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ORIGINAL ARTICLE

Inhibition assays of free and immobilized urease for detecting hexavalent chromium in water samples

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Abstract

The present work describes the inhibition studies of free as well as immobilized urease by different heavy metals. Porous silicon (PS) films prepared by electrochemical etching were used for urease immobilization by physical adsorption. The enzyme was subjected to varying concentrations of Cr^{6+}, Cr^{3+}, Cu^{2+}, Fe^{2+}, Cd^{2+} and Ni^{2+} and analyzed for the variation in the activity. To study the effect of other heavy metals on the interaction of urease and Cr^{6+}, free as well as immobilized urease was subjected to the combination of each metal ion with Cr^{6+}. Results proved the sensitivity of free as well as immobilized urease towards heavy metals by observed reduction in activity. Immobilized urease showed less degree of inhibition compared to free urease when tested for inhibition by individual metal ions and in combination with Cr^{6+}. IC_{50} values were found higher for inhibition by the combination of metal ions with Cr^{6+}. Interaction of heavy metal ions with functional groups in active site of urease and limitations of mass transfer are the two factors responsible for the variation in activity of urease. Relation between the variation of urease activity and amount of heavy metals can be applied in biosensor development for determining the concentration of Cr^{6+} present in the water samples.

Keywords Urease · Immobilization · Porous silicon · Chromium · Heavy metals · Urease inhibition

Introduction

Enzymes are known for their high catalytic activity and specificity towards the substrate and are in use for various industrial and day to day activities (Choi et al. 2015). Enzymes catalyze the reaction by decreasing activation energy and increase reaction rate (Benkovic et al. 2003). Enzyme activity is a function of active site structure where the substrate interacts with the enzyme. Interaction of substrate with enzyme involves formation and breaking of bonds resulting in energy release. The energy released lowers the activation energy and carries out reactions on the substrate. Optimum environmental conditions determine the extent of enzyme activity and immobilization may offer the advantages of enzymes applications in adverse conditions (Ali Khan and Alzohairy 2010). Immobilization provides enzyme stability against structural deformation and various unfavorable environmental conditions (Hartmann and Jung 2010; Zheng et al. 2015b). In addition, ease of separation and higher reusability by the means of immobilization offers economic benefits over free enzymes (Zheng et al. 2015a, 2017).

Porous silicon (PS) is one of the widely used support material used for enzyme immobilization in biosensors (Daneshjou et al. 2017; Fernandez et al. 2008; Khaldi et al. 2017; Li et al. 2009; Smith et al. 2008; Vemulachedu et al. 2009; Yun et al. 2012). PS offers a higher surface-to-volume ratio, photoluminescence at room temperature and economical alternative over the other support materials (Dhanekar and Jain 2013; Thust et al. 1996).

The activity of enzymes varies with the presence of organic compounds, heavy metals and substrate analogues. Heavy metals such as cadmium (Cd), chromium (Cr), copper (Cu), ferrous (Fe), mercury (Hg), nickel (Ni), and silver (Ag) affect activity of various enzymes as reported by numerous studies (Attar et al. 2014; Do and Lin 2016; Fopase et al. 2019; Moyo et al. 2014; Yue et al. 2017). Cr, a well-known abundant element on earth, its different oxidative forms Cr^{3+}

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and Cr^{6+} have shown influence on the activity of various enzymes. Studies have reported some adverse effects of Cr on humans and environment due to its cellular permeability after exceeding certain concentration (Biswas et al. 2017). Different enzymes reported for variation in activity in the presence of Cr are: catalase (Chen et al. 2018), glucose oxidase (Syshchyk et al. 2015), hydrogen peroxidase (Zhou et al. 2012), tyrosinase (Domínguez-Renedo et al. 2004) and urease (Fopase et al. 2019; Nepomuscene et al. 2007).

Among many of the enzymes, Jack bean urease (*Canavalia ensiformis*; EC 3.5.1.5) has shown sensitivity towards both oxidative forms of Cr (Behbehani et al. 2012; Zhang et al. 2011). Urease is well-studied enzyme and has been in use for the development of various biosensors (Li et al. 2018; Marchenko et al. 2018; Soni et al. 2018; Vaghela et al. 2018; Vaidya and Annapure 2019). Various studies have reported inhibition of urease in the presence of other heavy metals such as cadmium, nickel, silver (Gumpu et al. 2015), cobalt (Jing et al. 2016), copper (Pan et al. 2016), lead, mercury (Do and Lin 2016), mercury (Domínguez-Renedo et al. 2009), and zinc (Wieczorek et al. 2015). The sensitivity of urease towards the heavy metals makes the enzyme best suitable for the development analytical system for the contaminants. Direct and indirect sensing of analytes by urease is applied due to sensitivity of urease towards the inhibitor molecules. Reduced activity of urease by presence of Cr showed its sensitivity towards Cr that makes the enzyme best suitable for the development of analytical system for the Cr.

The present work illustrates the effects of different heavy metals (Cr^{6+}, Cr^{3+}, Cd^{2+}, Cu^{2+}, Ni^{2+} and Fe^{2+}) on urease activity in free as well as PS-immobilized form. The work emphasizes the interaction of urease with Cr^{6+} and also reports the influence of other heavy metal ions on the above stated interaction. The obtained relation between enzyme activities with varying metal ion concentrations is useful for establishing calibration plots for biosensing of respective metals ions.

Materials and methods

Materials

Commercial Jack bean urease (Sigma-Aldrich) solution was prepared in phosphate buffer (pH 7). Laboratory grade urea was purchased from Merck chemicals. Heavy metal solutions were prepared using their respective salts: potassium dichromate ($K_2Cr_2O_7$) for Cr^{6+}, chromium chloride ($CrCl_3$) for Cr^{3+}, copper sulfate ($CuSO_4$) for Cu^{2+}, ferrous sulfate (Fe_2SO_4) for Fe^{2+}, cadmium nitrate ($Cd(NO_3)_2$) for Cd^{2+}, and nickel chloride ($NiCl_2$) for Ni^{2+}.

Phenate method was used to detect NH^{4+} ions. Phenate reagent (100 mL) was prepared using 5 g phenol and 95% ethanol. Sodium nitroprusside (0.5%) and alkaline reagent (trisodium citrate 10 g and sodium hydroxide 0.5 g for 100 mL) were prepared using deionized water. Standard graph for NH_4^+ was prepared using $(NH_4)_2SO_4$.

Preparation of porous silicon

Electrochemical anodization method was used to prepare PS film. A crystalline silicon wafer of resistivity 0.01–0.02 Ω cm and an electrolyte comprising 48% hydrofluoric acid and pure acetic acid (1:4) were used for anodization. A constant current of 30 mA/cm^2 was supplied through a programmable power supply unit for 10 min. Porous layer formed on the wafer surface was lifted up and were kept in a vacuum until use. Figure 1 shows the schematic diagram of the electrochemical anodization cell.

Urease immobilization

Method of physical adsorption was applied for the immobilization of urease. Approximately 1 cm^2 of PS film was used for adsorption of urease. 20 µl of urease solution was poured on the PS surface and allowed to dry at room temperature.

