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Sustained release of lidocaine from Poloxamer 407 gels

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Abstract

In this work, we show that alteration of P407 gel content can affect drug release rates. The inorganic salts and PEG 400 commonly included in the formulation of P407 gels can also change the rate at which a drug is released. Lidocaine was selected as a model drug because, although widely used in the treatment of pain, its use is limited by short duration of its effects. The use of P407 gels prolongs the residence time of the lidocaine at the injection site, sustains drug release and increases therapeutic efficacy. Release studies were performed in a diffusion system. During release, data followed the Higuchi square root law time kinetic ($r > 0.98$). Increased polymer concentration in the gel increases viscosity and reduces lidocaine release rates and diffusion coefficients via extended gel dissolution time and prolonged drug diffusion through the gel matrix. Lidocaine release rates and diffusion coefficients increased in gels composed of NaCl or PEG 400 aqueous solution. Because these additives are hydrophilic, they reduce gel dissolution time, thereby accelerating drug diffusion. Poloxamer is biocompatible and the results support the possibility of using Poloxamer gel as a sustained release injectable formulation.
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Keywords: Poloxamer 407; Lidocaine; Sustained release; Diffusion coefficient; Biocompatibility

1. Introduction

Poloxamers are non-ionic polyoxyethylene–polyoxypropilene– polyoxiethylene (PEO_n–PPO_n–PEO_n) tri-block copolymers with many pharmaceutical applications. Poloxamer 407 (P407), which has a molecular weight of 12,000 Daltons and a PEO/PPO ratio of 2:1 by weight, has been the most widely used of these copolymers. Solutions of P407 at concentrations of 20% show in situ thermoreversible gelation behavior (Schmolka, 1972; Bohorquez et al., 1999; Moore et al., 2000), which permit them to be administrated in cold liquid form by syringe. After administration, P407 cold solution forms a gel in situ at the site of the injection. The P407 gel is biocompatible with cells and body fluids (Park and Park, 1996). Used in pharmaceutical

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preparations, its low toxicity and weak immunogenic properties make it a suitable vehicle for drug delivery (Johnston and Miller, 1985; Veyries et al., 1999; Paavola et al., 2000).

The transition temperature of a sol–gel is lower than body temperature. This has made sol–gel attractive in formulating thermoreversible gels for transdermal implants to control the delivery of various drugs (Craig, 1996; Dash and Cudworth, 1998). Drug delivery via P407 gel occurs by diffusion and dissolution of the gel at the administration site. The release profiles of several drugs delivered via P407 gels have been published: antibiotics (Veyries et al., 1999), anesthetics (Paavola et al., 1998, 2000), antipyretics (Scherlund et al., 1998, 2000), anti-inflammatory drugs (Kin et al., 2000) and peptides (Barichello et al., 1999a, 1999b). These studies have shown that release into aqueous media follows the Higuchi square root law (Higuchi, 1962). These studies have also shown that the drug diffusion coefficient in the gel decreases as P407 content increases, coinciding with an increase in gel viscosity. This has led the authors to propose that drug release rates are determined by gel viscosity (Pandit and Wang, 1998).

Most pharmaceutical P407 gels are formulated with buffer salts (Gilbert et al., 1987; Wang and Johnston, 1993), and may contain the salt form of a drug. Those studies show that salts and organic solvents can change the sol–gel transition temperature of P407 gels. Those studies also show that salts and organic solvents have a significant influence on drug release rates from P407 gels (Pandit and Wang, 1998).

In this study, we report the effects of several salts and polyethylene glycol 400 (PEG 400) on the release of lidocaine hydrochloride from 25% P407 gels. We evaluated the effect of alterations in polymer content, employing release studies of a local anesthetic, lidocaine hydrochloride, from P407 gels. We also examined release from a synthetic membrane into an aqueous receptor phase in order to simulate delivery by injection.

Lidocaine hydrochloride is a weak base and should be ionized under the conditions of our experiment (the pH of our system ranged between 5.3 and 6.5). Since the charged molecules are not expected to enter the hydrophobic interior of micelles, we believe the lidocaine in our gel is located in the outer aqueous region than inside the P407 micelles. Thus, a change in lidocaine release is expected to reflect a change in the viscosity and hydrophilicity of the region outside the micelles. Gel viscosity can be increased by raising the polymer concentration and hydrophilicity can be increasing by adding salts.

