New Pseudopeptidic Cross-Linker Containing Urea Bonds: Study of Its Degradation Routes in Aqueous Media Using Capillary Electrophoresis-Mass Spectrometry

Paloma Pérez,[†] Carolina Simó,[‡] Christian Neusüss,[§] Matthias Pelzing,[§] Julio San Román,[†] Alejandro Cifuentes,[‡] and Alberto Gallardo*,[†]

Institute of Polymer Science and Technology (CSIC), Institute of Industrial Fermentations (CSIC), Juan de la Cierva 3, 28006 Madrid, Spain, and Bruker Daltonik GmbH, Permoser Strasse 15, 04318 Leipzig, Germany

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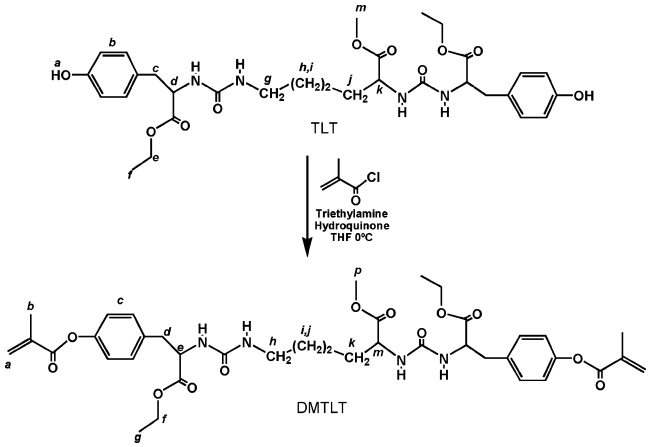
An accelerated degradation study has been performed on TLT, a pseudopeptide that includes esterified tyrosine and lysine linked by urea bonds, as well as on their derivatives, i.e., a dimethacrylic cross-linker (DMTLT) and a poly(dimethylacrylamide) cross-linked with DMTLT. The monitoring and analytical characterization has been carried out by capillary electrophoresis-mass spectrometry (CE-MS), using ion trap and time-of-flight MS analyzers. Several degradative species have been identified, and a kinetic analysis of the variation of their concentration with time has been obtained. During the initial stages of degradation, there is a competition between hydrolysis of the ester groups and cyclization by nucleophilic attack of the NHs of the urea groups to the carbonyl ester group. At higher degradation time (weeks or months), evidences of backbone breakdown, including urea hydrolysis, have been found.

1. Introduction

Lysine diisocyanate methyl ester (LDI) has attracted much interest as an amino acid-containing building block to prepare new biodegradable polymeric materials for biomedical applications. This structure reacts with hydroxyl or amino groups giving rise to urethane or urea bonds, respectively. The urethane groups have shown their degradative character when they are incorporated to polymeric chains.[1,2] However, data about degradation of urea bonds remains unclear in the literature[3,4] despite the important number of papers describing them. We have prepared a tyrosine−lysine−tyrosine pseudotripeptide (TLT) starting from LDI and containing urea bonds. The structure does not present free carboxylic acid, but ester groups, as indicated in Scheme 1. This TLT sequence has been functionalized with two methacrylic residues, to give the DMTLT structure, which is proposed as a biodegradable acrylic cross-linker. This compound can be used as an alternative to classical biostable diacrylic cross-linkers such as bisacrylamide. Accelerated degradation experiments in aqueous media (at pH 10 and 60 °C) of the monomer DMTLT, the parent pseudotripeptide TLT, and a polymer cross-linked with DMTLT (cross-linked poly(dimethyl acrylamide), poly-DMAA), have been carried out to identify possible hydrolytic mechanisms (and confirm the sensitivity of the urea bond). This polymer has been chosen as model hydrophilic hydrogel, since (linear) poly-DMAA itself is hydrosoluble, and therefore the cross-linker network is going to significantly swell, allowing a high accessibility of the hydrolytic agent (water).

We have demonstrated recently, that capillary electrophoresis-mass spectrometry (CE-MS) is a useful procedure to monitor the synthesis and degradation process of different biomaterials.[5,6]

Scheme 1. Schematic Representation of the Cross-Linker DMTLT and Its Parental Nonfunctionalized Pseudopeptide TLT



The on-line coupling of capillary electrophoresis (CE) with electrospray ionization-mass spectrometry (ESI-MS), yields a powerful method[7,8] in which CE offers high separation efficiency, while ESI-MS allows the determination of an accurate mass for a wide molecular mass range of molecules and macromolecules. CE can be coupled with different MS analyzers, i.e., with quadrupole, ion trap, time-of-flight, etc. Ion trap (IT) and time-of-flight (TOF) mass spectrometers are very powerful detectors for CE-ESI-MS due to their sensitivity and speed, while TOF-MS is especially useful due to its high mass resolution and mass accuracy.

The goal of this work is, therefore, to elucidate the degradation pathways of this new LDI-derived pseudotripeptide in aqueous media using CE-MS and to confirm or not the rupture of the urea bond.

2. Materials and Methods

2.1. Chemicals and Samples. All chemicals were of analytical reagent grade and used as received. Ammonia (30%) from Panreac

* Corresponding author. Phone: 34-91-5622900. Fax: 34-91-5644853. E-mail: gallardo@ictp.csic.es.
[†] Institute of Polymer Science and Technology (CSIC).
[‡] Institute of Industrial Fermentations (CSIC).
[§] Bruker Daltonik GmbH.