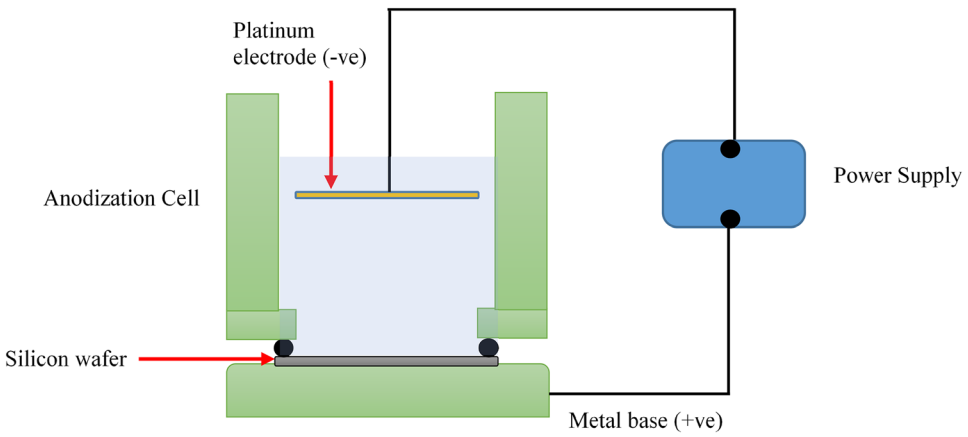

Fig. 1 Schematic diagram of electrochemical anodization cell

After drying, urease-immobilized PS films were kept at 4 °C until use. Adsorption of urease over the PS films was observed with the help of scanning electron microscopy (SEM) analysis.

Urease assays

Activity assays were performed to compare the activities of the free and PS-immobilized form of urease. For 10 ml reaction volume, 20 µl free urease and 1 ml urea were added and incubated for 15 min at 30 °C. Ammonium ions released by the action of urease on urea were detected by the phenate reagent method. Phenate reagent and sodium nitroprusside (400 µl each) were added to the reaction mixture and mixed well. One milliliter alkaline reagent was added to reaction volume and kept in dark incubation for 30 min. The color developed was analyzed spectrophotometrically at 630 nm. For immobilized assays, urease-immobilized PS films were directly dipped into the reaction mixture and removed after for 10 min of incubation. The samples were analyzed spectrophotometric at 630 nm.

Urease inhibition assays

Inhibition assays studied the effects of various metal ions on the urease activity. 1 ml of varying concentrations of different metal ions solution was added to the urease assay mixtures and analyzed spectrophotometrically at 630 nm.

To emphasize the effect of Cr^{6+}, urease activity was analysed in the presence of Cr^{6+} in combination with each of the stated metal ions. Varying concentrations of metal ions were added to the reaction mixture of urease with Cr^{6+} and analyzed spectrophotometrically. Cr^{6+} concentration used for coexisting ions studies was determined by observed urease inhibition pattern for Cr^{6+} individually. All inhibition assays were performed for free as well as for PS-immobilized urease.

Results and discussion

Physical adsorption

Porous silicon film is a porous material with the pores of a specific diameter range over the surface. Parameters used for PS preparation resulted in the formation of pores within the diameter range of 20–40 nm. Physical adsorption relies on the entrapment of enzyme molecules within the pores present on the surface of the film (Biswas et al. 2017). Urease has a molecular diameter of approximately 16 nm (Erickson 2009) which allows the entrapment of urease molecules within the pores obtained over the PS surface. Figure 2 shows the SEM micrographs for urease adsorbed on the PS surface. The porous structure of the PS films appears covered with the layer of urease molecules.

Studies have reported similar results for enzyme immobilized by physical adsorption methods (Chaudhari et al. 2005; Saleem et al. 2016). The change in the appearance of PS surface observed by SEM confirms the presence of enzyme molecules on PS film. Retention of enzyme molecules on PS surface is mainly due to van der Waal forces, hydrogen bonds and ionic charges present (Jesionowski et al. 2014; Saleem et al. 2016). Enzyme solution with less ionic strength shows the interaction of charged amino acids and OH groups of PS via electrostatic forces (Zhou and Hartmann 2013). While for immobilizing condition with the pH equals to the p*I* of the enzyme molecules, van der Waals forces play a crucial role in the retention of enzyme molecules. Van der Waals forces resulted in denser clusters of enzyme molecules over PS surface (Sang et al. 2011). The activity of the adsorbed enzyme depends on orientation of the active sites during immobilization process.

