

Nanopatterned Cadmium Selenide Langmuir–Blodgett Platform for Leukemia Detection

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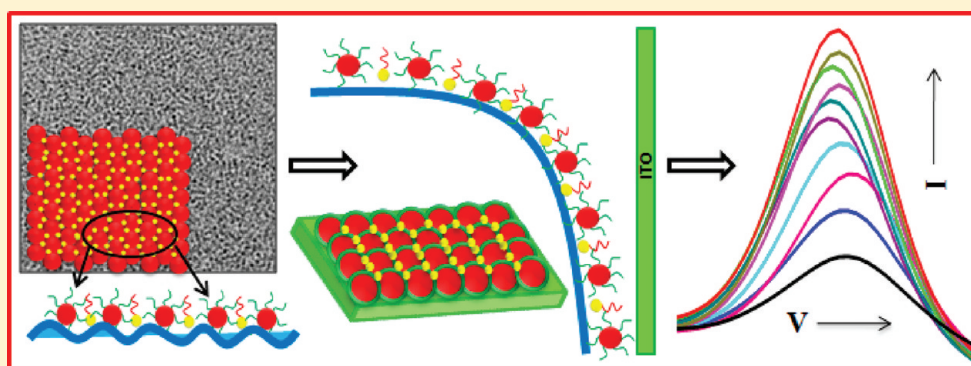
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Supporting Information



ABSTRACT: We present results of the studies relating to preparation of Langmuir–Blodgett (LB) monolayers of tri-*n*-octylphosphine oxide-capped cadmium selenide quantum dots (QCdSe) onto indium–tin oxide (ITO) coated glass substrate. The monolayer behavior has been studied at the air–water interface under various subphase conditions. This nanopatterned platform has been explored to fabricate an electrochemical DNA biosensor for detection of chronic myelogenous leukemia (CML) by covalently immobilizing the thiol-terminated oligonucleotide probe sequence via a displacement reaction. The results of electrochemical response studies reveal that this biosensor can detect target DNA in the range of 10^{-6} to 10^{-14} M within 120 s, has a shelf life of 2 months, and can be used about 8 times. Further, this nucleic acid sensor has been found to distinguish the CML-positive and the control negative clinical patient samples.

Chronic myelogenous leukemia (CML) is a hematological disorder caused by t(9;22)(q34;q11) reciprocal translocation, leading to generation of the *bcr–abl* oncogene, constitutively unregulated tyrosine kinase. It causes bone marrow to malfunction and produce leukemic cells that circulate in blood, leading to enlargement of the spleen, liver, and other organs, wherein they accumulate.¹ The prospects of enhanced survival rates by early detection of CML and its continuous monitoring have led to increased demand for highly sensitive, specific, and rapid biosensors. In this context, nucleic acid biosensors have aroused much interest, as these devices focus on direct detection of the oncogene.^{2–5} However, to achieve sensitive detection of specific nucleic acid sequences, precise control over surface coverage of probe molecules and their orientation are the key requirements that can perhaps be met by immobilization of these biomolecules onto ordered

molecular assemblies.^{6,7} Self-assembly is a natural basic building principle for producing organized monolayer arrays with controlled geometrical and physicochemical surface properties.⁸ Of all other rational approaches, the Langmuir–Blodgett (LB) technique holds appeal as it provides an ordered pattern of particulates onto the desired substrate with controlled thickness and interparticle spacing.⁹

The numerous reports available in literature to date pertain to investigations of the Langmuir monolayer behavior of various nanoparticles and their deposition onto different substrates by LB technique.^{7,10–15} These ultrathin films, however, have not been much explored for biosensing applications. Among the various

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nanoparticles, quantum dots (QDs) have been considered important since they provide a transducer surface with excellent surface activity and large specific surface area owing to their ultrasmall size. Besides this, QDs play an important role toward enhanced electron transfer between the immobilized biomolecule and the electrode surface, which may be attributed to the higher charge detaching efficiency of quantum dots arising from their quantum size effect, and thus improve performance of the electrochemical biosensor.^{16–19} However, owing to their dimensional similarities to DNA, a close packing of probe onto the nanopatterned surface is expected, subjecting the target DNA to both steric hindrance and strong electrostatic repulsion, thereby hampering the hybridization event. Thus, stringent control over the interparticle spacing is required to modulate the distance between adjoining probe molecules that can perhaps be achieved via incorporation of an amphiphilic molecule as the spacer unit.

We report results of the systematic studies relating to the Langmuir monolayer behavior of QCDSe in the presence of stearic acid (SA) molecules. Apart from acting as a spacer, SA has been found to improve the spreading property of QCDSe, thereby facilitating stable monolayer formation at the air–water interface. Subsequently, the QCDSe-SA monolayers deposition onto hydrophobized ITO substrate has been carried out by the LB technique to obtain a nanopatterned array of QCDSe. These thin films have been utilized for the fabrication of a nucleic acid sensor by covalent immobilization of the CML-specific thiol-terminated DNA as the capture probe. Electrochemical transduction of the hybridization reaction with complementary target sequence, identified from *bcr–abl* fusion gene, single-base mismatch, and noncomplementary sequence has been carried out to investigate response characteristics of this nucleic acid sensor. To the best of our knowledge, the present work is the first report on application of QDs-based LB film as the transducer surface for electrochemical detection of DNA hybridization.