Lidocaine hydrochloride is a local anesthetic and was selected as a model drug because its use is limited by the short duration of its effects. Use of P407 gels can prolong the release and thereby sustain the effectiveness of lidocaine.

1.1. Objectives

The purpose of the present work was to evaluate several P407 formulations designed for sustained delivery of lidocaine. The study can be divided into three parts: a physical characterization of the gel formulation, an in vitro release study and an in vivo biocompatibility test.

2. Materials and methods

2.1. Materials

Poloxamer 407 (Pluronic F-127®) was purchased from BASF. Lidocaine hydrochloride was purchased from Sigma Chemical (St. Louis, USA). Polyethyleneglycol (PEG), MW = 400, was obtained from Labsynth (São Paulo, Brazil). The inorganic salt (NaCl) was purchased from Merck (Darnistadt, Germany). The cellulose membrane (MWCO 12,000) was purchased from Sigma (St. Louis, USA).

2.2. Methods

2.2.1. Gel preparation

Gels were prepared on a weight basis using the cold method (Schmolka, 1972). Concentrations of P407 and of lidocaine hydrochloride reported here are expressed as percentage of weight/weight (% w/w). Amount of Poloxamer 407 sufficient to yield 20, 25 and 30% gels were slowly added to cold water (5 °C) maintaining constant stirring. Each dispersion was kept refrigerated until a clear solution was formed (6–12 h). Lidocaine hydrochloride sufficient to yield a 2% concentration was then dissolved in the cold solution. The P407 gels with 2% lidocaine hydrochloride were autoclaved at 120 °C for 30 min and then refrigerated. When PEG 400 (5%) or NaCl (0.9%) were used, they were dis-

solved in water. In both cases, the polymer was slowly added to the cold solvent as described.

2.2.2. Study of rheological properties

The rheological properties of the gels were studied using an ARES strain control rheometer (Rheometric Scientific, NJ, USA). The measuring system involved concentric cylinders (known as Couette geometry), each with an inner diameter of 25 mm, an outer diameter of 27 mm and a height of 32 mm. The cylinders are surrounded by an oven with electric resistance and the entire unit is capable of being heated to, and maintaining, a temperature of 37 °C.

Each cold polymer solution (10 ml per sample) was transferred to the cylinders. Measurements of dynamic viscosity (η') were carried out at 37 °C (normal body temperature), at shear rates (γ) between 0.1 and 400 s^{-1} (Ricci et al., 2002).

$\eta' = G''/\omega$ (Edsman et al., 1998)

$G'' = G^* \sin(\delta)$ (Edsman et al., 1998)

To measure the linear viscoelastic properties, the instrument was used in the oscillatory mode, in which the outer cylinder performs dynamic oscillations at a given frequency. To measure the shear steady state properties, the same geometry was used; in this case, the outer cylinder rotates at a given angular velocity (ω), which produces a shear rate (γ) gradient through the gap between the two cylinders. The studies of rheological properties were realized for determination of gel viscosity and in addition they were employed to establish a relationship between gel viscosity and drug release flow.

2.2.3. In vitro release studies

A diffusion system was employed to in vitro release studies. These studies are very important in evaluating the efficiency of an injectable delivery system (Gordon et al., 1995).

A dialysis tube (of cellulose membrane), previous washed in water, provided a cylindrical gel compartment. The exposed surface area of the gel was 4.5 cm^2. The cold P407 solution was introduced into the gel compartment (0.5 g), which was then placed in the 37 °C oven until the solution gelled and equilibrated. The exterior of the tube was washed with water and checked for leaks. Phosphate buffer sink (60 ml), at pH 7.4 and 37 °C was carefully added to the beaker. The acceptor solution was maintained in a 37 °C bath and stirred with a magnet bar at 100 rpm. The dialysis tube was immersed in the acceptor solution. At each time point (0, 0.5, 1, 2, 4 and 6 h), six systems ($n = 6$) were opened and one milliliter was withdrawn. The amount of lidocaine that had diffused into the acceptor solution was measured by High Performance Liquid Chromatography (HPLC).

