Photonic Crystal Hydrogel Material for the Sensing of Toxic Mercury Ion (Hg^{+2}) in Water

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Electronic Supplementary Information (ESI)

Experimental Details:

Preparation of PCCA: Acrylamide (AA, 0.100 g, 1.40 mmol, Sigma Aldrich), N,N′-methylenebisacrylamide (0.005 g, 0.01951 mmol), CCA (2.00 g, 8 wt %, 102 nm diameter crosslinked polystyrene particles prepared from previously reported recipe [1]), and AG 501-X8 ion-exchange resin (0.10 g, 20-50 mesh, mixed bed, Bio-Rad) were mixed together in a 10 ml screwcap glass vial by shaking in vortex mixer. 10% v/v diethoxyacetophenone (DEAP 7.7μL, 3.84 mol, Aldrich) in dimethyl sulfoxide (DMSO, Fisher) was added in to the above reaction mixture and mixed with the help of vortex mixer. This reaction solution (polymerization mixture) is nitrogen bubbled thoroughly to remove the any dissolved oxygen. The polymerization mixture was withdrawn from the vial using thin needle to avoid ion exchange resin and injected into a polymerization cell consisting of two quartz disks, separated by a 127 μm thick parafilm spacer. The polymerization cell was placed under mercury (Black Ray) lamp operating at 365 nm for 4 hours. After the completion of the reaction the quartz cell was opened in millipore water, and the obtained PCCA was washed thoroughly with millipore water several times.

Preparation of ions strength responsive PCCA (carboxylated PCCA): The hydrolysis of PCCA makes the PCCA ions responsive. Partial hydrolysis of amide groups of polyacrylamide backbone of PCCA yield carboxylate groups. These covalently attached carboxylate groups carries counter ions inside the hydrogel and the hydrogel (PCCA) swells owing to the osmotic pressure. Therefore, the PCCA becomes ionic strength responsive. The hydrolysis of PCCA was carried out as follows: a small piece of PCCA was treated with 25 mL of aqueous hydrolysis solution containing 0.3N NaOH (Merck) with 10% v/v *N,N,N′,N′*-tetramethylethylenediamine (TEMED, Sigma-Aldrich) for 1.5 hour. The hydrolyzed PCCA was washed thoroughly several times with Millipore water.

Preparation of urease coupled PCCA (UPCCA) sensor for mercury: 0.005g of urease (Jack Bean, 0.005g, MW 480 kDa SRL, India) and 0.005g of solid ethyl-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC, 0.005g, 0.026mmol, Aldrich) were dissolved in 1ml 0.1mM phosphate buffered saline (PBS) at pH 7.21. The urease-EDC solution was placed on top of the hydrolyzed PCCA for 12 hours. After 12 hours the resulting UPCCA mercury sensor was washed extensively with Millipore water.

Preparation of CCA free urease coupled hydrogel: CCA free hydrogel (blank hydrogel) was prepared using the similar recipe as described above (preparation of PCCA). In this case water (2g) was used instead of CCA. This blank hydrogel was hydrolyzed and urease coupled using the exactly identical experimental condition as described above for the determination of attached enzyme, free carboxylate concentrations and studies of enzyme-substrate-inhibition kinetics.

Determination of coupled enzyme concentration and free carboxylate concentrations: An UV-Visible spectrophotometer (Cary 100Bio, Varian) was used to record the absorption spectra of urease coupled blank hydrogel, urease solution in buffer and to study the urease-urea- inhibitor kinetics. An auto titrator (702 SM Titrino, Metrohm) was used for the determination of free carboxylate concentration in the blank hydrogel. The hydrogel was titrated against the standard 0.1N NaOH.

Measurement of UPCCA mercury sensor response: All the diffraction measurements were carried out in reflection mode with Ocean Optics (USB4000-UV-VIS-NIR) spectrophotometer. The sensing experiments of the UPCCA sensor were carried out as follows: A small piece of UPCCA was fixed in a petridish (made of polystyrene, Hi-Glass, India) and the diffraction was recorded in water. Then the

UPCCA was exposed to the different concentrations of urea solution in water and their diffraction spectra were collected. A gradual blue shift was observed with increasing urea concentration. Then the UPCCA was washed thoroughly with water and the original diffraction in water was obtained. Then the UPCCA was exposed to the 1mM urea solution in water which contains Hg^{2+} ion and the diffraction was recorded. Again the UPCCA was washed with water and got back the original diffraction in water. Then the UPCCA was exposed to the next higher Hg^{2+} concentration along with 1mM urea solution. After every exposure the UPCCA was washed with water and obtained the original diffraction in water.

