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ORIGINAL ARTICLE

Development of oral suspensions of microparticles of ethylcellulose with tramadol

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Abstract

Background: Although tramadol has less analgesic power than morphine, it presents fewer side effects and consequently is currently considered as a drug of choice in the treatment of chronic pain. The objective of this work was to obtain a sustained-release liquid preparation for oral administration, using pseudolatex of ethylcellulose as a delivery vehicle of the active principle. *Methods*: Once an appropriate microencapsulation had been achieved, different formulations with different viscosing agents were designed and subsequently subjected to in vitro release studies, using Franz-type diffusion cells. *Results*: The pseudolatex with tramadol showed an encapsulation efficiency of 82% but was found to be dependent on the quantity of the drug. The images obtained through scanning electron microscopy showed sphere-shaped particles with a porous surface and diameter sizes of 3.5 and 5.5 μm. Infrared spectrophotometry and calorimetric analysis revealed the formation of a drug–polymer complex. Of the formulations proposed, that with xanthan gum released 46% of the drug, whereas Carbopol®, sodium carboxymethylcellulose, and Avicel® gave 50% and 55%, respectively. All followed a release kinetic of cube root, with the release mechanism of the active principle occurring through anomalous transport. *Conclusions*: In accordance with the studies performed, we can confirm a liquid pharmaceutical preparation for oral use, capable of providing a sustained release of tramadol.

Key words: *Ethylcellulose; microencapsulation; oral administration; sustained release; tramadol*

Introduction

Although morphine continues to be considered as a prototype drug and the most commonly used treatment for chronic pain of any etiology, it cannot be forgotten that its adverse effects (nausea, vomiting, drowsiness and confusion, myosis, urine retention, constipation, respiratory depression, tolerance, and dependence)[1] are a direct consequence of its pharmacological activity. As a result, interest has arisen in the weaker opiates, while numerous revision articles and studies describing the favorable results obtained from their use have begun to appear[2,3]. Tramadol, because of its lower analgesic potency, has been categorized as a second-line analgesic drug. It has a double-action, opiate, and nonopiate mechanism, which allows the combination of the effects of the opiates and the neuronal reuptake of noradrenaline and serotonin[4,5] to be achieved in one single dose. Despite the lower analgesic potency of tramadol in comparison with morphine, tramadol has fewer side effects, while presenting the major advantages of lower degrees of addiction and tolerance. It can therefore be considered as a drug of choice in the case of chronic pain.

On the other hand, orally administered preparations have traditionally been the most widely used forms in the treatment of pain, above all in the case of opiates presenting acceptable levels of intestinal absorption, because of the numerous advantages associated with this type of administration, that is, convenience, autoadministration, greater patient independence, and so on[6,7]. Controlled release systems are currently considered as the most appropriate form of treatment of chronic pain, given that they ensure constant plasmatic levels of the drug, with neither peaks nor high accumulations. Their design and manufacture are often associated with the use of polymeric materials as transport

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vehicles of the active principle[8–11]. This research work is based on a colloidal suspension that was developed by our group[12], using a pseudolatex of ethylcellulose as transport vehicle, with the process of microencapsulation of the drug being achieved with the polymeric particles formed by this material. This suspension provides the basis for an orally administered liquid pharmaceutical formulation with sustained-release properties and presents considerable advantages for patients who have difficulties swallowing or digesting, such as children or the elderly.

Previous studies have shown pseudolatex particles to posses the capacity to microencapsulate morphine[12]. However, the objective of this study was to discover whether other drugs indicated for the treatment of chronic pain, such as tramadol, could be microencapsulated by these particles to an equally successful degree. Consequently, once appropriate microencapsulation of the drug had been achieved, pseudolatex was subjected to an exhaustive characterization process to assess homogeneity and reproducibility under laboratory conditions. Finally, a stable pharmaceutical preparation was designed, which proved to be durable and possessed appropriate organoleptic characteristics. In this study, the different formulations containing different viscosing agents were compared whereas the drug release profiles of each one were studied using Franz-type diffusion cells.