MATERIALS AND METHODS

All the reagents and solvents are of analytical grade and have been obtained from Sigma–Aldrich, India. Methylene blue (MB, 20 μ M) and buffer solutions have been prepared in deionized water (Millipore, 18.0 M Ω ·cm) and are autoclaved prior to being used. CML-specific probe oligonucleotide sequence (22 bases) identified from the *bcr–abl* gene, complementary, noncomplementary, and one-base mismatch target sequences have been procured from Sigma–Aldrich, Milwaukee, WI. This probe corresponds specifically to the P210 kDa *bcr–abl* tyrosine kinase fusion product obtained from the reciprocal translocation of 9;22 (Philadelphia chromosome). DNA sequences used for the studies are as follows:

probe DNA	thiol-5'-TGTCCACAGCATT- CCGCTGACC-3' (pDNA)
complementary	5'-GGTCAGCGGAATGCTGT- GGACA-3'
one-base mismatch	5'-GCTCAGCGGAATGCTG- TGGACA-3'
noncomplementary	5'-TACTCGCAATAACGTG- ATCTCC-3'

All oligonucleotide solutions have been prepared in TE buffer (1 M Tris-HCl and 0.5 M EDTA, pH 8.0) and stored at –20 °C.

Processing of Clinical Patient Samples. Extraction of RNA from the blood samples of CML-positive and -negative patients has been accomplished by use of QIAamp RNA blood

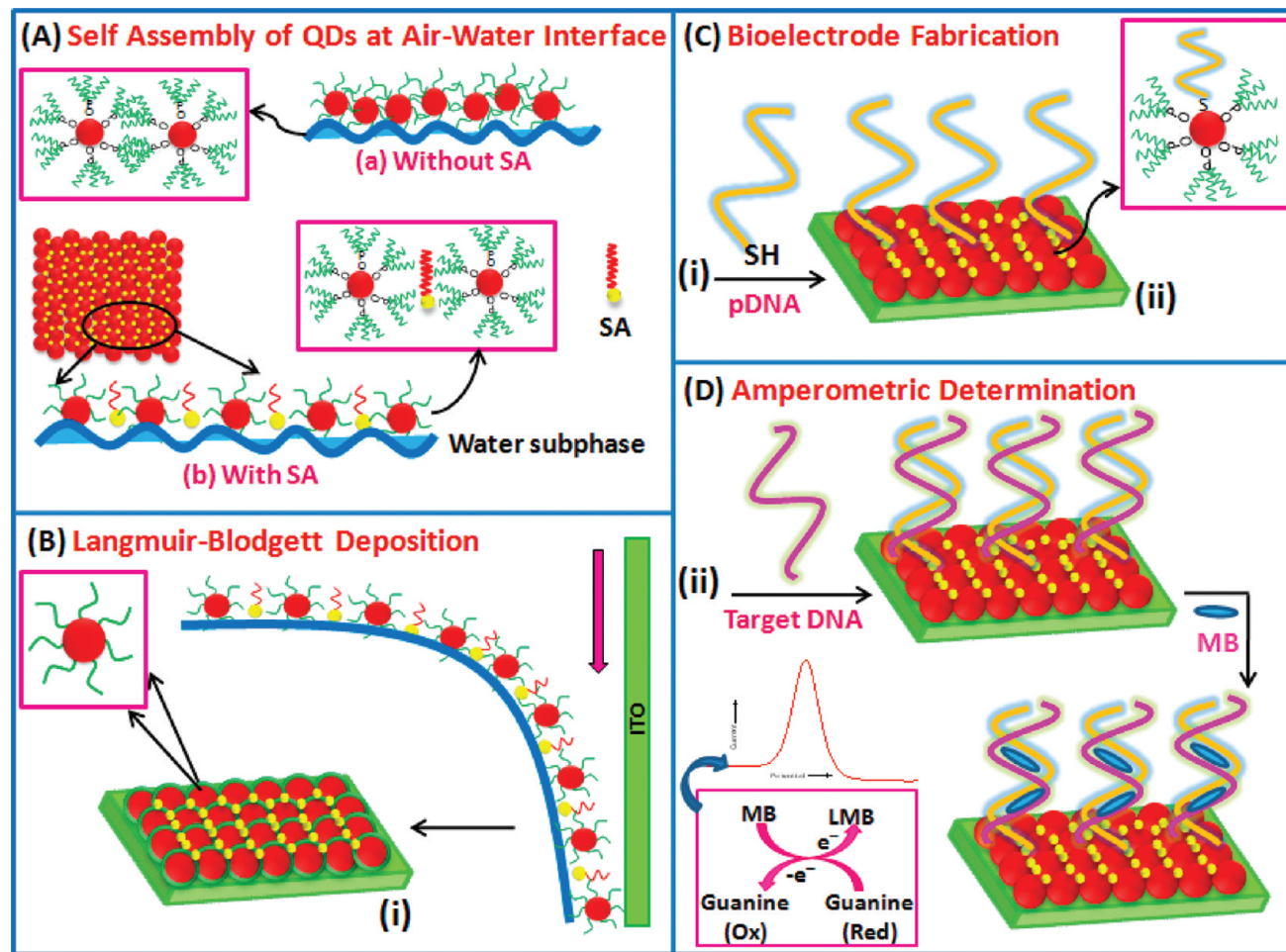
mini-kit (Qiagen). Leukocytes separated from lysed blood by selective erythrocyte lysis are subjected to repetitive homogenization and washing. The eluted RNA is reverse-transcribed to complementary DNA (cDNA) by use of a high-capacity cDNA reverse transcription kit (Applied Biosystems). For this, reverse transcription–polymerase chain reaction (RT-PCR) master mix is added to RNA and the mixture is placed in a thermal cycler, and optimized conditions programmed for the kit are used. The extracted cDNA is diluted 10 times in TE buffer and heated in a water bath (95 °C) for about 5 min, followed by chilling in an ice bath to obtain denatured single-stranded DNA. These aliquots of samples are subjected to sonication at 24 kHz frequency for 15 min using ultrasonic cleaner (Vibronics Pvt. Ltd., Mumbai, India) to break the long DNA strands into smaller fragments.²⁰

