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Controlled release of levamisole from poly-(ε-caprolactone) matrices
II. Effects of water-soluble polymer and iron powder incorporated into the matrices

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Abstract

Mixtures of hydrophobic-polymer/hydrophilic-polymer and hydrophobic-polymer/hydrophilic-polymer/iron powder were evaluated for the construction of rumino-reticulum devices (RRDs) containing levamisole hydrochloride as anthelmintic agent. A faster release profile of levamisole was achieved by incorporating a hydrophilic polymer such as polyethylene glycol 6000 and/or iron powder into a hydrophobic polymer such as poly-(ε-caprolactone) constituting the biodegradable matrix. In this hydrophobic/hydrophilic polymeric system, poly-(ε-caprolactone) maintained the integrity of the matrix, whereas polyethylene glycol 6000 dissolved from the matrix as the drug was released. Thus, the area-to-volume ratio of the device remained constant over the duration of the drug release. In vitro drug release studies were conducted at an ionic strength and pH as near as possible to those encountered in the rumen of cattle which generally varies from about 5.5 to 7.0. Drug release rates decreased as the matrix system:drug ratio increased. The drug release kinetics from these RRDs exhibit linearity with $t^{1/2}$ when the matrix was constituted with poly-(ε-caprolactone) and the release of the drug was determined as resulting from a diffusional mechanism following Higuchi's equation. When a part of the hydrophobic matrix was replaced with polyethylene glycol 6000 (5–15%), no linear correlation was observed with $t^{1/2}$ and the faster release of the drug was associated with the dissolution of the polyethylene glycol 6000. Complete dissolution of the drug at the typical pH encountered in the rumenal fluids and 39°C would ensure good bioavailability of the drug following oral administration.

Keywords: Biodegradable polymers; Controlled release; Oral drug delivery system; Levamisole; Rumino-reticulum devices

1. Introduction

Rumino-reticulum devices (RRDs) ensure a controlled release of an anthelmintic drug during

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the entire grazing season. The use of these devices is particularly well adapted for the treatment of parasitic diseases in ruminants (Praveen Tyle, 1987). The anatomy of the grazing animals digestive systems provides an opportunity for sustained release devices which would decrease the number of times that a drug must be administered.

During the last decade, some rumino-reticulum devices with anthelmintics were marketed. Different systems were reported: diffusion throughout a polymeric matrix (Ascher et al., 1988), osmotic pump (Pope et al., 1985; Zingerman et al., 1992) and intermittent devices (Whitehead and Shepherd, 1987; Duncan and Seymour, 1989; Cardinal and Witchey-Lakshmanan, 1992). Diffusion of drug from variable geometry to obtain the desired release has been studied by different research workers (Duncan and Seymour, 1989; Boettner et al., 1988; Conrad and Skinner, 1989).

Among the strategies proposed to obtain a sustained oral drug delivery, dispersion of drug in matrix-forming carrier substances provides a simple and inexpensive approach. We have investigated a drug delivery system where the drug is dispersed in a biodegradable polymer and is released by diffusion. A drug can be incorporated into a matrix consisting of hydrophilic gums, a melt of fats and waxes or a polymer in which the solubility of the polymer is pH dependent. The release of the drug from these systems may be controlled by diffusion/erosion of the matrix or a combination of both processes. In order to modulate the release rate of a drug from a polymer matrix, several investigators have developed different strategies. The modification of the release can be accomplished by: addition of acidic excipients into a biodegradable matrix system to increase the rate of degradation of bioerodible polymers such as poly(orthoesters) (Heller et al., 1990); basic drugs or excipients to increase the rate of degradation of polyesters such as poly-β-hydroxybutyric acid (Yoshioka et al., 1991) or bioerodible polymers such as polyanhydrides (Chasin et al., 1990). Autocatalytic degradation due to the presence of degradation products from the polymeric matrix (Choi and Heller, 1978; Heller et al., 1984); addition of osmotically active ingredients (sodium chloride, sodium carbonate, sodium lactate, etc.) which bring about swelling in polymers by an increase of osmotic pressure resulting in an acceleration of the release of the drug (Buri, 1985; Di Colo, 1992); copolymerization with monomers which allow a faster degradation of the polymer matrix (Mauduit and Vert, 1993; Feng et al., 1983; Pitt et al., 1981); and addition of water-soluble polymers to the hydrophobic matrix (Kyun et al., 1990) are other strategies to increase the release of a drug from a matrix system. This last strategy presents several advantages: ease of fabrication, ease of control for release by modification of the polymer ratio, potential to maintain mechanical properties similar to those observed without the presence of water-soluble polymers. Several investigators (Siegriest et al., 1970; Teel, 1978; Jones et al., 1983) have reported the possibility of using polyethylene glycol as an ingredient in the composition of rumino-reticulum devices. Increasing the density of these devices can be accomplished by two different approaches: either a density element independent of the matrix system or dispersion of iron powder in the matrix system. In both cases, a total density of 2.5 is necessary to construct a rumino-reticulum device without regurgitation problems. Previous work (Vandamme et al., 1995) demonstrated that the choice of the chemical nature (base or salt forms) of the levamisole, the anthelmintic drug incorporated into the RRDs, provided desirable release patterns. To release the anthelmintic drug during a period of 4–5 months, the salt form of levamisole was more appropriate than the base form. The drug loading in the RRDs was optimal at a content of 60% of levamisole hydrochloride. The aim of this study was to prepare oral controlled-release RRDs with 60% of levamisole hydrochloride and 40% of polymeric matrix and to investigate the influence of the incorporation of a water-soluble polymer such as polyethylene glycol 6000 and/or iron powder into the poly-(ε-caprolactone) matrix on the release of the anthelmintic drug.

