Improvement of the long-term stability of polyimide-coated fused-silica capillaries used in capillary electrophoresis and capillary electrochromatography

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Abstract

Swelling of the polyimide coating of fused-silica capillaries in acetonitrile-containing buffers was found to be the reason for several problems in capillary electrophoresis (CE) and capillary electrochromatography (CEC). Scanning electron microscopy photographs of the ends of raw fused-silica tubing showed that the coating becomes soft and increases its volume after longer contact with such buffers. As a consequence, separation efficiency can deteriorate, the capillary ends can clog or break off. To prevent swelling of the polyimide coating, fused-silica capillaries used in CE or CEC were heated at 300 °C for a longer period of time which improved their long-term stability in comparison to raw fused-silica tubing. © 2002 Elsevier Science B.V. All rights reserved.

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1. Introduction

During the last decade, capillary electrophoresis (CE) and related techniques became efficient tools in the circle of modern analytical separation methods. A joint feature of these ''electromigration'' techniques is the use of plain, surface modified or packed narrow-bore capillaries as the separation vehicle. So far, capillaries made of fused-silica (FS) and protected on the outside wall with a polyimide coating are mainly used as the material of choice, because they are flexible, show a high mechanical strength when having an intact coating, provide relatively high electroosmotic flows at appropriate

pH values; their chemical inertness is satisfactory for most applications, removal of the polyimide coating enables simple on-capillary UV detection; finally they are commercially available in a wide choice of inner diameters and in reproducible quality.

In practice, however, polyimide-coated fused-silica tubing also leads to troubles, which are caused by the adsorption activity of the silanol groups ([1] and literature cited therein) and by the tendency of the polyimide coating to become soft and to swell in certain organic solvents [2–4]. With our previous work in CE and capillary electrochromatography (CEC) [5] we were confronted from time to time with a rapid loss of separation efficiency on new capillaries and in the case of CEC, with the formation of cracks in the fused-silica wall close to the capillary ends, as well as the crumbling of small bits of the silica wall and of the frits. We attributed these processes, which often render the whole column

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useless within a short period of time, to a swelling of the polyimide coating and the subsequent loss of its protecting properties.

The aim of this work was to investigate the swelling behaviour of the polyimide coating of FS capillaries in detail, to demonstrate some of the problems that can be attributed to it, and to find a procedure which can reduce the extended swelling and improve the long-term stability of polyimide-coated fused-silica capillaries in CE and CEC.

2. Experimental

2.1. Materials, chemicals and buffers

FS tubing was purchased from MicroQuartz (Munich, Germany) and Polymicro Technologies (Phoenix, AZ, USA). To check the stability of the polyimide coating, fused-silica mono fibres from Fibertech (Berlin, Germany) were also included in the tests. If not otherwise specified in the figures, capillaries from MicroQuartz were used. To prepare capillary columns for CEC, FS capillaries were slurry-packed and equipped with sol–gel end frits as described by Schmid et al. [5]. Reversed-phase (RP) silicas from different producers were applied as packing material [5].

Analytical-grade solvents and test compounds were obtained from Promochem (Wesel, Germany). Tris(hydroxymethyl)aminomethane (Tris), 3-morpholinopropanesulfonic acid (MOPS), sodium borate and sodium dihydrogenphosphate were purchased from Fluka (Deisenhofen, Germany). Buffers were prepared by dissolving the appropriate amounts of chemicals in pure water (purified by Elgastat UHQ PS; USF Elga, Ransbach-Baumbach, Germany), followed by the adjustment of the pH with 1 mol/l hydrochloric acid or sodium hydroxide solution. If required, organic modifiers were added to the aqueous buffer solutions. Before use in CE or CEC, the buffer solutions were degassed by immersing the bottle in an ultrasonic bath (Bransonic 52; Branson, Danbury, USA) and purging it with helium for 10 min.
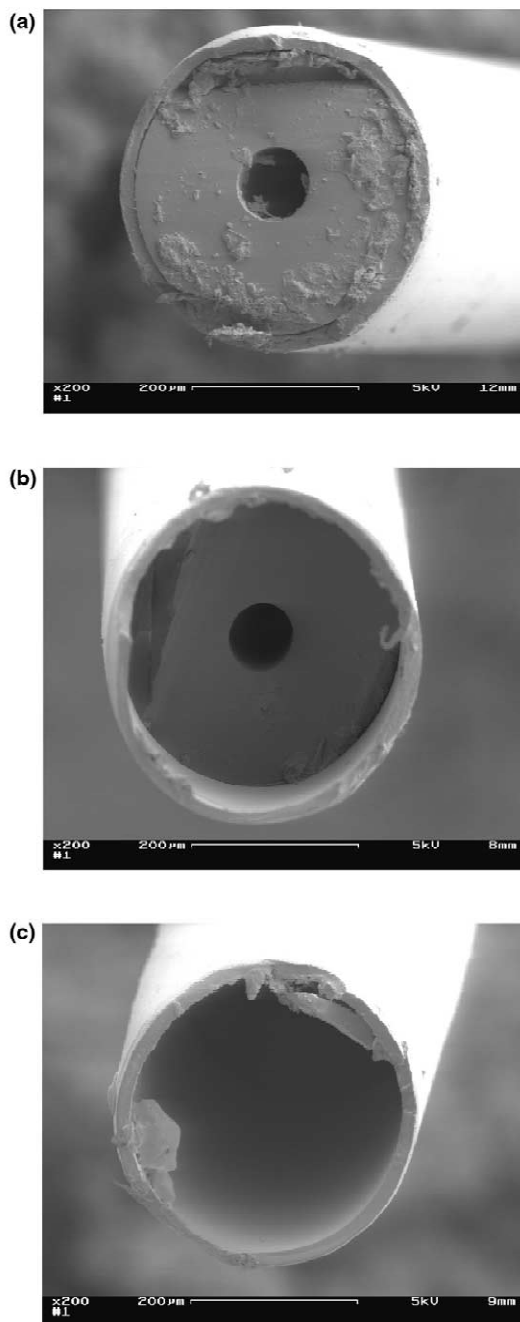

