CHAPTER IV

Results and Discussion
RESULTS AND DISCUSSION

TAILORING OF HIGHLY SPECIFIC NANOSTRUCTURED SORBENTS FOR DRUGS BASED ON MOLECULAR IMPRINTING TECHNOLOGY

Novel and effective methods for the purification and separation of drugs have always aroused great interest in the scientific community due to the ever-increasing demand to feed the pharmaceutical industry. Researches to develop more potent and cheaper methods to isolate chemical formulations with high purity percentage in an economical way gain huge investments not only due to philanthropic reasons but also because of major financial motives. Among the many methods available [1-3], molecular imprinting technique is a promising one in many aspects [4,5]. Apart from the general theoretical interest in this technique, potential practical applications are obvious as it is used in the diversified fields of drug separation, drug delivery, drug sensing etc. MIPs have had their commercial success as affinity sorbents in solid phase extractions. Such products are presently used for routine analysis by government laboratories, food producers, doping laboratories, and pharmaceutical and clinical analysis laboratories world-wide with unsurpassed performance in terms of sensitivity, selectivity and robustness. The robustness of MIPs together with their low cost is attractive not only for analytical scale separations but also for preparative or process scale separations. This can concern removal of toxic or unwanted by-products from process streams in the food industry, waste water treatment or biopharmaceutical separations. However, commonly used conventional polymers exhibit a bulkiness which can adversely affect the fabrication and accessibility of imprint cavities rendering poor MIP performance [6].
The spanking resurgence of interest in MIPs came along with the development of nanotechnology. The successful imparting of the “privilege” properties, like electrical [7], thermal [8], mechanical [9] etc., held by nanostructures onto the MIPs made the boost possible. Owing to the novel and better properties imparted to the bulk polymers by virtue of the incorporation of nanostructures, polymer-nanocomposites has emerged in itself as a new branch of research. Carbon nanotubes, possessing appreciably high surface-to-volume ratio, have proven quite flourishing in enhancing the properties of ordinary MIPs. Grafting of MWCNTs render increased surface-to-volume ratio for the otherwise agglomerated bulk polymer thereby creating template preferring cavities on or near the surface of the polymer. In effect, this leads to the enhancement of MIP properties as a whole.

The present work involves the tailoring of drug imprinted polymers on MWCNTs, which possess high selectivity and specificity towards the template molecules. Two chiral and two achiral drugs have been selected and imprinted on three different polymers each. The monomers were selected on the basis of their functionality such that one acidic (MAA), one basic (VIZ) and one amide (AAm) functional group bearing monomer was chosen. Various parameters affecting the rebinding capacity of the sorbent including the nature of the monomer has been studied in detail. For comparison, non-imprinted polymers with nanostructures grafted and bulk imprinted and non-imprinted polymers were also synthesised and evaluated. The kinetic and isotherm studies of the adsorption process were carried out. Further, the selectivity, specificity and reusability of the synthesised materials were computed.
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Figure IV.1. General procedure for the synthesis of drug imprinted and non-imprinted polymers on MWCNT

4.1. Synthesis of Cimetidine (CIM) Imprinted and Non-imprinted polymers on MWCNT

Majority of the reported procedures for non-covalent imprinting relies solely on the electrostatic interactions possible between the template and the monomer which concomitantly results in the use of non-polar, aprotic solvents. However, most of the biologically important chemicals are soluble in organic media only. But the usage of highly polar organic solvents may cause unwanted competition for binding sites resulting in poor template-monomer interaction. Only the right choice of a solvent can eliminate these problems. The solubility of the template and other components in the imprinting process also plays a crucial role in the selection of the solvent. In this work, three functional monomers with three different functionalities were deliberately selected to monitor the effects of nature of monomer on the imprinting and rebinding efficiency of the sorbents.
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4.1.1. Synthesis of vinyl functionalised MWCNT (MWCNT-CH=CH₂)

In sonication, the ultrasonic waves produce microscopic bubbles in the liquid; these microscopic bubbles collapse creating shock waves, which are highly effective in increasing the nanotube wetting. Therefore ultrasonication is now a common technique used to create homogeneous dispersions of CNTs. Acid-functionalised MWCNT, obtained from pristine carbon nanotubes after ultrasonication and refluxing with conc.HNO₃, on treatment with thionyl chloride under suitable conditions yielded acyl-functionalised MWCNTs. Surface vinyl functionalised multiwalled carbon nanotubes were obtained in good yield from this MWCNT-COCl by treatment with HEMA in presence of a strong base, pyridine.

4.1.2. Synthesis of imprinted and non-imprinted polymers

Nanostructured sorbents for the selective separation of CIM were synthesized on MWCNT-CH=CH₂ using AAm/MAA/VIZ as functional monomers, EGDMA as crosslinker and AIBN as the initiator (Figure IV.1). The bulk MIPs were synthesized in the same procedure as for the preparation of nano-MIPs only in the absence of MWCNT. Using the same synthetic strategy non-imprinted polymers was also prepared excluding CIM in the preparation. In the present study, functional monomers with different functionalities like AAm, MAA and VIZ were used to study the influence of the nature of monomers on template rebinding. The mechanism of rebinding/recognition of CIM by the imprinted polymers prepared using AAm/MAA/VIZ as functional monomer is believed to be the highly specific non-covalent interactions formed between the functional groups present in the template and the monomers.
4.1.3. Characterization of CIM imprinted and non-imprinted polymers on MWCNT

4.1.3(A) Fourier transform infrared spectroscopy (FT-IR)

The FT-IR spectra were used to characterize the functional changes of all intermediates and products formed during the synthesis of MWCNT-MIP. The spectrum of purified MWCNT showed a strong peak at 1737 cm\(^{-1}\) in correspondence to the C=O stretching vibration [10] and a peak at 2928 cm\(^{-1}\) related to the asymmetric stretching vibration of C-H. New peaks at 3300 and 1581 cm\(^{-1}\) were observed in the spectra of acid processed MWCNTs corresponding to the presence of functional groups –OH and –COOH. The peak at 1352 cm\(^{-1}\) is attributed to the C–O stretching vibrations of the –COOH group. MWCNT-CH=CH\(_2\) showed a strong peak associated with ether linkage around 1166 cm\(^{-1}\) which suggests that HEMA is coupled to MWCNT through the oxygen atom. Further the peak of -C=C present at 1630 cm\(^{-1}\) in vinyl functionalised MWCNT indicate the successful grafting of HEMA onto MWCNTs.

The FT-IR spectrum of conventional and nano-structure incorporated AAm-MIPs/NIPs showed a peak at 3450 cm\(^{-1}\) which can be assigned to the stretching vibration of N–H group. The characteristic C=O stretching vibration bands of amide have been observed at 1680 cm\(^{-1}\). Besides, an N-H bending peak was also observed at 1620 cm\(^{-1}\). There are also peaks at 1280 and 1180–1190 cm\(^{-1}\) which are characteristic of pure polyacrylamide. These peaks belong to the N-H stretching of amide groups in polyacrylamide. The nano and bulk MIPs and NIPs of MAA showed three characteristic IR bands corresponding to –COOH groups of the methacrylic acid units including its C=O stretching vibration, O–C–O absorption and –OH stretching vibration at 1689, 1201, 3440 cm\(^{-1}\).
respectively. All the VIZ-MIPs/NIPs showed the typical absorption bands of VIZ at 1646 cm\(^{-1}\) which have contributions from C=C (bend) and C=N (ring) stretching and at 959 and 730 cm\(^{-1}\) assigned to the C-H (ring) bending out-of-plane. The peak at 1225 cm\(^{-1}\) is due to the ring vibration. The peaks at 1371 and 1105 cm\(^{-1}\) are attributed to C-N stretching modes from the ring.

In addition to the above discussed IR bands, the nano-structure incorporated MIPs/NIPs showed the characteristic peaks of functionalised MWCNT as already discussed. The presence of CIM within the sorbents was also monitored using FT-IR spectroscopic techniques by comparing the spectra of sorbents before and after removal of CIM. The former showed all the characteristic peaks of CIM which confirmed the retention of CIM within the sorbents as these were absent in the latter. The FT-IR spectrum of MIPs before washing showed peaks at 3136 and 3216 cm\(^{-1}\) characteristic of N-H stretch and at 2176 and 1615 cm\(^{-1}\) of C \(\equiv\) N and \(>\)C=N– stretch, respectively of the cyano-guanidine unit present in CIM.
4.1.3(B) Powder X-ray diffraction (PXRD)

Figure IV.2. Powder X-ray diffraction patterns of (a) acrylamide (b) methacrylic acid, and (c) vinylimidazole systems

The X-ray diffraction technique was used to determine the crystallinity of MWCNT-MIPs (Figure IV.2.). The XRD pattern of MWCNT shows two crystalline peaks at $2\theta = 25.8^\circ$ and $2\theta = 43.7^\circ$ corresponding to the graphite (002) and (100) reflections of MWCNT (JCPDS No. 01-0646) [11]. The acid-functionalized carbon nanotubes were found to retain the crystalline nature. The conventional MIPs scatter the X-ray beams to give a
very broad peak ($\theta =10-15^\circ$) characteristic of its amorphous nature, while MWCNT-MIPs show sharp crystalline peaks imparted by the MWCNTs in addition to peaks of bulk MIPs; but with less intensity. This indicates the effective clubbing of the nanostructures into the polymer matrix.

### 4.1.3(C) Thermogravimetric analysis (TGA)

![Thermogravimetric analysis (TGA)](image)

**Figure IV.3.** TGA curves of (a) acrylamide (b) methacrylic acid, and (c) vinylimidazole systems (heating rate of 10°C min$^{-1}$ from room temperature to 800 °C under N$_2$ atmosphere)

To study the differences in thermal behavior of MWCNTs, MWCNT-COOH, MWCNT-MIPs and bulk MIPs, their TG analyses was done (Figure IV.3). Pristine MWCNT was found to be thermally stable up to 800°C without any substantial mass loss. Thermal decomposition pattern of
MWCNT-COOH was obtained with a continuous but not obvious mass loss due to the rupturing of carbonyl groups from the surface of MWCNT. From the residual yield, the percentage of acid formation on the surface of carbon nanotubes was found to be 8%. Thermogram of MWCNT-MIPs showed a similar profile to that of pure MIPs. However, the decomposition temperature and mass loss were lower and higher respectively for MIPs in comparison with MWCNT-MIPs which imply the greater thermal stability induced to the MWCNT-MIPs due to the incorporation of MWCNTs. From the residual mass, percentage of multiwalled carbon nanotubes was found to be about 15, 12 and 14% in MWCNT-AAm-MIP, MWCNT-MAA-MIP and MWCNT-VIZ-MIP respectively.

4.1.3(D) Scanning electron microscopy (SEM)
The morphological differences of intermediates and imprinted sorbents were observed using SEM techniques (Figure IV.4). The SEM micrographs of pristine MWCNT showed nanosized tubular moieties with an average diameter around 6 to 8nm. Functionalized MWCNTs showed a slight increase in diameter with the retainment of morphology. The MIPs generally showed more rough surface morphologies due to the leaching out of print molecules from the polymer matrix. Further, conventional MIPs exhibited an agglomerated morphology which was dramatically changed into tubular forms on the incorporation of MWCNTs into the polymer matrix.
4.1.3(E) High-resolution transmission electron microscopy (HRTEM)

For a more detailed morphological analysis, HR-TEM was taken (Figure IV.5). Pure MWCNTs showed nanotubes with a diameter ranging from 6 to 8nm and an approximate length of some micrometers. On polymerization, the thickness of the nanotubes was found to increase to about 24-27nm although it retained its nanofibrillar morphology which clearly indicates that the polymer was effectively wrapped around the carbon
nanotubes thereby providing the binding cavities on its surface. On the other hand, the bulk MIPs showed some agglomerated bundle-like structures with possibly more cavities in the bulk. This morphology also substantiates the higher homogeneity expressed by the MWCNT-polymer systems as seen later.