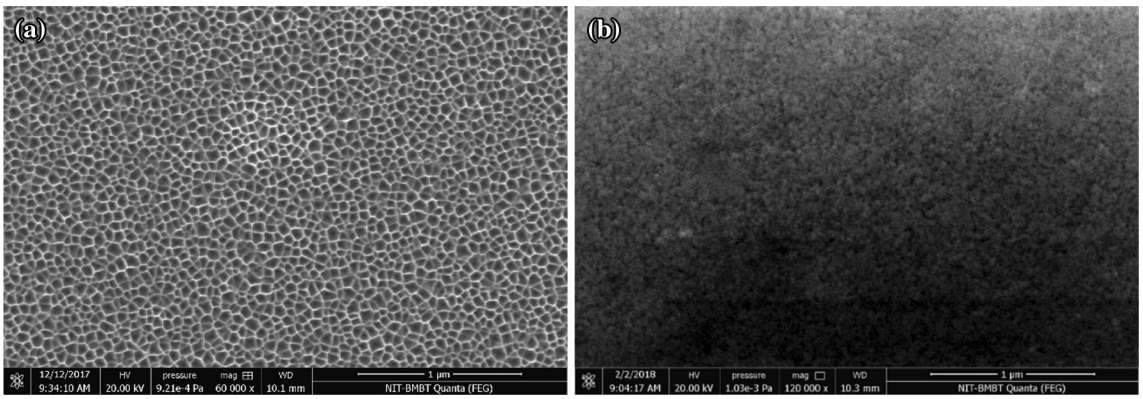

Fig. 2 **a** SEM micrograph of PS film. **b** Urease adsorbed over the PS films

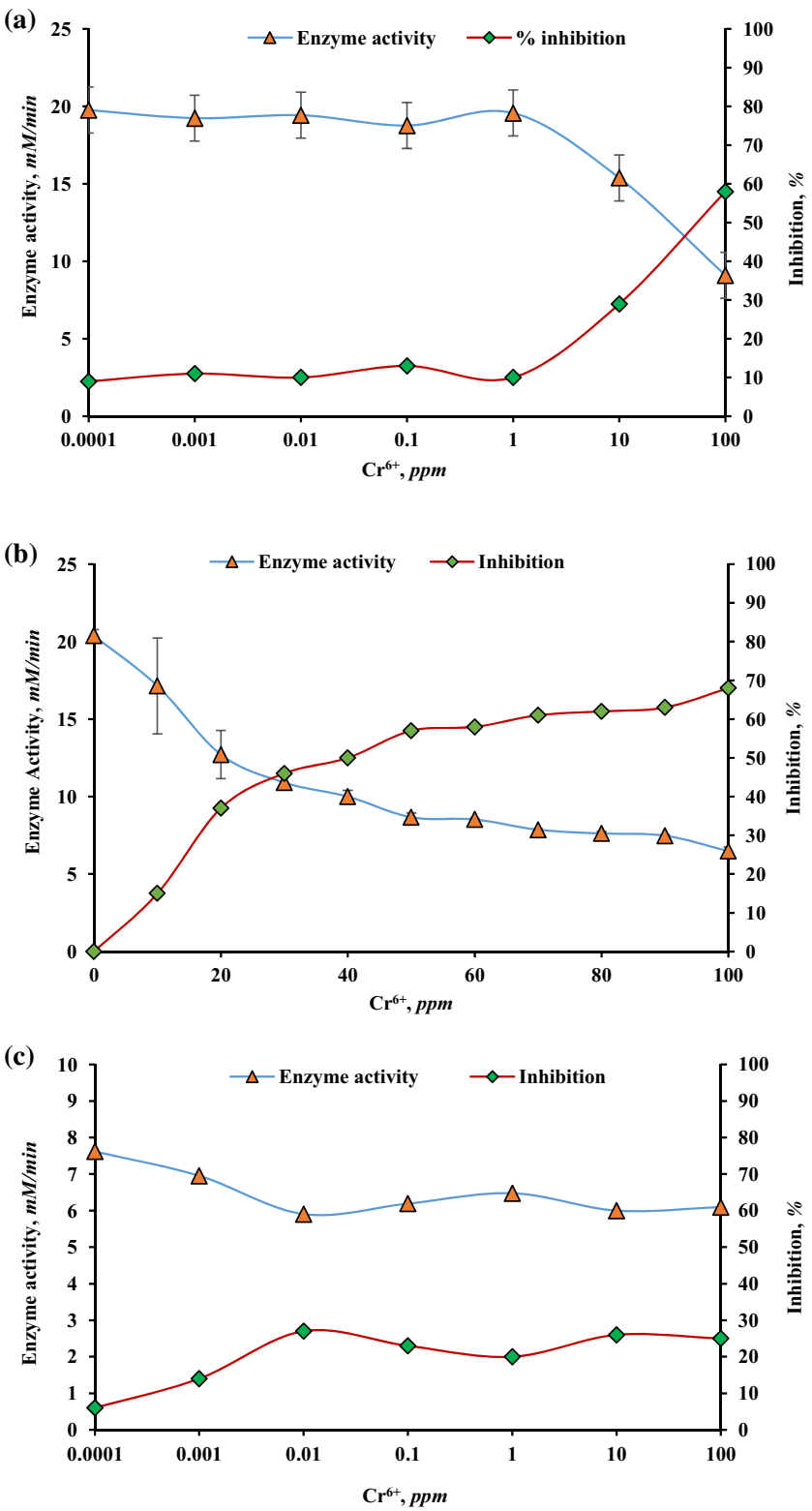

Fig. 3 Effect of Cr^{6+} on **a** free urease activity over 0.0001 to 100 ppm of Cr^{6+}. **b** Free urease activity over 0–100 ppm of Cr^{6+}. **c** Immobilized urease activity over 0.0001 to 100 ppm of Cr^{6+}

Urease inhibition assay

Enzyme inhibition assays provide the extent of inhibition of enzyme activity for the respective inhibitor concentrations. The relation between the degree of inhibition and inhibitor concentration gives a calibration plot to determine inhibitor concentration. Assays performed with various

concentrations of heavy metal ions showed the inhibition patterns of urease and influenced overall enzyme activity.

Cr^{6+}

Inhibition assays were performed for inhibitor concentrations ranging from 0.0001 to 100 ppm. Cr^{6+} concentration up to 1 ppm showed no significant effect on the activity of free urease (Fig. 3a). Above 1 ppm of Cr^{6+}, activity of urease linearly decreased with the increasing Cr^{6+} concentration and showed nearly 65% inhibition of free urease for 100 ppm Cr^{6+} concentrations (Fig. 3b). 40 ppm Cr^{6+} was the observed IC_{50} value for free urease. IC_{50} value is the inhibitor concentration for which activity reduces to half of its actual value. For immobilized urease, activity was affected by trace concentration of Cr^{6+}. However, the ultimate decline in activity for the highest tested concentration of Cr^{6+} was found as 60%. Compared to free urease, immobilized urease showed only 30% inhibition by 100 ppm of Cr^{6+} (Fig. 3c).

Cr^{6+} and Cr^{3+}

Free urease reported more sensitivity towards the Cr^{3+} compared to Cr^{6+} and showed a decrease in the activity by up to 44% for the concentration of 0.0001 ppm. Figure 4a represents inhibition pattern of free urease for varying Cr^{3+} concentrations. Free urease activity decreased with increasing Cr^{3+} concentrations and showed complete inhibition of the enzyme above 1 ppm of Cr^{3+}. 0.001 ppm of Cr^{3+} was the observed IC_{50} value of free urease. However, immobilized urease showed less sensitivity for trace Cr^{3+} concentrations with only 10% inhibition of activity. Inhibition was increased with increasing Cr^{3+} concentrations and approximately 90% loss of activity was observed (Fig. 4b) for the maximum inhibitor concentration. The observed IC_{50} value of immobilized urease for Cr^{3+} was 0.001 ppm.

Combine inhibition effect of Cr^{3+} and Cr^{6+} on free urease reported negligible effect by 0.0001 ppm Cr^{3+}. Increasing concentration of inhibitors combination resulted in decreasing activity and ultimately complete inhibition of

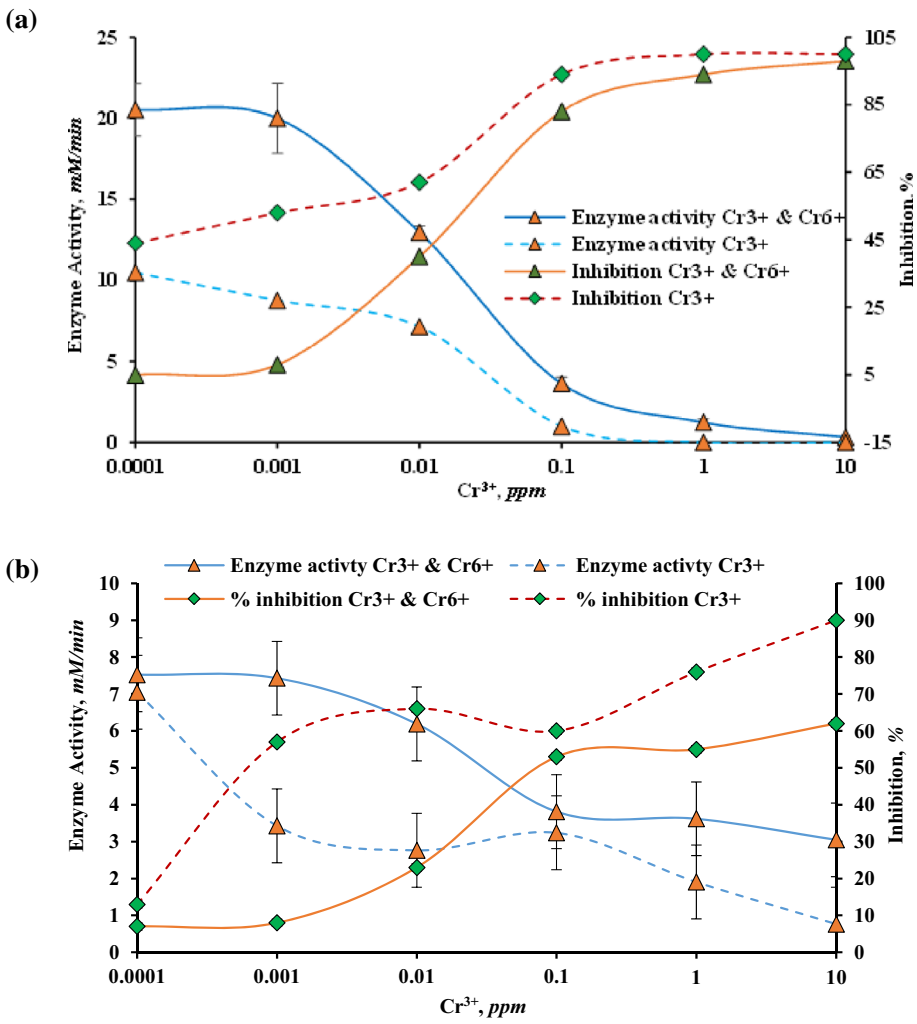

Fig. 4 Effect of Cr^{3+} and in combination with 1 ppm of Cr^{6+} on the activity of **a** free urease and **b** immobilized urease

free urease. The observed IC_{50} value of free urease was approximately 0.01 ppm of a combination of Cr^{3+} and Cr^{6+}. Study confirmed that free urease has higher sensitivity for Cr^{3+} alone compared to that of in combination of Cr^{3+} and Cr^{6+}. Immobilized urease showed a less degree of inhibition compared to free urease for initial concentrations of inhibitor combination. The activity of immobilized urease showed only 60% maximum inhibition with an IC_{50} value increased to 0.1 ppm. Combination of Cr^{3+} and Cr^{6+} affected the activity of immobilized urease comparatively lesser to Cr^{3+} alone.