2.2.4. Lidocaine measurement

Lidocaine levels were determined using a HPLC system consisting of a Shimadzu liquid chromatograph, model SPD 10A VP (with a variable wavelength UV detector operating at 210 nm), an LC-10 ADVP pump, a Rheodyne injector and a model CR6-A integrator. Separation was performed on a C_{18} reverse-phase column LichroCART® (Merck) 125 mm × 4 mm (5 μm) and a C_{18} pre-column 4 mm × 4 mm (5 μm), at room temperature (28 °C). A (35:65) acetonitrile:0.05 M sodium phosphate buffer, pH 6.0 mixture containing 0.05% diethylamide was used as the mobile phase, at a flow rate of 1 ml/min and a total injection of 20 μl (Ricci et al., 2001). This HPLC method was validated during 6 days and the coefficients of variation for precision and accuracy were below 3%. Detection limits was 0.25 μg/ml with precision of 5% (Causon, 1997).

2.2.5. Data treatment

The model represents drug release from the site of a semisolid implant, where the drug is completely dissolved in the vehicle. Such models follow the Higuchi square root law (Higuchi, 1962). The diffusion coefficient of the lidocaine was calculated from the Eq. (1) (Pandit and Wang, 1998):

$$Q = 2C_0\left(\frac{Dt}{\pi}\right)^{1/2} \quad (1)$$

where Q is the amount of drug release per unit area, C_0 is the initial drug concentration, D is the diffusion coefficient of the drug and t is time. If the rate of drug release obeys this law, the amount of drug release is a linear function of the $t^{1/2}$, and D can be calculated from the slope. The assumptions in this treatment are that the drug is the only component diffusing out of the vehicle, that sink conditions are maintained in the acceptor phase, and that D is constant with respect to time and position in the vehicle. Sink conditions were maintained in the experiments, in that the concentra-

tion of lidocaine at the end of the experiments was less than 10% of the solubility of the drug in the acceptor solution. The medium was maintained in a 37 °C bath and stirred with a magnet bar at 100 rpm.

2.2.6. In vivo biocompatibility studies

Twelve male rats each weighing about 250 g were divided in two study groups. Control group received a subcutaneous injection of saline (300 μl). The second group received a subcutaneous injection of 300 μl of formulation (20% Poloxamer 407 gel with 2% lidocaine hydrochloride). Rats were shaved to a small area on the dorsal part, and saline and formulation were injected in this area. The animals were sacrificed by decapitation at 12, 24 and 48 h. Tissue samples, skin and muscle, around the injection site were removed by a pair of scissors. Thin samples were placed in fixative solution of acetic acid–ethanol–chloroform (10:60:30) at 36 h. The samples were placed in 70% ethanol by 24 h. They were place in a sequence of 80, 90, 95% ethanol and 100% ethanol (three times) at 1 h in each solution. The samples were placed in a solution of ethanol–xilol (1:1) at 12 h and moreover they were placed in xilol at 3 h (Junqueira and Carneiro, 1995).

The samples were placed in paraffin and sliced by a microtome in slices with 5 μm. The samples were placed in xilol at 20 min to remove the paraffin, and in addition they were placed in a sequence of 90, 95, and 100% ethanol to remove the xilol (Junqueira and Carneiro, 1995).

The slices were washed with water and dyed with hematoxylin and eosin. The slices were washed, and in addition they were placed in a sequence of 100, 95, and 90% ethanol at 1 min in each solution. They were placed in xilol three times at 1 min. The slices were fixed in a blade and examined in light microscope (Junqueira and Carneiro, 1995).

3. Results

3.1. Rheological analysis of P407 gels

Shear rate viscosity was measured at 37 °C. Viscosity measurements are shown in Fig. 1. Gel viscosity decreased as shear rate (γ) increased. The gels were pseudoplastic, which facilitated the flow of the formulation. An increase in the concentration of the polymer results in gels becoming more viscous at 37 °C (Fig. 1).

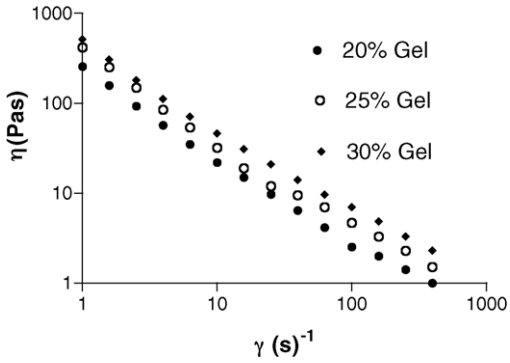

Fig. 1. Shear rate viscosity measurement at 37 °C.

