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Comblike Complexes of Poly(aspartic acid) and Alkyltrimethylamonium Cationic Surfactants

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Summary: A series of complexes from poly(aspartic acid) and alkyltrimethylammonium bromide surfactants with linear alkyl groups, containing even number of carbon atoms from 12 up to 22 were synthesized. The complexes were obtained in good yields, are soluble in organic solvents and stable up to temperatures near 170 °C. On the other hand, the paraffinic side chains are able to crystallize depending on the length of the surfactant *n*-alkyl chain being the main chain of the complexes is arranged in an extended conformation.

Keywords: comb-like polymers; ionic complexes; poly(aspartic acid)

Introduction

Comb-like polymers consisting of a stiff backbone chain with long flexible side groups are of prime interest not only on their own for their capacity to form supramolecular assemblies but also because they are promising materials for novel practical applications.[1–3] One distinguished class of these polymer systems is that comprising helical poly(α-peptide)s bearing long linear polymethylene side chains.[2,3] Such type of systems are unique in displaying a combination of properties that includes a high solubility in organic solvents and the ability to generate periodically layered structures at the nanometric scale. Very recently, similar structures have been shown to occur also in poly(α-alkyl β,L-aspartate)s[4,5] and poly(α-alkyl γ-glutamate)s,[6,7] two families of unconventional polypeptides that are able to take up regularly folded conformations of α-helix type.

Comb-like structures of ionic nature distinguish because they can be easily formed by complexation of polyelectrolytes with oppositely charged low molecular weight amphiphilic molecules. Stoichiometric complexes prepared from the sodium salt of both chemosynthetic poly(α,L-glutamic acid)[8,9] and microbial poly(γ,DL-glutamic acid)[10] and alkyltrimethylammoniun bromide salts have been reported to form layered supramolecular structures similar to those made of comb-like poly(alkyl glutamate)s. These systems are suitable to prepare hollow spheres useful for drug reservoirs by simply dissolution of the core in the appropriate solvent.[11,12] In this paper we report on the complexes synthesized from poly(aspartic acid) and alkyltrimethylammonium surfactants with the alkyl group being linear, and containing even number of carbon atoms from 12 up to 22. These complexes will be referred henceforth as nATMA · PAA, being n the number of methylenes of the alkyl group. The polyaspartic acid used in this work is a heterogeneous polymer consisting of a mixture of α- and β-units in D and L configurations randomly distributed.

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Experimental

Materials

L-aspartic acid and linear alkyltrimethylamonium bromide surfactants ($RN^+Me_3 \cdot Br^-$) with R=$C_{12}H_{25}$, $C_{14}H_{29}$, $C_{16}H_{33}$ and $C_{18}H_{37}$ were purchased from Aldrich Chemicals and those with R=$C_{20}H_{41}$ and $C_{22}H_{45}$ were synthesized according to literature procedures.[13] Organic solvents were of analytical grade and were used without further purification. Water used in the preparation of the complexes was distilled and deionized in a *Mili Q* water purification system and boiled before use.

Synthesis

Poly(aspartic acid) was prepared by thermal polycondensation of L-aspartic acid at 210 °C for 48 hour, according to previously reported methods.[14] Poly(aspartimide) firstly formed was then hydrolyzed to polyacid by treatment with sodium hydroxide and subsequent neutralization with 1% HCl. The polyacid was precipitated with methanol and washed several times with the same solvent followed with acetone and diethyl ether and dried under vacuum. The yield of the whole process was 75%. ^{1}H-NMR (in D_2O at 80 °C) δ(ppm) 2.8 (broad 2H CH_2 α and β units), 4.55 (broad 0.7 H CH β units), 4.75 (broad 0.3 H CH α units). ^{13}C-NMR (in D_2O at 80 °C) (ppm) 36 (CH_2), 51 (CH), 171 (CONH), 178 (COOH).

