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Grafting of molecularly imprinted polymer on multiwalled carbon nanotube for highly selective and sensitive determination of Thymine

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ABSTRACT

The molecular imprinting technology was used to synthesize vinyl functionalized multiwalled carbon nanotube imprinted polymer for thymine. The imprinted and non-imprinted polymers are characterized by using infrared spectrophotometer, thermo gravimetric analysis, x-ray diffraction analysis, transmission electron microscopy, and scanning electron microscopy. The adsorption behaviour was evaluated as a function of initial concentration, time, mass and solvent. The selectivity of the polymers evaluated with structurally similar analogues like 5-fluoro uracil and thymine. The approach would open the new opportunity to design of polymers with selective recognition properties.

Key words: multiwalled carbon nanotube, imprinting, thymine, adsorption, selectivity

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INTRODUCTION

Molecular imprinting is a technique to create template shaped cavities in the polymer matrices with the memory of template molecule. Molecularly imprinted polymers are highly selective towards the recognition sites. The polymerization is carried out monomers with the selective template molecules in the normal condition. After the polymerization the template molecule is removed from polymer matrix and leaving behind the cavity which is created by the template molecule. The template molecule designs the structure, shape and the selective recognition of the polymeric material. Imprinted polymer possesses potential application in the field of sensors, immune assay, catalysis and drug delivery etc [1-5]. The conventional bulk method of polymerization possesses number of disadvantages such as slow kinetic binding times, heterogeneous binding sites, poor accessibility of template molecules etc [6-9]. In order to overcome these problem scientists have made to effort to prepare nanostructured imprinted polymers with silica and multiwalled nano tubes are the supporting materials. The imprinted polymer is layered on the surface of a solid support monodipersion is take place as a result which improve the accessibility of the template molecule and vanish all the disadvantages possessed by the conventional method.

Multiwalled carbon nanotube (MWCNT) has widespread attention for their high electrical and thermal conductivity properties. MWCNT with unique mechanical properties and extremely large surface area should be an excellent candidate as the supported material, which would endow imprinted polymer with large surface area if the imprinted polymer were prepared onto the surface of MWCNT. Thus, the binding sites in the outer layer would improve the accessibility of template molecule and reduce the binding time.

The main intension of this work was the direct preparation and characterization of molecularly imprinted polymers on multiwalled carbon nanotubes (MWCNT MIP) with pyrimidine analogue thymine as template that could show molecular recognition properties very greatly [10-15]. Techniques such as Fourier transform infrared (FT-IR) spectroscopy, Transmission electron microscopy (TEM), Scanning electron microscopy (SEM), and Thermo gravimetric analysis (TGA) were used to confirm the formation of imprinted polymer on MWCNT.

MATERIALS AND METHODS

Materials

MWCNT, ethylene glycol dimethacrylate (EGDMA), and 2, 2' azoisobutyronitrile (AIBN) were purchased from Sigma-Aldrich (Germany). Thionyl chloride ($SOCl_2$), dimethlysulfoxide (DMSO), dimethylformamide (DMF), tetrahydrofuran (THF), triethylamine (TEA) were obtained from Merck (Germany). Triethylamine (TEA) and methacrylic acid (MAA) were obtained from SRL, India. 5-Fluoro uracil, thymine and uracil were purchased from Sigma-Aldrich (Germany) and used as received.

Methods

Absorption spectra of 5-FU, thymine and uracil were recorded by Shimadzu UV-Vis 2450 spectrophotometer. The FT-IR studies were carried out using Perkin-Elmer spectrum 400 FT-IR spectrophotometer. X-Ray diffractogram which was recorded by PAN analytic XPERT-PRO. The morphology was investigated by scanning electron microscopy (SEM) using a JEOL-JSM-6390 and JEOL-GEM 2100 transmission electron microscope.

Functionalization of multiwalled carbon nanotube

Crude carbon nanotube (0.05 g) is suspended in 60 mL of con HNO_3 in a round bottom flask under sonication with 10 minutes. After that the mixture was refluxed a5t 80 ^oC for 16 h. Cooled the mixture at room temperature and filtered through membrane and washed with distilled water till the pH reached to neutral. The solid was dried under vacuum for 24 h and obtained solid is carboxyl functionalized multiwalled carbon nanotube (MWCNT-COOH).

