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# Tailoring of Sorbents for the Selective Recognition of Tyramine based on Molecular Imprinting Approach on Multiwalled Carbon Nanotubes

**Rohini Ashok and Beena Mathew**
School of Chemical Sciences, Mahatma Gandhi University, Kottayam 686560, Kerala, INDIA

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## Abstract

*Core-shell nano structured molecularly imprinted polymers (MIPs) for the selective separation of tyramine have been synthesized by free radical polymerization technique using vinyl functionalized multiwalled carbon nanotubes as support material and tyramine as template molecule. Functional monomer and crosslinker used for the polymerization process are methacrylic acid and ethylene glycol dimethacrylate respectively. Core–shell molecularly imprinted polymer overcomes the problems associated with the template transfer and increases the binding capacity. Porogen used is a mixture solution of acetonitrile and toluene. Morphology of the synthesized MWCNT- MIP composite was characterized by Fourier transform infrared spectroscopy (FT-IR), X-ray diffraction technique (XRD) and scanning electron microscopy (SEM) which confirmed homogeneous formation of MIP on the surface of multiwalled carbon nanotubes. Investigation of adsorption and kinetic characteristics revealed that the MWCNT-MIP composites contain homogeneous binding sites and have high binding capacity. Selectivity of the synthesized polymer was evaluated using dopamine as the structurally related compound which demonstrated that imprinted polymer has high selectivity towards tyramine.*

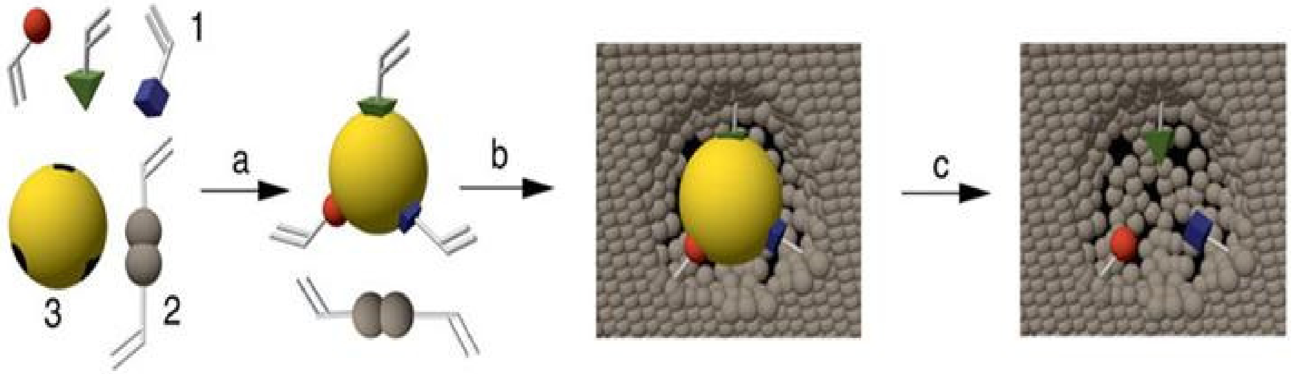
**Keywords**: Molecular imprinting, core-shell MIP, MWCNT, tyramine, molecular recognition.

## Introduction

Tyramine (p-hydroxyphenylethylamine) is a biogenic amine and it is participated in many of the metabolic processes of living organisms. In foods, it is often produced by the decarboxylation of tyrosine during fermentation or decay by microbial activity. Usually aged and fermented food like sauces, wine, beer, cheese, cured meat etc contains higher concentration of tyramine. The consumption of food containing higher concentration of tyramine may trigger migraine and increase blood pressure[1]. Degree of freshness or spoilage in food which is usually associated with the insufficient sanitary conditions during the production process is indicated by the concentration of tyramine in food samples[2]. Hence a highly sensitive method for the separation of tyramine from food samples would be valuable. High performance liquid chromatography (HPLC) and capillary electrophoresis, combined with different detection techniques like spectrophotometric, fluorometric, electrochemical and mass spectrometric methods are used for the detection and quantification of biogenic amines[3-5].

