Thermosensitive poloxamer-based injectables as controlled drug release platforms for veterinary use: Development and in-vitro evaluation

Bermudez J.M.[1]* and Grau R.[2]

[1]Instituto de Investigaciones para la Industria Química (INIQUI-CONICET), Universidad Nacional de Salta, Buenos Aires 177, A4402FDC Salta, Argentina
[2]Instituto de Desarrollo Tecnológico para la Industria Química (INTEC-CONICET), Universidad Nacional del Litoral, Güemes 3450, 3000 Santa Fe, Argentina

The aim of this work was to explore the potential of combining two different poloxamers (P407 and P188) with κ-carrageenan and sodium chloride for their utilization in the design of thermosensitive injectable depot systems for drug release. These delivery platforms were designed by combination of two different poloxamers (P407 and P188) with κ-carrageenan and sodium chloride. The addition of 10-15% w/w P188 in the poloxamer solution containing 28% w/w P407 allowed optimal gelation temperatures (24-28 °C) and a quick gelation process (30 s). The addition of 0.1% w/w of κ-carrageenan increased 3-fold the gel strength and did not change gelation temperature, compared with P407/P188 (28/15% w/w) alone. On the other hand, sodium chloride increased the gel strength and decreased the gelation temperature as a function of its concentration. In *in-vitro* release experiments, κ-carrageenan decreased the release rate of progesterone and reduced significantly the gel erosion in 48 h. This study demonstrates that addition of κ-carrageenan and sodium chloride into poloxamers blends can be considered a useful tool to design thermosensitive platforms, if added in suitable amounts. In conclusion, this system offers a promising alternative to development of injectable depot controlled drug release platforms for veterinary use.

Keywords: Drug delivery, *In situ* forming gel, injectable depot, poloxamer; veterinary use

INTRODUCTION

Polymeric drug delivery is the most widely studied area of drug delivery in recent years (Kwon and Kim 2005). Polymers can be manipulated to possess certain properties that can meet specific criteria for the designe of suitable delivery systems. Polymeric drug delivery systems may provide advantages such as (i) increased efficacy, (ii) reduced side effects and toxicity, and (iii) convenience (Kwon and Kim 2005). The success of the sustained release of a drug for the required duration of time with the optimum release mode depends on various factors, such as the physicochemical properties of the drug and the drug-carrier matrix in addition on the dosage form and administration route (Hosseinkhani et al., 2009, Subramani et al., 2009). Successful drug delivery will have high academic, clinical, and practical impacts on gene therapy, cell and molecular biology, pharmaceutical and food industries, and bio-production (Hosseinkhani and Hosseinkhani, 2009). Controlled release dosage forms are important products in veterinary medicine owing to several advantages which include better animal compliance, lower side effects and convenience for farmers. Controlled release parenteral formulations are classified by their physical form into liquids (aqueous-based or oily systems), *in situ* forming solids and solids (implants) (Medlicot et al., 2004). *In situ* forming systems for intramuscular use have been recently developed for the production of sustained release systems and represent an attractive alternative to replace implantations, temporary prosthesis or controlled release microspheres (Matscheke et al., 2002). The importance of *in situ* forming matrix systems is related to several advantages such as, for instance, easy application, use

of non-toxic carriers, simple and economical elaboration (Matschke et al., 2002), prolonged residence time and controlled drug release. Moreover these systems avoid painful surgical procedures to insert solid implants. For all these reasons, the development of these novel platforms in the veterinary field is of particular interest both from the scientific and industrial perspective. Particularly, a polymer solution capable of gelling *in situ* at a temperature close to the physiological one might be attractive for the development of drug delivery systems.

