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Plasmonic device based on a PAAm hydrogel/gold nanoparticles composite

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ABSTRACT: Stimuli-resposive hydrogels, such as poly(acrylamide), are smart materials that can be loaded with gold nanoparticles to explore the localized surface plasmon resonance effect to develop an optical device. Here we used electropolymerized poly(acrylamide) hydrogel for entrapped gold nanoparticles into gel structure (composite) to prepare a plasmonic device. Sensing tests were performed; for this bovine serum albumin molecules were placed into the composite by diffusion from an aqueous solution. The presence of the molecules alters the refractive index around the gold nanoparticles, changing its resonance conditions. The plasmonic band shifted ~3.8 nm when the composite was incubated at the 20 μg/mL bovine serum albumin solution, which is a result comparable to reports elsewhere using gold nanoparticles on glass substrates. The device showed that it was possible to detect significantly low concentrations up to 10 ng/mL of protein in aqueous solution. © 2015 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2015**, *132*, 42449.

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INTRODUCTION

Hydrogel is a hydrophilic 3D network of cross-linked polymer chains. These materials are very absorbent and exhibit swollen or shrunk characteristics depending on the environment. The volume change occurs in response to variations in factors such as solvent, pH, and temperature.[1,2] For example, the poly(acrylamide) (PAAm)-hydrogel captures a large quantity of water by swelling or become shrunken when placed in a hydrophobic media.[3] Over the years, the hydrogels have found great applications in sensors when loaded with metallic nanoparticles, combining the localized surface plasmon resonance (LSPR) effect with the stimuli-responsive characteristics of the hydrogels.[4–6]

LSPR is a collective oscillation of electrons induced by visible light and is excited using noble metal nanoparticles such as gold, generating an intense color observed with the naked eye.[7] Therefore, metal nanoparticles display a specific extinction band that depends on the shape, size, interparticle distance, and the environment of the nanoparticles.[8,9] The LSPR band undergoes changes in maximum wavelength or intensity by shifting the refractive index of the surrounding medium of the nanoparticles.[10] Alternatively, the LSPR band can be tuned using the stimuli-responsive properties of a hydrogel which contains entrapped metal nanoparticles, since gel swelling process can change the refractive index of the medium surrounding the nanoparticles. This may be done if the composite is placed in contact with a solution containing molecules that, after diffused into the material, show affinity to the nanoparticle's surface embedded into the gel.

Sensors based on composite metal nanoparticles/hydrogel (especially gold and silver nanoparticles) have attracted great attention since they allow efficient biochemical to optical signal transduction by exploiting the LSPR effect. Recent studies showed the detection of glucose-using biosensors based on ionic strength and pH-responsive hydrogels loaded with silver nanoparticles.[11,12] Similarly, a sensor was also produced using a solvent-responsive hydrogel with gold nanoparticles (AuNPs) trapped on its surface.[3]

In this context, herein we report the preparation of a optical device based on an AuNPs/cross-linked PAAm-hydrogel composite for the detection of bovine serum albumin (BSA) biomolecules. The PAAm-hydrogel film was electropolymerized by using the acrylamide monomer in presence of zinc chloride and *N,N′*-bis-acrylamide methylene on glass indium tin oxide (ITO) electrodes (glass-ITO). The PAAm-hydrogel shows swelling in water and shrunk in an aprotic medium such as acetone.[13] Then the composite was prepared by immersing the shrunken hydrogel in aqueous AuNPs solution as reported elsewhere.[14] The system detected different concentrations of BSA and the detection

mechanism is based on an associated interaction of the biomolecule (introduced inside the gel by diffusion) with the polymeric chain and by proximity with the nanoparticle surfaces.

EXPERIMENTAL

Materials

The ITO-glass slides (50.0 × 50.0 mm and thickness: 0.5 mm, Adafruit Inc.) used as working electrodes. Acrylamide (99%, Aldrich, USA), *N,N′*-bis-acrylamide methylene (96%, cross-linker, Acros Organics, USA) and zinc chloride (97%, $ZnCl_2$, Dinâmica Química Comtemporânea, Brazil) were used for hydrogel electrosynthesis. Hydrochloric acid (F.Maia, Brazil) was used for dissolving the metallic zinc precipitate. Acetone (99.5%, Casa da Química, Brazil) was used to shrink the hydrogel. Gold chloride (III) (30% w/v, $HAuCl_4$) and tribasic sodium citrate (99%, reducing agent) used for the gold nanoparticles synthesis and bovine serum albumin (BSA, 99.7%) were purchased from Sigma-Aldrich (USA).

