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In vitro release of new designs of modified-release tramadol hydrochloride included in a polymer matrix

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Abstract: Tramadol reaches therapeutic plasma concentrations in a time interval of 0.5 to 1.7 hours, so it is necessary to dose 4 times/day, which reduces compliance with the dose and the effectiveness of the treatment. Design formulations of tramadol that allow the release time to be prolonged, surpassing those obtained with the commercial product and tramadol without excipients. Several formulations of 5% tramadol hydrochloride were designed in a matrix system based on poloxamer 407 at different concentrations (10%, 14%, 17%, and 20%). *In vitro* release studies were performed, using a spectrophotometer at a wavelength of 273.15 nm; were compared the results with tramadol without polymeric supplements and with the commercial formulation samples were taken in a period of time from 0.25 to 72 hours, and also compared the use or absence of dialysis membrane with a porosity of 50 kilodaltons was. With the use of the membrane, the designed formulations had a release of 98%, 50%, 23%, 16% at 72 hours, respectively, different from the commercial product and the tramadol formulation without excipients released the 24 hours. Without using dialysis membranes, a 90-100% release was achieved in the 10% and 14% formulation at 36 hours. The 17% and 20% formulation at 48 hours and the commercial formulation and tramadol without excipient were released within 2 hours. Modified release formulations were obtained, which retain and prolong the release of tramadol compared to the commercial product. Therefore, we propose to conduct further *in vivo* model experiments to confirm our conclusion.

Keywords: Tramadol, release, polymer matrix, *in vitro*, spectrophotometer, membrane, concentrations.

INTRODUCTION

A veterinarian specialized in dogs and cats, as part of his responsibilities besides attacking the origin of the disease, controls pain to preserve the patient's quality of life (Subedi *et al.,* 2018, Giudice *et al.,* 2017). Pain is defined as an unpleasant sensory experience for the animal indicating that there is damage or alteration to the integrity of the tissues and is administered with the purpose to obtain analgesia in several species, both domestic (Taylor *et al.,* 2016; Wolfe and Kennedy, 2015) and zoo (Black *et al.,* 2010; Kilburn *et al.,* 2014; Souza *et al.,* 2012, Baker *et al.,* 2011).

Recently, among the analgesics available on the market, tramadol has been used in clinics and hospitals for dogs and cats due to the safety it generates to manage mild to severe pain with minimal adverse effects (Kögel *et al.,* 2014). Tramadol is characterized by being a bitter white crystalline white powder, which has a solubility of 0.75 mg/mL, is soluble in water and ethanol, has a molecular weight of 263.19 g/mol and a visible detection of 272-279 nm (KuKanich and Papich, 2017).

Papich and Kukanich (2004) refer to therapeutic concentrations of tramadol hydrochloride in the blood of 0.5-1.7 hours in plasma's samples in dogs, being that at 6 hours maximum therapeutic concentrations are obtained, so it is necessary to re-dose 3 or 4 times a day to cover over time in the therapeutic window (Schütter *et al*., 2017). For this reason, the approach of this work is to obtain a formulation that maintains therapeutic plasma concentrations for a longer time compared to those formulations of conventional and immediate release and thus once again used in the clinic of dogs and cats that pain management in patients do not persist and patients have a favorable quality of life (Barter, 2011; Giral *et al.,* 2014).

The advantage of tramadol is that its adverse effects are minimal or zero compared to other opioids (Vazzana *et al*., 2015). In this way a long-acting tramadol is justified for clinical cases in small species (KuKanich and Papich 2017; Schütter *et al*., 2017).

Studies of modified release have been published, based on tramadol hydrochloride with 0.5% chitosan, with carbopol 934-P at 0.7% and with poloxamer 407 at 10%, which reached plasma concentrations up to 12 hours in the formulation by using chitosan and carbopol and 24 hours

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within poloxamer 407 (Gaitán, 2010). Based on these results, it was decided to work with poloxamer 407, increasing the percentage in the formulation in order to achieve a prolonged release of the drug, which exceeds the predecessor.