The poloxamers, specially poloxamer 407 (P407) and poloxamer 188 (P188), are thermosensitive materials frequently used due to their advantages such as, availability, easy gel preparation methods, good compatibility with several drugs and pharmaceutical excipients (Koffi et al., 2006) and high solubilizing capacity of drugs (Albertini et al., 2009; Albertini et al., 2010). Poloxamer gels have been widely investigated in the human pharmaceutical field as drug release systems since they are relatively easy to obtain and generally recognized as safe (Mayol et al., 2008). It is well known that poloxamer solutions present the reverse thermal gelation phenomenon, which is characterized by a sol-gel transition temperature (Tgel). Below this temperature, the sample remains fluid and above it the solution becomes semi-solid (Matschke et al., 2002). This unique property depends on the concentration of the poloxamer and on the presence of additives such as salts and other polymers (Mayol et al., 2008, Liu et al., 2009, Gratieri et al., 2010). 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 (Nanjawade et al., 2007). Some efforts of poloxamer chemical modification reported in literature showed promising results like slower gel erosion when used as injectable systems, although chemical modifications usually incorporate toxic residues, so they are not widely accepted (Bromberg and Ron, 1998; Cohn et al., 2003). Addition of natural or semi-natural macromolecules to poloxamer's gels is another alternative to obtain gels systems with appropriate mechanical and release properties. Furthermore, they are more profitable, convenient and safe. To this aim, in this work κ-carrageenan (CA) was selected as natural polysaccharide due to its controlled released properties (Liu et al., 2009). Carrageenan hydrocolloid is obtained by extraction with water or aqueous alkali from some members of the *Rhodophyceae* class (red seaweed). Carrageenans are classified into three families (λ-, ι- and κ-) according to the position of sulfate groups and the presence or absence of anhydrogalactose.

The scope of the present contribution was to explore the potential of novel thermosensitive drug delivery platforms designed by combination of two different poloxamers (P407 and P188) with κ-carrageenan and sodium chloride for application as an injectable depot system for veterinary use. Rheological measurements were performed to characterize the viscoelastic properties, gelation temperature and gel strength. The effect of sodium chloride and κ-carrageenan on gelation temperature and gel strength of the injectable depot system was studied. Progesterone was used as a model drug to test the feasibility of these platforms as injectable depot systems. The gel erosion and in vitro release profiles were also evaluated.

MATERIALS AND METHODS

Poloxamers (P407, P188) were a gift of BASF (Germany), κ-carrageenan was kindly provided by Soriano (Buenos Aires, Argentina), progesterone was supplied by Farmabase, (Rovereto, Italy) and sodium chloride by Carlo Erba Reagents (Milan, Italy). All reagents were used without any further purification process. Bidistilled water was used throughout all the experiments.

Preparation of thermosensitive gel

Poloxamer gels were prepared by the “cold method” described by Schmolka (1972), which allows easier poloxamer dissolution and avoids possible alterations produced by heat. An appropriate amount of poloxamer was added to a cold water solution (5-10 °C) while keeping constant agitation with a magnetic stirring rod. To ensure complete dissolution, poloxamer solutions were kept in a refrigerator during 24 h. To prepare κ-carrageenan and sodium chloride solutions, appropriate amounts of these additives were weighed and dissolved in pure water at 80 °C and 25 °C, respectively.

Finally, polymeric platforms were loaded with micronized progesterone (1% w/w), by direct dispersion of the drug in the polymer blend. Magnetic continuous stirring was used to homogenize the mixture. The analyzed formulations are reported in Table 1.

Visual measurement of gelation temperature (Tgel)

A transparent- glass reactor containing 50 ml of poloxamer gel was coupled to a refrigerated circulator bath. A K-type temperature sensor was connected to a digital thermometer and immersed in the poloxamer gel, whith heating rate at 1 °C/min and continuous stirring rate at 80 rpm. Tgel was determined as the temperature displayed on the thermometer when the bar magnet stops moving due to gelation (Choi et al. 1998). This method is simple and easy to perform but shows significant limitations because the sol-gel transition occurs within a large temperature interval, thus the Tsol-

Table 1. Gelation temperature and gel strength of thermosensitive gels composed of poloxamer.

Formulation (P407/P188/CA/NaCl) % w/w	Gelation temperature (°C)		Gel strenght
	Visual determination	Rheology	F (s)
28	17.5 ± 1.3	18.2 ± 0.7	2.0 ± 1.1
28/10	26.6 ± 1.6	25.5 ± 1.0	13.3 ± 1.2
28/15	27.9 ± 1.9	28.2 ± 0.8	72.7 ± 4.3
28/10/0.1	24.6 ± 0.9	25.8 ± 0.7	64.0 ± 4.5
28/15/0.1	26.4 ± 0.9	27.7 ± 0.9	206.0 ± 6.2
28/15/0.1/0.2	26.1 ± 1.5	27.9 ± 0.7	245.8 ± 9.8
28/15/0.1/0.4	24.8 ± 1.6	23.1 ± 1.0	248.1 ± 7.4

gel value is greatly influenced by experimental set-up conditions (Durmotier et al., 2006). For this reason the rheological method, afterwards described, was also used to determine the gelation temperature of poloxamer formulations.