3.2. Release studies

The main purpose of our study was to examine the effects of P407 content alteration on lidocaine release from the gel. We also examined the effects of the inorganic salts and PEG 400 on drug release. Our results show that lidocaine release followed the Higuchi square root law (Higuchi, 1962), and the diffusion coefficient was calculated by Eq. (1) (Pandit and Wang, 1998). Fig. 2a and Fig. 3a show the lidocaine release profiles of the P407 gels. These profiles are similar, although the release rates are different (Fig. 2b and Fig. 3b).

3.3. Effect of P407 alteration content on lidocaine release rate

Fig. 2b shows a release rate graph for diffusion of lidocaine from the 20, 25 and 30% P407 gels. The fraction of drug release was plotted against the square root of the time. For the three formulations, the amount of increase in lidocaine release was linear to the square root of the time ($r > 0.98$). Increased polymer content in the gel retards the lidocaine release rate.

3.4. Effect of NaCl and PEG 400 on lidocaine release rates

Fig. 3b shows release rate graphs for the drug diffusion from the 25% P407 gels containing or not containing NaCl or PEG 400. In this case, a graph was made showing the fraction of drug release and the square root

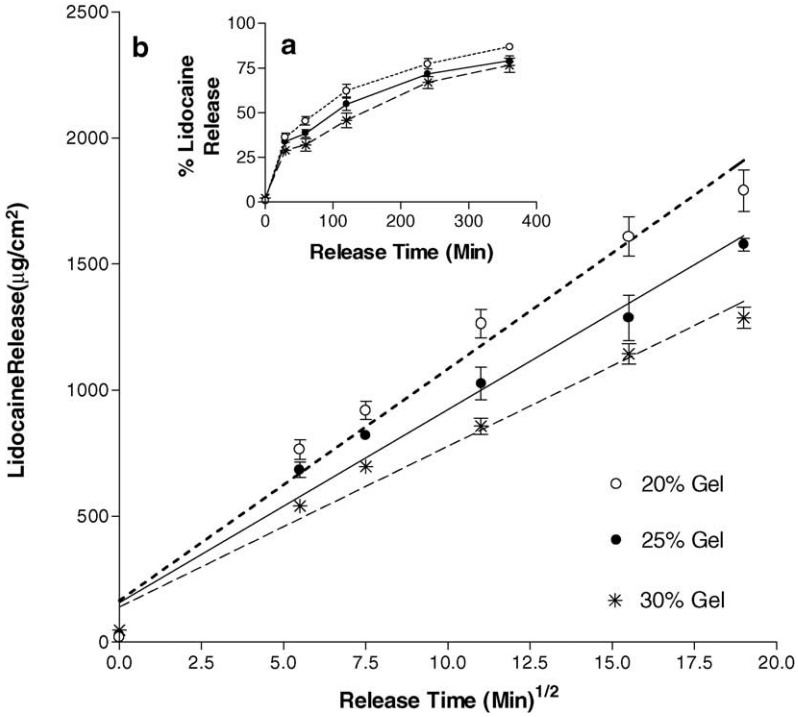

Fig. 2. In vitro lidocaine release profile and release rate from P407 gels. Values were measured by HPLC and expressed as: (a) lidocaine release (%) in the acceptor solution vs. release time or (b) μg/cm^2 (flow) release in the acceptor solution vs. the square root of the time (mean ± S.D.; $n = 6$). The formulations were: 500 mg of 20, 25 and 30% P407 gels with 2% lidocaine hydrochloride. Acceptor solution: phosphate buffer sink (PBS) (60 ml), at pH 7.4, maintained in a temperature at 37 °C, and stirred at 100 rpm.

of the time. For the three formulations, drug release increased linear to the square root of the time ($r > 0.98$). The presence of NaCl or PEG 400 increases drug release rates.

3.5. Effect of polymer content alteration and the presence of additives on diffusion coefficients

Diffusion coefficients for lidocaine were calculated from linear portions of the release rate graph. Table 1 shows lidocaine diffusion coefficients. The increase of P407 content in the gel reduces diffusion coefficient. The presence of either NaCl or PEG 400 increases diffusion coefficients.

