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Characterization of inner surface phenomena in capillary electrophoresis capillaries by electron microscopy, atomic force microscopy and secondary ion mass spectroscopy

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Abstract

Scanning electron microscopy (SEM), atomic force microscopy (AFM) and secondary ion mass spectroscopy (SIMS) were used to investigate the topography and physicochemical properties of fused-silica capillary inner surfaces. SEM is a quick and economical standard technique, e.g., it is used to investigate adsorption phenomena and to optimize the drawing processes. However, it is impossible to investigate coated capillaries, and vertical information is only vague. Here the use of AFM is advantageous, e.g., in deep grooves (down to 500 nm) using this technique. SIMS offers poor lateral resolution (1 μm), but a great deal of chemical information about adsorbates or immigration of ions into deeper glass layers. Other high-resolution techniques like X-ray photoelectron spectroscopy (XPS) and Auger electron spectroscopy (AES) are not or not yet [scanning tunneling microscopy (STM), near-field scanning optical microscopy (NSOM)] practical techniques for investigating capillary electrophoresis (CE) capillaries. © 1997 Elsevier Science B.V.

Keywords: Capillary columns; Atomic force microscopy; Scanning electron microscopy; Mass spectrometry; Adsorption

1. Introduction

Some phenomena in capillary electrophoresis (CE) are not yet fully understood, e.g., adsorption, stability of coatings and long-term changes of the electroosmotic flow (EOF). All these aspects are connected to surface processes. In the last two years it became possible to open CE capillaries [1–4] and to characterize their inner surfaces by atomic force microscopy (AFM) [1,2,4] and scanning electron microscopy (SEM) [3]. Bare fused-silica shows a number of surface defects, like bulged structures, cracks and grooves. Between these defects, there are areas that are rather smooth [1,3].

AFM also proved to be useful to characterize coatings. Oxidized silicon wafers were coated by the same method as CE capillaries. The coating procedure was optimized by using AFM in order to obtain a homogeneously covered coating. The final conditions led to a stable coating. The EOF was reduced to 10^{-4} compared to uncoated capillaries [2].

Microscopic techniques can also be useful to understand adsorption phenomena [3,4]. Adsorption and desorption of the protein ferritin (isoelectric point, p*I* 5.0–5.2) has been investigated by AFM at pH 4.6 and 7.0 [4]. Adsorption has been found at both pH values. Thus, protein adsorption cannot be prevented by using a buffer pH above the p*I*.

Both 1 *M* NaOH and sodium dodecyl sulfate (SDS) solutions are sufficient to restore most of the bare capillary, but some ridges of proteinaceous material are still found after rinsing. The desorption of the proteins was also directly measured. Most of

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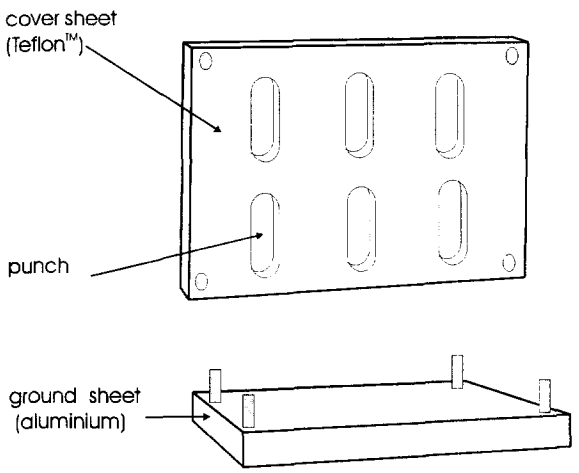

Fig. 1. Laboratory-built mould to embed capillaries.

the adsorbed ferittin passed the detector after the first rinsing, the second rinsing desorbed about 5% of the first. No signals of desorbed proteins have been detected in the following rinsing steps [4]. However, even after intensive rinsing, when the capillary has almost reached its old performance, still patchwork-like rests of adsorbate are occasionally found by SEM [3].

Surface analysis seems to offer a lot of information about CE. However, there are more than the two techniques for surface analysis used up to now. There are many more techniques available (e.g., [5–10]). Before a systematic investigation of surface processes is started, the most useful techniques should be identified.

The first requirement for a surface analysis technique is commercially available robust equipment. The spatial resolution is second-most important. Maximum resolution in horizontal and vertical direction is strongly desirable: from comparing theoretical and observed glass stability it is clear that there are always defects down to the molecular level [11]. Surface details of a size of about 1 nm have been already observed.

On the other hand, a flexible size for the cuts is beneficial. The maximum size possible should not be below 70 μm (about 1 capillary diameter); vertically 25 μm should be possible. From overview cuts it should be possible to quickly magnify details. Thus, scan speed is another important aspect.