Materials and methods

Materials

The ethylcellulose polymer (9004-57-3) was supplied by ICN Pharmaceuticals (Aurora, OH, USA) with an ethoxy content of 49.3% and viscosity of a 5% solution in toluene ethanol (4:1) at 25°C of approximately 45 cps. Tramadol hydrochloride was provided by Fluka Biochemica (Suiza, Switzerland) and met the requirements of the European Pharmacopoeia. Of the thickeners tested, xanthan gum was provided by SBI System Bio-Industries S.A. (Barcelona, Spain), Carbopol® 934-P by Quimidroga S.A. (Barcelona, Spain), and sodium carboxymethylcellulose 110–200 and Avicel® (microcrystalline cellulose) by Labor Tecnic (Barcelona, Spain).

All chemicals were of analytical quality and manufactured by Panreac (Barcelona, Spain). Water was used to prepare the solutions and suspensions were of Milli-Q quality (Milli-Q Academic, Millipore, France).

Methods

Ethylcellulose pseudolatex preparation

The pseudolatex was prepared using a modified version of the technique proposed by Vanderhoff et al.[13], with the omission of the use of the stabilizing emulsifier cetyl alcohol and an increase in the quantity of sodium lauryl sulfate. Given that the cellulose polymer is not soluble in aqueous media, it was therefore prepared through polymer emulsification. Synthesis was initiated by dissolving ethylcellulose in a suitable mixture of solvents (ethanol and benzene in a 15:85 proportion) and by emulsifying the polymers in water with sodium lauryl sulfate at a concentration of 0.4%. Emulsification was performed by mechanical stirring. The solvent mix benzene/ethanol has been totally eliminated from the final product following an evaporation method.

The determination of encapsulation efficiency

The quantity of encapsulated drug was determined by preparing different pseudolatex with different drug concentrations to discover what influence this factor had on the encapsulation capacity of the microparticles. The drug was incorporated to aqueous phase (continuous phase) during preparation process[12]. The samples of pseudolatex were centrifuged at 21,500 × *g* for 30 minutes, after which the supernatant obtained was collected and centrifuged once again for the same period of time and at the same speed. The sediment obtained from this second centrifugation process was removed and the resulting supernatant was filtered using a 0.2 Millipore filter so as to ensure that all microparticles had been removed. The free standing drug left suspended in the supernatant was then quantitatively determined using spectrophotometry.

Microphotographic study

The characterization of size, shape, and surface properties of the microparticles was carried out through scanning electron microscopy, using a Hitachi S-5-10 scanning microscope.

Infrared spectroscopy

The spectrum was obtained using an infrared spectrophotometer (Nicolet 20 SXB, Houston, TX, USA), with a resolution of 2 cm^{-1}.

Calorimetric analysis

Differential scanning calorimetry was carried out using a Mettler FP85 device, within the temperature range of 50–300°C, with measurements being taken at intervals of 5°C/min. The samples, each weighing between 5 and 6 mg, were obtained after centrifuging the polymeric suspensions (PS) with the drug at 16,640 × *g* for 30 minutes. The resulting sediment was left to dry at room temperature.

Design and preparation of the final suspension

Three different thickeners were tested: the natural polysaccharide xhantan gum, the synthetic thickener

Table 1. Components of the final formula.

Formulation	
Polymeric suspension	50%
$CaCl_2$ 10^{-2} M	c.s.
Anhydrous sodium sulfite	0.1%
Simethicone	0.5%
Kathon CG	0.1%
Thickener	50%

Carbopol® (for internal use), and the semi-synthetic thickener sodium carboxymethylcellulose/Avicel®. Each one was tested at a concentration of 1% to determine its effect on the pharmaceutical formula. Calcium chloride at a concentration of 10^{-2} M was added to all the thickeners to obtain a weakly flocculated suspension that was easily redispersible. In light of previous studies on the design of polymer suspensions for oral administration, simethicone was added to the final formula as an antifoaming and antibloating agent so as to counteract the effects of the emulsifier, together with anhydrous sodium sulfite as an antioxidant and Kathon CG® (Panreac, Barcelona, Spain) as an antimicrobial agent (Table 1).

