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Capillary zone electrophoretic analysis of positively charged poly(ethylene oxide) macromolecules using non-covalent polycation-coated fused-silica capillary and indirect UV detection

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Abstract

Capillary zone electrophoresis was used to show the coupling between NH_2-terminated poly(ethylene oxide) and oligomers of lactic acid activated by transforming carboxyl chain ends to acyl chloride ones. The demonstration was based on the use of fused-silica capillary physically modified by pre-adsorption of polycations in the reversed polarity mode. As poly(ethylene oxide) macromolecules are UV transparent, indirect UV detection was used. A creatinine solution at pH 4.8 was selected as background electrolyte. Commercially available polycations with different structures were tested. It was shown that the reversed electroosmosis could be modulated according to the structure of the polycation. The method was then applied to analyse a commercial α,ω-diamino poly(ethylene oxide), namely Jeffamine ED 600 characterised by a broad mass dispersion. Data showed that the method can detect and separate amino poly(ethylene oxide) of different structures. When applied to analyse post coupling products, no free NH_2-terminated poly(ethylene oxide) segments were detected. Moreover, the method allowed detection of water-soluble oligomers generated by partial degradation of lactic segments during the reaction.

Keywords: Capillary columns; Poly(ethylene oxide); Lactic acid oligomers

1. Introduction

Nowadays, increasing attention is being paid to diblock copolymers of lactic acid and ethylene oxide (PLA-b-PEO) as sources of smart hydrogels, nanosystems of the nanoparticle-type and macromolecular micelles, particularly for drug delivery devices [1–7]. While ethylene oxide blocks are biostable, lactic acid blocks are hydrolytically degradable regardless of the presence of enzymes or living systems.

The possibility of activating the carboxyl end groups by transformation to their acyl chloride form using thionyl chloride was reported a few years ago [8]. Such an activation offered a simple route to synthesise PLA-b-PEO by simple chemical coupling. However, the absence of residual polymeric reagent is always a source of problems because of the possibility of physical entrapment by the amphiphilic copolymers. In order to alter the degradation profile of PLA-b-PEO systems where the two blocks are usually linked through an ester bond, attempts have

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been made to replace this ester bond by an amide one.

It has been shown recently that capillary zone electrophoresis (CZE) can be a powerful tool to analyse water-soluble by-products issued from the hydrolytic degradation of aliphatic polyesters, especially when reversed electroosmotic flow separation using temporary coating of a fused-silica capillary (FSC) by a polycation (PC) is used [9–11]. This method reverses the electroosmotic flow of the FSC and decreases or even precludes the absorption of cationic entities. It requires a preliminary rinse of the capillary with a dilute PC solution. The polycation absorbs onto the inner surface of the FSC because of a ''polycation/polyanion'' interaction with the negative charges present at the surface of silica. This conditioning results in a new capillary referred to as PCFSC. The electrostatic interaction is so strong that the PCFSC can be rinsed with a separation buffer which remains PC-free. This particularity allows separation of the CZE type, i.e., using a background electrolyte that does not interact with the analyte. The method has been successfully applied to analyse various compounds, namely basic proteins, organic acids, end-charged oligomers produced by degradation of aliphatic polyester of the α- or β-type, etc. [10–16]. However, poor reproducibility of the separation is sometimes a source of limitation that makes the method unpopular despite its simplicity. Recently, it was shown that plotting data versus $-1/t$ is a means to rule out the problem of reproducibility [11,17].

In this paper, we wish to report on the analysis of amide linked PLA-b-PEO obtained by direct coupling of activated lactic acid polymer chains to the primary chain-end of amino poly(ethylene oxide) macromolecules (Me-OPEG-NH_2) using CZE with the aim of showing the absence of unreacted amino poly(ethylene oxide) segments. As poly(ethylene oxide) macromolecules are UV transparent, indirect UV detection was used [18]. A creatinine solution at pH 4.8 was selected as the background electrolyte (CBE). It is of value to notice that (Me-OPEG-NH_2) macromolecules are positively charged in CBE. This ionisation is a prerequisite for CZE.

A separation protocol of positively charged poly(ethylene oxide) macromolecules was defined. This protocol was based on the use of PCFSC. A commercial sample of aminated poly(ethylene oxide), namely Jeffamine ED 600 with a broad molecular mass dispersion was selected as a reference. The data were used to both discuss the limits of this CZE method and to monitor the advances of the coupling reaction.