The properties of the matter under investigation are important for the choice of the analytical technique. Fused-silica, coated or uncoated, is a poor conductor and relatively chemically inert. Chemical information of a microscopic technique can be very useful to characterize an adsorbate and to distinguish artefacts (e.g., dust and glass particles) from relevant surface details.

A number of microscopic techniques can be excluded, because the parameters they measure are not relevant for CE capillaries (e.g., magnetic force microscopy, scanning thermal microscopy).

Scanning tunnelling microscopy (STM) offers best resolution so far: orbitals of chemical bonds can be visualized. However, STM needs conductive matter as underground; non-conductive material can certainly be made conductive by sputtering, e.g., with a gold layer. However, an additional layer always worsens resolution. Thus STM is not very useful for the intended investigations [5].

Near-field scanning optical microscopy (NSOM) is a scanning probe microscopic technique; resolution and other characteristics are similar to AFM. This technique offers UV–Vis spectra of single molecules on a surface, that means chemical information together with high resolution [12]. This technique seems to be promising for the future. At the present time it is still very expensive.

Apart from SEM and AFM, secondary ion mass spectroscopy (SIMS), X-ray photoelectron spectroscopy (XPS) and Auger electron spectroscopy (AES) are promising techniques because of the chemical information they provide [13].

The aim of this paper is to evaluate which techniques are most useful for the investigation of CE capillaries. By doing this, some interesting facts are observed about uncoated and coated capillaries as well as about adsorption processes.

2. Experimental

2.1. Preparation of capillaries

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

The capillaries were cut to suitable length, sealed at the ends using silicon paste and then embedded in a laboratory-built mould. The dimensions of the mould or of the resulting epoxide blocks depend on the maximum sample size permitted for the respective analytical method. The mould consists of a cover sheet (Teflon) and a ground sheet (aluminum) (Fig. 1). They are put together and fixed by nuts at the corners. The ground sheet is covered by a thin film of paraffin to prevent the epoxide from pasting on it during the hardening. The pieces of capillary were put into the punches and weighted by e.g., screws to keep them completely to the bottom of the mould and to prevent them from floating during pouring the resin. The used embedding material was an epoxide resin (Epofix from Struers, Erkrath, Germany). The Epofix resin consists of two components: resin and hardener (25:3, w/w). Methylethylketone may be added to reduce the viscosity of the resin in order to improve the handling.

All components are mixed at 60°C (thermostatic bath) and stirred for 3 min to obtain a homogeneous liquid and to remove air bubbles. Afterwards the resin is poured into the mould. At about 60°C the resin had to hard out for 8 h. Due to surface tension, the surface of the obtained block is not smooth. Thus, the block is first ground to an exact square stone. Then the block is pasted on a pad and ground by a pot grinding machine (Conrad WOKO 50, Germany) using a diamond grinding pot D46 (D= diamond; 46=average particle size in μm) layer-by-layer in 10 μm steps (Fig. 2). Afterwards dust was blown away by a smooth air stream. No water was used for in the grinding process. For in-process control an optical microscope is necessary.

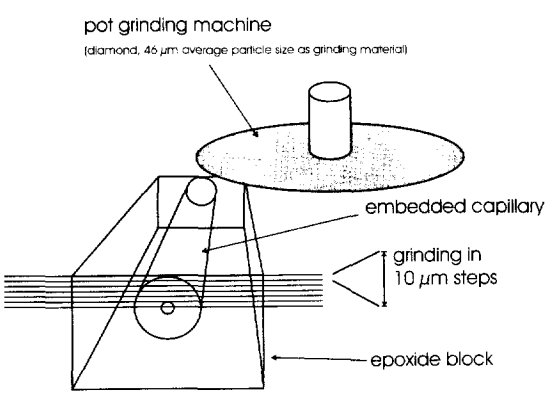

Fig. 2. Grinding of the embedded capillary.

2.2. AFM of coated capillaries

The atomic force microscope was a Dimension 3000 (Digital Instruments, USA). The sample was scanned in the contact mode using a silicon nitride (Si_3N_4) probe tip (Digital Instruments, base width: 200 μm, spring constant: 0.12 N/m).

2.3. AFM studies of uncoated fused-silica capillaries

A fused-silica capillary from the same batch as for the SIMS studies in Section 2.4 was used. The atomic force microscope was an ULTRAObjektiv Rastersondenmikroskop from Zeiss (Jena, Germany). It was measured in the contact mode. The horizontal and vertical resolution were 256 points and the scan speed 0.5 lines/s.

2.4. Stigmatic time of flight (TOF)-SIMS study of a contaminated fused-silica capillary

A fused-silica capillary (75 μm I.D., Polymicro) was pre-treated with 0.1 *M* NaOH at 5 kV for 2 h on a laboratory-built CE separation system. This capillary was equilibrated using 60 mmol/l borate buffer pH 10 (14 days at 7 kV). Finally the capillary was rinsed by Millipore water for 10 min. This capillary was opened and used for the SIMS studies.

