Ion-exchange electrokinetic capillary chromatography with Starburst (pamam) dendrimers: a route towards high-performance electrokinetic capillary chromatography

M. Castagnola[a,c,*], L. Cassiano[b], A. Lupi[c], I. Messana[b,c], M. Patamia[c],
R. Rabino[b], D.V. Rossetti[b], B. Giardina[b,c]

[a]*Dipartimento di Medicina Sperimentale e Scienze Biochimiche, Università di Roma "Tor Vergata", Rome, Italy*
[b]*Istituto di Chimica e Chimica Clinica, Facoltà di Medicina "A. Gemelli", Università Cattolica del S.C., Rome, Italy*
[c]*Centro di Studio per la Chimica dei Recettori e delle Molecole Biologicamente Attive, CNR, Rome, Italy*

Abstract

The use of different generations of Starburst (pamam) dendrimers provides a new opportunity for ion-exchange electrokinetic capillary chromatography. The uniform surface charge density and the structural homogeneity of these macroelectrolytes provide a decrease of plate height in comparison with micellar electrokinetic capillary chromatography with cationic surfactants. In addition, the use of different dendrimer generations allows the separation selectivity to be modulated.

1. Introduction

The presence of electroosmotic flow (f_{eo}) in capillary electrophoresis (CE) is the basis for a class of relatively new separation techniques collectively termed electrokinetic chromatography. The f_{eo} acts as a chromatographic eluent and the analytes are separated on the basis of their different interaction either with an electrolyte that migrates counterflow to the f_{eo} or with different stationary phases that are linked to the inner surface of the capillary. The best studied electrokinetic chromatography is micellar electrokinetic capillary chromatography (MEKC), originally developed by Terabe and co-workers [1,2]. In this case an anionic detergent [usually sodium dodecyl sulphate (SDS)] is added in the electrophoretic solution at a concentration greater than its critical micellar concentration (CMC); the negatively charged micelles migrate counterflow towards the anodic terminal of the capillary, such as a pseudo-stationary chromatographic phase. Another option for electrokinetic separations is the use of soluble polymer ions that migrate counterflow to f_{eo} [3]; in this instance the technique is termed ion-exchange electrokinetic chromatography (IEEKC).

Even though the separative potential of electrokinetic chromatography is impressive, the separation depends on many parameters which are difficult to optimize [4]. The counterflow electrolyte concentration, the pH of the solution,

* Corresponding author. Address for correspondence: Centro di Studio per la Chimica dei Recettori e delle Molecole Biologicamente Attive, c/o Istituto di Chimica e Chimica Clinica, Facoltà di Medicina e Chirurgia, Università Cattolica, Largo F. Vito 1, 00168 Rome, Italy.

the polarity and the percentage of organic solvents, the temperature and the applied voltage are important parameters that are mutually connected in the modulation of the selectivity and of the performance of the separation. In fact, the band broadening can be described, as for any chromatographic system, in term of plate height, which depends on several terms [5,6]; one of the most relevant is linked to the microheterogeneity of the counterflow electrolyte [4].

In order to modulate this microheterogeneity, recently the use of double-chain surfactants [7] or the modification of MEKC in microemulsion electrokinetic chromatography has been suggested [8]. In close comparison with chromatographic systems, one of the main reasons for the progress from conventional to high-performance separations was the availability of small, stable, uniform stationary phases; thus the availability of uniformly sized polyelectrolytes can contribute to obtaining electrokinetic separations characterized by high performance.

For this purpose, novel interesting dendritic molecules are available. They are obtained by "cascade" synthesis starting from an initiator core of a branched building block [9]. As a function of the starting block these substances were divided into several classes [10]; some of them were named arborols, silvanols, micellanes (or unimolecular micelles); recently, different generations of cascade dendrimers obtained from an initiation core of ethylenediamine became commercially available (Aldrich); they were collectively termed Starburst [poly(amidoamine); pamam] dendrimers (SPD). They are characterized by a surface with a uniform charge density. Tanaka et al. (11) reported that these substances can be utilized as good carriers for electrokinetic chromatography.

This study was aimed at establishing if the use of these compounds in electrokinetic chromatography provides a general increase in the separation performance. As the Starburst dendrimers utilized have terminal positive charges, the separations were carried out with an inverted electroosmotic flow with respect to the usual direction (anodic instead of cathodic electroosmotic flow) [12].