The MWCNT-COOH (0.04 g) was treated with thionyl chlorine for undergoing acylation. The mixture is refluxed at 80 ^oC for 20 h using chloroform as the solvent. As the refluxion is completed the solid was washed several times with tetrahydrofuran in order to remove the excess thionyl chloride. Then the solid was dried under vacuum, obtaining the acyl functionalized carbon nanotube MWCNT-COCl. The acyl functionalized MWCNT (0.04 g) is treated with allyl alcohol and triethyl amine in the presence of 4-DMAP as catalyst. The mixture is refluxed 50 ^oC for 24 h. After that the solid is washed with THF , filtered and dried under vacuum, obtaining vinyl functionalized MWCNT (MWCNT-$CH=CH_2$).

Synthesis of molecularly imprinted polymer on MWCNT

The polymerization was carried out by radical polymerization. Here thymine, methacrylic acid, AIBN was used as template, functional monomer and initiator respectively. The MWCNT-$CH=CH_2$ is mixed with the solvent mixture, chloroform and acetonitrile in a round bottom flask, purged with nitrogen and subjected to sonication for 10 minutes. Then thymine and MAA in acetonitrile was added to this mixture, initiator AIBN was also added. This mixture was refluxed at 70 ^oC for 10 h. The product was collected and washed with acetonitrile until no thymine was detected in the eluent. The polymer was dried under vacuum for 24 h. For the comparison the conventional imprinted polymer was synthesized without adding functionalized MWCNT and also the non-imprinted polymers for both new and conventional methods were also synthesized with and without adding functionalized nanotube (Table 1).

Table 1: Preparation of polymers

Polymer	MAA (mmol)	EGDMA (mmol)	Thymine (mmol)	AIBN (g)	MWCNT-$CH=CH_2$ (g)
MWCNT MIP	0.025	1.25	0.05	0.01	0.02
MWCNT NIP	0.025	1.25	0.00	0.01	0.02
MIP	0.025	1.25	0.05	0.01	0.00
NIP	0.025	1.25	0.00	0.01	0.00

Adsorption characteristics

The adsorption characteristics of thymine were determined by batch experiments. The adsorption isotherm, adsorption kinetics and selectivity of the imprinted and non-imprinted polymers were examined. The equilibrium concentration of the thymine after the treatment was analyzed by UV-vis. spectroscopy. The adsorption capacity was calculated using the equation.

$$Q = \frac{Co - Ce}{M} \times V \qquad (1)$$

Where Q (mg/g) is the amount of total adsorption of thymine, C_o and C_e are initial and equilibrium concentration of thymine in solution (mg/L), respectively. V (mL) is the volume of the solution and M (g) is the weight of imprinted and non imprinted polymers.

The dependence of adsorption characteristics on the concentration was estimated. The different initial concentrations in the range 1.6 - 2.2 $x10^{-7}$ mg/L were prepared. Stirred well and collected the supernatant and equilibrium concentration of solution was determined. The adsorption capacity was determined using above equation. The adsorption kinetics was determined for the imprinted and non-imprinted polymers at concentration 2.2 x 10^{-7} mg/L for different time intervals. The adsorption capacity was also determined.

The effect of change in polymer amount was studied by varying the amount of polymer used for template rebinding. The effect of various solvents on the recovery of thymine was also determined. The various eluents used are methanol, acetonitrile and chloroform.

The selectivity experiments were carried out using structurally similar pyrimidine analogues like 5-fluoro uracil and uracil. The binding capacity was calculated using the above equation. The selectivity factor (α) which is the ratio between the amount of template and the amount of the template analogue bound on the polymer and is calculated using the equation.

Separation factor = $\alpha_{template} = K_{MIP} / K_{NIP}$

K = template bound / template free
Selectivity factor = $\alpha_{template} / \alpha_{analogue}$

RESULTS AND DISCUSSION

Thymine imprinted and non-imprinted polymers with and without MWCNT were prepared. The vinyl functionalized carbon nanotube is used for selective polymerization with MAA and thymine which is the functional monomer and template respectively for introducing the imprinted polymer on vinyl group incorporated MWCNT. The synthesized polymers were characterized by the following characterization techniques.

