Fritless packed columns with great potential for use in capillary Electrochromatography

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Several approaches to prepare fritless packed columns for capillary electrochromatography are presented, including *in-situ* polymerization of organic and inorganic monomers and fusing the individual particles packed in a capillary.

The past several years have seen a significant resurgence of interest in capillary electrochromatography(CEC), a hybrid technique that combines the high efficiency owing to the flow characteristics related to electroosmosis with the selectivity of high performance liquid chromatography(HPLC). In CEC, separations are generally carried out using fused silica capillaries packed with HPLC

Article available at http://analusis.edpsciences.org or http://dx.doi.org/10.1051/analusis:199826050049

stationary phases with mobile phases containing supporting electrolytes. Electroosmotic flow (EOF) induced by an electric field drives the mobile phase through the packed bed. This results in a significant reduction of contribution to band broadening due to the plug-like flow profile.

However, in practice the production of packed capillaries can be problematic. The packing of narrow diameter (< 0.1 mm i.d.) capillary tubes is obviously troublesome. The crux of CEC is the manufacture of inlet and outlet frits to retain the stationary phase, since the quality of the frits contributes significantly to bubble formation and stability of the packed column bed. Although there are no clear explanations, bubbles appear to generate due to the flow differences which would exist at the packed-unpacked interface, i.e., at the retaining frits [1], which act as a nucleation site. The problem of bubble formation can be largely alleviated by pressurizing the both ends of the column [2,3], but it is unlikely that such a system makes CEC amenable to coupling with mass spectrometry. The use of a supplementary hydraulic flow is an alternative to suppress bubble formation in a column and to stabilize the flow conditions in CEC [4-7]. This variant of CEC is termed pressurized flow-driven CEC or the so-called pseudo-electrochromatography. Unfortunately, separation efficiency is sacrificed due to the parabolic velocity profile of the pressurized flow superimposed to the flat profile of EOF [7].

In this article, we will present the feasibility of using fritless packed columns for CEC separations of neutral compounds. This type of column, often referred to as a monolithic or rod column, could eliminate the problems associated with the particle packing, frit-making, and bubble generation during runs. Furthermore, it is anticipated that the fritless column preparation technique could yield continuous materials with a greater degree of perfusive character and therefore provide increased efficiencies as a result of remarkably reduced effective particle diameter [8].

Experimental

The CEC experiments were made using an apparatus as described in reference [13]. The high voltage power supply was a HCZE-30PN0.25-LD (Matsusada Precision Devices, Kusatsu, Japan). Detection was performed using a 870-CE (Jasco, Tokyo, Japan) absorption detector at 254 nm. The electrochromatograms were acquired on a Labchart 180 integrator (System Instruments, Tokyo, Japan). The fused silica capillaries used were obtained from GL Sciences (Tokyo, Japan). Chemicals were of reagent grade or higher.

Results and discussion

Approaches to prepare fritless packed columns

There seems several approaches to fabricate fritless packed capillary columns. The most common approach is based on a free-radical polymerization of a mixture of monomers, including functional monomers and crosslinking agents, in a capillary. As a rule, efficiencies of ~120000 plates/m are obtained with the in-situ polymerized columns for retained solutes [9,10]. Discussion on this approach will be presented later.

The second approach is to glue the individual particles in some way after the packing is completed. It is worthy to note that the silica particles in loosely packed regions of a CEC column which is fabricated in a conventional manner can change their positions upon application of voltage because silica particles carry negative charges [11]. Such a rearrangement of particles would negatively affect the efficiency and long-term stability of the column. The immobilization of silica particles may be performed by loading colloidal silica (or water glass) into the packed column and then heating the column with a movable heating coil [12].

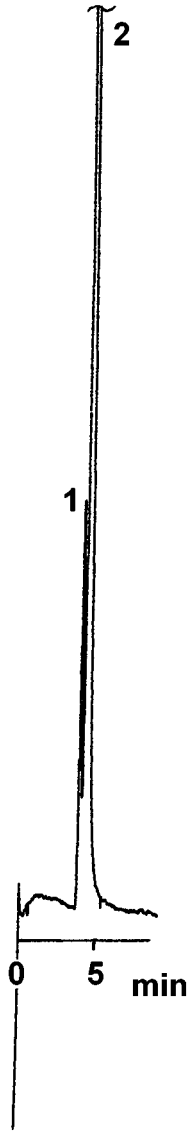

Figure 1. Electrochromatographic separation on a particle-fused column. Conditions: column, 0.150 mm i.d. × 37.0 cm (active length, 20.0 cm); mobile phase, 20:80 v/v mixture of acetonitrile and 2.5 mM phosphate buffer pH 6.8; voltage, 15 kV. Peaks: naphthalene (1) and phenanthrene (2).