Enzyme kinetics studies: The well known Berthelot reaction[2] was used to study the urease-urea hydrolysis kinetics and the inhibition of this hydrolysis by various heavy metal ions such as Hg^{2+}, Cu^{2+} and Ag^{+}. Briefly the method is as follows: Two reagents; reagent A [phenol (5 g, 53 mmol, Merck-India) and sodium nitroprusside (25mg, 0.084mmol, Merck-India) were dissolved in 500 ml of water] and reagent B [NaOH (2.5g, 0.0625mmol, Merck-India) and NaOCl contains 5% active chlorine (4.2 mL, Merck)] were prepared. A small piece of UPCCA (blank hydrogel) was taken in a disposable plastic cuvette. Reagent A (1.5 ml), urea solution of known concentration and reagent B (1.5 ml) were added to the cuvette subsequently. The production of indophenols blue dye started immediately after the addition of reagent B. The progress of the reaction was monitored with time by recording the absorption spectra of indophenols dye using a UV-Vis spectrophotometer (Cary100 Bio, Varian).The values of absorption correspond to the dye concentration which is equal to the produced ammonia concentration due to the urea-urease hydrolysis.[2] The experiment was carried out with various urea concentrations for the studies of inhibition kinetics in the presence of inhibitors (Hg^{2+}, Cu^{2+}, and Ag^{+}). We have also carried out the urease-urea hydrolysis kinetics experiments by varying the inhibitor
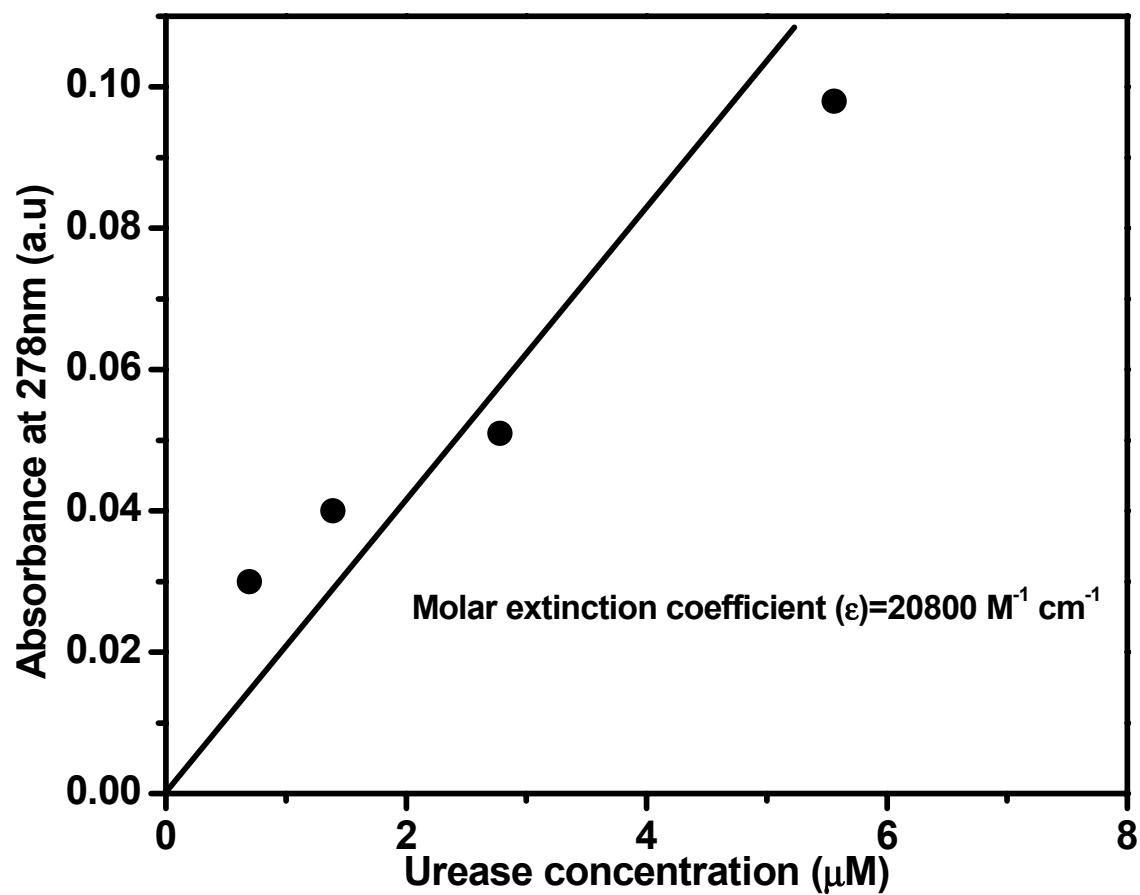
concentrations. The similar urea-urease hydrolysis and inhibition kinetics were done using free urease
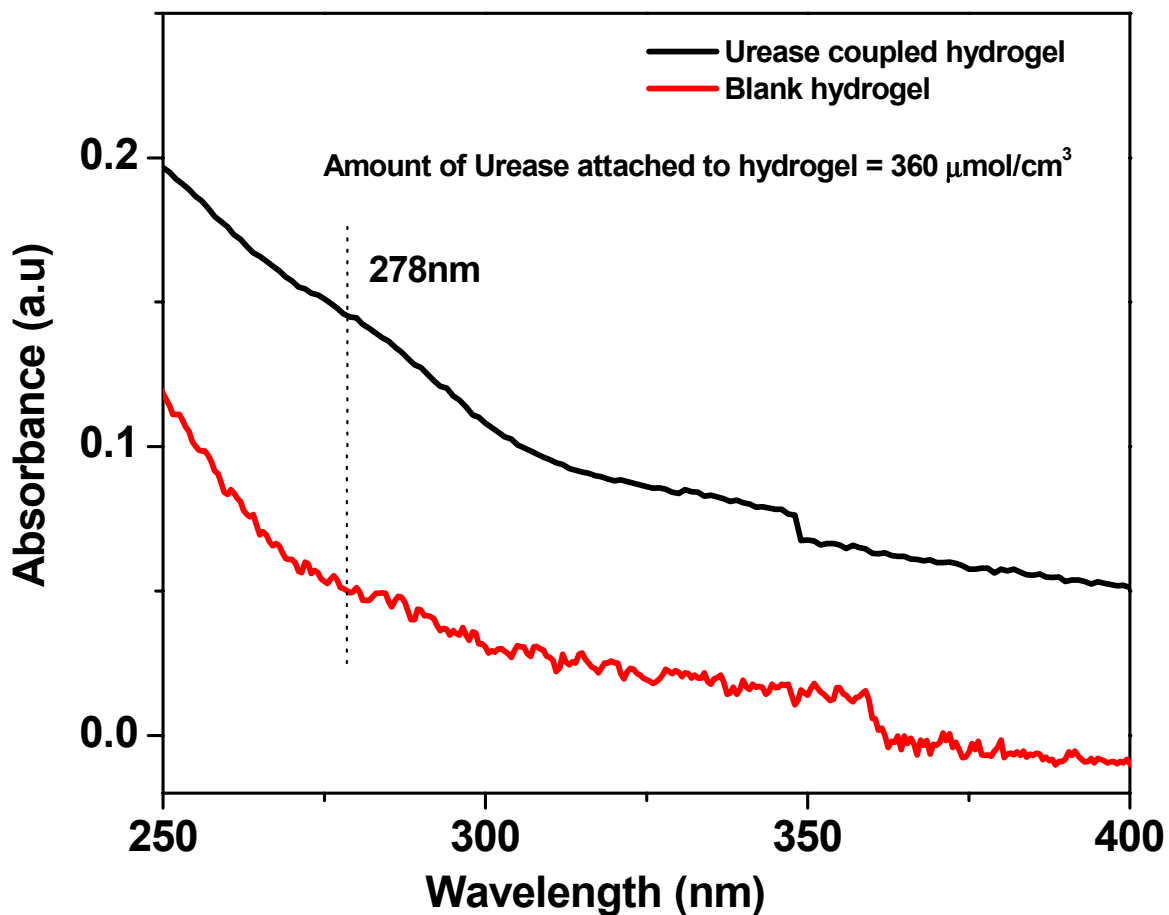
in the solution.