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(Barcelona, Spain), methanol, 2-propanol, and acetonitrile (HPLC grade), from Scharlau (Barcelona, Spain), ammonium hydrogen carbonate from Merck (Darmstadt, Germany), formic acid from Riedel-de Häen (Seelze, Germany), and acetic acid from Acros Organics (Geel, Belgium) were used for the CE running buffers and sheath liquids. Distilled water was deionized by using a Milli-Q system (Millipore, Bedford, MA). For the synthesis of the cross-linker DMTLT, L-tyrosine (Aldrich, Steinheim, Germany), lysine diisocyanate (LDI, Kyowa Hakko), and thionyl chloride (Scharlau, Barcelona, Spain) were used as received. Methacryloyl chloride (Aldrich, Steinheim, Germany), triethylamine, and *N*,*N*-dimethylacetamide (Scharlau, Barcelona, Spain) were previously distilled. Ethanol and tetrahydrofurane (Scharlau, Barcelona, Spain) were distilled over calcium chloride at atmospheric pressure. DMAA (Aldrich, Steinheim, Germany) was previously distilled under reduced pressure.

2.2. Capillary Electrophoresis. CE-UV analyses were performed in a PACE/2100 CE apparatus (Beckman, Fullerton, CA), equipped with a UV detector working at 200 and 254 nm. System Gold software was used for CE instrument control. Bare fused-silica capillaries with 50 μm i.d. were purchased from Composite Metal Services (Worcester, England). The detection length to the UV detector was 30 cm and the total length 37 cm. Injections were made at the anodic end using N_2 pressure of 0.5 psi for 2 s.

Before their first use, fused-silica capillaries were washed with 0.1 M sodium hydroxide for 20 min and deionized water for 20 min (all rinses were done using N_2 pressure at 20 psi).

Four different aqueous buffers were initially tested: 100 mM formic acid in water at pH 2.2, 50 mM acetic acid in water at pH 5, 50 mM ammonium hydrogen carbonate in water at pH 8, and 46.5 mM ammonium hydrogen carbonate at pH 10. The pH values where adjusted by using ammonia (30% in water).

2.3. Capillary Electrophoresis-Ion Trap-Mass spectrometry (CE-IT-MS). CE-ESI-MS analyses were carried out in a PACE/5500 CE apparatus (Beckman, Fullerton, CA) coupled to the MS detector using a commercial coaxial sheath-flow interface (see below). The capillary detection and total length was 87 cm. Between analyses, the capillary was rinsed with water for 5 min and with running buffer for 3 min. Running voltage was +25 kV, and injections were made at the anodic end using N_2 pressure of 0.5 psi for 4 s. The instrument was controlled by a PC running the System Gold software from Beckman.

MS experiments were performed on a Bruker Daltonik Esquire 2000 ion trap-mass spectrometer (Bruker Daltonik, Bremen, Germany), equipped with an orthogonal electrospray interface (model 61607A from Agilent Technologies, Palo Alto, CA). Electrical contact at the electrospray needle tip was established via a sheath liquid delivered by a 74900-00-05 Cole Palmer syringe pump (Vernon Hills, IL). The mass spectrometer was operated in both the positive and negative ion mode. The spectrometer was scanned at 15−1000 *m*/*z* range (with 500 *m*/*z* target mass and 50% compound stability) at 13 000 *m*/z/s during the separation and detection. The instrument was controlled by a PC running the Esquire NT software from Bruker Daltonik. ESI parameters were as follows: nebulizer pressure of 4 psi, dry gas flow equal to 8 L/h, dry gas temperature at 200 °C, a sheath liquid made of 2-propanol/water (50:50, v/v) with 0.5% acetic acid (in MS positive ion mode) and without acetic acid (in MS negative ion mode), at 4 μL/min flow rate.

2.4. Capillary Electrophoresis-Time-of-Flight-Mass Spectrometry (CE-TOF-MS). CE-TOF-MS was performed using a Hewlett-Packard ^{3D}CE (Agilent, Waldbronn, Germany) coupled to an ESI-orthogonal-accelerated-TOF-MS (micrOTOF, Bruker Daltonik GmbH, Bremen, Germany). Fused-silica capillaries of 61 cm × 50 μm (i.d.) were used. A coaxial sheath-liquid interface (Agilent, Waldbronn, Germany) is used for coupling the CE to the MS. The ESI voltage of the micrOTOF is applied at the end cap of the transfer capillary to the MS (−4.5 kV), with the spray needle being grounded. Sheath liquid is provided by a syringe pump of Cole Parmer (Vernon Hill, IL). The micrOTOF was operated to acquire spectra in the range of 200−800 *m*/*z* every 55 μs, 30 000 spectra being averaged for subsequent data analysis. Transfer parameters were optimized for high sensitivity while keeping the resolution to better 10000. The mass accuracy for external calibration is better than 5 ppm, enabling together with the true isotopic pattern (using SigmaFit) an unambiguous elemental composition determination of the analytes.

2.5. Synthesis of the Cross-Linker. The dimethacrylic pseudopeptide cross-linking monomer was prepared by the reaction of the pseudopeptide of tyrosine−lysine−tyrosine TLT, with methacryloyl chloride according to Scheme 1. TLT was obtained previously by the reaction of tyrosine ethyl ester with lysine diisocyanate in the presence of triethylamine, as has been described elsewhere.[6] Dry tetrahydrofurane THF and fresh distilled methacryloyl chloride were used as solvent and reactant at 0 °C. TLT and DMTLT were characterized by ^{1}H NMR giving the following chemical shifts (assignments correspond to the structures drawn in Scheme 1):

TLT ^{1}H NMR (500 MHz): δ 1.11 (t, 6H, f), 1.25 (dm, 4H, h,i), 1.55 (dm, 2H, j), 2.79 (m, 4H, c), 2.92 (m, 2H, g), 3.60 (s, 3H, m), 4.03 (m, 5H, e,k), 4.27 (m, 2H, d), 6.02 (d, 1H, NH), 6.09 (t,1H, NH), 6.19 (d,1H, NH), 6.57 (d, 1H, NH), 6.66 (dd, 4H, b), 6.94 (dd, 4H, b), 9.24 (s, 2H, a).

