Chapter 3

One step synthesis of AuNPs@MoS_2-QDs composite as a robust peroxidase- mimetic for instant unaided eye detection of glucose in serum, saliva and tear

3.1 Introduction

Nano enzymes have emerged as a significant tool for colorimetric detection with greater constancy and lower cost than protein enzymes due to their much higher stability, easy preparation, controllable structure, good tunability, catalytic activity and composition, [Zhang et al., 2017; Mondal et al., 2017]. Nanoenzymes have attracted considerable attention in several applications such as nanomedicine, drug delivery, bio sensing, agriculture, and food processing [Su et al., 2015; Patra et al., 2015; Zhu et al., 2013; Baranwal et al., 2016]. Up to now, various types of nanomaterials such as gold nanoparticles [Luo et al.,2010), metal oxides [Asati et al., 2009; Liu et al., 2016; Kumar et al., 2016], graphene quantum dots [Nirala et al., 2015; Chen et al., 2017; Kumar et al., 2014] etc. were exposed with peroxidase [Gao et al., 2007; Liu et al., 2015; Nirala et al., 2017], oxidase [Enache et al., 2006], catalase [Pirmohamed et al., 2010], laccase [Chiouet al., 2017] and superoxide dismutase mimicking activities [Korsvik et al., 2007]. Still, low cost, quick and sensitive colorimetric biosensors needs to be developed based on robust enzyme mimetic nano-enzymes.

Due to unique chemical and physical properties of new 2D nanomaterials, these are appearing as hot spot areas for scientists [Lin et al., 2014; Chhowalla et al., 2013]. Among the 2-D nanomaterials, researchers are interested towards Molybdenum disulphide. (MoS_2) has been increasing due to marvellous catalytic, electrical conductivity and biocompatibile properties [Guo et al., 2017; Wang et al., 2016]. New properties of 2D materials appear when transformed to zero-dimensional being reason for prominent edge and quantum confinement effects [Nirala et al., 2015]. In contrast to other MoS_2 nanostructures, MoS_2-QDs have higher surface area to volume ratio and

better electron transport facility. The remarkable properties of MoS_2-QDs such as optical and electronic properties have been used in various applications from electronics (photo detectors and FETs etc.) to biomedical (bio-imaging and biosensors etc.) [Wang et al., 2016; Ramasubramaniam et al., 2017].

Recently, gold nanoparticles (AuNPs) decorated inorganic semiconductors composite have enhanced catalytic activity and conductivity for development of highly sensitive biosensors [Polyakov et al., 2014; Kamat et al., 2001; Zhang et al., 2014; Roy et al.,2014]. Really, these materials have exciting properties like its optical and electronic properties which are originated from scarce hetero junctions and the exciton plasmon coupling [Su et al., 2015; Tran et al., 2017]. Other nanocomposites mostly carbon derivatives require functionalization to establish chemical bonding with the gold nanoparticles [Wang et al., 2012]. In contrast, layered transition metal dichalcogenides, like WS_2 and MoS_2 which have sulphur atom over the outer layer are suitable for metal ligand formation that is, formation of strong Au−S bonds. This chemical bonding can increase the stability of resulting nanocomposites and facilitate a charge transfer between sulphur and Au [Polyakov et al., 2014; Sun et al., 2014].

In the present work, firstly we have developed a simple and one step synthesis method of AuNPs@MoS_2-QDs composite. The composite instantly catalyzed the peroxidase substrate 3, 3', 5, 5'-tetramethylbenzidine in presence of H_2O_2 to develop bluish-green colour products. Based on the investigation, AuNPs@MoS_2-QDs composite is used to detect H_2O_2 and glucose using colorimetric method. We developed sensing system with an excellent selectivity, reproducibility and stability towards glucose sensing. The proposed method was used to develop a portable test kit to detect glucose with unaided eye in real human fluid samples through agarose hydrogel being used as visual platform.

3.2 Experimental Section

3.2.1 Chemicals and materials

Chemical reagents of analytical grade were used which were glucose, 30% hydrogen peroxide, horseradish peroxidase (HRP), 3, 3', 5, 5'-tetramethylbenzidine (TMB), Glucose oxidase (GOx,>100 units/mg), gold (III) tetrachloride trihydrate ($HAuCl_4.3H_2O$) andagarose, purchased from sigma Aldrich (USA). Sodium molybdatedihydrate ($Na_2MoO_4.2H_2O$) was bought from Moly Chem India (≥ 98% purity, Product code- 18600). L-cysteine, Hydrochloric acid (HCl), disodium hydrogen phosphate and sodium dihydrogen phosphate were purchased from Merck (Merck, India). We made all the chemical reagent solutions using ultrapure water. For the detection of glucose in human blood serum, the blood samples were collected from the authorized hospital.

3.2.2 Characterization tools

XRD of synthesized MoS_2-QD and AuNPs@MoS_2-QDs composite were recorded by a Miniflex 600 X-ray-diffractometer (Cu–Kα radiation, Kα =1.54056 Å, 3°/min scan rate) in the range between 5° to 80°. Change in the absorbance was analyzed by using UV–vis Epoch 2 microplate reader Biotech (USA) spectrophotometer in quartz cuvette (1 cm optical path length). Structural characterization of composite materials was done by using HR-TEM, the Energy Dispersive X-ray and mapping by using FEI, TECHNAI G^2 20 TWIN (Czech Republic) electron microscope operating with 200 keV accelerating voltage on a carbon-coated copper grid modified with 6µL solution of the MoS_2-QD and AuNPs@MoS_2-QDs composite. Atomic force microscope (AFM) of MoS_2-QDs and AuNP@MoS_2 were performed with NT-MDT, Russia on silicon wafer substrate. Cyclic voltammogram was recorded with Auto lab (PG STAT, 302, The Netherlands, NOVA 1.11 software) using a conventional three-electrode system. Zeta of MoS_2-QDs

and AuNPs@MoS_2-QDs composite were executed with Nanoparticles Analyzer SZ-100, Japan.

3.2.3 Experimental procedure for preparation of MoS_2-QDs

MoS_2-QDs synthesis was performed through one step hydrothermal method. Individual solution of 0.5 g of L-cysteine and 0.25 g of sodium molybdate ($Na_2MoO_4.2H_2O$) were prepared in 25 ml of distilled water (DI) with constant stirring for 15 minutes, then both the solutions were mixed followed by constant stirring at ~40°C and pH (3.0) maintained by concentrated HCl (12 N). Then to carry out hydrothermal process, the above solution was transferred into 100 ml capacity of Teflon autoclave (stainless steel lined) and left for 42 h at ~200°C. After the reaction, solution was left for cooling at room temperature to get yellow colloidal solution containing MoS_2-QDs. The final product was dialyzed by dialysis bag (retained molecular weight 2000 Da) for 3-4 days to get the product of MoS_2-QDs. (Figure 3.1).

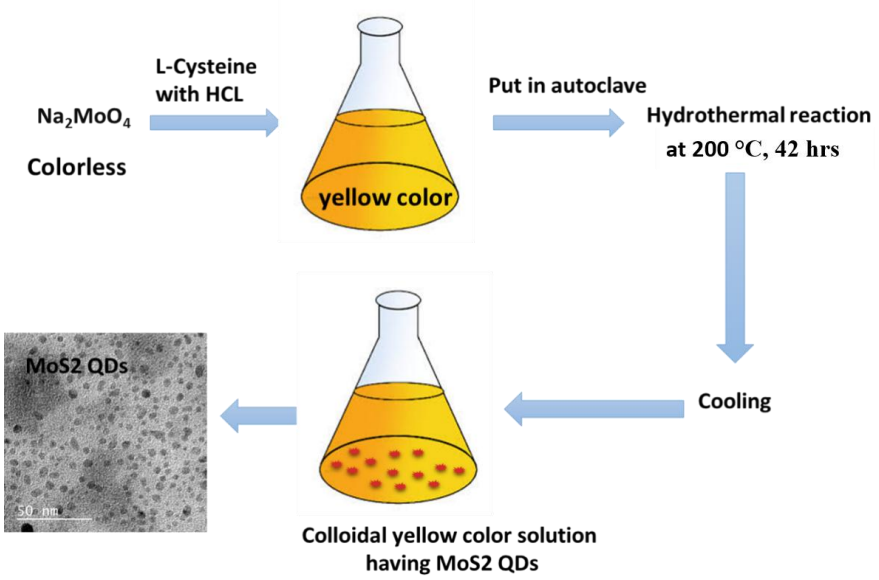

Figure 3.1 Synthesis of MoS_2-QDs.

3.2.4 Preparation of AuNPs@MoS_2-QDs composite

60 µl of MoS_2-QDs was added to 5 ml aqueous solution of $HAuCl_4$ (1mM) at boiling along with vigorous stirring for 30 minutes. The appearance of light purple colored solution indicates the formation of AuNPs@MoS_2-QDs composite formation.

3.2.5 H_2O_2 Detection

For the colorimetric sensing of H_2O_2, 10 µL of AuNPs@MoS_2-QDs composite (0.2µg/ml) was added in solution containing 50 µl of TMB (1mM) and 20 µL H_2O_2 of different concentration ranging from 0 µM to 200 µM in sodium acetate buffer (0.2 M) with pH 4.0, incubate for 35 min at 40°C. For the electrochemical detection of H_2O_2, glassy carbon electrode (GCE) was modified with 10 µL of AuNPs@MoS_2-QDs composite (0.2 µg/ml). Then Cyclic voltammetry was performed on developed electrode in phosphate buffer (pH 7) for the study of electrochemical response in presence of different concentration of H_2O_2 (Figure 3.13).