Table 1
Compositions of rumino-reticulum devices

Excipient	Formulation weight (g)						
	A	B	C	D	E	F	G
Levamisole HCl	22.05	22.05	22.05	22.05	22.05	22.05	22.05
Poly-(ε-caprolactone)	14.70	88.20	13.96	13.23	12.49	14.70	13.23
Polyethylene glycol			0.73	1.47	2.20		1.47
Iron powder						48.89	48.89

2. Materials and methods

2.1. Materials

The polymers used for this study were poly-(ε-caprolactone) (Tone® Polymer P767-E) obtained from Union Carbide Benelux NV (Antwerpen, Belgium); polyethylene glycol 6000 obtained from Merck (Darmstadt, Germany). Levamisole hydrochloride was obtained from Indis (The Netherlands). The iron powder (92% of particles having a size between 30 and 50 μm) used for incorporation in the rumino-reticulum devices was analytical grade obtained from Merck (Darmstadt, Germany). The methylene chloride used to solubilize the polymer was analytical grade obtained from UCB (Belgium). All reagents used for the preparation of the dissolution medium were of analytical grade or better.

The extruder consisted of a copper barrel with a 25 mm diameter, topped by a copper piston terminated by a piece of Teflon. The copper barrel was surrounded by a heating element and copper tubing to cool the system after extrusion. The system was placed in a Carver laboratory press (Wabash, USA) to push the mixture of the polymer and the drug into the barrel.

2.2. Preparation of rumino-reticulum devices

RRD formulations consisted of levamisole hydrochloride and excipients. The compositions of the RRD formulations investigated are listed in Table 1. For the construction of the devices (Fig. 1), the quantity of excipients and drug was 1.5 times that indicated in Table 1. After extrusion, the RRDs were cut to contain 22.05 g of the drug and the excipient. Excess was used to determine the quantity of residual solvent after extrusion and the loading of drug in the device.

First, poly-(ε-caprolactone) was dissolved in methylene chloride with polyethylene glycol 6000 for some preparations. Then, the iron powder was added for some preparations. Levamisole hydrochloride was added when all polymeric flakes were in solution and the iron powder was in

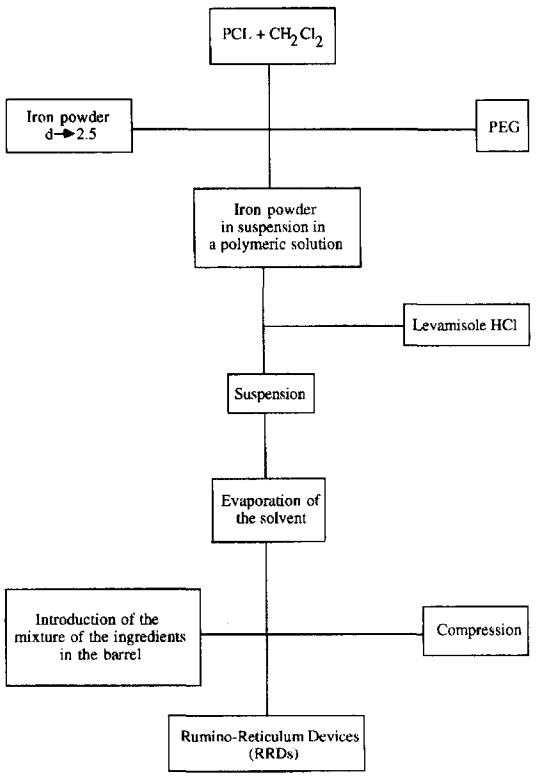

Fig. 1. Preparation of rumino-reticulum devices.

