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# Characterisation of inner surface and adsorption phenomena in fused-silica capillary electrophoresis capillaries

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**Abstract**

Method validation in capillary electrophoresis (CE) is substantial for the routine use in quality control. Thus all phenomena that can influence results in CE must be carefully characterised. Especially the inner surface of the fused-silica capillaries plays an important role. Sometimes there is a big difference in the properties of capillaries from different batches. Inner capillary surface can be pictured by scanning electron microscopy (SEM). Nature, pattern and number of cracks and other surface defects altered from batch to batch. These surface defects were no artefacts of sample preparation, which could be excluded by comparing different preparation techniques and results from literature. Treatments with hydrofluoric acid cannot completely remove the surface cracks and defects in subsurface areas. Hydrofluoric acid treatment not only changes the surface roughness but also the inner capillary diameter. A correlation between etching time and increase of diameter is investigated. SEM also pictures adsorption phenomena, e.g. proteins are not adsorbed as a homogenous layer. Clusters at different regions are observed. Thus the $\zeta$ potential differs considerably with space, leading to losses in separation efficiency. SEM can readily be used to analyse the effectiveness of rinsing procedures. There are residuals of adsorbed compounds even after using aggressive rinsing reagents.

*Keywords:* Capillary columns; Surface characterization; Adsorption; Scanning electron microscopy

## 1. Introduction

Capillary electrophoresis (CE) is a rapidly developing analytical separation techniques. The separation efficiency is excellent, thus incorrect results by peak overlapping are minimised. The precision of CE has improved very much during the last years. While five years ago values of 5 to 10% for R.S.D. of peak areas were given, now values of about 0.5% are obtained. This improvement has been achieved by advances in instrumentation, especially in automation of operations and in detection technologies, by optimising the sample concentration [1–6], and by using internal standards [3,7–9]. Thus CE may be preferred to other techniques, especially if a high separation efficiency is required.

Method validation is substantial for quality control. General guidelines to validate CE methods were already proposed [10]. However, there is still more experience in validation of high-performance liquid chromatography (HPLC), which is one of the last remaining disadvantages of CE compared to LC. Thus validation of CE methods is a main issue. Difficulties in transfering methods from one laboratory to another were reported in many cases. These difficulties may depend on changing the instrumentation, the purity of reagents [11] or differences in capillary material [10]. The properties of capillaries from four different suppliers were compared in a systematic study and found distinctly different [12].

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Significant differences between different batches were also reported [13].

Thus the closer investigation of these properties seems to be essential. The aim of this study was to test if scanning electron microscopy (SEM) [14] was a suitable tool to investigate capillary surfaces. Only few authors have tried to find out more about the inner surface of fused-silica capillaries. Gas chromatography (GC) capillaries were investigated using microscopy about ten years ago [15].

In recent studies Barberi et al. used atomic force microscopy (AFM). Topographic profiles of surface areas between 0.5×0.5 $\mu$m and 4×4 $\mu$m were shown [16]. Coated capillaries were also investigated [17].

## 2. Experimental

### 2.1. Preparation of capillaries

In these studies capillaries from different batches and I.D. were investigated (Polymicro, Phoenix, AZ, USA). All chemicals were reagent grade (Merck, Darmstadt, Germany).

Method (A): Twenty 1 cm long pieces of capillary (5 pieces each of 4 batches) were embedded in a home built mould filled with epoxide resin (Struers, Erkrath, Germany) and left to harden overnight. A thin layer of the epoxide kit and also a part of the capillaries were removed on a pot grinding machine (Fa. Mueller, Nürnberg, Germany), using a diamond grinding pot D46 (D=diamond; 46=average particle size in $\mu$m).

This sample preparation allowed us to look inside the capillaries over a range of several millimetres using an SEM S-900 (Hitachi, Japan). The voltage of the electron beam was 1 kV (to avoid excessive loading of the non conducting material). Thus it is not necessary to sputter the sample with gold vapour or gold particles as a conducting metal (The samples in Figs. 1, 4–8 and 12 were prepared by this method).

