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Highly sensitive bovine serum albumin biosensor based on liquid crystal

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A highly sensitive liquid crystal (LC) based bovine serum albumin (BSA) protein biosensor is designed. A uniform homeotropic alignment of nematic LC was observed in BSA free substrate which changed into homogeneous in presence of BSA. The change in the LC orientation is found to depend strongly on BSA concentration. This change in the LC alignment is attributed to the modification in the surface conditions which is verified by contact angle measurements. We have detected an ultra low concentration (0.5 μg/ml) of BSA. The present study demonstrates the utilization of LC in the realization of high sensitivity biosensors. © *2014 AIP Publishing LLC*. [http://dx.doi.org/10.1063/1.4863740]

Liquid crystal (LC) materials have been extensively utilized in various display as well as non-display devices due to their easy and fast response to the external stimuli such as electric, magnetic, and anchoring fields.[1] The high sensitivity of LC molecular orientation on anchoring interactions proved them suitable candidates to be utilized in sensing applications. The sensing devices based on LC materials have attracted particular attention for their unique characters as they permit label-free detection with high sensitivity even without any need of complex instruments and electrical power. This made them sufficiently simple and well suited for the primary screening assay of analytes performed away from central laboratories.[2,3] The highly sensitive anchoring responses (in the form of their modified orientation) to different substrates, biological molecules like proteins and DNA proved LCs as potential candidates to be utilized in extensive biosensors. Numerous studies on LC based sensors for the detection of DNA hybridization, protein binding event, enzymatic reaction, enzyme inhibitors, surface-active reagents, antigen−antibody, etc., have been reported.[4–12]

Most of the LC biosensors are based on the biological macromolecule binding events which can change the anchoring behaviors of LCs. The disruption of the orientations of a nematic LC supported on a surface patterned with glycine oligomers has been analyzed by observing the change in the optical birefringence, and the simultaneous detection of triglycine, tetraglycine, and pentaglycine is reported by Bi *et al*.[13] Park *et al*. demonstrated the detection of the specific interactions between biomolecules by manipulating the orientational behavior of LC to the printed protein surfaces.[14] Liao *et al*. utilized the phenomenon of biometallization to detect the presence of acetyl cholinesterase by monitoring the change in the optical transmission due to the disruption of LC molecular alignment.[15] The disruption in the orientational ordering of LC molecules due to changed surface characteristics could even been utilized for highly sensitive detection of heavy metal ions.[16] Recently, an efficient detection of bovine serum albumin (BSA) protein has been achieved by integrating LC dots on micro fluidic channels by monitoring the change in the orientational ordering of LC dots when interacted with BSA containing buffer solutions.[17] The authors used very sophisticated experimental techniques to achieve sensitive detection of BSA with a sensitivity of 4 μg/ml. However, we have designed a simple LC based BSA biosensor with a sensitivity of 0.5 μg/ml. In this paper, the results based on BSA detection by probing the change in the LC ordering interacting with BSA have been presented. The change in the LC ordering has also been analyzed by measuring the contact angles which LC material made with BSA immobilized indium tin oxide (ITO) substrates.

BSA was purchased from Bangalore Genei, India. N-(3-dimethylaminopropyl)- N′-ethyl carbodiimide hydrochloride (EDC), N-hydroxy succinimide (NHS), sodium tetrahydroboride ($NaBH_4$), (3-Aminopropyl)triethoxysilane (APTES), 3-mercapto propionic acid (MPA), and phosphate buffer saline (PBS) were obtained from Sigma-Aldrich Corp. Tetrachloroauric (III) acid ($HAuCl_4$) was obtained from Himedia Pvt. Ltd., India. MPA capped gold nanoparticles (GNP) solution was prepared by a procedure as reported earlier.[18] For that, 12 mg of $HAuCl_4$ was dissolved in 30 ml of ethanol and 0.70 mg of MPA was added in this solution and vigorously stirred for 30 min. Thereafter, a solution containing 10 mg $NaBH_4$ in 20 ml ethanol was added drop by drop to the above mixture under stirring for 1.5 h. A dark brown colloidal suspension was obtained which was washed with ethanol and centrifuged at 15 000 rpm for 30 min. The black precipitate so obtained was then dried under vacuum and re-dissolved in water to obtain aqueous MPA capped gold solution of desired concentration.

