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FULL PAPER

PLASMA PROCESSES AND POLYMERS

Argon–water DBD pretreatment and vapor-phase silanization of silica: Comparison with wet-chemical processes

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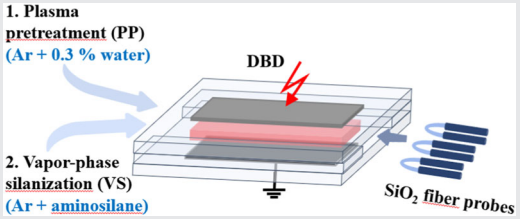
Abstract

A dielectric-barrier discharge (DBD) in an argon–water mixture is applied to plasma pretreatment (PP) of amorphous silica for subsequent vapor-phase silanization (VS) in the same reactor. Comparison of amino-silanization of silica fiber-optic biosensor probes using a PP/VS sequence with strategies involving wet-chemical pretreatment or silanization shows a considerable improvement in reproducibility by the completely dry process. Practical applicability is demonstrated by an immunoassay with human immunoglobulin G as analyte. Thanks to the advantages of shorter processing time, avoidance of washing steps, and improved reproducibility, PP/VS is highly promising for industrial use.



KEYWORDS
argon–water, biosensors, dielectric-barrier discharges, plasma pretreatment, silanization

1 | INTRODUCTION

This paper presents a new application of dielectric-barrier discharges (DBDs) in humid argon, the cleaning and hydroxylation of fused silica as a pretreatment for a subsequent vapor-phase silanization (VS). The specific application considered here is the amino-silanization of silica optical fibers to be used for fiber-optic biosensors (FOB). These types of biosensors, silanization of silica, and the required pretreatment will be outlined in the following sections before the role of plasma pretreatment as a replacement of state-of-the-art wet-chemical processes is explained.

1.1 | Fiber-optic biosensors

FOB constitute a subclass of optical biosensors, which offer several advantages such as miniaturization,

high-throughput analysis, and easy integration with various optical phenomena such as absorbance, fluorescence, and interferometry.[1] While there are several commercial applications using silica or polymer optical fibers as simple waveguide-based extrinsic sensors,[2,3] a significant quantum of research has been in progress toward the development of fiber-optic intrinsic sensors by exploiting the evanescent waves (EW) at the fiber core surface. A large number of studies using single or multimode silica fibers with modified core geometry or grating structures embedded within the core have also been reported.[4,5] FOB based on a modified fiber core geometry, especially the U-bent multimode optical fiber sensors, show distinct advantages of good reproducibility, ease in handling, and optical coupling; they are relatively robust and compact, in addition to having high EW absorbance sensitivity due to improved light–matter interactions.[6–8] Since the EW are a near-field phenomenon, it is important to immobilize the bioreceptors within the evanescent field on the sensor surface to realize an efficient FOB with high sensitivity, reliability, and reproducibility.[9–11]

1.2 | Silanization

The bioreceptors are typically immobilized covalently onto a functionalized fiber core surface to obtain uniform, stable, and reproducible coverage.[12,13] As functionalizing agents, organosilanes are often used for the attachment of bioreceptors such as antibodies, DNA, or cells on glass or silica surfaces. Silanes carrying amino groups such as (3-aminopropyl)-trimethoxysilane (APTMS, $(CH_3O)_3Si–(CH_2)_3–NH_2$) or -triethoxysilane (APTES, $(C_2H_5O)_3Si–(CH_2)_3–NH_2$) are generally preferred[14] due to the nucleophilic properties of amines, that is, their reactivity with carbonyl compounds, such as active esters or aldehydes, with isothiocyanates, and epoxides. With aqueous solutions of glutaraldehyde (GA, $O{=}CH–(CH_2)_3–CH{=}O$), the reactivity can easily be reversed into electrophilic, resulting in a surface which is able to bind biomolecules carrying amino groups.

During attachment of APTMS or APTES to a silica or glass surface, condensation reactions take place between surface silanol groups, ≡Si–OH, and silanol groups formed intermediately by hydrolysis of the aminosilane's methoxy or ethoxy groups with a small quantity of water. Providing (a) a contamination-free surface with a high density of silanols and (b) a controlled amount of water during the reaction are, therefore, vital to obtain reproducibly, a smooth, dense, and stable aminosilane layer. Although the term "self-assembly monolayer" is frequently applied to this kind of layers, it is in fact difficult to achieve, using APTMS or APTES with three alkoxy groups, a degree of order in an aminosilane layer as it may be done, for example, with monofunctional molecules such as thiols on gold surfaces. The reason is the ability of these silanes to polymerize, that is, to form siloxane linkages (≡Si–O–Si≡) with other silane molecules, a process which can happen in a silane solution in the presence of water, or on the surface.