4.1.4. Optimisation of conditions of rebinding

4.1.4(A) Concentration study

![Figure IV.6. Adsorption isotherms of imprinted and non-imprinted polymers](image)

Figure IV.6. Adsorption isotherms of imprinted and non-imprinted polymers (Amount of polymer, 10mg; volume, 8.0mL; concentration of CIM from 0.4 to 3.6mmolL$^{-1}$)

The effect of initial template concentration in the range of 0.4 to 3.6mmolL$^{-1}$ on adsorption (investigated under the specified conditions; contact time of 3h; adsorbent dosage of 10mg; and temperature of 28°C) is shown in figure IV.6. To compare the capacity of adsorption, bulk MIPs as well as MIPs formed on the surface of nanomaterials (MWCNTs) were chosen. As can be seen, an obvious difference in $Q_e$ between MWCNT-MIPs and bulk MIPs could be found even at very low initial concentration of CIM. It might be due to the differing quantity of available binding sites. It was observed that the
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adsorption capacity increased for both imprinted sorbents with an increase in initial template concentration and the adsorption capacity of the polymer on MWCNTs was higher than that of the imprinted bulk polymer. The increased affinity of the imprinted polymers towards the template molecule as compared to the non-imprinted polymers revealed that they had specific recognition ability for CIM, which could be ascribed to the formation of complementary cavities. The non-covalent forces, associated with cavity conformations, were the main cause for the affinity and specificity of imprinted polymers to the template. Further, the higher adsorption capacity of MWCNT-MIPs might be attributed to the fact that the incorporation of high surface-to-volume ratio nanostructures into the polymer matrix results in increased solid-liquid surface of contact thereby providing more accessible binding sites and higher valid collisions between MWCNT-MIP and template.

4.1.4(B) Time of rebinding

![Graph: Effect of time on the binding of template](image)

**Figure IV.7.** Effect of time on the binding of template (Amount of polymer, 10mg; volume, 8.0mL; concentration of CIM, 2.4mmolL⁻¹, binding time varies from 0 to 4h)
Figure IV.7 shows the dynamic adsorption of CIM onto the sorbents with respect to time. The saturation time is too small for MWCNT-MIPs than the bulk MIPs because in MWCNT-MIPs most of the rebinding cavities are at the surface or near the surface due to its high surface-to-volume ratio. MWCNT-MIPs showed rapid adsorption initially (less than 30 minutes) and then slowed down on approaching equilibrium. The results show that the rapid adsorption of CIM occurred onto the surface recognition sites of the MWCNT-MIPs. When the imprinted sites on the surface of MWCNT-MIPs were occupied, the adsorption rate decreased and complete saturation lead to an equilibrium. In the case of bulk MIPs, since the polymer matrices are in the agglomerated form, the rebinding cavities are also present in the inner part of the sorbents and thus took more time for rebinding. Also, the irregular trend of rebinding shown by bulk MIPs suggests their heterogeneous distribution of template cavities. In non-imprinted systems, the amount of template bound was very low indicating that there are no specific binding sites. So the binding was fast and random and reached saturation soon after incubation of sorbents on template solution.

4.1.4(C) Adsorbent dosage

![Figure IV.8. Effect of adsorbent dosage on the amount of template bound (Amount of polymer varies from 5 to 20mg; volume, 8.0mL; concentration of CIM, 2.4mmolL\(^{-1}\), binding time, 3h)](image)
The influence of imprinted polymer dosage on CIM sorption was examined by varying dosages from 5 to 20mg. Figure IV.8 presents typical set of results obtained for template sorption by varying adsorbent dosages. From the analysis of experimental data obtained, it was observed that the removal efficiency increased with increase in sorbent dosage owing to the increase in number of binding sites. The increase in effectiveness of adsorption with adsorbent dose was maximum for MWCNT-MIPs which may be due to the increase in number of available binding sites. The NIPs showed a much less marked difference in the amount adsorbed with increasing mass due to the absence of specific binding sites.

4.1.4(D) Effect of solvent

![Figure IV.9](image.png)

**Figure IV.9.** Effect of solvents on the amount of template bound (Amount of polymer, 10mg; volume, 8.0mL; concentration of CIM, 2.4mmolL\(^{-1}\); binding time, 3h)

The effect of solvent on the extent of rebinding was checked in acetonitrile, methanol and water. The results are shown in figure IV.9. The rebinding was found to be maximum in acetonitrile, which was used as the solvent for the MIP preparation due to “solvent memory effect” [12] i.e., the molecularly imprinted sorbents always exhibit maximum rebinding capacity
when the same porogen is used as the preparation and rebinding medium. The use of other solvents will cause the destruction of the three-dimensional structure of the molecular imprinted cavities which are shape-complementary to the target molecule. The solvent polarity is another important factor that affects the rebinding of the MIPs. More polar porogens tend to interfere in the non-covalent interactions between CIM and MIPs thus causing the template to remain in solution rather than rebind to the polymers, which is clear here from the minimum rebinding obtained in water.

4.1.4(E) Effect of monomer on rebinding

Figure IV.10. Effect of nature of monomer on the amount of template bound (Amount of polymer, 10mg; volume, 8.0mL; concentration of CIM, 2.4mmolL$^{-1}$; binding time, 3h)

The effect of nature of monomer on the extent of rebinding was determined by selecting three monomers with different functionalities. The maximum amounts of template bound by these polymers are compared in
Figure IV.10. Our results indicate that when CIM was used as the template, the MWCNT-MIP prepared using acrylamide as the functional monomer showed better recognition of CIM than the MWCNT-MIPs prepared in the same solvent using an acidic (methacrylic acid) or a basic (1-vinylimidazole) functional monomer. The shape, size and nature of the hydrogen-bonding groups of the monomer are all important factors in determining the substrate selectivity. The formation of stronger H-bonds by the amide groups with the template than the carboxyl group of MAA was supported by the large differences in dipole moment and dielectric constant between the carbonyl oxygen of the amide and carboxyl groups. The dielectric constant and dipole moment values for acetic acid are 6.20 and 1.70 D respectively, while for acetamide these two values are 67.6 and 3.76 D respectively [13]. Moreover, the pK_a value (relative to water) for the carbonyl oxygen of the amide is between -6 and -7 whereas that of carboxyl group is between -0.5 and -1.5. This also indicates the stronger tendency of the amide group to form H-bonds than the carboxyl groups. The improved performance of the amide MIP over those MIPs prepared using vinylimidazole may possibly be explained by the fact that VIZ has lesser number of interaction points and even the available ones are mobile and thus less likely to be in the optimum conformations for binding [14]. The higher hydrogen bonding ability of the amide group enhances the strength of the hydrogen-bonding interactions between the template molecule and the amide functional group creating specific recognition sites within the polymer matrix during imprinting and thus yielding imprinted polymers with better recognition properties. Based on the above results, MWCNT-AAm-MIP was chosen as the best system for CIM rebinding under the optimised conditions.
4.1.5. Studies of the optimised system

4.1.5(A) Adsorption isotherms

**Figure IV.11.** Langmuir plot for the adsorption of CIM by MWCNT-AAm-MIP and AAm-MIP (Amount of polymer, 10mg; volume, 8.0 mL; concentration of CIM, 0.4 to 2.4mmolL$^{-1}$; binding time, 3h)

**Figure IV.12.** Freundlich plot for the adsorption of CIM by MWCNT-AAm-MIP and AAm-MIP (Amount of polymer, 10mg; volume, 8.0mL; concentration of CIM, 0.4 to 2.4mmolL$^{-1}$; binding time, 3h)
TABLE IV.1
Adsorption isotherm parameters of MWCNT-AAm-MIP and AAm-MIP

<table>
<thead>
<tr>
<th>Sample</th>
<th>Langmuir parameters</th>
<th>Freundlich parameters</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( Q_m ) (mmolg(^{-1}))</td>
<td>( k_a ) (Lmmol(^{-1}))</td>
<td>( R^2 )</td>
</tr>
<tr>
<td>MWCNT-AAm-MIP</td>
<td>0.419</td>
<td>10.272</td>
<td>0.998</td>
</tr>
<tr>
<td>AAm-MIP</td>
<td>0.369</td>
<td>2.034</td>
<td>0.966</td>
</tr>
</tbody>
</table>

Equilibrium data, commonly referred to as adsorption isotherms, are basic requirements for the designing of adsorption systems. The adsorption isotherms represent the relationship between the amount adsorbed by unit weight of adsorbent and the amount of adsorbate remaining in the solution at equilibrium. The Langmuir isotherm model assumes a monolayer adsorption onto a surface containing a finite number of adsorption sites having uniform strategies of adsorption with no transmigration of adsorbate in the plane of surface. Based on the least square fit, adsorption isotherm data of MWCNT-AAm-MIP was well fit for the Langmuir model than for AAm-MIP (Figure IV.11). As illustrated in the figure, a linear plot with correlation coefficient (\( R^2 \)) value of 0.998 was obtained from Langmuir isotherm equation for MWCNT-AAm-MIP when plotting \( 1/C_e \) against \( 1/Q_e \) with a slope and intercept equal to \( 1/Q_m k_a \) and \( 1/Q_m \), respectively. Consequently, adsorption isotherm data provided that the adsorption process was mainly monolayer on a homogeneous adsorbent surface. The Langmuir constants \( Q_m \) and \( k_a \) were found to be 0.419mmolg\(^{-1}\) and 10.272Lmmol\(^{-1}\), respectively. It is also interesting to note that the CIM adsorption capacity calculated from Langmuir equation
(0.419 mmol g\(^{-1}\)) is closely associated with that experimentally obtained (0.414 mmol g\(^{-1}\)) from the concentration study.

Freundlich adsorption isotherm is an empirical one for nonideal adsorption on heterogeneous surfaces as well as for multilayer adsorption. The equilibrium data were further analyzed using the linearized form of Freundlich isotherm by plotting \(\ln Q_e\) versus \(\ln C_e\), as shown in figure IV.12. The calculated Freundlich isotherm constants and the corresponding correlation coefficients for MWCNT-AAm-MIP and AAm-MIP are shown in table IV.1. In the case of MWCNT-AAm-MIP the theoretical and experimental values of the Freundlich isotherm show a large deviation while those for the Langmuir isotherm do not. So it can be concluded that the Langmuir isotherm model was more suitable for the experimental data of MWCNT-AAm-MIP than Freundlich isotherm because of the high value of the correlation coefficient, suggesting that adsorption in MWCNT-AAm-MIP takes place at specific homogeneous sites and no further adsorption takes place at the site which has already been occupied by a template molecule. The well fit curve of Freundlich model obtained for AAm-MIP evidenced the heterogeneity of the bulk imprinted polymer.
4.1.5(B) Adsorption kinetics

Figure IV.13. Adsorption rate of CIM by MWCNT-AAm-MIP (Amount of polymer, 10mg; volume, 8.0mL; concentration of CIM, 2.4mmolL$^{-1}$; temperature, 301, 311 and 321K)

Figure IV.14. Pseudo-first-order kinetic plot for the adsorption of CIM by MWCNT-AAm-MIP (Amount of polymer, 10mg; volume, 8.0mL; concentration of CIM, 2.4mmolL$^{-1}$; temperature, 301, 311 and 321K)
Figure IV.15. Pseudo-second-order kinetic plot for the adsorption of CIM by MWCNT-AAm-MIP (Amount of polymer, 10mg; volume, 8.0mL; concentration of CIM, 2.4mmolL$^{-1}$; temperature, 301, 311 and 321K)

