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

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Supporting Information

ABSTRACT: A robust solid sensing platform for an on-site operational and accurate detection of heavy metal is still a challenge. We introduce chitosan based carbon dots rooted agarose hydrogel film as a hybrid solid sensing platform for detection of heavy metal ions. The fabrication of the solid sensing platform is centered on simple electrostatic interaction between the NH₃⁺ group present in the carbon dots and the OH⁻ groups present in agarose. Simply on dipping the hydrogel film strip into the heavy metal ion solution, in particular Cr⁶⁺, Cu²⁺, Fe³⁺, Pb²⁺, Mn²⁺, the strip displays a color change, viz., Cr⁶⁺ → yellow, Cu²⁺ → blue, Fe³⁺ → brown, Pb²⁺ → white, Mn²⁺ → tan brown. The optical detection limit of the respective metal ion is found to be 1 pM for Cr⁶⁺, 0.5 μM for Cu²⁺, and 0.5 nM for Fe³⁺, Pb²⁺, and Mn²⁺ by studying the changes in UV−visible reflectance spectrum of the hydrogel film. Moreover, the hydrogel film finds applicability as an efficient filtration membrane for separation of these quintet heavy metal ions. The strategic fundamental feature of this sensing platform is the successful capability of chitosan to form colored chelates with transition metals. This proficient hybrid hydrogel solid sensing platform is thus the most suitable to employ as an on-site operational, portable, cheap colorimetric-optical detector of heavy metal ion with potential skill in their separation. Details of the possible mechanistic insight into the colorimetric detection and ion separation are also discussed.

KEYWORDS: agarose hydrogels, chitosan carbon dots, solid sensing platform, heavy metal ions, ion separation

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,¹ colorimetric,²−⁶ optical,⁷,⁸ atomic absorption spectroscopy,⁹,¹⁰ 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.¹¹ To mention a few reports including modification of gold nanoparticle surfaces using dyes or cysteine,¹²,¹³ lead(II) induced allosteric G-Quadruplex DNAzyme for Pb²⁺ detection,¹⁴ sulfide ion detection of lead in the presence of stabilizing nonprecious nanoparticles,¹⁵ functionalized ruthenium sensitizers for mercury ions detections,¹⁶ a dual DNAzyme allosteric unimolecular Cu²⁺ ion detecting system,¹⁷ and cyclic N,O,S-donor crown ether based Ag⁺ chemosensors.¹⁸ 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...
sensing platform based on a three-dimensional nanoscale structure. These involve multiple heavy metal ions detection by immobilization of diphenylcarbazide (DPC), dithizone (DZ), tetraphenylporphine, tetrasulfonic acid (TPPS), and pyrogallol red (PR) molecular receptors with intrinsic mobility onto 3D nanoscale structures and monolithic cage cubic Fm3m mesostructures of a F108 (EO141PO44EO141) copolymer for Cd detection. In this regard, a hydrogel based sensing platform has also gained attention for effective detection of heavy metal ions. The art of hydrogel based sensing depends on the diffusion process of the analyte in a predictable 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. 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. There are reports of a voltammetric sensor for in situ detection of heavy metals by diffusion through the agarose hydrogel.

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 (Cr6+, Cu2+, Fe3+, Pb2+, Mn2+) 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. Thus, there is likely to be scope for utilization of these Agar/CD hydrogel films in industrial scale and also as a preliminary investigation of metal cations in salts with quick detection of the quintet heavy metal ions down to a concentration limit of 1 pM for Cr6+, 0.5 μM for Cu2+, and 0.5 nM for Fe3+, Pb2+, and Mn2+. The Ag/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 Ag/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 (Ag/CD) also offers convenient on-site handling and use in comparison to other sensing platforms as already mentioned. The Ag/CD hydrogel film has shown its capability not only in optical sensing of the quintet heavy metal ions (Cr6+, Cu2+, Fe3+, Pb2+, Mn2+) 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. Thus, there is likely to be scope for utilization of these Agar/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 (Merck), sodium chloride (NaCl) (Merck), potassium iodide (Merck), ferric nitrate (Fe(NO3)3) (Merck), manganese(II) chloride tetrahydrate (MnCl2·4H2O) (Merck), copper sulfate pentahydrate (CuSO4·5H2O) (Merck), lead nitrate (Pb(NO3)2) (Merck), zinc chloride (ZnCl2) (Merck), mercuric chloride (HgCl2) (Merck), cadmium chloride monohydrate (CdCl2·H2O) (Merck), cobalt(II) chloride hexahydrate (CoCl2·6H2O) (Merck), nickel(II) chloride hexahydrate (NiCl2·6H2O) (Merck), and chromium(VI) oxide (CrO3) (Merck) were used as received. All other reagents used were of analytical grade.

**Preparation of Agarose (Ag) 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 ~ 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 3:1 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 μL 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 gelatin 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.26

Preparation of Agrase Carbon Dots (Agr/CD) Hydrogel Film. This involved preparation of 2.5% (w/v) solution of agrase in CD solution. Accurately weighed 0.25 g of agrase 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 agrase, and film casting was done following the same protocol as done in the preparation of the agrase (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 ~ 1 × 1 cm and thickness 0.3 ~ 3 mm) in the respective metal ions solutions, viz., CrO3, CuSO4, Fe(NO3)3, MnCl2, Pb(NO3)2, HgCl2, CdCl2, ZnCl2, CoCl2, NiCl2, 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 x 1 cm hydrogel film piece of thickness ~ 0.3 mm was recorded in a UV–vis spectrophotometer from 800 to 190 nm wavelength and then kept immersed in 2 mL of 1 mM solution of each metal ion, Cr6+, Cu2+, Fe3+, Pb2+, and Mn2+, 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 (Cr6+: λ = 380 nm, Cu2+: λ = 290 nm, Fe3+: λ = 360 nm, Pb2+: λ = 215 nm, Mn2+: λ = 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 μM, 0.5 μM, 1 mM, 0.5 mM, and 1pM, 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., Cr6+, Cu2+, Fe3+, Pb2+, and Mn2+, through flame atomic absorption spectroscopy (AAS). A 10 ppm solution of the metal ions was prepared, and the hydrogel films of size ~ 1 x 1 cm and thickness ~ 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⁻¹ 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 (Zigma VP). UV–visible study was done using Shimadzu UV spectrophotometer-UV 2600 to study the absorbance of the hydrogel film (1 x 1 cm). For PL intensity analysis, measurements were done in Varian Carl Eclipse fluorescence spectrophotometer. The chitosan carbon dot solution and hydrogel films (1 x 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 Agrase/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. Agrase 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 ~ 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.26 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.31 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 ~ 2.7 nm) and the CDs were positively charged with zeta potential value ζ = +27.8 mV. The positive nature of the chitosan CD is due to the protonation of NH3+ groups to NH3+, which is also well explained in our previous report.26

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 ζ = −104.2 mV was observed in case of the Agr hydrogel film whereas on incorporation of carbon dots the ζ value shifted towards more positive reporting a zeta potential of ζ = +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

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incorporation of carbon dots into agarose was performed (Figure 1). The distribution of carbon dots all over the agarose 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 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, λ_{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 cm\(^{-1}\) in Agr/CD compared to that in Agr indicated extensive hydrogen bonding between –NH\(^2\) and –OH\(^-\) groups of chitosan and agarose. The peak at 2896 cm\(^{-1}\) due to –CH\(_2\) stretching vibration in Agr was shifted to 2882 cm\(^{-1}\), and the 2941 cm\(^{-1}\) peak was much more pronounced in Agr/CD indicating the presence of a chitosan moiety. The peak around 1647 cm\(^{-1}\) in Agr and Agr/CD was due to H–O–H, the stretching vibration of bound water. The peaks at 1154 and 1067 cm\(^{-1}\) in Agr were shifted to 1110 and 1036 cm\(^{-1}\), respectively, in Agr/CD specifying extensive C–O–C bending vibration of glycosidic linkage. The 3,6-anhydro galactose peak at 930 cm\(^{-1}\) shifted to 924 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 affected 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 °C. On the other hand, the Agr/CD hydrogel film was extremely stable under high temperature with only 7% degradation at 100 °C and maximum degradation was achieved at as high as 263 °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 °C.

**Colorimetric Signature of Quintet Heavy Metal Ions (Cr\(^{6+}\), Cu\(^{2+}\), Fe\(^{3+}\), Pb\(^{2+}\), 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 ~ 1 × 1 cm, thickness ~ 0.3 mm) were placed in 2 mL of 1 mM solutions of different metal ions, Cr\(^{6+}\), Cu\(^{2+}\), Fe\(^{3+}\), Pb\(^{2+}\), Mn\(^{2+}\), Co\(^{2+}\), Cd\(^{2+}\), Zn\(^{2+}\), Ni\(^{2+}\), Hg\(^{2+}\), Na\(^{+}\), and 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 Cr\(^{6+}\), Cu\(^{2+}\), Fe\(^{3+}\), Pb\(^{2+}\), and Mn\(^{2+}\). The color change of the Agr/CD hydrogel film was corresponding to the color of the respective metal ion solution, viz., Cr\(^{6+}\) → yellow, Cu\(^{2+}\) → blue, Fe\(^{3+}\) → brown, Pb\(^{2+}\) → white, and Mn\(^{2+}\) → 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 μM 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., \{Fe(NO\(_3\))\(_3\) + Pb(NO\(_3\))\(_2\}\), \{FeCl\(_3\) + CuCl\(_2\) + MnCl\(_2\}\}, and \{CuCl\(_2\) + MnCl\(_2\}\}, were prepared. A dominance of the Fe\(^{3+}\) hydrogel film was shown, thereby changing color of the film to dark brown. On the other hand, when the target solution containing \{CuCl\(_2\) + 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
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 NaOH; thus, when free 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.29,30,32,33

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 Cr (Agr/CD-Cr), the chromium experimental diffraction peaks were found to be at 2θ values of 25.1°, 26.6°, 30.8°, and 32.6° corresponding to d spacing values of 3.54 Å, 3.34 Å, 2.89 Å, and 2.74 Å, respectively (JCPDS file no. 01-072-9167, 00-012-0241, 00-006-0139, 00-059-0308). Similarly, for Agr/CD-Cu, the d spacing values 2.07 Å, 1.80 Å, and 1.27 Å corresponding to copper diffraction peak 2θ values

Figure 3. Photographs of colorimetric sensing test of heavy metal ions, exclusively Cr$^{6+}$, Cu$^{2+}$, Fe$^{3+}$, Pb$^{2+}$, and 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.
$43.48^\circ$, $50.62^\circ$, and $74.38^\circ$, 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 2θ 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 2θ 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 2θ 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

Figure 5. UV−visible spectrum of agarose (Agr) and agarose/CD (Agr/CD) hydrogel films before and after keeping in metal ion solutions (A) Cr$^{6+}$, (B) Cu$^{2+}$, (C) Fe$^{3+}$, (D) Pb$^{2+}$, and (E) 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 μM, 0.5 μM, 0.5 nM, and 1 pM) in Agr/CD hydrogel film. (Cr$^{6+}$, $\lambda$ = 380 nm, Cu$^{2+}$, $\lambda$ = 290 nm, Fe$^{3+}$, $\lambda$ = 360 nm, Pb$^{2+}$, $\lambda$ = 215 nm, Mn$^{2+}$, $\lambda$ = 250 nm). Inset: The enlarged view of the graph from 0.5 μM to 1 pM concentration range of metal ions and the photograph of the Agr/CD hydrogel film after binding with the respective metal ions.
metal ions. However, such diffraction peaks were not at all observed in Ag/CD 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 Ag/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 (Cr^{6+}, Cu^{2+}, Fe^{3+}, Pb^{2+}, Mn^{2+}).** To systematically study the colorimetric detection of the quintet metal ions, a detailed UV–visible spectroscopic analysis showing optical detection of Cr^{6+}, Cu^{2+}, Fe^{3+}, Pb^{2+}, and Mn^{2+} by Ag/CD was carried out simply by observing changes in the reflectance spectra on binding with the metal ion. The Ag and Ag/CD hydrogel films (size ~ 1 x 1 cm, thickness ~ 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 Ag/CD; however, such new peaks were found to be less prominent or absent in Ag/CD hydrogel films. The graphs showing change in the UV–visible spectrum of the hydrogel films before and after binding with the Cr^{6+}, Cu^{2+}, Fe^{3+}, Pb^{2+}, and Mn^{2+} are shown in Figure S5. 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 Ag/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 Ag/CD hydrogel film on binding with Cr^{6+} was 9.3 times more in comparison to that in the Ag hydrogel film. Similarly, the net change in absorbance of Ag/CD for Cu^{2+} = 11.25, Fe^{3+} = 7.09, Mn^{2+} = 9.72, and Pb^{2+} = 9.64 was higher in comparison to Ag hydrogel film. Hence it can be stated here that Ag/CD hydrogel film is an effective candidate of selective detection of quintet metal ions (Cr^{6+}, Cu^{2+}, Fe^{3+}, Pb^{2+}, Mn^{2+}).

To further investigate the sensitivity of the optical detection of these quintet metal ions by Ag/CD, a set of different concentration solutions (1 mM, 0.5 mM, 1 μM, 0.5 μM, 1 nM, 0.5 nM, and 1 pM) of each metal ion were prepared. The Ag/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 Ag/CD was found capable of optically detecting the Cr^{6+} metal ion down to a concentration limit of 1 pM, other quintet metal ions, i.e., Fe^{3+}, Pb^{2+}, Mn^{2+} to a concentration limit of 0.5 nM, and Cu^{2+} to a concentration limit 0.5 μM. A comparison plot of net change in absorbance of the Ag/CD on binding with the quintet metal ions of concentrations 1 mM, 0.5 mM, 1 μM, 0.5 μM, 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 Ag/CD on binding with Cr^{6+} of 1 pM concentration was almost 6.32 times more than that observed in the case of Ag binding with 1 mM Cr^{6+}. Thus, we can say here that the net change in absorbance of Ag/CD is much higher even for a concentration as low as 1 pM. Similar results were observed 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 Cu^{2+} (0.5 μM), Fe^{3+} (0.5 nM), Pb^{2+} (0.5 nM), and Mn^{2+} (0.5 nM) in comparison to 1 mM concentration treatment of Ag hydrogel film.

**Applicability of Ag/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 Cr^{6+}, 0.5 μM for Cu^{2+}, 0.5 nM for Fe^{3+}, Pb^{2+}, and 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 Ag and Ag/CD were allowed to remain immersed in a 10 ppm solution of Cr^{6+}, Cu^{2+}, Fe^{3+}, Pb^{2+}, and Mn^{2+} for 12 h followed by subsequent analyses of all the quintet heavy metal ions solutions from atomic absorption spectroscopy (AAS). It was evident from the AAS analysis (Figure 7) done in triplicate that the net removal of respective metal ions by Ag was much less in comparison to Ag/CD. For Cr^{6+} the net removal was found to be 27.75% by Ag/CD which was only 2.95% by Ag. Also, the net ion removal by Ag/CD for other metal ions was calculated to be 54.85%, 38.48%, 35.41%, and 83.97% for Cu^{2+}, Fe^{3+}, Mn^{2+}, and Pb^{2+}, respectively, in contrast to very low ion removal of 21.11%, 11.54%, 2.2%, and 47.35% by Ag for Cu^{2+}, Fe^{3+}, Mn^{2+}, and Pb^{2+}, respectively. We can now easily draw the conclusion from these experimental studies that Ag/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. In the case of Ag/CD hydrogel films (Scheme 2), the chitosan carbon dots were beautifully dispersed all over the agarose hydrogel matrix and also the free NH_{3}^{+} groups of chitosan CD were again deprotonated due to addition of NaOH. The deprotonated NH_{3}^{+} groups of chitosan form chelates with metal ions as per the already reported pendent model. This makes Ag/CD to be effective in detection of metal ions in their solution and also aids in their separation. However, the selectivity of Ag/CD toward the quintet metal ions Cr^{6+}, Cu^{2+}, Fe^{3+}, Pb^{2+}, and Mn^{2+} exclusively has to be answered yet. In Ag hydrogel films, on the other
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 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., Cr\(^{6+}\), Cu\(^{2+}\), Fe\(^{3+}\), Pb\(^{2+}\), and Mn\(^{2+}\). The color changes observed in Agr/CD hydrogel thin film correspond to the colors of the respective metal ion solution, viz., Cr\(^{6+}\) → yellow, Cu\(^{2+}\) → blue, Fe\(^{3+}\) → brown, Pb\(^{2+}\) → white, and Mn\(^{2+}\) → 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 Cr\(^{6+}\), 0.5 nM for Fe\(^{3+}\), Pb\(^{2+}\), and Mn\(^{2+}\), and 0.5 μM for 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 Agr and Agr/CD hydrogel films. The comparative plot showing net change in KM absorbance value after absorption of metal ions of Agr and Agr/CD hydrogel films. This material is available free of charge via the Internet at http://pubs.acs.org.
Machines.