Method (B): Samples of about 10 cm length from 6 capillary batches were treated with concentrated sulphuric acid overnight. By this method the polyimide layer was damaged. After rinsing with water this layer could be removed by peeling with a razor blade. The peeled capillaries were now smashed using mortar and pestle. The glass particles were now put on the sample tray and coated with gold particles (sputter Balzers, Wiesbaden, Germany, 108 s, 0.1 mbar, 160 V, 25 mA, 50 mm distance between anode and sample tray). At lower magnification the suitable fragments were selected. The shots were taken by a Zeiss SEM DSM 92 scanning electron microscope (Köln, Germany) at 15 kV. (The samples in Figs. 2, 3 and 9–11 were prepared by this method).

The capillaries in Figs. 11–13 were used to determine drug concentrations in human plasma [4]. Three samples of different capillaries were investigated.

### 2.2. Capillary etching

In order to etch capillaries, these were rinsed with hydrofluoric acid (HF) 10% (w/w) (Merck) using a vacuum pump (30 mbar) for 30 s, rinsed with 0.1 $M$ hydrochloric acid (HCl) for 2 min, and again HF 10% for 30 s. The further treatment was rinsing with 0.1 $M$ HCl for 5 min, 6 min with HPLC grade water (Millipore, Darmstadt, Germany) and drying by an air stream for 4 min. One etched sample was pictured (Fig. 8).

### 2.3. Measurement of the inner capillary diameter

The increase of the inner capillary diameter by HF etching was measured applying Hagen-Poiseuille's law (Eq. 2, see below). Therefore a flow meter was constructed (Fig. 13). The capillary (l approx. 7 cm) was linked to silicone hose by an adapter. The upper end of the hose was linked to an open dropping funnel. Its high capacity guarantees the constancy of the filling height ($h$=1.25 m) and so the constancy of the hydrostatic pressure ($\Delta p$). Thus all parameters like pressure difference, time, viscosity, capillary length and density were constant during these measurements. Hagen-Poiseuille's law is reduced to Eq. 1:

$$r^4 = c \times m_r \qquad (1)$$

Before the capillary was etched for the first time, the mass ($m_r$) of a liquid (HCl 0.1 $M$) that rinsed

through the capillary in a definite time ($t_r$=5 min) was determined ($n$=5 measurements). The declared value of the inner diameter was inserted in Eq. 1, thus the constant c could be obtained.

Now capillaries from Polymicro (1 approx. 7 cm) were etched several times as described above. After each step, $m_r$ was again determined (5 min, $n$=5). The new inner diameter was then calculated using Eq. 1.

### 2.4. Electroosmotic flow (EOF) measurements

The capillary was of 48/50 μm I.D. A home-built CE instrument was used, similar to the ones described previously [18,19]. The total capillary length was 40 cm, effective length 25 cm, and UV detection was performed at 200 nm. The capillary was rinsed with 0.1 *M* NaOH for 3 min, with running buffer for 3 min and equilibrated at 20 kV for 30 min. The running buffer was phosphate 0.08 mol/l, pH 7.4. It was prepared by filling up 9.073 g of $KH_2PO_4$ and 11.876 g of $Na_2HPO_4$, respectively, to 1000.0 ml with HPLC grade water (Millipore). Then 18.2 ml of the potassium phosphate and 81.2 ml of the sodium phosphate solution were mixed. Injection was done electrokinetically at 5 kV for 10 s. The EOF was determined at 15 kV, using acetanilide as marker.

EOF dependence on etching: the capillaries were rinsed with HF 10% (w/w) for 30 s and with 0.1 *M* HCl for 2 min. Afterwards the equilibration process described above was done before the EOF measurement.

## 3. Results and discussion

### 3.1. Opening of the capillaries and avoidance of artefacts

The drawing process conditions, e.g. the cooling rate and the quality of the bulkware influence the properties of the fused-silica capillaries [20]. In order to investigate this, samples for SEM were prepared. Therefore the capillaries must be opened (see Section 2). The danger of producing artefacts by SEM sample preparation must be discussed. The preparation technique of smashing the capillaries may change the original surface. Thus the peeled outer surface (see Section 2) was compared before and after smashing. No further cracks or phenomena were observed (no figures shown). Moreover parts without visible defects at the inner surface were discovered (Fig. 2).

