



## Full Length Article

# Determination of NH<sub>2</sub> concentration on 3-aminopropyl tri-ethoxy silane layers and cyclopropylamine plasma polymers by liquid-phase derivatization with 5-iodo 2-furaldehyde

Anton Manakhov<sup>a,b,\*</sup>, Jan Čechal<sup>c,d</sup>, Miroslav Michlíček<sup>b,e</sup>, Dmitry V. Shtansky<sup>a</sup><sup>a</sup> National University of Science and Technology "MISIS", Leninsky pr. 4, Moscow 119049, Russia<sup>b</sup> RG Plasma Technologies, CEITEC—Central European Institute of Technology, Masaryk University, Kotlářská, 2, 61137 Brno, Czech Republic<sup>c</sup> CEITEC—Central European Institute of Technology, Brno University of Technology, Purkyňova 123, 612 00 Brno, Czech Republic<sup>d</sup> Institute of Physical Engineering, Brno University of Technology, Technická 2896/2, 616 69 Brno, Czech Republic<sup>e</sup> Department of Physical Electronics, Faculty of Science, Masaryk University, Kotlářská 2, 61137 Brno, Czech Republic

## ARTICLE INFO

## Article history:

Received 16 February 2017

Received in revised form 14 April 2017

Accepted 17 April 2017

Available online 19 April 2017

## Keywords:

Amine layers

APTES

Derivatization

Plasma polymers

XPS

## ABSTRACT

The quantification of concentration of primary amines, e.g. in plasma polymerized layers is a very important task for surface analysis. However, the commonly used procedure, such as gas phase derivatization with benzaldehydes, shows several drawbacks, the most important of which are the side reaction effects. In the present study we propose and validate a liquid phase derivatization using 5-iodo 2-furaldehyde (IFA). It was demonstrated that the content of NH<sub>2</sub> groups can be determined from the atomic concentrations measured by X-ray photoelectron spectroscopy (XPS), in particular from the ratio of I 3d and N 1s peak intensities. First, we demonstrate the method on a prototypical system such as 3-aminopropyl tri-ethoxy silane (APTES) layer. Here the XPS analysis carried out after reaction of APTES layer with IFA gives the fraction of primary amines (NH<sub>2</sub>/N) of 38.3 ± 7.9%. Comparing this value with that obtained by N 1s curve fitting of APTES layer giving 40.9 ± 9.5% of amine groups, it can be concluded that all primary amines were derivatized by reaction with IFA. The second system to demonstrate the method comprises cyclopropylamine (CPA) plasma polymers that were free from conjugated imines. In this case the method gives the NH<sub>2</sub> fraction ~8.5%. This value is closely matching the NH<sub>2</sub>/N ratio estimated by 4-trifluoromethyl benzaldehyde (TFBA) derivatization. The reaction of IFA with CPA plasma polymer exhibiting high density of conjugated imines revealed the NH<sub>2</sub>/N fraction of ~10.8%. This value was significantly lower compared to 17.3% estimated by TFBA derivatization. As the overestimated density of primary amines measured by TFBA derivatization is probably related to the side reaction of benzaldehydes with conjugated imines, the proposed IFA derivatization of primary amines can be an alternative procedure for the quantification of surface amine groups.

© 2017 Elsevier B.V. All rights reserved.

## 1. Introduction

The quantification of primary amine concentration is an important technological step in bio-applications of amine layers including targeted drug delivery, biosensing, and tissue engineering because the density of functional groups affects the properties of materials [1,2]. The density of surface amine groups would affect the number of adsorbed biomolecules (e.g. antibodies, DNA), as well as the adhesion of vital proteins and peptides. As a result, the concentra-

tion of functional groups influences cell viability [3,4], the efficiency of biosensors [5], and the wound healing properties of engineered tissues [6].