Angew. Chem., Int. Ed. by Oligonucleotide − Cadmium Ion (Cd2+).

SERS-Active Nanoparticles for Sensitive and Selective Detection of 50th anniversary.

DEDICATION

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DEDICATION

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

REFERENCES


Novel carbon dot coated alginate beads with superior stability, swelling and pH responsive drug delivery†

Neelam Gogoi and Devasish Chowdhury*

Novel carbon dot coated alginate beads (CA-CD) exhibiting superior stability and swelling properties have been successfully prepared. CA-CD show exceptional stability in ambient condition and are stable at room atmosphere and temperature even after 60 days. Moreover, CA-CD show excessive swelling in comparison to calcium alginate (CA) beads. The beads were characterized by Fourier transform infrared spectroscopy (FTIR), thermogravimetric analysis (TGA), scanning electron microscopy (SEM) and optical microscopy. The CA and CA-CD beads were investigated for their use as pH dependent sustained drug delivery vehicles taking tetracycline (TC) and tetracycline associated with β-cyclodextrin (β-TC) as model drug systems. It was observed that TC loading was 35% and 77% with CA and CA-CD, respectively. Tetracycline associated with β-cyclodextrin (β-TC) shows 48% loading for CA and much greater loading (as high as 90%) for CA-CD. At pH 1, CA-CD and CA beads show maximum drug release with TC cumulative release of 70% and 37% at 96 h, respectively. However, the delivery rates at pH 1 were slower in case of tetracycline associated with β-cyclodextrin (β-TC) loading showing 61% release for CA-CD and 22% for CA after 96 h. Thus, CA-CD can be suitably used as an effective drug delivery vehicle with maximum release obtained at pH 1 emphasizing its use in the gastrointestinal tract where pH is low. Also, the use of β-cyclodextrin with the drug as an inclusion complex renders the CA and CA-CD beads useful for slow and long-term drug administration.

1. Introduction

In recent years, a great deal of research has been focused on biomaterials based on alginate and chitosan and their wide range applications such as in tissue engineering, cell culture, food preservation and drug delivery. Alginate and chitosan are natural polysaccharides that are biocompatible and biodegradable, and provoke no immunogenic toxicity on administration. Alginate derived from seaweeds and commercially available as a sodium salt of alginic acid is a linear anionic copolymer of alternating blocks of 1,4-linked α-L-guluronic (G) and β-D-mannuronic (M) acid residues. Sodium alginate forms an “egg-box” like gel network when combined in aqueous solution with divalent ions, such as Ca2+. Inter-chain cross linking results in a three dimensional calcium alginate hydrogel network due to Ca2+ ion coordination with the available O-atom of the two facing GG blocks. Chitosan obtained from shell fish is a linear cationic copolymer consisting of β-1,4-linked 2-amino-2-deoxy-D-glucose (D-glucosamine) and 2-acetamido-2-deoxy-D-glucose (N-acetyl-D-glucosamine) units and obtained by alkaline deacetylation of a naturally occurring polysaccharide, chitin.

In our previous report,† we introduced a simple method of preparation of carbon dots (CD) from chitosan gel. CDs are a new class of recently discovered fluorescent carbon nanomaterials attracting enormous attention due to their application in optoelectronic devices, biomedical imaging, biological labelling and drug delivery. These photoluminescent materials are chemically stable, and environmentally and biologically compatible compared to semiconductor quantum dots like CdS, CdSe, ZnS etc., which are known to be toxic and are potential environmental hazards as they contain heavy metals.

In this work, we report calcium alginate (CA) beads coated with carbon dots (CD) prepared from chitosan gel forming a protective coat over CA beads. There are reports in literature of biomaterials based on polyelectrolyte complex between chitosan and alginate but this is the first report of alginate beads

† Electronic supplementary information (ESI) available: Synthesis procedure of carbon dots, Solid PL emission spectra of CA, CA-CD and CA-CD/TC, photograph of CA and chitosan coated calcium alginate bead, SEM images of CA and CA-CD beads. 3-D plot of release of TCs in real time situation. BET plot of CACA-CD beads. Molecular structure of tetracycline hydrochloride (TC) showing different reactivity regions and FTIR spectra of tetracycline (TC), β-cyclodextrin (β-CD) and β-cyclodextrin–tetracycline inclusion complex (β-TC). See DOI: 10.1039/c3tb21835j

coated with carbon dots. The carbon dot coated calcium alginate (CA-CD) beads show extraordinary stability over more than 60 days in atmospheric conditions and excellent swelling properties. The CA-CD beads also show no sign of degradation unlike CA beads, which even after preservation under water degrade after a few days. This work focuses on all these properties of CA-CD that make it an exciting operational candidate for its application in various fields in comparison to CA and other reported chitosan–alginate materials.25,26 Carbon dots prepared from chitosan gel are positively charged as determined by zeta potential measurement (detail discussed later). On the other hand, calcium alginate has –OH and –COO– groups making it slightly negatively charged. We have used this electrostatic interaction for coating of carbon dot on calcium alginate (CA-CD). Moreover, chitosan is already in use as a drug delivery vehicle.27 In general, the formation of these polyelectrolyte complexes can be achieved through ionic interactions between carboxylate residues of alginate and protonated amino residues of chitosan. Such type of polyelectrolyte complexes have been widely used to obtain devices for controlled release of drugs such as antibiotics.28 Tetracycline (TC) is a well-known antibiotic having a broad spectrum range of bactericidal activity. It exhibits anti-inflammatory action, promotes attachment of fibroblasts and connective tissues to root surfaces29,30 and is also effective against proteinase imbalance diseases such as cancer metastasis, periodontitis and osteomyelitis.31,32 β-cyclodextrin (β-cd) is known to form inclusion complexes with many drugs by taking up the drug molecules into its central cavity33 and enhances the solubility, stability and bio-availability of drugs.34 It belongs to a class of cyclic oligosaccharides consisting of α-1,4-linked α-D-A glucopyranose units preferably in chair conformation.

Hence, taking clues from the literature we have also studied the applicability of the carbon dot coated calcium alginate hybrid beads (CA-CD) as a drug delivery vehicle taking tetracycline (TC) and β-cyclodextrin:tetracycline (β-TC) as model drug systems. It was observed from drug release profiles that CA and CA-CD show maximum release of TC and β-TC at pH 1, emphasizing its use in the gastrointestinal tract where pH is low. There is a report in the literature of encapsulation of Bifidobacterium breve into alginate matrix before coating in multilayers of alternating alginate and chitosan with the intention to improve the survival of B. breve during exposure to low pH.35 In this work, we show that carbon dot coated alginate beads (CA-CD) possess superior stability, swelling and are an efficient drug delivery vehicle in comparison to calcium alginate beads (CA).

2. Experimental

2.1. Materials

Sodium alginate (Sigma Aldrich), low molecular weight chitosan (Sigma Aldrich, degree of deacetylation determined 77.7%), glycerol (about 98% purified, Sigma Aldrich), hydrochloric acid (Merck, India), sodium hydroxide pellets (purified) (Merck, India), calcium chloride (Rankem Laboratory Reagent, India), and glacial acetic acid (Qualigens Fine Chemicals) were used as received. Tetracycline (TC) was obtained from tetracycline hydrochloride capsules (Aventis Pharma Ltd, Gujarat, India). All other materials used were of analytical reagent grade from commercial sources.

2.2. Preparation of calcium alginate beads

In a typical procedure, 10 mL of 1% (w/v) freshly prepared sodium alginate solution was added dropwise through a 10 mL syringe into 150 mL of 0.1 M CaCl2 solution under stirring conditions, resulting in instant formation of spherical beads due to cross-linking of sodium alginate with Ca2+. Calcium alginate (CA) beads thus formed were left in CaCl2 solution overnight to allow complete gelation before filtering and thoroughly washed with water to make them chloride free. Finally, CA beads were stored under water for further use. In all the experiments, Milli Q water with resistivity 18.2 MΩ was used.

2.3. Carbon dot coated calcium alginate beads (CA-CD beads)

To enhance the properties of CA beads, the beads were coated with carbon dots (CD) as shown in Fig. 1. For this purpose, CDs were synthesised from chitosan gel as per our previous report.15 The carbon dots (CDs) obtained were fluorescent and the emission of the CDs gradually shifted to higher wavelengths accompanied with decreased fluorescence intensity with an increase in the excitation wavelength from 300 nm to 430 nm.15 This type of red shift in the emission of CDs has been reported earlier.36 The size of the CDs was determined to be less than 10 nm from dynamic light scattering (DLS) data. Zeta potential study also confirmed a net positive zeta potential, ζ = 30.1, for CDs prepared from chitosan gel.15 We also studied the fluorescence properties of carbon dot coated alginate beads (CA-CD) and carbon dot coated alginate beads after tetracycline (TC) drug loading (CA-CD/TC). Solid fluorescence measurements were taken for CA, CA-CD and CA-CD/TC beads as shown in Fig. S1(A) (ESI).† As evident from the figure, PL emission is very weak. The weak PL emission is due to quenching of carbon dot emission upon coating on alginate beads. PL properties of carbon dots in the presence of TC and β-TC were studied and are shown in Fig. S1(B) (ESI).† PL emission shows that there is quenching of PL intensity on addition of TC. The same trend is also observed with β-TC. It is observed that quenching of PL intensity is more pronounced with β-TC than with TC. To coat the CA beads, the prepared CA beads were taken out from Milli Q water, air dried and immersed in CD solution for 3 h. After 3 h, the CA beads were removed from CD solution, dried on filter paper to remove excess unbound CDs and stored at room atmosphere in a closed Petri dish. The % coating yield was found to be 59.42%.

2.4. pH responsive behaviour and stability study of CA and CA-CD beads

CA and CA-CD beads were separately immersed in solutions of different pH to study their behaviour in varying pH. A set of five different pH solutions were prepared (pH 1: 0.1 M HCl, pH 3: 0.1 M acetic acid, pH 5: 0.005 M acetic acid, pH 9: 0.0015 M NaOH, pH 12: 0.1 M NaOH). The beads were kept in these solutions for
24 h and viewed under optical microscope. To check the stability of CA and CA-CD beads at room atmosphere, photographs of the prepared beads were taken at intervals for 2 months. A swelling study was also done for CA and CA-CD beads using a simple formula for the swelling ratio (SR):

\[
SR = \left(\frac{W_s - W_d}{W_d}\right)
\]

where \(W_s\) and \(W_d\) are the bead weights after swelling and in the dry state, respectively.

2.5. In vitro release study

Tetracycline HCl (TC) was used as a model drug to study the drug release property of CA and CA-CD beads through UV spectrophotometry. CA and CA-CD beads were kept immersed in 20 mL of 5 mg mL\(^{-1}\) solution of TC for 6 h. After 6 h, beads were taken out, air dried and immersed in 20 mL of Milli Q water to study their drug release behavior. pH responsive drug release of the beads was also studied in six different pH solutions (pH 1, pH 3, pH 5, pH 7, pH 9 and pH 12). Aliquots of 3 mL were withdrawn from the release medium periodically and added back after each sampling to maintain constant volume of the release medium. The amount of TC release from the beads was determined by measuring UV absorption at 275 nm (\(\lambda_{\text{max}}\) for TC) for each sampling. The drug loading capacity of the beads was calculated from the equation shown below:

\[
\text{Drug loading(\%) } = \frac{\text{weight of bead after drug loading} - \text{weight of bead before drug loading}}{\text{weight of bead before drug loading}} \times 100
\]

To check the role of \(\beta\)-cyclodextrin (\(\beta\)-cd) in drug release profiles, a 1 : 1 molar ratio \(\beta\)-cyclodextrin:tetracycline hydrochloride inclusion complex (\(\beta\)-TC) was prepared. The inclusion complex (\(\beta\)-TC) was prepared by mixing equal amounts of TC (5 mg mL\(^{-1}\)) and \(\beta\)-cd (5 mg mL\(^{-1}\)). This inclusion complex (\(\beta\)-TC) was then loaded into the beads and studied for the drug release profiles as per the above mentioned protocol.

2.6. Characterization

The CA and CA-CD beads were characterised by Fourier transform infrared spectrometry ( Nicolet 6700 FT-IR) to investigate the chemical changes in bead 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\(^{-1}\) over 32 scans. Thermogravimetric analysis (TGA) was done using a Perkin Elmer TGA 4000 from 35 \(^\circ\)C to 500 \(^\circ\)C at a heating rate of 10 \(^\circ\)C per minute and with a nitrogen flow rate of 20 mL per minute. The surface morphology of the beads was investigated using a scanning electron microscope (SEM) from Carl Zeiss (Sigma VP). Transmission electron microscopy was carried out in a JEOL JEM 2100 at an operating voltage of 200 kV. The TEM sample for CA-CD is prepared by dispersing CA-CD in 0.1 N NaOH and dropping onto a TEM grid.

3. Results and discussion

The carbon dots (CDs) form a protective coating over the CA bead, which increases the stability and hence the applicability of the carbon dots coated calcium alginate (CA-CD) beads. The stability of CA-CD beads over CA beads was monitored for 60 days (2 months) at atmospheric pressure and room temperature. Fig. 2 shows photographs of the CA and CA-CD beads kept at ambient conditions at the 0\(^{th}\), 1\(^{st}\), 10\(^{th}\), 30\(^{th}\) and 60\(^{th}\) day. It is evident from the photographs that CA beads when kept in ambient conditions were stable for only 1 day and dried out completely with time. In contrast, carbon dot coated CA-CD
beads show extraordinary stability even after 60 days (2 months). Hence, the CA-CD beads are stable in ambient conditions over months and therefore no extra measures are required to preserve the beads, unlike CA beads.

Here we want to add that we also tried to coat CA beads with chitosan following the same coating procedure. For this, 1% (w/v) chitosan solution was also prepared in 0.1 M acetic acid solution as with the CDs. In contrast to the extraordinary properties of CD coated CA beads (CA-CD), chitosan coated CA beads do not even show any swelling property. In fact, during coating with chitosan the CA beads showed complete shrinkage and thus any characteristic property of CA-CD was not observed in chitosan coated CA beads (photograph of chitosan coated CA bead, ESI (Fig. S2†)). Also the percentage coating yield was found to be less (31%) than that for CD coating.

Fig. 3 shows the representative TEM images of carbon dots (CD) and CA-CD beads. It is clear from the TEM images that CD particles are well dispersed inside the bead. Scanning electron microscopy (SEM) analysis is also done to study the surface morphology of CA and CA-CD beads (ESI, Fig. S3†). The SEM images show that CA-CD beads have a rough and porous surface, in comparison to the smooth surface of CA beads. At higher magnification the rough and porous surface of the CA-CD beads is more apparent. It has to be mentioned that carbon nanoparticles are not visible on the surface of the beads in the SEM image although in the TEM image of a CA-CD bead (Fig. 3(B)) the presence of carbon dots is vivid. A swelling study of CA and CA-CD beads was carried out in water as the swelling medium. The CA-CD beads showed excessive swelling with swelling ratio (SR) value of 8.2 in comparison to very low swelling of CA beads with SR determined to be 0.55. The high SR of CA-CD is a result of the porous surface of CA-CD in comparison to CA beads. The interaction of carbon dots with alginate was further investigated by determining the surface zeta potential of CA and CA-CD beads from Dynamic Light Scattering (DLS) measurements. It is well known that CA itself is anionic (i.e., negatively charged) due to the presence of COO−/CO3 groups engulfling the Ca2+ ion all over to form an “egg-box” like gel network. Surface zeta potential data also supported this fact showing ζ = −18.4 for CA beads. It is interesting to note that after coating CA beads with positively charged carbon dots (CDs), i.e., CA-CD beads, a net positive surface zeta potential of ζ = +18.6 is obtained. This clearly suggests that there is successful interaction between the negatively charged COO− groups of alginate and the positively charged NH3+ groups of CD making CA beads positively charged overall.