Cr^{6+} and Cu^{2+}

The presence of Cu^{2+} affected the activity of free urease up to 60% for a tested range of inhibitor concentrations (Fig. 5a). Lower Cu^{2+} concentration showed nearly 20% inhibition of free urease activity and IC_{50} value was observed as 0.01 ppm of Cu^{2+}. In combination with Cr^{6+}, Cu^{2+} showed a significant effect on free urease activity leading to complete inhibition by Cu^{2+} concentrations above 1 ppm. Concentration range of 0.01–0.1 ppm of Cu^{2+} showed the IC_{50} value of free urease for a combination of Cu^{2+} with 1 ppm of Cr^{6+}. For immobilized urease, highest Cu^{2+} concentration tested resulted in 60% inhibition, which was similar to the extent of inhibition of free urease. The IC_{50} value of immobilized urease for Cu^{2+} was 0.1 ppm.

Inhibition by a combination of varying Cu^{2+} concentrations and 1 ppm of Cr^{6+} showed different effects on free and immobilized urease (Fig. 5b). 0.1 ppm Cu^{2+} and higher concentrations in combination with Cr^{6+} completely inhibited the activity of free urease while for immobilized urease only 47% inhibition was achieved for the tested range. The observed IC_{50} values of free and immobilized urease were in the range of 0.01–0.1 ppm of Cu^{2+} and 10 ppm of Cu^{2+} in combination with 1 ppm of Cr^{6+}, respectively. Results signified that free urease has higher sensitivity towards an inhibitory combination of metal ions compared to Cu^{2+} alone.

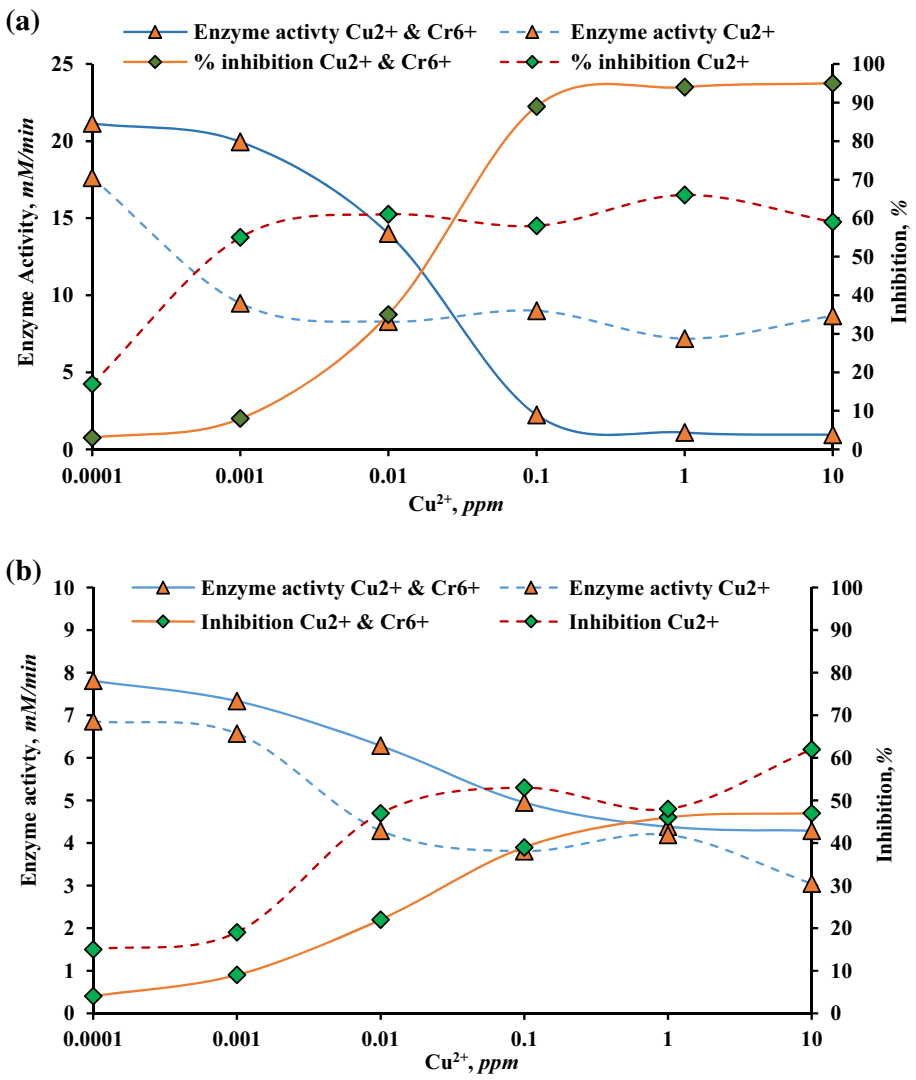

Fig. 5 Effect of Cu^{2+} and in combination with 1 ppm of Cr^{6+} on the activity of **a** free urease and **b** immobilized urease

Cr^{6+} and Ni^{2+}

Free urease showed high sensitivity for the presence of Ni^{2+} by showing 40% inhibition by trace amount of Ni^{2+} (Fig. 6a). The activity was decreased further with increasing Ni^{2+} concentration and reported complete inhibition of free urease. The observed IC_{50} of urease for Ni^{2+} was approximately 0.0001 ppm. For immobilized urease, no such sensitivity was observed and only 20% inhibition within the tested range of Ni^{2+} was found (Fig. 6b).

Study for effects of Ni^{2+} and Cr^{6+} combination on free urease showed negligible sensitivity for the concentration up to 0.1 ppm of Ni^{2+}. Further increase in Ni^{2+} concentration resulted in a rapid decrease in activity followed by complete inhibition of free urease. However, immobilized urease showed moderate sensitivity towards the inhibitor combination with the observed IC_{50} value of 1 ppm. The highest Ni^{2+} concentration used in combination of Cr^{6+} showed 70% inhibition of immobilized urease.

Cr^{6+} and Cd^{2+}

Free urease showed sensitivity towards Cd^{2+} in the tested range and the IC_{50} value was observed between 0.01 and 0.1 ppm of Cd^{2+} (Fig. 7a). Free urease activity was completely inhibited by 10 ppm and above concentrations of Cd^{2+}. Comparatively, Cd^{2+} showing only 20% inhibition of activity showed a small extent of inhibition of immobilized urease for the tested range (Fig. 7b).