Characterization. Ultraviolet–visible (UV–vis) absorption studies have been carried out on a UV–vis spectrophotometer (Phoenix 2200DPCV). The fluorescence spectra have been collected on a luminescence spectrometer (Edinburgh Instruments, F900) equipped with a xenon lamp. Monolayer depositions have been conducted in an LB trough (NIMA 601A). Fourier transform infrared (FTIR) measurements have been conducted in attenuated total reflection (ATR) mode, with germanium waveguide, on a Perkin–Elmer Spectrum BX II spectrophotometer. The FTIR signal is obtained by averaging 64 scans at the resolution of 4 cm^{–1}. Contact angle (CA) measurements have been taken on contact angle meter (Data Physics OCA15EC). The morphological characterization has been carried out on a field emission transmission electron microscope (TEM; JEOL JEM-2100F) at an accelerating voltage of 200 keV and on a scanning electron microscope (SEM, LEO 440). Electrochemical characterization has been conducted on an Autolab potentiostat/galvanostat (Eco Chemie, Netherlands) using a three-electrode system with ITO as the working electrode, Ag/AgCl as reference electrode, and platinum foil as counter electrode, in phosphate-buffer saline (PBS; 50 mM, pH 7.4, and 0.9% NaCl).

Preparation of Tri-*n*-octylphosphine Oxide (TOPO) Capped CdSe Quantum Dots. The QDs have been synthesized via a modified procedure reported in literature²¹ and previously used by our group for the synthesis of core CdSe nanocrystals.²² Typically, a 0.1 mmol portion of selenium is dissolved in 2 mL of tri-*n*-octylphosphine (TOP) to obtain TOPSe solution. A mixture of 0.1 mmol portion of cadmium oxide and 0.4 mmol of oleic acid is added to 5 mL of 1-octadecene and heated to 250 °C with continuous stirring under an argon atmosphere until a colorless solution is obtained. The temperature is lowered to 50 °C, followed by addition of 2 g of octadecylamine and 1.5 g of TOPO to the solution, which is then heated to 280 °C under argon atmosphere. TOPSe solution is injected swiftly to the Cd solution and the reaction is allowed to proceed for about 15 min at 270 °C. The solution is cooled and washed repeatedly with a mixture of hexane/methanol (1:1 v/v), allowing separation of the QDs and the unreacted precursor. Finally, the QDs are precipitated by addition of ethanol to the hexane layer and redissolved in chloroform to obtain the concentration of 2 mg·mL^{–1}. The average size of QCDSe, investigated by λ_{max} value (584 nm) of the UV absorption spectrum (Supporting Information, Figure S1), is found to be 4 nm.²³ If the particles are assumed to be spherical, the mean molecular weight of these QDs is estimated to be 151 494 Da that includes the molecular weight of surrounding TOPO molecules.^{13,14}

Organization of QCDSe-SA at Air–Water Interface. Surface chemistry measurements at the air–water interface have been conducted on an LB trough having dimensions of

Scheme 1. Steps Involved in Fabrication of pDNA/QCdSe-SA-LB/ITO Bioelectrode



10 cm $\times$ 30 cm. The computer-controlled symmetrically movable barrier has been employed to regulate the surface area, and constant temperature is maintained by use of a temperature controller (Julabo F5). Deionized water is used as the monolayer subphase, and the surface pressure is measured by the immersed Wilhelmy plate. Typically, 40 μ L of QCdSe (3 mg·mL⁻¹ in CHCl₃) is vortexed with 20 μ L of SA solution (1 mg·mL⁻¹ in CHCl₃) for about 5 min, which is then gently spread onto the subphase and is left for about 30 min to allow evaporation of CHCl₃ (Scheme 1A).

Langmuir–Blodgett Deposition of QCdSe-SA Monolayers. Prior to deposition of the QCdSe-SA-LB film, the precleaned ITO substrate (0.75 cm $\times$ 1 cm) has been hydrophobized by dipping it into 0.4 g·L⁻¹ octadecyltrichlorosilane (OTS) solution in cyclohexane for about 30 min, followed by washing with cyclohexane to remove any unbound OTS molecules.¹⁴ The hydrophobic substrate is positioned perpendicular to the air–water interface, and the monolayers are subsequently deposited by vertical dipping (Scheme 1B) at a rate of 10 mm·min⁻¹ to obtain Y-type multilayer film.

Fabrication of pDNA/QCdSe-SA-LB/ITO Bioelectrode. The thiol-terminated probe DNA has been immobilized onto QCdSe-SA-LB/ITO electrode via Cd–thiol affinity. For this purpose, 40 μ L of pDNA (1 μ M) is spread onto the electrode surface and incubated at 25 $^{\circ}$ C to allow displacement of surface TOPO molecules with the –SH terminal of pDNA (Scheme 1C). The pDNA/QCdSe-SA-LB/ITO bioelectrode is repeatedly rinsed with TE buffer (pH 8.0) to remove any unbound pDNA on the

electrode surface. The fabricated pDNA/QCdSe-SA-LB/ITO bioelectrode has been utilized for CML detection by subjecting it to varying concentration of complementary target DNA for about 30 min, and the corresponding change in current is measured by differential pulse voltammetry (DPV) with MB as a redox indicator (Scheme 1D).

