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Research Article

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Histamine H_2-receptor antagonist imprinted-poly (vinylimidazole) grafted multiwalled carbon nanotubes

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ABSTRACT

Functionalised surfaces of multiwalled carbon nanotubes were used for grafting cimetidine, a histamine H_2-receptor antagonist, imprinted poly(vinylimidazole) onto the nano matrix. Fourier Transform-Infrared spectroscopy, Powder X-ray Diffraction studies, Thermogravimetric Analysis and Scanning Electron Microscopy were used for the characterisation of the product. Different parameters affecting the rebinding process like initial template concentration, time of contact, mass of sorbent etc. were optimised. The nanosorbent exhibited excellent homogeneity as indicated by its high regression coefficient value (R^2 = 0.999) for Langmuir adsorption isotherm model. Selectivity studies further demonstrated the successful fabrication of template-specific cavities on the surface of the nanosorbent.

Key words: molecular imprinting; nanotubes; morphology; adsorption; thermogravimetric analysis

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INTRODUCTION

Molecular imprinting is the method of creating “memory cavities” using an analyte molecule as the imprint in a rigid polymeric matrix [1]. The target molecule is later removed without disturbing the geometry of the solid matrix. The cavities thus formed will be capable of selectively and specifically binding the analyte molecule. The use of a suitable cross linker also helps to maintain the rigidity of the polymer and its well defined recognition sites [2]. The molecularly imprinted polymer retains the ability to rebind the template because of its functional arrangement regarding shape selectivity and pre-organisation of functional groups [3]. However, deeply imprinted cavities, production of heterogeneous binding sites, entrapment of template molecules in the polymer matrix, poor site accessibility etc. poses serious disadvantages to traditional bulk imprinted polymers [4]. This hinders the performance of MIPs reducing their kinetics of binding target analyte. The incorporation of nanostructures, particularly carbon nanotubes, into the polymer matrix has proven quite successful in solving these problems to a great extent due to the high surface-to-volume ratio imparted by them to the polymer matrix. This can increase the surface area of polymers thereby creating more recognition sites near or in the surface of the sorbent for the analyte.
Cimetidine, approved by FDA for the inhibition of gastric acid secretion, is considered as the first ever blockbuster drug. Chemically it is a histamine H_2-receptor antagonist, which is used in the treatment of heartburn, peptic ulcers, gastric and breast cancer [5]. We present here an efficient nanostructure-based-sorbent technique making use of molecular imprinting technology for the selective recognition and separation of cimetidine from its closely related compounds. The molecularly imprinted sorbent (MWCNT-MIP) was prepared on surface vinyl functionalised multi walled carbon nanotubes with cimetidine as template, 1-vinylimidazole as functional monomer, EGDMA as cross-linker and AIBN as initiator. The sorbent rebinding process was optimised with regard to various parameters. The selective recognition of MWCNT-MIP towards CIM was evaluated using adsorption experiments. The adsorption isotherms of the MWCNT-MIP were also studied in detail. The composition of MWCNT-MIP was analysed using FT-IR, powder XRD and thermo gravimetric analysis and their morphologies were observed using scanning electron microscopic technique.

EXPERIMENTAL SECTION

Materials

Pristine multiwalled carbon nanotubes were purchased from Reinste Nano Ventures Private Limited, India. 1-Vinylimidazole (VIZ, 98%), 2-hydroxy ethyl methacrylate (HEMA 99%), ethylene glycol dimethacrylate (EGDMA, 98%) and all solvents (HPLC grade) were obtained from Merck, India. Cimetidine (CIM, 99%), Ranitidine (RAN, 99%), Famotidine (FAM, 99%), 2,2'-azo-bisisobutyronitrile (AIBN) and thionyl chloride ($SOCl_2$) were obtained from Sigma Aldrich. All chemicals and solvents were used as obtained without further purification.

Apparatus

The Fourier-transform infrared spectra (FT-IR) were recorded on a Perkin-Elmer 400 FT-IR spectrophotometer using direct sampling technique. Ultraviolet-visible (UV–vis) absorption spectra were investigated by a Shimadzu UV-vis. spectrophotometer model 2450. Thermogravimetric analysis (TGA) was conducted on a NETZSCHSTA449C instrument from room temperature to 600^{o}C at a heating rate of 10^{o}C/min under nitrogen atmosphere. Morphological studies were carried out on a JEOL-JSM-6390A scanning electron microscope (SEM).