Molecular imprinting is an advanced technique for the synthesis of artificial biological macromolecular receptors that are able to recognize and bind different target molecules with high affinity and specificity[6]. Molecularly imprinted polymers (MIPs) have many advantages, such as easy synthesis, low cost, physical robustness, chemical stability selectivity and reusability[7]. Because of its large number of advantages MIPs have been used for a wide variety of applications, such as solid-phase extractions, analytical separations, chemical sensing, drug delivery, catalysis and artificial antibodies[8-12]. The recognition sites are created in situ by a copolymerization process between functional monomer, an excess of crosslinker and template molecule[13]. After polymerization, the template is removed from the polymer matrix by washing it with a suitable solvent or by heating without affecting the polymer geometry. The removal of the template from polymer matrix leaves binding cavities complimentary to the template in size and shape (scheme-1). MIPs prepared by conventional bulk polymerization have several disadvantages such as poor accessibility, low-affinity binding and high diffusion barrier to the template, as the template molecules were embedded inside the thick polymer network[14]. To avoid these drawbacks, a core–shell approach has been developed in which a thin MIP layer is deposited on the surface of a solid support such as multiwalled carbon nanotubes, monodispersed polystyrene seeds and silica spheres etc[15].

In this work, a novel molecularly imprinted polymers based on multiwalled carbon nanotubes (MWCNT-MIP) for the separation of tyramine have been developed using tyramine (TA) as template, methacrylic acid (MAA) as functional monomer and ethylene glycol dimethacrylate (EGDMA) as crosslinker. In order to form uniform MIP layer on MWCNTs and to improve the interfacial interaction and bonding between carbon nanotubes and polymer, MWCNTs are modified with carboxyl functional groups which is then converted into vinyl functionalized MWCNTs via covalent reaction.



1. Functional monomers
2. Crosslinker
3. Template molecule

a. Assembly of the pre-polymerization complex
b. Polymerization
c. Extraction of the template liberating the binding site

**Scheme-1**
**Molecular imprinting technique**

Then, EGDMA and MAA would couple with the vinyl groups on the surface of MWCNTs, forming uniform MIP layer. It is not possible to graft the polymer layer on the surface of unfunctionalized MWCNTs.

## Material and Methods

MWCNTs (internal diameter 2–6 nm, outer diameter 10–15 nm, length 0.1–10 m, and purity > 90%), ethylene glycol dimethacrylate (EGDMA), 2,2'- azobisisobutyronitrile (AIBN), were purchased from Sigma-Aldrich (Germany)). Thionylchloride ($SOCl_2$), dimethlysulfoxide (DMSO), dimethylformamide (DMF) and tetrahydrofuran (THF) were obtained from Merck (India). Triethylamine (TEA) and methacrylic acid (MAA) were obtained from SRL (India). Tyramine and dopamine were purchased from Sigma-Aldrich (Germany).

The morphology was investigated by scanning electron microscopy (SEM) using a JEOL-JSM-6390. Absorption spectra of, tyramine were recorded by Shimadzu UV-vis 2450 spectrophotometer. The FT-IR studies were carried out using Perkin-Elmer 400 FT-IR spectrophotometer. X- Ray diffractogram was recorded by PAN analytic XPERT- PRO.

