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Application of capillary zone electrophoresis to the separation and characterization of poly(amidoamine) dendrimers with an ethylenediamine core

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Abstract

Generations 0 through 5 of ethylenediamine-core poly(amidoamine) dendrimers were synthesized and capillary zone electrophoresis has been applied to the separation of different generations of synthesized dendrimers and for the characterization of individual generations.

Keywords: Dendrimers; Poly(amidoamine) dendrimers; Ethylenediamine

1. Introduction

Dendritic polymers are a new class of recently discovered polymeric substances, whose molecular architecture may be considered as a construction, consisting of initiator core and repeating units with branching (Fig. 1). The main specific structural feature of dendritic polymers is that in the course of growing to each repeating unit several repeating units may be connected at the branching point.

The initiator core may be connected with one or several repeating units forming asymmetric dendrons or spherical symmetric dendrimers, respectively. In case of poly(amidoamine) (PAMAM) dendrimers the initiator core is represented by an ammonia or by an ethylenediamine (EDA) molecule, which have three or four repeating units attached to the core, respectively. Each repeating unit of PAMAM dendrimers $-CH_2CH_2CONHCH_2CH_2N<$ has a branching with multiplicity 2, to which repeating units or some special terminal groups may be attached. In a simple case two hydrogen atoms are bonded to the branching point and the outer surface of PAMAM dendrimer is covered then with amino groups. One can imagine the ideal dendrimer as a central core covered with spherical layers of repeating units and in each next layer the number of repeating units is doubled.

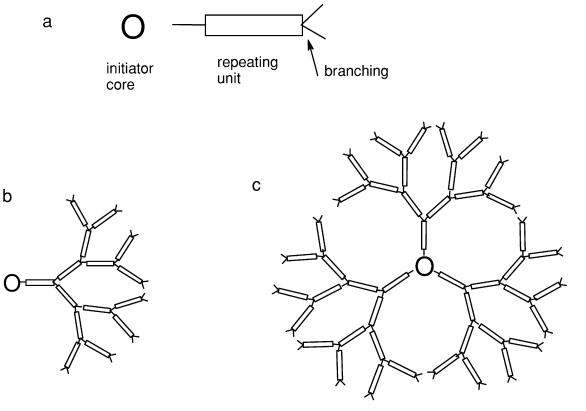

Fig. 1. Architecture of dendritic macromolecules: (a) structural elements, (b) dendron, (c) dendrimer.

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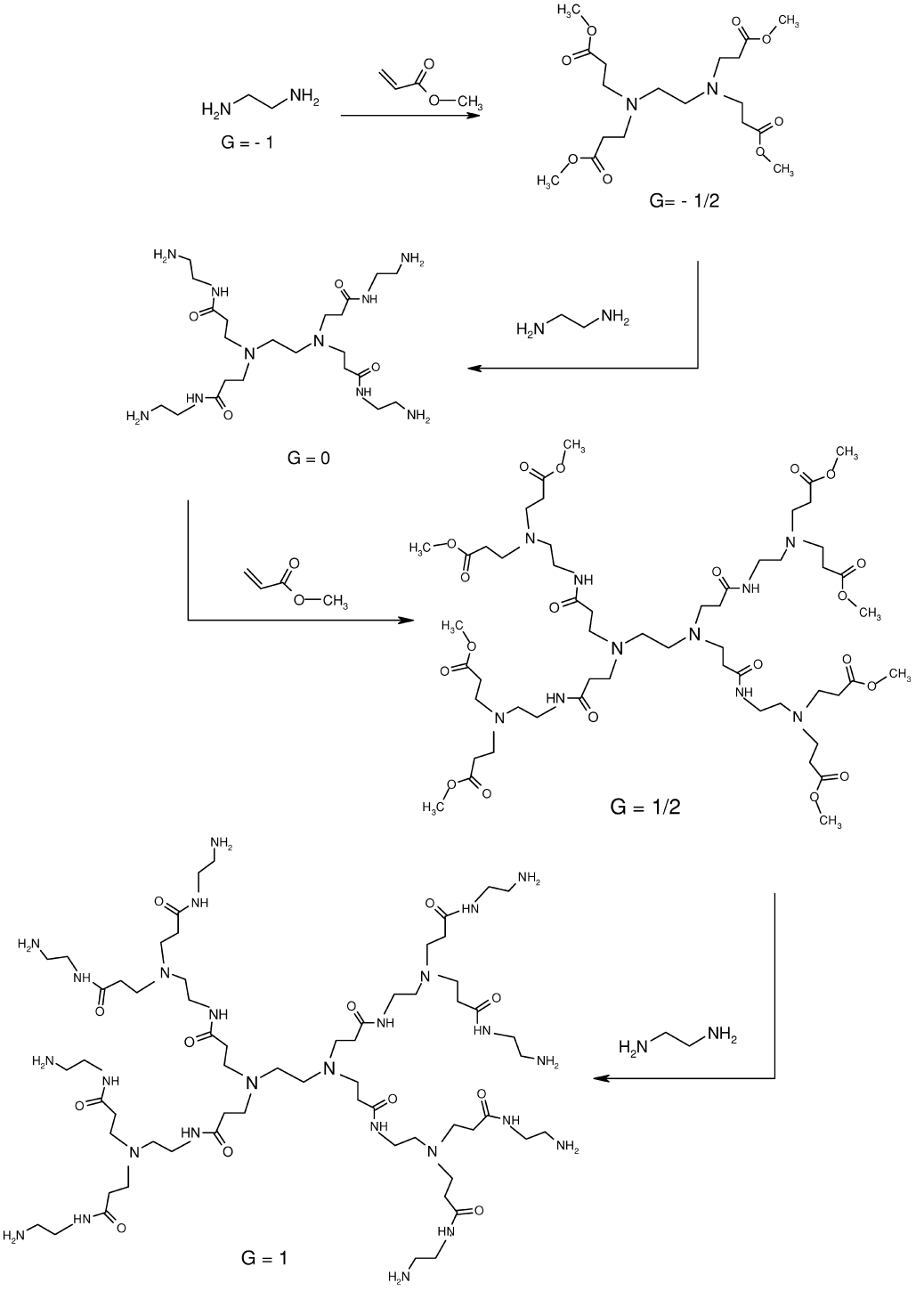

