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Surface Modification of Glass Beads with an Aminosilane Monolayer

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Surface modification of glass beads with a self-assembled monolayer of (3-Aminopropyl)-triethoxysilane (APTES) was investigated. Characterisation of the self-assembled monolayer was confirmed by a reaction between amine terminal groups and thionylchloride of Rhodamine-B dye using fluorescence microscopy. Quantitative 3-dimensional profiling was obtained for all modification processes by converting microscopic images to numerical values. The reaction steps were also analysed using infrared spectroscopy (FTIR).

Key Words: Glass beads, surface modification, self-assembled monolayer, silanisation.

Introduction

Self-assembled monolayers (SAMs) are monomolecular layers which are spontaneously formed upon immersing a solid substrate into a solution containing functional molecules. The most attractive feature of SAMs is molecular level control over the modification of surfaces, and incorporation of multiple or multilayer molecular components onto the monolayer leads to various functional properties. SAMs can be prepared using different types of molecules and different substrates. Commonly employed examples are alkylsiloxane monolayers, fatty acids on oxide materials and alkanethiolate monolayers[1−3].

SAM formation provides surface functionalisation by organic molecules containing suitable functional groups like –SH, –CN –COOH, $–NH_2$ and silanes[4]. These surfaces can be effectively used to build up interesting nano-level architectures. The first types of strongly bound chemisorbed monolayers using silane compounds were reported by Sagiv, where glass or oxidised silicon surfaces were etched to clean and coat them with active silanol (Si-OH) groups [5]. Recently, increasing effort has been directed towards potential applications of silane SAMs, particularly chloro and terminal amine groups which have the capability of attaching larger molecules[6−9]. Flexibility with respect to terminal functionalities of the organic molecules allows control of the hydrophobicity or hydrophilicity of surfaces.

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Silanisation with silane compounds has been used as a process for modification of glass surfaces. On siliceous surfaces attachment is typically mediated by first silylating the surface followed by immobilisation of biomolecules of interest. Aminosilanes such as (3-Aminopropyl)-triethoxysilane (APTES) are attractive for such applications, due in part to significant advances in the understanding of this class of surface modification agents[10]. Notably, aminosilanes have the advantage of catalytic activity by the amine group that facilitates formation of siloxane bonds with surface silanols[10−12], mitigating and potentially obviating the need for post-depositional curing.

In this paper, a 2-step procedure for the introduction of APTES on glass beads is described. After chemisorptions of APTES, the terminal amino group was reacted with Rhodamine-B. The mechanism of attachment between APTES monolayers and Rhodamine-B is investigated as a model for interfacial amine–Rhodamine-B reactions. Infrared spectroscopy serves as a main investigative tool for anchoring of terminal amine groups on the glass beads. The surface functionalised was characterised using quantitative 3-dimensional profiles by converting microscopic images to numerical values.

Materials and Methods

Materials

All chemicals used for the preparation of the surface functionalisation of glass beads were of analytical grade, obtained from Merck. Glass beads with average diameters of 2.8 mm were purchased from Ildam Kimya Ltd.

Surface modification

Prior to monolayer preparation, the glass beads were treated for 1 h in boiling piranha solution (3:1 concentrated H_2SO_4:H_2O_2). (**Caution**: Piranha solution is an extremely strong oxidant and should be handled very carefully!) After 1 h, the glass beads were removed from the cleaning solution, rinsed with high purity water (Ultrapure Milli-Q Reagent Water System Millipore) and dried in a stream of nitrogen until they appeared dry just prior to SAM deposition.

The freshly cleaned glass beads were immersed in 1% (w/w) APTES in anhydrous toluene for 24 h with agitation as previously described [13]. Toluene was freshly distilled from sodium. After the glass beads had been removed from the solution, followed by a sequence of 2 washes with anhydrous toluene, deionised water and ethanol were employed to remove any physisorbed APTES. The glass beads were dried in nitrogen atmosphere. It was previously pointed out that this attachment protocol results in partial monolayers of APTES with a coverage of about 1 residue/nm^2 [13], as compared to coverages of about 2 residues/nm^2 reported in most literature studies of APTES monolayers prepared under anhydrous conditions[14−17]. The lower coverages are chiefly attributed to washing of the glass beads with deionised water prior to curing, which is expected to remove silane molecules that are not already covalently bonded prior to curing.

