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Physico-chemical analysis of metronidazole encapsulation processes in Eudragit copolymers and their blending with amphiphilic block copolymers

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ABSTRACT

A physico-chemical analysis of metronidazole–Eudragit copolymers L100 and RLPO (a cationic polymeric matrix with an electrophilic character) was carried out in order to explore the drug–polymer interaction and its possible effects on the encapsulation and release profiles. An oil-in-oil encapsulation procedure was designed to obtain more intimate drug–matrix mixtures and to obtain a better insight into the details of the interaction. The encapsulation efficiency obtained in these cases was high (in the range of 85–95%), but the release rates were quite rapid. Solubility and interaction between metronidazole and copolymers are discussed in detail with a view to explaining the results. Amphiphilic block copolymers of poly(ethylene)-b-(polyethylene oxide) (20, 50 and 80% PEO) were tested as a matrix for metronidazole release in order to improve drug profiles. The performance of RLPO as the matrix for drug release was improved by blending it with amphiphilic block copolymer poly(ethylene)-b-(polyethylene oxide) (20% PEO). The release mechanism of metronidazole is governed mainly by the swelling of RLPO, yielding a better fit with the second-order Schott equation.

1. Introduction

Metronidazole (1-(β-hydroxyethyl)-2-methyl-5-nitroimidazole) (Scheme 1) is one of the most important drugs in the group of 5-nitroimidazoles and possesses toxicity to anaerobic micro-organisms. DNA represents the main target for its biological action, which is dependent upon the nitro group reduction process (La-Scalea et al., 1999; Cavalcanti et al., 2004).

Metronidazole is generally used in oral administration but is also prepared in other forms such as suppositories, ointments and gels for dermal formulations (Musial, 2007) and also for periodontal diseases (Newman et al., 1984; Gusberti et al., 1988). The problems observed in these applications are the adverse effects of metronidazole involving the gastrointestinal tract with high doses. Thus, their reduction through the use of controlled oral release devices is desirable (Ozyazici et al., 2006). In any case a prolonged or controlled release of the compound is necessary for a good compliance, although the drug released must possess the required bioactivity.

The procedure to circumvent this problem is to covalently bind metronidazole to a polymer. Generally the hydroxyl group of this molecule (see Scheme 1) is used for this purpose, forming a conjugate (Mocanu et al., 1993) as a means of delaying release by the slow hydrolysis of the bond polymer–drug formed. However, encapsulation with polymeric matrix has been the most common procedure.

Metronidazole has been encapsulated with methylcellulose, chitosan, carbopols, and lipid matrices and preferentially as gels or crosslinked gels (Musial, 2007; Ozyazici et al., 2006; Nakikeosza-Jarmolowska, 2006; Barat et al., 2007; Lahiani-Skiba et al., 2006). Crosslinked poly-acids such as carbopols are able to bind metronidazole irreversibly to the polymer structure (Musial, 2007).

However, its high solubility in water (10.61 mg/mL), particularly at a low pH in HCl 0.1 N (32.30 mg/mL), hinders the process of encapsulation and the subsequent release profile for an effective control of its performance (Di Martino et al., 2007; Wua and Fassihib, 2005).

On the other hand, metronidazole is relatively stable with little or negligible degradation in its liquid phase (Wua and Fassihib, 2005) and its hydrolysis occurs at a higher temperature and pH (Salo et al., 2003).

The presence of nitrogen atoms in the structure of metronidazole facilitates the interaction with an acid polymeric matrix such as acrylic polymers or their copolymers (Antonik and Khabibulina, 2008), since it reveals the basic characteristics of the molecules.

In particular, acid copolymers such as Eudragits are being extensively used for encapsulation purposes (their structures are shown in Scheme 2). The Eudragit copolymers have also been used for encapsulation of metronidazole (Chourasia and Jain, 2004; Yassin et al., 2001; Menendez and Skar, 2004; Pornsak et al., 2005).

