of 1–100nm disseminated in a green solution (plant extract). The AgNPs were impregnated in a Resorcinol-formaldehyde resin (RFR) by polycondensation method. The existence of AgNPs alters considerable properties of RFR in expressions of morphology and adsorption capacity for heavy metals retention. The AgNPs loaded RFR (RFR-AgNPs) exerted a significant Hg (II) adsorption and prominent antibacterial efficacy against the water-borne diseases causing gram-negative (*Escherichia coli, Salmonella typhi*) and gram-positive (*Bacillus subtilis* and *Staphylococcus aureus*) bacteria. The polymeric nanocomposites have great potential for utilization in the prevention and treatment of microbial diseases and heavy metals pollution for environmental applications.

*Keywords:* Zero valent silver nanoparticles; Resorcinol-formaldehyde resin; polymer nanocomposites; antibacterial; mercury (II) removal.

### 7.1. Introduction

The development of industries has exasperated the contamination of water bodies such as oceans, lakes, rivers and groundwater ensuing from heavy metal ions is a universal environmental problem. The existence of heavy metals in water bodies has increased apprehension for the eminent harmful effect of such contamination toward human beings, animals and plants. Among the heavy metals, mercury well-known as a type of extremely poisonous and non-biodegradable metal also possibly will cause health hazard while even at very low concentration in water [1]. Thus, the removal of
mercury from effluents has been a major apprehension in mainly industries because of monetary and ecological aspects. Several efficient methods for mercury removal from the aqueous solution are continuously followed such as ion-exchange, membrane filtration, reverse osmosis, chemical precipitation, adsorption and electrochemical treatment [2]. Among these techniques, ion-exchange is a better technique with numerous advantages related with high removal capacity, cheap and superior renewability. Among the materials utilized in this technique, synthetic polymeric ion exchangers are universally chosen as they are effective and economical. As a result they are unable to entrap the heavy metals to meet the standard regulation by latest strict rules mainly in the occurrence of other opposing ions (e.g. Ca$^{2+}$, Mg$^{2+}$, and Na$^+$).

Consequently, it demands the improvement of innovative definite adsorbents for selective removal of heavy metals from waters. Previously we have been exploited the AgNPs (zero valent metal) as specific adsorbents for heavy metals in wastewater and amended environmental quality [3]. Nonetheless, these nanoparticles are generally exhibit as fine or ultrafine particles [4] and cannot be utilized for direct apply in fixed-bed or any flow-through systems owing to the extreme pressure drop and meager mechanical strength. To beat the scientific problems, hybrid sorbents were subsequently built-up by impregnating these nanoparticles within the schematic significant porous materials like activated carbon [5] and porous polymer [6]. Among these, the porous polymer is predominantly selective due to their convenient pore
space, good mechanical strength and also the potential Donnan membrane effect exerted by the immobilized negatively charged sulfonic acid groups bound to the macroporous cation exchanger result in preconcentration and penetration enhancement of heavy metal ions prior to their effective segregation by the impregnated nanoparticles [7]. As we built-up a novel hybrid cation exchange resin by encapsulating AgNPs within a macroporous resorcinol-formaldehyde cation exchanger for the removal of mercury from aqueous solution. In the present work, the AgNPs was synthesized by green method (biological reduction method) using CRGE as per literature [8]. Moreover, AgNPs contain high surface area so they are extremely reactive species at nanometer scale as well as they have been a subject for a lot of antibacterial applications [9]. since sulfur and phosphorous are detected in large quantity all over cell membrane, AgNPs react with sulfur- incorporating proteins within or exterior the cell membrane, which in order affects cell capability [10]. one more assumption suggested that Ag$^+$ ions from AgNPs can interact with phosphorous medieties in DNA ensuing in deactivation of DNA replication and can react with sulfur- comprising proteins to bottle up enzyme roles [11]. As a result, the AgNPs are impregnated into a variety of matrix for instance macro porous polymer matrix, activated carbon, wound dressing materials and textiles [12]. Hence, this paper reports a tailored biological reduction method to synthesize AgNPs in a non-hazardous (plant extract) solution using CRGE and then the AgNPs are immediately loaded on RFR to synthesize RFR-AgNPs through
polycondensation method [13]. As well, we screened the biological characteristics of the RFR-AgNPs, RFR and AgNPs by targeting few harmful water borne diseases inducing bacterial strains *viz.*, *Escherichia coli*, *Salmonella typhi* (Gram-negative), *Bacillus subtilis* and *Staphylococcus aureus* (Gram-positive) and develops the selective Hg (II) adsorption of the RFR in the presence of competing alkali and alkaline earth metals (e.g. Ca$^{2+}$, Mg$^{2+}$, and Na$^+$) by loading the AgNPs.

