ELSEVIER

Journal of Chromatography A, 758 (1997) 191–197

JOURNAL OF CHROMATOGRAPHY A

Comparison of ODS-modified silica gels as stationary phases for electrochromatography in packed capillaries

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Received 20 May 1996; revised 18 July 1996; accepted 14 August 1996

Abstract

The electroosmotic mobilities of the eluent generated during electrochromatography with a range of commercial ODS-bonded silica gel stationary phases packed into fused-silica capillary columns of 50 μm I.D. have been determined using thiourea as an unretained marker compound. The electrochromatography was carried out using acetonitrile–25 m*M* 3-cyclohexylamino-2-hydroxy-1-propanesulfonic acid buffer (pH 9.53) (70:30, v/v) as the mobile phase with electric field strengths in the range of 0.2 to 1.5 kV/cm. The experiments were carried out without pressurisation of the column, but the temperature of the column was controlled with a liquid coolant. Nucleosil 5 C_{18} and LiChrospher RP-18 showed the highest electroosmotic mobilities. However, these stationary phases had low chemical stabilities in the alkaline medium and only retained their properties for 2–10 days. Spherisorb S5 ODS2, Zorbax BP-ODS and Hypersil ODS all demonstrated high chemical stability but showed only moderate or low electroosmotic mobilities. No electroosmotic flow was detected with Partisil 5 ODS3 or with Purospher RP-18, highly base-deactivated column materials. The electroosmotic mobilities have been compared with the carbon loading and surface areas of the stationary phases and with the silanol activities determined chromatographically on the same stationary phases packed into polyether ether ketone (PEEK) micro columns (0.5 mm I.D.) using an aniline/phenol test mixture and elution with methanol–water (45:55, v/v). However, there was little correlation between the electroosmotic flow-rate and the silanol activity or carbon loading of the stationary phases. It appeared that materials specially suited for good liquid chromatography, such as Purospher, may not be useful in electrochromatography because they may create only a limited electroosmotic flow-rate.

Keywords: Stationary phases, LC; Electrochromatography; Silica, bonded; Electroosmotic flow; Capillary columns, packed

1. Introduction

There has been increasing interest in capillary electrochromatography (CEC) with packed columns, because it can potentially offer a much higher separation efficiency than conventional pressure-driven liquid chromatographic separations [1,2]. Recent papers have described applications of CEC in the pharmaceutical industry for neutral and ionised analytes using reversed-phase and ion-exchange columns [3,4]. Much of the work has concentrated on the parameters effecting the speed and efficiency of separations, including the applied voltage, eluent pH, ionic strength and proportion of organic modifier [5–10]. A number of papers have reported methods for the preparation of the capillary columns

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PII S0021-9673(96)00718-2

[2,7,9,11,12] and have discussed the effects of particle size, column fragility and problems with bubble formation in the eluent [2,7,8,10,12].

Unlike the parabolic flow profile characteristic for pressure-driven chromatography, in electrochromatography there is a plug flow resulting from the movement of the electrical double layer at the solid–liquid interfaces in the column as a result of the electrical field applied along the column [2]. The velocity of this flow (v_{eo}) is inversely proportional to the viscosity of the eluent (v) (and thus the temperature), the thickness of the electric double layer, $1/v$ (or Debye radius), the surface charge density (σ) and the applied electric field strength (E):

$$v_{eo} = \frac{1}{\kappa}\frac{\sigma E}{\eta} \quad (1)$$

The double layer thickness ($1/\kappa$) depends on the concentration and nature of counter-ions in the bulk solution. The surface charge density is dependent on the number and acidity of free silanol groups and the extent of their ionisation, the effective surface area and the pH of the solution [5,6]. The movement of a particular eluent through a column can be characterised by the electroosmotic mobility (μ_{eo}), which is determined by normalising the velocity of the eluent for the strength of the applied electric field (Eq. (2)).

$$\mu_{eo} = v_{eo}/E = L_{col}/t_R E \quad (2)$$

where t_R is the retention time for a chromatographically non-retained compound ($k=0$), L_{col} is the length of the packed column and E is the strength of the electric field in the column. In the absence of exact values for the potential differences across the packed and open portions of the capillary, the electrical field strength can be approximated as $E = V/L_{total}$, where V is the potential difference applied to the capillary and L_{total} is the total length of the column. The mobility is a characteristic parameter of a particular stationary phase, eluent and temperature.

