Ionic Complexes of Biotechnological Polyacids with Cationic Surfactants

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Summary: Stoichiometric complexes of biotechnological poly(γ-glutamic) acid and poly(β,L-malic) acid with alkyltrimethylammonium surfactants of long alkyl chains could be readily prepared in aqueous medium. They adopt a biphasic layered structures in which the main chain and the side chain alternate with nanometric periodicity. Alkyl side chains show reversible melting that involves generation of mesophases. Complexes degraded by water by different mechanisms depending on the constitution of the main chain; the polymalic complexes underwent surface erosion whereas the polyglutamic ones degraded in bulk. Erythromycin could be homogenously loaded into the paraffinic subphase of the complexes and delivered upon incubation under physiological conditions in parallel to the hydrolysis of the polymer.

Keywords: poly(β malic acid); poly(γ glutamic acid); polyelectrolyte surfactant complexes; polyglutamic ionic complexes; polymalic ionic complexes

Introduction

Comb-like polymers made of a rigid main chain and a flexible side chain are able to self-assembly in biphasic layered structures sensitive to temperature changes.[1] As early as 1985, Watanabe described comb-like polymer structures made of long alkyl esters of polyglutamates and polyaspartates.[2] These are covalent systems in which the side chain is linked to the main chain by an ester bond. It is well established that in these comb-like polypeptides, the main chain is in helical conformation with hydrogen bonds intramolecularly formed. The side chains come out from the helix to fill up the interlayer space.[3] The symmetry of the helix is determined by the constitution of the polypeptide, poly-α-, poly-β- or poly-γ-aspartate or glutamate [4,5], but what is common to all of them is the helical conformation adopted by the main chain, which is stiff and stable.

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These systems show thermal transitions with particular features. Upon heating, the alkyl side chain melts but the helical main chain remains unaltered, *i.e.* it preserves the helical conformation and retains its position so that the layered structure is still present after melting. The interlayer spacing decreases however due to the slightly contraction undergone by the polymethylene chain. Such thermal behaviour has interesting consequences which potential technological applications as responsive temperature membranes or thermochromic sensors.[6,7]

Comb-like systems similar to those described above can be also prepared by ionic coupling between the main chain and the side chain. First work published on this type of systems was reported by Antonietti in 1994.[8] In this pioneering work, synthetic poly(α-glutamic acid) was coupled with cationic surfactants to produce ionic comb-like systems with a structure similar to that described for the covalent ones. The main advantage afforded by the ionic systems respect concerns the preparation work; whereas preparation of alkyl polyglutamates and polyaspartates implies

a laborious and long synthetic route, ionic complexes can be prepared by simple mixing of the corresponding components in aqueous medium.

Poly(γ-glutamic) acid (PGGA) and poly(β,L-malic) acid (PMLA) are biotechnological polyacids obtained by aerobic fermentation of organic substrates, the former with bacteria *B. licheniformis* and the latter with mixomicete *P. polycephalum*. These two polyacids are good candidates for preparing comb-like ionic complexes of the type previously described from poly(α-peptide)s. In this contribution, a summary of the results obtained in the synthesis, characterization properties and applications of the complexes derived from PGGA and PMLA is reported.

Experimental Part

Sodium poly(γ,DL-glutamate) with a D:L enantiomeric ratio of 59:41 and a weight-average molecular weight of about 300,000 Da and $M_w/M_n = 2.6$, which was kindly supplied by Dr. Kubota of Meiji Co. (Japan), was used in this work. The polymer was changed to the protonated form by acidification with HCl followed by precipitation in 2-propanol. Poly(β,L-malic acid) of microbial origin was used in this work. It was obtained by cultivation of *Phisarum polycephalum*. The polyacid was NMR pure and had a $M_w = 26{,}000$ Da and $M_w/M_n = 1.25$. Linear alkyltrimethylammonium surfactants of general formula $RN^+(CH_3)_3 \cdot Br^-$ with $R = C_{12}H_{25}$ (dodecyl), $C_{14}H_{29}$ (meristyl), $C_{16}H_{33}$ (cetyl), and $C_{18}H_{37}$ (stearyl) were purchased from Aldrich or Merck and used as received. Those with $R = C_{20}H_{41}$ (eicosyl) and $C_{22}H_{45}$ (docosyl) were synthesized by us according to literature procedures.[9]