### 7.2. EXPERIMENTAL

#### 7.2.1. Materials

*Cyperus rotundus* grass (*C. rotundus*) were locally collected and cleaned before use. Resorcinol and formaldehyde used in the current study were Fischer reagents (India). LR grade (purity: 98.3%) of concentrated Sulphuric acid (Sp.gr. = 1.82) was used. AR grade SD fine silver nitrate (AgNO$_3$) was purchased and its 0.1 M solution was prepared in stock and diluted to 1 mM solution. All the microorganisms were procured from Microbial Type Culture Collection, Chandigarh, India. All other chemicals and reagents were of chemically pure grade (AnalaR) procured from SD Fine Chemicals, India. Stock solutions of the RFR-AgNPs, the RFR and the AgNPs dispersed in deionized
water were prepared.

### 7.2.2. Methods

Resorcinol (10g) and Con. H\textsubscript{2}SO\textsubscript{4} (11.5mL) were mixed slowly with constant stirring and kept overnight [14]. 1\% and 0\% (w/w) of AgNPs were added to Resorcinol sulphonic acid and these represent the samples labeled as RFR-AgNPs and RFR. The blend was polymerized with formaldehyde (12.5mL) at 110\degree C and cured at this temperature for 3 h to yield a dark brown chunky mass which was ground, washed, dried and sieved (250– 300\mu m) using Jayant sieves (India) and preserved for characterization [15].

### 7.2.3. Characterization of the samples

The presence of AgNPs in the RFR-AgNPs was confirmed by using a UV–Vis spectrophotometer (UV-1800 SHIMADZU spectrophotometer) at the wavelength of 300-800 nm. FT-IR (SHIMADZU MODEL FT-IR spectrometer) spectra was used to the study the before and after Hg (II) adsorption on resins using the IR-grade KBr pellets in the ratio of 1:200 at the wave number ranging from 400 to 4000 cm\textsuperscript{-1}. The morphology of free and Hg (II) loaded resins were studied by SEM (Vega3 Tescan SEM instrument). For studying the elemental constitute of free and Hg (II) loaded adsorbent, EDX (Bruker machine) was used. The thermal degradation of the free and Hg (II) treated resins were found out by using TGA and DTA analyzer (SII MODEL 6000 thermal analyzer).
7.2.4. Column adsorption studies

Column experiment was performed with a fixed-bed glass column with 2.0 cm internal diameter and 35 cm height and packed with 2 cm (3.5 g) of RFR and RFR-AgNPs were packed within two separate columns. The column bed volume is 6.28 cm$^3$. The flow circulation was improved by an addition of glass wool beads. mercury (II) ions solution (initial concentration= 2 mg/L) and other competing ions were used as influent with high concentration than the target heavy metals and fed through the column at a constant flow rate of 5 mL/min in down-flow mode. The effluent solution was collected at various time intervals and evaluated for Hg (II) content using EDTA titration techniques. Breakthrough curves were calculated by plotting volume of the influent against the proportion of Hg (II) ions concentrations in the effluent throughout the column for the adsorption of Hg (II) from aqueous solution. In this work, desorption study was carried out by using the 10% (w/w) NaCl as the eluting agent.

% of Regeneration = \( \frac{\text{Amount of metal ions desorbed}}{\text{Amount of metal ions adsorbed}} \times 100 \) \hspace{1cm} (1)

7.2.5. In-vitro Antibacterial Studies

In order to explore their biological applications, the RFR-AgNPs, the RFR and the AgNPs were tested for in vitro antibacterial activity against few pathogenic bacterial strains. The in vitro antibacterial activity was carried out against *Escherichia coli*, *Salmonella typhi* (Gram-negative), *Bacillus subtilis* and *Staphylococcus aureus* (Gram-positive) by paper disk method, using nutrient agar as the medium and ciprofloxacin as
the standard (200 μg/disk). Each strain was swabbed uniformly on the plates using sterile cotton swabs. For the measurement of the activity, the samples were coated in punched disks (diameter) of 6 mm, and the activity was determined using a modified agar diffusion assay (disk test). The presence of clear zone around the paper disk on the plate medium was recorded as an indication of inhibition against the bacterial species. After incubation at 37 °C, the different levels of zone of inhibition of bacteria (for 48 h) were determined [16].

