

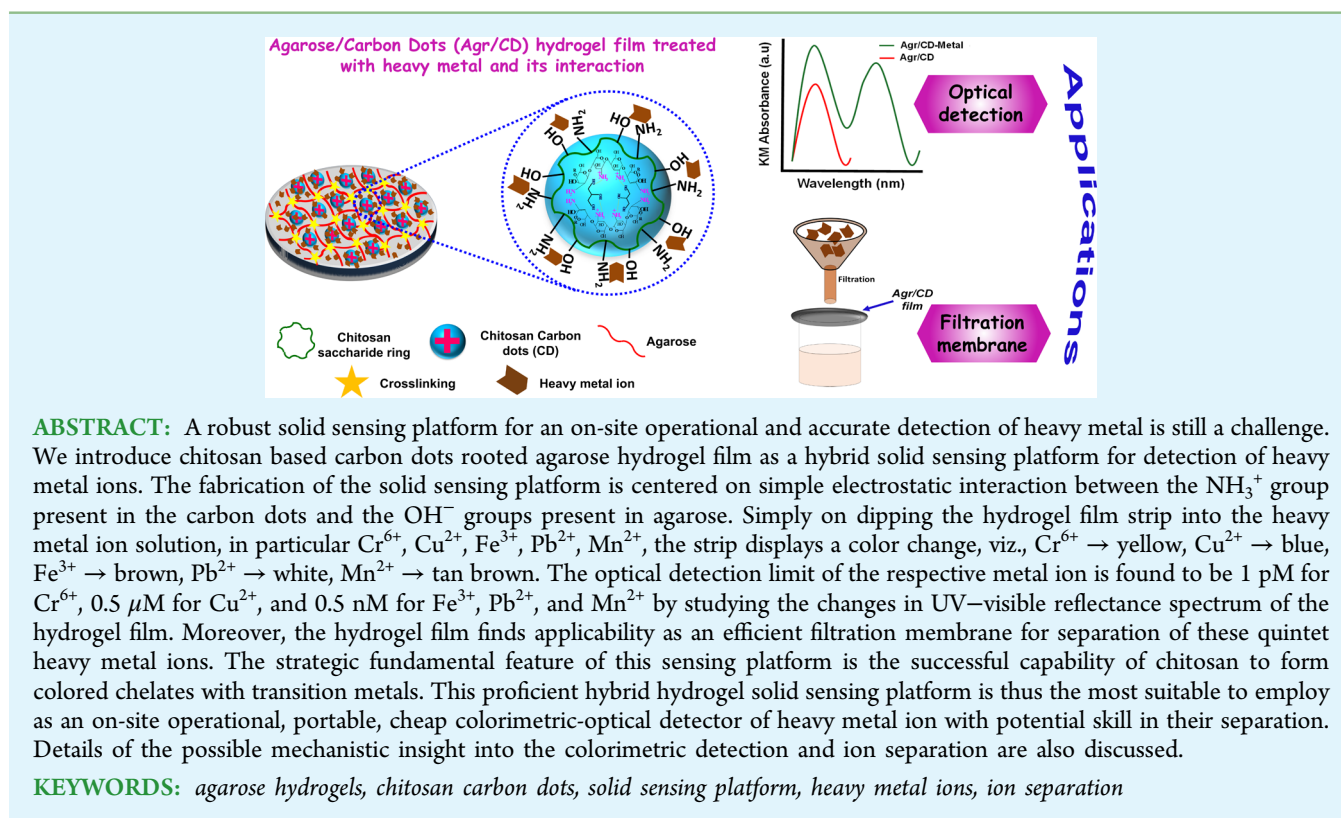
# Carbon Dots Rooted Agarose Hydrogel Hybrid Platform for Optical Detection and Separation of Heavy Metal Ions

Neelam Gogoi,<sup>†</sup> Mayuri Barooah,<sup>†</sup> Gitanjali Majumdar,<sup>‡</sup> and Devasish Chowdhury<sup>\*,†</sup>

<sup>†</sup>Material Nanochemistry Laboratory, Physical Sciences Division, Institute of Advanced Study in Science and Technology, Paschim Bora, Garchuk, Guwahati, 781035, India

<sup>‡</sup>Department of Chemistry, Assam Engineering College, Jalukbari, Guwahati, 781013, India

## S Supporting Information



## INTRODUCTION

Heavy metals and their critical detection have become one of the most popularized research domains owing to fastly growing urbanization and industrialization which has resulted in rampant pollution of the environment. Researchers are trying subtle approaches to dig out methods for selective and sensitive on-site detection of toxic heavy metals. There is evidence from studies that heavy metal ions pose serious threats to the environment and human health with a multitude of toxicities causing severe ailments and even death. To conquer these problems researchers are employing many techniques like fluorescence,<sup>1</sup> colorimetric,<sup>2–6</sup> optical,<sup>7,8</sup> atomic absorption spectroscopy,<sup>9,10</sup> and many more. Development of sensing material as per the requirement of these techniques is yet another issue for scientists. The employment of nanomaterials has been practiced in the past for detection of heavy metal ions.<sup>11</sup> To mention a few reports including modification of gold

nanoparticle surfaces using dyes or cysteine,<sup>12,13</sup> lead(II) induced allosteric G-Quadruplex DNAzyme for  $\text{Pb}^{2+}$  detection,<sup>14</sup> sulfide ion detection of lead in the presence of stabilizing nonprecious nanoparticles,<sup>15</sup> functionalized ruthenium sensitizers for mercury ions detections,<sup>7</sup> a dual DNAzyme allosteric unimolecular  $\text{Cu}^{2+}$  ion detecting system,<sup>16</sup> and cyclic N,O,S-donor crown ether based  $\text{Ag}^+$  chemosensors.<sup>17</sup> These sensing materials have permitted selective and sensitive detection of heavy metals.

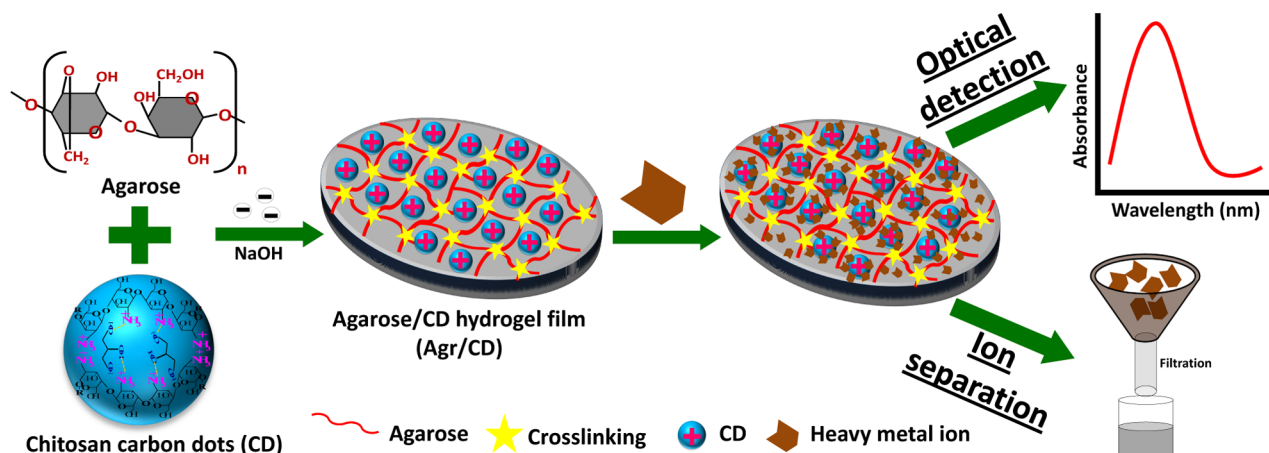
However, designing a robust solid sensing platform for an operational, on-site, quick, and accurate sensing of these heavy metals is still a challenge. Various studies as revealed in literature showed few design strategies for a solid heavy metal

Received: September 26, 2014

Accepted: January 8, 2015

Published: January 8, 2015

**Scheme 1.** Pictorial Representation of Fabrication of Agarose/CD (*Agr/CD*) Hydrogel Film and Its Applicability as Optical Sensor and Filtration Membrane for Separation of Quintet Heavy Metal Ions  $\text{Cr}^{6+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Fe}^{3+}$ ,  $\text{Pb}^{2+}$ , and  $\text{Mn}^{2+}$



sensing platform based on a three-dimensional nanoscale structure. These involve multiple heavy metal ions detection by immobilization of diphenylcarbazine (DPC), dithizone (DZ), tetraphenylporphine, tetrasulfonic acid (TPPS), and pyrogallol red (PR) molecular receptors with intrinsic mobility onto 3D nanoscale structures<sup>18</sup> and monolithic cage cubic *Fm3m* mesostructures of a F108 (EO141PO44EO141) copolymer<sup>19</sup> for  $\text{Cd}^{2+}$  detection. In this regard, a hydrogel based sensing platform has also gained attention for effective detection of heavy metal ions.<sup>20,21</sup> The art of hydrogel based sensing depends on the diffusion process of the analyte in a predictive concentration and time scale taking into account the chemical conversion of the analytical species. Agarose is a well-known candidate to form thermo reversible hydrogels by hydrogen bond linking.<sup>22</sup> It is a linear polymer made up of the repeating unit of agarobiose, which is a disaccharide of D-galactose and 3,6-anhydro-L-galactopyranose, and are extracted from seaweeds.<sup>23</sup> There are reports of a voltammetric sensor for in situ detection of heavy metals by diffusion through the agarose hydrogel.<sup>24,25</sup>