2. Experimental

2.1. Materials

The following polycations were commercial compounds (Fig. 1): PDDAC (polydiallyldimethylammonium chloride, medium molecular mass, Aldrich), DEAE Dextran (diethylaminoethyl dextran containing 40 mol % of quaternised amino groups as side reactions [19], Pharmacia), P(DEAE MA) (polydiethylaminoethyl methacrylate, Rohm & Haas), P(DMAE MA) (polydimethylaminoethyl methacrylate, Coatex), P-α-lys [poly-(α-L-lysine), hydrobromide, molecular mass: 15 000–30 000 g/mol, Sigma] and P-ε-lys [poly-(ε-L-lysine), Chisso]. Prior to use, they were purified by ultrafiltration against pure water by using an Amicon cell equipped with a YM10 ultrafiltration membrane.

Jeffamine ED 600 [*O,O′*-bis(2-aminopropyl)-polyethylene glycol 500, Fluka] was used as received.

Me-OPEG-NH_2 2000 (Shearwater Polymers Europe, The Netherlands) was re-crystallised (chloroform–cold diethyl ether) before use.

Oligomers of lactic acid o-PLA50 (average molecular mass, $\overline{M}_n$ 500 according to nuclear magnetic resonance analysis) were synthesised by polycondensation of D,L-lactic acid syrup (Sigma) promoted by vacuum distillation of water (0.1 mmHg) up to 150°C under a nitrogen atmosphere (1 mmHg = 133.322 Pa).

Thionyl chloride and tetrabutylammonium bromide (Aldrich) were used as received.

A one litre solution of 25 m*M* creatinine background electrolyte (CBE) was obtained by dissolving 2.827 g of creatinine (Sigma) in pure water, adjusted to pH 4.8 with acetic acid (Merck).

2.2. Synthesis

The coupling between o-PLA50 and Me-OPEG-NH_2 2000 was achieved in two stages. On the one

