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Catalytic signal amplification using [Fe^{III}(biuret-amide)]-mesoporous silica nanoparticles: visual cyanide detection†

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Catalytic signal amplification was used for the colorimetric detection of CN^- in aqueous media by using the enzyme catalase in tandem with mesoporous silica nanoparticle based synthetic HRP enzyme mimic Fe-MSNs. Signal amplification up to a maximum of eight fold was observed for the reporter ''oxidized TMB'' with respect to the added CN^- ion.

Signal amplification is a tool used over the last few decades for the ultrasensitive detection of analytes of interest.[1] The concept of signal amplification is well adopted by many biological assays such as in ELISA, where a molecular recognition event of the analyte and a surface bound receptor co-immobilizes an enzyme, which is used for the generation of an easy detectable signal. Recently, several efforts have been made by researchers to design chemical systems that mimic these enzymes and function as signal amplification catalysts for analyte sensing. However, unlike biological assays[2] the signal amplification technique is poorly exercised in the chemical approach of analyte sensing. For example, supramolecular allosteric catalysis,[3a] molecular wire approach,[3b] catalytic Heck reaction,[3c] and dendritic chain reactions[3d] have been used to amplify the signal for analyte detection. Recently gold nanoparticles have been used to improve biological assays by providing multivalency.[3e] Most of these chemical assays use either purely organic solvents or a mixture of organic and aqueous solvents, in contrast to biological assays, which are completely performed in aqueous medium. This limits the scope of these chemical assays since most biologically relevant analytes are present in water. Hence development of synthetic systems that detect analytes in aqueous media with high selectivity and sensitivity is extremely important from both biological and environmental perspectives.

One enzyme that is very routinely used for signal amplification is horseradish peroxidase (HRP) which in the presence of H_2O_2 converts a non-chemiluminescent molecule into a chemiluminescent molecule with several thousand turnovers, thus decreasing the detection limits by several fold.[4a] The increased concentration of reporter molecules produced upon catalytic reaction by HRP renders detection of very low amounts of analyte using UV-VIS and fluorescence spectroscopy feasible. Development of synthetic mimics of HRP which display improved properties in regards to stability and reactivity has been a frontier goal in biomimetic chemistry.[4b] We have recently developed [Fe^{III}(biuret-amide)]$^{2-}$ immobilized inside mesoporous silica nanoparticles (Fe-MSNs),[4c] that are excellent functional mimics of the enzyme HRP. Fe-MSNs have several attributes that make them very attractive as replacement for HRP: (i) Fe-MSNs exhibit excellent reactivity towards 3,3′,5,5′-tetramethylbenzidine (TMB) in the presence of H_2O_2 to generate a visually detectable green oxidation product at neutral pH; (ii) they exhibit very high stability, especially at low pH, high ionic strength and in the presence of anions such as CN^- which is known to inhibit HRP; (iii) [Fe^{III}(biuret-amide)]$^{2-}$ catalysts are mostly grafted inside the MSN pores (2–3 nm) which limits their interaction with other enzymes during biological assays; (iv) the amount of immobilized catalyst and the distance between them can be controlled to achieve varying reactivity.[5] Here, we show that Fe-MSNs can be used as catalyst in tandem with the enzyme catalase for visual detection of the toxic cyanide ion in water *via* signal amplification. We also demonstrate that using our protocol the maximum amount of reporter signal generated is approximately 8-fold that of the added CN^- ion (Scheme 1).

Cyanide ion (CN^-) is extremely toxic to human as it has a huge tendency to inhibit many heme containing enzymes.[6] In spite of its very high toxicity, cyanide is used widely for the industrial synthesis of nylon, plastics and in metallurgy. Even micromolar CN^- contamination from industrial effluents is dangerous for the mammalian world and aquatic life. Hence it is very important to have improved analytical detection techniques to quantify μM level CN^-. Most of the methods developed involve the interaction of CN^- with a receptor molecule which results in a direct signal transduction by either turning ''on'' or ''off'' the optical property of the switch.[7] The final read-outs of the signal include potentiometric,

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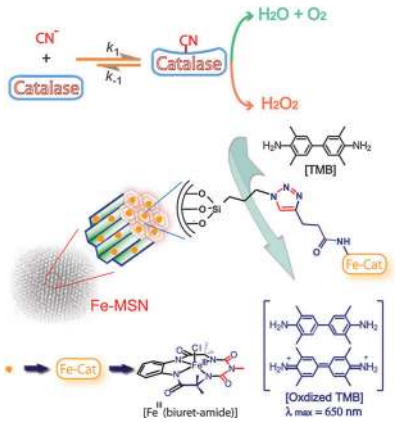

Scheme 1 Cyanide detection assay through signal amplification.