RESULTS AND DISCUSSION

Surface Measurements of QCdSe-SA Langmuir Monolayer. The pressure–area (π –A) isotherm of QCdSe-SA Langmuir monolayer has been recorded with varying concentrations of SA molecules (Supporting Information, Figure S2). In the absence of SA, development of QCdSe domains via aggregation is observed at the spreading stage that shrinks as the Langmuir monolayer is compressed. It is well-known that lesser particle–subphase (water) interaction results in poor spreading ability; apparently, the cohesive interaction between QCdSe is higher than the spreading force exerted by the subphase. Incorporation of SA molecules in QCdSe not only leads to reduction of the aggregate formation and improvement of its spreading behavior²⁴ but also prevents interdigitation of hydrophobic tails of TOPO molecules, thus acting as a spacer. The amount and concentration of SA has been optimized until QCdSe forms a stable monolayer at the air–water interface.

Figure 1A shows the π –A isotherm of QCdSe-SA Langmuir monolayer at the air–water interface obtained within the

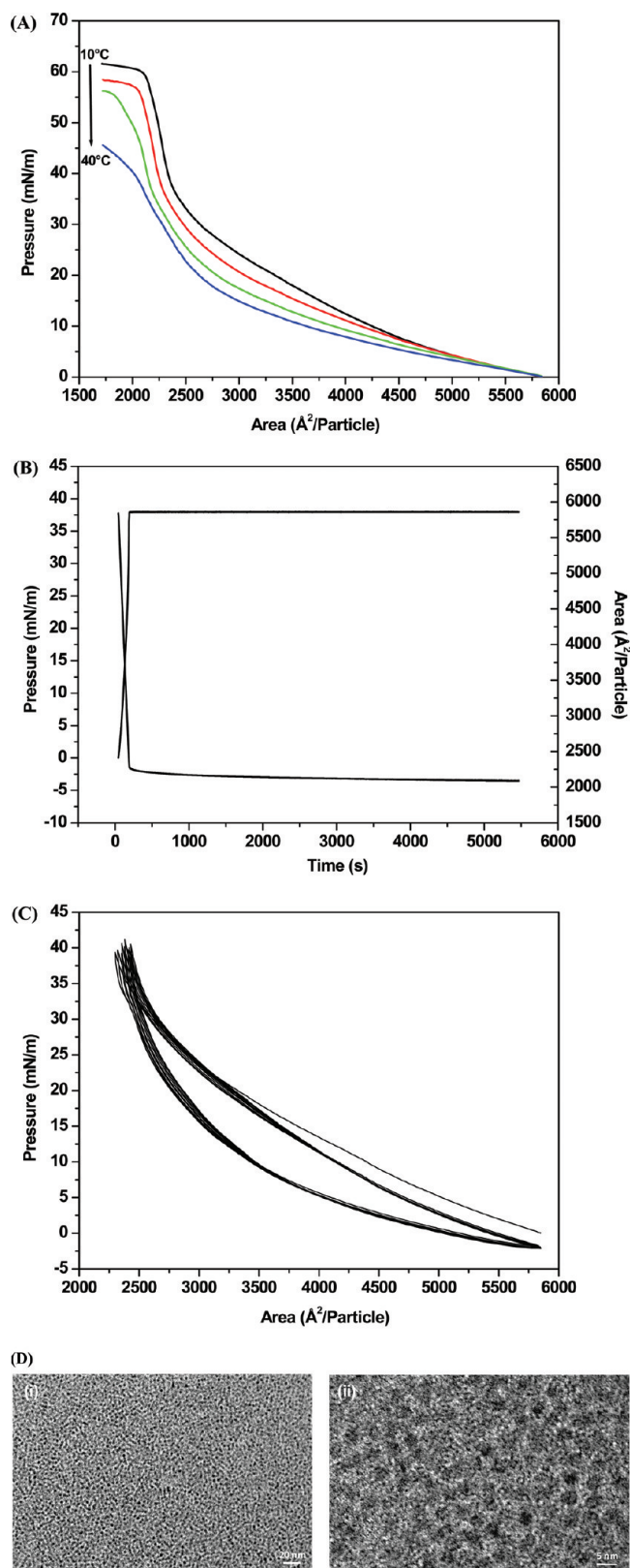


Figure 1. (A) Surface pressure–area isotherm of QCdSe-SA Langmuir monolayer at varying subphase temperatures (10–40 °C). (B) Kinetic measurements showing variation in the particulate surface area at the air–water interface as a function of time at a constant surface pressure of 38 mN·m^{−1}. (C) Multiple compression/expansion cycles of the Langmuir monolayer at a surface pressure of 38 mN·m^{−1}. (D) (i) TEM and (ii) HRTEM images showing LB deposition of film at target surface pressure.

temperature range from 10 to 40 °C. It has been observed that at each temperature the shape of π – A isotherm is typical of coexistence of the two-dimensional gas and liquid-expanded (LE) phases in the lower surface pressure range, and of the LE and liquid-condensed (LC) states thereafter (according to Harkins's classification).²⁵ However, as the subphase temperature is lowered to 20 °C (and below), π – A isotherm indicates a sharp kink (corresponding to LE–LC phase) followed by a pronounced plateau at a critical surface pressure (π_c) of 58 mN·m^{−1}, corresponding to collapse of the QCdSe-SA Langmuir monolayer. At 20 °C, the limiting area occupied by QCdSe-SA Langmuir monolayer is about 2600 Å²/molecule, which is obtained by extrapolating the linear region of the isotherm to zero surface pressure.¹⁰ With increasing temperature, enhanced flexibility of hydrocarbon chains of the TOPO molecule is expected, as a result of which a more compact arrangement is obtained, leading to reduced limiting area of the particulates at the interface.