The poloxamer 407 (Pluronic F127) is a triblock copolymer of the type polyethylene oxide (hydrophobic portion), polypropylene oxide (hydrophobic portion) and polyethylene oxide (hydrophilic portion) which when in contact with water forms a gel (Balakrishnan *et al.,* 2015; Youn, *et al*., 2021; Yu and Di, 2008). They are non-ionic surfactants that are used in the pharmaceutical industry as excipients in various pharmaceutical formulations (Wang *et al.,* 2016). Poloxamer 407 provides an excellent drug delivery system to be used by different routes of administration and is compatible with different substances.

The poloxamer at higher concentrations results in a multimolecular aggregate consisting of a hydrophobic core with polypropylene oxide chains and a hydrophilic crown (Peng *et al.,* 2016; Derakhshanden *et al.,* 2010; Maderuelo *et al.,* 2011). Micellization occurs only in dilute solutions of copolymer blocks above the Critical Micellar Concentrations. At higher concentrations above the Critical Micellar Concentration of the gel the micelles can be added within a network (Mabrouk *et al.,* 2018; Yap and Yang, 2016; Volkmer *et al.,* 2013). The P-407 polymer aggregation process is promoted y increasing the temperature and its concentrations. The micellization is presented above the Critical Micellar Concentration and the Critical Micellar Temperature thus having a slow release favorable to the pharmaceutical industry (Dos Santos *et al.,* 2015; Gao, *et al.,* 2016).

Considering that the short half-life of tramadol can be modified by increasing the exposure of the drug and avoiding re-dose 4 times a day, for cases of mild or moderate pain in patients who require it, it is necessary to design a modified release system while maintaining treatment within the therapeutic margin (Escobar-Chávez *et al.,* 2006; Galgatte and Chaudhari, 2014; Arranja *et al.,* 2016). Recently, among the analgesics available on the market, tramadol has been used in clinics and hospitals for dogs and cats due to the safety it generates to manage mild to severe pain with minimal adverse effects. (Dos Santos *et al.,* 2015; Guzálin *et al.,* 2009; Giorgi *et al.,* 2009).

Poloxamer 407 is a negative thermosensitive hydrogel, which below a temperature is in the liquid state and its gelation occurs in the heating process when the so-called Critical Micellar Temperature is reached, it has been reported to be 24°C (Deore *et al.,* 2010), which allowed aqueous solutions at low concentrations to have the ability to self-organize in the form of micelles above the Critical Micellar Concentrations and Critical Micellar Temperature (Subramanian and Vijayakumar, 2012; Arjunan *et al.,* 2014; Dumortier *et al.,* 2006; Klouda and Mikos, 2008; Al-Soufi *et al.,* 2012).

The objective of this work was to evaluate the *in vitro* release of a tramadol hydrochloride design that achieves a longer half-life compared to the commercial formulation. The results of the rheological properties of the designed formulations are also presented.

MATERIALS AND METHODS

Materials

Tramadol hydrochloride was donated from PISA Agropecuaria SA de CV (Mexico), poloxamer 407 commercially known as Pluronic F127 from Sigma Aldrich (Mexico) and 50 kDa dialysis were obtained through an IBI Scientific distributor, ® the solution of HEPES by Sigma Aldrich.

Equipment

An incubator with shaker and programmed temperature, model: ES-60/ES-60 + /ES-60E, serial number: MU36SM, located in the Faculty of Veterinary Medicine and Zootechnics, National Autonomous University of Mexico.

A rheometer of controlled efforts Discovery HR-3 (TA-Instruments) Controlled efforts geometry: concentric cylinders, located in the Faculty of Chemistry, building F, area of Pharmaceutical Technology, National Autonomous University of Mexico.

Equipment of Spectrohotometer S2000, DT-1000CE-BT tungsten light source (Ocean Optics, Inc., USA) and a quartz cuvette with a 10 mm optical spectrophotometer, located in the Faculty of Chemistry, building F, area of Pharmaceutical Technology, National Autonomous University of Mexico.

Experimental method, release tests

Formulation preparation with Pluronic 407 (F127)

The "cold method" (Gao, *et al*., 2016) was adopted for the preparation of the 5 % tramadol hydrochloride thermo-reversible formulations, the required amount of poloxamer 407 (Pluronic F127, Sigma Aldrich ®) for the formulation was 10%, 14%, 17% and 20% respectively, which was mixing according to Matthew's method (Matthew *et al.,* 2002) for a final volume of 100 mL with deionized water in the laboratory of Faculty of Chemistry, National Autonomous University of Mexico, Mexico city.