3.2.6 Optimization procedure of colorimetric sensing of glucose

Following procedure was adopted for colorimetric detection of glucose. 20 µL of GOx (5 mg/ml) was added into glucose solutions having different concentration in Phosphate buffer (0.1 M, pH 7.0), and incubated for 35 minutes at 40 °C to produce H_2O_2. Thereafter, 10 µL of AuNPs@MoS_2-QDs composite (0.2 µg/ml), 50 µL of TMB (1mM) and 300 µl of sodium acetate buffer (0.2 M, pH 4.0) were added to the above mixture solution, and incubated for 35 min at 40 °C.

3.2.7 Development of portable test kit for detection of diabetes in human using biological fluids (serum, saliva and tear)

A portable test kit was developed for checking glucose level in human biological fluids (serum, saliva and tear). All the samples were collected from healthy people (from our research group). Tear samples were collected by the use of onions through induced tearing and saliva samples were taken by psychological stimulation. Collected sample

were boiled for denaturation of extra protein present in the sample and centrifuged at 12,000 rpm for 10 min. The serum samples were taken from healthy people with the help of our Institute hospital (IMS, BHU, Varanasi). For development of test kit, 20 mg agarose was dissolved in 2 ml of water and placed in hot plate to dissolve properly then agarose solution was allowed to cool up to approx 40°C. Now, 50 μL of AuNPs@MoS_2-QDs composite solution (10 μM), 50 μL GOx (5 mg/ml), 100 μL TMB (5 mM) and 100 μL of acetate buffer (0.2 M, pH 4.0) were added to this cooled agarose solution. After gentle mixing of the solution, 200 μL of the above solution was transferred to the wells of ELISA plate and left at room temperature for 5 min to make a gel like structure. This ELISA plate with a gel like structure into each of its well is our test kit, which was developed and stored at 4 °C. Analysis of the sensing performance of the system was done by adding 30 μL of serum/saliva/ tear sample which was diluted 20 times in 0.1 M phosphate buffer (pH 7.0), to each well of the portable test kit and incubated for 30 min at 40 °C to permit the penetration of the reaction solution into the hydrogel. The hydrogel color was changed from colorless to bluish-green.

3.3 Results and Discussion

3.3.1 Structural and Morphological Analysis

The AuNPs@MoS_2-QDs composite obtained, is an urchin-like structure which is confirmed through UV-Vis, XRD, zeta, AFM, HR-SEM, TEM, SAED pattern and EDS mapping. First we characterized MoS_2-QDs by UV-Visible spectra and observed the two absorption maxima peaks appearing at ~247 nm and ~357 nm which is a characteristic feature of MoS_2-QDs shown in Figure 3.2. The result suggests that optical absorption for low dimensional QDs exhibit a strong blue shift when the lateral dimensions reduces to < 50 nm, ascribed to the quantum confinement.

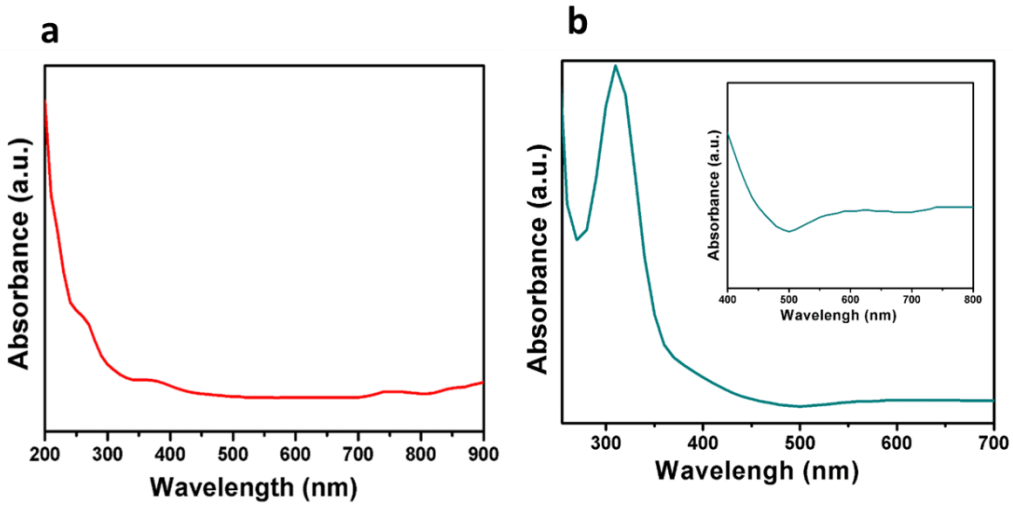

Figure 3.2 Absorption spectra of (a) MoS_2-QDs and (b) AuNPs@MoS_2-QDs, Inset of (b) shows the enlarged view of AuNPs@MoS_2-QDs peak. (524 nm)

The crystal structures of MoS_2-QDs and AuNPs@MoS_2-QDs composite were investigated by X-ray diffraction (XRD) measurements in Figure 3.3 (a and b). (002), (004), (101), (006), (106), (110), (108) faces are assigned to MoS_2-QDs and the AuNPs@MoS_2-QDs composite has been assigned (002), (004), (101) due to MoS_2-QDs and (111), (200), (220), (311) due to Au nanoparticles respectively (CAS no. 77-1716 and 7440-57-5).

AuNPs@MoS_2-QDs composite shows strong stability compared to MoS_2-QDs was confirmed by ZETA potential graph (-10.5 mV for MoS_2-QDs and -24.5 mV forAuNPs@MoS_2-QDs composite) in Figure 3.3c and d.

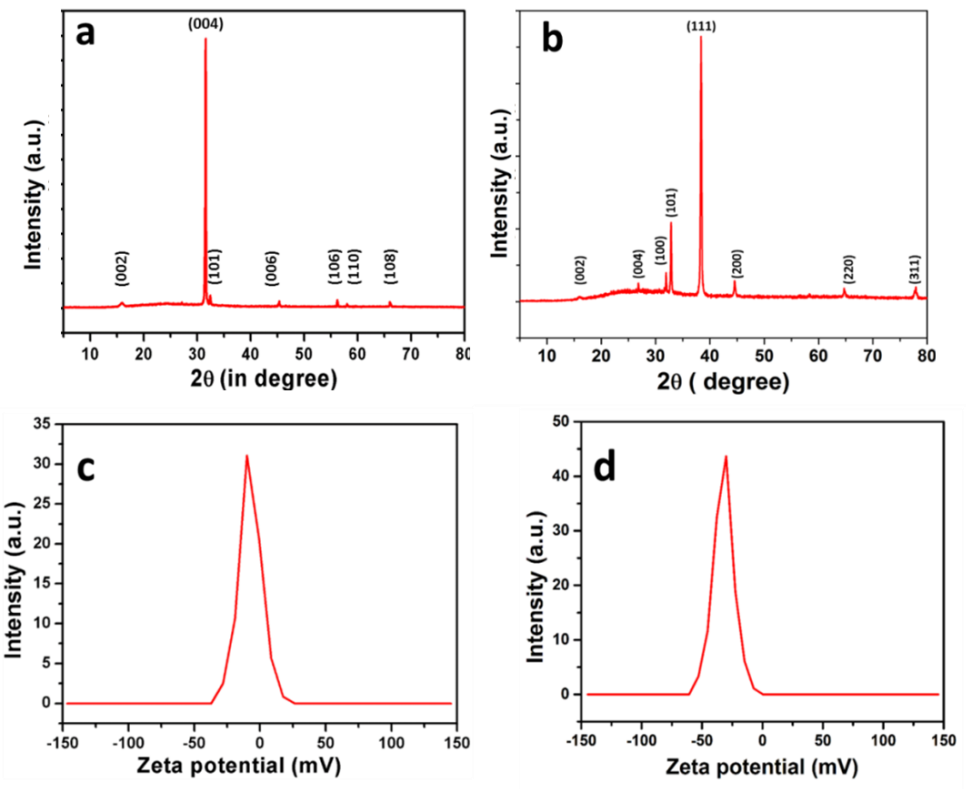

Figure 3.3 X-ray Diffraction of (a) MoS_2QDs (b) AuNPs@MoS_2-QDs obtained from JCPDS (CAS no. 77-1716 and 7440-57-5) file, (c) and (d) are the zeta potential graph of MoS_2 QDs and AuNPs@MoS_2-QDs respectively.

The structural morphology of synthesized nanostructure has been investigated by TEM. Figure 3.4 shows the uniform distribution of MoS_2-QDs with an average size 2-4 nm. The inset of Figure 3.4a shows the SAED pattern which suggests that MoS_2-QDs is crystalline in nature.

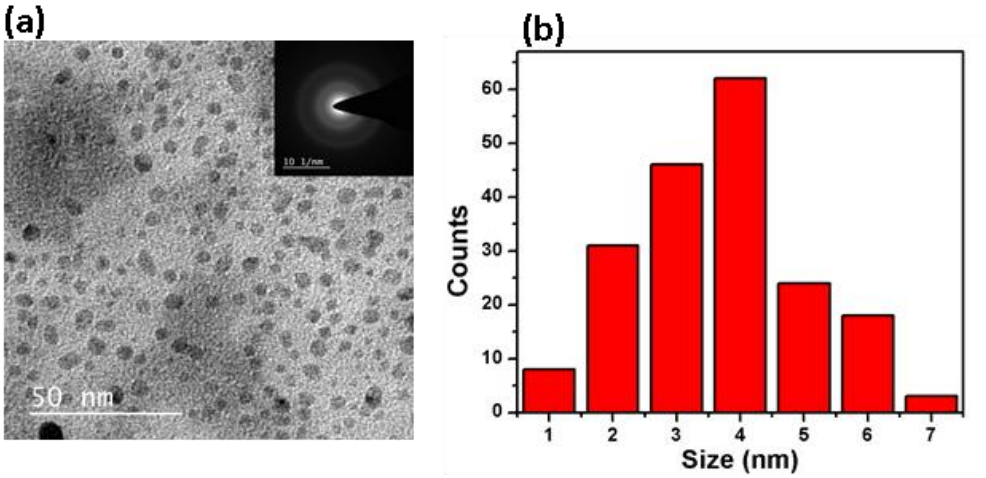

Figure 3.4 (a) TEM images of MoS_2-QDs and corresponding SAED pattern (inset), (b) particle size distribution of MoS_2-QDs.

