ESCUELA TÉCNICA SUPERIOR DE INGENIERÍA
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"Synthesis, characterization and biomedical applications of microbial polymalic and polyglutamic acids derivatives."

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Trabajo realizado bajo la dirección de los Drs.
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Barcelona, Febrero 2008

Ionic complexes of biosynthetic polymalic and polyglutamic acids as prospective drug delivery systems

Summary

The hydrolytic degradability and erythromycin release from stoichiometric ionic complexes of biotechnological poly(β,L-malic) and poly(γ,D-glutamic) acids with alkyltrimethylammonium surfactants were evaluated under diverse incubation conditions for linear alkyl side chains containing 14 and 18 carbon atoms. The influence of pH, temperature and antibiotic load on hydrolysis rate was comparatively examined. It was found that polymalic complexes degraded by a surface erosion mechanism at higher rate than polyglutamic complexes, which eroded in bulk. Erythromycin was lodged in the paraffinic subphase of the complexes and it was delivered in parallel to the complex hydrolysis according to a sigmoidal profile that appeared independent on the used antibiotic load.

10.1. Introduction

Development of controlled drug delivery systems constitutes one of the most active areas of science contributing to human health care that has grown and diversified rapidly in recent years. As a result, a good number of polymer backbones both biodegradable and non-biodegradable, and covering a diverse range of functionalities is available nowadays.[1] Controlled release systems aim to improve the effectiveness of drug therapy either by increasing therapeutic activity or avoiding the need for specialized drug administration.

A wide variety of drugs including among others hemotherapeutic drugs, antiinflammatory agents, antibiotics, analgesics and vaccines can benefit from this technology. A prime criterion in the designing of new drug delivery systems is the fate of the polymer after drug release. In this sense, polymers that are naturally excreted from the body, either directly via the kidneys or biodegraded into excretable or metabolizable small compounds, are most desirable. Bioassimilable systems have concentrated much interest since they do not need to be removed after their application.[2]

The drug delivery process is the direct consequence of degradation and erosion polymer processes. Surface and bulk erosion are two extreme mechanisms, both of them occurring simultaneously in most of biodegradable polymers. The relative extent in which they occur varies radically with the chemical structure of the polymer. Provided that the drug is distributed uniformly throughout the matrix, optimum release is achieved if the polymer is depleted by surface erosion. Most synthetic polymers display bulk erosion in which the polymer matrix becomes highly porous as time progresses.

The chemical structure of the polymer is the main factor that determines the mechanism by which erosion proceeds.[3] Poly(β,L-malic acid) (PMLA) and poly(γ,D-glutamic acid) (PGGA) are well-known naturally occurring polymers that may be produced by biotechnological methods based on the aerobic fermentation of organic substrates with fungi and bacteria, respectively.[4,5] Both are biodegradable compounds of well proved non-toxicity that are being currently explored for their potential use in the biomedical field. In this paper we wish to report on the prospective use as drug delivery systems of the stoichiometric ionic complexes that these polyacids are known to form withalkyltrimethylammonium surfactants (*n*ATMA, *n* being the number of carbon atoms contained in the alkyl group).
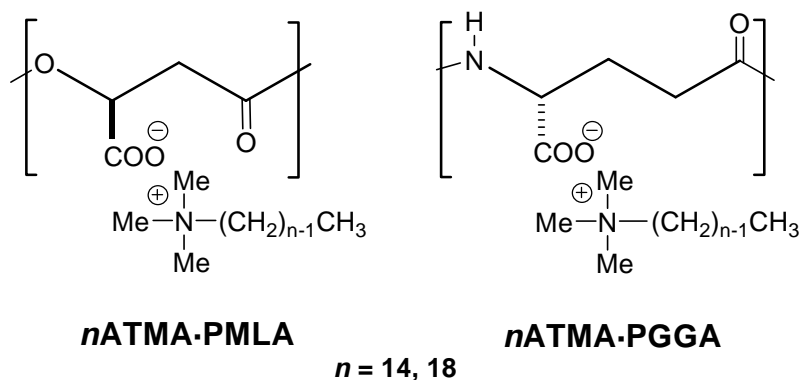
Scheme 1. Chemical structures of complexes studied in this work.

These complexes have been synthesized and fully characterized previously,[6,8] they are coded as nATMA·PMLA and nATMA·PGGA, respectively, and their chemical structures are represented in Scheme 1. These compounds display interesting features regarding their possibilities as drug carriers and systems for controlled drug release: a) they are easily produced by using water-based simple methods, b) they are constituted by biodegradable non-toxic components, and c) in the solid state, they adopt well organized nanostructures with the paraffinic phase segregated from the polymer backbone in a separated domain of lipophilic nature (Scheme 2).
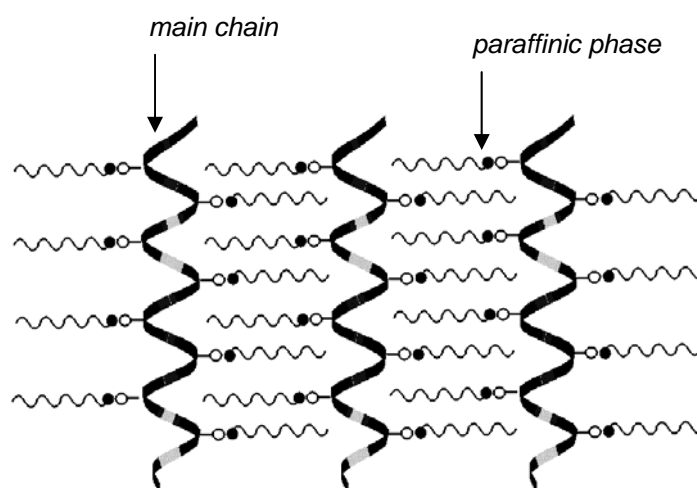
Scheme 2. Supramolecular structure of complexes.

Although nATMA·PMLA and nATMA·PGGA share the same pattern of supramolecular structure, they differ in chemical constitution and their response to biodegradation may be anticipated therefore to be significantly different.

