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Reaction of poly-L-lysine with aryl acetates and aryl methyl carbonates. A mechanistic study

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The pH profile (log *k* *vs*. pH) of the reactions of poly-L-lysine (PL) with a series of aryl acetates and aryl methyl carbonates in aqueous solution show the same conformational changes as those determined by potentiometric titrations. When PL is a random coil, the most probable mechanism for the reactions studied is through the formation of a tetrahedral intermediate and its breakdown to products as the rate-determining step. The tetrahedral intermediate is stabilized by a hydrogen bond interaction between the nitro groups in the substrate and the NH group of the principal chain or some NH_2 groups of the lateral chains. Copyright © 2007 John Wiley & Sons, Ltd.

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Keywords: kinetics; mechanism; aminolysis; poly-L-lysine; Brønsted plots

INTRODUCTION

The reactions of some substrates with macromolecules can be important models to understand the behaviour of complex enzymatic systems. In general, a polymer can concentrate or disregard a reactive molecule in its environment and if some catalytically active function is incorporated they can be able to react with substrates, showing a kinetic and mechanistic effect on the reaction.[1–3]

The hydrolysis of esters in the presence of polyamines is a model of enzymatic reactions, such as in those described for quimiotripsine.[4] In this model, the macromolecule that represents the enzyme with the active site is a polyelectrolyte, carrying out the hydrolysis according to a Michaelis–Menten mechanism.[4b]

In general, the kinetic and mechanistic studies of these reactions suggest pre-equilibrium, acylation and deacylation steps;[4b] nevertheless, the studies have been restricted mainly to the deacylation or hydrolysis step because the acylation or aminolysis steps are fast.[4a]

Some works with amino-functionalized polyelectrolytes[5,6] (with imidazole, benzylimidazole and pyridines among others) show an increase in the catalytic activity relative to the monomeric counterpart. In electrolytes such as poly(ethylenimine)[7] (PEI) and poly(allylamine)[5] (PAA), modified with hydrophobic groups (alkyl or benzyl), the results show that a hydrophobic environment increases the esters hydrolysis. In all these cases the bond formation between the substrate and the macromolecule is influenced by ionic, hydrogen bond and hydrophobic interactions.[5b]

A first approach toward the understanding of the reactions involving acetyl groups transfer was obtained by the kinetic and mechanistic studies of the aminolysis of esters using monomeric amines.[8]

A few works about the kinetics of the aminolysis of esters with polymeric amines have been described. At this respect, it is noteworthy the systematic study of Arcelli[9] on the aminolysis reactions of aryl acetates with PEI, both in the absence[9a] and presence[9b] of electrolytes, where different mechanisms were observed in both cases. A work on the reaction of PAA with a series of aryl acetates and aryl methyl carbonates shows a concerted aminolysis mechanism.[10]

Taking into account that polyamine basicity changes with the dissociation degree it is possible to obtain a basicity range of the nucleophilic groups without a change in the polymer structure. This allows the use of Brønsted-type plots for the mechanistic studies of reactions with polyelectrolytes.[9,10]

It is known that poly-L-lysine (PL) can exist in three different conformations: coil, α-helix and β-fold. In aqueous solution, at room temperature and acid or neutral pH, this polymer adopts a disordered statistic coil, whereas in basic media, when the lateral amino groups are not charged, it adopts an ordered α-helix conformation, stabilized by intramolecular hydrogen bonds.[11–17]

The aminolysis mechanisms of aryl acetates,[18] alkyl aryl carbonates[19–21] and diaryl carbonates[20b,22,23] with monomeric amines have been well established. Some of these reactions are

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known to proceed through a zwitterionic tetrahedral intermediate ($T^{\pm}$) and others by a concerted pathway (a single step). Structure–reactivity correlations, such as the Brønsted-type relationship, have helped to clarify these mechanisms.[18–23]

In this work, we report a kinetic study of the reactions in aqueous solution of 4-nitrophenyl acetate (NPA), 2,4-dinitrophenyl acetate (DNPA), 2,4,6-trinitrophenyl acetate (TNPA), 4-nitrophenyl methyl carbonate (NPC), 2,4-dinitrophenyl methyl carbonate (DNPC) and 2,4,6-trinitrophenyl methyl carbonate (TNPC) with PL of two different polymerization degrees (Scheme 1). This, together with the investigation on the dissociation behaviour of PL, will allow the determination of the mechanism and the assessment of the kinetic effects of (i) the neighbourhood (micro-environment surrounding) of the reaction site (ii) the PL polymerization degree and (iii) the leaving and non-leaving groups of the substrate.

