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Injectable Thermosensitive Gels Based in Poloxamer as Modified Drug Release Systems for Veterinary Use

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***SUMMARY*. The purpose of this work is to explore the potential of combining poloxamer 407 and carrageenan for its utilization in an injectable depot drug release system. Reverse thermal gelation of these formulations allow the local injection in liquid form, gelling *in situ* after its administration. Carrageenan reinforces the structure of poloxamer gels (after 50 h of testing only 20 % of the system is eroding) and allows to modulate the release rate of progesterone as a function of formulations composition. The elastic modulus of sole poloxamer gels (G' = 56 Pa) increases significantly in presence of carrageenan (G' = 1 347 Pa) at 10 °C. The gelation temperature of tested formulations is between 17 and 22 °C and the gelation process is very quick. Poloxamer-carrageenan systems offer a promissory alternative approach to development of injectable depot systems for veterinary use.**

INTRODUCTION

Injectable fluid systems, with *in situ* formation of polymeric flexible or rigid matrixes, represent an attractive alternative to replace implants, temporary prosthesis or platforms, preformed for drug release, in the field of human and animal medicine. Particularly, attractions of viscoelastic hydrogels, with micro and macroscopic properties conveniently designed, ensure prolonged residence time and controlled drug release. In this context, a polymer solution capable of gelling *in situ* at temperatures close to the physiological proves to be attractive for the development of drugs release systems.

The importance of these systems is increasing since they exhibit some advantages such as easy application, use for local anesthesia, easy programming in procedure, precision for the quantity of material to be injected, easier and more economic elaboration, and sometimes, as a possibility of extending the validity of drugs with invention patents to expire. Thus, the development of these applications in veterinary field has become of increasing commercial interest, generating intensive R&D work. The global market for advanced drug delivery systems amounted to $134.3 billion in 2008, and was forecast to grow to $139 billion in 2009. The estimate is $196.4 billion by 2014, for an annual growth rate of 7.2% in the 5-year period. The estimated sales for sustained-release systems are $ 36.1 billion in 2009 and $ 45.8 billion by 2014 for an annual growth rate of 4.9 % [1].

The poloxamers, specially the poloxamer 407 (Fig. 1), are thermosensitive materials more frequently used due to their advantages such like, easy availability, facile preparation gel methods and good compatibility with various drugs and pharmaceutical excipients [2]. Poloxamer gels have been widely investigated as drug

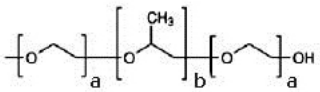

Figure 1. Chemical structure of poloxamer 407, a = 101, b = 56.

KEY WORDS: Injectable depot, Modified release, Thermosensitive gel.

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release systems since they are relatively easy to obtain and widely used in the pharmaceutical field. Moreover, they are generally like considered safe excipients [3]. It is well known that poloxamer solutions present the reverse thermal gelation phenomenon, behaving as solution at low temperature and gelling as temperature increases [4,5]. However there are also disadvantages associated to poloxamer's gel applications as drug delivery systems such as limited stability, poor mechanical properties and short residence times due to its rapid dissolution when placed within biological environments [6].

Some intents of poloxamer chemical modification reported in literature show promising results attaining slow gel erosion when used as injectable, although chemical modifications generally incorporate toxic residues, so they are not widely accepted [7,8]. Addition of natural or semi-natural macromolecules to poloxamer's gels is another alternative that rises the possibility to obtain gels systems with appropriate mechanical and release properties. Furthermore they are more profitable, convenient and safe.

In this work carrageenan is used as natural polysaccharide. Carrageenan hydrocolloid is obtained by extraction with water or aqueous alkali from some members of the Rhodophyceae class (red seaweed). Carrageenans are divided into three families according to the position of sulfate groups and the presence or absence of anhydrogalactose. These are: λ-Carrageenan (lambda-carrageenan), ι-Carrageenan (iota-carrageenan) and κ-Carrageenan (kappa-carrageenan). Previous works reported the use of this macromolecule to control drug release from polymeric matrixes [9-11].

Progesterone is a natural hormone used as contraceptive either alone or combined with an oestrogen. The reported solubility of progesterone in water under ambient conditions is 0.016 mg/ml. Residues resulting from the use of progesterone as a growth promotor in accordance with good animal husbandry practices are unlikely to pose a hazard to human health.

The scope of the present contribution is to explore the potential of poloxamer 407 and carrageenan combination as an injectable depot system for drug release to veterinary use. Progesterone is used as a model drug. The profiles of release and erosion *in vitro* are evaluated. Rheological measurements are performed to characterize resulting viscoelastic properties of prepared gels.