Fig. 2. Diagram of the synthesis of EDA-core PAMAM dendrimers.

For the synthesis of PAMAM dendrimers a well-working procedure has been developed [1,2]. The construction of EDA-core PAMAM dendrimer begins with alkylation of EDA with methyl acrylate according to the Michael addition reaction (Fig. 2). This results in a tetraester molecule the so-called generation −0.5 (G−0.5) dendrimer and the initial EDA itself may be considered as the generation G−1 dendrimer. The following amidation of tetraester with EDA yields the generation zero (G0) dendrimer with four terminal amino groups. The reiteration of this two-step procedure leads to the higher dendrimer generations. Such a strategy of constructing dendrimers, starting from the core, is known as divergent synthesis. The molecular mass of dendrimers and the number of terminal groups are rapidly increasing with the number of generations. For EDA-core PAMAM full generation G*N* the number of terminal groups is 4×2^{GN} (four is the number of repeating units linked to the core and two is the multiplicity of branching).

Dendrimers are the objects of extensive investigation. Owing to the unique properties, such as solubility in water, well-defined molecular architecture, spherical shape, and possibilities to be modified, dendrimers have found numerous applications in medicine, science and technology [3,4]. They may be used as calibration standards in size-exclusion chromatography (SEC) [5], as pseudostationary phases in electrokinetic chromatography [6], as drug and X-ray contrast agent delivery systems [7,8] or as carriers of genetic material into cells [9–11]. Some dendrimers have light-emitting properties that may provide new and unexpected applications [12]. Dendritic polymers may be applied as ingredients in molecular nanotechnology. Recently, some dendrimers became commercially available compounds (e.g., PAMAM and polypropylene imine dendrimers).

Most existing instrumental methods (e.g., IR spectroscopy, NMR, SEC, mass spectrometry with chemical ionization, electrospray ionization, matrix-assisted laser desorption ionization, atomic force microscopy, etc.) have been involved in the investigations of dendrimers [13–16]. During the synthesis of dendrimers, several side reactions may occur which lead to the formation of defective structures in all generations. In many cases the structure of the very first dendrimer generations and their defective impurities were thoroughly studied, but generational separation of dendrimers with higher molecular masses poses a serious problem. Capillary electrophoresis (CE) is one of the few separation techniques that can be used for this purpose [17,18], because in CE, separation is not sensitive to mass but to mass-to-charge ratio.

The aim of present study was to synthesize the first six generations (G0 through G5) of EDA-core PAMAM dendrimers and to apply capillary zone electrophoresis (CZE) to the separation of different generations and to the characterization of individual generations of synthesized dendrimers.