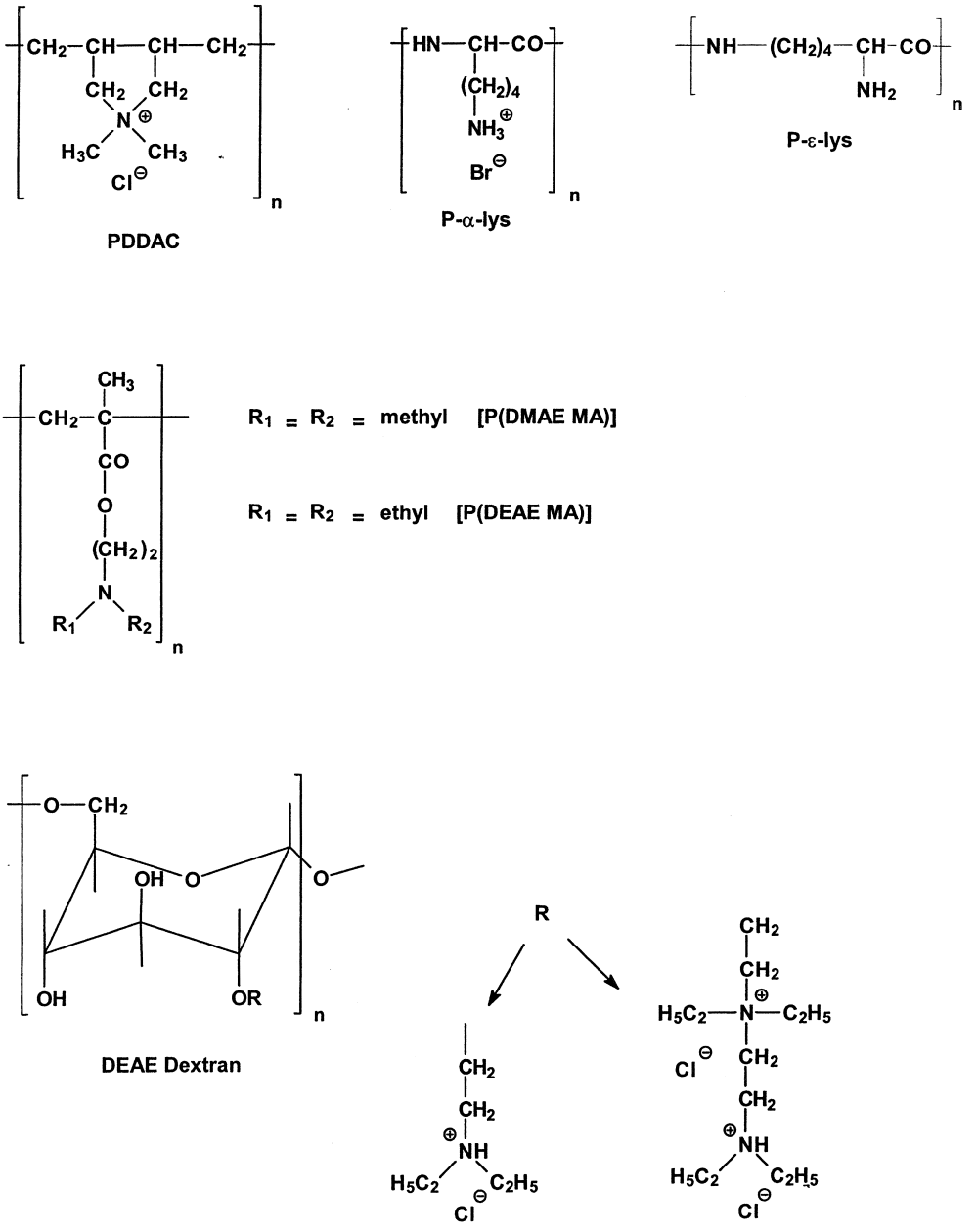


Fig. 1. Structures of the different polycations (PCs) used in this study.

side, o-PLA50 (0.4 g; 0.8 mmol) was introduced into a three-necked flask equipped with a magnetic stirrer. Thionyl chloride (40 ml) was added. The solution was stirred at room temperature under a nitrogen atmosphere for 12 h. Thionyl chloride was then removed by distillation under reduced pressure. The collected activated o-PLA50 was finally dissolved in dichloromethane (40 ml). In parallel, Me-OPEG-NH_2 2000 (0.98 g; 0.49 mmol) was dissolved in distilled water (70 ml). The pH was fixed at 9 by adding 1 *M* NaOH solution dropwise and finally, tetrabutylammonium chloride (15 mg) was added. To achieve the coupling, the activated o-PLA50 solution was introduce dropwise into the Me-OPEG-

NH_2 2000 solution under vigorous agitation (Ultraturax at 24 000 rpm). During the whole process, the pH was maintained at 9 by adding 1 *M* NaOH. The emulsion was further stirred for 5 min. The pH was then lowered to 3 by adding 1 *M* HCl. The organic phase and the aqueous one were separated. The solid samples issued from each phase, namely Cp-Aq and Cp-Org, were recovered by freeze–drying and solvent evaporation, respectively.

2.3. Methods

2.3.1. CZE

Data were collected using a P/ACE 5000 Beckman instrument equipped with UV detection at 230 nm. The FSC (57×50 μm I.D.) was used in the reversed polarity mode. The capillary was conditioned every day using the following rinses: 1 *M* HCl for 30 min, water for 1 min, 1 *M* NaOH for 30 min and CBE for 5 min. Furthermore, the capillary was rinsed before each run according to the following sequence: 0.1% PC in CBE for 5 min, water for 1 min and CBE for 3 min. Separations were performed at 25°C with an applied voltage of −20 kV. After the last run of the day, the capillary was rinsed with 1 *M* NaOH for 30 min, followed by water for 5 min before being stored wet. All the samples (sample concentration≈3 g/l) were initially dissolved in CBE, and injected at the cathodic side of the capillary by the hydrostatic pressure method (10 s).

2.3.2. Mathematical treatment of CZE data

The matrix UA=$f(t)$, where UA and t are the UV absorption signal and the migration time, respectively, was transformed into UA=$f(-1/t)$ by using Sigmaplot 4.0 for Windows that allowed a direct view of the transformation.

2.3.3. Mass spectrometry

Electrospray ionization (ES) MS data were carried out using an Autospec X EBEqQ mass spectrometer (Micromass) under a voltage of 8 kV. Injection of the analyte dissolved in a mixture of water–acetonitrile (2:1, v/v) proceeded via a 10-μl loop thermostated at 80°C. Calibration was performed with cesium iodide.

3. Results and discussion

In 1993, Bullock described a capillary electrophoretic separation with the normal injection mode of positively charged poly(ethylene oxide) macromolecules based on the use of a background electrolyte composed of creatinine and of neutral poly(ethylene oxide) additives under normal injection mode [20]. This method was tested without polymer additive but on checking it we found poor reproducibility from run to run. This weakness was probably due to adsorption of creatinine onto silica. Therefore, we decided to test the reversed mode with PCFSC by considering different PCs (Fig. 1).

The tested polycations were commercially available as polymers or as monomers which could be easily polymerised via radical polymerization as in the case of methacrylic polymers. PDDAC bore quaternary ammonium groups and was permanently charged regardless of the pH of the background electrolyte. In contrast, the other PCs bore weak basic groups whose ionization was pH-dependent, thus giving flexibility for separation performance. It must be noted that DEAE Dextran is actually a bifunctional polybase bearing both strong and weak basic side groups [19].