^1H and ^{13}C NMR spectra were recorded on a Bruker AMX-300 NMR instrument provided with a CP-MAS accessory for the analysis of solids and using TMS as reference. 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 °C min^{-1} and under a nitrogen atmosphere. Thermogravimetric analyses were performed under an inert atmosphere with a Perkin-Elmer TGA6 thermobalance at heating rates of 10 °C min^{-1}. Optical microscopy was carried out in an Olympus BX51 polarizing microscope equipped with a digital camera system and a Linkam THM5600 hot stage. Filmy samples of *n*ATMA · PMLA of complexes were prepared from 1% (v/v) chloroform solutions, which were left to evaporate slowly between two microscope cover slides. X-ray diffractograms were obtained in a diffractometer with geometry Debye-Scherrer INEL CPS-120. The Cu K_α radiation of wavelength 0.1541 nm was used in all cases.

For degradation studies, disc specimens were cut from films which were prepared by evaporation of halogenated solvent solutions containing a mixture of the complex with the appropriate amount of erythromycin (10 up to 30% w/w). Degradation of discs was performed in vials containing the buffered solutions at the selected pH (4.0, 11.0 and 7.4) at 37 °C.

Results and Discussion

Synthesis of *n*ATMA · PGGA Complexes

PGGA was produced by aerobic fermentation of medium E with bacteria *Bacillus licheniformis*. PGGA may be obtained with different molecular weight and different enantiomeric composition depending on the manganese concentration used in the incubation medium. The most common form is the DL form with a composition close to racemic and a molecular weight of about 400,000 Da. PGGA in the sodium form is a very hydrophilic compound that absorbs very large amounts of water to produce highly viscous gels.[10]

The *n*ATMA · PGGA complexes with the chemical structure depicted in Figure 1 were readily formed as a white fine powder when equal volumes of aqueous solutions of Na-PGGA and the corresponding

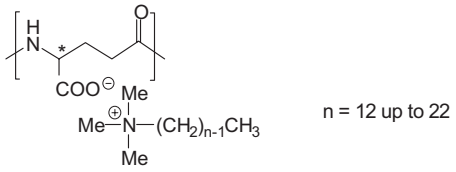

Figure 1.
Chemical formula of complexes of PGGA studied in this work.

*n*ATMA · Br, at the same molar concentration, were mixed and the mixture was left to stay at room temperature for a few hours.

Ionic complexes separated as a precipitate that could be easily isolated as a white powder. This powder was non-water soluble but soluble in chloroform. The results obtained in the preparation of PGGA complexes are given in Table 1. Complexes for even values of *n* from 12 to 22 were prepared. Yields oscillate between 50 and 90%, and the composition was less stoichiometric as the used surfactant alkyl chain was longer.[11]

Thermal Behaviour and Structure of *n*ATMA · PGGA Complexes

Figure 2 shows the TGA traces of *n*ATMA•PGGA complexes compared for the whole series. Thermal decomposition starts close to 200 °C for all the complexes and they decompose in two main steps, the first one at temperatures of approximately 270–280 °C and the second well above 300 °C (Table 1).

The degradation mechanism has been elucidated and it is depicted in Figure 3. In the first step, the main chain decomposes giving pyroglutamic acid and at the same time the ionic complex decomposes to give the surfactant hydroxide. The second step is

Table 1.
Synthesis results and thermal behaviour of *n*ATMA · PGGA complexes.