DMTLT ^{1}H NMR (500 MHz): δ 1.11 (t, 6H, g), 1.25(dm, 4H, i,j), 1.55 (dm, 2H, k), 1.97 (s, 6H, b), 2.91 (m, 6H, d,h), 3.58 (s, 3H, p), 4.02 (q, 4H, f), 4.08 (m, 1H, m), 4.36 (m, 2H, e), 5.86 (s, 2H, a), 6.06 (t, 1H, NH), 6.13 (d, 1H, NH), 6.24 (s, 2H, a), 6.31 (d, 1H, NH), 6.55 (d, 1H, NH), 7.06 (dd, 4H, c), 7.19 (dd, 4H, c).

From the chemical shifts of the ^{1}H NMR spectra of TLT and DMTLT, it can be concluded that the ester bond between the phenol and the methacrylic carbon was formed. In the DMTLT spectrum appear two new peaks at 5.86 and 6.24 ppm, which correspond to the acrylic protons. Besides, in the DMTLT spectrum the hydroxyl peak disappears and the aromatic peaks appear at higher chemical shifts than in TLT, corroborating the formation of the ester bond.

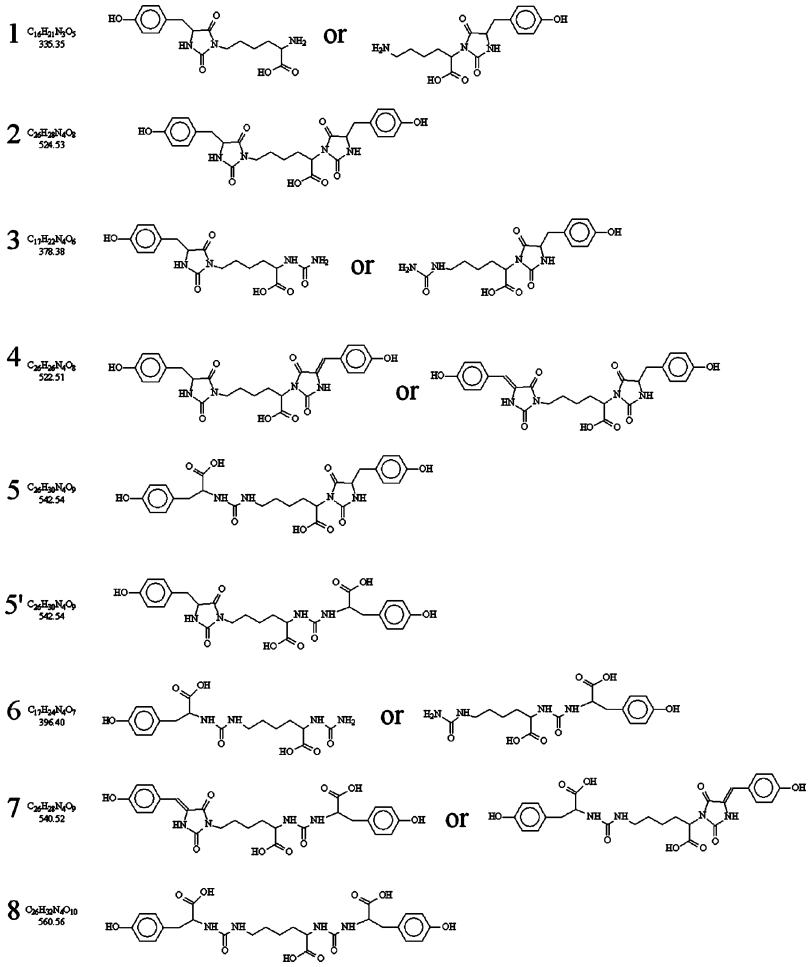
2.6. Synthesis of the Polymers. Copolymerization reactions of DMAA/DMTLT were performed in dioxane solutions at 60 °C for 24 h, using benzoyl peroxide as initiator (2×10^{-2} mol L^{-1}). DMTLT feed mol fractions were 0.01, 0.05, and 0.10. Reactions were carried out in the absence of oxygen by bubbling nitrogen for 10 min. The obtained gels were washed with water and acetone and dried at room temperature. A DMAA/bisacrylamide control copolymer with a 0.05 bisacrylamide feed mol fraction was prepared in the same conditions.

2.7. Hydrolysis Experiments. Hydrolysis of TLT, DMTLT, and the polymers prepared were carried out as follows: 10 mg of TLT, DMTLT, or the polymer were dispersed in 1 mL of a pH 10 buffered water solution and incubated at 60 °C. The color of the buffer was transparent to white at the beginning and turned into yellow while the products dissolved in the medium. Aliquots of the supernatant liquid phase were taken at different times to follow the degradation kinetics.