3.1. Influence of the nature of PCs on the electroosmotic flow of PCFSC

With indirect UV detection, analytes give negative peaks which are due to displacement of the absorbing background electrolyte [21]. In the present investigation, electroosmosis appeared as a positive peak without the presence of neutral marker (see Fig. 2). This peak was located between 5 to 8 min according to the nature of the polycation used to coat the capillary (Table 1). It is important to note that: (i) for a given PC, t_{EO} slightly increased from run to run, and (ii) the average t_{EO} value was strongly dependent on the nature of the polycation.

3.2. CZE separation of Jeffamine ED 600

Jeffamine ED 600 is a mixture of poly(ethylene oxide) bearing primary amino groups at both ends:

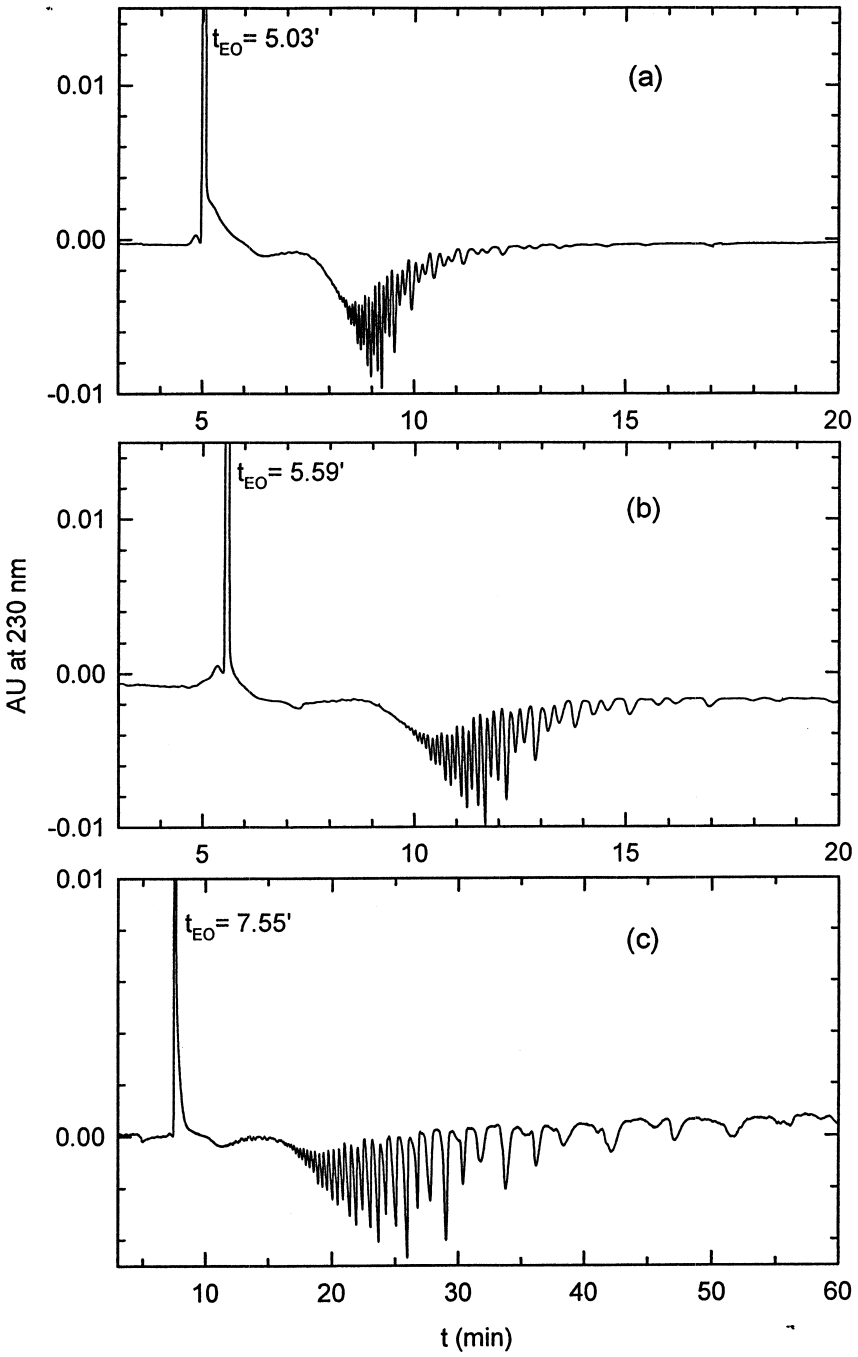
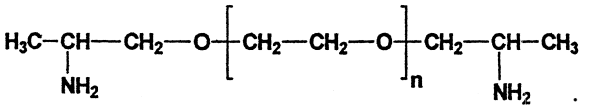


Fig. 2. Electropherograms of Jeffamine ED 600 as a function of the nature of the PC: (a) P(DEAE MA), (b) PDDAC, (c) DEAE Dextran. I.D. of the FSC = 50 μm; total length = 57 cm (total volume = 1120 nl); temperature = 25°C; reversed mode (V = −20 kV); background electrolyte = CBE; hydrostatic injection: 10 s (or 10.5 nl).