$R(CH_3)_3N^+$	Complex	Yield (%)	Composition	ρ (g mL^{-1})	^{o}Td [a)] (°C)	^{o}Tm [b)] (°C)
-$nC_{12}H_{25}$	12ATMA · PGGA	56	1.0:1	1.00	–	–
-$nC_{14}H_{29}$	14ATMA · PGGA	62	1.1:1	1.03	198	267/315
-$nC_{16}H_{33}$	16ATMA · PGGA	67	1.1:1	1.02	200	270/330
-$nC_{18}H_{37}$	18ATMA · PGGA	57	1.2:1	1.06	198	274/352
-$nC_{20}H_{41}$	20ATMA · PGGA	89	1.3:1	1.01	199	279/364
-$nC_{22}H_{45}$	22ATMA · PGGA	54	1.3:1	1.02	198	281/382

[a)] Onset decomposition temperature measured for 5% lost of the initial weight.
[b)] Maximum rate decomposition temperature for the two decomposition steps.

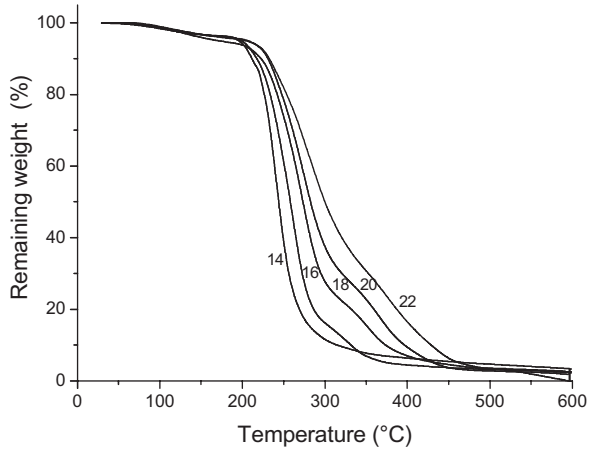

Figure 2.
TGA traces of *n*ATMA · PGGA complexes.

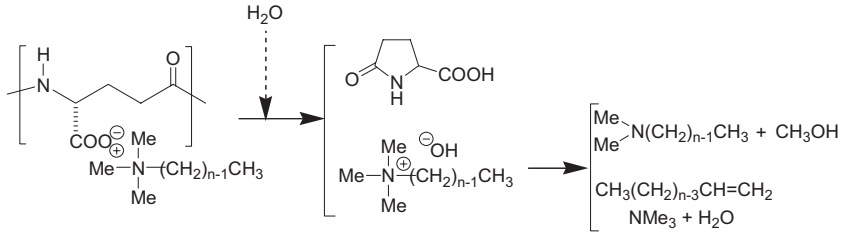

Figure 3.
Mechanism of thermal degradation of of *n*ATMA · PGGA complexes.

the decomposition of the surfactant by two ways, generating amines with releasing of methanol and undergoing the Hoffman degradation reaction producing an alquene, trimethyl amine and water.[12]

The melting behaviour of PGGA complexes was studied by DSC. Figure 4 (left) shows the heating, cooling and second heating DSC traces of 18ATMA · PGGA. The endothermal peak at 50 °C corresponds to the melting of the paraffinic phase made of alkyl side chains. The melting peak observed for the surfactant bromide appears at 107 °C, clearly distinguishable from the melting of the alkyl side chains. In the DSC traces for the whole series of complexes shown in Figure 4 (right), melting is only observed for $n = 16$ or higher, and the melting temperature is found to increase linearly with the length of the alkyl side chain.[11]

The texture observed for the films of these complexes is very dependent on the length of the alkyl side chain. Figure 5 shows the polarizing microscope photographs taken from three of them. The 12ATMA · PGGA displays a mosaic texture characteristic of smectic mesophase whereas the 20ATMA · PGGA displays a spherulitic texture typical of semicrystalline polymer. The texture observed for 16ATMA · PGGA is intermediate between the other two with the domains showing a spherulitic appearance as expected for a defectively crystallized material. [11]