3. Results and Discussion

3.1. CE-MS Method Development. To get a picture as complete as possible of the degradation products, two MS analyzers (an ion trap and a time-of-flight) were coupled via an electrospray interface with CE separation. As expected, the composition of CE buffer and sheath liquid was found to affect CE separation and MS sensitivity. Thus, four CE volatile buffers at pH values of 2.2, 5.0, 8.0, and 10.0 containing formic acid, acetic acid, ammonium hydrogen carbonate, and ammonium hydroxide were tested. It could be observed that the best results in terms of resolution and analysis speed were obtained at pH 10, with a 46.5 mM ammonium hydrogen carbonate aqueous buffer. However, resolution between some migrating peaks could still be improved. Therefore, three different organic solvents were tested as buffer additives; namely, methanol, 2-propanol, and acetonitrile were added to the buffer at percentages from 5% to 20% (at 5% steps). The best results in

Chart 1. Assigned Structures of the Degradation Products Obtained after Hydrolysis of TLT and DMTLT



terms of resolution and time of analysis were obtained adding 15% of methanol. Next, three different hydro−organic mixtures were tested (namely, methanol/water, 2-propanol/water, and acetonitrile/water) with or without acetic acid. A solution of 2-propanol/water (50:50, v/v) containing 0.5% acetic acid provided the best sensitivity in the positive ion mode, and a solution of 2-propanol/water (50:50, v/v) provided the best sensitivity in the negative ion mode.

According to the mass values provided by the two CE-MS instruments, the elemental compositions obtained by CE-TOF-MS and the results observed during ESI ionization, the molecular structures given in Chart 1 were assigned. These results are discussed in detail elsewhere.[6]

Figure 1 shows the IT-MS spectra under optimum conditions, corresponding to the analysis of TLT in the negative and positive ion mode, and DMTLT in the positive ion mode. It can be clearly seen that the positive MS ion mode provides much better sensitivity than the negative mode for the detection of TLT (compare the MS signal intensities). A similar result was observed for DMTLT.

3.2. Study of TLT and DMTLT by CE-IT-MS. TLT and DMTLT have shown characteristic ESI-MS patterns, which have been very useful in the elucidation of the initial degradation steps in the further accelerated hydrolytic study. Table 1 shows the experimental molecular ions obtained by CE-MS in the positive and negative ion mode and the main MS fragments for TLT and DMTLT. For comparison the theoretical mass values for TLT and DMTLT also are included. Although the $[M - H]^-$ ion could not be observed for DMTLT, there is a perfect match between the experimental mass values obtained by CE-MS and the expected theoretical mass values (i.e., 630.3 for TLT and 766.3 for DMTLT), which confirms the adequate synthesis of both products.

As shown in Table 1 and Figure 1, it has been observed that both compounds give rise to MS fragments with 32, 46, 78, and 92 *m*/*z* units less than the molecular ion. This pattern is consistent with cyclization processes as shown in Scheme 2. For simplicity, only the proposed structures resulting from TLT fragmentation during its ionization in the ESI chamber are given in this scheme. As can be seen, the nucleophilic attack of the NH groups of the urea on the carbonyl groups gives rise to several cyclic compounds whose proposed structures are in good agreement with the mass values determined by MS. Similar cyclizations can be found in the literature.[9] DMTLT presented the same behavior. Moreover, as can be deduced from the MS results for DMTLT in the positive ion mode (see Figure 1), apart from the mentioned loss of 32, 46, 78, and 92 units (giving rise to the MS fragments with 735.3, 721.3, 689.4, and 675.3 *m*/*z*, respectively), DMTLT also loses 68, 100, and 114 *m*/*z* units, giving rise to fragments of 699.3, 667.3, and 653.3, respectively. These fragments correspond to the rupture of the methacrylic ester bond (i.e., 68 *m*/*z*) plus the product from the mentioned cyclization processes ($68 + 32 = 100$ *m*/*z* or $68 + 46 = 114$ *m*/*z*) bond. The behavior of TLT and DMTLT during ESI ionization, gives interesting information about the expected stability of the different groups in these molecules, which has been taken into consideration when studying their degradation during the hydrolysis experiments.