Rheological tests

Oscillatory rheometry was performed for all the evaluated formulations using a rheometer MCR301 controlled stress (Anton Paar, Germany). Experiments were performed to assess changes in the rheological parameters as function of temperature, by oscillatory measurements at a fixed frequency of 10 Hz and with stress amplitude to ensure linear viscoelasticity (nondestructive dynamic conditions). The study was conducted using the cone-plate geometry (50 mm diameter) in a temperature range of 10 to 37 °C with a heating rate of 1 °C/min. The rheometer was also used to characterize the time-dependent changes in the elasticity modulus at 37 °C.

Two moduli were calculated from the rheological data: the storage modulus or elastic modulus (G´) and the loss modulus or viscous modulus (G´´). G' gives information about the stored elastic energy, while G'' describes the viscous character or the energy dissipated as heat. Gelation temperature is defined as the temperature at which the values of both moduli were equal, reflecting similar elastic and viscous properties ("G´ G´´ crossover" criterion) (Durmotier et al., 1991).

Measurement of gel strength

50 ml of poloxamer gel were poured in a 100 ml graduated cylinder and gelled in a thermostated bath at 37 °C. The device for measuring gel strength (weight 33 g) was placed onto poloxamer gel. The gel strength was determined as the time that the device took to sink 5 cm down through the poloxamer gel. If the time would exceeded five minutes, several weights would be placed on top of the device (Choi et al., 1998).

In vitro release experiment

The release experiments were performed using the membraneless model since this procedure allows direct contact between gel and release medium, and gel erosion can also be considered. In membraneless model, two phenomena are involved: the fickian diffusion of the drug and the dissolution of poloxamer. This model has been widely used for poloxamer based gels (Zhang et al., 2002; Liu et al., 2007; Cafaggi et al., 2008; Liu et al., 2009), and specially for injectable formulations (Moore et al., 2000; Paavola et al., 2000; Amiji et al., 2002).

Briefly, the vial containing the loaded gel with progesterone was placed in a thermostatic bath (37 ± 0.05 ºC) until a semisolid gel was formed. Then, 10 ml of release medium pre-equilibrated at 37 ± 0.05 ºC were carefully poured on the surface of the gel. To simulate physiological conditions sodium chloride 0.9% w/w was used. At predetermined time intervals, the release medium was completely replaced by fresh medium kept at 37 ºC and formulations were weighed to calculate the proportion of dissolved gel. The concentration of progesterone in the release medium was determined by UV spectrophotometry at maximum wavelength absorbance, 245 nm (UV2 Spectrometer Unicam, New York, USA).

The results are expressed as a percentage of progesterone released. Progesterone release profiles from each gel formulation were analyzed using the power law model,

$$\frac{Mt}{M\infty} = kt^n$$

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 system (Tarvainen et al., 2002). By fitting experimental results of M_t and M_∞ in the time domain, exponent n was

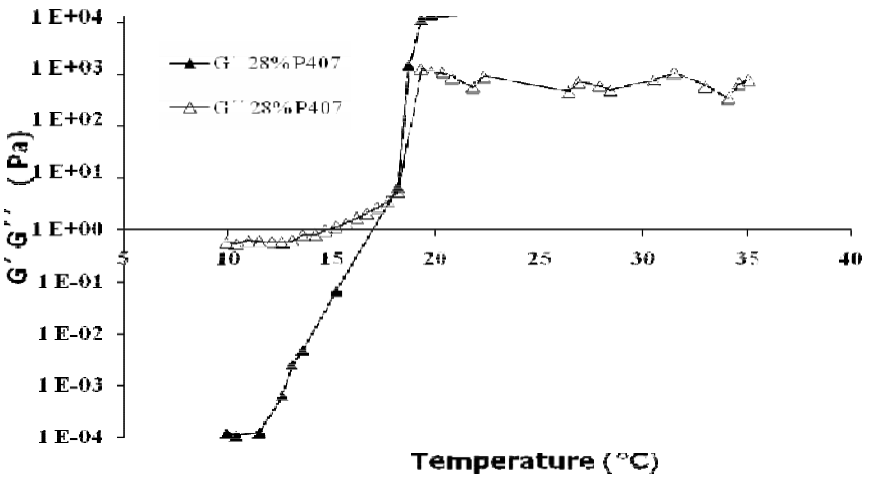

Figure 1. Elastic (G´) and viscous (G´´) moduli as a function of temperature for gel 28% poloxamer 407. Results are the means of three measurements. SD was always lower than 10%. Error bars are omitted for clarity purpose

calculated. This value provides information about the drug release mechanism from the platform (Takka et al., 2001; Bermudez et al., 2008).