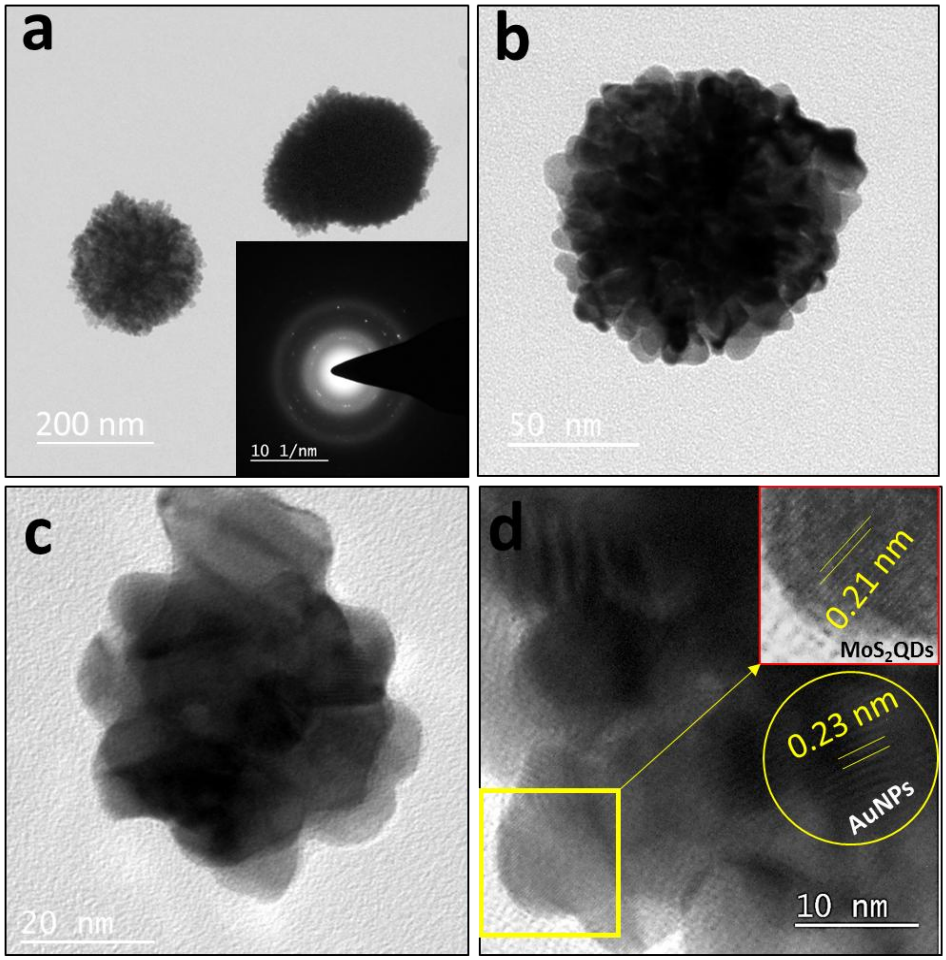

Figure 3.5 (a), (b) and (c) TEM image of AuNPs@MoS_2-QDs composite (d) HR TEM image of AuNPs@MoS_2-QDs showing lattice fringe spacing of AuNPs and MoS_2-QDs Inset of Figure (a) shows corresponding SAED pattern.

TEM image shows MoS_2-QDS nicely decorated over AuNPs to make urchin-like structure (Figure 3.5a, b, c). The HR-TEM image of AuNPs@MoS_2-QDs composite(Figure 3.5 d), shows the magnified image of the selected region, inside the yellow circle AuNPs have lattice fringe spacing ~0.23 nm, and inside the red box MoS_2-QDs layers are stacked with lattice fringe spacing ~0.21 nm, which is in good agreement with the previous available reports [Gu et al., 2016]. Energy dispersive X-ray spectroscopy (EDS) mapping of metal-semiconductor composite of AuNPs@MoS_2-

QDs, infer the presence of elemental composition such as sulphur, gold, molybdenum shown in Figure 3.6.

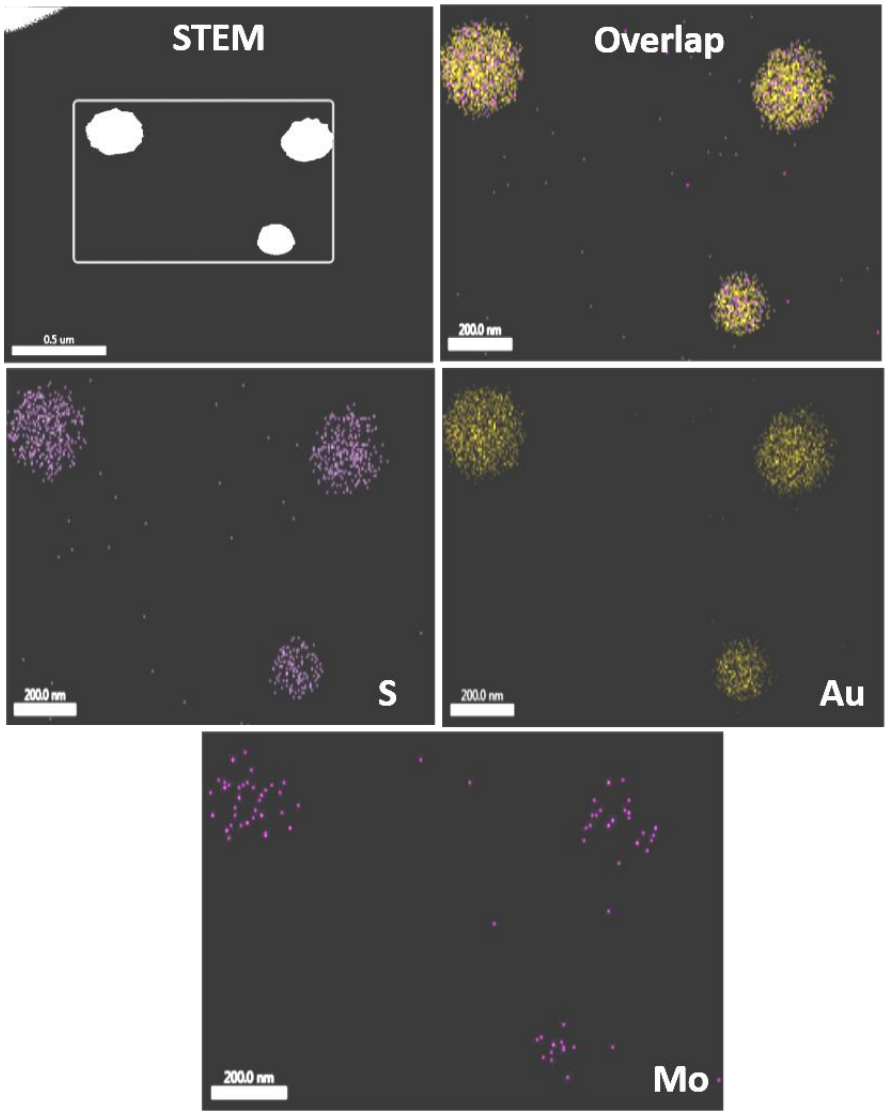

Figure 3.6 EDS mapping of AuNPs@MoS_2-QDs composite with overlapped image.

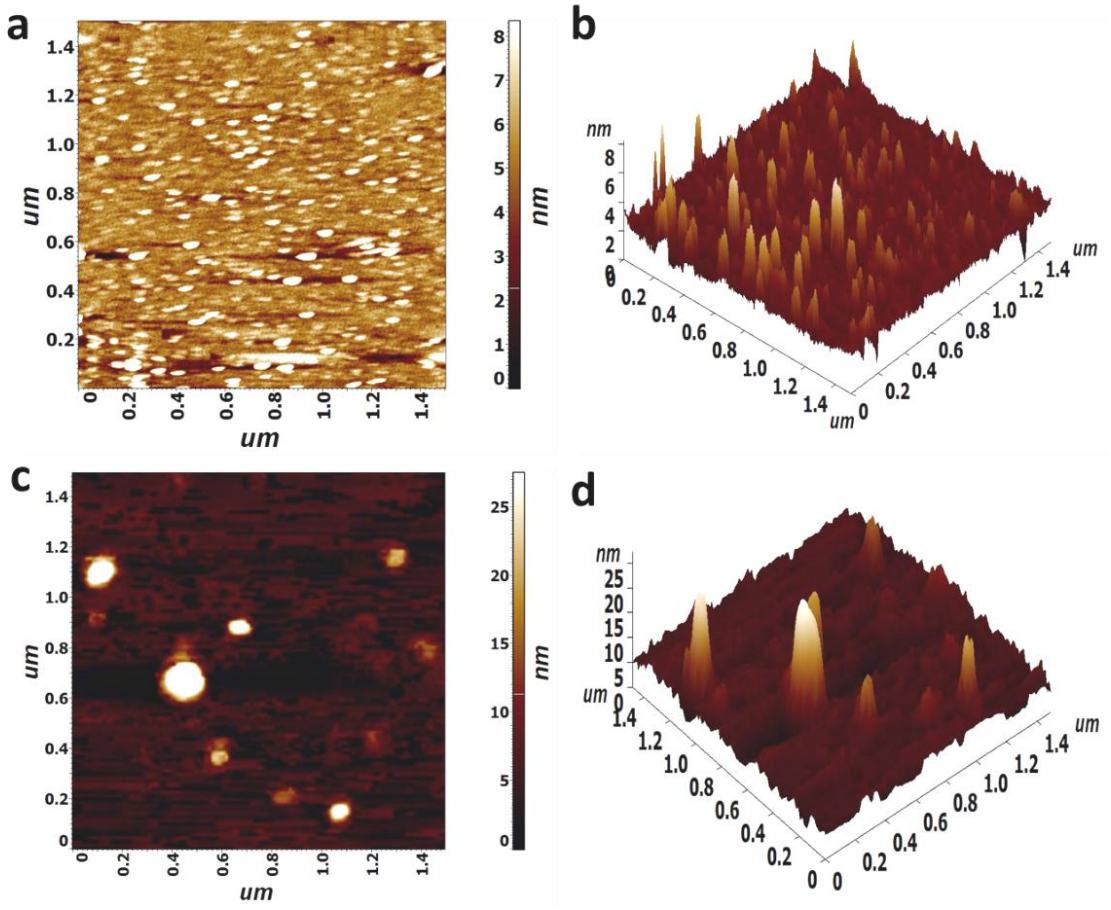

Figure 3.7 AFM images of MoS_2 QDs and AuNPs@MoS_2-QDs composites.