Research on polyelectrolyte-surfactant complexes has multiplied recently because their potential application to pharmaceutical and food sciences and for their importance in gene therapy.[9,10]

10.2. Experimental

10.2.1. Materials. PMLA was obtained by cultivation of Physarum polycephalum and isolated and purified by chromatography according to previously described methods.[11] The final polyacid sample was pure according to NMR and had a Mw =26 000 Da and a Mw/Mn =1.25. PGGA with a D:L enantiomeric ratio exceeding 9:1 was obtained by fermentation of B. *licheniformis* as reported in detail elsewhere.[12]

The initial polymer size (Mw$\approx 4X10^6$ Da, Mw/Mn $\approx$4) was cut down to 120 000 Da with Mw/Mn =1.5 by irradiation with microwaves.[13] Tetradecyltrimethylammonium bromide (14ATMA·Br) and octadecyltrimethylammonium bromide (18ATMA·Br) were purchased from Aldrich and used as received. Erythromycin assay 95% (NT) was supplied by Fluka, lipase from Candida *cylindracea* (EC 3.1.1.3, Type VII, 943 units ·mg^{-1}) and α-chymotrypsin from bovine pancreas (EC 3.421.1, Type II, 50 units ·mg^{-1}) were purchased from Sigma. Organic solvents were analytical grade and used without further purification. Water used in the preparation of the complexes was distilled and deionized in a ''Milli-Q'' system.

Ionic complexes of PMLA and PGGA were prepared according to procedures described recently by us.[6,8] In brief, 50 mL of an aqueous 0.01 M solution of surfactant were added to the same volume of a 0.01 M of polyacid solution in phosphate buffer pH=7.0 under stirring, and the mixture was left to rest at room temperature. A white precipitate appeared in a few minutes, which was isolated by centrifugation, washed several times with water, and dried under vacuum at 30 ºC.

The complexes were characterized by ^{1}H and ^{13}C NMR spectroscopy. Comparison of the areas measured for main chain and side chain protons signals observed in the ^{1}H NMR spectrum afforded an accurate estimation of the composition. Relevant characteristics of polyacids and complexes studied in this work are given in Table 1.

Table 1. Data of compounds subjected to study.

Compound	D:L (%)[a]	M_w[b]	PD[b]	T_m[c] (ºC)
PMLA	0:100	36,200	1.25	216
14ATMA·PMLA	0:100	106,000	1.25	n.o.
18ATMA·PMLA	0:100	123,800	1.25	48
PGGA	90:10	120,000	1.50	n.d.
14ATMA·PGGA	90:10	337,000	1.50	n.o.
18ATMA·PGGA	90:10	381,000	1.50	50

[a]Enantiomeric composition. [b]Weight-average molecular weight (M_w) and polydispersity (PD) determined by GPC. Values for complexes calculated from PGGA and PMLA data taking into account the increment of mass by stochiometric complexation. [c]Melting temperature determined by differential scanning calorimetry.

10.2.2. Preparation of Discs and Hydrolytic Degradation. Disc specimens used in this study were prepared by solvent evaporation in triplicates. 20 mg of the complex, either pure or mixed with the appropriate amount of erythromycin (10, 20 and 30 % w/w), were dissolved in chloroform (2 mL), and stirred for 1 h. The clean solutions were poured into rounded moulds of Teflon® of 0.5 cm-diameter and the solvent left to evaporate completely at room temperature. In the case of 18ATMA·PMLA, the complex was dissolved in CCl_4 and the solution evaporated at 80 ºC in a heated chamber. Molded films of 1 mm-height were removed and stored in desiccators at room temperature for 24 h before use. In all cases, discs displayed a milky appearance, high flexibility and very good mechanical resistance.

The degradation study was performed by placing the discs into vials and adding 10 mL of buffered solutions at the selected pH (citrate buffer for pH=4.0, carbonate buffer for pH=11.0 and phosphate buffer for pH=7.4). The sample vials were stored in sealing condition to avoid partial evaporation of the fluids at 37 ºC in a heated chamber. The temperature effect was evaluated by placing the discs into vials and adding 10 mL of phosphate buffered saline solution (pH=7.4) and stored at 20, 37, 50, 60 and 80 ºC in heated chambers.

The enzymatic degradation was carried out in a phosphate buffered saline solution (pH=7.4, 37 ºC) with lipase (1 mg in 10 mL) for PMLA complexes and α-chymotrypsin (10 mg in 10 mL) for PGGA complexes.[14] Discs were taken out from the vials at set times, washed with distilled water, dried under vacuum at room temperature and weighted. Since the degradation of the PMLA discs, and maybe that of PGGA too, should generate extra acid groups, the evolution of pH with incubation time in the experiment performed at pH=7.4 was measured. It was found that the decrease in pH is less than unity for PMLA complexes and much lesser for PGGA complexes.

Degradation was followed by NMR and gel permeation chromatography (GPC) analysis of both the remaining disk and the products released to the degradation medium. For the latter, discs were incubated in deuterated water at 37 ºC and ^{1}H NMR and GPC analysis carried out at set times. In vitro enzymatic degradation and drug release tests were carried out at scheduled times with the incubation medium being periodically removed from the vials with pipette and replaced with the same volume of fresh medium. In other tests the incubation medium was not replaced to avoid the destabilization and dissolution phenomena in the hydrolytic degradation. The delivery of antibiotic by the disks was monitored by UV spectroscopy of the incubating medium at a wavelength of 280 nm. The cumulative drug concentration was plotted against releasing time.

10.2.3. Measurements. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AMX-300 NMR instrument. Analysis in solution of the complexes was carried out with samples dissolved in CDCl$_3$ and using TMS as reference. The characterization of the complexes by NMR was done by dissolving 10 mg of the respective complex in 1 mL of CDCl$_3$ (1% w/v). 640 scans were recorded for ^{1}H with delay times of 10 s at 298.1 K. Composition was determined from the ratio of CH main chain and N–CH3 surfactant side chain peaks area. The study of the hydrolytic degradation by NMR was done by dispersing 10 mg of the corresponding sample complex in 1 mL of D$_2$O. The water soluble degradation products were then observed in the ^{1}H NMR spectra recorded at room temperature (298.1 K).

GPC was performed with a Waters equipment provided with a refraction-index detector. Chromatography of the soluble products of degradation was carried out in phosphate buffer pH 7.0 using two ultrahydrogel linear columns at a flow rate of 0.8 mL·min^{-1}. Chromatograms were calibrated against poly(ethylene oxide) (PEO) monodisperse standards.

The complexes were chromatographed with 0.005 M sodium trifluoroacetate-hexafluoroisopropanol (NaTFA-HFIP) using a polystyrene-divinylbenzene packed linear column with a flow rate of 0.5 mL·min^{-1}. Chromatograms were calibrated against poly(methyl methacrylate) (PMMA) monodisperse standards.