3.3. Mechanism and Kinetic Studies of TLT and DMTLT Hydrolysis. The kinetic of hydrolysis of TLT and DMTLT at

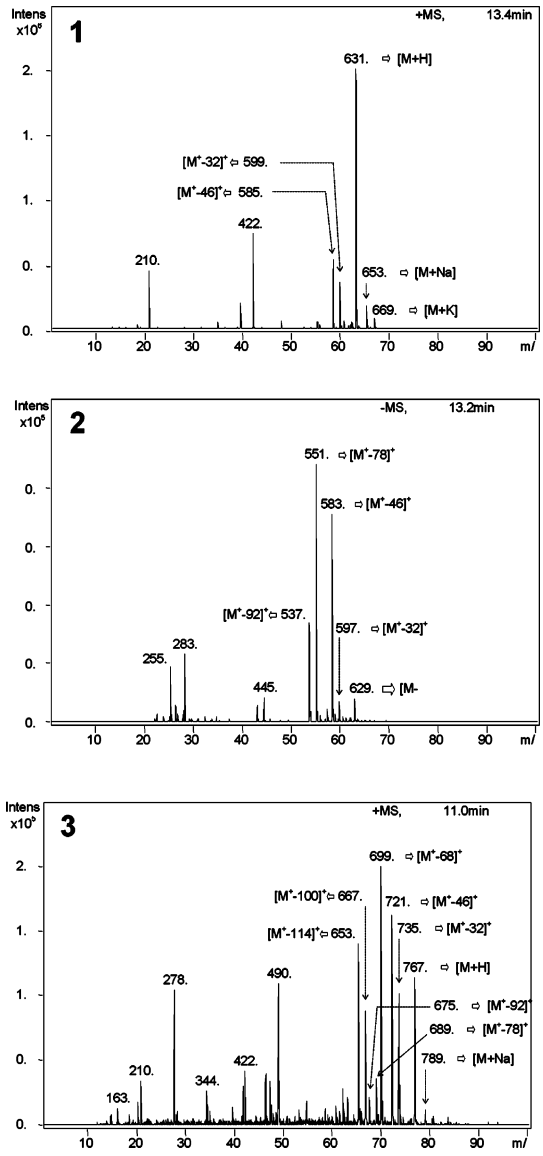


Figure 1. Mass spectra of TLT and DMTLT (0.5 mg/mL in acetone/water 1:1, v/v). TLT analysis in the (1) positive ion mode and (2) negative ion mode. DMTLT analysis in the (3) positive ion mode. CE-ESI-MS conditions: bare silica capillary (87 cm ld, 87 cm l_t, 50 μm i.d.); running buffer composition, 46.5 mM ammonium hydrogen carbonate with 15% methanol at pH 10; running voltage, +25 kV; injection, 4 s at 0.5 psi dry gas at a flow rate of 8 L/min and 200 °C; sheath liquid, water/2-propanol (50:50, v/v) with 0.5% acetic acid (in the positive ion mode analysis) and water/2-propanol (in the negative ion mode analysis) (50:50, v/v) at 4 μL/min flow rate; nebulizer pressure, 4 psi.

pH 10 and 60 °C was monitored by CE-IT-MS for ca. 70 days, to achieve a deeper knowledge on the degradation process of this cross-linker. The aromatic ester present in DMTLT is hydrolyzed during the first weeks as expected (see Figure 2). Similar aromatic esters have previously shown to be sensitive to enzymatic and nonenzymatic hydrolysis in aqueous physiological environment.[10,11] The slow cleavage of this group ensures the whole biodegradability or resorbability of the final polymeric material. Besides, DMTLT is converted, after the aromatic ester hydrolysis, in TLT and for this reason both molecules provide identical results (except for the described initial appearance of methacrylic acid in the case of DMTLT).

Table 2 and Figure 3 show the variation of the peak area of the nine degradation products that can be obtained from the common skeleton of TLT and DMTLT versus the hydrolysis time. Schemes 3−5 give the proposed degradation mechanism. Thus, the kinetic analysis of Table 2 indicates the appearance of several species during the initial stages of degradation (Figure 3), compound 2 being the most abundant ($[M + H]^+ = 525.2$). The isomeric species 5 and 5′ (with $[M + H]^+ = 543.2$) also appear in the first days but at lower quantities. Finally, there is another compound appearing at this initial period (although at very low concentration), compound 8 with $[M + H]^+ = 561.2$, obtained from the hydrolysis of the three esters. The comparative analysis of the areas of these four initial compounds is in agreement with a competition between the cyclization processes described previously (see Scheme 2) and the hydrolysis of the esters, as shown in Scheme 3. Taking into account that there is not any further structure compatible with the cycle obtained from the attack to the methyl ester (this cycle was observed in Scheme 2), we presume that this ester is hydrolyzed quickly, before any cyclization, in good agreement with the higher aqueous reactivity of this group compared with that of the ethyl ester. However, in the case of these ethyl esters, it seems that there is a higher selectivity toward the cycle formation than toward the ester hydrolysis, as corroborated by the clearly higher population of compound 2 compared to that of 5 and 5′ and 8 (see Figure 3). The kinetic analysis also suggests that there is no cyclization from the hydrolyzed form (from the carboxylic group). Besides, there is no conversion between 5 (or 5′) and 8 (neither apparently between 2 and 5 or 5′), and this shows clearly that there is no ring opening from these 2, 5, and 5′ compounds, concluding that the cycle formed is stable.

At higher degradation times (10 days), compound 4 appears (see Table 1, $[M + H]^+ = 523.2$). This is formed by oxidation of compound 2 because the benzylic position is highly activated. Compound 5 (or the 5′) also undergoes a similar process, giving rise to the small peak corresponding to compound 7 ($[M + H]^+ = 540$, see Table 1).