The apparatus was a TRIFT Charles Evans (USA). A pulsed 25 keV Ga^+-ion beam (10 kHz, 10 ns) at I=0.5 nA was used for primary bombardment.

2.5. Protein adsorption at laboratory glass surfaces

Human plasma was diluted to 5% in a freshly prepared solution of NaCl (0.9%). This solution was stored in a flask for 5 days at 6°C. The flask was cleaned using a 200 mmol/l SDS solution. Then it was smashed using mortar and piston. Some of the glass particles were sputtered by gold particles (sputter: Balzers, Wiesbaden, Germany, 108 s, 1

mbar, 160 V, 50 mm distance between anode and sample tray) to improve the surface conductivity and investigated by a scanning electron microscope S-900 (Hitachi, Tokyo, Japan) at 25 kV.

2.6. SEM study of uncoated capillaries

An SEM S-900 (Hitachi) was used in the electron microscopic study of uncoated capillaries. The voltage of the electron beam was 1 kV (to avoid excessive loading of the non conducting material, [3]). The gold sputtering described in Section 2.5 would improve the conductivity but cover interesting details.

3. Results and discussion

The new sample preparation described in Section 2.1 allows one to look inside the capillaries over a range of several millimetres. The capillary was embedded in parallel to the surface of a block. This block was sufficiently stable to grind away the surface together with the capillary. Layers of about 10 μm were removed in each step. By grinding layer-by-layer and subsequent cooling of the sample, a warming up could be hardly detected. Artefacts caused by heating coatings or adsorbed proteins can be excluded.

The choice of the embedding material is an important point. Many materials are available. However, most of them are only suitable for metals or soft materials. The hardness of the embedding material has to correlate with the hardness of the embedded object, in this case the glass capillaries. Otherwise the capillaries splinter during the grinding process. Volume contraction during the hardening out may change the parallel position of the capillary to the surface. Lengthwise opening would become impossible. The used epoxide resin corresponds to all these demands.

Preliminary experiments were carried out with XPS and AES. The electrostatic loading of the sample made the experiments very difficult. Only poor information was obtained: at the surface there are Si–O bonds with different chemical neighbourhood. In deeper layers all Si–O seem to be alike. Because of this low pay-off other techniques were preferred during this study.

SEM is a standard technique. It is available in many research centres, and is economical and quick. The lateral resolution is about 1 nm. The conductivity of bare fused-silica is still sufficient for analysis by SEM without sputtering, although there are some loading effects (Fig. 3). However, it is impossible to investigate coatings with SEM without sputtering (no Figures shown). Moreover, SEM only offers vague vertical information.

Protein adsorption on model glass surfaces has been further investigated. A measuring flask was exposed to a plasma solution and then cleaned (using SDS) to virtual cleanness. Adsorbed protein fibers, probably containing fibrin and albumin, were found

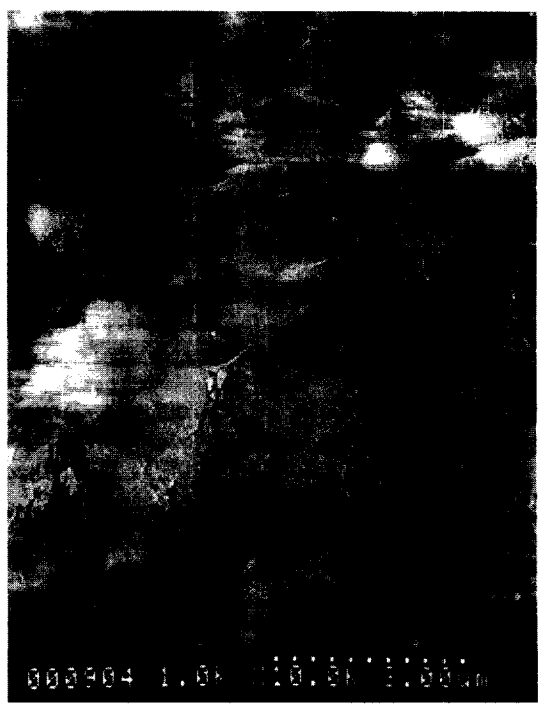

Fig. 3. Non sputtered, uncoated capillary investigated by SEM (magnification approx. 10 000×). A vertical groove can be clearly recognized, however the depth of this groove cannot be estimated. Though a voltage of 1 kV offers sufficient resolution and helps to avoid over-loading, some white spots with horizontal stripes indicate that loading is not completely prevented.

