Diffusion-Controlled Simultaneous Sensing and Scavenging of Heavy Metal Ions in Water Using Atomically Precise Cluster–Cellulose Nanocrystal Composites

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Supporting Information

ABSTRACT: Development of a system that can simultaneously sense and scavenge toxic heavy metal ions at low concentrations is an ideal solution for in situ monitoring and purification of contaminated water. In this paper, we report on the synthesis and application of a novel system, luminescent atomically precise cluster–cellulose nanocrystal composite, namely, bovine serum albumin-protected gold nanoclusters (Au@BSA NCs)-loaded cellulose nanocrystal–alginate hydrogel beads, that can simultaneously sense and scavenge heavy metal ions, specifically mercury ions in water. Characterization of the system performed using scanning electron microscopy coupled with energy dispersive spectroscopy and X-ray photoelectron spectroscopy elucidated the physical and chemical characteristics of the system. Additionally, we proposed a new method to visualize the diffusion phenomenon and calculate the effective diffusion coefficient of heavy metal ions in hydrogel beads by monitoring the fluorescence-quenching dynamics of Au@BSA NCs upon binding with mercury ions. Finally, practical applications of this nanocomposite were demonstrated using batch adsorption experiments as well as using a dip pen device loaded with the hydrogel beads for in situ monitoring of heavy metal ions in water.

KEYWORDS: Cellulose nanocrystals, Nanoclusters, Nanocomposites, Adsorption, Sensing, Hydrogels, Diffusion, Water Purification

INTRODUCTION

Water is being increasingly contaminated by a wide variety of pollutants from industrial, municipal, and agricultural sources like heavy metal ions, dyes, food additives, pharmaceuticals, detergents, agrochemicals, etc. Thus, access to clean water is becoming more challenging, and efficient technologies that can purify this contaminated water have become essential.1,2 Among all these pollutants, toxic heavy metal ions, which accumulate in the food chain continue to be a major concern. Various physical, chemical, and biological treatment methods can be employed to remove heavy metal ions from water. Of all these treatment methods, adsorption implemented using a well-designed system offers the best solution for producing high-quality treated water at affordable cost. This technique offers many advantages, such as simplicity in design and operation, low initial investment, effectiveness, and insensitivity to other substances present in the water stream. Researchers around the globe are searching for various low cost nonconventional adsorbent materials.3–7

Researchers have examined its feasibility.8–13 CNCs are rod-like nanoparticles obtained from cellulosic biomass, having diameters in the range of 5–20 nm and lengths of a few hundred nanometers. They can be produced at the industrial scale by acid hydrolysis of pulp fibers. Because of their high specific surface area, good mechanical strength, biodegradability, and high functionality,14 adsorbents based on these nanomaterials have the capacity to remove a wide variety of pollutants from wastewater.15,16 Even though these nanomaterials were found to outperform several conventional adsorbents used in developing countries, their practical application for large-scale water treatment systems is limited as they are difficult to separate from the water bodies after adsorption. This can be solved by incorporating them into

Special Issue: Building on 25 Years of Green Chemistry and Engineering for a Sustainable Future

Received: July 19, 2016
Revised: October 14, 2016
Published: October 16, 2016

DOI: 10.1021/acssuschemeng.6b01674
ACS Sustainable Chem. Eng. 2016, 4, 6167−6176

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matrices, like hydrogel beads, that can be easily used in batch as well as column adsorption processes.\textsuperscript{17–19}

Mercury, in both organic and inorganic forms, is one of the most hazardous environmental contaminants among all heavy metal ions.\textsuperscript{20} Different approaches have been used for the determination of trace levels of mercury. Fluorescence-based techniques are important in the determination of ionic mercury. However, for trace levels of heavy metals, ultrasensitive methods that are readily implementable in the field have to be developed, working at the limit of single ions.\textsuperscript{21}

Aspicules,\textsuperscript{22} atomically precise quantum clusters of noble metals, have been gaining attention in the field of sensor owing to their enhanced photostability, high quantum yield and ease of functionalization. Their discrete energy states, resulting from a size range corresponding to Fermi wavelength of electrons, endow them with luminescent properties in the visible range. Though a wide range of ligands have been utilized for synthesizing aspicules, biomolecules like proteins and peptides are emerging owing to their simplicity in synthesis, biocompatibility, and wide range of applications. Noble metal clusters protected with BSA, lysozyme, and lactoferrin are some notable examples.\textsuperscript{23–28} Xie et al.\textsuperscript{29} first reported the use of BSA-protected gold nanostructures (Au@BSA NCs) for sensing Hg$^{2+}$ ions. Several other groups have used the protein-protected quantum cluster systems for sensing heavy metal ions like Hg$^{2+}$, Cu$^{2+}$, and Pb$^{2+}$. Though highly sensitive, the colloidal stability of these clusters limit their application in the field, and thus, a suitable substrate to anchor them is required. We have previously demonstrated the ability of Au@BSA to bind with nanofoibers and have used it for detecting Hg$^{2+}$ ions. Such confinement on a substrate also leads to an increase in the luminescence intensity.\textsuperscript{30,31}

Until now there have been no reports on CNCs-based nanocomposites that can be used for the simultaneous sensing and scavenging of heavy metal ions in water. In this paper, we report on the synthesis and application of novel nanocomposite hydrogel beads, viz., Au@BSA NCs-loaded cellulose nanocrystal–alginate hydrogel beads that can simultaneously sense and scavenge heavy metals ions, specifically Hg$^{2+}$ ions in water. Several researchers have attempted to probe diffusion and calculate the effective diffusion coefficient using various techniques that are rather complex. Herein, we report a novel approach based on the fluorescence-quenching dynamics of Au@BSA NCs upon Hg$^{2+}$ mercury ion binding, which can be used to probe diffusion and determine the diffusion coefficient of Hg$^{2+}$ ions in the hydrogel beads. The sensing occurs as a result of the adsorption of Hg$^{2+}$ ions on Au@BSA NCs, which is controlled by several parameters like analyte concentration, porous and anionic nature of the hydrogel bead, etc. We performed batch adsorption experiments to elucidate the adsorption characteristics that are pertinent to the sensing application. At this stage of development, the practical application of this system for the removal of Hg$^{2+}$ ions in contaminated water is limited to small-scale operations. Finally, the practical application of these hydrogel beads is demonstrated using a dip pen device containing these hydrogel beads for \textit{in situ} monitoring of heavy metal ions in water.