Carboxyl functionalisation of MWCNT

The purchased MWCNT was purified as reported in the literature [6]. 0.75g purified MWCNT was sonicated with 50mL conc. HNO_3 for about 12 minutes in a bath type sonicator at 40 KHz. The mixture was then stirred at 80^{o}C for 7.5h followed by dilution with excess amount of deionised water and filtration through 0.2μm PTFE membrane to separate the solid component. The washing procedure was repeated until the pH of the filtrate became neutral. The obtained solid product was dried under vacuum at 60^{o}C for 24 hours, yielding MWCNT with carboxyl functionalisation.

Preparation of vinyl functionalized MWCNT (MWCNT-CH=CH$_2$)

0.5g MWCNT-COOH was added into 20mL $SOCl_2$ in 15mL THF and refluxed at 65^{o}C for 24h under vigorous stirring. After cooling, the mixture was repeatedly washed with THF (5x25mL). Centrifugation at 14000 rpm separated the solid product from THF solution which was then dried under vacuum at 60^{o}C for 18h. The obtained MWCNT-COCl treated with 3mL 2-hydroxy ethylmethacrylate yield the surface vinyl functionalised product, MWCNT-CH=CH_2 [7].

Preparation of cimetidine (CIM) imprinted polymer on multiwalled carbon nanotubes

0.075g vinyl functionalized MWCNT mixed with 0.5mmol of 1-vinylimidazole (VIZ), 0.1mmol CIM and 1mmol EGDMA in 25mL acetonitrile was sonicated for 20min for the better dispersion of MWCNTs. To this 50mg AIBN was added and refluxed at 70^{o}C for 24h under N_2 atmosphere. The polymer formed was separated and ground well. It was then washed repeatedly with methanol for the complete removal of drug molecules which was monitored using UV-vis. spectroscopy (λ_{max}=220nm). The imprinted polymer thus obtained was dried in a vacuum desiccator for 24h before use. For comparison, the non-imprinted polymers on MWCNT (MWCNT-NIP) were also prepared using the same procedure, but without using the drug molecules in the polymerization process.

Initial template concentration

The initial template concentration at which maximum amount of template is bound by a given mass of the adsorbent was studied and optimized through a batch equilibration process. For this, 0.01g of MWCNT-MIP/NIP was added into each of the 6 samples of 8mL analyte solution having different concentration (0.4, 0.8,…3.6 mmolL^{-1}). The system was then equilibrated and centrifuged. The maximum amount of template bound by the polymer can be calculated using a Uv-vis spectrophotometer at 220nm from the collected centrifugate using the equation:

$$Q_e = (C_o - C_e)V/M \quad (1)$$

where C_o (mmolL^{-1}) and C_e (mmolL^{-1}) are the initial and equilibrium concentration, V (L) is the volume of cimetidine solution and M (g) is the weight of the sorbent.

Time of contact

0.01g of MWCNT-MIP/NIP was added into each of a number of 8mL samples of 2.4mmolL^{-1} CIM solution. After equilibration at different time intervals, the mixture was centrifuged (1600rpm), filtered and the concentration of CIM in the supernatant was measured at 220nm using a UV-vis spectrophotometer. The adsorption amount bound at different time intervals was calculated using equation (1).

Adsorbent mass

The effect of increasing adsorbent dosage (5 to 20mg) was studied by equilibrating the respective weight of adsorbent with the optimum template concentration. The amount of template bound by the polymer sorbent was calculated using equation (1).

Adsorption experiment

The uniformity and distribution of binding sites on the nanosorbents were evaluated using Langmuir and Freundlich adsorption isotherms. 10mg of the synthesized sorbents (MWCNT-MIP) was added to 8mL each of template solution having different concentration ranging from 0.4 to 2.4mmolL^{-1}. It was then shaken for 4h. The equilibrated mixture was centrifuged and decanted. Using UV-vis spectra (220nm), concentration of the supernatant liquid was measured. From this data the adsorption capacity (Q_e) of the adsorbent was calculated (eqn 1). The experimental value of Q_e obtained from concentration study was compared with the theoretical values of Q_e obtained using the Langmuir (2) and Freundlich (3) equations:

$$(1/Q_e) = (1/Q_m) + (1/Q_m K_a) \times (1/C_e) \quad (2)$$

$$\log (Q_e) = \log K_F + 1/n \log C_e \quad (3)$$

where C_e (mmolL^{-1}) and Q_e (mmolg^{-1}) are CIM concentration and amount adsorbed at adsorption equilibrium, Q_m (mmolg^{-1}) and K_a (L$mmol^{-1}$) are the theoretical maximum adsorption capacity and Langmuir equilibrium constant related to the theoretical maximum adsorption capacity and energy of adsorption, respectively. K_F and n are the Freundlich constants, which denote the adsorption capacity and adsorption intensity respectively. All parameters of each model can be found from the slope and intercept of the different plots using regression analysis. The validity of isotherm models was compared by using correlation coefficient (R^2) value.

Selectivity studies

The ability of the nanostructure-incorporated sorbent to selectively and specifically bind the analyte molecule from a mixture of closely related compounds was tested by a batch equilibration process where 2.4mmol solutions of CIM, RAN and FAM were used. The procedure was same as that of adsorption studies. The separation and selectivity factors of MWCNT-MIP towards CIM over RA and FAM were calculated using the equations:

$$\text{Separation factor } (\alpha_{\text{Template}}) = K_{MIP} / K_{NIP} \quad (4)$$

$$K = \text{Template}_{\text{Bound}} / \text{Template}_{\text{Free}} \quad (5)$$

The selectivity of the imprinted polymers towards the template was calculated in terms of selectivity factor.

$$\text{Selectivity factor} = \alpha_{\text{Template}}/\alpha_{\text{Analogue}} \quad (6)$$

RESULTS AND DISCUSSION

FT-IR spectra were used to characterize the structural changes of purified MWCNT, MWCNT-COOH, MWCNT-$CH=CH_2$ and MWCNT-MIP before and after washing. Purified MWCNT shows a strong peak at 1737 cm^{-1} corresponding to the C=O stretching vibration, while the peak at 2928 cm^{-1} is related to the asymmetric stretching vibration of C-H. The presence of additional peaks at 3300 and 1581 cm^{-1} in the acid processed MWCNTs corresponded to the presence of functional groups –OH and –COOH. The peak at 1352 cm^{-1} and several other low intensity peaks were attributed to the C–O stretching vibrations of –COOH. The appearance of C=C peak at 1630 cm^{-1} after the coupling of HEMA with MWCNT-COCl confirms the successful grafting of vinyl functionalisation onto the MWCNT surface. Further, a strong peak associated with ether linkage around 1166 cm^{-1} confirmed the coupling of HEMA to MWCNT through the -O- atom. MWCNT-MIP before and after leaching out template, gave notably different spectra. Before washing spectrum showed peaks at 3136 and 3216 cm^{-1} for the N-H stretch and at 2176 and 1615 cm^{-1} for the –CN triple bond and the >C=N– stretch of cyano-guanidine unit of CIM respectively, which were absent in the after washing spectrum. This demonstrated that the template was held by the polymer before complete leaching out.

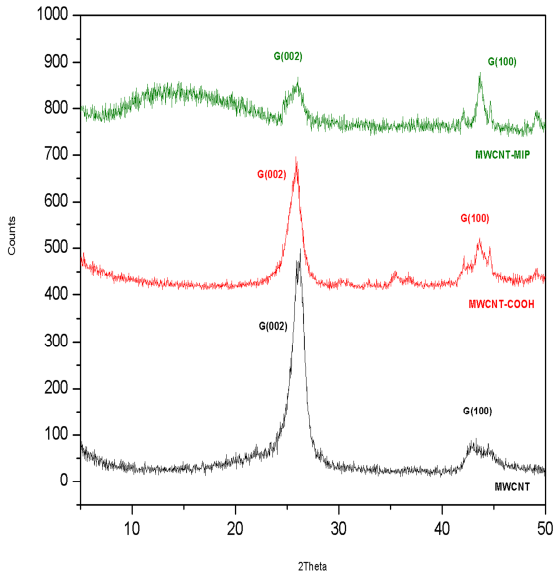

Figure 1. Powder X-ray diffraction patterns of (a) MWCNT, (b) MWCNT-COOH and (c) MWCNT-MIP

X-ray diffraction technique gives useful insight into the crystallinity of MWCNT-MIP (fig 1). The XRD pattern of MWCNT clearly shows two crystalline peaks at $2\theta = 25.8^{o}$ and $2\theta = 43.7^{o}$ corresponding to the interlayer spacing (d_{002}) and reflection (d_{100}) of MWCNT [8]. MWCNT-COOH was found to retain the crystalline nature of parent nanotubes. However, MWCNT-MIPs show sharp peaks imparted by the crystalline MWCNTs in addition to the broad peaks characteristic of the amorphous polymer. This further confirms the effective clubbing of nanostructures into the polymer matrix [4].

