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Characterization of the molecular heterogeneity of copolymers obtained via zwitterion by capillary zone electrophoresis

I. M. Perič (✉)[1], B. L. Rivas[2], S. A. Pooley[2], L. A. Basáez[1]

[1]Departamento de Química Analítica e Inorgánica. Facultad de Ciencias Químicas,
Universidad de Concepción Casilla 160-C, Concepción, Chile
[2]Departamento de Polímeros. Facultad de Ciencias Químicas, Universidad de
Concepción, Casilla 160-C, Concepción, Chile
e-mail: iperic@udec.cl Fax: (56)(41)245974

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Summary

Using capillary zone electrophoresis has made characterizations of different copolymers obtained via zwitterionic polymerization. Results demonstrate that this technique is able to put into evidence the molecular heterogeneity of the different copolymers. Analyses were performed in less than 10 minutes using a borate buffer. To our knowledge this the first time that free-zone capillary electrophoresis has been applied to the characterization of copolymers systems below the oligomer range obtained via zwitterion polymerization.

Introduction

It is known that the characterization of polyelectrolytes in size-exclusion chromatography (SEC) is problematic because these polymers are mainly soluble in aqueous media and may exhibit adsorption and exclusion effects with the stationary phase. In addition, aggregation effects of the analytes can interfere with the characterization [1]. Since the pioneering work of Jörgenson and Lukacs [2] capillary electrophoresis (CE) has found wide applications in the analysis of charged and uncharged compounds including biopolymers like DNA, proteins and carbohydrates [3-8]. The intrinsic advantages of CE are the principal use of aqueous buffers, the diminishing of adsorption effects on the capillary wall and the suppression of the electroosmotic flow (EOF) by special surface modification techniques. In this context CE has been shown to be an alternative for the separation of synthetic polymers [9]. Degree of polymerization, chain charge density and ionic strength dependences on electrophoretic mobilities have been studied for uniformly charged and well-characterized polyelectrolytes [10,11]. Recently, the separation of different series of the random copolymers like poly(2-acrylamido-2-methyl-1-propane-sulfonate-*co*-acrylamide), poly(acrylic acid-*co*-acrylamide), and poly(acrylic acid-*co*-N,N'-dimethylacrylamide) of variable compositions by free-zone CE have been described

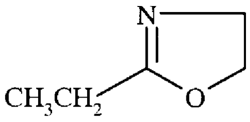
[12, 13]. Where the electrophoretic mobility increases as expected with the ionic monomer content. Encouraged by the high efficiency and rapid analysis that free-zone CE offers, we set out to investigate the potential of this technique for analysis of different water-soluble copolymers obtained by spontaneous polymerization. These were obtained in our labs by copolymerization via zwitterion using acrylic acid (AA) as electrophylic monomer and 2-ethyl-2-oxazoline (EtOx), thiazoline (Th) and 2-methyl-2-thiazoline (MTh) as nucleophylic monomers in different ratios [14, 15]. The main characteristic of these copolymers is their low molecular weight (Mn ≈ 2500), which is suitable to study their oligomer composition. To the authors' knowledge, there are no reports regarding the use of free-zone CE for the characterization of copolymers obtained via zwitterion. In this paper we report the results of the separation of these copolymers by using free-zone CE and direct UV detection. These results are analyzed by considering previous reports.

Experimental

Separations were conducted using a PrinCE 450 Capillary Electrophoresis System connected with a Lambda 1010 UV-Vis detector (Bishoff, Leonberg, Germany). System DAx software was used for data acquisition and analysis. Polymicro capillaries (Phoenix, AZ) were used with an inner diameter of 25-µm, an outer diameter of 375µm and a total length of 70-cm (55-cm to the detector). The experiments were performed at 30 kV and 25°C. Detection was by direct UV absorbance at 200 nm. Samples were injected hydrodynamically at 50 mbar for 15 s. The buffer was prepared using reagent grade boric acid (Aldrich). The buffer was adjusted to basic conditions using NaOH (Merck). Samples were prepared by dissolving copolymers at 1.0 mg/mL in 50-mM borate buffer. All solutions were filtered through 0.45-µm filters prior to being used.