\section*{Experimental Section}

\textbf{Materials.} CNCs supplied by the University of Maine Process Development Centre were used in this study. Sodium alginate (ALG) was purchased from FMC Biopolymer. Sodium chloride (NaCl), calcium chloride (CaCl$_2$), and tetrachloroauric acid trihydrate (HAuCl$_4$·3H$_2$O) were purchased from Sigma-Aldrich. Bovine serum albumin (BSA) was purchased from Sisco Research Laboratories Pvt. Ltd. Divalent acetates of mercury, copper, zinc, nickel, lead, cadmium, manganese, cobalt, iron, and magnesium were purchased from Merck. Chromium(III) nitrate nonahydrate (Cr(NO$_3$)$_3$·9H$_2$O) was purchased from Lobachemie, and sodium arsenite (NaAsO$_2$) was purchased from SD Fine Chemicals Limited. Purified water from a Milli-Q Millipore system (>18 MΩcm) was used for all the experiments. All chemicals were used directly without additional purification.

\textbf{Methods.} Preparation of Nanocomposite. Preparation of the nanocomposite was performed in two steps. In the first step, synthesis of Aug@BSA NCs-loaded CNC gel was carried out. During this step, 1 mL of 6 mM HAuCl$_4$·3H$_2$O aqueous solution was added dropwise to a 4 mL reaction mixture containing 25 mg BSA and 150 mg CNCs and stirred overnight. After 30 min, the pH of this solution was adjusted to \~12 by adding 200 μL of 1 M NaOH, and then, the whole reaction mixture was stirred overnight. The reaction mixture turned into a gel-like structure, and the formation of Au@BSA NCs was indicated by the characteristic color change from pale yellow to deep brown, with intense red fluorescence under UV light. In the second step, the final nanocomposite, viz. Au@BSA NCs-loaded CNC-ALG hydrogel beads, was prepared by further cross-linking the earlier synthesized Au@BSA NCs-loaded CNC gel using ALG by an ionotropic gelation method in the presence of Ca$^{2+}$ ions. During this step, the desired ratio of Au@BSA NCs-loaded CNC gel and ALG solution were thoroughly homogenized and loaded into syringes. This homogenized solution was then extruded through syringes mounted with a 24-gauge-sized needle using a syringe pump at a flow rate of 0.8 μL/min into 20 mL of 2 wt % CaCl$_2$ solution that was gently stirred to prevent the beads from adhering to each other. The hydrogel beads that were formed were then allowed to cross-link in a CaCl$_2$ solution for 15 min, after which they were washed with water several times to remove residual CaCl$_2$ and BSA and used for further experimentations.

\textbf{Batch Adsorption Studies.} In a typical batch adsorption experiment, 500 mg of water-swollen hydrogel beads were stirred overnight in a vial containing 5 mL of heavy metal ion solution of either Hg$^{2+}$ or Cu$^{2+}$ at neutral pH and 25 °C. The initial and final concentrations of the heavy metal ion solutions were measured using ICP-MS after acidification (5% HCl for Hg$^{2+}$ and 5% HNO$_3$ for Cu$^{2+}$). Removal % and uptake of heavy metal ions ($q_e$) by hydrogel beads were calculated using eqs 1 and 2.\textsuperscript{31,32}

\begin{equation}
\text{Removal} \% = \frac{(C_0 - C_e)}{C_0} \times 100
\end{equation}

\begin{equation}
\text{Uptake} \ (q_e) = \frac{(C_0 - C_e) V}{m}
\end{equation}

where $q_e$ is the amount of heavy metal ions adsorbed per g of adsorbent (mg/g) in equilibrium, $C_0$ is the equilibrium concentration of free heavy metal ions in the bulk solution (mg/L), $C_e$ is the initial heavy metal ion solution concentration (mg/L), $V$ is the volume of solution (L), and $m$ is the mass of adsorbent (g).

Adsorption capacity of this nanocomposite for Hg$^{2+}$ and Cu$^{2+}$ uptake was also evaluated using the Langmuir adsorption isotherm. This was performed by plotting the equilibrium heavy metal ion uptake ($q_e$) against the equilibrium concentration of heavy metal ion ($C_e$) for batch adsorption experiments performed with different initial heavy metal ion concentrations, ranging from 20 to 100 ppm. This equilibrium data was fitted using the linearized form of the Langmuir adsorption isotherm model mentioned in eq 3.

\begin{equation}
\frac{1}{q_e} = \frac{1}{K_L q_m C_s} + \frac{1}{q_m} \frac{1}{C_e}
\end{equation}

where $q_m$ is the maximum amount of heavy metal ions which can be adsorbed per gram of adsorbent (mg/g) in equilibrium, and $K_L$ is a constant related to the energy of adsorption (L/mg) which depicts the affinity between the heavy metal ion and adsorbent. Parameters $q_m$ and $K_L$ were determined from the intercept and slope of the plot of $1/q_e$ versus $1/C_e$, respectively.
Development of an Ideal Sensor–Scavenger System. Three hydrogel bead systems containing the CNC:ALG ratios of 1:2, 1:1, and 2:1 were prepared by mixing different ratios of Au@BSA NCs-loaded CNC gel and ALG solutions. Leaching of Au@BSA NCs from the hydrogel beads placed in water for 2 days were analyzed by inspecting the supernatant, both visually under UV light and measuring the absorbance at 280 nm using a UV–vis spectrophotometer. Also, the mercury removal percentages of the three systems were compared using batch adsorption studies.

