CHAPTER 3

A Sulphonated Carbon Dot–Chitosan Hybrid Hydrogel Nanocomposite As An Efficient Ion-Exchange Film for Removal of Ca$^{2+}$ and Mg$^{2+}$ Ions From Water
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Chapter 3

Water is the prime essential component of human life. However, the alarming rise in pollution issues originating from the improper disposal of hazardous chemical wastes from paper, textile, fertilizer or petrochemical industries, electroplating plants, tanneries, and slaughterhouses, etc. have led to the exacerbation of the safe drinking water crisis. Another major issue pertaining to water-related problems is the hardness of water caused primarily by the presence of dissolved Ca\(^{2+}\) and Mg\(^{2+}\) ions in water, the principal sources being percolation of water through deposits of chalk and limestone which largely comprises of calcium and magnesium carbonates.\(^{137}\) Although hard water is known to have moderate health benefits, it is a serious problem when it comes to the breakdown of costly equipment in industrial settings owing to corrosion. Therefore, the need for suitable water purification techniques to meet this global safe water crisis is in demand. Among the numerous methods available, ion exchange has unwaveringly received significant attention for water softening due to its high efficiency, superior regeneration power and low operational costs.\(^{138}\) The process of ion exchange consists of the interchange of ions between two phases viz. the ion-exchange resin which is a cross-linked polymer network and comprises the insoluble phase to which an ion is electrostatically bound and the solution phase comprising a solution containing ions of the same charge.\(^{139}\) Ion exchange resins are commonly fabricated from organic polymer substrates. Conventionally polymers such as polyaniline (PANI), polypyrrole (PPy), polythiophene (PT) and their derivatives have continued to gain much interest as
chemical adsorbents owing to their ease of synthesis, porous structure, tunable morphology, good electrorheological property, unique redox chemistry, nontoxicity, insolubility in water and reversible ion (especially cation) sorption/desorption capability.\textsuperscript{140} Exchange of ions occurs between the two phases with the extent of ion exchange depending on the concentration of ions in the solution phase and the respective affinity of the ions in solution towards the insoluble phase relative to the solution phase.

Basically, there are two types of ion-exchange resins: cation exchange resin and anion exchange resin as represented by Equation 3.1.1 and 3.1.2 respectively.

\begin{align*}
\text{Cation Exchange Resin} \\
{\text{A}^+} & \quad \text{B}^+ \\
\text{[Polymer]-X}^- & \quad \text{[Polymer]-X}^- + \text{A}^+ \quad \text{......................... 3.1.1} \\
\text{Anion Exchange Resin} \\
{\text{C}^-} & \quad \text{D}^- \\
\text{[Polymer]-Y}^+ & \quad \text{[Polymer]-Y}^+ + \text{C}^- \quad \text{......................... 3.1.2}
\end{align*}

Different ion-exchange resins consist of different polymers and different ligands covalently bound to the polymers. Ion-exchange resins owe their adequacy to insolubility of the resin phase, easy separation, and reusability due to which they have continued to find extensive applicability in water softening, removal of toxic metals from water in the environment, wastewater treatment, hydrometallurgy, sensors, catalysis, chromatography, and biomolecular separations.\textsuperscript{139} However, the increasing cost of their synthesis demands alternatives to draw the same benefits at an affordable price. Moreover, typical ion exchange resins used for water softening are derived
from polystyrene crosslinked with divinylbenzene which employs the use of harmful chemicals and harsh reaction conditions as divinylbenzene is carcinogenic and is found to cause respiratory and kidney disorders. In view of these disadvantages, there have been unabated efforts from the scientific community to harness the same potential in a more environmentally friendly way.

3.1 The Motivation

Nanoparticles have always continued to occupy the forefront of all facets of environmental remediation research ever since their discovery. However, the use of nanoparticles alone for water treatment is undesirable due to difficulties in regeneration, reuse and even possible risk to the ecosystem and human health. Therefore the most valuable approach has been to design hybrid nanocomposites using biocompatible polymers and non-toxic nanoparticles to replace the traditionally used toxic ones like titanates, Fe$_3$O$_4$ nanoparticles, etc. There are already a few reports available in the literature on use of hybrid polymeric nanocomposites for water treatment. For instance, Musico et al. demonstrated the use of the poly(N-vinyl carbazole)–GO nanocomposite for adsorption of Pb$^{2+}$ from waste water. Zhang et al. developed a simple and novel method for the synthesis of hierarchical PANI/GO nanocomposites using 1D (uniform aligned PANI nanorods) and 2D (GO nanosheets) nanocomponents by dilute polymerization and were used as a super adsorbent material for Cr(VI). However, the most important aspect to be noted here is that all the materials used were based on synthetic polymers. Contrarily, among the biocompatible and biodegradable polymers, chitosan, obtained from the deacetylation of chitin, a natural biopolymer that is present in the exoskeleton of crustaceans, has been able to draw significant attention due to its enhanced
biocompatibility, mechanical strength, excellent adhesion and absorption ability (due to the presence of hydroxyl (OH) and amine (NH$_2$) functional groups) due to which it can potentially bind to a variety of chemical compounds through electrostatic attraction or hydrogen bonding. Carbon dots (fragments of molecules below 10 nm in size) on the other hand are a nascent class of QDs possessing far-reaching potential like chemical inertness, biocompatibility, strong and tunable fluorescence and low toxicity. Therefore, fabrication of a hybrid nanocomposite material endowed with the advantages of both the polymer and the nanoparticle can always be a better alternative with anticipated potential for water softening applications. Our group has also developed a carbon dot rooted agarose hydrogel hybrid sensing material for the optical detection and separation of heavy metal ions (Cr$^{6+}$, Cu$^{2+}$, Fe$^{3+}$, Pb$^{2+}$, Mn$^{2+}$) from aqueous media.