We report herein the fabrication of an agarose hydrogel rooted with carbon dots (CD) as a thin film solid sensing platform for optical detection and successful ion separation of quintet heavy metal ions ( $\text{Cr}^{6+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Fe}^{3+}$ ,  $\text{Pb}^{2+}$ ,  $\text{Mn}^{2+}$ ). The carbon dots in our study are prepared from chitosan hydrogel in keeping with our previous report.<sup>26</sup> These chitosan carbon dots of size less than 10 nm showed beautiful blue fluorescence under UV light and were positively charged with zeta potential value of  $\zeta = +27.8$  mV. These positively charged chitosan carbon dots when rooted into the negatively charged agarose matrix ( $\zeta = -104$  mV, prepared in 0.1 N sodium hydroxide) resulted in a solid sensing platform via successful electrostatic interaction between the  $\text{OH}^-$  of agarose and  $\text{NH}_3^+$  groups present in carbon dots. Although there are reports of immobilization of quantum dots (QDs)<sup>27</sup> and carbon nanotube (CNTs)<sup>28</sup> into agarose hydrogel, to date there is no report of such carbon dots rooted agarose hydrogel film for heavy metal sensing as per our knowledge. The agarose/carbon dots (*Agr/CD*) hydrogel film offered selective and sensitive optical detection of the quintet heavy metal ions down to a concentration limit of 1 pM for  $\text{Cr}^{6+}$ , 0.5  $\mu\text{M}$  for  $\text{Cu}^{2+}$ , and 0.5 nM for  $\text{Fe}^{3+}$ ,  $\text{Pb}^{2+}$ , and  $\text{Mn}^{2+}$ . The *Agr/CD* hydrogel films when treated with any of these quintet heavy metal ions showed signaling changes in their reflectance spectra when

subjected under UV–visible analysis. Also, these metal treated *Agr/CD* hydrogel films showed an instant color change characteristic of that metal ion salt within a time of 5–10 s.

In this study, we endeavored to design a smart, green, cost-effective, and easy way to prepare a solid sensing platform for colorimetric-optical sensing of heavy metals. This carbon dots rooted agarose hydrogel film (*Agr/CD*) also offers convenient on-site handling and use in comparison to other sensing platforms as already mentioned. The *Agr/CD* hydrogel film has showed its capability not only in optical sensing of the quintet heavy metal ions ( $\text{Cr}^{6+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Fe}^{3+}$ ,  $\text{Pb}^{2+}$ ,  $\text{Mn}^{2+}$ ) but also its applicability as an efficient filtration membrane for these quintet metal ions (Scheme 1). The principle of membrane filtration lies in the ability of chitosan to form chelates with heavy metals.<sup>29,30</sup> Thus, there is likely to be scope for utilization of these *Agr/CD* hydrogel films in industrial scale and also as a preliminary investigation of metal cations in salts with quick response.

## EXPERIMENTAL SECTION

**Materials.** Agarose low gelling temperature (SRL, India), low molecular weight chitosan (Sigma-Aldrich, degree of deacetylation=77.7%), glycerol (about 98% purified, Sigma-Aldrich), sodium hydroxide (NaOH) pellets (purified) (Merck), glacial acetic acid  $\text{CH}_3\text{COOH}$  (Merck), sodium chloride (NaCl) (Merck), potassium iodide (KI) (Merck), ferric nitrate ( $\text{Fe}(\text{NO}_3)_3$ ) (Merck), manganese(II) chloride tetrahydrate ( $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ ) (Merck), copper sulfate pentahydrate ( $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ ) (Merck), lead nitrate ( $\text{Pb}(\text{NO}_3)_2$ ) (Merck), zinc chloride ( $\text{ZnCl}_2$ ) (Merck), mercuric chloride ( $\text{HgCl}_2$ ) (Merck), cadmium chloride monohydrate ( $\text{CdCl}_2 \cdot \text{H}_2\text{O}$ ) (Merck), cobalt(II) chloride hexahydrate ( $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ ) (Merck), nickel(II) chloride hexahydrate ( $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$ ) (Merck), and chromium(VI) oxide ( $\text{CrO}_3$ ) (Merck) were used as received. All other reagents used were of analytical grade.

**Preparation of Agarose (*Agr*) Hydrogel Film.** In a typical procedure, agarose hydrogels of 2.5% (w/v) were prepared by dissolving 0.25 g of agarose in 10 mL of 0.1 N NaOH solution followed by boiling in microwave condition for 30 s. The film casting was done by pouring the above solution in a petri dish and cooling to room temperature which upon keeping overnight (preferably 12–15 h) took the shape of a thin hydrogel film of thickness  $\sim 0.3$  mm.

**Preparation of Carbon Dots (CD) from Chitosan Hydrogel.** The precursor used for the preparation of carbon dots was chitosan hydrogel. The chitosan hydrogel was synthesized using a simple approach. 1% glacial acetic acid solution and glycerol was mixed in a ratio of 1 part of acetic acid solution to 3 parts of glycerol to form a

solvent. 0.1 g of low molecular weight chitosan (dried in oven at 65 °C for about 2 h) was taken in 10 mL of the aforesaid solvent and stirred for 2 h in magnetic stirrer to form a clear pale yellow solution. Thereafter, 300  $\mu$ L of 5 N NaOH solution was added for neutralization (to form a cross-link), and immediately a clear slightly tacky three-dimensional gel network was formed. No free water or glycerol was apparent.

For CD preparation, a 20 mL of 0.1 M solution of acetic acid was added to the prepared chitosan hydrogel and stirred to dissolution. The solution was then microwaved for 10–15 min, resulting in formation of beautiful blue fluorescent chitosan carbon dots when viewed under UV light.<sup>26</sup>

**Preparation of Agarose Carbon Dots (Agr/CD) Hydrogel Film.** This involved preparation of 2.5% (w/v) solution of agarose in CD solution. Accurately weighed 0.25 g of agarose was added to a 10 mL of 1:1 ratio mixture of CD solution and 0.1 N NaOH. The solution was then microwaved for 30 s to complete solubility of agarose, and film casting was done following the same protocol as done in the preparation of the agarose (Agr) hydrogel film.

**Colorimetric Signature of Quintet Heavy Metal Ions.** The presence of heavy metal ions was detected by the colorimetric signature of the respective metal ions. The detection was done by placing the Agr and Agr/CD hydrogel films (size  $\sim 1 \times 1$  cm and thickness 0.3  $\sim$  mm) in the respective metal ion solutions, viz., CrO<sub>3</sub>, CuSO<sub>4</sub>, Fe(NO<sub>3</sub>)<sub>3</sub>, MnCl<sub>2</sub>, Pb(NO<sub>3</sub>)<sub>2</sub>, HgCl<sub>2</sub>, CdCl<sub>2</sub>, ZnCl<sub>2</sub>, CoCl<sub>2</sub>, NiCl<sub>2</sub>, NaCl, and KI of concentration 1 mM. The hydrogel films were then removed from the metal ion solutions after 12 h and dried in vacuum. The photographs of each hydrogel film were also taken.

**Optical Sensing of Agr/CD Hydrogel Film in the Presence of the Quintet Heavy Metal Ions.** Optical sensing of the heavy metal ions for their selective and sensitive detection monitored by UV–vis spectrophotometer was studied in detail. Significant changes in the reflectance spectra of the hydrogel film in the presence of the metal ion were observed. For the analysis, the reflectance spectrum of a  $1 \times 1$  cm hydrogel film piece of thickness  $\sim 0.3$  mm was recorded in a UV–visible spectrophotometer from 800 to 190 nm wavelength and then kept immersed in 2 mL of 1 mM solution of each metal ion, Cr<sup>6+</sup>, Cu<sup>2+</sup>, Fe<sup>3+</sup>, Pb<sup>2+</sup>, and Mn<sup>2+</sup>, for 12 h. After this the film was recovered from the metal ion solution and the reflectance spectrum of the film was again recorded to study any signaling change in the hydrogel film. The net change in the absorbance (% reflectance converted to Kubelka–Munk (KM) absorbance, instrumentally) at the specific wavelengths of metal ions (Cr<sup>6+</sup>,  $\lambda = 380$  nm, Cu<sup>2+</sup>,  $\lambda = 290$  nm, Fe<sup>3+</sup>,  $\lambda = 360$  nm, Pb<sup>2+</sup>,  $\lambda = 215$  nm, Mn<sup>2+</sup>,  $\lambda = 250$  nm) was then calculated. For the sensitivity study, a set of seven different concentration solutions of each metal ion, viz., 1 mM, 0.5 mM, 1  $\mu$ M, 0.5  $\mu$ M, 1 nM, 0.5 nM, and 1 pM, was prepared, and the complete study mentioned above was repeated for each set of concentrations. The net change in absorbance was then plotted against each concentration of the metal ion solution.