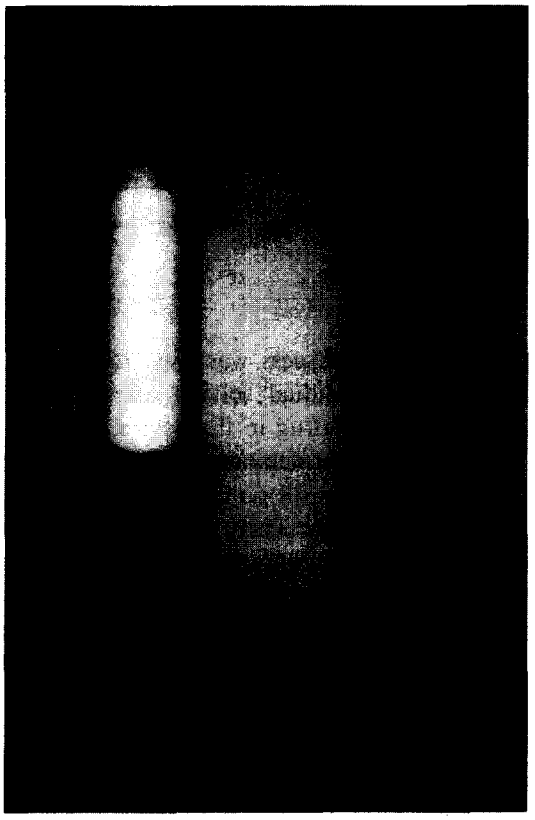

Fig. 2. Rumino-reticulum devices with a density element placed at one extremity (A) and containing iron powder in the matrix (B).

suspension. Incorporation of levamisole hydrochloride was performed in a dry room (R.H. < 30%) to avoid reaction between levamisole hydrochloride and the iron powder present in some preparations. The solvent was evaporated under heat on a Teflon plate. The mixture was introduced into the barrel (90 ± 0.2°C) of the extruder. The mixture was compressed at 2000 kg/cm^2; a water-circuit cooled the extruder at a rate of 5°C/min. The RRDs were driven out by pressure on the upper piston. Rumino-reticulum devices had a length of 10.5 ± 0.3 cm. The RRDs containing iron powder in the matrix (Fig. 2) were capped on each extremity; only one extremity was capped for the RRDs constructed with an iron density element. The covering was a 1 cm high hood to avoid release by the ends of the cylinder and allow release only by the longitudinal area. This construction was accomplished by cutting some polyethylene scintillation vials (Baxter, McGaw Park, USA) 1 cm from the bottom. For RRDs constructed with iron powder in the matrix, each end was pushed into a cap with cyanoacrylate glue applied to adhere the caps to the device and insure their attachment during the experiment. For the devices without iron powder in the matrix (Fig. 2) one extremity was pushed and set in a cavity of an iron density element during the extrusion phase; the other extremity of these devices was capped by a polyethylene cap as described above. RRDs were fabricated in triplicate for each formulation.

2.3. Solvent residual determination

The residual solvent in the constructed devices was evaluated by GC (Helwett Packard 5890 Series II gas chromatograph) using the excess material obtained after extrusion and cutting out the RRDs. A (6% cyanopropylphenyl) methylpolysiloxane column (DB™-1301), 30 m long × 0.328 mm i.d. with a film thickness of 0.25 μm, was used. The carrier gas was hydrogen at an isocratic flow rate of 2.0 ml/min (38.5 cm/s) and the injection volume was 1 μl and realized by split technique. The detector used was FID. The initial temperature was 30°C and the final temperature was 110°C with a 5°C/min rate of temperature increase. Each sample was run twice. Working standards, 1–20 ppm, were prepared for the dilution of the stock standard with methanol. Determination of residual solvent was accomplished as follows. A quantity of the rumino-reticulum devices containing an equivalent of 0.1 g of the polymer was dissolved in tetrahydrofuran (THF). When the polymer was dissolved, 4.0 ml of methanol was added to the suspension to precipitate the polymer. The suspension was then centrifuged (GS 15R centrifuge Beckman, Beckman instruments, USA) in glass centrifuges tubes at 10 000 rpm for 5 min. Supernatant (1 μl) was injected onto the GC. Only rumino-reticulum devices with a concentration of methylene chloride < 10 ppm were accepted for drug delivery studies.