Analytical methods

The concentration of tramadol hydrocloride was measured using UV spectrophotometry at 270 nm (λ_{max}). The method was previously validated and verified for accuracy, precision, and linearity. Standard solutions were prepared by diluting the stock solution (200 µm/mL) with phosphate-buffered saline at the following concentrations: 50, 100, 125, 150, 175, and 200 µm/mL. A Perkin-Elmer UV-Vis Lambda 40 UV spectrophotometer was used to take all measurements.

Diffusion experiments

Franz-type diffusion cells have been commonly used in these types of studies[14,15]. The FDC-400 cell (Vidra-Foc, Barcelona, Spain) used in this experiment consisted of two compartments with a membrane clamped between the donor and the receiver chambers. The receptor phase was phosphate-buffered saline, at a chosen pH of 6.2, considered appropriate, given that this is the pH encountered in the proximal portion of the small intestine, where the drug is absorbed after oral administration.

The membranes were 47 mm in diameter and 0.45 µm in pore size. Subsequent to tests on membranes of both methylcellulose (Teknokroma, Sant Cugat del Valles, Barcelona, Spain) and nylon (Waters Corporation, Barcelona, Spain) the nylon variety were selected for our diffusion studies, given that these were found to offer the least resistance to the diffusion of the active principle.

The release mechanism of the active principle from the microparticles

As a means to predicting the release mechanism from the microparticles, the semi-empirical model published by Peppas and Sahlin[16] was used in accordance with the following equation:

$$\frac{M_t}{M_\infty} = k \cdot t^n,$$

where M_t represents the quantity of drug released at time t, M_∞ the quantity of drug accumulated at infinite time, k the kinetic constant (an experimentally determined parameter), depending on the structural and geometric characteristics of the system, and n is an exponent that depends on the geometry of the system, associated with the release mechanism.

Results and discussion

Encapsulation efficiency

As shown in Table 2 the concentration of the drug in the medium has a direct influence on encapsulation efficiency. Eighty-two percent encapsulation of tramadol hydrochloride was achieved with an increase in active principle concentration during the continuous phase. From this point on, as can be observed, efficiency decreases concomitantly with an increase in drug concentration. This can be explained by the fact that it is at this point when the maximum load that the microparticles are capable of accommodating has been reached.

Microphotographic study

The images obtained through SEM of pseudolatex with tramadol show spherical particles with a porous surface (Figure 1). This porosity on the microparticles surface is a direct consequence of the preparation process, or more specifically, which occurs during the evaporation stage of organic solvents. This property has a decisive influence on its release kinetics[17]. Some studies have established a strong relationship between a higher

Table 2. Influence of drug concentration on percentage of tramadol hydrochloride encapsulated.

Concentration (µg/mL)	Percentage encapsulated	DE
50	30.55	8.96
100	41.32	1.42
250	82.14	1.97
450	60.44	0.45
600	43.77	1.02

Note: DE – Standard deviation (in english).