References

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2. M. Berthelot, *Repert. Chem. Applique*, 1859, **1**, 284; C. J. Patton, S. R. Crouch, *Anal. Chem.*, 1977**,**

    49, 464-469.
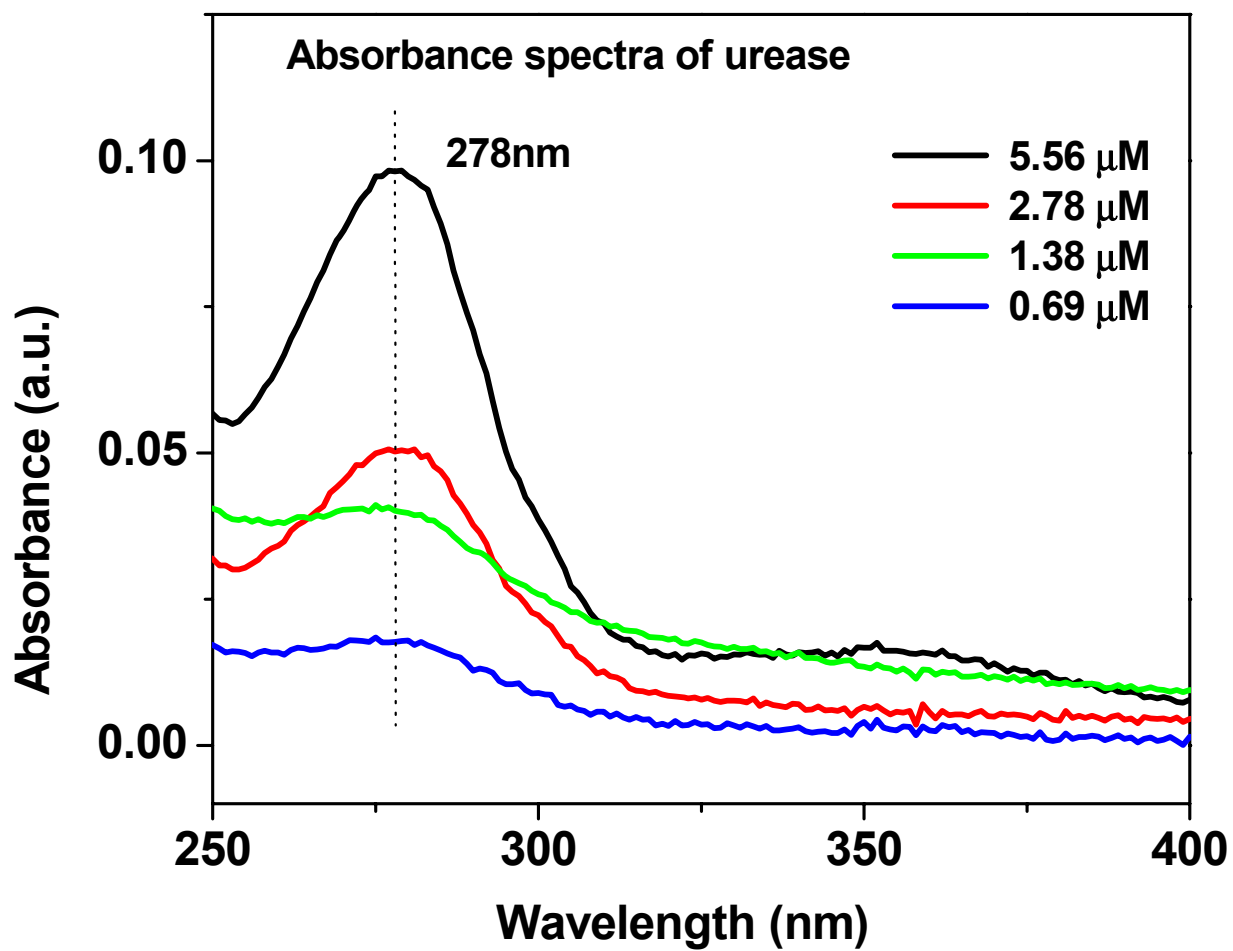
ESI Figure 1: Absorption spectra of urease enzyme in PBS medium

