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Evaluation of the parameters determining the performance of electrochromatography in packed capillary columns

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Abstract

Electrochromatography is a variant of reversed-phase liquid chromatography performed in capillary columns whereby transport of the eluent is accomplished by applying an electric field across the length of the column. Column-length restrictions arising from resistance to flow encountered with conventional pumping are absent in electrochromatography, allowing separation in capillaries packed with 1.5-μm stationary phases up to 50 cm, thus rendering efficiencies of more than 100 000 plates per column. Practical problems, however, have restricted the number of successful applications reported. A number of parameters determining the electrochromatographic performance are investigated: eluent, frits, packed columns and optional supplementary pressure. These factors are investigated separately and then combined stepwise. With thoroughly degassed eluent, frits made of sintered silica gel, and the use of supplementary pressure, stable and reproducible conditions may be readily obtained.

1. Introduction

High-performance liquid chromatography (HPLC) and capillary electrophoresis (CE) are methods which have been well evaluated and are now routinely used for a wide range of applications. Electrochromatography, on the other hand, is a new technique that can be considered as a combination of these two methods: reversed-phase liquid chromatography is performed in packed columns with an electric field applied across the length of the column.

The retention of neutral molecules is determined purely by chromatographic parameters, while the retention of charged analytes is additionally influenced by electrophoresis. Tsuda [1] used this approach for tuning the selectivity in HPLC separations of charged analytes. He also described the problem of excessive Joule heat, which results in bubble formation, and proposed overcoming this effect by the application of pressure.

Another approach to avoiding the problems related to heat development is the miniaturization of the inner diameter of the capillary column to provide increased heat dissipation. While Tsuda used columns of 200–500 μm inner diameter (I.D.), recent work on electrochromatography has been performed using capillary columns of 50–100 μm I.D. [2–9].

An important effect of the electric field is the transport of the eluent by electroosmotic flow, thus rendering the pressure of HPLC unnecessary. The phenomenon of electroosmotic flow provides a two-fold advantage over conventional pumped systems. First, the efficiency of the separation increases by a factor of approximately two as a consequence of the flat flow profile.

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Secondly, the electroosmotic flow is independent of the particle size of the stationary phase over a wide range, thus giving access to the high efficiency obtainable in 0.5-m columns packed with 1.5-μm particles [2–6].

Despite the enormous potential offered by this method, relatively few successful applications have been reported so far, mainly because of the difficulty in obtaining stable flow conditions in purely electro-driven systems. To date, few have approached these problems. In this paper we report our investigations upon the parameters influencing the electrochromatographic performance.

2. Experimental

2.1. Preparation of the frits

Three different types of frits are produced for testing in electrochromatographic columns. The first is formed by in situ polymerization of a potassium silicate solution with formamide according to the method of Cortes et al. [10]. The potassium silicate solution is prepared by adding 1.3 g potassium hydroxide and 2.4 g silicon dioxide to 12 ml water. Thermostatting for 2 h at 70°C yields a clear liquid. The solution is diluted in a ratio of 1:6 with formamide, resulting in a viscous liquid which begins to harden after ca. 10 min. During this time a small plug of this liquid is drawn by vacuum into the appropriate position inside the capillary. The material is completely polymerized after 1 h at 120°C.

Another type of frit is prepared by tapping the tip of a capillary into spherical silica gel (Gromsil, particle diameter $d_p = 5$ μm; Grom, Herrenberg, Germany) which is wetted with potassium silicate solution. The frit is sintered using an electrically heated hot iron wire.

The third type of frit is formed by sintering pure silica gel (Gromsil, $d_p = 5$ μm). It is filled into the capillary column either by slurry packing or by tapping the end of the capillary into silica gel wetted with water.

The frits were tested for mechanical stability by flushing the capillary with water and applying pressure. The pressure is increased in steps of 25 bar and each step lasts 30 s.