Fig. 1. SEM photographs of the ends of raw FS capillaries kept in (a) acetone, (b) methanol and (c) acetonitrile for 4 weeks.
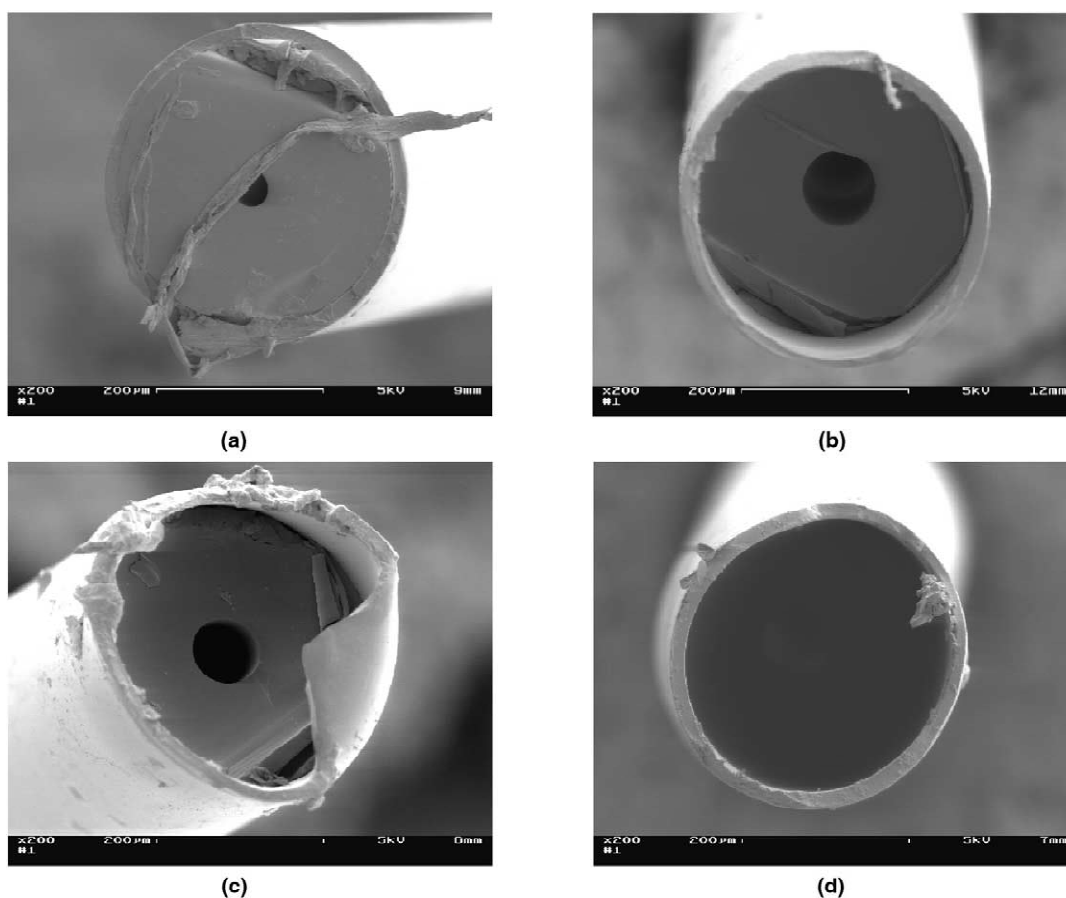
Fig. 2. SEM photographs of the ends of raw FS capillaries kept in (a) pure aqueous Tris buffer, 20 mM, pH 9.0 (buffer A), (b) buffer A+10% (v/v) acetonitrile (buffer B), (c) buffer A+50% (v/v) acetonitrile (buffer C), (d) buffer A+90% (v/v) acetonitrile for 2 weeks.