Depending on the history of the surface to be silanized, the density of silanol groups can be substantially lower than the maximum possible value, typically about 5 per nm^2.[15] During the manufacturing or, in the present case, bending of silica optical fibers, for example, the amorphous SiO_2 undergoes a thermal treatment at a temperature beyond 1,000°C. At this temperature, virtually all of the silanol groups, which are normally present on a silica surface in equilibrium with the humidity of ambient air, will be condensed to siloxane groups, rendering the surface relatively hydrophobic and inert.[16] The full rehydroxylation necessary for silanization is a relatively slow process, requiring hours or even days in water.[17] Electron-spectroscopic investigations reported by Wendt et al.[18] indicate that defect-free, well-ordered silica surfaces are unreactive toward adsorbed water at low temperatures and that certain point defects are required to make the formation of silanol groups possible. The role of surface defects for water reactivity at silica surfaces has also been demonstrated by molecular dynamics simulations.[19]

Many different protocols have been reported in the literature for the pretreatment of surfaces before silanization from the liquid or vapor phase.[14] Frequently, wet-chemical methods involving strongly oxidizing, acidic media are used such as "piranha solution" (a mixture of concentrated sulfuric acid and 30% hydrogen peroxide) or solutions of hexavalent chromates in concentrated sulfuric acid. These media are able to remove organic contaminations by the cleaning action of oxidative Cr^{6+} or H_2O_2, and simultaneously provide a strong acidic environment in which the catalytic action of protons, H_3O^+, helps "open" the siloxane bond and furnish the required silanol groups.[20] In addition to the wet-chemical treatment, exposure of the substrate to a plasma, mostly a low-pressure oxygen plasma, has also been frequently employed for surface cleaning or "activation."[14]

In addition to providing surface silanol groups, it is crucial to control the amount of water on the surface to avoid excessive condensation and polymerization of silanes, which typically results in multilayer formation. In contrast, it is also required to at least have a small amount of water on the surface for the formation of siloxane linkages. Thus, obtaining a uniform and reproducible monolayer is still a challenge and the formation of multilayers has repeatedly been reported

with wet-chemical silanization (WS) processes despite using nominally anhydrous organic solvents.[21–23]

As an alternative to wet-chemical protocols, VS has been investigated by a number of authors, see, for example, References [24,25] and solvent- and gas-phase protocols have been compared in several papers.[21,26] Quite generally, vapor-phase deposits have thicknesses closer to a monolayer and are smoother, because the deposition of silane polymer particles, formed even in "anhydrous" solvents by adventitious amounts of water traces, can be avoided.[21] The amount of water involved in the vapor-phase process can be controlled by the parameters (temperature, water vapor partial pressure) of a dehydration step inserted between substrate pretreatment and silanization.[24]

1.3 | Cleaning and hydroxylation of silica in an atmospheric-pressure Ar–water plasma

As already noticed by Sneh and George,[17] siloxane sites on the surface of (partially) dehydroxylated silica can be cleaved at low temperatures by H atoms and HO radicals, in their experiments provided by a low-pressure (40 Pa) water plasma.[17] At present, the detailed chemical mechanism of this process is still unknown, but there are reasons to believe that the initial reactions in Equations (1) and (2) both contribute to the overall process result, a virtually completely hydroxylated surface:

$$\equiv\mathrm{Si{-}O{-}Si} \equiv + \mathrm{H}\cdot \rightarrow \equiv\mathrm{Si}\cdot + \mathrm{HO{-}Si} \equiv \quad (1)$$

$$\equiv\mathrm{Si{-}O{-}Si} \equiv + \mathrm{HO}\cdot \rightarrow \equiv\mathrm{Si{-}O}\cdot + \mathrm{HO{-}Si} \equiv \quad (2)$$

Equation (1) represents the interaction of atomic hydrogen with strained bonds in the amorphous SiO_2 network, which, according to ab initio modeling, results in a so-called hydroxyl E′ center.[27,28] It is unclear if a subsequent reaction of this center with water plays a role as it is observed for a "broken bond defect" ≡Si· ·O–Si≡.[29,30] A reaction with HO radicals, however, should finally result in two neighboring silanols while a reaction with an H atom would yield a ≡Si–H group from which hydrogen could again be abstracted by another H atom[28] or by an HO radical to reform the hydroxyl E′ center.

However, there are also arguments for the reaction in Equation (2) to play a role as the first step in the siloxane cleavage mechanism: Aside from the mechanisms discussed in a theoretical paper on the interaction between HO radicals and various moieties on a silica surface,[31] cleavage of siloxane bridges induced by HO radicals have been shown to play a role in the crystallization of zeolites under hydrothermal conditions,[32] and HO radicals may also be responsible for the hydroxylation of siloxane bridges of silica bilayers in contact with a thin ice layer under electron-beam irradiation.[33] The radical center ≡Si–O· formed as an intermediate in Equation (2) will react with water by abstraction of hydrogen or by recombination with H atoms from the gas phase.

Apart from their role as reactive agents for the hydroxylation of silica, the strongly oxidizing HO radicals are able to remove, virtually, any organic contamination, including pyrolytic graphite, at room temperature. The collision efficiency (γ) for oxidation of the latter is >0.005 at 298 K and HO radicals are found to be much more reactive than free oxygen atoms.[34]

Summarizing, one may conclude that a water plasma is able to take over both functions of the conventional wet-chemical pretreatment (WP) of SiO_2 surfaces: removal of organic contaminants by oxidation as well as the catalysis of siloxane-bridge hydrolysis, resulting in full hydroxylation in short time at moderate temperatures.