Ionic complexes were obtained by slow mixing under magnetic stirring in a temperature range of 25 to 60 °C of equivalent amounts of 0.05 M aqueous solutions of the polyacid and the surfactant, following the procedure described by Pérez-Gamero, *et al*.[10] When the addition was completed, the mixture was allowed to rest at room temperature. The precipitated complexes were repeatedly washed with water and purified by slow precipitation with ether from chloroform solution. Chemical characterization was carried out by IR and NMR spectroscopy. Thermal properties were analyzed by TGA and DSC. The structure in the solid state was examined by X-ray diffraction of films obtained by casting.

Characterization

Infrared spectra were registered on a Perkin-Elmer 2000 instrument from KBr discs samples or films prepared by casting. Oriented films for Infrared dichroism were prepared in a poly(ethylene oxide) (PEO) matrix, $M_n = 3 \times 10^5$ Da, following the method described by Ingwall *et al*.[15]

NMR spectra were recorded on a Bruker AMX 300 spectrometer at the indicated temperatures from samples dissolved in deuterated solvents using tetramethylsilane (TMS) or trimethylsilylpropanesulfonate (TMPS) as internal references.

Calorimetric measurements were performed with a Perkin-Elmer DSC-7 calibrated with indium. Samples of about 5 mg were heated or cooled at rates of 10 °C min^{-1} under an ultra pure nitrogen atmosphere for temperatures ranging from −40 to 120 °C. Thermo-gravimetric analysis, TGA, were carried out on a Perkin-Elmer TGA-7 thermobalance under similar conditions as those used for DSC runs but within a temperature range of 25 to 500 °C. X-ray difractogram patterns were obtained in a Statton-type camera using nickel-filtered CuKα (radiation of wavelength 0.1542 nm) calibrated with molybdenum sulfide (d_{002} = 0.6147 nm). Optical microscopy was carried out in an Olympus BX51 polarizing microscope equipped with a digital camera system. Tinny film samples of *n*ATMA · PAA were prepared from 1% (p/v) chloroform solutions, which were left to evaporate slowly between microscope cover slides.

Results and Discussion

Synthesis and Characterization of *n*ATMA · PAA

The method used in this work for the synthesis of the poly(aspartic acid) was the same previously reported by Wolk, *et al.*,[14] which consisted of polycondensation of

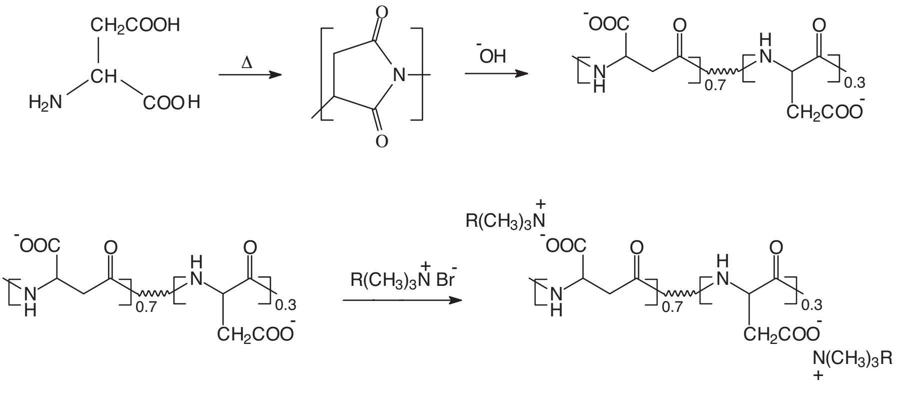
L-aspartic acid by heating to obtain poly (aspartimide) that was then hydrolyzed to poly(aspartic acid) with a number average molecular weight $\sim 5 \times 10^3$ by treatment with NaOH followed by acidification. According to ^{1}H-NMR spectroscopy data, poly(aspartic acid) was obtained with a 30% of α units that are produced during the basic hydrolysis of the poly(aspartimide). These results are close similar to those reported by Wolk, *et al.*[14] The whole synthetic route to the complexes is depicted in Scheme 1.