The pH responsive behavior of CA and CA-CD beads was also studied. CA and CA-CD beads were kept in five different pH solutions for 24 h as already described in the Experimental section. It was observed that the beads were stable in acidic pH and became dispersed in basic pH. At pH 1, the beads showed maximum shrinkage whereas at pH 3 and pH 5 the beads showed considerable swelling; maximum swelling was observed at pH 5. At pH 9 and pH 12, the beads were unstable and became completely dispersed. Both the beads, CA and CA-CD, showed the same trend in their pH responsive behavior. It is interesting to note here that even though for chitosan coating of CA beads the chitosan solution was prepared in 0.1 M acetic acid (pH 3), the chitosan coated CA beads showed complete shrinkage at pH 3 resulting in no swelling property. This suggests that chitosan coating is inefficient at making CA beads a better candidate with distinct properties.
Systematic study of the extent of swelling and shrinkage in CA and CA-CD when kept at different pH conditions for 24 h was done by measuring the size of the beads by optical microscope before and after storage in different pH solutions. For the study, 5 different sets of equal sized beads were immersed in different pH solutions for 24 h and the change in bead size was measured. Fig. 4 shows the optical microscope images with scaling at different pH and the corresponding average size distribution plot for CA and CA-CD beads. At pH 1, CA showed average shrinkage of 280 ± 17.83 μm in bead diameter and CA-CD showed shrinkage of 220 ± 25.99 μm. At pH 3, swelling was observed with an average increase in bead diameter of 430 ± 35.09 μm for CA and 670 ± 143.19 μm for CA-CD beads. At pH 5, the beads showed maximum swelling; the average increase in bead diameter for CA beads was 860 ± 58.71 μm, whereas CA-CD showed much more swelling with average increase in bead diameter of 1570 ± 67.08 μm.

A possible explanation for this kind of pH responsive behaviour of the beads is that it is the result of ionization of COO\(^-\) groups in acidic pH and ion exchange reaction between Na\(^+\) and Ca\(^+\) ions in basic pH.\(^{37,38}\) In the alginate bead system, two types of interactions mainly occur: charge repulsion between ionized COO\(^-\) groups and H-bonding between COOH and COO\(^-\) groups. At pH 1, the mutual charge repulsion between COO\(^-\) groups responsible for loosening of the network structure decreases considerably and instead intermolecular H-bonding between COOH and COO\(^-\) groups contributes to shrinkage of the beads. When the pH is in the range of 3–7, mutual charge repulsion of COO\(^-\) groups predominates over H-bonding between COOH and COO\(^-\) groups due to much less protonation of COO\(^-\) groups. Also, in this pH region a large osmotic pressure due to more mobile ions inside the bead causes loosening of network structure and hence swelling is observed. In basic medium, on the other hand, the beads follow a different mechanism. At pH ≈ 9, due to the presence of the non-gelling cation Na\(^+\) an ion-exchange reaction occurs between Na\(^+\) and Ca\(^+\), the gelling cation. Thus, “egg box” multimers get disrupted in aqueous NaOH solution resulting in dispersion of beads.

Thermal stability of CA and CA-CD beads was investigated by thermogravimetric analysis (TGA) under nitrogen flow (Fig. 5). CA beads showed about 90% degradation up to 100 °C. On the other hand, in CA-CD beads only 14% degradation was observed at 100 °C. At 200 °C CA-CD showed only 32% weight loss and 93% weight loss was observed at 266 °C. The extraordinary thermal stability of CA-CD beads was attributed to the coating of carbon dots.

The FTIR spectra of CA and CA-CD beads are shown in Fig. 6. The broadening of the peak at 3500 cm\(^{-1}\) in the FTIR spectra for the CA-CD beads in comparison to that of the CA beads.
indicates extensive hydrogen bonding between O–H groups of chitosan, glycerol and Ca-alginate. The peaks at 2940 cm\(^{-1}\) and 2879 cm\(^{-1}\) due to C–H group stretching are much more pronounced in CA-CD than CA bead showing presence of chitosan moiety. There is a shift and decrease in intensity of the peaks at 1631 cm\(^{-1}\) and 1410 cm\(^{-1}\) due to asymmetric and symmetric stretching of the COO\(^{-}\) group in the case of CA-CD beads. This can be due to the electrostatic binding of the positively charged \(\text{–NH}_3^+\) groups of chitosan with the negatively charged COO\(^{-}\) groups of Ca-alginate. A new peak at 1331 cm\(^{-1}\) due to C–N stretching frequency and one peak at 1220 cm\(^{-1}\) due to O–H bending vibration in the CA-CD spectrum are found to be absent in the CA spectrum. The region between 1150 cm\(^{-1}\) and 1000 cm\(^{-1}\) features strong signals caused by C–C and C–O stretching vibrations. The C–O stretching peak is at 1089 cm\(^{-1}\) in CA and is shifted to 1113 cm\(^{-1}\) in CA-CD, and the C–C stretching peak is at 1032 cm\(^{-1}\) in CA and is shifted to 1041 cm\(^{-1}\) in CA-CD. Out of plane bending of the ring C–H bond contributes to the peak around 900–675 cm\(^{-1}\). 

3.1. Tetracycline hydrochloride (TC) drug loading and pH responsive sustained release

The CA and CA-CD beads were investigated as drug delivery vehicles taking tetracycline and tetracycline associated with β-cyclodextrin as model drug systems. In order to study the drug release functioning of the beads at appropriate pH conditions, CA and CA-CD beads were first loaded by soaking in 20 mL tetracycline hydrochloride (TC) solution (5 mg mL\(^{-1}\)). The % drug loading for CA was found to be 35%, which was much lower than the 77% drug loading capacity observed for CA-CD. Fig. 7 shows the in vitro tetracycline (TC) release profiles of the studied beads at different pH conditions (pH 1, 3, 5, 7, 9, 12). It is evident from the drug release plots that at pH 1 there is maximum cumulative drug release observed for both CA and CA-CD beads. The CA-CD beads showed 70% drug release after 96 h at pH 1, whereas only 33%, 36%, 36% 35% and 27% was observed at pH 3, 5, 7, 9 and 12, respectively. In the case of the CA beads the drug release is slow with a maximum 37% release observed at pH 1.

Fig. 8(A) shows the comparison of cumulative drug release at pH 1 for CA and CA-CD beads. It clearly shows that CA-CD beads show higher drug release in comparison to CA beads. Thus, this vehicle showing sustained drug delivery at pH 1 will be suitable for gastrointestinal medium. We would like to add here that another method was investigated to increase the loading efficiency of CA-CD by premixing TC with alginate before preparing beads, but it was found that leaching of TC from alginate beads takes place on storage in water, indicated by yellow coloration of the solution. So, premixing of TC with alginate is not advisable. A systematic study of % cumulative drug release for three sets of CA-CD synthesised by keeping 10, 20 and 30 CA beads each in 10 mL CD solution was also done. All the three sets of CA-CD showed a similar trend and % cumulative release in their drug release profile is shown in ESI, Fig. S4.† It is evident from the drug release profile that CA-CD beads showed ~70% drug release after 96 h at pH 1, similar to what was obtained earlier. This may be due to the use of the higher concentration of CD used for coating with respect to one CA bead, which does not affect the coating yield of one bead irrespective of the number of beads coated.

3.2. β-Cyclodextrin:tetracycline hydrochloride (β-TC) and its pH responsive sustained release from CA-CD beads

It is well known that β-cyclodextrin (β-cd) forms an inclusion complex with tetracycline hydrochloride in a 1 : 1 molar
Fig. 7  pH dependent drug release profile up to 96 h of (A) CA beads loaded with TC (CA/TC) and (B) CA-CD beads loaded with TC (CA-CD/TC).

Fig. 8  Comparison of % cumulative drug release at pH 1 for (A) CA and CA-CD beads loaded with TC, i.e., CA/TC and CA-CD/TC, (B) CA and CA-CD beads loaded with β-TC, i.e., CA/β-TC and CA-CD/β-TC, (C) CA-CD beads loaded with TC (CA-CD/TC) and CA-CD beads loaded with β-TC (CA-CD/β-TC), and (D) CA beads loaded with TC (CA/TC) and CA beads loaded with β-TC (CA/β-TC). Inset: in each figure % drug release profile for the first 60 min is shown.
ratio\textsuperscript{31,34,39} (of $\beta$-cyclodextrin and tetracycline). It is also reported in the literature that the $\beta$-cyclodextrin:tetracycline hydrochloride inclusion complex ($\beta$-TC) shows good host-guest responsive drug release profiles with effective drug loading capacity. So, we investigated the efficiency of our systems CA and CA-CD in release of $\beta$-cyclodextrin:tetracycline hydrochloride inclusion complex ($\beta$-TC) at pH 1 where maximum drug release has been observed. In the first instance, higher $\beta$-TC drug loading efficiency was observed with 90% loading for CA-CD and 48% for CA, in comparison to TC loading (77% for CA-CD and 36% for CA). Interestingly, it was observed that delivery rates were slower in case of $\beta$-TC loading with 61% release for CA-CD and 22% release for CA after 96 h (Fig. 8(B)). Initially, CA-CD showed cumulative release of 18% for the first 10 min which increased up to 29% at 60 min for $\beta$-TC unlike TC which showed 28% and 38% cumulative release in 10 and 60 min, respectively (Fig. 8(C)). Similarly, $\beta$-TC loaded CA showed lower % cumulative release in comparison to TC loaded CA at different time intervals (Fig. 8(D)). The reason for the slower release can be attributed to the stable CA/$\beta$-TC and CA-CD/$\beta$-TC system. However, such type of $\beta$-cyclodextrin:drug inclusion complex system can be effective in enhancing the drug loading efficiency of a polymer and also helps in slower drug release rates, which can be suitable for slow and long-term drug administration. Hence, the carbon dot (CD) coated alginate bead drug delivery system was designed to meet a specific criterion, i.e., the system

![Fig. 9](image_url)

(A) Schematic representation of the electrostatic interaction between CA-CD and tetracycline (TC) and $\beta$-cyclodextrin:tetracycline ($\beta$-TC), (B) comparison of IR spectrum of CA-CD/TC and CA-CD/$\beta$-TC, and (C) comparison of IR spectrum of CA/TC and CA/$\beta$-TC.
shows sustained delivery at pH 1 suitable for gastrointestinal medium.

We have also tried to study the real time situation: release characteristics of the CA-CD/TC at different time intervals from acidic conditions in the stomach to basic conditions in the intestine. For this, TC release was studied by changing the pH at different time intervals. The 3-dimensional plot is shown in ESI, Fig. S5.† The plot clearly shows that the drug release is maximum at pH 1 and there is very little release at other pH conditions.

The loading of TC by CA-CD is a consequence of possible hydrogen bonding between –CH2OH groups present in carbon dots (CDs) and –OH and –CONH2 groups present in TC as shown in Fig. 9(A). The reason for higher loading efficiency of more than 90% of β-cyclodextrin:tetracycline (β-TC) shown by CA-CD beads can be attributed to the fact that the TC molecule enters the cavity of β-cyclodextrin (β-cd) to form a 1 : 1 inclusion complex. The interaction between β-cd and CA-CD is again through hydrogen bonding between the outer –OH group present in β-cd and the –CH2OH present on CDs as shown in Fig. 9(A). Moreover, the carbon dot on the alginate bead increases the surface area for attachment of the drug (surface area of CA is 1.477 m² g⁻¹ and that of CA-CD is 10.58 m² g⁻¹ (ESI, Fig. S6†)) and thus efficient drug loading is achieved. Hence, the small size of the carbon dot makes it an effective drug delivery system.

This is also well supported by IR spectra of CA-CD/TC and CA-CD/β-TC (Fig. 9(B)). The shift of the –OH peak at 3481 cm⁻¹ of CA-CD/TC to 3387 cm⁻¹ in CA-CD/β-TC, i.e., towards lower frequency, gives indication of H-bonding between the β-cd and CA-CD and thus supports the higher loading efficiency of β-cyclodextrin:tetracycline (β-TC) shown by the CA-CD beads. On the other hand, the presence of sharp –OH peaks at 3421 cm⁻¹ and 3402 cm⁻¹ for CA/TC and CA/β-TC, respectively, shows less interaction of the CA bead with TC and β-TC (Fig. 9(C)). The pH of the drug solution (TC and β-TC) is in the range of 3–5. In this pH range, repulsion between the COO⁻ groups of CA predominates over H-bonding between CA and TC, and CA and β-TC, similar to the pH dependant behaviour of CA. Also for CA/β-TC the shift of –OH peak at 3421 cm⁻¹ to the lower frequency of 3402 cm⁻¹ indicates extended H-bonding thus explaining the higher loading efficiency of CA/β-TC (48%) in comparison to CA/TC (37%). In spite of higher loading efficiency with β-TC for CA and CA-CD both, the slower release can be due to stronger interaction between β-cyclodextrin and tetracycline.
Tetracycline has two structural activity regions X and Y (molecular structure shown in Fig. S7, ESIF). It is known that functional group changes in region X do not cause a reduction or increase in activity but changes in Y region may result in drastically reduced activity. The pH responsive drug release profiles of CA and CA-CD beads can easily be explained on the basis of these two reactivity regions. In acidic medium at pH < 2, tetracycline eliminates a molecule of water with concomitant rearrangement of the ring to form anhydrotetracycline as depicted in Scheme 1. The fact that in pH 1 beads show a better drug release profile can be attributed to the shrinkage of beads because of lower network flexibility at this pH (as discussed earlier) which pushes the drug molecules out of the network structure and results in better drug release. In basic medium at pH > 9, the ring of tetracycline is opened up to form isotetracycline as shown in Scheme 2. Thus, it is not effective in basic medium.

4. Conclusions

In this article we fabricated calcium alginate beads (CA) and carbon dot coated calcium alginate beads (CA-CD). Exceptional stability in ambient condition even after 60 days is evident in the case of CA-CD beads. A swelling study indicated superior swelling behaviour of CA-CD in comparison to CA. The CA and CA-CD were investigated as drug delivery vehicles taking tetracycline (TC) as model drug systems. It was observed that TC loading was 77% with CA-CD and only 35% with CA. Interestingly, CA-CD beads showed a better drug release profile than CA beads with maximum release obtained at pH 1 emphasizing its use in the gastrointestinal tract where pH is low. A host-guest type inclusion complex of β-cyclodextrin:tetracycline (β-TC) showed greater drug loading efficiency but its release was slower as compared to TC. Hence, this type of β-cyclodextrin:drug inclusion complex can be effective in slower drug release rates applicable in slow and long-term drug administration.

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Notes and references

In this communication we report the preparation of fluorescent carbon dots (CDs) from chitosan gel. We also studied the photoluminescent (PL) properties of CDs prepared in different pH conditions. While CDs prepared at pH 3 give an intense sharp blue fluorescence, those prepared at pH 1 or 5 give a broad emission. In addition, we also investigated CDs prepared from chitosan/Ag and chitosan/Au nanocomposites. It was observed that although the emission was broad and less well defined, there was an enhancement of PL emission for the CDs prepared from chitosan/Ag or chitosan/Au nanocomposites.

In recent years there has been a huge interest in carbon nanomaterials primarily because of their unique properties. Carbon nanomaterials discovered previously which have become a hot research topic are carbon nanotubes (SWNT)\(^1\)\(^\text{-}^4\) and graphene.\(^5\)\(^\text{-}^8\) A new class of carbon nanomaterials that have been recently discovered are carbon dots (CDs), which have attracted enormous attention due to their application in optoelectronic devices, biomedical imaging,\(^9\)\(^\text{-}^11\) biological labelling\(^12\)\(^\text{-}^15\) and drug delivery.\(^16\)\(^\text{-}^18\) These photoluminescent materials are chemically stable and environmentally and biologically compatible\(^19\)\(^\text{-}^21\) compared to semiconductor quantum dots like CdS, CdSe, ZnS etc., which have known toxicity and are potentially environmentally hazardous as they essentially contain heavy metals.