ESI Figure 2: Plot for the calculation of molar extinction coefficient (ε) of urease in aqueous (PBS)

ESI Figure 3: Absorption spectra of blank hydrogel, urease coupled blank hydrogel (hydrogel made in the identical condition as PCCA without CCA) for the determination of urease loading in the hydrogel.
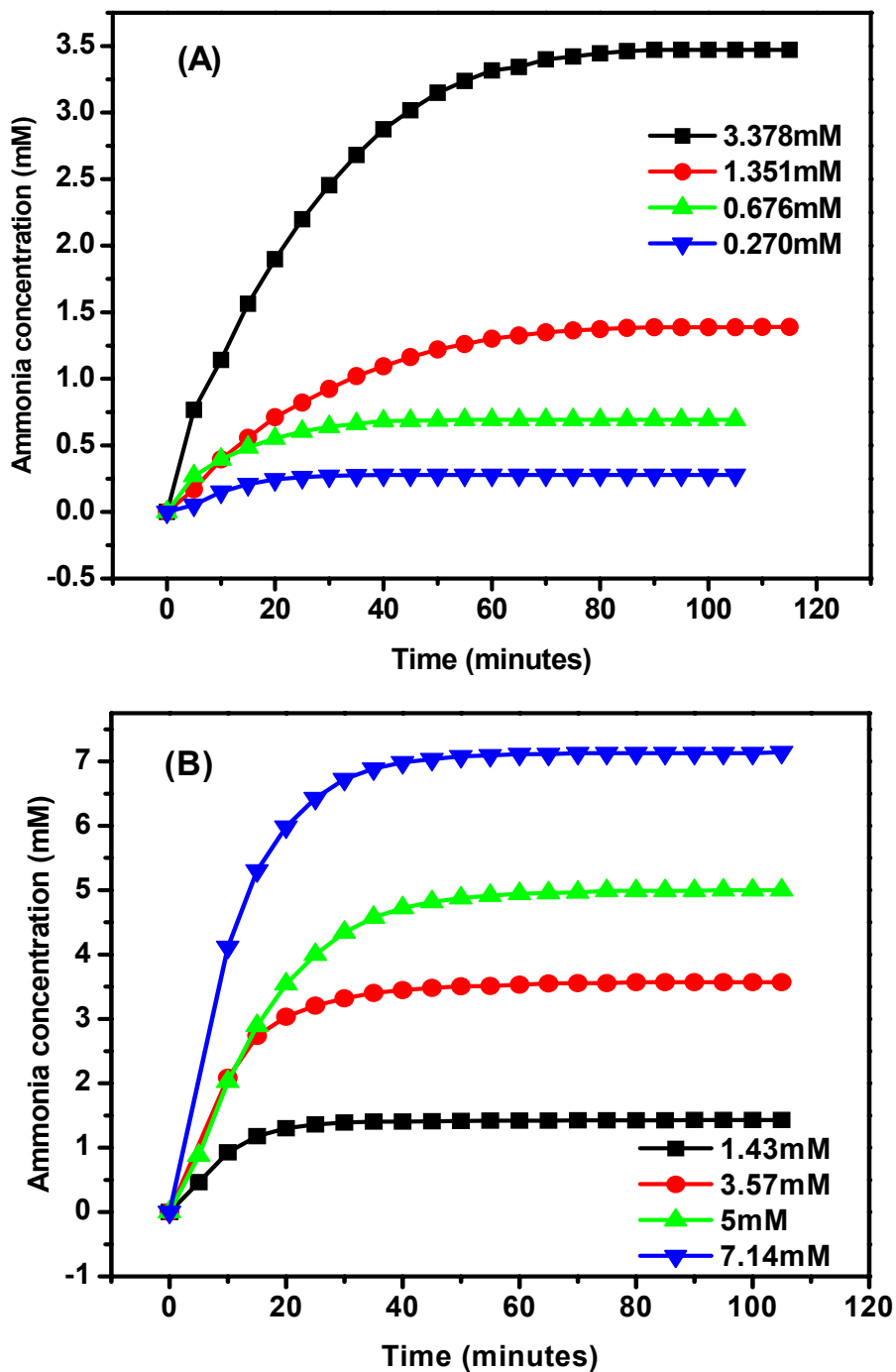
ESI Figure 4: Progressive curves for urease-urea hydrolysis for various urea concentrations (indicated in the figure) for a fixed enzyme concentration. The hydrolysis is monitored using Berthelot method.
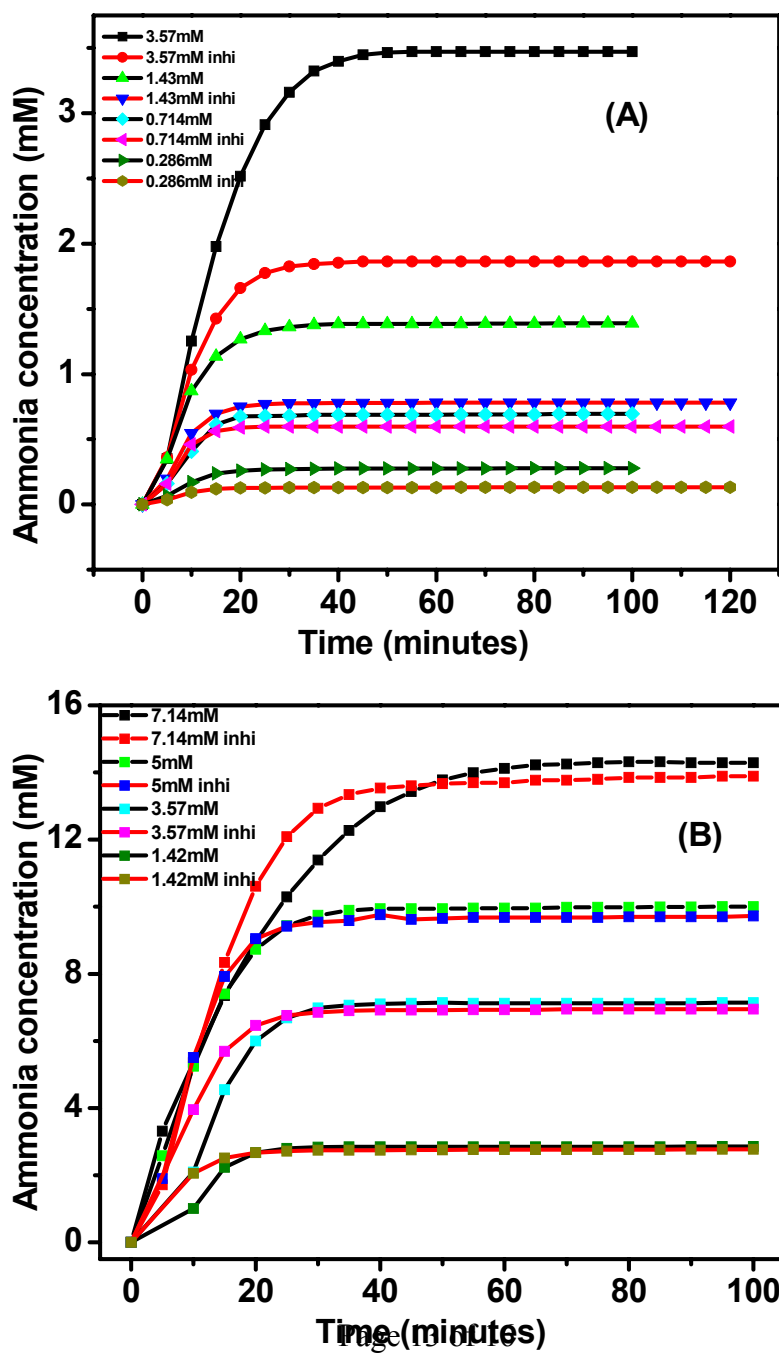
(A) solution state; enzyme concentration = $0.011\mu mol/cm^3$ (B) hydrogel state; enzyme concentration = $360\mu mol/cm^3$.
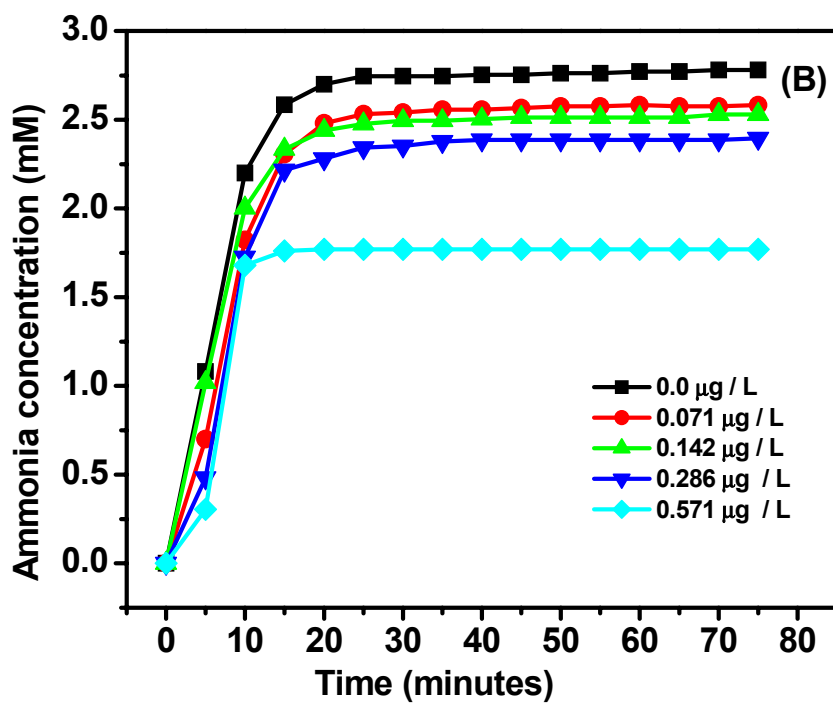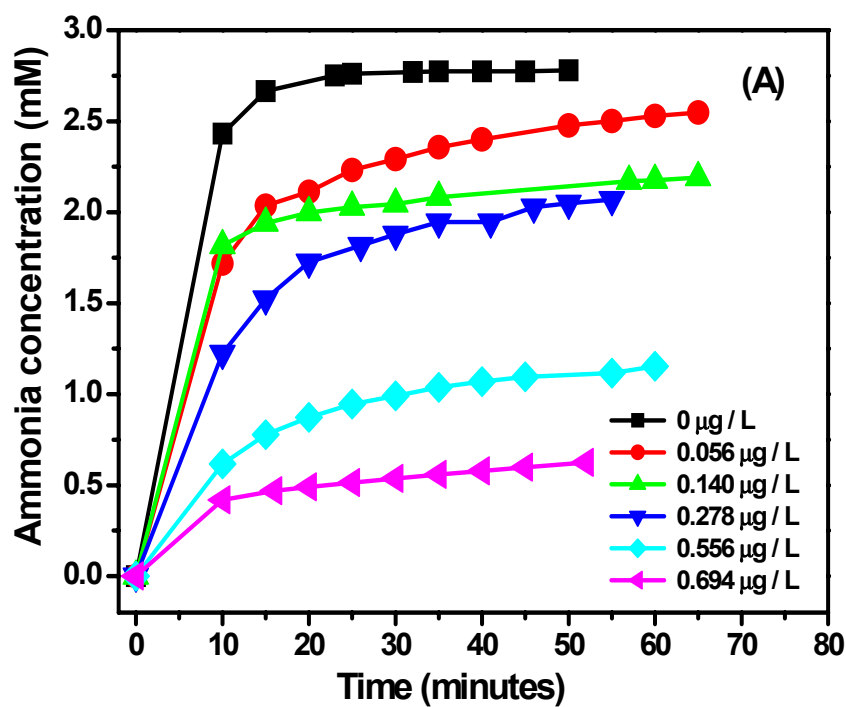