TABLE IV.2

<table>
<thead>
<tr>
<th>Concentration, (mmolL$^{-1}$)</th>
<th>Temperature, (K)</th>
<th>$Q_{e,exp}$ (mmolg$^{-1}$)</th>
<th>$Q_{e,cal}$ (mmolg$^{-1}$)</th>
<th>$K_1$ (min$^{-1}$)</th>
<th>$R^2$</th>
<th>$Q_{e,cal}$ (mmolg$^{-1}$)</th>
<th>$K_2$ (gmmol min$^{-1}$)</th>
<th>$R^2$</th>
</tr>
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<tr>
<td>2.4</td>
<td>301</td>
<td>0.415</td>
<td>0.185</td>
<td>0.050</td>
<td>0.973</td>
<td>0.421</td>
<td>0.742</td>
<td>0.999</td>
</tr>
<tr>
<td>2.4</td>
<td>311</td>
<td>0.383</td>
<td>0.282</td>
<td>0.060</td>
<td>0.984</td>
<td>0.392</td>
<td>0.689</td>
<td>0.999</td>
</tr>
<tr>
<td>2.4</td>
<td>321</td>
<td>0.343</td>
<td>0.235</td>
<td>0.053</td>
<td>0.984</td>
<td>0.355</td>
<td>0.643</td>
<td>0.999</td>
</tr>
</tbody>
</table>

So as to further expose the rate-controlling steps and sorption mechanism of CIM onto MWCNT-MIP, a kinetic investigation was conducted. Pseudo-first-order and pseudo-second-order equations have been used for testing experimental data. The rate of adsorption of CIM by MWCNT-AAm-MIP was measured as a function of time. The constants for pseudo-first-order equation were determined experimentally by plotting ln($Q_e-Q_0$) versus t and are
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listed in table IV.2. The data does not fall on a straight line and had a low correlation coefficient (Figure IV.14.), indicating the first order kinetic model as less appropriate. The theoretical values ($Q_{e,\text{cal}}$) were far deviated from the experimental ones ($Q_{e,\text{exp}}$) (Table IV.2), again implying that the adsorption process does not follow fully the pseudo-first-order rate expression for adsorption. The plot $t/Q_t$ versus $t$ ought to give a straight line if the second order kinetics is applicable and the values $Q_e$ and $k_2$ can be calculated from the slope and the intercept of the plot $t/Q_t$ vs $t$, respectively. Actually, the plot of $t/Q_t$ against $t$ resulted in a high correlation coefficient of 0.999 (Figure IV.15.) indicating that CIM adsorption by MWCNT-AAm-MIP follows a second order kinetics. The results reveal that the second-order kinetic equation provided a better estimation as compared to the first-order kinetic equation. When the temperature was increased, values of kinetic parameters ($k$ and $Q_e$) were decreased showing that the adsorption process was exothermic (Table IV.2.). When the temperature of the reaction was increased the adsorption was decreased and desorption of the template molecule from the adsorption site was increased at higher temperature [14].

4.1.5(C) Selectivity experiments

To check the selectivity of MWCNT-AAm-MIP and bulk MIP towards the CIM molecule, in addition to the template CIM, two other structurally similar compounds RAN and FAM were selected as interfering substrates (Figure IV.16). The binding capacities of MWCNT-AAm-MIP, AAm-MIP, and non-imprinted polymers of both sorbents are shown in Figure IV.17. MWCNT-AAm-MIP showed the maximum binding capacity for CIM molecule compared with bulk MIP because of the increased number of binding sites on the surface of the nanostructured MIP. The adsorption efficiency of NIPs for CIM was lower than that of both MIPs whereas the
adsorption capacities of all the four sorbents were found to be more or less same for the structural analogues RAN and FAM with very low $Q_e$ values. These results suggested that the molecular imprinting process created a micro-environment depending on the interaction, size, shape, and functionality of the template [15]. There were no proper cavities and recognition sites formed in the NIP, so that it binds compounds only by nonspecific adsorption [16] which led to its decreased adsorption capacity than the MIPs. Also, because of the lack of structure specific cavities there was no significant difference in adsorption efficiency for the interfering compounds. Table IV.3 summarizes the separation factor ($\alpha$) and selectivity factor (k) obtained from the selectivity studies.

![Figure IV.16. Chemical structures of (a) CIM, (b) RAN, and (c) FAM](#)

![Figure IV.17. Evaluation of the selectivity of MWCNT-AAm-MIP compared with AAm-MIP and both NIPs for CIM, RAN, and FAM](#)
TABLE IV.3
Separation and selectivity factors of MWCNT-AAm-MIP and AAm-MIP

<table>
<thead>
<tr>
<th>Drug</th>
<th>Separation factor ((\alpha) = \frac{K_{\text{MWCNT-AAm-MIP}}}{K_{\text{MWCNT-AAm-NIP}}})</th>
<th>Selectivity factor ((k) = \frac{\alpha_{\text{Template}}}{\alpha_{\text{Analogue}}})</th>
<th>Separation factor ((\alpha) = \frac{K_{\text{AAm-MIP}}}{K_{\text{AAm-NIP}}})</th>
<th>Selectivity factor ((k) = \frac{\alpha_{\text{Template}}}{\alpha_{\text{Analogue}}})</th>
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<tr>
<td>CIM</td>
<td>6.825</td>
<td>4.327</td>
<td>4.235</td>
<td>2.871</td>
</tr>
<tr>
<td>RAN</td>
<td>1.577</td>
<td></td>
<td>1.475</td>
<td></td>
</tr>
<tr>
<td>FAM</td>
<td>1.417</td>
<td>4.817</td>
<td>1.246</td>
<td>3.399</td>
</tr>
</tbody>
</table>

4.1.5(D) Regeneration and robustness

![Graph showing regeneration cycles of MWCNT-AAm-MIP and AAm-MIP](image)

**Figure IV.18.** Regeneration cycles of MWCNT-AAm-MIP and AAm-MIP using 2.4mmolL\(^{-1}\) CIM solution in CH\(_3\)CN as adsorbing medium and pure CH\(_3\)CN as desorbing agent (Amount of polymer, 10mg; volume, 8.0mL; binding time, 3h)

The regeneration capability of MWCNT-AAm-MIP/AAm-MIP was investigated in ten sequential cycles of adsorption–desorption. After incorporation of CIM onto the imprinted sorbents, both the MIPs were regenerated using the porogen CH\(_3\)CN. Figure IV.18. shows the adsorption efficiency of both MIPs for CIM in ten consecutive adsorption-regeneration cycles. The experiment
showed that MWCNT-AAm-MIP showed 100% adsorption regeneration upto 5 cycles. After that the regeneration capacity decreased slightly due to the destruction of some recognition sites in the network of MWCNT-MIP during the process of rewashing and thus, was not fit for the template molecule anymore. The bulk MIPs showed irregular trends of regeneration from the second cycle onwards because in addition to the destruction of some recognition sites, the leaching out of the entire template from the bulk during the regeneration process also became more difficult. So MWCNT-AAm-MIP can be considered a better adsorbent for the selective recognition and separation of cimetidine from its structural analogues than the bulk MIP.

4.2. Synthesis of Aspirin (ASP) Imprinted and Non-imprinted polymers on MWCNT

Tailoring of imprinted polymers for the selective recognition of ASP has been carried out. Three different functional monomers- acrylamide (AAm), methacrylic acid (MAA) and 1-vinylimidazole (VIZ) were used to monitor the effect of nature of monomer on the rebinding process. The non-covalent interactions between aspirin and the functional monomers result in highly template-specific cavities in the polymer matrix. Various parameters affecting the process of rebinding were optimised and applied for detailed investigations including the selectivity and reusability studies of the best sorbent system.

4.2.1. Synthesis of imprinted and non-imprinted polymers

MWCNT-MIP for the selective separation of ASP were synthesised on MWCNT-CH=CH\textsubscript{2} using AAm/MAA/VIZ as functional monomers, EGDMA as crosslinker and AIBN as the initiator (Figure IV.1). The bulk MIPs were also prepared following the same procedure but in the absence of MWCNT. The non-imprinted counter parts were also synthesised as above but excluding ASP.
4.2.2. Characterization of ASP imprinted and non-imprinted polymers on MWCNT

4.2.2(A) Fourier transform infrared spectroscopy (FT-IR)

FTIR spectra were used to analyse the functionality changes of all intermediates and products. The FTIR spectra of purified, acid and vinyl functionalized carbon nanotubes and MWCNT-MIPs with and without ASP were taken. In the spectrum of purified MWCNT, a strong absorption at 1737 cm\(^{-1}\) corresponds to the C=O stretching vibration, while the peak at 2928 cm\(^{-1}\) corresponds to the asymmetric stretching vibration of C-H. The spectrum of acid processed MWCNTs showed new peaks at 3300 and 1581 cm\(^{-1}\), which confirmed the presence of –OH and –COOH functional groups. The peak at 1352 cm\(^{-1}\) could be attributed to the C–O stretching vibrations of –COOH. Spectrum of vinyl functionalized MWCNT showed a strong peak associated with ether linkage around 1166 cm\(^{-1}\). The presence of this peak suggests that HEMA is coupled to MWCNT through the oxygen atom of the hydroxyl group.

The FT-IR spectrum of bulk AAm-MIPs/NIPs and MWCNT-AAm-MIPs/NIPs showed the following peaks with assignments given in parenthesis: 3450 cm\(^{-1}\) (stretching vibration of N–H group in AAm), 1680 cm\(^{-1}\) (characteristic C=O stretching vibration bands of amide), 1620 cm\(^{-1}\) (N-H bending), 1280 and 1180-1190 cm\(^{-1}\) (N-H stretching of amide groups in polyacrylamide). The nano and bulk MIPs and NIPs of MAA showed three characteristic IR bands at 1689 (C=O stretching vibration), 1201 (O–C–O absorption) and 3440 cm\(^{-1}\) (–OH stretching vibration) corresponding to –COOH groups of the methacrylic acid units. All the VIZ-MIPs/NIPs showed the typical IR absorption bands of VIZ at 1646 cm\(^{-1}\) (C=C (bending) and C=N (ring) stretching), 1225 cm\(^{-1}\) (ring vibration), 1371 and 1105 cm\(^{-1}\) (C-N stretching modes from the ring) and at 959 and 730 cm\(^{-1}\) (C-H (ring) bending out-of-plane). The MIPs before and after washing demonstrated some notable
changes in their spectra as the appearance of ASP-characteristic peaks in the former which point to the embedding of template ASP within the nanostructured sorbents. The spectrum of before washing MIPs showed broader peaks around $3300-2500\text{cm}^{-1}$ representative of the -OH stretching of the carboxylic acid in aspirin [18]. Also the peaks at 1682 and 1750 cm$^{-1}$ were due to the carboxylic acid carbonyl stretching and the carbonyl stretching of the ester unit of ASP respectively.

### 4.2.2(B) Powder X-ray diffraction (PXRD)

**Figure IV.19.** Powder X-ray diffraction patterns of (a) acrylamide (b) methacrylic acid, and (c) vinylimidazole systems

The X-ray diffractogram of MWCNT showed two crystalline peaks at $2\theta = 25.8^\circ$ and $2\theta = 43.7^\circ$ due to the presence of hexagonal graphitic structure.
(002) and (100) reflections of MWCNT, respectively (Figure IV.19). The crystallinity of MWCNT was not affected by acid functionalisation as is seen from its PXRD pattern. Normal polymers scatter X-ray beams completely thereby giving broad peaks which was observed in the case of bulk MIP samples \((2\theta = 15^\circ)\) that implies its amorphous nature. But the nanostructure-incorporated MIPs showed the two sharp peaks characteristic of MWCNT with less intensity, in addition to the broad peaks of MIP. This showed the effective incorporation of MWCNT into the polymer matrix.