In Figure 3.7, the surface topography of MoS_2 QD and AuNPs@MoS_2-QDs composite has been investigated by AFM. Average thickness of MoS_2-QDs is below 10 nm and after composite formation the thickness of AuNPs@MoS_2-QDs composites increases to ~25 nm.

3.3.2 AuNPs@MoS_2-QDs composite catalysis the oxidation of TMB as the peroxidase substrates

After successful synthesis, AuNPs@MoS_2-QDs composite system has been used to examine robust peroxidase mimetic activity through catalysis of TMB in the presence of H_2O_2 [Josephy et al.,1982]. The catalyzed TMB shows bluish-green colored oxidation product and yield directly proportional to the concentration of H_2O_2. It shows a typical absorbance peak at 652 nm. From Figure 3.8A, it is observed that there is no change in

absorbance at 652 nm after the addition of H_2O_2 or AuNPs@MoS_2-QDs composite to TMB because the oxidation reaction has not occurred. After the addition of AuNPs@MoS_2-QDs composite and H_2O_2 to TMB solution, it is found that the color of solution changed from colorless to bluish-green indicating TMB oxidation as evident from the absorbance at 652 nm. In Figure 3.8B the typical absorbance peak intensity at 652 nm with respect to time of AuNPs@MoS_2-QDs composite-TMB-H_2O_2 is higher and has faster catalytic activity than TMB–H_2O_2 and AuNPs@MoS_2-QDs composite–TMB system. Hence, it clearly indicates that AuNPs@MoS_2-QDs composite shows robust peroxidase mimetic activity towards the substrates.

It is expected that the possible catalytic mechanism of AuNPs@MoS_2-QDs composite arose from enhancement of electron transfer leads to increase electron density and mobility. In the current process, TMB molecules are adsorbed on the surface of AuNPs@MoS_2-QDs composite and donate lone-pair electrons of the amino groups to AuNPs@MoS_2-QDs composite system. This results in an increase electron density and mobility of the AuNPs@MoS_2-QDs composite. The P-type nature of the semiconductor has a tendency to receive electrons from the TMB molecules with lone pair of electrons [Xu et al., 2000]. Due to the shift of electron density towards the composite, it confers the higher Fermi level and also the involvement of the excess electrons in the conduction band of AuNPs which results in an easy electron transfer to H_2O_2, leading to its faster breakdown into H_2O and hydroxyl radicals catalyzed by AuNPs@MoS_2-QDs composite. The un-oxidized TMB combines with hydroxyl radicals to make charge transfer complex in the form of bluish green color which shows absorbance at 350 nm and 652 nm (Figure 3.8A). The enzyme mimetic activity of different catalytic system is also studied (MoS_2-QDs, AuNPs, simple mixture of AuNPs-MoS_2-QDs and AuNPs@MoS_2-QDs composite) by absorption spectra based on TMB reaction. It is

found that the composite system shows higher catalytic activity than other catalytic materials (Figure 3.9).

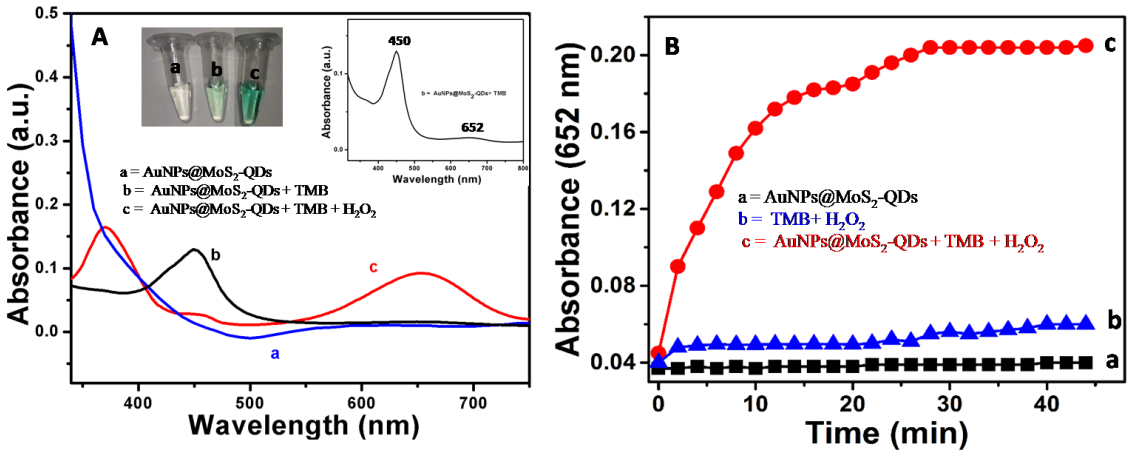

Figure 3.8 The absorbance spectra of TMB oxidation products for different reaction systems: (A) (a)AuNPs@MoS_2-QDs composite, (b) AuNPs@MoS_2-QDs composite + TMB, (Inset image shows enlarge view), (c) AuNPs@MoS_2-QDs composite +TMB + H_2O_2 and (B) time dependent study of (a) AuNPs@MoS_2-QDs composite, (b) TMB + H_2O_2 and (c)AuNPs@MoS_2-QDs composite + TMB + H_2O_2. (Inset image shows corresponding color image). The reaction system contains AuNPs@MoS_2-QDs composite (0.2 µg/ml), TMB (1mM) and H_2O_2 (0.15mM).

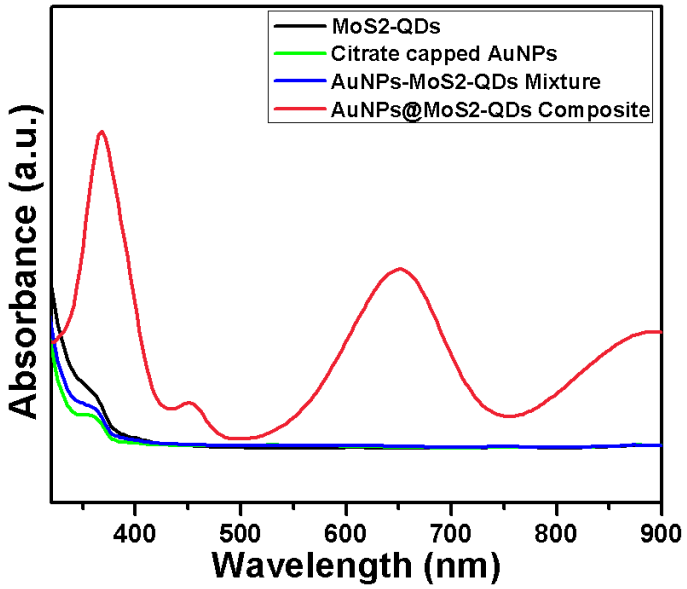

Figure 3.9 UV-Vis spectra of different catalytic system (MoS_2-QDs, AuNPs, simple mixture of AuNPs-MoS_2-QDs and AuNPs@MoS_2-QDs composite).

The catalyzed TMB oxidation showed significant peaks at 365 nm and 652 nm, which were due to the formation of a charge transfer complex with un-oxidized TMB molecules in the form of blue colored product. The developed sensing system was used for the analysis of H_2O_2 level which corresponds to the change in color and absorbance intensity of TMB oxidation product at 652 nm.

The catalytic mechanism ofAuNPs@MoS_2-QDs composite in the presence of OH radical scavenger such as ascorbic acid and $O_2^{\bullet}$ - scavenger i.e. Sodium Azide has been shown in Figure 3.10. However, the absorbance intensity decreased in the case of ascorbic acid as compared to blank, it also clearly shows that the OH radicals are required for the oxidation of TMB. Further, in case of Sodium Azide there was no change in the absorbance intensity, which indicates the presence of $O_2^{\bullet}$ - scavenger. These results suggest that OH radicals are important for TMB oxidation.

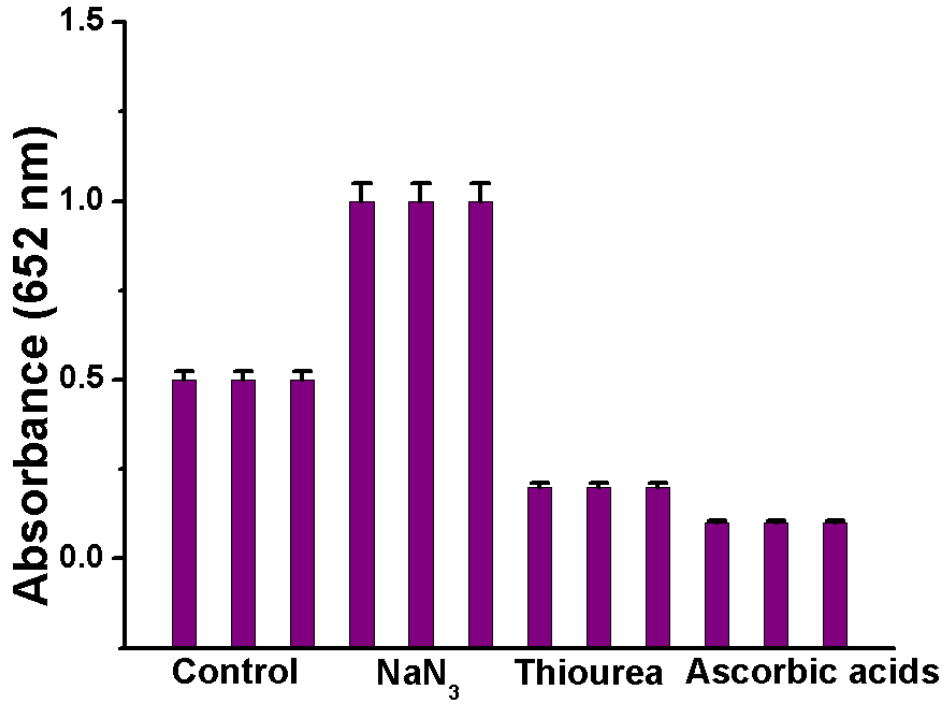

Figure 3.10 The catalytic reaction at the presence of different radical scavengers.

