



# SYNTHESIS AND CHARACTERIZATION OF MOLECULAR IMPRINTED POLYMER ON MULTIWALLED CARBON NANOTUBE FOR THE RECOGNITION OF 5-FLUORO URACIL

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## ABSTRACT

An artificial receptor for 5-fluoro uracil (5-FU) was synthesized by molecular imprinting on the surface of multiwalled carbon nanotube (MWCNT). This was done by *in situ* polymerization of methacrylic acid, ethylene glycol dimethacrylate in presence of 5-FU by free radical polymerization. After removing the template, the obtained material was able to rebind 5-FU and discriminate it among other interfering species. Recognition of 5-FU was confirmed by the non-rebinding ability displayed by non-imprinted materials, obtained without a template. FT-IR, SEM and X-ray diffraction techniques confirmed the surface modification of the MWCNT. The ability of this material to rebind 5-FU was confirmed by adsorption kinetics, adsorption isotherm, and selectivity. Good selectivity was observed against uracil, and thymine. It is expected that this work opens new horizons in the design of new artificial receptors.

**Key words:** MWCNT, Imprinting, 5-FU, Adsorption, Selectivity.

## INTRODUCTION

Molecular imprinting technique is a new method for preparing highly selective polymer receptors for given molecules<sup>1-4</sup>. Imprinted polymerization is a process, in which monomers are polymerized in a solution containing the specific analyte producing imprinted polymer, which is selective towards the target analyte. The ability to selectively recognize a target molecule in a pool of similar molecules is essential to biological and chemical processes. Imprinted polymers are easy to prepare, stable, inexpensive and can be reused and the polymers (MIP) can be used as materials of molecular recognition, such as separation, pre-concentration, constructing sensors, chromatography stationary phases, pseudo-immunoassay, catalysis<sup>5</sup>, and so on. The MIP synthesized by traditional bulk imprinting methods exhibit high affinity and selectivity but poor site accessibility to the target

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molecules. Meanwhile, the kinetics of the adsorption process is unfavorable and the mass transfer is slow<sup>6</sup>. Thus, in order to overcome these drawbacks, a few template-controlled imprinting techniques<sup>7,8</sup>, and techniques such as film surface imprinting technique have recently been reported<sup>9,10</sup>. These techniques try to place the imprinting cavities on the surfaces of solid particles (support matrix), which could provide MIPs with high selectivity, more accessible sites, faster mass transfer, and binding kinetics. The surface imprinting materials are more effective for recognizing the template molecules than the imprinting materials prepared with the conventional imprinting method. Nowadays, for different application requirements, many types of inorganic materials have been used as the support in surface imprinting techniques<sup>11-14</sup>. Nano-TiO<sub>2</sub>, as a multifunctional material, has increasingly attracted much attention.

The MIP can also be grafted on graphene by reversible addition fragmentation chain transfer polymerization. In these approaches the preparation of the composites of carbon nanotube and imprinted polymer, the good interfacial bonding and interaction between carbon nanomaterials and polymers were very important. In order to form uniform imprinted layer on the surface of MWCNT, carbon nanotube was modified with carboxyl group by strong acid treatment followed by vinyl group functionalization via covalent reaction, the monomer and crosslinker would couple with the vinyl group on the surface of MWCNT, forming uniform imprinted layer. Unfunctionized MWCNT could not be covered with the polymer layer<sup>15</sup>.

The main aim of this work is the direct preparation and characterization of 5-FU imprinted polymers on multiwalled carbon nanotubes (MWCNT MIP) that could exhibit better molecular recognition properties. Techniques such as Fourier transform infrared (FT-IR) spectroscopy, scanning electron microscopy (SEM), and X-ray diffraction techniques confirmed the surface modification of the MWCNT.

## **EXPERIMENTAL**

### **Materials and methods**

MWCNTs (internal diameter 2-6 nm, outer diameter 10-15 nm, length 0.1-10 nm, and purity > 90%), ethylene dimethacrylate (EGDMA), 2, 2' azoisobutyronitrile (AIBN), 2-hydroxy ethyl methacrylate (HEMA) were purchased from Sigma-Aldrich, Germany. Thionyl chloride (SOCl<sub>2</sub>), dimethylsulfoxide (DMSO), dimethylformamide (DMF), tetrahydrofuran (THF), triethylamine (TEA), and acetic acid (HOAc) 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.