4.2.2(C) Thermogravimetric analysis (TGA)

![TGA curves](image)

Figure IV.20. TGA curves of (a) acrylamide (b) methacrylic acid, and (c) vinylimidazole systems (heating rate of 10°C min\(^{-1}\) from room temperature to 800 °C under \(\text{N}_2\) atmosphere)

Comparison of the thermal behavior of MWCNT, MWCNT-MIPs and bulk MIPs were carried out by thermogravimetric analysis (Figure IV.20).
Thermal degradation pattern of purified MWCNT was linear without any loss in mass up to 800°C. MWCNT-COOH showed a continuous weight loss but not in a huge amount (8%) due to the elimination of carbonyl groups from the surface of MWCNT. MWCNT-MIPs showed a similar degradation pattern to that of pure MIPs. There occurs a small dip in the TGA curves of all imprinted sorbents corresponding to the removal of solvent molecules trapped inside the polymer matrix. The degradation temperature and mass loss were lower and higher respectively for MIPs than the corresponding MWCNT-MIPs which indicate the greater thermal stability induced by the clubbing of MWCNT to the MIP. The difference in residual mass of bulk and nanostructured MIPs showed that 13, 14, 13% of the initial weight of MWCNT-AAm-MIP, MWCNT-MAA-MIP and MWCNT-VIZ-MIP respectively, comprised of multiwalled carbon nanotubes.

4.2.2(D) Scanning electron microscopy (SEM)
Purified MWCNTs showed tubular morphology in the SEM micrographs (Figure IV.21). After acid functionalisation, the morphology was found to be retained with a very slight increase in diameter. Bulk MIPs showed a coagulated structure due to the agglomeration of polymer matrices. However, the incorporation of nanomaterials into the molecularly imprinted polymers led to an entirely different morphology for MWCNT-MIPs. For further clarification on the morphologies of the imprinted sorbents HR-TEM technique was made use of.
4.2.2(E) High-resolution transmission electron microscopy (HRTEM)

Figure IV.22. HRTEM images of (a) MWCNT, (b) MWCNT-AAm-MIP, and (c) AAm-MIP

MWCNTs showed tubular morphology with diameter ranging from 6 to 8nm and a length of several micrometers in TEM micrographs (Figure IV.22). Bulk AAm-MIP was found to have an agglomerated morphology whereas MWCNT-AAm-MIP retained the characteristic nanotubular morphology of carbon nanotubes. The effective wrapping of polymer around the carbon nanotubes in MWCNT-AAm-MIP was further demonstrated by
the increase in thickness to about 60-65nm. This resulted not only an increase in the template accommodation sites on the surface but also a higher homogeneity of the MWCNT-polymer system.

4.2.3. Optimisation of conditions of rebinding

4.2.3(A) Concentration study

Figure IV.23. Adsorption isotherms of imprinted and non-imprinted polymers (Amount of polymer, 10mg; volume, 8.0mL; concentration of ASP from 0.4 to 4.8mmolL\(^{-1}\))

Figure IV.23 shows the effect of initial ASP concentration in the range of 0.4 to 4.8mmolL\(^{-1}\) on adsorption by the imprinted and non-imprinted polymers. It was observed that the adsorption capacity increased for MWCNT-MIPs than MIPs with an increase in initial template concentration. The binding was rapid in the initial stages for all MWCNT-MIPs, which may be due to the presence of more surface binding sites, and attains equilibrium at 4mmolL\(^{-1}\). The variation in the amount of ASP adsorption between MWCNT-MIPs and MIPs was caused by the difference in the number of available binding sites. Also the incorporation of nanostructures into the polymer matrix resulted in increased
polymer-template surface of contact thereby providing higher valid collisions between the binding sites and template molecules. The increased affinity of the imprinted polymers towards the template molecule as compared to the non-imprinted polymers revealed the formation of template-complementary cavities in the imprinted polymers.

4.2.3(B) Time of rebinding

Figure IV.24. Effect of time on the binding of template (Amount of polymer, 10mg; volume, 8.0mL; concentration of ASP, 4mmolL$^{-1}$; binding time varies from 0 to 4h)

Figure IV.24 shows the dynamic curve of adsorption of ASP onto the sorbents with respect to time. MWCNT-MIPs showed rapid adsorption initially (less than 15 minutes) and then slowed down on approaching equilibrium. The results show that the rapid adsorption of ASP occurred onto the surface recognition sites of the MWCNT-MIPs. Once the imprinted sites on the surface of MWCNT-MIPs were occupied, the adsorption rate decreased. The saturation occurs faster in MWCNT-MIPs than the bulk MIPs due to its higher surface-to-volume ratio. The irregular trend of rebinding
shown by bulk MIPs suggests their heterogeneous distribution of template cavities. In non-imprinted systems, the amount of template bound was very low indicating that there are no specific binding sites. So the binding was fast and random and reached saturation soon after incubation of sorbents on template solution.

**4.2.3(C) Adsorbent dosage**

![Figure IV.25](image)

**Figure IV.25.** Effect of adsorbent dosage on the amount of template bound (Amount of polymer varies from 5 to 20mg; volume, 8.0mL; concentration of ASP, 4mmolL$^{-1}$; binding time, 3h)

Figure IV.25 presents the influence of varying adsorbent dosages on template sorption. The removal efficiency was found to increase with increase in sorbent dosage owing to the increase in number of binding sites. Further, MWCNT-MIPs showed much greater removal efficiency due to the presence of increased surface ‘memory cavities’ which were deeply-set in the case of bulk MIPs. Also, due to the absence of these specific binding sites, all the NIPs showed a much less marked difference in the amount of ASP adsorbed.
4.2.3(D) Effect of solvent

![Figure IV.26. Effect of solvent on the amount of template bound (Amount of polymer, 10mg; volume, 8.0mL; concentration of ASP, 4mmolL$^{-1}$; binding time, 3h)](image)

The porogenic effect on the extent of rebinding was determined in chloroform, 1,4-dioxane and water. The results are shown in figure IV.26. The rebinding was found to be maximum in chloroform, which was used as the solvent for MIP preparation. More polar porogens tend to interfere in the H-bonding between ASP and MIPs thus reducing the adsorption capacity which was the case when the more polar water was used as the rebinding medium, in which minimum adsorption was obtained.
4.2.3(E) Effect of monomer on rebinding

**Figure IV.27.** Effect of nature of monomer on the amount of template bound
(Amount of polymer, 10mg; volume, 8.0mL; concentration of ASP, 4mmolL⁻¹; binding time, 3h)

AAm, MAA and VIZ were selected to study the effect of nature of monomer on the extent of rebinding. The maximum amounts of template bound by these polymers are compared in figure IV.27. The results indicate that AAm was capable of forming stronger H-bonding interactions with ASP than MAA and VIZ. This led to increased adsorption capacities by AAm system when ASP was used as the template in the same solvent. The higher hydrogen bonding ability of the amide group enhances the strength of the hydrogen-bonding interactions between the template molecule and the amide functional group and thus yields imprinted polymers with better recognition properties. So MWCNT-AAm-MIP was chosen as the best system for ASP rebinding to carry out further studies.
4.2.4. Studies of the optimised system

4.2.4(A) Adsorption isotherms

Figure IV.28. Langmuir plot for the adsorption of ASP by MWCNT-AAm-MIP and AAm-MIP (Amount of polymer, 10mg; volume, 8.0mL; concentration of ASP, 0.4 to 4mmolL\(^{-1}\); binding time, 3h)

Figure IV.29. Freundlich plot for the adsorption of ASP by MWCNT-AAm-MIP and AAm-MIP (Amount of polymer, 10mg; volume, 8.0mL; concentration of ASP, 0.4 to 4mmolL\(^{-1}\); binding time, 3h)
TABLE IV.4
Adsorption isotherm parameters of MWCNT-AAm-MIP and AAm-MIP

<table>
<thead>
<tr>
<th>Sample</th>
<th>Langmuir parameters</th>
<th>Freundlich parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$Q_m$ (mmol g$^{-1}$)</td>
<td>$k_a$ (L mmol$^{-1}$)</td>
</tr>
<tr>
<td>MWCNT-AAm-MIP</td>
<td>0.710</td>
<td>51.888</td>
</tr>
<tr>
<td>AAm-MIP</td>
<td>0.862</td>
<td>0.392</td>
</tr>
</tbody>
</table>

A linear plot with $R^2$ value 0.999 was obtained from Langmuir adsorption isotherm for MWCNT-AAm-MIP (Figure IV.28), demonstrating that the adsorption process was mainly monolayer on a homogeneous adsorbent surface. The Langmuir parameters $Q_m$ and $k_a$ were found to be 0.710 mmol g$^{-1}$ and 51.888 L mmol$^{-1}$, respectively. Also, the experimental (0.7268 mmol g$^{-1}$) and theoretical (0.710 mmol g$^{-1}$) maximum adsorption capacities towards ASP were found to substantiate the Langmuir model for MWCNT-AAm-MIP. The calculated isotherm constants and the corresponding correlation coefficients for MWCNT-AAm-MIP and AAm-MIP are shown in table IV.4. The equilibrium data were further analyzed using the linearised form of Freundlich isotherm by plotting $\ln Q_e$ versus $\ln C_e$, as shown in figure IV.29. The theoretical and experimental values of the Freundlich isotherm show a large deviation in the case of MWCNT-AAm-MIP, while those for the Langmuir isotherm do not. However, bulk MIP was found to obey Freundlich adsorption isotherm ($R^2 = 0.992$) thus evidencing its heterogeneous binding sites and multilayer adsorption.
4.2.4(B) Adsorption kinetics

Figure IV.30. Adsorption rate of ASP by MWCNT-AAm-MIP (Amount of polymer, 10mg; volume, 8.0mL; concentration of ASP, 4mmolL$^{-1}$; temperature ranging from 301, 311 and 321K)

Figure IV.31. Pseudo-first-order kinetic plot for the adsorption of ASP by MWCNT-AAm-MIP (Amount of polymer, 10mg; volume, 8.0mL; concentration of ASP, 4mmolL$^{-1}$; temperature, 301, 311 and 321K)
Figure IV.32. Pseudo-second-order kinetic plot for the adsorption of ASP by MWCNT-AAm-MIP (Amount of polymer, 10mg; volume, 8.0mL; concentration of ASP, 4mmolL$^{-1}$; temperature, 301, 311 and 321K)

**TABLE IV.5**

Kinetic parameters of pseudo-first-order and pseudo-second-order equations for ASP adsorption onto MWCNT-AAm-MIP at 301, 311 and 321K

<table>
<thead>
<tr>
<th>$C_0$ (mmolL$^{-1}$)</th>
<th>Temperature (K)</th>
<th>$Q_{e,exp}$ (mmolg$^{-1}$)</th>
<th>Pseudo-first-order</th>
<th>Pseudo-second-order</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>$Q_{e,cal}$ (mmolg$^{-1}$)</td>
<td>$k_1$ (min$^{-1}$)</td>
<td>$R^2$</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>301</td>
<td>0.727</td>
<td>0.174</td>
<td>0.038</td>
</tr>
<tr>
<td>4</td>
<td>311</td>
<td>0.688</td>
<td>0.220</td>
<td>0.051</td>
</tr>
<tr>
<td>4</td>
<td>321</td>
<td>0.640</td>
<td>0.191</td>
<td>0.044</td>
</tr>
</tbody>
</table>

The kinetic studies at different temperatures showed that the rapid adsorption of ASP occurred onto the recognition sites of MWCNT-AAm-
MIP initially but slowed down with time (Figure IV.30). The maximum adsorption occurred after 1.75h at a capacity around 0.726mmolg\(^{-1}\). The initial rapid adsorption occurs due to the availability of more binding sites on the surface of the sorbent which on forming non-covalent interactions with the template molecules becomes unavailable for further template binding thereby decreasing the rate of adsorption.