**Successful Filtration Capability of the Hydrogel Films.** Agr/CD and Agr hydrogel films were investigated as filtration membrane in particular for heavy metal ions, viz., Cr<sup>6+</sup>, Cu<sup>2+</sup>, Fe<sup>3+</sup>, Pb<sup>2+</sup>, and Mn<sup>2+</sup>, through flame atomic absorption spectroscopy (AAS). A 10 ppm solution of the metal ions was prepared, and the hydrogel films of size  $\sim 1 \times 1$  cm and thickness  $\sim 0.3$  mm were kept immersed in 10 mL of each of these metal ion solutions for 12 h. The hydrogel films were then removed from the solutions, and then the concentration of the metal ions in the solutions was analyzed by AAS.

**Characterization.** The Agr and Agr/CD hydrogel films were characterized by Fourier transform infrared spectrometer (NICOLET 6700 FT-IR) to investigate the chemical changes in the film structure before and after treatment with carbon dots. Spectra were obtained in transmission mode from samples in KBr pellets in the range of 400–4000 cm<sup>-1</sup> over 32 scans. Size distribution and zeta potential of the CDs and the hydrogel films were done using Malvern Zetasizer NanoZS 90 to investigate the particle size of CD and zeta potential of the hydrogel film. Thermogravimetric analysis (TGA) was done using PerkinElmer TGA 4000 from 35 to 800 °C at a heating rate of 10 °C per minute with a nitrogen flow rate of 20 mL per minute. Surface

morphology of the chitosan carbon dots were investigated using scanning electron microscope (SEM) from Carl Zeiss (Sigma VP). UV–visible study was done using Shimadzu UV spectrophotometer-UV 2600 to study the absorbance of the hydrogel film ( $1 \times 1$  cm). For PL intensity analysis, measurements were done in Varian Carl Eclipse fluorescence spectrophotometer. The chitosan carbon dot solution and hydrogel films ( $1 \times 1$  cm) were excited at 340, 360, 380, 400, 420, and 440 nm excitation. The filtration capability of the hydrogel films was analyzed using an atomic absorption spectrometer in a AA-7000 Shimadzu model. XRD analysis was done in Bruker D8 Advanced Diffractometer.

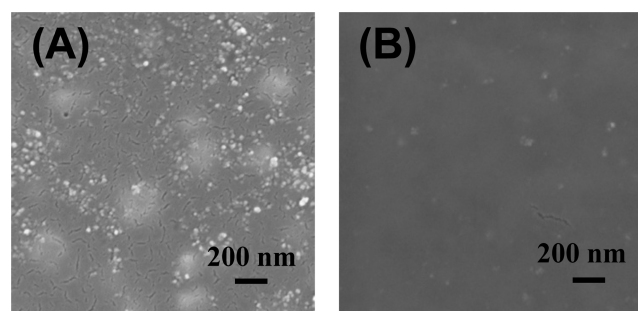
## ■ RESULTS AND DISCUSSIONS

### Fabrication of the Hybrid Agarose/CD Hydrogel Film.

A green hybrid hydrogel film of chitosan carbon dots rooted into agarose as a proficient on-site operational solid sensing platform for heavy metal ions was fabricated in a simple one step protocol. Agarose in 2.5% (w/v) was immersed in a 1:1 ratio mixture of chitosan carbon dots:sodium hydroxide followed by microwave heating until dissolution of agarose in the mixture and then poured into a petri dish for film casting. Owing to the “low temperature gelling” characteristic of agarose a beautiful thin hydrogel film of thickness  $\sim 0.3$  mm was formed upon keeping the petri dish at room temperature. The chitosan carbon dots used in the fabrication were prepared as per a previous report.<sup>26</sup> The chitosan CD was well-characterized by studying its photoluminescence, particle size, and zeta potential properties (Figure S1, in Supporting Information). The chitosan CD showed excellent photoluminescence properties with well-known excitation based emission spectrum.<sup>31</sup> The characteristic red shift in the emission wavelength was observed upon excitation of CDs to wavelengths 340, 360, 380, 400, 420, and 440 nm. Also, the dynamic light scattering (DLS) analysis showed that the particle size of CDs was less than 10 nm (size  $\sim 2.7$  nm) and the CDs were positively charged with zeta potential value  $\zeta = +27.8$  mV. The positive nature of the chitosan CD is due to the protonation of NH<sub>2</sub> groups to NH<sub>3</sub><sup>+</sup>, which is also well explained in our previous report.<sup>26</sup>

This positive zeta potential value of chitosan carbon dots encouraged the fabrication of a hybrid hydrogel from chitosan CD and agarose. For the successful electrostatic interaction of agarose with the positively charged CD the agarose was made negatively charged, hence explaining its dissolution in sodium hydroxide (NaOH). To study and compare the role of chitosan carbon dots in the hybrid agarose/CD (Agr/CD) hydrogel film, a simple agarose (Agr) hydrogel film was prepared by dissolution of 2.5% (w/v) agarose in sodium hydroxide only. The Agr/CD hydrogel film showed significant strength, flexibility, and stability (in terms of degradation) in comparison to Agr hydrogel film, making it a sustainable and easy-to-use soft hydrogel film. A zeta potential DLS analysis was done to study the net surface charge on the hydrogel films. A high negative zeta potential value of  $\zeta = -104.2$  mV was observed in case of the Agr hydrogel film whereas on incorporation of carbon dots the  $\zeta$  value shifted towards more positive reporting a zeta potential of  $\zeta = +51.4$  mV for Agr/CD hydrogel film. This suggested that owing to dissolution of agarose in NaOH initially the Agr hydrogel film showed such a high negative zeta potential but with incorporation of CD in Agr/CD there was successful electrostatic interaction between the agarose and the chitosan moieties which resulted in predominance of net positive zeta potential. Next, scanning electron microscopy analysis to study any perceivable morphological change on

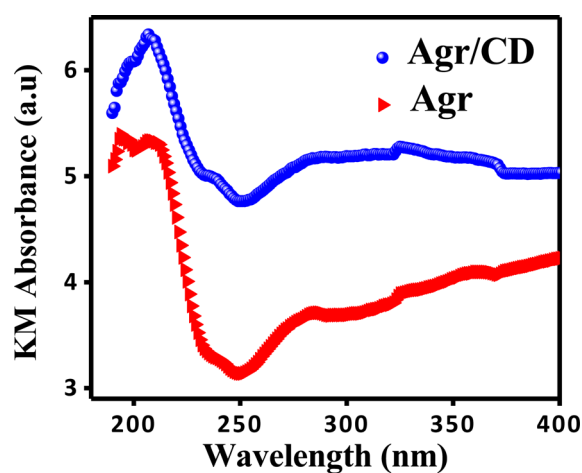
incorporation of carbon dots into agarose was performed (Figure 1). The distribution of carbon dots all over the agarose



**Figure 1.** Scanning electron microscope (SEM) image of (A) *Agr/CD* and (B) *Agr* hydrogel film.

hydrogel film was vivid from the SEM image of *Agr/CD*. However, such distribution was absent in *Agr* hydrogel film and in fact the film surface was found to be smooth in comparison to that of *Agr/CD*.

**Systematic Characterization of *Arg* and *Agr/CD* Hydrogel Films.** Any change in the optical property of *Agr* hydrogel film on incorporation of chitosan CD was investigated through UV–visible and photoluminescence spectrophotometry. Initially, the UV–visible analysis (Figure 2) was done in



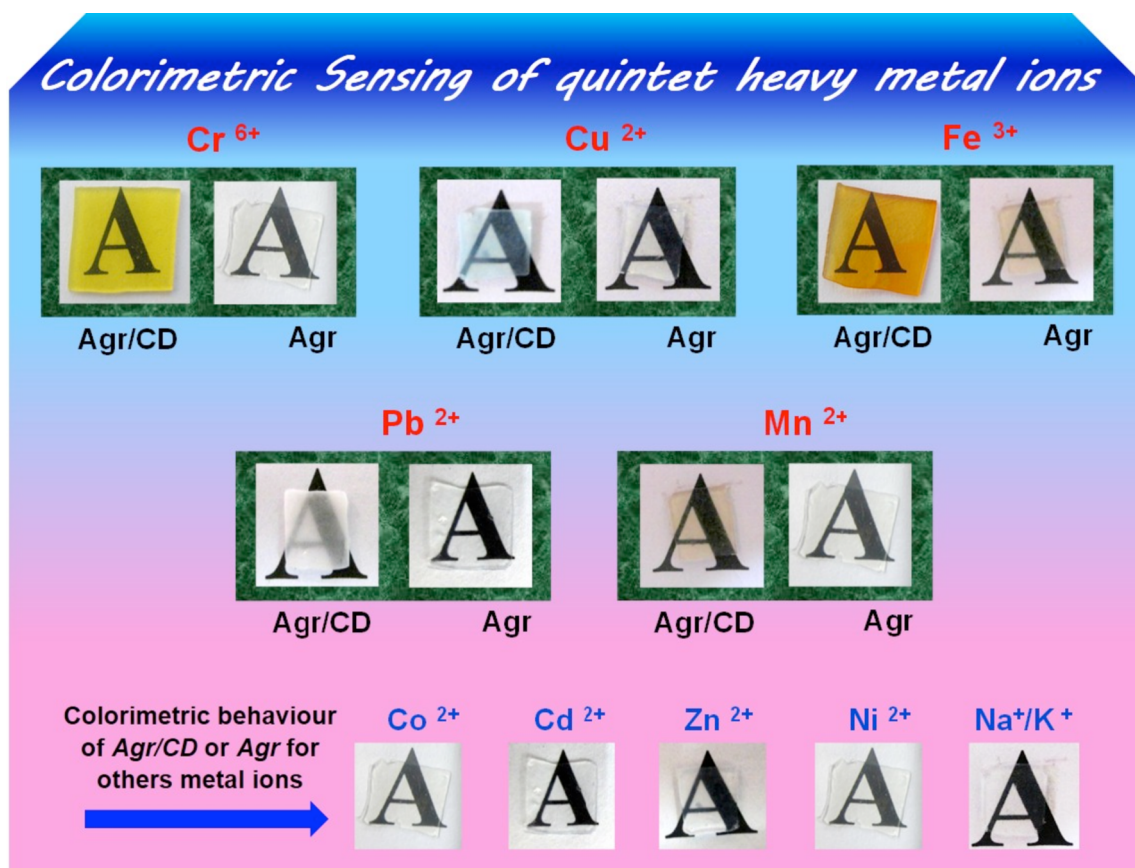
**Figure 2.** UV–visible spectrum of agarose (*Arg*) and agarose-carbon dots (*Agr/CD*) hydrogel films. (KM = % reflectance converted into absorbance by the Kubelka–Munk method (arbitrary units)).