2. Experimental

2.1. Chemicals

Starburst (pamam) dendrimers of different generations were purchased from Aldrich (Milwaukee, WI, USA). Dodecyltrimethylammonium bromide (DTAB) was obtained from Sigma-Chemie (Deisenhofen, Germany), triethylamine of Sequanal grade from Pierce (Rockford, IL, USA), acetanilide and methanol of HPLC grade from Carlo Erba (Milan, Italy) and dansylamino acids from Sigma (St. Louis, MO, USA). Other common reagents were of analytical-reagent grade from Merck (Darmstadt, Germany) and were used as received.

2.2. Instrumentation and method

The MEKC separations were performed with a P/ACE System 2100 (Beckman, Palo Alto, CA, USA), equipped with GOLD software (Beckman); the capillary was of bare silica, 56.5 cm in length (50.0 cm at the detection window) × 75 μm I.D., with the injection terminal corresponding to the cathodic solution. The applied voltage was 15 kV and the temperature of the separation was 25°C (unless indicated otherwise). The electrophoretic buffer was 40 mmol/l phosphoric acid – 43 mmol/l triethylamine adjusted pH 8.3 with HCl (or NaOH in the case of DTAB solution) and mixed with methanol (80:20) to obtain a final concentration 4.0% (w/v) of cationic detergent. This concentration corresponds to 30.0, 12.3 and 5.8 mmol/l for SPD of generations 1, 2 and 3, respectively, and to 130 mmol/l for DTAB (considerably greater than its critical micellar concentration [13]). The samples were prepared at a concentration 0.3 mmol/l in a buffer obtained by diluting the separation buffer (without the cationic surfactant) in the ratio 1:9 with ultrapure water. The injection time was 1.0 s, corresponding to about 4 nl of sample solution and to about 10 fmol of sample injected. Detection was performed at a wavelength of 254 nm. The capillary, before each run, was rinsed with 1.0 mol/l NaOH for 5 min, washed with ultrapure

water for 5 min and equilibrated with the separation buffer for 2 min.

3. Results and discussion

Starburst (pamam) dendrimers (SPD) are a class of branched molecules, usually starting from an initial core of ethylenediamine. The first synthetic step in their production involves Michael addition of methyl acrylate to the nucleophilic core; after a reaction with an excess of ethylenediamine, a nucleophilic surface of generation 0 is obtained. Repetition of these two steps leads to the following generations, which are characterized by a number of repeat units or degree of polymerization (N_{ru}), number of surface groups (Z_s) and molecular mass (M_r) as a function of the initiator core multiplicity ($N_c = 4$) and its molecular mass ($M_c = 60.1$), branch cell multiplicity ($N_b = 2$) and repeat unit molecular mass (M_{ru}), according to recurrent equations described by Tomalia [14]. The values of N_{ru}, M_r, Z_s and total charge (Z_t) for the dendrimers of generation 0 to 3, are reported in Table 1, where several other properties of the dendrimers are also shown, such as the values of the ratio between their total charge and the molecular mass to the power 1/3 (which can be assumed to be approximately proportional to the dendrimer radius) and the values of the ratio between their surface charge and the molecular mass to the power 2/3 (which can be assumed to be approximately proportional to the dendrimer surface area). The first ratio should be related to the dendrimer mobility and the second should be proportional to the surface charge density and

should be related to the interaction with other electrolytes.

As dendrimers can be considered to be polymer solutes with a uniformly charged surface, the electrokinetic separation should be considered as ion-exchange electrokinetic chromatography, although further interactions might contribute to the solute retention [11]. When higher SPD generations become available, this definition could be reconsidered as the thermodynamic properties of a solution containing a macromolecule uniformly charged on the surface should be established and compared with micellar solutions.

In order to produce a counterflow migration of the positively charged SPD, an anionic f_{eo} is necessary; this can be achieved by the use in the separation solution of a high concentration of triethylamine [12]; in addition, the presence of a cationic surfactant such as SPD itself contributes to the generation of anodic electroosmotic flow [13].

After testing several substances, acetanilide was chosen as a marker of the f_{eo} mobility (μ_{eo}). In fact, its migration always coincided with a small disturbance on the baseline (if present) due to a sample plug. The mobility of f_{eo} and the mobilities of several standard dansylamino acids are plotted as a function of the surface charge density of the dendrimer multiplied by its concentration (Fig. 1); μ_{eo} increases as a function of the SPD generation and decreases as a function of the product of the surface charge density and the concentration. Hence the concentration of the dendrimer (over a limiting value) is not relevant for the generation of μ_{eo}, while the most relevant parameter should be related to the dimensions of the SPD. A dendrimer of higher

Table 1
Properties of Starburst (pamam) dendrimers as a function of their generation