2. Experimental

2.1. Synthesis of PAMAM dendrimers

EDA was purified by distillation over CaH_2. Methyl acrylate was Aldrich 99% pure material and was used as received. Solvents were Aldrich HPLC grade. SEC was performed on a 5×50-cm column packed with Sephadex LH-20. For column chromatography silica gel from Merck was used.

For the preparation of dendrimers we utilized divergent synthesis that was carried out via an excess reagent method. Dendrimeric generations were synthesized by iterative sequence of Michael addition and amidation reaction, starting from EDA core [1,2]. For synthesis of ester-terminated PAMAM dendrimers G−0.5, G0.5, etc. (Fig. 2), full generation from the previous iteration (EDA in the very first step) was dissolved in methanol and methylacrylate was added. The excess of methylacrylate and solvent were removed under vacuum and then half-generations were purified using chromatographic techniques. Generations −0.5 through 1.5 were purified by column chromatography and for G2.5–G4.5 SEC was utilized.

For the synthesis of amine-terminated PAMAM dendrimers the methanol solutions of EDA and multiester were mixed at low temperature (below −25 °C.) and allowed to react for several days in argon. The excess of EDA was distilled off as an *n*-butanol azeotrope and a pale amber-colored syrup was obtained as a product.

2.2. Capillary zone electrophoresis

CZE separations of synthesized dendrimers were performed on a laboratory-made CE set-up consisting of a high-voltage power supply unit (International High Voltage Electronics), an UV detector ISCO CV^4 and an uncoated fused-silica capillary (Polymicro Technology, Phoenix, AZ, USA) of 75 cm (length to the detection window 50 cm)×75 μm I.D. UV absorption was measured at 212 nm and the detector signal was recorded through an ADC (Keithley) at frequency 4.2 Hz. The half-generations of dendrimers were analyzed in 0.02 *M* phosphate buffer (pH 7.8) and for full-generations 0.1 *M* phosphate buffer (pH 2.7) was utilized. In general, the methodology of analysis was similar to the procedure used by Brothers et al. [18] for the separation of ammonia-core PAMAM dendrimers. At the beginning of each day, the capillary was rinsed with 0.1 *M* NaOH for 5 min and then with the run buffer. Before each injection the capillary was rinsed with Milli-Q water and buffer. For full-generations the voltage of 18 kV was applied and in case of half-generations the voltage was 16 kV. Samples were introduced electrokinetically at the anodic end of the capillary and the direction of electroosmotic flow was towards the grounded cathode. The analyzed samples were prepared by dissolving 1–2 mg of dendrimer in 1 ml of buffer and were run immediately after dissolving.

3. Results and discussion

Separation in CZE occurs when the molecules of analytes are charged and have different apparent electrophoretic mobilities. A straightforward approach assumes that the resistance experienced by the ion when flowing through the liquid medium is approximately proportional to the ion's mass (or size) and that mutual separation of ions takes place according to the charge–mass ratio. Half and full generations of PAMAM dendrimers exhibit different electrophoretic behaviour that depends also on the pH of the running buffer.

Dendrimers' full generations have terminal amino groups which become protonated in an acidic medium and the electrophoretic mobility of positively charged molecules is in the usual direction of electroosmotic flow (i.e., towards cathode). With increasing generation number the calculated charge-to-mass ratio remains essentially constant (assuming the protonation of all amino groups) and electrophoretic velocities of different generations should be

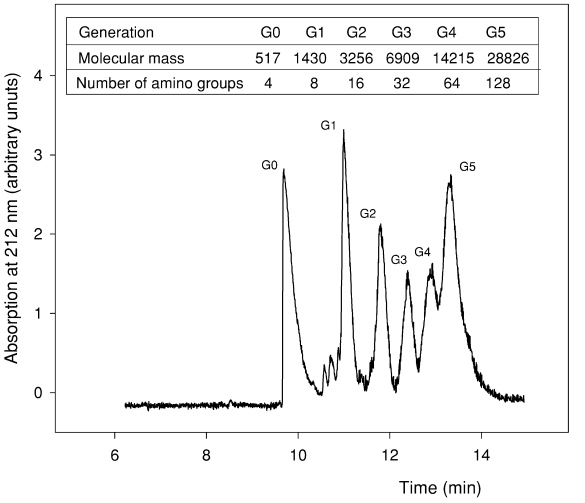

Fig. 3. Electropherogram of first six generations of EDA-core PAMAM dendrimers. (0.1 *M* phosphate buffer with pH 2.7).