In vitro release using of 50 kDa membranes

Based on the Xu *et al.,* (2012) technique, 50 kDa membranes (Sigma Aldrich ®) were used and 0.5 mL of each formulation containing 5% of tramadol

hydrochloride with 10% of poloxamer, 14%, 17% and 20%, named: TP10, TP14, TP17 and TP20 respectively, and were compared the results with tramadol without polymeric supplements (T) and with the commercial formulation samples (TC)

The release medium was into a baker with 333 mL of HEPES Buffer solution with a pH 7.4 at a temperature of 38°C with 100 rpm, then 3 mL of each of the formulations was taken at certain times that should be released in the solution with HEPES Buffer with a pH 7.4, the times of sampling were: 0.25 hours, 0.5 hours, 1 hour, 2 hours, 4 hours, 6 hours, 8 hours, 10 hours, 12 hours, 24 hours, 36 hours, 48 hours, 60 hours and 72 hours, they were still incorporated 3 mL to HEPES Buffer solution again into the baker (fig. 1).

Dialysis membrane of 50 kDa was used for the release tests of each of the formulations with their three respective repetitions, also considering the reference sample without the addition of the poloxamer (T) and the commercial product (TC) that presents an immediate or conventional release.

***In vitro* release without use of a membrane**

Based on the Marcos technique, 0.5 mL of each formulation: TP10, TP14, TP17 were placed in a 5 mL baker (Marcos, 2016), the release medium were 4 mL of HEPES Buffer solution with a pH 7.4 at a temperature of 38°C with 100 rpm, then 4 mL of each of the formulations was taken at certain times that should be released in the solution with HEPES Buffer with a pH 7.4, the times of sampling were: 0.25 hours, 0.5 hours, 1 hour, 2 hours, 4 hours, 6 hours, 8 hours, 10 hours, 12 hours, 24 hours, 36 hours, 48 hours, 60 hours and 72 hours, they were still incorporated 4 mL to HEPES Buffer solution again into the baker.

In this case no one membrane of dialysis was used for the release tests of each of the formulations, for this case we compared both types of formulations.

Spectroscopic technique

Samples of each of the formulations TP10, TP14, TP17, TP20, T and TC with and without membrane were analyzed through the Spectrophotometer S2000 (Ocean Optic, Inc, USA), the absorbance was measured in a wavelength of 273.13 nm. (fig. 2-3)

STATISTICAL ANALYSIS

The percentage of release of tramadol hydrochloride is reported with the average plus the standard deviation of the formulation of TP10, TP14, TP17, TP20, T and TC were calculated using one way ANOVA tests. The differences between the groups were obtained by the Tukey test. A value of $P < 0.05$ was considered statistically significant with the software JMP® (Version 14. SAS Institute Inc., Cary, NC, 1989-2019).

Data are reported as the mean ± standard deviation (SD). The normality of the data was determined by the Shapiro-wilks test and the homogeneity of the variations by the Tukey test comparisons were made with the ANOVA test and the differences between means by the Tukey test.

Experimental method, rheology tests

Simple shear test

Simple shear measurements were determined at temperatures of 38°C, over a cutting speed range of 0.1 to 1000 s^{-1}. For this, a geometry of concentric aluminum cylinders (double Gap, internal cylinders diameters, 20.38 mm; external cylinders diameters, 21.96 mm and 59.5 mm height) was used for samples of the different formulations. The viscosity was estimated as a function of the cutting speed, $\eta(\gamma)$.

RESULTS

Table 1, shows the statistical analysis of the different concentrations of the poloxamer: TP10, TP14, TP17 and TP20 in comparison with the formulation containing tramadol hydrochloride without the addition of the poloxamer (T) and the commercial formulation of tramadol hydrochloride (TC) in the different sampling hours, using or not a 50 kDa membrane.

Fig. 2, shows the calibration curve of tramadol hydrochloride in HEPES Buffer solution with pH 7.