7.3. Results and discussion

7.3.1 Spectral characterization

7.3.1.1. UV–Vis spectra

The UV–vis spectrum here Fig.1 indicated surface plasmon resonance (SPR) peak bands centered at 425 nm as well as these suggested the existence of silver particles in the RFR-AgNPs. Former studies showed that the spherical silver nanoparticles reveal absorption bands about 400–440 nm in the UV-Visible spectrophotometer [17] and exhibited the association of silver nanoparticles. Then the schematic structure of the RFR-AgNPs was shown in fig.1b, the sulfonate functional group and AgNPs have potential active sites for the withholding of Hg (II) from aqueous media, which were found out and discussed with additional information’s in the subsequent sections.
Fig. 1 (a) & (b) UV–Visible spectra & schematic structure of the RFR-AgNPs.

7.3.1.2. FT-IR, SEM, SEM-EDX, TGA and DTA analysis
Fig. 2 (a), (b), (c) & (d) IR spectra of the free & Hg$^{2+}$ loaded RFR & RFR–AgNPs. The FT-IR spectra of the free and Hg$^{2+}$ loaded adsorbents were shown in Fig.2a, 2b, 2c, 2d and table 1. These indicated a shift or disappear in wave number of dominant peaks related with the loaded metal and confirmed the metal binding process occurring at the exterior of resin [18]. This information gives confirmation that the functional groups of SO$_3^-$ are involved in binding the Hg (II) ions onto the ion exchange resins (IERs), which distorted the novel power of the sulphonic groups. SEM images of the free and Hg$^{2+}$ loaded adsorbents are shown in fig 3a, 3b, 3c and 3d respectively. As observed from Figures, a small number of solids and a visible change were detected on the surface of the Hg (II) loaded RFR and RFR- AgNPs exhibited that the adsorption of Hg (II) ions has happened on the resin. The SEM-EDAX spectrum of free and Hg (II) loaded adsorbent are shown in fig 4a, 4b, 4c and 4d. The presence of Hg (II) peaks in the
spectrum after adsorption confirms the adsorption of Hg (II) onto RFR and RFR-AgNPs. The TGA (Fig.5a and 5b) & DTA (Fig.6a and 6b) analysis of the free and Hg$^{2+}$ loaded RFR-AgNPs are indicated a shift in temperature dominant peaks correlated with the loaded metal. This shift in the temperature evidences that the metal binding process occurring at the surface of RFR-AgNPs.

Fig.3 (a), (b), (c) & (d) SEM images of free & Hg$^{2+}$ loaded RFR & RFR-AgNPs
Fig. 4 (a), (b), (c) & (d) EDX spectra of free & Hg\textsuperscript{2+} loaded RFR & RFR-AgNPs
Fig. 5 (a) and (b) TGA analysis of free and Hg$^{2+}$ loaded RFR-AgNPs.

Table 1 IR Spectral Studies (ν in cm$^{-1}$)

<table>
<thead>
<tr>
<th>Assignment</th>
<th>RFR</th>
<th>RFR-Hg</th>
<th>RFR-AgNPs</th>
<th>RFR-AgNPs-Hg</th>
</tr>
</thead>
<tbody>
<tr>
<td>S=O str</td>
<td>1030</td>
<td>1022</td>
<td>1026</td>
<td>1020</td>
</tr>
<tr>
<td>SO$_2$ str</td>
<td>1184</td>
<td>1198</td>
<td>1213</td>
<td>1203</td>
</tr>
<tr>
<td>C-S str</td>
<td>664</td>
<td>671</td>
<td>668</td>
<td>659</td>
</tr>
<tr>
<td>Bonded –OH str</td>
<td>3440</td>
<td>3447</td>
<td>3432</td>
<td>3428</td>
</tr>
<tr>
<td>C-C str</td>
<td>1465</td>
<td>1473</td>
<td>1462</td>
<td>1454</td>
</tr>
</tbody>
</table>
7.3.2. **Column studies:**

The breakthrough curve is especially main features for finding out the process and the dynamic reaction of adsorption in a fixed-bed column [19]. The uninterrupted adsorption of Hg$^{2+}$ on RFR and RFR-AgNPs was studied in the presence of competing ions like Na$^+$, Ca$^{2+}$ and Mg$^{2+}$ by a research laboratory scale in a fixed-bed. The breakthrough time and exhaustion time for Hg$^{2+}$ ion was found.