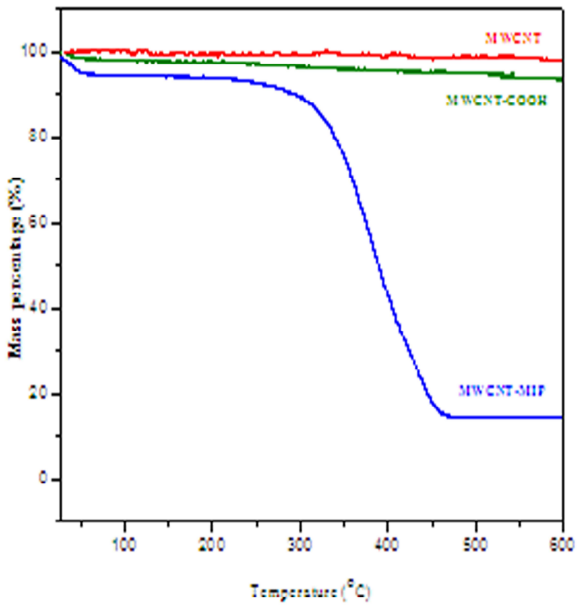

Figure 2. TGA curves of (a) MWCNT, (b) MWCNT-COOH and (c) MWCNT-MIP (heating rate of 10°C min^{-1} from room temperature to 600 °C under N_2 atmosphere)

In the thermogravimetric analysis (fig 2) pristine MWCNT was found to be thermally stable upto 600^{o}C without any significant mass loss. MWCNT-COOH underwent a continuous and less obvious mass loss which may be due to the decomposition of carbonyl groups from the surface of MWCNT. From the residual yield, the percentage of acid formation on the surface of carbon nanotubes was calculated to be 8%. The thermal decomposition pattern of

MWCNT-MIP showed an apparent mass loss in the temperature range 300° to 450°C due to the degradation of the polymer part. From the residual mass, percentage of multiwalled carbon nanotube in MWCNT-MIP was found to be about 14.75%.