2.2. Slurry packing of the capillary columns

Fused-silica capillaries of 50, 100 and 150 μm I.D. and 360 μm O.D. were obtained from Polymicro Technology (Phoenix, AZ, USA). For preparation of the packed capillary columns, only the frits consisting of sintered spherical silica gel were used. The slurry of either reversed-phase material (GromSil ODS-2, $d_p = 1.5$ μm) or silica gel (GromSil, $d_p = 5$ μm) in acetone (1:10, w/v) is ultrasonicated for 5 min and transferred into a stainless-steel slurry chamber (25 mm × 1 mm I.D.). The capillary protruded about 2 mm into the chamber, which was placed into an ultrasonic bath during packing. The slurry was pumped into the capillary at 400 bar using a liquid chromatographic pump (Model S1100; Sykam, Gilching, Germany).

A packed capillary column is prepared in the following steps. First, a temporary frit is produced by tapping an end of the capillary into silica gel wetted with water, drying for 12 h at room temperature and sintering. After packing the capillary with silica gel, the final outlet frit is formed by sintering at a distance of approximately 15 cm from the end of the capillary. The temporary end-frit is then cut-off and the capillary emptied on both sides of the final outlet-frit by flushing with water under ultrasonification. Removal of the polyimide coating by the electrical heater allows on-column UV absorbance detection. Packing of the capillary with the reversed-phase particles is followed by removal of ca. 1 cm stationary phase by heating the tip of the capillary, thus causing rapid solvent evaporation and ejection of slurry. Finally, this end of the capillary is filled up with silica gel and the inlet-frit sintered after drying.

2.3. Apparatus

The electrochromatographic system consists of a modular capillary electrophoresis system (Grom, Herrenberg, Germany). It can be combined with a HPLC system (Sykam, Gilching, Germany) for flushing and pressurizing the

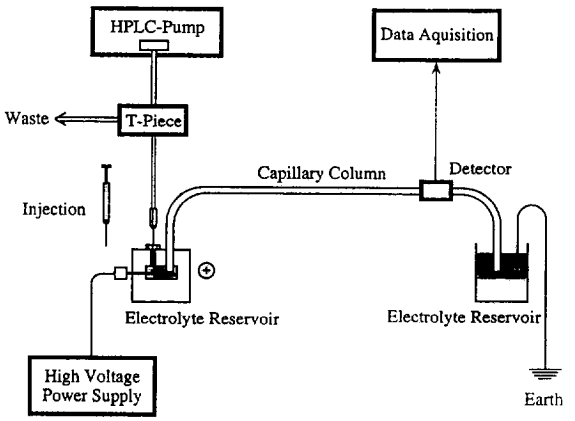

Fig. 1. Schematic representation of the setup for pressurized electrochromatography.

packed capillary columns as shown in Fig. 1. A T-piece and a capillary column of 50 μm I.D. and 30 cm length are used for solvent splitting. A Chromatopack C-R6A (Shimadzu, Kyoto, Japan) is used for data processing.

2.4. Electrochromatography

Pressurized electrochromatography is carried out in capillary columns of 100 μm I.D. packed to a length of 15 cm (GromSil ODS-2, $d_p = 1.5$ μm), 150 bar supplementary pressure and 20 kV. The overall length is 40 cm.

On-column UV absorbance detection (254 nm) is performed 0.1 cm behind the frit. The mobile phase is 80% acetonitrile–20% buffer containing 5 m*M* sodium phosphate at pH 8. Samples are injected electrokinetically for 5 s at a voltage of 10 kV.

The chemicals for eluent preparation were purchased from Merck (Darmstadt, Germany) and were of research grade.

3. Results and discussion

3.1. A practical approach to electrochromatography

Electrochromatography in packed capillary columns should ideally provide stable and reproducible electroosmotic flow without interfering with detection. In practice, it is often hampered by several phenomena such as variation of the electric current, the electroosmotic flow velocity or the detection signal. These effects can increase, causing the detection noise to rise until current and electroosmotic flow finally break down. When the capillary column is flushed, bubbles are swept out which have formed inside the capillary column.