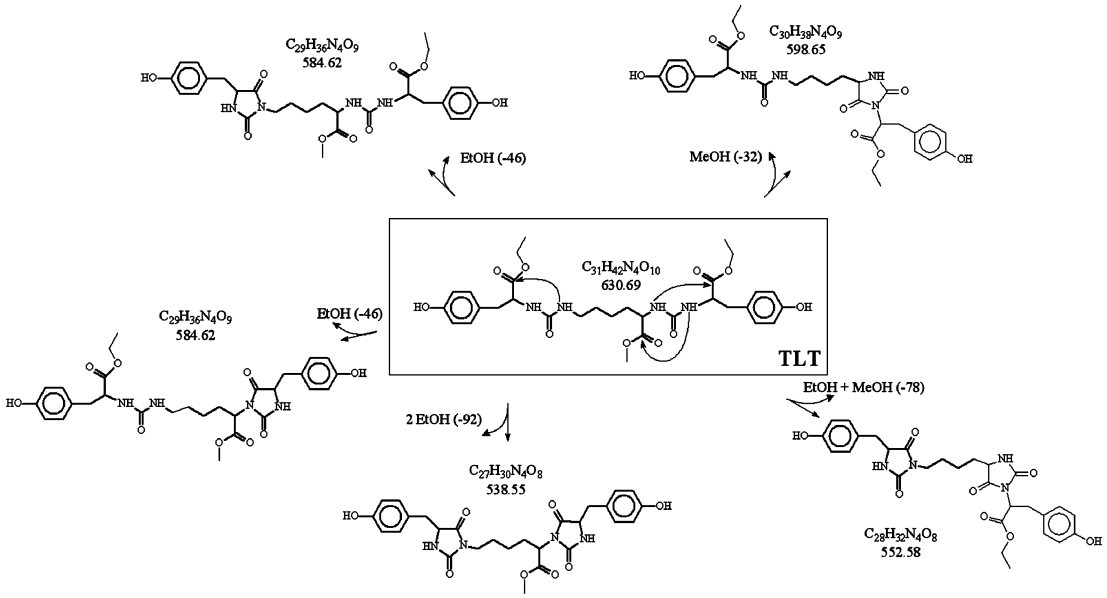
The most interesting result from the hydrolysis study is probably the appearance of compounds 1 and 3, which exhibit a continuous increase in intensity during all the degradation time (10 weeks, see Table 2). The relevance of these compounds lies on the fact that evidence the break of the pseudotripeptide at the urea bond (and the additional formation of tyrosine, Scheme 4) or at the adjacent bond (and the additional formation of cynnamic acid, Scheme 5). This result means that in a final polymeric material the residues formed upon the degradation of the aromatic esters may evolve toward the formation of small compounds that can even be active amino acids. In other words, this result shows, to our knowledge for the first time, a

Table 1. Experimental Molecular Masses and Main MS Fragments Obtained for TLT and DMTLT by CE-MS in the Positive and Negative Ion Mode (All the Conditions as in Figure 1)[a]

	TLT	DMTLT
theoretical mass (Da)	630.3	766.3
MS positive ion mode:		
$[M + H]^+$	631.3	767.3
main fragments	599.3, 585.3, 553.4, 422.2, 210.1	735.3, 721.3, 699.3, 689.4, 667.3, 653.3, 490.2, 278.2
MS negative ion mode:		
$[M - H]^-$	629.3	n.d.
main fragments	597.3, 583.4, 551.4, 537.4, 283.4	733.3, 719.3, 687.4, 673.4, 651.3, 619.4, 283.4

[a] Theoretical values for TLT and DMTLT were calculated according to the expected molecular structures (see Scheme 1).

Scheme 2. Molecular Structures of Some Representative Products Formed during the ESI Ionization of TLT



degradation mechanism of the urea bond derived from LDI, with the formation of very attractive amino acids of high activity in the human body, particularly in the transmission mechanism of the central nervous system.

From the kinetic analysis we propose a tentative degradation route for the formation of compounds 1 and 3 (Schemes 4 and 5, respectively). On one hand, compound 1 must be formed by the urea hydrolysis of compounds 5 and 5′. On the other hand, compound 3 probably is formed from the breakdown of compound 4, according to the following kinetic analysis. Assuming the stability of the cycles (in addition to the previous comment, species 1 and 3 seem to be stable because they exhibit a continuous concentration increase), compound 2 must be degraded only to compound 4. This compound 4 indeed increases its concentration during the first 2 weeks; after that it reaches a plateau during 20 days, and finally decreases. The concentration profiles of compounds 2 and 4 are in agreement with this degradation scheme, assuming that compound 4 is an unstable intermediate compound.

$$2 \Rightarrow 4 \Rightarrow \text{other compound}$$

A deep analysis of the kinetic results in Table 2 shows that compound 4 has to be converted into species 3, which is the only one that increases in a correlative way (see Figure 3). It seems that the oxidation of compound 2 to compound 4 "activates" the cycle, and makes it capable of hydrolytic breakdown. This degradation pathway would also explain the appearance of the small peak assigned to compound 6 that may be formed by a similar mechanism from compound 5 or 5′ passing through the unstable compound 7. In the previous discussion, we have to take into account that, although the relationship between area and concentration is quantitative in capillary electrophoresis and also in capillary electrophoresis-mass spectrometry, the ionization yield of different compounds is expected to be different. The differences in ionization yield would explain the apparent higher amount of 525 compared with that of the other two (the area under the curve for compounds 523 and 379 is about 70% of the area for compound 525).

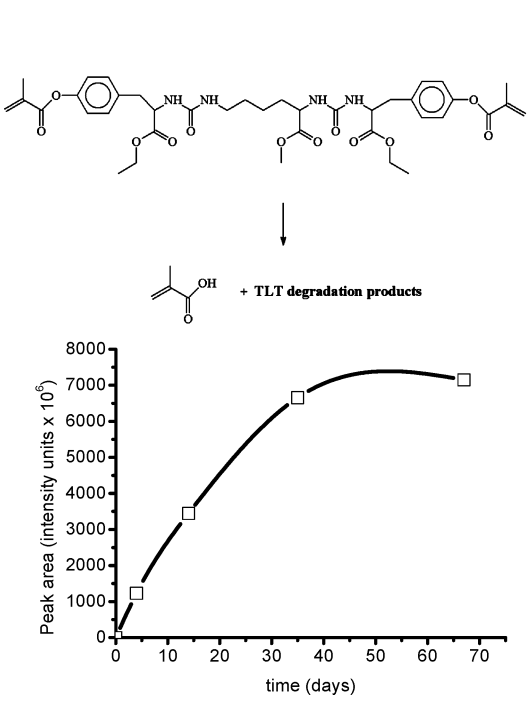


Figure 2. Hydrolytical scheme of the aromatic ester and the variation of the peak area of methacrylic acid.