In the present work, for the first time we introduced a novel carbon source (11-mercaptoundecanoic acid, MUA) for the one-step synthesis of sulphur-bearing carbon dots. These carbon dots bearing surface thiol (–SH) groups were then oxidized to convert the –SH groups into sulphonic acid (–SO$_3$H) groups. Subsequently, the oxidized carbon dots were blended with chitosan hydrogel to form carbon dot embedded hybrid chitosan nanocomposite thin films. The potential applicability of these films was then tested for the removal of Ca$^{2+}$ and Mg$^{2+}$ ions from water employing the principle of ion-exchange. Although a great deal of work has been done for the removal of toxic ions from water based on synthetic polymers, materials for hardness (specifically Ca$^{2+}$ and Mg$^{2+}$ ions) removal based on biopolymers are yet to be explored. Therefore, this material we report is the first of its kind with
tremendous potential to act as a biodegradable and green material for water-softening applications.

3.2 Materials

11-Mercaptoundecanoic acid (Sigma-Aldrich), sodium hydroxide flakes (Merck), hydrogen peroxide solution (50%, purified) (Merck), acetic acid (glacial, 99-100% for synthesis) (Merck), glycerol (anhydrous, ≥98%) (Merck), calcium chloride (Merck), magnesium chloride (Merck), and chitosan (pure, Mol. Wt. = 193400) (Sisco Research Laboratories Pvt. Ltd.) were used as received without further purification. The water used throughout the experiments was from a Milli-Q water purification system.

3.3 Experimental

3.3.1 Synthesis of Carbon Dots (CDs) from 11-Mercaptoundecanoic acid (MUA)

Blue photoluminescent CDs were prepared from 11-Mercaptoundecanoic acid (MUA). In this work for the first time we introduced MUA as a novel carbon source for CD synthesis. For synthesis of CDs, briefly, 0.1g of MUA was taken in a beaker and heated at 250°C for 45 minutes. The white solid mass melted into a colorless liquid which gradually turned yellow, then orange and finally dark brown in color. The brown liquid so obtained was then added dropwise to 10 mL of 1.25 M NaOH solution under vigorous stirring to obtain a pale yellow coloured dispersion containing the CDs. This dispersion was then centrifuged at 7000 rpm for 30 minutes in order to separate the CDs from the heavier microparticles or impurities present. The
supernatant solution obtained after centrifugation containing the fluorescent CDs was collected and systematically characterized and was termed as MUA-SH-CDs solution.

3.3.2 Oxidation of Carbon Dots (CDs) synthesized from 11-Mercaptoundecanoic acid (MUA)

The CDs prepared from MUA were subsequently oxidised in a single step in order to convert the surface –SH groups into –SO$_3^-$ groups using hydrogen peroxide as oxidising agent. Briefly, 10 mL of MUA-SH-CDs solution was taken in a beaker and to it 5 mL of 30% H$_2$O$_2$ solution was added and stirred magnetically for 3 hours at room temperature. It was observed that the original brown colour of the CD solution gradually faded away along with the evolution of vigorous effervescence immediately after addition of H$_2$O$_2$. The resulting solution obtained was the oxidized MUA-SH-CDs solution and was termed as MUA-SO$_3$Na-CDs solution.

3.3.3 Fabrication of Chitosan-MUA-SO$_3$Na-CDs nanocomposite thin films (Ch-SO$_3$Na-CDs films)

After successful oxidation of MUA-SH-CDs, the oxidized CDs were embedded into a solid platform in the form of a hybrid nanocomposite thin film via conjugation of CDs with the biopolymer chitosan. A hybrid hydrogel was accordingly synthesized from chitosan with the oxidized CDs being embedded in the hydrogel matrix via electrostatic interactions. Briefly, 0.1g of chitosan was dissolved in 10 mL of a mixture of two parts glycerol and three parts 0.1 M acetic acid under magnetic stirring at room temperature for 2 hours. After complete dissolution of chitosan in the solvent mixture, 6 mL of MUA-SO$_3$Na-CDs solution
was added dropwise to this solution. Immediately, a hydrogel material began to separate out from the liquid mixture. The beaker containing the hydrogel and the solvent mixture was allowed to stand overnight to ensure complete gel formation. This hydrogel material formed via conjugation of CDs to chitosan was termed as the Ch-SO₃Na-CDs hydrogel. Subsequently, the Ch-SO₃Na-CDs hydrogel material was filtered and washed thoroughly with milli pore water to remove all the excess ions and impurities. The hydrogel synthesized was found to contain an MUA-SO₃Na-CD concentration of 0.011 M i.e., 0.35 (wt%). The Ch-SO₃Na-CDs hydrogel was then spread onto glass slides and dried in a hot air oven for 2 hours at 50⁰C. Finally, the dried hydrogel films were peeled off from the glass slides and stored under desiccation for further characterization.