Differences are expected in the electroosmotic flow produced by different brands of ODS and other bonded-phase packing materials, as the manufacturers will often have used different underlying silicas with different purities, pore sizes and surface areas and will have employed different bonding chemistries, including the possible use of end-capping. Some stationary phase materials will also have been treated to reduce interactions with basic groups (base-deactivated columns). This will have an effect on the surface chemistry and thus on the effective surface charge. However, in most cases, previous workers have described electrochromatographic separations on only a single or small number of stationary phases and no work has been carried out to compare the suitability of different brands and grades of packing materials.

This work describes a method for packing stationary phases into fused-silica capillary columns and uses this method to prepare a range of columns with selected packing materials from different manufacturers. Similar commercial materials have been used in the majority of previous papers from other researchers, but, as an understanding of the properties required is developed, bonded phases specifically designed for electrochromatography are likely to be developed in the future. The velocity of the electroosmotic flow in these columns was then measured and compared with their chemical and physical properties, including their silanol activities [13,14], which were determined chromatographically for the same phases packed into polyether ether ketone (PEEK) microbore columns. This preliminary study was aimed at demonstrating the range of flows that could be obtained and to gain an indication of the factors that could be used to predict suitable materials to obtain a significant electroosmotic flow.

2. Experimental

2.1. Chemicals and materials

3-Cyclohexylamino-2-hydroxy-1-propanesulfonic acid (CAPSO) was supplied by Sigma (Poole, UK). Acetonitrile and methanol were of HPLC grade from Fisons (Loughborough, UK). Water of 18.2 MΩ resistance was prepared using Elga Pure Water (Elga, High Wycombe, UK) purification equipment.

The fused-silica tubing was obtained from Composite Metals Services (The Chase Hallow, UK). The Vespel frits 1/16 in. O.D.×0.3 mm I.D. (1 in.=2.54 cm), PEEK tubing, PEEK column end-fittings and porous titanium frits were obtained from Phase Separations (Deeside, UK).

The stationary phase materials were Nucleosil 5 C_{18} (Macherey-Nagel, Düren, Germany), Zorbax BP-ODS (DuPont, Analytical Instruments Division, Wilmington, DE, USA), Partisil 5 ODS3 (Whatman, Maidstone, UK), LiChrospher RP-18 and Purospher RP-18 (Merck, Darmstadt, Germany), Hypersil ODS (Shandon Southern Products, Runcorn, UK), Spherisorb S5 ODS2 and Spherisorb Diol (Phase Separations).

2.2. Apparatus

A Beckman P/ACE System 2050 (High Wycombe, USA) with System Gold software was used for the electrochromatography experiments. The columns were installed into standard Beckman capillary cartridges with a detector aperture of 50 μm and were maintained at 25.0°C by a liquid cooling bath.

Liquid chromatography was carried out using the microbore system described previously [15].

Elemental analysis of the stationary phases was carried out using a Perkin-Elmer 2400 elemental analyser (Perkin-Elmer, Norwalk, CT, USA).

2.3. Preparation of packed capillary columns

In the Beckman instrument, the column is installed in a capillary cartridge. Short columns (20–30 cm long) could be wound into the cartridge after packing. However, longer columns (40–50 cm long) were wound onto the cartridge drum before packing, as the strain in the capillary after packing made them brittle.