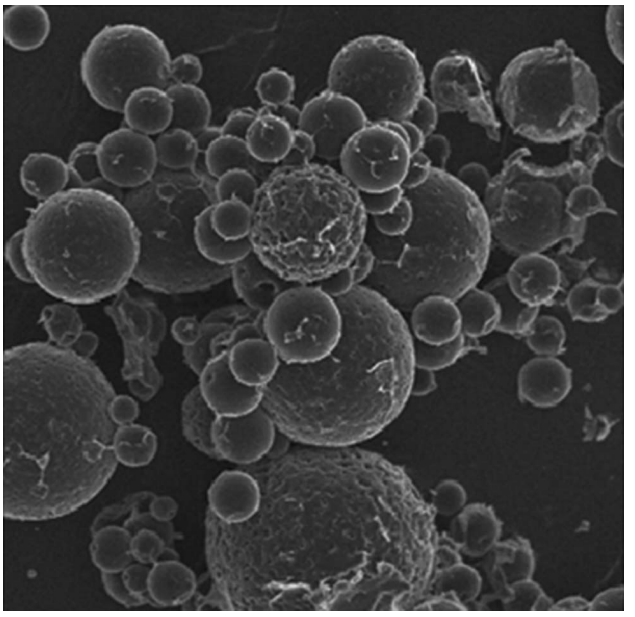

Figure 1. SEM micrographs of microparticles containing tramadol hydrochloride.

degree of porosity and a greater rate of active principle release[18,19].

This same microphotograph served to obtain a distribution of particle sizes. Figure 2 shows the distribution for pseudolatex with tramadol, with the most representative particles having diameter sizes between

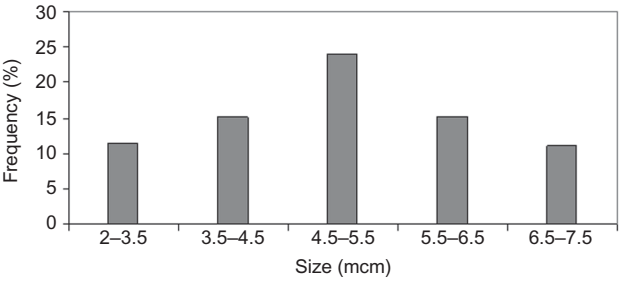

Figure 2. Particle size distribution of microparticles containing tramadol hydrochloride.

3.5 and 5.5 μm. Polydispersion with regard to size distribution is therefore clearly apparent.

Infrared spectrophotometry

Figure 3 shows the infrared spectrum of pseudolatex without the drug, pseudolatex with tramadol, and that of the drug only. Ethylcellulose contains groups of hydroxyl and ether groups, which have the capacity to interact with other molecules through the formation of hydrogen bridges and through electrostatic forces. An absorption peak can be observed at 3498 and 1083 cm^{-1} for pseudolatex without the drug, corresponding to the elongation vibration of the hydroxyl and carbonyl groups, respectively. This allows the functional groups of the polymer to be identified. In the case of the tramadol molecule, the groups that can interact with the polymer