Sensing Experiments. Further, the sensitivity of this nanocomposite to various heavy metal ions was also analyzed. For the single hydrogel bead-sensing experiment, one bead was placed in each HPLC vial containing 1 mL of 1 ppm concentration of various heavy metal ion solutions like Hg^{2+}, Cu^{2+}, Zn^{2+}, Ni^{2+}, Pb^{2+}, Cd^{2+}, Cr^{6+}, As^{3+}, Mn^{2+}, Co^{2+}, and Fe^{3+}. The fluorescence of Au@BSA NCs inside the hydrogel beads when immersed in these heavy metal ion solutions was monitored to evaluate the sensitivity and selectivity of the system. A hydrogel bead was also placed in the solution of Mg^{2+} and Ca^{2+} ions, which are the common ions contributing to the hardness of water, in order to study the effect of counterions.

Characterization of Nanocomposite. Photographs of the hydrogel beads were recorded, and the size distribution and mean diameter of hydrogel beads were measured using a vernier caliper. Morphology, elemental analysis, and elemental mapping studies of the hydrogel beads were performed using a scanning electron microscope (SEM) equipped with energy dispersive analysis of X-rays (EDAX) (FEI Quanta 200). For the SEM and EDX measurements, freeze-dried hydrogel bead samples were spotted on an aluminum sample stub. X-ray photoelectron spectroscopy (XPS) measurements were conducted using an Omicron ESCA Probe spectrometer with unmonochromatized Al Kα X-rays (energy = 1486.6 eV). High resolution transmission electron microscopy (HRTEM) of Au@BSA NCs was performed with JEOL 3010, a 300 kV instrument. The samples for HRTEM were prepared by dropping the dispersion on a carbon-coated copper grids and drying under ambient conditions. UV–visible absorption spectra and photoluminescence spectra of Au@BSA NCs were acquired using PerkinElmer Lambda 25 UV–vis spectrophotometer and Jobin Yvon NanoLog spectrofluorometer, respectively.

Quenching Dynamics to Study Diffusion. Dark field fluorescence microscopy was employed to monitor the quenching dynamics of Au@BSA NCs upon the diffusion of Hg^{2+} ions into the hydrogel beads. Fluorescence imaging and spectra of hydrogel beads were performed using the Cytoviva HSI system containing an Olympus BX-41 microscope equipped with a Dage high resolution camera. Dark field fluorescence microscopy was used in an excitation window corresponding to DAPI/FITC/Texas RED (DAPI, 452–472 nm; FITC, 515–545 nm; Texas RED, 600–625 nm), and emissions were collected using a triple pass emission filter DAPI/FITC/Texas RED (DAPI, 452–472 nm; FITC, 515–545 nm; Texas RED, 600–625 nm). We have chosen similar excitation and emission filters for simultaneous visualization of the fluorescent area along with the quenched area. The distinct red fluorescence of the cluster allows us to easily distinguish between the quenched and fluorescent part of the hydrogel bead.

Hydrogel beads of a size greater than 1 mm was imaged under a dark field fluorescence microscope. The intense red color of the bead indicates the uniform distribution of the clusters. Hydrogel beads were initially kept in Millipore water, and the fluorescence intensity was observed for some time to confirm that it did not change in the control condition. Then, a single bead was immersed in 100 ppm of Hg^{2+} ion solution, and time-dependent quenching of the red emission from Au@BSA NCs was observed. Images were captured at regular time intervals. By monitoring the fluorescence area from these images, the diffusion kinetics of the Hg^{2+} ions were studied. The rate of diffusion of ions corresponds to the rate of fluorescence quenching. As the time scale of reaction between the metal ion and the cluster which quenches the luminescence is much faster than ionic diffusion, the error due to the former event is insignificant. The diffusion of Hg^{2+} ions into the hydrogel bead was monitored by studying the reduction in the fluorescence diameter of the bead.

The diffusion coefficient (D) is an essential parameter to model diffusion by solving Fick’s second law:

$$\frac{\partial C}{\partial t} = D \frac{\partial^2 C}{\partial x^2}$$

(4)

where C is the concentration, x is the position, and t is the time. Solving this equation requires knowledge of flux and concentration varying over time. The rate of change of mercury concentration (dC/dt) can be equated to the rate of change in fluorescence intensity (dI/dt) using the following equation:

$$dI = dC$$

(5)

By substituting the above equation in Fick’s law, we obtain the coefficients for fluorescence quenching which is similar to D for Hg^{2+} ions. This was calculated by analyzing the variation of red intensity along the bead diameter at various time points. The variation in red intensity along the diameter of the bead was extracted using an image analysis code run using the open software called R. The code is included in the section S10 of the Supporting Information (SI). For ease of calculation, the equation representing the concentration was assumed to be a multiple of two independent functions, distance and time. The variation in red intensity for various time points was fitted to a sine curve representing the variation along the diameter. Then, the maximum intensity (taken from the center of the sine curve) was plotted against time to obtain the function describing the change of intensity with time. This was fitted to an exponential curve.

The solution for Fick’s equation under homogenization seems to fit the equation of fluorescence as a function of distance and time as given by

$$F = 0.66 \sin \frac{3.14 x}{1260} \left( e^{-0.1594} - 0.11 \right)$$

(6)

Also, the minimum measurable distance, i.e., length of one pixel, and the error from fitting the exponential decay for time constant were used to calculate error. The detailed calculations are included in section S11 of the SI.