All the attempts to improve the molecular weigh and diminish the proportion of α units, as for example hydrogenation of poly(α-benzyl β,L-aspartate) were fruitless. All complexes prepared in this work were obtained in yields up to 70% and were solids soluble in organic solvents as methanol or chloroform but insoluble in water. This behavior is characteristic of this type of ionic complexes.[8–10,16–18] Some results obtained for *n*ATMA · PAA are summarized in Table 1.

The constitution and composition of *n*ATMA · PAA were determined by ^{1}H-NMR and FTIR. Figure 1 shows the ^{1}H-NMR spectrum of *16*ATMA · PAA with the respective assignations, as representative of the whole series. In the spectrum all protons of the polyaspartate and surfactant moieties are present confirming the expected structure of the complex. The content of counter-ions in the complexes was precisely measured by ^{1}H-NMR by area quantification of the respective peaks. The surfactant to polyaspartate molar ratio, as it can be seen in Table 1, was near to 1 for $n = 12, 14$, and 16, whereas considerable greater values were found for higher values of *n*. This fact indicates that additional amounts of the surfactant are retained in the complexes made of long alkyl chain surfactants, which were not easy to remove by successive washes with hot water.

IR spectra of *12*ATMA · PAA, PAA and tetradecyltrimethylamonium bromide (*12*ATMA · Br) are compared in Figure 2. The spectrum of the complex exhibits the characteristic absorptions of both, the polyaspartate and alkyltrimethylammonium moieties. The shifting of the carbonyl band of the acid, from 1720 cm^{-1} to 1590 cm^{-1}, which is characteristic of the carboxylate group and the absence of the broad OH stretching acid signal at 3600 cm^{-1} in the spectrum of the complex corroborate its formation.

Like as in the poly(γ,D-glutamic acid) complexes,[10] water is also present in these complexes, as may be observed by the absorption centered at 3500 cm^{-1} in the infrared spectrum, overlapped with the amide A band and a signal at 4.3 ppm in the ^{1}H-NMR spectrum. Water could not be removed by drying under vacuum for days.



Scheme 1.
Synthesis of *n*ATMA · PAA.

Table 1.
Synthesis results and thermal behavior of *n*ATMA · PAA.

Complex	Yield	*n*ATMA · PAA	T_d [a]	T_m [b]	ΔH_m [b]	ΔS_m [c]	n_c [d]	d [e]
	%		°C	°C	KJ/mol	J/Kmol		nm
*12*ATMA · PAA	73.8	0.96:1	218	–	–	–	–	3.2
*14*ATMA · PAA	77.4	1.09:1	223	–	–	–	–	3.4
*16*ATMA · PAA	81.8	0.96:1	231	27	10.9	37.2	2.9/3.5	3.5
*18*ATMA · PAA	71.3	1.25:1[f]	236	48	17.9	56.0	4.7/5.4	3.7
*20*ATMA · PAA	77.4	1.54:1	276	59	25.5	76.5	6.6/7.4	3.8
*22*ATMA · PAA	82.9	1.75:1	287	71	33.9	98.2	8.7/9.5	4.0

[a] Decomposition temperature corresponding to the maximum of the DTGA curve.
[b] Measured by DSC.
[c] Estimated using he expression $\Delta H_m/T_m$.
[d] Estimated number of crystallized methylenes from DSC measurements using ΔH_m and ΔS_m values respectively.
[e] From X-rays.
[f] After repeated washing with hot water. Initial value 1.48:1.