spectrophotometric and fluorimetric techniques. Recently, cobinamide has been used for the detection of CN^- spectrophotometrically by monitoring absorbances at 580 nm (LOD 8 nM).[7b] Many of these methods are inadequate since they employ organic co-solvents and also suffer from considerable interference from other anions such as F^-, Cl^-, S^{2-} and OAc^-. We have developed a method based on the consecutive catalytic reaction of the enzyme catalase and our HRP mimic Fe-MSNs. This allows visual detection of cyanide in aqueous media with a lowest detection limit of 0.9 μM without interference from other anions. Our analytical method consists of three consecutive reactions: (i) inhibition of the enzyme catalase by cyanide; (ii) disproportionation of H_2O_2 to water and oxygen by cyanide-free catalase; and (iii) activation of the residual H_2O_2 by Fe-MSNs to oxidize TMB to produce a green color which can be visually detected and quantified. It is very well known in literature that CN^- binds to the enzyme catalase very strongly and the binding constant is much higher than other common ions present in water. We therefore envisaged that increasing the amount of CN^- to catalase would lead to high residual H_2O_2 in the catalytic disproportionation of H_2O_2, which can then be converted to a colorimetric reporter molecule using our HRP mimic Fe-MSNs. In short, when no CN^- is added to catalase, the fast reaction rates of the enzyme would lead to complete disproportionation of H_2O_2. Therefore, addition of Fe-MSNs and TMB would not lead to any detectable colorimetric signal as Fe-MSNs are capable of oxidizing TMB only in the presence of H_2O_2. However, when CN^- is added to catalase, its catalytic disproportionation reaction will be inhibited and this leads to incomplete disproportionation of H_2O_2. The residual H_2O_2 can then be utilized by Fe-MSNs to oxidize TMB to generate a colorimetric signal which can be co-related to the amount of CN^- added.

To achieve this, the kinetics of H_2O_2 disproportionation by catalase in the presence and absence of CN^- was studied first to estimate the residual H_2O_2 in the reaction as a function of time.

The catalytic disproportionation of H_2O_2 was measured spectrophotometrically by tracking the rate of decrease of absorbance at 230 nm that is typical for H_2O_2 (ε = 72.8 M^{-1} cm^{-1}).

The decay in the absorbance of H_2O_2 fitted very nicely according to the first-order rate law. It was determined that the half-life of the reaction ($t_{1/2}$) increased with increasing concentration of CN^- (Table S9†). From the $t_{1/2}$ values it was calculated that in a period of 5 min the number of half lives of the reaction would decrease from 10 to 5 upon addition of 0 to 10 μM of CN^- (Table S9†). To ascertain that the reactivity of catalase is unaffected by other ions, we performed control reactions where the disproportionation of H_2O_2 was performed in presence of other ions such as F^-, Cl^- and OAc^-. It was observed that the half-life of the disproportionation reaction remained unaltered in the presence of other ions. Hence, it was concluded that the inhibition of catalase under the assay conditions was specific for CN^- and this binding can be used to inhibit disproportionation of H_2O_2 in amounts which is proportional to the added CN^-. Finally, Fe-MSNs catalyzed oxidation of TMB with H_2O_2 has to be used to generate a visual signal that is proportional to the amount of H_2O_2 present. This would then lead to a colored reporter molecule that is several times higher in concentration than the added CN^- leading to signal amplification of the analyte. However, it must be first ascertained that CN^- does not bind to Fe-MSNs and inhibits its activity; otherwise a linear response for the presence of H_2O_2 will not be obtained. The titration of the complex [Fe^{III}(biuret-amide)] with CN^- shows virtually no change in its UV-VIS spectra indicating the CN^- did not bind to the Fe(III) complex (Fig. S5†). Further, the reactivity of the Fe-MSNs towards oxidation of TMB in the presence of H_2O_2 remained unchanged in the presence of CN^- as well as with a host of anions such as Cl^-, F^-, OAc^- among others (Table S8, Fig. S7†). Interestingly, when the same experiments were performed with the enzyme HRP, it was observed that CN^- did bind to HRP and the rates of TMB oxidation decreased up to 90% with increase in CN^- (Table S7, Fig. S6†). Therefore, our HRP mimic Fe-MSNs are much better suited for the estimation of CN^- by our methodology than the native enzyme HRP. We then proceeded to combine both these reactions to see if CN^- could be detected colorimetrically in a quantitative manner.