PAm Hydrogel Film Preparation

A PAAm hydrogel film was obtained by electropolymerization of the acrylamide monomer in the presence of $ZnCl_2$ and *N,N′*-bis-acrylamide methylene using cyclic voltammetry.[14] The glass-ITO (area of 2 cm^2) was used as a working electrode. A saturated calomel electrode and a platinum sheet were used as reference and counter-electrode, respectively. The glass-ITO slides were cleaned with acetone and chloroform in an ultrasonic bath for 10 min (each solvent) and dried in blowing N_2. The electropolymerization of acrylamide was performed using a 5.0 mol/L aqueous solution containing 0.2 mol/L $ZnCl_2$ and 0.1 mol/L *N,N′*-bis-acrylamide methylene. The cyclic voltammetries (6 cycles) were done using an Autolab PGSTAT30 potentiostat/galvanostat scanning from −1.5 V to 0.2 V, holding in −1.5 V for 20 s before starting the next cycle. The hydrogel film was left for 24 h in HCl solution for dissolving the zinc precipitate (formed during the voltammetric reduction step). A scanning electron microscope (SEM, Shimadzu - SS-550 super scan) was used to analyze the hydrogel morphology (samples lyophilized 24 h before) and to calculate the average diameter of pores (Image-Pro® PLUS software in combination with the program STATISTIC version 8.0). The thickness of the hydrogel was also measured from the SEM images.

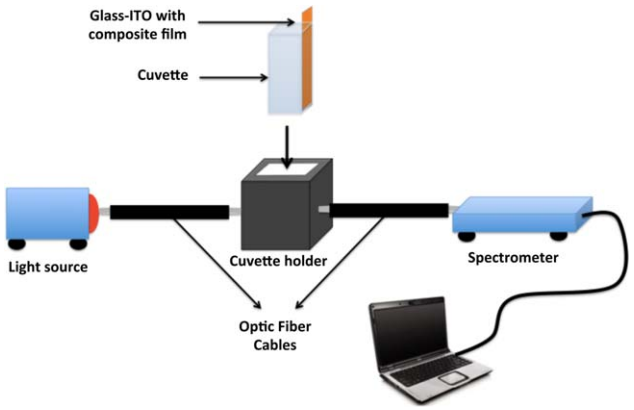

Figure 1. Experimental setup for measuring UV-Vis spectra of the AuNPs/PAAm-hydrogel composite. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

Gold Nanoparticles Synthesis

A gold chloride (III) solution was prepared in concentration of 0.01% (w/v). This solution was preheated until boiling in a reflux system and then 2.5 mL of 1% (w/v) sodium citrate solution was added to the system at constant heating for 15 min. After synthesis, the solution containing the gold colloid was poured into an ice bath, and finally stored in an amber vial under refrigeration.[15] The material was characterized by an UV-vis spectrometer (Ocean Optics, USB2000) and transmission electron microscopy (TEM, JOEL Shimadzu, JEM 1400). TEM images were also used to calculate the average diameter of AuNPs (Image-Pro® PLUS software with the program STATISTC version 8.0).

Gold Nanoparticles/Poly(acrylamide)-Hydrogel Composite

The composite was obtained in three steps. First, the swollen gel was kept in acetone for 1 min to remove water. Second, the gel was re-swollen by placing in the AuNPs aqueous solution for 24 h, so that the nanoparticles were introduced into the gel. Finally, nanoparticles weakly adsorbed on the gel were removed

