Short communication

Unexpected surface chemistry in capillaries for electrophoresis ☆

Stefan Kaupp[a], Henning Bubert[b], Leonhard Baur[d], Gary Nelson[c], Hermann Wätzig[d],*

[a]*Byk Gulden, D-78467 Konstanz, Germany*
[b]*Leibniz-Institut for Spectrochemistry and Applied Spectroscopy (ISAS), Bunsen-Kirchhoff-Strasse 11, D-44139 Dortmund, Germany*
[c]*Polymicro Technolgies Inc., 18019 N 25th Avenue, Phoenix AZ, 85023, USA*
[d]*University of Braunschweig, Institute of Pharmaceutical Chemistry, Beethovenstrasse 55, D-38106 Braunschweig, Germany*

Abstract

Good and reproducible capillary quality is needed to develop robust methods and to facilitate method transfer in CE. Physical surface defects no longer play a major role in variability of fused-silica capillaries. Nevertheless, problems are frequently being reported when buffers in the pH range between 4 and 7 are used. Thus the surface chemistry has been studied by X-ray photoelectron spectroscopy. Silicon–carbon bindings have been found on inner capillary surfaces for electrophoresis. This binding type is not completely removed by pre-conditioning with 1 M NaOH for 30 min. This corresponds to the result, that capillaries provide more stable migration times, especially in the pH range 4–7, when they are pre-conditioned for longer than 1 h. The origin of this Si–C bond is still not quite clear. They could be caused by graphite which is used during the fabrication of the raw cylinders prior to capillary drawing. Further investigations are intended in order to understand if there are any differences in surface carbon content from batch to batch and if this can influence experimental results in CE. A better understanding of the surface chemistry should not only improve robustness in CE, but also help to facilitate and accelerate capillary pre-conditioning and rinsing procedures to remove strongly adsorbed analytes or matrices. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: X-ray photoelectron spectrometry; Capillary columns; Surface composition

1. Introduction

Good and reproducible capillary quality is needed to develop robust methods and to facilitate method transfer in CE. Thus several groups have characterized the inner surface of capillaries for electrophoresis during the last years [1–8].

In earlier investigations, stronger surface defects have occasionally been observed [5,6]. Moreover,

the drawing temperature has been identified as a crucial parameter for capillary quality [7]. Meanwhile all parameters that control the microscopical capillary quality are clearly identified. If properly handled, physical surface defects no longer play a major role in variability of fused-silica capillaries. It is now possible to obtain capillaries with ''atomic flatness'', that means the remaining roughness is in the order of atomic distances [7]. Subsequently, difficulties related to capillary properties have significantly decreased during the last years.

However, problems are still frequently being reported when buffers in the pH range between 4 and 7 are used [9,10]. These problems are strongly changing migration times from run to run and from

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capillary to capillary during method transfer. These changes in migration times are always caused by changes in the electroosmotic flow (EOF). This is certainly due to a change in charge at the capillary wall, therefore this effect is most profound in the pK_a range of the surface silanol groups. If there are no structural differences between the capillaries on a microscopic scale, differences in surface chemistry must be the reason for these effects.

In order to investigate the microscopic differences between capillaries, methods have been developed to open capillaries without destroying the inner surface [6,7]. These methods should be of use for the investigation of the surface chemistry as well.

In this study, X-ray photoelectron spectroscopy (XPS) has been preferred among other surface analytical techniques to provide most information about chemical binding properties. The lateral resolution for XPS is only in the μm range. However, other techniques only offer very restricted chemical information (like secondary ion mass spectroscopy (SIMS), Auger electron spectroscopy (AES), transmission electron microscopy (TEM), transmission electron energy loss spectroscopy (TEELS)). Scanning near-field optical microscopy (SNOM) offers good spatial resolution and chemical information, but it requires absorbance in the UV or visible range. IR or Raman offer even less spatial resolution than XPS [11,12].