If the samples are prepared by embedding the capillaries and grinding the sample, the formation of artefacts is very unlikely. The capillaries are fixed in the epoxide kit and just the upper part is removed by the grinding pot. The embedded parts of the tubes are not touched by the grinding artefacts, because the rotation direction is parallel to the capillaries. Fig. 1 gives an overview of such a lengthwise opened capillary.

### 3.2. Observed surface structures

An extreme example of significant differences between batches is shown in Figs. 2 and 3. However, similar differences were also observed in only one batch when different parts of the same spool were investigated.

Capillaries were prepared for SEM before and after pretreatment with 0.1 *M* NaOH and buffer. Obviously the preconditioning does not change the microscopic properties very much. However, additional structures were observed, when used capillaries were investigated. These structures could be crystallised buffer constituents. If not stated otherwise, surfaces shown here were not pretreated.

If the sample was sputtered with gold, in some cases no surface defects or inhomogenities could be detected by SEM. However, this does not mean that there are none [16,21]. Comparing theoretical and practical stability of glasses it becomes clear that there are always surface defects [22]. These are probably covered by the gold layer (thickness: approximately 25 nm). Unsputtered samples always show surface defects by SEM, as well as by AFM [16].

Vertical stripes, perpendicular to the drawing direction of the capillary, were observed in two samples (Figs. 4–6). They are possibly caused by the drawing or the cooling process.

Bulged structures, like at the top of Fig. 7, were found in every sample. They are possibly crystallisation products.

Bending of capillaries does not increase the num-

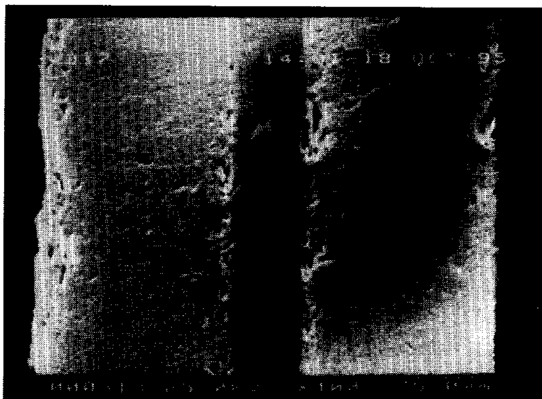

Fig. 1. Overview of a longitudinal opened capillary (sample not sputtered with gold, magnification approx. 400×).

Fig. 3. An outer surface area of a peeled capillary, showing very big surface defects. Sputtered sample, same magnification as Fig. 1.

Fig. 2. Capillary surface. No surface structures can be observed despite the presence of an dust particle, fixed by the gold sputtering. This particle was used to focus at the surface (magnification approx. 25 000×).

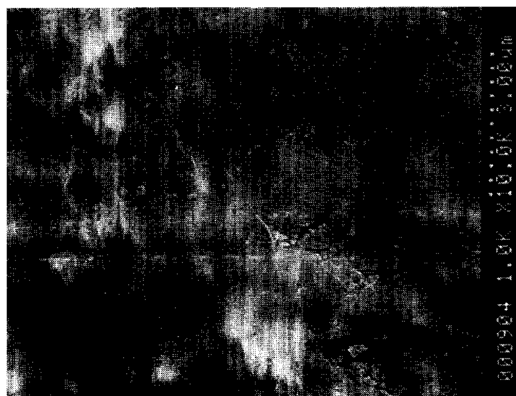

Fig. 4. A rough area of a non sputtered sample, showing several different surface structures like cracks and vertical stripes (magnification approx. 10 000×).

ber of surface defects. Several pieces of capillary from one spool, approximately 30 cm long were compared. Some of these pieces were coiled with a radius of 1 cm. No differences in the inner surfaces were observed (no Figure shown).

