH 4.5. In HA-PEG 400 matrix systems, PEG-400 concentrations were varied between 0 and 20% in order to study the effect of PEG-400 on drug release. HA-XG matrix systems at 3 differents concentrations of HA (0%, 0.5%, and 2%), while XG and PEG-400 kept constant (1% and 5% respectively), in order to observe the different release on structured (HA 0%-XG 1%-PEG 400 5% and HA 0.5%-XG 1%-PEG 400 5%) and non structured (HA 2%-XG 1%-PEG 400 5%) systems.

In Figure 6 percentages of drug released against time were plotted. In HA 2%-PEG 400 5% formulations more than 90% of the total amount of drug was released at 24 h [Figure 6(A)]. The release profiles are biphasic with an faster initial release (≈6 h) followed by a delayed drug release.

On the other hand, Figure 6(B) shows the XG presence in the matrix allowed a higher delayed drug release (35–60% at 24 h). Moreover, this release of metrodinazole was slightly lower when increasing HA concentration. As it was previously observed, those matrices are less structured and less viscous. The suggested interactions between XG and HA could decrease the metrodinazole release.

Drug release data obtained from the release studies was fitted using Korsmeyer-Peppas and Peppas-Sahlin, in order to predict the drug release mechanism. Kosmeyer-Peppas derived a simple

Table II. Parameters of Carreau Model Fits [eq. (1)] to Flow Curves for HA-XG-PEG 400 Matrix Systems at pH 4.5

% Polymers			Carreau model parameters[a,b]		
HA	XG	PEG-400	η_0 (Pa s)	$\dot{\gamma}_c$ (s^{-1})	c ± 0.01
2	0	0	1.12 ± 0.04	24.6 ± 1.5	0.25
2	0	5	1.35 ± 0.07	22 ± 1	0.20
2	0	10	2.3 ± 0.3	19.2 ± 2.8	0.24
2	0	20	3.25 ± 0.12	15.3 ± 1.3	0.25
0	1	5	4000 ± 100	0.0027 ± 0.0004	0.43
0.1	1	5	1400 ± 20	0.0022 ± 0.0001	0.38
0.25	1	5	1000 ± 20	0.0032 ± 0.0005	0.37
0.33	1	5	700 ± 10	0.0028 ± 0.0003	0.36
0.4	1	5	530 ± 10	0.0031 ± 0.0003	0.34
0.5	1	5	480 ± 6	0.0030 ± 0.0002	0.33
0.75	1	5	80 ± 2	0.0012 ± 0.0001	0.25
1	1	5	46.3 ± 0.9	0.0019 ± 0.0002	0.23
2	1	5	27.9 ± 0.6	0.0042 ± 0.0006	0.12

[a] Parameter values correspond to mean data ± standard deviation.
[b] The correlation coefficient was higher than 0.99 in all cases.

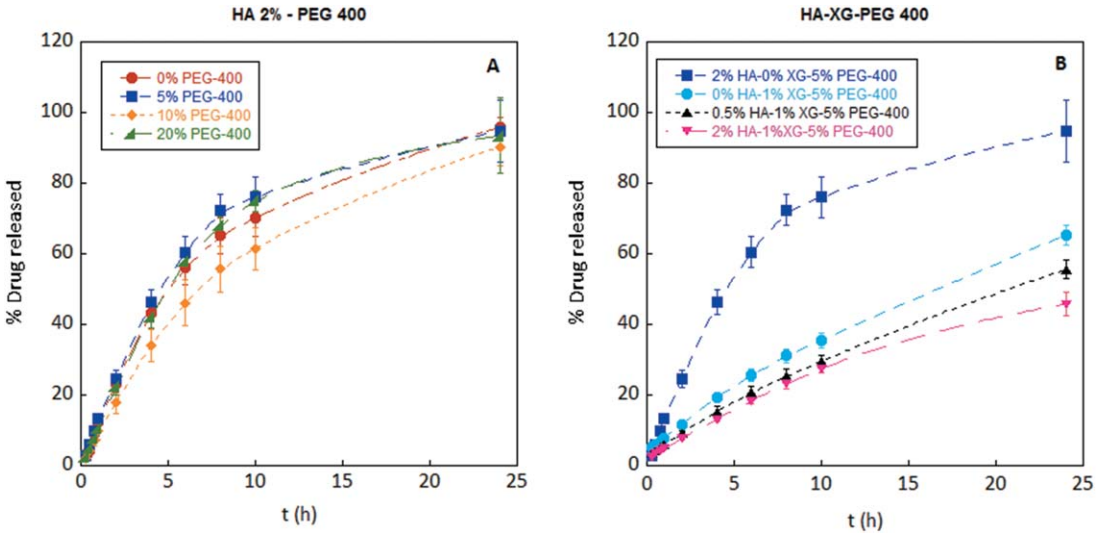

Figure 6. % Drug released versus time in kinetic tests of metronidazole for all formulations at pH 4.5. A: HA 2%-PEG 400 matrix systems for all PEG concentrations. B: HA-XG-PEG 400 polimeric mixtures. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

relationship which described drug release from a polymeric system[23]:

$$\frac{M_t}{M_\infty} = Kt^n \tag{2}$$

In this equation, M_t/M_∞ is the fraction of drug released, k is the kinetic constant, t is the release time and n is the diffusional exponent that depends on the release mechanism and the shape of the swelling device tested. For thin films, values of $n = 0.5$ indicate Fickian release (case-I transport), values of $0.5 < n < 1.0$ indicate anomalous (non-Fickian or coupled diffusion/relaxation) drug release, whereas values of $n = 1.0$ indicate case-II or zero-order release kinetics. Table III summarises the values of n and K for all the samples tested. In HA-PEG 400 matrix systems $n \approx 0.5$ while in HA-XG the diffusional exponent, n, is between 0.67 and 0.71. K was higher in HA-PEG 400 systems than in HA-XG-PEG 400 systems what could indicate a controlled diffusion in HA-XG-PEG 400 systems. The drug release corresponded to an anomalous transport since $0.5 < n < 1$, what means the drug release was governed by diffusion and polymer relaxation.

