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Available online at www.sciencedirect.com

ScienceDirect

Materials Today: Proceedings 00 (2018) 0000–0000

materialstoday: PROCEEDINGS

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SURFOCAP 2017

Sensitivity enhancement of a fluorescent pH sensor by double silanization of the sensing surface

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Abstract

The goal of this work concerns a new method to substantially increase the sensitivity of a fluorescence pH sensor. The method is based on a double silanization method. Two methods are compared: one using APTMS (3-Aminopropyl)trimethoxysilane) only and another one using both APTMS and APDMS (3-Aminopropyl)dimethoxymethylsilane). Using the second method, sensor's sensitivity is improved by more than 500 %.

Selection and/or Peer-review under responsibility of SURFOCAP 2017.

Keywords: Silanization; APDMS; APTMS; Fluorescence; pH sensor

1. Introduction

Surfaces functionalized with ligands which can bind specifically to molecules to be detected are used in a large number of sensors. For certain types of functionalization chemistries, amine groups located at the end of molecules which are grafted onto the sensor's surface are used to covalently attach ligands onto the surface. Depending on the sensor surface material, different chemistries can be used; thiol chemistry for gold coated surfaces [1] or silane chemistry for glass substrates [2]. In most cases, functionalization leads to the creation of a monolayer of amine terminated molecules. Therefore, the number of potentially grafted ligands onto the surface remains relatively low. Sometimes, thick aminosilane layers are grown on top of the sensing surface in order to increase the number of amine functions. The idea behind increasing the number of amine functions is that it is thought that the number of sensing molecules will be increased accordingly.

2214-7853
Selection and/or Peer-review under responsibility of SURFOCAP 2017.

In this conference, we show that increasing the number of amine function is not enough to increase the number of sensing molecules at the surface of the sensor. Although it is important to increase the number of these amine functions, it is also crucial that these amine functions remain available for grafting. We then developed a double silanization method used to make the amine functions available for grafting. Indeed, two silanization methods are compared in this study. The first one uses APTMS which produces a layer where only the amine functions at the silane layer surface are available for dye grafting. The second one uses first APTMS to create the layer grafted on the substrate and second APDMS which produces a volume brush-like structure where much more amine functions are available for grafting. In what follows, we first describe the two silanization methods we compare. We next estimate the surface concentration in amine function obtained using both silanizations. The sensor's performances enhancement is then presented. To this end, fluorescein molecules were grafted onto the silanized surface and pH measurement using fluorescence properties of this indicator are performed.

2. Sensitivity enhancement using a double silanization technique

As previously mentioned, this is not only the number of amine functions which dictates the sensitivity of a sensor but more importantly the number of amine functions available for grafting. To illustrate this we refer to fig. 1. It can clearly be seen that some of the amine function are hardly available for grafting (red circles) while other can easily bind to sensing molecules (green circles).

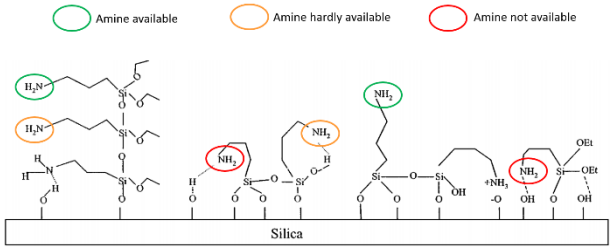

Fig. 1. Illustration of the need for amine functions available for grafting. (Adapted from [3].).

There exists a large number of aminosilane molecules which can be used for surface functionalization. In this study, we focus on APTMS and APDMS. In the next section we explain the structure of the silane layer obtained using the two silanization methods already mentioned.

2.1. Silane layer structure as a function of the silanization strategy

APTMS is a tri-functional silane molecule while APDMS is a di-functional molecule. When used alone, these aminosilane molecules lead to the creation of dense silane layers with a low number of amine function as depicted in fig. 2.

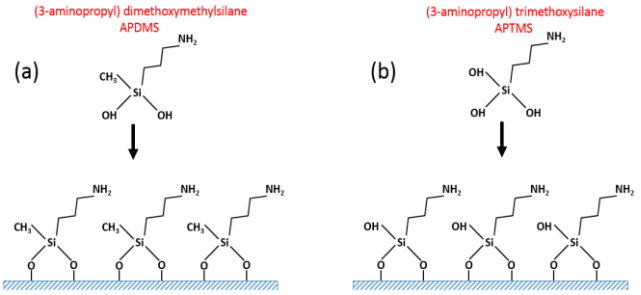

Fig. 2. Silane structure obtained when using either APTMS or APDMS.

In this work, we propose to use these two molecules sequentially in order to grow a brush-like structure. This is illustrated in fig.3. APTMS is used first to grow a dense monolayer at the sensor's surface. Then, APDMS is used to form long chains of aminosilane molecules. In this way, not only the number of amine functions is increase but also their availability for grafting. Note that the length of the APDMS chains, and therefore the potential sensitivity of the sensor can be tuned on demand by adjusting the silanization duration. Note that figure 3 is a schematic representation of the long silane chains which can be obtained using the double silanization method. The real situation is a bit more complicated because reticulation between chains is likely to happen. This does not change the principle of the method.