APTES-derivatised glass beads were placed in a solution of 0.1 mL of triethylamine and 15 mg of Rhodamine-B in 20 mL of acetonitrile. By modifying the procedure[7], the reaction was allowed to proceed for 24 h at room temperature, followed by washing 3 times with acetonitrile, twice with ethanol, and a last wash with dichloromethane, followed by overnight drying in nitrogen atmosphere.

An Olympus (BX51/BX2-FLB3-000) microscope was used to obtain digital microscopic images of the immobilised lavation step by step, with imaging of all the stages. The fluorescence was provided by a 100 W

tungsten lamp using a 550 nm peak (BP510-550 excitation filter), a 570 nm peak (DM570 emission filter) plus a 590 nm dichroic beam (BA590) splitter. Microscope images were collected using a CCD camera (DP70 12.5 million pixel resolution) giving a digital output of a maximum 356 x 300 pixels with 16-bit resolution of the light intensity in each pixel. The digital camera was connected to a PC and checked by digital image analysis, and then the images were saved. Scion Image (V.402) was used to convert the images from TIFF files to numerical image data, which were transferred to Microsoft EXCEL for subsequent conversion into absorbance values with short pixel by pixel height values.

Characterisation

Elemental analyses for carbon, nitrogen, and hydrogen content were performed with a Perkin Elmer Elemental Analyser Model 240 instrument (TÜBİTAK Ankara Test and Analysis Laboratory). Fourier transform infrared (FTIR) spectroscopy was performed in transmission on approximately 1 mg of modified powder sandwiched in a chamber created by a circular gasket and 2 KBr windows. Each spectrum was an average of 1000 scans collected at 2 cm^{-1} resolutions on a Perkin Elmer 1600 Series FTIR spectrometer.

Characterisation of the functionalised Rhodamine B was performed using a fluorescence microscope (OLYMPUS BX51/BX2-FLB3-000) equipped with a filter set for Rhodamine-B (ex 520–550/em 570–580), a CCD camera (DP70 Microscope Digital Camera) and image analysis software (DP Manager 1,2,1 107).

Results and Discussion

The initial experiments aimed at producing SAM of APTES on glass beads were prepared by exposing glass beads for 24 h to 11% (w/w) solution of the adsorbate in anhydrous toluene. Then the APTES monolayer was further functionalised, exploiting the reactivity of the primary amine in a subsequent reaction with sulphonyl chloride in Rhodamine-B dye. The structure and properties of the silane layer on an inorganic support depends on the functionality attached to the silane, but also on the method used to apply the silane to the surface. The ideal case would be to form a monolayer of coupling agent to the surface where all silanol groups have reacted either with surface hydroxyl groups or silanol groups on neighbouring silanes. The ideal reaction for all reaction stages is sketched in Figure 1.

In order to investigate the anchoring of terminal amino groups in APTES on glass beads, FTIR infrared spectroscopy gave a broad peak at 3451 cm^{-1} due to –NH stretching[18]. All silane groups on the glass bead were modified to silanol groups. IR spectra of glass beads showed a new and distinct peak at 1028 cm^{-1}, attributable to Si-O stretching of the silane group. Silanes, which are useful compounds for anchoring an organic layer to an inorganic surface and contain Si-O bonds, react with surface hydroxides and pendant hydrocarbon chains to link with organic overlayers, such as a polymer or protein molecules. Silanisation therefore gives us a way to tailor the adhesion properties of a surface or change its biocompatibility.