Stability of QCdSe-SA Langmuir Monolayer. Stability of the QCdSe-SA monolayer at the air–water interface has been examined by two different methods, kinetic measurements and compression/expansion cycling. While the temperature is kept constant (20 °C), Langmuir monolayer is compressed to different target surface pressures, ranging from LE to LC phase, revealing that the QCdSe-SA monolayer can withstand a maximum of 38 mN·m^{−1} pressure. At this pressure, area per QD decreases by about 144 Å² (to 2106 from 2250 Å²/QD) over a duration of about 90 min (Figure 1B) that corresponds to <7% of the limiting QD area of the Langmuir monolayer. The apparent slight decrease in the QD surface area can be attributed to the rearrangement of particles that reduces the empty space in the monolayer or a partial aggregation of the QCdSe at a surface pressure of 38 mN·m^{−1}.

The hysteresis curves shown in Figure 1C demonstrate the compression/expansion behavior of QCdSe-SA monolayer at the air–water interface. The decrease of about 300 Å² in the limiting nanoparticle area is observed after the first compression/expansion cycle, whereas nearly identical loops are obtained for nine successive cycles. This reveals that the QDs perhaps respread to initial state prior to each succeeding compression, indicating a long-term stability of Langmuir film at the air–water interface.

Particulate Arrangement Exploration in Langmuir Film. In order to gain better insight into the QCdSe-SA arrangement onto the subphase, the Langmuir monolayer is transferred to a 200-mesh carbon-coated Cu grid under optimized experimental conditions (20 °C, 38 mN·m^{−1}). The transmission electron microscopic (TEM) analysis indicates uniformly distributed particulates revealing that QCdSe-SA spreads homogeneously onto the subphase without formation of domains due to aggregation (Figure 1D, panel i), which is further confirmed by high-resolution transmission electron microscopy (HRTEM; Figure 1D, panel ii). Additionally, the TEM and HRTEM studies reveal that the ordered arrangement of QCdSe-SA at the air–water interface is preserved at the solid substrate.

Transfer Characteristics of QCdSe-SA-LB Film. Once the monolayer stability and deposition pressure are determined, multilayered (up to eight monolayers) thin films of QCdSe-SA are deposited onto ITO-coated glass substrates by the Langmuir–Blodgett technique. The single monolayer of crystallite is transferred onto the hydrophobic substrate in each upward and downward stroke. Transfer onto the substrate,

as estimated from the change in surface area at the air–water interface, is nearly the same for each monolayer except for the first two monolayers (Figure 2A). This may be assigned either

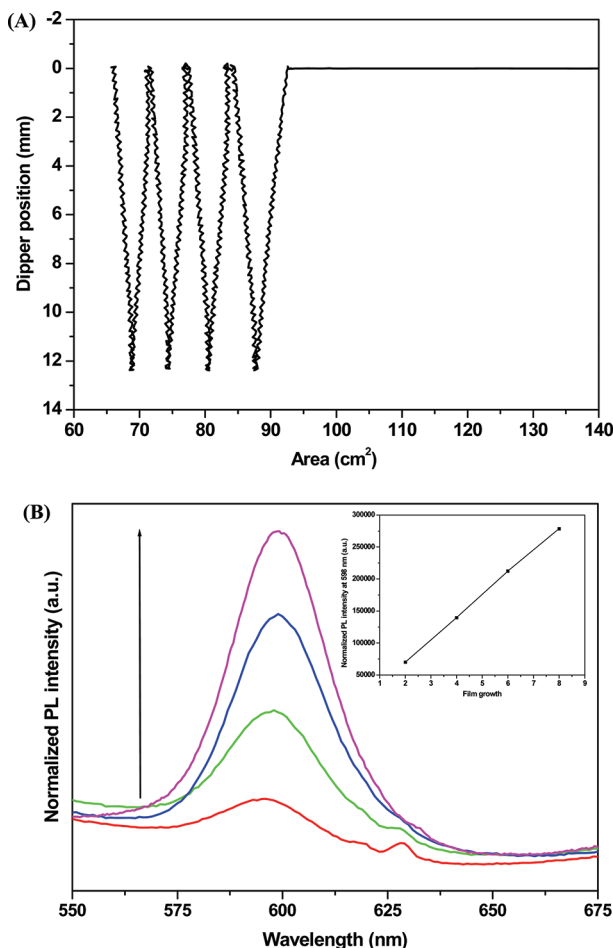


Figure 2. (A) Deposition curves of QCdSe-SA-LB monolayer onto ITO substrate. (B) Fluorescence spectra of the QCdSe-SA-LB film, deposited onto ITO substrate, as a function of the number of layers. The arrow indicates the increasing number of layers (from 2 to 8). (Inset) Fluorescence emission intensity at 598 nm as a function of the number of layers deposited.

to high surface roughness of OTS-functionalized ITO substrate or to large hydrophobic octadecyl chains that present better interaction with the TOPO chains compared to smaller octyl chains present on the surface of subsequent layers. After each bilayer is transferred, the substrate is air-dried for 600 s and is repositioned in the subphase to allow another bilayer to transfer on top of the previous one. It may be noted that we have studied only the bilayer system, as after each odd-numbered monolayer transfer the carboxylic group of the SA is expected to be pointed upward, which is undesired. The carboxylic moieties if present on the surface may interact with the amino groups of nucleoside bases, thus leading to nonspecific binding of the pDNA.

