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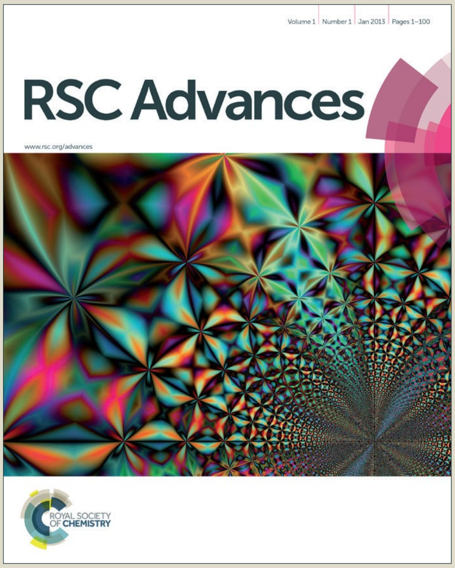

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Bifunctional monomers molecularly imprinted polymers based on the surface of multi walled carbon nanotubes for solid-phase extraction of tartrazine from drinks

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Abstract

A novel composite material of multi-walled carbon nanotubes (MWNTs) -molecularly imprinted polymers (MIPs) for tartrazine assay was synthesized by surface molecular imprinting technique. The molecularly imprinted polymers were prepared with Beta-Cyclodextrins and [2-(methacryloyloxy) ethyl] trimethylammonium chloride (DMC) as bifunctional monomers. The MWNTs–MIPs were characterized by transmission electron microscope (TEM) and Fourier transform infrared spectroscopy (FT-IR). Adsorption experiments showed that the MWNTs–MIPs exhibited high capacity and favourable selectivity toward tartrazine, the maximum adsorption capacity, q_m, and distribution coefficient, k_d, were estimated to be 135.15 mg g^{-1} and 14.72, respectively. A SPE-HPLC analytical method was developed and used to assay tartrazine in five drinks. The recoveries were in the range from 88.6-102.4% with relative standard deviation below 5.0%. The contents of tartrazine are all lower than the permitted maximum limit set by the European Commission and Ministry of Health of the People's Republic of China. t test demonstrated this method had no significant difference with China standard method of GB/T 5009.35-2003 at P=0.95.

Key words: Molecular imprinting; Multi-walled carbon nanotubes; Tartrazine; Bifunctional monomers; Solid-phase extraction; High performance liquid chromatography;

1. Introduction

Recently, molecular imprinted polymers (MIPs) have attracted tremendous interest owing to their distinctive advantages, including predetermined recognition ability, mechanical and chemical stability, durability, reusability and lower preparation costs.[1] MIPs can act as sorbents, are usually used in solid-phase extraction (SPE) for separations and enrichments.[2-4] Conventional bulk MIPs exhibit low-affinity binding and low-rate mass transfer, to overcome these disadvantages, the surface molecular imprinting techniques have been developed, the advantages of surface molecular imprinting include improving mass transfer, increasing affinity binding and decreasing high diffusion barrier of the template by fixing MIPs on a support substrate.[5-7] To date, various materials, such as magnetic nanoparticles,[8,9] hydroxyapatite,[10] stainless steel fiber,[11] silica gel,[12] and TiO_2 were used as the support matrixes for preparation of MIPs.[13] Multi-walled carbon nanotubes (MWNTs) with unique mechanical properties and extremely large surface areas can be an excellent candidate as available support material due to the binding sites in the outer layer of the composite will improve the accessibility of the template molecule and reduce the binding time.[14-16] In order to improve the weak interfacial adhesion, covalent and noncovalent functionalization of MWNTs is commonly used. Vinyl group and amine group are selected to link to the surface of MWNTs by covalent bonding resulting in strong interfacial interactions between the MWNTs and MIPs.[17]

In the process of MIPs synthesis, in order to strengthen multi-noncovalent interactions between the template and functional monomers, bifunctional MIPs were the best choice due to their high adsorption capacity and selectivity. Xu and co-workers synthesized parathion-methyl-templated MIPs using methacrylamide and 4-vinyl pyridine as bifunctional monomers, and the MIP was successfully used to detect the pesticide in the soil.[18] Zhang and co-workers adopted acryloyl-β-cyclodextrin and methacrylic acid as stimuli-recognition element toward erythromycin imprinting.[19] In our previous work, we had synthesized a novel bifunctional MIPs using Beta-Cyclodextrins-maleic anhydride and [2-(methacryloyloxy)ethyl] trimethylammonium chloride as co-functional monomers and utilized it as sorbents in SPE to extract congo red in food.[20]

Synthetic colorants are usually added to food products, most contain azo (N=N) functional groups and aromatic ring structures which are harmful to human health.[21] Tartrazine is one of the most common azo colorants, high performance liquid chromatography (HPLC) is the most frequently used method to determine.[22] Meanwhile, an enrichment procdure by molecularly imprinted solid-phase extraction (MISPE) is usually essential when especially for food sample analysis by HPLC method.[23]

In this paper, a novel MIPs with an improved selectivity for tartrazine on the surface of MWNTs was synthesised. Beta-Cyclodextrins (β-CD) and

[2-(methacryloyloxy) ethyl] trimethylammonium chloride (DMC) were choose as co-functionals, the performances of the MIPs utilized as solid phase extraction sorbents were investigated in detail, the results demonstrated that the MWNTs-MIPs could selectively recognize tartrazine. Combing SPE with HPLC techniques, the MWNTs-MIPs were used for selective preconcentration of tartrazine from five dink samples successfully.