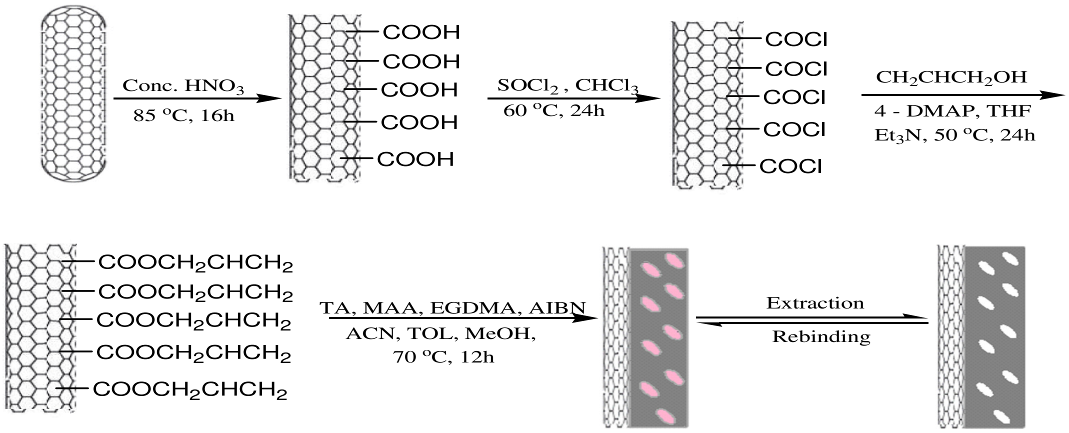
**Synthesis of MWCNTs-CH=CH$_2$.:** 0.5 g MWCNTs were added to 60 mL of $HNO_3$, sonicated for 10 min and refluxed at 85°C for 16 h. After cooling to room temperature, the mixture was filtered through a 0.22-μm polycarbonate membrane and washed thoroughly with distilled water for several times until the pH value of the filtrate was neutral. The filtered solid was dried under vacuum, obtaining carboxylic acid-functionalized MWCNTs (MWCNTs-COOH).

0.4 g MWCNTs-COOH was suspended in a mixture of 10 mL sulfoxide chloride ($SOCl_2$) and 30 mL chloroform and refluxed for 24 h at 60°C. The solid was washed by anhydrous tetrahydrofuran (THF) for several times to remove the excess $SOCl_2$ and dried under vacuum to obtain MWCNTs-COCl. Then 0.2 g MWCNTs-COCl in 30 mL of anhydrous THF was added with 1.16 g allyl alcohol, 0.244 g 4-DMAP and 6.06 g triethylamine. The mixture was refluxed 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 a vacuum desiccator, obtaining vinyl group functionalized MWCNTs (MWCNTs-CH=CH$_2$)[15].

**Synthesis of MIP on the surface of vinyl functionalized MWCNTs:** MWCNT-MIP composites with tyramine as a template molecule were prepared by selectively polymerizing MIP onto the vinyl group functionalized MWCNT surface (scheme-2). Molar amounts of MWCNT-CH=CH$_2$, TA, MAA and EGDMA taken for the synthesis of different polymers were given in the table-1. MWCNT-CH=CH$_2$ was added to the mixture solvent of 30 mL of acetonitrile and 5 mL of toluene in a 250-mL round-bottom flask and purged with $N_2$ under magnetic stirring. TA and MAA dissolved by 5 mL of methanol were added to the MWCNT-CH=CH$_2$ and stirred for 30 minute to form a complex of tyramine and MAA. Then EGDMA and AIBN were also added. Then the reaction temperature was increased to 70 °C, and the reaction was allowed to proceed for 12 hour. The obtained polymer was collected by centrifugation and washed with ethanol to remove the excess reagents. Then the composite was eluted by methanol for several times to extract the template molecules until no TA could be detected by UV-vis (at 277 nm) in the eluent. The obtained polymers were dried in the vacuum desiccator for 24 hour before use. For comparison, blank polymers (MWCNT-NIPs) were prepared by the same procedure, only without using the template molecule in the polymerization process. To get an insight into the role of MWCNTs, tyramine imprinted and non-imprinted polymers without using carbon nanotubes were also prepared[16].