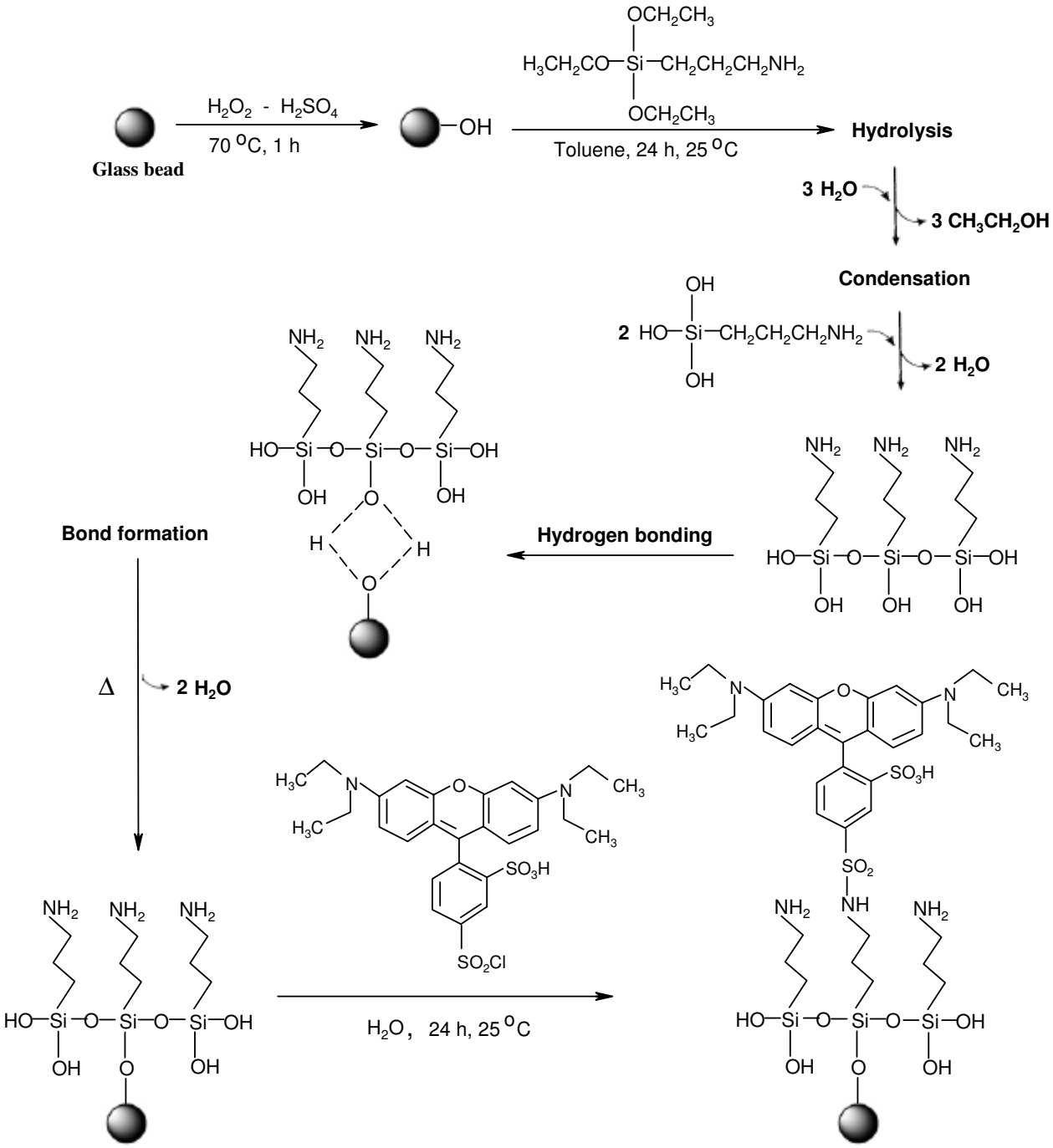

Figure 1. Surface modification of glass beads with SAMs.

The surface modification process for all stages was controlled using fluorescence microscopy. As shown in Figure 2, the piranha solution treated beads (1) and APTES treated glass beads (2) showed almost no fluorescence, in contrast to the strong fluorescence observed on the surface of the silane-coupled bead (3) when the glass beads were treated with Rhodamine B. The piranha solution glass beads were also treated with Rhodamine B solution. It was found that the images were the same as the original. These results suggested that amino groups were successfully introduced onto the surface of (2).

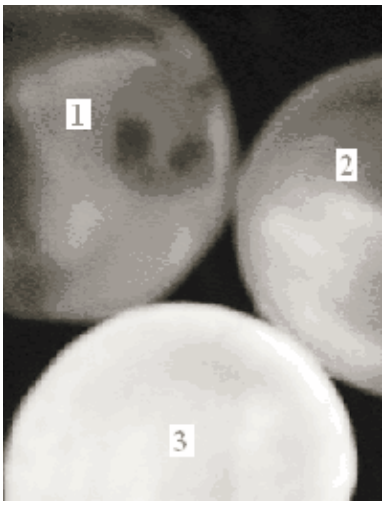

Figure 2. Fluorescence microscopy of resulting beads with average diameter of 2.8 mm. (1) Piranha solution treated glass bead. (2) APTES (silane coupling reagent) treated glass bead. (3) Rhodamine-B treated glass bead.