Most of the synthetic methods developed for the synthesis of CDs involve thermal decomposition, laser ablation, thermal oxidation or electro-oxidation of carbonaceous sources. To the best of our knowledge, the first report of the preparation of quantum sized carbon dots was reported by Sun et al., who produced bright colourful photoluminescent quantum sized carbon dots by laser ablation of a carbon target in the presence of water vapour, with argon as a carrier gas and using diamine terminated oligomeric poly(ethylene glycol) as a surface passivating agent.\(^19\) Mochalin et al.\(^20\) reported a route to produce hydrophobic blue fluorescent nanodiamonds by covalently linking octadecylamine to 5 nm nanodiamonds. Bourlinos et al.\(^21\) reported the synthesis of carbon dots by the thermal oxidation of a novel salt precursor made from the acid–base combination of tris(hydmethyl) aminomethane (Tris) and betaine hydrochloride into the corresponding ammonium carboxylate complex. Peng et al.\(^22\) prepared luminescent carbogenic dots (due to the presence of oxygen) using carbohydrates.\(^19\) Jaiswal et al.\(^23\) reported a one-step microwave mediated method for synthesizing carbon dots using PEG as a precursor and passivating agent.\(^20\) In a similar study, Wang et al.\(^24\) presented a one-step synthetic route for producing carbon dots using carbohydrates like glycerol, glycol, glucose, sucrose, etc., and a tiny amount of an inorganic ion. There are reports in the literature of producing carbon dots from activated carbon and candle soot.\(^25\)\(^26\) Zhang et al.\(^27\) prepared green luminescent carbon dots by carbonization of sucrose. The non-luminous carbon nanoparticles were separated by dialysis. They also showed that after surface functionalization with PEG, the non-luminous nanoparticles became blue emitters.\(^24\)

In this communication we report a simple method for the preparation of fluorescent carbon dots (CDs) from chitosan gel. It is the first reported method of obtaining CDs from chitosan gel. The advantage of using a gel is that it is stable for years without any degradation, so it will increase the shelf life of the source material for preparing CDs (i.e. chitosan). Moreover, the PL emission of chitosan gel is sharp, unlike that of chitosan solution where a broad peak is observed (details in the ESI†). We also studied the photoluminescent properties of the CDs in the presence of acetic acid (AA) and hydrochloric acid (of different pH). In addition we investigated CDs prepared from chitosan/Ag and chitosan/Au nanocomposites synthesized by in situ and ex situ methods, labelled as chitosan/Ag in situ (CH–Ag-I) or chitosan/Au in situ (CH–Au-I), and chitosan/Ag ex situ (CH–Ag-E) or chitosan/Au ex situ (CH–Au-E) nanocomposites, respectively (details of experimental procedure and nanoparticles (NPs) preparation is given in the ESI†).

The chitosan (CH) hydrogels were synthesized using a simple approach. 1% glacial acetic acid solution and glycerol were mixed in the ratio of 1 part acetic acid solution to 3 parts glycerol to form a solvent. 0.1 g chitosan was dissolved in 10 ml of the aforementioned solvent by stirring with a magnetic stirrer at room temperature for 1–2 h to form a clear pale yellow solution. The solution was neutralized by adding 5 N NaOH. Immediately a clear, slightly tacky gel was formed. The gel, which apparently results from the interaction of chitosan, glycerol and water, has a three dimensional structure, and no free water or glycerol is apparent.

To make the CDs, a small part of the CH hydrogel was taken and dissolved in 0.1 M acetic acid and heated in a microwave for 5 mins. Fig. 1(A) shows the schematic representation of the procedure used to obtain the carbon dots (CDs) from the chitosan gel. The
The zeta potential of the prepared CDs was found to be 27 mV, indicating the positive charge on the CDs. It is worth mentioning here that CDs are not formed by the microwave heating of glycerol and acetic solution and no fluorescence was observed, confirming that chitosan acts as a carbon source for the formation of CDs.

We also tried to make CDs at different pH values. It was observed that the colour of the CD solution became darker at lower pH. The colour of the CD solution is dark yellow at pH 1 (CDs prepared in 0.1 M HCl), pale yellow at pH 3 (CDs prepared in 0.1 M AA), and off white at pH 5 (CDs prepared in 0.005 M AA). This change in colour of the CD solution suggests a change in the electronic transition of π–π* and n–π* in the carbon nanodomain at lower pH. The PL spectra of CDs obtained using 0.1 M hydrochloric acid (pH 1) after excitation at different wavelengths from 310 nm to 400 nm is shown in Fig. 3(A). The emission spectra show that the spectrum profile is a little different when compared with that of the CDs produced using 0.1 M acetic acid, shown in Fig. 2 (pH 3). There is a sharp emission peak at a similar wavelength followed by a broad peak, with red shift and decrease in intensity observed on increasing the excitation wavelength from 310 to 400 nm. It was observed that excitation at 320 nm gave a sharp emission at 357 nm followed by broad peak at 396 nm. CDs prepared using 0.1 M acetic acid (pH 3) gave emission at 358 nm with same excitation wavelength. Fig. 3(B) shows the PL emission spectra of CDs prepared in presence of 0.005 M AA (pH 5) excited at different wavelengths from 310 nm to 400 nm. In this case too the PL emission spectra are broad and are more shifted with increased excitation wavelength. For example, excitation at 320 nm gave a sharp emission at 359 nm and a broad emission at 402 nm. It was observed that the CDs prepared at pH 3 showed a smaller particle size distribution. For the CDs prepared at pH 1 and pH 5, the particle size distributions as obtained from DLS showed a bigger distribution of particles, and hence the PL emission was also broad and two emission peaks were obtained. In fact it was also found that it was not possible to measure the zeta potential of the CDs obtained at pH 1, perhaps because of the polydisperse nature of the particle size. Hence the pH value affects the fluorescence properties of the CDs, and the emission can be tuned by changing the pH.

It is now well known that the interaction of fluorophores like CDs with metal NPs results in increased photostability, fluorescence enhancement and decreased lifetime. This can be attributed to increased rates of system radioactive decay. The fluorescence enhancement observed in this system can be derived from surface plasmon resonance. The absorption/emission spectra of the CDs

representative scanning electron microscope image of the CDs is shown in Fig. 1(B). The particle size of the CDs prepared from the chitosan gel was also determined by dynamic light scattering (DLS) measurements. The DLS data showed that the particle sizes of CDs are in the range of 0.6 nm to 8.7 nm (Fig. 1(C)). Fig. 2 shows the photoluminescence (PL) spectra of CDs excited at different wavelengths. It was observed that with an increase in the excitation wavelength from 300 nm to 400 nm, the emission of the CDs gradually shifted to higher wavelengths accompanied with decreased fluorescence intensity. This red shift in the emission of CDs has also been reported previously.20 It was observed that excitation at 300, 310, 320, 330, 340, 350 and 400 nm resulted in emission at 334, 347, 358, 371, 384, 397 and 461 nm, respectively. As far as the PL mechanism of CDs is concerned, it is believed that both the quantum size effect and surface defects contribute to the PL. The inset of Fig. 2 shows a photograph of a CDs solution when viewed under a UV lamp. The zeta potential of the prepared CDs was found to be 27 mV, indicating the positive charge on the CDs. It is worth mentioning here that CDs are not formed by the microwave heating of glycerol and acetic solution and no fluorescence was observed, confirming that chitosan acts as a carbon source for the formation of CDs.

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It is now well known that the interaction of fluorophores like CDs with metal NPs results in increased photostability, fluorescence enhancement and decreased lifetime. This can be attributed to increased rates of system radioactive decay. The fluorescence enhancement observed in this system can be derived from surface plasmon resonance. The absorption/emission spectra of the CDs
show excellent overlap with the silver scattering spectrum, enabling enhanced fluorescence. So we then tried to prepare CDs from ex situ and in situ chitosan-Ag nanocomposites and chitosan-Au nanocomposites. In CH–Ag-I, Ag NPs are incorporated while making the chitosan gel, and as a consequence the Ag NPs are electrostatically adsorbed in the 3-dimensional gel structure, and are thus quite stable. In such a structure Ag NPs are strongly bound to the gel structure. On the other hand, the CH–Ag-E nanocomposite is formed by placing the chitosan gel in an Ag NPs solution, resulting in electrostatic adsorption of the NPs onto the already formed gel structure. So, the electrostatically adsorbed Ag NPs are loosely bound on the surface.25

Fig. 4(A) and (B) show the photoluminescence emission spectra of CDs formed from chitosan gel, CDs obtained from CH–Ag-I and CH–Ag-E nanocomposites, termed as Ag-I/CDs and Ag-E/CDs, respectively, and CDs obtained from CH–Au-I and CH–Au-E nanocomposites, termed as Au-I/CDs and Au-E/CDs, respectively. It is evident from the spectra that there is an enhancement of emission intensity in the case of Ag-I/CDs and Au-I/CDs when compared with the CDs obtained from chitosan gel. However the emission peaks are broad and less well defined. The reason for the broad peak is that CH–Ag-I, CH–Ag-E, CH–Au-I and CH–Au-E give more polydisperse CDs (bigger size) than those prepared from CH gel. The degree of metal–fluorophore interaction strongly depends on the distance between the fluorophore and the metal surface.27,28 We think that the enhancement of photoluminescence intensity shown by Ag-I/CDs and Au-I/CDs is due to the fact that the distance between the CDs and Ag is shorter in Ag-I/CDs than in Ag-E/CDs. As Ag and Au are loosely bound in CH–Ag-E and CH–Au-E, the interaction between the CDs and Ag or Au is weaker. More experiments are required in this regard. We are working on finding the answers to the following questions: (1) what is the interaction with different sizes of metal nanoparticles, and (2) whether the interaction of Ag NPs with chitosan, which is of course different in CH–Ag-I and CH–Ag-E, has any role when preparing CDs from the CH–Ag-I and CH–Au-E nanocomposites (in CH–Ag-I the electrostatically adsorbed Ag NPs form a 3-dimensional gel structure which is quite stable. On the other hand, in CH–Ag-E the electrostatically adsorbed Ag NPs are loosely bound). Moreover, the particle size distributions of the Ag-I/CDs, Ag-E/CDs, Au-I/CDs and Au-E/CDs reveal that the Ag-E/CDs or Au-E/CDs have bigger particle sizes than Ag-I/CDs or Ag-I/CDs (details given in the ESI†). Photoluminescence emission and absorption spectra obtained with progressively longer excitation wavelengths from 300 nm to 400 nm of the Ag-I/CDs, Ag-E/CDs, Au-I/CDs and Au-E/CDs are shown in the ESI.† It is also interesting to note that the net surface charge on the Ag-I/CDs and Ag-E/CDs obtained from the zeta potential was determined to be positive, like the CDs obtained from chitosan gel (details provided in the ESI†).

Conclusions

In this communication we successfully prepared fluorescent carbon dots (CDs) from chitosan gel. The particle sizes of the CDs were in the range of 0.6 nm to 8.7 nm. PL studies on the CDs prepared at different pH conditions showed that, while the CDs prepared at pH 3 gave an intense sharp blue fluorescence, those prepared at pH 1 and pH 5 show broad emission. In addition, CDs prepared from CH–Ag-I, CH–Au-I, CH–Ag-E, and CH–Au-E showed enhancement of emission intensity in the case of Ag-I/CDs and Au-I/CDs when compared with Ag-E/CDs, Au-E/CDs, and CDs obtained from chitosan gel. This enhancement is attributed to the stronger interaction of Ag or Au in the Ag-I/CDs and Au-I/CDs. This work demonstrates a simple strategy for preparing CDs from chitosan gel, which possess good PL properties, low cytotoxicity and biocompatibility, and can find applications in biological labelling, biological imaging and in biosensors.

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Fragmentation of supported gold nanoparticles@agarose film by thiols and the role of their synergy in efficient catalysis†‡‡

Neelam Gogoi and Devasish Chowdhury*

Thiols are known for stabilizing gold nanoparticles (Au NPs). Herein, an intriguing feasibility of a group of thiols to non-conventionally fragment supported Au NPs in the absence of any auxiliary applied energy is demonstrated. The Au NPs are studded into an agarose hydrogel film as a solid support (Au@Agr), and the group of thiols studied includes thioglycolic acid (TGA), cysteine (CS), 2-mercaptopethanol (ME), L-methionine (MET), thiourea (TU), and 1,3-propanedithiol (PDT). By simply keeping the Au@Agr film immersed into thiol solution for 1 h at room temperature, fragmented nanoparticles of much smaller sizes were obtained. Interestingly, only TGA, CS, ME and MET were found to be capable of successfully fragmenting Au@Agr. A mechanistic interpretation suggests that a prompt transfer of electron density from thiols to Au@Agr can be credited for the fragmentation behaviour of thiols. In addition, the efficacy of such a film in showing an efficient catalytic activity regulated by synergism between thiols and supported Au NPs (Au@Agr) is also presented. TGA and CS fragmented Au@Agr, i.e., TGA-Au@Agr and CS-Au@Agr films, work as an effective catalyst taking ~20 to 30 seconds for the complete reduction of p-nitrophenol (p-NP), an industrial pollutant with sluggish removal properties. A pseudo first-order rate for the catalytic p-NP reduction reaction is followed by TGA-Au@Agr as well as CS-Au@Agr with rate constant values determined to be $1.6 \times 10^{-1} \text{s}^{-1}$ and $1.1 \times 10^{-1} \text{s}^{-1}$, respectively. The exclusivity of TGA and CS to swiftly catalyze p-NP reduction and also the individual role of Agr, Au@Agr and TGA/CS-Au@Agr operating in synergy for the successful catalysis was also studied in detail.

Introduction

The fate and pre-conceptualised nature of gold nanoparticles (Au NPs) took a new turn after the report of thiol-derivatised gold nanoparticles by Brust et al.¹ in 1994. They introduced a two-phase liquid–liquid system for the preparation of Au NPs attached with self-assembled thiol monolayers behaving unusually as a simple chemical compound with moderate polydispersity. Consequently, thiol-capped Au NPs have been extensively studied over the two decades reporting a various number of papers establishing the role of thiols in stabilizing as well as regulating the size of Au NPs in a solution.²–⁹

Due to persistence of aggregation¹⁰–¹³ related problems, scientists next reported a photophysical fragmentation of these thiol-capped Au NPs induced by a laser.¹⁰ It is believed that this non-conventional perspective of Au NPs fragmentation by thiols is due to the photo-ejection of electrons, which is followed by charging of the nanoparticle surface. Novo et al.¹⁴ in a report showed charge induced fragmentation of gold nanorods by sodium borohydride. As a follow-up, Wang et al.¹⁵ showed fragmentation of Au NPs by the cysteine biomolecule. They demonstrated that upon mixing gold chloride with cysteine and excess of sodium borohydride, Au NPs of 3–5 nm size are first obtained, which are then converted into ultrasmall nanoparticles sized <1.5 nm after 6 h of aging at room temperature. In other reports, Riaz et al. demonstrated thiol-ubiquinone induced fragmentation¹⁶ and thioanisole induced size-dependent fragmentation of Au NPs.¹⁷ The role of alkanethiol fragmented Au NPs in controlling the fluorescence properties of Au NPs for the sensitive and selective detection of Hg(II) was shown by Huang et al.,¹⁸ and thiol induced core etching of lysozyme type VI (Lys VI)-stabilised luminescent Au₈₈ clusters was shown by Ke et al.¹⁹ However, the feasibility of such non-conventional thiol-mediated fragmentation of Au NPs when supported on a solid platform is still an unexplored area of research.
In this study, we investigated the feasible role of thiols in fragmenting Au NPs supported on an agarose (Agr) hydrogel film. Addressing within the limits of our knowledge, it is the first report to show the non-conventional thiol-mediated fragmentation of Au NPs studded into an Agr hydrogel film solid support as a hybrid material. The Au NPs, unlike other reported methods, are generated inside the agarose hydrogel film through adsorption of 5 mM tetrachlorauric salt (HAuCl₄) solution by a preformed Agr hydrogel film, which is subsequently reduced by only 10 min exposure to 365 and 254 nm wavelengths UV radiation. The role of a agarose hydrogel as a solid support in facile and quick generation of Au NPs is also considered here. This hybrid Au@Agr film is then investigated to study the feasibility of such a system to undergo the recently popularized non-conventional fragmentation of Au NPs by a group of thiols (viz., thioglycolic acid (TGA), cystine (CS), 2-mercaptoethanol (ME), l-methionine (MET), thiourea (TU), and 1,3-propanedithiol (PDT)). The surface plasmon resonance (SPR) peak of Au NPs detected by UV-visible spectrometry strongly depends on its particle size, and any change in the size of Au NPs is reflected by a shift in the SPR peak wavelength either towards lower (blue shift) or higher (red shift) wavelength values. Accordingly, to study the fragmentation of Au@Agr by thiols such a blue/red shift in the SPR peak of Au NPs is closely monitored by UV-visible spectrometry. Among all the six thiols, TGA, CS, ME and MET were found to be capable of showing fragmentation of Au@Agr displaying a considerable 6 to 11 nm blue shift in the SPR peak of Au@Agr. Other thiols of the group, i.e., TU and PDT, do not show any sign of fragmentation, instead they have been found to cause red shifts in the SPR peak of Au NPs.