The morphology was investigated by scanning electron microscopy (SEM) using a JEOL-JSM-6390. 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.

### **Synthesis of MWCNT-CH=CH<sub>2</sub>**

Crude MWCNT (0.5 g) were added to 60 mL of HNO<sub>3</sub> under sonication for 10 min. Then the mixture was stirred under 85°C for 16 h after cooling to room temperature, the mixture was filtered through polycarbonate membrane and washed thoroughly with distilled water for several times until the pH of the filtrate was neutral. The filtered solid was dried under vacuum, obtaining carboxylic acid functionalized MWCNT (MWCNT-COOH).

MWCNT-COOH (0.4 g) was suspended in the mixture of 10 mL of thionyl chloride and 30 mL chloroform at 60°C for 24 h under reflux. The solid was washed by anhydrous tetrahydrofuran for several times to remove excess SOCl<sub>2</sub> and dried under vacuum to give MWCNT-COCl. Then MWCNT-COCl (0.2 g) in 30 mL of anhydrous THF was added with HEMA (1.16 g), 4-DMAP (0.244 g), and tetraethyl amine (6.06 g). The mixture was stirred at 50°C for 24 h and then collected by centrifugation and washed with anhydrous THF. After washing and centrifugation, the resulting solid was dried overnight in vacuum desiccator, obtaining vinyl group functionalized MWCNT (MWCNT-CH=CH<sub>2</sub>).

### **Calculation of acid capacity**

MWCNT (50 mg) was taken in a round bottom flask and 10 mL of NaOH solution was added to this. It is stirred for about 4 h. The resultant solution was centrifuged and titrated against the 0.0055 N HCl solution, using phenolphthalein as indicator. The acid capacity of the functionalized MWCNT indicates the amount of functional group attached on the surface of MWCNT. So these functionalities can interact with other monomer units for imprinting process and bind the target molecule more effectively.

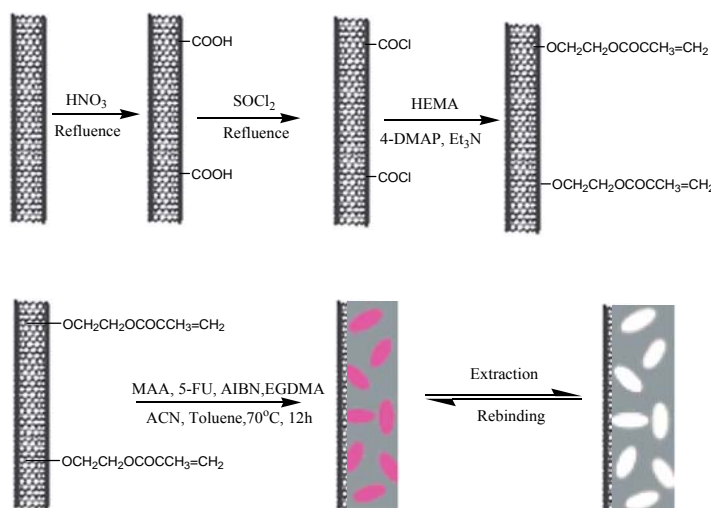
### **Synthesis of MWCNT-imprinted polymer**

MWCNT-imprinted polymer with 5-fluoro uracil as template molecule was prepared by selective polymerization of the imprinted polymer onto the vinyl functionalized MWCNT surface. In brief, MWCNT-CH=CH<sub>2</sub> was added to the solvent mixture of 15 mL of acetonitrile and 2 mL of toluene in a 250 mL round bottom flask and purged with N<sub>2</sub> under magnetic stirring. 5-FU and MAA dissolved in methanol (5 mL) was added to the reaction flask and sonicated for 10 min. AIBN was added and the temperature was increased to 70°C,

and the reaction was allowed to proceed for 12 h. The resulting product was collected by centrifugation and washing. The template was eluted by methanol until no 5-FU could be detected by UV-vis (266 nm) in the eluent. The obtained polymers were dried in the vacuum desiccator for 24 h. For comparison, blank polymers (MWCNT-non-imprinted polymer) were prepared by the same procedure, only without using the template molecule in the polymerization process. In order to reveal the distinctly formulated MWCNT, imprinted and non-imprinted polymers were also prepared without carbon nanotube by following the similar procedure as described above.