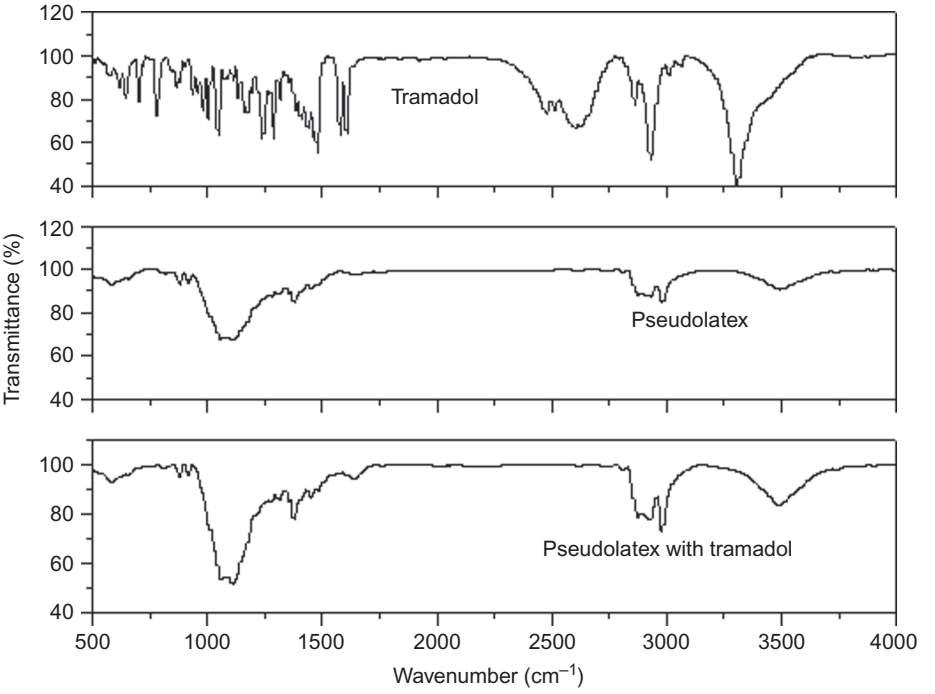

Figure 3. Infrared spectra of latex, latex with tramadol hydrochloride, and tramadol hydrochloride.

are the hydroxyl, methoxy, and amino groups, which may be identified at 3311, 2934, and 1051 cm^{-1}, respectively. Consequently, the formation of hydrogen bridges between tramadol and the polymer occurs among the hydroxyl and carbonyl groups of the polymer and the hydroxyl, methoxy, and amino groups of the drug. These interactions may cause a decrease in the electronic density of oxygen, bringing about the appearance of less-intense bands at the same wave number in the spectrum of pseudolatex with the drug. Consequently, the changes in the infrared spectrum of pseudolatex with tramadol, in comparison with pseudolatex without the drug, show that an interaction between tramadol and ethylcellulose takes places.

Calorimetric analysis

The thermogram of tramadol hydrochloride (Figure 4) shows a well-defined endothermic peak at a temperature of around 184°C, representing the point at which fusion with the active principle occurs. On having obtained the thermogram for the drug, a comparison of pseudolatex without active principle and pseudolatex with the drug can be made. In Figure 5, the thermogram of pseudolatex without the drug shows an endothermic peak at around 180°C whereas that for pseudolatex with tramadol can be seen to occur at 209°C.

It is therefore clear that the thermogram of pseudolatex with the drug does not coincide with either that for the active principle or that for the pseudolatex only. Consequently, it can be established that a polymer–drug interaction has taken place. Tramadol hydrochloride is a salt that is readily soluble in water. For this reason, the molecules that remain free in the medium can easily be separated from those that are bound to the microparticles through centrifugation, after which the molecules of free drug will remain within the supernatant, although the sediment will be made up exclusively of the polymer–drug complex. It can therefore be confirmed that the sediment is not just a physical mixture of both but rather that a real drug–polymer complex has been formed.

Study of the drug release mechanism

As a previous step to the drug release study, membrane selection processes were studied to arrive at a reliable assessment of the influence of the suspension in tramadol hydrochloride release. Two types of synthetic membranes were tested: nylon and methylcellulose. In Figure 6, it is clear that the nylon membrane is that which offers least resistance, allowing a greater quantity of the drug to pass through it. This membrane was therefore chosen for the ensuing release study of formulations with tramadol hydrochloride. In all cases, the quantity of added tramadol hydrochloride was 2400 μg, subsequent to which, and in accordance with predetermined time intervals, 400 μL was extracted from receptor compartments, being subsequently replaced with the same volume of receptor solution. The quantities of drug, estimated spectrophotometrically for each sample, were corrected on the basis of the number of preceding

