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Short communication

Comparison of different packing methods for capillary electrochromatography columns

Stephanie Roulin[a], Richard Dmoch[a], Richard Carney[a], Keith D. Bartle[a,*], Peter Myers[a], Melvin R. Euerby[b], Christopher Johnson[c]

[a]*School of Chemistry, University of Leeds, Leeds LS2 9JT, UK*

[b]*AstraZeneca R&D Charnwood/Lund, Pharmaceutical and Analytical R&D, Bakewell Rd, Loughborough, Leicestershire LE11 5RH, UK*

[c]*AstraZeneca R&D Avlon/Charnwood, Process R&D, Bakewell Rd, Loughborough, Leicestershire LE11 5RH, UK*

Abstract

A study was carried out in which 50 μm I.D. fused-silica capillaries were packed with 3 μm octadecylsilane bonded silica, from the same batch, by four methods; liquid slurry and carbon dioxide supercritical carrier, each with and without the use of an ultrasonic probe. A neutral test mixture was analysed by capillary column in reversed-phase mode, and the reproducibility of the electroosmotic flow and of migration time, column efficiency and retention factors, was determined. Initially results suggested that there was no significant difference between properties of columns packed by different methods, and a more thorough statistical evaluation confirmed this; differences observed in the column performance were attributed to random variations between replicate columns, and not between packing methods. However, the variation was least when applying the ultrasonication during liquid slurry. © 2000 Published by Elsevier Science B.V.

Keywords: Electrochromatography; Electroosmotic flow; Column packing; Packing methods

1. Introduction

The most important part of the capillary electrochromatograph is the column, which represents the heart of the separation. The increasing interest in capillary electrochromatography (CEC) has generated a demand for efficient and reliable columns, and a number of procedures have been proposed [1–4]: among these, the use of liquid carriers with ultrasonication [2] have been pre-eminent, although significant advantages for supercritical carbon dioxide as carrier in producing highly efficient columns have been claimed [3]. Other procedures include electrokinetic [5] and centrifugal [6] packing.

In this paper, we compare the properties of CEC columns packed with the octadecylsilane (ODS) bonded silica from the same batch by four different methods: with liquid or supercritical CO_2 carrier, with and without ultrasonic agitation.

2. Experimental

2.1. Reagents and test compounds

The test mixture contained thiourea, anisole, benzamide, benzophenone and biphenyl (Sigma–Aldrich, Poole, UK). HPLC-grade acetonitrile and methanol were purchased from Riedel-de Haen (Seelze, Germany). Distilled water was used

*Corresponding author.

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throughout, and mobile phase was filtered through a 0.2-μm PTFE syringe filter (Acrodisc, Fisher Scientific, Loughborough, UK). Carbon dioxide was CP grade.

3 μm particle size Spherisorb ODS1 from a single batch (No. 95/219) with a surface area 190 $m^2 g^{-1}$, pore volume 0.45 ml g^{-1}, pore size 8 nm, with a bonded monofunctional C_{18} silane carbon loading of 7% (w/w), was kindly provided by Phase Separations (Deeside, UK). Fused-silica was purchased from Composite Metal Services (Worcester, UK). Mobile phase for CEC contained Tris buffer (Sigma–Aldrich) and hydrochloric acid (Fisher Scientific).

2.2. Packing the CEC columns

2.2.1. Supercritical carbon dioxide packing

Fifty cm lengths of 50 μm I.D.×375 μm O.D. fused-silica capillary were connected to the packing reservoir, which was previously cleaned and sonicated thoroughly with methanol and dried completely prior to packing. A 0.3-g amount of packing material was placed in the reservoir (Fig. 1). The end of the reservoir was connected to an Isco 26057 syringe pump (Jones Chromatography, Hengoed, UK) via a high-pressure valve to allow the introduction of carrier phase liquid carbon dioxide [3]. The end of the column was connected by a Valco union containing a metal screen (2 μm) to a linear restrictor (25 cm×30 μm I.D. fused-silica tubing). Both the capillary and the restrictor were immersed in an ultrasonic bath containing warm water (60–70°C). This temperature, together with the increase of the pressure allowed the liquid carbon dioxide to reach its supercritical state [7]. The liquid carbon dioxide was introduced into the system by opening the high-pressure valve. The column was sonicated and the pressure increased from 50 to 300 bar in steps approximating to 50 bar per minute. The pressure was then maintained at 300 bar. Continuous tapping of the reservoir was required to promote transfer of the packing particles into the capillary. After the capillary was packed to the required length (usually a packed length of 23 to 25 cm), sonication and tapping were stopped and the high pressure main-