Calorimetric measurements were performed with a Perkin-Elmer Pyris DSC instrument calibrated with indium. Sample weights of about 2–5 mg were used at heating and cooling rates of 10 K min^{-1} under a nitrogen atmosphere. Scanning electron microscopy (SEM) was used to examine the surface/cross-sectional morphology of samples before and after degradation. Gold coating was accomplished by using a Balzers SDC-004 Sputter Coater. The SEM microphotographs were taken with a JEOL SSM-6400 instrument.

10.3. Results and discussion

10.3.1. Hydrolytic Degradation of Complexes. The susceptibility of the complexes to hydrolysis was comparatively examined in order to evaluate the influence of the chemical structure of both main chain and surfactant moiety on their hydrolytic degradability.

Different pH values and temperatures were used to delineate the effect of environmental conditions. No differences were observed in the physical appearance of the discs when prepared with 10, 20 and 30 % w/w of erythromycin and degradation essays were performed with disks containing 20% of antibiotic. The weight losses with time observed for the four studied complexes incubated under physiological conditions (pH=7.4 at 37 ºC) are comparatively plotted in Figure 1.
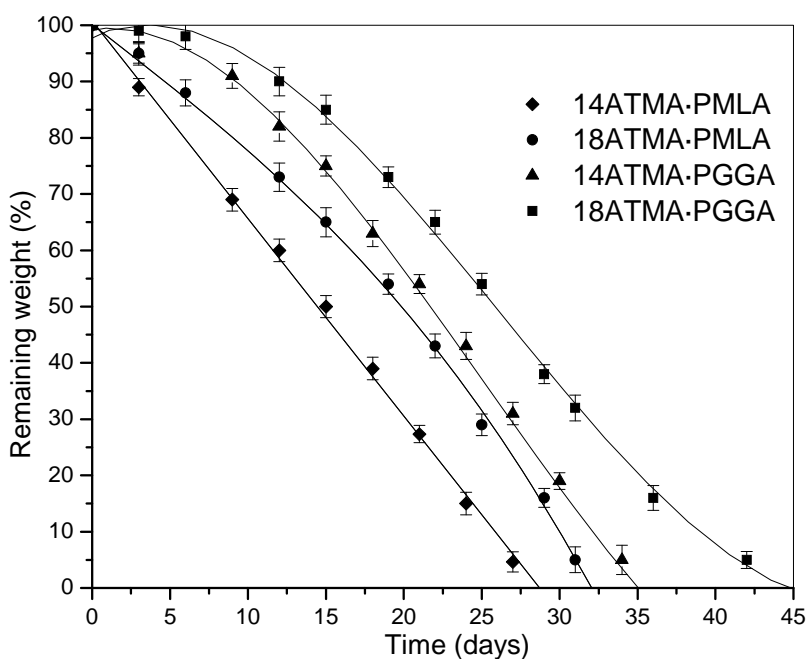

Figure 1. Compared mass loss of complexes discs incubated at pH 7.4 and 37°C.

The results reveal that the complexes made of poly(malic acid) hydrolyzed faster than those made of poly(glutamic acid) and that hydrolysis is delayed when the tetradecyl side chain is replaced by the octadecyl chain. Such effects of constitution on degradability were invariably found under all the other conditions that were essayed indicating the general validity of the trend displayed in Figure 1.

The higher degradability of nATMA·PMLA complexes respect to nATMA·PGGA complexes is doubtlessly the direct consequence of the weakness of the polymalate main chain ester group to water attack compared to the amide group of polyglutamate. On the other side, the influence of the length of the surfactant alkyl chain on hydrolysis rate may be rationalized simply in terms of hydrophobicity. Furthermore, crystallinity present in the 18ATMA complexes should be taken as an additional factor contributing to hinder the water attack.

This factor will operate only in complexes with n≥16, which is the minimum length required for side chain crystallization. The conclusion drawn from this study is that the hydrolytic degradability of these complexes can be finely adjusted by adequate constitutional changes in either the main chain or the surfactant alkyl side chain.

It is worth noting that the hydrolytic degradation of the parent polyacids, PMLA and PGGA, takes place at much higher rates than for the complexes. In fact, we have observed that PMLA becomes degraded near 90% in 3 d at pH=7.4 and 37 ºC (unpublished results) and PGGA near 60% in 2 d under the same conditions.[15] It becomes clear therefore that the paraffinic counterpart of the complex structure acts as a hydrophobic barrier that prevents the access of water to the polyester or polyamide main chain. This shielding effect is also reflected in the response to enzymes.

The hydrolytic degradation with the concourse of enzymes under physiological conditions was explored using lipase and α-chymotrypsin for PMLA and PGGA complexes, respectively, with no significant modifications in the weight loss-time profiles shown in Figure 1. These results reveal the insensitivity of these complexes to the enzymatic catalysis at difference to what is known to happen in the case of the uncomplexed polyacids.

10.3.2. Effect of pH and Temperature. The influence of pH on hydrolysis was evaluated for both acidic and basic pH values and results obtained for 14ATMA complexes of PMLA and PGGA are shown in Figure 2. Comparison of data presented in this Figure with those shown in Figure 1, which were obtained at pH =7.4, leads us to conclude that deviation of pH from neutrality enhances significantly the hydrolysis of PMLA and PGGA complexes.

It should be noted however, that the pH influence appears to be of opposite sign in the two cases, i.e. PGGA complexes hydrolysis is favored at acidic pH whereas PMLA complexes hydrolyze faster under basic conditions.

