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Research Article
In vitro Release of New Modified-Release Tramadol HCl Designs and Their Rheological Characterization

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Abstract

Background and Objectives: Tramadol is a widely used analgesic due to its efficacy and lower incidence of adverse effects, but it has a short time of action. In this study, various formulations of 5% tramadol HCl in a poloxamer 407-based matrix system were designed at different concentrations (10, 14, 17 and 20%) to achieve a modified release formulation. **Materials and Methods:** Rheological-characterization and release *in vitro* using an Ultraviolet (UV) spectrophotometer. The results were compared with tramadol salts without excipients (T) and reference medicine (R). The follow-up time was 72 hrs and the use or absence of a dialysis membrane with a porosity of 50 kDa was also compared. **Results:** When the membrane was used, the formulations name TP_{10}, TP_{14}, TP_{17} and TP_{20} had a release of 98, 50, 23 and 16% each at 72 hrs, exceeding 3 times the release time of T and R. When membranes are not used, the TP_{17} and TP_{20} formulations achieved this in 48 hrs instead of the 2 hrs required by the T and R formulations. The use of the 50 kD dialysis membrane was more discriminating as it allowed to differentiate both the quantity and the speed of the tramadol release process. **Conclusion:** Modified-release formulations were obtained, which retain and prolong tramadol hydrochloride release to the reference medicine, which could reduce the daily dose frequency and help to comply with the analgesic treatment in patients with pain.

Key words: Opioids, analgesic, rheology, spectrophotometer, dialysis membrane, pain, poloxamer

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

One of the responsibilities of a veterinarian specialized in dogs and cats, besides attacking the origin of the disease, is to preserve the patient's quality of life, controlling pain[1,2]. 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[3,4] and zoo[5-8].

Within the analgesics, the tramadol has recently been used in clinics and hospitals of dogs and cats due to the safety it generates to manage mild pain to severe pain with minimal adverse effects[9]. Tramadol is characterized by being a bitter white crystalline white powder, which has a solubility of 0.75 mg mL^{-1}, is soluble in water and ethanol, has a molecular weight of 263.19 g mol^{-1} and a visible detection of 272-279 nm[10].

Papich and Kukanich[11] refer to therapeutic concentrations of tramadol hydrochloride in the blood of 0.5-1.7 hrs in plasma´s samples in dogs, being that at 6 hrs maximum therapeutic concentrations are obtained, so it is necessary to re-dose 3 or 4 times a day to cover overtime in the therapeutic window[12]. 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 favourable quality of life[13,14].

Opioids are potent painkillers, such as morphine, fentanyl, buprenorphine and butorphanol, to name a few, but they have some adverse effects: Constipation, nausea, vomiting, sedation, itching and respiratory depression[15]. The advantage of tramadol is that its damaging effects are minimal or zero compared to other opioids. In this way, tramadol's long-acting is justified for clinical cases in small species[10,16].

In vitro, modified-release studies have been published based on tramadol hydrochloride formulated with various polymers, including chitosan, carbopol, or poloxamer (407 or 188) added in different concentrations. In these studies, as the polymer concentration increases, the drug release time increases; however, it has only been possible to reach up to 24 hrs to release the active principle, compared to tramadol formulation without polymer[17-20]. Based on these published results, it was decided to work with poloxamer 407, increasing the percentage in the formulation ($\geq$10%) to achieve a prolonged release of the drug at least two days, surpassing the predecessor and characterizing the formulations rheological viscosity analysis.

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[18,21]. They are non-ionic surfactants that are used in the pharmaceutical industry as excipients in various pharmaceutical formulations[22]. Poloxamer 407 provides an excellent drug delivery system to be used by different routes of administration and is compatible with other substances.

Poloxamer 407 is a negative thermosensitive hydrogel, which below a temperature is in the liquid state and its gelation occurs in the healing process when the so-called Critical Micellar Temperature is reached, it has been reported to be 24°C[23], 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[24-28].

This work's objective was to evaluate the *in vitro* release of a tramadol hydrochloride design that reaches a longer half-life compared to the commercial immediate-release formulation and then administer it in dogs in subsequent work. In this paper, the results of the rheological properties of tramadol formulations at different concentrations with poloxamer 407, which were carried out to predict their end-use properties, are also presented.