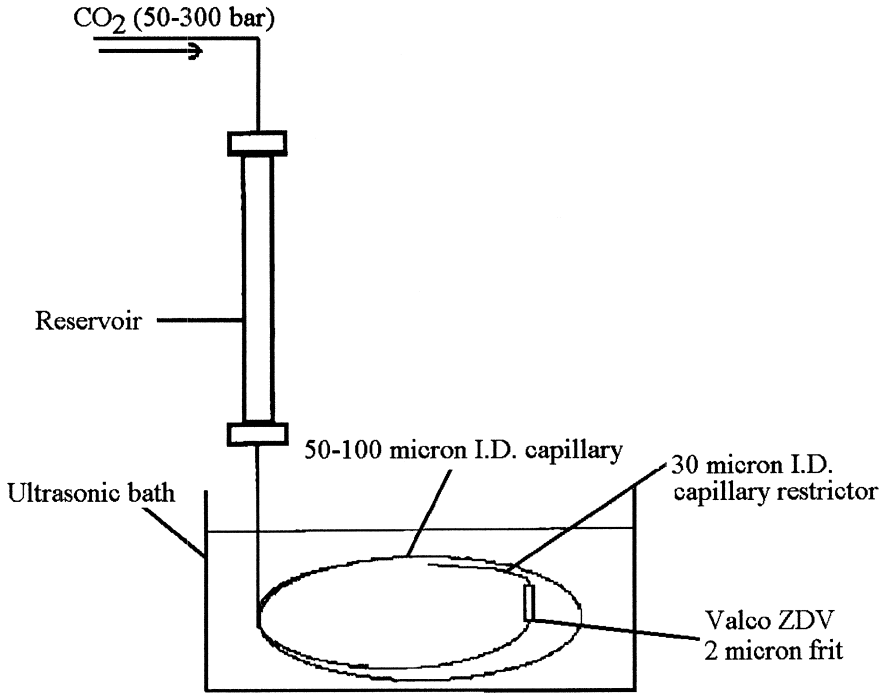

Fig. 1. Schematic of the apparatus for packing capillary columns using the supercritical CO_2 method.

tained for approximately 30 min to consolidate the packed bed. The high-pressure valve was closed, the reservoir disconnected from the pump and the capillary left to depressurise overnight.

2.2.2. Supercritical carbon dioxide with ultrasonic probe packing

The capillary was connected to the chamber of a MPSST ultrasonic miniprobe [2], (Kerry Ultrasonics, Hitchin, UK) used as a packing reservoir. An identical amount of the same packing material used in the previous method was placed in the chamber of the probe. One side of the reservoir was connected to the Isco pump via a high-pressure valve allowing the introduction of the carrier phase, while the other side was connected to the capillary, which incorporated a linear restrictor (25 cm×30 μm I.D. fused-silica capillary) at its end. Both the capillary and the restrictor were immersed in an ultrasonic bath containing warm water (60–70°C). Liquid carbon dioxide was introduced into the system by opening the high-pressure valve. The initial pressure was 50 bar, with the sonication at a ''medium'' setting, rapidly increasing to a ''high'' setting over 8 s. The pressure was also increased from 50 bar up to 300 bar in steps of 50 bar per minute, and finally a pressure of 300 bar was continuously applied. When the capillary was packed to the required length, ultrasonication was stopped and the pressure held constant for approximately 30 min. The high-pressure valve was then closed, the probe disconnected from the pump, and the column left to depressurise overnight.

2.2.3. Liquid slurry packing

Columns were packed with a Shandon slurry packer (Shandon Southern, Runcorn, UK). A slurry mixture composed of 0.1 g of packing material in 2 ml of acetonitrile was prepared and sonicated for 20 min in order to obtain good dispersion and achieve a homogeneous suspension of the packing material. The slurry medium was then introduced into a previously cleaned and dried reservoir (diameter 0.5 cm) (Fig. 2). One side of the reservoir was connected to a valve allowing the introduction of the carrier mobile phase, while the other side was connected to the fused-silica tubing (50 cm×50 μm I.D.) to be packed which incorporated a linear restrictor at its end (25 cm×30 μm I.D. fused-silica tubing) containing a metal screen (2 μm). The reservoir was immersed in an ultrasonic bath containing cold water. Acetonitrile was introduced into the system at 300 bar. The reservoir was then sonicated and the