EXPERIMENTAL

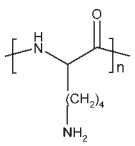
Materials

Poly-L-lysine hydrobromide, from Sigma, of polymerization degrees 402 (PL-402) and 277 (PL-277), was used without further purification. NPA, DNPA and TNPA were synthesized as described previously.[24] NPC, DNPC and TNPC were synthesized by a standard procedure.[25] All other reagents were of analytical grade.

pK_{app} determination

The potentiometric titration of PL was carried out in aqueous solution, at $25.0 \pm 0.1^{\circ}C$, under nitrogen, by means of a Radiometer autotitrator equipped with a PHM-62 pH-meter, an ABU-11 autoburette, a TTT-60 titrator, an REA-160 recorder, a TTA-60 thermostatic support and a G-2040 glass and a K-4040 calomel electrodes. In each experiment 10 mL of the polymer solution at different concentrations (5.1×10^{-4}–4.24×10^{-3} M, expressed as amine groups), and an ionic strength of 0.1 M, maintained with KCl, was titrated with NaOH (0.01–0.1 M). The polymer concentration range in the potentiometric titration was similar to that in the kinetic measurements. The apparent dissociation constant (pK_{app}) was calculated according to Eqn (1),[26] where α is the dissociation degree. The latter is the quotient between the free amine groups and the total PL concentration (expressed as monomeric units); the concentration of free amino groups was determined from the added NaOH volume at each pH.[26,27]

$$pK_{app} = pH + \log(1-\alpha)/\alpha \qquad (1)$$



Poly-L-lysine

NPA: $R_1 = CH_3$, $R_2 = C_6H_4(NO_2)$
DNPA: $R_1 = CH_3$, $R_2 = C_6H_3(NO_2)_2$
TNPA: $R_1 = CH_3$, $R_2 = C_6H_2(NO_2)_3$
NPC: $R_1 = OCH_3$, $R_2 = C_6H_4(NO_2)$
DNPC: $R_1 = OCH_3$, $R_2 = C_6H_3(NO_2)_2$
TNPC: $R_1 = OCH_3$, $R_2 = C_6H_2(NO_2)_3$

Scheme 1. Structures of the polymer (PL) and the substrates used in this work

Since the α values for PL for the two polymerization degrees were closely similar, in the concentration range stated at each pH, these values were averaged as α_m. Table 1 (in Supplementary Material) shows the pK_{app} and α_m values of PL at different pH and at various polymer concentration ranges.

Kinetics

PL solutions were prepared freshly in aqueous solutions of the corresponding external buffer 0.01 M, ionic strength 0.1 M (KCl) and the desired pH. The reactions were initiated by addition of 30 μL of a stock solution of the corresponding substrate into 2.5 mL of the polymer solutions thermostated at $25.0 \pm 0.1^{\circ}C$. The initial concentration of the substrates was 5×10^{-5} M. The kinetic measurements were carried out spectrophotometrically by following the production of 4-nitrophenoxide (400 nm), 2,4-dinitrophenoxide (355 nm) and 2,4,6-trinitrophenoxide (355 nm) anions, by means of a Hewlett-Packard 8453 diode array spectrophotometer. Under amine excess, pseudo-first-order rate coefficients (k_{obs}) were found for all reactions. The plots of k_{obs} *versus* total amine concentration were linear, with k_{Nobs} as slope. Phosphate, borate and carbonate buffers (0.01 M) were used at appropriate pH ranges.

The experimental conditions and the values of k_{obs} and k_{Nobs} for the reactions of PL with TNPA, DNPA, NPA, TNPC, DNPC and NPC are summarized in Tables 2–5 (in Supplementary Material).