The deposited QCdSe-SA-LB film has been analyzed via fluorescence spectroscopy. The ITO substrates containing multilayered thin film of various deposition layers are subjected to excitation at 300 nm, and the corresponding PL intensity is measured (Figure 2B). The PL intensity at 598 nm is found to increase linearly with the number of QCdSe-SA-LB bilayers

(Figure 2B, inset), demonstrating a uniform and lamellar film deposition up to eight monolayers.

Characterization of pDNA/QCdSe-SA-LB Bioelectrode.

If the fact that thiols have a higher affinity for CdSe quantum dots is kept in view, displacement of hydrophobic TOPO moieties with a –SH terminated DNA is expected, resulting in covalent immobilization of probe molecules onto the electrode surface. Also, with the progress of immobilization process, the surface becomes more hydrophilic, owing to presence of the phosphodiester backbone of pDNA, which is indicated by the variation in CA of water at different time intervals (Supporting Information, Figure S3). Initially, the value of CA for QCdSe-SA-LB/ITO electrode is found to be 113.7° (Figure S3, inset i), reflecting the presence of highly hydrophobic TOPO capping ligands on the surface. The CA value subsequently decreases with time and becomes constant at 76.6° after about 16 h (Figure S3, inset ii), revealing that complete immobilization of probe occurs in this duration.

The FTIR spectrum of pDNA/QCdSe-SA-LB/ITO bioelectrode has been recorded in ATR mode and compared with that of QCdSe-SA-LB/ITO electrode to confirm the immobilization of probe. The FTIR spectrum of QCdSe-SA-LB/ITO electrode (Figure 3A, panel i) displays well-defined peaks at 2922 and 2854 cm^{-1} that can be assigned to C–H stretching and less intense peaks at 1458 and 1084 cm^{-1} corresponding to CH_2 bending and $\text{P}=\text{O}$ stretching vibration modes, respectively, in the TOPO moiety.^{26,27} Further, a low-intensity peak at about 1706 cm^{-1} , corresponding to carbonyl stretching vibrational mode of SA molecules, has been observed that appears at about 1720 cm^{-1} after immobilization of pDNA (Figure 3A, panel ii). The additional peaks seen in the FTIR spectrum of bioelectrode at 1600 and 1018 cm^{-1} , corresponding to N–H bending and deoxyribose sugar, respectively, and enhanced intensity of the peak at about 1091 cm^{-1} , assigned to $\text{P}=\text{O}$ stretching vibration, can be correlated with successful immobilization of the probe onto the QCdSe-SA-LB/ITO electrode surface.^{6,28,29}

Morphological studies of the bioelectrode have been carried out to investigate packing of the probe molecules onto the electrode surface, and the results of SEM studies are displayed in Figure 3B. A regularly arranged globular structure in the micrometer scale (Figure 3B, panel i) indicates that the ordered arrangement of quantum dots onto the transducer surface provides a conducive environment for immobilization of pDNA in an oriented manner. Furthermore, a close view of the bioelectrode surface (Figure 3B, panel ii) reveals the existence of isolated globules that exclude the probability of steric hindrance to target oligonucleotide and hence play an important role in determining the performance of this nucleic acid sensor.³⁰

Electrochemical Response Studies of the Bioelectrode.

Electrochemical response of the fabricated pDNA/QCdSe-SA-LB/ITO bioelectrode has been investigated via DPV technique with MB as the redox hybridization indicator (Figure 4A). MB undergoes reduction by oxidizing the unpaired nucleoside bases of single-stranded probe DNA, thereby providing a means to quantify the extent of hybridization.³¹ On incubation with the complementary DNA, the MB peak current is found to decrease significantly (curve iv, 0.18 μA) with respect to that of the pDNA/QCdSe-SA-LB/ITO bioelectrode (curve i, 0.45 μA). This may be attributed to the fact that interaction between the MB molecules and nucleoside residues of the probe is prevented by hybrid