3.6. Histology

Fig. 4A is presenting edematous tissue on injection site of the formulation (at 12 h). The edema is a mild inflammatory response. No signs of tissue injury, necrosis, and severe inflammation were observed in the microscopy examination, and addition we did not observe neutrophyles accumulation (Fig. 4B). After 24 h of injection of gel some mastocytes were observed but no signs of skin and muscle damage were evident (Fig. 4C). The most important change was observed at

Table 1
Effect of polymer content alteration and presence of the additives on the diffusion coefficient (D) of lidocaine at 37 °C

Formulation	D ($\times 10^{-6}$, cm^2 s^{-1})
20% P407 gel	2.22
25% P407 gel	1.64
30% P407 gel	1.24
25% P407 gel formed in 0.9% NaCl solution	2.96
25% P407 gel formed in 5% PEG 400 solution	2.70

P407 gels have 2% lidocaine concentration.

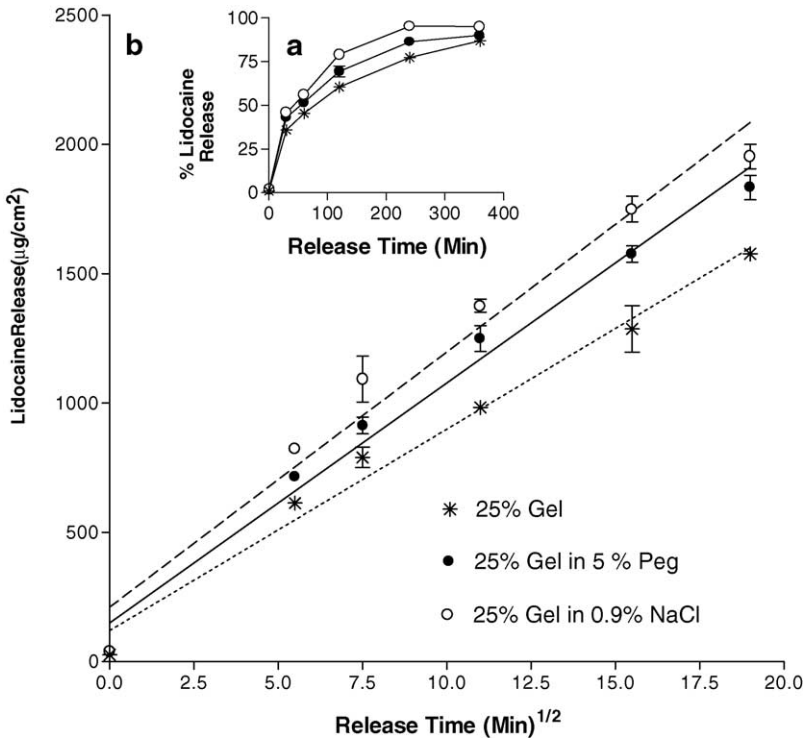

Fig. 3. In vitro lidocaine release profile and release rate from P407 gels. Values were measured by HPLC and expressed as: (a) lidocaine release (%) in the acceptor solution vs. release time (min) or (b) $\mu g/cm^2$ (flow) release in the acceptor solution vs. the square root of the time (mean ± S.D.; $n = 6$). The formulations were: 500 mg of 25% P407 gels formed in water and 25% P407 gels formed in 0.9% NaCl, or 5% PEG aqueous solution with 2% lidocaine hydrochloride. Acceptor solution: phosphate buffer sink (PBS) (60 ml), at pH 7.4, maintained in a temperature at 37 °C, and stirred at 100 rpm.

48 h from rats which had been injected with formulation. Some lymphocytes, plasmocytes, and mastocytes appeared in the tissue, but serious inflammation and necrosis were not evident (Fig. 4D).

4. Discussion

Sustained release drug delivery systems are receiving considerable attention from the pharmaceutical industries because they offer advantages over conventional drug delivery systems. The proponents of these systems claim benefits such as prolongation of drug action, reduction or elimination of side effects, reduction of frequency of administration, better patient compliance and generally more efficient delivery of the drugs (Danckwerts and Fassihi, 1991; Pang, 1998; Pepas, 2000).

The purpose of this work was to investigate lidocaine release from the P407 gels, focusing on prolonging the residence time of the drug at the injection site and thereby increasing its therapeutic efficacy. The combination of lidocaine with the biocompatible P407 vehicle showed sustained release in vitro. In some cases, such as minor surgery, sutures and tooth extraction, P407 cold solutions with lidocaine can be injected and form in situ gels. The gel sustains lidocaine release, prolonging the anesthetic effect and reducing the need for more frequent administration.