Product studies

The presence of 4-nitrophenoxide, 2,4-dinitrophenoxide and 2,4,6-trinitrophenoxide anions as products of the reactions was determined spectrophotometrically by comparison of the UV–Vis spectra at the end of the reactions with those of authentic samples under the same experimental conditions.

RESULTS AND DISCUSSION

Potentiometric titrations

Due to the complexity of macromolecular systems and to the great number of ionizing groups the potentiometric behaviour of polyamines is quite different than those for monomeric amines. The ionization of a group is affected by the proximity of other ionizing groups and by the presence of hydrophobic moieties and hydrogen bonds in others.[27]

A difference with monomeric amines is that, in polyamines[27–29] and polyaminoacids[11,12] the dissociation equilibrium of the ionizing groups in a polyacid (including the conjugate acid of a polybase) is cooperative and complex due to the strong electrostatic interactions between the charged groups in the chain.

Figures 1 and 2 show the titration curves of PL-402 and PL-277, as pK_{app} *versus* α plots. It can be observed that pK_{app} is a function of both concentration and dissociation degree. The dependence of pK_{app} on the polymer concentration has been described for PEI,[28] PAA[10] and polyacids.[27] On the other hand, the comparison between both figures shows an independence of the polymerization degree.

For low dissociation degrees ($\alpha < 0.2$) Figs. 1 and 2 show that pK_{app} increases as α increases; for α values in the 0.2–0.6 range a relatively constant pK_{app} is observed and for $\alpha > 0.6$ a decrease in pK_{app} is observed as α increases. This can be

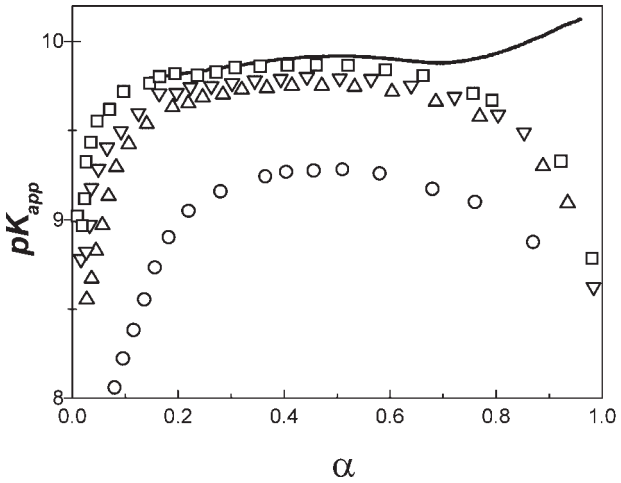


Figure 1. Titration curves of PL-402 in aqueous solution at 25°C and ionic strength 0.1 M (KCl). 0.51×10^{-3} M (○), 1.69×10^{-3} M (△), 3.05×10^{-3} M (▽), 4.24×10^{-3} M (□). The solid line is from Reference [12]

explained by the presence of a transition or conformational change. The regions described correspond to random coil, the random coil to α-helix transition and α-helix, respectively, in accordance with literature.[11,12] The transition zone has been reported in the α 0.2–0.5 range for PL (MW 110 000) in KBr 1 M[11] and α 0.4–0.75 range for PL (PD 450) 1.91–2.87×10^{-2} M in KCl 0.1 M.[12] The differences in the assignment of zones are related to the experimental conditions, such as pH, solvent, temperature, polymer concentration and type and concentration of added salt.[29]

The results are also in accordance with those reported by circular dichroism,[13,16] optical rotatory dispersión[15] and ultrasonic studies,[17] which describe random coil and transition in the 8–10 pH range.

Kinetic study

A linear dependence of the pseudo-first-order rate constant (k_{obs}) on PL concentration at each pH was observed, according to Eqn (2), where k_0 and k_{Nobs} are the rate coefficients for spontaneous hydrolysis and aminolysis of the substrates, respectively. These results suggest that there is no substrate–polyelectrolyte association because the presence of this association shows limiting plots, such as those described for PEI in the absence of KCl.[9a]

$$k_{obs} = k_0 + k_{Nobs}[N]_{tot} \quad (2)$$

No differences in k_{Nobs} values were observed when reactions were carried out with PL of different polymerization degrees (refer to Tables 2–5 in Supplementary Material).