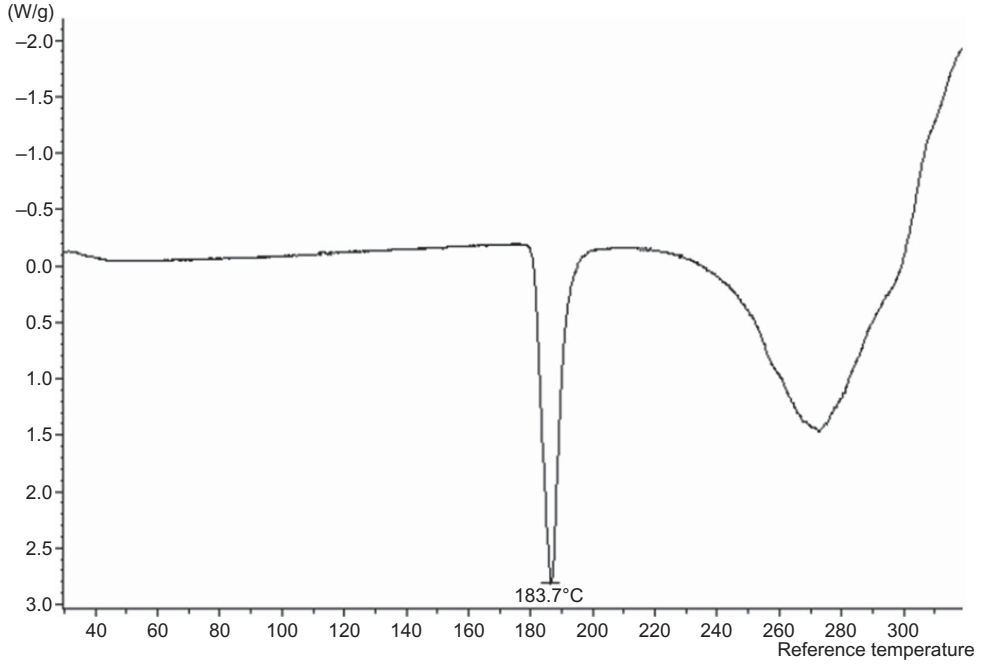

Figure 4. DSC thermogram of tramadol hydrochloride.

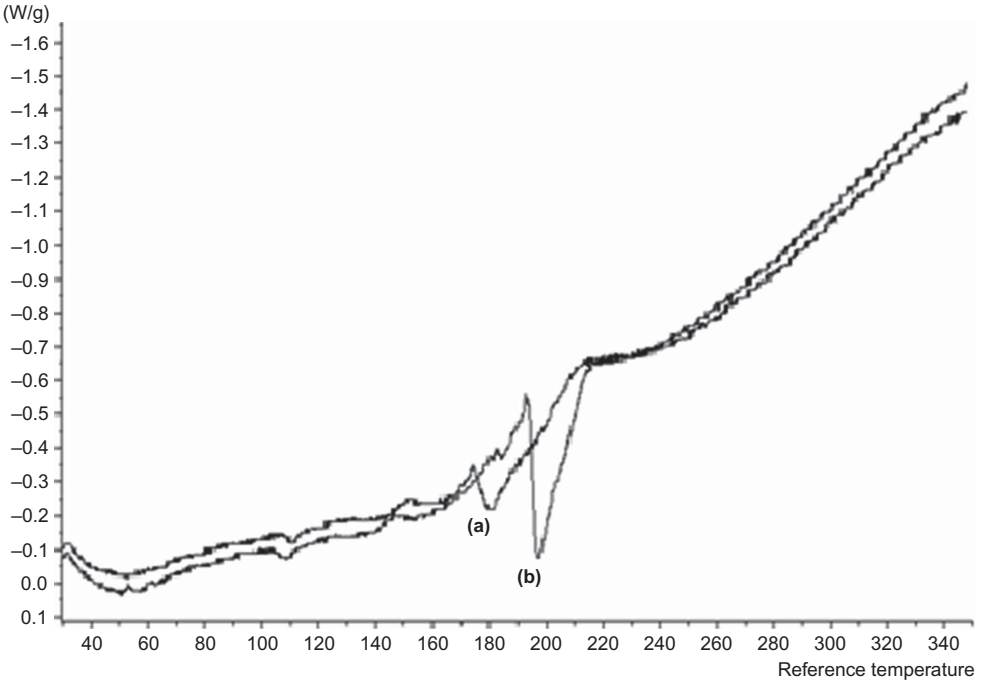

Figure 5. DSC thermograms of (a) latex without drug and (b) latex with tramadol hydrochloride.

