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PVA-based nanobiosensor for ultrasensitive detection of folic acid by fluorescence quenching

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ABSTRACT

The present work demonstrates *in vitro* folic acid sensing with a polyvinyl alcohol (PVA) based hybrid hydrogel as an efficient and cost effective fluorescence quenching based sensor. This new sensor PVA-tryptophan-CdTe QDs (PTQ), exhibited better sensing efficiency with an excellent limit of detection (0.57 pg/ml) compared to commercially available ELISA kits. The excellent sensitivity was attributed to a combination of a strong Photoinduced Electron Transfer process and an Inner Filter Effect in the sensor-folic acid interaction. The real time sensing applications of the sensor was investigated for folic acid present in the blood serum samples of healthy mice and human; and cancer infected mice and human. Our sensor exhibited efficient sensing for folic acid in the blood serum samples of acute myeloid leukemia [limit of detection (LOD) 42.29 ng/ml] and ovarian cancer effected patients (LOD 365 ng/ml). The LOD value indicates that our sensor is highly efficient toward sensing of FA in acute myeloid leukemia as its LOD value lies below 110 ng/ml. Such works will help to bring together material chemists, biologists and clinicians in a single platform to develop cost effective, photostable and specific assays for diagnostic purposes.

1. Introduction

Folic acid (FA) is the synthetic form of the naturally occurring folate, a member of Vitamin B family. It is a water soluble compound which is present in the substrates and coenzymes involved in the acquisition, transport and enzymatic processing of one carbon unit for aminoacid and nucleic acid metabolism [1]. Some previous studies suggest that FA together with vitamin B_{12} can participate in the synthesis of DNA and RNA [2]. In addition, it plays pivotal role in copying RNA from DNA. Thus, it is essential for accurate replication of DNA [3]. It is well known that FA concentration varies significantly in a number of health disorders like in cancer, cardiovascular disease, alzheimer's disease, depression, reduced cognition and neural tube defect (NTD) [4–7]. Low or high level concentrations of FA in blood serum, plasma and red blood cells are exploited as an efficient biomarker for diagnosis of various disease conditions.

Since FA is essential for proper functioning of numerous biological functions in human, it is pertinent to develop simple and sensitive methods for detection of FA in biological systems. In this regard, researchers round the globe have developed different kinds of sensor arrays viz. electrochemical sensors, ELISA etc. ELISA kit, which is widely used immunoassay technique with many advantages like high specificity and high sensitivity [8]. But such kits have certain disadvantages like long reaction time, effect of temperature, unstable upon exposure to light etc. [8]. However, in recent years, fluorescence based assays have emerged as an attractive alternative choice for such purposes. In this regard, fluorescence based assays have their own advantages and disadvantages. The main advantages are ability of signal mulitiplication and amplification, low cost and fast response time while disadvantages are less specificity, temperature effect, ionic concentration effect, light scattering etc. [9]. Keeping these in mind, an effort has been made toward the design of an efficient fluorescence based assay for FA sensing. There also exist few reports where fluorescence quenching based processes has been employed for FA sensing [10–17]. Hu et al. reported the fabrication of graphene oxide and Ag nanoparticles for the detection of FA, Yan et al. reported the use of gold nanoclusters for FA and Zhang et al. reported the application of polyethylenenimine-capped silver nanoclusters for FA [14–16]. However, most of the reported works suffer from many drawbacks like high fabrication cost, expensive precursors, devoid of long term stability, poor detection limit and poor selectivity. Taking these and the cost effectiveness of the sensor into consideration, we have selected a cheap and readily available precursor

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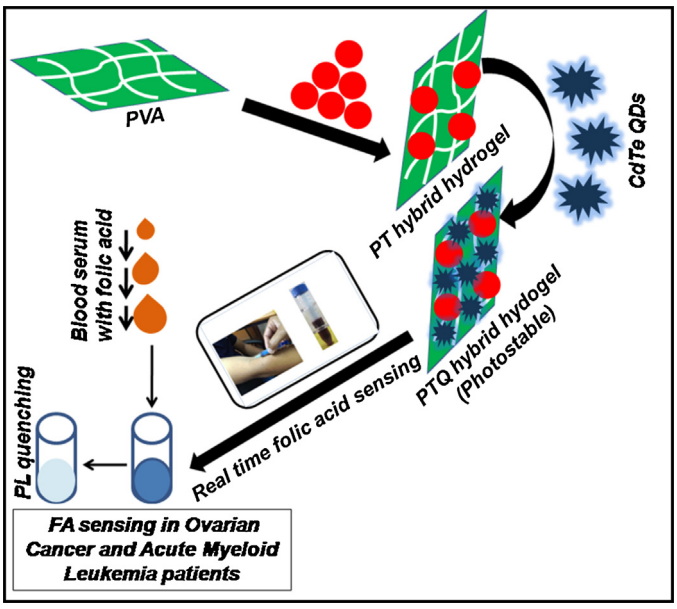

Fig. 1. Schematic representation of the biosensing process.

