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'Turn-on' fluorescence assay for inorganic phosphate sensing

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ABSTRACT

Phosphorus (in the form of phosphate) is the second most abundant mineral in the human body and is essential for normal body activities. Abnormal concentrations of phosphate can cause serious disorders and may be fatal if not treated. One of the drawback of current phosphate sensing method is its lack of sensitivity and specificity. This issue can be overcome by using photoinduced electron transfer (PET) based fluorescent on/off sensors. Semiconductor quantum dots (QDs) are used for the purpose of phosphate sensing because of their excellent size tunable optical properties (fluorescence and absorbance). Here we report the synthesis and characterization of thiol capped cadmium telluride (CdTe) QDs and their use in 'turn on' assay for inorganic phosphate sensing. The assay utilizes europium nitrate which forms complex with CdTe QDs (QD-Eu complex) and quenches fluorescence of QDs through photoinduced electron transfer (PET) process. Addition of phosphate solution results in recovery of fluorescence intensity of TGA capped CdTe QDs. Fluorescence recovery was quantified in terms of the concentration of inorganic phosphate. The fluorescence intensity versus Pi concentration curve fits to the sigmoid shape after optimization for the desired range of phosphate concentration. This 'turn on' sensing assay method is reliable, repeatable and might be useful for determination of physiological inorganic phosphate level.

1. Introduction

Phosphate sensing is essential as it play many important roles in human physiology. Phosphate in free ionic form i.e. inorganic phosphate, is the second most abundant mineral present in human body (after calcium) [1,2]. Phosphate is necessary for bone mineralization, energy storage in the form of ATP, muscle fatigue, acid–base balance through its buffer action, cellular signaling etc. [3–12]. In cells and tissues, phosphorus exists in organic forms such as sugar phosphate, phosphor-proteins, phospholipids and nucleic acids [13,14]. While in body fluids, it is present in inorganic (Pi) form with the ratio $H_2PO_4^-/HPO_4^{2-}$ being 1:4 at physiological pH [15–17]. Pi concentration in serum depends on its absorption in intestines, reabsorption in kidney and consumption by the body [18]. The normal range of serum Pi changes from neonatal (1.88–2.4 mM) through childhood (1.45–1.8 mM) to adulthood stages (0.85–1.44 mM) [19]. Deficiency of Pi may lead to hypophosphatemia and other serious biological disorders such as bone mineralization defects resulting osteomalacia or rickets, CNS dysfunction, abnormal cell function, muscle weakness and cardiac dysfunction. On the other hand, hyperphosphatemia decreases life expectancy leading to seizures, muscle weakness, decreased visual acuity, renal failure and eventually death [15,20]. Therefore, detection and quantification of Pi concentration is important to regulate and monitor human health.

Literature reports on phosphate sensing include use of colorimetric, potentiometric ion-selective electrode, amperometric and potentiometric enzyme electrode, amperometric plant-tissue electrode and other devices in the form of integrated probe based methods [21–24]. Various sensing methods and techniques have been reviewed in literature [25]. Enzyme and chemiluminescence based detection techniques have also been reported in literature [26,27]. Although the reported techniques show efficient sensing, the interference due to presence of other anions in analyte may result in altered interpretations. This issue of sensitivity and specificity can be overcome by using photoinduced electron transfer (PET) based fluorescent sensors, whose response can be made very specific to the analyte of interest by choosing appropriate fluorophore and other intermediate moieties.

Abbreviations: QDs, quantum dots; CdTe, cadmium telluride; Pi, inorganic phosphate; CNS, central nervous system; PET, photoinduced electron transfer; TGA, thioglycolic acid; SBF, simulated body fluid; HRTEM, high-resolution transmission electron microscope; EDS, energy-dispersive X-ray spectroscopy; NaHTe, sodium hydrogen telluride; FWHM, full width at half-maximum; ANOVA, analysis of variance; FRET, Förster (fluorescence) resonance energy transfer.

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A 'turn-on' PET-based sensor consists of a luminescent species or fluorophore, which is negatively (or positively) charged moiety that can form a complex with a positively (or negatively) charged recognition group by donating (or accepting) the lone pair of the electrons. The luminescence of the fluorophore quenches due to the process of complex formation. The moiety to be sensed has to be negatively (or positively) charged in order to bind with the recognition group attached to the fluorophore. Upon binding with the moiety of interest, the lone pair of electrons (or the positive charge) in the recognition group can no longer serve as quenchers for the luminescent part of the molecule thereby regaining fluorescence. Recently, many such schemes have been developed for anion sensing [28–31] but Pi sensing using semiconductor quantum dots (QDs) PET based sensors is not reported till date. Colloidal TiO_2 nanoparticle semiconductor based 'turn-off' surface-enhanced Raman scattering sensor (SERS) has been developed and phosphate concentration was detected quantitatively [32]. Different type of semiconductor nanocrystals such as CdSe, CdSe-ZnS, CdS, CdTe are used in detection of small molecules and ions such as Cu^{2+}, Ag^{+}, Fe^{3+}, Zn^{2+}, Mn^{2+}, Ni^{2+}, Co^{2+}, I^{-}, CN^{-} [33]. Europium has been used in Pi detection by fabricating off-on fluorescent probe using carbon dots [34]. Studies have illustrated use of europium in detection of phosphate containing compound as Eu^{3+} forms complex with phosphate group and luminescence quenching or enhancement is observed [35–38]. Cadmium telluride (CdTe) QDs has been used in 'turn on' fluorescence sensing of various anions corroborated with different mechanisms [30]. Numerous luminescent species such as organic dyes are available for sensing of various analytes, however, CdTe QDs due to their excellent optical properties act as ideal fluorophore. In this work, thioglycolic acid (TGA) capped CdTe QDs are used for development of 'turn-on' PET based sensing element for detection of inorganic phosphate in aqueous solution with good repeatability and reproducibility. Europium (Eu^{3+}) is used as an intermediate species that quenches fluorescence of QDs; it has been demonstrated that addition of phosphate solution of different concentrations recovers the fluorescence. This simple approach may prove useful in detection of physiological level of Pi explicitly.

