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## Polymer films with size-selected silver nanoparticles as plasmon resonance-based transducers for protein sensing

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### ABSTRACT

Transducers based on the phenomenon of localised surface plasmon resonance (LSPR) are fabricated using silver nanoparticles (NPs) imbedded into thin poly (methyl methacrylate) films. The particles are produced by magnetron sputtering, size selected with a precision  $\leq 10\%$  of the diameter and deposited on the films in vacuum. Immersion of NPs is controlled by post-deposition thermal annealing providing very good adhesion, in particular, resistance against following wet chemical procedures. LSPR properties of silver NPs are exploited for protein detection using a classical antibody-antigen scheme through covalent attachment of antibody molecules to the particles, followed by incubation with antigen (chicken egg albumin). Atomic force microscopy studies reveal that antibodies are predominantly attached to NPs providing binding sites for the antigens of interest. Specific changes in the LSPR band are observed for each step of protein incubation on NPs. Thus, the transducers demonstrate good sensitivity and selectivity in detection. Flexibility of polymer substrates paves a way for making devices of required configuration.

**Keywords:** *silver nanoparticles, polymer films, localised surface plasmon resonance, optical transducers, protein sensing.*

### 1. INTRODUCTION

Detection of biomolecules based on surface plasmon resonance is one of the advanced label free and real-time detection approaches [1]. One of the most important elements of this sensing technique is an optical transducer which can be realised through the formation of thin metal films giving rise to propagating plasmons or nanostructures utilizing localised surface plasmon resonance (LSPR). In particular, coinage metal nanoparticles (NPs) are known to provide enhanced LSPR [2] allowing for significantly improved detection sensitivity. Therefore, preparation of functional plasmonic transducers utilizing metal NPs is under extensive study [3]. Despite the numerous research publications on this topic, there is a number of challenges which needs to be addressed to design efficient and reliable detection schemes, to develop appropriate surface immobilisation chemistry providing selectivity of sensing as well as to ensure data registration and analysis. One good example demonstrating this complexity can be the development of biomarkers for Alzheimer's disease, in particular a suggested route where LSPR nanostructures were used to monitor the antibody-antigen interaction requiring appropriate functionalisation and synthesis techniques [4]. One should also consider possible negative effects of NPs and surface chemistry on biological systems under detection [5]. Thus, the spectrum of problems is still wide and in particular an optimisation of parameters of nanoscale transducers is a hot topic.

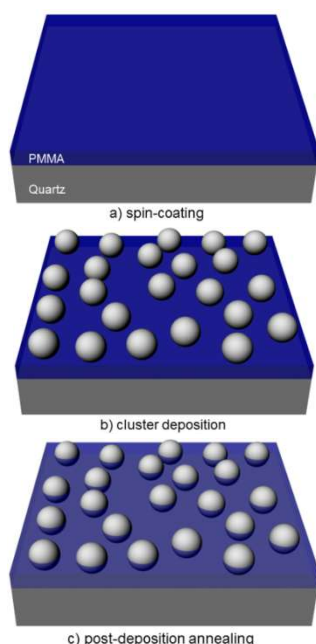
For example, the importance of appropriate particle size in obtaining a good plasmon sensing signal was demonstrated with gold NPs [6]. Attachment of Au NP to CdTe nanowires allows preparing biosensors through coupling of excitons and plasmons

[7]. Another important direction of study is the formation of guided self-assembly of noble metal NPs allowing to tune and optimise parameters of LSPR [8, 9]. In this content, the formation of polymers with partly embedded NPs of controlled size seems to be a promising option for the preparation of transducers. As plastic and flexible polymer materials are easy to form, a wealth of systems with various geometries and dimensions can be easily achieved. Recently, it has been shown that utilization of cluster beam technique, i.e. deposition of NPs in low-energy regime is an efficient method for patterning of polymers and the formation of composite films with stable plasmonic properties [10-12].