All the experiments were carried out at least in triplicate and the results are expressed as the mean ± standard deviation.

RESULTS AND DISCUSSION

The interest of new injectable drug delivery systems has been sparked by the advantages these delivery systems possess. Injectable polymeric matrices, which are already widely used as sustained drugs release formulations, have also been applied in tissue engineering for its potential use as a cell delivery carrier or supportive matrix (Hosseinkhani et al., 2006a,b,c). Interest in injectable scaffolds is mainly related to the fact that they eliminate the need for surgical implantation, increasing patient compliance (Quaglia, 2008). The field of tissue engineering has been benefited significantly by controlled release research and technology. Recently, gels exhibiting a sol-gel thermosensitive behavior have been reported to act as carriers of cells for tissue regeneration (Quaglia, 2008). Gelation temperature is the temperature at which the liquid phase undergoes the transition from sol to gel. If the gelation temperature of the injectable depot system is lower than 25 °C, gelation occurs at room temperature leading to difficulty in manufacturing, handling and administering. If the gelation temperature is higher than 37 °C, the injectable depot still remains as a liquid at body temperature, resulting in loss of the gel immediately after administration.

The goal of this work was to formulate injectable thermosensitive gels made up of poloxamer blends (P407 and P188) and κ-carrageenan with suitable erosion and controlled drug release properties. The viability of these platforms as injectable depot systems for veterinary use was assessed by studying the gelation temperature, the gel strength and the viscoelastic properties. The effect of sodium chloride on the rheological properties of poloxamer gels was also evaluated. Besides the visual evaluation of the studied formulation upon gelification, a proper rheological analysis is a valuable technique to investigate the gelling process and the viscoelastic properties of thermosensitive gels. The oscillatory measurements using low oscillating angle became appropriate because the gel structure remains intact during measurements. This assay gives information about dynamic properties, G´, the elasticity modulus, and G´´, the viscous modulus. In this study, the moduli were measured within the linear region, where the shear force of gel depot is small, and then the results might be close to the *in vivo* situation. The gelation temperature (Tgel) was identified as the temperature at which G´ and G´´ curves intersect each other.

The gelification temperatures obtained by visual determination and by the rheological analysis are reported and compared in Table 1.

Gelation temperature of poloxamer solution containing 28% of P407 (28) alone was 18.2°C (Figure 1). The results obtained indicated that P407 alone could not provide the suitable gelation temperature. Gelation temperature increased along with the concentration of P188. With the addition of 10% and 15% of P188, the gelation temperatures increased about 7°C (25.5°C) and 10°C (28.2°C), respectively (Figure 2). The thermogelation results from interactions between different segments of the copolymers. As temperature increases, poloxamer copolymer molecules aggregate into micelles, due to the dehydration of hydrophobic polyoxypropylene blocks, which represents the very first step in the gelling process. These micelles are spherical with a dehydrated polyoxypropylene core with an outer shell of hydrated

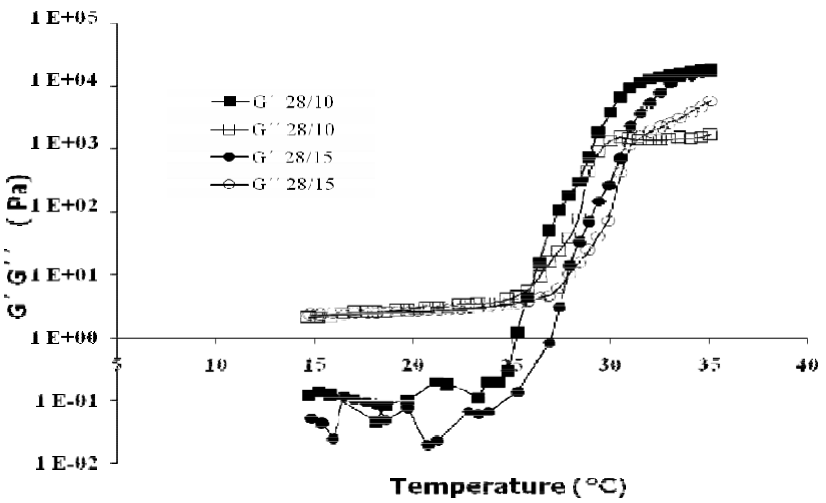

Figure 2. Elastic (G´) and viscous (G´´) moduli as a function of temperature for gels composed of poloxamer 407/poloxamer 188 with different concentrations weight / weight. Results are the means of three measurements. SD was always lower than 10%. Error bars are omitted for clarity purpose.