2.2. Equipment

CE experiments were performed with an Agilent [3D]CE system (Agilent Technologies, Waldbronn, Germany). CEC tests were carried out on a laboratory-made set-up comprising a high-voltage power supply (CZE 1000 PN 30; Spellman, NY, USA), a UV–Vis filter photometer (WellChrom K-2000; Knauer, Berlin, Germany) and an acrylic glass box with pressure-stable polymer vessels which hold the separation capillary, the electrodes, and 1.8 or 4 ml standard vials. In CE and CEC, high voltage was always reached via a 2–6 s time ramp to avoid stress to the capillaries. A scanning electron microscope Model DSM 962 (Zeiss, Oberkochen, Germany) and a stereo microscope Model Stemi SV8 (Zeiss) were

used to inspect the capillary ends. For the heat treatment of the capillaries a gas chromatograph from Carlo Erba (Milan, Italy) was used.

2.3. Procedures

For the swelling experiments, 4-ml vials with PTFE-lined septa were filled with 3 ml of the solvent or buffer. 2 cm long ends of the capillaries investigated were immersed into this liquid. After different periods of time, the ends were inspected by scanning electron microscopy (SEM).

The CE tests were automatically run on the Agilent [3D]CE system according to the following programmed sequence. After installation of the capillaries: (i) pressure-driven flushing with acetonitrile
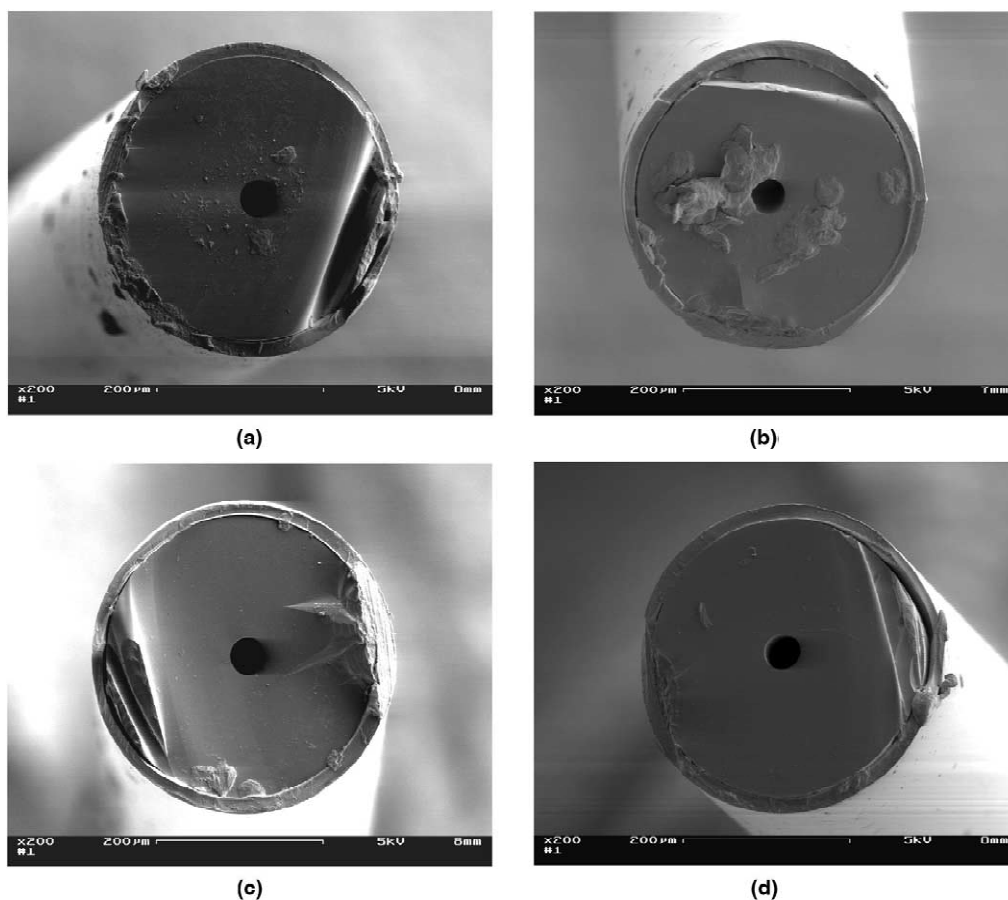
Fig. 3. SEM photographs of the ends of raw FS capillaries kept in pure aqueous buffers for 4 weeks; (a) 20 m*M* borate, pH 9.4, (b) 20 m*M* MOPS, pH 7.0, (c) 20 m*M* phosphate, pH 7.0 and (d) 20 m*M* phosphate, pH 3.0.