MATERIALS AND METHODS

The release study was carried out at the Department of Physiology and Pharmacology, Veterinary Medicine Faculty, National Autonomous University of Mexico, Mexico City, Mexico, from January-November, 2018.

The rheological study was carried out at the Department of Pharmacy, Chemistry Faculty, National Autonomous University of Mexico, Mexico City, Mexico, from January-July, 2019.

Materials: Tramadol hydrochloride was donated from PISA Agropecuaria SA de CV (Mexico), poloxamer 407 commercially known as Pluronic F127 and the solution of HEPES from Sigma Aldrich® (St. Louis, MO) and Float-A-Lyzer (5 mL, 50 kDa MWCO cellulose ester) were obtained from Biotech (Mexico).

Equipment: An incubator with a shaker and programmed temperature (Hanchen, ES-60, Shanghai, China) was used in this study. This incubator was set to deliver a temperature of 38.0°C. The incubator's door was closed and samples were shaking to 100 rpm for 72 hrs (Fig. 1).

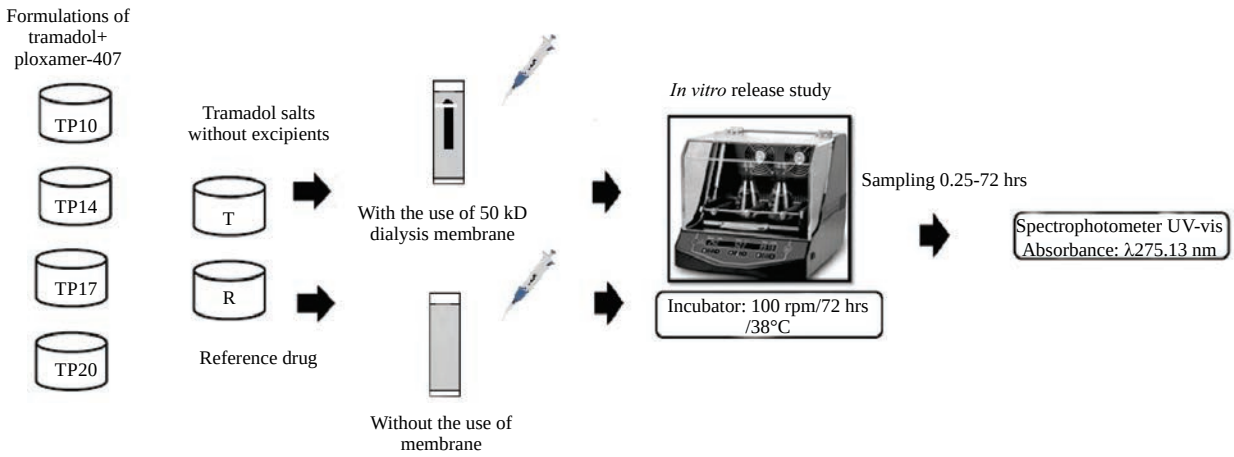

Fig. 1: *In vitro* release studies of tramadol formulations in the matrix system and compared with tramadol salts and reference drug

TP_{10}, TP_{14}, TP_{17}, TP_{20}: Formulations with poloxamer 407 at 10, 14, 17 and 20%, T: Without poloxamer, R: Reference drug

Equipment of Spectrophotometer S2000 (Ocean Optics, Inc., NY, USA) and a quartz cuvette with a 10 mm optical spectrophotometer. Obtaining a spectrum requires manually measuring the transmittance (see the Beer-Lambert Law) of the sample and solvent at each wavelength. The double-beam design greatly simplifies this process by measuring the transmittance of the sample and solvent simultaneously. The detection electronics can then manipulate the measurements to give the absorbance (Fig. 1).

A rheometer of controlled efforts Discovery HR-3 (TA-Instruments, DE, USA) Controlled efforts geometry: Concentric cylinders. Solutions were tested under linear viscoelastic time sweeps with simultaneous ultrasound application to monitor the gelation process. The resulting gel was characterized under linear oscillatory frequency sweeps and stress relaxation tests at room temperature (25, 38 and 45°C) (Fig. 1).