(Fig. 4a Fig. 4b). This result is in agreement with earlier findings [3,4]. Most of the adsorbed proteins are washed out instantly, but even long rinsing times do not lead to complete desorption. However, small amounts of adsorbed proteins may influence experiments due to catalytic effects. This study shall be

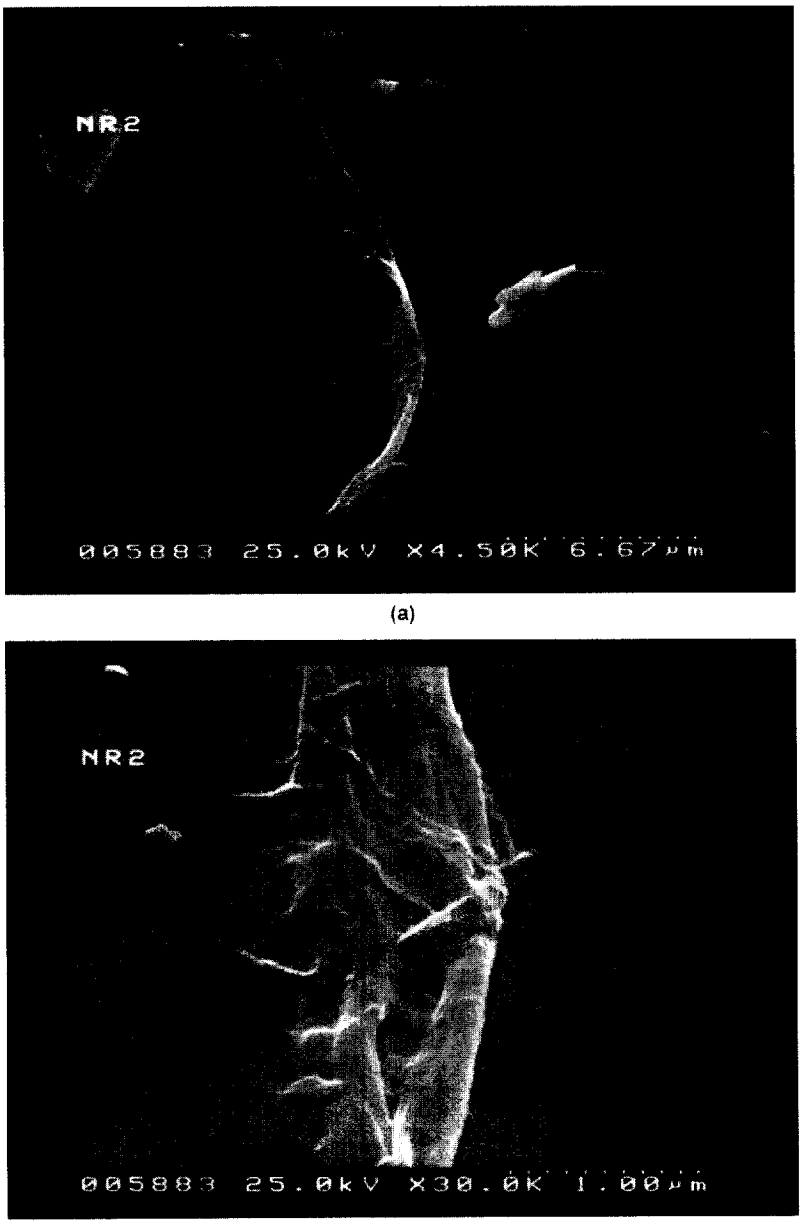

Fig. 4. (a) Virtually clean glass after protein exposure and cleaning with brush and SDS-containing water. In some regions adsorbates are still observed. (b) Magnification of (a).

continued with respect to more efficient cleaning procedures.

AFM was one of the first scanning probe microscopic techniques. The principle is simple: a tip probes the surface like a stylus of an old phonograph used to do with a vinyl disc. Because the movements of the tip are very small, they have to be magnified, e.g., by optical systems [2,4].

AFM instruments offer good lateral and vertical resolution (about 1 nm). In principle, atomic and even subatomic resolution is possible [5]. In practise, resolution is limited by the probing tip. A tip of only a few atoms, ideally one, is necessary. It has been demonstrated that these tips can be prepared; however, they are still not available for routine analysis. The resolution of 10 nm in this study was easily obtained.

In contrast to SEM, AFM allows precise determination of the depth of surface structures. In Fig. 5 an overview AFM scan of a bare fused-silica capillary is shown. Radial grooves, probably grinding traces from the raw cylinder used for drawing, can be observed. These can also be seen by SEM (Fig. 3), but using AFM their depth can be precisely measured using profile graphs (Fig. 6). Usually there are no surface defects deeper than 100 nm (like found in [1]). However, the grooves are more than 500 nm deep. It has been postulated that these grooves are cleavages as they look different (no Figures shown).