Table 1: Statistical analysis through the ANOVA test to determine the differences between means of the different concentrations of poloxamer 407 in concentration 10%, 14%; 17% and 20% contained in a dialysis membrane of 50 kDa and without the use of the membrane, compared to the tramadol hydrochloride without addition of the poloxamer (T) and with the commercial formulation (TC) with a significant difference of $P<0.05$ by means of JMP Software. The literals (a-c) within the column, without a common letter, differ significantly ($P<0.05$).

Differences between means of the release time of tramadol			
With the use of a dialysis membrane of 50 kDa		Without the use of dialysis membrane	
TC	a	TC	a
T	a	T	a
TP10	b	TP10	b
TP14	c	TP14	c
TP17	d	TP17	c
TP20	d	TP20	c

In Fig. 3, the release percentages of the formulations of tramadol with poloxamer 407 (TP10, TP14, TP17 and, TP20) were compared with the formulation of tramadol without the addition of excipient (T) and with the commercial product (TC), using or not a 50 kDa membrane.

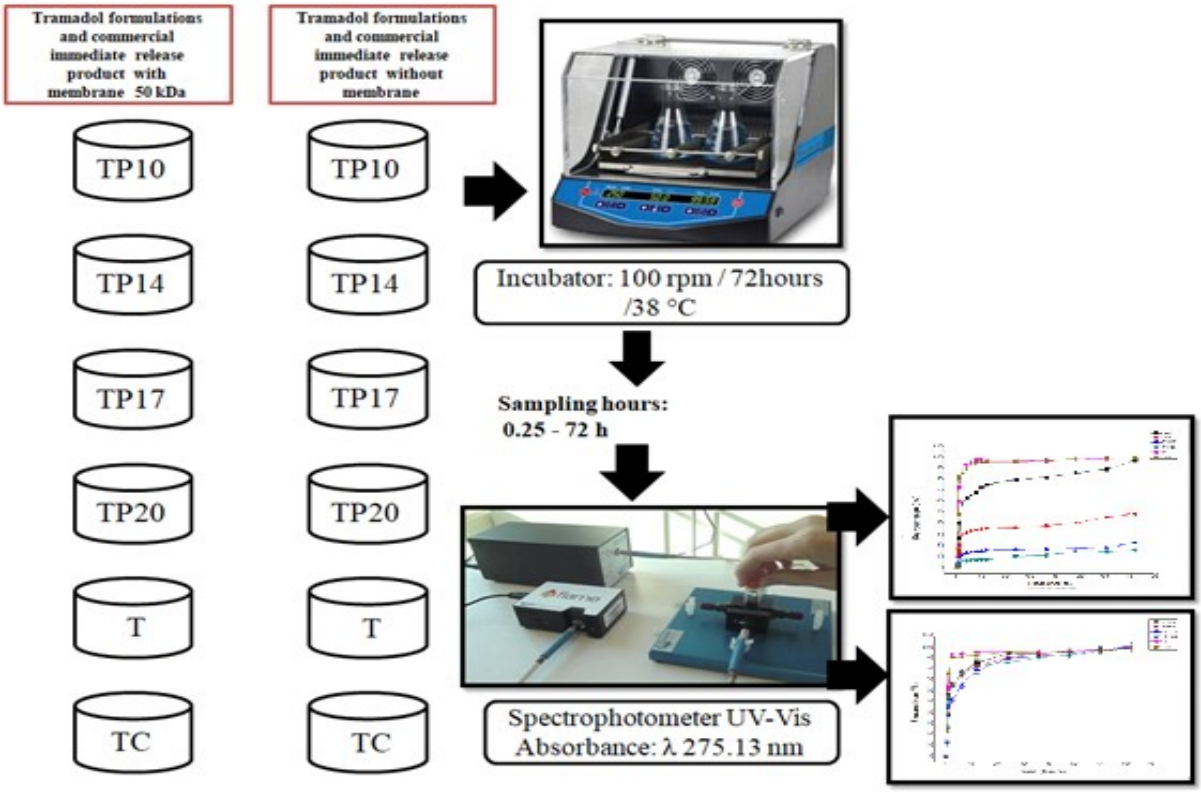

Fig. 1: Design of the *in vitro* release study where 6 formulations were administered within a 50 kDa dialysis membrane at different concentrations of the poloxamer in addition to tramadol, which have to be compared with each other and the same concentrations were also designed without using dialysis membranes at the respective sampling hours to later read its absorbance in the spectrophotometer.