### *3.3. Deepness of cracks and influence on the EOF*

The deepness of the cracks can also be estimated by AFM. The maximum depth measured was about 60 nm [16]. However, only limited surface areas were scanned in this work. Using SEM, it is easier to get an overview over a wider surface segment (Fig. 4). The number of shots per sample is unlimited, only a few minutes per shot are required. In this work about 10 shots per sample were taken. Interesting details can be easily magnified (Figs. 5 and 6). Probably the defects are deeper than 60 nm in this example.

Cations are preferably adsorbed at surface defects.

Fig. 5. Magnification of Fig. 4 (20 000×).

Fig. 7. Surface defects and bulged structures, which were found in every sample (non sputtered sample, magnification approx. 13 000×).

Fig. 6. A further magnification (50 000×) of Fig. 4.

Fig. 8. Inner surface treated with hydrofluoric acid (see Section 2). Some of the above described surface structures, e.g., the bulged structures and the vertical stripes, are not found anymore, but the cracks could not be removed completely. Sample not sputtered (magnification 5000×).

Thus the Stern layer and the $\zeta$ potential locally change [23], the EOF is locally different as well. Locally different bulk velocities lead to band broadening like in LC. Therefore the separation efficiency is affected by the surface structures of the capillary.

The significance of these effects depends on the scale of the structures. If the structures were much smaller than the diffuse double layer, as assumed in [16], these effects would be negligible.

### 3.4. Effect of HF etching on the capillary surface

The standard reagent to etch glassy materials is HF. A concentration of 10% (w/w) HF proved to be optimal. Lower concentrations show only minor effects, whereas concentrated HF is too aggressive: the etching velocity cannot be properly controlled.

If the capillary is pretreated with HF, some of the surface structures described above, e.g., the bulged structures and the vertical stripes, are not found anymore (Fig. 8). The etched surface shows less defects. Although it was impossible to remove all the cracks, the local deviation of the $\zeta$ potential should be decreased. Moreover the amount of the $\zeta$ potential and hence EOF should increase [23].

In order to prove this, the EOF was measured after etching the capillary several times with HF 10% for 30 s (Fig. 9). The EOF did not increase significantly after etching for 30 s, which corresponds to a layer of about 0.5 $\mu$m thickness (compare Table 1). However, there was a significant increase after the second and third etching period of 30 s, which is consistent with the assumptions made above. After four or more etchings no further increase of the EOF was found.

Table 1
Calculated changes of inner capillary diameter using Hagen-Poiseuille's law. The mass of the liquid $m_r$ was measured five times (compare Section 2)

| $m_r$ (mg) | I.D. ($\mu$m) | $\Delta$I.D. (nm) | $m_r$ R.S.D. (%) |
|---|---|---|---|
| 89.8 | 75.00 | 0 | 1.50 |
| 99.98 | 77.04 | 2039.80 | 1.44 |
| 101.28 | 77.30 | 250.00 | 1.23 |
| 102.10 | 77.46 | 156.00 | 1.05 |
| | | | |
| 97.34 | 75.00 | 0 | 0.56 |
| 99.02 | 75.32 | 321.60 | 0.40 |
| 106.34 | 76.68 | 1355.00 | 1.26 |
| 106.66 | 76.74 | 57.60 | 1.26 |
| | | | |
| 86.75 | 75.00 | 0 | 0.92 |
| 88.70 | 75.56 | 541.80 | 1.54 |
| 90.80 | 75.86 | 318.60 | 0.88 |
| 91.20 | 75.94 | 83.40 | 2.15 |
| 91.40 | 75.98 | 41.60 | 0.23 |
| | | | |
| 99.40 | 75.00 | 0 | 0.86 |
| 101.70 | 75.44 | 430.20 | 1.65 |
| 102.20 | 75.52 | 92.40 | 1.53 |
| 102.80 | 75.64 | 110.60 | 0.86 |
| 103.20 | 75.70 | 73.60 | 1.34 |
| 106.40 | 76.30 | 583.80 | 0.95 |