**Table 1: Preparation of various 5-FU imprinted and non-imprinted polymers**

Polymer	MAA (mmol)	EGDMA (mmol)	5-FU (mmol)	AIBN (g)	MWCNTCH = CH <sub>2</sub> (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



**Scheme 1: Synthesis of MWCNT-MIP**

### Binding experiments

Binding of 5-fluoro uracil from methanol solutions was investigated in batch experiments. Adsorption isotherm, kinetics and selectivity of the fabricated imprinted and

non-imprinted polymers were examined. The concentration of 5-fluoro uracil after desired treatment periods was analyzed using UV-vis. spectroscopy. The adsorption capacity was calculated as below –

$$Q = \frac{C_o - C_e}{M} \times V \quad \dots(1)$$

Where Q (mg/g) is the total adsorption of 5-fluoro uracil,  $C_o$  and  $C_e$  are initial and equilibrium concentration of 5-fluoro uracil in solution (mg/L). V (mL) is the volume of the solution and M (g) is the weight of imprinted and non-imprinted polymers.

The adsorption kinetics was studied by stirring imprinted and non-imprinted polymers in  $2.2 \times 10^{-7}$  mg/L 5-fluoro uracil for different time intervals. The adsorption isotherms were studied by treating imprinted and non-imprinted polymers in 5-fluoro uracil solution at different concentration ( $1.6 \times 10^{-7}$  -  $2.2 \times 10^{-7}$  mg/L). The effect of various solvents on the recovery of 5-fluoro uracil was studied by stirring imprinted and non-imprinted polymers in 5-fluoro uracil solution in various solvents such as methanol, acetonitrile and DMSO.

The selectivity experiments were carried out using thymine and uracil as the structurally similar pyrimidine analogue of 5-fluoro uracil in the binding process. The rebinding experiment was carried out by the incubation of MWCNT-imprinted polymers or MWCNT-non imprinted polymers in 5-fluoro uracil, thymine, and uracil solutions ( $2.2 \times 10^{-7}$  mg/L). After 3 h, the supernatant was transferred into the UV-vis. spectrophotometer cell to measure the absorbances of 5-fluoro uracil, thymine, and uracil at 266, 255, and 456 nm. The binding capacity was calculated using the above equation. The selectivity factor ( $\alpha$ ) 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.

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

$$K = \text{template bound}/\text{template free}$$

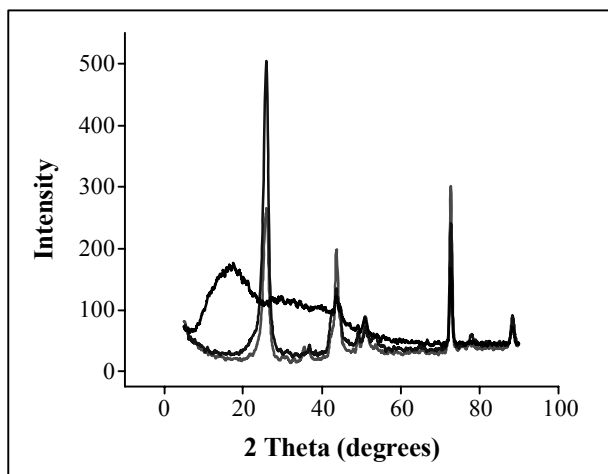
$$\text{Selectivity factor} = \alpha_{\text{template}} / \alpha_{\text{analogue}}$$

## RESULTS AND DISCUSSION

Before the polymerization vinyl group is introduced on the surface of MWCNT by treating the acid functionalized MWCNT with HEMA. This is polymerized with methacrylic acid and EGDMA by free radical polymerization<sup>16</sup>.