Another advantage of AFM over SEM is that the disturbing effects caused by electrostatic loading do not occur. Non-conducting coated surfaces can well be characterized. The inner surface of a capillary is strongly curved, because the radius is very small. It was assumed that this could cause problems when AFM is used, but the curvature can easily be compensated. The distance of the probe to the surface can be regulated with very high resolution but allows high deflections at the same time.

Fig. 7a shows an overview of an opened capillary, coated with polyacrylamide. A few larger elevations are detected; their origin is not yet clear. Possibly this is dust. Although dust was not found on uncoated capillaries, it may be attached to the much more sticky coated surface. Two magnifications of the same surface are given in Figs. 7b and 7c. The

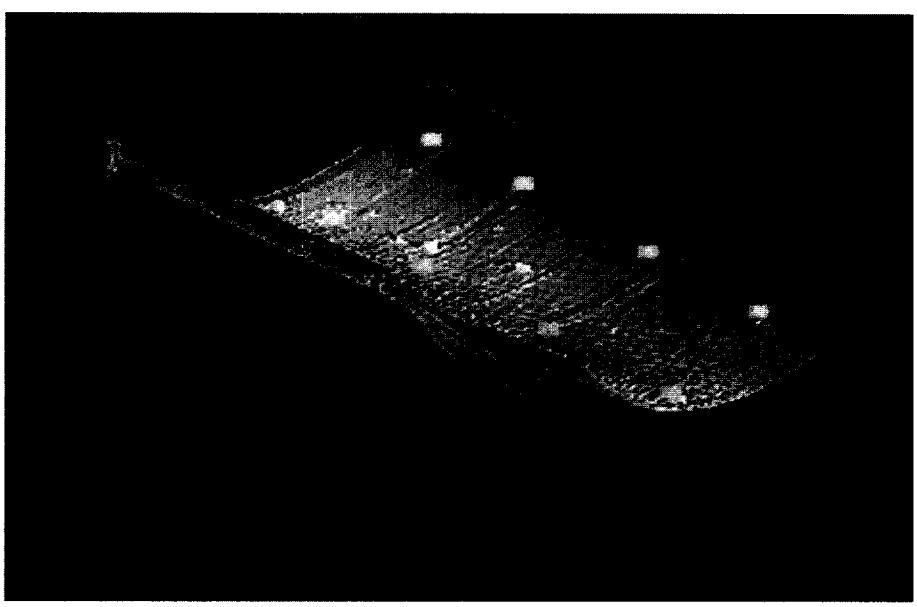

Fig. 5. AFM surface overview of bare fused-silica capillary (8 μm×4 μm). Deep grooves were detected as in Fig. 3. The two lines of four rhombi mark the positions of the profiles given in Fig. 6.