Experimental method

Preparation of the formulation with pluronic 407 (F127): The "cold method" was adopted for the preparation of thermo-reversible formulations of 5% tramadol hydrochloride and the required amount of poloxamer 407 (Pluronic F127, Sigma Aldrich®, St. Louis, MO), according to the required formulation (10, 14, 17 and 20%) respectively, which was mixed according to Matthew's method for a final volume of 100 mL with deionized water[29].

A total of 6 formulations were prepared: R is the Reference drug (Immediate Release 5% Tramadol Commercial Product). T (Tramadol without the addition of poloxamer), 5 g of tramadol was weighed on a precision analytical balance PA124C (Ohaus, NJ, USA), then added to a 100 mL beaker in which 20 mL of deionized water was also added.

TP_{10}, the above procedure was developed, then 10 g of poloxamer 407 (Plutonic F127) was weighed and carefully poured into the solution while stirring with a magnetic stirrer (IKA®, Staufen, Germany) deionized water was added to reach a 100 mL solution. The procedure was carried out in a cold room, using the "cold method"[29].

For TP_{14}, TP_{17} and TP_{20}, the same procedure was carried out; only the concentrations of poloxamer 407 (Pluronic F127) were varied at 14, 17 and 20% (Fig. 1).

***In vitro* release using 50 kDa membranes:** Based on the Xu *et al.*[30] technique, Float-A-Lyzer (5 mL, 50 kDa MWCO cellulose ester) were used and 0.5 mL of each formulation containing 5% of tramadol hydrochloride with 10% of poloxamer, 14, 17 and 20%, named: TP_{10}, TP_{14}, TP_{17} and TP_{20} respectively.

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, 0.5, 1, 2, 4, 6, 8, 10, 12, 24, 36, 48, 60 and 72 hrs, they have still incorporated 3 mL to HEPES Buffer solution again into the baker.

Dialysis membrane of 50 kDa was used for the release tests of each formulation with their three respective repetitions, considering the reference sample without the addition of the poloxamer (T) and the commercial product (R) presents an immediate or conventional release (Fig. 2a).

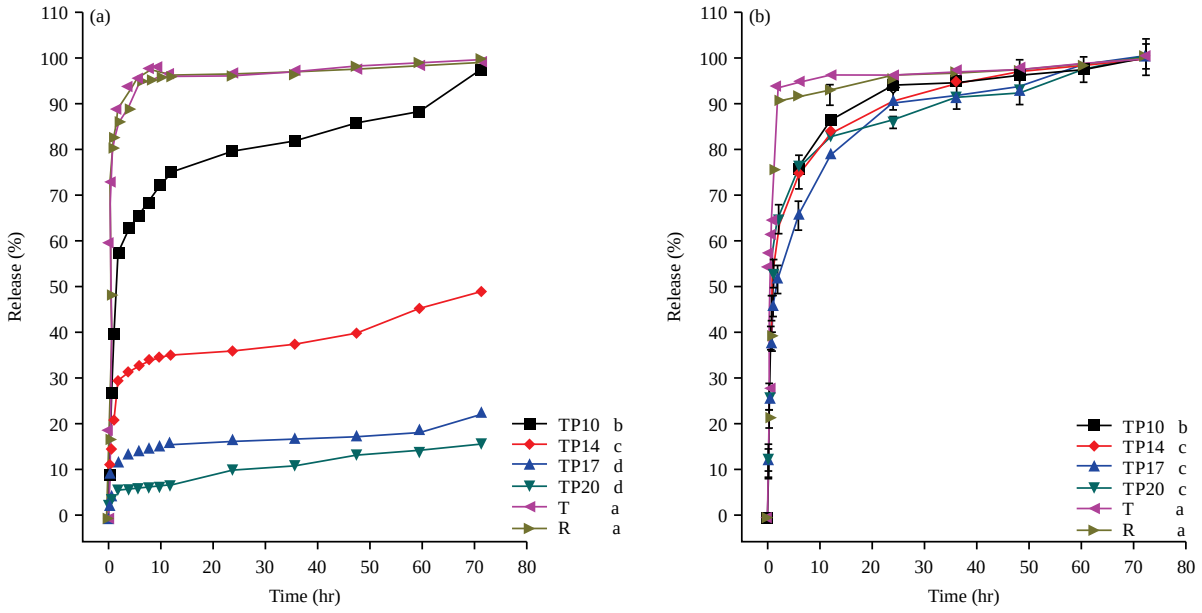

Fig. 2(a-b): Mean±SD release of tramadol HCL

(a) By using a Float-A-Lyzer (5 mL, 50 kDa MWCO cellulose ester and (b) Without using a membrane. The values (a-d) show the difference between groups, without a common letter, they differ significantly ($p<0.05$), obtained from the Tukey test