3.4. Polymer Degradation. Three cross-linked polymeric samples were prepared with 1%, 5%, and 10% of DMTLT mol percentage. The materials overcame a continuous increasing of swelling during the whole experiments, which is in agreement with a slow hydrolysis of the aromatic ester linking the main backbone to the pseudetripeptide. A clear influence of the cross-linker content was observed: the higher DMTLT content, the slower is the swelling increase, in fact, the 1% sample becomes soluble after approximately 20 days (see Figure 4).

Besides, the degradative species characterized in Chart 1 were detected using the CE-MS protocol developed previously. Figure 5 shows, for the 5% and 10% DMTLT cross-linked samples (with the 1% DMTLT, most of the species were below the detection limit), the peak concentration (at several weeks) of the more populated degradative compounds 2, 3, 4, 5, and 5′. These results demonstrate that the degradation pathway of the cross-linker into the polymer is the same as that the observed in the TLT and DMTLT, i.e., hydrolysis of the aromatic ester, competition between cyclization-side ester hydrolysis, backbone

Table 2. Corrected Peak Area (Area/Migration Time) versus Time of Hydrolysis for the Different Compounds Described in Chart 1

	no. of days										
	0.3	1	2	4	8	14	21	35	49	56	67
compound 1 (336.3)	0	0	0	4[a]	12	43	84	231	384	457	754
compound 2 (525.2)	2133	2658	3264	3778	3376	3348	1923	1022	487	353	326
compound 3 (379.3)	2	5	7	34	97	249	397	621	731	798	934
compound 4 (523.2)	0	0	27	82	254	667	789	739	432	343	312
compound 5 (543.2)	247	360	470	604	624	693	518	361	260	225	242
compound 5′ (543.2)	193	226	296	368	409	482	383	354	294	294	366
compound 6 (397.1)	0	0	0	0	4	10	20	45	63	76	115
compound ∗ (451.2)	0	0	6	9	24	43	43	55	41	35	39
compound 7 (541.1)	0	0	0	4	16	56	76	121	98	99	113
compound 8 (561.2)	13	18	25	35	38	44	38	37	35	31	43

[a] Peak area in arbitrary units × 10^4.

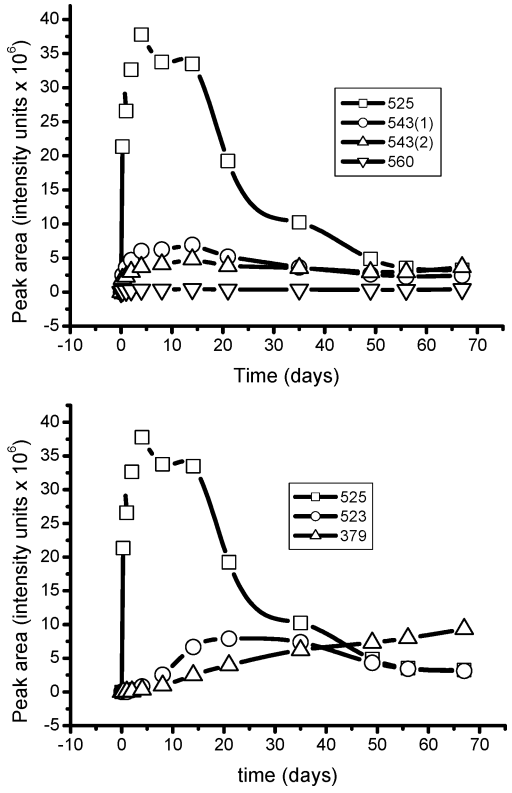


Figure 3. Variation of the peak area of the degradation products 2, 5, 5′, and 8 (above) and 2, 4, and 3 (bottom) from TLT against the time of hydrolysis.

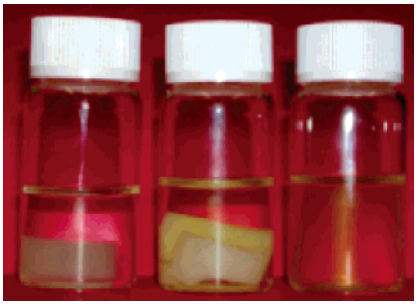


Figure 4. Aqueous degradation steps of the dimethylacrylamide-based polymeric network cross-linked with the new biodegradable cross-linker DMTLT: nondegraded disk (left sample), highly swollen and fragmented disk after a few days of degradation (center sample), and material solubilized after several weeks of aqueous degradation (right sample).