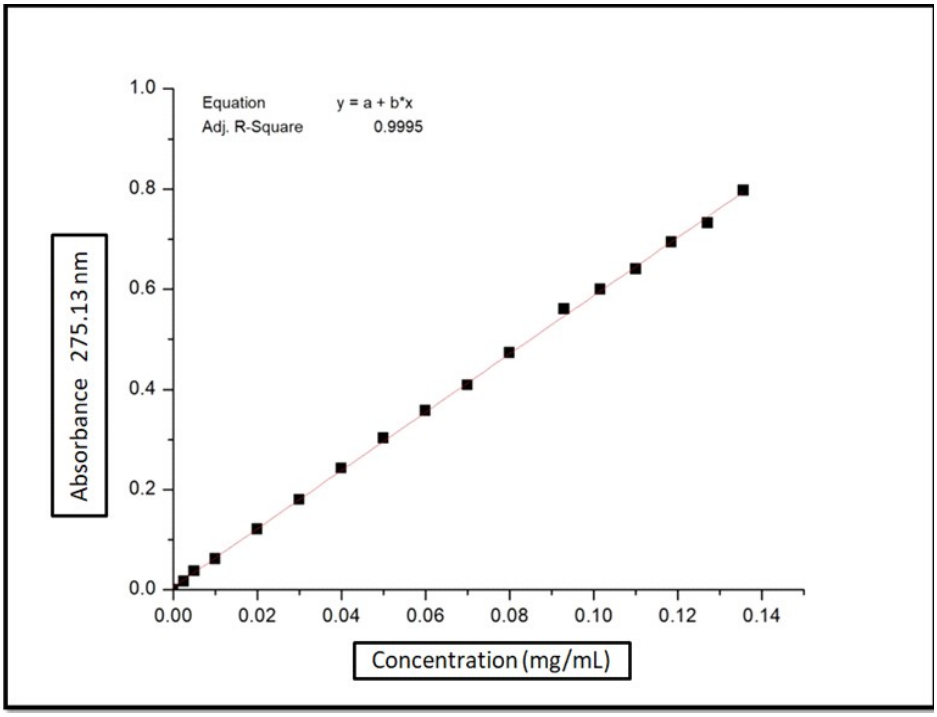

Fig. 2: Calibration curve of tramadol hydrochloride in HEPES Buffer solution with pH 7.4