Fourier transform infrared spectroscopy (FT-IR)

The synthesized polymers were characterized using important spectroscopic tool like FT-IR [16-17]. The vinyl functionalized carbon nanotube MWCNT-$CH=CH_2$ showed a sharp peak at 1729 cm^{-1}, which is the stretching vibration of C=O group of the ester group formed on allylation. The absorbance at 1634 cm^{-1} is C=C stretching vibration. From the result it is clear that the vinyl group is successfully introduced on the surface of multi walled carbon nanotube. In the imprinted polymer the main characteristic peaks are at 3610, 1722, 1250, and 1139 cm^{-1} which correspond to O-H, C=O and C-O symmetric and asymmetric stretching vibrations of the incorporated functional monomer methacrylic acid and crosslinking agent EGDMA. In MWCNT-imprinted polymer all these peaks are present which confirm the presence of the imprinted layer on the surface of vinyl functionalized MWCNT.

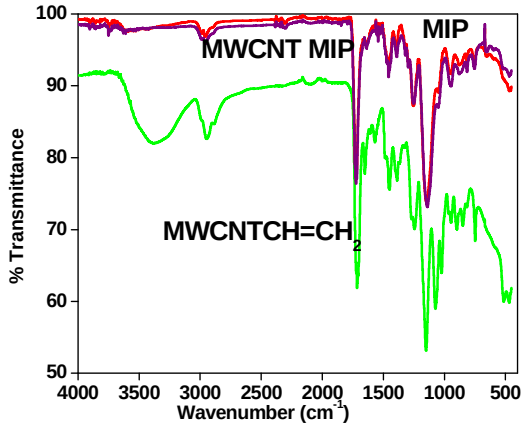

Figure 1. FT-IR spectra of MWCNT $CH=CH_2$, MWCNT-imprinted polymer and imprinted polymer

Thermogravimetric analysis

The thermal stability of the synthesized polymer was estimated by thermogravimetric analysis. Figure 2 shows the TG curves of MWCNT, MWCNT-imprinted polymer and imprinted polymer respectively. The crude multiwalled carbon nanotube is stable without any weight lost up to 600 ^oC. But MWCNT-imprinted polymer was steady up to 260 ^oC when the temperature was increased the wrapped layer of polymer is degraded which shows the weight loss and then increase the temperature 460 ^oC there is no weight lost. The results cleared that comparison with MWCNT

and MWCNT-imprinted polymer 50 % of weight lost was happen in MWCNT-imprinted polymer. This loss is because of the polymer layer grafted on the surface of MWCNT. Also compared with conventional imprinted polymer the weight loss in MWCNT-imprinted polymer is low and obviously MWCNT-imprinted polymer is thermally stable than conventional one.

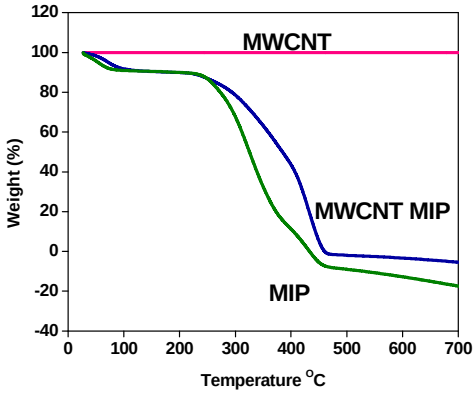

Figure 2. TGA of MWCNT, MWCNT-imprinted polymer and imprinted polymer

X-ray diffraction analysis

The XRD pattern of the MWCNT, MWCNT-imprinted polymer and imprinted polymer are shown in the Figure 3. In MWCNT the important graphitic peak is observed in (002) of MWCNT at 2 theta value 26.4^{o}. But in the case of MWCNT-imprinted polymer the number of peaks are increased due to the grafting of imprinted layer on the surface. The diffraction peaks at 26^{o}, 72^{o}, and 88^{o} from MWCNT-imprinted polymer are assigned. In the case of imprinted polymer no peak is observed only the broad halo amorphous peak.