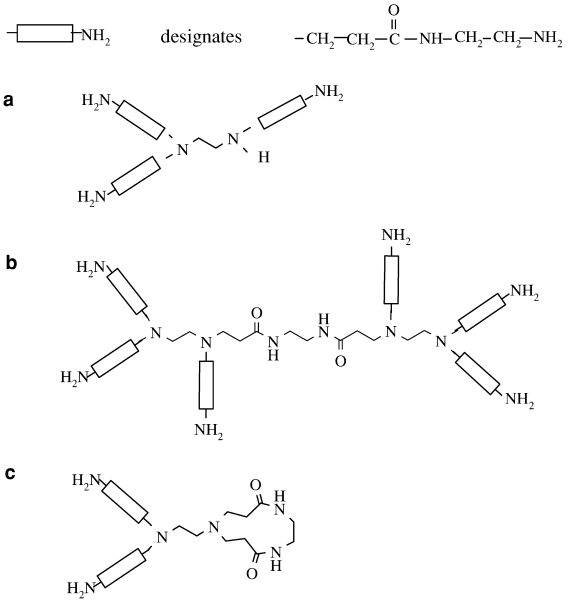

Fig. 4. Defective structures of G0 dendrimer: (a) missing arm, (b) dimer, (c) intramolecular cyclization.

close. Nevertheless our experiments with different generations of PAMAM dendrimers showed that in an acidic (pH 2.7) phosphate buffer, where the electroosmotic flow is practically suppressed, the separation occurs. Fig. 3 demonstrates separation of the first six generations of EDA-core PAMAM dendrimers with molecular masses ranging from 517 to 28 826. Similar results were obtained earlier for ammonia-core PAMAM dendrimers [18]; however, the profile of possible ''structural errors'' for EDA-core dendrimers is more complicated.

Electropherograms of single generations allow to characterize the homogeneity and the presence of side products in the synthesized dendrimers. There are three main types of side reactions that may occur during the synthesis of PAMAM dendrimers. The first one is the *retro*-Michael reaction, giving rise to asymmetrical missing arm structures (Fig. 4a). The second side reaction is the formation of dimers (or oligomers) in amidation step (Fig. 4b) and the third side reaction that also occurs during the amidation is the intramolecular cyclization (Fig. 4c). The presence of many functional groups in the outer shell provides different cyclization possibilities. Incomplete Michael addition also leads to the asymmetrical product in Fig. 4a.

Starting from the first generation all purified full generations contain the previous generation and its side products as the impurities (Fig. 5) (the same was observed with commercial Starburst dendrimer