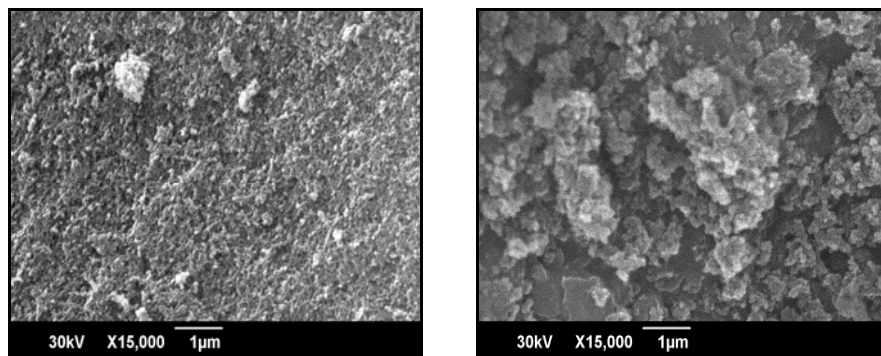
The FT-IR spectrum of MWCNT-CH<sub>2</sub>=CH<sub>2</sub> there is a C = O stretching peak at 1730 cm<sup>-1</sup> and C = C stretching vibration at a peak at 1632 cm<sup>-1</sup>. This confirmed that the vinyl group is successfully introduced on the surface of multiwalled carbon nanotube. In the case of MIP the main peaks are 3512, 1724, 1269 and 1145 cm<sup>-1</sup>, which correspond to OH stretching, C = O stretching and ester symmetric and asymmetric stretching respectively. All these prominent peaks are observed in the case of MWCNT-imprinted polymer. It suggests that imprinted polymer is grafted successfully on the surface of MWCNT.

The XRD patterns of the crude MWCNT, MWCNT-imprinted polymer and imprinted polymer without MWCNT are given in the Fig. 1. The X-ray diffractogram spectrum of the crude MWCNT shows the peak corresponding to the graphitic hexagonal planes (002) of MWCNT at 2 theta value 26.4°. In comparison with both spectra it is found that the main peaks of crude multiwalled nanotube almost overlap with multiwalled nanotube supported imprinted polymer. The diffraction peaks at 26°, 72°, and 88° from MWCNT-imprinted polymer are assigned. No diffraction peaks were observed in imprinted polymer. These suggested that the polymer was successfully fabricated on the MWCNT surface. The primary particle size calculated by Sherrer formula is about 8.9 nm<sup>17</sup>.



**Fig. 1: XRD Patterns of MWCNT, MWCNT-imprinted polymer and imprinted polymer**

The morphological structure of the MWCNT particles was observed with SEM, as given in Figure 2. This result could be used as a confirmation of the successful formation of MWCNT-imprinted polymer and revealed that the imprinted layer was successfully attached to the MWCNT surface. Furthermore, the surface of the MWCNT-imprinted polymer was thick due to the covering by EGDMA-crosslinked methacrylic acid polymer on its surface.