reflectance mode from 800 to 190 nm wavelength range to study the behavioral change in *Agr* and *Agr/CD* hydrogel films. In the *Agr/CD* UV–visible spectrum a sharp peak at 206 nm (characteristic of chitosan) was evident which was found to be absent in *Agr*, thus supporting the presence of chitosan moiety in the *Agr/CD* hydrogel network. Following this a photoluminescence (PL) analysis was carried out, and the hydrogel films were subjected to excitation wavelengths of 340, 360, 380, 400, 420, and 440 nm. Both the hydrogel films were found to show maximum PL intensity at 360 nm excitation wavelength. Supporting Information Figure S2 shows the comparative PL emission spectra of *Agr* and *Agr/CD* at excitation wavelength,  $\lambda_{\text{excitation}} = 360$  nm. It was evident from the figure that *Agr/CD* showed an increase in PL intensity of about ~25% from that of

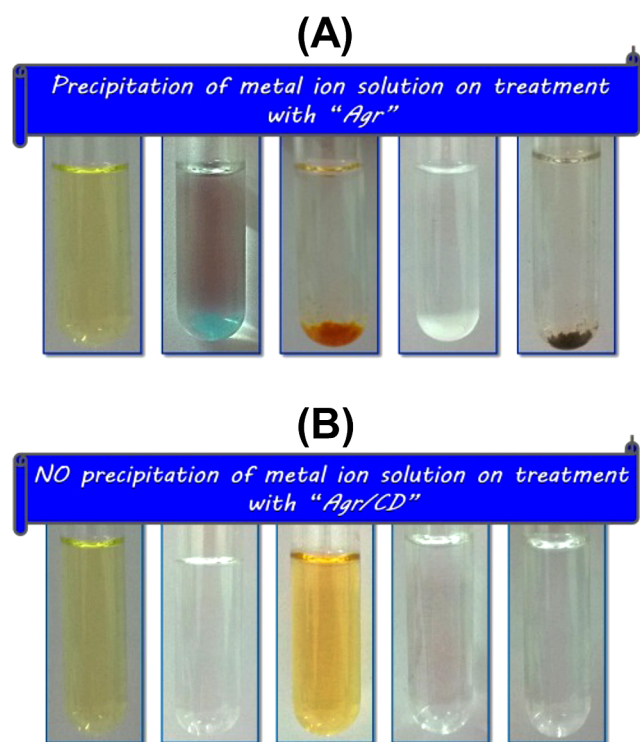
*Agr*, hence justifying the presence and role of chitosan carbon dots in the hydrogel films.

FTIR analysis was done to systematically investigate the electrostatic interaction between agarose and chitosan CD. Figure S3A in Supporting Information shows the FTIR spectrum of the hydrogel films. The broadening and shift of the peak around  $3500\text{ cm}^{-1}$  in *Agr/CD* compared to that in *Agr* indicated extensive hydrogen bonding between  $-\text{NH}_3^+$  and  $-\text{OH}^-$  groups of chitosan and agarose. The peak at  $2896\text{ cm}^{-1}$  due to  $-\text{CH}_2$  stretching vibration in *Agr* was shifted to  $2882\text{ cm}^{-1}$ , and the  $2941\text{ cm}^{-1}$  peak was much more pronounced in *Agr/CD* indicating the presence of a chitosan moiety. The peak around  $1647\text{ cm}^{-1}$  in *Agr* and *Agr/CD* was due to  $\text{H}-\text{O}-\text{H}$ , the stretching vibration of bound water. The peaks at  $1154$  and  $1067\text{ cm}^{-1}$  in *Agr* were shifted to  $1110$  and  $1036\text{ cm}^{-1}$ , respectively, in *Agr/CD* specifying extensive C–O–C bending vibration of glycosidic linkage. The 3,6-anhydro galactose peak at  $930\text{ cm}^{-1}$  shifted to  $924\text{ cm}^{-1}$  with considerable decrease in intensity also confirmed the successful interaction between chitosan and agarose moieties. These extensive electrostatic interactions between agarose and chitosan CD also effected the thermal behavior of hydrogel films investigated by thermogravimetric analysis (TGA) under nitrogen flow (Figure S3B, Supporting Information). The *Agr* hydrogel films showed about 45.4% degradation at  $100\text{ }^\circ\text{C}$ . On the other hand, the *Agr/CD* hydrogel film was extremely stable under high temperature with only 7% degradation at  $100\text{ }^\circ\text{C}$  and maximum degradation was achieved at as high as  $263\text{ }^\circ\text{C}$ . This extremely high temperature stability in the *Agr/CD* hydrogel film was thus attributed due to added interaction of agarose with chitosan carbon dots. In *Agr* hydrogel films, the maximum degradation occurred at  $136\text{ }^\circ\text{C}$ .

**Colorimetric Signature of Quintet Heavy Metal Ions ( $\text{Cr}^{6+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Fe}^{3+}$ ,  $\text{Pb}^{2+}$ ,  $\text{Mn}^{2+}$ ).** The *Agr* and *Agr/CD* were studied for their capability in successful detection of heavy metal ions. At the onset, *Agr* and *Agr/CD* (size  $\sim 1 \times 1$  cm, thickness  $\sim 0.3$  mm) were placed in 2 mL of 1 mM solutions of different metal ions,  $\text{Cr}^{6+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Fe}^{3+}$ ,  $\text{Pb}^{2+}$ ,  $\text{Mn}^{2+}$ ,  $\text{Co}^{2+}$ ,  $\text{Cd}^{2+}$ ,  $\text{Zn}^{2+}$ ,  $\text{Ni}^{2+}$ ,  $\text{Hg}^{2+}$ ,  $\text{Na}^+$ , and  $\text{K}^+$ , for 12 h. Within 5–10 s of loading of metal ions into the *Agr/CD* hydrogel film (Figure 3), there was distinct color change observed particularly in the case of  $\text{Cr}^{6+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Fe}^{3+}$ ,  $\text{Pb}^{2+}$ , and  $\text{Mn}^{2+}$ . The color change of the *Agr/CD* hydrogel film was corresponding to the color of the respective metal ion solution, viz.,  $\text{Cr}^{6+} \rightarrow$  yellow,  $\text{Cu}^{2+} \rightarrow$  blue,  $\text{Fe}^{3+} \rightarrow$  brown,  $\text{Pb}^{2+} \rightarrow$  white, and  $\text{Mn}^{2+} \rightarrow$  tan brown. Interestingly, such color change was not observed in the case of any other metal ions. This color change could be distinguished up to a concentration limit of  $1\text{ }\mu\text{M}$  through naked eyes. In contrast, in the *Agr* hydrogel film (Figure 3) such a color change was not at all observed for any of the metal ions. Even upon keeping the hydrogel films in the metal ion solutions for 12 h to ensure maximum absorption and coloration, the *Agr* hydrogel films did not show any sign of color change. A colorimetric analysis examining the behavior of the *Agr/CD* film toward a mixture of metal ion solutions was also conducted (Figure S4, Supporting Information). For the analysis, three target solutions, viz.,  $\{\text{Fe}(\text{NO}_3)_3 + \text{Pb}(\text{NO}_3)_2\}$ ;  $\{\text{FeCl}_3 + \text{CuCl}_2 + \text{MnCl}_2\}$ , and  $\{\text{CuCl}_2 + \text{MnCl}_2\}$ , were prepared. A dominance of the  $\text{Fe}^{3+}$  hydrogel film was shown, thereby changing color of the film to dark brown. On the other hand, when the target solution containing  $\{\text{CuCl}_2 + \text{MnCl}_2\}$  as the components was treated with *Agr/CD*, the film changed color to blue. Figure 4A shows the photographs of the test tube containing the *Agr* film in different metal ion solutions. Interestingly, *Agr* film that was