Dip Pen Experiments. A concept dip pen device was fabricated to demonstrate the application of these hydrogel beads for in situ monitoring of heavy metal ions in water. A capillary column of inner diameter 2 mm and length 5 cm was filled with hydrogel beads of diameter 1.9 mm along the length of the column. This capillary column was dipped into a glass vial containing 5 mL of 50 ppm mercury ion solution. Photographs of the dip pen device placed in the mercury ion solution were taken at regular intervals to monitor fluorescence quenching of Au@BSA NCs, as a result of the capillary rise of mercury ion solution through the hydrogel beads.

RESULTS AND DISCUSSION

Preparation of Nanocomposite. Highly fluorescent Au@BSA NCs with a characteristic red emission are formed as a result of in situ reduction of Au ions encapsulated in BSA protein upon adjusting the reaction pH to ~12 by the addition of NaOH. When the synthesis of these nanoclusters takes place in the reaction mixture containing CNCs, it leads to the formation of a gel-like structure due to the ion-mediated gelation of CNCs by the cationic Au ions. Chau et al. and Shafei-Sabet et al. have reported that CNCs at a particular concentration tend to form gel-like structures upon the addition of cations due to stronger propensity of CNCs for side-by-side association. Gelation of CNCs can be induced by the increasing ionic strength of CNC suspension with the addition of salts. Salt addition reduces the Debye length of CNCs, and as a result, the electrostatic repulsion between them is suppressed and dominant attractive forces like van der Waals forces and hydrogen bonding come into play.
When the nanocomposite solution containing Au@BSA NCs, CNCs, and ALG was introduced to a CaCl₂ solution, hydrogel beads were instantaneously formed, as a result of ionotropic gelation of ALG by Ca²⁺ ions. ALG is an anionic polysaccharide composed of (1→4)-linked β-D-mannurionate (M) and α-L-guluronate (G) units which in the presence of divalent cations, such as Ca²⁺, form hydrogels via ionic cross-linking of calcium bridges between the M and G units of the adjacent chains.³⁵ Figure 1 shows the Au@BSA NCs-loaded CNC gel and Au@BSA loaded CNC-ALG hydrogel beads under white light and UV light.

Au@BSA clusters were studied independently in the group, and previous studies including ours³⁶,³⁷ have established that the clusters showing emission at 660 nm are composed of 30 atoms of Au. They also exhibit well-defined mass spectra, measured using matrix-assisted laser desorption ionization (MALDI). However, as the focus here is on sensing and scavenging, we are not discussing these aspects in greater detail.

Development of an Ideal Sensor–Scavenger System. Among the three nanocomposite hydrogel bead systems, the 1:2 CNC:ALG system was found to be an ideal sensor–scavenger system. This system showed lesser leaching of Au@BSA NCs as compared to 1:1 and 2:1 systems when placed in water for 2 days. Figure 2(a) shows the photographs of the vials containing the three systems, taken at the start and end of the leaching experiment. More intense red luminescence was observed for the supernatant for the 1:1 and 2:1 system compared to the 1:2 system, due to the higher leaching of Au@BSA NCs from these systems. This is further confirmed by the higher UV absorbance of the supernatants from the 1:1 and 2:1 systems compared to the 1:2 system measured at 280 nm, as shown in Figure 2(b). Mechanical integrity tests performed by stirring the beads vigorously overnight showed that the 1:2 system was more robust compared to other systems as shown in Figure 2(c). Figure 2(d) shows that the 1:2 system also displayed higher Hg²⁺ removal compared to the 1:1 and 2:1 systems. Because of their high surface area and negative charge of CNCs, they are also capable of absorbing heavy metal ions. A control batch adsorption experiment performed by stirring 10 mg/mL CNCs and 50 ppm of Hg²⁺ overnight showed that CNCs themselves were capable of removing 50% Hg²⁺ (Figure S1). The carboxylate groups present along the polymeric chains of ALG coupled with the porous nature of the hydrogel beads also contribute to the adsorption of heavy metal ions.³⁸,³⁹ Thus, the synergistic adsorption by the CNCs and ALG in the nanocomposite contributed to an increased adsorption performance of the 1:2 system compared to the other two systems.

Sensing Experiments. Au@BSA NCs fluoresce with a deep red emission, which when entrapped inside these hydrogel beads also impart the characteristic red fluorescence to the beads under UV light. A single hydrogel bead sensitivity experiment, performed using 1 ppm concentration of all the major heavy metal ions such as Hg²⁺, Cu²⁺, Zn²⁺, Ni²⁺, Pb²⁺, Cd²⁺, Ca²⁺, As³⁺, Mn²⁺, Co²⁺, and Fe²⁺ employing the 1:2 CNC:ALG system showed that the nanocomposite is highly selective to Hg²⁺ ions. The nanocomposite itself was prepared using CaCl₂ that dissociates into Ca²⁺ and Cl⁻ ions, where Ca²⁺ ions will aid in the cross-linking of alginate chains and Cl⁻ ions remain free in the solution or diffuse into hydrogel beads. No fluorescence quenching of Au@BSA NCs was observed when the nanocomposites were stored in this solution for several hours, and hence, it is clear that Au@BSA NCs are not sensitive to Cl⁻ ions. No sensitivity to Ag⁺ was seen for this cluster system.²⁸ In addition, Xie et al.²⁸ and Hu et al.²⁵ have reported on the selectivity of Au@BSA NCs using various chemical
species and have demonstrated that they are highly selective to Hg$^{2+}$ ions.