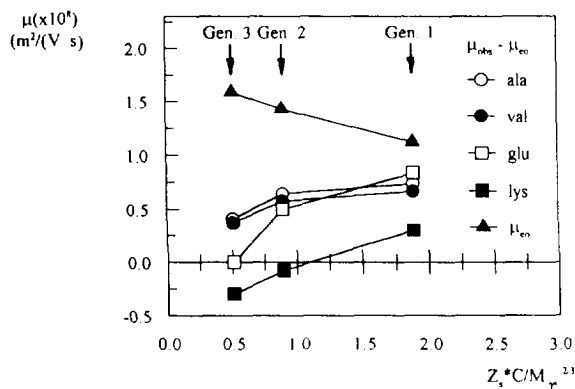| Generation	N_{ru}	M_r	Total Z (Z_t)	Surface Z (Z_s)	$Z_t/(M_r)^{1/3}$	$Z_s/(M_r)^{2/3}$
0	4	516	6	4	0.75	0.062
1	12	1430	16	8	1.42	0.063
2	28	3256	32	16	2.16	0.073
3	60	6909	64	32	3.36	0.088

For explanation of the symbols, see the text.

Fig. 1. Mobility of the electroosmotic flow (μ_{eo}) and intrinsic mobility [$\mu_{obs} - \mu_{eo}$, (Eq. 1)] of several dansyl-amino acids as a function of the product of the surface charge density and concentration for different SPD generations. The IEEKC conditions are given in Section 2.2.

generation has a greater effect on the inversion of the surface ζ potential, which is probably connected to the higher positive charge presented towards the solution by the higher dendrimer bound to the inner capillary wall.

As outlined by Terabe and Isemura [3], the observed mobility (μ_{obs}) in IEEKC is given by

$$\mu_{obs} = \mu_{eo} + x_f\mu_f + (1 - x_f)\mu_p$$

$$= \mu_{eo} + \frac{1}{1 + K[P]} \cdot \mu_f + \frac{K[P]}{1 + K[P]} \cdot \mu_p \qquad (1)$$

where μ_f and x_f are the mobility and the molar fraction of the analyte ion free from the polymer ion, respectively, μ_p is the mobility of the polymer ion, [P] its concentration and K the ion-pair association constant. It must be noted that μ_f and μ_p have opposite signs. This equation assumes that μ_p does not change significantly on interaction. Further, Eq. 1 does not account for any other interaction and it assumes that μ_{eo} does not change significantly as a function of [P]. As previously outlined, with the use of SPD this condition is approximately respected.

The results reported in Fig. 1 indicate that the intrinsic mobilities ($\mu_{obs} - \mu_{eo}$) of several standard dansylamino acids (Ala, Val, Glu, Lys) generally decrease as a function of the dendrimer generation (or increase as a function of the

product between the surface charge density and the concentration). This observation indicates that the last term of Eq. 1 increases as a function of SPD generation, either for a large increase in the ion-pair association constant and/or an increase in μ_p as a function of the SPD generation (see Table 1). This is particularly evident for the intrinsic mobility of dansyl-Glu, which in the case of SPD of generation 1 is the highest measured, owing to its negative charge, whereas in the case of SPD of generations 2 and 3 it is lower than the mobility measured for dansyl-Ala and dansyl-Val as a consequence of a strong increase in its ion-pair association constant as a function of dendrimer generation. The intrinsic mobility of dansyl-Lys is the lowest, obviously owing to its positive charge. Further, the modification of its mobility as a function of the SPD generation indicates that the interaction increases according to SPD dimensions more for dansyl-Lys than for dansyl-Ala and dansyl-Val. Thus mechanisms different from simple ion-pair interaction operate, as already observed by Tanaka et al. [11].