swollen polyoxyethylene chains. This micellization is followed by gelation when the samples are enough concentrated. This gelation was attributed to the ordered packing of micelles (Durmotier et al., 2006). The addition of 0.1% w/w of κ-carrageenan (28/10/0.1) practically did not change the gelation temperature with respect to the values of the 28/10 and 28/15 poloxamer solutions (Table 1). In addition, Figure 3 clearly shows the intersection of the elastic and viscous moduli of 28/10 and 28/10/0.1 formulations. Besides a suitable gelation temperature, an injectable gel intended for both *in situ* gelification and controlled release should exhibit an adequate consistency and strength. The value of gel strength, which measures the viscosity of the poloxamer gel at physiological temperature, was determined using the device described by Choi et al. (1998) and the results are reported in Table 1. The addition of P188 increased the strength of poloxamer gel. With the addition of 10% P188, the gel strength increased from 2 to 13 s and adding 15% P188, it was 73 s. It can be observed that a little increase in the concentration of P188 in the poloxamer gel leads to a great change in the gel strength. The gel strength increased also considerably when κ-carrageenan was incorporated in the formulation. In 28/15 formulation, the gel strength increased from 73 to 206 s with the addition of 0.1% carrageenan (Table 1). Therefore, κ-carrageenan macromolecules might be able to interact with micelles through secondary bonds, such as hydrogen bond, reinforcing the structure and therefore the mechanical properties of the gel.

These results indicated that 28/15/0.1 formulation resulted the most appropriate one as drug delivery platform since it exhibited suitable gelation temperature (about 28 °C) and gel strength (about 206 s). Therefore, 28/15/0.1 was used to study the effect of sodium chloride on the mechanical properties of poloxamer gels. The formulations P407/P188/CA/NaCl were prepared in the following proportions: 28/15/0.1/0.2 % w/w (abbreviated 28/15/0.1/0.2) and 28/15/0.1/0.4 % w/w (abbreviated 28/15/0.1/0.4). It was also prepared a formulation 28/15/0.1 containing 0.9% w/w sodium chloride but it was excluded from further investigation because its high viscosity at room temperature would make difficult its administration as injectable depot.

It was supposed that sodium chloride could strengthen the bonding of cross-linked reticular in the poloxamers systems by placing them in the gel matrix, because it would interact with poloxamer through strong cross-linking bonds. This behavior could result in an increase in gel strength and a decrease in gelation temperature (Choi et al., 1999). With the addition of 0.2% sodium chloride, the gel strength of 28/15/0.1 increased from 206 to 245 s while gelation temperature did not change. When 0.4% of sodium chloride was added, the gel strength did not further increase significantly (Table 1) but the gelation temperature considerably decreased (from about 27 to 23 °C), which is undesirable. Therefore it is possible to prepare a suitable injectable depot system with a liquid form at room temperature and a gelation temperature in the range 25-28 °C, by adjusting the contents of sodium chloride at 0.2% w/w.

Another important characteristic for *in situ* gelling injectable systems to be considered is the gelation time. In fact, after intramuscular application, a short gelation time would be advantageous, since the risk of dilution with physiological fluid and the possibility of local drainage at the site of application are reduced. Gelation process for the evaluated systems was found to occur very quickly. Gelation time describes time-dependent changes occurring during the gelation. It is defined as the