**Fig. 2: SEM photographs of the MWCNT and MWCNT-imprinted polymer**

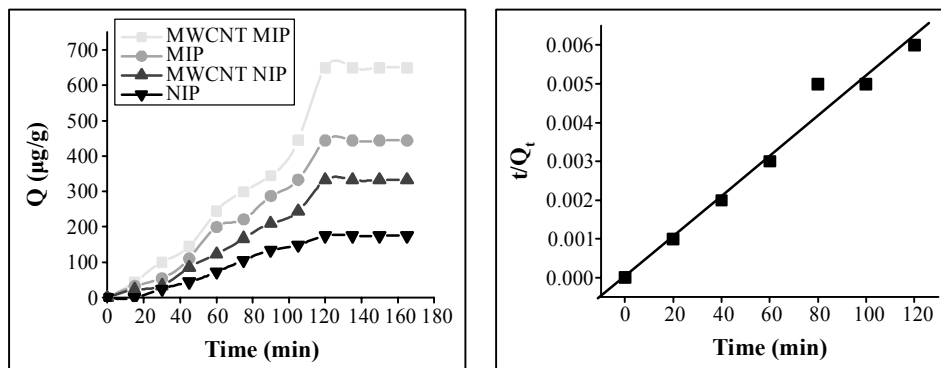
### Adsorption characteristics

Figure 3 shows the time dependence on the adsorption capacities of 5-FU towards imprinted polymer. As seen here, 5-FU adsorption increased with time during the first 20 min and then levels off as equilibrium was attained. This is due to high complexation between 5-FU and 5-FU cavities in the imprinted polymer structure. The removal of the template from the polymeric matrix leaves cavities of complementary size, shape and chemical functionality to the template. Maximum adsorption capacity is 650  $\mu\text{g/g}$ . It implies that MWCNT-imprinted polymer possesses high affinity for 5-FU. The sorption kinetic data of 5-FU was analyzed using the Langergen rate model.

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

where  $q_e$  and  $q_t$  are the amounts of 5-FU adsorbed ( $\text{mg g}^{-1}$ ) on the adsorbent at the equilibrium and at time  $t$  respectively.  $k_1$  ( $\text{min}^{-1}$ ) and  $k_2$  ( $\text{g mg}^{-1} \text{min}^{-1}$ ) are the adsorption rate constants, respectively. The value of  $k_1$  was calculated from the plots of  $\log(q_e - q_t)$  versus  $t$  by first-order, and  $k_2$  was obtained from plotting  $(t/q_t)$  versus  $t$  by the second-order.

As seen from Fig. 3 the correlation coefficients ( $R^2$ ) given by the two kinetic models are 0.9667 and 0.9993, respectively. Obviously, the second-order kinetic model shows a good correlation for the adsorption of 5-FU molecules on MWCNT-imprinted polymer. The adsorption kinetic constants and linear regression values are summarized in Table 2. These values show that there is a higher correlation coefficient between the experimental data with the second-order kinetic model. Thus, the adsorption behavior of 5-FU onto MWCNT-imprinted polymer follows the second-order kinetic model, and this indicates that the adsorption process is chemical in nature.



**Fig. 3: Effect of time on the adsorption of 5-FU on MWCNT-imprinted polymer and corresponding kinetics**

**Table 2: Kinetic parameters of the rate equation for 5-FU adsorption onto MWCNT-MIP**

First order kinetics		Second order kinetics	
$k_1$ ( $\text{min}^{-1}$ )	$R^2$	$k_2$ ( $\text{g mg}^{-1} \text{min}^{-1}$ )	$R^2$
0.0177	0.9667	2.0017	0.9993

The dependence of the initial concentration of 5-FU on its adsorption by the imprinted polymer was investigated. The adsorption values increased with increasing concentration of 5-FU, and saturation was achieved at an initial concentration of  $2.2 \times 10^{-7}$  mg/L. The level off represents saturation of the active binding cavities on the imprinted polymer.

During the batch experiments, adsorption isotherms were used to evaluate adsorption properties. For the systems considered, the Langmuir model was found to be applicable in interpreting 5-FU adsorption on the imprinted polymer. The Langmuir model is probably the best known and most widely applied sorption isotherm. It has produced good agreement with a wide variety of experiment data and may be represented as follows :

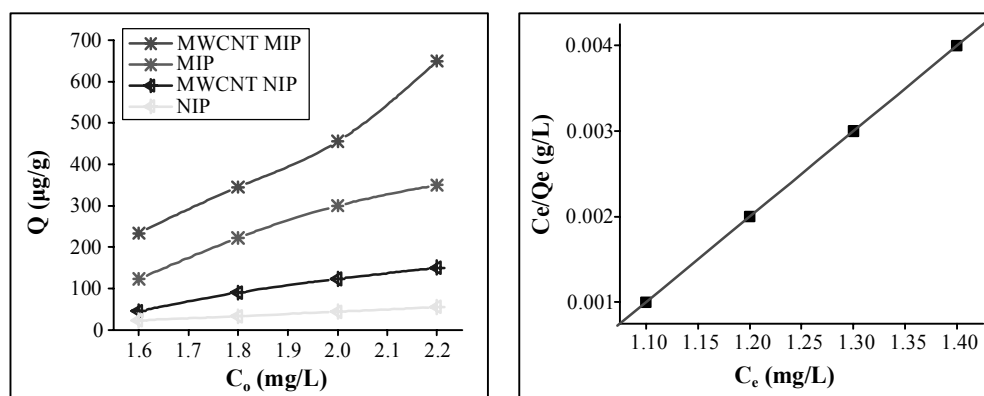
$$\log q_e = \frac{1}{n} \log C_e + \log K_F \quad \dots(3)$$