3.3.4 Fabrication of blank chitosan thin films (Ch-films)

Similar to the hybrid Ch-SO₃Na-CDs hydrogel films, blank chitosan thin films were fabricated and characterized for control experiments. Typically, 0.1g of chitosan was dissolved in 10 mL of a mixture of 2 parts glycerol and 3 parts 0.1 M acetic acid under continuous stirring at room temperature for 2 hours. After complete dissolution of chitosan in the solvent mixture, 6 mL of 1.25 M NaOH was added dropwise to it. Immediately, a hydrogel material began to separate out from the liquid mixture. The beaker containing the hydrogel and the solvent mixture was allowed to stand overnight to ensure complete gel formation. It is to be noted here that the same procedure was being followed for formation of blank chitosan hydrogel (without incorporation of MUA-SO₃Na-CDs) except that 1.25 M NaOH solution was used instead of MUA-SO₃Na-CDs solution for hydrogel
formation as in the previous case. The chitosan hydrogel (Ch-hydrogel) formed was then filtered and washed thoroughly with milli pore water to remove all the impurities. The hydrogel was then spread onto glass slides and dried in an oven for 2 hours at 50°C. Finally, the dried hydrogel films were peeled off from the slides and stored under desiccation for further characterization.

3.4 Characterization

The CDs prepared from MUA, subsequently oxidized with H₂O₂, the Chitosan-MUA-SO₃Na-CDs hybrid nanocomposite thin films, and the bare chitosan thin films prepared were subjected to extensive stepwise and systematic characterization by various analytical techniques. The particle size distribution and zeta potential analyses were performed in a Malvern Zetasizer NanoZS 90 instrument. The surface morphology of the prepared CDs was investigated via Scanning Electron Microscope (SEM) and Transmission Electron Microscopy (TEM) using a Carl Zeiss (Sigma VP) instrument and JEOL, Model: JEM 2100 respectively. Fluorescence microscopy images of the prepared nanocomposite films were recorded in a fluorescence microscope LEICA DMI 3000 B. The PL emission properties of CDs were studied on a Jasco Spectrofluorometer FP-8300. In order to confirm the presence of different functionalities on the CDs before and after oxidation and also after film fabrication, infrared spectra were recorded in Fourier Transform (FTIR) mode and Attenuated Total Reflectance (ATR) mode respectively on a Nicolet-6700 FTIR spectrophotometer. The FTIR spectra were recorded using KBr pellet samples in transmission mode over 32 scans in the range of 400-4000 cm⁻¹. All the UV-visible spectral studies were carried out on a
Shimadzu UV spectrophotometer-UV2600. The spectra of the thin films were initially recorded in percentage reflectance mode from 800 to 190 nm which were then converted into absorbance mode using Kubelka–Munk (KM) transformation. Optics Technology UV lamp (365 nm) was used to check the fluorescence intensity of CDs. Powder XRD spectra were collected on a Bruker D8 Advance diffractometer. The TGA thermograms were recorded in a Perkin Elmer 4000 in the temperature range of 35–800°C at a heating rate of 10°C/min under nitrogen flow rate of 20 mL/min. Centrifugation of the CD solution was carried out on a Spinwin microcentrifuge. Atomic Absorption Spectroscopy analysis was carried out on a Shimadzu AA-7000 Atomic Absorption Spectrophotometer. The concentration of Ca²⁺ ions in solution was determined employing a calcium combination ion selective electrode in an Accumet Excel Dual Channel pH/Ion/Conductivity Meter, XL 50 (Fischer Scientific) and the measurements were recorded in millivolt mode.

3.5 Results and Discussion
Scheme 3.1 Schematic representation of the protocol followed for synthesis of thiol functionalized CDs from MUA followed by their oxidation to form sulphonated CDs and subsequent fabrication of hybrid polymeric nanocomposite films via conjugation of the sulphonated CDs with the biopolymer chitosan.

Thiol-functionalized CDs were prepared for the first time from MUA followed by their oxidation to form sulphonated CDs and the subsequent fabrication of hybrid polymeric nanocomposite films via conjugation of the sulphonated CDs with the biopolymer chitosan by the formation of a Chitosan-MUA-SO₃Na-CD hydrogel as illustrated in Scheme 3.1.

3.5.1 Characterization of CDs prepared from MUA before and after oxidation

The CDs prepared from MUA and subsequently oxidized with H₂O₂ were subjected to extensive stepwise and rigorous characterization by various analytical techniques as discussed below:

(a) Inference from Dynamic Light Scattering (DLS), Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM) Analysis

The particle size and zeta potential of CDs synthesized from MUA were determined both before and after oxidation using Dynamic Light Scattering Technique.
**Table 3.1** The respective weight percentages of different elements present on the surface of CDs.

<table>
<thead>
<tr>
<th>Element</th>
<th>Weight %</th>
<th>Atomic %</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>10.026</td>
<td>14.03</td>
</tr>
<tr>
<td>O</td>
<td>57.793</td>
<td>61.11</td>
</tr>
<tr>
<td>Na</td>
<td>31.913</td>
<td>24.57</td>
</tr>
<tr>
<td>S</td>
<td>0.273</td>
<td>0.29</td>
</tr>
<tr>
<td>Total</td>
<td>100.00</td>
<td></td>
</tr>
</tbody>
</table>

**Figure 3.1** (A) Particle size graph and zeta potential value of CDs prepared from MUA (B) SEM and (C) TEM micrograph of CDs with –SH surface functionality before oxidation (D) Particle size graph and zeta potential value of CDs (E) SEM and (F) TEM micrograph of CDs with –SO₃⁻ surface functionality after oxidation.