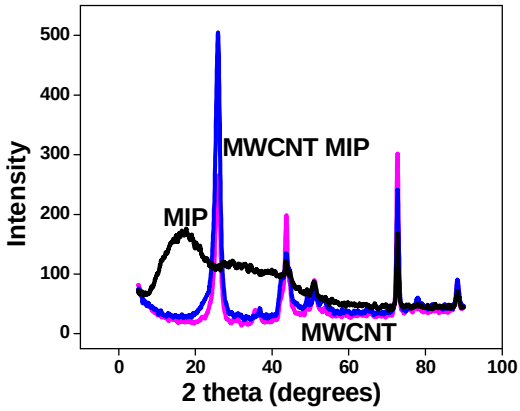

Figure 3. XRD pattern of MWCNT, MWCNT-imprinted polymer and imprinted polymer

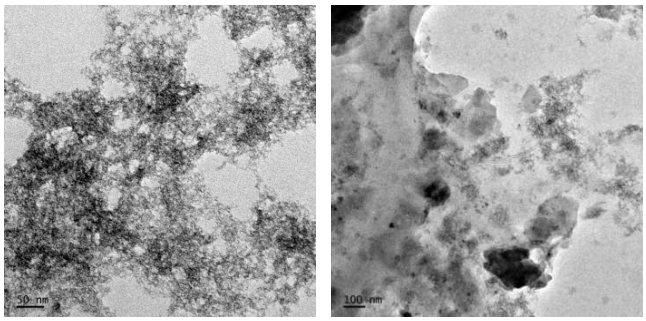

Figure 4. TEM photographs of the MWCNT and MWCNT-imprinted polymer

Transmission electron microscopy

The structure of MWCNT and MWCNT-imprinted polymer are morphologically revealed by TEM. In MWCNT, thread like structure is observed. Obviously the results clear that MWCNT-imprinted polymer shows the successful

grafting of imprinted layer on the surface of MWCNT whereas in unfunctionalized MWCNT, no polymer coating is observed.

Scanning electron microscopy

This result could be used as a support for the successful incorporation of imprinted polymer layer on the surface of MWCNT. Furthermore, the surface of the MWCNT-imprinted polymer was thick due to the covering by the EGDMA-crosslinked methacrylic acid polymer.

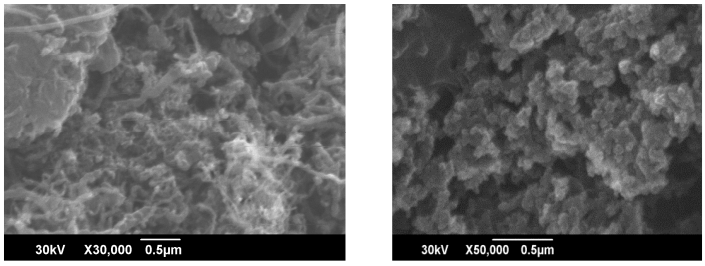

Figure 5. SEM photographs of the MWCNT and MWCNT-imprinted polymer

Adsorption characteristics

Adsorption isotherm

The adsorption characteristics were investigated by batch experiments. The maximum adsorption capacity was observed with the initial concentration 2.2 x 10^{-7} mg/L. The results cleared that as the initial concentration is increased the adsorption capacity is also increased. As concentration of thymine solution increases, the cavities in the imprinted polymer traps the template molecule easily making efficient adsorption of the template molecule. In the case of non-imprinted polymers the adsorption is very low because of the unavailability of such imprinted cavities.

Langmuir and Freundlich adsorption isotherms were used to evaluate the adsorption characteristics. Langmuir model is represented as follows

$$q_e = \frac{q_m b C_e}{1+bC_e} \qquad (2)$$

The above equation can be rearranged to the following linear form:

$$\frac{C_e}{q_e} = \frac{C_e}{q_m} + \frac{1}{bq_m} \qquad (3)$$

Freundlich equation used is:

$$\log q_e = \frac{1}{n} \log C_e + \log K_F \qquad (4)$$

Where C_e is the equilibrium concentration, q_e the amount of uracil adsorbed at equilibrium, q_m is the amount of thymine adsorbed for a complete monolayer, b is a constant related to the energy or net enthalpy of sorption. The sorption data were analyzed using the linear form of the Langmuir isotherm. The plots of specific sorption, C_e/q_e, against the equilibrium concentration, C_e for MWCNT-imprinted polymer are shown in Figure 6. The correlation coefficient (R^2) is 0.9933. It can be confirmed that the thymine sorption by MWCNT-imprinted polymer follow the Langmuir model with the maximum sorption capacity (Table 2).