Figure 3 shows that the fluorescence of hydrogel beads were completely quenched in the presence of Hg$^{2+}$ ions, and slight fluorescence quenching also occurred in the presence of Cu$^{2+}$ ions. There are several proposed mechanisms for the fluorescence quenching of Au@BSA NCs by Hg$^{2+}$ ions, such as (a) high affinity metallophilic bonding between the d$^{10}$ centers of Hg$^{2+}$ and Au$^+$ that disrupts the fluorescence from Au$^+$–BSA interactions and (b) photoinduced electron transfer process wherein the Hg$^{2+}$–S bonds are formed with BSA which causes the reduction of Hg$^{2+}$ to Hg$^+$ and the latter species creates interference during excitation, thereby quenching the fluorescence. Apart from this, at a similar heavy metal ion concentration, the binding affinities of Hg$^{2+}$ with Au$^+$ are much stronger compared to other toxic metal ions, which also makes Au@BSA NCs more sensitive to Hg$^{2+}$ ions. The selectivity of the nanocomposite to heavy metal ions is based on the type of gold nanoclusters incorporated in them. For example, Pb and As selectivity can be imparted to the nanocomposite by incorporating gold nanoclusters reported earlier by Zhu et al. and Subhasish et al., respectively. Earlier reports have also shown that Cu$^{2+}$ ions can also quench the fluorescence of Au@BSA NCs via ion-induced aggregation as they possess a strong affinity toward BSA and histidine. Hence, the slight fluorescent quenching of hydrogel beads observed in the case of Cu$^{2+}$ ions can be attributed to this fact.

The fluorescence of the hydrogel beads after exposure to varying concentrations of Hg$^{2+}$ was examined. The experiment showed that hydrogel beads are sensitive to as low as 1 ppm. Even though the sensitivity of this nanocomposite has been studied using a much lower concentration of Hg$^{2+}$ ions, the minimum sensitivity reported in this study is 1 ppm. The quenching of Au@BSA NCs fluorescence upon binding to Hg$^{2+}$ ions is limited by the diffusion of Hg$^{2+}$ ions into the hydrogel beads, which in turn is controlled by several other factors, such as size, charge, concentration gradient of diffusing ions, cross-linking density of hydrogels, interfacial properties of hydrogel matrix materials with the solute, etc. At a lower concentration of Hg$^{2+}$ ions, the rate of diffusion will be drastically reduced, and visual detection of the fluorescence quenching of Au@BSA NCs upon Hg$^{2+}$ ions diffusion may require a sufficiently longer time for the current nanocomposite. For the dynamic quenching experiments, we have conducted studies using 100, 10 and 1 ppm of Hg$^{2+}$ and noticed that the fluorescence of Au@BSA NCs was completely quenched in 45 min, 3 h and 24 h, respectively. Further studies are underway to develop systems that can detect lower concentrations of Hg$^{2+}$ within a shorter time span. Our preliminary experiments using photoluminescence spectroscopy have shown that the fluorescence of nanocomposite is sensitive down to 1 ppb Hg$^{2+}$.

Adsorption of Heavy Metal Ions. Among all the heavy metal ions tested for the sensing experiments, the adsorption capability of the nanocomposite for the most sensitive ion, namely, Hg$^{2+}$, was estimated using the Langmuir adsorption isotherm. Figure 4(a) shows the fitting of equilibrium data using the linearized form of Langmuir adsorption isotherm, and the maximum adsorption capacity ($q_m$) was calculated to be 26 mg/g. Figure 4(b) displays the photographs of the crushed and freeze-dried nanocomposite material before and after adsorption, under white light and UV light. After binding to Hg$^{2+}$, the color of the nanocomposite changed from yellowish brown to deep brown. Also, the fluorescence of the nanocomposite was completely quenched confirming the higher binding of Hg$^{2+}$ to the nanocomposite. Adsorption studies were also carried out using Cu$^{2+}$, which also showed some fluorescent quenching during the single hydrogel bead-sensing experiment (Figure S2).

Characterization of Nanocomposite. Average diameter of the hydrogel beads measured using a vernier caliper was 2.70 mm, which was illustrated in Figure S3. The average size distribution of the 10 samples were used for this measurement. The SEM image of the freeze-dried hydrogel bead (Figure 5a) shows undulations and folds on the bead surface. The inset of the SEM image shows undulations and folds on the bead surface.
The homogeneous adsorption of Cu\(^{2+}\) throughout the nano-nanocomposite before and after adsorption of Hg\(^{2+}\) are shown in Figures S4, S5, and S6, respectively. A comparison of the results from Figures S4 and S6 reveals the homogeneous adsorption of Hg\(^{2+}\) throughout the nanocomposite. Similarly, comparison of Figures S4 and S5 confirm the homogeneous adsorption of Cu\(^{2+}\) throughout the nanocomposite. Au@BSA NCs, which gives the characteristic red fluorescence as well as the sensing property to the nanocomposite, have also been characterized. The TEM image (Figure S7a) shows that these nanoclusters are around 2 nm in size. The UV−vis spectrum (Figure S7b) shows a peak at ∼280 nm, and the photoluminescence spectrum shows an emission maximum at 660 nm, when excited at 365 nm. These are the characteristic features for BSA-protected Au nanoclusters. Although we have studied such clusters through mass spectrometry previously,\(^{27}\) this was not possible in view of the nanocomposite nature of the present material that prevents ionization and desorption of the cluster. XPS analysis of the nanocomposite was performed to elucidate the fluorescence quenching as well as to confirm the adsorption of Hg\(^{2+}\) by the nanocomposite. XPS spectra in the Au 4f region of the nanocomposite before and after adsorption of Hg\(^{2+}\) are shown in Figure S8a. Comparing the XPS spectra, it is observed that the adsorption of Hg\(^{2+}\) on the nanocomposite has led to an increase in the binding energy of Au from 84.5 to 84.9 eV. This is reportedly due to oxidation of the Au core by the Hg\(^{2+}\) ion, which causes quenching of red fluorescence.\(^{30}\) Also, the peak of Hg 4f\(_{7/2}\) seen at 101.8 eV (Figure S8b) implies that there is a reduction of Hg\(^{2+}\) to Hg\(^{0}\), which occurs when Hg\(^{2+}\) ions are adsorbed on the nanocomposite.\(^{30}\)