Such an opposite response of hydrolysis rate to pH is according to what should be expected from the mechanism operating in the water cleavage of ester and amide groups. Although both acidic and basic conditions favor the hydrolysis, the relative degradability of PMLA to PGGA complexes is not altered, the latter's being more resistant than the former's at any pH value. Similar results were obtained with 18ATMA complexes confirming the conclusions drawn for the 14ATMA series.
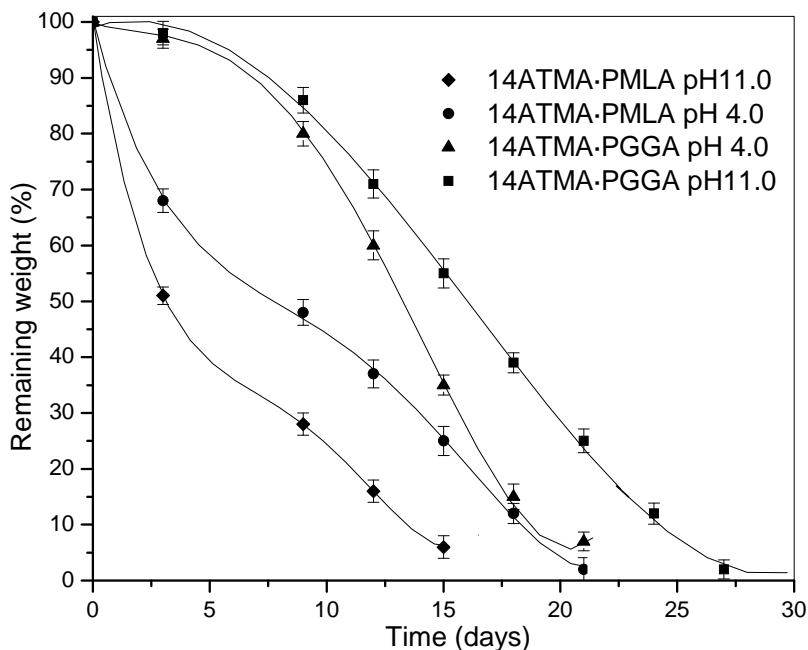

Figure 2. Mass loss of 14ATMA·PMLA and 14ATMA·PGGA disks at 37 ºC and the indicated pH.

Since both water diffusion and hydrolytic reaction rate increase with temperature, it can be anticipated that hydrolysis of the complexes will be favored by heating. Additionally, special effects should be expected in the case of complexes made of 18ATMA, which have the alkyl side chains crystallized in a separated paraffinic phase melting at 48–50 ºC. The weight loss of 18ATMA·PMLA with time at different incubation temperatures is shown in Figure 3.
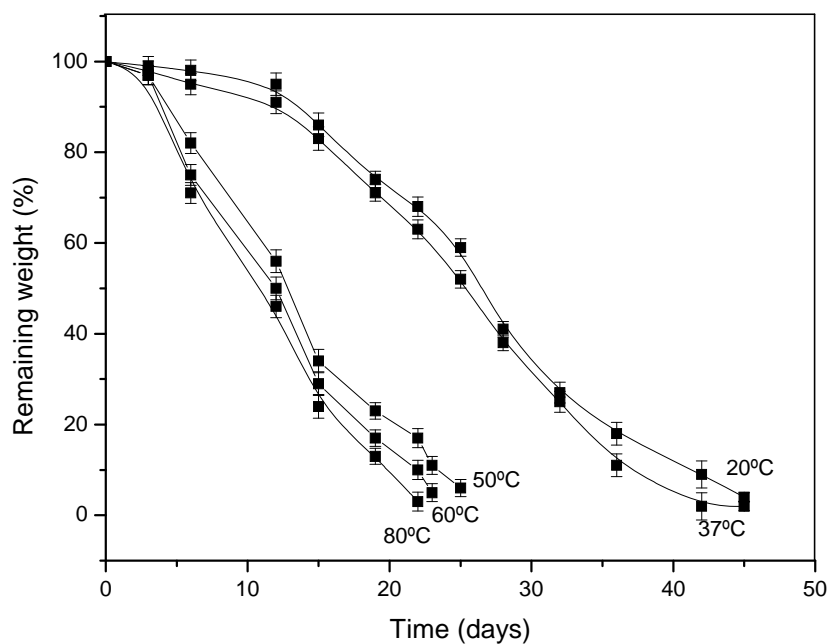
Figure 3. Mass loss of 18ATMA·PMLA disks at pH 7.4 and the indicated temperatures.

It is seen that the degradation rate increases with temperature showing a jump at 50 ºC, which is the melting temperature of the complex. Obviously the fusion of the paraffinic phase makes the complex more permeable to water which can reach the hydrolysable main chain ester groups more easily. A similar response to temperature was observed for 18ATMA·PGGA in full agreement with its thermal behavior. On the other hand, the hydrolysis rate of both 14ATMA·PMLA and 14ATMA·PGGA was found to increase continuously with temperature not showing any abrupt change, which is consistent with the amorphous nature displayed by these two compounds.

10.3.3. Degradation Mechanisms. The decrease in the weight-average molecular weight (Mw) with time of the complexes subjected to hydrolytic degradation under physiological conditions is plotted in Figure 4. It is seen that after an incubation period of one month the PGGA complexes are almost completely degraded whereas the molecular weights of PMLA complexes become reduced by only 10–20% of their initial value. These results are rather striking when compared to those obtained in the sample weight measurements (Figure 1), which showed that material depletion upon incubation happened much faster for PMLA than for PGGA complexes. The GPC analysis revealed that the products released from PGGA complexes upon degradation consisted of medium-size polymer of high polydispersity. On the contrary, no products of appreciable chain size were detected in the incubation medium of PMLA complexes.
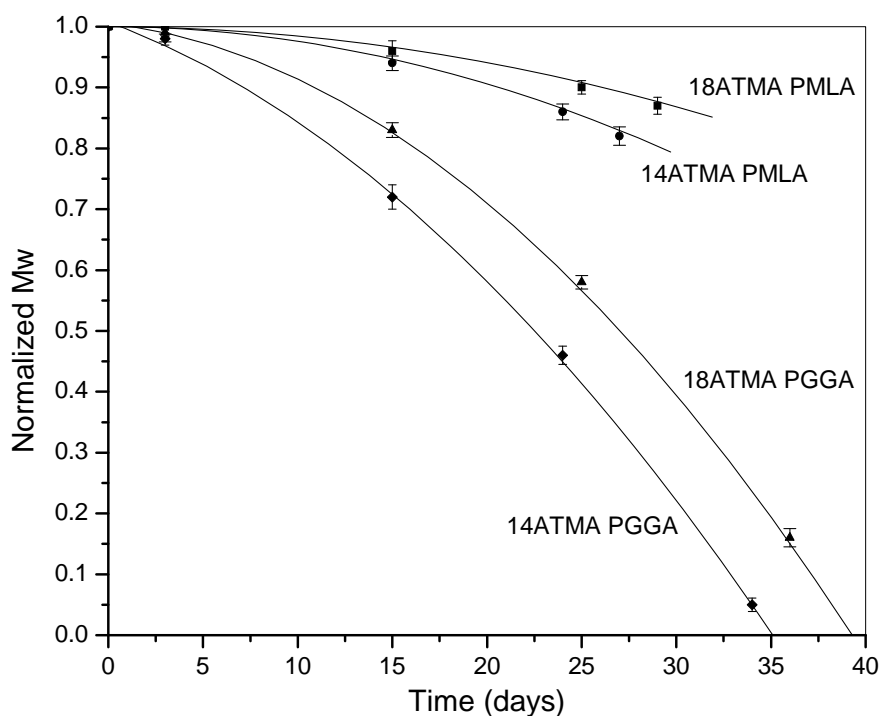
Figure 4. Normalized molecular weight of complexes discs degraded at pH 7.4 and 37°C.