Procedures: 20 μL AuNPs@MoS_2-QDs (0.2 μg /ml), 100 μL Sodium acetate buffer (0.2 M, pH 4.0), 30 μL TMB (2mM) and 15 μL of different radical scavenger were mixed. The volume of the mixture was adjusted to 200 μL with water, and then 100 μL H_2O_2 (1.0 mM) was added. After that the reaction was carried out at room temperature for 30 min. The absorbance at 652 nm was recorded. The original concentrations of NaN_3 and ascorbic acid were 1 mM respectively.

3.3.3 Temperature, pH and H_2O_2 dependent catalytic activity of AuNPs@MoS_2-QDs composite

The pH, Temperature and H_2O_2 dependent catalytic activity of AuNPs@MoS_2-QDs composite have been studied and shown in (Figure 3.11). The activity of HRP is largely affected by the change in pH condition (lower to higher), temperature (above 60 °C) and H_2O_2 concentration. AuNPs@MoS_2-QDs composite shows good catalytic activity in wide range of temperature (25-80 °C) and pH (2.0-12.0) as compared to HRP (Figure 3.11a, b). The optimum catalytic activity of AuNPs@MoS_2-QDs composite was observed in acidic medium (pH 4.0) than other peroxidase mimetic system. The wide range of pH would be beneficial for sensing application. The catalytic activity of AuNPs@MoS_2-QDs composite system has also been checked, which showed higher catalytic activity in wide range of H_2O_2 concentration in contrast to HRP (Figure 3.11c). At optimum concentration the catalytic activity of AuNPs@MoS_2-QDs composite is stable because there are no available active sites of the catalyst.

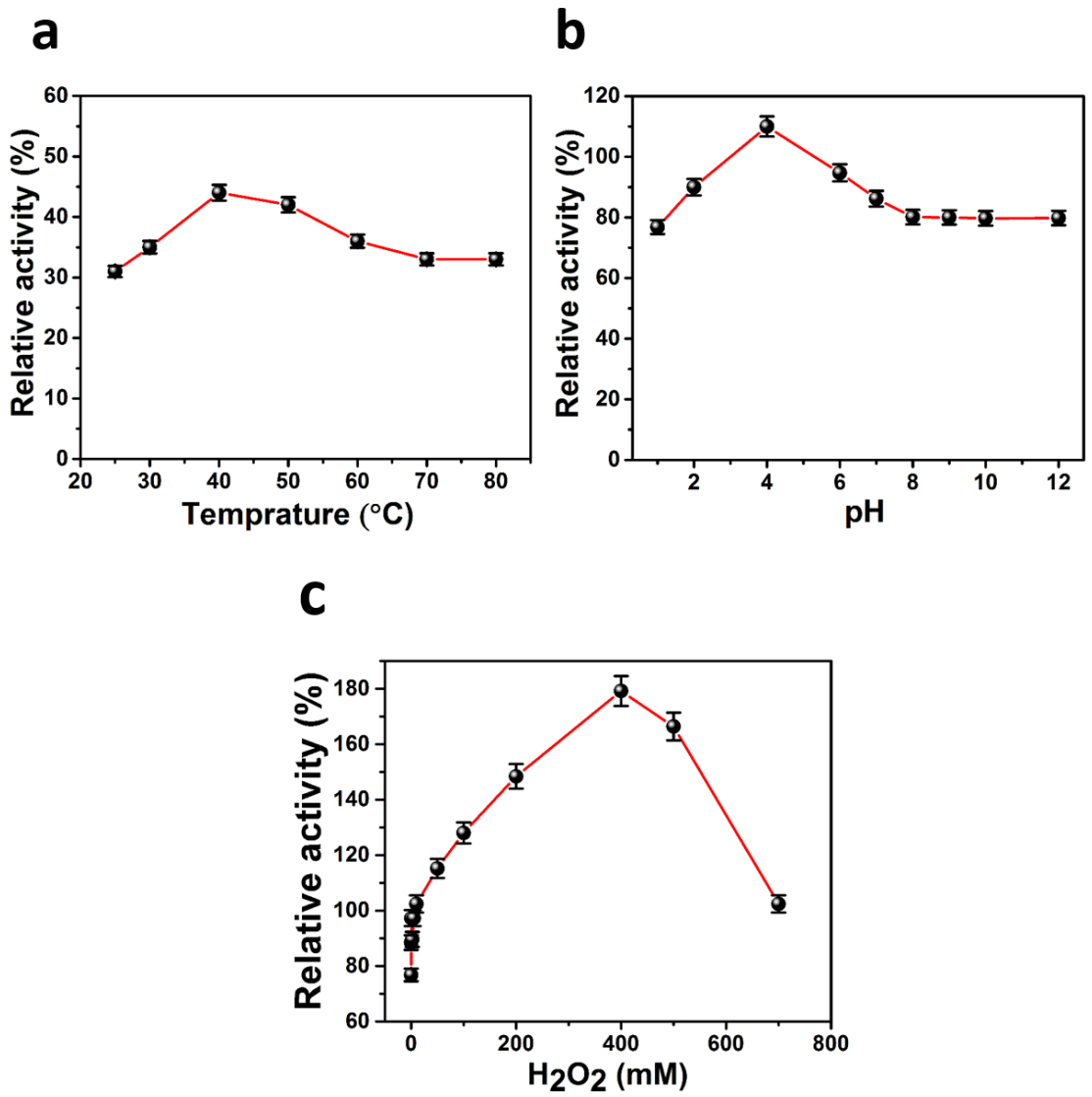

Figure 3.11 Dependency of AuNPs@MoS_2-QDs composite catalytic activity on (a) Temperature, (b) pH and (c) H_2O_2 concentration. Optimize reaction conditions are AuNPs@MoS_2-QDs composite (0.2 µg/ml), TMB (1mM), H_2O_2 (0.15 mM), 1ml of acetate buffer (0.2 M, pH 4.0) at 40°C.

3.3.3 Steady state kinetics of AuNPs@MoS_2-QDs composite

The steady state kinetics of AuNPs@MoS_2-QDs composite system was investigated by maintaining the concentration of TMB or H_2O_2 constant. The initial rate (V_0) of catalytic reaction (Table 3.1) was calculated by using the Beer–Lambert Law equation (3.1).

$$C = A\varepsilon / b \qquad \text{Eq.3.1}$$

In this equation, C denotes substrate concentration, A is the absorbance; b is thickness of the solution. The TMB oxidation derived product concentration was calculated through the molar absorption coefficient ε, value 39,000 $M^{-1}cm^{-1}$ [Shi et al., 2011]. The AuNPs@MoS_2-QDs composite was used towards TMB and H_2O_2 as substrate follows Michaelis–Menten equation at suitable concentrations. The maximum initial velocity (V_{max} = 10.6 × 10−6 Ms^{-1}) and Michaelis–Menten constant (K_m = 0.06) was calculated from the Michaelis–Menten equation (Figure 3.12a, b Eq.3.2).

$$V_0 = \frac{V_{max}\,[S]}{K_m + [S]} \qquad \text{Eq.3.2}$$

Where, V_{max} denotes maximum initial velocity, *Km* is the substrate concentration (S) at (V_{max})/2. Higher V_{max} of AuNPs@MoS_2-QDs composite was obtained which reveals good catalytic activity towards TMB substrates. Higher apparent K_m value of AuNPs@MoS_2-QDs composite was obtained with respect to H_2O_2 than HRP (Figure 3.12a), from the experimental observation, higher concentration of H_2O_2 is needed for maximum activity. The apparent K_m value of AuNPs@MoS_2-QDs composite with TMB as substrates was found smaller than HRP (Figure 3.12 b). The results suggested that AuNPs@MoS_2-QDs composite shows higher affinity towards TMB substrates than HRP. The kinetic parameter of AuNPs@MoS_2-QDs composite was compared from literature and observed that developed system showed good kinetics with low K_m and higher V_{max} than other catalytic nanomaterials because of higher surface area allowing more interaction between glucose oxidase and TMB molecules (Table 3.2).

Table 3.1 An illustration for calculation of initial reaction rate.