2. Materials and methods

2.1. Reagent and chemicals

Hydroxyl Multi-wall carbon nanotubes (95%, outer diameters≤8nm, lengths ranging from 10μm to 30μm) were purchased from Chengdu institute of organic chemistry, Chinese academy of sciences. Tartrazine, carmine and sunset yellow were supplied by Pure Crystal Shanghai Reagent Co. Ltd. (Shanghai, China). Their chemical structures are shown in **Fig. 1**. DMC, N,N–methylenebisacryl amide (MBA), methyl methyl acrylate (MMA), dimethyformamide (DMF) and ammonium persulfate (APS) were obtained from Gaoyu Special Chemical Co. Ltd (Tianjin, China). Beta-Cyclodextrins sodium (β-$CD^{-}Na^{+}$), γ-(2,3-epoxypropoxy)propyltrimethoxysilane (KH-560) were bought from Nanjing Shuguang Chemical Group Co, Ltd. (Jiangsu, China). Methanol, acetic acid, ammonia solution (28.0%) and ammonium acetate were obtained from Guangzhou Chemical Reagent Factory (Guangzhou, China). HPLC-grade methanol and water were purchased from Burdick & Jlllackson (USA) and Hangzhou Wahaha Group (Zhejiang, China), respectively.

Fig. 1

2.2. Instruments

Tecnai G2 F20 S-TWIN microscope was used to obtain transmission electron microscopy (TEM) images (USA). The solid phase extraction experiment was conducted with an ASE-12 solid phase extraction system from Automatic Science Instrument Co. Ltd (Tianjin, China). The spectrums were obtained by Shimadzu UV-2550 spectrophotometer. 10.0 μL of solution was analyzed by an Agilent 1100

HPLC system (USA). The separations were carried out on a Germil-p C18 column (150 mm × 4.6 mm, 5 μm) from Wu-Ben Biotechnology Co. Ltd (Xi'an, China). Isocratic elution was carried out with methanol-0.02 M ammonium acetate buffer solution (10:90, v/v) as the mobile phase at a flow rate of 1.0 mL/min, and filtered through a 0.45 μm membrane filter prior to use. Column oven temperature was set at 30℃. The detection of analytes was carried out with the photodiode-array UC detector at 254 nm.

2.3. Preparation of MWNTs grafted epoxy (MWNTs-epoxy)

Hydroxyl MWNTs (4.00 g) was dispersed in 30.0 mL of DMF and sonicated for 30 min, 1.0 mL of acetic acid was added slowly to the slution under the vigorously stirring and the mixture was heated to 90℃, then KH-560 slution (2.8 mL KH-560 in 20.0 mL DMF) was dropped under mechanical stirring and nitrogen protection, the reaction mixture was stirred for 12 h to obtain epoxy groups functionalized MWNTs.

2.4. Synthesis of MWNTs-methyl methyl acrylate/Beta-Cyclodextrins (MWNTs-MMA/β-CD)

β-CD$^-$ Na$^+$ (4.80 g) was transferred into MWNTs-epoxy solution and reacted at 90℃ for 12 h. Then 1.8 mL of MMA and 80 μL of $BF_3{\cdot}Et_2O$ were added to the mixture, which was incubated 12 h under stirring for prepolymerization at 45℃. A black precipitate obtained was filtrated and washed orderly several times with DMF, methanol, acetone, the abtained MWNTs-MMA/β-CD was dried under vacuum at 30℃ for preparation of MWNTs-MIPs.

2.5. Preparation of tartrazine imprinted polymers（MWNTs-MIPs）

MWNTs-MMA/β-CD (4.00 g), tartrazine (0.25 g) and 50.0 mL of 0.05M sodium phosphate buffer of pH=7.0 were mixed under sonication for 0.5 h and stirred for 24 h at 60℃. Then 120 μL DMC was added to the previous pre-polymerization mixture, after the process of 4 h self-assembly, APS (0.05 g) and MBA (0.75 g) were added and the mixture was purged with nitrogen to remove oxygen for 30 min. Next, the flask was sealed and left polymerizing at 60℃ for 24 h. The resulting particles were washed by methanol-ammonia (7:3, v/v) solution in a Soxhlet extraction system until no tartrazine could be detected by HPLC. For comparison, non-imprinted polymers (NIPs) were prepared by the same procedure, only without using the template molecule in the polymerization process.