For most of the reactions studied k_0 is negligible in Eqn (2), except for those at higher pH media, where the two terms are important.

The nucleophilic rate constant (k_N) is obtained by the quotient between the k_{Nobs} values (Tables 2–5 in Supplementary Material) and the corresponding α_m values (of Table 1 in Supplementary Material). Tables 1 and 2 show the values of pK_{app} (of Table 1 in Supplementary Material) and k_N for the reactions of PL with NPA, DNPA, TNPA, NPC, DNPC and TNPC at the different pH values.

The nucleophilic rate constants for the reaction of PL with carbonates are smaller than those for the corresponding acetates at the same pH values. This result can be explained through the larger electron-donating effect exerted by MeO in the carbonates relative to Me in the acetates, rendering the former carbonyl carbon atom less positively charged and, therefore, less prone to amine attack. This is in accordance with what has been found for the reactions with PAA[10] and monomeric amines.[18f,g,19a]

pH profile

The behaviour of log k_N *versus* pH for the PL reactions (both fractions) with the series of acetates and methyl carbonates are shown in Figs. 3 and 4, respectively.

It can be observed in Figs. 3 and 4 that for pH values lower than ≈9 the log k_N values increase with pH, probably due to the increase in free amine fraction. In this region, the presence of the protonated ε-amine lateral groups leads to the formation of a statistical coil. The pH region ranged 9–10 is characterized by a

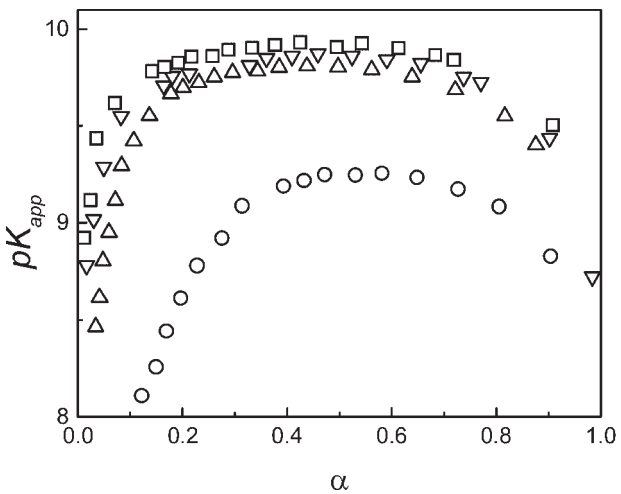


Figure 2. Titration curves of PL-277 in aqueous solution at 25°C and ionic strength 0.1 M (KCl). 0.51×10^{-3} M (○), 1.69×10^{-3} M (△), 3.05×10^{-3} M (▽), 4.24×10^{-3} M(□)

Table 1. Nucleophilic rate constants (k_N) for the reactions of NPA, DNPA, TNPA, NPC, DNPC and TNPC with PL-402 at different pH values, in aqueous solution, 25.0°C, ionic strength 0.1 M

		k_N ($s^{-1} M^{-1}$)					
pH	pK_{app}	NPA	DNPA	TNPA	NPC	DNPC	TNPC
7.0	8.39	0.179	2.05	6.67	0.069	0.309	1.39
7.5	8.71	—	3.73	12.4	0.110	0.610	2.37
8.0	9.05	—	7.95	25.5	0.675	2.29	6.14
8.5	9.46	1.72	18.0	54.7	0.859	3.67	12.6
9.0	9.63	1.38	24.4	54.5	1.11	6.77	18.9
9.5	9.63	1.50	—	77.7	0.85	8.01	14.1
10.0	9.60	1.64	14.3	49.1	0.87	5.34	18.0
10.5	—	2.15	19.3	57.9	1.48	10.9	48.7
11.0	—	4.60	40.0	145	1.86	21.5	152
11.5	—	7.60	72.0	—	3.50	30.0	230