The author tried to use alkaline solution to fuse silica particles in a capillary. A 0.150 mm i.d. fused silica capillary attached to a microcolumn end fitting with a metal frit was slurry packed with irregular silica gel (Chemcosorb I-5Si, Chemco, Osaka, Japan) for a length of 20 cm. Subsequently, 0.01 M NaOH was pumped into the capillary. The silica gel inside the capillary was dried over nitrogen at an elevated temperature and then reacted with *n*-octadecyltriethoxysilane. Figure 1 shows a chromatogram as obtained on a column prepared in this way. It was possible to use the column over a week in a reproducible manner.

The third possible, but not well-demonstrated approach is to use an external field to retain the separation medium in a capillary. For instance, if magnetized particles can be used as the stationary phase, they may be fixed by use of a magnet. This approach should allow the easy replacement of the stationary phase.

Soft (swollen) gels

The authors, for the first time, used highly swollen crosslinked gels for CEC separations of uncharged compounds [13-15]. Columns were prepared in aqueous solution by copolymerization of *N*-isopropylacrylamide (IPAAm), 2-acrylamido-2-methylpropanesulfonic acid (AMPS), and *N,N'*-methylenebisacrylamide [15]. High efficiencies (~160000 plates/m) were obtained with a 4.0% total monomer concentration (%T), 10.0% degree of crosslinking (%C), and 10.0% mole fraction of AMPS in the total monomer (%S). Figure 2 shows the separation of a mixture on a 4.1%T, 9.7%C, 7%S poly(AMPS-co-IPAAm) column. The inclusion of a functional monomer, AMPS, into the polymerization mixture enables the generation of EOF in the resulting stationary phase. Increasing the AMPS content in the mixture results in an increase in the magnitude of EOF, and at the same time decreases the hydrophobicity of the stationary phase. The pore structure would also change with the AMPS content because of the electrostatic repulsion between the sulfo groups of AMPS. The EOF is reversed by using tertiary amines or quaternary ammonium bases in place of AMPS. The capacity factor of solute may depend on the voltage applied on the column. The separation of polycyclic aromatic hydrocarbons were examined using a 7.7%T, 3.9%C, 9.4%S poly(AMPS-co-4-acryloylmorpholine-co-allyl *n*-octyl ether) column (the crosslinker, piperazine diacrylate). Figure 3 shows that the retention factors increase with increasing the field strength. This tendency is more pronounced for more retained solutes. Probably, the heat generated in the column is excessive above a certain point of field strength, which results in an increase in the hydrophobicity of the column. Soft gel columns of high hydrophobicity have been developed by others [16,17].

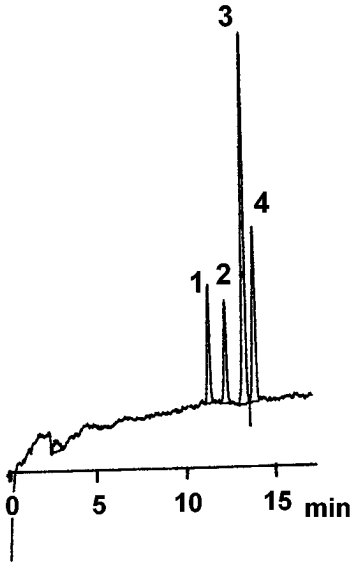

Figure 2. Electrochromatographic separation on a soft gel column. Conditions: column, 0.075 mm i.d. × 48.5 cm (active length, 24.0 cm); stationary phase, 4.1%T, 9.7%C, .7%S poly(AMPS-co-IPAAm); mobile phase, 20:80 v/v mixture of acetonitrile and 2.5 mM phosphate buffer (pH 6.8); voltage, 16 kV. Peaks: benzyl alcohol (1), resorcinol (2), methylparaben (3) and β-naphthol