1.4 | Content and organization of the present paper

From a practical and economical point of view, it was of interest to investigate if a simultaneous cleaning and hydroxylation of silica might be achievable using an atmospheric-pressure plasma treatment in a water-containing atmosphere, providing HO radicals for efficient removal of carbonaceous contaminations, H atoms and HO radicals for the generation of catalytically active defects, and water for full hydroxylation.

In the present paper, the results of corresponding investigations are reported and compared with the results of state-of-the-art wet-chemical processes. We show the feasibility of an integrated process consisting rehydroxylation of a high-temperature-annealed silica fiber in an atmospheric-pressure DBD and immediate subsequent vapor-phase amino-silanization in the same chamber. A DBD in humid argon, as it is used here, is an efficient tool to produce highly reactive species H and HO in a near-room-temperature gas at atmospheric pressure.

In the main part of the paper, results of experimental studies are reported in which different combinations of pretreatment and silanization steps were investigated to assess the feasibility of replacing the commonly applied combination of a WP in strongly oxidizing solutions (piranha or chromate/H_2SO_4) and WS (the entire process abbreviated as WP/WS) by a completely dry procedure, utilizing a DBD for the pretreatment (PP) with subsequent VS in the same reactor (PP/VS). To compare and evaluate the three procedures, including combinations of the wet and dry process steps, WP/WS, PP/WS, and

PP/VS, fluorescein isothiocyanate (FITC) was used as a reporter molecule. FITC is highly electrophilic and can react with nucleophiles such as amines, thiols, and hydroxyls, forming thioureas, thiocarbamates, and carbamates, respectively.[35] With carboxylates, amides can be formed at elevated temperatures.[36] In the present case, however, where organic contaminations can safely be assumed to be absent on the silica surface before silanization, FITC is amino-selective. As a crucial comparative test, a direct immunoassay was carried out with FITC-tagged human immunoglobulin G (HIgG) as an analyte.

For the major part of the plasma treatment, a mixture of Ar with 0.3% H_2O was applied. When the corresponding experimental work had largely been completed, chemical-kinetic results obtained with a simplified model of the gas-phase chemistry, subject of an accompanying paper,[37] became available which suggested that it could be of interest to do experiments with substantially lower water vapor fraction. Results of the corresponding experiments, yielding a significantly larger binding of FITC by the silanized surface, are reported in chapter 3.4.

2 | EQUIPMENT, MATERIALS, AND METHODS

Protocols used for the preparation of U-bent fiber probes, buffers, and other solutions, as well as for secondary functionalization can be found in the Supporting Information Data.

2.1 | Electrical and optical equipment

The reactor used for the DBD treatment of the bent fibers and the VS is shown schematically and not to scale in Figure 1, together with the electrical and gas supply system.

The DBD reactor consists of two 0.23-cm thick quartz plates (length in gas flow direction, 13 cm), separated by two 0.23-cm thick quartz bars, leaving a 2.1 cm wide-open channel of $d = 0.23$ cm height, through which the gas flows. The electric field is applied by two strips of a self-adhesive metal foil, with 10-cm length and 2.1-cm width, glued onto the opposite faces of the reactor as shown in Figure 1. The fibers are inserted into the discharge volume (4.8 cm^3) about 1 cm deep.

To work at elevated temperatures, the reactor is heated from below and from the sides, using a well-shaped, home-made temperature-controlled heater, and thermally insulated on top by a 2-cm thick styrofoam plate with a central hole through which the high-voltage connection is made. The heater base-plate is made from two plates of copper soldered together, both carrying half-cylindrical, milled grooves (0.6 cm diameter), forming, after soldering, a meander-shaped channel used to preheat the gas stream before entering the reactor. The bottom plate had two drilled holes (0.8 cm diameter) for the heating elements and one (0.11 cm diameter) for a thermocouple.

In several experiments, the DBD reactor was used after the plasma had been switched off for VS of the fibers at a controlled temperature. The same unit was used for all experiments reported here. To put it into a