Table 2. Langmuir and Freundlich constants for sorption of thymine

Langmuir isotherm			Freundlich isotherm		
Q_o(mmol/g)	b (mol/g)	R^2	K_F	n (mmol/g)	R^2
356.2	0.056	0.9933	16.32	1.90	0.84

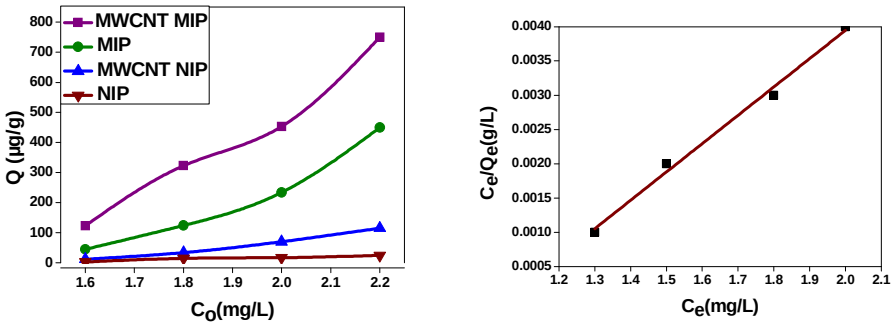

Figure 6. Effect of initial concentration on the adsorption imprinted and non- imprinted polymers, Langmuir isotherm of MWCNT-imprinted polymer

Adsorption kinetics

The adsorption kinetics of thymine was investigated as function of time. The initial concentration of the standard solution was 2.2 x 10^{-7} mg/L. The adsorption of template thymine is high at initial stage and after 130 minutes it reaches saturation. In order to analyze the adsorption of thymine onto the surface of polymers, a second-order (5) model is employed.

$$\frac{t}{q_t} = \frac{1}{k_2 q_e^2} + \frac{t}{q_e} \qquad (5)$$

Where q_e and q_t are the amounts of thymine adsorbed (mg g^{-1}) on the adsorbent at the equilibrium and at time t, respectively. k_2 (g mg^{-1} min^{-1}) is the adsorption rate constants and k_2 was obtained from plotting (t/q_t) versus t by the second-order approach. As seen from Figure 7, the correlation coefficient (R^2) given by the kinetic model is 0.9988. Obviously, the second-order kinetic model shows a good correlation for the adsorption of thymine molecules on MWCNT-imprinted polymer. The adsorption kinetic constants and linear regression values are summarized in Table 3.

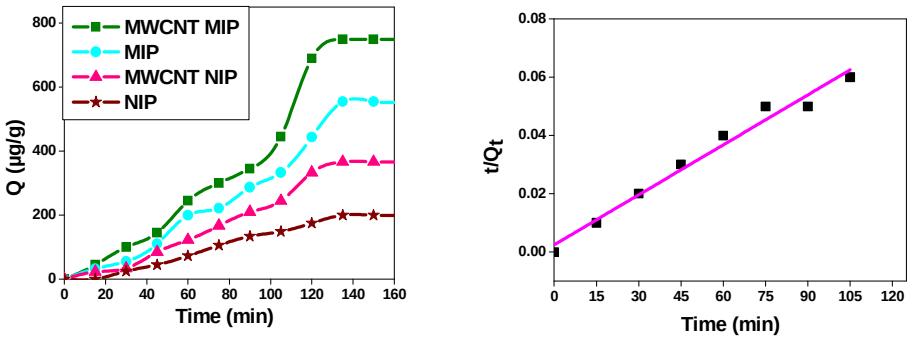

Figure 7. Effect of time on the adsorption imprinted and non-imprinted polymers and second order kinetics of MWCNT-imprinted polymer

Table 3. Kinetic parameters of the rate equation for thymine adsorption onto MWCNT-MIP

Kinetics	k	R^2
First order kinetics	0.1034	0.9645
Second order kinetics	5.4509	0.9988

Amount of polymer

The optimization of synthesized polymer was investigated by changing the amount of polymer. Different amounts of polymer, ranging from 10-50 mg was taken. The concentration of the template solution was kept at 2.2.x 10^{-7} mg/L. Obviously with increasing amount of polymer the adsorption capacity also increased (Figure 8).

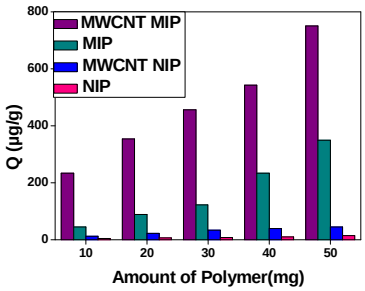

Figure 8. Effect of amount of polymer on the adsorption of imprinted and non-imprinted polymers

Solvent Effect

The effect of solvent on thymine adsorption was also noted. Acetonitrile is the best solvent to elute thymine. Chloroform also successfully retained thymine from imprinted polymer, and recovery values were satisfactory due to non-polar aprotic properties. The polar methanol disturbed the elution which leads to elution very low. In view of the above, acetonitrile was selected as the elution solvent for eluting thymine from the imprinted polymers (Figure 9).