The uniformity of SPD chemical structure can contribute to a higher performance of the electrokinetic separation. The band broadening in MEKC [5,6] can be expressed in terms of several factors contributing to the overall plate height (H_{tot}), similarly to the common Van Deemter equation for chromatographic separations. The principal factors are the plate heights generated by longitudinal diffusion (H_{ld}), sorption–desorption kinetics in micellar solubilization (H_{mc}), intermicelle mass transfer in the aqueous phase (H_{aq}), radial temperature gradient effect on the electrophoretic velocity of the micelles (H_{tm}) and dispersion of electrophoretic mobilities of the micelles (H_{ep}). Among these factors, H_{ld}, H_{mc} and H_{ep} are those which, in MEKC, have the greatest effect on H_{tot}. In particular, H_{ep} is connected with the differences in mobilities of the micelles and, as a consequence, is linked to the microheterogeneity of the micellar phase. A similar treatment for IEEKC was not described. Nevertheless, the principal factors in IEEKC should be H_{ld} and H_{ep}, while the others can be considered negligible or unsuitable, e.g., H_{mc}
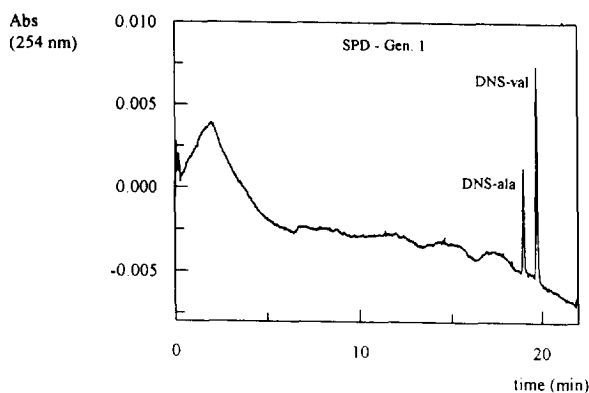
Fig. 2. Separation of dansyl-Ala and dansyl-Val in the presence of SPD of generation 1. The IEEKC conditions are given in Section 2.2.

should be described as a factor linked to the sorption–desorption kinetics of interaction of the analytes with the dendrimer (H_{ki}).

In Figs. 2–4, separations of dansyl-Ala and dansyl-Val in the presence of SPD of generations 1–3 are shown. In Fig. 5, for comparison, the separation of the same pair in the presence of DTAB at a concentration greater than its CMC is presented. This comparison is not completely pertinent since the electrokinetic DTAB separation is under micellar conditions, but, in the absence of an appropriate reference, is the best that can be proposed. In Table 2 the performance of these separations in terms of mean
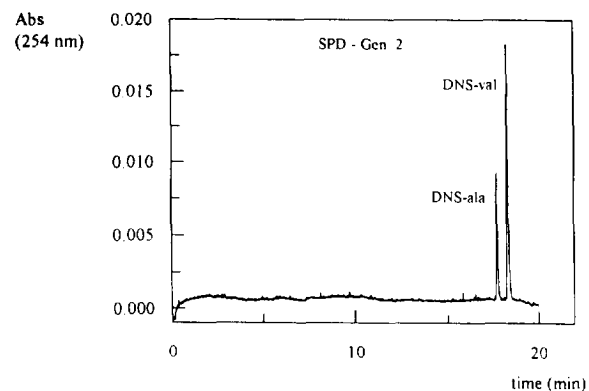

Fig. 3. Separation of dansyl-Ala and dansyl-Val in the presence of SPD of generation 2. The IEEKC conditions are given in Section 2.2.
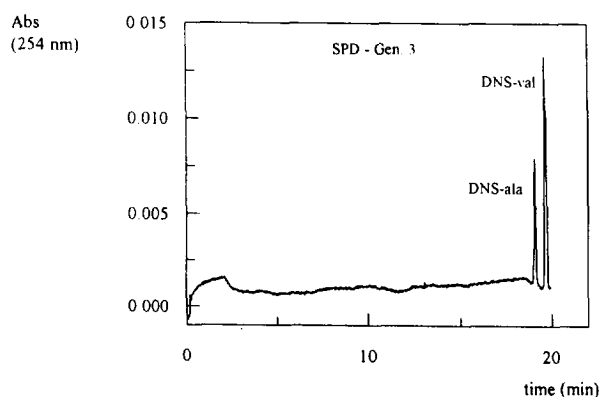

Fig. 4. Separation of dansyl-Ala and dansyl-Val in the presence of SPD of generation 3. The IEEKC conditions are given in Section 2.2.
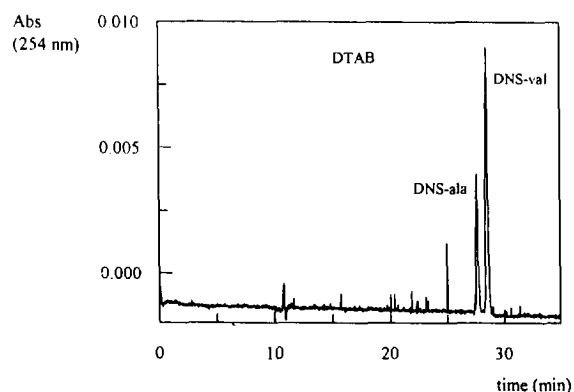

Fig. 5. Separation of dansyl-Ala and dansyl-Val in the presence of DTAB. The IEEKC conditions are given in Section 2.2.