To overcome these problems we investigated some of the factors determining stability and reproducibility of the electrochromatographic performance. The electrochromatographic system is split into the factors eluent, frits, columns and optional supplementary pressure. These are tested separately for suitable performance and then combined stepwise to obtain an appropriate system.

3.2. Degassing of the eluent

An eluent composition similar to those described in [3,4] with 80% acetonitrile and 5 m*M* phosphate buffer at pH 8 was chosen to test the eluent in an open tubular column of 150 μm I.D. This diameter is large compared with those used in other electro-driven systems, thus problems related to insufficient heat dissipation are likely to occur; however, it provides a sensitive system for the investigation of related phenomena.

Degassing of the eluent proved to be the crucial factor in this test system. Insufficient degassing led to increased baseline drift, unstable current and finally breakdown of electroosmotic flow as a result of bubble formation. These symptoms can be overcome by thoroughly degassing the eluent. The best results are obtained by a combination of purging with helium and applying vacuum under ultrasonification.

3.3. Selection of appropriate frits

The ideal frit should have sufficient mechanical stability to withstand the pressure of the packing procedure, should be easily prepared and, most importantly, must not interfere with the electrochromatographic process. Three different types of frits, described in the Experimental section, are evaluated.

The frit prepared by condensation of potassium silicate and formamide displayed the best mechanical properties. Even with pressures up to 400 bar it was not possible to remove it from its position in the capillary column. The frit is easily prepared and can be placed at any desired position inside the capillary column. It has a three-dimensional network of connected channels forming pores of ca. 1 μm diameter [10]. Thus, both good mechanical stability and sufficient permeability to the eluent are provided. However, current and baseline signal were unstable under electrochromatographic conditions.

The same problems, although not as severe, are encountered with frits made by sintering spherical silica gel wetted with potassium silicate. In addition, these have only moderate mechanical stability, being displaced at pressures above 100 bar.

It is presumed that these problems originate in the freshly polymerized silicate surface which is common to both systems. The formation of a homogenous electrical double-layer on the surface area is presumably disturbed. The electron micrograph of this frit (Fig. 2A) shows silica gel spheres connected and partly covered by the silicate polymer. The covering is inhomogeneous so that irregular channels are formed. This observation coincides with results of Rebscher and Pyell [8] using similar frits made of silica gel and potassium silicate. They measured band-broadening in electrochromatography and explained the high contribution of the inlet frit by inhomogeneities of the flow within the frits.

The frit prepared by sintering of pure spherical silica gel proved to be better suited for the electrochromatographic system. It allows stable conditions of electroosmotic flow and a stable baseline. The electron micrograph of this frit (Fig. 2B) shows a section of packed particles. The silica gel spheres are melted together at their contact points. Unfortunately, the sintering of the frit destroys the polyimide coating, making these parts of the column susceptible to breakage. Frits of this type are removed by pressures in excess of 50 bar in capillaries of 150 μm I.D., so that care must be exercised during the slurry packing of these capillary columns. Fortunately, the stability of the frits increases