For Cd^{2+} and Cr^{6+} combination, free urease showed no effect for Cd^{2+} concentrations less than 0.1 ppm. With further increases in metal ion concentrations, rapid decrease in activity of free urease was observed. IC_{50} value of free urease for Cd^{2+} and Cr^{6+} combination was in the range of 1–10 ppm of Cd^{2+}. For immobilized urease, Cd^{2+} and combination of Cd^{2+} with Cr^{6+} showed a similar trend of inhibition until the concentration of 0.1 ppm of Cd^{2+}. Activity was further decreased for the combination of inhibitors. Inhibition studies showed around 60% inhibition of immobilized

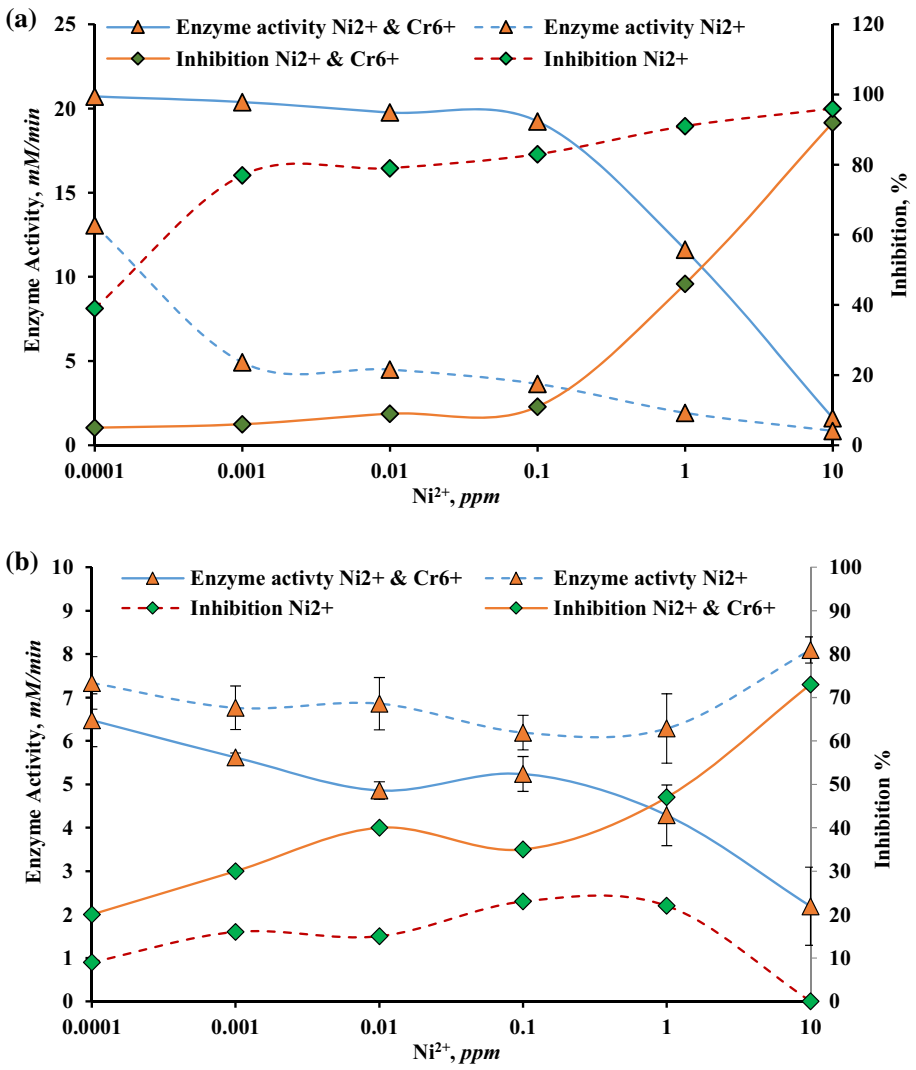

Fig. 6 Effect of Ni^{2+} and in combination with 1 ppm of Cr^{6+} on the activity of **a** free urease and **b** immobilized urease

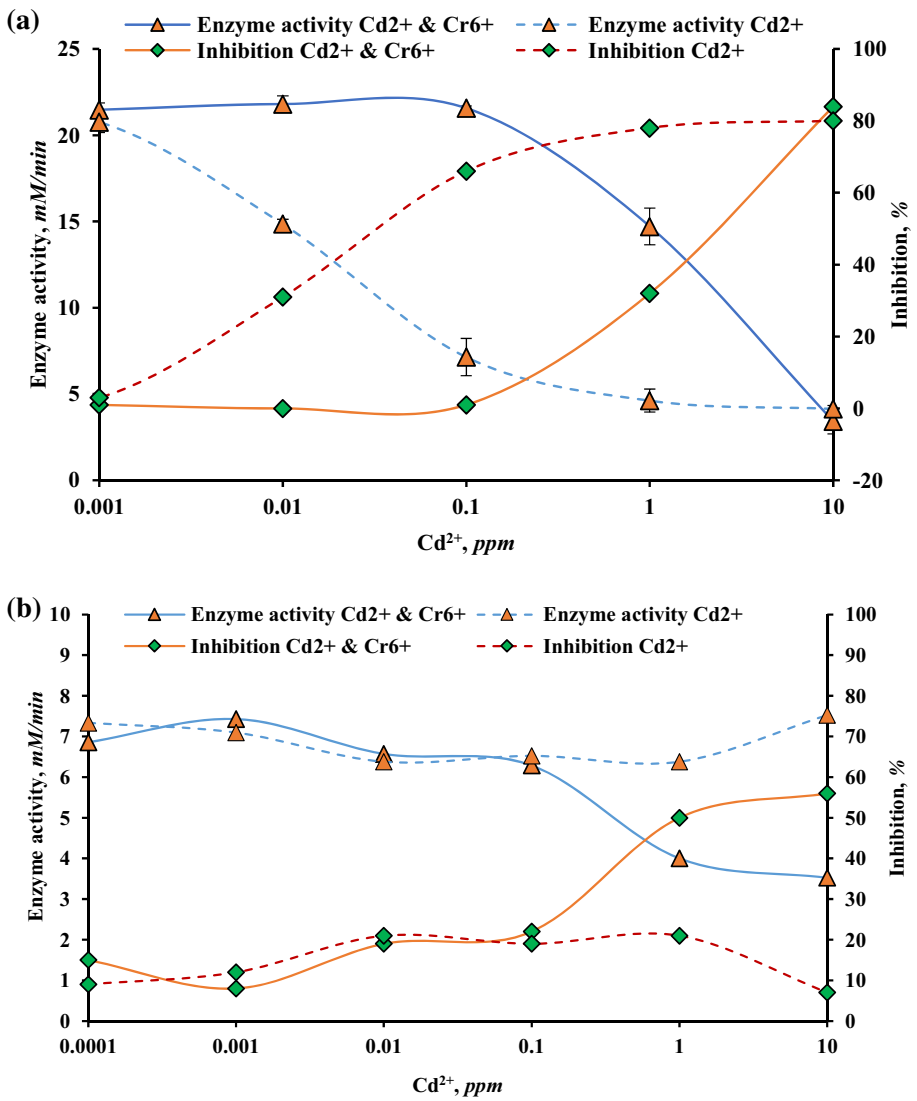

Fig. 7 Effect of Cd^{2+} and in combination with 1 ppm of Cr^{6+} on the activity of **a** free urease and **b** immobilized urease

urease by inhibitors combination with the IC_{50} value of 1 ppm of Cd^{2+}.

Cr^{6+} and Fe^{2+}

The presence of Fe^{2+} reported strong inhibition of free urease for concentration higher than 0.1 ppm (Fig. 8a). The IC_{50} value of free urease for Fe^{2+} was observed as 0.01 ppm, approximately. Immobilized urease showed around 60% inhibition for the tested range of Fe^{2+} (Fig. 8b). However, the extent of inhibition for immobilized urease was less than that of free urease. The observed IC_{50} value of immobilized urease for Fe^{2+} was 0.1 ppm, approximately. Combined effect of Fe^{2+} and Cr^{6+} showed only 20% inhibition of free urease and 30% inhibition of immobilized urease for the tested range.