The most widely used method for the quantification of primary amines is based on the reaction with a marking agent, predominantly the gas phase reaction of amine containing layers with vapours of benzaldehydes (pentafluorobenzaldehyde or 4-trifluoromethyl benzaldehyde). The concentration of primary amines is then obtained by evaluation of concentration of fluorine atoms in respect to the residual nitrogen atoms in the layer. However, the use of benzaldehydes was recently criticized by several research groups [7–9]. For example, Klages et al. demonstrated that benzaldehydes displays side reactions with imines leading to the overestimation of concentration of primary amines [8]. These

\* Corresponding author at: National University of Science and Technology "MISIS", Leninsky pr. 4, Moscow 119049, Russia.

E-mail address: [ant-manahov@ya.ru](mailto:ant-manahov@ya.ru) (A. Manakhov).

results raised a critical question regarding the applicability of the method for plasma polymerized amine layers because all amine plasma polymers (regardless of the monomer nature) exhibit the characteristic imine peaks in their IR spectra. Hence, the primary amines concentration can be significantly overestimated. It is also worth noting that the reaction of 4-trifluoromethyl benzaldehyde (TFBA) vapours with amine layers proceeds only within the near-surface area leading to the heterogeneous depth profile with high fluorine content in topmost layers (2–3 nm) and a decreasing concentration in deeper portion of the sample. At a depth of 9–10 nm, the concentration of fluorine decreased by ~60% compared to the topmost layer [9,10]. This effect is likely related to the limited penetration of the relatively large TFBA molecule into the dense structure of plasma polymer. Furthermore, the TFBA is prone to oxidation to 4-trifluoromethyl benzoic acid by atmospheric oxygen and, therefore, the surface analysis should be performed in Ar atmosphere. However, even small oxygen contamination could lead to the precipitation of 4-trifluoromethyl benzoic acid onto the sample surface, hereby affecting the XPS results.

In view of the above arguments, the alternative compounds for the primary amine derivatization appear to be necessary. It can be expected that the reaction of aliphatic or cyclic aldehydes with a weak aromaticity avoids the problem with the side reaction with conjugated imines and helps to eliminate the oxidation towards the derivatives of benzoic acid. However, small aliphatic aldehydes such as 2-(Trifluoromethyl) propionaldehyde are highly toxic and dangerous. In this context, the derivatives of furaldehyde would be better candidates for the primary amine derivatization due to their lower toxicity, better stability, slightly smaller size (compared to TFBA), and a weaker aromaticity. Within the group of furaldehydes, 5-iodo 2-furaldehyde (IFA) appears to be the most appropriate compound due to smaller size compared to TFBA and a weak aromaticity of furan ring. Importantly, the subsequent chemical analysis by X-ray photoelectron spectroscopy (XPS) will benefit from very high relative sensitivity factor (RSF) of I 3d peak. However, the reaction between IFA and amine layer cannot be carried out in a gas phase due to low vapour pressure of IFA and, therefore, the derivatization must be carried out by liquid phase reaction in a solution of IFA.

To validate the IFA derivatization reliability, a standard amine-rich layer with a well-defined chemistry should be prepared. The self-assembled monolayers of amine terminated thiols (e.g. cysteamine) that are often used in biosensor functionalization, should be excluded due to very small thickness of the layer leading to a low signal of targeted elements (C and N) and the strong influence of carbon contaminations [12]. A more appropriate materials are the 3-aminopropyl tri-ethoxy silane (APTES) layers because such films can be deposited with adjustable thickness, roughness and well-defined chemistry [13,14]. Although APTES silanization is studied for decades and the APTES layers are used for many emerging technologies including cancer treatment [15,16], drug delivery [17,18], fire retarding agent [19], and nano-engineering [13,20,21], there are many discrepancies in the characterization of the chemical structure of APTES layer. For example, it still unclear how much  $\text{NH}_2$  groups are available at the surface of APTES layers. Xue et al. [22] reported a high value of  $\text{NH}_2/\text{N} = 51.5\%$  estimated by XPS N 1s curve fitting, whereas Acres et al. [23] considered that the concentration of amine groups in the outermost APTES layer is very small. Note that the chemical environments of nitrogen and silicon are strongly dependent on the parameters of the APTES silanization process, especially on water residue [24,25]. It was found that the water residue in APTES solution could lead to the formation of silanol groups resulting in the protonation of 30–60% of nitrogen in the formed APTES layer. Hence, the density of primary amines on deposited APTES layer varies as a function of silanization process.