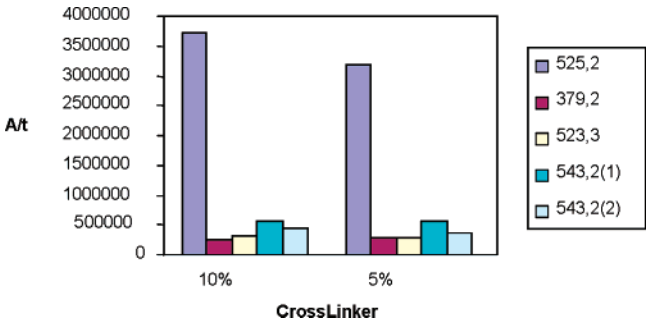


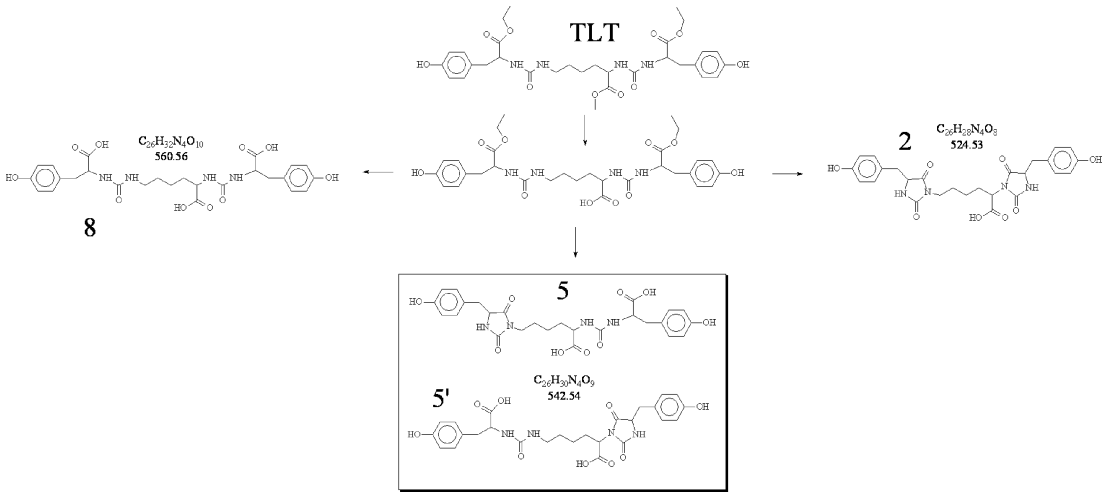
Figure 5. Peak areas of compounds 2, 3, 4, 5, and 5′ as obtained after 67 days of degradation of the polymer samples prepared with 5 and 10 mol % of DMTLT.

breakdown, etc. The poly-DMAA cross-linked with bisacrylamide and used as reference material did not show any degradative peak. As indicated in Figure 5, there are significant differences between the three polymeric samples: there is a balance between hydrophilicity and concentration of cross-linker. The lower the cross-linker mol content is, the higher is the swelling, the faster is the degradative process, and the lower is the amount of degradative species detected.

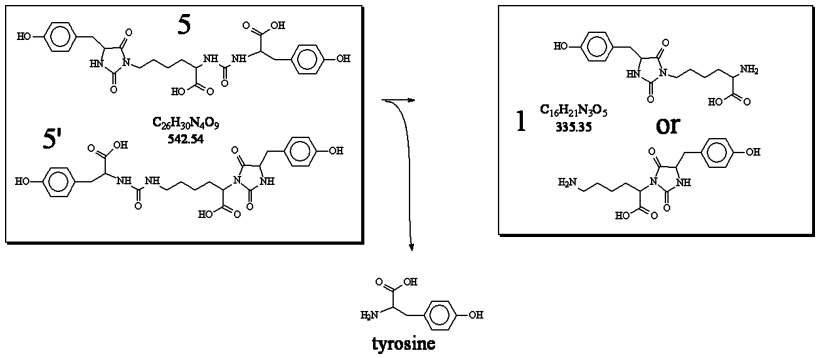
4. Conclusion

The experiments of accelerated degradation in aqueous environment at pH 10 and 60 °C, over the pseudopeptide TLT

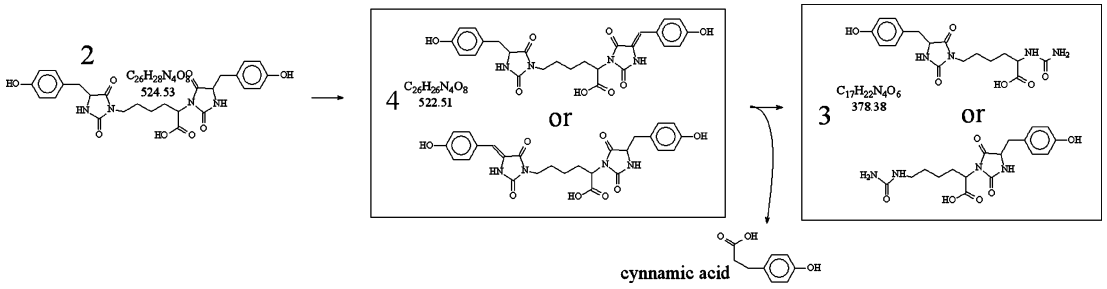
Scheme 3. Proposed Degradation Mechanism of TLT (and DMTLT) at the First Stages of Hydrolysis Giving Rise to Compounds 2, 5, 5′, and 8



Scheme 4. Proposed Degradation Mechanism of Compound 5 and 5′ ([M + H]$^+$ = 543.2) during Hydrolysis and Formation of Compound 1



Scheme 5. Proposed Degradation Mechanism of Compound 2 ([M + H]$^+$ = 525.2) during Hydrolysis and Formation of Compounds 4 and 3



and its derivative DMTLT (the difunctional monomer) and a model poly-DMAA cross-linked with this structure, have shown new degradation pathways. A competition between hydrolysis and cyclization has been observed during the first days of degradation. At longer times, new species related to the breaking of the main chain have been detected clearly. One of this new species is formed by hydrolysis of the urea bond.

The use of DMTLT as a biodegradable cross-linker instead of bisacrylamide provides resorbable sensitive hydrogels which becomes soluble after the hydrolysis of the cross-linker agent. This is of great importance for the preparation of supports for tissue engineering and pH or temperature-sensitive drug delivery systems. In addition, the biodegradation of DMTLT contained in the cross-linked hydrogels gives rise to the release of interesting bioactive amino acids such as tyrosine (a natural precursor of dopamine).

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