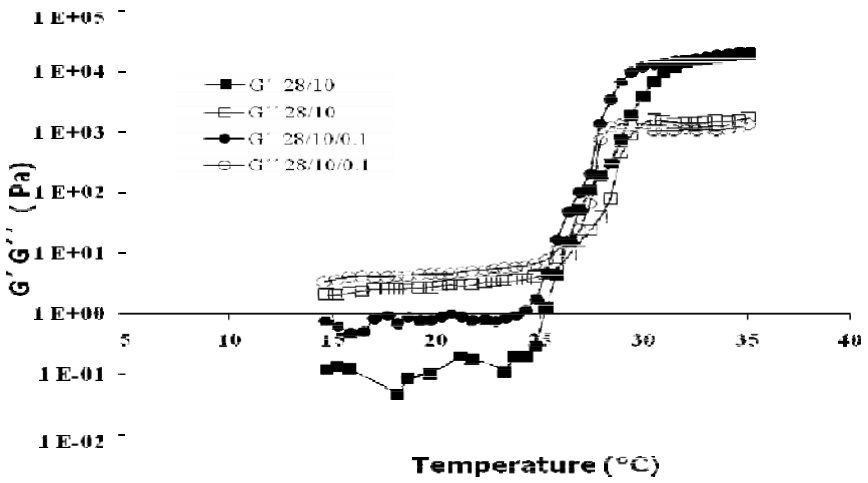

Figure 3. Elastic (G´) and viscous (G´´) moduli as a function of temperature for gels composed of poloxamer 407/poloxamer 188/carrageenan with different concentrations weight / weight. Results are the means of three measurements. SD was always lower than 10%. Error bars are omitted for clarity purpose.

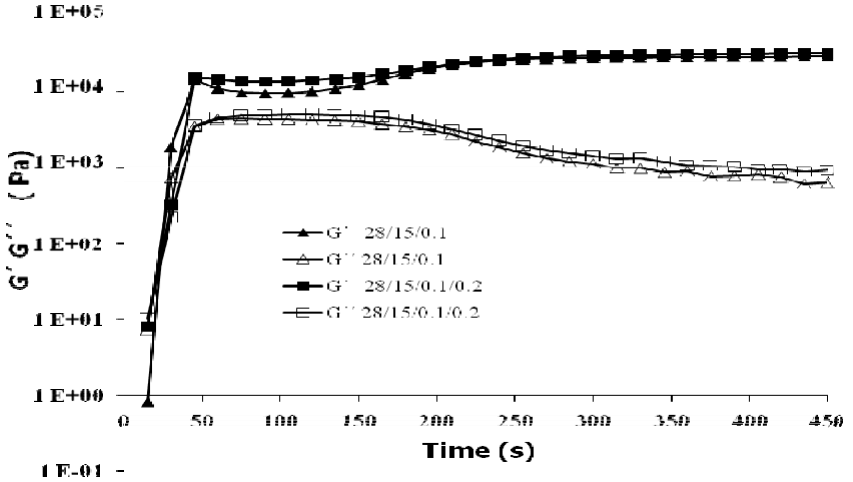

Figure 4. Elastic (G´) and viscous (G´´) moduli at 37 °C as a function of time for gels composed of poloxamer 407/poloxamer 188/carrageenan/sodium chloride with different concentrations weight / weight. Results are the means of three measurements. SD was always lower than 10%. Error bars are omitted for clarity purpose.

time after which the elasticity modulus becomes higher than the viscosity modulus at a constant temperature above the transition. For example, the gelation time of P407/P188/CA (28/15/0.1) and P407/P188/CA/NaCl (28/15/0.1/0.2) was observed at about 30 s (Figure 4).

Another chemical-physical property useful to evaluate the behavior of the gel is its viscosity. It was found that the viscosity of the formulations was significantly influenced by the content of κ-carrageenan and sodium chloride. As shown in Figure 5, the addition of these components, especially sodium chloride, greatly increased the viscosity of the poloxamer formulations at low temperature. Moreover, the curves of viscosity versus temperature obtained for the different formulations exhibited the typical behavior of poloxamer gels and varied linearly at low temperatures but increased substantially in a temperature range of 25-28 °C for poloxamer systems. These results are related to the analysis of sol-gel transition previously discussed.

The addition of progesterone to the studied formulations did not modify the sol-gel transition temperature and the gelation time (data not shown).

With regard to gel dissolution (erosion) studies and progesterone release, physiological solution was used as release medium to simulate the environment after the intramuscular administration.