In the current paper, we describe the developed methodology for plasmonic transducers with good quality signal generation using cluster beam technique for the formation of size-selected silver NPs and deposition on thin films of poly (methyl methacrylate) (PMMA) followed by controllable immersion of the NPs. Such composites are found to be stable against wet chemical procedures suited for incubation with proteins. Flexibility and plasticity of polymer substrates pave a way for making the transducers of required configuration that can facilitate biomolecule injection and drainage. We also address the problem of protein interaction with NPs which is a rather complex issue and requires special routes. We propose a way for functionalisation of silver surfaces to provide suitable linkers for antibodies with following detection of an antigen. As a model system, chicken egg albumin is used for detection and proving the concept of the transducers. This antigen belongs to the large family of albumins among which, for instance, human serum albumin is of significant practical importance [13].

## 2. EXPERIMENTAL SECTION

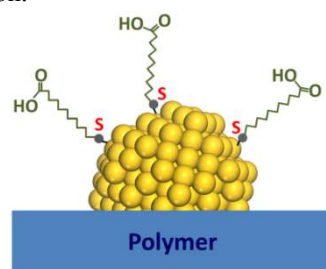
Thin (25-50 nm) PMMA films were prepared by a standard spin coating procedure from a 1% solution (molecular weight 950K PMMA C 9, MicroChem, America) in toluene onto cleaned quartz substrates of 10x10 mm<sup>2</sup>. After the spin coating, the samples were annealed at 100°C for 10 min. to evaporate the solvent and solidify the polymer. According to earlier studies this preparation scheme provides the best conditions for immersion of deposited silver NPs [12]. For the formation of size-selected NPs, magnetron sputtering cluster apparatus (MaSCA), which is described in more detail elsewhere [14], has been used. For the experiments described in this paper, clusters of mean size (diameter) around 13 nm with a relative standard deviation of 10% are selected and deposited on the PMMA films. After deposition, the samples are annealed at 180°C for 5 min. This annealing is found to favour the partial immersion of NPs into the polymer as will be shown in the next section. The procedure for the formation of PMMA composites with Ag NPs is schematically shown in Fig. 1.



**Figure 1.** Schematic image of samples preparation including spin-coating of PMMA, cluster deposition and partial immersion using thermal annealing. See text for details.

For incubation of proteins, surfaces of silver NPs should be functionalised. The procedure developed earlier [15] is followed. In particular, the samples with NPs are incubated with a 1 mM 11-mercaptoundecanoic acid (11-MUA) solution in ethanol for 30 minutes and subsequently washed with pure ethanol to remove residual not reacted 11-MUA. After that the substrates have been

dried under a stream of nitrogen. 11-MUA is expected to become selectively bound to silver NP by the thiol-containing end and it provides terminal carboxylic acid groups for the coupling of proteins (see Fig. 2). To activate these groups the samples were incubated with a freshly prepared 1-Ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride/ N-Hydroxysuccinimide mix ratio 1:1 for 20 minutes. Subsequently, the mix has been removed from the substrates and a protein solution has been added on top of the activated substrates. The incubation period for protein solutions was 30 min. After the incubation, the samples have been thoroughly rinsed with deionised water. The used proteins (anti-chicken egg albumin antibody (Ab) and chicken egg albumin as antigen (AG)) are commercially available and they have been utilized without further purification.



**Figure 2.** Schematic picture of functionalisation of silver surface using 11-MUA. See text for details.

To study the efficiency of coupling of AG to Ab and incubation on NPs, albumin is deposited at two different concentration ratios with respect to Ab. In one case it is 1:1 (Ab-AG), in the other 1:5, i.e. too few Ab molecules compared to AG.