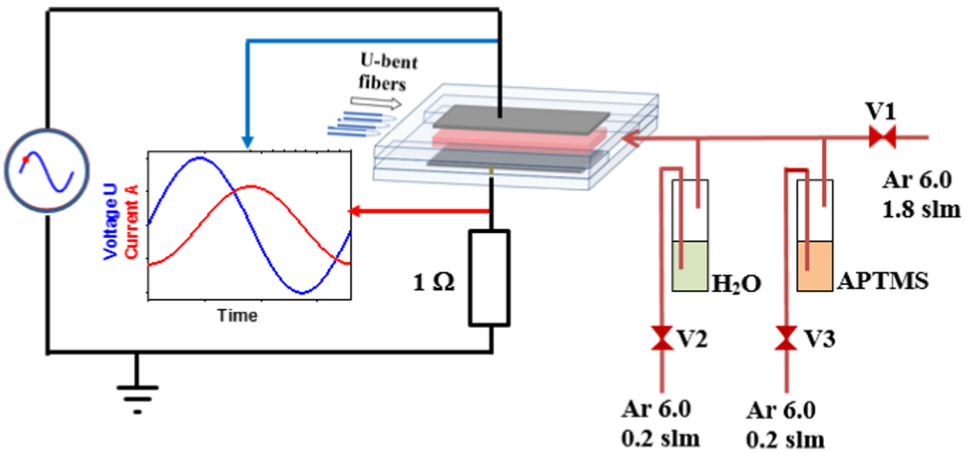


FIGURE 1 Center: Not-to-scale view of the dielectric-barrier discharge treatment unit applied in this study. Light blue: quartz, gray: metal strips used as electrodes, red: discharge (see text for some further details). Blue and red arrows in the electrical circuit, powered by an AC high-voltage generator, indicate measurement of voltage at the reactor and current through the discharge using an oscilloscope. The current is determined from the voltage drop at a 1 Ω resistor. For plasma treatment, valves V1 and V2 in the gas line are on, valve V3 is off; for silanization valve V3 is on, valves V1 and V2 are off; for purging valves V1 is on, valves V2 and V3 are off. APTMS, (3-aminopropyl)-trimethoxysilane

reproducible state for every experiment, it was cleaned after each experiment with a 5-min treatment of an Ar–H_2O discharge to destroy organic residues on the wall and convert any silicon-containing deposits into silica.

To power this reactor, a medium frequency voltage generator 7010R and a high-voltage transformer AT 7010R from SOFTAL Corona & Plasma GmbH (Hamburg, Germany) were used. Gas flows were metered by a multigas flow programmer MKS 647C and flow controllers 1179 (MKS Instruments Deutschland GmbH, Germany). Figure 1 schematically shows the electrical circuit (black) and the gas line (brown) with bubblers for H_2O and APTMS, respectively.

Cleaning of U-bent fiber probes by sonication was performed in an EMMI 280 HC ultrasonic bath (EMAG AG, Mörfelden-Waldorf, Germany) with 1,000 W, 28 kHz, at room temperature (also abbreviated RT). Optical transmission measurements on U-bent fibers were done using equipment from Avantes BV (Apeldoorn, the Netherlands): a spectrometer AvaSpec-2048L and a light source AvaLight-DHc, or a custom-made white light-emitting diode (LED) 1 W, 400–800 nm.

2.2 | Materials

Silica fibers purchased from Thorlabs Inc. (FT200UMT; core diameter: 200 μm) were used for experiments reported in chapters 3.1 to 3.3, and chapter 3.5 while fibers from CeramOptec®, Germany (Optran UV; 200 μm core) were applied for investigating the impact of the water vapor fraction x_{H_2O} on the relative density of reactive amino groups obtained (chapter 4.4). Boric acid, sodium tetraborate, phosphate-buffered saline (PBS) tablets, APTMS, bovine serum albumin (BSA), capture antibody solution (goat anti-human IgG, specific to F_{ab} of human IgG, GaHIgG, 2 mg/ml), FITC-tagged HIgG, procured from Sigma-Aldrich (Merck KGaA, Darmstadt, Germany), sulfuric acid (98%), and aqueous GA solution (25%) from Fisher Chemical (Fisher Scientific Company LLC, Waltham, MA), hydrogen peroxide (30%) from Roth (Carl Roth GmbH+Co. KG, Karlsruhe, Germany) were of analytical grade.

2.3 | Procedures

2.3.1 | Pretreatment and silanization of U-bent silica fibers

Wet pretreatment/wet silanization

The U-bent silica fiber probes were cleaned by sonication in acetone (15 min, 1,000 W, 28 kHz). The cleaned U-bent regions (referred to as sensor) of the fiber probes were treated with piranha solution (20 min, 60°C) to oxidize organic contaminations and generate silanol groups on the sensor surface. Thereafter, the fiber probes were washed with deionized water and dehydrated for 1 hr at 115°C to remove physisorbed water.

For amino-silanization, the fiber probes were dipped in a 1% solution of APTMS in a 5:2 (v/v) mixture of ethanol and acetic acid (5 min). Finally, the fiber probes were washed three times in ethanol, sonicated (15 min), and dried (100°C, 1 hr).

Plasma pretreatment/wet silanization

After cleaning in acetone (see above), three to five U-bent silica fiber probes were simultaneously plasma-treated in the DBD chamber at 80°C, using a 2 SLM (2 L/min at standard temperature and pressure) argon gas stream containing 0.3 mol% water vapor, obtained by mixing 0.2 SLM Ar, saturated with water at room temperature, with 1.8 SLM pure Ar. Amplitude of applied voltage and frequency were maintained at 5 kV and 18.6 kHz, respectively. The average power, calculated from integrating the current–voltage product $I(t) \times U(t)$, was 7.5 W, resulting in an average power density, $p_V = 1.5\,\mathrm{W/cm^3}$; the residence time of the gas in the reactor was 0.14 s. The plasma treatment was carried out at varying durations to find the optimum conditions. After switching off the plasma, the sensors were purged with dry argon for another minute to remove physisorbed water. Then, the plasma-treated fiber probes were silanized wet-chemically as described in the preceding paragraph.