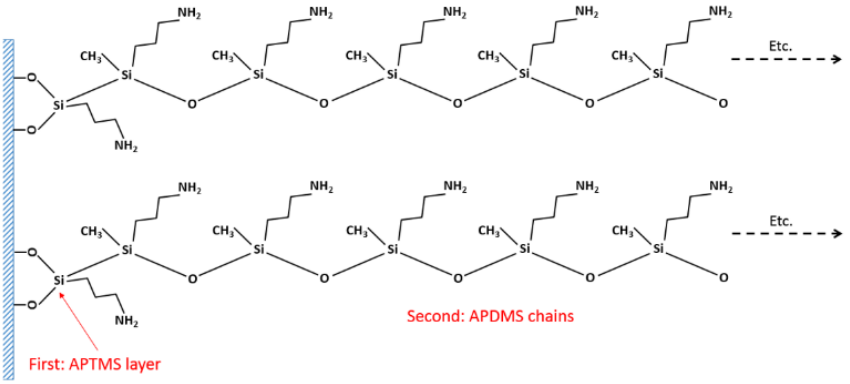

Fig. 3. Brush-like structure obtained using both APTMS and APDMS.

2.2. Comparing both silanization methods performing pH measurements using grafted fluorescein

The two above described silanization methods have been compared. Overall silanization times were equal for both methods (2 days). Fluorescein molecules were grafted onto the silane layers grown on silicon samples. The natural nanometric silica layer present at the surface of any silicon sample makes these sample behaving like pure silica surfaces. However, using silicon allows calculating the amine function density using Fourier Transform Infrared Spectroscopy. Details of the experimental processes for silanization, grafting and amine quantification can be found in [4]. Using only APTMS, we measured a concentration of 365 molecules/nm^{-2}. Using both APTMS+APDMS, the amine density raises to 492 molecules/nm^{-2}. The number of amine functions is then increased by about 30% between the single and the double silanization methods.

Fluorescein molecules were then grafted to the amine functions and fluorescence spectra were recorded for various pH values. The result is given in fig. 4.

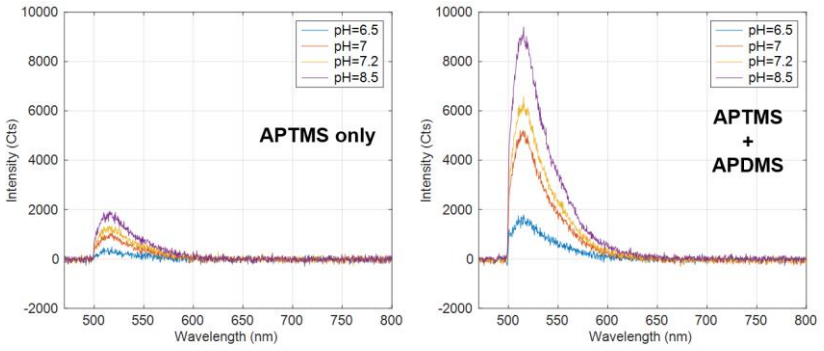

Fig. 4. pH dependent fluorescence spectra obtained using both APTMS and APDMS (from [4]).

We can see that the intensities of the fluorescence signals are much higher using the double silanization. We can now fit the intensities of the spectra maxima as a function of pH using sigmoid functions. The result is shown in fig. 5(a) and is in accordance with data from literature [5] in fig. 5(b). Note that the working range of the sensor is ± 1 or 2 upH around the pK_a value as it is the case for fluorescent indicators. Fluorescein, and other indicators with pK_a around 7, is usually employed to sense pH values in the biological pH range.

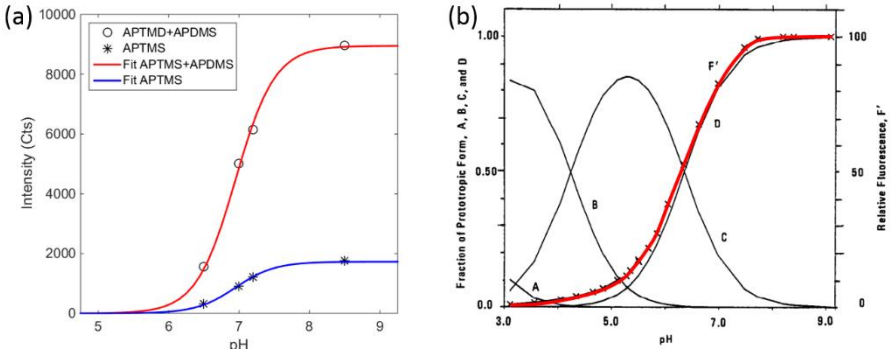

Fig. 5. Fitting the fluorescence behavior as a function of pH. (a) Result obtained when comparing the two silanization methods (from [4]). (b) Data from [5].

The slope of these sigmoids at the center of the linear region represents the sensitivity of the sensor. Using only APTMS, the slope is equal to 1280 Cts/upH. It raises up to 6620 Cts/upH for the double silanization. This represents an increase of 518 % of the sensor's sensitivity.

Extracting the noise from fig. 4 and comparing it to the intensity measured at pH = 7 allows calculating the improvement in Signal to Noise Ratio: 518 %.

The most interesting feature concerning the SNR is what it means in terms of pH measurement accuracy. For the single silanization, the accuracy in terms of pH is 0.24 pH units. This accuracy becomes equal to 0.05 pH units using the double silanization, *i.e.* an improvement of 480 %.

These results demonstrate that this is not only the number of amine functions present in a silane layer which leads to a high sensitivity of such sensors, but the number of amine functions actually available for grafting.

3. Conclusion

In this conference, we have presented a double silanization which allows improving performances of sensors based on fluorescent indicators grafted on a surface. Demonstration is proposed using fluorescein molecules used for pH sensing. Although the number of amine function is increase of only 30 %, the fact that they are actually more available for fluorescein grafting leads to an increase of the sensor's performance of about 500 %.

Furthermore, the sensitivity enhancement being related to the silanization time using APDMS, the sensor's performances can be tuned on demand by adjusting the silanization duration. Also, it should be noted that this double silanization method can directly be applied to any sensing application based on indicators grafted onto a surface.

Acknowledgements

This work was supported by the European Commission (grant number FE2007/2013, operation 36381).

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