Figure 3.1 (A) and (D) shows the particle size graphs and zeta potential values of the CDs before and after oxidation respectively. The particle sizes of the CDs were found to be below 10 nm thereby confirming the successful synthesis of CDs from MUA. Moreover, the CDs being synthesized from MUA would possess –SH and –COOH as the surface functional groups. It was observed that the zeta potential of the CDs (-34.266 mV) was found to become more negative (-52.233 mV) after oxidation which gave a sure indication of incorporation of oxygenated sulphonate groups into the CD surface thereby confirming the successful oxidation of –SH groups to –SO₃⁻ groups using H₂O₂. Further, in order to ascertain the presence of particles below 10 nm, SEM and TEM images of CDs were recorded both before and after oxidation. Figure 3.1 (B) and (E) shows the SEM and (C) and (F) show the TEM micrographs of the CDs before and after oxidation. Interestingly, both the SEM and TEM micrographs revealed the presence of particles below 10 nm in diameter which is very well in accordance with the DLS result. Thus, it could be inferred that the CDs prepared from MUA were of size below 10 nm as evident from DLS, SEM and TEM analysis.

**Figure 3.2** (A) The EDX Spectrum of CDs prepared from MUA along with the respective weight percentages of different elements present on the CD surface as tabulated in Table 3.1.
Furthermore, the evidence for the fact that the prepared CDs possessed sulfur-containing groups on the surface could be drawn from the EDX spectrum of the CDs. Figure 3.2 (A) shows the EDX Spectrum of CDs prepared from MUA and Table 3.1 shows the respective weight percentages of the different elements present.

(b) Inference from UV-Visible and FT-IR Spectroscopic Analyses

In order to ascertain the presence of surface functionalities on the prepared CDs, UV-Visible and FTIR Spectra of the CDs were recorded both before and after oxidation of CDs.

Figure 3.3 (A) The comparative UV-Visible absorption spectra and (B) the comparative FT-IR spectra of the CDs prepared from MUA before and after oxidation.

Figure 3.3 (A) shows the comparative UV-Visible spectra of the CDs both before and after oxidation. From the UV-Visible spectra, it was evident that the MUA-SH-CDs exhibited an absorption peak at 245 nm owing to the n-π* transitions of the –COOH groups on the CDs prepared from MUA. Upon oxidation of CDs with H₂O₂, this characteristic absorption peak was found to be hypsochromically shifted to 231 nm which could be attributed to the increased electron density (π-π* transitions of –COO⁻ and –SO₃⁻ groups) on the CDs due to oxidation of the –SH groups to -SO₃⁻ groups.
Figure 3.3 (B) shows the comparative FTIR spectra of the CDs both before and after oxidation. The characteristic peaks appearing in the FTIR spectrum of MUA-SH-CDs were found to occur at 3380 (O-H stretch carboxylic acid), 2918, 2849 (C-H stretch alkanes), 2497 (S-H stretch), 1767 (C=O stretch carboxylic acid), 1558, 1433 (C-H bend alkanes) and 865 cm\(^{-1}\) (C-H rock alkanes). On the other hand, the characteristic peaks for MUA-SO\(_3\)Na-CDs were found to appear at 3418 (O-H stretch carboxylic acid), 2917, 2849 (C-H stretch alkanes), 1563, 1427 (C-H bend alkanes), 1179 (S=O stretch sulfonates) and 865 cm\(^{-1}\) (C-H rock alkanes). The most important inference that could be drawn from FTIR spectroscopy was to observe that the peak appearing at 2497 cm\(^{-1}\) (S-H stretch) for MUA-SH-CDs was not present in the FTIR spectrum of MUA-SO\(_3\)Na-CDs. Further, the peak appearing at 1179 cm\(^{-1}\) (S=O stretch sulfonates) for MUA-SO\(_3\)Na-CDs was absent for MUA-SH-CDs. Thus FTIR spectroscopy could provide a clear indication of the successful oxidation of the –SH groups of MUA-SH-CDs to –SO\(_3\)Na groups in MUA-SO\(_3\)Na-CDs.

(c) *Inference from Photoluminescence (PL) Spectroscopic Analysis*

![Figure 3.4](image.png)

**Figure 3.4** The comparative PL emission spectra of CDs before and after oxidation at two different excitation wavelengths viz. at (A) 300 nm and (B) 360 nm.
As the CDs prepared from MUA were of size below 10 nm, they were found to exhibit bright blue fluorescence as observed under a UV-lamp at 365 nm wavelength. Figure 3.4 shows the comparative PL emission spectra of CDs before and after oxidation at two different excitation wavelengths viz. at (A) 300 nm and (B) 360 nm. The PL emission spectra revealed that the prepared CDs exhibit strong excitation-dependent emission property. However, the maximum PL emission intensity was observed at different excitation wavelengths for MUA-SH-CDs and MUA-SO$_3$Na-CDs. As evident from figure 3.4 (A), the emission intensity was found to be higher for MUA-SH-CDs at $\lambda_{\text{exc}} = 300$ nm and that it was higher for MUA-SO$_3$Na-CDs at $\lambda_{\text{exc}} = 360$ nm. PL analysis, therefore, could provide convincing evidence for the fact that the CDs were fluorescent both before and after oxidation and that fluorescence is a characteristic property of CDs.

3.5.2 Characterization and comparative analysis of Ch-SO$_3$Na-CDs and Blank Ch-films

Hybrid hydrogel nanocomposite thin films were fabricated via conjugation of MUA-SO$_3$Na-CDs with the biopolymeric chitosan by way of forming a hydrogel in order to incorporate the CDs into the hydrogel matrix via electrostatic interactions. Nanocomposite thin films of thickness $\sim 0.07$ mm were successfully prepared and subjected to extensive stepwise and systematic characterization in order to ascertain the successful incorporation of CDs.