In XPS, solids are irradiated by X-rays of a characteristic energy $h\nu$ and photoelectrons may be ejected by ionisation of atoms in the solid. The discrete kinetic energy E_{kin} of these electrons is measured. The binding energy E_B of the electrons can be determined using Eq. (1):

$$E_{kin} = h\nu - E_B - \Phi_S \qquad (1)$$

where Φ_S is the "work function" which depends on both the spectrometer and the sample. The chemical surroundings of an atom influences the binding energies of its electrons and, therefore, the measured photoelectron energies give information about neighbouring atoms.

A better understanding of the surface chemistry should substantially reduce the need for sample pretreatment in CE. Thus costs in CE will be further cut, making this technique even more attractive compared to other analytical separation techniques.

2. Experimental

All the capillaries were provided by Polymicro Technologies (Phoenix, AZ, USA). Their dimensions were 363 μm O.D.×51 μm I.D. Pieces of capillary were pre-treated with 1 *M* NaOH for 0, 30 and 300 min, respectively. Then the samples were prepared by embedding and grinding the capillaries like described in [6].

Photoelectron spectra were recorded on an X-ray photoelectron spectrometer AXIS-HS (Kratos, Manchester, UK), using non-monochromatic Al Kα radiation for excitation, a take-off angle for the electrons of 0° with respect to the surface normal analysis. The pass energy of the concentric hemispherical analyser (CHA) has been set to 10 eV. For depth profiling an Ar$^+$ ion beam was used, ion energy 2 keV, angle of incidence 45° with respect to the surface normal.

The samples showed low conductivity and the emerging charge could only partly be dissipated to the conductive sample carrier. Therefore, a low electron energy flood gun was used to compensate charging to a large extent. The remaining charge shifting of the spectra was corrected relative to the saturated hydrocarbon line (C1s at 285.0 eV). The spot investigated was about 30 μm in diameter.

In order to get a spectral survey of the samples, wide-scan spectra were taken using only 1 eV per step. For quantitative analysis, narrow-scan spectra were recorded for each visible line using 0.2 eV per step. The quantification of the last mentioned spectra was carried out by applying a standard procedure, i.e. linear background subtraction, fitting the measured lines by means of a set of Gaussian curves and converting the intensities into atomic concentrations by using atomic sensitivity factors [13].

3. Results and discussion

In order to investigate the chemical surface properties of fused-silica capillaries for electrophoresis, they have been compared with the well-characterized reference silica material (SiO$_2$) Herasil. Capillaries have been prepared by a method described recently [6].