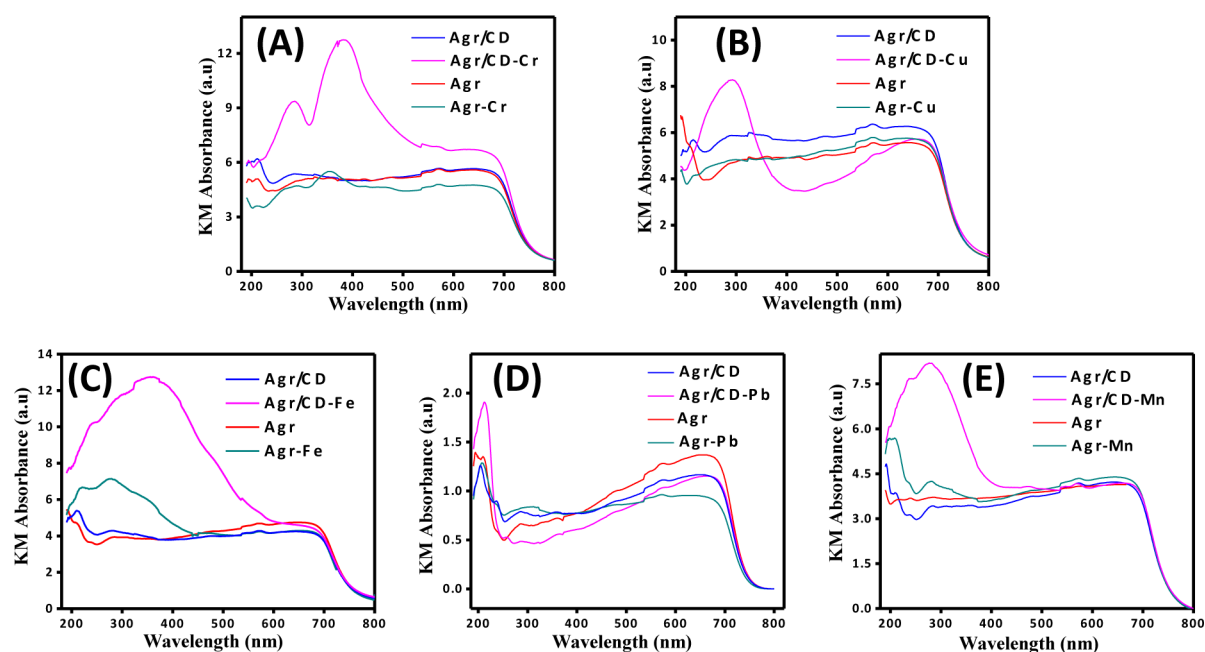
**Figure 3.** Photographs of colorimetric sensing test of heavy metal ions, exclusively  $\text{Cr}^{6+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Fe}^{3+}$ ,  $\text{Pb}^{2+}$ , and  $\text{Mn}^{2+}$ , when loaded into the hydrogel films *Agr/CD* and *Agr*.



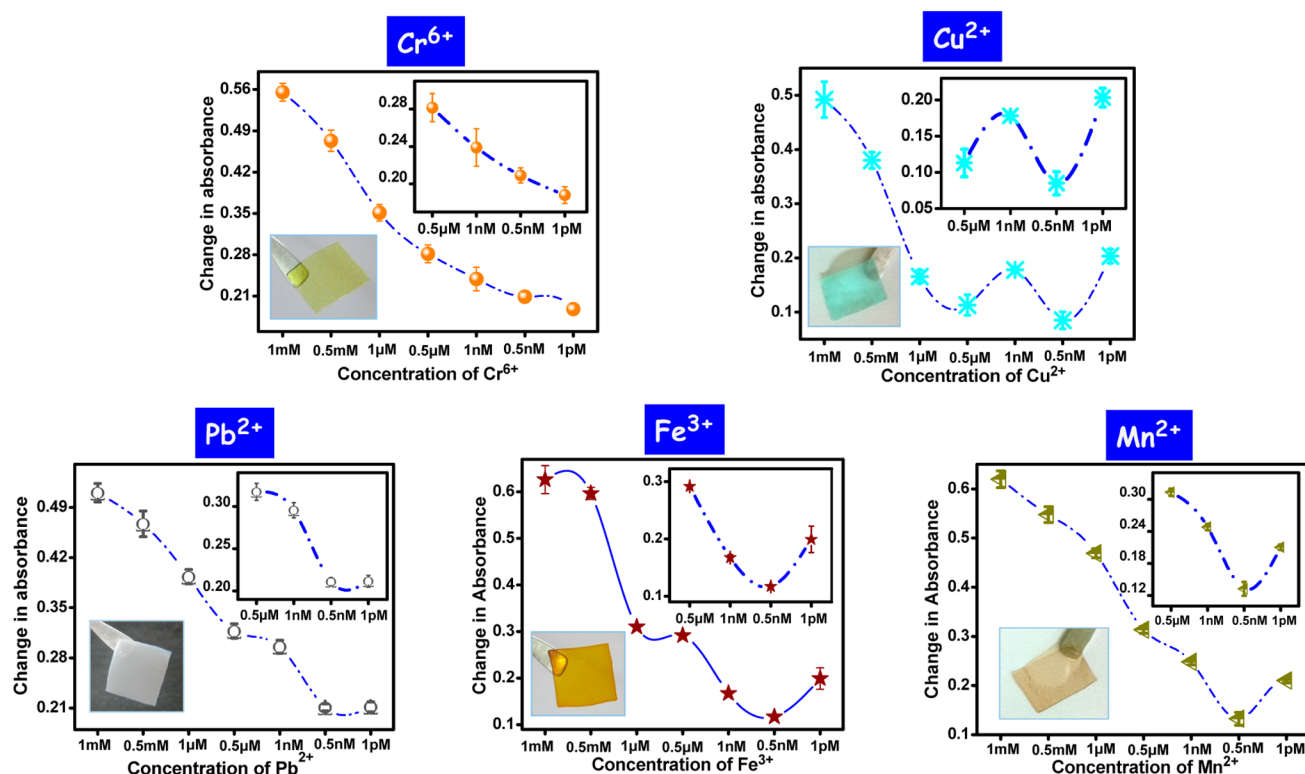
**Figure 4.** Fate of the quintet heavy metal ion solutions after loading into (A) *Agr* and (B) *Agr/CD*.

incapable of showing any coloration resulted in colored precipitation from the respective metal ion solution when subjected to same treatment (placing in metal ion solution). This occurrence of precipitation can be explained due to the formation of respective metal hydroxide when *Agr* was placed in the metal ion solution. The *Agr* hydrogel film was prepared by dissolution of agarose in  $\text{NaOH}$ ; thus, when free  $\text{OH}^-$  ions happened to come in close proximity with the metal ions, this eventually resulted in formation of metal hydroxides which were finally precipitated out in the metal ion solution. However, in the case of *Agr/CD* (Figure 4B), no precipitation was apparent, which was obviously due to the well reported affinity of chitosan for transition metal ions forming chitosan–metal chelates.<sup>29,30,32,33</sup>

To further explain and provide experimental proof of the precipitation of metal hydroxides and formation of chitosan–metal chelates, the *Agr* and *Agr/CD* hydrogel films were investigated by X-ray diffractometer (XRD), Figures S5 and S6 (Supporting Information). The presence of the quintet metal ions in the *Agr/CD* hydrogel film was found to be very prominent, showing the characteristic diffraction peaks of the metal ions. But such representative diffraction peaks of the quintet metal ions were not evident in the *Agr* hydrogel film. For *Agr/CD* treated with  $\text{Cr}$  (*Agr/CD*- $\text{Cr}$ ), the chromium experimental diffraction peaks were found to be at  $2\theta$  values of  $25.1^\circ$ ,  $26.6^\circ$ ,  $30.8^\circ$ , and  $32.6^\circ$  corresponding to  $d$  spacing values of  $3.54 \text{ \AA}$ ,  $3.34 \text{ \AA}$ ,  $2.89 \text{ \AA}$ , and  $2.74 \text{ \AA}$ , respectively (JCPDS file no. 01-072-9167, 00-012-0241, 00-006-0139, 00-059-0308). Similarly, for *Agr/CD*- $\text{Cu}$ , the  $d$  spacing values  $2.07 \text{ \AA}$ ,  $1.80 \text{ \AA}$ , and  $1.27 \text{ \AA}$  corresponding to copper diffraction peak  $2\theta$  values



**Figure 5.** UV–visible spectrum of agarose (*Agr*) and agarose/CD (*Agr/CD*) hydrogel films before and after keeping in metal ion solutions (A)  $\text{Cr}^{6+}$ , (B)  $\text{Cu}^{2+}$ , (C)  $\text{Fe}^{3+}$ , (D)  $\text{Pb}^{2+}$ , and (E)  $\text{Mn}^{2+}$ . (KM = % reflectance converted into absorbance by the Kubelka–Munk method (arbitrary units).)



