Chapter 3

EXPERIMENTAL
3.1. General

3.1.A. Source of chemicals

All the polymers involved in the present work were synthesized in the laboratory. The 4-vinylpyridine, ethylene glycol dimethacrylate (EGDMA) and azo-bis-isobutyronitrile (AIBN) were purchased from Sigma Aldrich (Germany). N,N’-methylene-bis-acrylamide (NNMBA), acrylamide and potassium persulphate (K$_2$S$_2$O$_8$) were purchased from SRL (Mumbai). Alginic acid was obtained from Merck (India). All the metal salts were of AR grade and purchased from Merck (India). Solvents used were of AR grade and purified by standard methods. 4-Vinylpyridine was distilled under reduced pressure prior to use. Millipore water was used throughout.

3.1.B. Analysis and characterization

The following instruments were used for the analysis and characterization of various polymers.

(i) FT-IR spectrophotometer: Fourier transform infrared (FT-IR) spectra of the metal ion imprinted, non-imprinted, and the ion bound polymers were recorded between 4000-400 cm$^{-1}$, using a Perkin Elmer 400 FT-IR spectrophotometer.

(ii) UV-vis. spectrophotometer: UV-vis. spectral measurements were carried out on a Shimadzu UV-vis. spectrophotometer model 2450.

(iii) EPR spectrophotometer: EPR spectra of Cu(II) ion bound polymers were recorded on Varian E-12 instrument at room temperature.

(iv) X-ray diffraction patterns: XRD patterns were recorded on PAN Analytic XPERT PRO analyser.
(v) **Scanning electron microscope:** Scanning electron micrographs of the imprinted and non-imprinted polymers were taken on JEOL-JSM-840A scanning electron microscope. SEM-EDAX of metal bound polymers were recorded in nitrogen atmosphere using the same instrument.

(vi) **Atomic absorption spectrophotometer:** Atomic absorption measurement of metal ion solution were recorded using Perkin Elmer Atomic Absorption Analyzer 300.

(vii) **Thermogravimetric analyzer:** Thermogravimetric analysis of various polymers were recorded using Shimadzu D 740 TG Analyser.

(viii) **Surface area:** Surface area measurements were carried out by BET method using Micrometrics TriStar 3000 sorptometer.

3.2. **Synthesis of 25 mol % NNMBA-cross-linked metal ion imprinted and non-imprinted interpenetrating polymer networks : General procedure**

The metal ion imprinted polymer networks were synthesized using alginic acid as functional polymer, acrylamide as functional monomer and NNMBA as crosslinker. Potassium persulphate was used as initiator and the polymerisation was carried out at 70°C with constant stirring. The bulk polymer obtained was washed with water to remove unreacted monomer and with dil. HCl to remove metal ions. The polymer after acid treatment was washed several times with water to remove the presence of any acid. The polymer was dried, crushed and sieved. Non-imprinted polymer networks were also prepared without the template metal ion. The composition of the functional monomers, crosslinking agent, template metal ions and the yield obtained are given in Table III. 1.
Table III. 1. Synthesis of metal ion imprinted and non-imprinted interpenetrating polymer networks

<table>
<thead>
<tr>
<th>Metal ions (g)</th>
<th>Acrylamide (g)</th>
<th>Alginic acid (g)</th>
<th>NNNBA (g)</th>
<th>Yield (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>IIP</td>
</tr>
<tr>
<td>Pb(II) (0.796)</td>
<td>10.66</td>
<td>7.5</td>
<td>7.71</td>
<td>15.8</td>
</tr>
<tr>
<td>Cd(II) (0.64)</td>
<td>10.66</td>
<td>7.5</td>
<td>7.71</td>
<td>9.5</td>
</tr>
<tr>
<td>Mn(II) (0.82)</td>
<td>10.66</td>
<td>7.5</td>
<td>7.71</td>
<td>12.0</td>
</tr>
<tr>
<td>Fe(III) (0.97)</td>
<td>10.66</td>
<td>7.5</td>
<td>7.71</td>
<td>13.0</td>
</tr>
<tr>
<td>Co(II) (0.57)</td>
<td>10.66</td>
<td>7.5</td>
<td>7.71</td>
<td>17.7</td>
</tr>
<tr>
<td>Ni(II) (0.571)</td>
<td>10.66</td>
<td>7.5</td>
<td>7.71</td>
<td>16.2</td>
</tr>
<tr>
<td>Cu(II) (0.96)</td>
<td>10.66</td>
<td>7.5</td>
<td>7.71</td>
<td>17.0</td>
</tr>
<tr>
<td>Zn(II) (0.691)</td>
<td>10.66</td>
<td>7.5</td>
<td>7.71</td>
<td>14.6</td>
</tr>
</tbody>
</table>