Thermal Properties

The thermal stability of the complexes was evaluated by TGA. All the complexes appeared to be stable up to 170 °C with a decomposition maximum in the range of 218 to 287 °C which increased with the length of the side chain, as may be appreciated in Table 1. All TGA traces produced decomposition patterns similar to those shown in Figure 3 for *16* and *18*ATMA · PAA. Below 200 °C the little weight loss shown in the TGA traces is attributed to the release of hydration water which was previously observed in the IR and NMR spectra. On the other hand, thermal stability of these complexes is lower than the observed for the covalent analogs.[19]

As it is common to covalent comblike polymers, it is well known that *n*-alkyl side

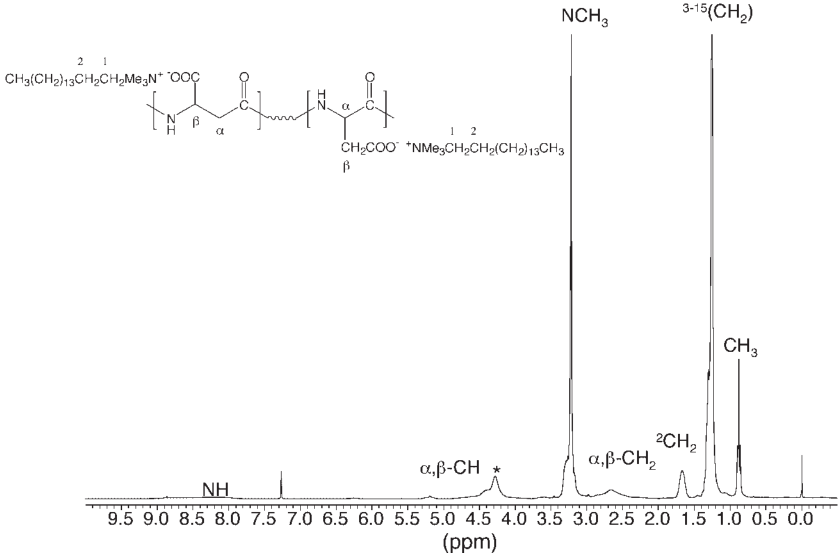


Figure 1.
^{1}H-NMR of *16*ATMA · PAA in $CDCl_3$. * Peak of water.

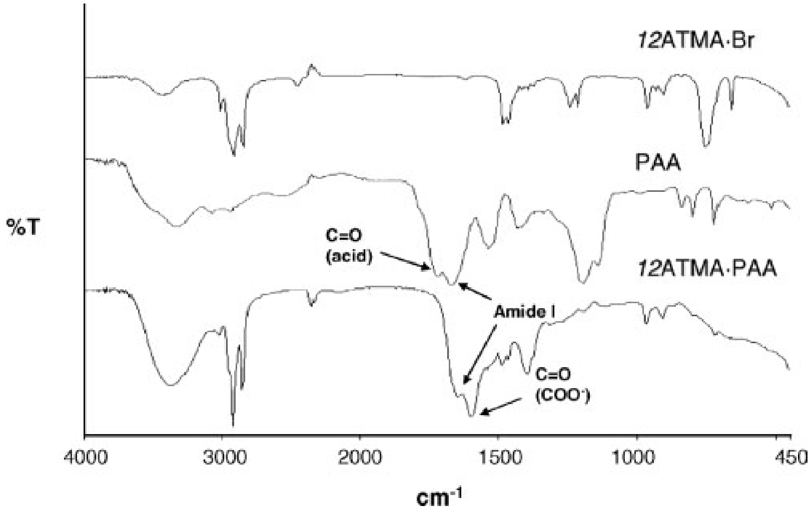


Figure 2.
Infrared spectra of *12*ATMA · Br, PAA and *12*ATMA · PAA.

chains are able to crystallize when they reach a minimum length.[8,10] In order to determine if the *n*-alkyl chains of the surfactant moiety are able to crystallize in *n*ATMA · PAA complexes, a DSC study was carried out. As it can be observed in Figure 4, DSC heating scan recorded for *12* and *14*ATMA · PAA do not show any endotherm, whereas for *16*ATMA · PAA to *22*ATMA · PAA endothermic peaks at temperatures between 27 to 71 °C and attributed to the fusion of the paraffinic phase made of *n*-alkyl side chains were displayed. As it illustrated in the insert of Figure 4 for *20*ATMA · PAA, these transitions were reversible on cooling and reheating. As it happens with the melting temperature, T_m, the enthalpy involved in the fusion also increased with the value of *n*. The values of both T_m and ΔH_m obtained for the whole series are summarized in Table 1.