Table 2. Nucleophilic rate constants (k_N) for the reactions of NPA, DNPA, TNPA, NPC, DNPC and TNPC with PL-277 at different pH values, in aqueous solution, 25.0°C, ionic strength 0.1 M

		k_N ($s^{-1} M^{-1}$)					
pH	pK_{app}	NPA	DNPA	TNPA	NPC	DNPC	TNPC
7.0	8.39	0.205	2.05	7.17	0.077	—	1.28
7.5	8.71	0.424	—	—	0.132	—	1.73
8.0	9.05	0.651	—	31.3	0.675	—	7.71
8.5	9.46	2.19	18.7	55.4	0.891	3.20	9.38
9.0	9.63	1.66	18.9	48.8	1.01	6.36	15.0
9.5	9.63	1.85	16.5	52.9	—	7.48	—
10.0	9.60	1.46	13.8	35.1	1.19	5.20	25.3
10.5	—	1.53	13.3	51.8	1.12	8.53	56.8
11.0	—	—	—	—	2.80	—	76.0
11.5	—	6.70	63.0	369	3.80	—	210

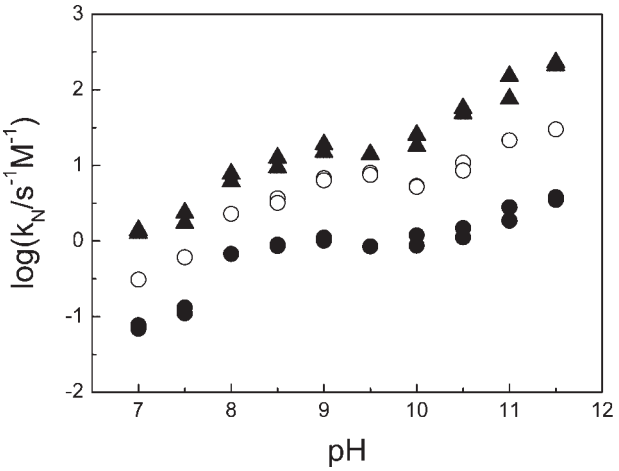


Figure 4. Plots of log k_N *versus* pH for the reactions of TNPC (▲), DNPC (○) and NPC (●) with PL, in aqueous solution, at 25°C and ionic strength 0.1 M (KCl)

small decrease in log k_N, due to the presence of a less reactive specie.[11,12,14–17] Above pH ≈ 10 the log k_N values increase with pH, probably due to the fact that the polymer adopts its α-helix conformation.[11,12,14–17]

These pH profiles are in agreement with those described in our studies for the Schiff base formation between 5′ pyridoxal phosphate and PLs of several polymerization degrees.[30]

The leaving group effect on k_N for the series of acetates and methyl carbonates studied is in accordance with those discussed for the reactions of the same substrates with PAA[10] and monomeric amines,[18c,e,f,g,19b,20b] which show a k_N increase with the decrease in basicity of the leaving group. On the other hand, the change of CH_3 by OCH_3 as the non-leaving group reduces the k_N value due to the electron donation of the latter that renders a less reactive carbonyl group in the same way as observed for the reactions with PAA,[10] pyridines[18c,d,f,19a,b] and secondary alicyclic amines.[18e,g,20a]

Mechanism

The Brønsted-type plots for the reactions studied are linear in the 7.0–9.0 pH range (Figs. 5 and 6). In this pH range, the PL is a statistical coil, in accordance with the titration study (refer to above). The slope (β_{nuc}) values found are 1.1, 1.0 and 0.9 for NPC, DNPC and TNPC, respectively, and 0.9, 0.9 and 0.8 for NPA, DNPA and TNPA, respectively; these values are subject to an error of ±0.1.

Considering the β_{nuc} values found (0.8–1.1), in the same range of those obtained for the reactions of the same and similar substrates with monomeric amines[18–23] and with that found for the reaction of NPA with PEI[9b]; the most probable mechanism for the reactions studied in this work is that shown in Scheme 2 (shown for a lysine group), where a tetrahedral intermediate ($T^{\pm}$) is formed and its breakdown to products is the rate-determining step, when PL is a random coil.