Table 1 illustrates the IC_{50} values of free and immobilized urease for the above-mentioned heavy metal ions and in combination with 1 ppm of Cr^{6+}. Based on IC_{50} values, the order of sensitivity for free urease was observed as $Ni^{2+} > Cu^{2+} > Cr^{3+} > Fe^{2+} > Cd^{2+} > Cr^{6+}$. Sensitivity of urease towards heavy metals is previously reported by various other researchers (Krajewska and Brindell 2016; Preininger and Wolfbeis 1996; Volotovsky and Kim 1997). Reported order of degree of inhibitions for soil urease by various heavy metals is: Ag > Hg > Cu > Ni > Co > Cd > Fe > Zn > Pb (Preininger and Wolfbeis 1996). Another study suggested the inhibition order as $Cu^{2+} > As^{3+} > Cr^{3+} > Cd^{2+} > Zn^{2+} > Pb^{2+}$

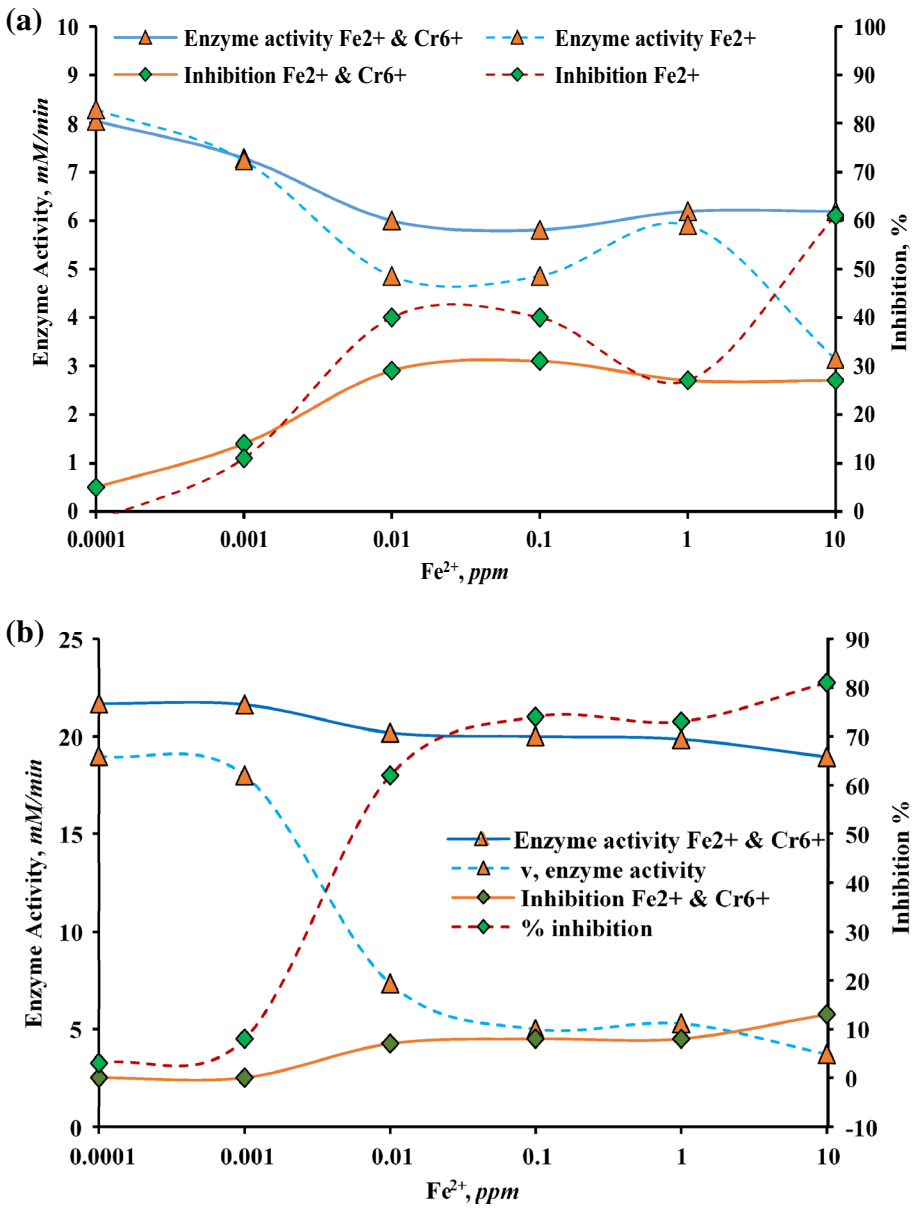

Fig. 8 Effect of Fe^{2+} and in combination with 1 ppm of Cr^{6+} on the activity of **a** free urease and **b** immobilized urease

Table 1 IC_{50} values of free and immobilized urease for individual different heavy metal ions and in combination with 1 ppm Cr^{6+}

Metal ion	IC_{50} (ppm) for free urease		IC_{50} (ppm) for immobilized urease	
	In individual	With 1 ppm of Cr^{6+}	In individual	With 1 ppm of Cr^{6+}
Cr^{6+}	40	NA	< 50% inhibition	NA
Cr^{3+}	0.001	≈ 0.01	0.001	0.1
Cu^{2+}	0.001	≈ 0.01	0.01	1
Cd^{2+}	0.01–0.1	1–10	< 50% inhibition	1
Ni^{2+}	0.0001–0.001	1	< 50% inhibition	1
Fe^{2+}	≈ 0.01	< 50% inhibition	1	< 50% inhibition

in case of urease extracted from soybeans (Magomya et al. 2017).

Enzyme inhibition by heavy metals is a well-studied research area. Interaction of metal ions with the active site results in the decreased activity of urease. Interaction of heavy metal ions with –SH groups of Cys-592 in the active site is analogous to the formation of metal sulfides (Zaborska et al. 2001). Resulting insoluble metal sulfides

are toxic to the enzyme thus, the inhibition (Shaw and Raval 1961). Studies correlating toxicity and insolubility of metals sulfides have confirmed the formation of metal sulfides at active sites of urease (Preininger and Wolfbeis 1996; Shaw and Raval 1961; Toren and Burger 1968). Some of the studies have reported few evidences for involvement of some other functional groups of enzyme in addition to –SH groups. Nitrogen- (from histidine) and oxygen- (from aspartic and glutamic acid) are the other significant groups which may involve the binding of metal ions (Follmer and Carlini 2005; Krajewska 2008). The choice of binding site for individual metal ion is based on the concept of hard and soft acids-bases concept which includes the strength of electronegativity (Pearson 1968). Sulfhydryl groups present in the active site are responsible for catalytic structure of urease active site. Binding of metal ions to –SH groups results in the change of catalytic structure of active site leading to variation in enzyme activity. Most of the metal inhibition reactions of urease are non-competitive and slow binding processes (Liu et al. 1995; Oehlschläger et al. 1998; Zaborska et al. 2001).

In overall, the sensitivity of immobilized urease was observed comparatively lesser than free urease. Sensitivity here is the function of variation in enzyme activity by least amount of metal ion concentration. Decrement in activity of urease is due to involvement of –SH groups in active site with metal ions as discussed previously. There are two possible explanations for the reduced sensitivity of immobilized urease: first, the active site of immobilized urease may not readily available for the binding with metal ions because of unfavorable orientations (with regarding inhibitors). Second, mass transfer limitations might have restricted passage of metal ions to active sites, which is reflected in reduced enzyme activity. Based on the two possible reasons, reduced sensitivity resulted in the higher IC_{50} values of metal ion concentrations.

Conclusion

With the parameters used for anodization of crystal silicon wafer, thin porous films with average pore size of 40 nm were obtained. Urease molecules with molecular size of approximately 16 nm were immobilized on the surface of PS films. SEM analysis confirmed the physical adsorption of urease molecules on PS surface based on the change of surface morphology of PS films.

Inhibition assays with Cr^{6+}, Cr^{3+}, Cu^{2+}, Fe^{2+}, Cd^{2+} and Ni^{2+} for urease in free and immobilized form were carried out and the results showed the higher sensitivity of free urease towards trace amount of Cr^{3+} compared to Cr^{6+}. Based on the IC_{50} values, the order of inhibition of free urease by tested metals was $Ni^{2+} > Cu^{2+} > Cr^{3+} > Fe^{2+} > Cd^{2+} > Cr^{6+}$. In the case of immobilized urease, Cr^{3+} and Cu^{2+} showed higher inhibition capability compared to all other metal ions tested.