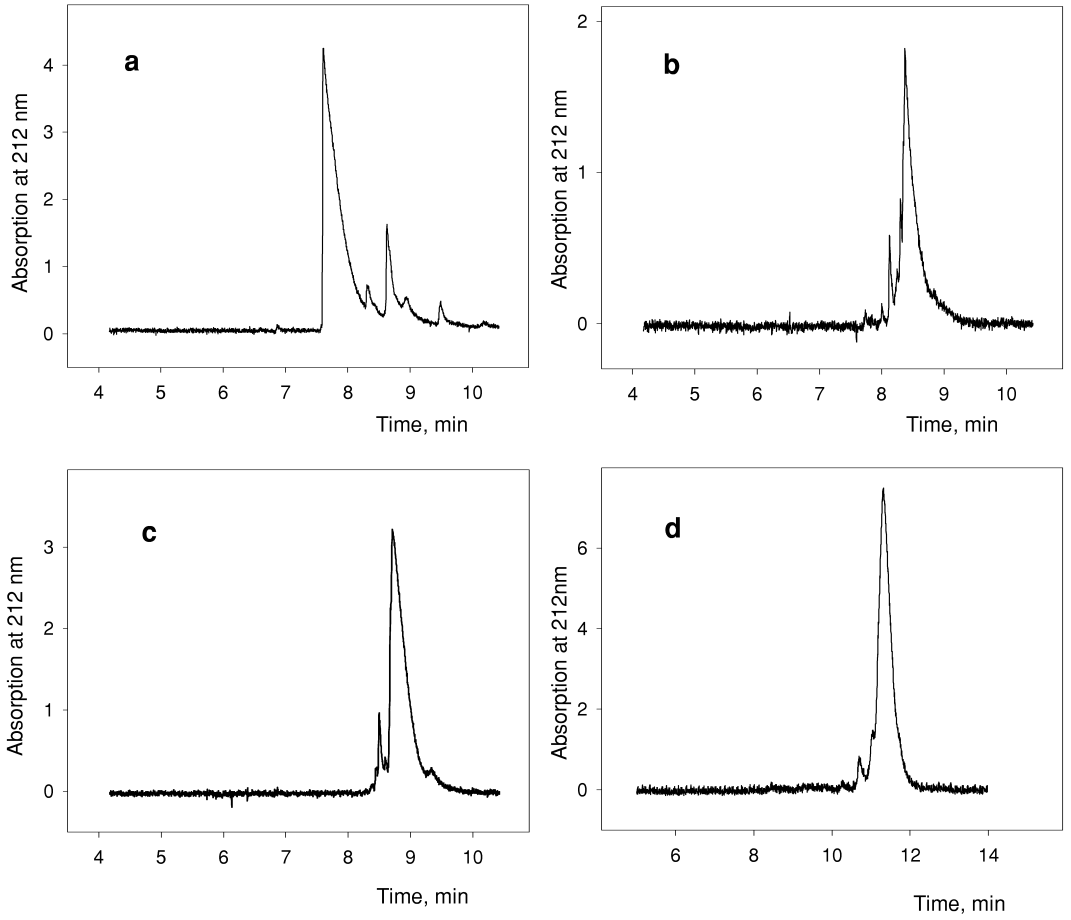

Fig. 5. Electropherograms of individual full generations of EDA-core PAMAM dendrimers. (a–c) Synthesized generations G0, G1 and G2; (d) Starburst G4 dendrimer from Aldrich.

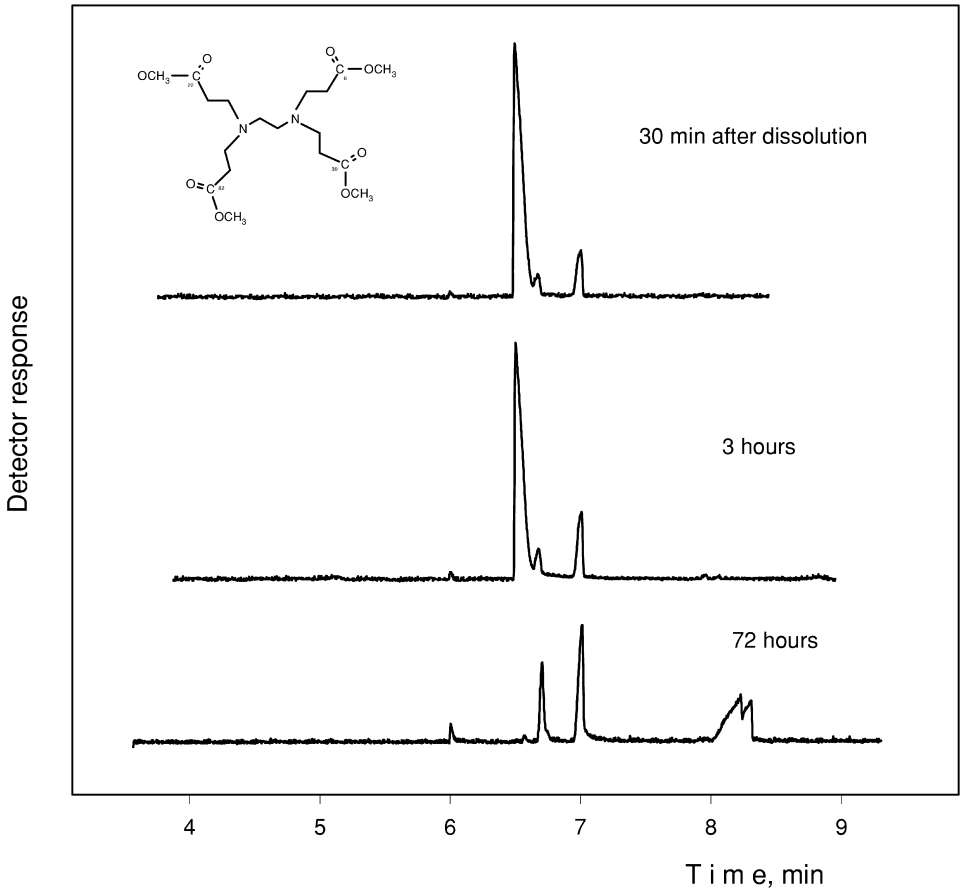

Fig. 6. Electropherograms of the generation of G−0.5 PAMAM dendrimer demonstrating the hydrolysis of half generations in phosphate buffer at pH 7.8.