***In vitro* release without uses of a membrane:** Based on the technique of Marcos *et al.*[31], 0.5 mL of each formulation: TP_{10}, TP_{14}, TP_{17} and TP_{20} were placed in a 5 mL beaker, the release medium was 4 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 were taken at certain times that must be released into the solution with HEPES Buffer, the sampling times were: 0.25, 0.5, 1, 2, 4, 6, 8, 10, 12, 24, 36, 48, 60 and 72 hrs.

As each of the samples was being obtained, 3 mL were added to the HEPES Buffer solution medium with a pH of 7.430 (Fig. 2b).

In this case, not a single dialysis membrane was used for the release tests of each of the formulations (Fig. 2b).

Note: The 3 mL sampling was carried out to recover the total concentration released from the formulation at each moment and to avoid supersaturation of the solvent. It was sought to control the Sink's conditions of the dissolution medium's volume and avoid that the concentration gradient controlled it[20,28,32,33].

Spectroscopic technique: The tramadol calibration curve was prepared with concentrations of 0.001-0.15 mg mL^{-1} in a HEPES buffer solution with a pH of 7.4.

Samples of each formulations TP_{10}, TP_{14}, TP_{17}, TP_{20}, T and R with and without membrane were analyzed through the Spectrophotometer S2000 (Ocean Optic, Inc, USA), the absorbance was measured at a wavelength of 273.13 nm (Fig. 1 and 2a-b).

Based on the study of Dos Santos *et al.*[34] to obtain the release efficiency concentration of tramadol, the next equation was considered:

$$\text{Release drug (\%)} = \frac{\text{Release group amount}}{\text{Actual drug content}} \times 100$$

Rheology tests: A rheological study was carried out using 6 formulations of tramadol at different temperatures: 25, 38 and 45°C with a concentration of poloxamer at 17 and 20% named as TP_{17} (25), TP_{17} (38), TP_{17} (45), TP_{20} (25), TP_{20} (38) and TP_{20} (45°C). Subsequently, samples of approximately 1 mL of the formula were collected at each of the referred temperatures (25, 38 and 45°C), consecutively, they were stored in previously identified glass test tubes with a capacity of 10 mL, which were stored for later analysis. All rheological measurements were performed with controlled efforts geometry: Concentric cylinders, (Discovery HR-3. TA-Instruments) located in the Faculty of Chemistry, building F, area of Pharmaceutical Technology, National Autonomous University of Mexico, was used.

Sample preparation for rheological analysis: All formulations have 5% tramadol, as already mentioned. The drug's dosage was carried out individually according to the manufacturer's instructions and was calculated based on a proportional dose derived from what corresponds to a parenteral dose. in the dog (the target species): 5 mg kg^{-1} [2].