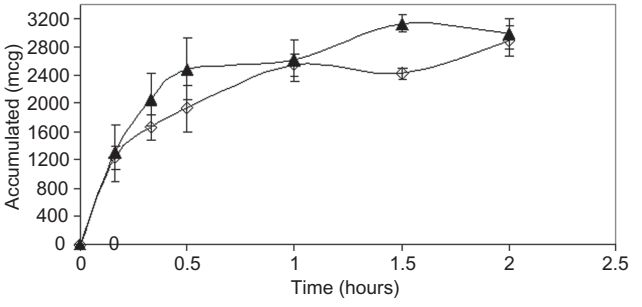

Figure 6. Amount of drug solution (μg) transferred with each type of membrane: nylon (▲) and methylcellulose (◆).

samples, and for each formulation tested, four replicas were performed. The study was carried out over a period of no longer than 8 hours. On the other hand, it should be taken into account that some of the components that constitute the formula may be soluble within the buffer, and as in the case of tramadol, the components will pass through the membrane. In parallel, release tests using a control formulation without the drug were carried out, in which components were observed to pass through the membrane and to contribute to absorbency of the samples at 270 nm. However, its contribution was found to be identical within the time interval studied, and the appropriate corrections were carried out. In such a way, it was possible to be sure that the quantities obtained only corresponded to quantity of released tramadol. Finally, all extracted samples were analyzed spectrophotometrically to determine the quantities released.

Figure 7 shows that the formula with carbopol® and sodium carboxymethylcellulose/Avicel® present practically identical behavioral patterns during the first 3 hours of the test. However, after this time, sodium carboxymethylcellulose/Avicel® begins to differ slightly from carbopol® by releasing tramadol at a higher rate. On the other hand, the formula with xhantan gum at 1% is that which releases the active principle at a slower rate, although at the end of the testing period, it can be seen that the quantities of released drug are in fact similar for all three formulae.

The percentages of the quantities of released tramadol hydrochloride for the formulations with each viscosing agent can be compared with the polymeric suspension without the viscosing agent. It is therefore possible to make an accurate assessment of the influence of the viscosing agent on release behavior. On completion of the test, it can be seen that the formula with xhantan gum at 1% releases 46% of tramadol, a much lower percentage than that released by the polymeric suspension without the viscosing agent. This behavior can be explained by the fact that xhantan gum confers the formulations with the highest viscosity, as confirmed by previous rheological studies[7]. The fact that release is retarded at higher viscosity levels has already been confirmed by numerous studies[20]. The formulation with carbopol® at 1% (at 8 hours) releases approximately 50% of the drug, whereas in the case of

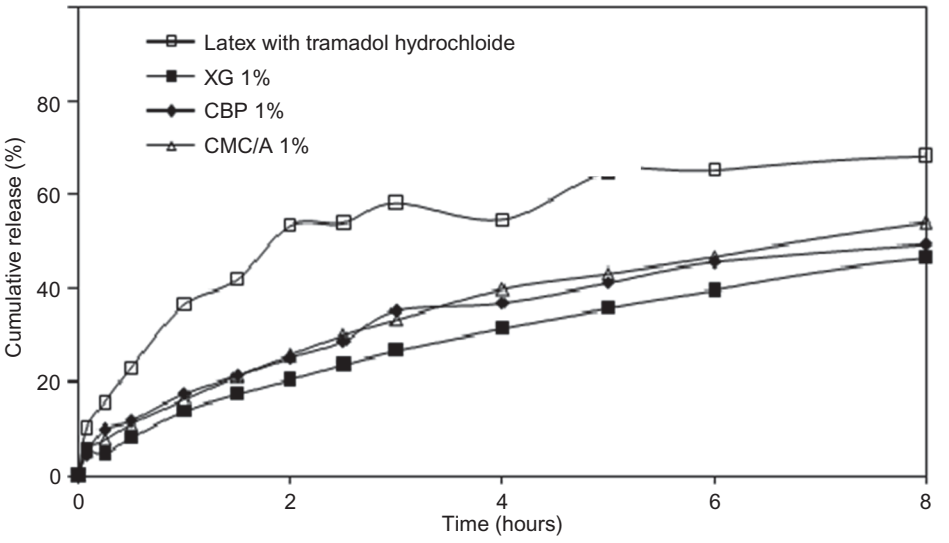

Figure 7. Percentage of tramadol hydrochloride released from formulas with xanthan gum at 1%, carbopol® at 1% and sodium carboxymethylcellulose/Avicel® at 1%, compared with the suspension without thickener.