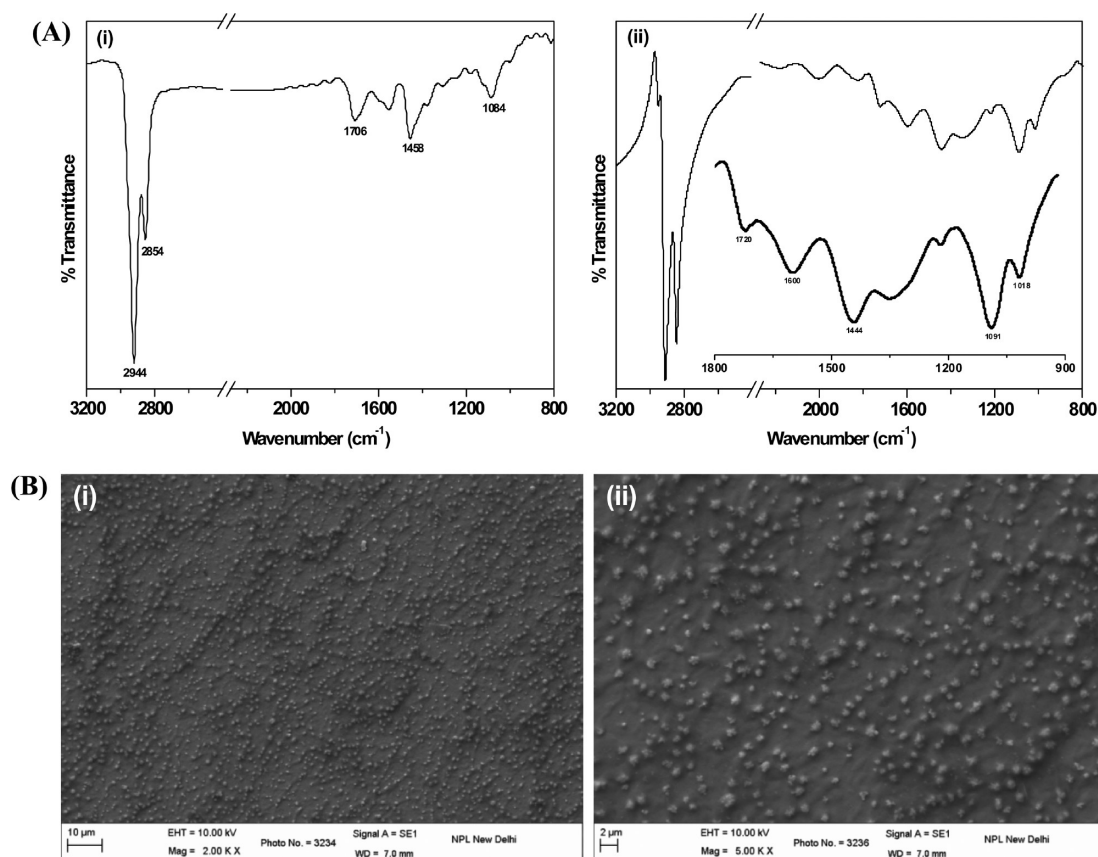


Figure 3. (A) FTIR spectra of (i) QCdSe-SA-LB/ITO electrode and (ii) pDNA/QCdSe-SA-LB/ITO bioelectrode. (B) SEM images of pDNA/QCdSe-SA-LB/ITO bioelectrode at (i) low magnification (2000×) and (ii) high magnification (5000×).

formation on the electrode surface.^{32,33} In other words, after hybridization, the signal from the MB is suppressed, resulting in a much decreased signal compared with that from direct interaction with the nucleoside bases of single-stranded pDNA. However, slight enhancement of the MB peak current (curve ii, 0.49 μA, with SD across four repetitive experiments 4%) has been observed upon incubation of the bioelectrode with non-complementary DNA that may be attributed to its nonspecific adsorption onto the bioelectrode surface. For the DNA sequence having a one-base mismatch, a significant decrease in MB reduction peak (curve iii, 0.29 μA, with SD across four repetitive experiments 4%), as compared to that of the non-complementary DNA, indicates that the fabricated DNA sensor can discriminate even a single nucleotide variation in target DNA sequence.

Figure 4B exhibits a typical DPV response of pDNA/QCdSe-SA-LB/ITO bioelectrode with varying concentration of complementary DNA in the presence of MB. It has been found that incubation time of 120 s is sufficient for interaction of the MB molecules with probe DNA. The experimental results indicate that the current signal, pertaining to the reduction of MB, is minimum at 10⁻⁶ M complementary target concentration, revealing that all the immobilized probes on surface participate in the hybridization process at this concentration. Furthermore, the observed increase of DPV current obtained by varying the concentration of complementary DNA (1.0 μM to 10 fM) reveals detection limit of 10 fM, indicating that this sensor can be used to detect as low as ~115 × 10³ DNA molecules in a 40 μL sample.³⁴ However, a linear relationship between the peak current density and the target concentration on logarithmic

scale is obtained in the range from 10⁻⁸ to 10⁻¹⁴ M (Figure 4B, inset) and follows eq 1:

$$I_{\text{pDNA/QCdSe-SA-LB/ITO}} = (1.1671 \times 10^{-7}) - (2.1714 \times 10^{-8}) [\log (\text{target DNA concentration})] \quad (1)$$

with a standard deviation of 7.2859 nA and correlation coefficient of -0.9901.

A control experiment has been performed by immobilizing the pDNA onto (3-glycidoxypyril)trimethoxysilane-modified ITO substrate, which is found to detect up to 0.1 pM target DNA in the absence of QCdSe (Supporting Information, Figure S4), that is, 3 orders of magnitude lower than that of pDNA/QCdSe-SA-LB/ITO bioelectrode.

Reusability of the Bioelectrode. It has been found that the pDNA/QCdSe-SA-LB/ITO sensing surface can be easily regenerated by immersing it into buffer solution (pH 8.0) containing Tris-HCl (10 mM) and EDTA (1 mM) at 100 °C for 5 min, followed by cooling in ice bath for about 30 min, which completely removes hybridized DNA via thermal denaturation.²⁰ The regeneration of the bioelectrode, followed by hybridization process, has been performed for several consecutive cycles and the corresponding variation of MB peak current is recorded. The MB peak current value of pDNA/QCdSe-SA-LB/ITO bioelectrode (0.45 μA) has been found to decrease with each regeneration process and decreases to 0.38 μA after eight regeneration cycles. The results obtained clearly indicate that the pDNA/QCdSe-SA-LB/ITO bioelectrode