2.4. Physico-chemical characterizations

Polymer molecular weights (M_w) were determined by size exclusion chromatography (SEC) using a Waters 510 pump. Two ultrastyragel linear columns (Part. No. 10681, Waters®, Milford, USA) were used with THF as the eluting solvent at a flow rate of 0.5 ml/min at room temperature with a refractive index detector (Waters 410). The SEC procedure was calibrated using polystyrene standards of different molecular weights (Polymer Laboratories Ltd.). The polymers were dissolved in THF (2 mg/6 ml) and filtered through a 0.45 μm filter, after which 100 μl were injected.

DSC analysis was carried out with a Dupont 2000. Samples of 10 mg were put into aluminium pans. The pans were pierced in order to permit gas to leave during the heating process. The instrument was calibrated with an indium standard and measurements were carried out from 20 to 90°C under nitrogen at a scanning rate of 10°C/min. The crystallinity of the samples was calculated from totally crystalline PCL for which the enthalpy of fusion is 139.5 J/g (Crescenzi et al., 1972).

The weight (%) of remaining RRDs was determined as follows. The collected RRDs from in vitro experiments were washed with water, dried and stored for 48 h in a desiccator with P_2O_5 as dehydrating agent in the bottom. The devices were weighed to obtain the solid dry weight (M_d). The weight (%) remaining was calculated by dividing solid dry weight by the initial weight (M_0):

$$\text{weight \% remaining} = (M_d/M_0) \times 100\% \quad (1)$$

2.5. Release methodology

Release experiments were conducted with an in vitro medium as near as possible to that encountered in the rumen of cattle. The composition of this medium is listed in Table 2. The study was conducted in 1000 ml of the medium in 1200 ml polyethylene flasks maintained during the experiment at 39 ± 0.5°C. Each flask was double capped to prevent the escape of carbon dioxide which would modify the pH of the medium during the experiment. The shaking rate of each flask was 60 strokes per minute. Samples were withdrawn from the release medium at preset time intervals, filtered through 589[3] Blue ribbon Schleicher & Schuell filter paper circles and assayed for levamisole hydrochloride by a spectrophotometer method or high pressure liquid chromatography (HPLC).

2.6. Analytical method

The levamisole hydrochloride concentration was determined by a spectrophotometric method or by an HPLC method when interactions of drug excipients did not permit use of the spectrophotometer. The spectrophotometric method consisted of reading of the samples on a spectrophotometer (Unicam 8625 UV/vis) operated at a wavelength of 247 nm. The HPLC assays of levamisole concentrations (Vandamme et al., 1995) were performed as follows. The quantitative determination of levamisole was accomplished with a HPLC Waters delivery system (pump M45, injector U6K, detector M481, integrator baseline 810, sensitivity at 0.01 AUFS). An analytical column (C18 Nova-Pak®: 3.9 mm i.d. × 150 mm, 4 μm particle size and a porosity of 60 Å) was used for analytical determination. The mobile phase consisted of 15% (v/v) acetonitrile and 85% (v/v) aqueous solution of 0.05 M potassium dihydrogen phosphate. The paired-ion agent (1-pentane sulfonic acid sodium salt) was used at 0.005 M in the aqueous phase. The pH of the mobile phase was adjusted to 3 with phosphoric acid. The flow rate was maintained at 1.0 ml/min and the quantita-

Table 2
Composition of the medium for in vitro release

$Na_2HPO_4 \cdot 12H_2O$	9.3 g
$NaHCO_3$	9.8 g
NaCl	4.7 g
KCl	5.7 g
$CaCl_2 \cdot 2H_2O$	53×10^{-3} g
$MgCl_2 \cdot 6H_2O$	128×10^{-3} g
$FeSO_4 \cdot 6H_2O$	75×10^{-3} g
$MnSO_4 \cdot H_2O$	4×10^{-2} g
Urea	7×10^{-1} g
Aqua ad 1000 ml	
pH fixed at 6.9 with CO_2	