In Figure 6, side view schemes (normal and parallel to the orientation of the alkyl side chain) of the layered structure of the

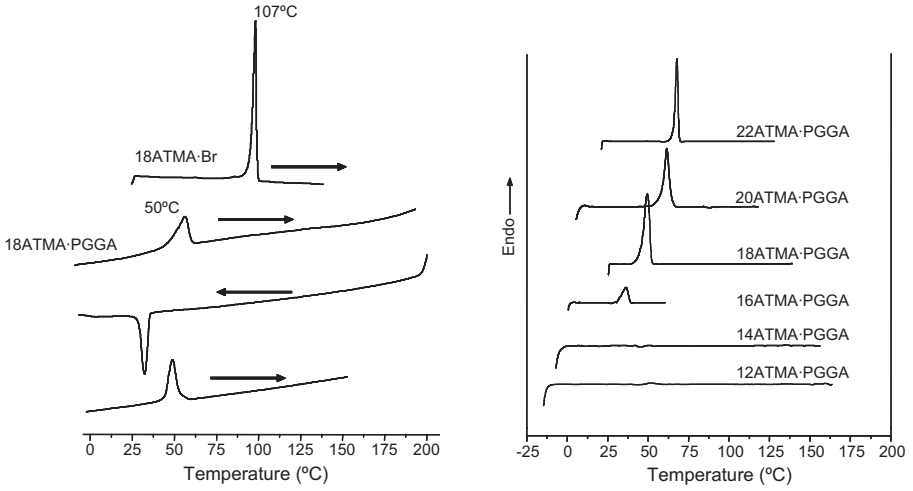

Figure 4.
DSC traces of *n*ATMA · PGGA complexes.

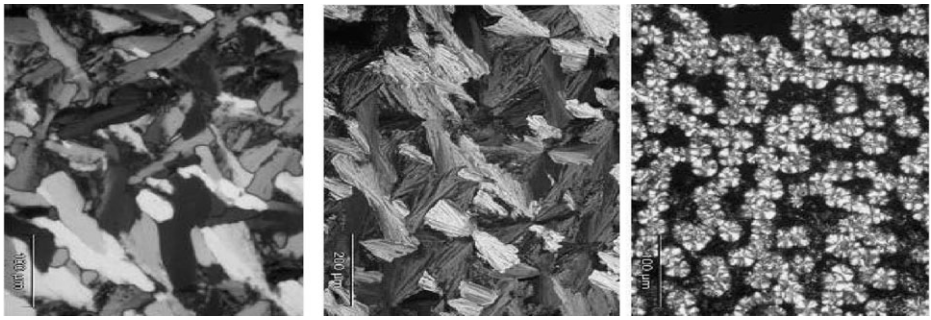

Figure 5.
Polarizing optical micrographs of *n*ATMA · PGGA. (Left: 12ATMA · PGGA, mosaic texture. Centre: 16ATMA · PGGA, fanlike texture. Right: Spherulites of 20ATMA · PGGA.)

crystalline complexes according to data afforded by X-ray and electron diffraction patterns are depicted. Polypeptide layers are separated a fixed distance by the hexagonally crystallized paraffinic phase in which the chains are extensively interdigitated and oriented perpendicular to the layers.

The long spacings of the layered structure against *n* are plotted in Figure 7 for the PGGA complexes and the alkyl glutamate esters (PAAG-n).