**Figure 6.** Net change in absorbance value of chitosan-metal chelate peak on binding of metal ion of different concentrations (1 mM, 0.5 mM, 1  $\mu\text{M}$ , 0.5  $\mu\text{M}$ , 1 nM, 0.5 nM, and 1 pM) in *Agr/CD* hydrogel film. ( $\text{Cr}^{6+}$ ,  $\lambda = 380$  nm,  $\text{Cu}^{2+}$ ,  $\lambda = 290$  nm,  $\text{Fe}^{3+}$ ,  $\lambda = 360$  nm,  $\text{Pb}^{2+}$ ,  $\lambda = 215$  nm,  $\text{Mn}^{2+}$ ,  $\lambda = 250$  nm). Inset: The enlarged view of the graph from 0.5  $\mu\text{M}$  to 1 pM concentration range of metal ions and the photograph of the *Agr/CD* hydrogel film after binding with the respective metal ions.

43.48°, 50.62°, and 74.38°, respectively (JCPDS file no. 00-003-0307, 00-004-0836), for *Agr/CD-Fe* the  $d$  spacing values 6.90 Å, 3.19 Å, 3.34 Å, and 3.24 Å corresponding to iron diffraction peak  $2\theta$  values 12.81°, 27.89°, 26.58°, and 27.4°, respectively (JCPDS file no. 00-013-0458, 00-008-0097, 00-005-0499), for *Agr/CD-Mn* the diffraction peak at  $2\theta$  value 31.35° with  $d$

spacing 2.85 Å (JCPDS file no. 00-008-0171), and for *Agr/CD-Pb*, the  $d$  spacing values 3.59 Å, 3.06 Å, 2.63 Å, 3.26 Å, and 1.58 Å corresponding to lead diffraction peak of  $2\theta$  values 24.77°, 29.08°, 34.01°, 27.26° and 58.2°, respectively (JCPDS file no. 00-018-0701, 01-076-1796, 00-041-1493), were reported in the XRD spectrum of *Agr/CD* hydrogel film treated with quintet

metal ions. However, such diffraction peaks were not at all observed in *Agr* hydrogel film treated with the quintet metal ions. Along with the XRD analysis, SEM and EDX (energy dispersive X-ray) analyses (Figure S7, Supporting Information) were also done to draw conclusions about the formation of respective metal ion chelates on *Agr/CD* hydrogel film as a support to other experimental evidence described earlier. The SEM images are showing the presence of cluster type structures on the hydrogel film surface which on inspecting by EDX shows the signature of the quintet metal ions.

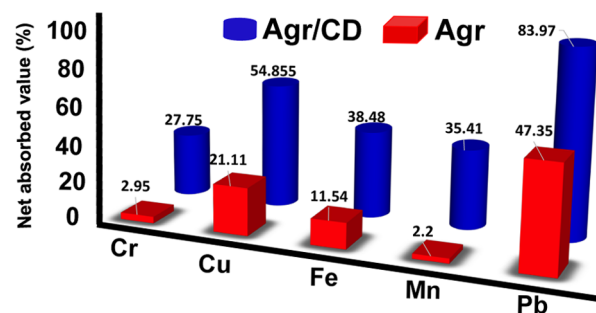
**Optical Detection of Quintet Heavy Metal Ions ( $\text{Cr}^{6+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Fe}^{3+}$ ,  $\text{Pb}^{2+}$ ,  $\text{Mn}^{2+}$ ).** To systematically study the colorimetric detection of the quintet metal ions, a detailed UV–visible spectroscopic analysis showing optical detection of  $\text{Cr}^{6+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Fe}^{3+}$ ,  $\text{Pb}^{2+}$ , and  $\text{Mn}^{2+}$  by *Agr/CD* was carried out simply by observing changes in the reflectance spectra on binding with the metal ion. The *Agr* and *Agr/CD* hydrogel films (size  $\sim 1 \times 1$  cm, thickness  $\sim 0.3$  mm) were kept immersed in 2 mL of all five metal ion solutions initially of 1 mM concentration for 12 h. In the first instance the only occurrence of new peaks after binding with the metal ion solutions was witnessed in the reflectance spectrum of the *Agr/CD*; however, such new peaks were found to be less prominent or absent in *Agr* hydrogel films. The graphs showing change in the UV–visible spectrum of the hydrogel films before and after binding with the  $\text{Cr}^{6+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Fe}^{2+}$ ,  $\text{Pb}^{2+}$ , and  $\text{Mn}^{2+}$  are shown in Figure 5. It can be seen from the figure that there is change in the spectral pattern with occurrence of new peaks with respect to the metal ion indicating successful binding and formation of chitosan–metal chelate in *Agr/CD*. The UV–visible spectral band is characteristic of the chitosan–metal chelate formation in the presence of different metal ions. Therefore, a net change in the absorbance of the hydrogel films before and after binding with the metal ion solution was calculated with respect to the respective peak of the chitosan–metal complex in the UV–visible spectrum (Figure S8, Supporting Information). It was observed that the net change in absorbance of the *Agr/CD* hydrogel film on binding with  $\text{Cr}^{6+}$  was 9.3 times more in comparison to that in the *Agr* hydrogel film. Similarly, the net change in absorbance of *Agr/CD* for  $\text{Cu}^{2+} = 11.25$ ,  $\text{Fe}^{2+} = 7.09$ ,  $\text{Mn}^{2+} = 9.72$ , and  $\text{Pb}^{2+} = 9.64$  was higher in comparison to *Agr* hydrogel film. Hence it can be stated here that *Agr/CD* hydrogel film is an effective candidate of selective detection of quintet metal ions ( $\text{Cr}^{6+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Fe}^{3+}$ ,  $\text{Pb}^{2+}$ ,  $\text{Mn}^{2+}$ ).

To further investigate the sensitivity of the optical detection of these quintet metal ions by *Agr/CD*, a set of different concentration solutions (1 mM, 0.5 mM, 1  $\mu\text{M}$ , 0.5  $\mu\text{M}$ , 1 nM, 0.5 nM, and 1 pM) of each metal ion were prepared. The *Agr/CD* hydrogel films were kept immersed in these different concentration solutions for 12 h, and then any signaling change in the reflectance spectrum of the film was monitored. Excitingly, the *Agr/CD* was found capable of optically detecting the  $\text{Cr}^{6+}$  metal ion down to a concentration limit of 1 pM, other quintet metal ions, i.e.,  $\text{Fe}^{3+}$ ,  $\text{Pb}^{2+}$ ,  $\text{Mn}^{2+}$  to a concentration limit of 0.5 nM, and  $\text{Cu}^{2+}$  to a concentration limit 0.5  $\mu\text{M}$ . A comparison plot of net change in absorbance of the *Agr/CD* on binding with the quintet metal ions of concentrations 1 mM, 0.5 mM, 1  $\mu\text{M}$ , 0.5  $\mu\text{M}$ , 1 nM, 0.5 nM, and 1 pM is plotted in Figure 6.

As can be witnessed from Figure 6, the net change in absorbance of *Agr/CD* on binding with  $\text{Cr}^{6+}$  of 1 pM concentration was almost 6.32 times more than that observed in the case of *Agr* binding with 1 mM  $\text{Cr}^{6+}$ . Thus, we can say

here that the net change in absorbance of *Agr/CD* is much higher even for a concentration as low as 1 pM. Similar results were obtained for other metal ions of the quintet showing 6.62, 4.80, 6.17, and 6.88 times more change in the absorbance value when subjected to  $\text{Cu}^{2+}$  (0.5  $\mu\text{M}$ ),  $\text{Fe}^{3+}$  (0.5 nM),  $\text{Pb}^{2+}$  (0.5 nM), and  $\text{Mn}^{2+}$  (0.5 nM) in comparison to 1 mM concentration treatment of *Agr* hydrogel film.

**Applicability of *Agr/CD* Hydrogel Film as a Filtration Membrane.** We have shown so far the selective colorimetric–optical detection of quintet heavy metal ions down to a concentration limit 1 pM for  $\text{Cr}^{6+}$ , 0.5  $\mu\text{M}$  for  $\text{Cu}^{2+}$ , 0.5 nM for  $\text{Fe}^{3+}$ ,  $\text{Pb}^{2+}$ , and  $\text{Mn}^{2+}$ . After detection of any contamination in the environment, what becomes important is its separation. So, the next step in our study was to test the applicability of these hydrogel films as an efficient filtration membrane for separation of the quintet heavy metal ions. To achieve this both the hydrogel films *Agr* and *Agr/CD* were allowed to remain immersed in a 10 ppm solution of  $\text{Cr}^{6+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Fe}^{3+}$ ,  $\text{Pb}^{2+}$ , and  $\text{Mn}^{2+}$  for 12 h followed by subsequent analyses of all the quintet heavy metal ion solutions from atomic absorption spectroscopy (AAS). It was evident from the AAS analysis (Figure 7) done in triplicate that the net removal of respective