Table 3
Molecular weights and crystallinity of the RRDs after 138 days

Formulation	M_w	δ	Crystallinity (%)
PCL alone	100 948	1.96	49.3
Levamisole HCl/PCL 60:40	100 883	2.02	49.4
Levamisole HCl/PCL 60:40 with 10% of PEG	100 978	1.97	49.6
Levamisole HCl/PCL 60:40 with iron powder	100 987	2.04	49.4
Levamisole HCl/PCL 60:40 with 10% of PEG and iron powder	100 896	1.98	49.5

tion was performed using peak area ratios (UV absorbance detection, 225 nm) of levamisole to quinine, the internal standard. A 100 μl aliquot of sample was placed in a 2.0 ml capped plastic tube. Quinine hydrochloride, 100 μl of 100 μg/ml solution, was added to each sample to serve as an internal standard. An alkaline pH was achieved with the addition of 0.125 ml of 0.1 N NH_3 to each sample. Some precautions were necessary for the extraction: the pH of the solution was not allowed to exceed 10, because above pH 10, the levamisole is unstable and rapidly degraded to [(−)-2-oxo-3-(2-mercapto-ethyl)-5-phenylimidazolidine] (OMPI) (Rousseau et al., 1981). The samples were mixed by vortexing and each was added to a reverse-phase cartridge (C18 bonded phase and a volume of 1 ml, Sopachem, Belgium). Each cartridge was then washed with 2.0 ml of water to eliminate the hydrosoluble substances followed by 3.0 ml of acetonitrile to elute levamisole and quinine. The eluant was collected in a disposable glass tube. The sample was evaporated to dryness using a gentle nitrogen stream at 50°C. The residue was reconstituted with 1 ml of acetonitrile and mixed by vortexing. The recoveries for the levamisole and quinine were respectively 73.10 ± 0.25% and 87.20 ± 0.36%. A 25 μl aliquot was injected onto the HPLC. Inter- and Intra-day coefficients of variations for the quantitation of the levamisole were respectively less than 3.8% and 4.2%.

2.7. Statistical analysis

All data are expressed as cumulative release % ± SD. Statistical evaluation of the data used either Student's t-test (where comparisons were made between two groups) or analysis of variance (ANOVA) (for comparisons between more than two groups). These tests allowed comparison of the percent released drug from the formulations with and without polyethylene glycol 6000 and/or iron powder. $p \leq 0.05$ was considered significant.

3. Results and discussion

3.1. Physico-chemical determinations

The initial molecular weight of the poly-(ε-caprolactone) used for the construction of the RRDs was 101 000 and the polydispersity (δ) was 2.0. The initial crystallinity of the polymer was 49.3%. The molecular weights of the matrices with and without iron powder or polyethylene glycol 6000 incorporated into the RRDs, determined at the end of the experiments, were not affected by the release of the drug and by the in vitro medium ($p > 0.05$). The crystallinity of the poly-(ε-caprolactone) was not significantly increased ($p > 0.05$). The values of these molecular weights and these crystallinities are reported in Table 3.

All of the RRDs showed an expansion or decrease of the size (length and diameter) < 5% at the end of the experiments. These data support previous results obtained from in vitro degradation studies of poly-(ε-caprolactone) (Vandamme et al., 1995).

3.2. Effect of addition of water-soluble polymer to the hydrophobic matrix on drug release

The simplest method of preparing a sustained-release system is to mix the drug with a release-controlling agent followed by compression or extrusion. Because the poly-(ε-caprolactone) is a hydrophobic polymer, the release rate of the drug from a matrix drug:polymer is very slow (Pitt, 1990).

Di Colo et al. (1982) incorporated a number of water-soluble liquid agents into hydrophobic matrices to increase the rate of release of the drug. In that study, glycerin, ethylene glycol, polyethylene glycol 200 and polyethylene glycol 400 were mixed with silicone polymer (PDMS) as potential drug-release enhancers. All of these additives produced a significant matrix swelling and the drug release rate was proportional to $t^{1/2}$. Furthermore, in this work, the drug release was proportional to $t^{1/2}$ and the rate constant. Di Colo's work has shown also that with these liquid osmotic agents, the cavities created in the polymer by the dispersed agent droplets are expected to be rather round shaped, so the swelling stress is better distributed and, therefore, polymer cracking is more strongly resisted than with the irregularly shaped particles of solid osmotic agents.