A 50 μm I.D. capillary column was fitted with a PEEK 1/16 in. column end-fitting (7) containing a titanium frit 1/16 in. (6) (Fig. 1A). The capillary column was held into the fittings using a Vespel 300 μm I.D. ferrule (5). The ends of the capillary tube were mounted in PTFE sleeves [1/16 in. O.D. and 0.250 mm I.D.; (4)], in order to reduce the dead volume. The packing pump was filled with methanol and the stationary phase, suspended in methanol–benzyl alcohol–toluene (33:27:40, v/v), was drawn up into the packing bomb (150×0.5 mm I.D.) (2). The assembled column was then connected (Fig. 1B) and packed using a constant pressure of 500 bar. The column was agitated in an ultrasonic bath and mechanical vibration was applied to the interface

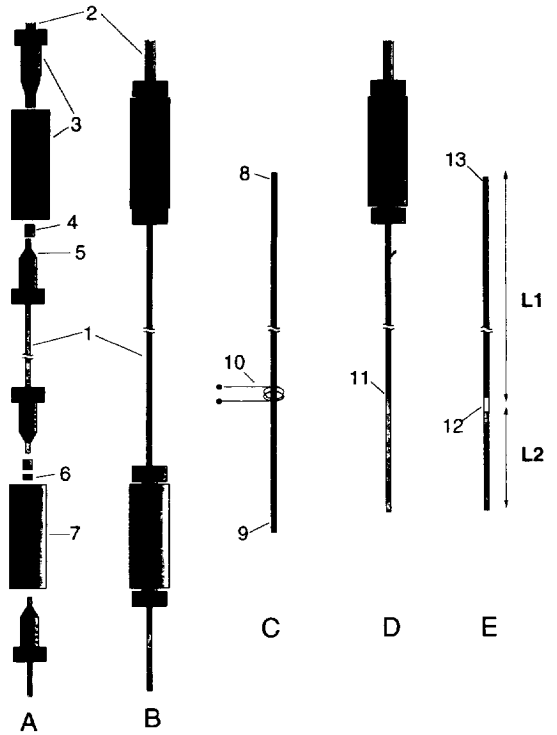

Fig. 1. Packing of capillary fused-silica columns for electrochromatography. (A) Assembly of the capillary column and its connections to the packing bomb: 1=capillary (0.05 mm I.D.); 2=packing bomb (150×0.5 mm I.D.); 3=Valco end-fitting; 4=PTFE sleeve (1/16 in.×0.250 mm I.D.); 5=Vespel ferrule (300 μm I.D.); 6=Valco porous titanium frit (1/16 in.) and 7=PEEK column end-fitting (1/16 in. I.D.). (B) Assembled capillary column prepared for packing; (C) 8 and 9=frits formed with sodium silicate solution; 10=sintering of the detector frit with an electric heater; (D) Set-up for flushing out packing material below detector frit (11); (E) final of the capillary column, 12=window for detection; 13=inlet frit; L1, L2=distances from the ends of the capillary column and the detector.

between the packing bomb and the column to prevent blockage of the column. After the suspension solvent had been eluted, methanol was flushed through the column and it was then allowed to relax. After depressurisation, the column was cut at both ends and allowed to dry at the ends. Sodium silicate solution [9] was sucked 3–4 mm into the ends of the column by capillary action (8 and 9 in Fig. 1C) to moisten the surface of the silica particles. The column was heated for 1 h at 40°C or left overnight at room temperature to form interstitial frits at both

ends. The column detector frit was then sintered in place using a heating element (10) (Fig. 1C) [9].

The outlet frit (9) was removed and the lower part of the column bed (below frit 11) was flushed out using a pump with the high pressure inlet end-fitting described earlier (Fig. 1D). The detector window (12) was then burnt in the polyimide coating and the packed capillary was cut to its final length (L1). The distance L2 should not be less than 7 cm, to fit into the column cartridge. If necessary, the inlet frit (13) was remade using sodium silicate (Fig. 1E). The column was installed in the cartridge and the working eluent was pumped through the column at a low flow-rate before it was installed in the CE instrument).

2.4. Measurement of electroosmotic mobility

The packed capillary columns were flushed with acetonitrile–25 m*M* CAPSO buffer, pH 9.53 (70:30, v/v), and placed in the electrophoresis instrument. A solution of thiourea in methanol was injected by application of voltage (3 kV) for 2 s and was eluted at 15–30 kV with detection at 214 nm. The electroosmotic mobility (μ_{eo}) of the eluent was calculated from Eq. (2), from the retention time of thiourea, the packed column length and the electric field strength in the column. The voltage was taken as the nominal value from the power source. Typical currents observed during a run were 0.7 to 1 μA at an electrical field strength of 1.5 kV/cm and changed little with time.