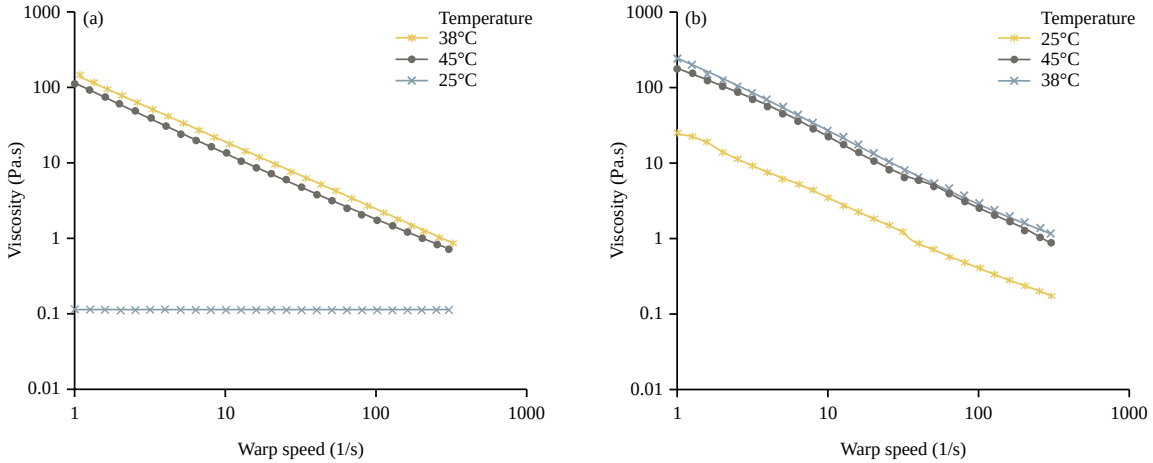

Fig. 3(a-b): Viscosity relationship at different temperatures for a poloxamer at different concentrations
(a) Concentration at 17% and (b) Concentration at 20%

It was necessary to compare the analysis results with the temperatures of 25, 38 and 45°C because the tramadol formulation's viscosity is temperature-dependent. It is necessary to determine if gel formation will occur from the poloxamer 407 in the dogs' temperature (the target species), 38-39.5°C[1]. What did not happen with the other formulations (TP_{10} and TP_{14})?

Simple shear test: Simple shear measurements were determined at temperatures of 38°C, over a cutting speed range of 0.1-1000 s^{-1}. For this, a geometry of concentric aluminium cylinders (double Gap, internal cylinders diameters, 20.38 mm; external cylinders diameters, 21.96 and 59.5 mm height) was used for samples of the different formulations. The viscosity was estimated as a function of the cutting speed, η (γ). Only the results obtained from the formulations TP_{17} and TP_{20} are presented, which presented a more prolonged release of tramadol in the chromatographic analysis (Fig. 3a-b).

For the TP_{17} and TP_{20} formulations, which had a sol-gel transition at 38°C, which is the body temperature of the dog (the target species), Frequency sweeps of the gels, were performed with tramadol and poloxamer with a concentration of 17 and 20% at 38°C to know the behaviour of the elastic modulus (G') compared to the viscous modulus (G'') (Fig. 4a-b).

Statistical analysis: The release percentage of tramadol hydrochloride is reported through the mean and the standard deviation of formulations TP_{10}, TP_{14}, TP_{17}, TP_{20}, T and R were calculated using ANOVA tests. The Tukey test obtained the differences among the groups. A value of $p<0.05$ was considered statistically significant with the software JMP®.

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

RESULTS

The data of Fig. 1 shows the design of the *in vitro* release study where 6 formulations of tramadol hydrochloride 5% were designed in a matrix system based on poloxamer 407 at different concentrations (10, 14, 17 and 20%, T: no poloxamer and R: immediate-release tramadol commercial product). The formulations were administered inside a Float-A-Lyzer (50 kDa MWCO cellulose ester), which must be compared with each other. The release study was also carried out without using dialysis membranes in the respective sampling hours to read their absorbance in the spectrophotometer later.

The calibration curve was used to determine tramadol concentration and verify the analytical instrument's correct functioning, with a correlation coefficient of 0.9995, after applying the linear regression by least-squares adjustment.

In Fig. 2a, the release percentages of the tramadol formulation without the addition of excipient (T) and with the different concentrations of poloxamer (TP_{10}, TP_{14}, TP_{17} and TP_{20}) and with the commercial product (R) were compared, using a 50 kDa membrane. With the use of dialysis membranes, TP_{20} and TP_{17} formulations released 10-20% tramadol in 72 hrs, while the TP_{14} formulation released 40% tramadol at 72 hrs and the TP_{10}% formulation released 70-80% at 72 hrs in comparison with the T and R formulations that reached 90-100% after 4 hrs. The statistically significant