The enthalpy associated with the melting process can be used to estimate the fraction of methylene units participating in the paraffinic crystalline phase using the following equation:[20]

$$\Delta H_m = \Delta H_{m(e)} + nk$$

Where n is the number of methylene units and methyl terminal group in the alkyl chain, $\Delta H_{m(e)}$ is the contribution from the chain ends and k is the fusion enthalpy per mol of CH_2 unit.

When the measured fusion enthalpies (ΔH_m) were plotted *vs. n* a linear correlation was found as it can be seen in Figure 5, and the approximate number of methylene units that are crystallized (n_c) can be estimated by using the following equation:

$$n_c = \Delta H_m / k$$

Values obtained for *n*ATMA · PAA are listed in Table 1. In this case the number of crystallized methylenes, are lower than those calculated for their covalent analogs which show crystallization from side chains of twelve carbon atoms.[4]

Conformation and Structure

With the aim to determinate the main chain conformation, some experiments of polarized infrared spectroscopy were carried out. For many years polarized infrared spectroscopy or infrared dichroism (DIR) have been used successfully in the determination of the conformation in polypeptides by

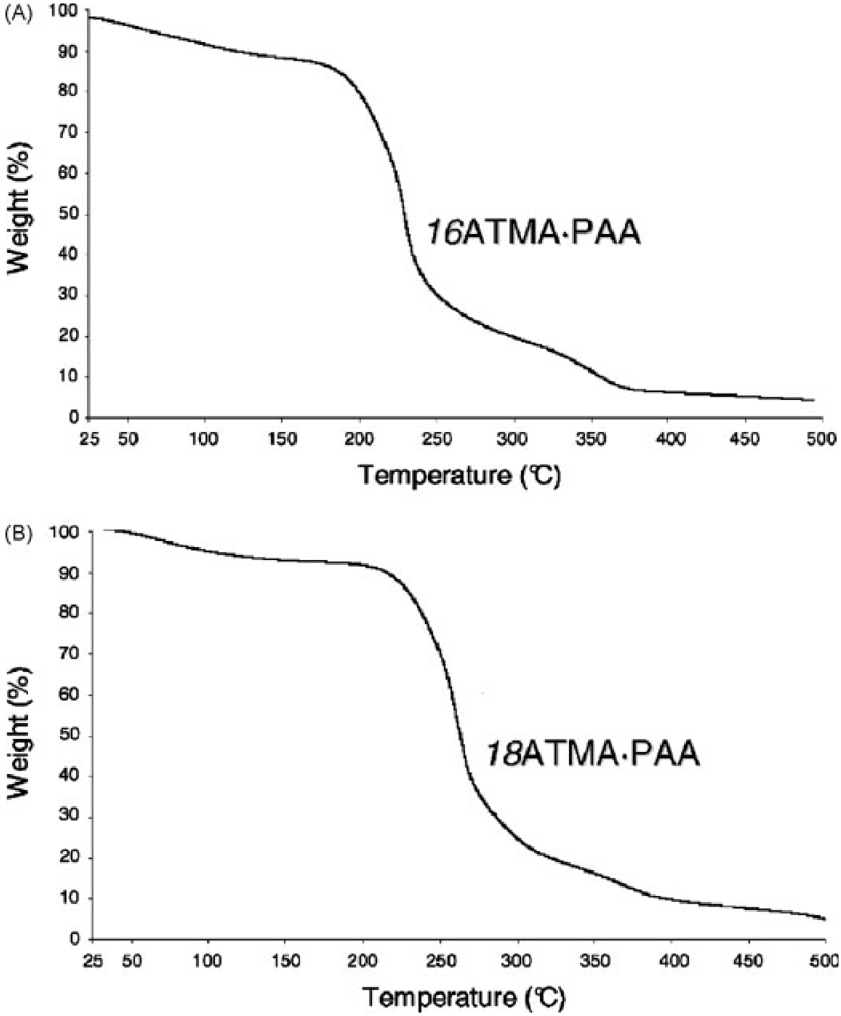


Figure 3.
TGA traces of (A) *16*ATMA · PAA and (B) *18*ATMA · PAA.