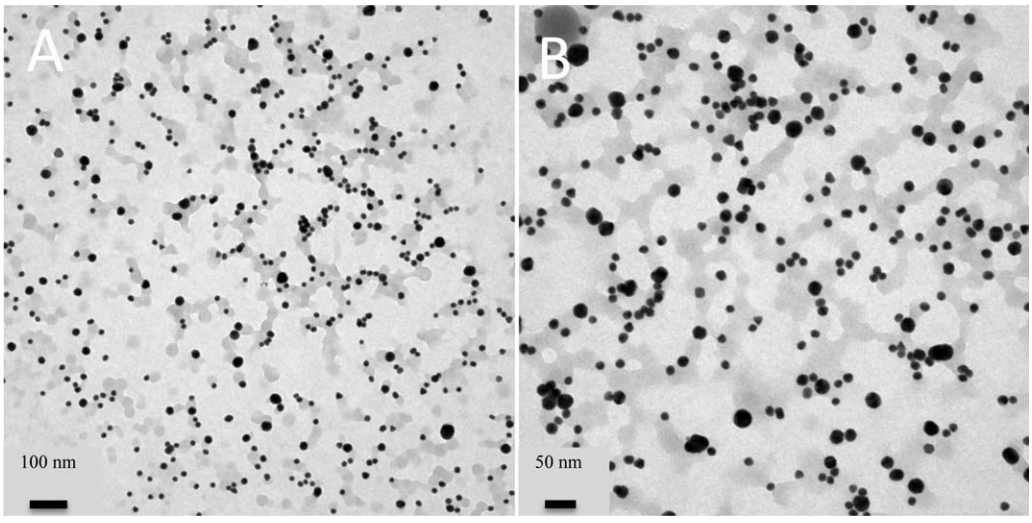

Figure 2. TEM images of AuNPs prepared in aqueous solution at different magnifications.

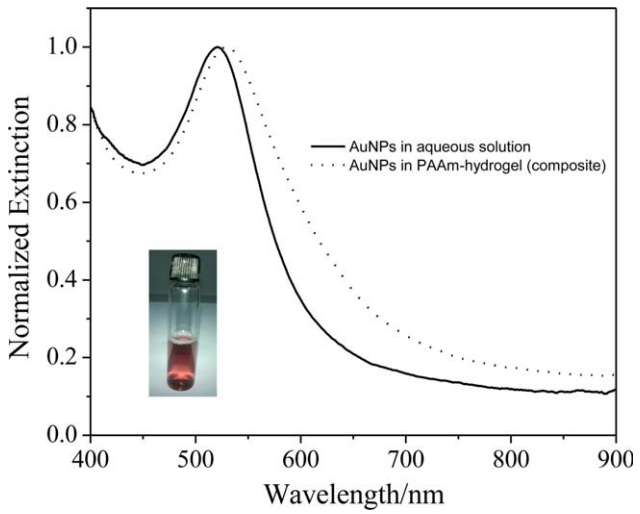

Figure 3. Extinction spectra for gold nanoparticles in aqueous solution and into hydrogel poly(acrylamide) chain. Inset: photographic image of the AuNPs suspension. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

by rinsing water. The composite was characterized by TEM and atomic force microscopy (AFM, Shimadzu, SPM-9500). AFM images were obtained from the lyophilized samples and TEM images from pulverized samples.

BSA Detection

A molecular detection was carried out through the introduction of the analyte within the gel. Aqueous solution of BSA was prepared at concentrations of 0.01, 0.05, 0.1, 0.5, 1, 5, 20, and 50 μg/mL. Simply, these solutions were left in contact with the same swollen AuNPs/PAAm-hydrogel composite for 24 h. Before measurement, the composites were extensively rinsed with water to remove weakly adsorbed molecules. UV-Vis spectra were obtained for the composite using the experimental setup depicted in Figure 1.

RESULTS AND DISCUSSION

Characterization of AuNPs Suspension, PAAm-Hydrogel, and Composite

Figure 2 shows the TEM images of AuNPs at different magnifications. In general, the particles were uniform (with