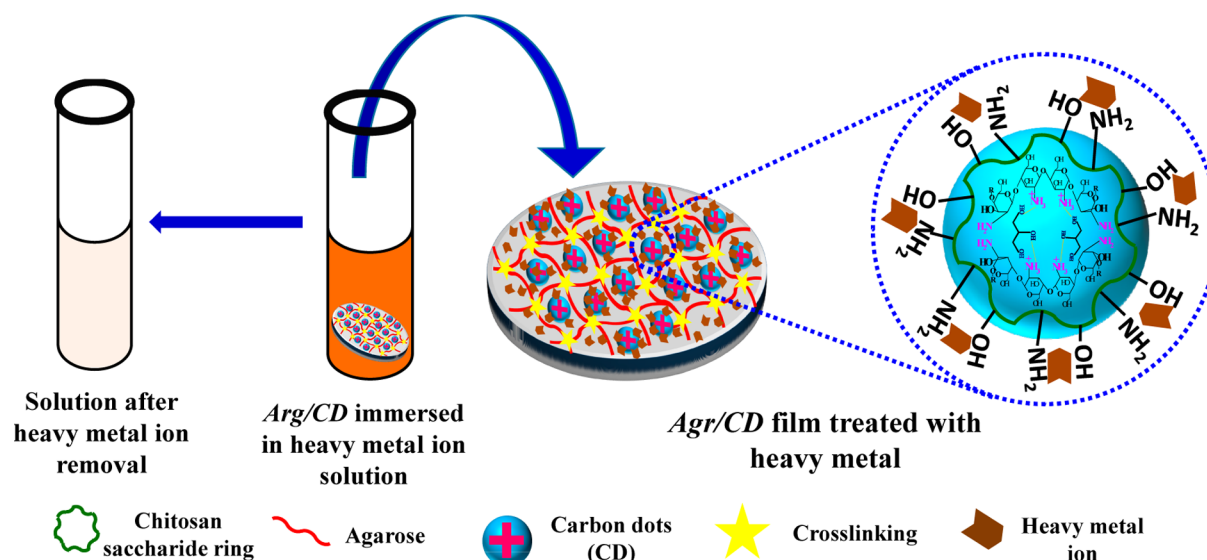


**Figure 7.** Net absorbed value (%) of the quintet metal ions by *Agr/CD* and *Agr* hydrogel film calculated by atomic absorption spectroscopic (AAS) analysis.

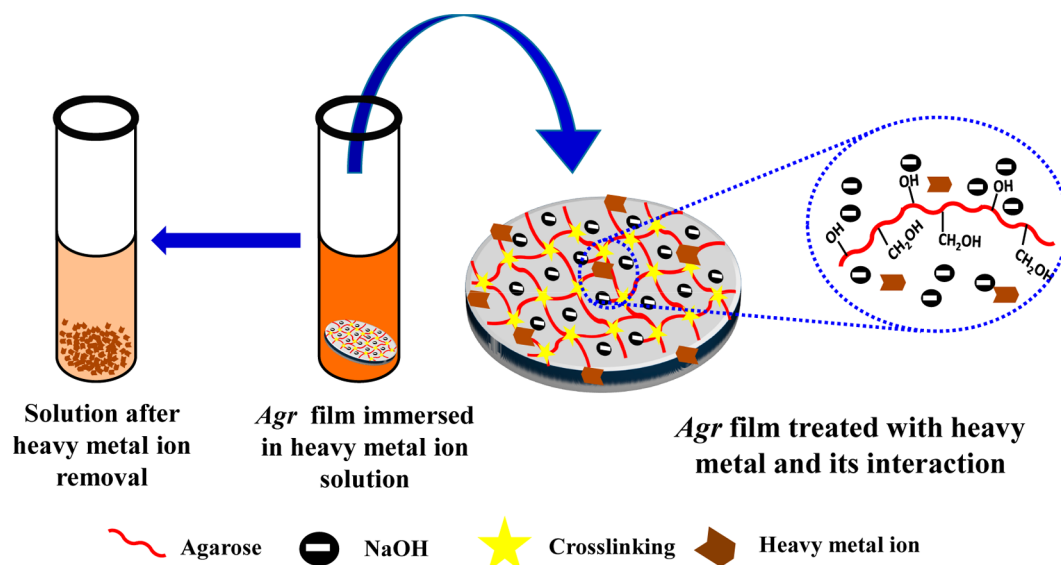
metal ions by *Agr* was much less in comparison to *Agr/CD*. For  $\text{Cr}^{6+}$  the net removal was found to be 27.75% by *Agr/CD* which was only 2.95% by *Agr*. Also, the net ion removal by *Agr/CD* for other metal ions was calculated to be 54.85%, 38.48%, 35.41%, and 83.97% for  $\text{Cu}^{2+}$ ,  $\text{Fe}^{3+}$ ,  $\text{Mn}^{2+}$ , and  $\text{Pb}^{2+}$ , respectively, in contrast to very low ion removal of 21.11%, 11.54%, 2.2%, and 47.35% by *Agr* for  $\text{Cu}^{2+}$ ,  $\text{Fe}^{3+}$ ,  $\text{Mn}^{2+}$ , and  $\text{Pb}^{2+}$ , respectively. We can now easily draw the conclusion from these experimental studies that *Agr/CD* shows operative efficiency to be used as a model filtration membrane for the separation of these quintet heavy metal ions.

A possible mechanistic explanation speculated for the detection and separation of these quintet heavy metal ions is due to the well reported tendency of chitosan to form chelates with transition metals.<sup>30,32</sup> In the case of *Agr/CD* hydrogel films (Scheme 2), the chitosan carbon dots were beautifully dispersed all over the agarose hydrogel matrix and also the free  $\text{NH}_3^+$  groups of chitosan CD were again deprotonated due to addition of NaOH. The deprotonated  $\text{NH}_2$  groups of chitosan form chelates with metal ions as per the already reported pendent model. This makes *Agr/CD* to be effective in detection of metal ions in their solution and also aids in their separation. However, the selectivity of *Agr/CD* toward the quintet metal ions  $\text{Cr}^{6+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Fe}^{3+}$ ,  $\text{Pb}^{2+}$ , and  $\text{Mn}^{2+}$  exclusively has to be answered yet. In *Agr* hydrogel films, on the other

Scheme 2. Pictorial Representation Showing the Possible Mechanism of Metal Binding with Agr/CD



Scheme 3. Pictorial Representation Showing the Possible Mechanism of Metal Binding with Agr



hand, the absence of any such functional group capable of forming chelates with metal ions makes *Agr* inapplicable for heavy metal ion detection. Instead in *Agr* hydrogel films formation of metal hydroxide takes place in the presence of metal ions due to existence of unbounded  $\text{OH}^-$  ions in the hydrogel matrix which in turn explains the occurrence of precipitates on treatment of *Agr* with the metal ion solutions (Scheme 3).

## CONCLUSIONS

In this report we successfully demonstrate the fabrication of agarose hydrogel rooted with carbon dots (CD) as an exciting sensing platform for colorimetric-optical detection of quintet heavy metal ions, viz.,  $\text{Cr}^{6+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Fe}^{3+}$ ,  $\text{Pb}^{2+}$ , and  $\text{Mn}^{2+}$ . The color changes observed in *Agr/CD* hydrogel thin film correspond to the colors of the respective metal ion solution, viz.,  $\text{Cr}^{6+} \rightarrow$  yellow,  $\text{Cu}^{2+} \rightarrow$  blue,  $\text{Fe}^{3+} \rightarrow$  brown,  $\text{Pb}^{2+} \rightarrow$  white, and  $\text{Mn}^{2+} \rightarrow$  tan brown. This color change can be attributed due to strategic formation of colored chitosan-metal

chelates. The minimum detection limit was found to be 1 pM for  $\text{Cr}^{6+}$ , 0.5 nM for  $\text{Fe}^{3+}$ ,  $\text{Pb}^{2+}$ , and  $\text{Mn}^{2+}$ , and 0.5  $\mu\text{M}$  for  $\text{Cu}^{2+}$ . In conclusion, this hybrid hydrogel solid sensing platform has potential applications as a ready, portable, cheap colorimetric detector of heavy metal ions. The hybrid hydrogel sensing platform also has an interesting advantage of its implementation as an efficient membrane for separation of heavy metal ions.

## ASSOCIATED CONTENT

### Supporting Information

Characterization of the chitosan carbon dots, FTIR, TGA, and XRD spectra of *Arg* and *Agr/CD* hydrogel films. The comparative plot showing net change in KM absorbance value after absorption of metal ions of *Arg* and *Agr/CD* hydrogel films. This material is available free of charge via the Internet at <http://pubs.acs.org>.



## ■ AUTHOR INFORMATION

## Corresponding Author

\*E-mail: devasish@iasst.gov.in. Fax: +91 361 2279909. Tel.: +91 361 2912073.

## Author Contributions

The manuscript was written through contributions of all authors.

## Notes

The authors declare no competing financial interest.

## ■ ACKNOWLEDGMENTS

The authors would like to thank Council of Scientific and Industrial Research (CSIR), New Delhi, for Project No. 01(2488)/11/EMR-II, All India Council For Technical Education, New Delhi, for Project No. 8023/RID/RPS-3/(NER)2011-12, and Science and Engineering Research Board (SERB), New Delhi, for Project Grant SR/S1/PC/0058/2010 and SB/S1/PC-69/2012. N.G. wants to thank CSIR, New Delhi, for a fellowship.

## ■ DEDICATION

Dedicated to Prof. Arun Chattopadhyay on the occasion of his 50th anniversary.

## ■ REFERENCES

(1) Ye, B. C.; Yin, B. C. Highly Sensitive Detection of Mercury (II) Ions by Fluorescence Polarization Enhanced by Gold Nanoparticles. *Angew. Chem., Int. Ed.* **2008**, *47*, 8386–8389.