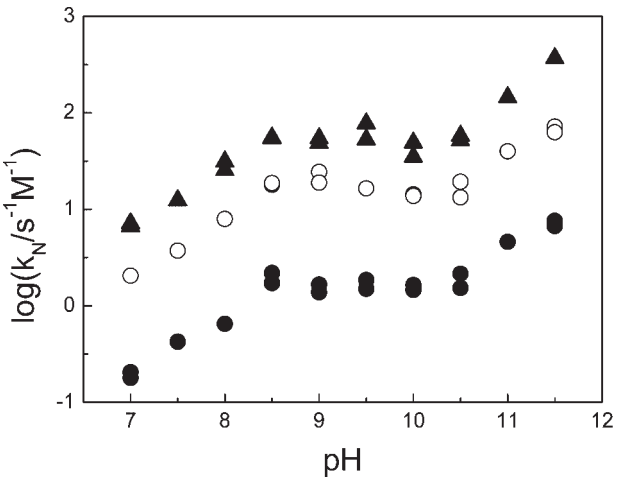


Figure 3. Plots of log k_N *versus* pH for the reactions of TNPA (▲), DNPA (○) and NPA (●) with PL, in aqueous solution, at 25°C and ionic strength 0.1 M (KCl)

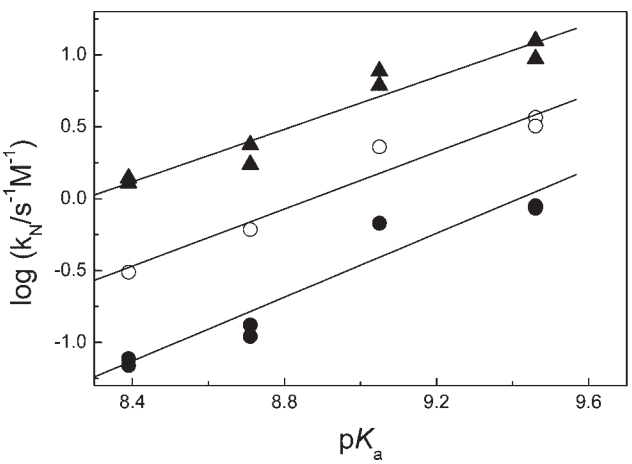


Figure 5. Brønsted-type plots (log k_N *vs.* pK_{app}) for the reactions of TNPC (▲), DNPC (○) and NPC (●) with PL, in aqueous solution, at 25°C and ionic strength 0.1 M (KCl)

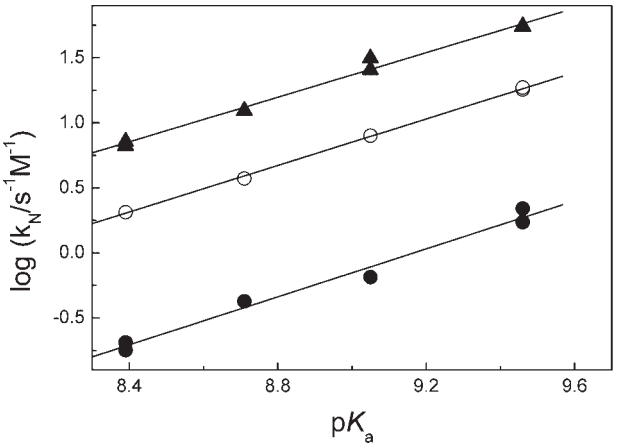


Figure 6. Brønsted-type plots (log k_N *vs.* pK_{app}) for the reactions of TNPA (▲), DNPA (○) and NPA (●) with PL, in aqueous solution, at 25°C and ionic strength 0.1 M (KCl)

Stepwise aminolyses of aryl acetates (with monomeric amines) show values of pK_a^0 (pK_a at the curvature centre of the Brønsted-type plot) about 4–5 pK_a units greater than the pK_a of the conjugate acid of the leaving group.[18] However, when the amine group is in the backbone of the polymer this difference is about 3–3.5 pK_a units.[9b] Considering the corresponding pK_a of the leaving groups (around 7, 4 and 0.3 for 4-nitro-, 2,4-dinitro- and 2,4,6-trinitro-phenols, respectively),[31] we should expect pK_a^0 values around 4.3–5.3 for TNPA, 8.0–9.0 for DNPA and 11–12 for NPA; similar ranges can be expected for methyl carbonates.