(a) Inference from Scanning Electron and Fluorescence Microscopy
In order to ratify the fact that MUA-SO$_3$Na-CDs have successfully been embedded into the chitosan hydrogel film matrix, the Ch-SO$_3$Na-CDs nanocomposite film was subjected to Scanning Electron and Fluorescence Microscopy analysis. Figure 3.5 illustrates the photograph of the hybrid Ch-SO$_3$Na-CDs nanocomposite film prepared (A), the representative SEM micrograph of Ch-SO$_3$Na-CDs nanocomposite film (B) clearly showing the presence of Ch-SO$_3$Na-CDs (size below 10 nm) embedded in the biopolymeric nanocomposite film and the uniform distribution of MUA-SO$_3$Na-CDs in the chitosan film as evident from the fluorescence microscopy image of the film (C) which shows the presence of beautiful tiny blue fluorescent CD particles distributed all over the film. Therefore Scanning Electron Microscopy and Fluorescence Microscopy could provide convincing evidence for the successful incorporation of MUA-SO$_3$Na-CDs into the chitosan hydrogel matrix.

(b) Inference from UV-Visible Spectroscopy and Powder XRD Analysis

Evidence in support of the successful incorporation of MUA-SO$_3$Na-CDs into the chitosan hydrogel film could also be drawn from the comparative UV-visible spectroscopy and powder XRD analysis of blank chitosan and Ch-SO$_3$Na-CDs films.
Figure 3.6 (A) shows the comparative UV-Visible spectral analysis of blank chitosan and Ch-SO$_3$Na-CD nanocomposite films.

![Figure 3.6](image)

**Figure 3.6** Comparative (A) solid state UV-visible spectra and (B) powder X-ray diffractogram of blank chitosan and Ch-SO$_3$Na-CDs nanocomposite thin films.

From the comparative plots, it was observed that the UV-visible spectrum of Ch-SO$_3$Na-CDs nanocomposite film exhibited a blue-shift in the absorption maxima from 275 nm to 263 nm of the characteristic peak of the blank chitosan film arising from n–σ* transitions of the –OH groups of chitosan. The hypsochromic shift observed could probably be ascribed to the increase in electron density (due to π–π* transitions of the –COO$^-$ and –SO$_3^-$ groups) in the chitosan hydrogel matrix owing to the successful incorporation of sulphonated CDs. Figure 3.6 (B) shows the comparative powder XRD analysis of blank chitosan and Ch-SO$_3$Na-CD nanocomposite films. Comparative powder XRD analysis of both the films showed that the blank chitosan film exhibits two distinct peaks at 2θ = 10.4 (002) and 20.1 (004) degrees. On the other hand after incorporation of MUA-SO$_3$Na-CDs into the chitosan hydrogel matrix, a number of peaks were found to appear along with those appearing for the blank chitosan film. The respective peaks appearing at 2θ = 10.18 (002), 13.7 (111), 16.6 (–211), 18.5 (300), 19.9 (004) and 25.36 (–313) were found to match the powder
XRD spectrum of \((2,4\text{-dihydroxy-3-(phenylazo)phenylazo})\text{benzenesulphonic acid sodium salt, which crystallizes in the monoclinic system (JCPDS number 00-056-1960). Since the MUA-SO}_3\text{Na-CDs prepared were in the form of the sodium salt of sulphonic acid, hence the powder XRD spectrum could furnish quite convincing evidence for the successful oxidation of the MUA-SH-CDs to MUA-SO}_3\text{Na-CDs and also for their successful incorporation into the chitosan hydrogel matrix to form the nanocomposite films.}

(c) Inference from ATR Spectroscopy and Thermogravimetric Analysis (TGA)

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure3.7.jpg}
\caption{(A) Comparative ATR spectra of Ch-SO}_3\text{Na-CDs films and Blank Ch-films (B) Stacked TGA thermograms of Ch-SO}_3\text{Na-CDs and Blank Ch-films.}
\end{figure}

Incorporation of MUA-SO}_3\text{Na-CDs into the chitosan hydrogel matrix in the form of hybrid nanocomposite thin films could be inferred from Attenuated Total Reflectance (ATR) Spectroscopic and Thermogravimetric Analysis (TGA). The prepared thin films viz. Ch-SO}_3\text{Na-CDs and Blank Ch-films were subjected to ATR Spectroscopic analysis and Thermogravimetric Analysis. Figure 3.7 (A) shows the comparative ATR spectra of Ch-SO}_3\text{Na-CDs and Blank Ch-films. The characteristic peaks in the ATR spectra of Ch-SO}_3\text{Na-CDs nanocomposite film were found to appear at 3355 (O-H}
stretch Hydrogen bonded), 2872 (C–H stretch alkanes), 1587 (N–H bend, amines), 1421 (C–C stretch, (in–ring), 1374 (C–O stretch, carboxylic acid), 1150, 1075, 1025 (C–N stretch, amines) and 891 cm\(^{-1}\) (N–H wag, amines), whereas for blank chitosan films the peaks were found to appear at 3362 (O–H stretch of chitosan), 2873 (C–H stretch alkanes), 1590 (N–H bend, amines), 1420 (C–C stretch, (in–ring), 1371 (C–O stretch, ethers), 1259, 1199, 1077, 1025 (C–N stretch, amines) and 893 cm\(^{-1}\) (N–H wag, amines). From the ATR spectra and the peak assignments it was evident that while all other peaks remaining the same for both the films, it was observed that the peak appearing at 1374 cm\(^{-1}\) for the Ch-SO\(_3\)Na-CDs nanocomposite film, was found to appear at 1371 cm\(^{-1}\) for the blank chitosan film. Furthermore, the peak appearing at 1259 cm\(^{-1}\) for the blank film was found to be absent for Ch-SO\(_3\)Na-CDs nanocomposite film. Also, the peak appearing at 1199 cm\(^{-1}\) for blank Ch-film was found to be shifted to 1150 cm\(^{-1}\) after incorporation of MUA-SO\(_3\)Na-CDs i.e., for Ch-SO\(_3\)Na-CDs nanocomposite films. All these observations could make way to provide evidence for the successful incorporation of MUA-SO\(_3\)Na-CDs into the chitosan hydrogel matrix via electrostatic interaction.