**Table-1**
**Preparation of MWCNT-MIP composites**

| Polymer | MWCNT-CH=CH$_2$ (g) | TA (mmol) | MAA (mmol) | EGDMA (mmol) | AIBN (mg) |
|---|---|---|---|---|---|
| MWCNT-MIP | 0.02 | 0.05 | 0.25 | 1.25 | 10 |
| MWCNT-NIP | 0.02 | 0 | 0.25 | 1.25 | 10 |
| MIP | 0 | 0.05 | 0.25 | 1.25 | 10 |
| NIP | 0 | 0 | 0.25 | 1.25 | 10 |



**Scheme-2**
**Schematic representation of fabrication of MWCNT-MIP**

**Binding experiment:** In binding isotherm experiments, 10 mg of the polymer particles were equilibrated with 10 mL of tyramine solutions with different initial concentrations ranging from 0.2 to 1.2 mmolL$^{-1}$ for 2 h at room temperature. After static adsorption, the solutions were centrifuged and the concentration of free tyramine in the supernatant was determined with a UV–vis spectrometer at 277 nm. The amount of tyramine bound to the polymer was calculated by subtracting the concentration of the unbound template molecule from the initial concentration. Similarly, the adsorption dynamics of the MWCNT-MIP was performed by detecting the free tyramine concentration in the supernate at different adsorption time intervals. Before binding experiments, a calibration curve was obtained from the UV–vis spectra of the tyramine solutions with different concentrations. The adsorption capacity (Q) was calculated from the equation

$$Q = \frac{(C_0 - C_e)}{m} \times V \quad \longrightarrow \quad (1)$$

where Q (µmol g$^{-1}$) is the adsorption capacity, V (mL) is the volume of the solution, m (g) is the weight of polymer, C$_0$ and C$_e$ (mmol L$^{-1}$) are the concentrations of template before and after adsorption, respectively.

The adsorption kinetics studies were performed by changing the adsorption time from 0 to 90 min while keeping the initial concentration of tyramine constant. The special selectivity was studied by using dopamine (DA) as a structurally related compound. The chemical structures of tyramine and dopamine are given in figure-1.

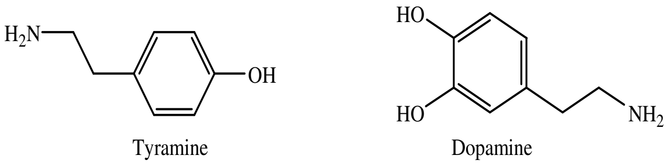


**Figure-1**
**Chemical structures of tyramine and dopamine**

The separation factor (α) represents the effect of the imprinting process which is the ratio of the amount of substrate bound by the MIP to that bound by the corresponding NIP. The selectivity of the imprinted polymers towards the template was calculated in terms of selectivity factor (a). Separation and selectivity factor were calculated using the equations

Separation factor, $\alpha_{Template} = \frac{K_{MIP}}{K_{NIP}}$, $\longrightarrow$ (2)

where, $K = \frac{Template_{Bound}}{Template_{Free}}$ $\longrightarrow$ (3)

Selectivity factor , $a = \frac{\alpha_{Template}}{\alpha_{Analogue}}$ $\longrightarrow$ (4)

## Results and Discussion

**Characterization:** MWCNT-CH=CH$_2$, MWCNT-MIPs, and pure MIPs were characterized by FT-IR spectroscopy (Figure-2). Vinyl functionalized MWCNTs have peaks at 1735 cm$^{-1}$ and 1628 cm$^{-1}$ which were assigned to C=O and C=C stretching vibrations respectively. These results clearly indicate that the vinyl group was successfully introduced onto the MWCNTs surface. Besides the characteristic peaks of MWCNT-CH=CH$_2$, the pure MIPs characteristic peaks also appeared in the spectrum of MWCNTs-MIPs. The peaks of pure MIPs situating around 1728, 1262, and 1147 $cm^{-1}$ were assigned to the C=O stretching vibration of carboxylic, C-O stretching vibration of symmetric and asymmetric ester, respectively. Figure-3 shows typical XRD patterns of MWCNT, MWCNT-MIP and MIP. The reflection peaks at 43.6°, 49.1° and 72.6° were observed at the same 2θ values in both MWCNT and MWCNT-MIP polymer. All these results suggested the synthesis of MIP on the surface of MWCNTs. In order to know the surface morphology, MWCNT and MWCNT-MIP were characterized by SEM (figure-4). SEM images suggest that MWCNT-MIP have a rough surface compared to MWCNT and this may be due to the formation of binding sites on the surface of MWCNTs.