Eudragit L100 is an enteric pH-dependent copolymer possessing methacrylic acid groups in its structure, being used for oral

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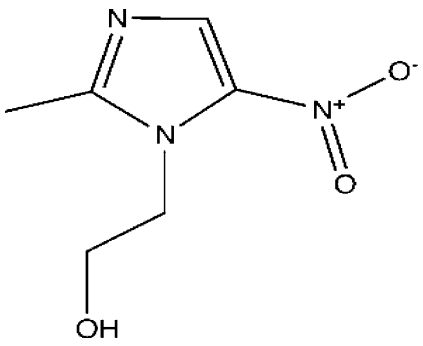


Scheme 1.

controlled administration (Gonzalez et al., 2008a,b; Dupeyron et al., 2005), since it is insoluble at acid pH and soluble in neutral or alkaline solutions. This copolymer can deliver drugs as it passes through the gastrointestinal tract, where the pH is around 6, slowly releasing the bioactive component.

Eudragit RLPO is a matrix that provides pH-independent drug release. Its structure is quite interesting because it is formed by units of methacrylates or acrylates, one of them containing trimethyl ammonium groups as salts (~10%) that impart an electrophilic character. This electrophilicity should favor the interaction of such structures with metronidazole.

This copolymer swells and presents permeability to water due to its quaternary salt component. It has been used in release formulations and also in blends with hydrophobic polymers for this purpose (Kuksal et al., 2006; Ganti et al., 2008; Ferreira et al., 2003), since the use of hydrophilic matrix alone for highly water-soluble drugs is restricted, due to the rapid diffusion of the dissolved drug.

Thus, taking into consideration the importance of this drug for medical purposes and the importance of retaining its properties during the encapsulation process, we consider the first aim of this study to be the detailed analysis of the solubility of drug and copolymers, as well as the possibilities of drug–polymer interaction for a successful encapsulation and controlled release of this drug.

The second aim is the study of the effect of amphiphilic block copolymers on metronidazole release from RLPO, thereby allowing the development of new polymeric drug carriers.

2. Materials and methods

Eudragit RLPO and the enteric copolymer (Eudragit L100) were purchased from (Röhm Pharma, Germany). Poly(ethylene)-b-poly(ethylene oxide) (80% PEO) Mn = 2250; poly(ethylene)-b-poly(ethylene oxide) (50% PEO) Mn = 920, HLB = 10, CMC = 28.8 mg/L (25 °C); and poly(ethylene)-b-poly(ethylene oxide) (20% PEO), Mn = 875, HLB = 4, were purchased from Aldrich (USA) and used as received. Metronidazole was supplied by Laboratories MedSol, Havana, Cuba.

Poly(ethylene)-b-poly(ethylene oxide) (80% PEO) with a carboxylic acid group as end group (PE-b-PEO (80% PEO)–COOH) was obtained and characterized in our laboratory and is part of a paper that is being considered for publication. All other materials and solvents were of analytical reagent grade.

2.1. Characterization techniques

The pH of solutions was measured using the pH meter Digimed (Digimed, Brazil).

Kinetic measurements and absorption spectra in the UV–vis region were obtained from a Hach DR 5000 UV–vis spectrometer using quartz cuvettes with a 1-cm optical path.

FTIR spectra were recorded in a FTIR Prestige 21-Schimadzu from 450 to 4500 cm^{-1} at a resolution of 1 cm^{-1}. Polymer samples were prepared on KBr pellets.

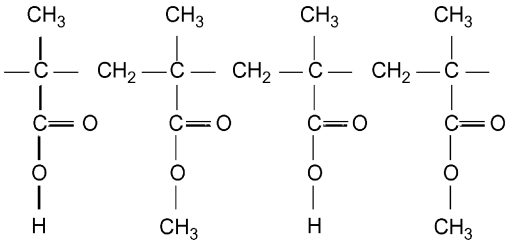
Optical images were recorded with an OPTON optical microscope, model TNB-04T-P, using magnification 10× lens.