MATERIALS AND METHODS

Chemicals

Poloxamer 407 (trade name Pluronic® F127), kindly provided by BASF, κ-carrageenan (Soriano, Buenos Aires, Argentina), progesterone (Farmabase, Rovereto, Italy) and sodium chloride (Cicarelli, Santa Fe, Argentina). All reagents were used without any further purification process.

Preparation of thermosensitive gel

Poloxamer gels are prepared by "cold method" described by Schmolka [12]. An appropriate amount of poloxamer (20, 24, 28 and 32 % w/ w) is added to a cold water solution (5-10 °C) while keeping constant agitation with a magnetic stirring rod. Later, poloxamer solutions are kept in a refrigerator during 24 h to ensure complete dissolution. To prepare solutions of carrageenan, appropriate amounts are weighed and dissolved in pure water (70-80 °C). Finally, polymeric platforms are loaded with progesterone (1 % w/w), by direct dispersion of the drug in the polymer blend. To homogenize the mixture, magnetic continuous agitation is used.

In vitro release experiment

To simulate physiological conditions sodium chloride 0.9 % w/w is used. The release experiments are performed using the membraneless model since this procedure allows direct contact between gel and release medium, and gel erosion can also be considered. This model being widely-used in the research of the systems of gels based in poloxamer [13-16], and is important to assess the poloxamer-based systems in which gel erosion usually plays a key role, or is the control step in the drug process release.

Briefly, the vial containing the gel loaded with progesterone is placed in a thermostatic bath (38 ± 0.05 °C) until obtaining a semisolid gel. Then, 10 ml of release medium pre-equilibrated at 38 ± 0.05 °C is carefully placed on the surface of the gel. At predetermined time intervals, the release medium is completely replaced by fresh medium kept at 38 °C and formulations are weighed to calculate the proportion of dissolved gel. The concentration of progesterone in the release medium is determined by UV spectrophotometry at maximum wavelength absorbance, 245 nm (Genesys 10 UV, Spectronic Unicam, New York, USA).

Reported experimental data is the average of 3 experiments and is expressed as a percentage

of progesterone released. Progesterone release profiles from the gel were analyzed using the power law model, $M_t / M_\infty = kt^n$, where M_t and M_∞ are the absolute cumulative amounts of progesterone released at time t and infinite time, respectively. The k is a parameter dependent upon structural and geometric characteristic of the device [17]. By fitting experimental results of M_t, M_∞ and t with this equation, for each formulation, exponent n is calculated providing insight into the drug release mechanism [18,19].

Rheological tests

Oscillatory rheometry is also performed for all formulations prepared using a rheometer AR-G2 controlled stress (TA Instrument, New Castle, USA). Experiments are performed to assess changes in the rheological parameters as function of temperature, by means of oscillatory measurements at a fixed frequency of 0.1 Hz and with a stress amplitude to ensure linear viscoelasticity. To evaluate the systems parallel plate geometry (25 mm diameter) is used. The temperature range investigated is 10 to 30 °C using a heating rate of 1 °C/min. The oscillatory experiments are performed with the purpose of finding temperature sol-gel transition, by means of the measurement of temperature at which G' showed a significant variation [2,20].

RESULTS AND DISCUSSION

Dissolution of the gel and drug release

Physiological solution is used as release medium to simulate close real circumstances under which gel is found after the intramuscular administration.

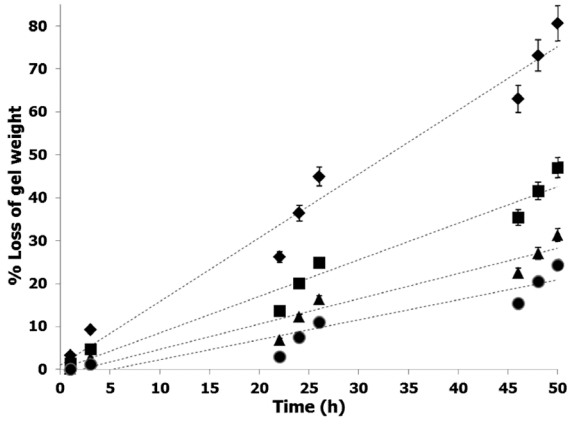

Figure 2. Erosion of thermosensitive gels composed of poloxamer 407 with different concentrations weight / weight: (◆) 20 %, (■) 24 %, (▲) 28 % and (●) 32 %.

Dissolution of the gel

To evaluate the influence of poloxamer concentration in the erosion of the release platform, poloxamer gels are prepared at different concentrations (20, 24, 28 and 32 % w/w). From obtained results it can be observed that erosion is significantly influenced by poloxamer concentration (Fig. 2). By increasing from 20 to 24 % w/w, erosion decreases about 30%, while when increased from 28 to 32 % w/w, the decrease is around 10 %. Based on the obtained results, poloxamer 20% w/w gels because erode quickly, and of poloxamer 32 % w/w because practically is gelled at room temperature making it difficult its administration as injectable depot, are excluded from further investigations.