In both cases, the long spacings are aligned in a straight line with a slope near to 0.12 nm, corresponding to the projection of the methylene unit. The ordinate difference between the two series of data is consistent with the interlayered additional space introduced by the ionic pair carboxylate-trimethylammonium. Long spacings for

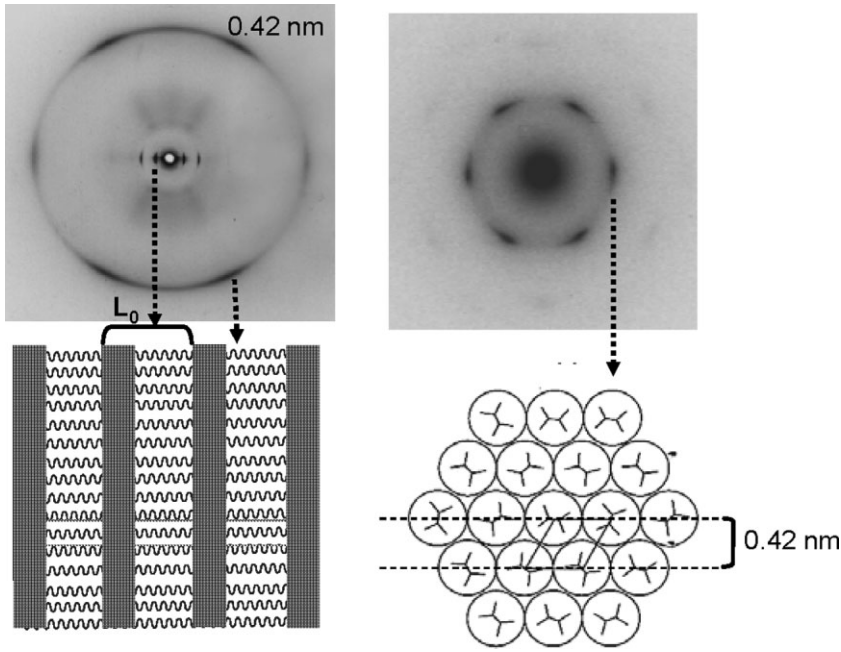

Figure 6.
X-ray (left) and electron (right) diffraction patterns of 18ATMA · PGGA and the corresponding schematic representations of the structure.

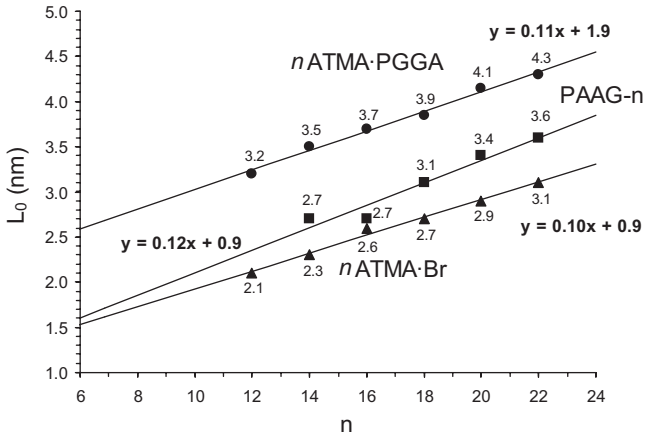

Figure 7.
Interlayer distance of the biphasic structure of PGGA complexes and PAAG-*n* as a function of the number of carbon atoms in the alkyl side chain. Data for surfactants bromides are also plotted for comparison.

the surfactant series are also aligned in a straight line with a similar slope indicating the occurrence of a similar structure. In this case however there is not evidence of the existence of a layered structure.[5,11]

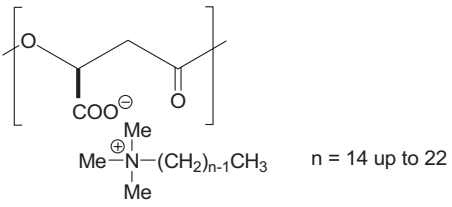

Figure 8.
Chemical formula of complexes of PMLA studied in this work.

Synthesis of *n*ATMA · PMLA Complexes

PMLA was produced by aerobic fermentation of the fungi *P. polycephalum* using a glucose enriched medium.[13]. PMLA was obtained with a molecular weight about 30,000 Da and a polydispersity close to 1.2 and contained 100% of L-enantiomer. Preparation of the complexes followed the same protocol as in the case of the PGGA complexes (Figure 8).[14] The two aqueous solutions were mixed together and the complex separated as a white precipitate with a composition nearly stoichiometric (Table 2).