Plasma pretreatment/vapor-phase silanization

The acetone-cleaned sensor probes were plasma-treated over a duration of 2 min under the conditions described above. Following the plasma treatment, the fiber probes were silanized in the vapor-phase, using a 200-sccm gas stream of argon, saturated at room temperature with APTMS. To find optimum conditions, temperatures of 60°C, 80°C, and 100°C and durations of 5, 10, 15, 20, 25, 30, and 60 min were used for this step. Finally, the samples were purged in dry Ar for another minute.

2.3.2 | Optical measurements

The amino-silanized fiber probes were inserted between a white LED light source and the spectrometer using SMA connectors and bare fiber adapters. The time-dependent absorbance response due to binding of FITC molecules to the amino groups was recorded in real-time. For this purpose, the fiber probes were dipped into 100 μl of 0.1 mM FITC solution prepared in 100 mM borate buffer. Borate buffer was used as a reference to record the

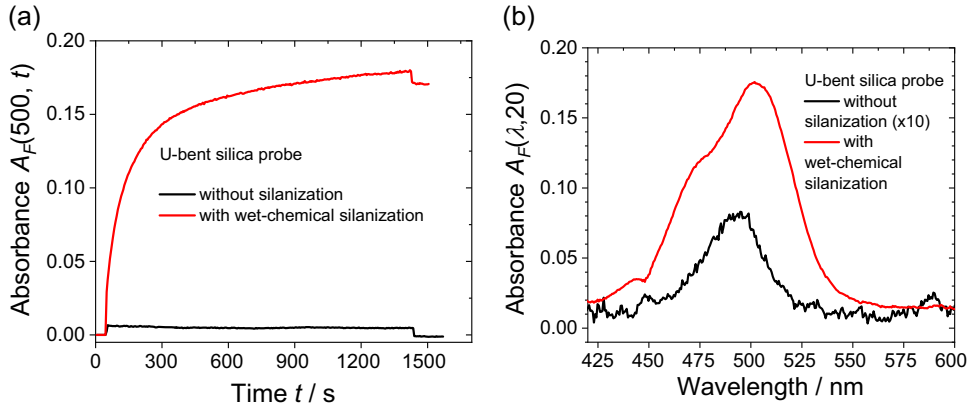


FIGURE 2 (a) Time-dependent absorbance at 500 nm, $A_F(500, t)$, and (b) absorbance spectrum after 20 min following a wash, $A_F(\lambda, 20)$, obtained using bare (black) and silanized (red) silica fiber probes with and without pretreatment, due to binding of FITC from a buffered solution (100 µl, 0.1 mM; procedures: black: −/−, red: −/WS. Note that data for the unsilanized fiber probes were multiplied by a factor of 10). The shift of the absorbance maximum for the silanized fiber probe in comparison to the bare fiber confirms the binding of FITC to the sensor surface. FITC, fluorescein isothiocyanate; WS, wet-chemical silanization

absorbance response. For optical measurement of the amount of FITC-tagged HIgG on the sensor surface, PBS solution was used as a reference.

3 | RESULTS AND DISCUSSION

3.1 | Solvent-phase silanization

The quantitative assessment of amino-group densities on the silanized sensor fiber probes was carried out using FITC as a reporter molecule. The binding of FITC to these groups on the sensor surface leads to an increase of the EW absorbance $A(\lambda) \equiv \log_{10}(1/T(\lambda))$ of the fiber (T = fiber transmission) that was recorded in real-time using the fiber-optic spectrometer. The absorbance due to FITC bound within a time t, $A_F(500, t)$, obtained after subtraction of the buffer solution spectrum as a reference, is directly proportional to the number of FITC molecules bound to the sensor surface. Figure 2a exemplarily shows the temporal increase at 500 nm, $A_F(500, t)$, measured at the maximum of the absorption spectrum of FITC at 500 nm and the absorbance spectrum (Figure 2b) obtained after 20 min following a wash, $A_F(\lambda, 20)$.[1]

In an experiment with nine samples each for three different procedures, the following results were obtained for $A_F(500, 20)$:

- Procedure − /−: 0.006 ± 0.002.
- Procedure − /WS: 0.153 ± 0.06.
- Procedure WP/WS: 0.214 ± 0.064.

[1]The symbol $A_F(n, m)$ signifies the EW absorbance due to FITC, that is, after subtraction of the reference, obtained at $\lambda = n$ nm after m min.

The wet-chemical silanization with preceding wet-chemical pretreatment (WP/WS) showed an enhancement by a factor of about ~35 in $A_F(500, 20)$ in comparison to the bare fiber probe, clearly indicating that the silanized probe surface bears a significant density of reactive amino groups. To examine the necessity of a pretreatment, a set of experiments were carried out with sensor probes silanized without pretreatment, the results showed a ~25-fold enhancement in $A_F(500, 20)$, which is inferior to fiber probes with pretreatment. In addition to an enhanced response, pretreated fiber probes are also considered to be more stably functionalized than probes on which a mere adsorption of a silanized layer on a contaminated, nonactivated silica surface has taken place.