Absorbance	Time (sec)	The concentration of TMB-derived oxidation products (M) (c=A/εb, where ε=39000 M^{-1} cm^{-1}, b=1 cm)	Average rate (Ms^{-1})
0.1	70	2.5 x 10^{-6}	
0.2	140	5.1 x 10^{-6}	
0.32	220	8.2 x 10^{-6}	2.4x 10^{-4}
0.38	280	9.7 x 10^{-6}	
0.4	350	10 x 10^{-6}	

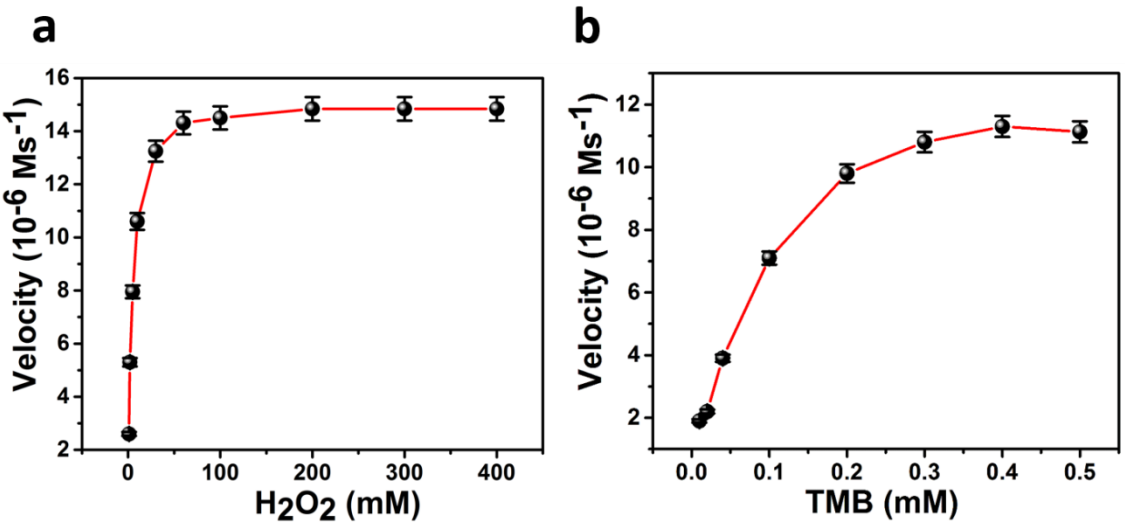

Figure 3.12 The enzyme kinetic parameter of AuNPs@MoS_2-QDs composite system toward substrates (a and b). Optimized reaction condition of AuNPs@MoS_2-QDs composite (0.2 µg/ml) in 1ml of 0.2 M sodium acetate buffer at 40°C (pH 4.0). (a) Kinetics of AuNPs@MoS_2-QDs composite for H_2O_2 in fix TMB (0.1 mM) (b) kinetic for TMB in fix H_2O_2 concentration (1mM).

Table 3.2 Comparative table of steady state kinetics of various catalytic substrates and HRP though TMB oxidation.

Catalytic substrate	K_m **(mM)**		V_{max} **(M s^{-1})**		**References**
	TMB	**H_2O_2**	**TMB**	**H_2O_2**	
C-Dots	0.039	26.77	3.61×10^{-8}	30.61×10^{-8}	[Shi et al., 2011]
MoS_2	0.525	0.0116	5.16×10^{-8}	4.29×10^{-8}	[Lin et al., 2014]
HRP	0.434	0.065	14.72×10^{-8}	5.65×10^{-8}	[Zheng et al., 2013]
Au NPs	0.023	-	2.15×10^{-8}	-	[Drozd et al., 2015]
AuNPs@ MoS_2-QDs	**0.06**	**5**	**10.6×10^{-6}**	**14.2×10^{-6}**	**Present work**

3.3.5 H_2O_2 detection based on AuNPs@MoS_2-QDs composite system by colorimetric and electrochemical method

H_2O_2 detection based on the color variation of TMB oxidation catalyzed by AuNPs@MoS_2-QDs composite is shown in Figure 3.13 a. The composite showed significant response in wide range of 0 to 200 µM of H_2O_2 with good linear relationship in 0.1 M PBS, (pH, 7.0). The linear regression equation obtained was A = 0.333 + 0.002C H_2O_2 with a correlation coefficient of 0.998. H_2O_2 detection was also carried out on the basis of electrochemical technique. Three electrode system, counter electrode of Pt plate, reference electrode Ag/AgCl and glassy carbon electrode modified with AuNPs@MoS_2-QDs composite as working electrode were taken and activity was measured in 0.1 M PBS, (pH, 7.0). The modified electrode was used for the study of catalytic activity of AuNPs@MoS_2-QDs composite in presence of H_2O_2 (3.13b), a significant increase in current response was observed when H_2O_2 was taken from 1mM to 12 mM and the current response was linear with concentration.

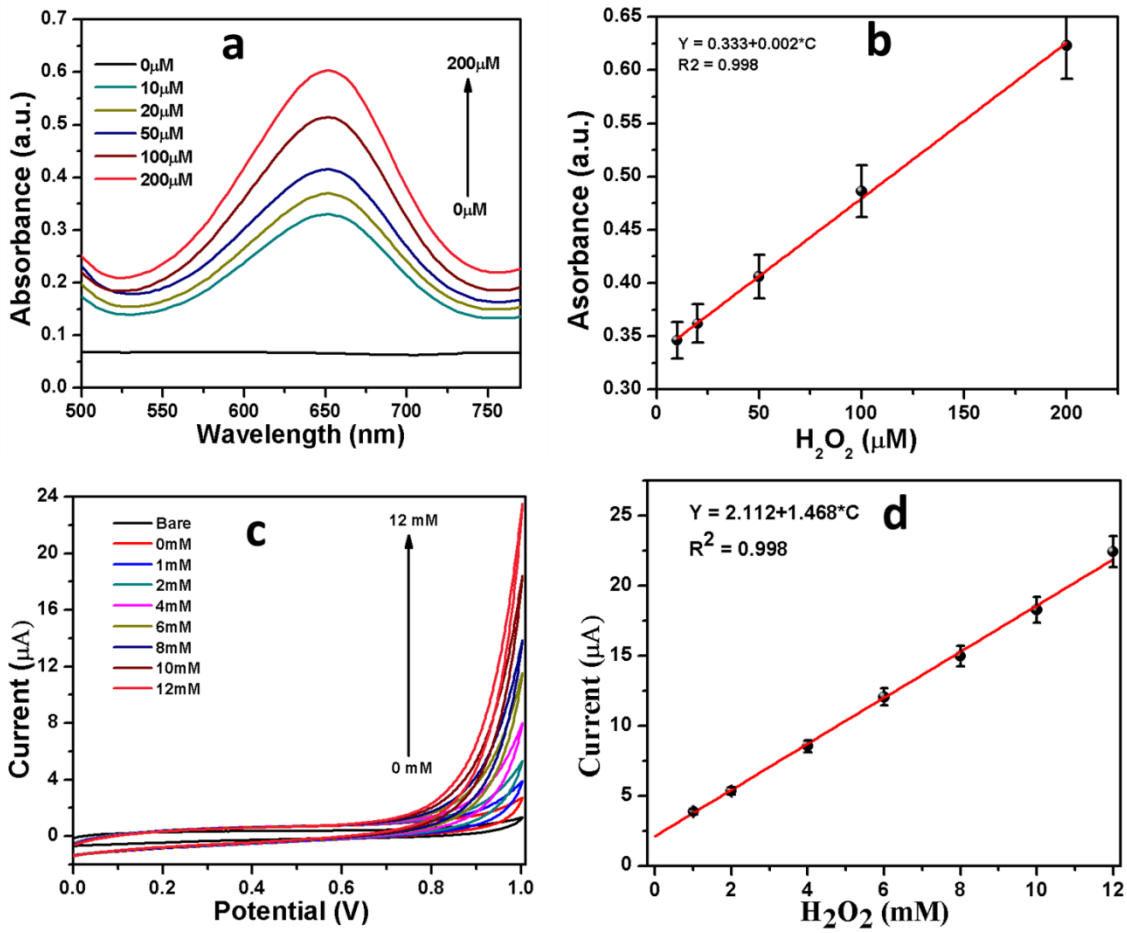

Figure 3.13 (a) Colorimetric response, (b) corresponding calibration plot, (c) Electrochemical response and (d) corresponding calibration plot of AuNPs@MoS_2-QDs modified electrode catalysis of H_2O_2 in 0.1 M PBS, (pH, 7.0)

3.3.6 Colorimetric detection of glucose

The AuNPs@MoS_2-QDs composite system was used for the detection of glucose. A sensing mechanism through schematic for colorimetric detection of glucose is proposed. The TMB substrates oxidized in the presence of H_2O_2 catalyzed by AuNPs@MoS_2-QDs composite to produce blue color. H_2O_2 is the indigenous product of redox reaction of GOx and glucose (Figure 3.14).

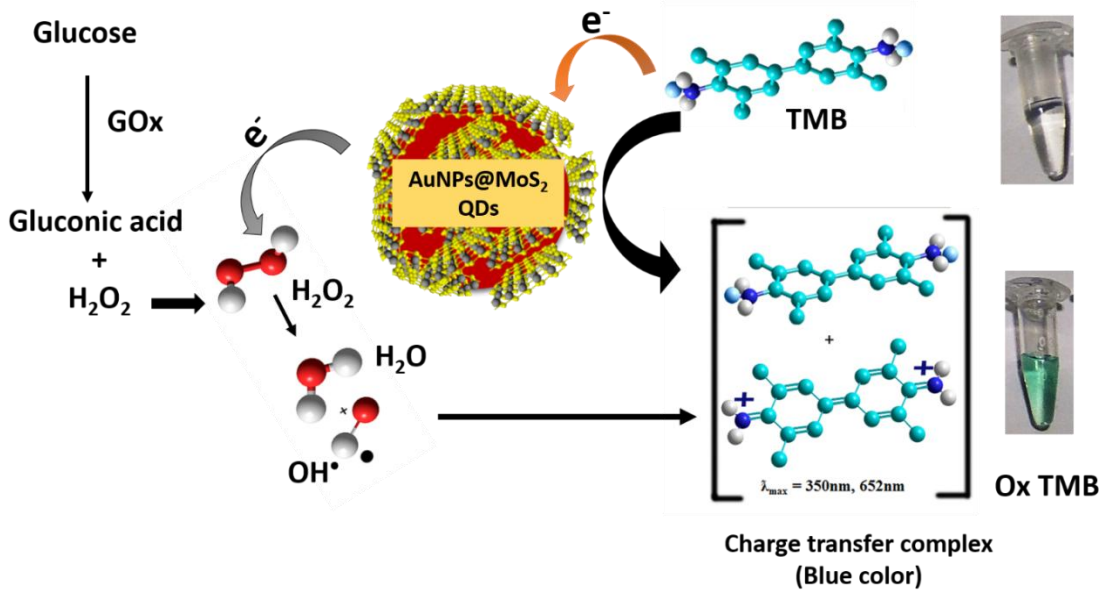

Figure 3.14 The catalytic mechanism of TMB oxidation in presence H_2O_2 catalyzed by AuNPs@MoS_2-QDs composite.