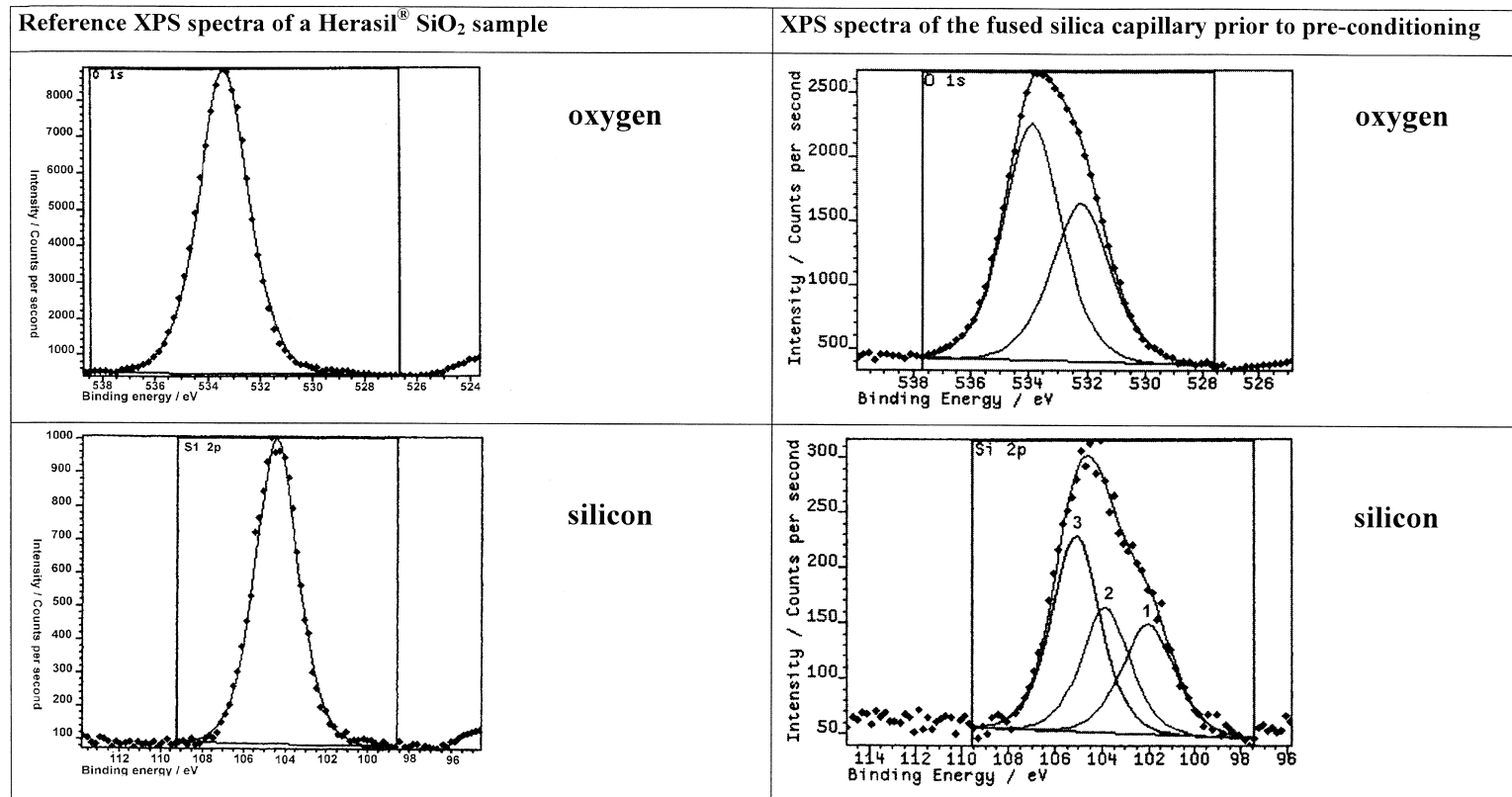| Reference XPS spectra of a Herasil® SiO₂ sample	XPS spectra of the fused silica capillary prior to pre-conditioning

Fig. 1. Reference XPS spectra of a Herasil SiO₂ sample and of fused-silica capillaries for electrophoresis prior to pre-conditioning. The reference spectra show one Gaussian peak at 533.5 eV and 104 eV, respectively. The spectra of the fused-silica capillary show asymmetric peaks. These can be deconvoluted to two Gaussian peaks with maximum at 532 and 534 eV for oxygen and to three peaks at 102, 103.3 and 105 eV for silicon.

The reference XPS spectra show the expected signals for SiO$_2$ (Fig. 1, left). The peaks appear at characteristic kinetic energies. Note the peak symmetry which indicates one predominant binding type.

The inner surface of CE capillaries has been investigated using the same set-up. At first, capillaries have been investigated directly after drawing without any pre-conditioning. A typical result is shown in Fig. 1, right. Two Gaussian peaks are necessary to deconvolute the oxygen spectrum, indicating two types of oxygen bindings, due to siloxane (Si–O–Si) bonds and silanol groups (Si–OH), which are known to be present on the surface ([8,14] and literature cited therein).

The silicon spectrum however is very surprising. Two signals can again be related to siloxane and silanol. However, the additional signal with maximum at 102 eV (No. 1 in Fig. 1) corresponds to a bond of silicon to a much less electronegative element [13]. On the same sample XPS signals corresponding to carbon were found on the capillary surface. Carbon was also found on the inner capillary surface in an earlier study using secondary ion mass spectroscopy (SIMS) [6]. Thus this signal at 102 eV should correspond to a silica–carbon bond.