In the present work, in order to increase the release rate of the drug, 5, 10 and 15% of the poly-(ε-caprolactone) phase were replaced with a water-soluble polymer, polyethylene glycol 6000. Under identical release conditions (Fig. 3), the drug release rate was significantly increased when some polyethylene glycol 6000 was incorporated into the matrix ($p < 0.05$) compared with the same devices without water-soluble excipient. For the formulations containing 10 and 15% of polyethylene glycol 6000 in the matrix, no difference was observed on the drug release rate ($p > 0.05$). A significant difference in drug release rate was observed for the RRDs containing 5% of polyethylene glycol 6000 ($p > 0.05$). The RRDs containing 5, 10 and 15% of polyethylene glycol 6000 released respectively 76.96, 78.52 and 70.60% of the drug after 31 days of the experiments. During the same period, the formulation without water-soluble polymer released only 34.02% of the drug. As mentioned above, all of the RRDs tested for the experimentations showed an expansion or decrease of the structure of $< 5\%$.

To determine the influence of the polyethylene glycol 6000 on the release rate of the drug, additional experiments were conducted to determine the weight loss (%) of these devices. For this, different formulations containing the drug (60%) with various amounts of polyethylene glycol 6000 were fabricated. Some RRDs were prepared without drug to be used as standards. These RRDs were placed in an in vitro medium and taken at a preset time to determine the weight losses (%). The RRDs fabricated without drug and polyethylene glycol 6000 showed a weight loss of 1.3% after 138 days, whereas the RRDs fabricated with levamisole hydrochloride (60%) but without polyethylene glycol 6000 showed after 138 days a weight loss of 37.10% due to the release of the drug (Fig. 4). During the same interval of time, these devices released 61.83% of the total drug.

Significant differences in weight losses ($p < 0.05$) were observed for the RRDs containing polyethylene glycol 6000 in the matrix compared with those without the water-soluble polymer. The RRDs constructed with 10% of polyethylene glycol 6000 but without drug lost 9.42% after 31 days and 9.65% after 138 days (Fig. 4). The weight loss occurring during these periods of time can be attributed to the dissolution of the water-

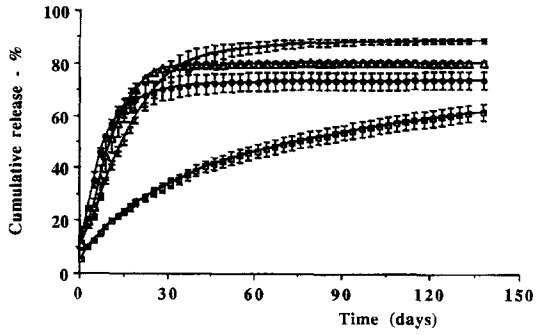

Fig. 3. Effect of polyethylene glycol 6000 on drug release rate from rumino-reticulum devices: (○) formula A, levamisole HCl/PCL 60:40; (+) formula C, levamisole HCl/PCL 60:40 with 5% of PEG; (△) formula D, levamisole HCl/PCL 60:40 with 10% of PEG; (●) formula E, levamisole HCl/PCL 60:40 with 15% of PEG ($n = 3$).

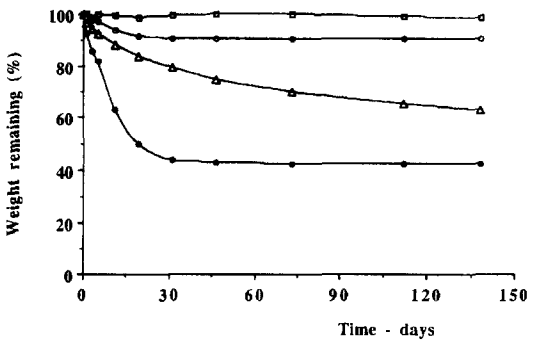

Fig. 4. Weight remaining (%) of the RRDs vs time: (□) poly-(ε-caprolactone) alone; (O) poly-(ε-caprolactone) with 10% of PEG; (△) levamisole HCl/PCL 60:40; (●) levamisole HCl/PCL 60:40 with 10% of PEG.

soluble polymer. The RRDs containing the drug (60%) and polyethylene glycol (10%) showed a 56.53% weight loss during this interval of time and the total weight loss after 138 days was only 57.90%. From the fact that a negligible weight loss was observed after 31 days, by which time all polyethylene glycol 6000 had been depleted, one can conclude that the increase of drug release rate from the RRDs with polyethylene glycol 6000 was facilitated by the dissolution of the water-soluble excipient.