![Fig.6 (a) and (b) DTA analysis of free and Hg$^{2+}$ loaded RFR-AgNPs.](image)
[20] and shown in Fig. 7 and the RFR was also needed for reference. It was indicated that the Hg (II) breaks through rapidly on the RFR due to its scanty selectivity towards mercury and the effective treatment volume is almost 600 bed volumes (BV). On the contrary, appropriate breakthrough results were determined for RFR-AgNPs as about 8000BV in identical conditions due to the immobilized negatively charged sulfonic acid groups bound to polymer matrix would significantly improved the permeation and preconcentration of the Hg (II) from aqueous media to interior plane of the polymer [21] as well as successively

![Graph showing Hg(II) concentration vs BV]
Fig. 7 comparison of breakthrough curves of Hg (II) adsorption by RFR-AgNPs and RFR. 

Fig. 8 A column regeneration capacity of the RFR-AgNPs and RFR.

make favorable conditions for Hg (II) retention by AgNPs. This effect is called as potential donnan membrane effect. Besides, Hg (II) can be selectively withdrawn by AgNPs particles by inner-sphere complexation of Hg (II) with AgNPs [22]. The mercury concentration in the effluent reduced considerably from 2 less than 0.001 mg/L, this
limit was allowed according to WHO and Environmental Bureau of Investigation. As the exhausted RFR and RFR-AgNPs column was regenerated by a 10% (w/w) NaCl solution and the results are shown in Fig.8. It was shown that the 15-20BV regenerates can be efficiently regenerated the preloaded mercury from the resin. Also, we carried out a continuous adsorption–regeneration cycle runs for RFR-AgNPs bed-column to confirm its feasibility for future application. The imbrications of mercury breakthrough curves for the 1st and 3rd cycle confirmed that RFR-AgNPs can be utilized for repetitive use without noticeable capacity loss after regeneration by 10% (w/w) NaCl solution. Note down that the dynamic adsorption capacities of RFR-AgNPs and RFR column towards mercury with opposing ions (Na\(^+\), Ca\(^{2+}\), and Mg\(^{2+}\)) was around 171.68 and 153.104 mg g\(^{-1}\).

7.3.3. Comparison of maximum adsorption capacity of RFR-AgNPs adsorbent with some other adsorbents

The variations of metal intake are caused by the properties of adsorbents like structure, functional groups and surface area. A comparison of the adsorption capacity of RFR-AgNPs adsorbent with the various other adsorbents reported in literature [23-27] is specified in Table 2.

7.3.4. In vitro antibacterial screening

The bacterial inhibiting efficiency of the silver nanoparticles proved to be of great interest for the prevention of adherence and proliferation activities of some bacteria on the materials surface [28, 29]. Further, bacterial invasion was one of the undisputed
functions of their protective role [30]. This showed their presence in constitutive antibacterial agents in response to bacterial attack. Hence, we put forth the in vitro antibacterial measurements of our RFR-AgNPs, RFR and AgNPs against microorganisms which cause water-borne diseases viz., Escherichia coli, Salmonella typhi (Gram-negative), Bacillus subtilis and Staphylococcus aureus (Gram-positive) [31]. Escherichia coli and Salmonella typhi were the most common bacteria among all. The results of qualitative antibacterial paper disk method tests illustrated the significant efficiency of RFR-AgNPs on all the microorganisms

Table 2 Comparison of adsorption capacities of different adsorbents with RFR-AgNPs