Several attempts have been made to sustain local drug levels and thereby increase their therapeutic effect. Many authors have recognized the potential of P407 gels for sustained topical drug delivery in various therapeutic situations. Gels have been tested as drug delivery systems for external application of

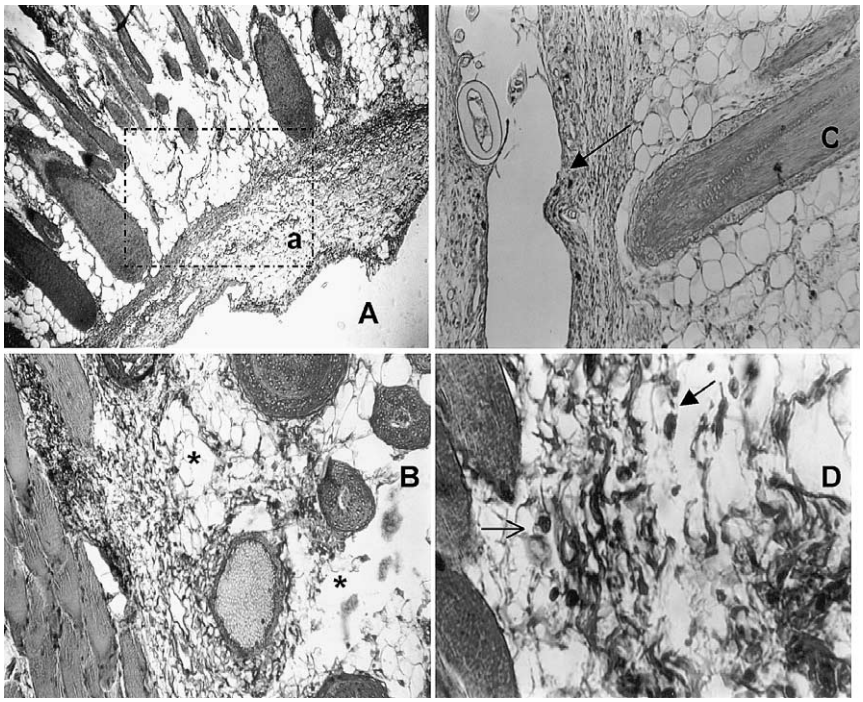

Fig. 4. Microphotography of slices (5 μm) of rat tissue stained with hematoxylin and eosin: (A) rat had been injected with formulation and (a) lump tissue (magnification 100×), (B) rat treated with lidocaine Poloxamer 407 gel (12 h), lump (*) (magnification 600×), (C) rat treated with lidocaine Poloxamer 407 gel (24 h), mastocyte (◀—) (magnification 600×) and (D) rat treated with lidocaine Poloxamer 407 gel (48 h), mastocyte (◀—) and plasmocyte (→) (magnification 600×).

anticancer drugs (Miyazaki et al., 1995), burn treatment (Schmolka, 1972), and anti-inflammatory drugs (Miyazaki et al., 1995; Shin et al., 2000). They have also been investigated as injectable formulations for peptides (Johnston and Miller, 1989; Pec et al., 1992), interleukin-2 (Johnston et al., 1992) and antibiotics (Veyries et al., 1999).

There is good compatibility between P407 and lidocaine hydrochloride. Poloxamer 407 undergoes thermoreversible gelification and, in aqueous solutions with increasing temperature, it aggregates in micelles to minimize the free energy of the solution. In the low-temperature region, P407 exists as monomers in the solution. Upon warming, equilibrium between monomers and micelles is established, and, at higher temperatures, aggregates are formed (Cabana et al., 1997; Moore et al., 2000). It is generally accepted that these micelles are spherical and consist of a PPO core with a water-swollen PEO shell. This conformation is attributed to the fact that PPO is virtually insoluble in water and PEO is highly soluble in aqueous solvent (Cabana et al., 1997; Moore et al., 2000).

In vitro diffusion of lidocaine through the P407 gel was slow. As the P407 consists of a large population of micelles in aqueous phase, the incorporated drug may be released by diffusion through gel matrix. Drug release can be affected by the viscosity of the gel, the size of the aqueous channels and the distribution of the drug between the micelles and the aqueous phase (Anderson et al., 2001).

Increased polymer concentration increases gel viscosity. At a shear rate of 1 s^{-1} (Fig. 1), the increase in polymer concentration causes an increase in gel viscosity. However, as shear rate increases, gel viscosity decreases and the solutions become more fluid, which facilitates the flow of the formulations. Poloxamer 407 gels are pseudoplastic and, therefore, when shear rate deforms, their viscosity decreases.