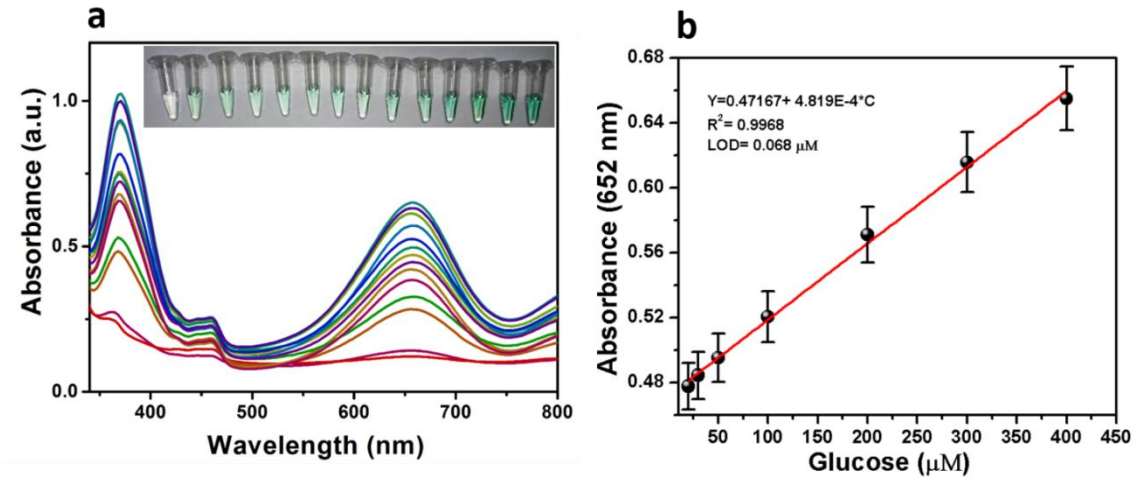

Figure 3.15 (a) UV-Visible spectra of colorimetric sensing of glucose based on AuNPs@MoS_2-QDs composite + TMB + GOx in buffer. Inset shows visible color in presence of glucose (1, 2, 5, 10, 15, 20, 30, 50, 100, 150, 200, 300 and 400 μM) (b) calibration curve.

Figure (3.15 a) shows an increase in absorbance intensity with addition of different concentrations of glucose in the developed sensing system. A better detection limit (0.068 μM) of sensing system is calculated by calibration curve (Fig 3.15b) compared to glucose detection based on various catalytic systems reported in literatures. On the basis

of comparison table one can say that AuNPs@MoS_2-QDs composite system shows better catalytic efficiency as compared to others (Table 3.3).

Table 3.3 Comparison of various nanoparticles- based methods for detection of glucose.

Catalytic system	**Technique**	**Detection limit(μM)**	**Linear range(M)**	**References**
V_2O_5 Nanozymes	Colorimetry	1	1×10^{-6}-500×10^{-6}	[Sun et al., 2016]
Pd_1Pt_3-graphene	Electrochemistry	5	1.0×10^{-3}-2.3×10^{-2}	[Zhang et al., 2013]
MoS_2Nanosheet	Colorimetry	1.2	5.0×10^{-3}-150×10^{-3}	[Lin et al., 2014]
WS_2 naosheets	Colorimetry	2.9	5.0×10^{-6}-3.0×10^{-4}	[Lin et al., 2014]
Au Nanoparticles	Colorimetry	-	1×10^{-3}–20×10^{-3}	[Jv et al., 2010]
MFe_2O_4 MNPs	Colorimetry	0.45	9.4×10^{-7}-2.5×10^{-5}	[Su et al., 2016]
AuNPs@MoS_2-QDs composite	**Colorimetry**	**0.068**	**20.0×10^{-6} 400×10^{-6}**	**This work**

3.3.7 Selectivity and Interference study

Interference study is a useful method to check the selectivity of developed biosensor towards glucose detection based on AuNPs@MoS_2-QDs composite catalytic system. The behaviour of developed sensing system is also studied in the presence of interference. First the concentration of glucose (200 μM) was fixed and interference consisting of ascorbic acid, cysteine, uric acid, urea, and cholesterol (5mM) was analysed. The developed sensor shows good selectivity for glucose shown in (Figure 3.16b). Further, calculated the percentage of interference and the data shows sufficiently

low interference with sensor in the presence of cysteine (10 %), ascorbic acids (8 %), urea (7%), uric acids (8%) and cholesterol (5%), (Figure 3.16a).

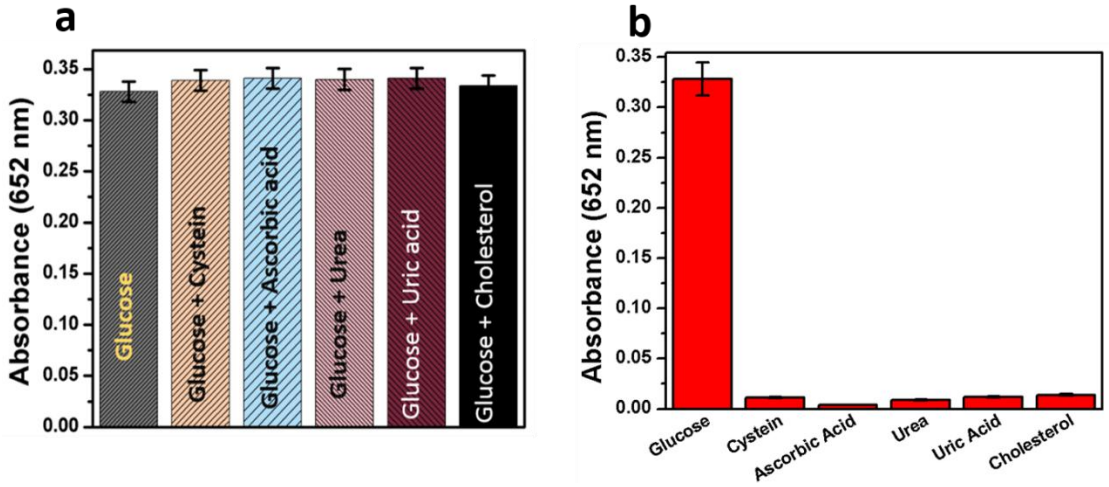

Figure 3.16 (a) Interference study and (b) Selectivity of developed sensor system towards glucose. Reaction condition are 0.5 ml of sodium acetate buffer (0.2 M, pH 4.0) with TMB (1 mM), 0.2 µg /ml, AuNPs@MoS_2-QDs composite and 1 mg/ml, glucose oxidase with different interference.

3.3.8 Study of repeatability of biosensor system

The sensing experimental results were validated through the study of reproducible test results of the developed system. The experiments for both intra (0 - 12 hrs.) and inter-day (0-3 days) was performed for repeatability study under the same set of conditions for detection of glucose (1 µM to 400 µM) shown in Figure 3.17a and Figure 3.17b. The findings suggested that the developed system (AuNPs@MoS_2-QDs composite) is reproducible and stable for detection of glucose.

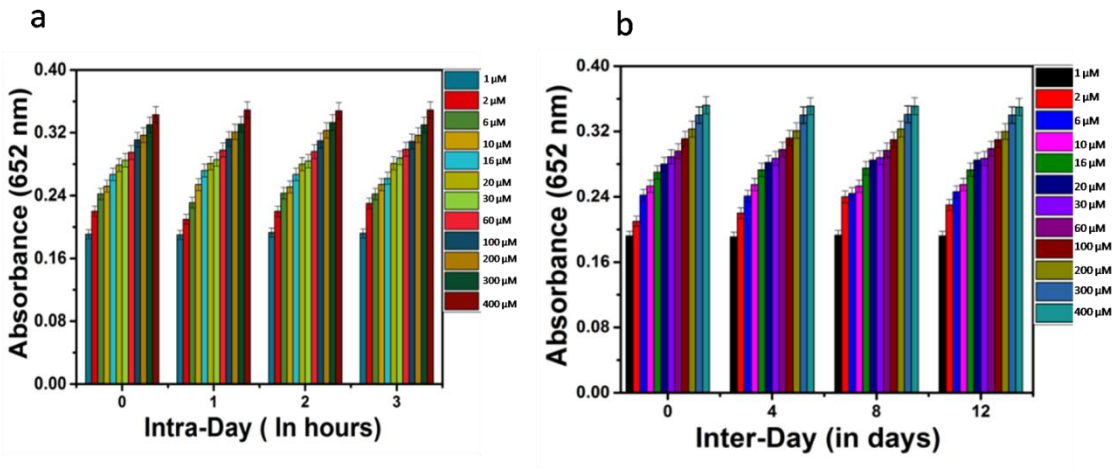

Figure 3.17 The reproducibility test of developed sensing method (a) Intra-day and (b) Inter-day repeatability for same setup condition for glucose (1µM to 400 µM) detection.

3.3.9 Testing of the glucose level through portable test kit in human biological fluids (serum, saliva and tear)

The proposed biosensing system has been used for the detection of glucose in human biological fluids (serum, saliva and tear). Normal range of glucose in healthy person in serum, tear and saliva is 4-6 mM, 0.1–0.6 mM and 0.008–0.21 mM respectively but exceeding the concentration range causes various complications i.e. diabetes etc.