The study also demonstrates application of these films in rapid catalysis of an industrially important reduction reaction of p-nitrophenol (p-NP) to p-aminophenol (p-AP). p-NP is a known pollutant that is very noxious and sluggish, while p-AP is of great importance serving as an intermediate for synthesis of many analgesic, antipyretic drugs, and polymers. Moreover, p-NP reduction to p-AP is one of the most ideal reactions studied for establishing catalytic efficiency of a catalyst due to its easy mode of monitoring with UV-visible spectroscopy and occurrence of no side reactions. The films (especially, TGA-Au@Agr & CS-Au@Agr) are successfully used as catalysts in the reduction of p-NP to p-AP in the presence of excess sodium borohydride (NaBH₄). The calculated rate constant was found to be $1.6 \times 10^{-1}$ s⁻¹ and $1.1 \times 10^{-1}$ s⁻¹ for TGA-Au@Agr and CS-Au@Agr catalysed reduction reactions, respectively, following pseudo first-order kinetics. To the best of our knowledge, this rate constant value of the order $10^{-1}$ s⁻¹ is one of the fastest catalytic rates observed for any solid supported catalyst. A thriving synergism between TGA or CS and Au@Agr stimulating a smooth relay of electrons from borohydride ions (BH₄⁻) to p-NP is credited for such a rapid catalysis shown by the TGA-Au@Agr and CS-Au@Agr films. Hence, this report is an endeavour to investigate and establish an intriguing feasibility for non-conventional behaviour of a group of thiols to fragment Au NPs supported in a solid Agr hydrogel film in absence of any auxiliary applied energy. The study also explores the synergy functioning inside the film to make it an efficient catalytic material for reduction of p-NP to p-AP.

**Experimental**

**Materials**

Agarose low gelling temperature (SRL, India), tetrachlorauric acid (Sigma Aldrich), thioglycolic acid (Sigma Aldrich), l-cysteine hydrochloride monohydrate (Merck), l-methionine (Sigma Aldrich), 2-mercaptoethanol (Himedia), thiourea (Merck), 1,3-propanedithiol (Sigma Aldrich), p-nitrophenol (Lobal Chemie), and sodium borohydride (Merck) were used as received. All other reagents used were of analytical grade.

**Methodical step-by-step protocol to investigate thiol-mediated fragmentation of gold nanoparticles (Au NPs) studded into agarose hydrogel film**

**Agarose hydrogel film (Agr).** Agarose hydrogel of 2.5% (w/v) was prepared following the conventional method of mixing 0.25 g agarose in 10 mL of Milli Q water and allowing microwave dissolution for 25 s. The film casting of the microwaved solution was done by pouring it in a Petri dish (10 mm diameter) and cooling to room temperature, which was then kept for 4 hours (at room temperature) to obtain a thin Agr hydrogel film.

**Au NPs studded agarose hydrogel film (Au@Agr).** To the Petri dish containing the prepared Agr hydrogel film, 10 mL of 5 mM HAuCl₄ solution was poured and kept as such for 1 h 15 min. After this, the HAuCl₄ solution was poured out from the Petri dish, and the film was washed thoroughly with plenty of Milli Q water to remove any unreacted HAuCl₄ if present. Then, the HAuCl₄ treated Agr film was exposed to 365 nm and 254 nm wavelength UV light for 10 min. The ruby red coloration of the Agr film after UV exposure indicates the formation of Au nanoparticles studded into the agarose hydrogel film, i.e., Au@Agr.

**Thioglycolic acid (TGA) mediated fragmentation of Au nanoparticles studded into agarose film (TGA-Au@Agr).** Au@Agr film was treated with thioglycolic acid (TGA) to investigate any fragmentation of Au NPs studded inside the Agr film. 2% (v/v) of TGA in Milli Q water was prepared, 10 mL of which was poured into a Petri dish containing the Au NPs studded agarose (Au@Agr) film and kept for 1 h at room temperature. The 2%TGA-Au@Agr film was then investigated with UV-visible analysis to ensure any fragmentation of Au NPs by TGA. The UV-visible spectrum was taken in reflectance mode, which is then converted instrumentally to absorbance through the Kubelka–Munk equation. Likewise, cysteine (CS), 2-mercaptoethanol (ME), l-methionine (MET), thiourea (TU), and 1,3-propanedithiol (PDT) were also used to prepare other thiol-fragmented Au@Agr films, and named as 2%CS-Au@Agr, 2%ME-Au@Agr, 2%MET-Au@Agr, 2%TU-Au@Agr, and 2%PDT-Au@Agr, respectively. The complete step-by-step methodical formulation of thiol-Au@Agr film is presented in a pictorial form in Scheme 1. The images of Agr, Au@Agr, and thiol-
Au@Agr films prepared in the laboratory are also included in Scheme 1.

Investigation of thiol-Au@Agr film as a catalyst for p-NP reduction

All the thiol-Au@Agr films were investigated for catalytic reduction of p-nitrophenol (p-NP) to p-aminophenol (p-AP) in the presence of sodium borohydride (NaBH4). For the process, 20 μL of 1 mM p-NP was mixed with 100 mL of 5 mM NaBH4 solution. The resulting solution showing λmax at 400 nm due to the formation of p-nitrophenolate ion (p-NP(r)) was then monitored for the complete catalysis study. A small piece of thiol-Au@Agr film (1 × 1 cm, thickness ~ 0.2 mm) was immersed in 2 mL of p-NP(r) followed by easy stirring, and then the UV-visible spectrum of the p-NP(r) monitoring its 400 nm peak was taken in regular interval of time, viz., 2.5 s, 5 s, 10 s, 15 s up to 60 s.

Characterization

The hydrogel films were characterized by UV-visible spectroscopy (Shimadzu-UV 2600) to study changes in the absorbance pattern of Agr, Au@Agr and thiol-Au@Agr films (each film strip used was sized ~1 × 1 cm, thickness ~0.2 mm). The spectra were first obtained in the reflectance mode in the 800–190 nm range, which was then converted into its absorbance value instrumentally through the Kubelka–Munk equation. The surface morphology and fragmentation of Au@Agr were investigated using a scanning electron microscope (SEM) from Carl Zeiss (Sigma VP). The energy dispersive X-ray spectroscopy (EDX) connected to SEM was used to ascertain the elements present in all the thiol-Au@Agr films. For the SEM and EDX analysis, a ~1 × 1 cm strip was placed on a stub, dried at 50 °C for 1 h and used for imaging. The particle size distribution analyses of freshly prepared Au NPs solution and all thiol-Au solutions were done with a Malvern Zetasizer NanoZS 90.

Results and discussion

Fragmentation of Au@Agr film by selective thiols

An investigation of the proficiency of a group of thiols to mediate a non-conventional fragmentation of Au NPs, which are studded into agarose hydrogel film, is presented here. The peculiar role of agarose in assisting a facile fragmentation of Au NPs and the candidature of such a film in rapid reduction catalysis of p-nitrophenol (p-NP) is also demonstrated. For the study, Au NPs are first studded into the Agr film, as detailed in the experimental section (Scheme 1). Briefly, a 2.5% (w/v) Agr film was treated with 10 mL of 5 mM HAuCl4 solution, and then Au3+ was reduced to Au0 by exposing the film to 365 and 254 nm wavelength UV radiation for only 10 min. The completion of the Au3+ → Au0 reduction process was indicated by ruby red coloration of the Agr film resulting in the formation of an Au NPs studded Agr film (Au@Agr). The formation of the Au@Agr film was experimentally proved by UV-visible spectrophotometry analysis. In the UV-vis spectrum of Au@Agr film, a sharp
peak at a wavelength 540 nm is distinguishably observed suggesting the successful formation of Au NPs of size 40–60 nm with UV exposure of Au$^{3+}$ loaded Agr (Fig. 1). A distinct variance in the spectral patterns of the Agr and Au@Agr films was also observed through UV-visible analysis. Furthermore, the actual loading of Au$^{3+}$ into the Agr film was checked and found to be 45% (Fig. S1, ESI†). An excess loading of Au$^{3+}$ above 45% resulted in the formation of aggregated Au NPs of sizes >60 nm when subjected to UV treatment. Therefore, this loading capacity was taken as the standard for the complete study.

After ascertaining the successful formation of Au NPs studded into the Agr film (Au@Agr) through UV-visible analysis, the feasibility of interaction of thiols towards such Agr supported Au NPs was studied. A group of thiols selected for the study includes thioglycolic acid (TGA), cysteine (CS), 2-mercaptethanol (ME), L-methionine (MET), thiourea (TU), and 1,3-propanedithiol (PDT). Each thiol was allowed to interact with Au@Agr film for 1 h at room temperature after which the film was analysed through UV-visible spectroscopy to check any perceivable changes in the spectral pattern of Au@Agr. Interestingly, it is observed that after interaction with thiol, the Au@Agr film showed a blue shift in the SPR peak of Au NPs observed earlier in its UV-visible spectrum. However, such kind of blue shift was found only in case of Au@Agr treated with TGA, CS, ME and MET. In TGA-Au@Agr, the blue shift observed in the SPR peak of Au NPs was 7 nm from 540 nm → 533 nm. In CS-Au@Agr, this shift was from 545 nm → 539 nm, a 6 nm shift. Moreover, in ME-Au@Agr and MET-Au@Agr, an 11 nm blue shift in both was observed showing 540 nm → 529 nm & 545 nm → 534 nm shifts, respectively. The PDT and TU treated Au@Agr, i.e., PDT-Au@Agr and TU-Au@Agr, respectively, failed to show any such blue shift in the SPR peak of Au NPs. Instead, a 14 nm red shift (534 nm → 548 nm) was observed in PDT-Au@Agr, and in TU-Au@Agr, no shift was evident. This peculiar behaviour of different thiols towards Au@Agr and also the selectivity of thiols to show blue or red shifts of the SPR peak of Au NPs studded into Agr (Au@Agr) detected by a UV-visible spectrophotometer is shown in Fig. 2.

It is well known that a blue/red shift in the SPR peak of a metal nanoparticle is an optical manifestation of a significant change in its particle size. In order to further examine this change in the particle size of Au@Agr on binding with thiols, a systematic scanning electron microscopy (SEM) analysis is done before and after treating Au@Agr with thiols. As illustrated in the SEM images (Fig. 3), the change in particle size of Au@Agr (50–60 nm) on treatment with thiols is vividly perceptible in accordance with the blue shifts of the SPR peak in the UV-visible spectrum of Au@Agr. In the SEM images of TGA-Au@Agr and CS-Au@Agr, a clear dense distribution of Au NPs having a particle size ≤30 nm is observed, contrary to the sparsely distributed Au NPs of size ≤30 nm in ME-Au@Agr as well as in MET-Au@Agr.

It would be informative to have a clear picture of Au NPs size distribution in all the films simply by assessing their SEM images. As shown in Fig. 4, in Au@Agr, a very broad particle size distribution with Au NPs ranging from 30 to 60 nm was obtained. In TGA-Au@Agr, the observation of very small and densely distributed fragmented Au NPs depicts the highest density of <10 nm Au NPs with fewer numbers of <20 nm AuNPs, and in CS-Au@Agr, the Au NPs density is highest in the particle size range of 11–20 nm with comparatively lesser density in the particle size range of 1–10 nm. On the other hand, the sparse distribution of Au NPs in ME-Au@Agr shows particles in 11–20 nm size range with very few in 21–30 nm size range. The Au NPs in this similar size range is also determined in MET-Au@Agr, but the number of particles is further less in

![Fig. 2](image_url) The UV-visible spectra showing the blue/red shift in SPR peak of Au NPs studded into agarose film (Au@Agr). (A) 2%TGA-Au@Agr, (B) 2%CS-Au@Agr, (C) 2%ME-Au@Agr, (D) 2%TU-Au@Agr, (E) 2%PDT-Au@Agr, and (F) 2%MET-Au@Agr.
support of their sparser distribution shown in the SEM image. For PDT-Au@Agr and TU-Au@Agr, the other thiols of the group, no sign of fragmentation is evident in their SEM images (Fig. S1, ESI†). In fact, bigger sized Au NPs (60–70 nm) were seen in PDT-Au@Agr, supplementing the 14 nm red shift in SPR peak of Au NPs observed in its UV-visible spectrum. Moreover, a distribution of 50–60 nm sized Au NPs in the SEM image of TU-Au@Agr is observed confirming no shifting of the SPR peak of Au@Agr upon binding with TU, which was detected in their UV-visible spectra.

The peculiar and intriguing behaviour of thiols to successfully fragment supported Au NPs (Au@Agr) can be credited to electron ejection on Au NPs by chemisorbed thiols.15,39 A shift in the SPR band wavelength caused by an electron donor or acceptor in close proximity can be comprehended due to alterations in the density of free electrons available on the nanoparticle. These alterations in turn cause difference in SPR energy making it spectroscopically perceptible. Thiol being a strong nucleophile can easily donate electron density to Au NPs, which accumulates at their surface.15,40,41 The stabilisation of a nanoparticle strongly depends on the surface charge density which upon reaching a critical value causes excess surface tension on the nanoparticle.14,15,42 When the surface charge density exceeds this critical value, increased disturbance leads to fragmentation of the nanoparticle as witnessed in TGA-Au@Agr, CS-Au@Agr, ME-Au@Agr and MET-Au@Agr. A lesser density of Au NPs in ME-Au@Agr and MET-Au@Agr can be attributed to the presence of terminal –OH groups in ME and –S–CH₃ groups in MET. It is reported that presence of –SH as well as –COOH groups together is the crucial factor for effective fragmentation of Au NPs, where –SH group plays the role of an electron donor and –COOH group aids in hydrogen bonding between adsorbed thiols and free thiols contributing to local concentration around the Au NPs.15 Due to the absence of one favouring factor among these two in ME and MET, a lower degree of fragmentation processes results in reduced density of fragmented Au@Agr. The contradictory behaviour of no fragmentation observed in TU-Au@Agr and PDT-Au@Agr can also be accounted for by the lack of suitable functional groups. TU does not have a terminal S-atom making donation of free electrons to Au NPs difficult, and also the presence of the NH₂ group at both ends makes interaction further difficult.15 Thus, TU-Au@Agr does not show any shift of the Au@Agr SPR peak. PDT-Au@Agr, on the other hand, shows a 14 nm red shift of SPR peak of Au@Agr which might be due to lowering of electron density on Au NPs by PDT resulting in aggregation and a red shift.40 Therefore, the experimental analyses by UV-visible spectroscopy and SEM imaging provide significant legitimate observations confirming the fact that thiols (TGA, CS, ME and MET) are capable of playing the chemistry with supported Au NPs (Au@Agr) in a non-conventional way, resulting in successful fragmentation of these supported Au NPs.