Table 1
Variation of the time of electroosmosis (in min) as a function of the nature of the PC, and for a given PC of the run number in an analysis sequence. I.D. of the FSC = 50 μm; total length = 57 cm (total volume = 1120 nl); temperature = 25°C; reversed mode (V = −20 kV); background electrolyte = CBE; hydrostatic injection: 10 s (or 10.5 nl)

PC	Run				
	1	2	3	4	5
P(DEAE MA)	5.03	5.13	5.28	5.40	5.54
P-α-lys	5.21	5.37	5.54	5.79	5.92
PDDAC	5.59	5.80	5.98	6.10	6.26
P(DMAE MA)	5.78	5.93	6.06	6.20	6.43
P-ε-lys	6.81	6.87	6.94	7.05	7.27
DEAE Dextran	7.49	7.60	7.65	7.68	7.85



It has a broad molecular mass dispersion as demonstrated by capillary electrophoresis [20].

Fig. 2 shows the CZE electropherograms obtained for Jeffamine ED 600 using different PCFSCs. The t_{EO} value which is characteristic of a given PC directly affected the separation of the positively charged analytes. The resolved signals appeared as negative peaks that were assigned to polymers whose charge/mass ratio increased as the migration time increased. The time of the separation and the shape of the electropherogram [AU = $f(t)$] strongly depended on t_{EO}.

When plotted as AU = $f(-1/t)$ (Fig. 3), data reflected the mobility of the species directly. Using the same x-axis scale mobility plots could be compared just after shifting along the x-axis [11,17]. It must be pointed out that resolution was improved for greater t_{EO}, which led to higher analysis time. The choice of PC was crucial since it governed resolution and analysis time in opposite trends, thus compelling compromises. Giving priority to resolution, the PCFSC with DEAE Dextran was chosen for subsequent studies.

It is noteworthy that a broad negative peak was observed at around -0.09 min^{-1} in Fig. 3c. It corresponds to an effective mobility of $\mu = +1.0 \cdot 10^{-4}$ cm^2 V^{-1} s^{-1}. This peak was assigned to the presence of a small amount of monoamine compounds in the family of polymers forming commercial Jeffamine, an observation that had already been reported in the literature [20].

3.3. Monitoring of the coupling of lactic acid oligomers onto a Me-OPEG-NH_2 sample

The coupling reaction is shown in Fig. 4. The carboxylic end-groups of o-PLA50 (I) were transformed to acyl chloride end-groups (II) in the presence of thionyl chloride. In fact, I was a mixture of oligomers of different polymerisation degree, a common feature in polycondensation. The resulting mixture of activated o-PLA50 in mol excess was allowed to react onto the NH_2 groups of Me-OPEG-NH_2 (III) to yield block copolymer (IV). III was also a mixture whose polydispersity was reflected through its ES-MS spectrum (Fig. 5).

Fig. 5 reveals a dispersion in molecular mass. The major peaks corresponded to the expected Me-OPEG-NH_2 structure and reflected a population of macromolecules with a maximum at 1700 g/mol. This value is slightly smaller than that given by the supplier, namely 2000 g/mol. Signals were composed of several peaks because of the presence of isotopes. Each signal corresponded to the $[M+H]^+$ species issued from Me-OPEG-NH_2 derivatives.

CZE of III did not show such a polydispersity (Fig. 6a). The negative peak centred at -0.122 min^{-1} had a monomodal shape corresponding to an effective mobility of $+0.6 \cdot 10^{-4}$ cm^2 V^{-1} s^{-1}. This value, that was lower than that of the monoamine derivatives of Jeffamine ED 600, agreed well with a decrease of mobility related to a smaller charge/mass ratio ($\approx 1/2000$ vs. $\approx 1/600$).