The sample cells for the present study were prepared using ITO coated glass plates. The ITO coated glass plates (1×1 cm^2) were cleaned by sequential ultrasonic cleaning in soapy water, acetone, ethanol, isopropyl alcohol, and distilled water for 10 min each and then dried in vacuum. The cleaned ITO glass plates were put in plasma chamber and exposed to oxygen plasma for 5 min. The plasma cleaned ITO glass plates were immersed in 2% APTES solution prepared in ethanol for 1.5 h, under ambient conditions. The APTES treated ITO plates were then washed with ethanol

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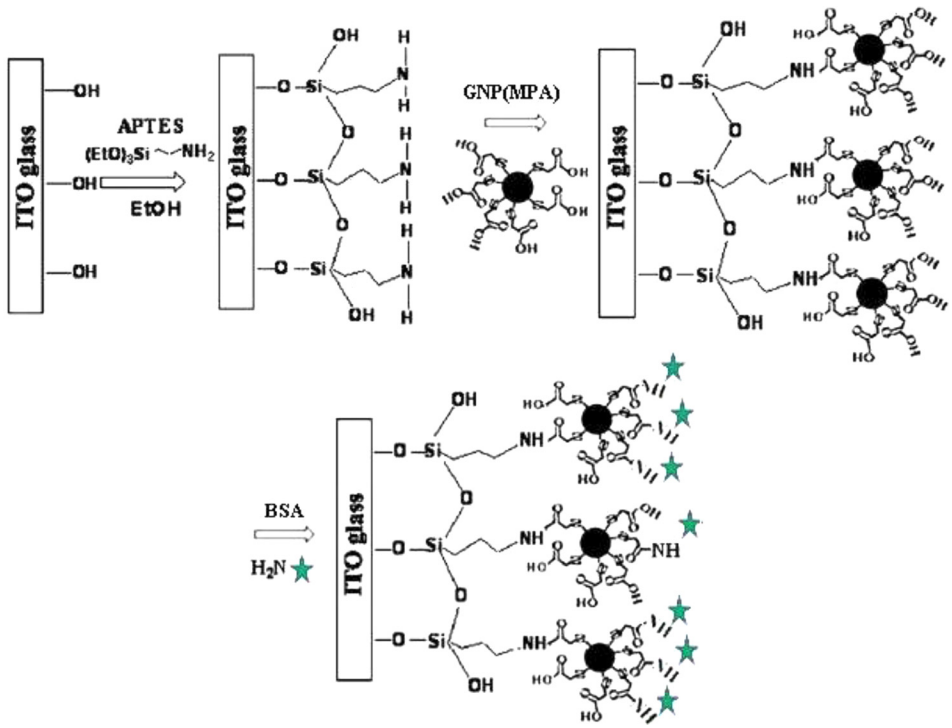

FIG. 1. Schematic illustration of the step wise surface modification of ITO glass plate with functionalized gold nanoparticles and immobilization of BSA.

and dried under N_2 flow. The APTES modified ITO glass plate were immersed in 1.0 mg/ml aqueous solution of GNP (MPA) containing 0.1 M EDC and 0.05 M NHS for 2 h and then thoroughly rinsed with double distilled water and dried under N_2 flow. Further, the ITO plates were covalently immobilized by distinct concentration of BSA prepared in PBS at 4 °C for 4 h. These BSA modified plates were then washed with PBS and finally dried under N_2 flow at room temperature. The scheme illustrating each step of surface modification of ITO glass plate and immobilization of BSA is shown in Figure 1. The untreated and BSA immobilized ITO glass plates were assembled to form the liquid crystal sample cell having sandwiched geometry. The thicknesses of the sample cells were maintained by using 4 μm thick Mylar spacers. The nematic LC material, namely, 4-pentyl-4′-cyanobiphenyl (5CB) is used for the present study. The orientation of LC molecules in BSA treated/untreated sample cells was analyzed by observing their optical micrographs by using polarizing optical microscope (POM, Carl Zeiss, Germany). The water contact angle of LC material on different concentrations of BSA treated ITO substrates and on untreated ITO substrates was measured by Drop Shape Analysis System (DSA10MK2, Krüss GmbH, Germany).