The role of agarose (Agr) as a support to Au NPs, and a favourable condition for fragmentation as well was inquired about. The reduction of Au³⁺ → Au⁰ by UV exposure in only 10 min was made possible by Agr without adding any reducing agent. However, a UV exposure to a solution of Au³⁺ ions could not reduce it to Au⁰ even after 5 h. This clearly indicates the role
of Agr as a stimulating condition for obtaining Au NPs simply by quick UV exposure. In addition, to check whether the fragmentation feasibility of thiols works for Au NPs in a solution also (i.e., when Au NPs are not supported by Agr), another investigation based on dynamic light scattering (DLS) was done. Herein, the fragmentation of freshly prepared Au NPs in solution by the group of thiols (TGA, CS, ME, MET) was investigated. For the analysis, a 0.1% solution of each thiol was prepared, and then mixed with freshly prepared Au NPs (2 mL of 0.1% thiol solution + 40 μL of Au NPs solution) followed by DLS investigation for all the samples (Fig. S3, ESI†). Excitingly, the thiol solutions could fragment the 60 nm sized Au NPs up to a maximum of 35 nm sized particles only, which was observed in case of CS-Au. For ME-Au and MET-Au, the Au NPs sizes observed were 38 nm and 40 nm, with TGA-Au showing minimum fragmentation reporting 57 nm sized Au NPs. Clearly, the fragmentation by thiols is less feasible if the Au NPs are present in the solution as the UV-visible and SEM studies (discussed before) clearly show that fragmentation of Au NPs are efficient when Agr acts as a solid support. This is probably because Agr restricts the movement of thiol molecules as well as Au NPs empowering an easy and effective electron density transfer leading to their successful fragmentation into much smaller sizes.

### Synergistic role of thiols and Au@Agr for catalytic reduction of p-nitrophenol

Understanding of non-conventional chemistry occurring between the thiols and Au@Agr led to another exploration of the viability of the catalytic property of Au NPs. Even after fragmentation of Au NPs, the residence of excess electron density on their surface is quite logical. Therefore, utilization of this electron density for effective and rapid reduction of one of the most refractory pollutant, p-nitrophenol (p-NP), to p-aminophenol (p-AP) was the next goal in this study.

An aqueous solution of p-NP has a characteristic maximum absorption peak appearing sharply at 320 nm in its UV-visible spectrum. Upon addition of freshly prepared NaBH₄ solution in large excess, the 320 nm peak red shifts to 400 nm due to the

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**Fig. 4** The particle size distribution plot assessed from SEM images (Fig. 3) of Au@Agr, 2%TGA-Au@Agr, 2%CS-Au@Agr, 2%ME-Au@Agr and 2%MET-Au@Agr.

**Fig. 5** The UV-visible absorption spectral analysis showing catalysis of the reduction reaction of p-nitrophenol (p-NP) to p-aminophenol (p-AP) using 2%TGA-Au@Agr, 2%CS-Au@Agr, 2%ME-Au@Agr and 2%MET-Au@Agr as catalysts. p-NP(r) is p-nitrophenol ions produced by adding freshly prepared excess solution of NaBH₄ to p-NP solution.
showing lm 20 (the reported standard reduction potential of a catalyst, this 400 nm peak of with no sign of thermodynamically favoured reduction to As mentioned in various reports, noted change in the colour of fresh strip of 2%TGA-Au@Agr exciting to observe that in the presence of 2%TGA-Au@Agr, the ined every 5 s by UV-visible spectroscopy (Fig. 5). It is very emerging peak appearing around 300 nm substantiating the conversion of /C24 a small strip of 2%TGA-Au@Agr 101866 | 1.6 2%TGA-Au@Agr Thioglycolic acid 2 1.6 10^{-3} \text{s}^{-1} 2%TGA-Au@Agr Thioglycolic acid 4 6.4 10^{-3} \text{s}^{-1} 2%CS-Au@Agr l-Cysteine 2 1.1 10^{-3} \text{s}^{-1} 2%CS-Au@Agr l-Cysteine 4 2.2 10^{-3} \text{s}^{-1} 2%ME-Au@Agr 2-Mercaptoethanol 2 3.3 10^{-4} \text{s}^{-1} 2%MET-Au@Agr l-Methionine 2 1.6 10^{-5} \text{s}^{-1} \text{formation of } p\text{-nitrophenolate ion in the alkaline surrounding triggered by the addition of NaBH}_4. \text{When unattended by a catalyst, this 400 nm peak of } p\text{-nitrophenolate ion stays stable with no sign of thermodynamically favoured reduction to } p\text{-AP (the reported standard reduction potential of } p\text{-nitrophenol/} p\text{-aminophenol is } -0.76 \text{ V and that of } \text{H}_2\text{BO}_3/\text{BH}_4^- \text{ is } -1.33 \text{ V). As mentioned in various reports,}^{25-29} \text{this } p\text{-nitrophenolate ion peak does not alter even after several days, indicating that the conversion of } p\text{-nitrophenol} \text{to } p\text{-AP is a very sluggish process. With an intent to catalyse this reduction reaction of } p\text{-NP, a small strip of 2%TGA-Au@Agr film (size } \sim 1 \times 1 \text{ cm, thickness } \sim 0.2 \text{ mm) was inserted into a 2 mL solution of } p\text{-NP(r)}(p\text{-NP(r)} = \text{a stock solution prepared by mixing freshly prepared 20 } \mu\text{L of 1 mM } p\text{-NP and 100 mL of 5 mM NaBH}_4 solutions showing } \lambda_{\text{max}} = 400 \text{ nm). Amazingly, an instant decolorization of the } p\text{-NP(r)} \text{ solution within a few seconds of time was noted as the first observation. Therefore, to observe precisely, another fresh strip of 2%TGA-Au@Agr film was inserted again, and the noted change in the colour of } p\text{-NP(r)} \text{ solution was then examined every 5 s by UV-visible spectroscopy (Fig. 5). It is very exciting to observe that in the presence of 2%TGA-Au@Agr, the sluggish peak at 400 nm corresponding to } p\text{-nitrophenolate ion showed a complete decrease in only 20 s of time with an emerging peak appearing around 300 nm substantiating the formation of } p\text{-AP. Moreover, since the reaction is monitored every 5 s, the presence of any Au NPs peak would have been seen in the UV-visible spectrum of } p\text{-NP(r), if any leaching of Au NPs from the film to the } p\text{-NP(r)} \text{ solution would have occurred, which validates that the catalytic reduction reaction is progressing only on the surface of the 2%TGA-Au@Agr film. The same catalytic reduction analysis of } p\text{-NP(r)} \text{ solution was examined again using other films, viz., } 2\%\text{CS-Au@Agr, } 2\%\text{ME-Au@Agr, and } 2\%\text{MET-Au@Agr (2%TU-Au@Agr and 2%PDT-Au@Agr were discarded as they do not show any fragmentation of Au@Agr). An instant catalysis for complete reduction of } p\text{-NP(r)} \text{ to } p\text{-AP was observed in the presence of } 2\%\text{CS-Au@Agr in only 30 s of time, as shown in Fig. 5, featuring degradation of 400 nm peak of } p\text{-NP(r)} \text{ accompanied by occurrence of a new 300 nm peak for } p\text{-AP. Contradictorily, even after the presence of fragmented Au@Agr, } 2\%\text{ME-Au@Agr and } 2\%\text{MET-Au@Agr were found to be incapable of showing effective reduction reaction catalysis for } p\text{-NP(r)} \text{ to } p\text{-AP even after 10 min (Fig. 5). This discrepancy was expected because of the presence of a much lower number of Au NPs in } 2\%\text{ME-Au@Agr as well as in } 2\%\text{MET-Au@Agr, as is clearly evident from their SEM images shown in Fig. 3. The reduction reaction of } p\text{-NP to } p\text{-AP is considered to be a pseudo-first order reaction owing to presence of NaBH}_4 \text{ in large excess.}^{26-37} \text{ As shown in Fig. 6(A), a graph of } \ln(C_t/C_0) \text{ is plotted against time to study the reaction when catalysed by all the different catalytic films, viz., } 2\%\text{TGA-Au@Agr and } 2\%\text{CS-Au@Agr. Herein, } C_t \text{ denotes the concentration of } p\text{-NP(r)} \text{ at time } 't' \text{ seconds in the presence of the catalytic film, and } C_0 \text{ is the concentration of } p\text{-NP(r)} \text{ at time 'zero' seconds. The value of each concentration was calculated from the observed values of absorbance at } \lambda_{\text{max}} = 400 \text{ nm after every 5 s during } p\text{-NP(r)} \text{ catalytic cycling. Upon plotting } \ln(C_t/C_0) \text{ values vs. time, a linear relationship is observed for both the } p\text{-NP catalytic reactions catalysed by } 2\%\text{TGA-Au@Agr and } 2\%\text{CS-Au@Agr. The rate constant obtained from linearly fitted curve reports a value of } 1.6 \times 10^{-1} \text{ s}^{-1} \text{ and } 1.1 \times 10^{-1} \text{ s}^{-1} \text{ for } 2\%\text{TGA-Au@Agr and } 2\%\text{CS-Au@Agr catalysed } p\text{-NP reduction reactions, respectively. To}

![Fig. 6](A) A comparative plot of ln(C_t/C_0) vs. time showing a pseudo-first order rate of reduction reaction of p-NP to p-AP catalysed by (A) 2%TGA-Au@Agr and 2%CS-Au@Agr films and (B) 4%TGA-Au@Agr and 4%CS-Au@Agr films. All graphs display linearly fitted values.

<table>
<thead>
<tr>
<th>Film</th>
<th>Thiol</th>
<th>% of thiol</th>
<th>Rate constant (k) Equal to</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agr</td>
<td>None</td>
<td>0</td>
<td>0.01 \times 10^{-1} s^{-1}</td>
</tr>
<tr>
<td>Au@Agr</td>
<td>None</td>
<td>0</td>
<td>0.05 \times 10^{-1} s^{-1}</td>
</tr>
<tr>
<td>2%TGA-Au@Agr</td>
<td>Thioglycolic acid</td>
<td>2</td>
<td>1.6 \times 10^{-3} s^{-1}</td>
</tr>
<tr>
<td>4%TGA-Au@Agr</td>
<td>Thioglycolic acid</td>
<td>4</td>
<td>6.4 \times 10^{-3} s^{-1}</td>
</tr>
<tr>
<td>2%CS-Au@Agr</td>
<td>l-Cysteine</td>
<td>2</td>
<td>2.2 \times 10^{-1} s^{-1}</td>
</tr>
<tr>
<td>4%CS-Au@Agr</td>
<td>l-Cysteine</td>
<td>4</td>
<td>1.1 \times 10^{-3} s^{-1}</td>
</tr>
<tr>
<td>2%ME-Au@Agr</td>
<td>2-Mercaptoethanol</td>
<td>2</td>
<td>3.3 \times 10^{-4} s^{-1}</td>
</tr>
<tr>
<td>2%MET-Au@Agr</td>
<td>l-Methionine</td>
<td>2</td>
<td>2.2 \times 10^{-3} s^{-1}</td>
</tr>
</tbody>
</table>
Table 2  The comparison table between the rate constant values of reduction reaction of p-NP to p-AP when catalysed by different materials reported in the literature and our proposed film (4%TGA-Au@Agr and 4%CS-Au@Agr).

<table>
<thead>
<tr>
<th>S. No.</th>
<th>System</th>
<th>Rate constant (k)</th>
<th>Time taken</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4%TGA-Au@Agr film (in solid phase)</td>
<td>$6.4 \times 10^{-1}$ s$^{-1}$</td>
<td>5 s</td>
<td>Our proposed material</td>
</tr>
<tr>
<td>2</td>
<td>4%CS-Au@Agr film (in solid phase)</td>
<td>$2.2 \times 10^{-1}$ s$^{-1}$</td>
<td>15 s</td>
<td>Our proposed material</td>
</tr>
<tr>
<td>3</td>
<td>Platinum nanoparticles stabilised by guar gum (in solution)</td>
<td>$8.3 \times 10^{-3}$ s$^{-1}$</td>
<td>420 s</td>
<td>S. Pandey and S. B. Mishra, Carbohydrate Polymers, 2014, 113, 525–531</td>
</tr>
<tr>
<td>4</td>
<td>Au(0)@TpPa-1 (in solution)</td>
<td>$5.35 \times 10^{-3}$ s$^{-1}$</td>
<td>240 s</td>
<td>P. Puchfule, S. Kandambeth, D. Diaz Diaz and R. Banerjee Chem. Commun., 2014, 50, 3169–3172</td>
</tr>
<tr>
<td>5</td>
<td>Ellipsoid-like MCM-41 supported Ag nanocomposite (in solution)</td>
<td>$4.3 \times 10^{-3}$ s$^{-2}$</td>
<td>180 s</td>
<td>N. Hao, L. Li and F. Tang, J. Mater. Chem. A, 2014, 2, 11365–11368</td>
</tr>
<tr>
<td>7</td>
<td>Fe$_3$O$_4$@TiO$_2$/Au@Pd@TiO$_2$ (in solution)</td>
<td>1.06 min$^{-1}$</td>
<td>4 min</td>
<td>W. Hu, B. Liu, Q. Wang, Y. Liu, Y. Liu, P. Jing, S. Yu, L. Liua and J. Zhang, Chem. Commun., 2013, 49, 7596–7598</td>
</tr>
<tr>
<td>9</td>
<td>Ag(0.7)–SBA-15 composites (in solution)</td>
<td>$9.0 \times 10^{-3}$ s$^{-1}$</td>
<td>360 s</td>
<td>J. Han, P. Fang, W. Jiang, L. Li and R. Guo, Langmuir, 2012, 28, 4766–4775</td>
</tr>
<tr>
<td>10</td>
<td>Au NPs prepared from 0.5 mL Breynia rhamnoides (in solution)</td>
<td>$4.65 \times 10^{-3}$ s$^{-1}$</td>
<td>600 s</td>
<td>A. Gangula, R. Podila, Ramakrishna M, L. Karanam, C. Janardhana, and A. M. Rao, Langmuir, 2011, 27, 15268–15274</td>
</tr>
<tr>
<td>12</td>
<td>$\alpha$-Cyclodextrin capped Au nanoparticles (in solution)</td>
<td>$4.65 \times 10^{-3}$ s$^{-1}$</td>
<td>600 s</td>
<td>T. Huang, F. Meng, and L. Qi, J. Phys. Chem. C, 2009, 113, 13636–13642</td>
</tr>
</tbody>
</table>

the best of our knowledge, this rate constant value of the order of $10^{-1}$ s$^{-1}$ is one of the fastest rate constant shown by a solid supported catalyst for the p-NP reduction reaction to date. For a comparative study, the p-NP reduction catalytic reaction was also studied using Agr and Au@Agr film as catalysts. As shown in Fig. S4 (ESI†), the catalytic behaviour of Agr and Au@Agr is insignificant with stagnant rate constant values of $0.01 \times 10^{-1}$ s$^{-1}$ and $0.05 \times 10^{-1}$ s$^{-1}$ when compared to 2%TGA-Au@Agr and 2%CS-Au@Agr.