2.2. Encapsulation of metronidazole and preparation of microparticles

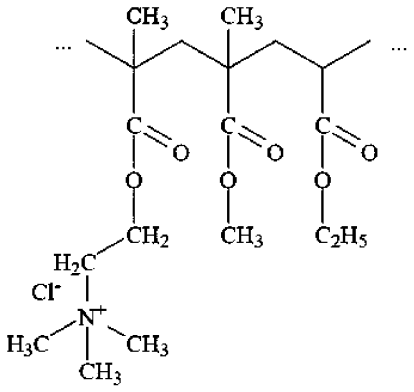
In the oil-in-oil emulsification, the drug is dissolved in a mixture of different oils. Firstly, the material is dispersed in the organic solution, forming an inner oil phase. The introduction of the second oil phase (immiscible with the first) promotes the creation of an outer oil phase. The organic solvent in the inner phase is completely eliminated from the evaporation, characterizing this as a completely anhydrous process.

The convenient choice of the outer oil phase allows that the diffusion of hydrophilic drug from the inner phase can be minimized, improving the entrapment efficiency of drug (Kobaslija and McQuade, 2006; Mana et al., 2007; Yang et al., 2005).

Using this procedure (as indicated in Scheme 3) a solution containing 10 mg of metronidazole and 90 mg of the Eudragit in 5–10 mL of chloroform (inner oil phase) was added dropwise to 80 mL of liquid paraffin. The mixture was stirred at 200 rpm at room temperature for 4 h and during this time the chloroform was evaporated. Microparticles were washed several times with hexane and dried under vacuum at room temperature for 12 h.

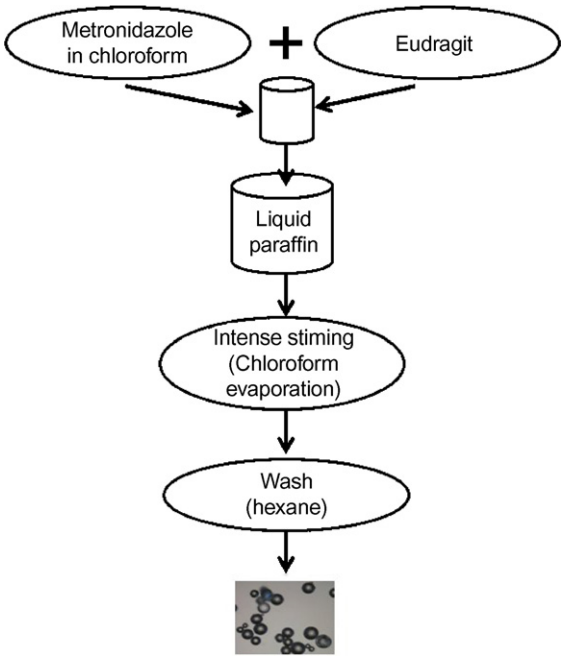


Eudragit L-100



Eudragit RLPO

Scheme 2.



Scheme 3.

The metronidazole was blended with appropriate amounts of RLPO and block copolymers poly(ethylene)-b-poly(ethylene oxide) (20, 50 and 80% PEO), and the latter with a carboxylic end group by a wet method using hexane, dried and compressed to obtain a uniform size using a tabletting machine.

The amounts of drug and copolymers used in formulations for encapsulation and blending are shown in Table 1. All experiments were carried out in duplicate.

The release measurements were carried out in sink conditions taking an amount of particles or tablets (50–100 mg) and adding them to 150 mL of water under stirring at 200 rpm at 25 °C.

The filtered aliquots were withdrawn at time intervals and measured, maintaining a constant volume. Release was followed by the value of the absorption peak at 318 nm and the concentration estimated using a previously prepared standard curve.

3. Results and discussion

3.1. Eudragit RLPO and L100 behavior

The Eudragit RLPO is sparingly soluble in water and it swells decreasing the pH of the solution. This fact may be explained by the reaction of interchange between the halide of the salt group and a hydroxyl from the water:

$$-(CH_3)_3N^+Cl^- + H_2O \leftrightarrow -(CH_3)_3N^+OH^- + Cl^- + H^+ \quad (1)$$