2.5. Preparation of PEEK microcolumns

PEEK microbore columns (150×0.5 mm I.D) containing the stationary phases were prepared as described previously [15], using a slurry medium comprising an aqueous solution of sodium dodecyl sulfate with methanol and sodium chloride under constant pressure (350 kg cm^{-2}).

2.6. Test procedure for silanophilic activity of stationary phases

The test mixture of aniline, phenol and thiourea [13] in methanol was chromatographed using methanol–water (55:45, v/v) at 40°C.

3. Results and discussion

Problems in the reproducible packing and handling of capillary columns have inhibited the ready adoption of electrochromatography and have been the subject of a number of studies [2,7,9,11,12]. Considerable skill has often been required and failure rates and breakages have been high, particularly once the frits and detector windows have been burnt through the polyimide coating. Most workers have firstly formed a sintered silica gel frit at the injection end of the column and then packed the bed into the column [2,9]. However, it is difficult to form a reproducible frit by this method and the frit can limit the linear flow velocity during the packing process, preventing the formation of a highly packed bed. In this study, a titanium frit held in a PEEK end-fitting was used during the packing stage, providing a consistent flow resistance. After the column bed had been formed, a terminating frit was created by controlled heating [9]. However, this method produces a carbon layer on the frit, which might produce a non-specific interaction with some analytes. We have also examined the use of supercritical carbon dioxide to dry-pack the column, which simplified the removal of the excess packing materials at the detector end of the column [16].

Because of the problems frequently encountered with localised heating in packed capillaries and degassing [2,8,10], an electrophoresis instrument was chosen, which employed a liquid cooling system to provide good thermal regulation. However, this was fitted with a column cartridge, which meant that all but the shortest columns had to be coiled. The procedure of installation of the filled column into the cartridge demanded extreme care, due to the low mechanical strength of the capillary in the region of the frit and the window. Improved results were obtained by packing the capillary column after it had been wound around the cartridge drum. Using this method, a number of different ODS-bonded silica gels from different manufacturers and a diol-bonded phase were packed into capillary columns (Table 1). The stationary phases differed in the ease with which they could be packed. Zorbax BP-ODS, which gave an efficient column, was very difficult to pack and had a tendency to block the capillary. In contrast, Purospher RP-18 and LiChrospher RP-18 possessed

Table 1
Properties of stationary phase materials, elemental analysis, retention ratio for aniline and phenol in liquid chromatography and electroosmotic mobilities of the eluent (μ_{eo}) obtained in packed capillaries and surface areas[a]

Stationary phase material	End-capping	Elemental analysis C (%)	Silanol test $k_{phenol}/k_{aniline}$	Surface area $m^2\ g^{-1}$	Electroosmotic mobility $\mu_{eo} \times 10^4\ cm^2\ V^{-1}\ s^{-1}$
Nucleosil 5 C_{18}	Fully capped	13.6	1.23	350	1.56
LiChrospher RP-18	Uncapped	21.7	1.25	450	1.45
Spherisorb Diol	Uncapped	1.9	not tested	220	0.80
Zorbax BP-ODS	Fully capped	14.5	1.00	350	0.68
Spherisorb S5 ODS2	Fully capped	10.8	1.24	220	0.50
Hypersil ODS	Fully capped	11.0	1.38	170	0.14
Partisil 5 ODS3	Fully capped	10.9	1.34	350	<0.01
Purospher RP-18	Chemically treated	17.8 N (%)=0.45	1.25	500	<0.01

Electrophoretic conditions as in Section 2.
[a] Published values from manufacturers literature.

excellent packing characteristics, probably due to their smaller particle size and good geometry. The columns typically had efficiencies of 40 000 to 60 000 plates, similar to those obtained for equivalent columns by other research groups.