A more reliable and informative analysis can be obtained by considering that drug release in swellable matrices depends on two processes: (i) drug diffusion into the swollen polymer, and (ii) relaxation contribution. Calculation of the approximate contribution of the diffusional and relaxational mechanism to the anomalous release process is carried out by fitting the data to the heuristic model proposed by Peppas and Sahlin[24]:

$$\frac{M_t}{M_\infty} = K_1 t^m + K_2 t^{2m} \tag{3}$$

where K_1, K_2, and m are constants.The first term of the right-hand side represents the Fickian contribution, and the second term is the case-II relaxational contribution. In this model, drug release from swellable matrices is described as the result of two transport mechanisms, i.e diffusion across the gel layer (F) and relaxation of the polymeric chains (R). F is linked to the coefficient of the diffusional contribution (square-root dependence on time) and R to the coefficient of the relaxational contribution (linear dependence on time) of the binomial equation

Table III. Kinetic and Statistical Parameters Obtained when Fitting $(M_t/M\infty) = f(t)$ to Korsmeyer-Peppas [eq. (2)] and Peppas-Sahlin Models [eq. (3)] for Systems at pH 4.5

% Polymers			Korsmeyer-Peppas[a]		Peppas-Sahlin[a]			
HA	XG	PEG	n	K (h^{-n})	K_1 (h^{-n})	K_2 (h^{-n})	m	R/F
2	0	0	0.55 ± 0.03	0.19 ± 0.02	0.14 ± 0.02	−0.050 ± 0.001	0.87 ± 0.03	≈0
2	0	5	0.52 ± 0.03	0.22 ± 0.02	0.15 ± 0.02	−0.005 ± 0.001	0.88 ± 0.01	≈0
2	0	10	0.56 ± 0.06	0.18 ± 0.03	0.10 ± 0.02	−0.002 ± 0.001	0.95 ± 0.06	≈0
2	0	20	0.52 ± 0.04	0.22 ± 0.04	0.14 ± 0.03	−0.005 ± 0.001	0.94 ± 0.03	≈0
0	1	5	0.67 ± 0.02	0.12 ± 0.01	0.12 ± 0.01	0.014 ± 0.003	0.52 ± 0.01	0.66 ± 0.13
0.5	1	5	0.71 ± 0.04	0.11 ± 0.01	0.11 ± 0.01	0.0020 ± 0.0006	0.66 ± 0.03	0.15 ± 0.05
2	1	5	0.68 ± 0.05	0.12 ± 0.02	0.10 ± 0.01	−0.0020 ± 0.0009	0.84 ± 0.07	≈0

[a] Kinetic parameters shown correspond to mean data ± standard deviation.

describing the time dependence of fractional drug release.[24] The ratio of relaxational (R) and Fickian (F) contributions can be calculated as:

$$\frac{R}{F} = \frac{K_2 t^m}{K_1} \quad (4)$$

In Table III, a negative value of K_2 for HA-PEG 400 matrix systems was obtained, what could be attributed to an insignificant effect of relaxation process (ratio $R/F \approx 0$) on drug release compared to Fickian diffusion.[25] In XG-PEG 400 systems a higher contribution of the relaxation process was obtained and when HA was incorporated to the XG-PEG 400 matrix system, the contribution of relaxation process was decreasing when increasing HA concentration, and became negligible for XG 1%-HA 2%-PEG 400 5% systems, predominating in this case the Fickian diffusion (as in HA-PEG 400 matrix systems) against relaxation process.

This behaviour could be attributed to swelling behaviour of the polymer, shape of the matrices, diffusion and erosion properties of the polymer and dissolution characteristics of the drug as drug release rate depends on these properties.[26]

CONCLUSIONS

The rheology of HA was influenced by the pH and PEG-400 concentration. However, the drug release in HA-PEG 400 matrix systems showed there were not statistically significant differences for diffusion coefficients when increasing PEG-400 concentration. When XG was incorporated to the HA-PEG 400 5% polimeric mixture, zero-shear viscosity increased several orders of magnitude, but it decreased when increasing HA concentration on these formulations. On the other hand, a full drug release in HA-PEG 400 formulations compared to a delayed drug release in XG-PEG and HA-XG-PEG 400 systems was observed. Therefore, XG-PEG 400 and HA-XG-PEG 400 mixtures could be an excellent topical vehicle of controlled drug release, because the released fraction at 24 h was between 35 and 60% according to the HA concentration used on the HA-XG polymeric mixture.

ACKNOWLEDGMENTS

The authors express their very great appreciation to Dr. Manuel Dolz Planas for his valuable and constructive suggestions during the planning and development of this research work. His willingness to give his time so generously has been very much appreciated.

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