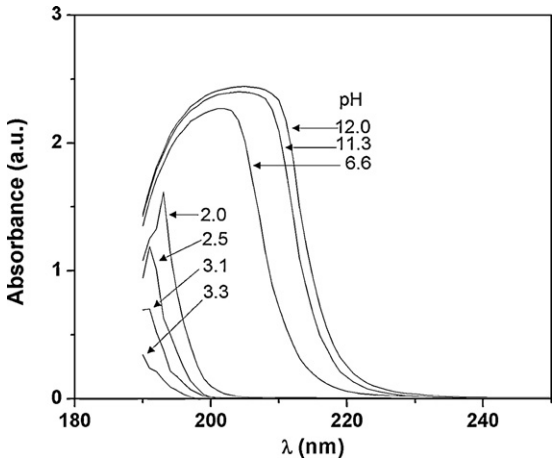


Fig. 1. Spectrophotometric UV–vis behavior of the soluble oligomer portion of Eudragit RLPO at different pH values.

This change can be detected by the small portion of oligomers soluble in solution that gave a UV–vis spectrum that depends on the pH, as shown in Fig. 1. The peak of maximum absorbance begins at 196 nm and shifts to 210 nm with the increase in the value of pH. The oligomer solution was tested with silver nitrate and the silver halide precipitated. The carboxylic acid groups would interact effectively with metronidazole in solid phase as well as in solution (Antonik and Khabibulina, 2008). This was what led us to use Eudragit L100 in the search for drug–polymer interaction. The behavior of Eudragit L100 at different pHs had previously been thoroughly studied by us (de Oliveira et al., 2009).

3.2. The encapsulation procedure

It is recommended that soluble water drugs should be encapsulated by double emulsion (water–oil–water) (Schugens et al., 1994). However, the internal morphology of the resulting particles is usually mono-nuclear or poly-nuclear vesicles (Nihant et al., 1994, 1995). Thus, in order to obtain an internal compacted and more intimate drug–matrix mixture that could facilitate interaction, the encapsulation technique used for metronidazole encapsulation with Eudragit L100 and RLPO was an oil-in-oil procedure (as described in Section 2.2). The complete exclusion of water from the process enabled drug loss to be kept to a minimum. Microparticles obtained from the RLPO–metronidazole system are shown in Fig. 2.

3.3. Release of metronidazole from Eudragit L100

Eudragit L100 encapsulates the metronidazole at a low pH and releases it in water at a neutral pH, as expected for an enteric copolymer. Furthermore, at a low pH (pH 1.0) a low release occurs, indicating that the polymeric matrix is not able to retain the entire

Table 1
Amounts in milligrams of copolymers and metronidazole used in all formulations: A and B (oil-in-oil procedure) and, C–I (tablets).

Compound	Formulation								
	Encapsulation		Blends						
	A	B	C	D	E	F	G	H	I
Metronidazole	30	30	36	36	36	36	36	36	36
Eudragit L100	300	–	–	–	–	–	–	–	–
Eudragit RLPO	–	300	–	–	–	–	180	252	108
PE-b-PEO (20% PEO)	–	–	360	–	–	–	180	108	252
PE-b-PEO (50% PEO)	–	–	–	360	–	–	–	–	–
PE-b-PEO (80% PEO)	–	–	–	–	360	–	–	–	–
PE-b-PEO (80% PEO)–COOH	–	–	–	–	–	360	–	–	–

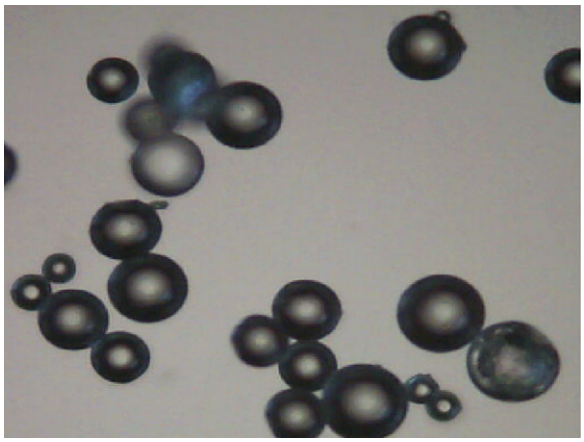


Fig. 2. Optical micrograph of microparticles obtained by the procedure of oil-in-oil emulsion for the RLPO–metronidazole system.