Effect of other heavy metal ions on the interaction of Cr^{6+} with urease showed the increase in IC_{50} values for urease in free as well as immobilized form. In addition, the sensitivity was observed to decrease for all the metal ions. In overall, urease has shown higher sensitivity towards Cr^{3+} in free, immobilized and in combination with Cr^{6+} followed by Cu^{2+}.

Variation in the activity of urease showed a relation with metal ion concentration. The relation may apply for the development of an analytical system to determine the respective concentration metals. Effects of other different heavy metals on the inhibition of urease by Cr^{6+} may offer crucial help while applying the concept for determination of dissolved Cr^{6+} present in the water samples.

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest to disclose.

References

Ali Khan A, Alzohairy MA (2010) Recent advances and applications of immobilized enzyme technologies: a review. Res J Biol Sci 5(8):565–575. https://doi.org/10.3923/rjbsci.2010.565.575

Attar A, Emilia Ghica M, Amine A, Brett CMA (2014) Poly(neutral red) based hydrogen peroxide biosensor for chromium determination by inhibition measurements. J Hazard Mater 279:348–355. https://doi.org/10.1016/J.JHAZMAT.2014.07.019

Behbehani GR, Barzegar L, Mohebbian M (2012) Strong inhibition of jack bean urease by chromium (III). Biosci Biotechnol Res Asia 9(2):2–6

Benkovic SJ, Hammes-Schiffer S, Karplus M, Truhlar DG (2003) A perspective on enzyme catalysis. Science 301(5637):1196–1202. https://doi.org/10.1126/science.1085515

Biswas P, Karn AK, Balasubramanian P, Kale PG (2017) Biosensor for detection of dissolved chromium in potable water: a review. Biosens Bioelectron 94:589–604. https://doi.org/10.1016/j.bios.2017.03.043

Chaudhari PS, Gokarna A, Kulkarni M, Karve MS, Bhoraskar SV (2005) Porous silicon as an entrapping matrix for the immobilization of urease. Sens Actuators B Chem 107:258–263. https://doi.org/10.1016/j.snb.2004.10.009

Chen L, Zhang J, Zhu Y, Zhang Y (2018) Interaction of chromium(III) or chromium(VI) with catalase and its effect on the structure and function of catalase: an in vitro study. Food Chem 244:378–385. https://doi.org/10.1016/j.foodchem.2017.10.062

Choi J-M, Han S-S, Kim H-S (2015) Industrial applications of enzyme biocatalysis: current status and future aspects. Biotechnol Adv 33:1443–1454. https://doi.org/10.1016/J.BIOTECHADV.2015.02.014

Daneshjou S, Dabirmanesh B, Rahimi F, Khajeh K (2017) Porous silicon nanoparticle as a stabilizing support for chondroitinase. Int J Biol Macromol 94:852–858. https://doi.org/10.1016/j.ijbiomac.2016.10.077

Dhanekar S, Jain S (2013) Porous silicon biosensor: Current status. Biosens Bioelectron 41:54–64. https://doi.org/10.1016/j.bios.2012.09.045

Do JS, Lin KH (2016) Kinetics of urease inhibition-based amperometric biosensors for mercury and lead ions detection. J Taiwan Inst Chem Eng 63:25–32. https://doi.org/10.1016/j.jtice.2016.03.011

Domínguez Renedo O, Lomillo A, Arcos Martinez MA, M.J (2004) Optimisation procedure for the inhibitive determination of chromium(III) using an amperometric tyrosinase biosensor. Anal Chim Acta 521:215–221. https://doi.org/10.1016/J.ACA.2004.06.026

Domínguez-Renedo O, Alonso-Lomillo MAA, Ferreira-Gonçalves L, Arcos-Martínez MJJ (2009) Development of urease based amperometric biosensors for the inhibitive determination of Hg (II). Talanta 79:1306–1310. https://doi.org/10.1016/j.talanta.2009.05.043

Erickson HP (2009) Size and shape of protein molecules at the nanometer level determined by sedimentation, gel filtration, and electron microscopy. Biol Proced Online 11:32–51. https://doi.org/10.1007/s12575-009-9008-x

Fernandez RE, Bhattacharya E, Chadha A (2008) Covalent immobilization of Pseudomonas cepacia lipase on semiconducting materials. Appl Surf Sci 254:4512–4519. https://doi.org/10.1016/j.apsusc.2008.01.099

Follmer C, Carlini CR (2005) Effect of chemical modification of histidines on the copper-induced oligomerization of jack bean urease (EC 3.5.1.5). Arch Biochem Biophys 435:15–20. https://doi.org/10.1016/j.abb.2004.12.001

Fopase R, Nayak S, Mohanta M, Kale P, Balasubramanian P (2019) Inhibition assays of urease for detecting trivalent chromium in drinking water. In: Green buildings and sustainable engineering. Springer, Singapore, pp 313–323

Gumpu MB, Sethuraman S, Krishnan UM, Rayappan JBB (2015) A review on detection of heavy metal ions in water—an electrochemical approach. Sens Actuators B Chem 213:515–533. https://doi.org/10.1016/j.snb.2015.02.122

Hartmann M, Jung D (2010) Biocatalysis with enzymes immobilized on mesoporous hosts: the status quo and future trends. J Mater Chem 20:844–857. https://doi.org/10.1039/B907869J

Jesionowski T, Zdarta J, Krajewska B (2014) Enzyme immobilization by adsorption: a review. Adsorption 20:801–821. https://doi.org/10.1007/s10450-014-9623-y

Jing C, Wang C, Yan K, Zhao K, Sheng G, Qu D, Niu F, Zhu H, You Z (2016) Synthesis, structures and urease inhibitory activity of cobalt(III) complexes with Schiff bases. Bioorganic Med Chem 24:270–276. https://doi.org/10.1016/j.bmc.2015.12.013

Khaldi K, Sam S, Lounas A, Yaddaden C, Gabouze NE (2017) Comparative investigation of two methods for acetylcholinesterase enzyme immobilization on modified porous silicon. Appl Surf Sci 421:148–154. https://doi.org/10.1016/j.apsusc.2016.12.169

Krajewska B (2008) Mono- (Ag, Hg) and di- (Cu, Hg) valent metal ions effects on the activity of jack bean urease. Probing the modes of metal binding to the enzyme. J Enzyme Inhib Med Chem 23:535–542. https://doi.org/10.1080/14756360701743051

Krajewska B, Brindell M (2016) Thermodynamic study of competitive inhibitors' binding to urease. J Therm Anal Calorim 123:2427–2439. https://doi.org/10.1007/s10973-015-5145-4

Li HL, Zhu Y, Xu D, Wan Y, Xia L, Zhao XS (2009) Vapor-phase silanization of oxidized porous silicon for stabilizing composition and photoluminescence. J Appl Phys 105. https://doi.org/10.1063/1.3133209

Li G, Xiao F, Liao S, Chen Q, Zhou J, Wu Z, Yu R (2018) Label-free 2D colloidal photonic crystal hydrogel biosensor for urea and urease inhibitor. Sens Actuators B Chem 277:591–597. https://doi.org/10.1016/j.snb.2018.09.059

Liu D, Yin A, Chen K, Ge K, Lihua L, Nie N, Yao S (1995) Determination of trace levels of mercury(II) based on the inhibition of urease using a saw/impedance enzyme transducer. Anal Lett 28:vii–xiii. https://doi.org/10.1080/00032719508006396

Magomya A, Barminas J, Osemeahon S (2017) Assessment of metal-induced inhibition of soybean urease as a tool for measuring heavy metals in aqueous samples. IOSR J Appl Chem 10:61–70. https://doi.org/10.9790/5736-1006026170