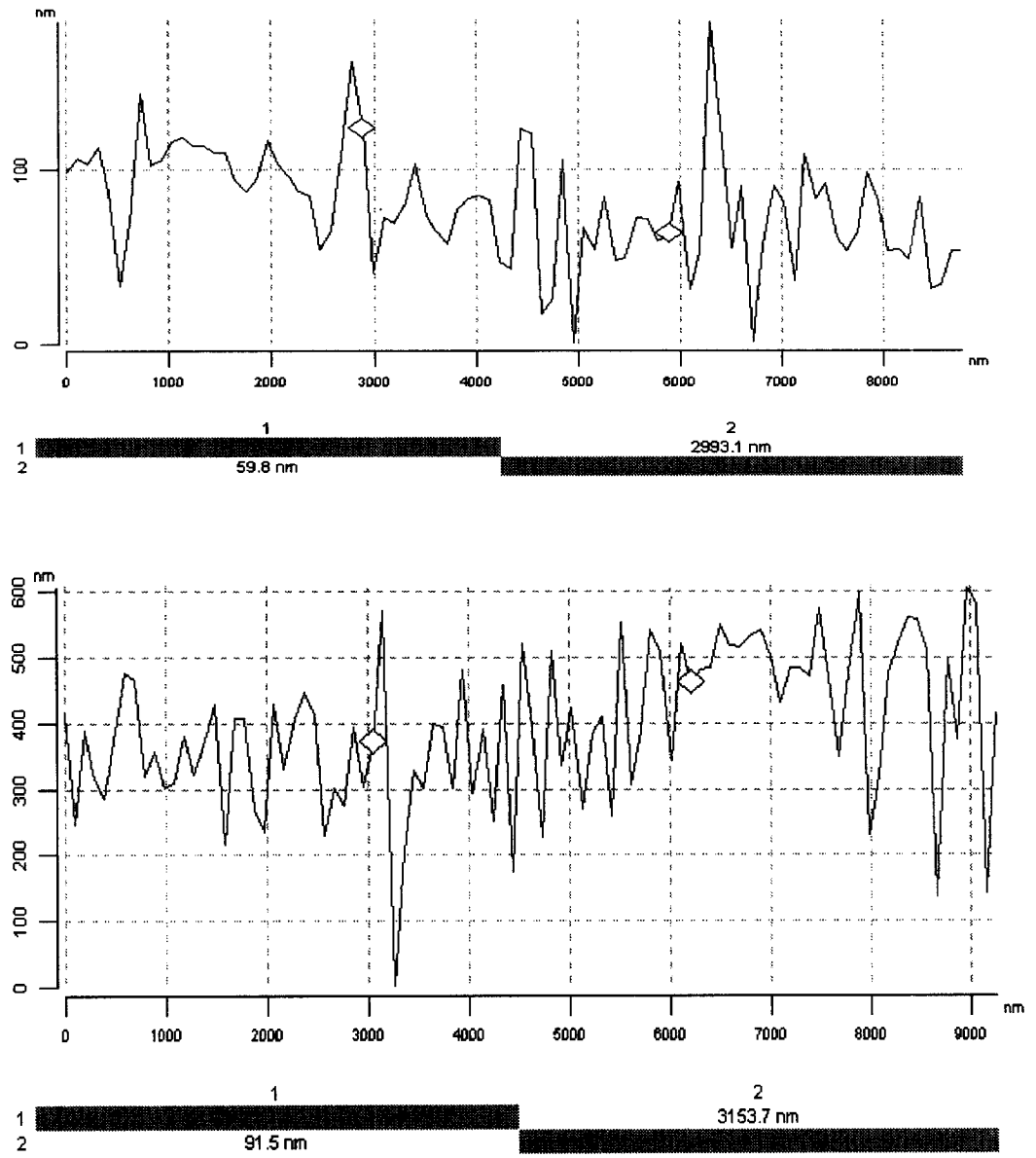

Fig. 6. AFM surface profile of bare fused-silica capillary. The upper and lower profiles correspond to the upper and lower lines of rhombi, respectively (compare to Fig. 5). Beginning and end are marked by the outer rhombi, the inner two rhombi correspond to the inner rhombi drawn in the profile. At the grooves, height differences of more than 500 nm were found.

maximum resolution achieved was about 10 nm. A veined structure of the linear polymers attached to the surface can be seen. Moreover, some elevations (white spots) are eye-catching. There scale is again estimated by a profile (Fig. 8). Compared to bare fused-silica, the roughness is rather decreased. Remaining irregularities on this scale are probably just caused by defects of the fused-silica underground.