Fig. 6b and c show the electropherograms of the compounds issued from the reaction of II with III. The absence of the negative signal, which was shown as typical of III, revealed that no residual III was present. It was considered as a proof that the reaction progressed as expected. The population of neutral block copolymer molecules (IV) dispersed in water followed the electroosmotic flow. Moreover, these amphiphilic species could transport unreacted II molecules by solubilisation. The negative peaks located in the detection range of negative species were assigned to the oligomers of lactic acid, that were formed by hydrolysis of II and/or IV in the

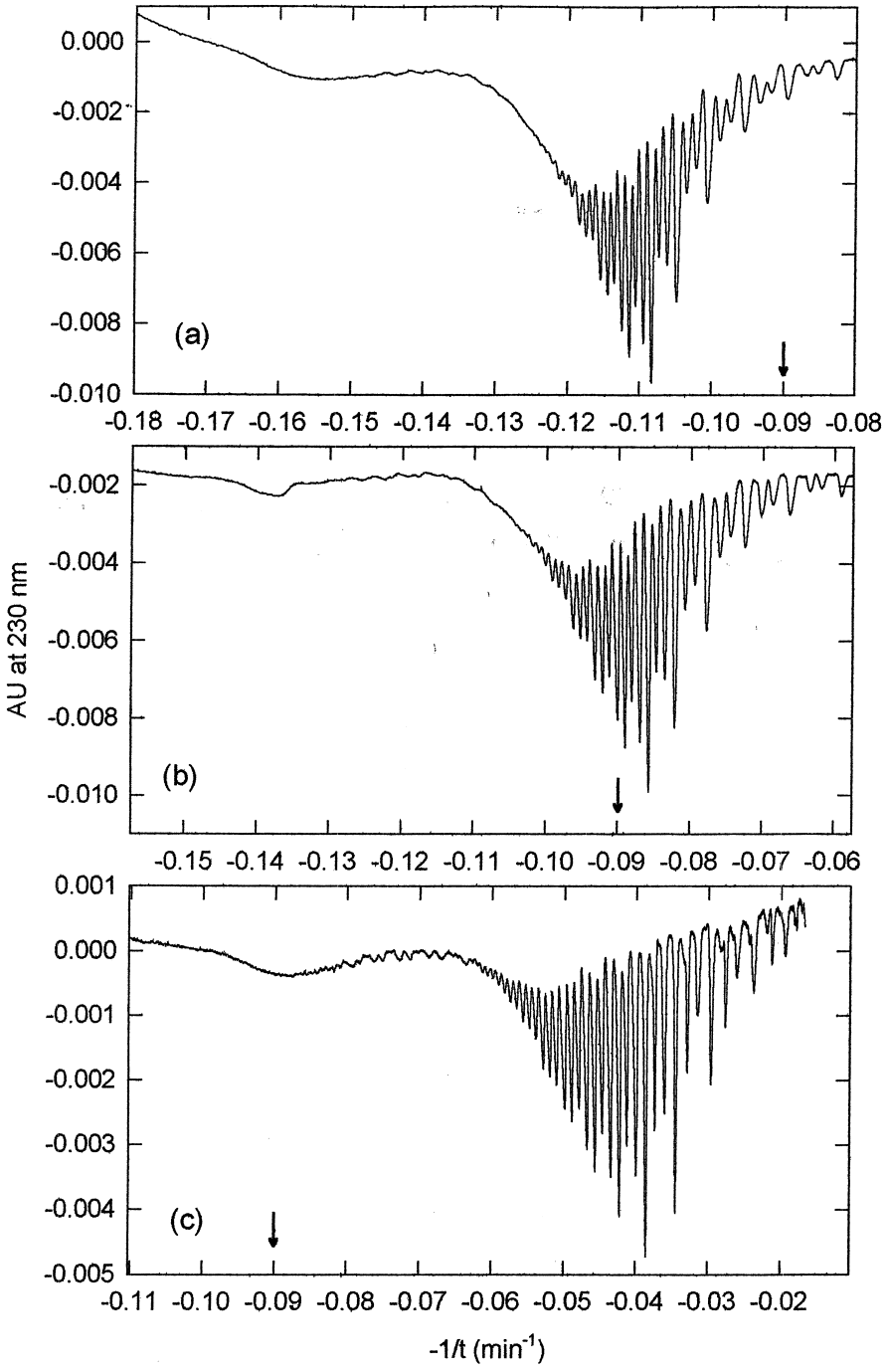


Fig. 3. Plots AU=$f(-1/t)$ of data of Fig. 2: (a) P(DEAE MA), (b) PDDAC, (c) DEAE Dextran.