theoretical plates, plate height and resolution is reported. Their values were calculated according to the usual relationships:

Table 2
Performance of IEEKC separation of dansyl-Ala and dansyl-Val by Starburst (pamam) dendrimers (SPD) of different generations

SPD generation	N	H (μm)	R
1	614 000	0.81	7.5
2	586 000	0.85	5.9
3	463 000	1.08	5.0
DTAB	277 000	1.81	4.0

$$N = 16(t_m/w)^2 \qquad (2)$$

$$H = L/N \qquad (3)$$

$$R = 2\,\Delta t/(w_1 + w_2) \qquad (4)$$

where w is the peak width at the peak base, t_m is the migration time and L is the length from the injection-point to the detection window of the capillary. As expected, the performance of the separations in the presence of SPD is about twice as good as that with DTAB. This can be linked to a strong decrease in H_{ep} due to the SPD microhomogeneity. The plate height increases with increase in generation. This can derive from a slight increase in H_{ep} as a function of SPD generation since, from the last term of Eq. 1, either multiple interactions of dansylamino acids can contribute to a greater dispersion of the mobility of the counterflow SPD polymer or greater molecular flexibility of the lateral chain in the SPD of higher generation can generate a variability of the ion-pair association constants.

Fig. 6 shows the dependence of the plate height on temperature. The separations in the presence of SPD of generations 1 and 2 showed a slight decrease in performance as a function of temperature increase; this is easily ascribed to an increase in H_{ld} and/or H_{tm}. The separation in the presence of SPD of generation 3 and in the presence of DTAB showed a minimum in the plate height at 30°C that suggested a complex
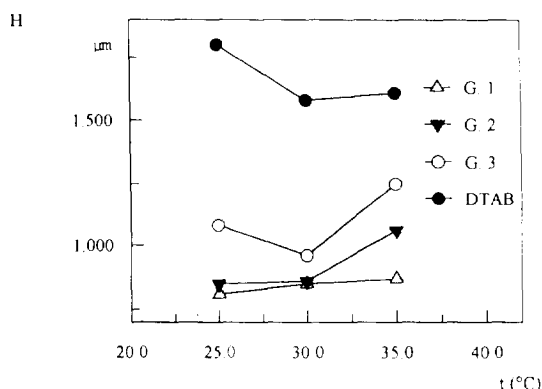
contribution of temperature to the performance of the separation; in DTAB separations the increase in performance as a function of increase in temperature is probably linked to stabilization of the micellar phase [4].

The greater complexity of MEKC with DTAB compared with IEEKC with SPD is evidenced by the performance of the separations as a function of the applied voltage (Fig. 7); in IEEKC with SPD the plate height decreased linearly when the voltage increased, according to the usual electrophoretic properties; in MEKC separation a strong increase in plate height was noted. With SPD of generation 1 and DTAB, the applied voltage was not set above 18 kV owing to the too strong current increase. As with generation 1 SPD the increase in voltage is linked to an increase in the separation performance, the decrease in performance observed with the use of DTAB cannot be ascribed only to a Joule effect.

In conclusion, IEEKC in the presence of SPD seems to be governed by general properties simpler than those observed in MEKC separations. The uniformity of their structure seems to provide an improvement in the separation performance. In addition, the different ion-pair interactions of the analytes as a function of SPD generation offer a new option for modification of the separation selectivity.

The future availability of SPD of generation

Fig. 6. Dependence of the plate height on temperature using different SPD generations and DTAB. The other IEEKC conditions are given in Section 2.2.
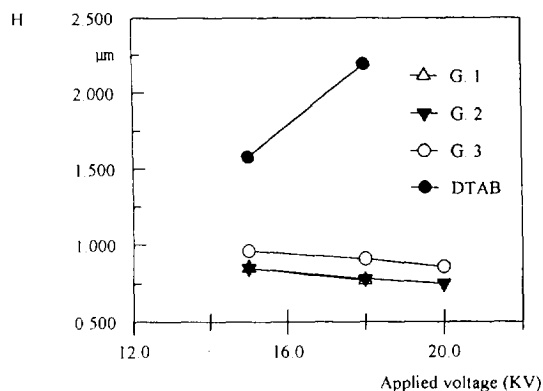

Fig. 7. Dependence of the plate height on applied voltage using different SPD generations and DTAB. The other IEEKC conditions are given in Section 2.2.

higher than 3 will permit the observations to be extended to a wider range and the thermodynamic properties of macrosolutes with a uniformly charged surface to be established.

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