drug (see Fig. 3). However, this Eudragit L100 behavior has not been observed in encapsulation for other drugs (Gonzalez et al., 2008a,b). This result may be due to the solubility of metronidazole in acidic solution, as mentioned in Section 1. It occurs in spite of the possible drug–polymer interaction between the carboxylic acid of the polymer and the nitrogen on position 3 of the nitroimidazole (see Scheme 1) that could occur as follows:

$$-\mathrm{COOH}\cdots\mathrm{Nring} \rightarrow -\mathrm{COO}^- + \mathrm{HN}^+\mathrm{ring} \quad (2)$$

The methacrylic acid moieties of the copolymer do not seem able to completely retain metronidazole through this interaction with this nitrogen atom of the imidazole ring. The release profile in water presents a sudden release at the beginning. This is another indication that solubility overcomes the drug–copolymer interaction in the solid state.

In addition, the polymer dissolved in water seems to be free of the drug. As a result, the full spectra recorded during release (see Fig. 4) did not present the absorption of metronidazole at 280 nm (typical of the protonated nitroimidazole ring). There is thus no evidence of the drug–polymer interaction in the solution. Samples with a 1:1 ratio of metronidazole and Eudragit L100 were grounded with KBr and the FTIR recorded. From the results, any band shift on metronidazole spectra was observed. Secondary forces of interaction have been suggested between metronidazole and acrylic polymers (Antonik and Khabibulina, 2008); however, for the system metronidazole–Eudragit L100 there was no evidence of any such interaction, either in solution or in solid phase.

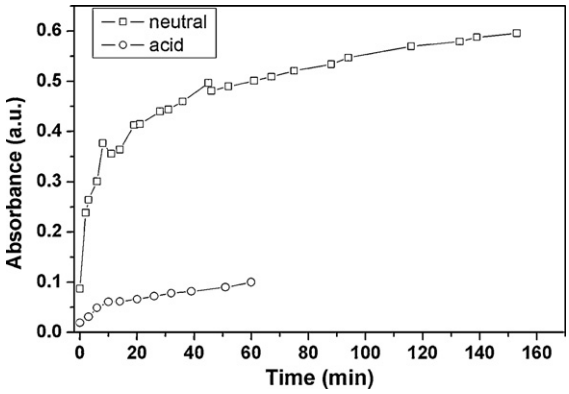


Fig. 3. Enteric behavior during the release of metronidazole from Eudragit L100 (upper curve in water recorded at 318 nm and lower curve at pH 2.35 recorded at 280 nm).

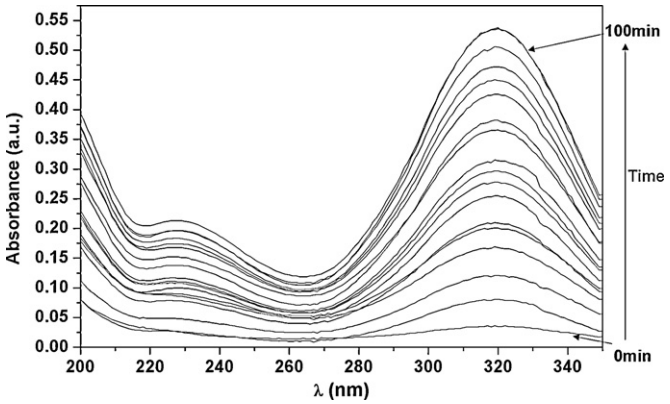


Fig. 4. UV–vis spectra of metronidazole during its releasing process in water from Eudragit L100.

Eudragit L100 was used in this study mainly to reveal the drug–matrix interaction between the carboxylic acid groups and metronidazole. Any other modification of the polymer, such as blending, could inhibit its enteric character. Finally, the results obtained are in agreement with those of other reports using this copolymer with other drugs (Gonzalez et al., 2008a,b).