Figure 3 shows the effect of carrageenan (0.10 % w/w) when combined with poloxamer (28% w/w). The gel constituted by poloxamer 28 %, as sole component, dissolves at a higher proportion, as reported in previous works [14-16]. Compared with the control gel, erosion decrease is significant for gels containing poloxamer 28 % - carrageenan 0.10 %. Possibly carrageenan would reinforce the structure of the gel, since after 50 hours of testing only 20 % of the system is eroded. This discussion also arises during rheological properties evaluation.

Drug release

As shown in Figure 4, carrageenan 0.10 % decreases the rate of release of progesterone from poloxamer gels 28 %. Kinetic data are processed using the power model and results are reported in Table 1. The n values were found to be close to one, suggesting that other factors besides the drug diffusion are involved in the kinetic control. Relaxation polymer processes could be involved in drug release mechanism.

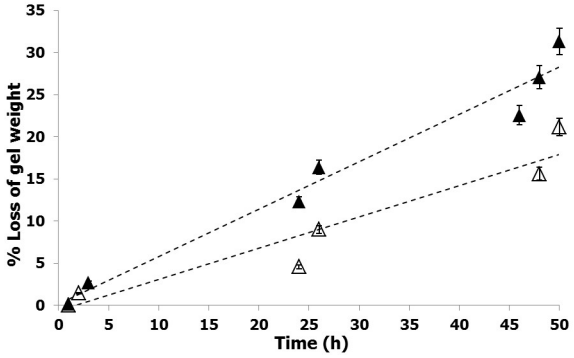

Figure 3. Erosion of thermosensitive gels composed (▲) poloxamer 28% w/w and (△) poloxamer 28%-carrageenan 0.10% w/w.

Concentration of carrageenan (w/w)	*n*	*K*	Fitting equation	Regression coefficient square (r^2)
0	0.847 ± 0.116	0.036 ± 0.003	$M_t/M_\infty = 0.036t^{0.847}$	0.985
0.05	0.817 ± 0.176	0.043 ± 0.004	$M_t/M_\infty = 0.043t^{0.817}$	0.980
0.10	0.897 ± 0.188	0.022 ± 0.002	$M_t/M_\infty = 0.022t^{0.897}$	0.981
0.15	0.938 ± 0.192	0.016 ± 0.002	$M_t/M_\infty = 0.016t^{0.938}$	0.982

Table 1. Data obtained using the fitting model to evaluate the release profiles of progesterone from gels poloxamer 407 (28 % w/w) with different concentrations of carrageenan.

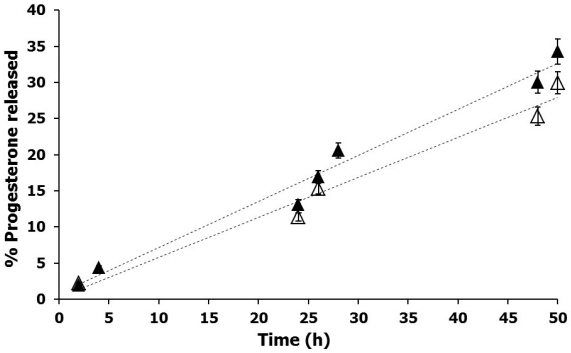

Figure 4. Profiles of progesterone release from thermosensitive gels compounds (▲) poloxamer 28 % w/w and (△) poloxamer 28 %-carrageenan 0.10 % w/w in the presence of sodium chloride 0.9 % w/w at 38 ± 0.05 °C.

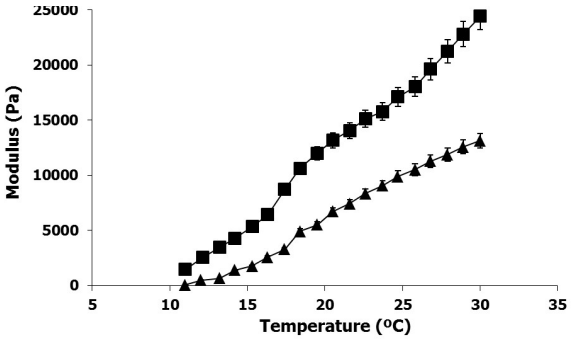

Figure 5. Elastic modulus for formulation (▲) poloxamer control 28 % and formulation (■) poloxamer 28 % - carrageenan 0.10 % w/w.

Poloxamer-carrageenan gels appear to be versatile release platforms providing a range of release rates and erosion in terms of composition. Carrageenan incorporation would reinforce the integrity of the gel. This could allow new injectable *in situ* forming matrices with optimal release and erosion properties.

Rheological characterization

Proper rheological analysis is a valuable technique to investigate and evaluate the gelling process and viscoelastic properties of thermosensitive gels [21-23].