carboxymethylcellulose/Avicel® at 1% the percentage of released drug is 55%. These two results are very similar, and after being subjected to statistical analysis, the release profiles among all three formulations did not show any statistically significant differences ($P > 0.05$).

The kinetics of the drug release mechanism

When the intrinsic mechanism of release for a given formula is unknown, mathematical models can be used to choose that which most reliably explains the kinetics of release. We fitted the experimental data to several models, then used the Akaike information criterion (AIC)[21] to find the function that best explained the drug release process by calculating

$$\mathrm{AIC} = n \cdot \ln \mathrm{SSQ} + 2p,$$

where n is the number of pairs of experimental data, SSQ is the residual sum of the squares, and p is the number of parameters in the fitting function. The model that best fits the data was that with the lowest AIC. Table 3 shows the AIC values for the different formulations. All three formulations followed cube root kinetics. According to the laws of geometry, this type of kinetics is found when drug particles are spherical or when the sample contains a surfactant that forms micelles in the solution[22]. In our samples, the ethylcellulose microparticles used to encapsulate tramadol hydrochloride were in fact spherical, and we thus assume that cube root kinetics was the function that best described drug release.

Table 3. Akaike discriminatory criteria values for formula with each thickener and type of kinetics.

	Zero order	Single order	Square-root order	Cube-root order
GX 1%	62.141	47.016	61.133	33.084
CBP 1%	61.584	47.767	61.825	34.923
CMCNa/Avicel 1%	61.802	47.077	61.441	33.869

The release mechanism of the active principle from the microparticles

The Peppas et al. equation[16] allows an exact analysis of the results to be made when the first fraction of the release curve, that is, that corresponding to the first 60% of released drug, is applied. From the experimental data gathered and the linear least squares adjustment method, the value of n for each suspension could be determined. In the case of pseudolatex with tramadol, the n value obtained was 0.48. Given that our system consisted of spherical colloidal particles, we can conclude that the release mechanism occurs through anomalous transport, including both diffusion and case II processes.

However, given that this value is lower for polydisperse particles than monodisperse particles, we can conclude that most of the drug is released through diffusion (rather than through swelling and erosion), as would be expected from hydrophobic systems[23,24].

Conclusion

Finally, we can conclude that a liquid pharmaceutical preparation for oral administration capable of provid-

ing a sustained release of tramadol was successfully obtained, using pseudolatex of ethylcellulose as transport vehicle. Using a simple and easily reproducible method in the laboratory, we have synthesized a polymeric suspension of tramadol, capable of achieving an encapsulation efficiency of 82%, depending on the concentration of the active principle used. Microphotographic studies reveal spherical particles with porous surfaces, which facilitate the release of the drug. The most representative diameter sizes were of 3.5 and 5.5 μm.

In accordance with the results obtained from the infrared spectra, we can confirm that there are interactions between determined functional groups of the microparticles and the active principle. Furthermore, the differences observed between the thermograms of microparticles with encapsulated drug in comparison with those for pseudolatex and tramadol reveal once again that there is no physical mixture between the two but rather the formation of a real drug–polymer complex.

Finally, the release studies carried out on the three proposed formulations did not show any statistically significant differences ($P > 0.05$) among the profiles studied with each type of viscosing agent. Additionally, the formulation tested followed a release kinetic of cube root and a release mechanism through anomalous transport, including diffusion and Case II processes. However, the results obtained suggest that most of the drug is released through diffusion, as is to be expected from hydrophobic systems.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this paper.

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