This increased gel viscosity could alter the dissolution and the process of lidocaine release from the gel.

The process of drug release from the preparation depends of the dissolution from the gels in the aqueous media. More viscous gels release the lidocaine slower than less viscous gels.

In vitro release studies are important in evaluating whether the formulations sustain the lidocaine release. These studies were made in a closed diffusion system. Isotonic phosphate buffer, at pH 7.2 and 37 °C, was used as the acceptor solution. This study employed a cellulose membrane that did not present a barrier to acceptor solution and drug diffusion.

The P407 gels at 20, 25 and 30% prolong lidocaine release and have sustained release profiles (Fig. 2a). Release profiles are similar, but the lidocaine release rate is different (Fig. 2b). The increase in P407 concentration in the gel increases its viscosity. The increase in gel viscosity reduces the lidocaine release rate because gel dissolution time is extended and drug diffusion through the gel matrix is prolonged.

The P407 gels at 25% formed in 0.9% NaCl or 5% PEG 400 aqueous solution prolong drug release and also show sustained release profiles. Release profiles are similar, but release rates are different (Fig. 3b). Gels formed in NaCl or in PEG 400 solutions increase drug release rates when compared with 25% P407 gels formed in water. NaCl and PEG 400 are hydrophilic compounds. Thus, they contribute to increase gel hydrophilicity because they create an osmotic pressure between the gel and the acceptor solution. We believe that the osmotic pressure difference between the gel with NaCl and PEG and the acceptor solution result in reducing dissolution time, causing an increase of lidocaine release rate. When a gel is more hydrophilic its dissolution time in the aqueous acceptor solution is smaller, therefore the drug release is higher.

Lidocaine release from the gel follows the Higuchi square root law (Higuchi, 1962) because drug release concentrations increase with the square root of the time ($r > 0.98$). In this kinetic model, the release process is dependent on formulation characteristics. Lidocaine release from P407 gels is controlled by gel dissolution in the acceptor solution and by drug diffusion from the gel matrix.

The lidocaine diffusion coefficient (D) can be affected by polymer content alteration and by the presence of additives (Pandit and Wang, 1998; Veyries et al., 1999). Diffusion coefficient is reduced by increased polymer concentration (Fig. 1) because the gels are more viscous. When a gel is more viscous its dissolution time in the aqueous acceptor solution is higher, therefore the diffusion coefficient is lower.

Lidocaine diffusion coefficients of the 25% P407 gels formed in inorganic salts and PEG 400 solution are higher than drug diffusion coefficients of the 25% gels formed in water. NaCl and PEG 400 are hydrophilic what increase the gel hydrophilicity. When a gel is more hydrophilic the dissolution is faster, therefore the diffusion coefficient is higher.

Poloxamer 407 gel is biocompatible which do not cause damage in various types of tissues. Johnston et al. (1993) did not observe damage in muscle cells after injection of Poloxamer 407 and they concluded P407 is a promising vehicle for extravascular injection of drugs.

The inflammatory changes in the tissue around the injection site of formulation (lidocaine Poloxamer 407 gel) were generally mild. No signs of tissue damage were evident. Inflammatory changes were also similar after the injection of physiologic saline, the main reason for the changes observed seems to be a mechanical irritation caused by physical presence of the gel. The gel in contact with tissue attracted lymphocytes and plamocytes besides mastocytes, but no signs of serious inflammation were evident. Poloxamer 407 is biocompatible and these cells were attracted for tissue by physics presence of formulation on the tissue.

5. Conclusions

We observed sustained release of the lidocaine from the P407 gels. The release profiles are similar, but lidocaine release rates are different. Lidocaine release follows the Higuchi square root law and is controlled by gel dissolution in the acceptor solution and by drug diffusion from gel matrix. The release rate is dependent on gel viscosity and presence of pharmaceutical additives such as salts and organic solvents. The concentration of P407 in the gel has a strong influence on the drug release rate. Increasing the amount of polymer in the gel increases its viscosity and reduces the drug release rate and diffusion coefficient. The presence of hydrophilic additives in low concentrations increases drug release rates and diffusion coefficients. Poloxamer is biocompatible and because only slight inflammatory changes in the injection site were observed, the results support

the possibility of using Poloxamer gel as a sustained release, injectable extravascular formulation. Poloxamer 407 gel proved to be a promising carrier for sustaining release of lidocaine.

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