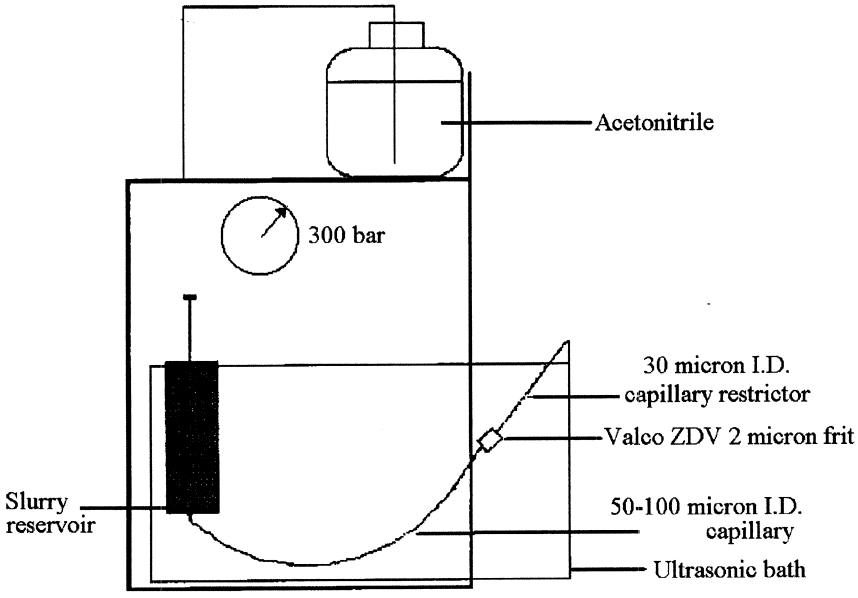

Fig. 2. Schematic of the apparatus for packing capillary columns using the slurry method.

pressure held constant at 300 bar throughout the packing process. A microscope was required to check the passage of particles into the column. When the capillary was packed to the required length, sonication was stopped and constant pressure maintained for approximately for 30 min. The valve was then closed and the column depressurised for 1 h.

2.2.4. Liquid with ultrasonic probe packing

The apparatus used for the liquid slurry packing and the ultrasonic probe used in the supercritical CO_2 method were combined. The liquid slurry mixture was sonicated for at least 20 min prior to being placed in the chamber of the probe [4]. An identical fused-silica capillary was connected to the packing reservoir while the end of the column was connected to the same type of linear restrictor as described above. The other side of the reservoir was connected to a valve allowing the introduction of the carrier phase. Acetonitrile was introduced into the system with an initial pressure of 300 bar, which was kept constant throughout the whole packing procedure. Sonication was applied as described for the CO_2. A microscope was again used in order to establish that packing was proceedings, the packing process being difficult to observe. Once the capillary was packed to the required length, the sonication process was stopped and the valve closed. The packed capillary was left to depressurise for 1 h.

2.2.5. Column wetting and fit insertion

Wetting of the columns was carried out as described elsewhere [3]. The capillary was first flushed with acetonitrile–water (80:20, v/v) and then distilled water. The first frit was formed a few centimetres from the end of the packed bed by using the hot filament method described by Boughtflower et al. [2]. The capillary was reversed and flushed with acetonitrile–water (80:20) overnight to consolidate the bed, and then with distilled water. The second frit was then inserted 25 cm from the first. Excess packing material was removed by flushing with the above solvent. A detection window was then made as close as possible to the first frit by burning off the polyimide with the hot filament and cleaning. The column was then placed in the capillary electrochromatograph cartridge for testing.

2.3. Capillary electrochromatography

CEC was carried out on a HP^{3D} CE system (Hewlett-Packard, Cheadle Heath, UK) using HP ChemStation software for system control, data acquisition and data analysis. Test mixtures were made up in acetonitrile–water (60:40, v/v) (0.1 g l^{-1} of each component). The mobile phase buffer was acetonitrile–water (80:20, v/v) containing 50 m*M* Tris, adjusted to pH 7.8 by the addition of hydrochloric acid. Prior to analysis, conditioning of the column was carried out [3] with buffer at 10 kV with an inlet pressure of 8 bar nitrogen for 15 min. A stable current of approximately 1 μA indicated that the capillary was ready for use. Samples were injected electrokinetically by placing the sample vial at the anodic end of the capillary and applying 5 kV for 15 s. The mobile phase vial was then replaced at the anodic end and the voltage ramped up to the operating voltage (30 kV) over a period of 0.5 min. Analytes were detected using the diode array with a rise time of 1 s. The temperature of the cartridge was maintained at 15°C.