2. Method

2.1. Reagents

Cadmium chloride monohydrate ($CdCl_2{\cdot}H_2O$, 201.32 g/mol, ≥98%), sodium hydroxide (NaOH, 40 g/mol, ≥97%) and disodium phosphate (Na_2HPO_4, 141.96 g/mol, 99%) were purchased from Merck, India. Tellurium powder (Te, 127.6 g/mol, 99.8%) and europium nitrate ($Eu(NO_3)_3$, 428.06 g/mol, 99%) were purchased from Sigma–Aldrich, India. Thioglycolic acid (TGA, 92.19 g/mole, density 1.32 g/ml, >98%) was purchased from Lancaster, and sodium borohydride ($NaBH_4$, 37.83 g/mole, 98.0%) was purchased from S D Fine-Chem Ltd, India. Monosodium phosphate (NaH_2PO_4, 119.98 g/mol, 99%) was purchased from Sisco Research Laboratories Pvt. Ltd. India. Simulated body fluid (SBF) was prepared as per the procedure reported by Kokubo et al. [39]. All reactions were carried out in deionized water.

2.2. Instrumentation

Lambda 25 UV-Vis spectrophotometer was used to record the UV-Vis spectra (PerkinElmer, India). Fluorescence spectra were measured using F-2500 fluorescence spectrophotometer (Hitachi, Japan), if not mentioned specially, the samples were excited at 375 nm; excitation and emission slits were 5 nm. Size determination of QDs was performed using a high resolution transmission electron microscope (Jeol JEM-2100F, USA, accelerating voltage 200 kV), for HRTEM analysis QDs were suspended in organic solvent by phase transfer reaction [40]. EDS data was obtained on a scanning electron microscope JSM-7600F (Jeol, Japan) system. X-ray diffraction (XRD) measurements were performed on a powder diffractometer PW3040/60 (Philips). Zeta potential was measured using zeta analyzer ZetaPALS (Brookhaven Instruments Co., USA). All the experiments were performed at room temperature in triplicate. To find out the extent of agreement among the triplicates, ANOVA (Analysis of Variance) was performed. Acceptance of null hypothesis would support the equality of three means while the rejection will show at least one of the means was different (alternative hypothesis). F_{stat} and $F_{critical}$ values will be compared for the level of significance of 0.05 (p value).

2.3. Preparation of CdTe quantum dots

TGA capped CdTe QDs were synthesized in aqueous phase with certain changes in method reported by Rogach et al. [41]. Briefly, 301.98 mg of $CdCl_2{\cdot}H_2O$ was dissolved in 140 ml of water followed by addition of 209.52 μl of TGA with stirring. pH of the reaction mixture was adjusted to 11.5 by adding 2 M NaOH solution dropwise. This reaction mixture was deaerated by N_2 bubbling for 30 min.

For preparation of sodium hydrogen telluride (NaHTe), 95.7 mg Te powder and 56.74 mg $NaBH_4$ were added in 10 ml of water with stirring. The reaction mixture was heated to 70 °C and deaerated by N_2 bubbling for 45 min. This freshly prepared NaHTe was immediately added to the above-mentioned Cd solution. Reaction mixture was heated to 100 °C and refluxed for 24 h. The molar ratio of Cd:TGA:Te was kept at 1:1.5:0.5. The aliquots of reaction mixture were taken out at regular intervals. The fluorescence was observed under UV lamp and the fluorescence spectra were measured with excitation wavelength 375 nm. As synthesized CdTe QDs were washed twice using ethanol precipitation and resuspended in water, which were stored in dark for further use. The molar concentration of TGA-CdTe QDs was calculated by the absorbance value at the first absorption peak of QDs and molar extinction coefficient according to the method described by Yu et al. [42].

2.4. Turn-on sensing

2.4.1. Preparation of artificial phosphate solution

Phosphate solution was prepared considering the highest and lowest concentrations of Pi that could be present in the human plasma at any stage of life. Monosodium phosphate and disodium phosphate were added to water to prepare the desired ratio. The ratio of NaH_2PO_4 to Na_2HPO_4 was kept at 1:4 for all the dilutions.

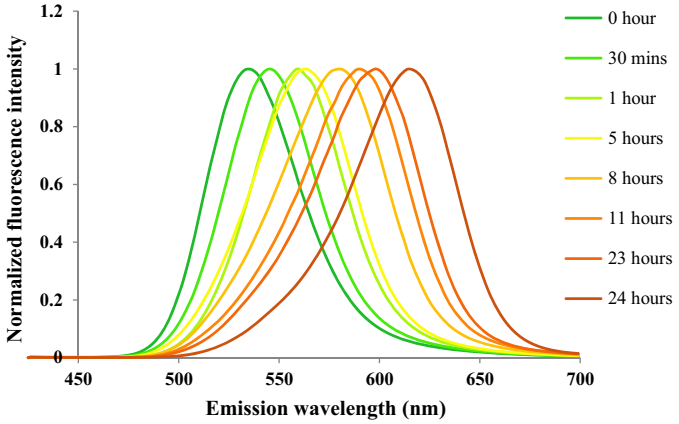

Fig. 1. Normalized fluorescence emission spectra of as synthesized TGA capped CdTe QDs.