for 1 h; (ii) pressure-driven flushing with water for 1 h; (iii) pressure-driven conditioning with buffer for 15 min; (iv) electroosmotic flow conditioning with buffer at 10 kV for 15 min; (v) sample injection (10 mbar, 2 s) and two runs each at 10 kV for 10 min and 20 kV for 5 min, pressure-driven flushing with buffer between each run for 2 min; (vi) replenishment of the buffer in the inlet and the outlet vial after four runs, continuation of step (iii). The flushing pressure was approximately 900 mbar.

Packed capillaries in CEC were run with different acetonitrile-containing buffers [5].

Heat curing of the capillaries was performed as follows: coiled fused-silica capillaries (5–10 m) were suspended in the oven of a gas chromatograph (GC) using a metal rod as holder. Then, they were heated at a rate of 5 °C/min to the final temperature of 300 °C which was held for 200 to 240 h. After switching off the heating, the capillaries were allowed to slowly cool down to room temperature while the oven door was kept closed. Capillaries were stored with closed ends until use in CE and CEC.
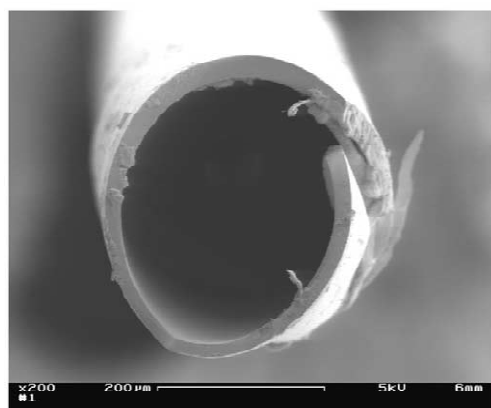
3. Results and discussion

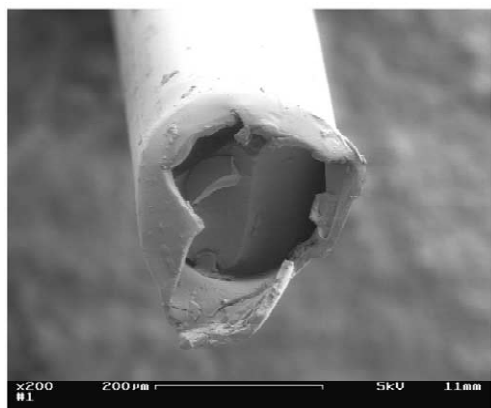### 3.1. Swelling behaviour of the polyimide coating in different solvents and buffers

To study the swelling behaviour of the polyimide coating, 2 cm of 5 cm long pieces of fused-silica
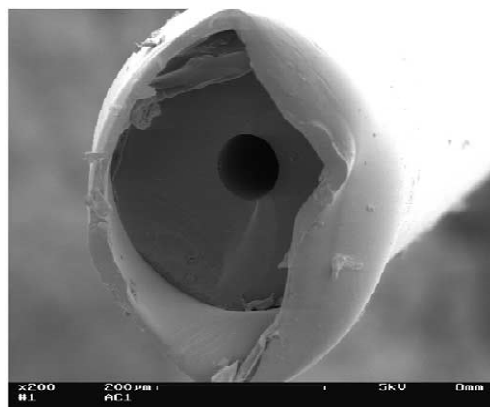
(a)

(b)

(c)

Fig. 4. SEM photographs of the ends of two FS capillaries and a FS fibre obtained from different suppliers kept in buffer C for 1 week; (a) MicroQuartz, (b) Polymicro Technologies, and (c) Fibertech.

tubing (MicroQuartz) were brought into contact with the appropriate solvents or buffers (see Experimental). After 1, 2 and 4 weeks of storage, the capillary ends were inspected by SEM.

Methanol, acetonitrile and acetone were chosen as organic solvents because they are typically used in CE, micellar electrokinetic chromatography and CEC as organic modifiers or as packing and slurry liquids. To rule out the possibility that plain aqueous buffers also affect the stability of the polyimide coating, different buffers such as phosphate (20 m*M*, pH 3.0 and 7.0), borate (20 m*M*, pH 9.4), Tris (20 m*M*, pH 9.0) and MOPS (20 m*M*, pH 7.0) were included in these tests. The influence of the acetonitrile content of the buffers was tested by exposing the fused-silica capillaries to mixtures of a Tris buffer (20 m*M*, pH 9.0) with different volume fractions of acetonitrile (0, 10, 50 and 90%, v/v).