ESI Figure 5: Progressive curves for urease-urea hydrolysis for various Hg^{2+} concentrations (indicated in the figures) for a fixed enzyme and substrate (2.78mM, urea) concentration. The hydrolysis is monitored using Berthelot method. (A) solution state; enzyme concentration = 0.011µmol/cm^3 (B) hydrogel state; enzyme concentration = 360µmol/cm^3.
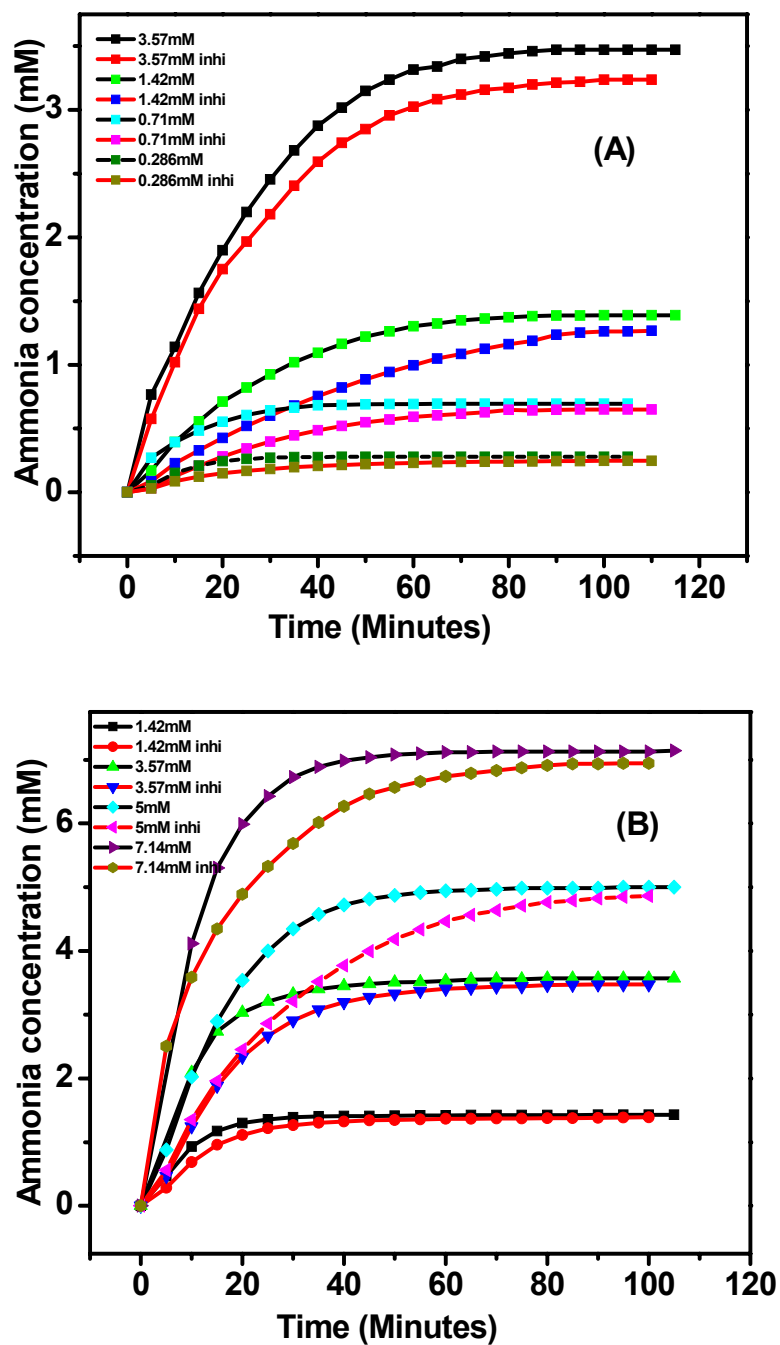
ESI Figure 6: Progression curves of urease-urea hydrolysis monitored by Berthelot method with and without inhibitor (Hg^{2+}) both in solution and hydrogel states. In all the cases urease enzyme and inhibitor (Hg^{2+}) concentration are kept constant. (A) solution state; enzyme concentration= $0.011 \mu mol/cm^3$ and inhibitor concentration = 0.556 ppb (B) hydrogel