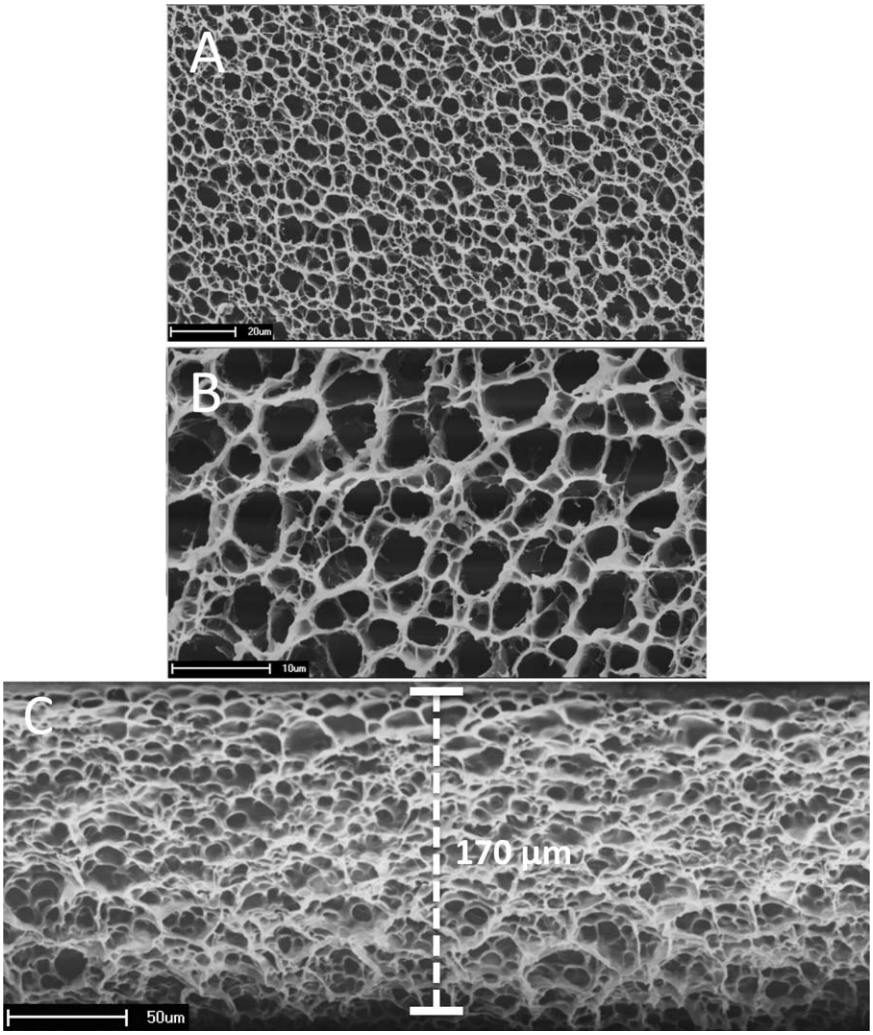

Figure 4. (A) and (B) show SEM images of PAAm-hydrogel surface at different magnifications. (C) shows a side view of the 170 μm-thick gel film.

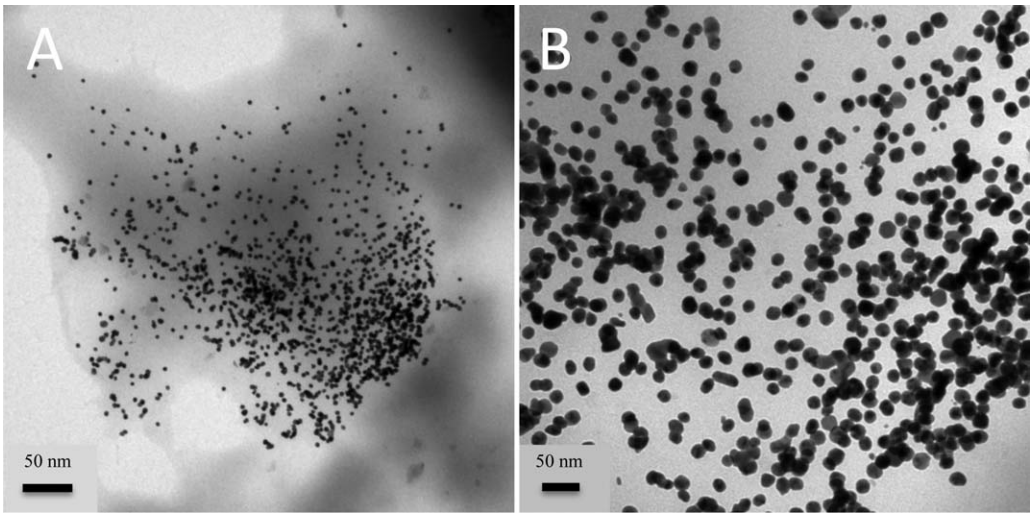

Figure 5. TEM images of AuNPs/PAAm-hydrogel composite at different magnifications.

some occasional agglomerates) with an approximately spherical shape and average diameter of 17 ± 4 nm. A colloidal suspension of AuNPs showed a red color and an absorption spectra with maximum ca. $\lambda_{máx} = 521$ nm which is characteristic of this size of gold nanoparticles.[16] The absorption spectrum of AuNPs in aqueous solutions is shown in Figure 3. The inset in Figure 3 shows a photographic image of the AuNPs suspension.