3.2 | Optimization of plasma treatment duration

From the previous experiment, it was clear that pretreatment is necessary to obtain enhanced amino-group densities. To determine the optimum duration of a DBD-plasma exposure as an alternative to the piranha treatment, the fiber probes were plasma-treated for a duration t_P of 0, 15, 30, 60, and 120 s, respectively, after which they were silanized using the standard WS process. Then, they were optically characterized using FITC solution. The results due to FITC binding to the silanized surface (see Figure 3) showed an increasing trend up to a virtual saturation at a plasma treatment duration of 120 s. The value of $A_F(500, 20)$ obtained after this time is the same, within the error limits, like after a wet-chemical

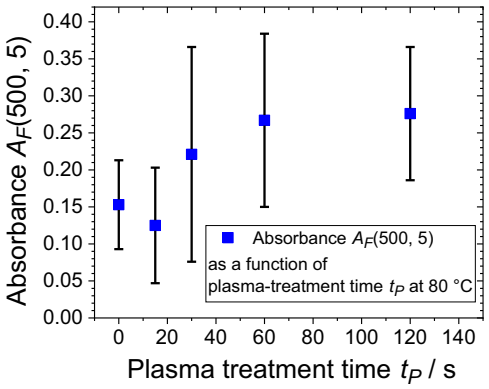


FIGURE 3 Procedure PP/WS: The influence of the plasma pretreatment time duration t_P on the silica surface functionalization determined by the relative amount of FITC bound to the fiber probe surface, measured by $A_F(500, 20)$ ($n = 9$ experiments for each data point except for $n = 6$ for $t_P = 15$ s). FITC, fluorescein isothiocyanate

piranha pretreatment. The large standard deviation for 30 and 60 s plasma treatment time, respectively, is an evidence of different durations needed for the individual specimens in the ensemble to "transit" to the cleaned and activated state.

To see if a completely dry silanization procedure (PP/VS) can also compete with the established wet-chemical sequence, the samples were silanized from the vapor phase, right after the plasma treatment, using the same reactor. For the optimization of the process, the same temperature T was used for both, the PP and the VS steps. It can be presumed that the influence of T on the plasma step, being a nonequilibrium process, is much smaller than on the silanization.

3.3 | Optimization of VS duration and temperature

First, the fiber probes were plasma-treated for 2 min and then silanzed in the vapor phase for varying durations: 5, 10, 15, 20, 25, and 30 min at 80°C, respectively. The results are shown in Figure 4a. Similarly, fiber probes were silanized using different temperatures including $T = 60$°C, 80°C, and 100°C, while the durations of plasma treatment and VS were kept constant as 2 and 20 min, respectively. The results are shown in Figure 4b.

The results show that there is a significant, maximum amount of FITC attached to the surface at a silanization duration of 15 min and a temperature of 80°C. Further increase in deposition time and temperature resulted in a drop in the absorbance response, suggesting the unavailability of free amino groups on the surface. This could be probably due to the polymerization of amino-silanes on the U-bent surface that reduces the availability of functional amino groups.[11,22,35]

The maximum value of $A_F(500, 20)$ obtained within this time is somewhat smaller than what can be achieved by WS. The standard deviation of the absorbance Δ, however, is significantly smaller than that for WS, 0.03 compared with 0.1 or more for the procedure PP/WS, or 0.06 for WP/WS (Figure 5). This interesting result indicates that the completely dry silanization procedure may not only have advantages under the aspects of workplace safety and environmental sustainability, but it may also be a remedy for frequently reported lack of reproducibility of WS.[24]

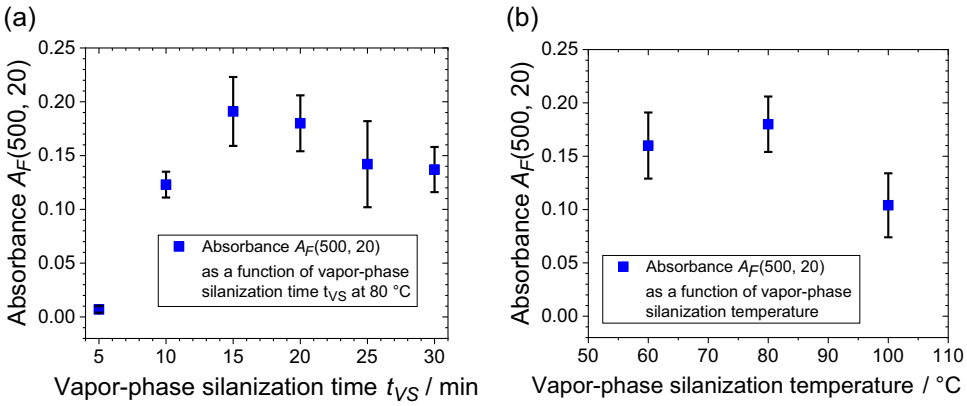


FIGURE 4 Dry procedure PP/VS: (a, left) The influence of the duration t_{VS} ($n = 3$ for 5, 10, and 30 min; $n = 9$ else) and (b, right) temperature T during the VS on the silica surface functionalization determined by the relative amount of FITC bound to the fiber probe surface, measured by $A_F(500, 20)$. The duration of the plasma pretreatments was 2 min ($n = 9$). FITC, fluorescein isothiocyanate