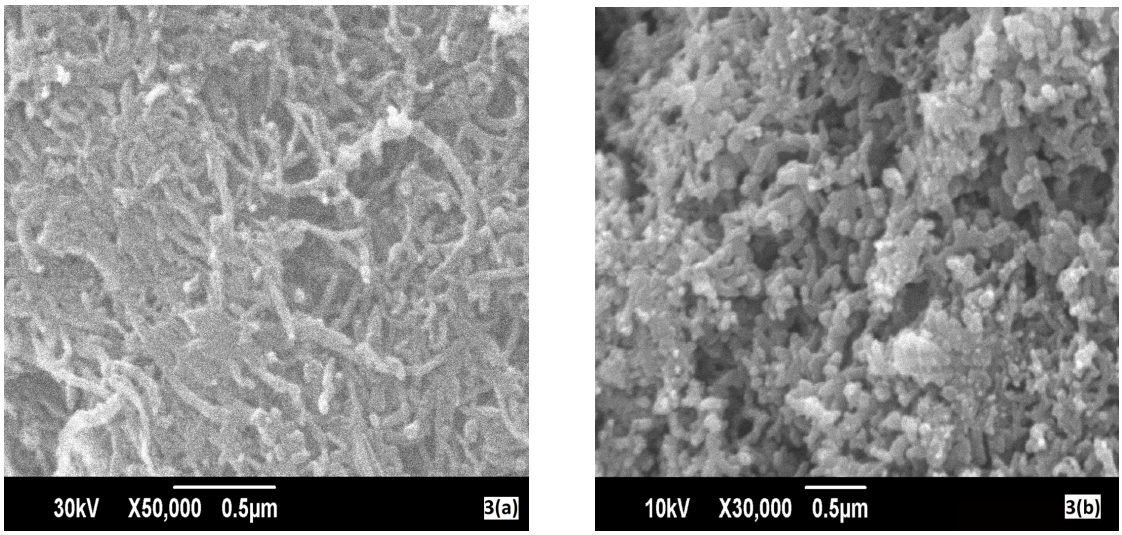

Figure 3. Scanning electron micrographs of (a) MWCNT and (b) MWCNT-MIP

The SEM micrographs (fig 3) of pristine MWCNT showed nanosized tubular moieties with an average diameter around 6 to 8nm. MWCNT-MIPs showed an increase in diameter with the retainment of nanofibrillar morphology thus illustrating the incorporation of MWCNTs into the polymer matrix. Moreover, the MIP showed a rough surface morphology which may be due to the leaching out of print molecules from the polymer matrix leading to the formation of template-specific cavities on its surface. This visibly substantiates the higher homogeneity expressed by the MWCNT-polymer system.

Concentration study

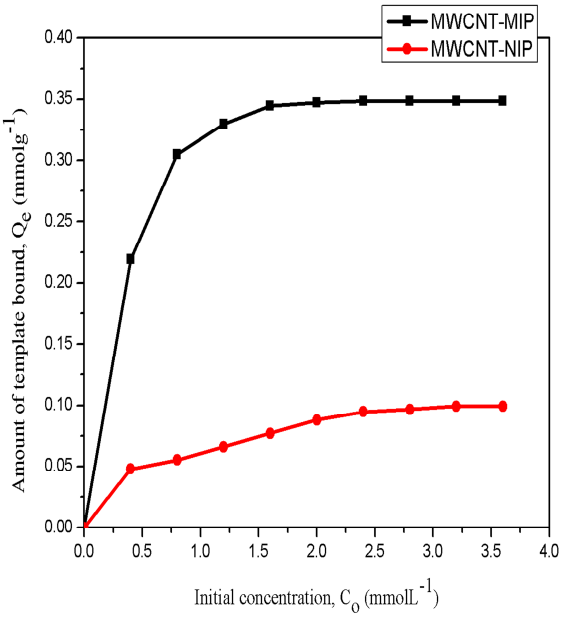

Figure 4. Adsorption isotherms of imprinted and non-imprinted polymers (Amount of polymer, 10mg; volume, 8.0 mL; concentration of CIM from 0.4 to 3.6 mmolL^{-1})

The maximum amount of template bound by the polymer sorbent for different initial template concentrations (investigated under the specified conditions; contact time of 4h; adsorbent dosage of 10mg; and temperature of 28°C) is shown in fig 4. As can be seen, the amount of template bound Q_e of MWCNT-MIP tends to increase with an increase in initial template concentration. However, Q_e increases only upto an optimum initial concentration

beyond which Q_e of MWCNT-MIP remains almost a constant. The initial rise in Q_e value is due to the availability of a large number of easily available surface binding sites which becomes saturated as template concentration increases. However, the non-imprinted polymer shows no particular affinity or specificity towards the analyte molecule due to the lack of template complementary cavities. The non-covalent forces, associated with cavity conformations, direct the monomer functionalities so that "memory cavities" with high affinity, selectivity and specificity towards the template molecule are created.

Adsorbent dosage

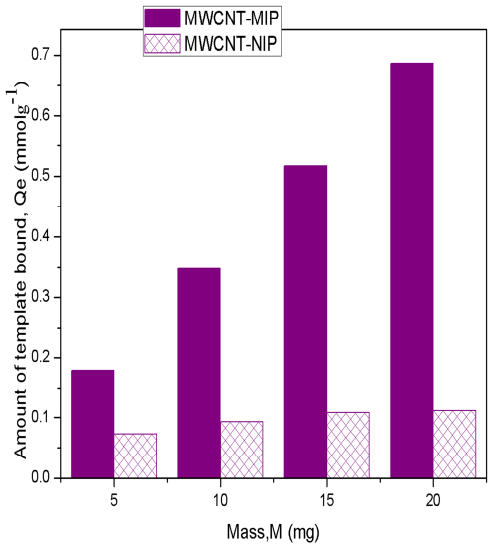

Figure 5. Effect of adsorbent dosage on template bound (Amount of polymer varies from 5 to 20mg; volume, 8.0 mL; concentration of CIM, 2.4 mmolL^{-1}, binding time, 4h)

As expected, the removal efficiency of adsorbent increased with an increase in its weight used owing to the availability of more number of binding sites (fig 5). Although both MWCNT-MIP and MWCNT-NIP have almost similar surface-to-volume ratio due to the incorporation of same amount of carbon nanotubes, the absence of template-specific binding sites in MWCNT-NIP expresses itself as very low Q_e values.