A high pH eluent based on a 25 m*M* CAPSO buffer, pH 9.53, was chosen to ionise a high proportion of the surface silanols and to generate a strong surface charge [5,6]. The larger size of the biological buffer ions compared with the more frequently used borate buffer have low inherent mobility and reduced the current through the column and hence the heating effects. Similar buffers have been used in earlier liquid chromatographic studies in our laboratories, without significant degradation of either the column materials or buffer salts [17]. The columns were thermostated at 25°C and very few problems with degassing or bubbling were encountered, unless the temperature of the instrument was raised to 40°C. However, at this pH and under these conditions, the stabilities of the columns differed. The LiChrospher RP-18 and Nucleosil 5 C_{18} columns only retained their chromatographic properties for two-to-ten days before the efficiency and peak shapes degraded. The remaining column materials, in particular the Hypersil ODS and Spherisorb ODS columns, were much more stable and showed little change with time. The electroosmotic flow of the eluent in each of the columns was determined by measuring the retention time of thiourea, as a neutral marker compound. However, even after an extended elution time, no thiourea peak was detected for the Purospher RP-18 and Partisil 5 ODS3 materials and they appeared to have no (or a negligible amount of) electroosmotic flow. In order to compare the columns independently of the applied voltage and column length, the electroosmotic mobilities (μ_{eo}) were calculated from the velocities according to Eq. (2) (Table 1). The values differed markedly and the highest mobilities were found for the LiChrospher RP-18 and Nucleosil 5 C_{18} columns, with moderate mobilities for the Zorbax BP-ODS, Spherisorb S5 ODS2 and the Spherisorb Diol columns and a low mobility for the Hypersil ODS column.

These values were compared with mobilities for the same or similar phases calculated from published retention times for thiourea or reported eluent flow-rates (Table 2). Sometimes, only approximate values could be derived, as the full column dimensions were not always cited, so that the field strength could only be estimated and the column temperature was not always defined. It can be seen that the mobilities for the same stationary phases are of a similar order to the present work. However, a significantly higher value was found by Boughtflower et al. [9] for the Hypersil ODS column.

As dissociated silanol groups are believed to be the primary source of the negative charges on the surface of silica gel, the silanophilic activities of the stationary phases in the present work were tested, according to the procedure described by Engelhardt and Jungheim [13] and Schmitz et al. [14]. Each of the stationary phase materials was packed into a microbore PEEK column and the ratio of the relative

Table 2
Calculated electroosmotic mobilities for different stationary and mobile phases from reported flow-rates or retention times for thiourea

Stationary phase	Aqueous solution	Acetonitrile–buffer ratio (v/v)	Electroosmotic mobility $\mu_{eo} \times 10^4$ cm^2 V^{-1} s^{-1}	Reference
LiChrospher RP-18	2 m*M* Sodium tetraborate, pH 9.0	70:30	2.2	[8]
Spherisorb ODS	4 m*M* Sodium tetraborate, pH 9.1	80:20	0.3	[10]
Hypersil ODS	4 m*M* Sodium tetraborate, pH 9.1	80:20	0.8	[10]
Hypersil ODS	2 m*M* Sodium tetraborate, pH 8.7	80:20	1.8	[7]
Hypersil ODS	50 m*M* Tris buffer	80:20	1.5	[11]
Spherisorb ODS2	50 m*M* Tris buffer	80:20	1.6	[11]
Spherisorb ODS2	2 m*M* Borate buffer, pH 7.6	50:50	1.0	[9]

retention factors of aniline and phenol were determined using a methanol–water (45:55, v/v) unbuffered eluent. The measured values (Table 1) were similar to those reported previously (for example $k_{phenol}/k_{aniline} = 1.15$ for LiChrospher RP-18 and 1.36 for Hypersil ODS [14]). However, there was little correlation between this measure of silanol activity and the electroosmotic solvent mobility. The most obvious example is the marked difference between the LiChrospher RP-18 and Purospher RP-18 columns, which behaved almost identically in the silanol activity test but had almost the highest and lowest electroosmotic solvent mobilities, respectively. One reason is that the activity test effectively measures the degree of ionisation in a neutral uncontrolled pH solution and will be dominated by the presence of small amounts of very acidic silanol groups. Whereas, the electroosmotic flow is dependent on the much higher proportion of silanol groups that can be ionised at pH 9.53. However, the results suggest that, with the exception of ODS Zorbax, all of the phases examined should have a low silanophilic activity, as the aniline was eluted before phenol.