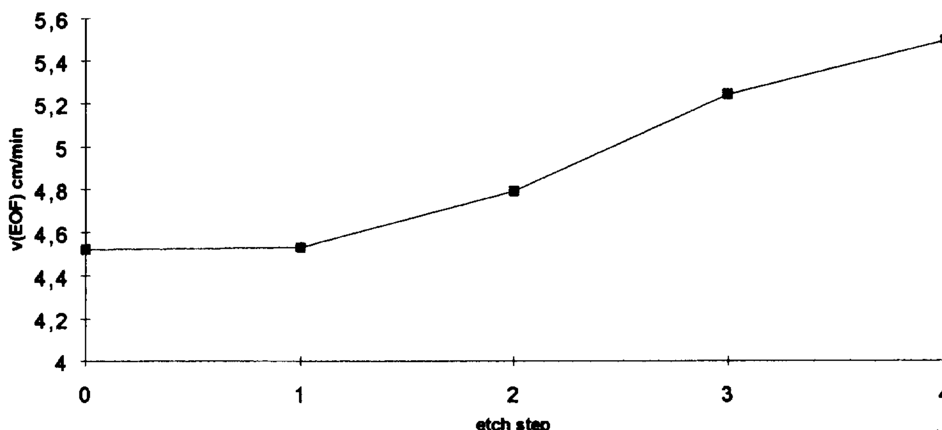


Fig. 9. Capillaries were etched several times with HF 10% for 30 s. The EOF did not increase significantly after etching for 30 s, which corresponds to a layer of about 0.5 $\mu$m thickness (compare Table 1). A significant increase was found after the second and third 30 s, after four or more etchings no further increase of the EOF was measured.

### 3.5. Measurement of the increase of the capillary diameter

The increase of the capillary diameter and thus the decrease of the layer was measured using a flow meter (see Section 2 Fig. 10). The mass flow depends on the diameter of the capillary. Very small changes in the diameter cause significant changes of the mass flow, because the radius is set to the forth power in Hagen-Poiseuille's law (Eq. 2). This method is highly sensitive and reproducible (C.V.% ($m_r$)< 3%).

$$\eta = \frac{\Delta p \times \pi \times t_r \times r^4}{8 \times 1 \times \frac{m_r}{\rho}} \qquad (2)$$

The etching has less effect on deeper layers. The first cracked layer does not resist against the aggressive reagent. The increase in the inner diameter was usually maximal for the first etching steps (Table 1). This suggests that there are differences in glass properties in a layer of about 0.5 μm from the surface compared to deeper layers.

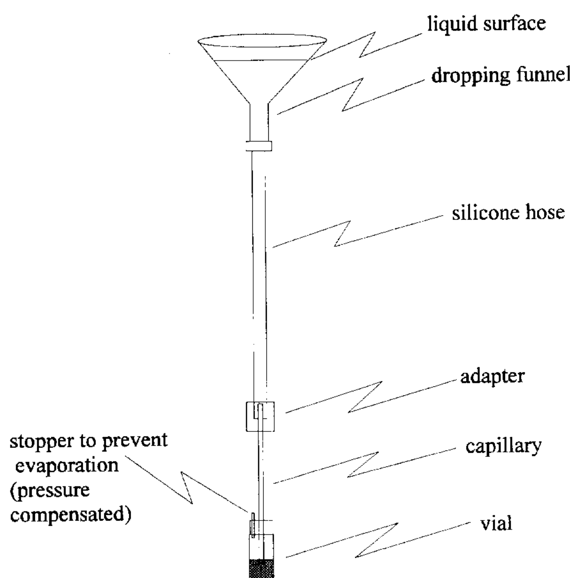


Fig. 10. Scheme of flow meter. Proportions not true to scale: capillary length, approx. 7 cm; filling height, 125 cm (distance of liquid surfaces in funnel and vial).

### 3.6. Analysis of adsorption phenomena

SEM can also be used to study adsorption phenomena in CE. Capillaries were used to analyse drugs from plasma, as described in [24,25]. The buffer used was borate pH 10.0, 60 mmol/l, containing 200 mmol/l sodium dodecyl sulfate (SDS). This separation system managed to separate paracetamol and salicylic acid from the plasma proteins and led to reproducible results.