using oriented samples of the materials.[15,21,22] While the samples of the *n*ATMA · PAA complexes were not able to be oriented by stretching, probably due to their low molecular weight, orientation was tried by incorporating the samples in a matrix of PEO. It is well known that polypeptides of low molecular weight incorporated in PEO are suitable for infrared dichroism analysis since PEO induces the partial orientation of the samples without interfering in the infrared absorptions.[15,22] Figure 6 shows the polarized infrared spectra of an oriented film in PEO of *14*ATMA · PAA recorded with the polarization vector parallel and perpendicular to the stretching direction in the region of 1500–1750 cm^{-1} where the bands of amide I and II appear. The region of amide A and B (3000–3700 cm^{-1}) is overlapped with the strong water signal (Figure 2) impeding to extract useful information from this region.

In this spectrum it can be observed a weak perpendicular dichroism in amide I which strongly suggests that the main chain

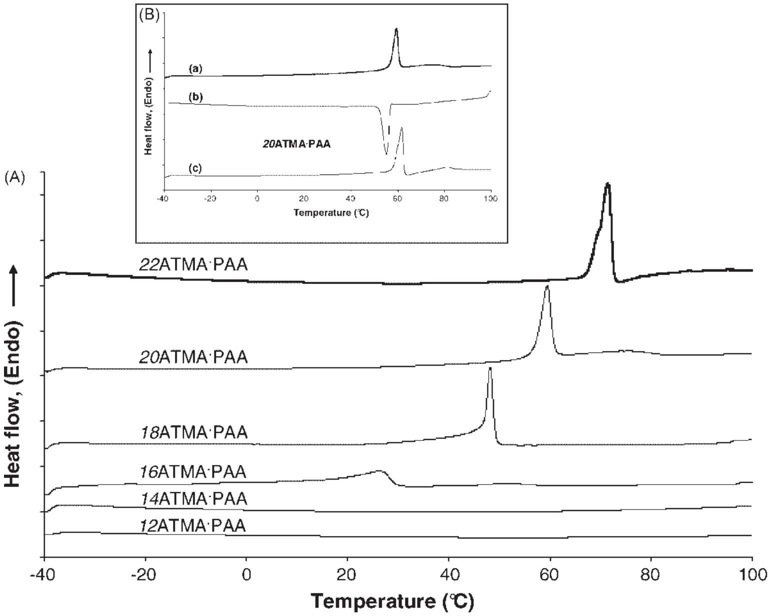


Figure 4.
(A) DSC heating scans at 10 °C · min^{-1} for series of *n*ATMA · PAA. (B) Insert 20ATMA · PAA (a) first heating, (b) cooling, (c) second heating.

adopts an extended conformation. This result is opposite to the observed for their covalent analogs[4] or poly(α,L-glutamic acid) complexes[8,9] which adopt an helical conformation. On the other hand, the spectrum is quite similar to the previously observed for *n*ATMA · PGGA which are thought to be arranged with the polypeptide chains in an extended conformation,[10] probably due to the neighboring interaction between the surfactant and the polar groups of the main chain.