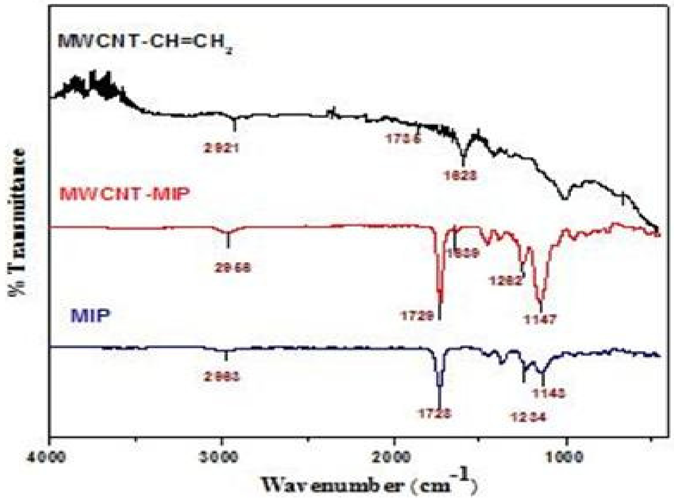


**Figure-2**
**FT-IR spectra of MWCNT-CH=CH$_2$, MWCNT-MIP and MIP**

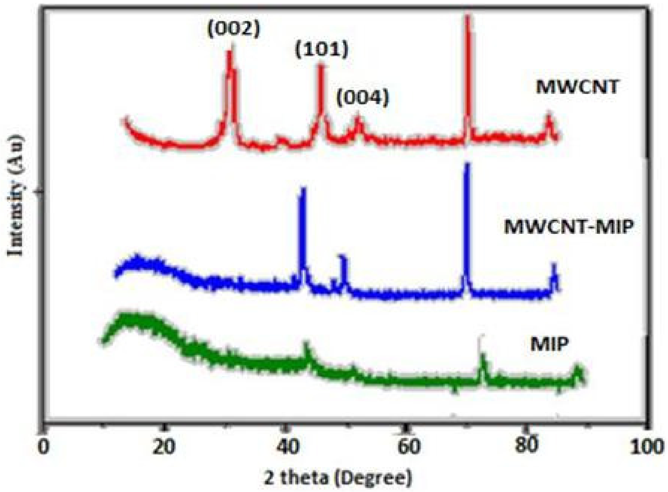


**Figure-3**
**XRD pattern of MWCNT, MWCNT-MIP and MIP**

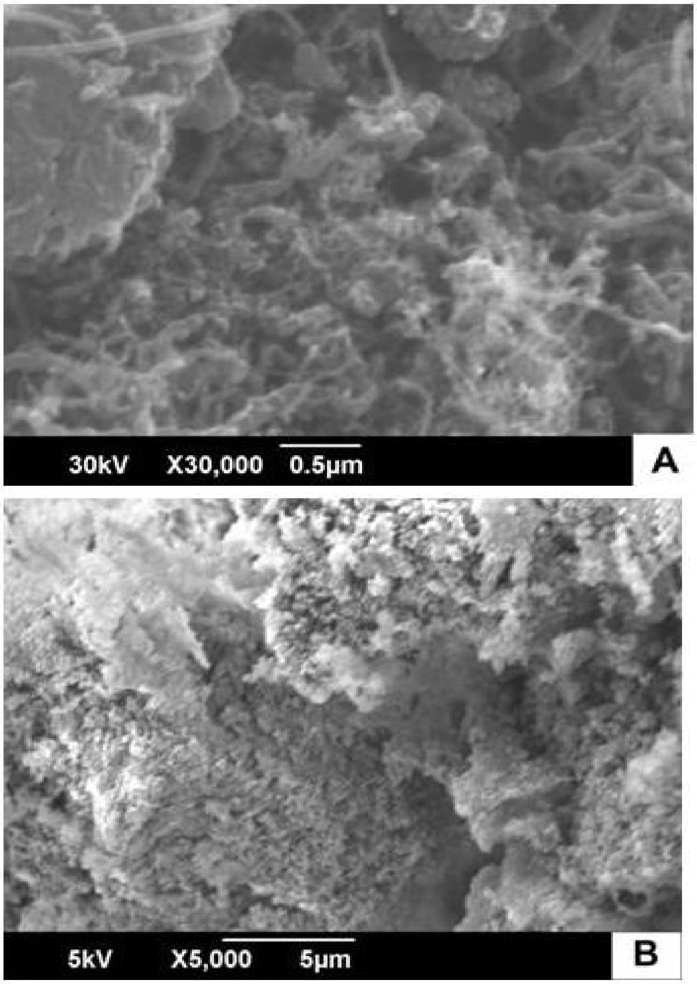


**Figure-4**
**SEM images of (A) MWCNT (B) MWCNT-MIP**

**Binding studies:** Binding studies show that the amounts of tyramine bound to MWCNT-MIP at equilibrium concentration increased along with the increase of the initial concentration of tyramine, but the binding capacity of MWCNT-MIP for tyramine was greater than that of MIP, MWCNT-NIP and NIP in the whole concentration range (figure-5a). In MWCNT-MIP most of the binding sites are situated on the outer MIP layer on the surface of MWCNT, which is in the nanometer range and hence the binding sites are easily accessible and homogeneous. The weak adsorption of MWCNT-NIP and NIP may be due to the non-specific interaction of template with the polymer matrix due to the lack of recognition cavities.

The tyramine adsorption isotherm better fitted for the Langmuir isotherm model (figure-5b). According to Langmuir's isotherm equation

$$\frac{C_e}{Q} = \frac{1}{K_m Q_m} + \frac{C_e}{Q_m} \qquad (5)$$

where $C_e$ is the equilibrium concentration, Q is the amount of template bound per unit weight of MIP and $Q_m$ is the theoretical maximum adsorption capacity and $K_m$ is the adsorption equilibrium constant. Langmuir model of isotherm indicate the homogeneous distribution of binding sites and the monolayer coverage of adsorbent on the MWCNT-MIP composites.