For detection of CN^-, 28 nM of catalase was first incubated with varying amounts of cyanide and then H_2O_2 (0.8 mM) was added to it. The amount of H_2O_2 was optimized to 0.8 mM to obtain a maximum signal to noise ratio. After 5 min of incubation with H_2O_2, Fe-MSNs and TMB were added and the green color observed was recorded in a UV-VIS spectrophotometer after 10 min (Fig. 1, inset). Control experiments when no CN^- was added remained colourless since nearly all the H_2O_2 was consumed by enzyme catalase in 5 min (10 half-lives by kinetic studies). Fig. 1 represents the increase in absorbance of the reporter TMB at 650 nm as a function of added CN^-. The plot shows that the OD increases with increasing CN^- and finally reaches a saturation level. A linear dependence of absorbance *vs.* cyanide concentration was obtained in the range of 0 to 156 ppb with a limit of detection (LOD) of 23.4 ppb (0.9 μM). The allowed limit of cyanide (1.9 μM) in drinking water by the World Health Organization falls in the linear range of detection illustrated in this method, so rendering it suitable for analysis of real-life samples. A plot of the concentration of oxidized TMB *vs.* the concentration of added CN^- produces a straight line with a slope of approximately 8 (Fig. 2). This indicates that on average the output signal was amplified 8-fold with respect to the CN^- ion present. We believe that this represents a first report of detection of CN^- anion in water by catalytic signal amplification.

This methodology has several attributes which makes it exciting. Since this analytical method is dependent on the cascade of reactions, changes in the concentration of catalase, H_2O_2 and Fe-MSNs would lead to different output signals. For example, when 80 nM of

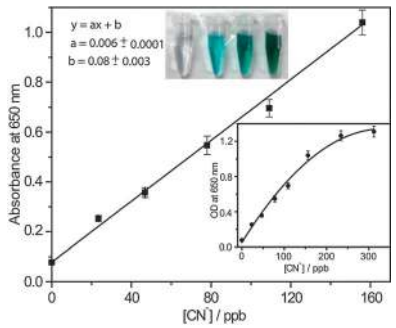

Fig. 1 Linear calibration plot of CN^- *vs.* OD at 650 nm. Inset plot shows dependence of OD on CN^- concentration. Inset: photos for different concentration of CN^-; from left: 0 ppb, 23.4 ppb, 156 ppb and 234 ppb.

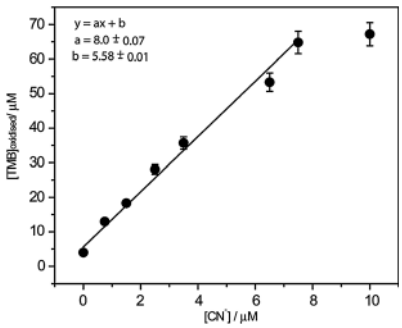

Fig. 2 Concentration of cyanide incorporated *vs.* concentration of TMB oxidized. The slope indicates 8-fold signal amplification.

catalase is used instead of 28 nM, the observed signal remained linear from 0 to 5.2 ppm although the LOD increases to 1.3 ppm (Table S3, Fig. S2†). Hence by simply varying kinetic parameters this method can be utilized to suitably detect CN^- over a broad range of concentration. However, in this case no signal amplification was achieved because of limited solubility of TMB in water. Since catalase binds to CN^- very strongly, this method displayed very high selectivity towards cyanide ion over other common anions present in wastewater. Even the usage of hundred fold higher concentration of various anions (F^-, Cl^-, Br^-, I^-, SCN^-, $HCOO^-$, NO_3^-, OAc^-, SO_4^{2-} and HCO_3^-) did not elicit any response, as is indicated by the plots and accompanying photographs (Fig. 3). Since

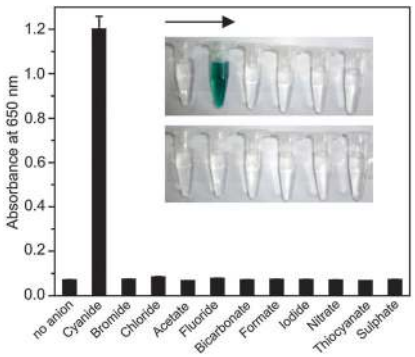

Fig. 3 Bar plot showing selectivity for 5 μM CN^- in comparison to 500 μM of other common anions. The inset shows corresponding photos in the same order of the bar plot.

all the experiments were done in DI water, we wanted to see if this method can be extended to regular tap water that is known to contain metal ions that may decompose H_2O_2. Therefore, known amounts of CN^- were spiked into tap water and quantification using the calibration curve performed in DI water afforded accurate results (Fig. 1).

In summary, the HRP mimic Fe-MSNs developed by us has been used in tandem with the enzyme catalase for the colorimetric detection of CN^- in aqueous media through signal amplification. This represents one of the very few examples in which catalytic signal amplification has been used for the detection of anions in water. Although the LOD was determined to be rather high at 0.9 μM, we believe it can be lowered several fold by using substrates which generate reporter molecules that either have very high extinction co-efficient or generate fluorescence upon oxidation. Also, improvement in the catalytic activity of $[Fe^{III}(\text{biuret-amide})]^{2-}$ would also help to lower detection limits. Such efforts are underway in our laboratory.

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