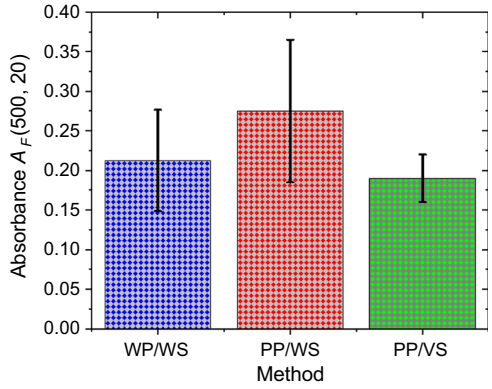


FIGURE 5 Comparison between three methods of silanization including (a) optimized dry procedure PP/VS with 2-min plasma pretreatment and vapor-phase silanization (PP/VS) for 15 min at 80°C, (b) 2 min of plasma pretreatment at $T = 80$°C followed by wet-chemical silanization (PP/WS), and (c) wet-chemical pretreatment and silanization (WP/WS)

3.4 | Influence of the water vapor fraction

The experiments reported in preceding chapters were obtained using a water vapor mole fraction of 0.3%. To investigate if an increase of the amino-group density could be achieved by using smaller amounts of water in the gas phase during DBD treatment, a series of additional experiments was performed with x_{H_2O} between 0% and 0.3%. The results are shown in Figure 6. As a different kind of fiber was used for these experiments, the absorbances are not comparable with previously reported data without taking different fiber geometries into account. Although the errors are still relatively large and more investigations are needed to come to safe conclusions, it appears that the PP/VS process with x_{H_2O} of <0.1% results in an improved amino-functionalization—an observation which is not unexpected in view of the modeling results reported in Reference [37] showing a substantial increase of n_H while the water fraction falls below 0.3%. In view of its practical relevance, more experiments are needed to confirm the relation between x_{H_2O} and results of subsequent silanization, especially for very small water vapor fractions. The result obtained for $x_{H_2O} = 0$ is probably due to water permeation through tubes and residual adsorbed water on chamber walls.

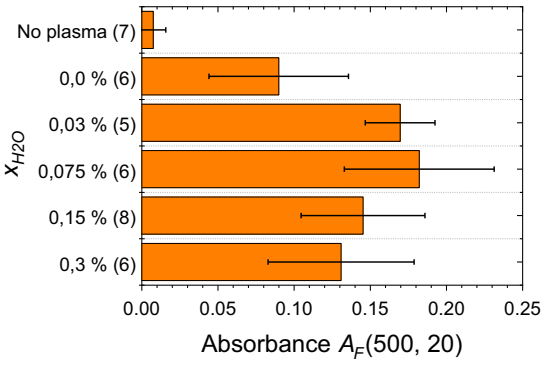


FIGURE 6 Effect of a variation of water vapor fraction, x_{H_2O}, on the absorbance at 500 nm, measured after 20 min of silanization. (x_{H_2O}, given in mol% on the vertical axis, was calculated from the fraction of admixed water-saturated Ar in the total gas flow). Figures in parentheses are numbers of experiments

3.5 | Fiber-optic immunosensor: Comparison of silanization strategies

The ultimate test which the new, completely dry amino-silanization procedure had to pass was an immunoassay, using a capture antibody immobilized to the silica surface. Corresponding experiments were performed applying the standard wet procedure WP/WS and the new PP/VS process, respectively. In addition, a control experiment was carried out using a fiber probe without any surface treatment. In brief, a direct immunoassay utilizing GaHIgG and FITC-tagged HIgG was performed utilizing the control and silanized (WP/WS and PP/VS) fiber probes. The fiber probes silanized using both the methods (WP/WS and PP/VS) were treated with GA to establish aldehyde groups on the U-bent silica surface, facilitating the binding of capture antibody (GaHIgG, F_{ab}-specific) through amine–aldehyde interaction.