3.4. Release of metronidazole from RLPO

The release of metronidazole from RLPO microparticles presented an exponential type of profile (see Fig. 5). A short burst was observed and the release rate was quite rapid (approximately 100% in 1 h), even when the encapsulation efficiency for this sample was high (95%). The oil-in-oil formulation procedure worked very well, yielding encapsulation efficiencies for several releasing runs between 85 and 95% (not shown). These results could suggest that the interaction in solid phase favors the intimate mixture of the drug with the copolymer. It is known that RLPO swells to a certain extent, due to the presence of quaternary salts on its side groups; this property does not permit the complete retention of the drug, and bursts ranging between 4 and 11% were also detected. A profile with a better controlled release in time was not obtained in any of the runs performed and all of them had results similar to those shown in Fig. 5. As mentioned above, the solubility of the drug, and the nature of the polymer joint together produced this profile. As a result, the drug–polymer interaction is not strong enough to retain the metronidazole and produce a less rapid release profile. A polymer such as halide or base (Eq. (1)) does not interact sufficiently

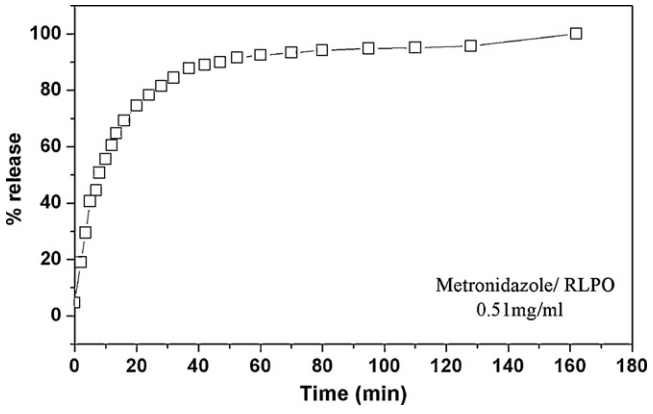


Fig. 5. Release profile of metronidazole from Eudragit RLPO in water recorded at 318 nm.

strongly or effectively with metronidazole and the solubility of the drug predominates.

The release profile shown in Fig. 5 is similar to that reported for metronidazole released from a lipid matrix (Ozyazici et al., 2006). The use of stearic acid as matrix produced profiles with 60–80% released in 100 min. For several fatty acid esters or waxes as matrix the metronidazole is released in the range of 40–60% in 100 min. In this paper the kinetic runs are reported to fit the Higuchi plot in spite of the exponential-type profiles obtained.

However, the trend reported for the metronidazole released from chitosan appears to be that of zero-order kinetics (Mocanu et al., 1993; Nakikeosza-Jarmolowska, 2006), whether using crosslinker or not. In this latter case the gel-like solution produced a slower linear release over 24 h before attaining a plateau that is useful for dental purposes.

On the other hand, polyacrylic acid gels for metronidazole encapsulation (Musial, 2007) gave a kinetic trend of the first order and a release of around 30–40% in 100 min.

As can be observed from the above examples, there is no unique kinetic trend for metronidazole-controlled release suitable for practical purposes (with the exception of chitosan), since, as a rule, the amount released is quite high for this compound. Thus, the solubility of metronidazole overcomes any drug–polymer interaction and governs the release of the drug.

This behavior suggests that factors other than a chemical interaction are operating. A physical factor related to diffusion, swelling, viscosity or physical entanglements or all of them seem to be present. In terms of release mechanisms it corresponds to passing from a quasi-exponential type of release profiles to more smooth or quasi-linear ones.

3.5. RLPO blending with amphiphilic block copolymers

For the purpose to improving the performance of RLPO as a matrix for metronidazole, amphiphlic block copolymers containing a hydrophilic block and a hydrophobic one that might affect the process of metronidazole release were considered. The copolymers used were the 20% PEO, 50% PEO and 80% PEO and also their 80% PEO with a carboxylic acid end group.

It must be borne in mind that block copolymers are used as compatibilizers between incompatible polymers in blends (Billmeyer, 1984), favoring their compatibility in nano-level domains. The same effect could be operating for this blend, making it a better material than the polymer RLPO alone.