Figure 3.7 (B) shows the stacked TGA thermograms of Ch-SO\(_3\)Na-CDs nanocomposite films and blank chitosan films. From the graphs, it was observed that the blank Ch-film and Ch-SO\(_3\)Na-CDs nanocomposite films did undergo degradation at two stages viz. at 272\(^{\circ}\)C and 532 \(^{\circ}\)C. However, the interesting fact to be noticed was that after incorporation of MUA-SO\(_3\)Na-CDs into the chitosan hydrogel matrix, the Ch-SO\(_3\)Na-CDs nanocomposite films were found to undergo somewhat lesser extent of degradation compared to the blank Ch-films. It was observed that at 272\(^{\circ}\)C
and 532 °C while the blank Ch-film would undergo 30.7% and 31% degradation, the Ch-SO$_3$Na-CDs nanocomposite films underwent only 24% and 28% degradation respectively. Thus, it was very clear that the chitosan films could acquire greater thermal stability upon formation of nanocomposites with MUA-SO$_3$Na-CDs thereby indicating successful incorporation of the CDs into the chitosan hydrogel matrix.

3.5.3 Removal of Ca$^{2+}$ and Mg$^{2+}$ from aqueous solution by the Ch-SO$_3$Na-CDs hybrid nanocomposite film

After convincing evidence being obtained from the extensive characterization of the Ch-SO$_3$Na-CDs nanocomposite films, the potential applicability of the films was subsequently tested in the form of a hybrid platform for the removal of Ca$^{2+}$ and Mg$^{2+}$ ions from water based on the principle of ion-exchange. The strategy adopted for the same was a very simple one involving a dip-stand-measure technique. Ch-SO$_3$Na-CD nanocomposite films of size 2 cm × 2 cm were cut and dipped in $5 \times 10^{-4}$ M solutions (in accordance with WHO standard limits for water hardness) of calcium chloride (CaCl$_2$), magnesium chloride (MgCl$_2$) and also a mixture of both. The concentration of Ca$^{2+}$ ions in solution was determined by an easy, quick and effective technique employing a calcium combination ion selective electrode in an Accumet Excel Dual Channel pH/Ion/Conductivity Meter, XL 50 (Fischer Scientific) and the measurements were recorded in millivolt mode. The millivolt potentials of standard solutions of Ca$^{2+}$ ($10^{-5}$, $10^{-4}$, $10^{-3}$, $10^{-2}$ and $10^{-1}$M) were measured using a Calcium Combination Ion Selective Electrode in an Accumet Excel Dual Channel pH/Ion/Conductivity Meter, XL 50 (Fischer Scientific). The electrode was immersed into the standard solutions and the millivolt readings were recorded once it had attained stabilization. Subsequently, a calibration curve was constructed by plotting
the concentration of Ca\(^{2+}\) on the X-axis and the corresponding millivolt reading on the Y-axis. The data was then fitted logarithmically to obtain the calibration curve as shown in figure 3.8 (A). The potential value of the sample solution (unknown) was then measured in a similar way under identical conditions. The concentration of the unknown sample (the solution of Ca\(^{2+}\) after dipping-in of the Ch-SO\(_3\)Na-CDs nanocomposite film and blank chitosan film) was then determined by using the mV reading and plotting it in the equation obtained from logarithmic fitting of the standard data. Likewise, the concentrations of Ca\(^{2+}\) in the unknown sample were determined at different time intervals starting from 0\(^{th}\) hour to 24\(^{th}\) hour (although the time interval monitored was up to 60\(^{th}\) hour, significant absorption of Ca\(^{2+}\)/Mg\(^{2+}\) took place within 24 hours and therefore the plot of Ca\(^{2+}\)/Mg\(^{2+}\) removal has been shown up to 24 hours) and the concentration values were plotted against the respective time intervals.

Figure 3.8 (A) Standard calibration curve (logarithmically fitted) for the determination of Ca\(^{2+}\) in solution. (B) Plot showing the comparative decrease in the concentration of Ca\(^{2+}\) in solution with time for both Ch-SO\(_3\)Na-CD nanocomposite films and blank Ch-film dipped solutions.

Figure 3.8 (B) depicts the gradual decrease in Ca\(^{2+}\) concentration in solutions with dipped-in Ch-SO\(_3\)Na-CD nanocomposite films and blank Ch-films recorded at various time intervals. This procedure was followed for both samples with dipped-in Ch-
SO$_3$Na-CDs nanocomposite film and blank chitosan film. As evident from the figure, a decrease in Ca$^{2+}$ concentration in solution was found to be faster and higher for solutions with dipped-in Ch-SO$_3$Na-CD nanocomposite films as compared to those with dipped blank Ch-films thereby focusing on the role played by the sulphonated CDs (MUA-SO$_3$Na-CDs) embedded in the nanocomposite film in Ca$^{2+}$ removal.