The probes were dipped in the capture antibody solution, including control probes without any treatment, anticipating the immobilization through physical interaction. Then, the fiber probes with immobilized capture antibody were subjected to 75 µl of 1 mg/ml analyte solution (FITC-tagged HIgG) and the absorbance response due to the binding of analyte with the capture antibody was monitored and recorded. In all the cases, a concentration of 5 mg/ml of BSA solution was used to block the free aldehyde groups on the sensor surface before subjecting the sensor probes to the analyte solution, to reduce non-specific binding of the analytes. The results are shown in Figure 7, both WP/WS and PP/VS silanized fiber probes gave a significant increase in the absorbance response due to binding of FITC-tagged HIgG (A_{FH}(500, 30)) in comparison to the control fiber probes. This could be attributed to the density of capture antibody bound to the functionalized surface in comparison to the untreated surface. Further, WP/WS and PP/VS showed a similar response

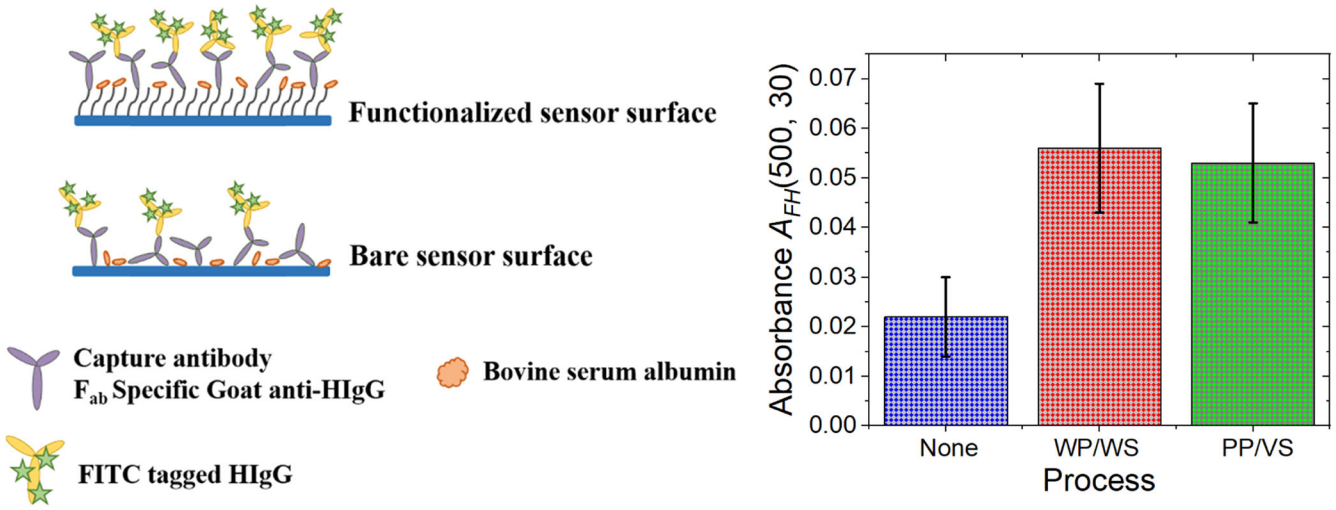


FIGURE 7 Comparison of immunoassay response from the fiber probes without silanization or subjected to wet-chemical pretreatment and silanization WP/WS, or the optimized dry procedure PP/VS ($n = 6$ experiments each): Absorbance response $A_{FH}(500, 30)$ due to binding of FITC-tagged HIgG (1 mg/ml, 75 µl) onto the U-bent silica sensor probe with immobilized GaHIgG (50 µg/ml, 50 µl, 4°C, overnight) and blocked using BSA (5 mg/ml, 15 min). BSA, bovine serum albumin; FITC, fluorescein isothiocyanate; HIgG, human immunoglobulin G

showing the efficacy of PP/VS with the existing wet-chemical method, despite having slightly lower amine density as evident in FITC binding studies.

4 | CONCLUSIONS

A DBD discharge in Ar with a small amount of water vapor (mole fractions in the range of 0.05% to 1%) is an efficient tool to simultaneously generate H atoms and strongly oxidizing HO radicals, species which are able to remove organic contaminants from the surface (HO) and to catalyze the gas-phase hydroxylation of silica (H).

DBD pretreatment in an Ar–H_2O gas mixture with 0.3% water vapor, combined with an immediately subsequent VS in a flowing gas stream of Ar, saturated with APTMS at room temperature, was applied for the amino-silanization of U-bent silica fiber-optic sensors. Both processes were run in the same process chamber, a low-cost DBD reactor made from quartz plates.

In a subsequent study of the impact of a reduced $x$$H_2O$ on the attainable density of amino groups, it was found that the density of these groups could be increased by a factor of 1.4 at 0.075% water vapor, in qualitative agreement with results from the model.

In an immunoassay-based comparison, virtually the same results were achieved with the new vapor-phase procedure (with 0.3% H_2O) on the one hand, and the standard wet-chemical sequence—etching in piranha solution and silanization in solution—on the other, although about 30% less reactive amino groups were still generated on the surface by the dry procedure. More important for biochemical applications of the new procedure appears to be, instead of achieving the maximum absolute value of reactive amino group density, its standard deviation, which was found to be smaller by a factor of 3 than for WS.

Aside from the better reproducibility, the new procedure has the advantage, compared with the wet-chemical procedure, that no hazardous chemicals must be used. In contrast to low-pressure plasma pretreatment, frequently applied to clean and activate the silica surface before silanization, it requires less investments and process times and it can straightforwardly be transferred to an industrial in-line process.

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SUPPORTING INFORMATION

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