(2) Zhang, J. F.; Zhou, Y.; Yoon, J.; Kim, J. S. Recent Progress in Fluorescent and Colorimetric Chemosensors for Detection of Precious Metal Ions (Silver, Gold and Platinum ions). *Chem. Soc. Rev.* **2011**, *40*, 3416–3429.

(3) Hossain, M. Z.; Brennan, J. D.  $\beta$ -Galactosidase-Based Colorimetric Paper Sensor for Determination of Heavy Metals. *Anal. Chem.* **2011**, *83*, 8772–8778.

(4) Kim, Y.; Johnson, R. C.; Hupp, J. T. Gold Nanoparticle-Based Sensing of “Spectroscopically Silent” Heavy Metal Ions. *Nano Lett.* **2001**, *1*, 165–167.

(5) Ye, B.-F.; Zhao, Y.-J.; Cheng, Y.; Li, T.-T.; Xie, Z.-Y.; Zhao, X.-W.; Gu, Z.-Z. Colorimetric Photonic Hydrogel Aptasensor for the Screening of Heavy Metal Ions. *Nanoscale* **2012**, *4*, 5998–6003.

(6) Ye, B.; Rong, F.; Gu, H.; Xie, Z.; Cheng, Y.; Zhao, Y.; Gu, Z. Bioinspired Angle-Independent Photonic Crystal Colorimetric Sensing. *Chem. Commun.* **2013**, *49*, 5331–5333.

(7) Collins, C. G.; Peck, E. M.; Kramer, P. J.; Smith, B. D. Squaraine Rotaxane Shuttle as a Ratiometric Deep-Red Optical Chloride Sensor. *Chem. Sci.* **2013**, *4*, 2557–2563.

(8) Li, C.; Shi, G. Polythiophene-Based Optical Sensors for Small Molecules. *ACS Appl. Mater. Interfaces* **2013**, *5*, 4503–4510.

(9) Li, Y.; Jiang, Y.; Yan, X. P.; Peng, W. J.; Wu, Y. Y. A Flow Injection On-Line Multiplexed Sorption Preconcentration Procedure Coupled with Flame Atomic Absorption Spectrometry for Determination of Trace Lead in Water, Tea, and Herb Medicines. *Anal. Chem.* **2002**, *74*, 1075–1080.

(10) Tsalev, D. L.; Lampugnani, L.; Georgieva, R.; Chakarova, K. K.; Petrov, J. Electrothermal Atomic Absorption Spectrometric Determination of Cadmium and Lead with Stabilized Phosphate Deposited on Permanently Modified Platforms. *Talanta* **2002**, *58*, 331–339.

(11) Yin, J.; Wu, T.; Song, J.; Zhang, Q.; Liu, S.; Xu, R.; Duan, H. SERS-Active Nanoparticles for Sensitive and Selective Detection of Cadmium Ion ( $\text{Cd}^{2+}$ ). *Chem. Mater.* **2011**, *23*, 4756–4764.

(12) Li, D.; Wieckowska, A.; Willner, I. Optical Analysis of  $\text{Hg}^{2+}$  Ions by Oligonucleotide–Gold-Nanoparticle Hybrids and DNA-Based Machines. *Angew. Chem., Int. Ed.* **2008**, *47*, 3927–3931.

(13) Darbha, G. K.; Rai, U. S.; Singh, A. K.; Ray, P. C. Selective Detection of Mercury (II) Ion using Nonlinear Optical Properties of Gold Nanoparticles. *J. Am. Chem. Soc.* **2008**, *130*, 8038–8043.

(14) Li, T.; Wang, E.; Dong, S. Lead (II)-Induced Allosteric G-Quadruplex DNzyme as a Colorimetric and Chemiluminescence Sensor for Highly Sensitive and Selective  $\text{Pb}^{2+}$  Detection. *Anal. Chem.* **2010**, *82*, 1515–1520.

(15) Yan, J.; Indra, E. M. Colorimetric Method for Determining  $\text{Pb}^{2+}$  Ions in Water Enhanced with Non-Precious-Metal Nanoparticles. *Anal. Chem.* **2012**, *84*, 6122–6127.

(16) Yin, B. C.; Ye, B. C.; Tan, W.; Wang, H.; Xie, C. C. An Allosteric Dual-DNzyme Unimolecular Probe for Colorimetric Detection of Copper(II). *J. Am. Chem. Soc.* **2009**, *131*, 14624–14625.

(17) Shamsipur, M.; Alizadeh, K.; Hosseini, M.; Caltagirone, C.; Lippolis, V. A selective Optode Membrane for Silver Ion Based on Fluorescence Quenching of the Dansylamidopropyl Pendant Arm Derivative of 1-aza-4,7,10-trithiacyclododecane ( $[\text{12}]_{\text{aneNS}_3}$ ). *Sens. Actuators, B* **2006**, *113*, 892–899.

(18) El-Safty, S. A.; Ismail, A. A.; Matsunaga, H.; Hanaoka, T.; Mizukami, F. Optical Nanoscale Pool-on-Surface Design for Control Sensing Recognition of Multiple Cations. *Adv. Funct. Mater.* **2008**, *18*, 1485–1500.

(19) El-Safty, S. A.; Prabhakaran, D.; Ismail, A. A.; Matsunaga, H.; Mizukami, F. Nanosensor Design Packages: A Smart and Compact Development for Metal Ions Sensing Responses. *Adv. Funct. Mater.* **2007**, *17*, 3731–3745.

(20) Hong, W.; Li, W.; Hu, X.; Zhao, B.; Zhang, F.; Zhang, D. Highly Sensitive Colorimetric Sensing for Heavy Metal Ions by Strong Polyelectrolyte Photonic Hydrogels. *J. Mater. Chem.* **2011**, *21*, 17193–17201.

(21) Leeuwen, H. P. V.; Town, R. M.; Buffle, J.; Cleven, R. F. M. J.; Davison, W.; Puy, J.; Riemsdijk, W. H. V.; Sigg, L. Dynamic Speciation Analysis and Bioavailability of Metals in Aquatic Systems. *Environ. Sci. Technol.* **2005**, *39*, 8545–8556.

(22) Golmohamadi, M.; Davis, T. A.; Wilkinson, K. J. Diffusion and Partitioning of Cations in an Agarose Hydrogel. *J. Phys. Chem. A* **2012**, *116*, 6505–6510.

(23) Firouzabadi, H.; Iranpoor, N.; Gholinejad, M.; Kazemi, F. Agarose Hydrogel as an Effective Bioorganic Ligand and Support for the Stabilization of Palladium Nanoparticles. Application as a Recyclable Catalyst for Suzuki–Miyaura Reaction in Aqueous Media. *RSC Adv.* **2011**, *1*, 1013–1019.

(24) Belmont-Hébert, C.; Tercier, M. L.; Buffle, J. Gel-Integrated Microelectrode Arrays for Direct Voltammetric Measurements of Heavy Metals in Natural Waters and Other Complex Media. *Anal. Chem.* **1998**, *70*, 2949–2956.

(25) Keller, O. C.; Buffle, J. Voltammetric and Reference Microelectrodes with Integrated Microchannels for Flow through Microvoltammetry. 1. The Microcell. *Anal. Chem.* **2000**, *72*, 936–942.

(26) Chowdhury, D.; Gogoi, N.; Majumdar, G. Fluorescent Carbon Dots Obtained from Chitosan Gel. *RSC Adv.* **2012**, *2*, 12156–12159.

(27) Liu, J.; Yang, X.; Wang, K.; Wang, Q.; Ji, H.; Wu, C.; Li, J.; He, X.; Tang, J.; Huang, J. Combining Physical Embedding and Covalent Bonding for Stable Encapsulation of Quantum Dots into Agarose Hydrogels. *J. Mater. Chem.* **2012**, *22*, 495–501.

(28) Lewitus, D. Y.; Landers, J.; Branch, J. R.; Smith, K. L.; Callegari, G.; Kohn, J.; Neimark, A. V. Biohybrid Carbon Nanotube/Agarose Fibers for Neural Tissue Engineering. *Adv. Funct. Mater.* **2011**, *21*, 2624–2632.

(29) Krajewska, B. Diffusion of Metal Ions Through Gel Chitosan Membranes. *React. Funct. Polym.* **2001**, *47*, 37–47.

(30) Onsosyen, E.; Skaugrud, O. Metal Recovery using Chitosan. *J. Chem. Technol. Biotechnol.* **1990**, *49*, 395–404.

(31) Jaiswal, A.; Ghosh, S. S.; Chattopadhyay, A. One Step Synthesis of C-dots by Microwave Mediated Caramelization of Poly (ethylene glycol). *Chem. Commun.* **2012**, *48*, 407–409.

(32) Mekahlia, S.; Bouzid, B. Chitosan-Copper (II) Complex as Antibacterial Agent: Synthesis, Characterization and Coordinating Bond-Activity Correlation Study. *Phys. Procedia* **2009**, *2*, 1045–1052.

(33) Ogawa, K.; Oka, K.; Yui, T. X-ray Study of Chitosan-Transition Metal Complexes. *Chem. Mater.* **1993**, *5*, 726–728.