The PAAm-hydrogel film obtained by electropolymerization on glass-ITO showed good homogeneity and a three-dimensional network with pores in a circular honeycomb-like shape (Figure 4). The pores had an average diameter of 3.8 μm and the gel film was ca. 170 μm thick [Figure 4(C)], which are large enough to allow the molecules to diffuse and be adsorbed within the three-dimensional structure of the gel.

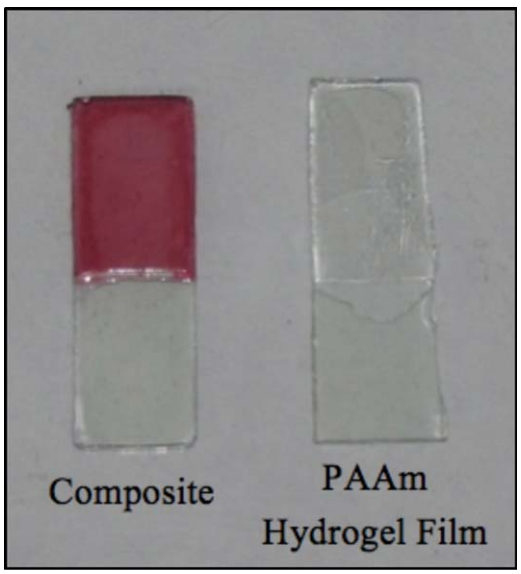

Figure 6. Photographic image of PAAm hydrogel (right) and AuNPs/PAAm composite (left). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

TEM images at different magnifications (Figure 5) confirm the presence of gold nanoparticles within the hydrogel. The shadowed regions in Figure 5 are assigned to the hydrogel. The included nanoparticles kept good homogeneity with an average diameter of 19 ± 2 nm. This suggests that the general features, such as the shape and size of the nanoparticles into the hydrogel, are very similar with respect to the aqueous suspension.

The reddish color of the hydrogel after immersing into the AuNps solution evinces the nanoparticles inclusion, Figure 6. The wavelength of maximum extinction of the nanoparticles within the gel was ca. $\lambda_{máx} = 530$ nm (Figure 3), that is near to that obtained for the AuNps in suspension (ca. 521 nm). This small increase in maximum extinction is probably due to the fact that nanoparticles are embedded in the polymer chains of the hydrogel which causes a small increase in the refractive index of the surrounding environment of AuNPs, so that there is a small change in the resonance condition of LSPR.[8]

Sensing Test

To test the BSA detection, the composite was loaded with BSA molecules from aqueous solutions of different concentrations, as showed in the Figure 7(A). When the shrunken hydrogel is placed in an aqueous solution of gold nanoparticles it absorbs large amounts of water. This causes a relaxation in the polymer three-dimensional chains that allows the entry of AuNPs. Nanoparticles then remain adsorbed into the gel matrix due to electrostatic interactions with the polymer chains caused to the presence of primary amines in the polymer (interaction between primary amines and gold nanoparticles have been reported elsewhere[17]). Then, the swollen composite was placed in contact with BSA solution so that the protein molecules may diffuse into the hydrogel.

The BSA molecules that were diffused into the hydrogel caused a maximum wavelength red-shift in the absorption spectra [Figure 7(B), inset]. This occurred since the biomolecules attached to the polymeric structure are probably near to the nanoparticles surface, which are present in high density into the gel. Due to the proximity, there is a physical interaction between