3.3. Synthesis of copper ion imprinted and non-imprinted polymers

3.3.A. Synthesis of (60-80%) EGDMA-crosslinked Cu(II) ion imprinted and non-imprinted polymers: General procedure

For the synthesis, 4-vinylpyridine was used as the functional monomer, EGDMA as cross-linker and Cu(II) ion as the template. The 4-vinylpyridine and crosslinking agent along with the initiator AIBN were dissolved in 40:10 methanol-water mixture and the mixture was heated on a water bath at 60°C for 6 h. The composition of the monomer, crosslinking agent and the template Cu(II) ion used for the synthesis of 60-80% EGDMA-crosslinked Cu(II) ion imprinted and non-imprinted polymers are given in Table III.2. Non-imprinted polymers were synthesized without the template Cu(II) ion. The resultant bulk
polymer was collected by filtration and washed several times with methanol. The ion imprinted polymer was treated with dil. HCl to remove template Cu(II) ion. After the complete removal of template Cu(II) ion the polymers were washed with distilled water and dried in vacuum.

Table III. 2. Synthesis of 60-80% EGDMA-crosslinked Cu(II) ion imprinted polymers

<table>
<thead>
<tr>
<th>Crosslinking (%)</th>
<th>Functional monomer (4-vinyl pyridine) (mL)</th>
<th>Template Cu(II) ion (mg)</th>
<th>Crosslinker (EGDMA) (mL)</th>
<th>Solvent (methanol-water) (mL)</th>
<th>Initiator AIBN (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>60</td>
<td>4.20</td>
<td>0.95</td>
<td>6.8</td>
<td>50</td>
<td>100</td>
</tr>
<tr>
<td>80</td>
<td>2.06</td>
<td>0.99</td>
<td>10.6</td>
<td>50</td>
<td>100</td>
</tr>
</tbody>
</table>

3.3.B. Synthesis of (60-80%) TTEGDA-crosslinked Cu(II) ion-imprinted and non-imprinted polymers: General procedure

For the synthesis of 60-80% TTEGDA-crosslinked Cu(II) ion-imprinted and non-imprinted polymers, 4-vinylpyridine was used as the functional monomer, TTEGDA as crosslinker and Cu(II) ion as the template. The 4-vinylpyridine and crosslinking agent along with the initiator AIBN were dissolved in 40:10 methanol-water mixture and the polymerization mixture was heated on a water bath at 60° C for 6 h. The composition of the monomer, crosslinking agent and the template metal ion used for the synthesis of 60-80% TTEGDA-crosslinked Cu(II) ion imprinted polymer are given in Table III.3. Non-imprinted polymers were also synthesized without the template Cu(II) ion. The resultant bulk polymer was collected by filtration and washed several times with methanol. The ion imprinted polymer was washed with dil. HCl to remove
template Cu(II) ion. After the complete removal of template metal ion the polymer was washed with distilled water and dried in vacuum.

**Table III.3. Synthesis of 60-80% TTEGDA-crosslinked Cu(II) ion imprinted polymers**

<table>
<thead>
<tr>
<th>Crosslinking (%)</th>
<th>Functional monomer (4-VP) (mL)</th>
<th>Template (Cu(II) ion) (g)</th>
<th>Crosslinker (TTEGDA) (mL)</th>
<th>Solvent (methanol-water) (mL)</th>
<th>Initiator (AIBN) (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>60</td>
<td>4.2</td>
<td>1.99</td>
<td>20.11</td>
<td>50</td>
<td>100</td>
</tr>
<tr>
<td>80</td>
<td>2.06</td>
<td>0.99</td>
<td>26.6</td>
<td>50</td>
<td>100</td>
</tr>
</tbody>
</table>