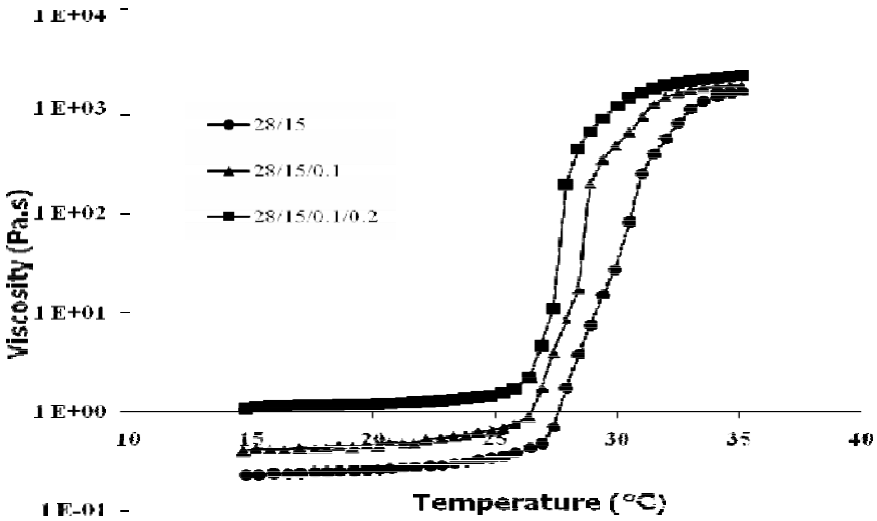

Figure 5. Viscosity as a function of temperature for gels composed of poloxamer 407/poloxamer 188/carrageenan/sodium chloride with different concentrations weight / weight. Results are the means of three measurements. SD was always lower than 10%. Error bars are omitted for clarity purpose

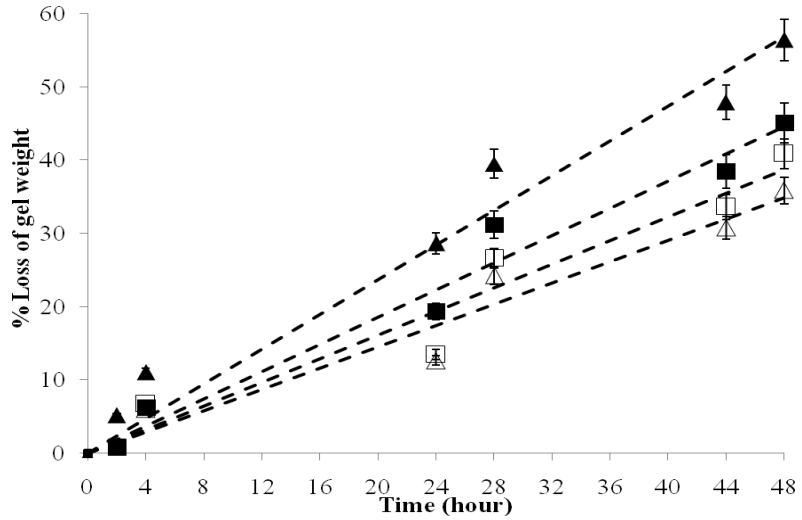

Figure 6. Erosion of thermosensitive gels composed of poloxamer 407/poloxamer 188/carrageenan with different concentrations weight / weight: (▲) 28/10%, (■) 28/15%, (Δ) 28/10/0.1% and (□) 28/15/0.1% (n=3, means±SD).

To asses the influence of the concentration of poloxamer and carrageenan in the erosion of the release platform, gels of poloxamer 407, poloxamer 188 and κ-carrageenan in different proportions (formulations 28/10, 28/15, 28/10/0.1 and 28/15/0.1) were studied. It was observed that erosion was significantly influenced by the P188 concentration, decreasing about 12% when increasing 5% its concentration for gels without CA (Figure 6). This result could be attributed to the increase in the number and size of the micelles formed at the higher polymer concentration. Besides, higher poloxamer concentrations could result in a shorter intermicellar distance, leading to greater number of cross-links between neighboring micelles and a greater number of micelles per unit volume (Zhang et al., 2002).

The erosion decreased significantly for both gels containing κ-carrageenan, compared to the poloxamers gels 28/10 and 28/15 and it was about 35-40% in 48 h in both cases (Figure 6).

Progesterone is a natural hormone used as

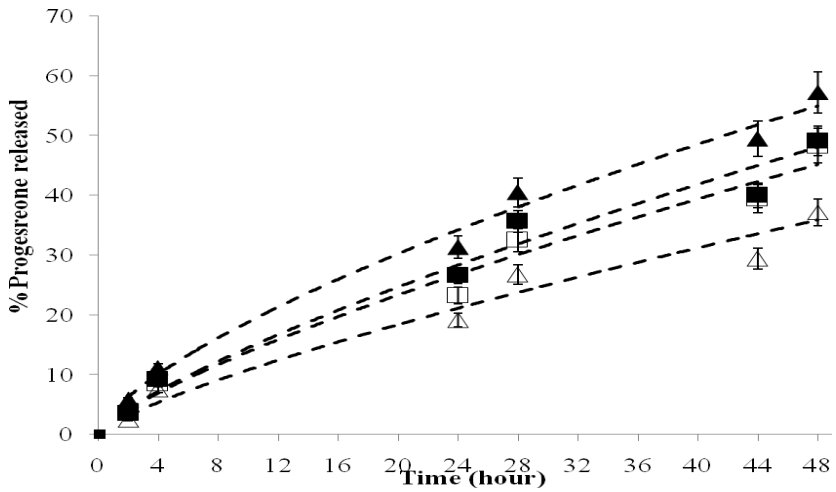

Figure 7. Profiles of progesterone release from thermosensitive gels composed of poloxamer 407/poloxamer 188/carrageenan with different concentrations weight / weight: (▲) 28/10%, (■) 28/15%, (Δ) 28/10/0.1% and (□) 28/15/0.1% in the presence of sodium chloride 0.9% w/w at 38 ± 0.05°C (n=3, means±SD).