Each amphiphlic block copolymer was used for preparing tablets with metronidazole. The release profiles of these devices are shown in Fig. 6. It can be observed that the order of profiles varies directly according to their hydrophobic–hydrophilic composition. Thus, the copolymer PE-b-PEO (80% PEO)–COOH shows a meager interaction with metronidazole despite the presence of a carboxylic group in its structure, whereas PE-b-PEO (20% PEO) resulted in a suitable profile. Those copolymers alone are not suitable for release purposes because, being amphiphilic materials, the dispersion occurs at times in the order of 1–2 h.

Blends containing metronidazole and RLPO were prepared with PE-b-PEO (20% PEO) at different percentages (30, 50 and 75%). Their release profiles are shown in Fig. 7. It may be noted that all profiles are slower than the mixture metronidazole–RLPO without block copolymer. The best result was obtained for the blend containing 50% of the block copolymer. Devices with compositions of 30 and 50% maintained the physical integrity all the time with a minimal swelling in comparison with RLPO. In contrast, a blend with 75% of block copolymer begins to disintegrate with time.

Block copolymers did not completely arrest the RLPO swelling but did decrease its rate. It may occur through physical entanglements between both polymers, producing a web mimicking a chemical crosslinked material able to control the drug diffusion. To our knowledge, the physical crosslinking between PE-b-PEO and RLPO was not reported yet in the literature. However, the chemical crosslinking processes of PEO with other polymers have been reported to be the interaction between PEO and chitosan by genipin (Jin and Song, 2006), creation of poly(ethylene oxide) networks crosslinked with methane or siloxane functions (Kweon and Noh, 2003), and PEO/PVA from the application of a electron beam (Yoshii et al., 1999).

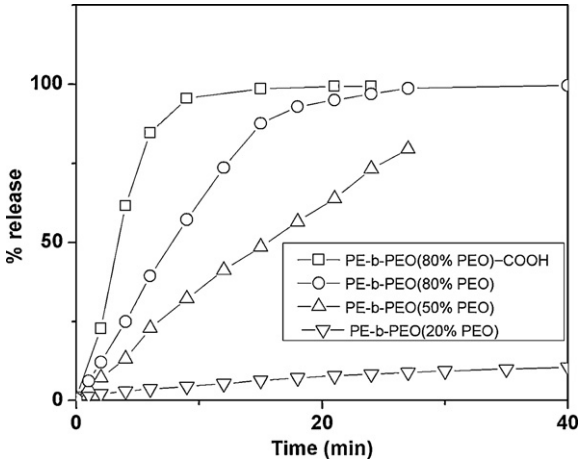


Fig. 6. Release profile of metronidazole from poly(ethylene)-b-poly(ethylene oxide) copolymers in water recorded at 318 nm.

As a result, block copolymers seem to isolate metronidazole from the water. In principle, metronidazole should be rejected by the highly hydrophobic PE-b-PEO (20% PEO), but the bursts are not so pronounced as expected. The internal structure of these devices is unknown, but at least one copolymer layer must be decreasing the drug motion and the slow swelling could be controlling the release. The blocks of these copolymers presented a rather low glass temperature for both blocks (−105 and −57 °C, respectively) (Brandrup and Immergut, 1989), but at the same time are semicrystalline polymers, a factor that leads to a decrease in diffusion (Vieth, 1991). For example, the PEO has a melting temperature of around 62 °C, depending on the molecular weight, and a high degree of crystallization (Machado et al., 2007).

The release kinetic for blends of RLPO with PE-b-PEO (20% PEO) at 30 and 50% did not fit either first- or zero-order kinetics. Also, a plot of percentage released versus square root of time (see Fig. 8)

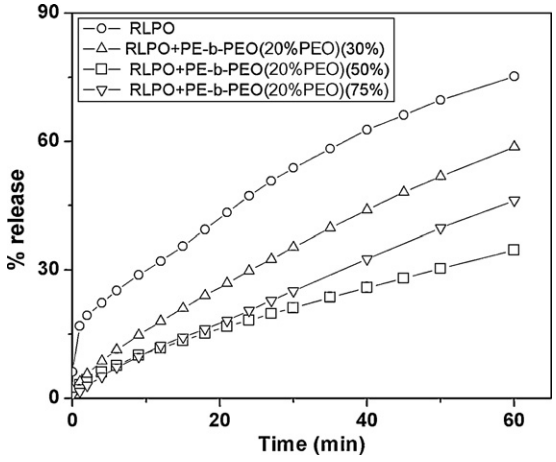


Fig. 7. Release profile of metronidazole of blends of RLPO + poly(ethylene)-b-poly(ethylene oxide) (20% PEO) in water recorded at 318 nm.