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

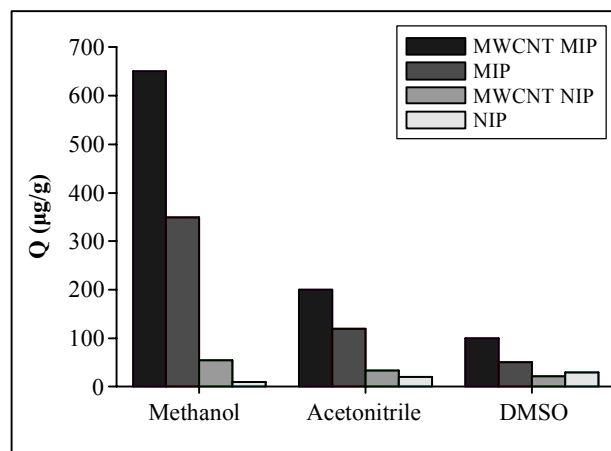
$$\frac{C_e}{q_e} = \frac{C_e}{q_m} + \frac{K_L}{q_m} \quad \dots(4)$$



where  $q_e$  ( $\text{mg g}^{-1}$ ) and  $C_e$  ( $\text{mg L}^{-1}$ ) are the amounts of 5-FU adsorbed on MWCNT-imprinted polymer and the concentration of 5-FU in the solution at equilibrium,  $q_m$  is the theoretical maximum adsorption capacity at monolayer ( $\text{mg g}^{-1}$ ),  $K_L$  ( $\text{mg L}^{-1}$ ) is the Langmuir constant (related to the affinity of adsorption sites), and  $K_F$  and  $n$  are Freundlich constants demonstrating adsorption capacity and intensity, respectively.  $K_F$  and  $n$  can be determined from a linear plot of  $\log q_e$  versus  $\log C_e$ . The data in Fig. 4 are treated using the Langmuir adsorption equation, and the straight line is displayed. The Langmuir equation is usually used to describe monolayer adsorption. In the present study, the Langmuir adsorption equation gave a better fit to the equilibrium adsorption data. Thereby, it indicates that the adsorption of 5-FU onto MWCNT-imprinted polymer occurs via a mono layer process.



**Fig. 4: Effect of initial concentration on the adsorption of 5-FU and Langmuir isotherm**

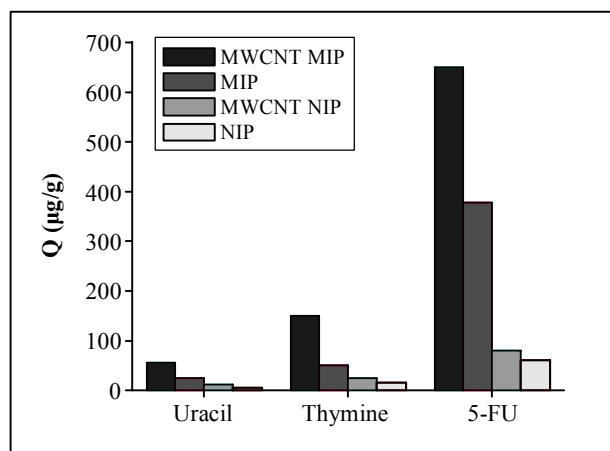


**Fig. 5: Effect of eluents on the adsorption of MWCNT-imprinted polymer**

In the study of the effect of solvent on 5-FU binding, the best result was noted in respect of methanol, whereas in other solvents 5-FU was retained by imprinted polymer. Relatively successful in eluting 5-FU from the imprinted polymers, however, the specific interactions between 5-FU and the MAA were stronger than the interactions between the template and the solvent, and recovery values were satisfactory due to polar protic properties. The non polar DMSO and acetonitrile also directly disrupted the recognition ability of the imprinted polymers, leading to a loss in the elution step for imprinted sorbents. In view of the above, methanol was selected as the elution solvent for eluting 5-FU from the imprinted polymers.