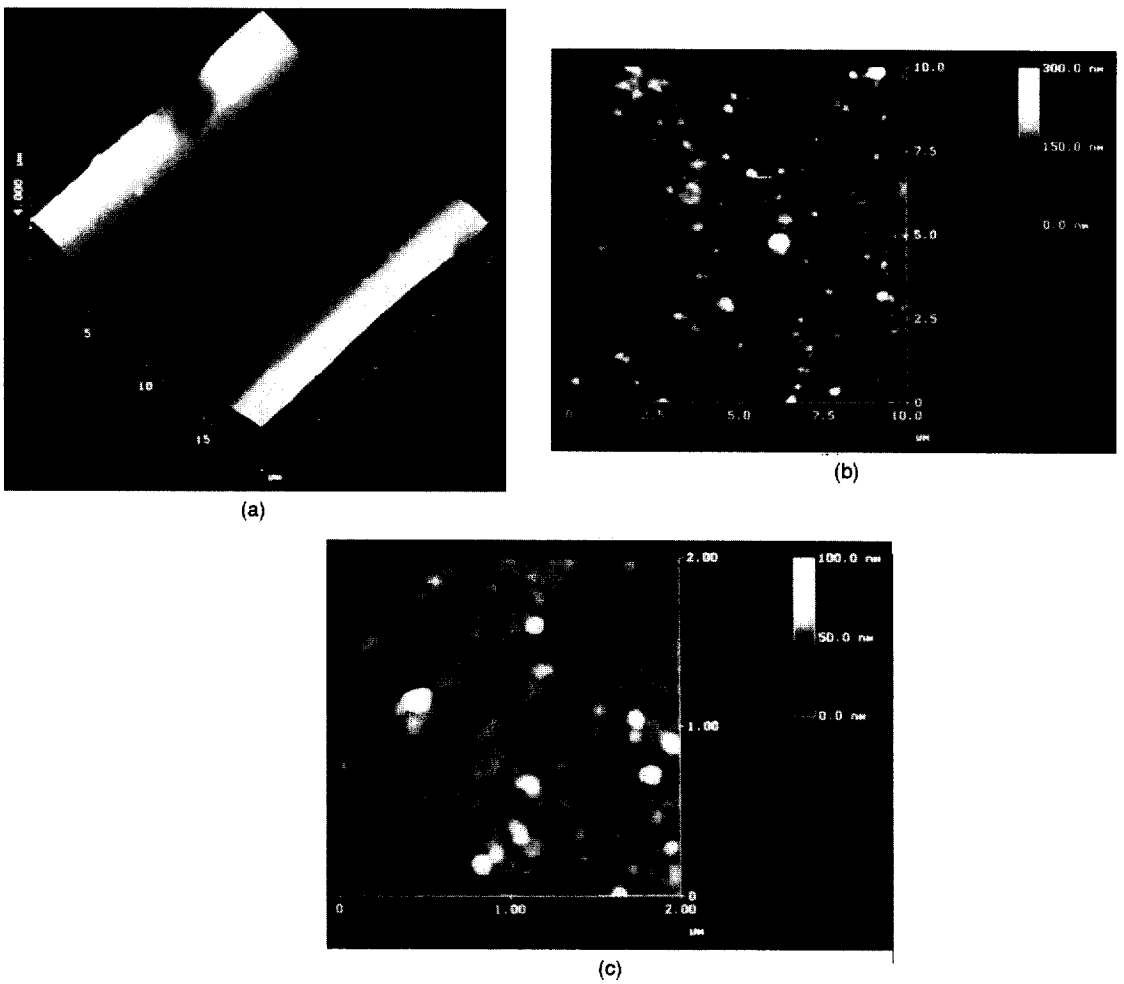

Fig. 7. (a) Segment of a polyacrylamide coated capillary (20 x 20 μm). (b) Magnification of (a). The coating seems to cover the surface completely. A veined structure of the coating is shown. A number of elevations can be observed; their height can be estimated using profiles (Fig. 8). (c) Magnification of (b). Reticular structure of the coating with small rises but no holes. The lateral resolution is limited to about 10 nm.

There are other scanning probe microscopic techniques with similar performance like AFM, e.g., lateral force microscopy (LFM) and NSOM.

SIMS offers sufficient vertical resolution (about 5 nm), but a lateral resolution of only about 1 μm.

This shortcoming is compensated by a lot of valuable chemical information. Fig. 9 shows cuts of a opened capillary. In the middle the inner surface can be seen.

Immigration of ions into glass (up to 200 nm) is always observed when glass electrodes are used. Here it takes hours, sometimes days, until the equilibrium is reached [14,15]. In order to investigate if this phenomenon can be found for CE capillaries as well, a capillary was exposed to a sodium borate solution for 14 days. After removing a layer of about 5 nm, the signal of sodium becomes much weaker (Fig. 9), whereas the signal of silicon remained constant (data not shown). In general, sodium does not immigrate into the bulk silica deeper than 5 nm.

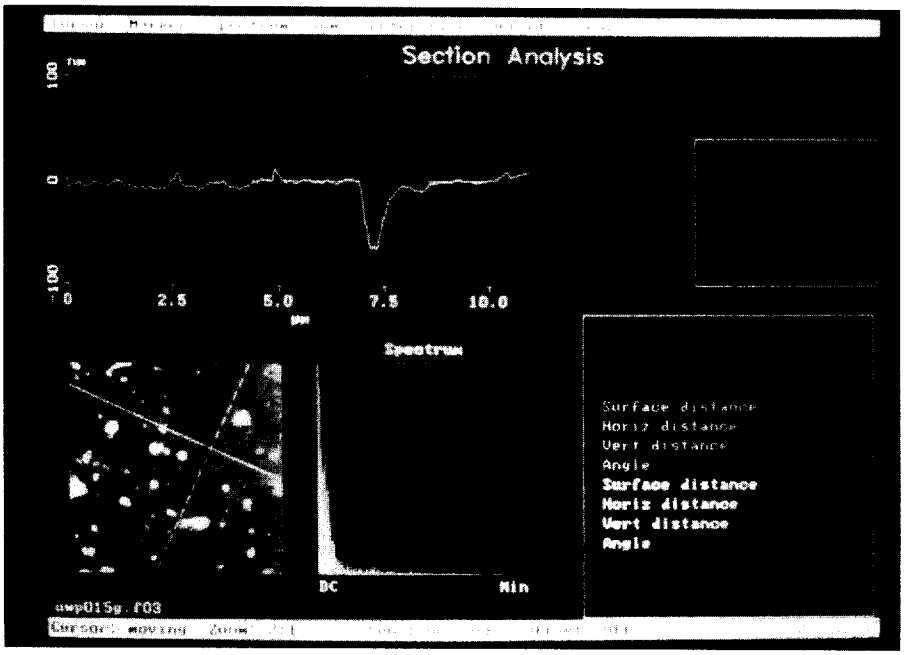

Fig. 8. In a surface profile the heights of the small rises can be precisely determined. At this section of the capillary a hole is detected, possibly caused by a underlying groove. The observed roughness is smaller than with bare fused-silica.