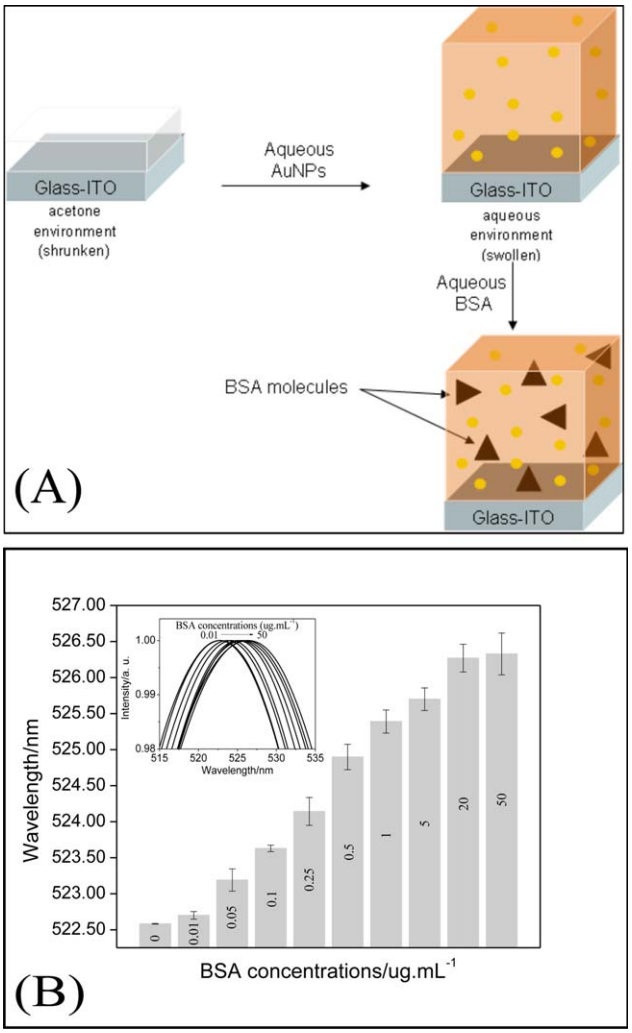

Figure 7. (A) Scheme to preparation of AuNPs/ PAAm hydrogel composite loaded with BSA molecules and (B) absorption spectra of AuNPs/ PAAm-hydrogel composite as function of the concentration which the composite was immersed. Inset: absorption spectra of AuNPs/PAAm-hydrogel composites after immersion in aqueous solution with different concentrations of BSA (spectra taken in pure water). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

BSA molecules and the nanoparticles surface,[18,19] which makes it possible to monitor the biomolecules into the hydrogel by LSPR effect. This was achieved since the resonance conditions were changed causing a shift of the maximum extinction of AuNPs.[8] Thus, the hydrogel 3D matrix is a suitable support to keep BSA molecules close enough to the nanoparticles surface to affect the electromagnetic field of surface plasmons. This was achieved without the need of a nanoparticles chemical modification to chemically attach the protein on their surface, as commonly used in some nanoparticle-based plattforms.[20–22]

The results showed in the Figure 7(B) inset also shown that the system was able to monitor the increasing BSA concentration in contact with the composite. Increasing shifts to longer wavelength in extinction band were observed, as there was an increase in the solution concentration. This occurred because a greater amount of molecules were diffused into the composite, which causes a gradual increase in refractive index surrounding the nanoparticles.

Figure 7(B) shows the extinction maximum wavelength of the AuNPs/PAAm-hygrogel composite as a function of the BSA concentration in which the composite was immersed. The minimum solution concentration that causes a displacement of the extinction band (shift ca. 0.2 nm) was a 0.01 μg/mL BSA solution (lower concentrations were tested but no shifts were detected). This limit of detection is slightly smaller than that reported by Zhu *et al.*, which worked with a similar plasmonic system.[23] A limit of detection of 0.02 μg/mL was achieved exploring the BSA physical adsorption on gold nanoparticles. However, limits of detection on the order of pg/mL have been achieved using traditional SPR sensors based on the configuration proposed by Kretschmann, operating with chips modified with graphene oxide.[24] Although the conventional SPR arrangement is more sensitive, the analysis using the proposed composite in this work is only based on absorbance measurements in collinear mode instead of a complex arrangement based on attenuated total internal reflectance used in conventional SPR.

The maximum wavelength shift was recorded in the 20 μg/mL solution, which was ca. 3.8 nm. Solutions with concentrations higher than 20 μg/mL did not significantly alter the maximum shift, which suggests that this hydrogel reached a maximum of trapped BSA molecules into polymeric matrix.

CONCLUSIONS

The polyacrylamide hydrogel loaded-gold nanoparticles composite was found to successfully detect BSA molecules absorbed from aqueous solutions. Plasmonic devices with this configuration have not been reported yet for the detection of proteins (such as BSA). Our results are comparable to published works regarding detection of BSA using functionalized gold nanoparticles on glass substrates.[25] In addition, the optical system proposed in the paper presents great potential for application in plasmonic sensors, whether a molecular recognition agent is used in the construction of the device.

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