Some SEM photographs taken after 4-week-long storage of the capillary ends in acetone, methanol and acetonitrile are shown in Fig. 1a–c, respectively. The tube-like extension of the polyimide coating as a symptom of swelling indicates that acetone has practically no effect, methanol a little effect and acetonitrile the strongest effect on swelling. In acetonitrile, swelling was already visible after 24 h of storage. The high aggressiveness of acetonitrile can be clearly seen in the photographs of Fig. 2 which show results of a 2-week-long storage in Tris buffers of increasing acetonitrile content. Whereas in the pure aqueous Tris buffer solution (buffer A) (Fig. 2a) no swelling can be seen, even an acetonitrile content of 10% (v/v) (buffer B) causes swelling (Fig. 2b) which is further enhanced when the acetonitrile content is increased to 50% (v/v) (buffer C) (Fig. 2c) and 90% (v/v) (buffer D) (Fig. 2d). Pure aqueous buffers cause no perceptible swelling as shown in Fig. 3 for some of the aqueous buffers. Fig. 4 demonstrates that fused-silica capillaries or fibres from different suppliers (MicroQuartz, Polymicro Technologies and Fibertech) behave similarly poorly with respect to swelling of the polyimide coating.

The electropherograms in Fig. 5 demonstrate that the swelling and the changes of the inlet and outlet conditions of the capillary (see Fig. 4) may strongly influence the peak shape and hence the separation efficiency and peak resolution even after a short use in a CE instrument. Using buffer C [Tris buffer, 10
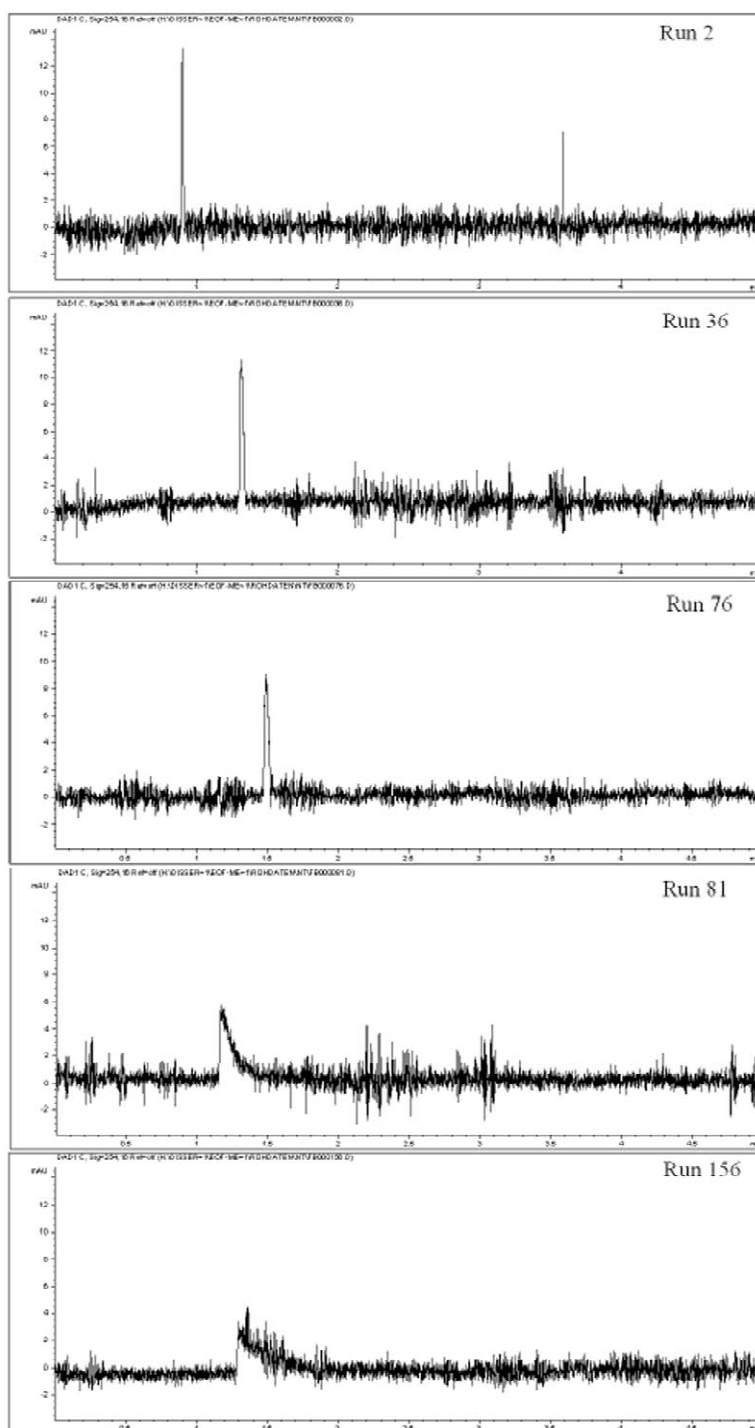
Fig. 5. Electropherograms of thiourea as a test compound on a raw FS capillary, 35 cm (effective length 26.6 cm)×50 µm I.D.; buffer: 10 m*M* Tris, pH 9.0–acetonitrile (50:50, v/v).
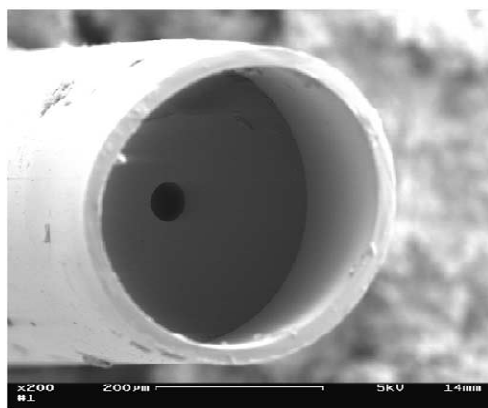
Fig. 6. SEM photograph of the anodic injection end of the raw FS capillary after the tests shown in Fig. 5.