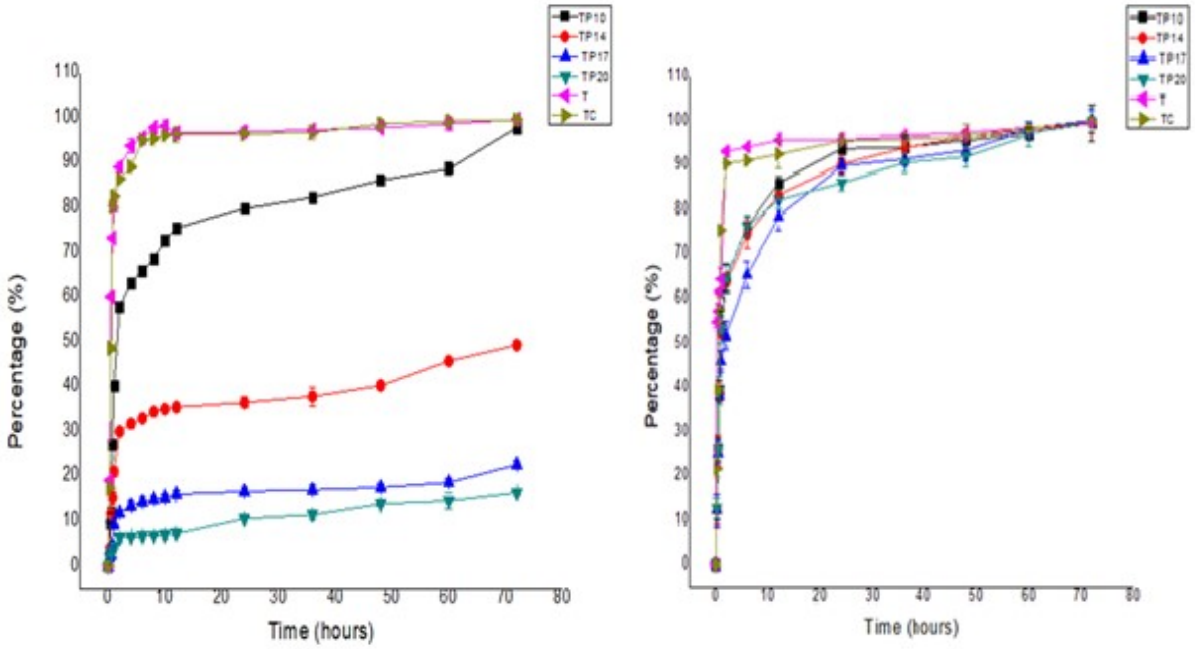

Fig. 3: Release concentration of tramadol hydrochloride in percent of a 50 kDa membrane (left) and without membrane (right) of the tramadol hydrochloride formulations with poloxamer 407 at the concentrations of TP10, TP14, TP17, TP20, T and TC plus the standards deviations.

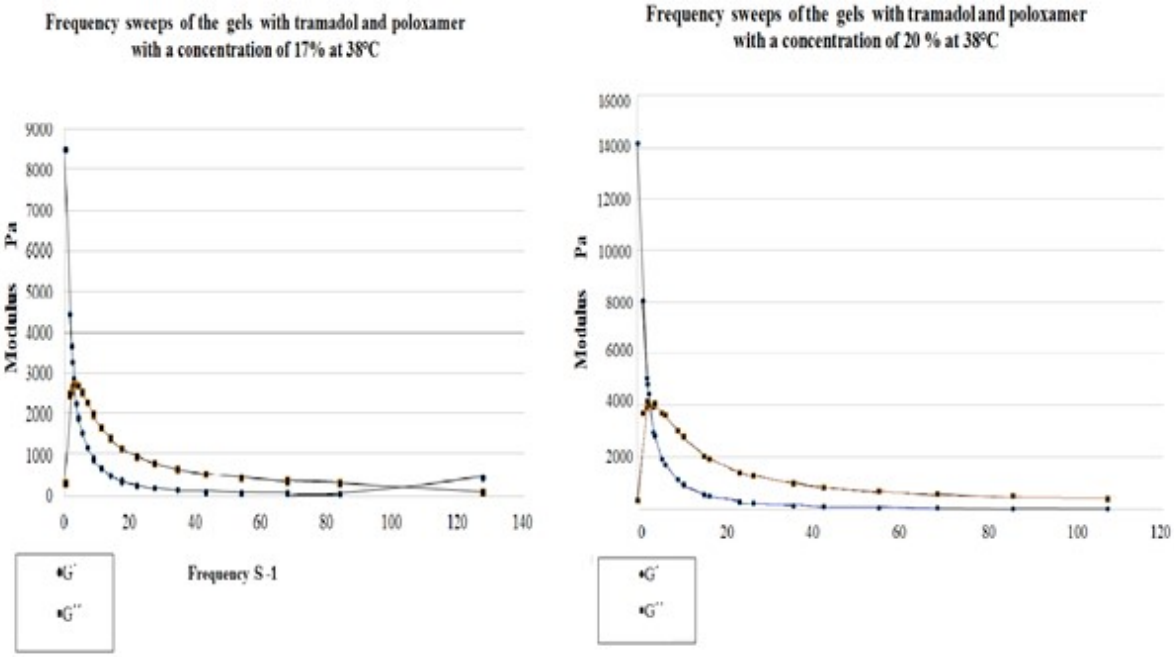

Fig. 4: Rheological analysis of the formulations TP17 (Left) and TP20 (Right) is observed at a temperature of 38 °C where the modulus of elasticity (G′) is compared with the viscous modulus (G′′), where G′ ′> G′, this indicates a viscous behavior, that is, the material flows at that concentration and temperature.