Pseudo-first and pseudo-second-order equations have been used for testing the kinetic data. In the case of pseudo-first-order equation, the constants were experimentally determined by plotting \(\ln(Q_e - Q_t)\) versus \(t\) (Figure IV.31) and are listed in table IV.5. The low correlation coefficient indicated the first order kinetic model as less appropriate. The theoretical values \((Q_{e, \text{cal}})\) exhibited large deviations from experimental data \((Q_{e, \text{exp}})\) (Table IV.5), again implying that the adsorption process does not follow fully the pseudo-first-order adsorption rate expression. The plot \(t/Q_t\) versus \(t\) (Figure IV.32) gave a straight line \((R^2 = 0.999)\) suggesting that second order kinetics is applicable and from the slope and intercept of the plot, \(Q_e\) and \(k_2\) were calculated respectively. Even as the temperature changes, the experimental and theoretical values for the amount of template bound \((Q_e)\) were found to be compatible. These results authenticated that the second-order kinetic equation provided a better estimation as compared to the first-order kinetic equation for MWCNT-AAm-MIP. When the temperature was increased, values of kinetic parameters \((k_2\) and \(Q_e)\) decreased, showing that the process of adsorption was exothermic and the increased temperature resulted in the desorption of the template molecule from the adsorption sites.

4.2.4(C) Selectivity experiments

To check the selectivity of MWCNT-AAm-MIP, AAm-MIP and non-imprinted polymers of both sorbents towards ASP molecule, two structural
analogues salicylic acid (SA) and benzoic acid (BA) (Figure IV.33) were selected as interfering substrates, in addition to ASP. The binding capacities are shown in figure IV.34. The nanostructured MIP had maximum binding capacity for ASP molecule as compared with bulk AAm-MIP owing to the increased number of specific binding sites on the surface of MWCNT-AAm-MIP whereas the adsorption capacity of non-imprinted polymers towards aspirin was lower than that of both imprinted polymers. NIPs bind compounds only by non-specific adsorption due to the lack of formation of complementary three-dimensional spaces in the polymer matrix which led to its decreased adsorption capacity than the MIPs. Also, the adsorption capacities of all four sorbents were found to be almost comparable for the structural analogues SA and BA due to the lack of structure specific cavities for the interfering compounds. These results suggest that depending on the interaction, size, shape and functionality of the template, the molecular imprinting process created a micro-environment in the polymer matrix. Table IV.6 summarizes the values obtained for separation factor ($\alpha$) and selectivity factor ($k$) from the selectivity studies.

![Chemical structures](attachment:Figure_IV.33.png)

*Figure IV.33. Chemical structures of (a) ASP, (b) SA and (c) BA*
Figure IV.34. Evaluation of the selectivity of MWCNT-AAm-MIP compared with AAm-MIP and both NIPs for ASP, SA and BA

TABLE IV.6
Separation and selectivity factors of MWCNT-AAm-MIP and AAm-MIP

<table>
<thead>
<tr>
<th>Drug</th>
<th>Separation factor ( (\alpha) = \frac{K_{MWCNT-AAm-MIP}}{K_{MWCNT-AAm-NIP}} )</th>
<th>Selectivity factor ( (k) = \frac{\alpha_{\text{Template}}}{\alpha_{\text{Analogue}}} )</th>
<th>Separation factor ( (\alpha) = \frac{K_{AAm-MIP}}{K_{AAm-NIP}} )</th>
<th>Selectivity factor ( (k) = \frac{\alpha_{\text{Template}}}{\alpha_{\text{Analogue}}} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>ASP</td>
<td>7.859</td>
<td>7.727</td>
<td>6.179</td>
<td>6.203</td>
</tr>
<tr>
<td>SA</td>
<td>1.017</td>
<td>7.868</td>
<td>0.996</td>
<td>6.306</td>
</tr>
<tr>
<td>BA</td>
<td>0.998</td>
<td></td>
<td>0.979</td>
<td></td>
</tr>
</tbody>
</table>
4.2.4(D) Regeneration and robustness

![Figure IV.35](image.png)

**Figure IV.35.** Regeneration cycles of MWCNT-AAm-MIP and AAm-MIP using 4mmolL⁻¹ ASP solution in CHCl₃ as adsorbing medium and pure CHCl₃ as desorbing agent (Amount of polymer, 10mg; volume, 8.0mL; binding time, 3h)

The reusability of the regenerated molecularly imprinted polymers was studied in seven sequential adsorption-desorption cycles (Figure IV.35). The MIPs were regenerated from all the rebinding studies using chloroform as solvent. The experiment showed that after five adsorption-regeneration cycles the regeneration capacity of MWCNT-AAm-MIP slightly decreased but not perceptibly. This may be attributed to the destruction of some recognition sites in the polymer matrix during the time of desorption of template, and thus, were not fit for the template molecules anymore. The bulk AAm-MIP showed irregular trends of regeneration from the second cycle onwards because of the entrapment of template molecules inside the bulk thereby making it entirely difficult to leach out the template from the bulk during the regeneration process. In addition, the destruction of some
recognition sites owing to the reduced robustness of the non-nano sorbents also led to reduced regeneration. Thus it was shown that the synthesized MWCNT-AAm-MIP is a better adsorbent for the selective separation of aspirin from its structural analogues than the bulk AAm-MIP under the specified conditions used.

4.3. Synthesis of S-ibuprofen (S-IBU) Imprinted and Non-Imprinted polymers on MWCNT

The non-covalent interactions between the template and monomers were found to affect the rebinding process of the polymers. So three different nano-sorbents bearing three different polymers with specific S-IBU binding sites were designed and synthesized. Various other parameters affecting the rebinding were also studied in detail and optimised. The optimised conditions were applied to study the best sorbent system.

4.3.1. Synthesis of imprinted and non-imprinted polymers

S-ibuprofen (S-IBU) specific MWCNT-MIPs were synthesized by the polymerization of AAm, MAA or VIZ as functional monomer, S-IBU as template, EGDMA as crosslinker and AIBN as initiator on the vinyl functionalized surface of MWCNT (Figure IV.1). The mechanism of rebinding/recognition of S-IBU by the imprinted polymers is believed to be the non-covalent interactions between the functional groups present in the template and the monomers. The bulk MIPs were synthesized following the above procedure but without using MWCNTs. The non-imprinted polymers were also prepared as above but in the absence of S-IBU.
4.3.2. Characterization of S-IBU imprinted and non-imprinted polymers on MWCNT

4.3.2(A) Fourier transform infrared spectroscopy (FT-IR)

The FT-IR spectrum of purified MWCNT exhibits a strong band at 1737 cm$^{-1}$ corresponding to the C=O stretching vibrations and a peak at 2928 cm$^{-1}$ due to the asymmetric stretching vibration of C-H. The spectra of acid processed MWCNTs showed new peaks at 3300, 1581 and 1352 cm$^{-1}$ which were due to the functional groups –OH, –COOH and the C–O stretching vibrations of the carboxyl group respectively. The presence of a strong peak around 1166 cm$^{-1}$, associated with ether linkage, observed in the spectrum of vinyl functionalized MWCNT suggests the successful coupling of HEMA to MWCNT through the oxygen atom of hydroxyl group.

The FT-IR spectrum of MWCNT-AAm-MIP/NIP and AAm-MIP/NIP exhibited the peaks: 3450 cm$^{-1}$ (stretching vibration of N–H group), 1680 cm$^{-1}$ (C=O stretching vibration bands of amide), 1620 cm$^{-1}$ (N–H bending peak), 1280 and 1180-1190 cm$^{-1}$ (N–H stretching of amide groups) which are characteristic of pure polyacrylamide. MWCNT-MAA-MIP/NIP and MAA-MIP/NIP showed three characteristic IR bands corresponding to –COOH groups in methacrylic acid including its C=O stretching vibration, O–C–O absorption and –OH stretching vibration at 1689, 1201, 3440 cm$^{-1}$ respectively. All the VIZ-MIPs/NIPs showed the bands at 1646 cm$^{-1}$ which have contributions from C=C (bend) and C=N (ring) stretching and at 959 and 730 cm$^{-1}$ assigned to the C–H (ring) out-of-plane bending. The peak at 1225 cm$^{-1}$ is due to the ring vibration whereas the peaks at 1371 and 1105 cm$^{-1}$ are attributed to C–N stretching modes from the ring. MWCNT-MIP before washing showed all the characteristic peaks of S-IBU which were absent in the spectrum of MWCNT-MIP after washing. The spectrum
showed the characteristic peak at 1704 cm$^{-1}$ representative of the carboxylic acid carbonyl stretching unit in ibuprofen. Further, a peak at 2951 cm$^{-1}$ was observed which correspond to the stretching of –OH group linked with the acidic carbonyl moiety. All this pointed to the successful embedding of S-IBU within the nanostructured sorbents.

4.3.2(B) Powder X-ray diffraction (PXRD)

Figure IV.36. Powder X-ray diffraction patterns of (a) acrylamide (b) methacrylic acid, and (c) vinylimidazole systems
The X-ray diffraction technique was used to determine the crystallinity of MWCNT-MIPs (Figure IV.36). The XRD pattern of MWCNT showed two crystalline peaks at $2\theta = 25.8^\circ$ and $2\theta = 43.7^\circ$ corresponding to the graphite (002) and (100) reflections of MWCNT. The acid-functionalized carbon nanotubes were found to retain the crystalline nature. The conventional MIPs scattered the X-ray beams completely to give a very broad peak ($2\theta = 10-15^\circ$) characteristic of its amorphous nature, while MWCNT-MIPs showed sharp peaks imparted by MWCNTs in addition to the peaks of bulk MIPs; but with less intensity. This indicates the incorporation of nanostructures into the polymer matrix.

4.3.2(C) Thermogravimetric analysis (TGA)

![Figure IV.37.](image)

TGA curves of (a) acrylamide (b) methacrylic acid, and (c) vinylimidazole systems (heating rate of 10°C min$^{-1}$ from room temperature to 800°C under N$_2$ atmosphere)
Thermal behavior of MWCNTs, MWCNT-COOH, MWCNT-MIPs and bulk MIPs were studied using TG analyses (Figure IV.37). High thermal stability of pristine MWCNT was slightly decreased with the introduction of acid groups. A continuous mass loss was observed in the case of MWCNT-COOH due to the rupturing of carbonyl groups from the surface of MWCNT. The 8% weight loss of MWCNT-COOH corresponds to the amount of acid functionalisation which is calculated from the residual weight. MWCNT-MIPs and MIPs showed similar degradation pattern. However, in MWCNT-MIP the degradation started only at a higher temperature range and also the mass loss was not very severe. This supported the suggestion of increased thermal stability as a result of the incorporation of MWCNTs. From the residual mass, percentage of multiwalled carbon nanotubes was found to be about 15, 14, 14% in MWCNT-AAm-MIP, MWCNT-MAA-MIP and MWCNT-VIZ-MIP respectively.

4.3.2(D) Scanning electron microscopy (SEM)
Figure IV.38. Scanning electron micrographs of a) MWCNT, (b) MWCNT-COOH, (c) MWCNT-AAm-MIP, (d) AAm-MIP, (e) MWCNT-MAA-MIP, (f) MAA-MIP, (g) MWCNT-VIZ-MIP, and (h) VIZ-MIP

The morphological studies were carried out using scanning electron microscopy (Figure IV.38). Pristine MWCNT showed nanosized tubular moieties with an average diameter around 6 to 8nm. A slight increase in diameter with retention of tubular morphology was observed in the case of acid-functionalised MWCNTs. MWCNT-MIPs showed nanotubular forms while their bulky counterparts exhibited an agglomerated morphology. All the imprinted sorbents also showed rough surfaces as compared to non-imprinted ones which might be due to the formation of surface imprinted cavities.
4.3.2(E) *High-Resolution Transmission electron microscopy (HRTEM)*

To get a clearer picture of the morphology, HRTEM analysis was done (Figure IV.39). Pristine MWCNTs showed tubular morphology with diameter ranging from 6 to 8nm and a length of several micrometers. MWCNT-AAm-MIP exhibited the characteristic nanotubular morphology with a substantial increase in their thickness (60-65nm) whereas the bulk AAm-MIP showed an agglomerated morphology. This nanotubular morphology of MWCNT-MIP resulted in the high homogeneity expressed by the system.