mM, pH 9.0–acetonitrile (50:50, v/v)], the peak of thiourea showed a very strong tailing after as few as 80 runs, which corresponds to a buffer contact time of approximately 13 h. After 170 runs (contact time of about 25 h) the peak disappeared completely. After dismounting the capillary, SEM photographs of the capillary ends were taken. In Fig. 6, the swollen tube-like extension of the polyimide coating (anodic injection end of the capillary) can be seen. This extension forms an enormous dead volume and a type of a sorptive phase, which may cause the observed effects. In many cases, the soft and swollen outer coating does not only form this tube-like
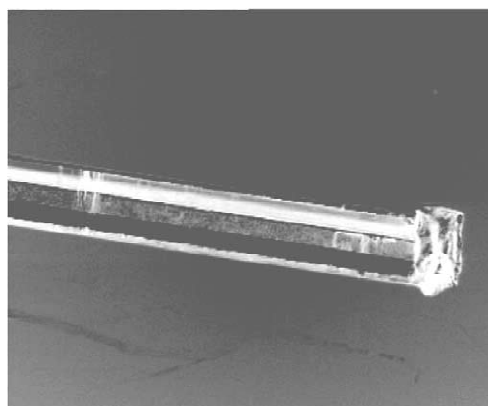
extension, but also undergoes other changes in shape, such as retraction or collapse of the coating during penetration of the vial cap at one or both extremities. Even clogging of the capillary may happen as shown in Fig. 7 for a packed capillary, which had been used with an acetonitrile containing buffer in CEC.

Because acetonitrile is indispensable as an organic modifier or as a solvent in many a capillary electrophoretic or electrochromatographic application, some users remove a piece of the polyimide coating at the ends of the capillary. This way, peak shape distortion and a loss of separation efficiency caused by the effects described above can be diminished or even
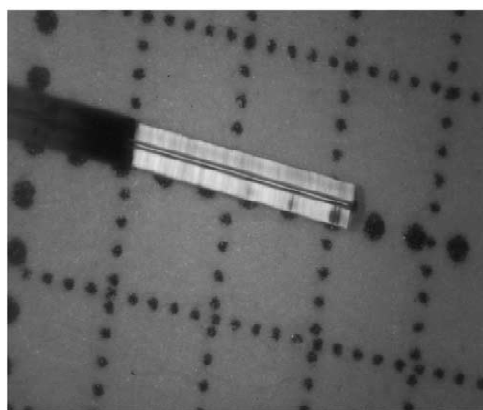
(a)
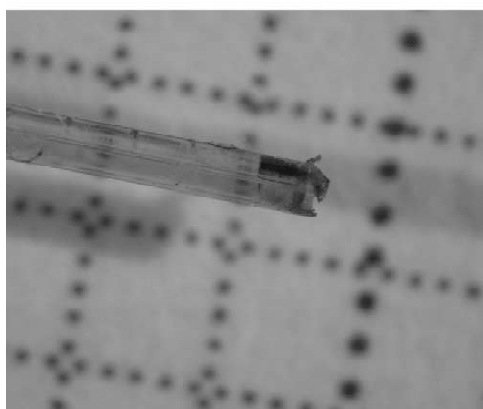
(b)