The expected pK_a^0 value for the nitro derivatives is larger than the largest pK_a of the measured range and, therefore, the β values correspond to β_2 ($T^{\pm}$ breakdown to products as rate limiting). Nevertheless, if the mechanism is that of Scheme 1, for the pK_{app} studied, the Brønsted plot for the dinitro derivatives should be biphasic and the β for the trinitro derivatives should be β_1 ($T^{\pm}$ formation as rate limiting), with an expected β value range of 0.1–0.3.[18–23]

The absence of a biphasic plot for the dinitro derivatives and the β values found for the trinitro and dinitro derivatives suggest that for all these reactions breakdown of $T^{\pm}$ to products is the rate-limiting step. This means that for these reactions $k_{-1} > k_2$ in Scheme 1. In order to explain these results, we propose that the intermediate $T^{\pm}$ could be stabilized by a hydrogen bond interaction between the nitro groups in the substrate and the NH group of the main chain or some NH_2 groups of the lateral chains. Due to this interaction, the rate constants k_{-1} and k_2 would be smaller than those expected for the reactions of these substrates with a monomeric amine. Nevertheless, this interaction should diminish the rate of expulsion from $T^{\pm}$ of the leaving group (k_2) more than that of the amine (k_{-1}), so that for the reactions of these substrates with PL, k_{-1} is still larger than k_2.

CONCLUSIONS

(i) The mechanism of the aminolysis (PL) of the series of aryl acetates and aryl methyl carbonates studied is stepwise, with the formation of a tetrahedral intermediate and its breakdown to products as the rate-determining step, when PL is a random coil. (ii) The presence of the nitro groups in the substrates stabilize the tetrahedral intermediate by a hydrogen bond interaction between the nitro groups in the substrate with the NH group of the principal chain or some NH_2 groups of the lateral chains.

Acknowledgements

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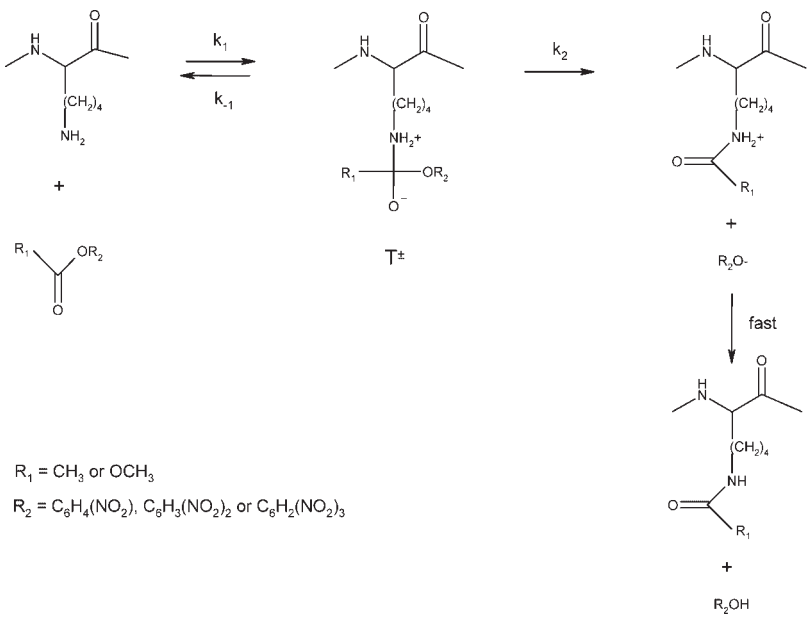

Scheme 2. Mechanism for the reactions of PL with aryl acetates and aryl methyl carbonates

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