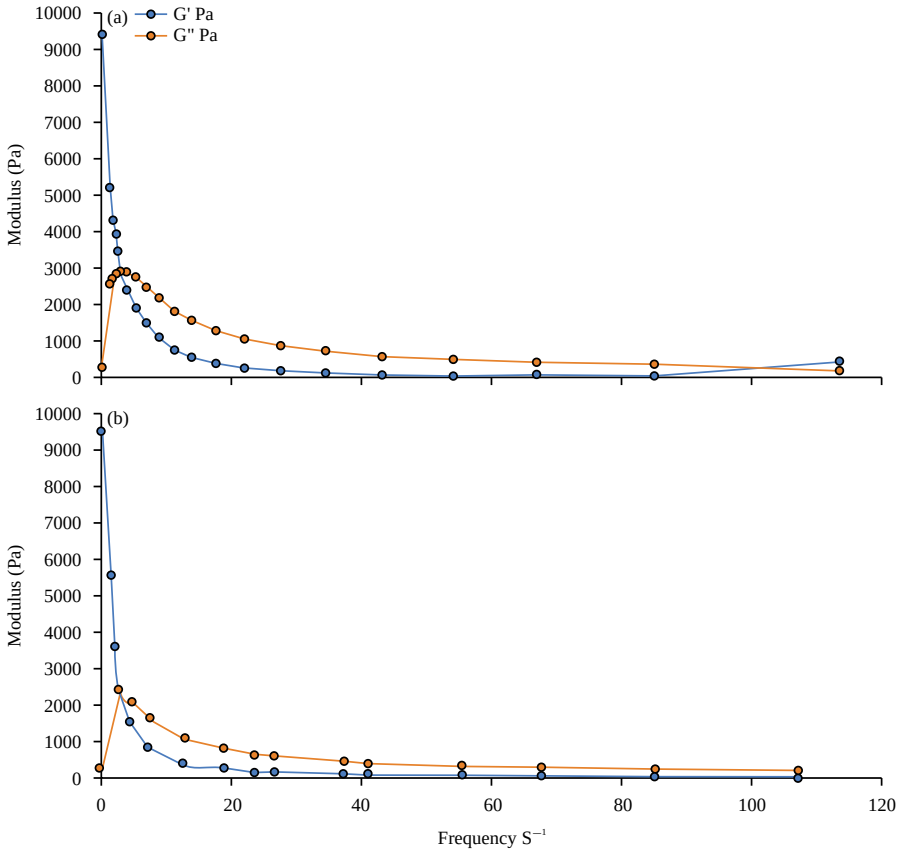

Fig. 4(a-b): Frequency sweeps of the gels with tramadol and poloxamer at 38°C
(a) With a poloxamer concentration at 17% and (b) With a poloxamer concentration at 20%

difference between the groups is shown employing the Tukey test ($p<0.05$) through JMP Software® (Version 14. SAS Institute Inc., Cary, NC, 1989-2019): The four formulations with poloxamer 407 show a difference for T and R, they also offer a statistically significant difference between them, except for the TP_{17} and TP_{20}.

In Fig. 2b, the release study was performed without dialysis membrane, aliquots were only taken to check if the membrane material retained the release of tramadol hydrochloride in addition to poloxamer 407, the graph shows that the TP_{20} and TP_{17} formulations released 95-100% at 72 hrs and the TP_{14} and TP_{10} formulation achieved a release at 48 hrs in comparison with the T and R formulation that reached 98% after 2 hrs. Based on the results of Fig. 2b, the concentration of tramadol hydrochloride release in percentage shows that the concentration of poloxamer 407 is inversely proportional to the percentage release of each formulation. The figure shows the statistical analysis through the ANOVA test. The differences between means of the different concentrations of poloxamer 407 without dialysis membrane compared to tramadol hydrochloride without the addition of poloxamer and with the commercial formulation with a significant difference of $p<0.05$; meanwhile, TP_{10} is the only formulation with significant differences compared with the rest of formulations, using JMP Software®.

Rheological results: The result of Fig. 3a-b show the rheological analysis and the oscillatory flow of the formulations TP_{17} and TP_{20}, which occurred at the different manipulated temperatures (25, 37 and 45°C).

The result of Fig. 3a shows the relationship with the deformation rate and viscosity for the concentration at the different temperatures managed for the 17% poloxamer concentration; the behaviour of the simple shear flow is shown, which the formulation sample exhibits, with a poloxamer concentration of 17% at a temperature of 38 and 45°C, the sol-gel transition is formed, the result is a non-newtonian fluid, indicating that, the viscosity increases, when the temperature deformation rate increases, which does not occur with the formulation at a temperature of 25°C, therefore the formulation with a poloxamer concentration of 17% at 25°C behaves like a Newtonian fluid, which is a fluid where the representation of the shear stress is a function of the reaction rate, creating a straight line as shown.