### Selectivity studies

Figure 6 shows the binding capacity of MWCNT-imprinted polymer and non-imprinted polymer towards 5-FU, thymine, and uracil respectively. It can be seen that the saturated adsorption capacity of 5-FU on MWCNT-imprinted polymer is higher than that on non-imprinted polymer. The binding capacity of the imprinted particles towards thymine is almost equal to that of the corresponding non-imprinted particles, but lower than those of 5-FU. In addition, the imprinting particles exhibited a lower adsorption capacity towards uracil than 5-FU. The corresponding non-imprinted particles even exhibited a higher binding capacity towards the two analogues. This suggests that the uptakes of thymine and uracil by non-imprinted polymer particles are predominately attributed to the non-specific adsorption. The above facts demonstrate that MWCNT-imprinted polymer has high affinity, high recognition ability, and special selectivity to 5-FU.



**Fig. 6: Selectivity studies of 5-fluoro uracil with thymine and uracil**

Competitive adsorption tests of 5-FU/thymine, and 5-FU/uracil on MWCNT-imprinted polymer were studied in static experiments. Table 3 summarizes the data of the distribution coefficients, selectivity coefficients and relative selectivity coefficients. It can be seen that the selectivity coefficients of MWCNT-imprinted polymer of 5-FU/Thymine, and 5-FU/Uracil increases due to the imprinting process. This suggests that the adsorption abilities of MWCNT-imprinted polymer for 5-FU are stronger than that for thymine, and uracil. Two parts determine the selective recognition and separation of 5-FU. One part is the functional groups left after the imprinting process, and these groups can interact with certain sites of 5-FU. Another part is the cavities with specific shape and size left after the imprinting process, because the cavities imprinted by 5-FU are not very well matched to the other three analogues in size, shape, and spatial arrangement of combining sites thymine are bigger than 5-FU in size, so they could not easily enter into the cavity imprinted by 5-FU, and also it is difficult for them to produce chemical bond interactions with combined sites. Uracil has a smaller molecular diameter than 5-FU but no specific sites; thereby it shows relatively smaller steric hindrance and a greater adsorption by MWCNT-imprinted polymer than other two analogues.

**Table 3: Separation factor and selectivity factor**

Polymer	Separation factor ( $\alpha$ ) of 5-FU	Separation factor ( $\alpha$ ) of Thymine	Separation factor ( $\alpha$ ) of Uracil	Selectivity factor = $\frac{\alpha_{5-FU}}{\alpha_{Thymine}}$	Selectivity factor = $\frac{\alpha_{5-FU}}{\alpha_{Uracil}}$
MWCNT MIP	6.22	1.02	1.10	6.09	5.65
MIP	3.02	1.0	1.01	2.99	3.13

## CONCLUSION

In the present work, we proposed a simple surface molecular imprinting method to prepare surface-modified vinyl-functionalized imprinted polymer on the surface of multiwalled carbon nanotube, in which MWCNT particles were used as the support matrix, MAA as the functional monomer, EGDMA as the cross-linker, and 5-FU as the template molecule. MWCNT-imprinted polymer exhibited an imprinting effect and was applied to the adsorption of pyrimidine analogues such as thymine and uracil. The kinetic adsorption data were best described by the second order kinetic model. The isotherm study showed that the experimental equilibrium data could be well fitted by the Langmuir adsorption model. Also high specific and selective binding of uracil is possible in the MWCNT support than the conventional imprinted polymer (MIP). Thus the nanostructured uracil imprinted EGDMA-

crosslinked methacrylic acid can be effectively used for the specific and selective concentration of 5-fluoro uracil.

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