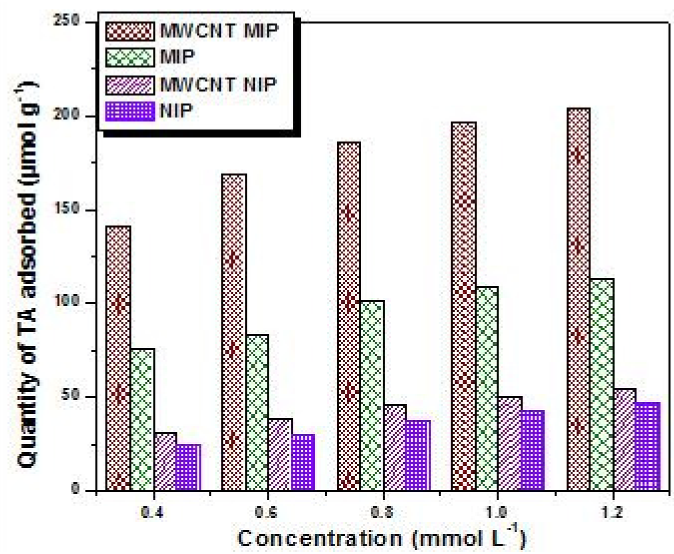


**Figure-5a**
**Binding isotherm of MWCNT-MIP, MIP, MWCNT-NIP and NIP**

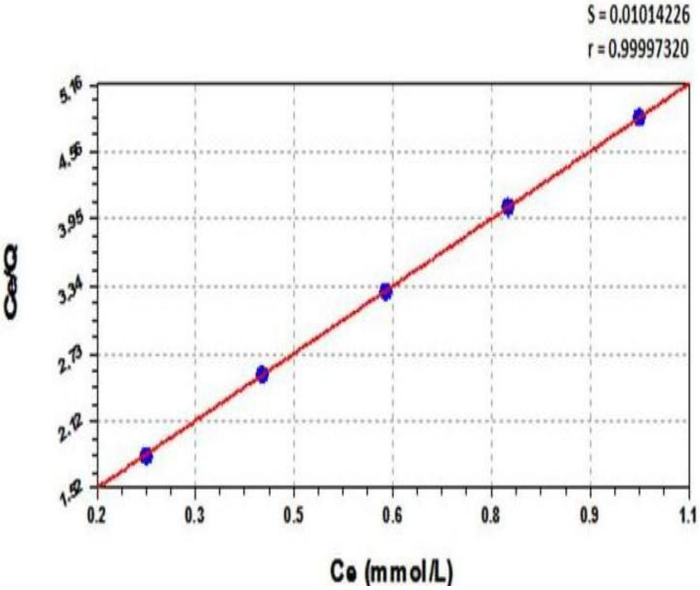


**Figure-5b**
**Adsorption isotherm of tyramine on the MWCNT-MIP (Langmuir)**

**Adsorption kinetics:** The kinetics of adsorption of tyramine on to the MWCNT-MIP was evaluated by measuring the absorbance as a function of time. Kinetic study shows that in the initial stage MWCNT-MIP composites have large no of unoccupied binding sites in the surface which help the template to bind easily and the adsorption rate increases rapidly within 20 min. As the time increases, adsorption rate slowed down because tyramine occupied most of the binding sites and and finally the maximum adsorption capacity was reached within 75 min (figure-6a). Kinetic studies revealed that adsorption of tyramine on MWCNT-MIP follows linear form of second order rate equation[17] (figure-6b).

$$\frac{t}{Q_t}=\frac{1}{k_2 Q_e^2}+\frac{t}{Q_e} \longrightarrow \quad (6)$$

where $Q_e$ and $Q_t$ are the amount of template adsorbed at equilibrium and at a given time t respectively and $k_2$ is the second order rate constant.