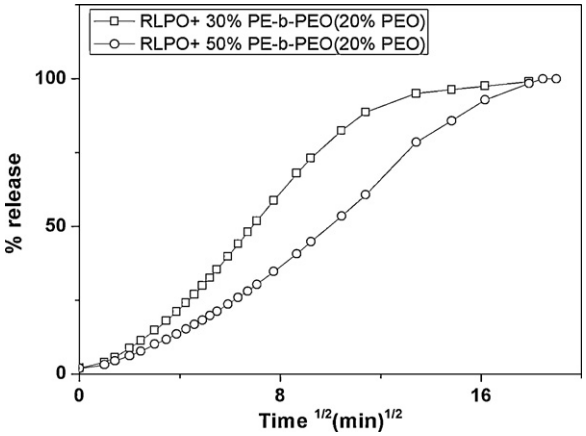


Fig. 8. Release kinetics of metronidazole versus square root time in RLPO blends with PE-b-PEO (20% PEO) at 30 and 50%.

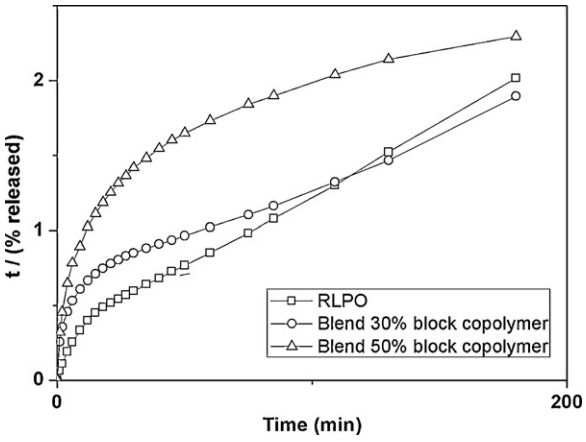


Fig. 9. Swelling according to second-order Schott equation for blends RLPO + PE-b-PEO (20%).

did not demonstrate any linear trend. Kinetic models based on release devices of constant geometry (Donbrow, 1992), including the Higuchi model (Higuchi, 1963) were a total failure. However, a sigmoid curve is observed in Fig. 8, indicating that an auto-accelerated process is taking place. This suggests that the increasing swelling of RLPO is governing the release process of metronidazole. The effect of swelling was tested using the Schott equation suitable for this purpose (Schott, 1992), as shown in Fig. 9. At the beginning of the curve some bias of data is observed but the curve subsequently becomes linear. This trend is more evident for the blend with 30% of block copolymer and for RLPO alone. This result confirms the main role of swelling in the release process of metronidazole from RLPO and its blends.

4. Conclusions

The release obtained for metronidazole devices, particles and tablets using Eudragit L100 and Eudragit RLPO gave exponential-type profiles in short times. The polymer–drug interaction of acid–base type does not provide an explanation for the release profiles obtained. The swelling of polymer and the solubility of metronidazole play a role in the process.

Several amphiphilic block copolymers poly(ethylene)-b-poly(ethylene oxide) were tested for a better control of metronidazole release and of these the poly(ethylene)-b-poly(ethylene oxide) (20% PEO) proved suitable for this purpose.

The blending of RLPO with poly(ethylene)-b-poly(ethylene oxide) (20% PEO) delayed metronidazole release, providing a more suitable profile for practical purposes for their use in the development of new drug carriers.

Kinetic measurements cannot be fitted by models that consider constant geometry for the devices involved. However, the second-order Schott equation for the swelling of polymers worked fairly well, indicating that swelling plays a major role.

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