In Fig. 3b, for the 20% poloxamer concentration, there is a relationship between the strain rate and the viscosity, which indicates that it increases and so does the sol-gel transition. Therefore, at a higher concentration and even at lower temperatures below average body temperature<38°C, the sol-gel transition occurs, it shows the relationship between the viscosity of the designed formulation (Pascals per second) and the cutting speed (1 s^{-1}) at three different temperatures (25, 38 and 45°C) with a poloxamer concentration of 20%. In this case, the simple shear flow behaviour exhibited by the formulation sample with a poloxamer concentration of 20% at the three temperatures: 25, 38 and 45°C are non-Newtonian.

Rheological results with sol-gel optimum temperature and viscosity measurements demonstrated that poloxamer 407 gels are pseudoplastic; therefore, when the strain sweep flow deforms it, its viscosity decreases.

Time sweeps were performed with blank solutions with and without an active agent (tramadol), recording the elastic modulus as a function of time. The resulting gel was characterized under linear oscillatory frequency sweeps and stress relaxation tests at room temperature (25°C) with a controlled-stress rheometer (AR-1000 TA Instruments).

The result of Fig. 4a-b show the oscillatory flow analyzes at a temperature of 38°C of the TP_{17} and TP_{20} formulations, respectively, recording the modulus of elasticity as a function of time.

The result of Fig. 4a shows a predominant viscous behaviour at the start of the test, with the viscous modulus (G") dominating the elastic modulus (G'), as expected for a viscous liquid.

The concentration of poloxamer 407 with 17% shows that the sol-gel transition temperature is the point where the dynamic modules G' and G" intersect, unlike Fig. 4b, which presents the TP_{20}, where it is not possible to observe the intersection.

DISCUSSION

According to the results obtained during this study, tramadol hydrochloride, in addition to poloxamer 407, forms micelles that are due to the sol-gel transition, depending on the concentration and temperature of each formulation[33]. This is confirmed with the viscosity results obtained with the 17% formulation at temperatures of 38 and 42°C and with the 20% formulation at a temperature of 25°C (Fig. 3a-b).

The commercial formulation of tramadol for dogs is known to have a concise analgesic effect, around 4-6 hrs. Researchers have tried to reformulate tramadol to find a prolonged-release. In studies published by Papini and Barati, they used 10% tramadol, when it used carbopol and chitosan for its formulation, it did not achieve the prolonged release of the active principle; however, when using poloxamer 407 at a 10% concentration, it was able to maintain therapeutic concentrations for 24 hrs[17,18].

On the other hand, in the study by Mendonca, who formulated tramadol with 20% poloxamer, 60% of the active principle was released; in the 30% poloxamer formulation, it released 30% tramadol and in the 35% poloxamer formulation, it released 24% tramadol. All of the above was achieved at 24 hrs[34].

Considering these published results, in this project, it was decided to use poloxamer but with concentrations of 10, 14, 17 and 20% to find prolongation of the release of tramadol to 5% since it is the required dose for dogs that is the target species.

In the present *in vitro* release study, a 50 kDa dialysis membrane was used, with which the release was quantified up to 72 hrs, obtaining the following results: Formulations TP_{20} and TP_{17} released about 10-20% tramadol at 72 hrs. The TP_{14} formulation released 40% of tramadol at 72 hrs and the TP_{10} formulation released about 70-80% at 72 hrs compared to the T and R formulations, which reached about 90-100% after 4 hrs (Fig. 2a).

Meanwhile, formulations in which dialysis membranes were not used, such as TP_{20} and TP_{17}, released around 95-100% at 72 hrs. The TP_{14} and TP_{10} formulations achieved a release at 48 hrs compared to T and R, which reached 90% after 2 hrs. Therefore, this study's results are in agreement with those established by Papini and Mendonca[18,34] (Fig. 2b).

The positive results found in the present work can be explained because a prolonged release of tramadol was achieved by varying the concentrations of poloxamer and exposing them to different temperatures; the poloxamer is thermoreversible since at low temperatures, both the polymer fractions, called: PPO like PEO are soluble in water. When the temperature is increased, the PPO units dehydrate and add, creating a micellar nucleus while the PEO units are hydrophilic, forming the micellar nucleus and remaining hydrated[14,35,36].