state; enzyme concentration = 360 $\mu mol/cm^3$ and inhibitor concentration = 0.571 ppb . Black lines are non inhibition processes and red lines are inhibition processes. Urea concentrations are mentioned in the figures.
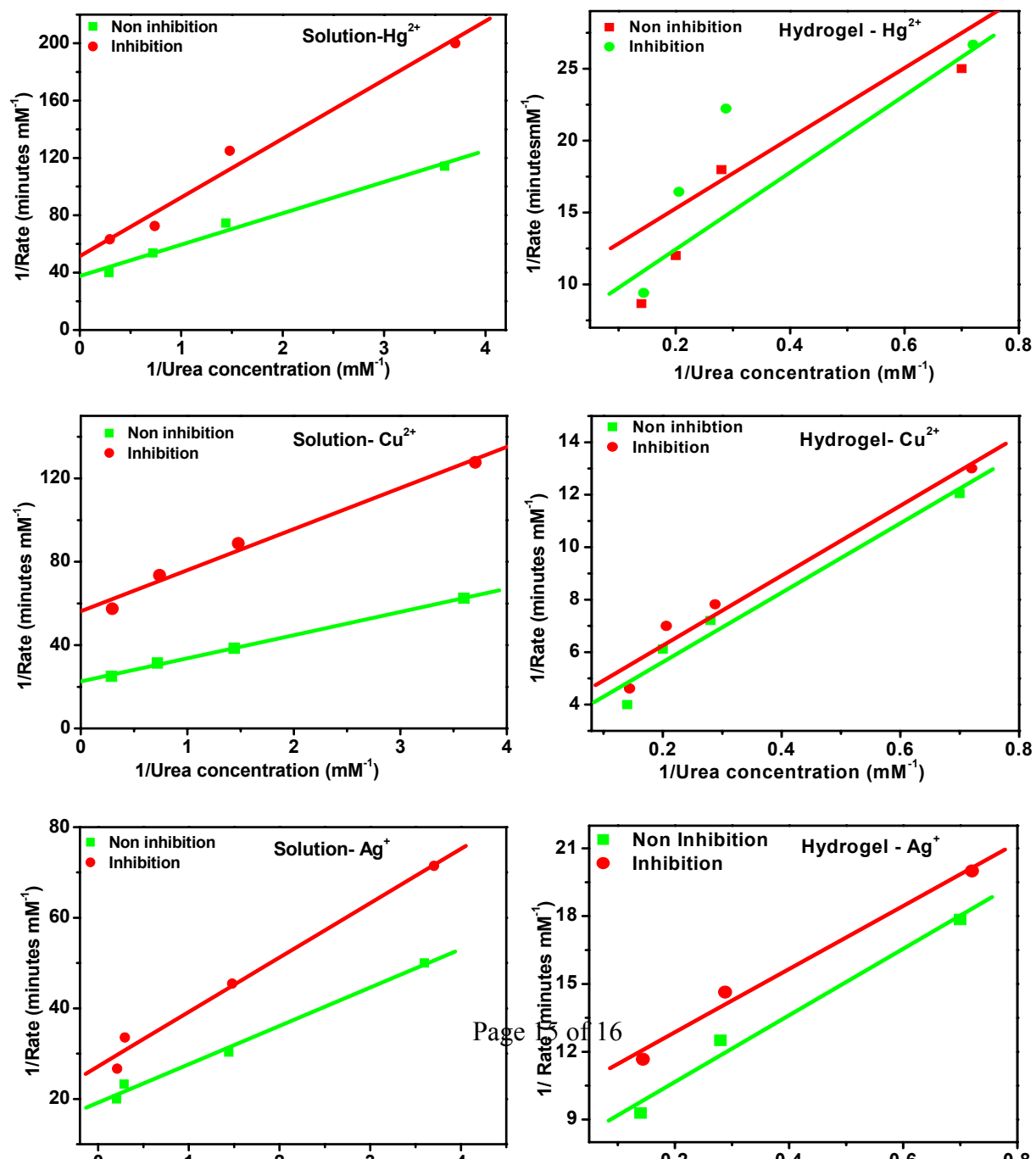
ESI Figure 7: Progression curves of urease-urea hydrolysis monitored by Berthelot method with and without inhibitor (Cu^{2+}) both in solution and hydrogel states. In all the cases urease enzyme and inhibitor (Cu^{2+}) concentration are kept constant. (A) solution state; enzyme concentration = $0.011\mu mol/cm^3$ and inhibitor concentration = 0.556 ppb (B) hydrogel state; enzyme concentration = $360\ \mu mol/cm^3$ and inhibitor concentration = 0.571 ppb. Black lines are non inhibition processes and red lines are inhibition processes. Urea concentrations are mentioned in the figures.