<table>
<thead>
<tr>
<th>Adsorbent</th>
<th>Hg (II)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>polyacrylamide-grafted iron(III) oxide</td>
<td>155</td>
<td>[23]</td>
</tr>
<tr>
<td>cross-linked magnetic chitosan-phenylthiourea resin</td>
<td>135</td>
<td>[24]</td>
</tr>
<tr>
<td>Ethylenediamine modified peanut shells</td>
<td>30.78</td>
<td>[25]</td>
</tr>
<tr>
<td>Rice straw</td>
<td>27.7</td>
<td>[26]</td>
</tr>
<tr>
<td>Thiol containing polymer encapsulated magnetic Nanoparticles</td>
<td>16.02</td>
<td>[27]</td>
</tr>
<tr>
<td>RFR-AgNPs</td>
<td><strong>171.68</strong></td>
<td>Present work</td>
</tr>
</tbody>
</table>
compared to others. The average inhibition zones observed for our products listed in Table 3 indicated that after 48 h of incubation, the zones of inhibition for RFR-AgNPs composite versus bacteria were significantly outlined (12 – 15 mm). It can be seen that the lower inhibition zone was obtained in the case of Bacillus subtilis and Staphylococcus aureus (about 9 mm), whereas the inhibition halos measured for Escherichia coli and Salmonella typhi were higher suggesting that for these situations, the silver composite showed superior bactericidal activities than its precessor. This proved that the existence of silver nanoparticles impregnated within the polymeric matrix demonstrated to be effective in inhibiting bacterial growth. Although the exact role of silver nanoparticles in the immediate surrounding of the

Table 3 In vitro antimicrobial investigation of the RFR-AgNPs

<table>
<thead>
<tr>
<th>no</th>
<th>Synthesized Compounds</th>
<th>Antibacterial activity</th>
<th>B. Subtilis</th>
<th>S. Aureus</th>
<th>S. Typhi</th>
<th>E. coli</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AgNPs</td>
<td></td>
<td>+++</td>
<td>+</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>RFR</td>
<td></td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>RFR-AgNPs</td>
<td></td>
<td>+++</td>
<td>+++</td>
<td>++++</td>
<td>++++</td>
</tr>
<tr>
<td></td>
<td>Ciprofloxacin</td>
<td></td>
<td>++++</td>
<td>+++</td>
<td>++++</td>
<td>++++</td>
</tr>
<tr>
<td></td>
<td>Deionized water</td>
<td></td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

inhibition (<3 mm); +, clear zone of 3-6 mm; ++, clear zone of 6-9 mm; ++++, clear zone of 9-12 mm; ++++, clear zone of 12-15 mm)
composite film is not very clear, the literature data explain the huge zones of inhibition produced by other films through the same mechanism of release of silver, which implies a oxidation reaction at the nanoparticle surface and diffusion of the silver ions thus causing structural changes and finally, bacterial death [32]. In agreement with these results, the above hybrid composites manifest a real potential for antimicrobial coatings in a wide variety of bioapplications. Ciprofloxacin acts as the standard while deionized water as a negative control which doesnot involve in any significant inhibition. Here, the bacterial activity of RFR-AgNPs may be due to their interaction with protein, which leads to the inactivation of protein and direct interaction with DNA. This interaction generates the mutation and stops the replication ability of DNA. Further, these nanoproducts can also undergo a cell wall passage easily due to their smaller size, thus inducing the cell lysis. It is also believed that the RFR-AgNPs after penetration into the microbes inactivate their cellular proteins/enzymes by possibly interacting with the DNA leading to bacterial cell death [33]. Besides, the results realized from this investigation show a good harmony with the antibacterial outlook of our RFR-AgNPs.

7.4. Conclusion

In the recent study prepared polymer supported silver nanoparticles (RFR-AgNPs) exhibited a selective adsorbent for Hg^{2+} retention from aqueous medium and waste water. As compared to RFR, RFR-AgNPs showed more favorable Hg^{2+} adsorption from aqueous media in the presence of competing ions due to the potential donnan
membrane effect exerted by loading AgNPs. Fixed-bed column consequences
demonstrated that mercury retention onto a RFR-AgNPs could answer in a obvious
reduce of this toxic metal from 2 less than 0.001mg/L. Besides, the spent RFR-AgNPs
beads are able to readily regenerated by 10% (w/w) NaCl solution for frequent use with
no any considerable capacity loss. Additionally, the incorporation of silver particles into
RFR present their antibacterial properties against water-borne diseases causing S.
aureus, S. typhi, E. coli and B. Subtilis. Therefore, these materials have great potential
for utilization in the fabrication of antibacterial coatings and textiles. These results
indicated that the RFR-AgNPs can be applied in prevention and treatment of diseases
caused by microbes and mercury retention for environmental applications.

7.5. References:


from aqueous solution on low-temperature exfoliated grapheme nano sheets, Langmuir.