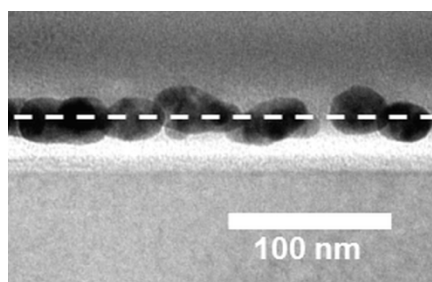
The samples are characterized using several methods, in particular, atomic force microscopy (AFM), transmission electron microscopy (TEM) and optical spectroscopy. AFM measurements are carried out in tapping mode using Ntegra Aura nanolaboratory (from NT-MDT). Commercial cantilevers with sharp silicon tips (radius of curvature < 10 nm and force constant  $k = 1.45\text{--}15.1$  N/m) are used. To obtain cross-sectional images of PMMA with embedded clusters, focused ion beam milling utilizing FEI-Versa is applied to prepare the samples and TEM measurements are carried out using FEI-Talos microscope operating at 200 kV. To avoid damage introduced by  $\text{Ga}^+$  ion beam during sample preparation, an additional  $\text{SiO}_2$  layer of 50 nm thicknesses is deposited on the PMMA with clusters by electron beam evaporation method. The extinction spectra are obtained from transmission measurements using a double beam Perkin Elmer High-Performance Lambda 1050 Spectrometer in standard configuration.

## 3. RESULTS SECTION

Annealing of the samples with deposited metal clusters leads to their immersion into the polymer. The driving force for this process is a huge difference in surface free energy between silver and PMMA. Surface tension ( $\gamma$ ) for silver is known to be  $\approx$

1200 mJ/m<sup>2</sup> [16], while  $\gamma \approx 30\text{--}40$  mJ/m<sup>2</sup> for PMMA [17]. Annealing at the temperature above glass transition  $T_g$ , which is 105 °C for PMMA, softens the material and increases the flexibility of polymer chains allowing the particles “to sink”. AFM

studies of the samples with as-deposited NPs and after annealing reveal that the mean particle height is decreased approximately twice evidencing that NPs become embedded into the polymer for approximately half of diameter. This immersion is directly proved by TEM (see Fig. 3) where one can also see that NPs preserve almost spherical shape. The polymer composites with size-selected silver NPs demonstrate excellent plasmonic properties characterized by the narrow extinction band and high quality factor of LSPR [12]. It is also found that the composites preserve stable plasmonic properties for a number of weeks due to high purity and monocrystalline structure of NPs.

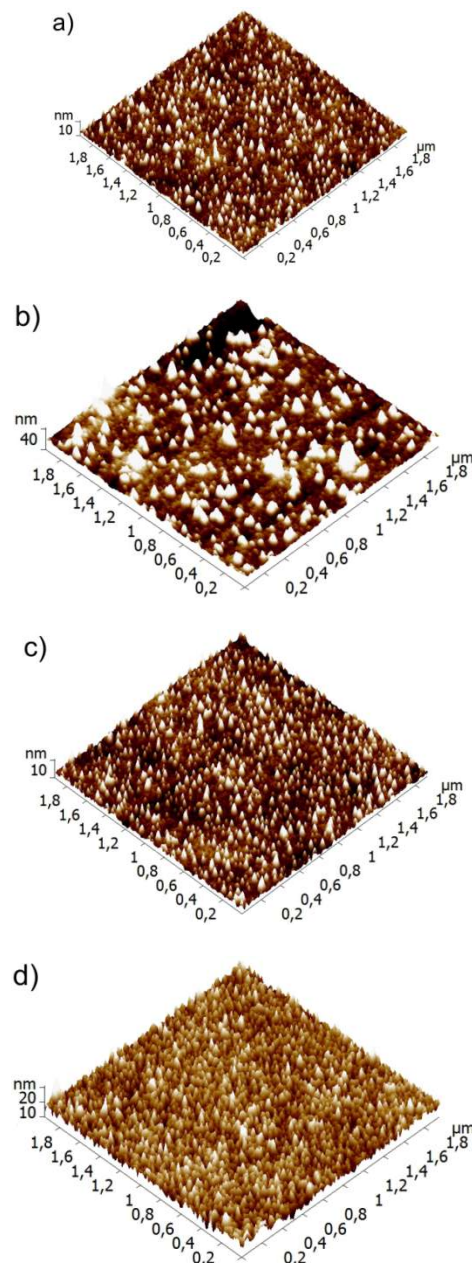


**Figure 3.** TEM cross-sectional image of PMMA with embedded by annealing silver NPs. Dashed line shows polymer surface for visual guidance.