Marchenko SV, Soldatkin OO, Kolomiets LA, Kornelyuk OI, Soldatkin AP (2018) Cyclodextrins application in urease-based biosensor for urea determination. Sens Lett 16:298–303. https://doi.org/10.1166/sl.2018.3955

Moyo M, Okonkwo JO, Agyei NM (2014) An amperometric biosensor based on horseradish peroxidase immobilized onto maize tassel-multi-walled carbon nanotubes modified glassy carbon electrode for determination of heavy metal ions in aqueous solution. Enzyme Microb Technol 56:28–34. https://doi.org/10.1016/J.ENZMICTEC.2013.12.014

Nepomuscene NJ, Daniel D, Krastanov A (2007) Biosensor to detect chromium in wastewater. Biotechnol Biotechnol Equip 21:377–381. https://doi.org/10.1080/13102818.2007.10817477

Oehlschläger K, Hüttl R, Wolf G (1998) Calorimetric investigations of urease inhibition by heavy metal ions. Thermochim Acta 310:185–189. https://doi.org/10.1016/S0040-6031(97)00395-X

Pan L, Wang C, Yan K, Zhao K, Sheng G, Zhu H, Zhao X, Qu D, Niu F, You Z (2016) Synthesis, structures and *Helicobacter pylori* urease inhibitory activity of copper(II) complexes with tridentate aroylhydrazone ligands. J Inorg Biochem 159:22–28. https://doi.org/10.1016/j.jinorgbio.2016.02.017

Pearson RG (1968) Hard and soft acids and bases, HSAB, Part I fundamental principles. J Chem Educ 45:581–587

Preininger C, Wolfbeis OS (1996) Disposable cuvette test with integrated sensor layer for enzymatic determination of heavy metals. Biosens Bioelectron 11:981–990. https://doi.org/10.1016/0956-5663(96)87657-3

Saleem M, Rafiq M, Seo S-Y, Lee KH (2016) Acetylcholinesterase immobilization and characterization, and comparison of the activity of the porous silicon-immobilized enzyme with its free counterpart. Biosci Rep 36:e00311–e00311. https://doi.org/10.1042/BSR20150154

Sang LC, Vinu A, Coppens MO (2011) General description of the adsorption of proteins at their iso-electric point in nanoporous materials. Langmuir 27:13828–13837. https://doi.org/10.1021/la202907f

Shaw WHR, Raval DN (1961) The inhibition of urease by metal ions at pH 8.9. J Am Chem Soc 83:3184–3187

Smith RG, D'Souza N, Nicklin S (2008) A review of biosensors and biologically-inspired systems for explosives detection. Analyst 133:571. https://doi.org/10.1039/b717933m

Soni A, Surana RK, Jha SK (2018) Smartphone based optical biosensor for the detection of urea in saliva. Sens Actuators B Chem 269:346–353. https://doi.org/10.1016/j.snb.2018.04.108

Syshchyk O, Skryshevsky VA, Soldatkin OO, Soldatkin AP (2015) Enzyme biosensor systems based on porous silicon photoluminescence for detection of glucose, urea and heavy metals. Biosens Bioelectron 66:89–94. https://doi.org/10.1016/j.bios.2014.10.075

Thust M, Schöning MJ, Frohnhoff S, Arens-Fischer R, Kordos P, Lüth H (1996) Porous silicon as a substrate material for

potentiometric biosensors. Meas Sci Technol 7:26–29. https://doi.org/10.1088/0957-0233/7/1/003

Toren EC, Burger FJ (1968) Trace determination of metal ion inhibitors of the glucose-glucose oxidase system. Mikrochim Acta 56:1049–1058. https://doi.org/10.1007/BF01224060

Vaghela C, Kulkarni M, Haram S, Aiyer R, Karve M (2018) A novel inhibition based biosensor using urease nanoconjugate entrapped biocomposite membrane for potentiometric glyphosate detection. Int J Biol Macromol 108:32–40. https://doi.org/10.1016/J.IJBIOMAC.2017.11.136

Vaidya AM, Annapure US (2019) Enzymes in biosensors for food quality assessment. Enzym Food Biotechnol 32:659–674. https://doi.org/10.1016/B978-0-12-813280-7.00038-4

Vemulachedu H, Fernandez RE, Bhattacharya E, Chadha A (2009) Miniaturization of EISCAP sensor for triglyceride detection. J Mater Sci Mater Med 20:S229–S234. https://doi.org/10.1007/s10856-008-3534-y

Volotovsky V, Kim N (1997) Urease-based biosensor for mercuric ions determination. Sens Actuators B Chem 42:233–237. https://doi.org/10.1016/S0925-4005(97)80340-1

Wieczorek K, Wyszkowska J, Kucharski J (2015) Sensitivity of soil urease to soil contamination by zinc, copper, nickel, cadmium and lead. Fresenius Environ Bull 24:2496–2504

Yue L-M, Lee J, Lü Z-R, Yang J-M, Ye Z-M, Park Y-D (2017) Effect of Cd^{2+} on tyrosinase: Integration of inhibition kinetics with computational simulation. Int J Biol Macromol 94:836–844. https://doi.org/10.1016/J.IJBIOMAC.2016.09.001

Yun D, Song MJ, Hwang S, Hong SI (2012) Fabrication and electrochemical characterization of nanoporous silicon electrode for Amperometric urea biosensor. Jpn J Appl Phys 51:06FG02. https://doi.org/10.1143/JJAP.51.06FG02

Zaborska WX, Krajewska B, Leszko M, Olech Z (2001) Inhibition of urease by Ni^{2+} ions. Analysis of reaction progress curves. J Mol Catal B Enzym 13:103–108. https://doi.org/10.1016/S1381-1177(00)00234-4

Zhang HM, Zhang GC, Wang YQ (2011) The interaction of chromium(VI) with urease in solution. Biol Trace Elem Res 141:53–64. https://doi.org/10.1007/s12011-010-8718-x

Zheng M, Mao L, Huang F, Xiang X, Deng Q, Feng Y (2015a) A mixed-function-grafted magnetic mesoporous hollow silica microsphere immobilized lipase strategy for ultrafast transesterification in a solvent-free system. RSC Adv 5:43074–43080. https://doi.org/10.1039/C5RA05611J

Zheng M, Zhu J, Huang F, Xiang X, Shi J, Deng Q, Ma F, Feng Y (2015b) Enzymatic deacidification of the rice bran oil and simultaneous preparation of phytosterol esters-enriched functional oil catalyzed by immobilized lipase arrays. RSC Adv 5:70073–70079. https://doi.org/10.1039/C5RA11533G

Zheng M, Xiang X, Wang S, Shi J, Deng Q, Huang F, Cong R (2017) Lipase immobilized in ordered mesoporous silica: a powerful biocatalyst for ultrafast kinetic resolution of racemic secondary alcohols. Process Biochem 53:102–108. https://doi.org/10.1016/J.PROCBIO.2016.12.005

Zhou Z, Hartmann M (2013) Progress in enzyme immobilization in ordered mesoporous materials and related applications. Chem Soc Rev 42:3894–3912. https://doi.org/10.1039/c3cs60059a

Zhou Y, Li Y-S, Tian X-L, Zhang Y-Y, Yang L, Zhang J-H, Wang X-R, Lu S-Y, Ren H-L, Liu Z-S (2012) Enhanced ultrasensitive detection of Cr(III) using 5-thio-2-nitrobenzoic acid (TNBA) and horseradish peroxidase (HRP) dually modified gold nanoparticles (AuNPs). Sens Actuators B Chem 161:1108–1113. https://doi.org/10.1016/J.SNB.2011.12.035