Thermal Behaviour and Structure of *n*ATMA · PMLA Complexes

The thermal degradation of these complexes takes place in three well resolved steps, the first one happening at 240–245 °C and corresponding to the quantitative decomposition of the polymalate main chain by a depolymerization mechanism similar to that operating in the decomposition of poly(malic acid). The Hoffman

Table 2.
Synthesis results and thermal behaviour of *n*ATMA · PMLA complexes.

$R(CH_3)_3N^+$	Complex	Yield (%)	Composition	ρ (g mL^{-1})	^oT_d [a] (°C)	^oT_m [b] (°C)
-$nC_{14}H_{29}$	14ATMA · PMLA	78	1.0:1	1.00	219	242/250/416
-$nC_{16}H_{33}$	16ATMA · PMLA	88	1.1:1	1.00	224	242/261/416
-$nC_{18}H_{37}$	18ATMA · PMLA	93	1.0:1	1.01	232	245/271/416
-$nC_{20}H_{41}$	20ATMA · PMLA	99	1.1:1	1.01	234	243/290/414
-$nC_{22}H_{45}$	22ATMA · PMLA	91	1.3:1	1.02	236	245/299/415

[a] Onset decomposition temperature measured for 5% lost of the initial weight.
[b] Maximum rate decomposition temperature for the three decomposition steps.

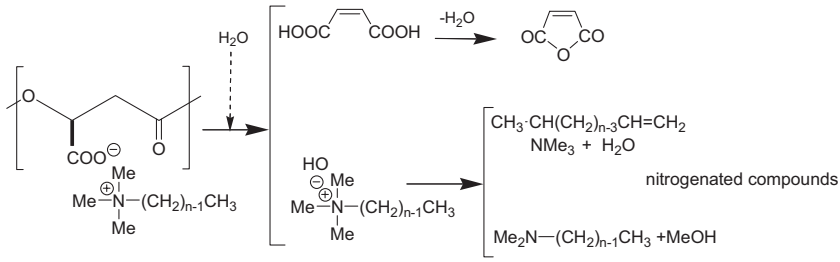

Figure 9.
Mechanism of thermal degradation of of *n*ATMA · PMLA complexes.

elimination of the quaternary trimethylalkyl ammonium hydroxide formed in the first stage and the pyrolysis of nitrogenated compounds accounts for the other two stages observed in the thermal decomposition of these complexes (Figure 9). [15]

DSC analysis of *n*ATMA · PMLA complexes revealed an endothermic peak at increasing temperatures from 40 up to 70 °C for $n = 16$ to 22 due to the fusion of the polymethylene side chain. This peak was not observed for 14ATMA · PMLA indicating absence of crystallinity in this complex.

Discrete X-ray diffraction peaks revealing the existence of order were observed in the scattering profiles registered from powdered samples complexes with $n \geq 16$, which is in full agreement with the DSC results. The sharp diffraction peak with a spacing of 0.415 nm is associated to the partially crystallized alkyl side chains. In the small angle region, all the complexes showed discrete diffraction scattering corresponding to the first and second order of a main spacing, L_0, that increases steadily from 3.6 to 4.2 nm (Figure 10). [14] According to what is known for other comb-like ionic complexes made of anionic polyelectrolytes and cationic surfactants, such spacing, L_0, is associated to the periodicity of a layered biphasic arrangement in which the paraffinic phase made of alkyl side chains alternates to the phase made of polymalate main chains.