Micellization occurs in dilute solutions of block copolymers in selected solvents above the critical micellar concentration at a given temperature. At higher concentrations, above a critical gel concentration, micelles can be arranged in a network[23,37].

It can be said that the poloxamer concentration is inversely proportional to the release of the drug in the medium, resulting in a release of almost 70% at 72 hrs for the formulation containing 17% poloxamer, so for future studies *in vivo*, this formulation should be used as prolonged-release was achieved.

The data of Fig. 3a-b show the formulations' deformation sweep flow with poloxamer concentration of 17 and 20%. The result is a non-Newtonian fluid, which indicated that the viscosity at 38 and 45°C decreases when the deformation speed increases. However, having a formulation at 25°C said fluid behaves like a Newtonian fluid. A fluid with representative shear stress works as a function of the deformation sweep speed, developing a straight line, as indicated in Fig. 4, comparing the generally observed behaviour. Coincide with that published by Wszolek[38] and Wang[22] means that the apparent viscosity decreases with increasing strain sweep flow.

On the other hand, taking into account Ricci's research, which shows his rheological results with measurements of viscosity and optimum sol-gel temperature and it is shown that poloxamer 407 gels are pseudoplastic; therefore, when the strain sweep flow deforms it, its viscosity decreases. Furthermore, taking into account Ricci's work in conjunction with the results of the same investigation, it was determined that the sol-gel transition temperature decreases with increasing polymer concentration, in this case, the concentration of poloxamer 407 with 17 and 20%[39,40].

Poloxamer concentrations of 17 and 20% at temperatures of 38 and 45°C showed changes in viscosity, due to the rupture of the internal structure of the fluid, so the viscosity is affected from 38°C, which is an optimal temperature for the transition from the formulation to sol-gel, according to rheology and release studies, the body temperature of the dogs (>38°C).

The viscosity alteration determines that the transition of the formulation from liquid to sol-gel meets the established objective of being a thermo-reversible gel to form micelles related to temperatures and citric concentrations. The same results were observed in the *in vitro* release and rheology[41,42]. (Fig. 3a-b).

With the present study, it was possible to predict, through *in vitro* release studies, to find a prolonged-release formulation of tramadol hydrochloride before conducting *in vivo* tests.

A modified-release formulation of tramadol was obtained from a polymeric matrix that achieved a prolonged release of the active ingredient compared to the formulation designed without poloxamer and the reference formulation. The release profiles of all the test formulations obtained in the present work were not considered similar to the reference drug profile and the formulation without excipient ($p<0.05$). With the administration of a single dose of the designed prototype, the analgesic's therapeutic concentrations are expected to remain for 72-100 hrs, which reduces the animal's assistance and allows compliance with the treatment.

This project involved the performance of characterization tests of new formulations of modified release tramadol, the release results and rheological properties of the formulations were also presented. The applications of the studies developed are to predict the end-use properties of the designed formulations. This study contributed to discovering new modified-release opioid formulations that will help the researcher discover critical areas in the design, development and characterization of small pet pain relievers that many researchers have failed to explore.

The authors recommend that it is convenient to carry out *in vivo* studies to establish the proposed release methodology's predictive capacity. In further research, selected formulations can be evaluated in the target species, which could confirm and overcome the limitations that *in vitro* results have.

CONCLUSION

This study's objective was to design an *in vitro* system before conducting *in vivo* tests on the target species to ensure the sustained release behaviour of tramadol hydrochloride.

According to the results obtained, a modified-release formulation of tramadol was achieved from a polymeric matrix that performs a prolonged release of the active ingredient compared with the formulation designed without poloxamer and the formulation of the commercial product, which could allow with a single dose of the formula. The therapeutic concentrations of the analgesic remain for more than 72 hrs and it can even last up to 100 hrs (4 days), which reduces the handling of the animal and allows compliance with the treatment.

SIGNIFICANCE STATEMENT

This study reveals that the 5% tramadol HCl formulations incorporated into poloxamer 407, in concentrations of 17 and 20%, allowed a prolonged release of the drug up to 72 hrs, exceeding 3 times the release time of tramadol without excipient (T) and the reference drug (R). ($p<0.05$). This study will help researchers to discover critical areas in the formulation of drugs with associated excipients.

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