The storage or elastic modulus *(G')* provides information about the elasticity or the energy stored in the material during deformation, while the viscous or loss modulus *(G")* describes the viscous character or the energy dissipated as heat. The higher the value of *G',* the elastic character is more pronounced, and conversely the higher *G",* viscous properties are more pronounced.

Viscoelastic properties of systems containing poloxamer and the sol-gel transition of these formulations were significantly influenced by the presence of carrageenan. Figure 5 shows the variation of *G'* in terms of temperature for 28 % poloxamer gels with and without carrageenan. Values of elastic modulus obtained for carrageenan formulations are much higher compared with those for pure poloxamer formulations. Thus carrageenan significantly increases the elastic properties of the gels. The elasticity modulus *G'* is relatively small at low temperatures and increases significantly at higher temperatures for both formulations (Table 2). Gelling temperatures for poloxamer 28% and poloxamer 28% - carrageenan 0.10% systems are 18.4 °C and 17.4 °C, respectively. Kinetic gelation process for the evaluated systems is very quick. This is beneficial to prevent loss of gel immediately after administration. The addition of progesterone does not modify the sol-gel transition.

The curves of viscosity versus temperature obtained for different formulations exhibit the typical behavior of poloxamer gels [24]. The viscosity of formulations varies linearly at low temperatures (Fig. 6) but increases substantially in a temperature range of 20-22 ° C for poloxamer gel containing 24 % and from 15-19 °C for poloxamer gel containing 28 %. These results

Formulation (% w / w) poloxamer/carrageenan	10 °C		30 °C	
	G' (Pa)	G" (Pa)	G' (Pa)	G" (Pa)
28/0	56 ± 3	94 ± 4	13160 ± 790	7108 ± 355
28/0.10	1474 ± 73	1057 ± 52	26540 ± 1 592	11730 ± 469
24/0.10	131 ± 5	186 ± 9	15960 ± 798	7021 ± 316

Table 2. Rheological parameters of formulations containing 0.10 % carrageenan and different concentrations of poloxamer 407. All samples were a viscous liquid at 10 °C and an elastic gel at 30 °C.

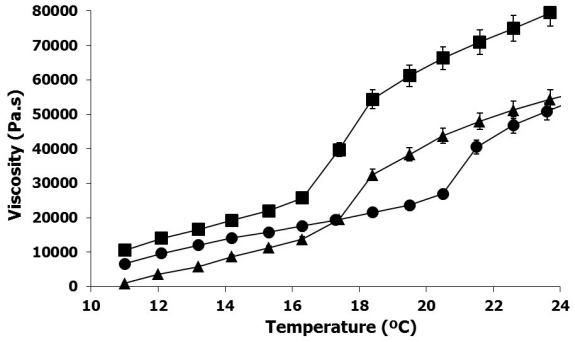

Figure 6. Apparent viscosities of formulations (▲) poloxamer 28 %, (●) poloxamer 24 % -carrageenan 0.10 % and (■) poloxamer 28 % - carrageenan 0.10 % w/w.

relate to the analysis of sol-gel transition previously discussed.

The presence of carrageenan leads to strong changes in the rheological properties of poloxamer, which could indicate possible interactions with the micelles. El Kamel [25] and Miller & Drabik [26] showed that the addition of hydroxylated organic compounds (glycerol and sorbitol) increases the elastic behavior of poloxamer gels while gelation temperature decreases. In fact, these compounds are known to promote the dehydration of poloxamers and therefore hydrophobic interactions between blocks of poly (oxypropylene). Carrageenan, due to its functional hydroxyls groups could induce the same dehydration phenomenon. When poloxamer is the only component in aqueous solution, gelation is explained by the organization of the poloxamer micelles in a micellar cubic phase [27,28]. The gelation mechanism might be different in presence of carrageenan. For example, carrageenan macromolecules might be able to interact with the micelles through secondary bonds, such as hydrogen, reinforcing the structure and therefore the mechanical properties of the gel. This phenomenon could occur before the organization of micelles in a cubic phase. However, additional studies are needed on the forming poloxamer micelles process to better understand the gelation mechanism in formulations studied in this contribution.

CONCLUSIONS

Poloxamer-carrageenan systems are easy to prepare and could be used to carry most of the drugs with applications in veterinary medicine. Very significant modulations of the viscoelastic properties of poloxamer 407 gels were obtained with the addition of a small amount of carrageenan (0.10 %). The low viscosity of poloxamer-carrageenan systems at low temperature facilitates administration in animals and enables intimate contact between the formulation and tissues, besides it should be possible to use these formulations in warm climates due to the reversibility of gelation process. Additionally, these systems need not be removed at the end of therapy because they gradually dissolve. Carrageenan can reduce the rapid erosion of the gels based in poloxamer, so offering new alternative to develop injectable depot modified drugs release systems for veterinary use.

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