This may be explained by the different glass types and networks. For glass electrodes different lithium or aluminium containing silicates are used.

However, in a few areas the sodium signal is less reduced. Perhaps sodium can migrate into the deeper layers via small cracks.

Moreover, a significant signal of carbohydrogens on the silica surface was detected using SIMS, probably contaminants from the polyimide coating or the embedding (data not shown).

4. Conclusions

SEM, AFM and SIMS proved be useful techniques to analyse inner capillary surfaces. SEM is a quick and economical standard technique. It should be preferred to study adsorption phenomena and to optimize the drawing processes. However, it is impossible to investigate coated capillaries using SEM, and vertical information is only vague. Here the use of AFM is advantageous. Grooves were found that were down to 500 nm deep using this technique. Coatings could be characterized with respect to completeness of coverage and homogeneity. SIMS offers a lot of chemical information. It was shown that there is no bulk immigration of sodium ions into deeper glass layers.

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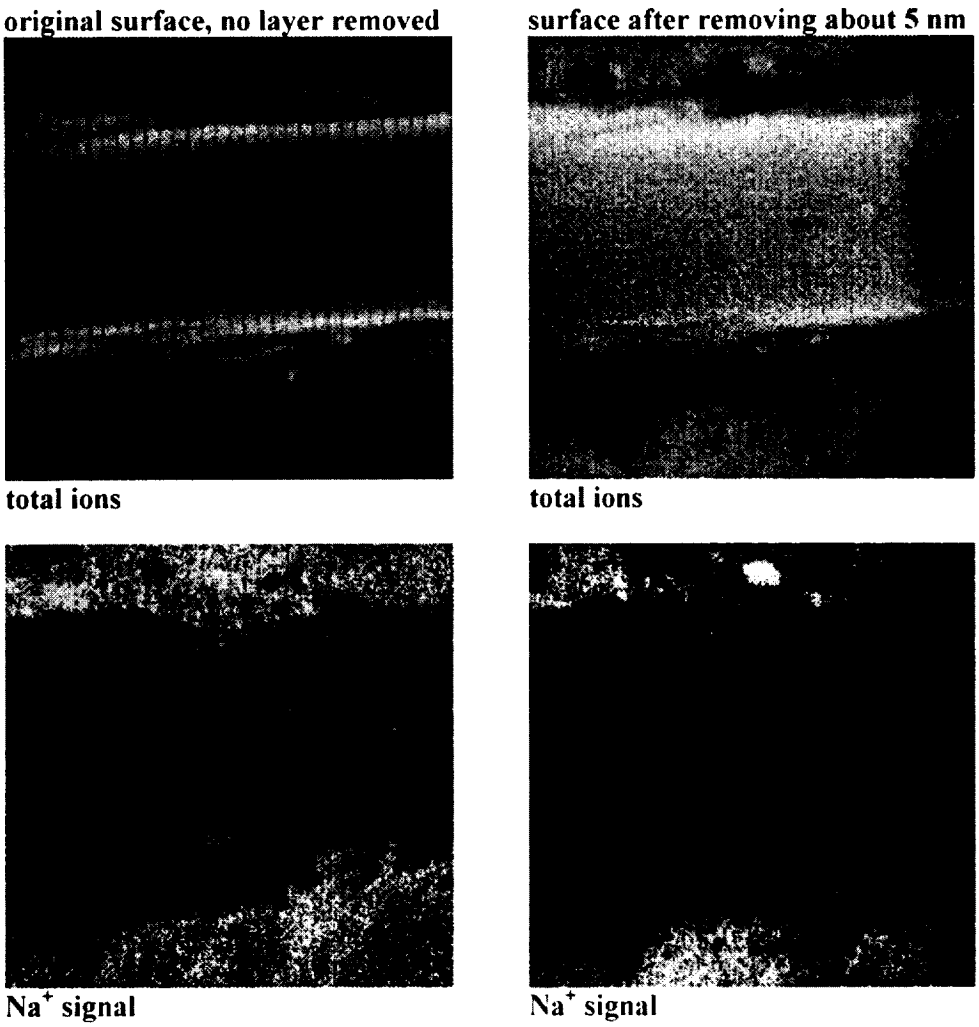

Fig. 9. TOF-SIMS study of an uncoated capillary before and after ion sputtering (cut: 140 μm×140 μm). The capillary was pre-treated with sodium borate buffer (see Section 2.4). Sodium ions are adsorbed at the surface. However, after removing a layer of 5 nm by sputtering, the sodium signal strongly decreases indicating that ions do not immigrate into the bulk silica.

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