As an alternative estimate of the degree of surface coverage, the carbon loading of the stationary phases was measured (Table 1), but there seemed to be no correlation with the electroosmotic mobility. However, it seemed that a significant factor was the presence or absence of end-capping. The uncapped phases, LiChrospher RP-18 and Spherisorb Diol, both had significant electroosmotic flow. However, there was still a considerable variation among the end-capped materials: Nucleosil 5 ODS, in particular, and Zorbax BP-ODS and Spherisorb S5 ODS had significant solvent mobilities, Hypersil ODS showed a low electroosmotic mobility, whereas the Partisil ODS3 and Purospher RP-18 had negligible flow-rates. However, Hypersil ODS has been used successfully in other studies (Table 2).

The surface areas of the stationary phases (Table 1) were also compared with the mobilities (Fig. 2). Because the Purospher ODS appeared to be anomalous (see below), it was excluded. The remaining columns showed a limited correlation but there was concern that the published surface areas represent total areas, including the internal area of the pores, whereas Knox and Grant [2] have suggested that these internal areas should not contribute to the overall electroosmotic flow because of overlap between the wall effects in capillary tubes.

The Purosphere RP-18 material appeared to be

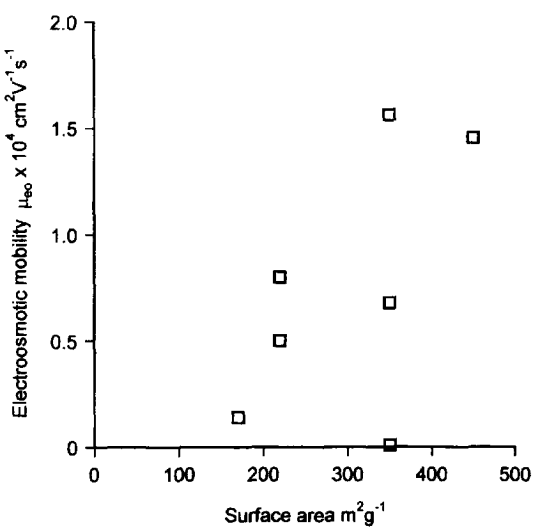

Fig. 2. Relationship between electroosmotic flow and reported surface areas of stationary phases (from Table 2).

anomalous, it had a similar silanol activity and carbon content to LiChrospher RP-18, but apparently generated a negligible electroosmotic flow. The elemental analysis of this phase revealed the presence of 0.45% N, suggesting that the column may have been base-deactivated by bonding positively charged ammonium groups to the surface. These would create a negative double layer and the electroosmotic flow would have been reversed. Under these conditions, the electroosmotic flow marker would not have been observed, giving the appearance of a negligible flow.

4. Conclusions

These results suggest that the liquid chromatographic properties of stationary phases may be a poor guide to their ability to generate significant electroosmotic mobilities on electrochromatography. Column materials specifically designed to give good liquid chromatography with a minimum of base interactions, such as the Purospher RP-18 column, are likely to have limited value in electrochromatography, as they may create minimal electroosmotic flows. In contrast, stationary phases that can acquire surface charges by ionisation at high pH, as in the uncapped phases, appear to be desirable in electrochromatography as they will provide significant electroosmotic flows. A permanent surface charge may have a further positive benefit, as indicated by the recent extremely high efficiencies reported by Smith and Evans [4] on an ion-exchange column, presumably partly because the strong electrical double layer inhibits or minimises analyte interactions with the stationary phases.

The variation between the different reported mobilities in this and earlier work also emphasises the need for the direct comparison of stationary phase materials under the same eluent conditions and temperatures.

Acknowledgments

Thanks to BDH Merck for a gift of Purospher RP-18 and LiChrospher RP-18 and to Beckman for a loan of a P/ACE 2050 capillary electrophoresis system.

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