**Figure IV.39.** HRTEM images of (a) MWCNT, (b) AAm-MWCNT-MIP and (c) AAm-MIP
4.3.3. Optimisation of conditions of rebinding

4.3.3(A) Concentration study

The effect of initial template concentration in the range of 0.4 to 5.2 mmolL$^{-1}$ on adsorption (investigated under the specified conditions; contact time of 3h; adsorbent dosage of 10mg; and temperature of 28°C) is shown in Figure IV.40. Even at very low initial concentrations there is an obvious difference in the amount of template bound by MWCNT-MIPs and bulk MIPs. This might be due to the availability of more surface recognition cavities in the nano-sorbents. Bulk MIPs showed much decreased adsorption capacity due to the presence of inaccessible binding sites in the bulk. The non-imprinted polymers adsorbed S-IBU to similar extents even after incorporation of nanostructures. This clearly indicated the formation of specific S-IBU recognition cavities in the imprinted polymers.

**Figure IV.40.** Adsorption isotherms of imprinted and non-imprinted polymers (Amount of polymer, 10 mg; volume, 8.0mL; concentration of S-IBU from 0.4 to 5.2mmolL$^{-1}$)
4.3.3(B) **Time of rebinding**

![Graph showing the effect of time on the binding of template](image)

Figure IV.41. Effect of time on the binding of template (Amount of polymer, 10mg; volume, 8.0mL; concentration of S-IBU, 4mmolL⁻¹; binding time varies from 0 to 4h)

The effect of time of contact between the template and the sorbent on the amount of template bound was studied (Figure IV.41). The maximum adsorption by MWCNT-MIPs was given within the first fifteen minutes and then slowed down on approaching equilibrium. The rapid adsorption rate is an indication of the availability of surface recognition sites in the nano-MIPs. The irregular trend shown by bulk MIPs substantiates their inherent heterogeneity. As expected, both the NIPs showed much decreased adsorption capacity due to the lacking of template-specific cavities.
4.3.3(C) Adsorbent dosage

Figure IV.42. Effect of adsorbent dosage on the amount of template bound (Amount of polymer varies from 5 to 20mg; volume, 8.0mL; concentration of S-IBU, 4mmolL$^{-1}$; binding time, 3h)

Varying amounts of adsorbent was added into optimum template concentrations to study the effect of imprinted polymer dosage on S-IBU sorption. As the amount of imprinted sorbent increased, the amount of template bound also increased simultaneously owing to the availability of more binding sites (Figure IV.42). However, the adsorption capacity was greater for MWCNT-MIPs than bulk MIPs due to their homogeneous distribution of binding sites. All the NIPs adsorbed almost similar amounts of template inspite of the increase in the amount of adsorbent. This again clarifies the non-specific binding followed by NIPs.
**4.3.3(D) Effect of solvent**

![Graph showing the effect of different solvents on the amount of template bound]

**Figure IV.43.** Effect of solvents on the amount of template bound (Amount of polymer, 10mg; volume, 8.0mL; concentration of S-IBU, 4mmolL⁻¹; binding time, 3h)

Three different solvents with different polarities (chloroform, ethanol and water) were selected to study the effect of porogen on the adsorption process. The use of same porogen as that used in the synthesis step will be favoured by the system due to “solvent memory” effect. Thus maximum adsorption capacity by all sorbents was shown in chloroform (Figure IV.43). Moreover, the use of more polar porogens might interfere with the weak non-covalent interactions between template and monomer, thus reducing their adsorption capacity. This was in accordance with the results obtained, as the minimum adsorption capacity was shown by systems in water.
4.3.3(E) Effect of monomer on rebinding

Figure IV.44. Effect of nature of monomer on the amount of template bound
(Amount of polymer, 10mg; volume, 8.0mL; concentration of S-IBU, 4mmolL$^{-1}$; binding time, 3h)

Among the three polymer systems studied, AAm systems showed enhanced affinity towards S-IBU due to the stronger H-bonding interactions possible in the system (Figure IV.44). MAA and VIZ were also capable of forming interactions with the template but to a lesser extent. Though made up of the same monomers, the nano-counter parts showed increased adsorption capacity than the bulk MIPs which is attributed to the increase in surface area as a result of infusion of nanostructures.
4.3.4. Studies of the optimised system

4.3.4(A) Adsorption isotherms

Figure IV.45. Langmuir plot for the adsorption of S-IBU by MWCNT-AAm-MIP and AAm-MIP (Amount of polymer, 10mg; volume, 8.0mL; concentration of S-IBU, 0.4 to 4mmolL$^{-1}$; binding time, 3h)

Figure IV.46. Freundlich plot for the adsorption of S-IBU by MWCNT-AAm-MIP and AAm-MIP (Amount of polymer, 10mg; volume, 8.0mL; concentration of S-IBU, 0.4 to 4mmolL$^{-1}$; binding time, 3h)
TABLE IV.7

Adsorption isotherm parameters of MWCNT-AAm-MIP and AAm-MIP

<table>
<thead>
<tr>
<th>Sample</th>
<th>Langmuir parameters</th>
<th>Freundlich parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$Q_m$ (mmol/g)</td>
<td>$k_a$ (L/mmol)</td>
</tr>
<tr>
<td>MWCNT-AAm-MIP</td>
<td>0.726</td>
<td>139.821</td>
</tr>
<tr>
<td>AAm-MIP</td>
<td>0.860</td>
<td>0.853</td>
</tr>
</tbody>
</table>

Langmuir and Freundlich adsorption isotherm models were used to study the nature of adsorption onto the sorbent surface (Figure IV.45 and Figure IV.46). A high correlation coefficient of $R^2 = 0.999$ was obtained for the Langmuir model in the case of MWCNT-AAm-MIP. Also, the agreement between the calculated ($Q_m = 0.726$mmol$^{-1}$) and experimental parameters ($Q_m = 0.738$mmol$^{-1}$) led to the conclusion that MWCNT-AAm-MIP was well-fit with Langmuir adsorption isotherm. This means that the nature of adsorption was monolayer on a homogeneous surface with no exchange of molecules across the surface. Freundlich adsorption isotherm model for MWCNT-AAm-MIP gave decreased correlation coefficient and parameters that deviated from the experimental value. On the other hand, bulk MIPs fitted well with Freundlich isotherm model expressing their heterogeneity. The calculated isotherm constants and the corresponding correlation coefficients for MWCNT-AAm-MIP and AAm-MIP are shown in Table IV.7.
4.3.4(B) Adsorption kinetics

Figure IV.47. Adsorption rate of S-IBU by MWCNT-AAm-MIP (Amount of polymer, 10mg; volume, 8.0mL; concentration of S-IBU, 4mmolL$^{-1}$; temperature, 301, 311 and 321K)

Figure IV.48. Pseudo-first-order kinetic plot for the adsorption of S-IBU by MWCNT-AAm-MIP (Amount of polymer, 10mg; volume, 8.0mL; concentration of S-IBU, 4mmolL$^{-1}$; binding time, 3h; temperature, 301, 311 and 321K)
Figure IV.49. Pseudo-second-order kinetic plot for the adsorption of S-IBU by MWCNT-AAm-MIP (Amount of polymer, 10mg; volume, 8.0mL; concentration of S-IBU, 4mmolL⁻¹; binding time, 3h; temperature, 301, 311 and 321K)

TABLE IV.8

Kinetic parameters of pseudo-first-order and pseudo-second-order equations for S-IBU adsorption onto MWCNT-AAm-MIP at 301, 311 and 321K

<table>
<thead>
<tr>
<th>$C_0$ (mmolL⁻¹)</th>
<th>Temperature (K)</th>
<th>$Q_{e,exp}$ (mmolg⁻¹)</th>
<th>Pseudo-first-order</th>
<th>Pseudo-second-order</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>$Q_{e,cal}$ (mmolg⁻¹)</td>
<td>$k_1$ (min⁻¹)</td>
</tr>
<tr>
<td>4</td>
<td>301</td>
<td>0.738</td>
<td>0.188</td>
<td>0.029</td>
</tr>
<tr>
<td>4</td>
<td>311</td>
<td>0.712</td>
<td>0.243</td>
<td>0.039</td>
</tr>
<tr>
<td>4</td>
<td>321</td>
<td>0.692</td>
<td>0.282</td>
<td>0.047</td>
</tr>
</tbody>
</table>

The kinetics of adsorption by MWCNT-AAm-MIP was obtained from the time studies done at different temperatures (Figure. IV.47). The amount of template adsorbed by the polymer was found to be decreasing with increase in temperature. This suggested that the adsorption of S-IBU by MWCNT-AAm-
MIP might be an exothermic process. The kinetic data of the sorbent was fitted with both pseudo-first-order and pseudo-second-order equations. The high correlation coefficient \( R^2 = 0.999 \) at all temperatures (Figure IV.49) and matching experimental and theoretical values for amount of template bound indicated that the kinetics of S-IBU adsorption by the sorbent was pseudo-second-order. Conversely, pseudo-first-order plot gave a very low correlation coefficient and did not fall on a straight line (Figure IV.48) signifying the less appropriateness of the model. The constants determined from the two models are listed in Table IV. 8.

4.3.4(C) Selectivity experiments

The non-covalent interactions formed in the pre-polymerisation complex are the driving force for molecular recognition in imprinted polymers. When the functional groups of monomers are arranged around the template and fixed through polymerisation and crosslinking, the functional groups are gaining memory of not only the size and shape of the template but also its optimal spatial fit. This property of MIPs makes them useful in enantiomeric separation. To check the enantioselectivity of S-IBU imprinted polymer, its enantiomer R-IBU was taken as the interfering substance (Figure IV.50). MWCNT-AAm-MIP, AAm-MIP and their corresponding NIPs were checked for their selectivity towards the imprint molecule. It was observed that both MIPs selectively and specifically bound the template molecule though MWCNT-AAm-MIP showed greater adsorption capacity (Figure IV.51). This indicates the formation of template-specific binding sites in both the MIPs and more number of binding sites in MWCNT-AAm-MIP. The increased surface area of contact between the polymer and template during imprinting resulted in the increase in number of binding sites in MWCNT-AAm-MIP which again corroborates the homogeneity of the system. The separation factor \((\alpha)\) and selectivity factor \((k)\) of MWCNT-
AAm-MIP and AAm-MIP are summarised in Table IV.9. The enantioselectivity of MWCNT-AAm-MIP and AAm-MIP was further confirmed by polarimetric studies. The specific rotation for the optimum concentrated racemic mixture solution of ibuprofen (4mmolL⁻¹) was found to be zero as it contained both enantiomers in equal proportions. 10mg of the imprinted sorbents was added into the racemic mixture followed by equilibration under the optimised conditions and centrifuged. The specific rotation of the supernatant solution was checked in the solvent chloroform at \( \lambda = 589\text{nm} \), \( T = 298\text{K} \) and \( l = 1\text{dm} \). The solution showed specific rotation values \(-55^0 \pm 1\) characteristic of the non-imprinted counterpart in the enantiomeric pair, R-IBU, thus revealing the selective binding of the sorbents towards S-IBU.