3. Results and discussion

3.1. General evaluation of the packing procedures

All the packing methods had similar inherent problems mostly associated with the fragility of the fused-silica material, especially at the frit and detection window, where the polyimide coating had been removed. Installation of capillaries both in (a); the packing apparatus (involving connections involving a zero-dead volume union) and (b) the HP^{3D} CE cassette also brought about breakages. A less commonly met effect was the production of the columns either with beds too densely or too loosely packed, or with gaps. In the first instance the pressures during wetting were prohibitively high, while the latter gave rise to unstable currents; again, none of the packing procedure was significantly more prone than others to such problems. A summary of the success rates for the four packing methods is shown in Table 1. The liquid slurry packing method has the advantage that depressurisation after packing was much more rapid than for those using supercritical

Table 1
Success rate for packing of capillaries by different methods

Packing method	Percentage of capillaries successfully packed and tested	Percentage of packed and tested columns giving satisfactory CEC
Supercritical CO_2	70	75
Supercritical CO_2+ultrasonic probe	70	80
Liquid slurry	75	75
Liquid slurry+ultrasonic probe	70	80

CO_2 (1 h, against overnight). However, the CO_2 packing process can be followed with the naked eye, whereas an inspection by microscope is necessary for liquid slurry packing. This also means that short packed length columns for short-end injection [7] are more easily prepared using CO_2.

3.2. Capillary electrochromatographic properties of the columns

Sets of six columns drawn at random from groups packed by each of the four different methods using the same batches of ODS packing material and fused-silica tubing, and identically wetted and preconditioned were evaluated by measuring EOF linear velocity (thiourea marker), and migration time, efficiency and retention factor with identical CEC conditions of buffer, test sample solution, voltage and detection. Six consecutive runs were performed on each column. The packed length was kept constant as to maintain similar contribution from the unpacked length of fused-silica tubing. Previous investigations have shown how for SCX (strong cation-exchange) columns of varying proportions of packed section (25–100% of the total length) at pH 7.5 the field strengths are similar in both sections and that the linear velocity changes little with the packed section length. In contrast, at pH extremes the field strengths were greater in the packed section and the velocity decreased with length [8].

In agreement with previous CEC repeatability measurements [3], relative standard deviations (RSDs) between runs on the same column in the same day were small, and for electroosmotic flow (EOF) and retention time less than the approximately 1% necessary for routine application in analysis. These values were, however, slightly increased for 30 consecutive runs on the same day (Table 2).

The mean values and RSDs of measured CEC parameters for a given packing method are compared in Table 3. The mean values are remarkably similar, although the RSDs within a given column set are much greater than those observed for tests on the same column; while CEC is clearly repeatable on a given column, there is much greater variability between columns prepared by the same method using the same materials, with clear implications for method transfer. The RSDs of between 5 and 22% for the variation of migration time are particularly significant.

The spread of values of CEC parameters for columns prepared by a given method is, in fact, apparently greater than differences between the means for columns packed differently. Significantly improved reproducibility was observed, however, when ultrasonication was applied during liquid slurry packing, although there was no corresponding improvement for packing with supercritical CO_2 carrier.

More stringent comparisons were now made of the sets of columns prepared by the four packing methods by carrying out *t*-tests, *F*-tests and analysis of

Table 2
Relative standard deviation (%) of CEC measurements

	EOF	Benzophenone		
		Retention time	Efficiency	Retention factor
6 consecutive runs	0.3	0.5	8.1	0.5
30 consecutive runs	2.0	1.7	9.1	2.5

Table 3
Properties of columns packed by four different methods

	Migration time[a] (mm)			EOF (mm s^{-1})			Efficiencies (plates m^{-1})			Capacity factors		
	Mean	SD	RSD (%)	Mean	SD	RSD (%)	Mean	SD	RSD (%)	Mean	SD	RSD (%)
Supercritical CO_2	4.17	0.42	10.2	1.65	0.14	8.5	208 100	28 400	13.7	0.644	0.038	5.9
Supercritical CO_2+ ultrasonic probe	3.73	0.38	10.3	1.74	0.09	5.0	199 200	28 500	14.3	0.533	0.158	29.6
Liquid slurry	3.99	0.87	21.9	1.61	0.22	14.0	219 900	58 700	26.7	0.51	0.134	26.3
Liquid slurry+ ultrasonic probe	3.69	0.20	5.4	1.69	0.12	7.3	213 900	27 000	12.8	0.501	0.046	9.1

[a] Migration time of benzophenone and calculated for a standard effective length of 25 cm.

variance (ANOVA). These tests generally confirmed the above conclusions, that no one packing method is superior to another as far as CEC properties are concerned. The tantalising possibility cannot be ruled out that all the columns are equally badly packed, with significant contributions to EOF from the open-tube part of the column, and any variability of the bed packing masked by the influence of plug-flow.

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