The NMR analysis provided information that was of invaluable help to understand the weight loss and GPC results described above. The ^{1}H NMR spectra of the products released to the incubation medium upon degradation by 14ATMA·PMLA and 14ATMA·PGGA are shown in Figure 5. In the former case, the ^{1}H NMR spectra show the presence of signals at 4.4 and 2.7 ppm, corresponding to malic acid and free surfactant from the earlier incubation time. It seems therefore that degradation of 14ATMA·PMLA occurs from the beginning by hydrolysis of PMLA to malic acid and simultaneous dissociation of the complex.

On the contrary, the ^{1}H NMR spectra recorded from the incubation medium of 14ATMA·PGGA do not show significant signals until 25 days after, the spectra then observed corresponding to medium size complex polymer chains. In this case, degradation of 14ATMA·PGGA appears to happen therefore by cleavage of the polyglutamate main chain in large segments that remain insolubilized until they are cut down to the size required to pass into solution.

On the other hand, the ^{1}H NMR analysis of the residual disk samples recorded along incubation gave similar results in the two cases, the spectra showing no differences with those of their respective original complexes.

All the results described above lead to the conclusion that different degradation/erosion mechanisms must operate for PMLA and PGGA complexes. In the former case, surface erosion motivated by fast hydrolysis rate of the polymer and poor water diffusion would be the prevailing mechanism.
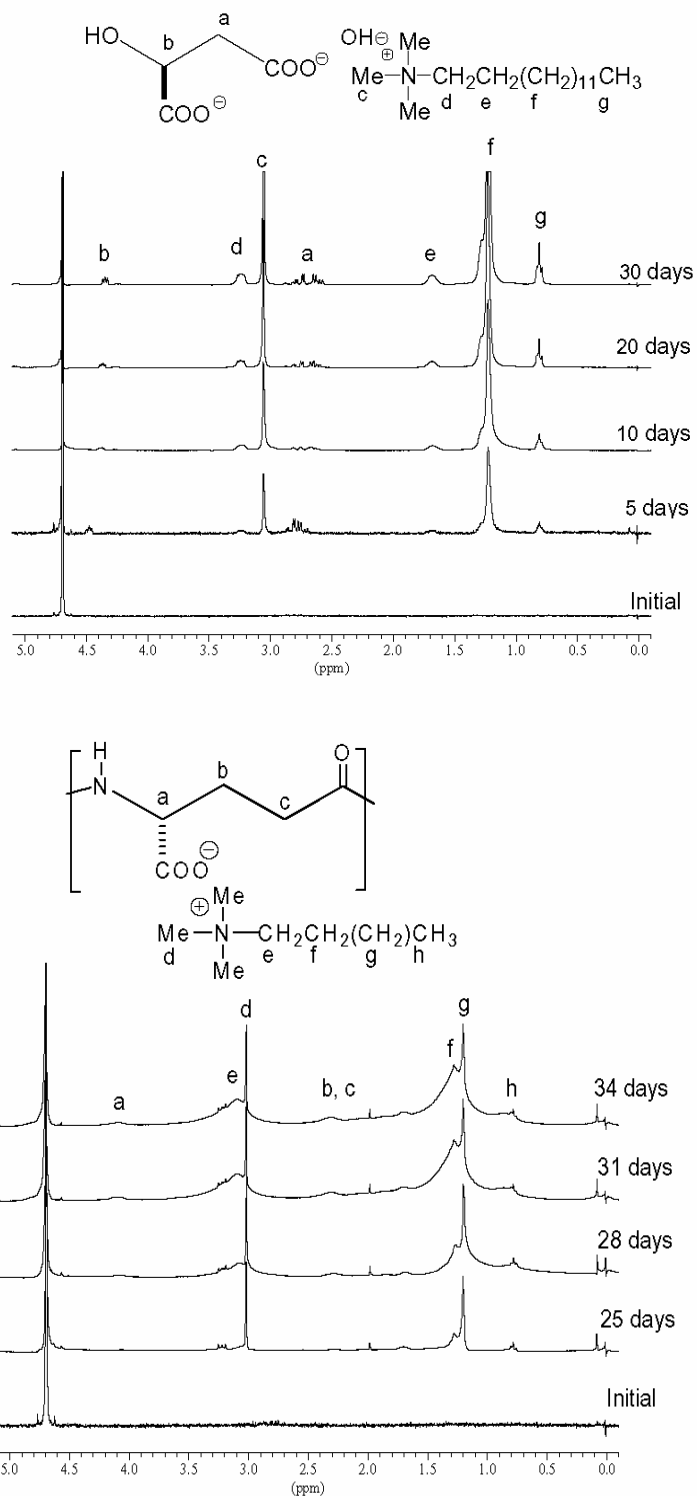

Figure 5. ^{1}H NMR spectra of degradation water of 14ATMA·PMLA (top) and 14ATMA· PGGA (bottom) complexes recorded at 25 ºC in deuterated water.