With an idea to check whether the concentration of thiol while preparing the films has anything to do with the catalysis rate, next catalytic films using a higher concentration of thiol were prepared (which is 4%, earlier it was 2%). From Fig. 6(B), it is quite clear that 4%TGA-Au@Agr and 4%CS-Au@Agr films show a relatively faster catalysis of p-NP reduction reaction with respective rate constant values of $6.4 \times 10^{-1}$ s$^{-1}$ (an approximately four times higher value) and $2.2 \times 10^{-1}$ s$^{-1}$ (an approximately two times higher value). The complete UV-visible analysis of p-NP reduction by 4%TGA-Au@Agr and
4%CS-Au@Agr is shown in Fig. S5, ESI.† Thus, an increase in thiol concentration does show a faster catalytic rate for the p-NP reduction reaction. A comparison table of different rate constant values obtained for the p-NP reduction to p-AP when catalysed by different films prepared is shown in Table 1. Another table showing the comparison between the rate constant values of reduction reaction of p-NP to p-AP when catalysed by different films prepared is also included in Table 2. Followed by this, a reusability test on 2%TGA-Au@Agr, 4%TGA-Au@Agr, 2%CS-Au@Agr, and 4%CS-Au@Agr was also conducted (Fig. S6, ESI†). For the study, a fresh strip of thiol-Au@Agr film (1 × 1 cm, thickness ~ 0.2 mm) was taken and the 1st catalytic cycle of the reduction reaction of p-NP was carried out. After the completion of 1st catalytic cycle, the film strip was recovered from the solution, washed several times with Milli Q water, dried on a filter paper and then used again for the 2nd catalytic cycle. It was observed that the activity of 2%TGA-Au@Agr and 2%CS-Au@Agr decreases after its first use, but 4%TGA-Au@Agr was equally effective as a catalyst in its second use; whereas, 4%CS-Au@Agr was found to show relatively lower activity in its second use. A SEM image (Fig. S7, ESI†) of the used catalytic film was taken, which shows that there are assemblies of aggregated Au NPs in the used film, which was otherwise absent in the fresh thiol-Au@Agr film. The aggregation of Au NPs in the film after its first catalytic cycle could be due to the presence of excess NaBH₄ in the p-NP(r) catalytic reaction solution. This aggregation of Au NPs probably caused a decrease in the catalytic activity of the film after the 1st use.

An explicit insight into the mechanistic behaviour for the catalysis reaction by the films was done (Scheme 2). The reduction catalysis starts only after adsorption of p-NP and NaBH₄ on the surface of Au NPs. In the present case, each component Agr, Au@Agr and TGA-Au@Agr or CS-Au@Agr plays a specific and favouring role in aiding a faster catalytic reduction of p-NP. Agr: (i) the process of adsorption of reactants made using an Agr hydrogel film as a solid support (ii) once adsorbed, the solid support also limits the movement of the adsorbed reactants making the electron transfer process swift. Au@Agr: (i) it provides a perfect and aligned surface with extra electrons for the catalysis reaction to play on with a swift relay of electrons from BH₄⁻ to p-NP; (ii) supported Au NPs also dilute the chance for aggregation, which is well known to be harmful for a catalysis reaction. TGA-Au@Agr or CS-Au@Agr: (i) first and foremost, TGA and CS prosecute the fragmentation of Au NPs delivering much smaller nanoparticles on a solid support for catalysis; (ii) second, the presence of TGA or CS stimulates adsorption of p-NP much easily due to the probability of forming hydrogen bonding between them; (iii) third, such interactions in conjunction with the agarose hydrogel nature make maximum and quick adsorption of p-NP for effective and swift catalysis. Hence, all these insights give a clear picture of the synergistic roles of Agr, Au@Agr and TGA/CS for such an efficient catalytic activity towards p-NP to p-AP reduction reaction in just ~20 to 30 seconds.

**Conclusion**

This study presents a physical experimentation and demonstration of the capability of a group of thiols to fragment an Au@Agaro film in the absence of any auxiliary applied energy, an unexplored non-conventional chemistry between thiols and supported Au NPs. A group of thiols (TGA, CS, ME and MET) were found to be very proficient in fragmenting 50–60 nm sized Au@Agr into well dispersed ≤30 nm sized nanoparticles. The fragmentation process can be easily explained by transfer of
electron density from thiols to Au@Agr causing an excess of charge on Au NPs, thereby undergoing fragmentation to relieve the surface tension. The TGA-Au@Agr and CS-Au@Agr films studded with such fragmented Au NPs have proven to be an efficient catalytic material for rapid reduction of p-NP to p-AP in just ~20 to 30 s. The rate constants for 2%TGA-Au@Agr and 2% CS-Au@Agr catalysed p-NP reduction are found to be 1.6 × 10⁻¹ s⁻¹ and 1.1 × 10⁻¹ s⁻¹, respectively. A faster catalytic rate for the reduction reaction is evident for increasing the concentration of TGA as well as CS with two reuses of the same film strip. Such a fast catalysis by TGA-Au@Agr and CS-Au@Agr can be accounted for from a synergistic role of Agr, Au@Agr and TGA or CS complementing each others role and contributing towards an efficient and rapid catalytic behaviour. On all these notes, it can concluded that this study opens up new insights about the chemistry of thiols towards supported Au NPs as well as the effectiveness of such a system to serve as an amazing catalyst for industrial noxious and lethargic pollutants.

Conflict of interest

The authors declare no competing financial interest.

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In-Situ and Ex-Situ Chitosan-Silver Nanoparticle Composite: Comparison of Storage/Release and Catalytic Properties

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In this work storage of silver nanoparticles (Ag NPs) in chitosan gel and its subsequent release for catalytic reduction processes is investigated. The generation of small sized metal nanoparticles which acts as catalyst is prerequisite to progress of a catalytic reaction. We show that Ag NPs extracted from chitosan gel are less than 5 nm so very effective in catalysis. Chitosan-Ag nanocomposite gels were prepared from two different approaches. The first approach involves in-situ incorporation of Ag nanoparticles into the reaction mixture while preparing the chitosan hydrogel and termed as chitosan-Ag-in-situ (CH-Ag-I) nanocomposite gel. And, in second approach already prepared chitosan hydrogel was placed in Ag NPs solutions, resulting in adsorption of Ag NPs and thus forming chitosan-Ag-ex-situ (CH-Ag-E) nanocomposite gel. The prepared gels were characterized by UV-Visible spectroscopy, Fourier transformed infra-red spectroscopy (FTIR), Thermogravimetric analysis (TGA) and Scanning electron microscopy (SEM). Swelling studies showed that the CH-Ag-E exhibits efficient water absorption property compared to that of CH-Ag-I. In addition to efficient swelling properties the CH-Ag-E can also act as store house of Ag NPs that can be used to catalyze the reduction of 4-Nitrophenol (4-NP) to 4-Aminophenol (4-AP) as Ag NPs of this composite can be easily extracted just by treating with sodium borohydride which is not possible in case of CH-Ag-I. The rate of the reaction increases upto 10 fold when CH-Ag-E nanocomposite gel is used as catalyst in comparison to CH-Ag-I. The reduction reaction catalyzed by such Ag NPs follow zero order kinetics and dependent on the size of the Ag NPs loaded in the gel (CH-Ag-E) as well as on the amount of the gel used. We found that smaller is the size of the loaded Ag NPs in CH-Ag-E, more effective it is in catalyzing the reduction reaction. The CH-Ag-E gel also showed reusability with efficient catalysis.

Keywords: Nanocomposites, Gel, Ag Nanoparticles, Catalysis.

1. INTRODUCTION

Among many materials that have been studied in last few years biopolymers have been investigated as soft matrices that can accommodate a variety of metal nanoparticles (NPs). The most characteristic feature of such bio-nanocomposites is that they can offer intrinsic functionalities of both metal NPs and biointerfaces of polymers. Out of many application fields, these bio-nanocomposites have gained increasing attention in catalytic activities. The polymers not only provide support to the catalytic metal nanoparticles but their structure also induces special properties such as enhanced reaction rate and stereoselectivity. Thus a proper selection of the polymer and metal nanoparticle can offer quite large opportunities and applications credited to the biopolymer.

Chitosan is a polysaccharide that is obtained by alkaline deacetylation of a naturally occurring polysaccharide, chitin. Chitosan is a linear co-polymer polysaccharide consisting of β (1→4)-linked 2-amino-2-deoxy-D-glucose (D-glucosamine) and 2-acetamido-2-deoxy-D-glucose (N-acetyl-D-glucosamine) units. Chitosan is a biopolymer that has been extensively studied in recent years because of its biocompatibility, low toxicity, enzyme immobilization, drug delivery, gene delivery, implant surgeries, and topical ocular application. It has found numerous applications in agriculture, food industry, cosmetics, and increasingly so in pharmaceutical sciences. It is a versatile polysaccharide due to the presence of amino and hydroxyl functionality and thus

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could be potentially modified with enhanced physical and biological properties. Chitosan based hydrogels are extensively studied.\textsuperscript{18,19} Most of the studies on chitosan hydrogel pertained to stimuli-responsive properties of the hydrogel.\textsuperscript{20} For example its response towards pH, voltage,\textsuperscript{21,22} interfacial properties\textsuperscript{23} etc. Various composites of chitosan and its derivatives were also reported. Baek et al. reported highly ordered chitosan-Au core–shell nanostructures.\textsuperscript{24} Various chitosan composite include iodine stabilized Cu nanoparticle chitosan composite,\textsuperscript{25} CdS QDs/chitosan composite,\textsuperscript{26} CaCO\textsubscript{3} and chitosan to produce well defined inorganic–organic nanoboxes and nanoframes,\textsuperscript{27} chitosan/lactic acid nanofibre,\textsuperscript{28} chitosan/silk hydrogel,\textsuperscript{29} and chitosan/glycine chloride.\textsuperscript{30} Chitosan has also found to be effective in adsorbing humic acid,\textsuperscript{31} mercury ions,\textsuperscript{32} acid dyes,\textsuperscript{33} toluene from aqueous solution\textsuperscript{34} etc. There are growing number of papers on synthesis of chitosan for supported catalysis due to its high sorption capabilities, stability of metal NPs on chitosan and physical versatility of the biopolymer. Among many metal NPs, recently, Ag nanoparticles (Ag NPs) have attracted a great deal of attention in biomedical and catalytic applications. Thus aminopolysaccharide chitosan can uniquely equip hierarchical assembly of nano-scale silver particles with important applications especially when synthesised benignly. Although there are methods available for such chitosan-Ag composite and its cytotoxic efficacy,\textsuperscript{35} molecular relaxation,\textsuperscript{36} catalytic properties\textsuperscript{37} is also studied and in-situ and ex-situ preparation of chitosan-Ag nanocomposite and its unique storage/release of Ag NPs and catalytic properties have not been studied before.

Herein, we report a benign and unique synthesis process for Chitosan-Ag nanocomposite. In this work we have synthesized chitosan-Ag nanocomposite by two approaches named in-situ and ex-situ process. In the first protocol Ag NPs were incorporated while preparing the chitosan hydrogel and is termed as chitosan-Ag-in-situ (CH-Ag-I) in the paper. In the second approach chitosan hydrogel was put in Ag NPs solution resulting in adsorption of Ag nanoparticles (NPs) in the chitosan hydrogel matrix. We term this chitosan-Ag-ex-situ nanocomposite (CH-Ag-E) in the paper. We have investigated the properties of these nanocomposites made by two different approaches. We also found that the Ag NPs adsorbed in CH-Ag-E can be extracted by adding sodium borohydride. Moreover it was observed that CH-Ag-E nanocomposite gel can effectively catalyze the reduction of 4-Nitrophenol (4-NP) to 4-Aminophenol (4-AP) than CH-Ag-I nanocomposite gel and the reaction follow zero order kinetics.

2. EXPERIMENTAL DETAILS

2.1. Materials

Commercially available chitosan of low molecular weight was purchased from Sigma Aldrich. Glycerol (about 98% purified), sodium borohydride, trisodium citrate-2-hydrate, silver nitrate and sodium hydroxide pellets (purified) were purchased from Merck. Glacial Acetic acid was purchased from Qualigens Fine Chemicals and 4-Nitrophenol was purchased from Loba Chemie. All other materials used were of analytical reagent grade from commercial sources.

2.2. Preparation of Chitosan Hydrogel

The chitosan gel (CH) 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 mixture. 0.1 gm chitosan was dissolved in 10 ml of the aforesaid solvent by stirring with a magnetic stirrer at room temperature for 1–2 hours to form a clear pale yellow solution. The solution was neutralized by adding 5 N NaOH. Immediately a clearly slightly tacky gel formed. The gel, which apparently results from the interaction of chitosan, glycerol and water, has a three dimensional structure, and no free water or glycerol is apparent.

2.3. In-Situ Process: Incorporation of Ag NPs Into the Hydrogel

The Chitosan-AgNPs hydrogels (CH-Ag) were prepared using the same procedure but in two different ways; in-situ and ex-situ incorporation of AgNPs into the gel. The in-situ incorporation of silver nanoparticles (AgNPs) was done prior to stirring by adding 3 ml of prepared Ag NPs solution to the chitosan-acetic acid-glycerol mixture. After 2 hours of stirring a light brown coloured solution was obtained, this was then neutralized by 5 N NaOH resulting in formation of the hydrogel.

2.4. Ex-Situ Process: Embedding and Releasing the Silver Nanoparticles from the Hydrogel

In this process, a small piece of dry hydrogels prepared by above methods was immersed in already prepared Ag NPs for 60 minutes. The Ag NPs were prepared separately by reduction of AgNO\textsubscript{3} (5 mM) using citric acid (5 mM) under boiling condition. This step allows loading of Ag NPs from the NPs solution to the hydrogel network. The process was also monitored with UV-Visible spectrophotometer. The loaded Ag NPs can be released by adding 5 mM of NaBH\textsubscript{4} solution.

2.5. Catalytic Reduction of 4-Nitrophenol (4-NP) by CH-Ag-I and CH-Ag-E Nanocomposites

The reduction of 4-Nitrophenol (4-NP) to 4-Aminophenol (4-AP) by sodium borohydride (NaBH\textsubscript{4}) catalyzed by CH-Ag-I and CH-Ag-E nanocomposite gels was monitored spectrophotometrically. Approximately 2 ml of 5 mM NaBH\textsubscript{4} and 20 \textmu l of 0.05 mM 4-NP was mixed together.
in a 3 ml cuvette. It was observed that the absorbance peak of aqueous solution of 4-NP (at 317 nm) got red shifted to 400 nm due to the reduction of 4-NP to 4-nitophenolate ion by NaBH₄. The new peak at 400 nm remained stable for few weeks in absence of any other reagent. For the catalytic study, 0.05 g weighed amount of CH-Ag-E nanocomposite gel was immersed into the cuvette solution containing 2 ml of 5 mM NaBH₄ and 20 μl of 0.05 mM 4-NP. The UV-Visible spectrum of the cuvette solution showed gradual decrease in the absorbance value around 400 nm (λₘₐₓ) with time. This time-dependent catalytic reduction of 4-NP to 4-AP by CH-Ag-E nanocomposite gel took 45 minutes to show maximum reduction of absorbance value at λₘₐₓ. The whole procedure was repeated with CH-Ag-I nanocomposite gel keeping the reaction condition and gel amount (0.05 g) identical. Unlike CH-Ag-E nanocomposite, the maximum reduction of absorbance value in CH-Ag-I nanocomposite was found to be only 0.01.

2.6. Characterization

Transmission electron microscopy was carried out using JEOL JEM 2100 with operating voltage at 200 kV. Scanning electron microscopy was carried out using Carl Zeiss Sigma VP using EHT 5 kV. Energy Dispersive X-ray Spectroscopy (EDX) was done using EDX detector from Oxford Inc. UV-Visible spectra were taken using Shimadzu (UV1601PC) spectrophotometer. FT-IR spectroscopic measurements of chitosan gel and Ag NPs loaded chitosan gel were recorded in Bruker FT-IR spectrometer. Samples for FT-IR measurements were prepared in the form of pellets by mixing 20 mg of IR spectroscopic grade potassium bromide with 2 mg of dried samples. The spectra were recorded in transmission mode over 256 scans with resolution of 4 cm⁻¹. Thermogravimetric analysis (TGA) was carried out in Perkin Elmer TGA 4000.

3. RESULTS AND DISCUSSION

3.1. Chitosan–Ag In-Situ and Ex-Situ Nanocomposite Gels

Chitosan of low molecular weight having DDA³₈ = 77.7% (Detail in electronic supporting information) was used to study the effect of in-situ and ex-situ incorporation of silver nanoparticles (Ag NPs) on the formation and characteristic properties of nanocomposite gel. The characteristic color of the Ag nanoparticles is visible in chitosan-Ag-in-situ nanocomposite gel. The morphology of chitosan hydrogel was changed due to incorporation of Ag NPs into the gel matrix. Thin layer of prepared hydrogels was placed on silicon wafer and coated with Au/Pd before SEM imaging. The SEM images (Fig. 2) show that while the chitosan hydrogel has fibrous network, the network structure improves and is very much evident in the presence of Ag NPs when chitosan-Ag nanocomposite gel is made in-situ. Beautiful fibrous network is visible in chitosan-Ag nanocomposite prepared by in-situ method (CH-Ag-I). It can also be seen from the SEM pictures that in CH-Ag-E (chitosan-Ag ex-situ nanocomposite gel) Ag NPs are beautifully more adsorbed on the surface of gel in comparison to CH-Ag-I (chitosan-Ag in-situ nanocomposite gel). The schematic of these two different methods used for the preparation of nanocomposite gel is shown in Figure 1.