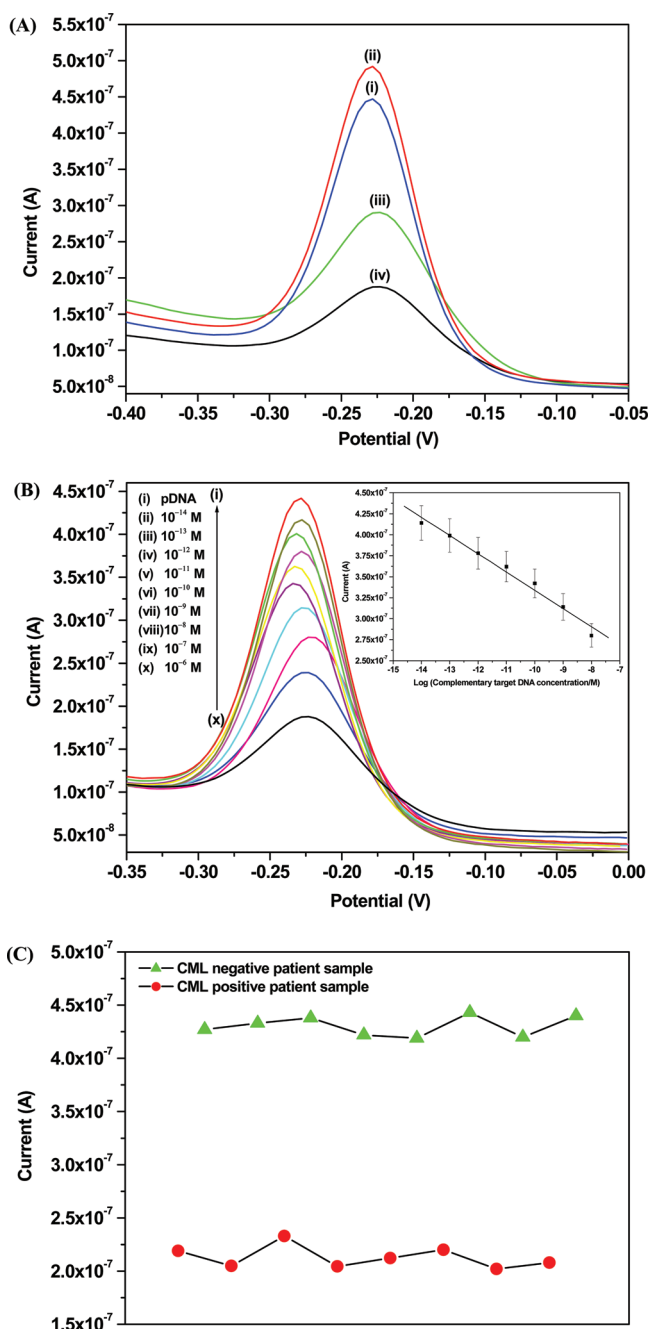


Figure 4. (A) Differential pulse voltammetric curves of (i) pDNA/QCdSe-SA-LB/ITO bioelectrode with (ii) non-complementary, (iii) one-base mismatch, and (iv) complementary target DNA sequence in PBS solution (50 mM, pH 7.4, and 0.9% NaCl) containing 20 μ M MB. (B) Sensing characteristics of pDNA/QCdSe-SA-LB/ITO bioelectrode as a function of target DNA concentration in PBS solution (50 mM, pH 7.4, and 0.9% NaCl) with MB (20 μ M) as a hybridization indicator. (Inset) Linearity plot for the variation in MB peak height with respect to target DNA concentration. (C) Plot showing DPV response of the pDNA/QCdSe-SA-LB/ITO bioelectrode with respect to CML-positive and -negative patient samples.

retains $\sim 85\%$ of original performance after eight regeneration cycles (Supporting Information, Figure S5). The slight decrease in the observed MB peak current for the pDNA/QCdSe-SA-LB/ITO bioelectrode after each regeneration cycle may perhaps be due to partial loss of pDNA from the bioelectrode surface during the process.^{35,36}

Detection of Target from Clinical Patient Samples.

The specificity of the sensor for cDNA samples has been studied by recording the change in the electrochemical signal after incubation of the bioelectrode with eight different samples of CML-positive and -negative patients. The sensor shows no response to CML-negative patient samples, as indicated by negligible change in the MB peak current of pDNA/QCdSe-SA-LB/ITO bioelectrode. However, with CML-positive patient samples, a significant decrease in MB peak current is obtained (Figure 4C). The fabricated nucleic acid sensor presents excellent scope for discrimination of the positive real samples from negative real samples.

CONCLUSIONS AND OUTLOOK

We have devised a novel high-performance electrochemical DNA sensor that relies on the ordered CdSe quantum dots array as the transducer surface. The covalently immobilized thiolated capture probe has been found to specifically hybridize with its complementary DNA, allowing the biosensor to readily detect target oligonucleotide concentration as low as 10 fM with a response time of 120 s. The selectivity of the biosensor has been demonstrated by discrimination of single-nucleotide mismatch and non-complementary DNA sequence. The analysis performed with clinical patient samples reveals its suitability for the monitoring of minimal residual disease in CML patients. This ultrathin film-based nucleic acid sensor is adaptable to a wide variety of nucleic acid detection applications and may have implications in point-of-care diagnostics.

ASSOCIATED CONTENT

Supporting Information

Five figures showing normalized absorption spectra of QCdSe in chloroform; pressure–area isotherm of QCdSe Langmuir monolayer; CA measurement of QCdSe-SA-LB/ITO electrode as a function of time; and variation in MB peak current as a function of log [DNA] and with repeated cycling. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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Notes

The authors declare no competing financial interest.

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