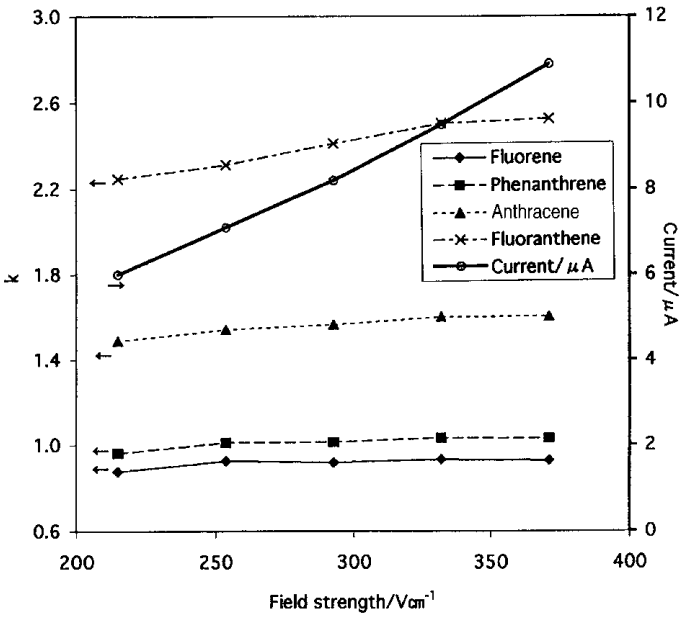

Figure 3. The retention factor, *k*, of polycyclic aromatic hydrocarbons on a 7.7%T, 3.9%C, 9.4%S poly(AMPS-co-4-acryloylmorpholine-co-allyl *n*-octyl ether) column as a function of the field strength. Conditions: column, 0.050 mm i.d. × 51.2 cm (active length, 25.2 cm); mobile phase, 20:80 v/v mixture of acetonitrile and 0.10 M Tris-0.15 M boric acid buffer (pH 8.2).

Rigid (incompressible) gels

In contrast to the soft gels, rigid gels permit rapid mobile phase exchange, as well as easy regeneration of the columns by hydrodynamic pumping [18]. The control of the porous properties is very critical for their use in CEC. The large size of pores results in a small surface area which is insufficient to retain analytes, while a decrease in pore size leads to decreased flow rates, resulting in excessively long retention times of analytes [9]. The pore structure varies with the

nature and content of the porogen (pore former), in addition to the polymerization temperature and the degree of crosslinking. A variety of organic polymer columns can be prepared from water-soluble and water-insoluble monomers.

Fritless column supports can be made of inorganic polymers. The authors prepared fritless silica supports by means of the acid-catalyzed hydrolysis of alkoxysilane as reported by Tanaka and co-workers [19]. A mixture of tetramethoxysilane, acetic acid, and poly(ethylene glycol) 10000 was filled in a fused silica capillary and allow to react for a few hours. After that, the capillary was threaded through a hole in the heated tip of a thermal wire stripper. The column was washed with methanol and dried to react with *n*-octadecyldimethoxysilane dissolved in toluene. Figure 4 shows an electrochromatogram for the separation of a mixture of ketones on the C_{18}-silica column. The column performance should be improved after the conditions in preparation are optimized.

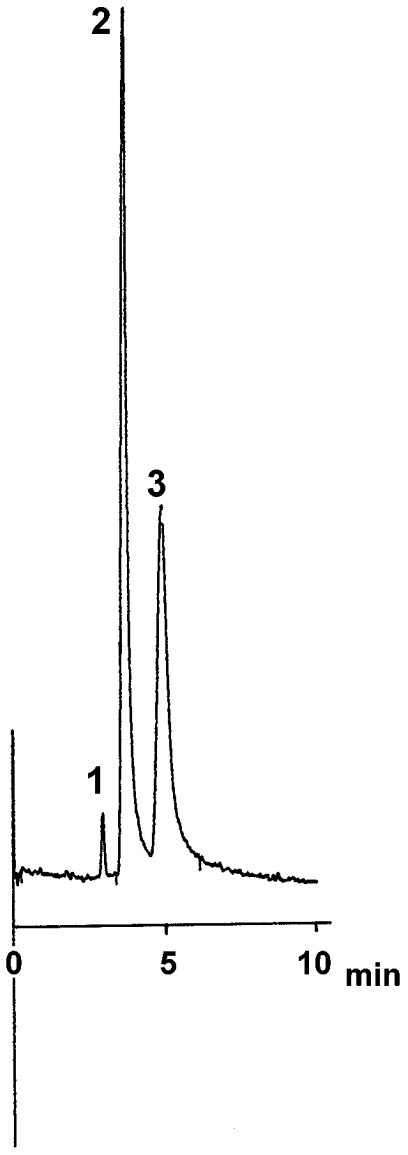

Figure 4. Electrochromatographic separation on a sol-gel-derived column. Conditions: column, 0.075 mm i.d. × 50.5 cm (active length, 26.0 cm); stationary phase, 4.1%T, 9.7%C, .7%S poly(AMPS-co-IPAAm); mobile phase, 20:80 v/v mixture of acetonitrile and 2.5 mM phosphate buffer (pH 6.8); voltage, 20 kV. Peaks: acetone (1), acetophenone (2), and propiophenone (3).

Conclusions

Fritless packed column CEC is a developing technique and much remains to be done before the technique is routinely used in analytical laboratories. In this paper, several approaches are shown which enables more reliable production of CEC columns. An advantage of fritless columns which is not mentioned here is that a further decrease in the i.d. of the column and therefore, a further reduction in analysis time under a higher field strength would become possible with this technique. In the near future, the fritless packed column technology will become the key to the successful construction of micro total analysis systems.

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