The analysis by polarizing optical microscopy afforded further information about the crystalline nature of the complexes. Micrographs of 18ATMA · PMLA are depicted in Figure 11. The spherulitic

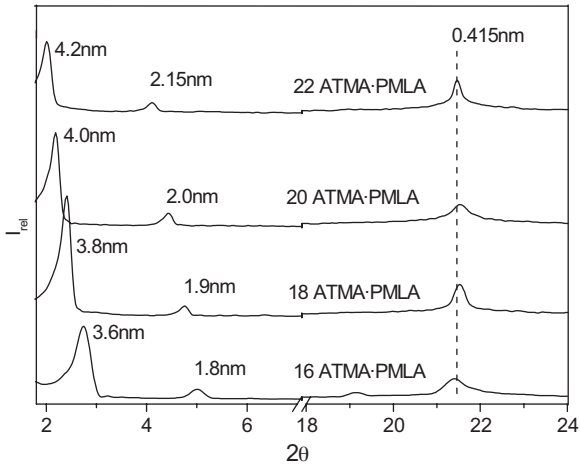

Figure 10.
Diffractograms of *n*ATMA · PMLA complexes at registered at room temperature.

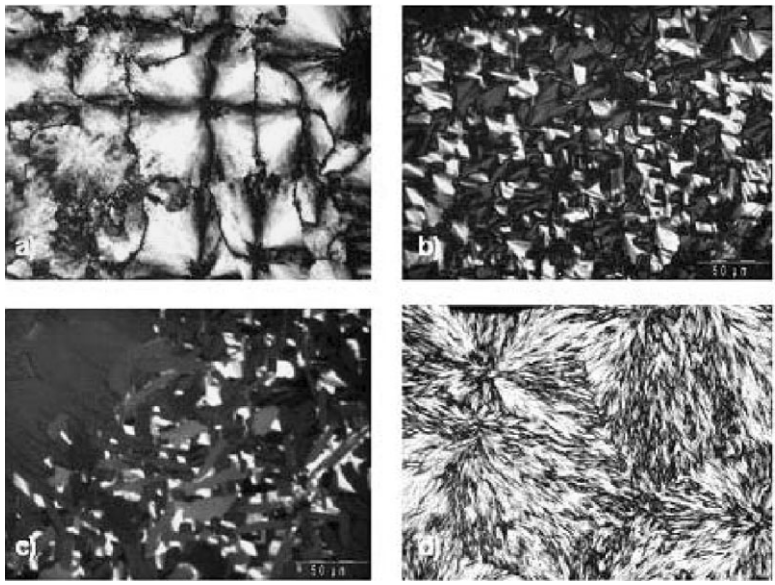

Figure 11.
Polarizing optical micrographs of a film of 18ATMA · PMLA crystallized from the melt. a) 20 °C, texture of the initial film; (b) 85 °C, smectic LC texture displayed between the melting point and the isotropic temperature; c) 85 °C, Smectic LC texture reappeared upon cooling from the isotropic phase; d) 20 °C, spherulitic texture reappeared upon cooling.

texture present in the initial sample changed upon heating into a well defined mosaic texture characteristic of a smectic mesophase. This texture disappeared upon further heating. Upon cooling down the smectic structure was readily recovered and crystallization took place generating well developed spherulites. [14]

Ionic Complexes as Biodegradable Drug Delivery Systems (DDS)

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 a variety of incubation conditions for linear alkyl side chains containing 14 and 18 carbon atoms.[16] The weight losses with time observed for the four studied complexes incubated under physiological conditions (pH 7.4 at 37 °C) are comparatively plotted in Figure 12. The results revealed that the complexes made of polymalic acid hydrolyzed faster than those made of polyglutamic acid, which is the direct consequence of the weakness of the polymalate main chain ester group to water attack compared to the amide group of polyglutamate. Hydrolysis was delayed when the tetradecyl side chain is replaced by the octadecyl chain. The conclusion is that the hydrolytic degradability of these complexes can be finely adjusted by adequate changes in the chemical structure, either in the main chain or in the surfactant alkyl side chain. On the other side, both deviation of pH from neutrality and increasing temperatures enhance significantly the hydrolysis of PMLA and PGGA complexes.