Table 2. Data obtained using the fitting model to evaluate the release profiles of progesterone from gels composed of poloxamer 407/poloxamer 188/carrageenan

Formulations (P407/P188/CA) % w/w	*n*	*k*	Fitting equation	Regression coefficient square (r^2)
28/10	0.716 ± 0.065	0.189 ± 0.095	$M_t/M_\infty = 0.189t^{0.716}$	0.995
28/15	0.744 ± 0.082	0.132 ± 0.084	$M_t/M_\infty = 0.132t^{0.744}$	0.993
28/10/0.1	0.755 ± 0.106	0.091 ± 0.074	$M_t/M_\infty = 0.091t^{0.755}$	0.989
28/15/0.1	0.774 ± 0.106	0.098 ± 0.080	$M_t/M_\infty = 0.098t^{0.774}$	0.990

n = diffusional exponent; *k* = kinetic parameter; M_t and M_∞ are the absolute cumulative amounts of progesterone released at time *t* and infinite time using the power law model

contraceptive either alone or combined with an estrogen. The reported solubility of progesterone in water in ambient conditions is 0.016 mg/ml. Drug release curves of progesterone from thermosensitive gels composed of poloxamer 407/poloxamer 188/carrageenan are shown in Figure 7. As it can be seen, all the formulations were able to sustain progesterone release for more than 48 h, which is a time period of interest for an intramuscular administration for veterinary use. Anyway, the release of the drug from these gels was complete within 96 h (data not shown).

The release profile of progesterone from 28/10 gel formulation was higher than that of 18/15. The addition of κ-carrageenan decreased the release of progesterone from the gels containing 10% of P188, but this effect was negligible in the gels with 15% of P188 (Figure 7). Release data were then processed using the power model and results are reported in Table 2. The *n* values obtained were between 0.5 and 1 for all the formulations, suggesting that, besides the diffusion mechanism, other mechanisms are involved in the kinetic control of drug release (Table 2). Therefore, dissolution and relaxation polymer processes could be involved in drug release mechanism. The poloxamer system is one of the swelling-controlled systems, which functions by a process of continuous swelling of the polymer carrier that is associated with simultaneous or later dissolution of the polymer. Poloxamer-carrageenan gels seem to be versatile release platforms providing a wide range of release rates and erosion varying their composition. This could allow new *in situ* injectable platforms with optimal drug release and erosion properties.

Several important issues must be considered in the drug delivery in the veterinary field. The main and most important is the overall product cost, which includes administration expense. The main driving force for the development of novel drug delivery systems intended for livestock is given by the reduction of the frequency of veterinary services and drug administration, the major control and extension of their effects, the reduction of the

motions of roundups and the decrease of the stress of the animals.

According to these features, *in situ* gelling injectable systems based on poloxamers P188 and P407 could be interesting delivering platforms to study. Poloxamer solution containing 28% of P407 alone did not possess the appropriate gelling temperature for its application as drug delivery system. The addition of 10-15% of P188 allowed obtaining optimal gelation temperatures. The results showed that the addition of κ-carrageenan led to an improvement of gel mechanical properties with an increase of gel strength and a decrease of gel erosion. Sodium chloride reinforced the gel strength and reduced the gelation temperature of the gels. Depending on the composition of the poloxamer blend, κ-carrageenan slowed the progesterone release. This study demonstrates that the addition of κ-carrageenan and sodium chloride into poloxamers blends can be considered a useful tool to design thermosensitive injectable depot systems, if added in suitable amounts. Finally, the low viscosity of poloxamers systems at low temperature makes them easy to be injected intramuscularly into the animal body via a syringe, and they could be used in warm climates due to the reversibility of the gelation process. These encouraging results, that need to be further assessed by *in vivo* experiments, show the possibility of using these novel platforms as injectable depot systems for veterinary use.

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