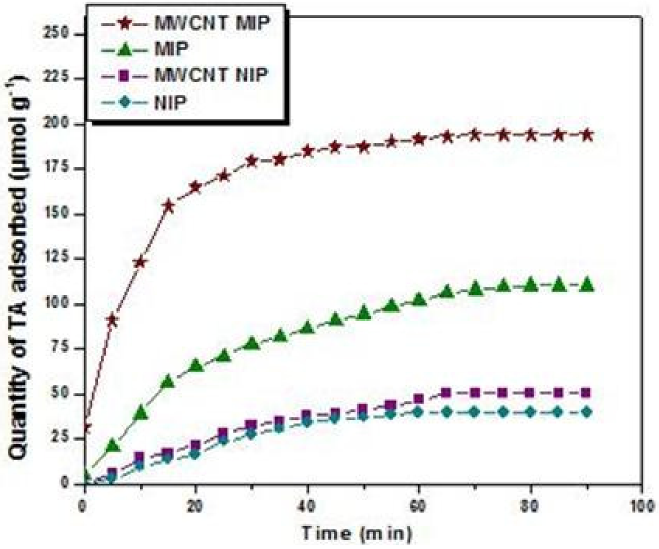


**Figure-6a**
**Effect of time on polymer**

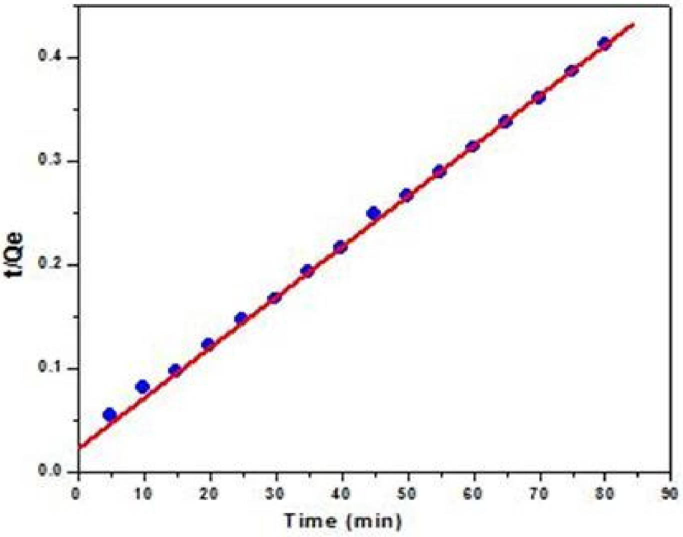


**Figure-6b**
**Adsorption kinetics of MWCNT-MIP**

**Table-2**
**Separation factor and selectivity factor**

| | **Separation factor (α) of Tyramine** | **Separation factor (α) of Dopamine** | **Selectivity factor,** $\alpha = \frac{\alpha_{Template}}{\alpha_{Analogue}}$ |
|---|---|---|---|
| $\alpha_{Template} = \frac{K_{CNT\ MIP}}{K_{CNT\ NIP}}$ | 4.586 | 1.136 | 4.038 |
| $\alpha_{Template} = \frac{K_{MIP}}{K_{NIP}}$ | 2.747 | 1.387 | 1.981 |

**Selectivity:** Adsorption experiment was performed to estimate the selectivity of MWCNT-MIP composites using dopamine as the structure related compound. The selectivity experiment shows that the binding capacity of MWCNT-MIP towards dopamine is much lower than that of tyramine, which confirmed that the MWCNT-MIP have a good selectivity for recognition of tyramine (figure-7). The selective recognition of MWCNT-MIP for tyramine may be attributed to the shape selective fitting of tyramine into complementary cavities created in the MWCNT-MIP during the imprinting procedure. The separation and selectivity factor were calculated for MWCNT supported MIPs and normal MIPs and the results showed that MWCNT supported MIP has higher separation factor in the case of tyramine which is low for normal MIP system and MWCNT-MIP composites have higher selectivity factor also (table-2). This suggests that the synthesized MWCNT-MIP can effectively separate tyramine from a mixture of closely related compounds.