Results and Discussion

Different copolymers obtained via zwitterion [14, 15] were analyzed using free-zone CE in order to determine the feasibility of using this technique for copolymer characterization. Acrylic acid (AA) was used as electrophilic monomer and 2-ethyl-2-oxazoline **1** (EtOx), thiazoline **2** (Th) and 2-methyl-2-thiazoline **3** (MTh) as nucleophilic monomers:



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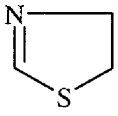

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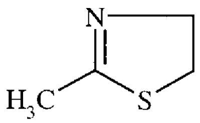

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It is known that zwitterion copolymerization result from spontaneous interactions of nucleophilic (M_N) and electrophilic (M_E) monomers without any additions of catalysts. Zwitterions form in the process that subsequently leads to formation of polymers. The step-growth polymerization mechanism can be depicted as follows:

$$M_N + M_E \rightarrow {}^{+}M_N\text{-}M_E^{-}$$
$${}^{+}M_N\text{-}M_E\text{-} + {}^{+}M_N\text{-}M_E\text{-} \rightarrow {}^{+}M_N\text{-}M_E\text{-}\,M_N\text{-}M_E^{-}$$
$${}^{+}M_N\text{-}M_E\text{-}\,M_N\text{-}M_E^{-} + {}^{+}M_N\text{-}M_E^{-} \rightarrow {}^{+}M_N\text{-}\,(\text{-}M_E\text{-}M_N\text{-})_2\,\text{-}M_E^{-} \rightarrow \rightarrow\rightarrow \text{polymer}$$

As the concentration of macrozwitterions becomes high and the monomer concentration decreases, the macrozwitterions reacts preferentially with each other. Alternating propagation occurs when dipole-dipole interactions between M_N and M_E are higher than ion-dipole reactions between ion centers of zwitterions and monomers, which in extreme cases lead to formation of homopolymers:

$$nM_N + {}^{+}M_N\text{-}M_E^{-} \rightarrow {}^{+}M_N\text{-}(\text{-}M_N\text{-})_n\text{-}M_E^{-}$$
$${}^{+}M_N\text{-}M_E^{-} + nM_E \rightarrow {}^{+}M_N\text{-}(\text{-}M_E\text{-})_n\text{-}M_E^{-}$$

IR, ^{1}H-NMR (90 and 400 MHz) spectra and elemental analysis have shown that among the obtained copolymers, only the copolymer AA-*co*-EtOx possesses an alternating structure, while others have a random structure [14, 15]. However, different types of heterogeneities may become important, depending on the composition of the monomer feed and the polymerization procedure. The structural complexity of synthetic polymers can be described using the concept of molecular heterogeneity meaning the different aspects of molar mass distribution, distribution in chemical composition, functional type distribution and molecular architecture distribution [16].

The electrophoretic migrations of the oligomers belonging to the alternating copolymer AA-*co*-EtOx (Fig.1a) are very closed, where band broadening effects can

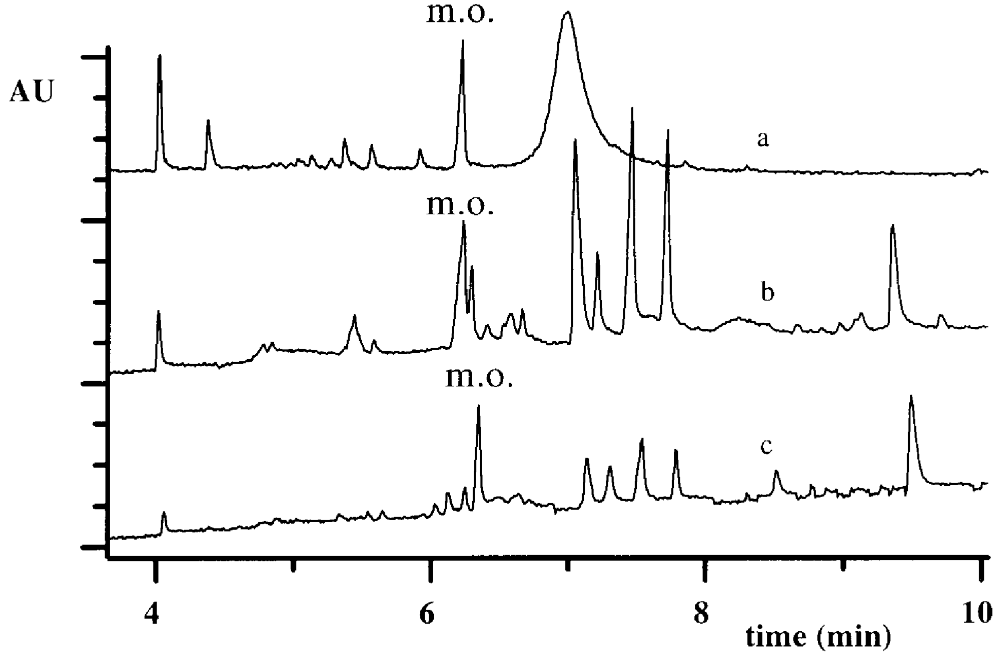


Figure 1 Electropherograms of copolymers AA-*co*-EtOx (alternate) (a), AA-*co*-MTh (random) (b) and AA-*co*-Th (random) (c). Carrier electrolyte: 50 mM borate buffer pH 10.0; voltaje: 30 kV; detection: direct UV at 200 nm; temperature: 25°C; capillary: 70 cm · 25 μm i.d.; Hydrodynamic inyection: 25 mbar for 12 sec; neutral marker: mesityl oxide (m.o.)

be the reason for the width of the main peak. Structural characteristics of this copolymer have been shown that these oligomers should not have any charge (Fig. 2a).