Fig. 8. Stereo microscopic photographs of a raw FS capillary; (a) end with polyimide coating burned off prior to use in CE, (b) the same end (the non-coated piece is broken off) after it was used in CE for a few days with buffer C (the graph paper in the background is shown for better clarity).
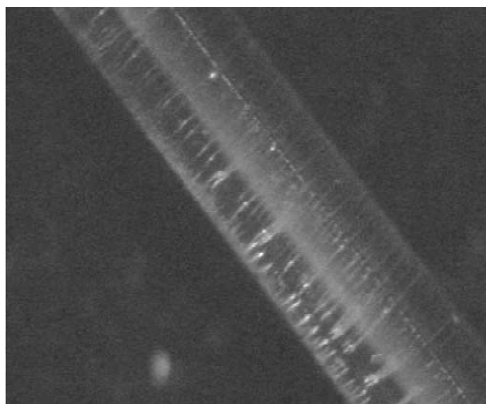

Fig. 7. Stereo microscopic photograph of the end of a raw FS capillary packed with Nucleosil C_{18}, 100 Å, 3 μm (Macherey & Nagel, Dueren, Germany), after the use in CEC for 3 days; buffer: 20 mM Tris, pH 8.0–acetonitrile, (30:70, v/v).

Fig. 9. Formation of cracks in a bare FS capillary packed with Nucleosil C_{18}, 100 Å, 5 μm (Macherey & Nagel, Dueren, Germany), after it was used in CEC for 2 weeks; buffer as in Fig. 7.

avoided. However, this procedure makes the capillary ends more fragile, at the very spot where they are heavily strained as they slide through the vial caps. Therefore, bare ends often lead to breakage of the ends of the fused-silica capillary. Unfortunately, in most cases, breakage is non-rectangular and results in a non-uniform electrical field at the capillary entrance which may cause band spreading [6–8].

Capillaries whose polyimide coating had been burned-off for a few millimeters showed no swelling of the carbonized coating after a longer storage in buffer C. When these capillaries were used in a commercial CE instrument, in most cases the bare end (Fig. 8a) was broken off after some time (Fig. 8b) which greatly affected the separation efficiency. Bare ends are especially dangerous in the case of packed capillaries for CEC. We often observed the formation of cracks, as shown in Fig. 9, and a peeling-off of silica bits, when the capillary was not protected by an intact polyimide coating. Astonishingly, to our knowledge, no such effects have ever been reported in the literature on CEC capillary columns. On the contrary, long lifetimes were found for the majority of CEC capillary columns [9] with acetonitrile-containing buffers. This may be explained by considering that the end frits of these columns were produced by means of heat treatment

[10] which effects the polyimide coating as well (see below).

3.2. Improvement of the coating stability by heat treatment

While looking for a solution to improve the stability of the polyimide coating, we remembered that the coating became dark brownish and more rigid when FS capillary columns had been used in GC for longer periods of time at high temperatures. We therefore imitated this aging process by heat-treating the CE and CEC capillaries in a GC oven at 300 °C. Indeed, heat treatment strongly diminished the tendency of the coating to swell in acetonitrile-containing buffers. This is illustrated by the SEM photograph in Fig. 10 which shows the nearly unchanged end of a capillary heat-cured at 300 °C for 240 h and stored in buffer C for 4 weeks. The long-term CE test with buffer C, which had already been applied to check the capillaries that were not cured by heat, showed that the separation efficiency of heat-cured capillaries is not reduced, even after a longer period of use, as illustrated by the electropherograms in Fig. 11. The slight increase in the noise and a reduction of the peak height with an increasing number of runs may reflect a contamination of the capillary and/or the difficulty to keep the
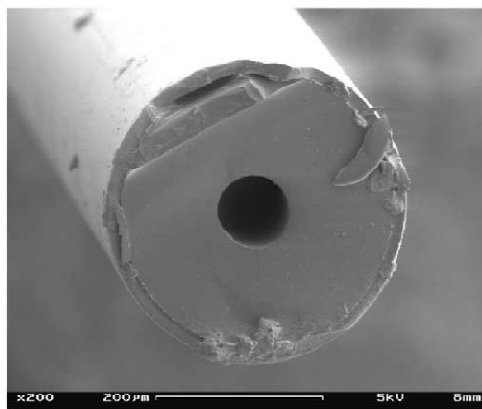