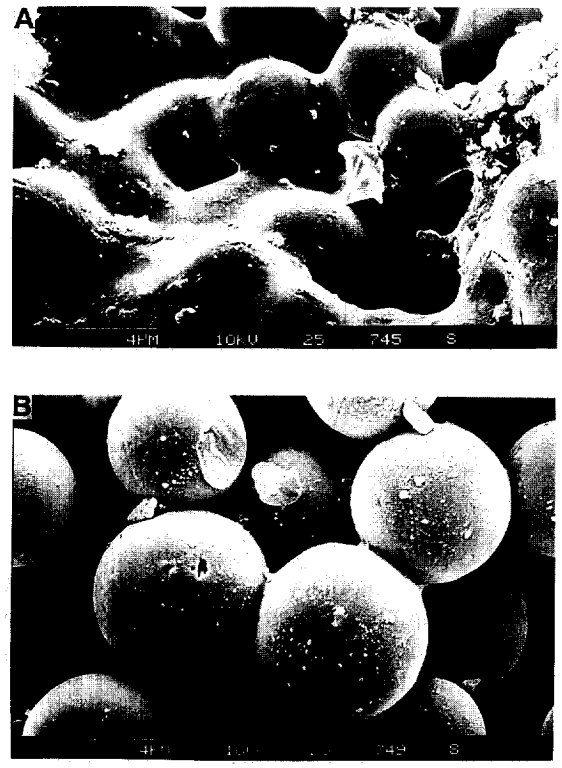

Fig. 2. Electron micrographs of frits used in packed capillary columns. (A) Sintering spherical silica gel that is wetted with potassium silicate solution yields packed spheres covered with irregular layers of polymer coating. (B) The spheres in a packing of silica gel are melted to each other by sintering with an electrical heater. During the preparation for the electron micrograph small fragments of polish fell onto the frit. In addition, a silica sphere broke off, leaving marks where it had been attached (see centre of the upper part).

with decreasing I.D., so that capillaries of 50 μm I.D. with sintered silica gel frits can be flushed at pressures up to 400 bar without removal of the frits. The preparation of these frits within the column requires several steps.

In spite of the laborious preparation, well-packed columns and satisfactory electrochromatographic performance are obtained with these frits.

3.4. Design of the chromatographic columns

In packed capillary columns for liquid chromatography in both the pressure and electro-driven

mode or in combinations of these, the stationary phase of slurry-packed columns must be contained between two frits: the outlet frit prevents removal of the particles when using the pump, while the inlet frit retains the particles which would otherwise migrate upstream in an electrical field as a result of their negative charge. On-column UV detection requires a detection window behind the packing material. The alternative in-column detection as described in [9] results in decreased sensitivity because of light scattering.

These requirements can be satisfied by two different column designs: a coupled and an uncoupled system, as shown in Fig. 3. The coupled system consists of two capillary columns connected by a Teflon tube. The chromatographic column is filled over its full length and limited by two frits. It is adjoined to an open tubular capillary which carries the detection window ca. 5 mm behind the connection to the outlet frit. With this setup the packed columns are readily prepared. In the case of breakage at the window, the open tubular capillary is easily exchanged. Unfortunately, the use of this system is restricted to a low electrical field strength in our experimental setup. The capillary is fastened by a metal support in the detection device of the capillary electrophoresis system. A current to the photocell arises at an electrical field strength higher than 10 kV m^{-1}. This prevents the application of higher field strengths in our setup because of an increase in detection noise. This effect can probably be reduced by the use of an insulating material as support in the detection device.

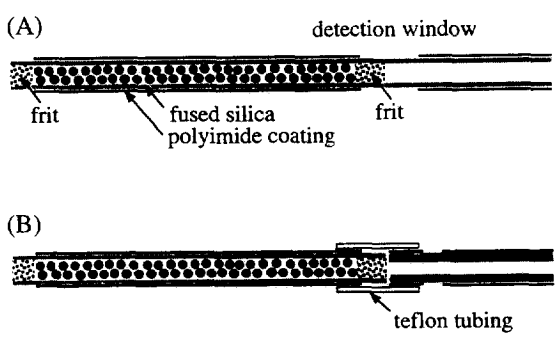

Fig. 3. Packed capillary columns for electrochromatography and micro-HPLC as (A) an uncoupled and (B) a coupled system.

The uncoupled column system consists of a single fused-silica capillary. Here, the outlet frit is several centimetres from the end of the capillary, and directly behind the outlet frit is the detection window. The minimized dead volume between the chromatographic column and the detection window reduces band-broadening. Disadvantages include the more difficult preparation of the columns and the susceptibility to mechanical breakage at the detection window. Despite these drawbacks, this column design is considered superior for capillary electrochromatography.