In this work the chemical reaction between IFA and several types of amine layers was studied. Firstly, the reaction between IFA and APTES layer grown by silanization was thoroughly analyzed. The XPS and Fourier transform infrared spectroscopy (FT-IR) data were carefully evaluated to determine the fraction of the reacted amine groups. Two complementary approaches to determine the amine group fractions were compared: (1) N 1s curve fitting for samples before derivatization and (2) measurement of the intensities of I 3d and N 1s peaks, which ratio is proportional to the desired primary amine fraction. We have found that the dispersion of the values obtained by these methods was less than 10%, which could be related to the uncertainties of the N 1s curve fitting. We also have analyzed the influence of conjugated imines presented in the cyclopropylamine (CPA) plasma polymers on the IFA and TFBA derivatization results.

## 2. Materials and methods

### 2.1. Materials

Anhydrous toluene, ethanol, sulfuric acid, 3-aminopropyl tri-ethoxy silane (APTES), hydrogen peroxide, cyclopropylamine (CPA), 5-iodo 2-furaldehyde (IFA), 4-trifluoromethyl benzaldehyde (TFBA), and isopropanol (all from Sigma Aldrich) were used without additional purification except isopropanol, which was further purified to minimize water contaminations. Argon (99.998%) was supplied by Messer. Silicon substrates were cut from double-side polished Si(111) wafer (N-type, phosphorus doped, resistivity  $0.5 \Omega \text{ cm}$ ) purchased from ON Semiconductor.

### 2.2. Silanization of APTES layers

The methodology for deposition of APTES layers was adapted from the literature [24]. First, silicon wafer was cut into  $10 \times 15 \text{ mm}^2$  pieces and cleaned by immersion into piranha solution (2:1 sulfuric acid to hydrogen peroxide) for 1 h. Then the samples were thoroughly rinsed in de-ionized water and dried under stream of nitrogen.

The silanization was performed in a 2% APTES solution in anhydrous toluene at room temperature (RT). In order to obtain thick APTES layers suitable for FT-IR analysis, the chemical treatment was carried out for 72 h. Then the samples were removed from the APTES solution and rinsed first under flowing toluene and then in ethanol. The thickness of APTES samples was around  $70 \pm 10 \text{ nm}$ . The difference in the layer thickness comparing to [24] can be related to the generally observed low homogeneity of the APTES film, influence of the silicon substrate pre-treatment. Same samples were also used for XPS analysis. The samples were stored in vacuum desiccator prior to the analyses or derivatization.

### 2.3. CPA plasma deposition

Based on our previous experience on the CPA plasma polymerization, two types of CPA plasma polymers were selected and used in this work. The CPA1 was prepared in a stainless steel parallel plate radio frequency (RF) plasma reactor as described elsewhere [4]. The CPA1 was polymerized in pulsed wave CPA/Ar plasma at a pressure of 50 Pa with the pulse repetition frequency (PRF) of 500 Hz. The plasma on and off times were set to 660 and 1340  $\mu\text{s}$ , respectively, i.e. the duty cycle (D.C.) was 33%. The discharge power was set to 100 W. The flow rate of Ar was controlled at 28 sccm and regulated by an electronic flow controller whereas the flow rate of CPA vapours was set to 2 sccm by a needle valve. The CPA2 sample was prepared in tubular glass reactor [26]. The flow rate of Ar and CPA was set to 28 and 1 sccm, respectively, whereas the pressure in the reactor was maintained at 120 Pa. The discharge power

Download English Version:

<https://daneshyari.com/en/article/5350786>

Download Persian Version:

<https://daneshyari.com/article/5350786>

[Daneshyari.com](https://daneshyari.com)