![Figure IV.50. Chemical structures of (a) S-(+)-IBU, and (b) R-(-)-IBU](image)

![Figure IV.51. Evaluation of the selectivity of MWCNT-AAm-MIP compared with AAm-MIP and both NIPs for S-(+)-IBU and R-(+)-IBU](image)
TABLE IV.9

Separation and selectivity factors of MWCNT-AAm-MIP and AAm-MIP

<table>
<thead>
<tr>
<th>Drug</th>
<th>Separation factor ((\alpha) = \frac{K_{\text{MWCNT-AAm-MIP}}}{K_{\text{MWCNT-AAm-NIP}}})</th>
<th>Selectivity factor ((k) = \frac{\alpha_{\text{Template}}}{\alpha_{\text{Analogue}}})</th>
<th>Separation factor ((\alpha) = \frac{K_{\text{AAm-MIP}}}{K_{\text{AAm-NIP}}})</th>
<th>Selectivity factor ((k) = \frac{\alpha_{\text{Template}}}{\alpha_{\text{Analogue}}})</th>
</tr>
</thead>
<tbody>
<tr>
<td>R-IBU</td>
<td>1.000</td>
<td>0.979</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

4.3.4(D) Regeneration and robustness

![Figure IV.52](image.jpg)

Figure IV.52. Regeneration cycles of MWCNT-AAm-MIP and AAm-MIP using 4mmolL\(^{-1}\) S-IBU solution in CHCl\(_3\) as adsorbing medium and pure CHCl\(_3\) as desorbing agent (Amount of polymer, 10mg; volume, 8.0mL; binding time, 3h)

The reusability and robustness of MWCNT-AAm-MIP and AAm-MIP was checked by eight sequential cycles of adsorption-desorption experiments (Figure IV.52). After the MIPs attained adsorption saturation they were desorbed using chloroform, dried and used again for adsorption.
MWCNT-AAm-MIP exhibited excellent regenerative capacity up to four cycles of adsorption-desorption. The mechanical stability induced by the nanostructure helps to maintain the rigidity of the cavities thereby making them fit for template molecule. AAm-MIP showed irregular trend in adsorption behaviour even after first cycle due to the destruction of cavities and presence of inaccessible binding sites making them less useful as reusable systems.

4.4. Synthesis of Levodopa (L-DOPA) Imprinted and Non-imprinted polymers on MWCNT

An attempt is made to tailor sorbents capable of separating the enantiomeric drug (L-DOPA) making use of molecular imprinting technology. For this, three monomers which can interact to different extents with the template L-DOPA were selected. In order to further investigate the effect of various other parameters on the rebinding process, they were studied in detail and optimized.

4.4.1. Synthesis of imprinted and non-imprinted polymers

Following the general procedure shown in Figure IV.1, nanostructured sorbents were synthesized on vinyl functionalized MWCNTs using AAm/MAA/VIZ as functional monomers, EGDMA as crosslinker and AIBN as initiator. For comparison, the conventional imprinted polymers were also synthesized making use of the same procedure but without using MWCNTs. The non-imprinted polymers were synthesised following the same methodology but in the absence of L-DOPA.
4.4.2. Characterization of L-DOPA imprinted and non-imprinted polymers on MWCNT

4.4.2(A) Fourier transform infrared spectroscopy (FT-IR)

FTIR spectra was used to monitor the changes of MWCNTs, MWCNTs-COOH, MWCNT-CH=CH$_2$ and MWCNT-MIPs with and without L-DOPA. The spectrum of purified MWCNT showed peaks at 1737 and 2928 cm$^{-1}$ characteristic of C=O stretching vibration and the asymmetric C-H stretching vibration respectively. Acid processed MWCNTs displayed the characteristic bands of –OH and –CO groups of carboxylic acid at 3300 and 1581 cm$^{-1}$ respectively. The peak at 1352 cm$^{-1}$ could be attributed to the C–O stretching vibrations of the –COOH. A strong peak associated with ether linkage around 1166 cm$^{-1}$ was observed in the spectrum of MWCNT-CH=CH$_2$, which suggested the oxygen atom linkage of HEMA to MWCNT.

The FT-IR spectrum of bulk AAm-MIPs/NIPs and MWCNT-AAm-MIPs/NIPs showed the following peaks with assignments given in parenthesis: 3450 cm$^{-1}$ (stretching vibration of N–H group in AAm), 1680 cm$^{-1}$ (characteristic C=O stretching vibration bands of amide), 1620 cm$^{-1}$ (N-H bending), 1280 and 1180–1190 cm$^{-1}$ (N-H stretching of amide groups in polyacrylamide). The nano and bulk MIPs and NIPs of MAA showed three characteristic IR bands at 1689 (C=O stretching vibration), 1201 (O–C–O absorption) and 3440 cm$^{-1}$ (–OH stretching vibration) corresponding to –COOH groups of the methacrylic acid units. All the VIZ-MIPs/NIPs showed the typical IR absorption bands of VIZ at 1646 cm$^{-1}$ (C=C (bend) and C=N (ring) stretching), 1225 cm$^{-1}$ (ring vibration), 1371 and 1105 cm$^{-1}$ (C-N stretching modes from the ring) and at 959 and 730 cm$^{-1}$ (C-H (ring) bending out-of-plane). Also MIPs with and without the template L-DOPA showed a significant change in their spectra. The FT-IR spectrum of L-DOPA
imprinted polymer before extraction of the template showed a characteristic broad peak around 3436 cm\(^{-1}\) for the –OH stretch in L-DOPA. This confirmed the embedding of L-DOPA within the polymer.

**4.4.2(B) Powder X-ray diffraction (PXRD)**

![X-ray diffraction patterns](image)

**Figure IV.53.** Powder X-ray diffraction patterns of (a) acrylamide (b) methacrylic acid, and (c) vinylimidazole systems

X-ray diffraction patterns of pure and acid functionalized MWCNT, MWCNT-MIPs and bulk MIPs are given in figure IV.53. The XRD pattern of purified MWCNT shows two characteristic crystalline peaks of MWCNT at 20 values 25.8° and 43.7° corresponding to the graphite (002) and (100) reflections of MWCNT. In the case of MWCNT-COOH the XRD patterns
are found to be the same as that of pure MWCNT which indicates the unaltered crystallinity of MWCNT even after acid functionalisation. The conventional bulk MIPs scatter the X-ray beams to give a very broad peak ($2\theta = 15^\circ$) due to its amorphous nature, while the incorporation of MWCNT on the polymer matrices for the synthesis of MWCNT-MIPs imparted sharp crystalline peaks corresponding to the characteristics of MWCNTs in addition to the peaks of bulk MIPs; but with less intensity, revealing that the resultant MIP were effectively clubbed with MWCNT.

4.4.2(C) Thermogravimetric analysis (TGA)

![TGA curves](image)

**Figure IV.54.** TGA curves of (a) acrylamide (b) methacrylic acid, and (c) vinylimidazole systems (heating rate of 10°C min$^{-1}$ from room temperature to 800 °C under N$_2$ atmosphere)
Figure IV.54 shows the TG curves of purified MWCNT, MWCNT-COOH and molecular imprinted polymers with and without MWCNT. Purified MWCNT was found to be thermally stable up to 800°C without any mass loss. Thermal decomposition pattern of carboxyl functionalized MWCNT was obtained with a continuous but not obvious mass loss due to the thermal degradation of organic molecules (carboxyl groups) attached to the surface of MWCNT. The TG curve of MWCNT-MIPs has a similar degradation pattern as that of the pure MIPs. However, the initial decomposition temperature increased after incorporating MWCNT onto MIPs, probably due to the covalent attachment of thermally stable nano material with the MIP. From the non-decomposed product, amount of MWCNT in nanostructured MIPs was found to be about 13, 12, 13% for MWCNT-AAm-MIP, MWCNT-MAA-MIP and MWCNT-VIZ-MIP respectively.

4.4.2(D) Scanning electron microscopy (SEM)
Figure IV.55. Scanning electron micrographs of a) MWCNT, (b) MWCNT-COOH, (c) MWCNT-AAm-MIP, (d) AAm-MIP, (e) MWCNT-MAA-MIP, (f) MAA-MIP (g) MWCNT-VIZ-MIP and (h) VIZ-MIP

The SEM micrographs gave an idea about the morphological differences of MWCNT after functionalisation and polymer attachment and are illustrated in figure. IV.55. The MWCNT showed its characteristic nanosized tubular structure. The tubular morphology was found to be retained by the acid functionalised MWCNT with a slight increase in diameter. After the coupling of polymer and carbon nanotubes, the tubular morphology still remained unaltered though the diameter increased manifold whereas the conventional bulk MIPs exhibited agglomerated shape. Both nano structured and bulk MIPs have rough surface morphology than NIPs due to the cavity formed during the time of extraction of the template molecule from the surface of the polymer matrix. The morphologies were further studied by TEM analysis.
4.4.2(E) **High-resolution transmission electron microscopy (HRTEM)**

![HRTEM images of (a) MWCNT, (b) MWCNT-AAm-MIP and (c) AAm-MIP](image)

**Figure IV.56.** HRTEM images of (a) MWCNT, (b) MWCNT-AAm-MIP and (c) AAm-MIP

The HRTEM micrographs of MWCNT, AAm-MIP-immobilized CNT and bulk AAm-MIP are given in figure IV.56. Pure MWCNTs showed tubular morphology with a diameter ranging from 6 to 8nm and an approximate length of some micrometers. After the immobilization of polymers on the surface of MWCNT the thickness was found to increase to about 70-75nm retaining its nanotubular morphology, which clearly indicated that a layer of MIP effectively covered the surface of MWCNT
without changing the innate morphology of CNT. At the same time, the conventional MIP showed bulky structure due to the agglomeration of polymer matrices.

4.4.3. Optimisation of conditions of rebinding

4.4.3(A) Concentration study

Figure IV.57. Adsorption isotherms of imprinted and non-imprinted polymers (Amount of polymer, 10mg; volume, 8.0mL; concentration of L-DOPA from 0.4 to 5.2mmolL\(^{-1}\))

The effect of initial template concentration ranging from 0.4 to 5.2mmolL\(^{-1}\) on the adsorption capacity of both conventional MIPs and MWCNT-MIPs under specified conditions, (time of incubation: 4h, mass of sorbent: 10mg and temperature: 28°C) are shown in figure IV.57. It is observed that even at lower initial concentrations of the template solution, MWCNT-MIPs and MIPs exhibit significant differences in the amount of template bound (\(Q_e\)) which continues to increase with the increase in initial template concentration. This clearly shows the increased number of ‘surface memory cavities’ on MWCNT-MIPs which became evident in the presence
of template molecules. The high binding capacity shown by all the MIPs towards L-DOPA as compared with the NIPs emphasizes the formation of complementary cavities which also account for the increased recognition capacity for the template. The non-covalent interactions, coupled with cavity conformations were the main reason for the affinity, specificity and selectivity of MIPs to L-DOPA.

4.4.3(B) **Time of rebinding**

![Figure IV.58](image-url)

**Figure IV.58.** Effect of time on the binding of template (Amount of polymer 10mg; volume, 8.0 mL; concentration of L-DOPA, 4.4mmolL\(^{-1}\); binding time varies from 0 to 4h)

The minimum time required for the maximum amount of adsorption to take place was determined and optimized (Figure IV.58). The maximum adsorption for MWCNT-MIPs took place at around 1.25h after which no significant adsorption took place, though the major portion of adsorption was complete even under the first fifteen minutes. The presence of binding sites on the surface of polymer caused the initial rapid adsorption. Heterogeneous binding nature of bulk MIPs expresses themselves as irregular binding with
increase in time. Non-specific adsorption caused the adsorption in NIPs to be random and fast with very low $Q_e$ values.

### 4.4.3(C) Adsorbent dosage

The mass studies were carried out by varying the adsorbent mass (5 to 20mg) to understand the effect of sorbent dosage on the amount of template bound (Figure IV.59). From the experimental data it was found that the binding efficiency of MWCNT-MIPs/MIPs towards L-DOPA increased with increase in sorbent mass due to the increase in availability of rebinding cavities. The result showed that in all systems, the template binding capacity was always maximal for MWCNT-MIPs than the bulk MIPs which may be attributed to the increase in surface-to-volume ratio. The NIPs had minimal template binding capacity and showed almost same amount of L-DOPA adsorbed even with increasing mass because of the absence of specific rebinding cavities.