3.3. Release mechanism

In the above formulations, levamisole hydrochloride powder is homogeneously dispersed throughout the polymeric matrix. Drug release from homogeneous matrix dosage forms has been described by Higuchi (1961) as follows:

$$Q = \sqrt{D(2W - C_s)C_s t} \quad (2)$$

In Eq. (2), D is the diffusion coefficient of the drug in the matrix, W is the total amount of the drug per unit volume of matrix, C_s is the solubility of the drug in the matrix, and t is the drug release time. When $W \gg C_s$, the above equation can be simplified to the following:

$$Q = \sqrt{2WDC_s t} \quad (3)$$

This equation indicates that the amount of drug released is proportional to the square root of the time for the diffusional release of a drug from a homogeneous matrix-type delivery system. In Fig. 5, the rate of drug release is reported according to the square root of time. The formulation containing only poly-(ε-caprolactone) shows a linear relation when the percentage of drug released is plotted vs the square root of time. This fact supports the conclusion that the drug is released throughout the poly-(ε-caprolactone) by a diffusion process. Conversely, formulations containing polyethylene glycol 6000 as a part of the matrix do not exhibit linearity with $t^{1/2}$. This fact can be attributed to the fast dissolution of polyethylene glycol 6000. The release of the drug is not due to a diffusion process throughout the matrix but due to the dissolution of the polyethylene glycol 6000.

3.4. Effect of addition of iron powder to the hydrophobic matrix on drug release

To increase the density of the RRDs to allow the RRDs to remain in the rumino-reticulum during the time for the release of the drug, two strategies were adopted. The first strategy was to attach, during the extrusion, a density element to a matrix system (Fig. 2). The second strategy was to disperse iron powder in the matrix system (Fig. 2). In both cases, a total density of 2.5 was reached. For this reason, the quantity of iron powder in the matrix was maintained constant for a given matrix system:drug ratio.

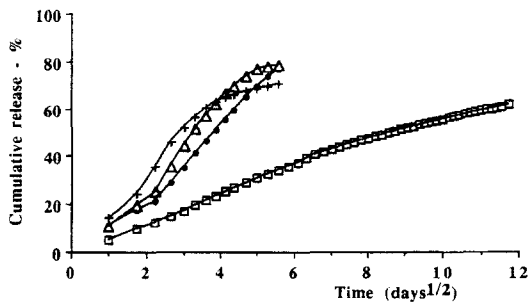

Fig. 5. Effect of polyethylene glycol 6000 on drug release rate from rumino-reticulum devices according to the square root of time: (□) formula A, levamisole HCl/PCL 60:40; (●) formula C, levamisole HCl/PCL 60:40 with 5% of PEG; (△) formula D, levamisole HCl/PCL 60:40 with 10% of PEG; (+) formula E, levamisole HCl/PCL 60:40 with 15% of PEG ($n = 3$).

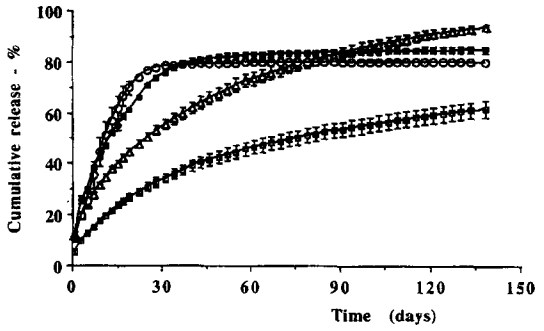

Fig. 6. Effect of iron powder on drug release rate from rumino-reticulum devices: (○) formula A, levamisole HCl/PCL 60:40; (△) formula F, levamisole HCl/PCL 60:40 with iron powder; (O) formula D, levamisole HCl/PCL 60:40 with 10% of PEG; (●) formula G, levamisole HCl/PCL 60:40 with 10% of PEG and iron powder (total density of the RRDs: 2.5, $n = 3$).