3.5. Electrochromatographic performance

Without supplementary pressure, electrochromatographic analysis of neutral compounds (alkylbenzenes) in capillary columns of 100 μm I.D. is moderately successful. Separations with a reduced plate height of 1.6 for butylbenzene are obtainable with an optimized buffer (5 m*M* phosphate). Both higher and lower salt concentrations produced unstable electrochromatographic conditions. Knox and Grant [3] investigated the dependency of the buffer salt concentration on the reduced plate height with a 40 μm I.D. packed capillary column. They described a minimum at ca. 4 m*M* phosphate buffer and increasing plate height with lower or higher concentrations. Corresponding to this, we observed that with columns of 50 μm I.D., electrochromatographic analysis is possible over a wider range of buffer concentrations than with the 100-μm column. However, phenomena like variance of current and temperature inside the column as indicated by a baseline drift still occurred in numerous separations. Thus, with this approach no satisfactory reproducible results are obtained.

3.6. Pressurized electrochromatography

The problems associated with electrochromatography driven solely by electroosmotic flow can be overcome by applying pressure to the

system so that electrochromatographic separations can be performed under stable and reproducible conditions [1,5,6]. This can be accomplished by two different approaches.

Smith and Evans [5] pressurized the buffer reservoirs of their capillary electrophoresis system on both the inlet and outlet sides by approximately 40 bar.

However, it is advantageous to couple an HPLC system to the capillary electrophoresis setup, allowing the capillary column to be pressurized up to 400 bar. This is necessary for filling the capillary column with solvent and for changing the eluent when a different composition is required or when the electroosmotic flow breaks down by bubble formation due to insufficient heat dissipation. Furthermore, a gradient elution mode can easily be realized [6]. Injection can be accomplished both electrokinetically and by pressure. In contrast to electrokinetic injection, injection by pressure does not discriminate the analytes according to their charge, and the injected sample amount is independent of the charge of the sample molecules.

In pressurized electrochromatography, thorough degassing is less important than in pure electrochromatography: purging with helium is found to be sufficient. Furthermore, analyses can be performed over a wider range of buffer and modifier concentrations [6].

A separation of alkylbenzoates is shown in Fig. 4. The analysis was carried out in a capillary column packed to a length of 15 cm with 1.5-μm stationary phase; 50 000 plates are obtained for pentylbenzoate within 4 min analysis time. The flow velocity of 3 mm s^{-1} is far higher than that of 0.4 mm s^{-1} obtained by application of the same pressure but without an electrical field. It is apparent that the eluent is transported primarily by electroosmotic flow and that the pressure has merely a stabilizing function.

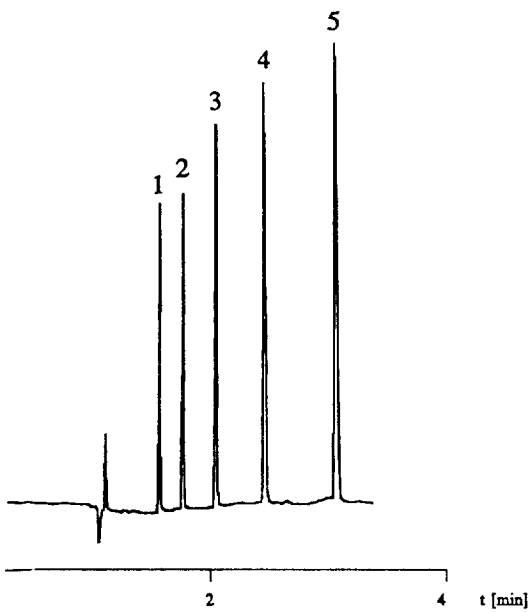

Fig. 4. Separation of alkylbenzoates by pressurized electrochromatography. The column of 100 μm I.D. is packed for a length of 15 cm with 1.5-μm reversed-phase particles. Other conditions as described in the Experimental section. The peaks 1–5 correspond to methyl-, ethyl-, propyl-, butyl- and pentylbenzoate, respectively.

4. Conclusion

Several parameters influencing the electrochromatographic performance are evaluated and experimental conditions are described to overcome the major problems encountered so far related to electrochromatography, opening this powerful separation technique to a wide field of applications.

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