polyvinyl alcohol (PVA) as a template for the design of a hybrid sensor. PVA is a synthetic environmentally benign polymer having unique properties for such applications. It is highly hydrophilic with long term thermal and pH stability [18]. It also possesses excellent thin film forming capability. In addition, it has the potential to be blended with various materials like nanomaterials, peptides, aminoacids etc. for use as a biosensor. In this regard, aminoacids having intrinsic fluorescence like tryptophan presents a very good choice that can be blended with PVA. The aminoacid tryptophan is widely used as a tool in the studies of protein structure, conformational changes and functions owing to its spectral properties. Its indole chromophore depicts high sensitivity to the interactions and changes of the environment [19–21]. But, for long term applications of a hybrid biosensor, photostability emerges as a key concern. In order to address this issue, researchers have tried to incorporate quantum dots (QDs) into polymeric substrates. To date, only few reports are available which emphasizes interaction of QDs with FA [22–24]. In the present work, we have reported the synthesis of two hybrid hydrogels i.e. PVA-tryptophan (PT) and PVA-tryptophan-CdTe QDs (PTQ). A comparative study of the PTQ hybrid hydrogel with PT hybrid hydrogel for FA has been carried out.

In a nutshell, we have reported a novel, simple, selective and rapid sensor system based folic acid detection pro-kit, which will be helpful in fast and efficient quantitative estimation of folic acid in blood serum with a detection limit up to 42.29 ng/ml.

The schematic representation for the process is depicted below (Fig. 1).

2. Experimental

2.1. Preparation of sensing materials

All the chemicals and solvents used in the process were of analytical grade and used without further purification. For the analysis, Milli Q water was used as a solvent.

2.1.1. Synthesis of green hybrid hydrogel PVA-tryptophan (PT)

PT hydrogel was prepared using a simple protocol-PVA (1.742 g) was weighted into a beaker and dissolved in Milli Q water (50 ml) with constant stirring at room temperature for half an hour followed by heating the solution using a heating mantle to 80 °C for another half an hour. Thereafter, half of the solution was disposed of and replaced with 25 ml tryptophan (0.0001 M). The reaction mixture was then stirred for one hour with constant heating at 80 °C. The mixture so obtained was cooled to 40 °C with stirring and poured into a PE plate containing a quartz slide. It was left for 3 days to allow the film to form. The film was removed from the plate and cut to obtain the quartz slide with thin PT film on the surface. The above procedure was repeated for 0.0005 M tryptophan solution. The synthetic route of PT hydrogel is provided in the Supplementary Information (Fig. S1, Supplementary Information).

2.1.2. Synthesis of quantum dots (CdTe-MSA QDs) doped hybrid hydrogel: PVA-tryptophan quantum dots (PTQ)

Herein, preformed CdTe QDs co-stabilized by mercaptosuccinic acid (MSA) [25] was incorporated into the PVA-tryptophan matrix. The detailed synthesis is given in Supplementary Information. The synthetic scheme of the process is depicted below (Fig. 2).

2.2. Characterization of sensing materials and CdTe QDs

The sensing materials (PT and PTQ) were extensively characterized using Field Emission Scanning Electron Microscopy (FESEM) (SIGMA-VP (ZEISS)), Fourier Transform Infrared Spectrometer (FTIR) with an ATR (Attenuated Total Reflectance) attachment (Nicolet 6700, Thermo Fischer), Thermogravimetric Analysis (TGA) (Perkin Elmer, TGA 4000), Differential Scanning Calorimetry (DSC) (Perkin Elmer DSC 6000) and Gel Permeation Chromatogram (GPC) (WATERS 2414).

The formation of CdTe QDs was confirmed by Transmission Electron Microscopy (TEM). The sample preparation was done by dispersing few drops of QDs sample into a 3 mm copper grid covered with a continuous carbon film and dried at room temperature. Subsequently, TEM characterization was performed on a JEOL JEM 2100 transmission electron microscope operating at 200 kV.

2.3. Optical measurements

2.3.1. Photoluminescence study (PL)

Fluorescence spectra were recorded in VARIAN CARY Eclipse with an excitation and emission slit width of 5 nm. Predefined quantities of the hydrogels (0.071 g) were weighted properly and dissolved in a 20 ml water and the solutions were then subjected to ultra high sonication. The dispersed solutions so obtained were used for analysis with proper dilution. A 20 ml stock solution of FA of 2.27×10^{-6} M concentration was prepared. Aliquot from the stock solution was added in a sequential manner (40 μl–525 μl) to the hydrogel solution (2 ml) for sensing studies.

For real time applications of our sensor PTQ for FA, ground commercial FA tablets were weighted and dissolved in Tris-HCl buffer (pH 8). The solution was filtered, washed repeatedly and diluted to a constant volume for the next dual functional detection. For the analysis, a reported protocol was adopted [15]. The initiation of experiment was done with the collection of drug free mice blood serum samples (A). The blood samples were centrifuged at 10,000 rpm for 10 min after standing for 2 h at room temperature. Due to high protein binding in the blood plasma, some pre-treatments were done to eliminate the interferences and then 1.5 ml acetonitrile was added into 1 ml serum sample. The mixture was centrifuged at 10,000 rpm for 10 min at 4 °C after vigorously shaking for 15 min. The obtained supernatant was adjusted to pH 8, and then diluted by 100 times with deionized water.

Similarly, drug treated mice serum samples (B) and drug free human serum samples (C) were collected and similar procedure was adopted for making the desired solutions. The blood serum samples of two cancer infected patients (acute myeloid leukemia and ovarian cancer) (D) were also collected and the same procedure