Temperature dependant degradation of the prepared gels was assessed by TGA analysis. The thermogram clearly shows that thermal profile is similar for chitosan hydrogel and chitosan-Ag-in-situ (CH-Ag-I) nanocomposite gel except that the amount of adsorbed water is more in CH-Ag-I. The thermal stability of chitosan-Ag-ex-situ (CH-Ag-E) nanocomposite gel is vastly improved as compared to chitosan hydrogel (CH) and CH-Ag-I. The degradation also starts at much higher temperature ~ 250 ºC. The swelling study showed that swelling ratio (SR) for CH and CH-Ag-in-situ nanocomposite (CH-Ag-I) gels was found to be 1.11. The SR for CH-Ag-ex-situ (CH-Ag-E) nanocomposite gel was determined to be 2.44 which is almost double than CH and CH-Ag-I. The high SR for CH-Ag-E could be because of freer amine groups in comparison to CH-Ag-I. That is also the reason why CH-Ag-E is able to absorb Ag NPs more than CH-Ag-I (detail given later).

3.2. Storage and Release of Ag NPs from CH Gel Matrix

The reversible binding of Ag NPs to the CH gel matrix was monitored UV spectrophotometrically. UV-Visible spectra of CH, CH-Ag-I and CH-Ag-E nanocomposite gels were taken and shown in Figure 3(A). It is evident from the spectra that CH-Ag-I has characteristics Ag Plasmon peak centered at 409 nm which is missing in both CH and CH-Ag-E nanocomposite gels. The storage of Ag NPs...
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Fig. 2. Scanning electron microscope (SEM) image of (A) chitosan hydrogel (CH) (B) Chitosan-Ag-in-situ (CH-Ag-I) and (C) Chitosan-Ag-ex-situ (CH-Ag-E) nanocomposite gel. TEM images of (D) prepared Ag NPs (E) After release from CH-Ag-E nanocomposite gel (F) Size distribution plot of before and after release of Ag NPs from CH-Ag-E.

Fig. 3. (A) Uv-vis spectrum of chitosan hydrogel (black), CH-Ag-I (red) and CH-Ag-E (green) nanocomposite gel (B) Uv-vis spectrum of as prepared Ag NPs (black), Ag NPs solution after putting in chitosan hydrogel for 60 minutes (red), Ag NPs after released from the chitosan hydrogel by adding sodium borohydride (green).

inside chitosan hydrogel matrix (CH-Ag-E) and subsequent release of the Ag NPs is depicted in Figure 3(B). UV-Vis spectrum of as prepared Ag NPs was shown in Figure 3(B) (black line) having the plasmon resonance peak centered at 407 nm. After putting the chitosan hydrogel in this Ag NPs solution for 60 minutes, Uv-vis absorption spectrum of the solution was again taken (Fig. 3(B) (red line)). It is evident from the spectrum that there is considerable decrease in absorption value which indicates the loading of Ag NPs in the chitosan hydrogel matrix. The subsequent release of Ag NPs by NaBH₄ results in emergence of Plasmon resonance peak at 386 nm as shown in Figure 3(B) (green line).

The schematic representation of ex-situ process of formation of hydrogel (CH-Ag-E), loading and release of Ag NPs is as shown in Figure 4. The UV-Visible data was supported by transmission electron microscope (TEM) data. Figures 2(D) and (E) shows the representative TEM image of prepared Ag NPs and Ag NPs released from CH-Ag-E gel, respectively. The size distribution plot is depicted in Figure 2(F). It is evident from size distribution that while the size of prepared Ag NPs are in the range of 15–20 nm, the extracted Ag NPs are smaller and majority of the particles are less than 5 nm in size. It is well supported by the blue shift in plasmon resonance peak of Ag NPs obtained from UV-Visible studies. Moreover, the extracted Ag NPs can be again loaded in the chitosan hydrogel and re-extracted (detail given in electronic supporting information, Fig. S3). The quantitative analysis of the amount of loading of Ag NPs was also
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Fig. 4. Schematic representation of loading of the Ag NPs in the hydrogel matrix (i.e., formation of CH-Ag-E nanocomposite gel) and subsequent release of Ag NPs from CH-Ag-E gel by addition of sodium hydroxide.

Done and determined to be 0.0164 M/gm of chitosan gel. It is worth mentioning here that a noted difference was observed between the Ag NPs release from CH-Ag-E and CH-Ag-I nanocomposite gels. The extraction of Ag NPs from CH-Ag-I does not take place with the addition of sodium borohydride. We also want to add here that loading of Au NPs takes more time unlike Ag NPs and it was not possible to recover the loaded Au NPs (Detail given in electronic supporting information, Fig. S4).

Scanning Electron Microscopy (SEM) was also used to investigate the storage of Ag NPs and subsequent release of Ag NPs. Figure 5(A) shows the SEM images of chitosan gel. After a small piece of chitosan hydrogel was put in a solution of Ag NPs, SEM image of CH-Ag-ex-situ nanocomposite shows Ag NPs deposited all over the hydrogel matrix. It was also confirmed by energy-dispersive X-ray spectroscopic (EDX) analysis shown in Figure 5(B)’ where it shows the presence of Ag. Subsequently after release of Ag NPs by addition of sodium borohydride, SEM image shows that the Ag NPs have completely been removed from the hydrogel matrix and no Ag nanoparticles visible in majority of the regions in the hydrogel. The EDX analysis also confirms that there is no Ag present in the chitosan gel matrix. The quantitative estimation of elements as obtained from EDX data in each step is tabulated and given in electronic supporting information, Figure S5.

FTIR analysis was carried out on chitosan gel (CH), chitosan-Ag-in-situ (CH-Ag-I), chitosan-Ag-ex-situ (CH-Ag-E) nanocomposite gels and stacked FTIR is shown in Figure 6(A). In case of CH gel, 1651 cm−1, 1568 cm−1, 1375 cm−1 are assigned to amide I, amide II and amide III respectively. 1152 cm−1 is characteristics of saccharide structure. 1320 cm−1 indicates C-N bond stretching. CH-Ag-I and CH-Ag-E nanocomposite gel showed similar peak positions. 3400 cm−1 peak is attributed to O-H stretching vibration, 2930 cm−1 and 2868 cm−1 represent the presence of C-H aliphatic chain. CH-Ag-E shows the C-H aliphatic peak at 2930 and 2879 cm−1 respectively. The characteristics amide I, amide II and amide III peaks at 1651, 1568, 1375 cm−1 respectively are present in CH-Ag-I and CH-Ag-E, 1153 cm−1 peak of saccharide structure and 1320 cm−1 peak of C-N bond stretching vibration is present in both CH-Ag-I and CH-Ag-E. The structural changes due to storage of Ag

Fig. 5. Representative scanning electron microscope image of (A) chitosan hydrogel (B) chitosan-Ag-ex-situ (CH-Ag-E) nanocomposite gel (C) After release of Ag NPs on addition of NaBH₄, CH-Ag-E after release. The corresponding energy-dispersive X-ray spectroscopic (EDX) is shown in (A’), (B’) and (C’) respectively.

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NPs inside chitosan hydrogel matrix, i.e., chitosan-Ag-

ex-situ (CH-Ag-E) nanocomposite gel and subsequent release of the Ag NPs was also monitored by FTIR and depicted in Figure 6(B). While the chitosan hydrogel (CH) didn’t have well resolved O—H and C—H aliphatic stretching vibrations, after loading of Ag NPs O—H stretching peaks at 3400 cm$^{-1}$ and C—H stretching peaks at 2930 and 2879 cm$^{-1}$ was distinctly visible. Other peaks are all present after loading of Ag NPs. After release of Ag NPs there is slight shift in the amide-II peak, the amide-I, amide-II and amide-III peaks were obtained at 1651, 1594, 1375 cm$^{-1}$ respectively.

3.3. Efficient Catalytic Reduction of 4-NP to 4-AP by Nanocomposite Gels

The superior catalytic property of CH-Ag-E and CH-Ag-I nanocomposite gels compared with those of CH hydrogels can be demonstrated by reduction of 4-nitrophenol (4-NP) with sodium borohydride as a model reaction. The reduction of 4-Nitrophenol (4-NP) to 4-Aminophenol (4-AP) by sodium borohydride (NaBH$_4$) catalyzed by CH-Ag-I and CH-Ag-E nanocomposite gels was monitored spectrophotometrically (Fig. 7). An aqueous solution of 4-NP shows a distinct spectral profile with an absorption maximum at 317 nm. Addition of sodium borohydride solution results in the shifting of the peak position to 400 nm ($\lambda_{\text{max}}$) which is due to the formation of 4-nitrophenolate ions in alkaline condition caused by the addition of NaBH$_4$. The BH$_4^-$ in the absence of catalyst is incapable of reducing 4-nitrophenolate ions to the corresponding amino compound. As evident from the Figure 7(A), in the presence of the CH-Ag-E nanocomposite gel the absorbance peak at 400 nm gradually decreased with time confirming the reduction of 4 NP. In the presence of CH-Ag-I nanocomposite gel, the decrease of nitrophenolate ions peak is slow (reaction monitored for 45 minutes) as compared with CH-Ag-E as shown in Figure 7(B). Figure 7(C) shows the combined plot of fate of nitrophenolate ions peak ($\lambda_{\text{max}} = 400$ nm) in the presence of CH-Ag-I and CH-Ag-E as catalyst after 45 minutes of reaction. The plot clearly shows that the rate of the reduction is faster when CH-Ag-E nanocomposite gel is being used as catalyst than CH-Ag-I nanocomposite gel.

3.4. Effect of Size of the Loaded Ag NPs Solution and Determination of Rate Law

The role of size of the Ag NPs loaded in chitosan-Ag-

ex-situ (CH-Ag-E) nanocomposite gel was also investigated. In this study three gel pieces individually loaded with three different sized Ag NPs 26 nm, 56 nm and 67 nm (sizes determined using DLS) with Plasmon band at wavelengths 410, 421, 431 nm respectively were used in the catalytic reduction of 4-NP to 4-AP UV spectrophotometrically as shown in Figure 8. It was observed that in presence of gel loaded with 26 nm Ag NPs corresponding to Plasmon band at wavelength 410 nm showed maximum decrease in 4-NP ion peak as compared to other two gel pieces loaded with Ag NPs of sizes 56 nm and 67 nm. Thus better catalytic activity can be obtained by decreasing the size of the loaded Ag NPs. So, smaller size of Ag NPs effectively catalyze reduction of 4-NP to 4-AP. It is very interesting to note that larger sizes of Ag NPs
The rate law for the overall catalytic reduction reaction of 4-NP to 4-AP was also determined. The rate constant \( k \) for the reaction was found to follow the zero order kinetics, obeying the zero order rate law:

\[
k = \frac{1}{t} \left( [C_0] - [C] \right)
\]

A plot of \( ([C_0] - [C]) \), i.e., concentration change as a function of time for the reduction of 4-NP to 4-AP is shown in Figure 9(A). Rate constant can be obtained from its slope and the rate of the reaction was found to be \( 4.00 \times 10^{-5} \) mol L\(^{-1}\) min\(^{-1}\), which remains constant throughout different intervals of time. The plot of concentration v/s time was also constructed to draw conclusion about the difference in rate of reduction of 4-NP catalysed by the two different nanocomposite gels CH-Ag-I and CH-Ag-E (Fig. 9(B)). For this study 0.05 g of each of the nanocomposite gels, i.e., CH-Ag-I and CH-Ag-E was used in catalysis. The rate of reduction reaction of 4-NP catalysed by
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In this work chitosan-Ag nanocomposite gel was prepared by two different approaches. In the first approach chitosan-Ag was made (CH-Ag-I) and in the other approach chitosan hydrogel was placed in Ag NPs resulting in absorption of Ag NPs thus forming chitosan-Ag-ex-situ (CH-Ag-E). It was found that the while Ag NPs adsorbed in CH-Ag-E can be subsequently extracted by addition of sodium borohydride; it was not possible to extract the Ag NPs from CH-Ag-I. The reason of this observation is due to the fact that in CH-Ag-I the electrostatically adsorbed Ag NPs forms a 3-dimensional gel structure which is quite stable and so it is not easy to remove the Ag NPs. While in CH-Ag-E nanocomposite gel Ag NPs are electrostatically adsorbed onto the already formed gel structure and thus these loosely bound Ag NPs can be extracted by addition of sodium borohydride. This is also the reason that CH-Ag-E nanocomposite gel is effective in catalyzing reduction of 4-Nitrophenol (4-NP) to 4-Aminophenol (4-AP) with a rate constant 10-fold greater than CH-Ag-I. It was also found that the rate of the reduction reaction is also depended on the size of the Ag NPs loaded in chitosan gel (CH-Ag-E) as well as the amount of the gel taken. Smaller size loaded Ag NPs in chitosan gel (CH-Ag-E) are more effective in catalyzing the reaction. Moreover the same CH-Ag-E nanocomposite gel can be used several times for the catalytic reduction of 4-NP to 4-AP demonstrating the reusability potential of CH-Ag-E nanocomposite gel. These notable properties and reusability of CH-Ag-E nanocomposite gel.

4. CONCLUSION

CH-Ag-I and CH-Ag-E was determined to be $3 \times 10^{-7}$ mol L$^{-1}$ min$^{-1}$ and $1 \times 10^{-6}$ mol L$^{-1}$ min$^{-1}$ respectively. It is evident that there is 10 fold increase in the rate of the reaction when CH-Ag-E nanocomposite gel is used as catalyst.

The mechanism of binding of citrate stabilized Ag NPs to chitosan is due to electrostatic adsorption of negatively charged citrate stabilized Ag NPs into cationic main chain. Chitosan in aqueous solution is positively charged in the form of R-NH$_3^+$. The Ag NPs can have affinity towards R-NH$_3^+$ group of chitosan through van-der Waals forces. Similar mechanism is also valid for Au NPs although the adsorption of Au NPs onto chitosan hydrogel is slow and it takes more than 1 hour for substantial adsorption of Au NPs (discussed earlier). However, the reason for this observation is not clear. Another interesting observation was that while it was possible to extract the Ag NPs from the CH-Ag-E nanocomposite gel by addition of sodium borohydride, in the case with CH-Ag-I nanocomposite gel it was not possible to extract the loaded Ag NPs. The reason can be explained by the fact that in CH-Ag-I, Ag NPs is incorporated while making the chitosan gel as a consequence the Ag NPs electrostatically absorbed forms a 3-dimensional gel structure which are thus quite stable (Fig. 10(A)). So, in such a structure it is not easy to remove the Ag NPs. On the other hand CH-Ag-E nanocomposite gel which is formed by placing the chitosan gel in Ag NPs solution results in electrostatic adsorption of Ag NPs onto the already formed gel structure. So, the Ag NPs that are being electrostatically adsorbed are loosely bound and thus can be extracted by addition of sodium borohydride (Fig. 10(B)). This also explains why CH-Ag-E is effective in catalyzing reduction of 4-Nitrophenol (4-NP) to 4-Aminophenol (4-AP) than CH-Ag-I. The CH-Ag-E nanocomposite gel which has loosely bound Ag NPs helps to transfer electrons from BH$_4^-$ to nitrophenolate and thus acting as a better catalyst for the purpose. We also want to add here that although in CH-Ag-E the Ag NPs are electrostatically adsorbed on chitosan hydrogel, sonication of the CH-Ag-E cannot remove the loaded Ag NPs showing the interactions between Ag NPs and chitosan hydrogel are quite strong.
nanocomposite gel are promising in many industrial applications.

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