The alignment of 5CB LC molecules in BSA untreated/treated sample cells has been analyzed by observing their optical micrographs, which have been shown in Figure 2. A uniform homeotropic (HMT) alignment of 5CB molecules has been observed in case of BSA untreated sample cell which is clear from Figure 2(a). In case of a uniform HMT alignment, the LC molecules orient uprightly to the substrate and a complete dark field of view appears under the crossed polarizers. The appearance of completely dark field of view verifies the uniform HMT alignment in BSA free sample cells [Figure 2(a)]. For BSA treated sample cells, HMT alignments prevails even for BSA concentrations <0.5 μg/ml [Figure 2(b)]. However, for 0.5 μg/ml of BSA concentration, a

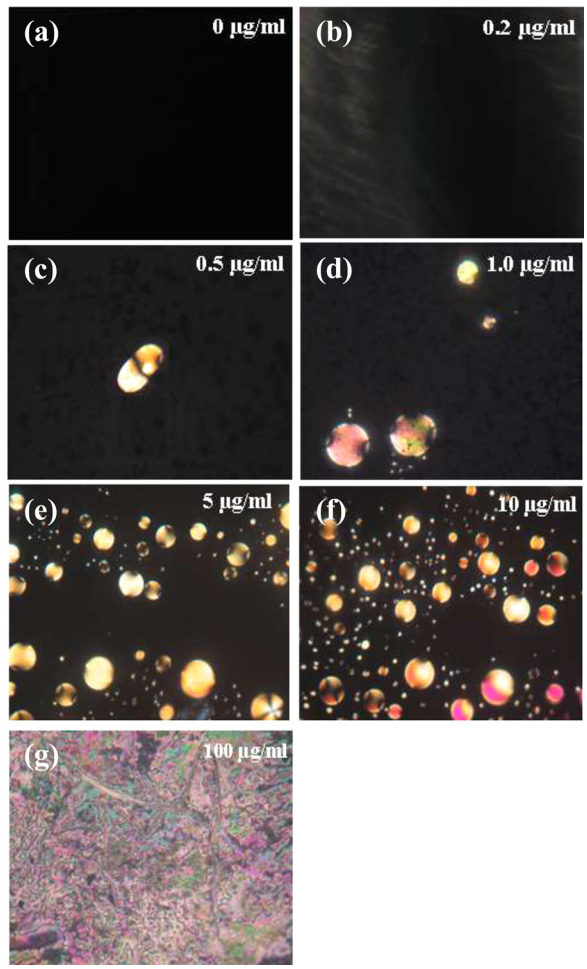

FIG. 2. Optical micrographs showing the configuration changes caused by different concentrations of BSA.

small portion having homogeneous (HMG) orientation of LC molecules is appeared indicating observable interaction between BSA and LC molecules. It suggested that the presence of 0.5 μg/ml BSA could sufficiently disrupt the LC ordering in some portion which is resulted in the form of observable light transmission through the LC sample cell [Figure 2(c)]. The number of portions having HMG orientations has been increased indicating the dominating interaction between BSA and LC ordering on increasing the concentration of BSA [Figures 2(d)–2(f)]. A complete HMG alignment in the whole sample cell is observed in case of 100 μg/ml BSA concentration where the interaction between BSA and LC molecules is sufficient enough to orient all the LC molecules in HMG ordering [Figure 2(g)]. It is worth to mention here that the minimum concentration of BSA that could be detected is found to be ~0.5 μg/ml, which is almost 4-fold lower than proposed earlier by involving sophisticated techniques.[17] The disruption in the LC ordering on interacting with different concentration of BSA has been further confirmed by contact angle (θ_C) measurement using the static sessile drop method. The water drop image was stored and an image analysis system calculated the value of θ_C from the shape of the drop. Figure 3 shows the variation in θ_C on untreated ITO substrate and different concentrations of BSA treated ITO substrates. The inset of Figure 3 depicts the microscopic images recorded during contact angle measurement. It is clear from the figure that the θ_C is found to be comparable (~65.4°) for untreated and low concentration BSA treated substrates. Such values of the θ_C are indicative of favorable HMT orientation of 5 CB LC when filled into the corresponding sample cells. Recently, Joshi *et al.* have explained the favorable HMT alignment of ferroelectric LC caused by ferro-fluid by measuring the water contact angles on substrates with and without ferrofluid.[19] Contact angle is found to decrease rapidly on increasing the concentration of BSA and found minimum (~30°) for substrate having BSA concentration of 100 μg/ml. The smaller value of θ_C indicated the expected change in the orientation of LC molecules. The change in θ_C on BSA treated substrates is clearly reflected from the corresponding microscopic images [inset of Figure 3] The LC droplets is greatly been spread over the substrate in case of 100 μg/ml BSA concentration, suggesting the complete change in the LC orientation from HMT to HMG. It is clear from the above discussion that the detection of BSA may also be achieved by calibrating the variation of contact angle with that of the concentration of BSA.