The rheology results of the TP17 and TP20 formulations are shown in figure 4. The oscillatory flow analyzes are appreciated at a temperature of 38°C

DISCUSSION

In agreement with the results obtained during this study, that tramadol hydrochloride in addition to poloxamer 407

forms micelles that are due to the sol-gel transition depending on the concentration and temperature of respective formulation (Dos Santos *et al.,* 2015; Guzálin *et al.,* 2009), which is confirmed with the viscosity results obtained with the 17% formulation at a temperature of 37°C and 42°C and with the 20% formulation at a temperature from 25 C°C (fig. 4-5).

Gaitan's study mentions that commercial formulation used for dogs have an extremely short analgesic effect, being 4 to 6 hours (Gaitan, 2010). Regarding to the experimental preparations made in Gaitan´s proyect when they were using and comparing different formulations, it were concluded that carbopol and chitosan don't obtained a long-action effect of tramadol .

While comparing the concentrations of carbopol and chitosan, it is observed that serum concentrations of tramadol hydrochloride in the preparation based on the poloxamer at concentration of 10% generate concentrations within the therapeutic range, maintaining the concentrations for more than 24 hours, leading to a true long-acting pharmacokinetics (Gaitán, 2010).

Based on the results analyzed, we write the following information:

In the Gaitan's study the concentration with 10% poloxamer with was used and 100% was released at 24 hours, for this reason it was decided to use poloxamer in the present study but with a concentration of 14%, 17% and 20%, although the release of tramadol was also evaluated with a concentration of 10% poloxamer (Gaitán, 2010).

While in the Dos Santos's study were used the formulations of 20% poloxamer a 60% of tramadol was released after 24 hours, in the formulation with 30% poloxamer a 30% of tramadol was released at 24 hours and for the formulation of 35% poloxamer was released 24% at 24 hours (Dos Santos *et al.,* 2015).

In the Dos Santos's study it was only possible to measure at 24 hours but in the present study for formulations in a dialysis membrane of 50 kDa the release was quantified until 72 hours, obtaining the following results:

TP20 and TP17 formulations released tramadol from 10-20% in 72 hours, being that the TP14 formulation released 40% of tramadol at 72 hours and the TP10% formulation releases 70-80% at 72 hours compared to the T and TC formulations that reach the 90-100% after 4 hours.

While in the formulations where dialysis membranes weren't used, the TP20 and TP17 formulation released 95-100% at 72 hours and the TP14 and TP10 formulation reached a release at 48 hours compared to T and TC formulation that reached 90% after 2 hours, it can be corroborated that the results of our study are in accordance to the established by Gaitan and Dos Santos (Gaitán, 2010; Dos Santos *et al.,* 2015).

Just as we evaluated results of our study and compare it with the studies of Gaitan and Dos Santos, the effect of assembling the formulations is due to the temperature and the concentration of the poloxamer, since at low temperatures, both PEO and PPO units are soluble in water, likewise when the temperature increases the PPO units are dehydrated and added thus creating a micellar core while PEO units are hydrophilic forming the micellar crown remain hydrates (Gaitán, 2010; Dos Santos *et al.,* 2015; Escobar-Chávez *et al.,* 2006).

Micellization occurs in dilute solutions of block copolymers in selected solvents above the critical micellar concentration, at a certain temperature. At higher concentration, above a critical gel concentration, micelles can be ordered in a network (Guzálin *et al*., 2009).

It can be said the concentration of the poloxamer is inversely proportional to the release of the drug in the medium, thus resulting in a release of almost 70% in 72 hours for the formulation containing the 17% poloxamer, therefore for subsequent *in vivo* studies will use this formulation as it is mostly eliminated and therapeutic plasma concentrations are expected to be found around this time.

The sol-gel transition temperature for gels containing 5% P-407 of tramadol and as mentioned periodically the gelation temperature is the point where the dynamic modules G´ and G´´ intersect as we saw it in figure 5 with the concentration of 17%, while in concentration with 20% of poloxamer we can not saw the intersection (Zhao and Zhang, 2017; Alvarez-Lorenzo *et al*., 2007).

CONCLUSION

A modified release formulation of tramadol was achieved, from a polymer matrix that achieves a prolonged release of the active ingredient compared to the formulation designed without poloxamer and also with the formulation of the commercial product, which could allow with a single dose of the formulated the therapeutic concentrations of the analgesic remain for more than 72 hours, it can even last up to 100 hours (4 days), which reduces the handling of the animal and allows compliance with the treatment.

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