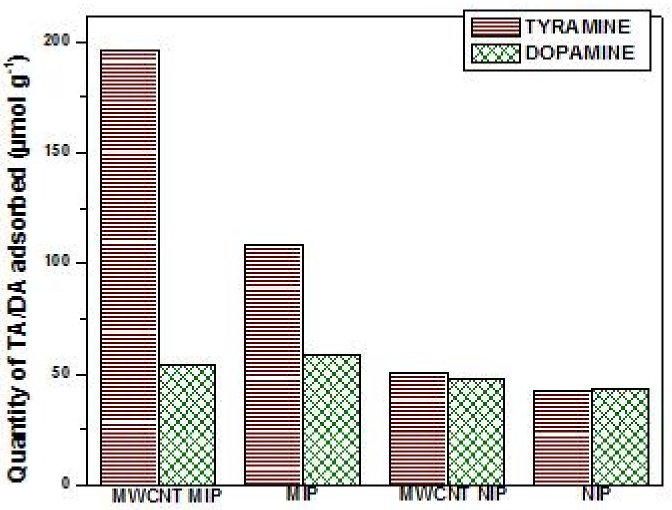


**Figure-7**
**Selectivity of the tyramine imprinted polymer**

## Conclusion

A novel MWCNT-MIP composite for the selective separation of tyramine has been developed. Compared to bulk polymerization preparation of nanolayer MIP on the MWCNT surface could improve the porous site availability. Vinyl group introduced on the surface of MWCNTs can act as a coupling backbone for the polymerization of functional monomers and cross linkers in the presence of tyramine. Homogeneous formation of MIP on the surface of MWCNTs was confirmed by FT-IR spectroscopy, SEM and XRD. Adsorption isotherm suggests the well conformity of experimental data with Langmuir isotherm model which confirms the monolayer adsorption of tyramine on the surface of MWCNT-MIP. Kinetic studies revealed that adsorption of tyramine on MWCNT-MIP follow a second order kinetics. The resulting MWCNT-MIP composites exhibited higher binding capacity, faster adsorption dynamics and higher selectivity for tyramine and hence it can be used in chromatographic separation as stationary phases, sensors and solid phase extraction etc.

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