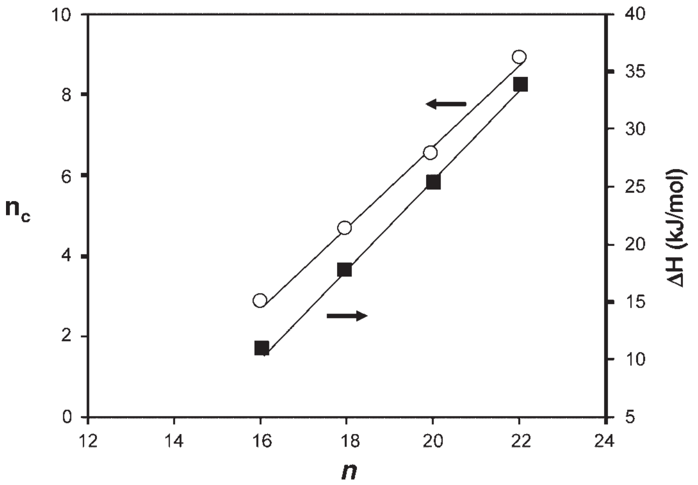


Figure 5.
Variation of the number of crystallized CH_2 (○) and enthalpy (■) *vs. n* for *n*ATMA · PAA.

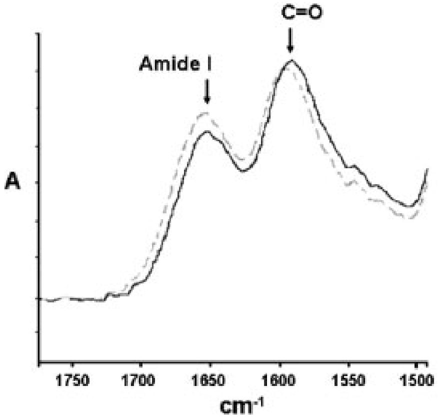


Figure 6.
Infrared spectra of partially oriented sample of *14*ATMA · PAA complex in a PEO matrix recorded with the polarization vector parallel (solid line) and perpendicular (dotted line) to the orientation axis.

In Figure 7 are shown the powder X-ray diffraction patterns of *12*ATMA · PAA and *18*ATMA · PAA as representative examples of the whole series.

The diffractograms exhibit two main reflections, the first one corresponding to the long spacing (L_o) between 3.2 and 4.0 nm which increase linearly with the length of the alkyl chain of the surfactant moiety, as may be seen in Figure 8. This reflection is associated with the interlayer distance between the main chains that increases due to the size of the paraffinic phase.

The second one appears at 0.42 nm as a sharp ring for *n*ATMA · PAA with n = 16–22 or at 0.45 nm as a broad signal for *12* and *14*ATMA · PAA. The sharp reflection that appears at 0.42 nm corresponds to an interplanar spacing characteristic of a crystalline paraffinic hexagonal phase while the broad scattering observed for *12* and *14*ATMA · PAA is characteristic of the amorphous paraffinic phase. These results are in agreement with those observed by DSC confirming that *n*-alkyl side chains with less than 16 carbon atoms are not able to crystallize in the *n*ATMA · PAA complexes.

Supramolecular Structure

When chloroform-cast films of the complexes were examined under the polarized optical microscope they showed birrefringent textures varying according to the length of the alkyl side chain as it can be seen in Figure 9. All textures were close similar to those reported for *n*ATMA · PGGA.[10] *12*ATMA · PAA and *14*ATMA · PAA showed mosaic-type textures characteristic of smectic mesophases displaying nonplanar orientation as illustrated in Figure 9a. Grainy textures of difficult interpretation were found for *16*ATMA · PAA and a close-to-spherulitic texture were observed for the higher members of the series (Figures 9b and 9c).