Next the influence of the capillary preconditioning on the XPS spectra has been investigated. After 30 min pre-conditioning with 1 M NaOH, the third peak corresponding to the Si–C binding has been strongly reduced but could still be detected. After 6 h pre-conditioning, the third peak at 102 eV was no longer observed. This finding corresponds to the result, that longer equilibration time improve the EOF long term stability, especially in the pH range between 4 and 7 (Fig. 2, from [10,15]).

This experiment has also served as a control. All three capillary samples have been opened by the same method. Thus it is unlikely that the carbon on the surface is due to a contamination during or after the embedding or the opening of the capillaries, because this was done after the NaOH treatment.

The covalently bound carbon on the surface could originate from the preparation of the raw cylinder prior to the capillary drawing process. These are obtained from synthetic silica rods that have been core drilled. During this drilling process, graphite is used as a tool to form the inner bore. Graphite
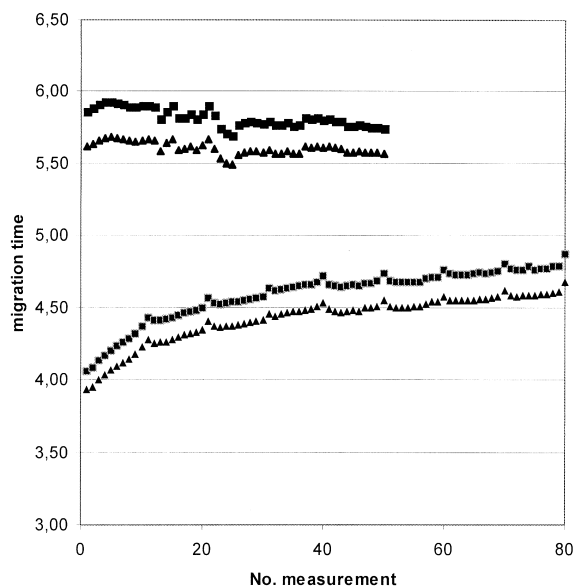

Fig. 2. Variation of the migration time after standard capillary pre-conditioning (1 h, 0.1 M NaOH, normal symbols) and after 6 h preconditioning using 1 M NaOH (bold symbols) [10,15]. The analytes 3- and 1-methyl esters of *cis*-camphoric acid (triangles and squares, respectively) have been completely separated using a 40 mM sodium acetate buffer, pH 4.0. The longer pre-conditioning times led to significantly improved migration time reproducibility (3-methyl ester: migration time, t_M, ranging from 5.48 to 5.68 min (RSD 0.8%), compared to t_M from 3.93 to 4.64 min (RSD 3.8%) after standard pre-conditioning; 1-methyl ester: t_M ranging from 5.69 to 5.93 min (RSD 1.1%), compared to t_M from 4.06 to 4.83 min (RSD 4.0%) after standard pre-conditioning).

contains phenol-like and epoxide-like moieties on its surface. These could react with the silanol or siloxane at the silica surface at the elevated temperatures during drilling. Finally, this raw cylinder is etched (HF) and then drawn to create the tubes used in the capillary draw.

4. Conclusions and outlook

Silicon–carbon bindings have been found on inner capillary surfaces for electrophoresis by XPS. This result has been confirmed by SIMS analysis. This binding type can be removed by extensive NaOH

pre-conditioning, but this needs longer than 30 min. This corresponds to the result, that capillaries provide more stable migration times, especially in the pH range 4 to 7, when they are pre-conditioned for longer than 1 h.

The origin of this Si–C bond is still not quite clear. They could be caused by graphite which is used in creating the raw cylinders prior to capillary drawing. Further investigations seem necessary and interesting, in order to understand if there are differences in surface carbon content from batch to batch and if this can influence the electroosmotic flow and derived parameters such as migration times and thus peak areas. A better understanding of the surface chemistry will also improve our ideas about capillary cleaning and pre-conditioning.

Acknowledgements

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