**Quenching Dynamics to Study Diffusion.** Practical knowledge of the mass transfer characteristics of the sorption process, especially diffusion occurring through porous media during this sorption process, is essential for various applications, especially from an industrial standpoint. It is necessary to determine the optimal flow rate and residence time in columns and reactors. Numerous methods and experiments have been devised to estimate the value of D, which is an essential parameter for modeling diffusion. However, all the methods are elaborate, involving diffusion cell set up, Couette flow setup, radio tracers, rotation disc measurement, and scanning electrochemical micrograph. The coefficient can also be estimated by monitoring the bulk solution concentration, but it often contains many uncertainties.\(^{48}\) In this study, a novel and relatively simpler method was used, wherein the quenching dynamics of the Au@BSA NCs was observed under dark field fluorescence microscopy upon diffusion of Hg\(^{2+}\) into the hydrogel bead.

Figure 5a also shows these investigations at a higher magnification. The cross-sectional image of the hydrogel bead (Figure 5b) shows the porous nature of the nanocomposite which can also contribute to the increased surface area of the binding as well as diffusion of heavy metal ions. SEM-EDS elemental analysis and elemental mapping of the control, Hg\(^{2+}\)-saturated nanocomposite, and Cu\(^{2+}\)-saturated nanocomposite are presented in Figure S4, S5, and S6, respectively. A comparison of the results from Figures S4 and S5 confirms the homogeneous adsorption of Hg\(^{2+}\) throughout the nanocomposite. Similarly, comparison of Figures S4 and S6 reveal the homogeneous adsorption of Cu\(^{2+}\) throughout the nanocomposite. Au@BSA NCs, which gives the characteristic red fluorescence as well as the sensing property to the nanocomposite, have also been characterized. The TEM image (Figure S7a) shows that these nanoclusters are around 2 nm in size. The UV−vis spectrum (Figure S7b) shows a peak at ∼280 nm, and the photoluminescence spectrum shows an emission maximum at 660 nm, when excited at 365 nm. These are the characteristic features for BSA-protected Au nanoclusters. Although we have studied such clusters through mass spectrometry previously,\(^{27}\) this was not possible in view of the nanocomposite nature of the present material that prevents ionization and desorption of the cluster. XPS analysis of the nanocomposite was performed to elucidate the fluorescence quenching as well as to confirm the adsorption of Hg\(^{2+}\) by the nanocomposite. XPS spectra in the Au 4f region of the nanocomposite before and after adsorption of Hg\(^{2+}\) are shown in Figure S8a. Comparing the XPS spectra, it is observed that the adsorption of Hg\(^{2+}\) on the nanocomposite has led to an increase in the binding energy of Au from 84.5 to 84.9 eV. This is reportedly due to oxidation of the Au core by the Hg\(^{2+}\) ion, which causes quenching of red fluorescence.\(^{30}\) Also, the peak of Hg 4f\(_{7/2}\) seen at 101.8 eV (Figure S8b) implies that there is a reduction of Hg\(^{2+}\) to Hg\(^{0}\), which occurs when Hg\(^{2+}\) ions are adsorbed on the nanocomposite.\(^{30}\)

**Quenching Dynamics to Study Diffusion.** Practical knowledge of the mass transfer characteristics of the sorption process, especially diffusion occurring through porous media during this sorption process, is essential for various applications, especially from an industrial standpoint. It is necessary to determine the optimal flow rate and residence time in columns and reactors. Numerous methods and experiments have been devised to estimate the value of D, which is an essential parameter for modeling diffusion. However, all the methods are elaborate, involving diffusion cell set up, Couette flow setup, radio tracers, rotation disc measurement, and scanning electrochemical microscopy. The coefficient can also be estimated by monitoring the bulk solution concentration, but it often contains many uncertainties.\(^{48}\) In this study, a novel and relatively simpler method was used, wherein the quenching dynamics of the Au@BSA NCs was observed under dark field fluorescence microscopy upon diffusion of Hg\(^{2+}\) into the hydrogel bead.

Figure 6 shows the quenching of nanocomposite fluorescence with respect to the diffusion of Hg\(^{2+}\) ions at various time intervals. It is shown that the intensity of red emission was slowly quenched from the surface of the bead and progressed inward. The blue and green intensities, also shown in the images, were found to be constant over time. The plot showing the variation in red intensity along the bead diameter at time t = 100 s for 100 ppm concentration of Hg\(^{2+}\) is given in Figure S9a. It was observed that the fluorescence of the hydrogel bead was completely quenched after time t = 2500 s. The red intensity from a completely quenched bead (t = 2500 s) was subtracted from the other images, thus ensuring that the intensity used for calculation was only from the fluorescent cluster. Our previous reports indicate that the reaction of Au@BSA NCs with the Hg\(^{2+}\) ion is generally fast and uniform,\(^{30}\) and hence, we also assume that the reaction rate far exceeds the diffusion rate. Figure S9b shows the sine curve fitting of the variation in red intensity for various time intervals. The maximum intensity (taken from the center of sine curve) plotted against time as shown in Figure S9c follows a typical exponential decay. The

![Figure 5. SEM image showing the (a) surface morphology and (b) porous nature of the hydrogel bead, after drying.](Image)

![Figure 6. Quenching of nanocomposite fluorescence with respect to the diffusion of Hg\(^{2+}\) ions at various time points (scale bar shown in the pictures is 200 μm). Initial concentration of Hg\(^{2+}\) in the solution 100 ppm.](Image)
variation of the concentration as a function of distance and time (eq 6) was obtained by multiplying the individual equations of distance and time, respectively. We solved for $D$ by inserting this function into Fick’s equation (eq 4).