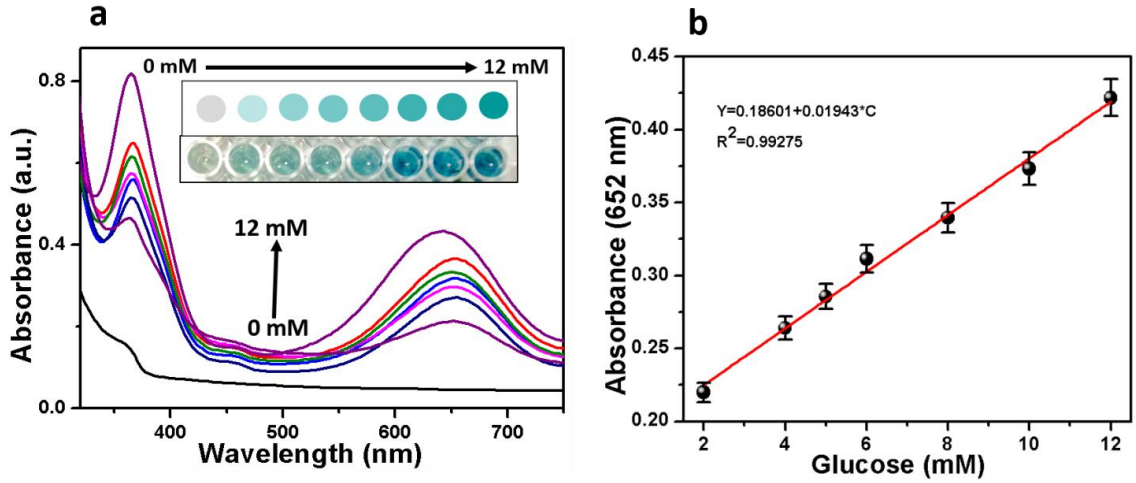

Figure 3.18 (a) UV-Vis spectra of test of glucose level in serum by portable test kit (based on AuNPs@MoS_2-QDs composite) and inset change the color of wells hydrogel with presence glucose level, with typical colorimetric chart with level glucose (0, 2, 4, 5, 8, 10, 11, 12 mM), (top) and (b) calibration curve.

On the basis of the developed sensing platform based on AuNPs@MoS_2-QDs composite, an agarose hydrogel based stable visual platform in ELISA plate is prepared and checked the glucose level in serum samples by using this portable test kit. Observing the change in color and absorbance of agarose hydrogel provides a stable visual sensing of serum glucose. In the testing process, 20 μL of serum sample was diluted 20 times with phosphate buffer (0.1 M, pH 7.0) and glucose was added to get various known concentrations. From Figure 3.18, hydrogel color change from colorless (blank) to light blue/greenish along with absorbance at 652 nm revealing glucose in normal range but after increasing the concentration of glucose (as diabetic person), the color changes to dark blue/greenish color, which discloses higher level of glucose i.e. (8-12 mM).

The developed portable test kit also shows an excellent linearity with better detection limit for tear and saliva diluted samples. The technique is explored for detection of glucose in tear and saliva in order to prove the concept of proposed non-invasive detection method which shows an excellent detection of the glucose levels in wide range of concentration in tear and saliva samples in Figure 3.19 and 3.20.

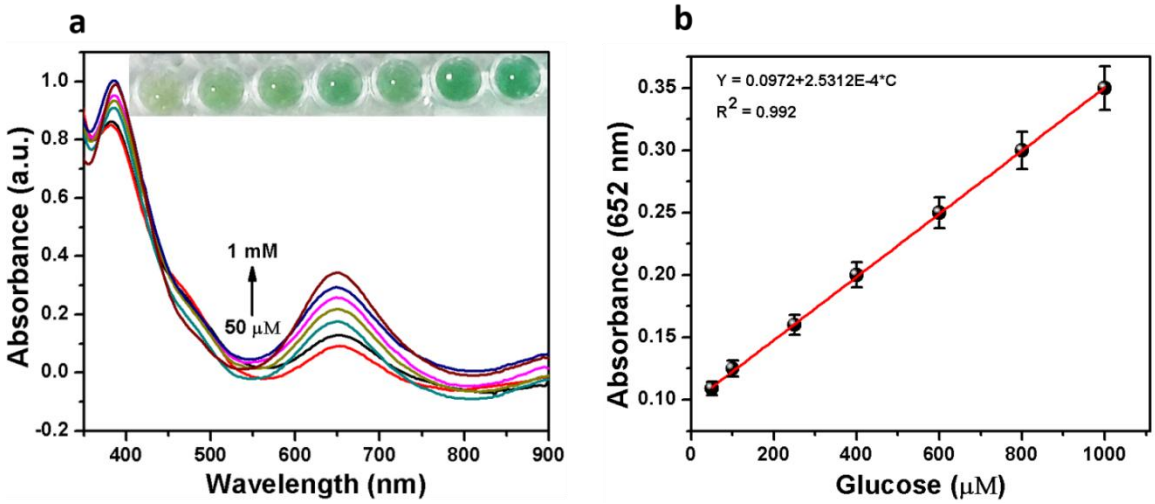

Figure 3.19 Absorption spectra of glucose level test in tear by portable test kit and inset change the color of wells hydrogel with presence glucose level (50 µM, 100, 250, 400, 600, 800 and 1mM) in tear (b) corresponding calibration curve.

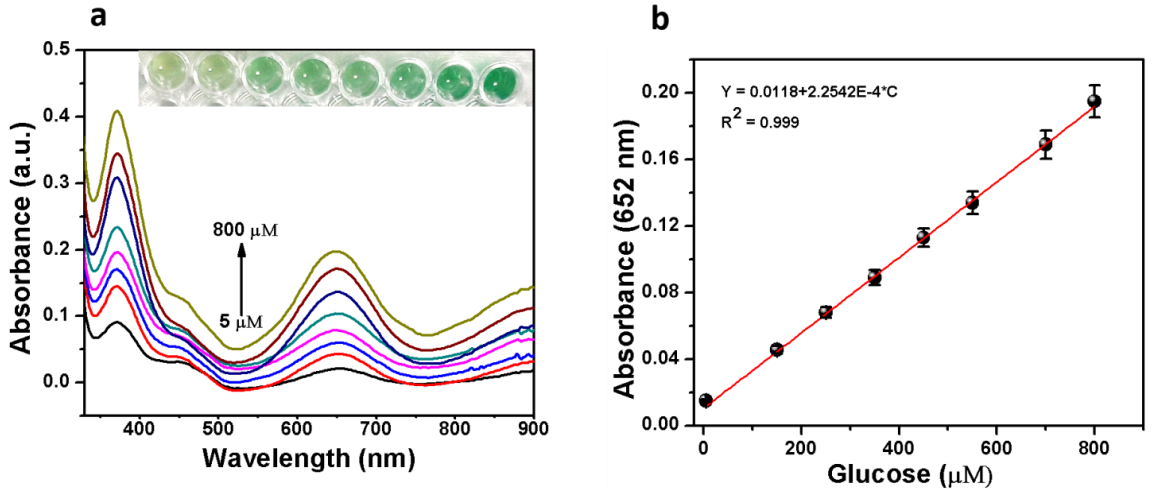

Figure 3.20 Absorption spectra of glucose level test in saliva by portable test kit and inset shows change in the color of wells hydrogel with presence glucose level (5 µM, 150, 250, 350, 450, 550, 700 and 800 µM) in saliva (b) corresponding calibration curve.

The AuNPs@MoS_2-QDs composite based biosensor system gave an excellent response for glucose detection in tear and saliva (Figure 3.19 and 3.20). The developed portable test kit can be used for unaided eye detection of glucose without using any expensive instrumentation. The glucose level in serum, saliva and tear samples was also compared

by determining it with the developed method and conventional technique, Auto analyzer (based on enzymatic reaction). On the basis of results, it is found that the proposed method is comparable to Auto analyzer for determination of glucose level (Table 3.4).

Table 3.4 Glucose determinations in serum, saliva and tear samples.

S.No.	Auto-analyzer method (mg/dL) [a]			Proposed method (mg/dL) [b]			Relative Error (%)		
	Serum (Se)	Tear (T)	Saliva (Sa)	Serum (Se)	Tear (T)	Saliva (Sa)	Se	T	Sa
1	95	6	3.2	90 ±1[c]	5.90±0.1[c]	3.1±0.1[c]	5	2	3
2	165	12	8	162 ±1[c]	11.60±0.1[c]	7.7.±0.1[c]	2	3	5
3	205	16	12	198±1[c]	15.4±0.1[c]	11.6±0.1[c]	3	4	3

[a]The concentration of glucose in samples have been analyzed by using conventional Auto-analyzer, based on enzymatic reaction. (Institute of medical science, Banaras Hindu University, Varanasi.)
[b]The confidence level was 3.33%.
[c]Number of samples = 3

3.4 Conclusions

Summarizing, the AuNPs@MoS_2-QDs composite have been successfully synthesized which showed a robust peroxidase mimetic activity towards peroxidase substrates to produce colored product in the presence of H_2O_2. The AuNPs@MoS_2-QDs composite ideally follows the enzyme kinetics i.e. Michaelis–Menten kinetics with peroxidase mimetic activity against wide range of pH and temperature. It is believed that AuNPs@MoS_2-QDs composite facilitates electron transfer for instantaneous breakdown of H_2O_2 into $OH^{\bullet}$ which combines with TMB to produce bluish-green color. The composite system is utilized for successful detection of glucose level in buffer solution with excellent selectivity and sensitivity. The proposed method further is used to

develop a portable test kit which is simple, inexpensive, for the detection of glucose level in human biological fluids (serum, saliva and tear). The designed bio-sensor is cable for non-invasive determination of glucose levels in tear and saliva. The developed colorimetric system based on peroxidase mimetic activity can be applicable in biotechnology and clinical diagnoses field.