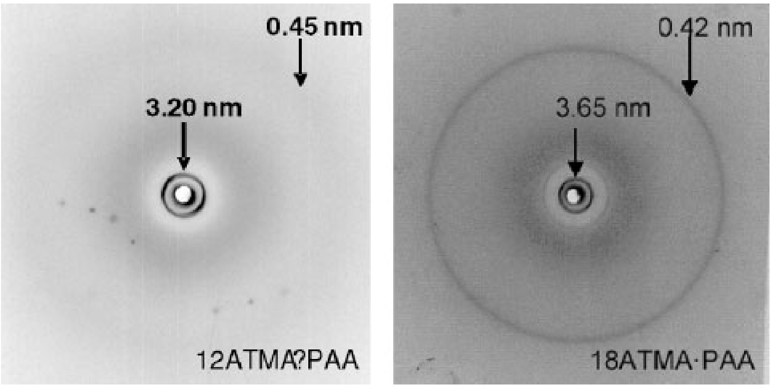


Figure 7.
Powder X-rays diffraction patterns of *12*ATMA · PAA and *18*ATMA · PAA.

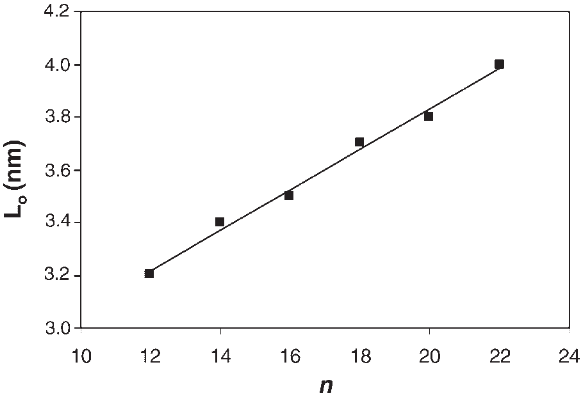


Figure 8.
L_o spacing versus the number of carbon atoms in the *n*-alkyl chain.

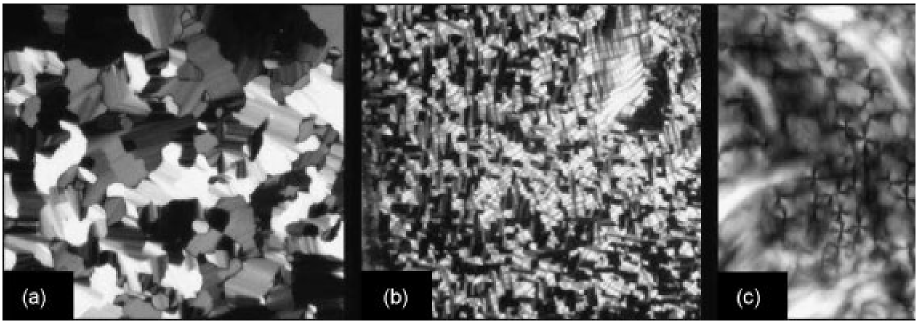


Figure 9.
Polarizing optical micrographs of (a) *12*ATMA · PAA, (b) *16*ATMA · PAA, (c) *22*ATMA · PAA.

Conclusions

In this work it has been shown that thermally produced polyaspartic acid of low molecular weight is able to form ionic complexes with alkyltrimethylammonium bromides bearing long alkyl chains in relative good yields. The complexes tend to retain an excess of surfactant, this trend being more pronounced as the length of the alkyl chain increased. All complexes are soluble in organic solvents, insoluble in water and stable up to temperatures near 170 °C. In solid state, the alkyl side chains of the complexes are able to crystallize when they contain at least 16 carbon atoms. The melting temperature of the paraffinic phase oscillated between 27 and 71 °C, which is in the same range observed for comb-like ionic complexes of poly(glutamic acid). DIR spectra suggest that the polypeptidic main chain adopts an extended conformation in these complexes, similar to that observed for comb-like ionic complexes of poly(γ-glutamic acid). Finally, in agreement with other comb-like polypeptide systems, the structure of these complexes can be interpreted as a layered assembly of alternating layers of polypeptide and paraffinic side chains.

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