Inert particles dispersed into a matrix system can influence the release of the drug: (i) an increase of the diffusional path for the solute around the insoluble particles, and (ii) a decrease of the available volume for diffusion because the diluting agent is waterproof to the solute drug. These effects should reduce the release rate of the drug in comparison with an identical system without the iron. However, the opposite phenomenon was observed (Fig. 6). The total increase of the surface area for diffusion from the matrix system, after the incorporation of iron powder, overrides the two previously described effects. Furthermore, in the present case, the iron powder particles dispersed in the matrix were not completely inert. In fact, the iron powder in the matrix interacted with drug during the release and led to the formation of levamisole base and iron (II and III) chloride. The presence of iron salts and levamisole base were confirmed as follows. Some rumino-reticulum devices were constructed with iron powder and levamisole hydrochloride and others were constructed with iron powder alone or with drug alone. These RRDs were submerged in a similar buffer to that used for the release experiments which did not contain any iron salts. After a preset time, the solutions were filtered and 1 N sodium hydroxide was added. Black precipitate of $Fe(OH)_3$ formed in the solutions from the RRDs containing levamisole hydrochloride and iron powder in the matrix. Extractions ($n = 3$) with methylene chloride of the in vitro medium after incubation of the RRDs, followed by evaporation of the solvent and redissolution in methanol, revealed the presence of levamisole only for the RRDs containing iron powder and levamisole hydrochloride dispersed together in the matrix. Thus, the appearance of a water-soluble salt of iron during the release modified the composition of the medium for the in vitro release. For this reason, it was necessary to quantify the drug release by HPLC.

To evaluate the effect of incorporation of iron powder into the matrix on the drug release, the iron was incorporated in some formulations (Table 1; formulae F,G) and excluded from some formulations (Table 1; formulae A,D). As seen from Fig. 6, the levamisole hydrochloride release rate of formulations containing polyethylene glycol 6000 as hydrosoluble additive was not influenced by the presence of iron powder dispersed homogeneously in the matrix and no significant differences have been observed on the drug release ($p > 0.05$). For the formulations containing polyethylene glycol 6000, after 34 days, ca. 78% of the drug was released. Additional iron powder did not increase significantly the release rate during this period of time ($p > 0.05$). Thus, incorporation of iron powder into the matrix did not cause a rapid release of the remaining part of the drug. Conversely, the formulations without polyethylene glycol 6000 showed a drug release rate influenced by the incorporation of iron powder ($p < 0.05$). After 138 days, a release of 61.84% of the drug was observed for the formulations which contained no iron powder, whereas the same formulations containing iron powder dispersed homogeneously released 93.68% of the drug. In this last case, the transformation of a part of the drug to a lipophilic form also contributes to slow the rate of the release of the drug.

4. Conclusions

Incorporation of a water-soluble polymer such as polyethylene glycol 6000 in a biodegradable

matrix constituted of poly-(ε-caprolactone) was shown to modulate significantly the drug release profile ($p < 0.05$). The water-soluble polymer maintains the integrity before the drug release. Its presence in the polymer matrix increases the release rate of drug in the external medium allowing the release of 80.42% of the total drug content after 138 days of experiment when 10% of the matrix was polyethylene glycol 6000. Only 61.83% of the total drug content was released when the matrix of the RRDs was only poly-(ε-caprolactone). This was attributed to the dissolution of the water-soluble additive. However, no significant difference in rates of drug release were observed between the RRDs containing 10 and 15% polyethylene glycol 6000 ($p > 0.05$). A significant difference ($p > 0.05$) in rate of drug release was observed for the RRDs containing 5% of the water-soluble polymer. Iron powder dispersed in the matrix increased the release rate significantly ($p < 0.05$) for RRDs containing only poly-(ε-caprolactone) as matrix agent. When polyethylene glycol 6000 was incorporated in the matrix, additional incorporation of iron powder did not significantly increase the release rate of the drug ($p > 0.05$). Furthermore, it was shown that dispersion of iron powder into the matrix resulted in the drug reacting with the iron during the release phase and changed the levamisole hydrochloride to levamisole base and some iron (II and III) chloride appeared in the in vitro medium. RRDs constructed with polyethylene glycol 6000 as a part of the matrix agent should be good candidates to release other drugs (antibiotics, growth-promoting compounds, vitamins and vaccines). Anthelmintics which require drug release during a more prolonged time will have to be fabricated without addition of a water-soluble polymer in the matrix system. Additional studies such as coating of the RRDs described in the present studies with biodegradable polymers to decrease the burst effect, to prolong the time of the release of the drug and to induce a lag time at the beginning of the release, desirable for induction of immunity in cattle, are in progress. In vivo gastric retention time of these devices, study of the bioavailability of the levamisole from these constructed RRDs and coprological studies will determine the real efficacy of these systems on grazing animals.

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