purchased from Aldrich, Fig. 5d). In all generations there were also some impurities that migrated more slowly than the main component. Those impurities may be attributed to the missing arm defects, formation of dimers (or oligomers) or to intramolecular cyclization. At each looping, as in Fig. 4c, the molecule looses 60 u of mass and two terminal amino groups. In a perfect dendrimer molecule each $–NH_2$ group ''carries'' roughly 200 u of mass and it is obvious that because of increased mass-to-charge ratios the cyclic products should have lower electrophoretic mobilities. These slowly migrating products can be seen especially clearly in the electropherogram of the zeroth generation, where they form several peaks (Fig. 5a). When two G0 molecules form a dimeric adduct, the mass-to-charge ratio also increases (the loss of two NH_2 groups and 60 u of mass) giving rise to more slowly migrating species. Compared to the ammonia-core G0 PAMAM dendrimer with one cyclization possibility, in case of an EDA-core several loopings are possible. The branches of one nitrogen atom of central EDA may be connected (*N*,*N*-cyclization) or branches on different nitrogen atoms of central EDA may be bridged (*N*,*N′*-cyclization), forming *cis*- or *trans*-isomers.

Half generation amido esters are not ionized at conventional for CZE pH values and we did not succeed in separating the freshly prepared mixture of two different half generation dendrimers. However, in a moderately basic phosphate buffer with pH 7.8

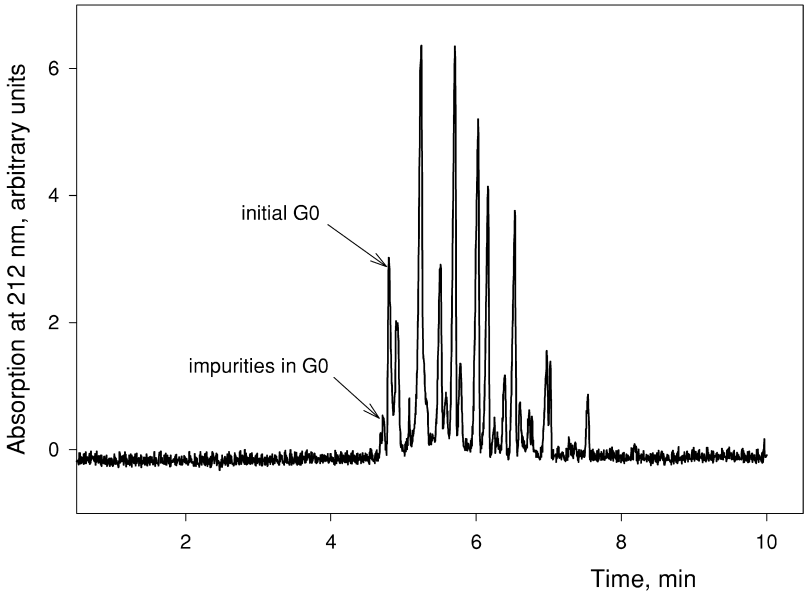

Fig. 7. Electropherogram of G0.5 hydrolysis products. Sample stayed in phosphate buffer (pH 7.8) for 24 h.

half generation dendrimers are markedly hydrolyzed. So, we could observe various carboxylate anions and kinetics of hydrolysis (Fig. 6).

Compared to the separation of full generation dendrimers, the cathode-directed electroosmotic flow in this case is relatively fast and electrophoretic migration of carboxylate anions is against the electroosmotic flow. The electropherograms in Figs. 6 and 7 show that for smaller G−0.5 and G0.5 generations hydrolysis products are totally separated. Hydrolysis products of generation G1.5 could be only partly separated and G2.5 revealed only one unresolved peak. For low generations the pattern of hydrolysis products in the electropherogram may be used for the elucidation of errors in the dendrimers' structures and for the characterization of individual half generations.

4. Conclusions

Synthesis of PAMAM dendrimers always generates ''structural errors''. CZE may serve as a suitable tool for quantitative estimation of the amounts of these ''errors'' in the first generations and for the assessment of homogeneity of single generations of PAMAM dendrimers. CZE allows the generational separations of EDA-core PAMAM dendrimers up to the fifth generation.

Acknowledgements

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