Typical AFM images corresponding to the NPs embedded in PMMA by the annealing process are shown in panels (a) and (c) of Fig. 4. These samples are further used for the Ab-AG incubations in two different concentration ratios as described above. Fig. 4(b) shows the topography of the sample after protein incubation at a ratio of 1:1. It reveals that there is no noticeable decrease in NPs' coverage demonstrating that partially embedded into the film NPs are stable against wet chemistry at all stages: functionalization of NP surfaces and following incubation with Ab and AG. One can see that the bumps corresponding to NPs are increased in size after the protein incubation while there are no features on the bare polymer surface (between the particles). Contrary to this image, Fig. 4(d), corresponding to a ratio of 1:5, demonstrates complete coverage of the surface with many small features. Analysis of these images suggests that for a 1:1 ratio Abs are bound to NPs and AGs are predominantly coupled to them. A few larger bumps in Fig. 4(b) could correspond to agglomerates of Abs and AGs around some surface defects or agglomerated NPs but they are not essential for the methodology of detection. However, for a 1:5 ratio, there are too many albumin molecules compared to available Abs incubated on NPs. Therefore, AGs are located everywhere on the sample surface (see Fig. 4(d)).

In order to estimate number of proteins attached to a single NP, one has to look into the tertiary (or 3 dimensional) structure of the proteins used in this study. Both of them have a globular structure, therefore, one can deduce the average size of a protein from its molecular weight using a simple formula  $R_{min} = 0.066M^{1/3}$  [18], where  $R_{min}$  is the minimal radius of a "smooth" sphere in nm that could contain the protein with given molecular weight  $M$  in Da. From known molecular weights (42, 7 kDa for albumin and 45 kDa for main constituent of the corresponding Ab), sizes (diameters) of both proteins are estimated to be around 4-5 nm. Thus, one can suggest that only very few or maybe only one Ab molecule can couple to an individual NP. Thereafter, AGs are

incubated with these NP-antibody conjugates. This prediction correlates well with the increase of bump sizes in the AFM images. In particular, for the case presented in Fig. 4(a) and (b) mean heights of bumps are increased from roughly 5-6 nm to approximately 15-20 nm.



**Figure 4.** AFM images of (a), (c) silver NPs embedded into PMMA and (b), (d) the same samples after Ab-AG incubation for ratios 1:1 and 1:5, respectively.

Optical spectra of the samples with Ag NPs before and after Ab-AG incubations are shown in Fig. 5. In order to minimize the substrate contribution and visualize LSPR signal alone, a reference spectrum of PMMA spin-coated quartz substrate is subtracted from every spectrum. Substrates with NPs show a relatively narrow plasmon band with a maximum at wavelength  $\lambda = 412$  nm for all prepared samples demonstrating a sharp size selection of the deposited particles. The intensity of LSPR band is high and very similar for both figures indicating almost identical surface coverages by NPs, which is also can be seen from the AFM images Figs. 4(a) and (c)). It needs to be stressed that good



control of NPs coverage is one more advantage of the cluster beam technique compared to other methods. Functionalisation of NPs with 11-MUA causes a red shift of the band and peak broadening with an appearance of a weakly pronounced shoulder at around 500 nm. This shoulder can be attributed to the residual ethanol trapped in the polymer film which changes the dielectric environment of the NPs. The presence of ethanol most probably also causes the decrease in the plasmon band intensity.