The diffusion coefficient of the bead calculated using this technique was found to be $1.01 \pm 0.3 \times 10^{-8}$ cm$^2$/s. There were no data in the literature on the diffusion of mercury through these beads, and hence, we were unable to make any comparison. However, the diffusion coefficient for Cu$^{2+}$ in water was measured by Marcinkowsky and Phillips using radioactive tracers and by Quickenden and Jiang using a rotating disk electrode. Both these investigators measured the diffusion coefficient in the range from $6.2 \times 10^{-6}$ to $7.5 \times 10^{-6}$ cm$^2$/s. Jang et al. measured the diffusion coefficient of Cu$^{2+}$ for 3.2% calcium alginate beads to be in the range from $1.0 \times 10^{-5}$ to $1.4 \times 10^{-5}$ cm$^2$/s. However, for small analytes, the diffusion rate through a bead is similar to that of the diffusion in water. Hg$^{2+}$ ions being heavier than Cu$^{2+}$ ions seemingly diffuse slower through the hydrogel bead. The lower diffusion rate could also be due to the binding of Hg$^{2+}$ ions with the clusters and high affinity of this reaction.

To ensure that the diffusion coefficient can be equated to the fluorescent intensity of the bead, we have placed a single hydrogel bead in control solution (Millipore water) for nearly 24 h and monitored any reduction in nanocomposite fluorescence as a result of leaching of Au@BSA NCs. No visible change in color of the control solution was observed, indicating no leaching of the Au@BSA NCs. However, in the sample, the complete quenching of the Au@BSA fluorescence was observed within 2500 s when we used 100 ppm of Hg$^{2+}$. In general, we have placed the hydrogel bead in water for at least 15 min before the start of experiment to ensure that there is no visible leaching of Au@BSA NCs. Within the time span of 1 h of the experiment, we do not believe that there would be any leaching of clusters to impact our calculation of diffusion coefficient.

The analysis and calculations reported here can be improved through better mathematical models. The use of confocal fluorescence microscopy can yield detailed real time three-dimensional visualization of the diffusion process. Here, we have presented a simpler procedure that allows for the direct visualization of the diffusion in porous beads. The scope can be extended to study diffusion of other molecules, especially proteins and DNA, which can be tagged to a fluorescent moiety.

**Dip Pen Experiments.** The hydrogel beads that were placed along the length of a dip pen device quenched its red fluorescence gradually when it was dipped in the mercury solution. The capillary rise of the mercury solution through the capillary column promoted the diffusion of Hg$^{2+}$ ions through the hydrogel beads that contained Au@BSA NCs. Figure 7 shows the photographs of the dip pen device and gradual change in the fluorescence of the hydrogel beads upon exposure to the mercury solution.

Even though the use of Au@BSA NCs in our nanocomposite raises questions regarding its appropriateness for commercial applications, there are several reasons that make this nanocomposite promising for use in water treatment applications, namely, the following:

(a) The Au@BSA NCs provide excellent fluorescence signals even at reduced concentration; hence, only less than 19.7 μg of Au is needed to produce one hydrogel bead. Thus, in most cases, cost may not be an issue. This quantity could be reduced further, depending on the fluorescence intensity required for detection.

(b) Au@BSA NCs have very good selectivity and sensitivity toward Hg$^{2+}$ ions as demonstrated in several earlier reports, and their incorporation into the current nanocomposite makes it also selective toward Hg$^{2+}$ ions.

(c) Au@BSA NCs when incorporated into this nanocomposite were found to be stable at high temperature without much loss in their characteristic red fluorescence. This was evident when we oven-dried the nanocomposite at a temperature of 100 °C. The freeze-dried nanocomposite containing Au@BSA NCs was stable, and they fluoresce with bright red emission even after six months when stored at room temperature. This improved stability is due to the protective environment provided by the alginate polymer chains wrapping around the fluorescent Au@BSA NCs. Figure S12 also shows the photograph of the freeze-dried nanocomposite hydrogel beads which have been stored for more than six months under UV light. Thus, the practical applicability of the nanocomposites containing Au@BSA NCs with respect to storage and usability in high temperature conditions, such as in mining effluents, is possible.

(d) The sensing and adsorption of Hg$^{2+}$ are controlled by diffusion; hence, this nanocomposite can be used as a probe to visualize the diffusion phenomena, allowing us to determine the diffusion coefficient. This is the first time such a method was proposed, and this was possible
because of several properties of the nanocomposite hydrogel bead, such as three-dimensional spherical structure, high porosity, hydrophilic and anionic matrix that drives the diffusion of cationic Hg\textsuperscript{2+} ions via electrostatic interaction, and homogeneous nanocluster distribution giving uniform florescence intensity throughout the nanocomposite. (e) The protein-templated nanocluster such as Au@BSA NCs can be synthesized in situ via a one-pot synthesis procedure. In contrast, most metallic clusters are synthesized separately and then incorporated in the substrate. Here, leaching of nanoclusters can become a challenging problem when used in a continuous process. On the other hand, the synthesis of Au@BSA NCs takes place in a reaction mixture containing CNCs. Due to the ion-mediated gelation of CNCs by the cationic Au ions, a gel-like structure is formed, and hence, the leaching of nanoclusters is drastically reduced. Apart from gold, only biomolecule (BSA), biodegradable polymers (CNCs, alginate), and eco-friendly chemicals (NaOH, CaCl\textsubscript{2}) were used in the synthesis when compared to systems prepared from electrospun nanofibers based on petroleum-based polymers, etc.\textsuperscript{30} (f) Also, the binding affinity of mercury did not vary after incorporating the cluster into the matrix. This was examined by comparing the sensitivity of the clusters alone and cluster-incorporated nanocomposites to different concentrations of Hg\textsuperscript{2+} ions using photoluminescence spectroscopy. Figure S13 shows the variation of the normalized fluorescence intensities of Au@BSA NCs and Au@BSA NCs CNC-ALG nanocomposite after binding with Hg\textsuperscript{2+} ions at regular time intervals. A similar trend observed for Au@BSA NCs and Au@BSA NCs CNC-ALG nanocomposites reveals that the affinity of mercury did not vary after incorporating the cluster into the matrix. (g) In terms of response and sensitivity, there are greater possibilities of improvement of this system compared to other reported systems. Because this nanocomposite can be manipulated into smaller-sized hydrogel beads and also into various shapes such as film, stick, or candle, depending on the end use, diffusion of Hg\textsuperscript{2+} ions can be enhanced using forced diffusion processes such as vacuum suction. In terms of selectivity, there is also an ability to incorporate different types of gold nanoclusters into the nanocomposite to make them selective to other heavy metal ions species of interest. Selectivity can be brought in also through molecular functionalization of the clusters. In summary, a novel nanocomposite that can simultaneously sense and scavenge toxic heavy metal ions like Hg\textsuperscript{2+} in contaminated water was developed and demonstrated. Nanostructures of the components, namely, cellulose nanocrystals and protein protected clusters, allowed the creation of this fast-responsive system. The sensing and scavenging property of this system is controlled by the diffusion of ions. At this stage of development, the system was not reusable due to the poor recovery of fluorescence of Au@BSA NCs upon Hg\textsuperscript{2+} binding. Better analysis and calculations may be performed to improve the modeling of quenching dynamics to study the diffusion of Hg\textsuperscript{2+} ions in hydrogels.