UV-visible spectroscopic analysis provided additional experimental evidence in support of the absorption of the Ca$^{2+}$ ion from solution by the Ch-SO$_3$Na-CD nanocomposite film. Figure 3.9 shows the stacked UV-visible absorption spectra of the blank chitosan film and Ch-SO$_3$Na-CD nanocomposite film before and after Ca$^{2+}$ absorption. As evident from the spectra, in both the cases the single absorption peak of the native films viz. at 275 nm for the blank Ch-film and at 263 nm for the Ch-SO$_3$Na-CD nanocomposite film was found to be split into three absorption peaks appearing at 306 nm, 258 nm and 203 nm for the blank Ch-film and at 300 nm, 258 nm and 204 nm for the Ch-SO$_3$Na-CD nanocomposite film after being dipped in Ca$^{2+}$ solution (i.e., after Ca$^{2+}$ ion absorption). The plausible explanation for the splitting pattern might be attributed to the interaction of –OH groups of cationic chitosan with the Ca$^{2+}$ ions of the blank chitosan film and to the interaction of the negatively charged carboxylate and sulphonate groups of the Ch-SO$_3$Na-CD nanocomposite film with Ca$^{2+}$ ions.

In order to study the possible effect of other ions such as Mg$^{2+}$ usually co-present with Ca$^{2+}$ in water we carried out the same experiment for pure Mg$^{2+}$ ions in solution and
also for a mixture of both Ca\(^{2+}\) and Mg\(^{2+}\) in solution. 5\times 10^{-4} \text{ M} solutions each of CaCl\(_2\) and MgCl\(_2\) were prepared and mixed together. 2cm x 2cm Ch-SO\(_3\)Na-CDs nanocomposite films were dipped in the mixture for 24 hours and the corresponding concentrations of Ca\(^{2+}\) and Mg\(^{2+}\) were determined using a Calcium Combination Ion Selective Electrode and via Atomic Absorption Spectroscopy (AAS) respectively. The initial concentrations (zeroth hour) of Ca\(^{2+}\) and Mg\(^{2+}\) in solution were equivalent to 20.04 ppm and 12.15 ppm respectively. After dipping in of the Ch-SO\(_3\)Na-CDs films (at the end of the 24\(^{th}\) hour) the concentration of Ca\(^{2+}\) was found to be 6.812 ppm as determined using the Calcium Combination Ion Selective Electrode. Further, the concentration of Mg\(^{2+}\) was found to be 3.50 ppm (24\(^{th}\) hour) as obtained from Atomic Absorption Spectroscopy. Therefore, from this experiment, it was very well evident that the Ch-SO\(_3\)Na-CDs films prepared could effectively remove both Ca\(^{2+}\) and Mg\(^{2+}\) from solution in the pure state and even when they were present together in a mixture.

Water hardness as classified by the U.S. Department of Interior and the Water Quality Association in accordance with WHO report and the respective concentrations of Ca\(^{2+}\) and Mg\(^{2+}\) in solution both before and after dipping of Ch-SO\(_3\)Na-CDs nanocomposite films is tabulated in Table 3.2.

<table>
<thead>
<tr>
<th>Standard Classification of Hardness</th>
<th>mg/L or ppm</th>
<th>Concentration of ions in solution before dipping of the film (ppm)</th>
<th>Concentration of ions in solution after dipping of the film (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soft</td>
<td></td>
<td>Ca(^{2+})</td>
<td>Mg(^{2+})</td>
</tr>
<tr>
<td>Slightly hard</td>
<td>0 – 17.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moderately hard</td>
<td>17.1 – 60</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hard</td>
<td>60 – 120</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Very hard</td>
<td>120 – 180</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>20.035</td>
<td>12.15</td>
</tr>
</tbody>
</table>
Table 3.2 Standard classification of water hardness based on concentration according to WHO report and respective concentrations of Ca$^{2+}$ and Mg$^{2+}$ in solution both before and after dipping of Ch-SO$_3$Na-CDs nanocomposite films.

For consistency, concentrations are generally converted to the equivalent concentration as CaCO$_3$ and expressed in terms of hardness as CaCO$_3$. However, Magnesium in hard water limits to 50 ppm. In our case, we have used $5 \times 10^{-4}$ M solutions of Calcium chloride (CaCl$_2$) and Magnesium Chloride (MgCl$_2$) for our experiments which were equivalent to 20.035 ppm of Ca$^{2+}$ and 12.15 ppm of Mg$^{2+}$. These values fall within the range where the water can be considered as slightly hard as evident from the above table. Therefore, we think that it was quite logical to consider this concentration for the experimental design that we present herein.

3.5.4 Analysis of Real Environmental Sample (Pond Water)

The potential applicability of the Ch-SO$_3$Na-CDs nanocomposite film as an efficient ion-exchange film for removal of calcium and magnesium from real environment water sample had been successfully tested. The water sample was collected from a local area pond and filtered using ordinary filter paper to remove all suspended matter. The presence of calcium and magnesium in the sample was confirmed via measurement using Calcium Combination Ion Selective Electrode and Atomic Absorption Spectroscopy (AAS) respectively. 2cm x 2cm pieces of the Ch-SO$_3$Na-CDs nanocomposite film were cut and dipped in the water sample and allowed to stand for 24 hours. The procedure for detection of calcium by the Calcium ion-selective electrode and magnesium by Atomic Absorption Spectroscopy (AAS) were performed as described earlier. It was found that the pond water sample contained 20.2 ppm of Ca$^{2+}$ and 3.26 ppm of Mg$^{2+}$ before dipping of the nanocomposite film.
After dipping of the Ch-SO$_3$Na-CDs nanocomposite film the respective concentrations were found to be 6.46 ppm (Ca$^{2+}$) and 1.42 ppm (Mg$^{2+}$) in solution. It was observed that the removal of Ca and Mg from the real sample by the nanocomposite film was found to be quite satisfactory.