These capillaries were opened after running some measurement series. Figs. 11 and 12 show typical clusters obtained. There is no continuous layer but a patchwork-like cover on the surface. Fig. 13 demonstrates the thickness of such adsorption layers.

These layers are probably formed by plasma proteins for the most part. Similar structures were obtained when serum or human serum albumin (HSA) samples were injected (no figures shown). However, the thicker layers are obtained when plasma is injected compared to serum or HSA. Possibly constituents of the clotting system play a role in the layer formation, too.

Figs. 11–13 are shots of a plasma component adsorption after washing with an SDS–methanol–NaOH mixture. Fig. 14 also shows an adsorbed layer but the sample was not covered by a gold layer before (compare the contrast to Figs. 11–13).

The ζ potential will be different over adsorbed layers compared to free silica surface sections. This will strongly affect the local EOF (compare above). Hence, if the capillary surface looks like a patchwork with adsorbed matrix components, the separation efficiency of low molecular mass components is considerably decreased, although they are not absorbed themselves (as observed in [26]).

## 4. Conclusions

SEM can be used to characterise cracks, vertical stripes, bulged structures and adsorbed compounds at capillary surfaces. Working at low voltages it is possible to study the non conducting silica surface without sputtering with gold vapour or gold particles and thereby more surface details can be observed. In combination with a gentle sample preparation, the original surface can be seen in every detail.

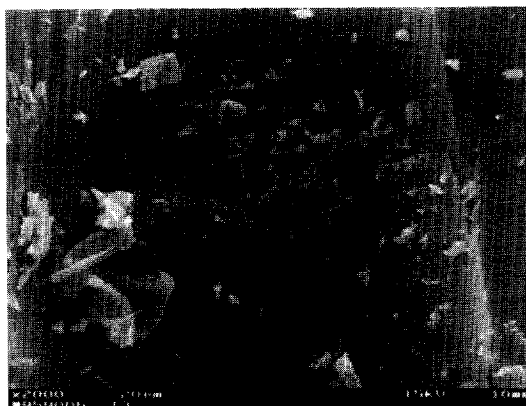

Fig. 11. Adsorbed plasma components. This capillary was used to analyse drugs from plasma, as described in [24,25]. The buffer used was borate (pH 10.0, 60 mmol/l), containing 200 mmol/l sodium dodecyl sulfate (SDS). Despite of the high concentration of detergent, the surface is covered with an inhomogeneous layer of proteins (non sputtered, 2000×).

Fig. 12. Magnification of Fig. 9 (10 000×).

Fig. 13. A view of adsorbat from the edge. The change from the broken part to the inner surface can be seen at the right. Thickness of protein layer: about 0.5 $\mu$m, although 200 mmol/l SDS was present in the buffer (sputtered, 10 000×).

Fig. 14. Protein adsorption on inner capillary wall. Sample not covered by a gold layer (compare the contrast to Figs. 11–13 which were sputtered before).

A gold layer may cover some interesting details. However, it may be advantageous to increase the contrast and thus the quality of shots (compare Figs. 11–13 to Fig. 14).

The treatment with HF can be used to smooth the capillary surface, but also to investigate surface properties. A top layer of about 0.5 $\mu$m thickness is different compared to deeper layers.

SEM in addition to the AFM [16,17] enables an accurate characterisation of glass surfaces and adsorption phenomena. It is easier to estimate the relation between rough and smooth surface areas using AFM. However, SEM provides a better overview of surface structures.

Microscopic studies can be used to test the quality of capillaries. This diagnostic tool can help to optimise manufacturing conditions. The effectiveness of rinsing procedures can be judged. It is supposed that the quality of the coated capillaries can be similarly controlled. In their latest work, Barberi et al., took shots of coated capillaries by AFM, which helped to optimise the coating conditions [17].

Not all differences from capillary to capillary can be explained by surface cracks and inhomogenities.

Their dimension is very small compared to the inner diameter. Further properties may be important: the quality of the bulkware [20], the number of active surface silanol groups, the surface density and the surface contamination.

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