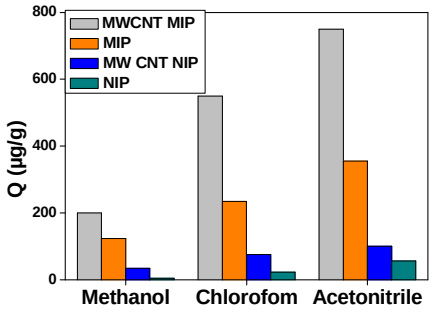

Figure 9. Effect of eluents on the adsorption of imprinted and non-imprinted polymers

Evaluation of selectivity

Figure 10 shows the selective thymine binding property of imprinted polymers compared with structurally similar pyrimidine analogues like 5-fluoro uracil and uracil. Here the adsorption capacity is maximum for imprinted polymers than non-imprinted polymers. The adsorption capacity of thymine imprinted polymer is greater than that of other pyrimidine analogues. The other two pyrimidine analogues show the adsorption capacity similar to that of non-imprinted polymer. This is attributed to the non-specific adsorption of other analogues. The above results suggest that MWCNT-imprinted polymer for thymine shows maximum adsorption and highly selective and specific towards thymine. The selective determination depends on two factors. One of the important factor is the functional group which leaving behind the imprinted material, which can interact with the specific template molecule strongly. The other part is the size and shape leaving behind the imprinted material which recognizes the specific template during the rebinding process.

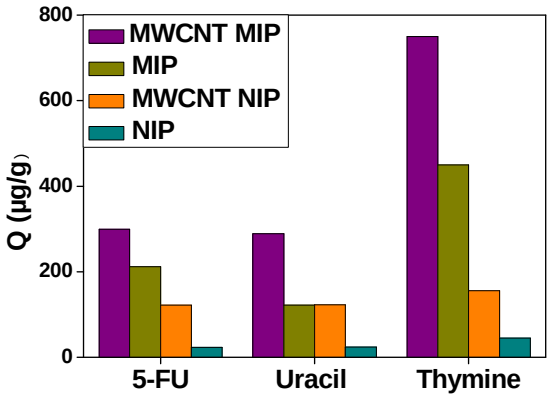

Figure 10. Selectivity studies of thymine with 5-fluoro uracil and uracil

Table 4. Separation factor and selectivity factor

Polymer	Separation factor (α) of Thymine	Separation factor (α) of 5-FU	Separation factor (α) of Uracil	Selectivity factor $= \frac{\alpha_{Thymine}}{\alpha_{5-FU}}$	Selectivity factor $= \frac{\alpha_{Thymine}}{\alpha_{Uracil}}$
MWCNT-MIP	5.11	1.12	1.01	4.56	5.05
MIP	3.13	1.10	1.00	2.84	3.13

The separation and selectivity factors calculated for MWCNT- imprinted polymer and normal imprinted polymer showed that MWCNT-supported imprinted polymer has high separation factor for the template thymine than 5-FU and uracil, the structural analogues of thymineb(Table 4). The normal imprinted polymer showed less selectivity characteristics compared to the imprinted system on MWCNT. This suggests that the synthesized MWCNT-imprinted polymer can effectively separate thymine from a mixture of closely related compounds.

CONCLUSION

The thymine imprinted polymer on MWCNT was synthesized and characterised with the conventional imprinted and corresponding non-imprinted polymers. The synthesized polymers were characterized by FT-IR, TGA, XRD, TEM and SEM characterization techniques. These techniques confirmed that imprinted polymer layer is successfully grafted on the surface of vinyl functionalized MWCNT. The adsorption characterization revealed that MWCNT-imprinted polymer adsorbed more thymine than conventional imprinted polymer. The adsorption isotherm obeys Langmuir model suggesting monolayer distribution of polymer on the surface of MWCNT. In the adsorption kinetic data are in agreement with second order kinetics. The selectivity characteristics of the imprinted polymer tailored on the MWCNT are superior to the conventional imprinted polymer. All these results suggest that molecular imprinting on MWCNT could improve the adsorption and selectivity characteristics of imprinted polymers.

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