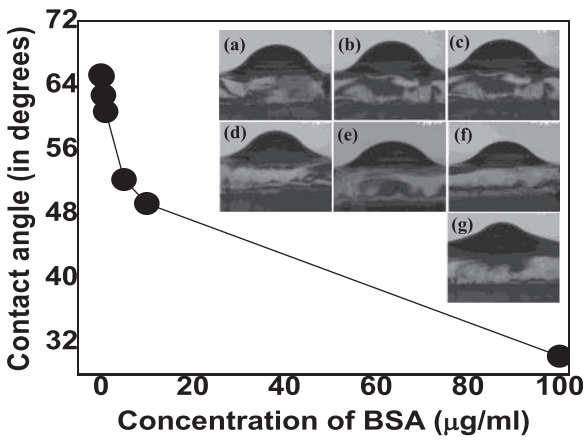

FIG. 3. Variation of contact angle subtended by water on untreated and BSA treated ITO substrates with concentration of BSA. Inset of the figure shows the microscopic images for different BSA concentrations of (a) 0 μg/ml, (b) 0.2 μg/ml, (c) 0.5 μg/ml, (d) 1.0 μg/ml, (e) 5 μg/ml, (f) 10 μg/ml and (g) 100 μg/ml recorded during the contact angle measurements.

This highly sensitive detection could be attributed to the modification in the anchoring conditions of LC material due to the presence of BSA. The nature of orientation of LC material critically depends on the surface characteristics of the substrates. The kind of orientation of a given LC material on a given substrate surface could be understood as a consequence of the competition between surface energies of the substrates and LC material.[19] In case of BSA untreated substrate, the LC molecules are in direct contact with MPA capped GNPs, and LC molecules preferred an upright (HMT) orientation on these surfaces. The preferred HMT orientation was clearly reflected from contact angle (a manifestation of surface energy) measurements, where greater values of the same were observed.[20] However, the presence of BSA immobilization on the MPA capped GNPs surfaces increased the surface energy, reflected from lower values of contact angles, which in turn resulted the HMG alignment of LC molecules. It is worth to mention here that change in LC orientation due to the presence of BSA has not took place uniformly, instead it appeared in the form of homogeneously aligned islands or domains and their number has been increased on increasing the concentration of BSA. This could be explained by taking into account the spreading of BSA on GNPs surfaces. In the low concentration regime of BSA, smaller number of GNPs surfaces were covered with BSA and their interaction is nucleated in the form of HMG islands. BSA covered surface area has been increased on increasing the BSA concentration and hence the number of HMG islands are increased with BSA concentration. At higher BSA concentration (100 μg/ml), a uniform spread of BSA over GNPs surfaces took place to give HMG orientation in the whole sample.

The results based on sensitive detection of BSA by utilizing LC material have been presented. It has been observed that the presence of BSA changed the orientation of LC material from HMT to HMG configuration. The disruption in the LC alignment caused by BSA is found to strongly depend on the concentration of BSA. This modification in the LC alignment is attributed to the change in the surface conditions due to the presence of BSA and verified by contact angle measurements. The present study explores the possibility of extensive utilization of LC materials in the realization of highly sensitive biosensors.

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