Time of rebinding

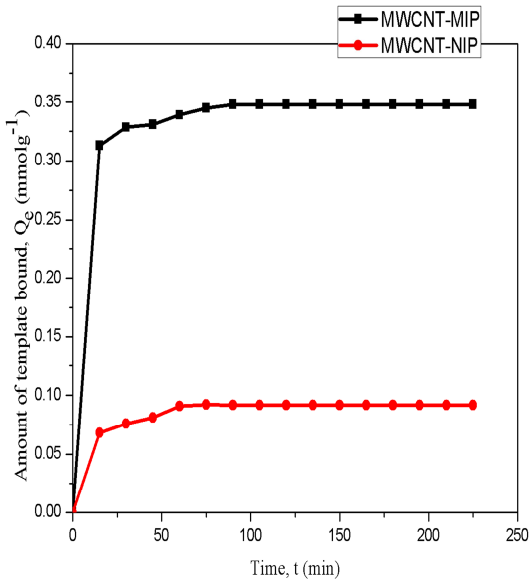

Figure 6. Effect of time on template bound (Amount of polymer varies from 10mg; volume, 8.0mL; concentration of CIM, 2.4 mmolL^{-1}, binding time, 4h)

The effect of increasing time of contact between the adsorbent and adsorbate in the process of rebinding is depicted in fig 6. The maximum rebinding by MWCNT-MIP is almost completed within the first 15 minutes indicating that the majority of the binding sites are located on the surface of the sorbent. As time increases, the extent of Q_e increase decreases and the reaches a constant. The very slight increase points to the saturation of binding sites near to the surface whereas the constant value shows that no further adsorption occurs after an optimum time period.

Adsorption isotherms

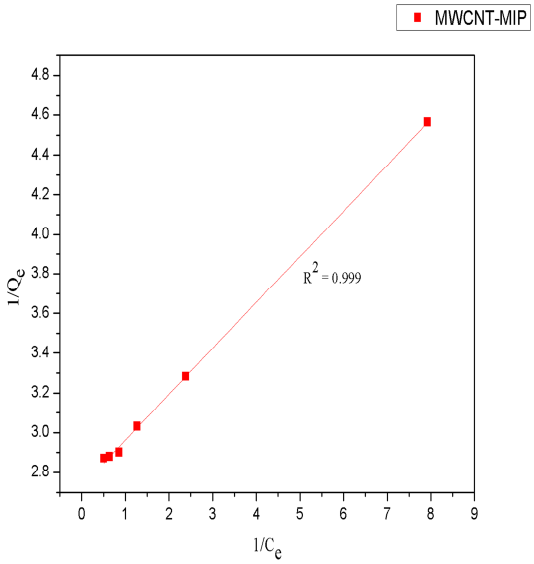

Figure 7. Langmuir plot for adsorption of CIM by MWCNT-MIP (Amount of polymer 10mg; volume, 8.0 mL; concentration of CIM 0.04 to 2.4 mmolL^{-1}, binding time, 4h)

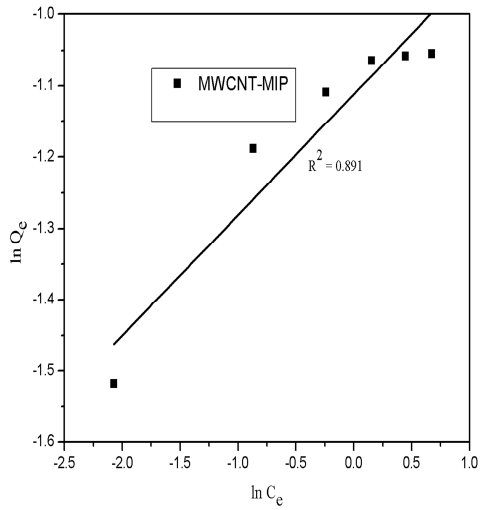

Figure 8. Freundlich plot for adsorption of CIM by MWCNT-MIP (Amount of polymer 10mg; volume, 8.0 mL; concentration of CIM 0.04 to 2.4 mmolL^{-1}, binding time, 4h)