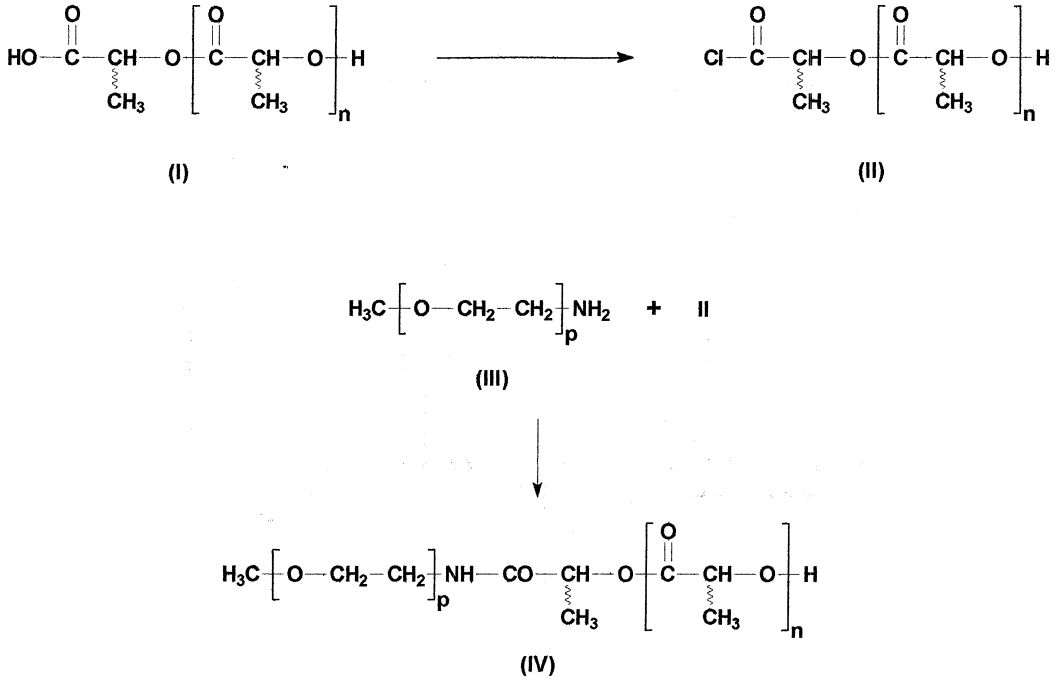


Fig. 4. Route to amide linked PLA-b-PEO.

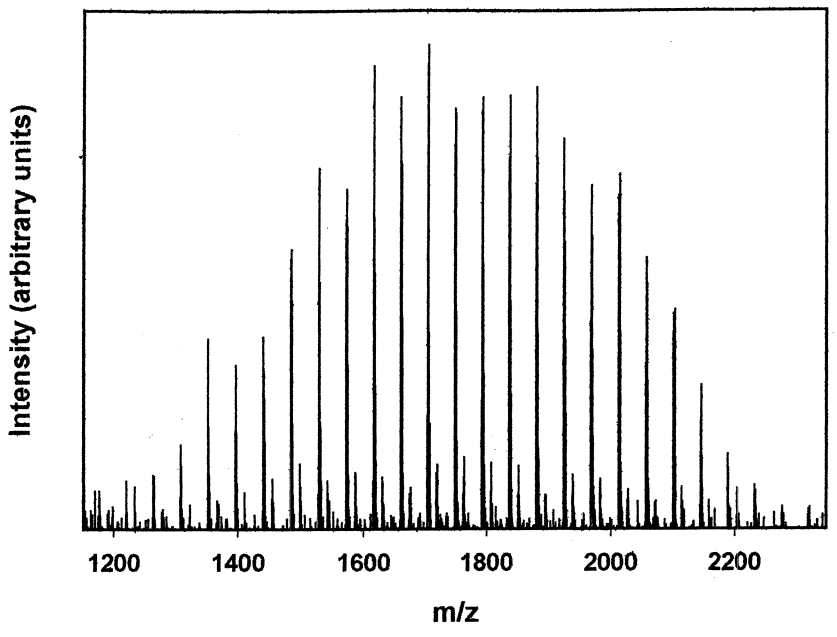


Fig. 5. ES-MS spectrum of the commercial sample of Me-OPEG-NH_2 (III).