Conversely, PGGA would erode in bulk due to the greater resistance of the polyamide chain to water cleavage and relative faster penetration of water in the polymer mass. Confirming evidence on the occurrence of these mechanisms was provided by SEM, the illustrating micrographs being shown in Figure 6 and 7 for 18ATMA·PMLA and 18ATMA·PGGA complexes, respectively.
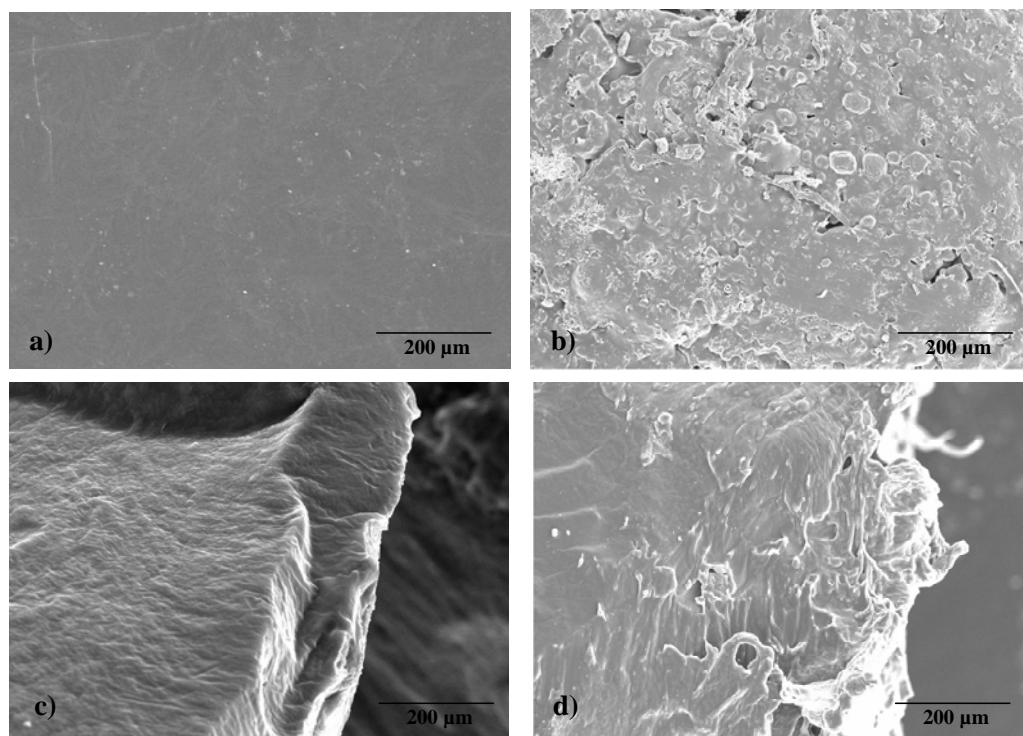

Figure 6. SEM micrographs of 18ATMA·PMLA discs. a) and c) surface and edge views of original disk, respectively. b) and d) same views of the disk after loosing around 50 % of its initial weight.

In these sets of pictures, surface and edge disc views are compared for the original disc (a and c) and for the disc after loosing around 50% of the initial weight (b and d). In the case of 18ATMA·PGGA both surface and edge appear similarly deteriorated indicating that a homogeneous erosion mechanism was operating. On the other hand, degradation of 18ATMA·PMLA is much clearer perceivable in the disc edge, revealing that a surface process must be the main mechanism through which this compound preferentially erodes.

The approach used by Burkersroda[16] to predict the mechanism of polymer erosion, surface or bulk, taking place upon hydrolysis has been applied to these complexes in order to assess the conclusions drawn from the analysis of the experimental results.

The semiempirical Equation (1) relates polymer features and sample shapes factors with the erosion coefficient ε. ε values lower and higher than 1 are made to correspond to bulk and surface mechanisms, respectively, the prediction being more uncertain as the ε value becomes closer to 1.
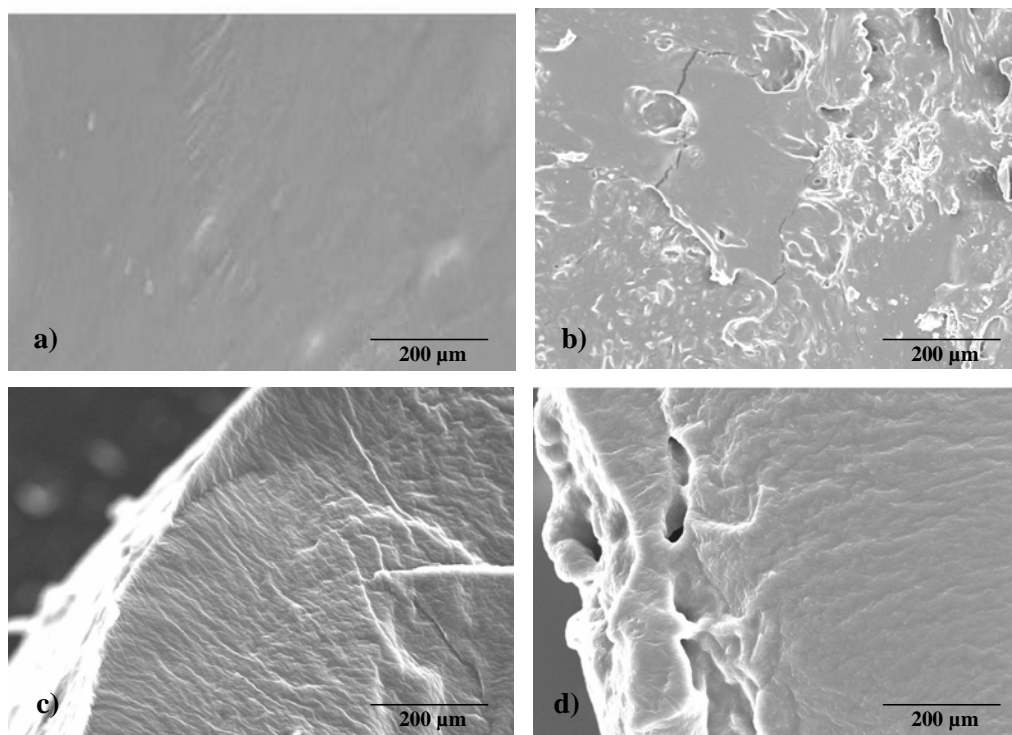

Figure 7. SEM micrographs of 18ATMA·PGGA discs. a) and c) surface and edge views of original disk, respectively. b) and d) same views of the disk after loosing around 50 % of its initial weight.

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$$\varepsilon = \frac{h^2 \lambda \pi}{4D(\ln h - \ln \sqrt[3]{M_n / N_A (x_n - 1)\rho}} \qquad (1)$$

where h is the disk thickness, λ the hydrolysis kinetic constant of the polymer, D the water diffusion constant, M_n the number-average molecular weight of the polymer, x_n the number-average degree of polymerization, ρ the density and N_A Avogadro's constant. To use Equation (1), the water diffusion constant of complexes was experimentally determined by using Fick's equation:

$$\frac{M_t}{M_\infty} = 4\sqrt{\frac{Dt}{\pi h^2}} \qquad (2)$$

where M_t and M_∞ are the disk weights at time t and at constant value weight. The disks were placed in phosphate buffer at pH=7.4 and 37 ºC, removed at 4 h intervals, dried on filter paper and weighed (M_t).