**CONCLUSIONS**

In conclusion, we have developed a novel nanocomposite that can simultaneously sense and scavenge toxic heavy metal ions in contaminated water. Au@BSA NCs leaching experiments and batch adsorption experiments using Hg\textsuperscript{2+} ions suggested that a nanocomposite with a 1:2 CNC:ALG ratio is an ideal sensor—scavenger system. The sensitivity of this nanocomposite was found to be highly selective to Hg\textsuperscript{2+} ions among all other heavy metal ions tested. The fluorescence of the nanocomposite was found to be completely quenched in the presence of Hg\textsuperscript{2+} due to high affinity metallophilic Hg\textsuperscript{2+}/Au\textsuperscript{2+} interaction on the Au@BSA NCs surface. Adsorption of Hg\textsuperscript{2+} ions by the nanocomposite showed a visible change in their color, and the maximum adsorption capacity measured using Langmuir adsorption isotherm was 26 mg/g. We also reported a novel method to probe diffusion and calculated the diffusion coefficient by visualizing the dynamic fluorescence quenching of Au@BSA NCs as the Hg\textsuperscript{2+} ions diffuse into the hydrogel beads. The diffusion coefficient calculated using this novel method was 1.01 (±0.3) × 10\textsuperscript{-6} cm\textsuperscript{2}/s, which is considered to be in the same range as reported for other ions in polymeric matrices using various other techniques. Further, the practical application of this nanocomposite was demonstrated using a dip pen experiment, wherein the fluorescence of the capillary column containing these hydrogel beads was quenched as the Hg\textsuperscript{2+} ions got adsorbed and diffused through them. Similar types of novel nanocomposites selective to other heavy metal ions can be designed. Systems incorporating these nanocomposites can be used as a simultaneous sensor—scavenger system and also as a tool to probe diffusion. The possibility to create various sustainable formulations with simultaneous sensing and scavenging offers new possibilities of applications for such materials.

**ASSOCIATED CONTENT**

Supporting Information

Supporting Information is available free of charge on the ACS Publications Web site. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acssuschemeng.6b01674.

Advertisements of Hg\textsuperscript{2+} by CNCs; Langmuir adsorption isotherm and photograph showing the change in the nanocomposite color before and after Cu\textsuperscript{2+} adsorption; photograph showing the vernier caliper used for measurement of hydrogel bead diameter and to average size distribution of 10 hydrogel beads used to measure the bead diameter; SEM-EDS elemental analysis and elemental mapping of control, Hg\textsuperscript{2+}-saturated and Cu\textsuperscript{2+}-saturated nanocomposite; TEM image, UV—vis absorption spectrum, and photoluminescence spectrum of Au@BSA NCs; XPS spectra of the Au 4f region for the nanocomposite before and after Hg\textsuperscript{2+} adsorption; XPS spectra of the Hg 4f region of the nanocomposite after Hg\textsuperscript{2+} adsorption; plot showing the variation in red intensity along the bead diameter at t = 100 s for 100 ppm concentration of Hg\textsuperscript{2+}; plot showing the fitting of the variation in red intensity for various time points to a sine curve; plot of the maximum intensity (taken from the center of sine curve) plotted against time; R-code to obtain the variation of red intensity along the bead diameter; error calculation for diffusion coefficient estimation; photograph of freeze-dried nanocomposite
hydrogel beads which have been stored for more than six months under UV light; plot showing the variation of normalized fluorescence intensities of Au@BSA NCs and Au@BSA NCs CNC-ALG nanocomposite after binding with Hg$^{2+}$ ions at regular time intervals. (PDF)

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**Notes**

The authors declare no competing financial interest.

### ACKNOWLEDGMENTS

Nishi Mohammed wishes to acknowledge the support from the MITACS Globalink Research Award for carrying out this research project at Indian Institute of Technology Madras, Chennai, India. Kam Chiu Tam wishes to acknowledge the funding from Canada Foundation for Innovation (CFI), National Sciences and Engineering Research Council of Canada (NSERC), and Grand Challenges Canada for their support for his research programs on nanomaterials for water treatment. T. Pradeep wishes to acknowledge the Nanomission of the Department of Science and Technology (DST), Government of India, for their constant support for his research programs on nanomaterials. The authors also wish to thank Chennu Sudhakar, E. Sundar Raj, and A. A. Arokia Gnani Loyola for their assistance in performing XPS and SEM-EDS experiments.

### REFERENCES