A quantitative 3-dimensional profiling of micron sized channel networks within transparent "lab-on-a-chip" microreactors was developed by Fletcher et al.[19]. The described method is in principle similar to spectroscopic imaging techniques such as UV-vis, IR, and Raman. These methods have been adopted for particular applications[20−22]. In the method developed, the microscope image is converted to a numerical value with a maximum transmission wavelength matching the wavelength of the absorbance maximum of the dye solution. Digitised images of glass beads functionalised with APTES and Rhodamine B dye solution are analysed pixel by pixel to yield a spatially resulted array of absorbance values. The array is then converted to optical path length values using the Beer-Lambert law, thereby providing a 3D profile of the network. Similarly, we converted the images of the surface functionalised glass beads with aminosilane monolayers step by step, taking all stages of the modification process[19].

The 3-D profiles procedure can be described as follows:

Absorbance of A solution is represented by the Beer-Lambert law:

$$A = \log \left\{ \frac{I_{ref} - I_{dark}}{I_{sol} - I_{dark}} \right\}$$

where I_{ref} and I_{sol} are the transmitted light intensities of the reference and the dye solutions, respectively. I_{dark} is the detector signal recorded in the absence of incident light, c is the concentration of absorbing species in the solution, d is the optical length and ε is the molar absorption coefficient[19].

We investigated the surface modification of glass beads with silane monolayers and their 3D profiles using fluorescence images of Rhodamine-B dye. This study will be followed by further studies using SAMs containing molecules of different chain lengths with functional groups that will act as chemical tethers for a polymer or a protein overlayer. By this procedure our aim was to have a uniform distribution of long chain molecules in the mixed layer, and give the most uniform surfaces.

In Figure 3 are illustrated the resulting 3D profiles and in Figure 4 the resulting 2D profiles of the functionalised surfaces for all stages. It is seen from these figures that the surface modification process for SAM of APTES on glass beads was successful. On the basis of these findings, the functionalisation of amine-linked beads by treating with different heterobifunctional groups can be prepared for various applications.

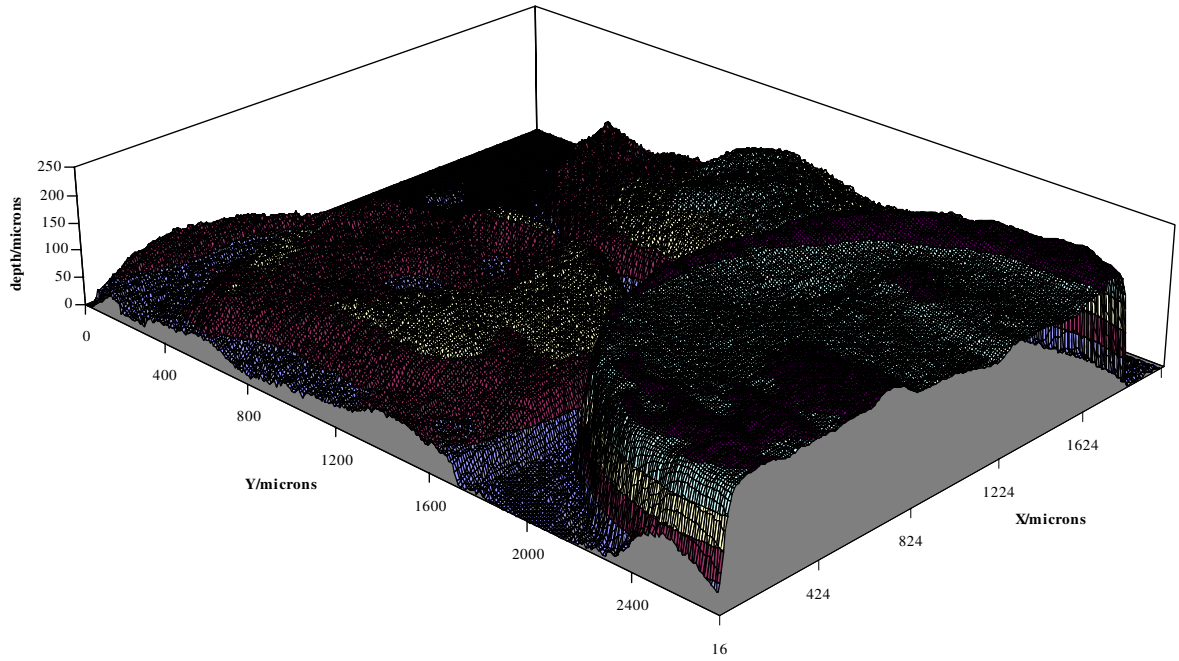

Figure 3. 3D profile images of all the reaction stages for the modification process (the profiles represent the images in Figure 2).