Table 1 Adsorption isotherm parameters of MWCNT-MIP

Sample	Langmuir parameters			Freundlich parameters		
	Q_m (mmolg^{-1})	k_a (Lmmol^{-1})	R^2	n	k_f [(mmol/g)(L/mmol)$^{1/n}$]	R^2
MWCNT-MIP	0.36	32.3	0.999	5.9	0.33	0.891

Adsorption isotherms represent the relationship between the amount adsorbed by unit weight of the adsorbent and the amount of adsorbate remaining in the solution at equilibrium. Adsorption isotherm data of MWCNT-MIP was found to be well-fit for Langmuir model than Freundlich based on least square fit (fig 7 and 8). The regression

coefficient value tending towards unity (R^2 = 0.999) and the agreeability between the calculated Q_e (from Langmuir, Q_e = 0.36) and experimental Q_e (from concentration study, Q_e = 0.36) render the Langmuir model as the best suitable one for explaining template rebinding in MWCNT-MIP (Table 1). Consequently, in MWCNT-MIP the adsorption process is mainly monolayer adsorption on a homogeneous adsorbent surface with no transmigration of adsorbate in the plane of surface. This corroborates with the high homogeneity expressed by the system.

Selectivity Experiments

MWCNT-MIP exhibited high selectivity towards the template molecule CIM as compared to its closely related structural analogues (fig 9 and table 2). This again evidences the formation of template-specific recognition sites on the surface of MWCNT-MIP. However, MWCNT-NIP showed almost similar adsorption capacity towards all three molecules irrespective of their size, shape and functionality due to the lack of specific recognition sites. Also, MWCNT-NIP showed much lower Q_e values than MWCNT-MIP as it binds compounds by very weak non-specific adsorption only.

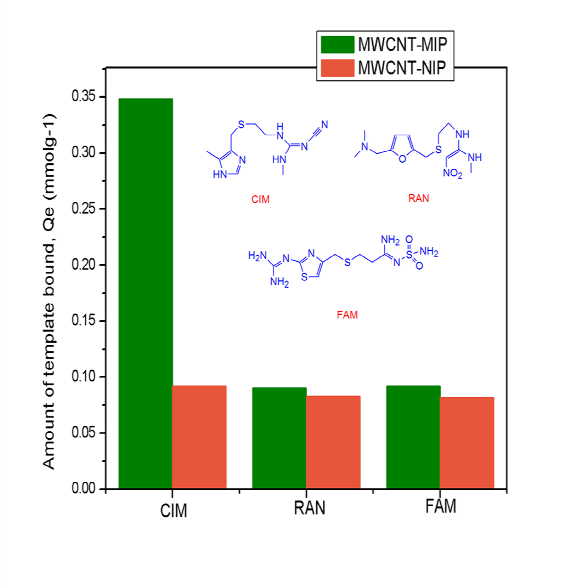

Figure 9. Evaluation of the selectivity of MWCNT-MIP/NIP for CIM, RAN and FAM

Table 2 The selectivity and separation factors of the MWCNT-MIP

Drugs	Separation factor ($\alpha_{Template}$) = K_{MIP} / K_{NIP}	Selectivity factor = $\alpha_{Template}/\alpha_{Analogue}$
CIM	4.264	-
RAN	1.089	3.916
FAM	1.130	3.774

CONCLUSION

Cimetidine, a histamine H_2-receptor antagonist, imprinted poly(vinylimidazole) was successfully grafted onto the surface of vinyl functionalised MWCNTs resulting in a novel composite sorbent capable of selectively and specifically rebinding the target analyte. The products were completely characterised using FT-IR, PXRD, TGA and SEM analyses. Various parameters such as template concentration, time of contact, adsorbent dosage etc. which can affect the rebinding process were optimised. From adsorption isotherm experiments, MWCNT-MIP was found to follow Langmuir adsorption model of monolayer adsorption on a homogeneous surface. The excellent selectivity and specificity of the imprinted polymer towards CIM as compared to its structural analogues RAN and FAM were further confirmed by selectivity studies.

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