The NMR analysis revealed that complex degradation took place by hydrolysis of either the polyester or polyamide backbone accompanied by dissociation of the ionic complex structure. The SEM analysis gave support to such interpretation and a selection of pictures illustrating such mechanisms is depicted in Figure 13. In these sets of pictures, surface and edge disc

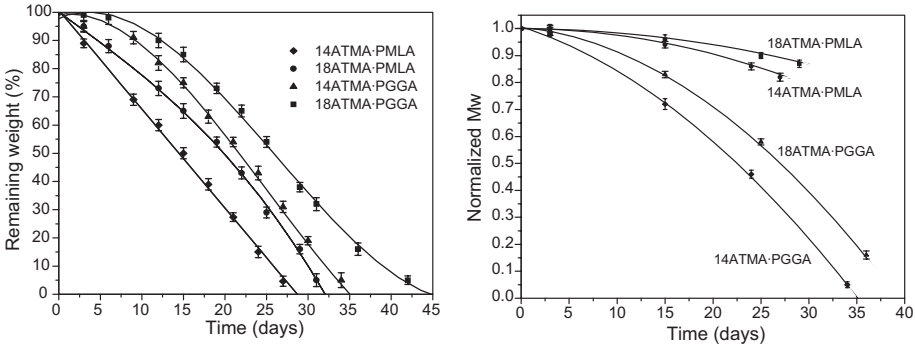

Figure 12.
Compared mass loss and normalized molecular weight of complexes disks degraded at pH = 7.4 and 37 °C.

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). Polymalic complexes underwent surface erosion whereas polyglutamic ones eroded in bulk.

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 disks indicating a good compatibility of the two components. DSC analysis revealed that erythromycin was homogenously dispersed in the hydrophobic subphase of the complexes and upon water incubation was delivered according to the same hydrolysis profile followed by the respective complexes with independence on the amount loaded.

Conclusion

Ionic complexes with a nearly stoichiometric composition can be prepared from PGGA and PMLA with alkyltrimethyl ammonium surfactants. The complexes display a layered biphasic structure with the alkyl side chains crystallized in a separated paraffinic phase. The complexes undergo reversible thermal transition consequent to melting of the paraffinic phase and involving generation of mesophases. They are thermally stable up to temperatures near 200 °C and decompose in several steps, the first one being depolymerization

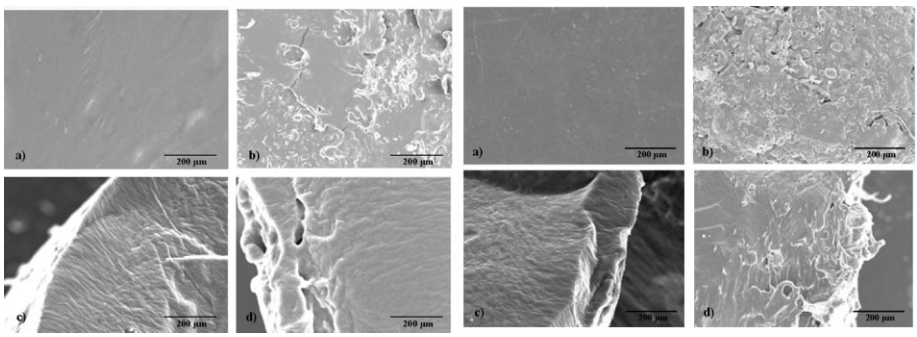

Figure 13.
SEM micrographs of 18ATMA · PMLA (left) and 18ATMA · PGGA (right) discs. a) and c) surface and edge views of original disc; b) and d) same views of the disc after loosing around 50% of its initial weight.

of the main chain. Complexes degraded by water under physiological conditions; it is possible to tune the degradability by chemical designing of the complexes. Drugs may be homogenously dispersed in the complexes and delivered upon water incubation following a releasing profile parallel to the hydrolysis of the polymers.

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