**Figure IV.59.** Effect of adsorbent dosage on the amount of template bound (Amount of polymer varies from 5 to 20mg; volume, 8.0mL; concentration of L-DOPA, 4.4mmolL$^{-1}$; binding time, 3h)
4.4.3(D) Effect of solvent

The porogen used for the synthesis of imprinted polymers will be preferred as binding medium and will give much better results in terms of adsorption. Here all the imprinted polymers showed maximum adsorption capacity in N, N-dimethylformamide which was used as the synthesis medium (Figure IV.60). Also, increased polarity of the sorbents tends to decrease the amount of template adsorbed by interfering with the formation of H-bonds between monomers and template. Thus the lowest adsorption capacities were shown by all imprinted systems when water was used as the rebinding medium.

**Figure IV.60.** Effect of solvent on the amount of template bound (Amount of polymer, 10mg; volume, 8.0mL; concentration of L-DOPA, 4.4mmolL\(^{-1}\); binding time, 3h)
4.4.3(E) Effect of monomer on rebinding

![Bar chart showing effect of monomer on rebinding](image)

**Figure IV.61.** Effect of nature of monomer on the amount of template bound (Amount of polymer, 10mg; volume, 8.0mL; concentration of L-DOPA, 4.4mmolL$^{-1}$; binding time, 3h)

AAm-L-DOPA system showed strongest H-bonding interactions as confirmed by comparing the $Q_e$ value of the three imprinted polymers. Hence MWCNT-AAm-MIP was found to be the best sorbent for L-DOPA under the investigated conditions (Figure IV.61).

4.4.4. Studies of the optimised system

4.4.4(A) Adsorption isotherms

![Langmuir plot for adsorption isotherms](image)

**Figure IV.62.** Langmuir plot for the adsorption of L-DOPA by MWCNT-AAm-MIP and AAm-MIP (Amount of polymer, 10mg; volume, 8.0mL; concentration of L-DOPA, 0.4 to 4.4mmolL$^{-1}$; binding time, 3h)
Results and Discussion

Figure IV.63. Freundlich plot for the adsorption of L-DOPA by MWCNT-AAm-MIP and AAm-MIP (Amount of polymer, 10mg; volume, 8.0mL; concentration of L-DOPA, 0.4 to 4.4mmolL⁻¹; binding time, 3h)

TABLE IV.10
Adsorption isotherm parameters of MWCNT-AAm-MIP and AAm-MIP

<table>
<thead>
<tr>
<th>Sample</th>
<th>Langmuir parameters</th>
<th>Freundlich parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Qₘ (mmolg⁻¹)</td>
<td>kₐ (Lmmol⁻¹)</td>
</tr>
<tr>
<td>MWCNT-AAm-MIP</td>
<td>0.801</td>
<td>19.334</td>
</tr>
<tr>
<td>AAm-MIP</td>
<td>0.496</td>
<td>0.754</td>
</tr>
</tbody>
</table>

The Langmuir adsorption isotherms of MWCNT-MIPs and MIPs were studied by plotting 1/Cₑ values against 1/Qₑ. The results showed that Langmuir adsorption isotherm model is more fit to MWCNT-MIP (Figure IV.62) with a correlation coefficient (R²) value of 0.999 than MIP (R² = 0.965) and supported the idea that the adsorption process occurring at the surface of MWCNT-MIP was homogeneous in nature. The Langmuir
constants $Q_m$ and $k_a$ were found to be 0.801mmol g$^{-1}$ and 19.334Lmmol$^{-1}$, respectively. It is very interesting to note that the adsorption capacity of the sorbent towards L-DOPA calculated from Langmuir equation (theoretical value) 0.801mmol g$^{-1}$ was almost same as the experimental value (0.796mmol g$^{-1}$) from the concentration study. Freundlich adsorption isotherms were obtained by plotting $\ln Q_e$ versus $\ln C_e$, as shown in figure IV.63. The Freundlich isotherm constants and the corresponding $R^2$ values for MWCNT-MIP/MIP are given in table IV.10. The results confirmed the suitability of Langmuir adsorption isotherm model for MWCNT-MIP system. The Freundlich isotherm model of MWCNT-MIP showed much deviation between the theoretical and experimental values while those for the Langmuir isotherm do not. The high correlation coefficient obtained for bulk MIP from Freundlich model further evidenced its inherent heterogeneity.

4.4.4(B) Adsorption kinetics

![Figure IV.64. Adsorption rate of L-DOPA by MWCNT-AAm-MIP (Amount of polymer, 10mg; volume, 8.0mL; concentration of L-DOPA, 4.4mmolL$^{-1}$; temperature, 301, 311 and 321K)
Results and Discussion

Figure IV.65. Pseudo-first-order kinetic plot for the adsorption of L-DOPA by MWCNT-AAm-MIP (Amount of polymer, 10mg; volume, 8.0mL; concentration of L-DOPA, 4.4mmolL\(^{-1}\); binding time 3h, temperature, 301, 311 and 321K)

Figure IV.66. Pseudo-second-order kinetic plot for the adsorption of L-DOPA by MWCNT-AAm-MIP (Amount of polymer, 10mg; volume, 8.0mL; concentration of L-DOPA, 4.4mmolL\(^{-1}\); binding time, 3h; temperature, 301, 311 and 321K)
TABLE IV.11

Kinetic parameters of pseudo-first-order and pseudo-second-order equations for L-DOPA adsorption onto MWCNT-AAm-MIP at 301, 311 and 321K

<table>
<thead>
<tr>
<th>C₀ (mmol L⁻¹)</th>
<th>Temperature (K)</th>
<th>Qₑ,exp (mmolg⁻¹)</th>
<th>Qₑ,cal (mmolg⁻¹)</th>
<th>k₁ (min⁻¹)</th>
<th>R²</th>
<th>Qₑ,cal (mmolg⁻¹)</th>
<th>k₂ (gmmol min⁻¹)</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.4</td>
<td>301</td>
<td>0.796</td>
<td>0.231</td>
<td>0.061</td>
<td>0.588</td>
<td>0.801</td>
<td>1.396</td>
<td>0.999</td>
</tr>
<tr>
<td>4.4</td>
<td>311</td>
<td>0.764</td>
<td>0.328</td>
<td>0.088</td>
<td>0.873</td>
<td>0.774</td>
<td>1.329</td>
<td>0.999</td>
</tr>
<tr>
<td>4.4</td>
<td>321</td>
<td>0.731</td>
<td>0.424</td>
<td>0.106</td>
<td>0.931</td>
<td>0.741</td>
<td>1.268</td>
<td>0.999</td>
</tr>
</tbody>
</table>

The kinetic studies were conducted to investigate the sorption mechanism of L-DOPA onto MWCNT-AAm-MIP. In this study pseudo-first-order and pseudo-second-order rate equations were used to check the adsorption kinetics of L-DOPA on the surface of nano sorbent. Figure IV.64 shows the adsorption pattern of L-DOPA onto MWCNT-AAm-MIP at optimum template concentration (4.4mmolL⁻¹). The adsorption was rapid in the initial 15min but rather slow towards equilibrium. The rapid adsorption of L-DOPA onto the surface of MWCNT-MIP was attributed to the increased number of binding cavities on its surface. After most of the imprinted cavities on MWCNT-MIP were occupied by the template, the adsorption rate of L-DOPA decreased due to the lack of specific binding cavities. The maximum rebinding occurred after 75min at a capacity about 0.7963mmolL⁻¹.

The plot t/Qₑ(t) versus t gave a straight line indicating that second order kinetics is applicable and the value of Qₑ calculated from the slope of the plot matched with the experimental value. Further, the plot of t/Qₑ against t
Results and Discussion

resulted in a high correlation coefficient (0.999) (Figure IV.66). This indicated that L-DOPA adsorption by MWCNT-AAm-MIP follows a second order kinetics. The disagreement of $R^2$ values and calculated parameters rendered the pseudo-first-order rate model to be less appropriate (Figure IV.65). With increasing temperature, the values of kinetic parameters ($k$ and $Q_e$) decreased showing that the adsorption process was exothermic (Table IV.11). At higher temperatures, adsorption decreased and desorption of the template molecule from the adsorption sites increased.

4.4.4(C) Selectivity experiments

L-DOPA and D-DOPA (Figure IV.67) were used to study the enantioselectivity of MWCNT-AAm-MIP and AAm-MIP. Once imprinted with a molecule, a good MIP should show a selective and specific binding towards the template even if it differs from other molecules only in its spatial arrangement. Such sharp selectivity and affinity was observed in the case of both MIPs, though the nanosorbent showed enhanced adsorption capacity (Figure IV.68). Wrapping of polymer around CNT resulted in a reduction of the innate bulkiness of MIP and consequently increased its surface area. Thus MWCNT-AAm-MIP possesses large number of surface binding sites which resulted in higher separation and selectivity factors. Table IV.12 summarizes the separation factor ($\alpha$) and selectivity factor (k) of the imprinted sorbents. The enantioselectivity of MWCNT-AAm-MIP and AAm-MIP was further confirmed by polarimetric studies. The specific rotation for the optimum concentrated racemic mixture solution of DOPA (4.4mmolL$^{-1}$) was found to be zero as it contained both enantiomers in equal proportions. 10mg of the imprinted sorbents was added into the racemic mixture followed by equilibration under the optimised conditions and centrifuged. The specific rotation of the supernatant solution was checked in the solvent N, N’-
dimethylformamide at \( \lambda = 589 \text{nm} \), \( T = 298 \text{K} \) and \( l = 1 \text{dm} \). The solution showed specific rotation values \( +12^\circ \pm 1 \) corresponding to D-DOPA which suggested the enantioselective binding of L-DOPA by the imprinted sorbents.

**Figure IV.67.** Chemical structures of (a) L-DOPA and (b) D-DOPA

**Figure IV.68.** Evaluation of the selectivity of MWCNT-AAm-MIP compared with AAm-MIP and both NIPs for L-DOPA and D-DOPA

**TABLE IV.12**

Separation and selectivity factors of MWCNT-AAm-MIP and AAm-MIP

<table>
<thead>
<tr>
<th>Drug</th>
<th>Separation factor ((\alpha) = K_{\text{MWCNT-AAm-MIP}} / K_{\text{MWCNT-AAm-NIP}})</th>
<th>Selectivity factor ((k) = \alpha_{\text{Template}} / \alpha_{\text{Analogue}})</th>
<th>Separation factor ((\alpha) = K_{\text{AAm-MIP}} / K_{\text{AAm-NIP}})</th>
<th>Selectivity factor ((k) = \alpha_{\text{Template}} / \alpha_{\text{Analogue}})</th>
</tr>
</thead>
<tbody>
<tr>
<td>D-DOPA</td>
<td>1.013</td>
<td>1.011</td>
<td>1.011</td>
<td>1.011</td>
</tr>
</tbody>
</table>
4.4.4(D) Regeneration and robustness

Figure IV.69. Regeneration cycles of MWCNT-AAm-MIP and AAm-MIP using 4.4mmolL$^{-1}$ L-DOPA solution in DMF as adsorbing medium and pure DMF as desorbing agent (Amount of polymer, 10mg; volume, 8.0mL; binding time, 3h)

The reusability of both MIPs was checked in eight sequential cycles of adsorption-desorption (Figure IV.69). The regeneration of the imprinted sorbents by the removal of L-DOPA was done using DMF as the solvent. The results showed that the nanosorbent retains 100% adsorption capacity up to six adsorption-regeneration cycles which then decreased slightly but not obviously which may be due to the damage of some rebinding cavities in the sorbent network during the time of regeneration (solvent extraction) thereby losing their template recognition capacity. However, bulk AAm-MIP showed irregularity in regeneration even from the second cycle onwards due to the damage of the recognition cavities and also due to the difficulty in leaching out the entire L-DOPA from the bulk polymer matrix during the time of regeneration. Hence MWCNT-AAm-MIP was found to be a better adsorbent for selective recognition and separation of L-DOPA from its enantiomeric pair than the conventional AAm-MIP.
References


Chapter V

Conclusion