The extents of removal of Ca$^{2+}$ and Mg$^{2+}$ from solutions (Ca and Mg containing solutions as well as real environmental samples) is depicted as a histogram plot of the percentage removal of the Ca$^{2+}$/Mg$^{2+}$ ion versus the type of film used as shown in figure 3.10 (A) and (B). From the plot (A) it was clear that the Ch-SO$_3$Na-CD nanocomposite film has the potential to remove up to 94% of Ca$^{2+}$ and 70.80% of Mg$^{2+}$ from solution, in contrast, the blank Ch-film could remove only 32.9%. The adsorption capacities of the Ch-SO$_3$Na-CD film were determined to be 0.15 g g$^{-1}$ and 0.218 g g$^{-1}$ for Ca$^{2+}$ and Mg$^{2+}$ respectively. This could very well signify the role played by the sulphonated CDs embedded in the chitosan nanocomposite film in the absorption of Ca$^{2+}$ and Mg$^{2+}$ from solution in comparison with the blank chitosan film. Further, most importantly the material developed, i.e., the Ch-SO$_3$Na-CD nanocomposite film had the potential to remove both Ca$^{2+}$ and Mg$^{2+}$ from solution and when both were present together in a mixture thereby justifying the potential of this material to be applicable for water softening purposes.
Figure 3.10 Histogram plots showing (A) the percentage removal of Ca$^{2+}$/Mg$^{2+}$ from solution by Ch-SO$_3$Na-CD nanocomposite films and blank Ch-films and (B) the percentage removal of Ca$^{2+}$/Mg$^{2+}$ from a pond water sample by the Ch-SO$_3$Na-CD nanocomposite film.

The histogram plot for the calcium and magnesium removal from the real environmental sample by the nanocomposite film is shown in figure 3.10 (B). From the plot, it was seen that the percentage removal of calcium and magnesium from pond water by the Ch-SO$_3$Na-CD nanocomposite film was 68.01% and 56.35% respectively. Therefore it was very well justified that the material developed has appreciable potential for applicability as a water softening material based on the principle of ion-exchange.

3.5.5 Mechanistic Insight

Scheme 3.2 Schematic representation of the proposed mechanism for absorption of Ca$^{2+}$/Mg$^{2+}$ from solution by the Ch-SO$_3$Na-CD nanocomposite film based on the principle of ion exchange.

The probable mechanism underlying the entire process of absorption of Ca$^{2+}$ and Mg$^{2+}$ from solution by the biopolymeric nanocomposite films is believed to follow the principle of ion exchange. The schematic representation of the proposed mechanism for absorption of M$^{2+}$ (M$^{2+}$ = Ca$^{2+}$/Mg$^{2+}$) from solution by the Ch-SO$_3$Na-CD nanocomposite film is shown in Scheme 3.2.
The amine groups of chitosan remain protonated in acidic media and therefore were proposed to interact electrostatically with the negatively charged sulphonate groups on the surface of the CDs. As the sulphonated CDs were dispersed in sodium hydroxide, the CD surface beared sodium sulphonate groups existing as an ion-pair (Na$^+$SO$_3^-$). Thereby Na$^+$ ions remained bound to the surface of the sulphonated CDs which were embedded in the chitosan film as discussed previously. The Ch-SO$_3$Na-CD nanocomposite film on being dipped in aqueous M$^{2+}$ (M$^{2+}$ = Ca$^{2+}$/Mg$^{2+}$) solution enabled the exchange of Na$^+$ ions in the hydrogel nanocomposite film matrix with the M$^{2+}$ ions in solution. In this manner, Na$^+$ ions from the nanocomposite film were released into the solution and in exchange M$^{2+}$ ions are absorbed into the hydrogel film matrix thereby assisting in the removal of M$^{2+}$ ions from solution. The observed decrease in M$^{2+}$ concentration in solution with blank chitosan films dipped in was proposed to be due to the fact that there were some weak interaction between the –OH groups on the chitosan film with the positively charged M$^{2+}$ ions owing to which a percentage of ions got absorbed into the blank chitosan film.

### 3.6 Conclusion

In this work, for the first time, we have successfully demonstrated the synthesis of carbon dots (CDs) bearing thiol (–SH) functional groups using a very simple one-step method from a novel carbon precursor viz. 11-mercaptoundecanoic acid. The oxidation of CDs and their subsequent dispersion in sodium hydroxide led to the incorporation of sodium sulphonate groups on the CD surface. These oxidized CDs were then used to form a hydrogel with the biopolymer chitosan and fabricated into hybrid nanocomposite thin films. The oxidized CD-chitosan
nanocomposite films were then successfully exploited as potential platforms for the separation of Ca\(^{2+}\) and Mg\(^{2+}\) from solution based on the principles of ion exchange. The prepared film was also successfully used to remove Ca\(^{2+}\) and Mg\(^{2+}\) from a real environmental sample (pond water). The sulphonated carbon dot–chitosan hybrid hydrogel nanocomposite film is biocompatible and can be an alternative material to toxic conventional polystyrene crosslinked with divinylbenzene generally used for ion exchange. However, the material developed was of one-time use owing to the disruption of the three-dimensional structure of the hydrogel upon acid treatment thereby limiting its reusability. Therefore, for the first time we could successfully report a very simple, easy, green and effective method for designing a hybrid polymer nanocomposite material employing a carbon nanomaterial (carbon dots) embedded in a biopolymeric (chitosan) hydrogel matrix as a promising platform with anticipated potential for applicability as a water-softening ion-exchange film for the removal of Ca\(^{2+}\) and Mg\(^{2+}\) from water.