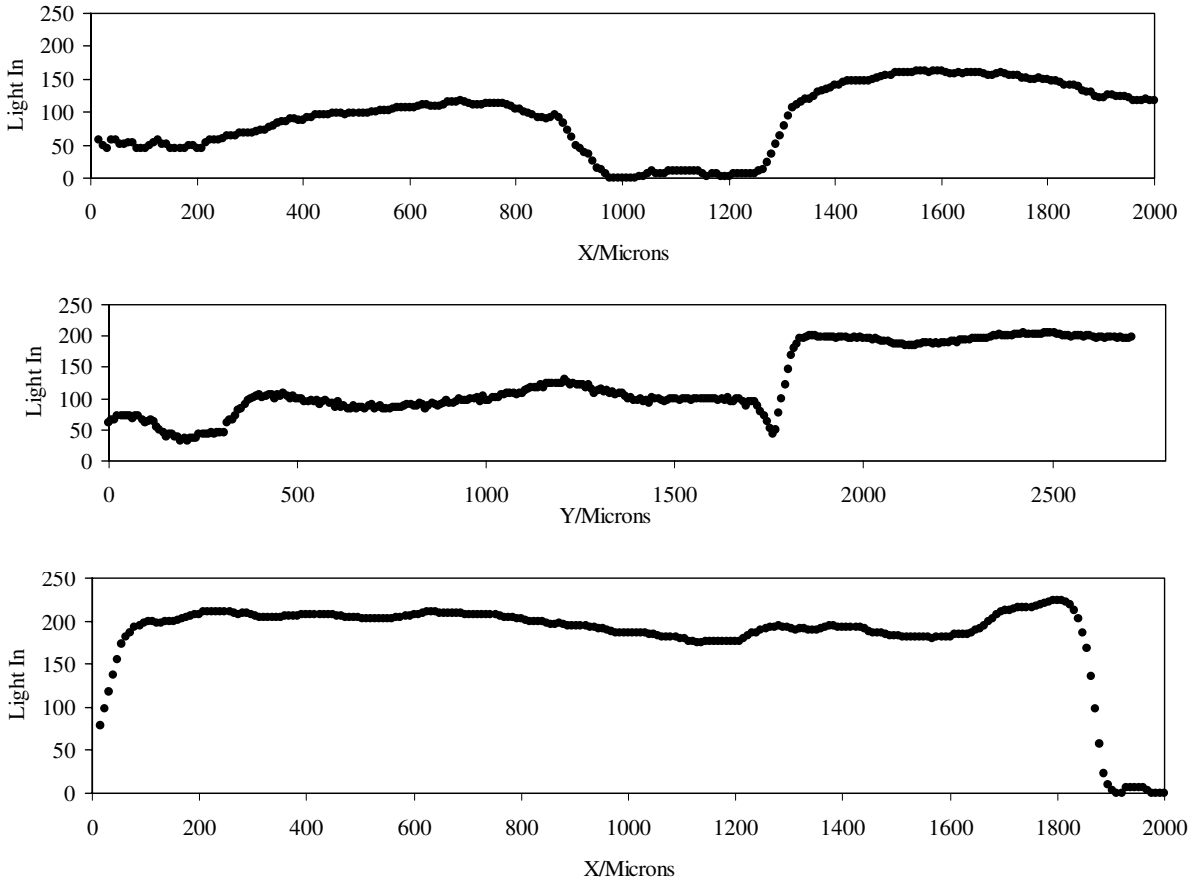

Figure 4. 2D profile images of all the reaction stages for the modification process (the profiles represent the images in Figure 2).

Conclusion

SAM of APTES as well as other silane compounds on glass can be used for the immobilisation of a variety of functional groups via surface reactions, since the amine groups have high reactivity towards many functional groups which can be incorporated to SAMs. Extensive characterisation of amine groups of the APTES has been carried out to convince us that APTES undergoes surface reactions. The further work is underway to immobilise biomolecules onto SAMs for various applications. Fluorescence microscopy has proven to be a valuable technique for the easy detection of interactions among the fluorophic groups in the SAM. In particular, the quantitative dimension of 3D profiles has elucidated immobilisation reactions sequentially.

Acknowledgements

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