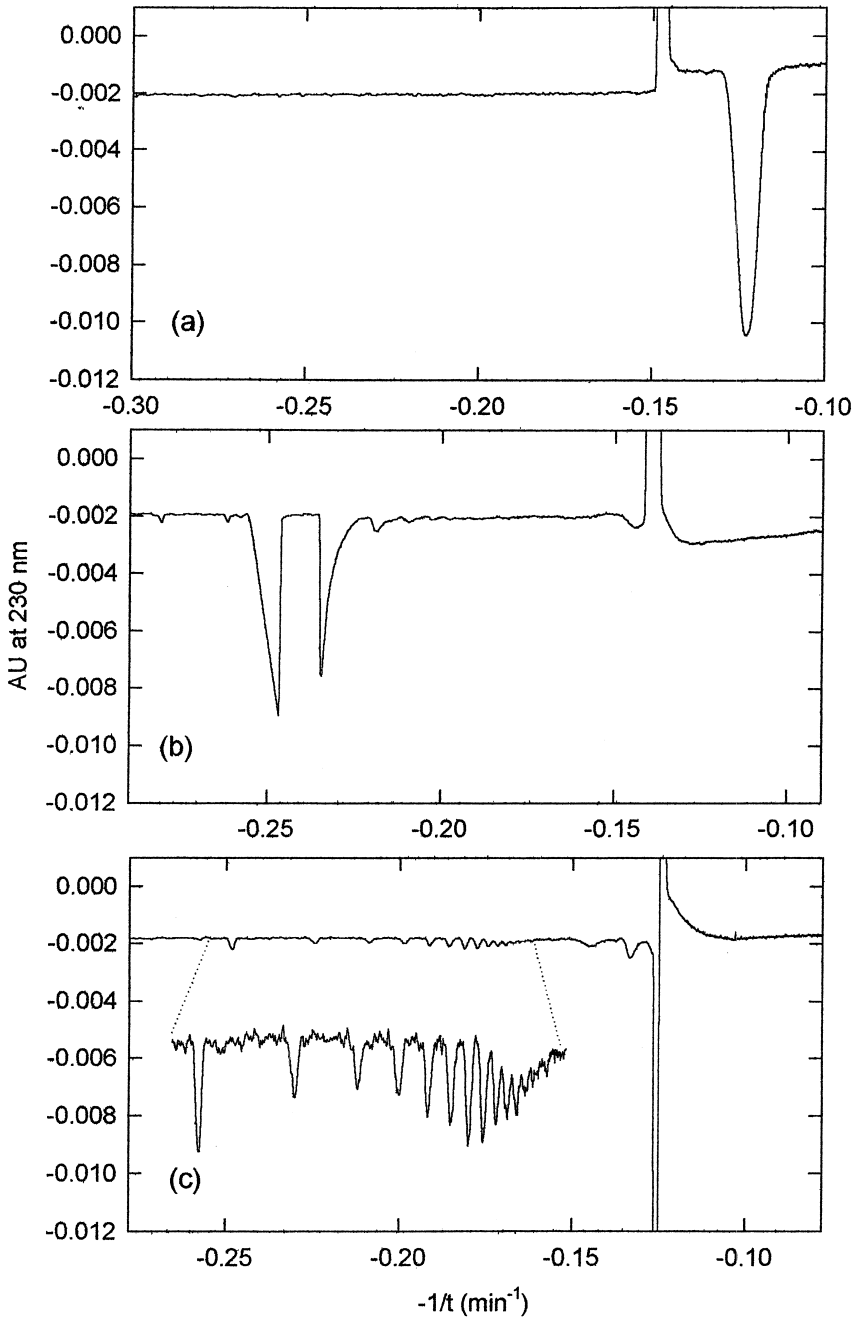


Fig. 6. Electropherograms showing the coupling efficiency of degree of Me-OPEG-NH_2 (III) onto activated lactic acid oligomers (II): (a) III before reaction; (b) aqueous phase of IV; (c) organic phase of IV. Fused-silica capillary coated with DEAE Dextran; I.D. of the FSC = 50 μm; total length = 57 cm (total volume = 1120 nl); temperature = 25°C; reversed mode ($V = -20$ kV); background electrolyte = CBE; hydrostatic injection: 10 s (or 10.5 nl).

presence of the water and the acidic media involved at the stage of the purification of IV. The whole series of oligomers was detected in organic extract (Fig. 6b). In the aqueous extract (Fig. 6c) only lactic acid, its dimer and its trimer were observed in higher amounts than in Fig. 6b, a feature which was yet observed for in vitro degradation on other poly(lactic acid) containing materials. It was due to the rapid hydrolysis of the higher water-soluble oligomers in acidic aqueous medium [10].

4. Conclusion

This study shows the potential of PCFSC for separation of polymers bearing a cationic charge at one or at both ends of the main chain. In the case of poly(ethylene oxide) derivatives, the separation method has to include indirect UV detection using creatinine as an absorbing cationic background electrolyte. Under these conditions, separations were strongly dependent on the electroosmotic flow which vary from run to run. The shape and the migration time values of the peaks were thus affected. Although the origin of the variation is still unknown, the separation did depend on a capillary zone process. Plotting data as $UA = f(-1/t)$ allowed a more precise presentation of the separation. A low electroosmotic flow is beneficial for a higher resolution while the analysis time is higher. Of the commercially available PCs, DEAE Dextran seems to be the most convenient compound.

The method allowed us to show that after the coupling of activated lactic acid oligomers and MeOPEG-NH_2, no free NH_2-terminated poly(ethylene oxide) segments remained. Moreover, the method showed the presence of water-soluble oligomers generated by partial degradation of lactic segments during the reaction. As such, it could be of interest to monitor the degradation of other related aliphatic polyesters in aqueous media.

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