These measurements were performed repeatedly until constant weight (M_∞). The plot of M_t/M_∞ against $t_{1/2}$ results in a straight line, the slope being the diffusion coefficient D. The ε values resulting from the application of Equation (1) for the four complexes investigated in this work are compared in Table 2, in which the other parameters used for calculation, have also been detailed. Values much smaller than one according to an erosion process taking place homogeneously throughout the whole mass of the sample, were found for PGGA complexes. On the contrary, values above one were calculated for PMLA complexes in agreement with the occurrence of a surface erosion mechanism as evidenced by experimentation.

It is worthy to note that D values obtained by sorption measurements are similar for all four complexes, indicating that this factor has not a decisive influence on the relative values obtained for ε. Apparently, the type of erosion mechanism operating in the complexes becomes essentially determined by the susceptibility of the polymer backbone to water attack.

Table 2. Erosion mechanism parameters of complexes**.**

	14ATMA· PMLA	18ATMA· PMLA	14ATMA PGGA	18ATMA· PGGA
h	1.0	1.0	1.0	1.0
λ^a	6.6×10^{-9}	6.6×10^{-9}	2.6×10^{-13}	2.6×10^{-13}
D^b	7.8×10^{-11}	6.6×10^{-11}	8.4×10^{-11}	7.0×10^{-11}
M_n	20,800	20,800	80,000	80,000
x_n	208	208	124	124
ρ^c	1.00	1.00	1.03	1.06
ε^d	4	4.7	1.5×10^{-4}	1.7×10^{-4}
Erosion	surface	surface	bulk	bulk

[a]Values taken from literature for polyesters and polyamides [14]. [b]Values determined by equation 2. [c]Density experimentally determined by the flotation method using aqueous glycerin solutions. [d]Erosion coefficient determined by equation 1.

10.3.4. Complexes as Drug-Delivery Systems. Incorporation of erythromycin in the complexes was carried out by casting at room temperature from chloroform solutions of the corresponding mixtures of polymer and antibiotic. No apparent changes were detected in either the texture or the optical features of the discs indicating a good compatibility of the two components. DSC traces recorded from 30% erythromycin loaded and unloaded 18ATMA·PGGA complex along with that of pure erythromycin are shown in Figure 8. Endothermic peaks characteristic of melting are seen around 50 and 190 ºC for the unloaded complex and antibiotic, respectively.

In the loaded disc, the melting peak of erythromycin is moved to much lower temperatures, indicating that it must be finely dispersed in the matrix of the complex. On the other hand, the melting peak associated to the paraffinic phase of the complex remains at its original position although showing significant broadening. It seems therefore that the rather lipophilic antibiotic is microcrystallized and lodged in the amorphous paraffinic subphase causing a notable increase in the dispersity of the paraffinic crystallite sizes. Similar results were obtained for the complex 18ATMA·PMLA although in this case the erythromycin crystallites did not appear so finely dispersed.

Such preference of the drug for the paraffinic phase, which is walled by the polyester or polyamide main chains, will make its release to the aging media by diffusion more difficult.
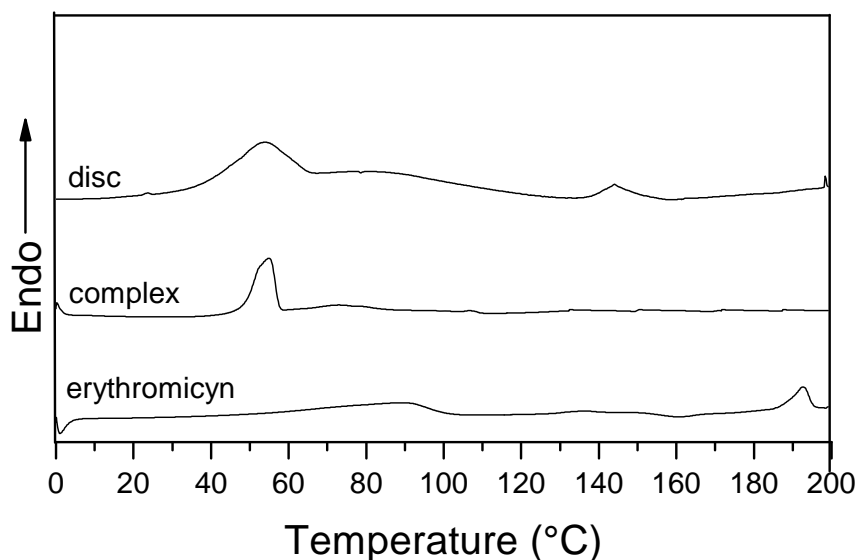

Figure 8. DSC traces of 18ATMA·PGGA disk and their components.

Erythromycin release from discs of the four complexes was evaluated under physiological conditions for antibiotic loads of 10, 20 and 30 % w/w. The releasing plots for the 10%-erythromycin loaded complexes are compared in Figure 9. In all cases approximately one tenth of the loaded antibiotic is already released after a few hours of incubation.

This is interpreted as simple detachment of compound that was merely adhered to the disc surface. In agreement with the degradation results described above, drug release was found to be faster for PMLA complexes than for PGGA complexes and for 14ATMA than for 18ATMA in both types of complexes. Additional differences of interest in the releasing behavior were found between PMLA and PGGA complexes.

Whereas drug release in PMLA complexes is noticeable from the beginning of incubation, a lag time that extends beyond the fifth day of incubation is observed for the PGGA complexes. Such delay in delivering the drug corresponds approximately to the time needed for observing appreciable weight loss in these complexes, suggesting that erythromycin release is subsequent to solubilization of the polymer segments that originate by hydrolysis.