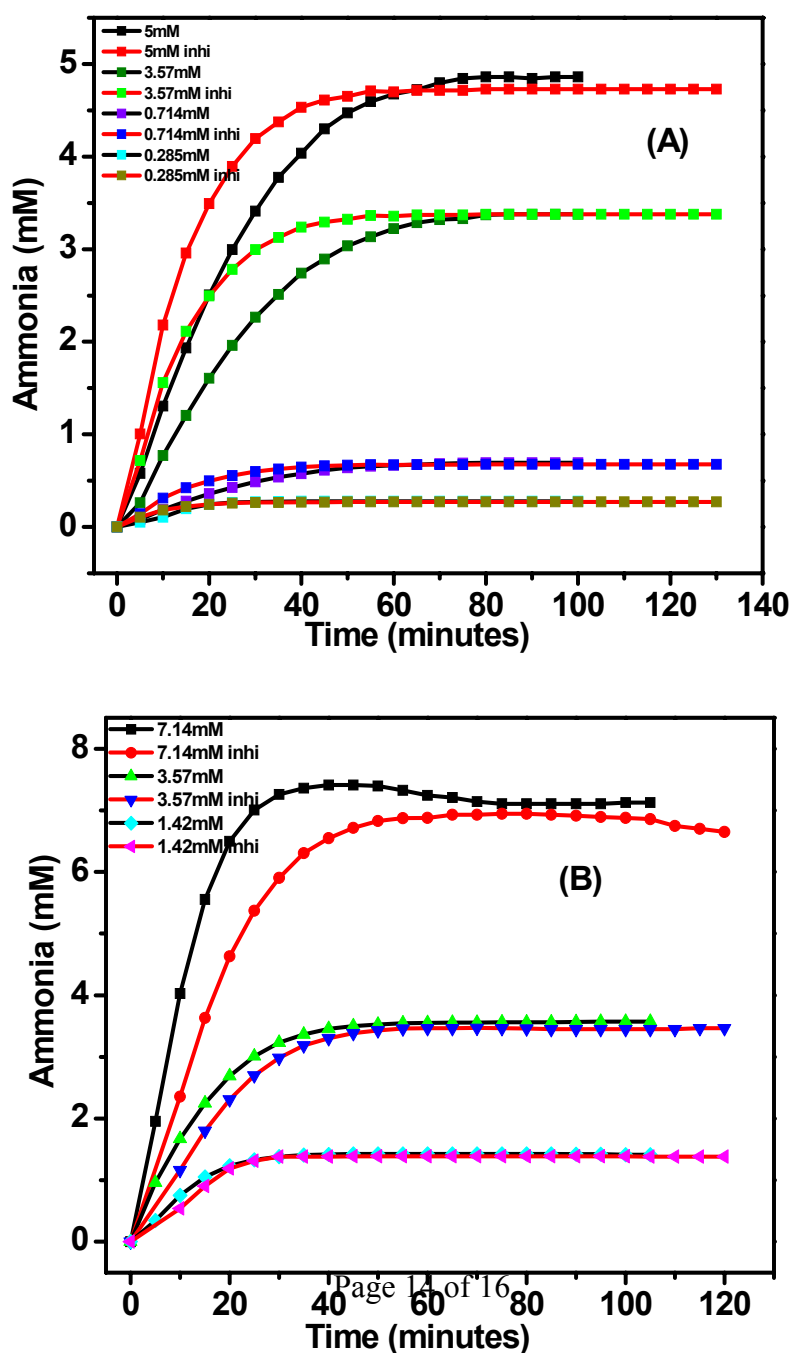
ESI figure 8: Progression curves of urease-urea hydrolysis monitored by Berthelot method with and without inhibitor (Ag^+) both in solution and hydrogel states. In all the cases urease enzyme and inhibitor (Ag^+) concentration are kept constant. (A) solution state; enzyme concentration= $0.011\mu mol/cm^3$ and inhibitor concentration = 0.556 ppb (B) hydrogel state; enzyme concentration = 360 $\mu mol/cm^3$ and inhibitor concentration = 0.571 ppb . Black lines are non inhibition processes and red lines are inhibition processes. Urea concentrations are mentioned in the figures.

ESI Figure 9: Lineweaver-Burk (L-B) plots obtained from the progression curves (Supporting information Figures 6-8) of urease-urea hydrolysis in presence and absence of inhibitors. The left and right rows subfigures are for the solution and hydrogel states, respectively. Lines in the figures are the best linear fit lines obtained from the data points.

ESI Table 1: The slopes and intercepts obtained from the L-B Plots presented in supporting information Figure 9.

	Without Hg^{2+}	With Hg^{2+}	Without Cu^{2+}	With Cu^{2+}	Without Ag^{+}	With Ag^{+}
Hydrogel State						
Slope	26.73	24.05	13.22	13.31	14.73	13.98
Intercept	7.1	10.41	2.98	3.59	7.72	10.07
Solution State						
	Without Hg^{2+}	With Hg^{2+}	Without Cu^{2+}	With Cu^{2+}	Without Ag^{+}	With Ag^{+}
Slope	21.86	40.98	11.14	19.72	8.47	12.01
Intercept	37.69	51.47	22.52	56.25	19.17	27.19