3.4. Determination of equilibrium water content (EWC)

A fixed amount of the polymer particles were packed into a sintered crucible which was filled with water. After 24 h of equilibration, the excess water was removed from the polymer by applying reduced pressure for 1 min. and the weight of the swollen polymers were taken. The EWC(%) of the polymer was then calculated from the following equation.

$$EWC(\%) = \frac{\text{Weight of wet polymer} - \text{Weight of dry polymer}}{\text{Weight of dry polymer}} \times 100$$

3.5. Rebinding studies of the metal ion imprinted and non-imprinted polymers

After extraction of the template, complementary binding sites are revealed in the imprinted polymer. Due to this molecular memory, the imprinted polymer can rebind the template with high specificity. The molecular recognition abilities of the imprinted polymers were assessed by various analytical methods.
3.6. Sorption capacity

All polymers were evaluated for sorption of the imprinted metal ion in the original imprinting solvent used during the imprinting process. 500 mg each of the imprinted and non-imprinted polymers were subjected to batch equilibration with equal volume (7mL) of metal ion solution of known concentration in a shaker kept at 30°C for 2 h. The concentrations of the template solutions before and after binding were followed by AAS. The difference in binding between the imprinted and non-imprinted polymers is the specificity in rebinding.

3.7. Factors affecting rebinding

The metal ion binding was found to vary with the concentration of template solution, time and pH of the metal ion solution.

(i) Concentration of template solution

The influence of concentration of template solution on binding performance of imprinted polymers was evaluated by batch-wise binding experiments. The batch method was used to study the metal ion sorption from aqueous solution. About 50 mg of IIP / NIP was suspended in 7 ml of aqueous metal ion solution of varying concentration (0.001-0.05M) and (1-5ppm) in the case of ion imprinted interpenetrating polymer networks. The samples were mechanically shaken at room temperature for 2 h. The polymer was filtered and estimated by different analytical methods.

(ii) Time of rebinding

The time required for optimum binding of metal ion was determined by batch equilibration method using metal ion solution. 50 mg of imprinted and non-imprinted polymers were added to 10 mL of metal ion solution. The metal ion bound at regular intervals were determined by AAS.
(iii) pH on metal ion binding

About 50 mg of imprinted and non-imprinted polymers were subjected to rebinding using metal ion solution at different pH. The amount of metal ion bound at different pH was determined by atomic absorption spectrophotometry.

3.8. Sorption studies

Sorption studies were carried out by batch equilibration method. Aqueous solution of metal ions (5ppm, 10mL) was added to imprinted and non-imprinted polymers (100mg) at its natural pH. The solutions were shaken in stoppered bottles. At regular time intervals the concentration of metal ion solution was found out by AAS. Sorption capacity was investigated using Langmuir and Früindlich isotherms and the order of the reaction was found out using Lagergren equation.

3.9. Kinetic studies

The effect of temperature on the imprinting processes of metal ion imprinted and non-imprinted interpenetrating polymer networks were carried out by kinetic studies. The temperature was varied from 30 to 60°C and the concentration of metal ion were found out by AAS. From the results the thermodynamic parameters such as free energy change ($\Delta G^\circ$), enthalpy change ($\Delta H^\circ$) and entropy change ($\Delta S^\circ$) were determined using the van’t Hoff plots.

3.10. Recyclability studies

In order to test the reusability of IIP, it was subjected to several loading (50mg/10mL of metal ion) and elution (4 mL of 3N HCl) operations.

3.11. Selectivity studies

Selectivity studies were carried out by column experiment. 1 g of metal ion imprinted or non-imprinted polymer was slurred with demineralised water (DMW) and then poured into a pyrex glass column (id.40 mm) plucked with
small portion of glass wool at the bottom. The column was pre conditioned by passing DMW and then mixture of metal ion solution (1x $10^{-3}$ M, 10 mL) was passed through the column at a flow rate of ~0.5 ml min$^{-1}$. The eluted solution was collected and the amount of metal ion bound was determined by atomic absorption spectrophotometric method.