In this sense it should be mentioned that carboxylic groups generated upon degradation will likely form erythromycin salts and that this mechanism could be determinant for the release of the drug to the aqueous medium.
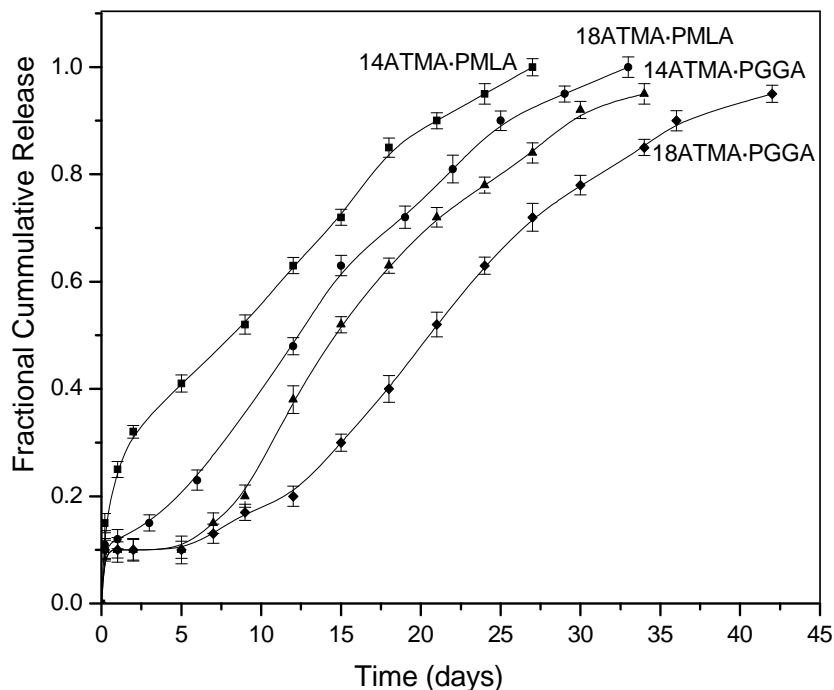

Figure 9. Erythromycin release profiles from disks of complexes loaded with 10% w/w of drug and incubated at pH 7.4 and 37°C.

Since PGGA degrades in the bulk with initial generation of long non-soluble polymer segments, release of the erythromycin salts in these systems will be delayed until the polymer chain attains a water-soluble size.

The delivering dependence on the amount of antibiotic loaded in the disc was estimated by comparing the releasing plots for 10, 20 and 30 % w/w, corresponding to 2, 4 and 6 mg of load of drug, respectively, in the 18ATMA·PMLA complex.

Results are shown in Figure 10 revealing that the release pattern is essentially unaffected by the concentration of the drug in the disc, the fractional amount of liberated erythromycin being almost the same for all the essayed loads. Such a behavior leads to consider the contribution of diffusion processes to the operating releasing mechanism to be negligible.

Temperature and pH effects on erythromycin releasing rate were also evaluated for all the complexes. The number of days needed for releasing 50% of the initial load under different selected conditions is compared in Table 3. These data indicate that the response to pH and temperature follows the trend observed for degradation, confirming that drug delivery is a process concomitant to polymer hydrolysis.

The influence of lipase and chymotrypsin enzymes on the erythromycin releasing was evaluated finally, the obtained results being as those expected from the hydrolytic degradation study, i.e. no enzymatic activity was detected in all cases.
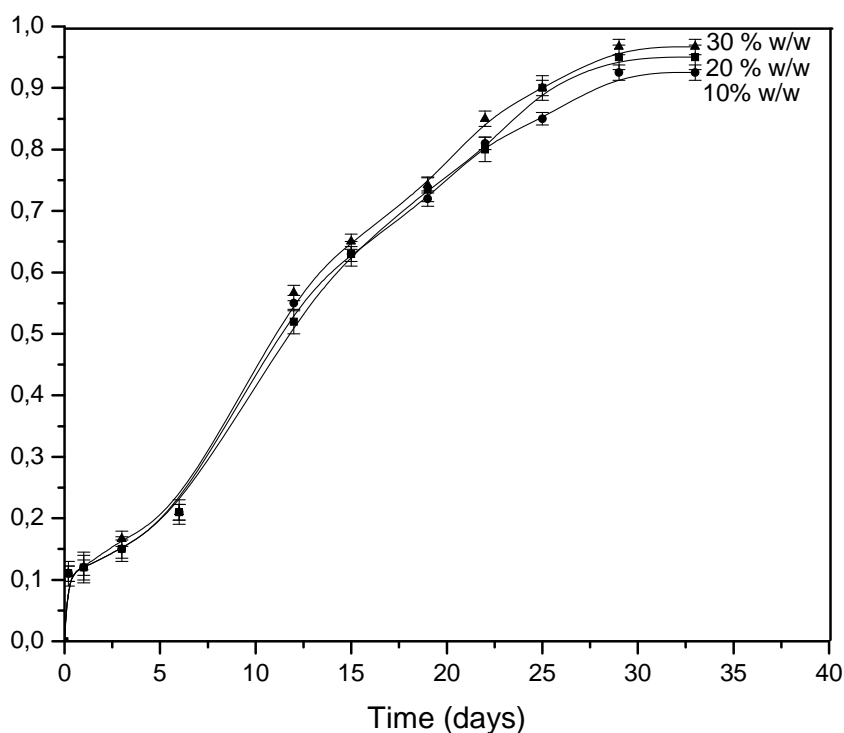

Figure 10. Erythromycin release profiles from 18ATMA·PMLA discs loaded with the indicated % of drug and incubated at pH 7.4 and 37°C.

Table 3. Erythromycin releasing half-time from complexes under different conditions.[a]

	14ATMA· PMLA	18ATMA· PMLA	14ATMA· PGGA	18ATMA· PGGA
pH 7.4 37 ℃	7	11	15	21
lipase	7	10	-	-
α-chymotrypsin	-	-	15	21
pH 7.4				
20 ℃	7	11	15	21
50 ℃	10	3	12	10
60 ℃	11	3	11	10
80 ℃	8	3	10	10
pH 4.0 37 ℃	6	5	8	15
pH 11 37 ℃	2	6	10	13

[a]Time (days) at which 50% of the initially loaded antibiotic is released to the incubation medium.

10.4. Conclusions

This work is a pioneer in the study of the hydrolytic degradability and drug releasing properties of ionic complexes composed of biotechnological polymers and cationic surfactants, a class of compounds distinguished by its chemical and supramolecular structure. The following outstanding conclusions may be drawn from this study:

a) Both poly(malic acid) and poly(glutamic acid) /alkyltrimethylammonium complexes degraded completely by water within one-to-two month's periods of time depending on pH, temperature and length of the alkyl side chain attached to the surfactant. It appears possible to tune the degradability of these compounds by appropriate chemical design of the complex.

b) Complex degradation took place by hydrolysis of either the polyester or polyamide backbone accompanied by dissociation of the ionic structure. Poly(malic acid) complexes underwent surface erosion whereas those of poly(glutamic acid) eroded in bulk.

c) Erythromycin could be homogenously dispersed in both complexes for loads of up to 30% w/w. The antibiotic was found to be lodged in the hydrophobic subphase of the complexes and upon water incubation was delivered according to the hydrolysis profile followed by the respective complexes with independence on the amount loaded.

10.5. References

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