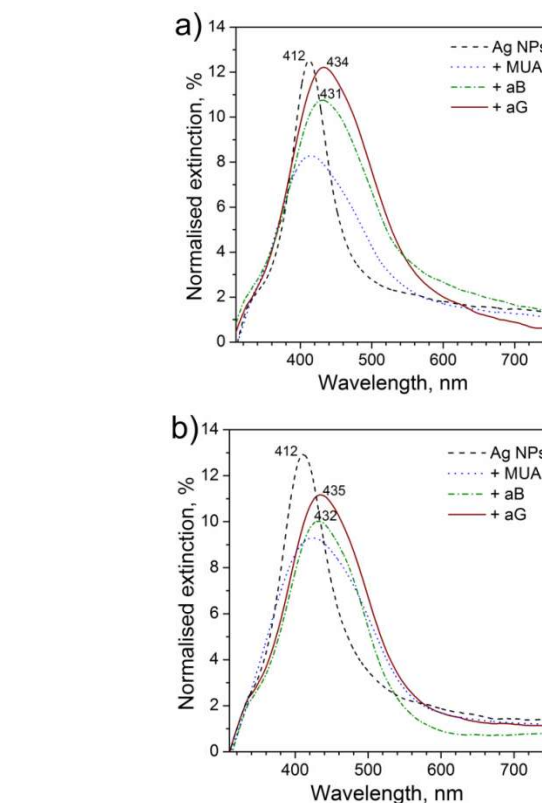
Following incubation of Ab causes further changes in plasmon band position, width and intensity. In both cases (two different concentration ratios), red shift of the band and some decrease of intensity are found (see Fig. 5). Abs are supposed to form covalent bonds with activated carboxyl ends of 11-MUA, thus, affecting the dipole characteristics of NPs leading to the observed changes of LSPR. Incubation of AG on Ab results in a further small but repeatable shift of the plasmon band towards longer wavelengths and a small but considerable increase of the resonance intensity. These changes are very similar for spectra in both panels despite the difference in albumin concentration which is 5 times higher for the case presented in panel (b) of Fig. 5 compared to that in panel (a) of the same figure. This observation indicates that “extra” albumin molecules, which are located randomly on the surface for the case with a ratio of 1:5 (see Fig. 4(d)), do not affect LSPR band because they are not coupled to Abs located on NPs. In other words, one can conclude that only AGs incubated on Abs can be detected through the corresponding change of the plasmonic properties of NPs. Thus, the proposed methodology shows good perspective for selective sensing of proteins and also control of concentration.

#### 4. CONCLUSIONS

Plasmonic transducers based on size-selected silver NPs partially embedded into thin PMMA films are fabricated and studied. NPs produced by the magnetron sputtering method are found to preserve stable optical properties in ambient environment for a number of weeks and PMMA films provide good adhesion of NPs, i.e. stability of the composites against wet chemistry procedures. LSPR properties of silver NPs are exploited for protein detection using a classical Ab-AG scheme through covalent linkage of Ab molecules to the particles and following incubation of AG. Specific changes in LSPR band are observed for functionalised NPs and after each step of protein incubation on them. AFM images of the samples reveal that Abs are

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**Figure 5.** Normalized extinction spectra for the samples with NPs followed by functionalisation with 11-MUA and incubated with Ab-AG for (a) ratio 1:1 and (b) ratio 1:5. Numbers near the spectra give exact wavelengths for the extinction maxima.

predominantly attached to the NPs providing binding sites for the antigens of interest. Increasing the AG concentration well above the Ab concentration does not affect the detection because the AG molecules are not bound to Abs located on NPs.

Thus, our work demonstrates a platform for the formation of plasmonic transducers where embedded into PMMA silver NPs are stable against wet chemistry and appropriate proteins incubation protocol provides a way for good selectivity in sensing and detection of different concentrations of AG. However, the latter (concentration) approach requires further investigation. Currently, it is only a proof of concept that the observed signal depends solely on the Ab-AG interaction.

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