Fig. 10. SEM photograph of the end of a heat-cured FS capillary kept in buffer C for 4 weeks.
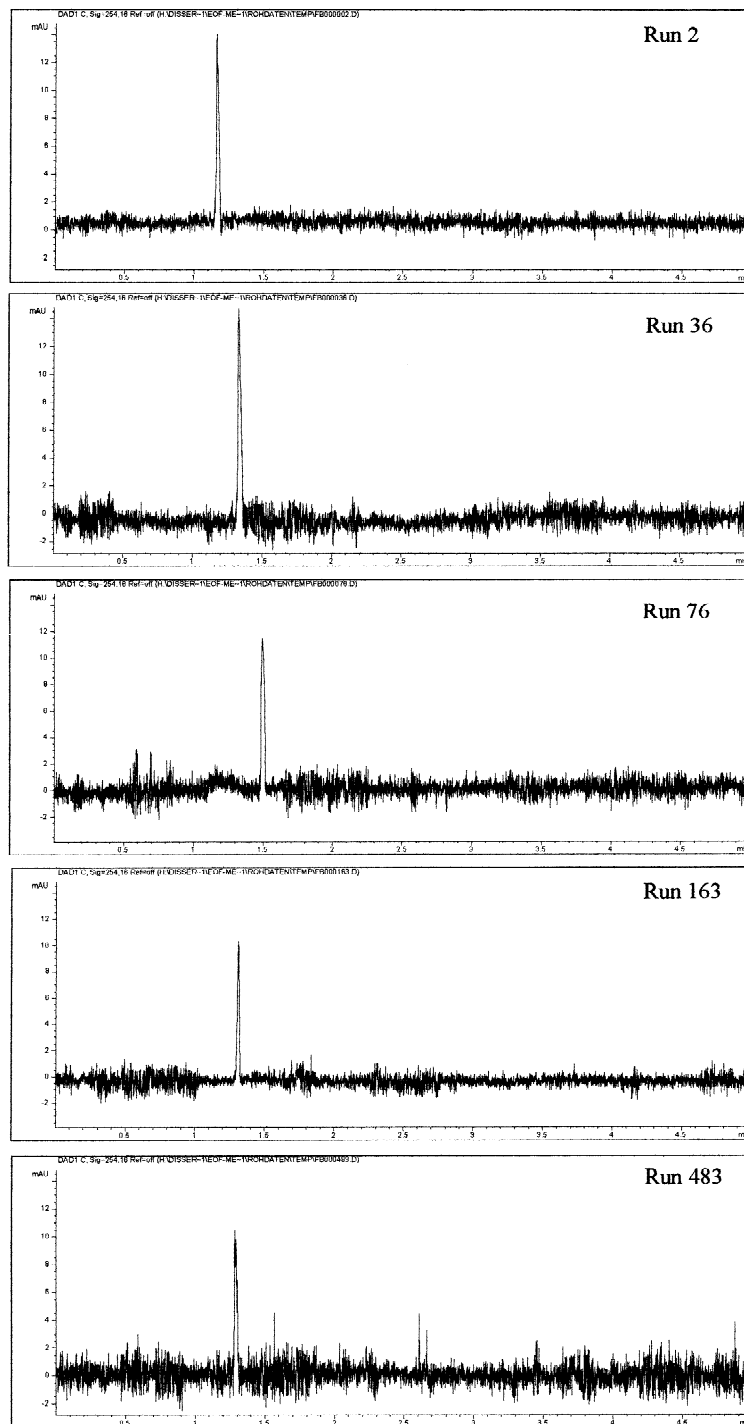
Fig. 11. Electropherograms of thiourea as a test compound on a heat-cured FS capillary, 35 cm (effective length 26.4 cm)×50 μm I.D., buffer C [20 m*M* Tris, pH 9.0–acetonitrile (50:50, v/v)].

test conditions absolutely constant for so many runs. The loss in tensile strength and the increase in the elasticity modulus upon thermal aging [11] did not affect the mechanical properties of the capillaries investigated.

Heat-cured fused-silica tubing proved to be superior as tube material for packed capillaries in CEC as well. The average lifetime of capillary columns packed with Nucleosil C_{18} silica of different particle diameters and equipped with sol–gel end frits according to [5] has improved tremendously when fused-silica tubing heat cured at 300 °C for at least 200 h was used. We have not investigated yet whether a higher temperature and a shorter period of heat treatment has the same effect or increases the fragility of the capillaries.

4. Conclusions

The time-dependent loss of separation efficiency in CE when using raw polyimide-coated fused-silica capillaries can be attributed to a swelling of the coating in acetonitrile-containing buffers. Swelling of the capillary coating is often also responsible for shorter column lifetime, which is caused by the formation of cracks, clogging and breaking of the capillary ends. The tendency of the polyimide coat-

ing to swell can strongly be diminished by a heat treatment of the capillaries at a temperature of 300 °C for a longer period of time. The heat treatment makes the fused-silica capillaries more robust and considerably prolongs their lifetime in CE and CEC in acetonitrile-containing buffers.

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