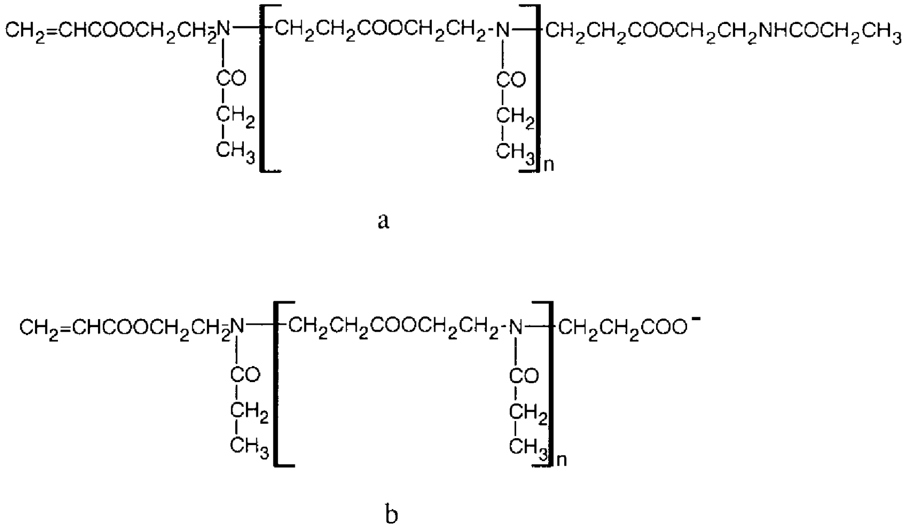
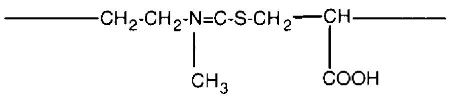


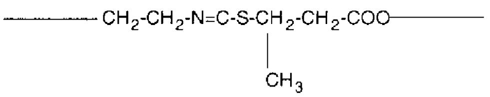
Figure 2 Chemical structure of poly(acrylic acid-*co*-2-ethyl-2-oxazoline) according to literature [14]. (a) neutral, (b) charged

Nevertheless, the main fraction of oligomers migrate slower towards the cathode than the internal neutral marker, mesityl oxide (m.o.), which migrates with the electroosmotic flow. This support one of the proposed chain termination mechanisms where a terminal carboxylic end group results, which should be negatively charged at alkaline conditions (Fig 2b)[14]. Moreover, it shows that molecular weight, as expected, is substantially below the normal oligomer range because a high molecular weight copolymer with one negative charge will not cause such a big mobility difference towards the electroosmotic flow.

On the other hand, the statistical copolymers AA-*co*-MTh (Fig. lb) and AA-*co*-Th (Fig. lc) show at least five negatively charged fractions. According to ^{1}H-NMR (90 and 400 MHz) and ^{13}C-NMR analysis these copolymers are richest in acrylic acid, where AA units are present in the backbone in two different forms according to the generated oligozwitterion [15]:



$$\text{—}CH_2\text{-}CH_2\text{-}\underset{CH_3}{N}\text{=C-S-}CH_2\text{—}\underset{COOH}{CH}\text{—}$$



$$\text{—}CH_2\text{-}CH_2\text{-N=}\underset{CH_3}{C}\text{-S-}CH_2\text{-}CH_2\text{-COO—}$$

The low molecular weight of these copolymers, the different acrylic acid content and the absolute electrophoretic mobility according to the number of carboxylic pendant groups, can explain the observed fractions (Figs. 1b and 1c). This in turn make evident a functional type distribution which is not clear from IR, ^{1}H-NMR and ^{13}C-NMR analysis. Consequently, the higher content of the carboxylic pendant group the most negative fraction and the slower absolute electrophoretic mobility towards the cathode results.

Conclusions

In conclusion, free-zone CE has shown to be useful in the study of these copolymer systems obtained via zwitterion polymerization, permitting the differentiation between the alternating copolymer AA-*co*-EtOx and the random copolymers AA-*co*-MTh and AA-*co*-Th. Moreover, it is shown that CZE presents a great advantage over conventional chromatographic techniques in characterizing copolymers by operating in a single aqueous phase system, thus excluding any unwanted interaction with a solid stationary phase. Also, CZE can be considered as a useful tool to evaluate the functional type distribution with similar copolymers systems. Consequently, a more extensive study of these and other copolymer systems is under further detailed investigation in our laboratory.

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