2.6. Preparation of SPE column

The SPE columns dry-packed with 100.0 mg of the MWNTs-MIPs or MWNTs-NIPs were leached with methanol (10 mL) and water (10 mL) orderly, stored in a vacuum oven. Prior to loading the sample, the column was preconditioned with 5.0 mL of methanol and 5.0 mL of water in succession.

2.7. Adsorption study

An amount of 50.0 mg of the MWNTs-MIPs, MWNTs-NIPs or raw MWNTs was suspended in 25.0 mL of water solutions with initial concentration of tartrazine ranging from 0.1 to 6.0 mmol L^{-1} for 3 h at 25 and the tube was sealed and oscillated at regular time interval, the mixture was centrifuged and the concentration of free tartrazine in the supernate was measured by HPLC.

Competitive adsorption of tartrazine and its structurally similar compound carmine and sunset yellow from their mixture was investigated in a batch system. A solution (25.0 mL) containing 6.0 mmol L^{-1} tartrazine, carmine and sunset yellow was adsorbed with MWNTs-MIPs, MWNTs-NIPs and raw MWNTs (50.0 mg) at 25 for 3 h and then centrifuged, the concentration of tartrazine, carmine and sunset yellow were determined by HPLC.

2.8. Sample Pretreatment

Five drinks products including different types of canned soft drinks were purchased from several supermarkets in Xian City. Samples were degassed by sonication for 30 min, and centrifuged for 15 min at 15,000 rpm to separate insoluble substance and the supernatant was mixed as loading solution. For the spiking experiments, analyte standard was added. For the recovery test, tratarzine standard was added to the samples.

3. Results and discussion

3.1. Characterization of the MWNTs-MIPs

FT-IR spectrometry was used to detect the surface chemical functional groups at different processing stages. The FT-IR spectra of MWNTs-MIPs, MWNTs-MMA/β-CD and raw MWNTs were shown in **Fig. 2**. The broad peak at the range of 3200-3700 cm^{-1} in spectra a, b and c was related to the stretching vibrations of O-H. The absorbance band at 1564 cm^{-1} in spectrum c was corresponding to the stretching vibration of C=C bonds and the peaks at 1195 cm^{-1} were resulted from C-O vibration belonging to the structure of hydroxyl MWNTs. Contrast to spectrum c, the absorbance peaks at 1037, 1656 and 2920 cm^{-1} in spectra b were attributed respectively to the stretching vibrations of Si-O-Si, C=O, and C-H. The absorbance intensities of peaks at 3363 cm^{-1} was strengthed due to the the structure of β-CD, These confirmed the successful coating of MMA/β-CD layer on MWNTs. Compared with spectrum b, the absorbance intensities of peaks at 1564 cm^{-1} in spectrum a was weakened attributed to the broken of C=C, new absorption bands appear at 1454 cm^{-1} was CH_2-N bond and the stretching vibration C=O (1718 cm^{-1}) revealed the existence of MBA. All these demonstrated that tartrazine MIPs had been grafted on MWNTs. The synthetic route of introducing silicon-oxygen group and further grafting MIPs onto the MWNTs surface was illustrated in **Fig. 3**.

Fig. 2

Fig. 3

TEM images of the raw MWNTs, and MWNTs–MIPs are shown in **Fig. 4a** and **Fig. 4b**. The average diameter of MWNTs-MIPs composite is larger than that of raw MWNTs, in addition, the MWNTs-MIPs exhibited a slightly rough surface compared to pristine raw MWNTs, which may assist tartrazine to be rebinded rapidly. The result indicated that the MIP layer was modified on the surface of MWNTs.

Fig. 4

3.2. Adsorption properties

It could be seen from **Fig. 5** that the adsorption capacities of all absorbents increased with increasing tartrazine concentration. As for MWNTs-MIPs and raw MWNTs, their maximum binding capacities were slightly different. The maximum binding capacity of MWNTs-MIPs was higher than that of the MWNTs-NIPs. The Freundlich isotherm model and Langmuir isotherm model were used to estimate the binding properties of MIPs.

Freundlich model: $\log q_e = \log K_f + \frac{1}{n}\log C_e$ (1)

Langmuir model: $q_e = \frac{Kq_m C_e}{1+KC_e}$ (2)

Where q_e (mg g^{-1}) was the amount of adsorbed analyte per unit of polymer mass at equilibrium, and C_e (mg L^{-1}) was the concentration of the analyte in solution at equilibrium, K_f and n were the two Freundlich constants, q_m was maximal amount of adsorbed analyte per unit of polymer mass, K was the Langmuir constant.

Fig. 5

According to the Langmuir model, adsorption performed uniformly on the active spots of the adsorbent. Once a template molecule occupied the site, no further adsorption could take place at this site. Freundlich model belongs to multilayer adsorption model and the surface of adsorbent is not uniform. The fitting relation coefficients of the MWNTs-MIPs and MWNTs-NIPs were given in **Table 1**. The statistics showed that absorption properties of MWNTs-MIPs and MWNTs-NIPs were better for the Langmuir model and the value of R^2 were 0.9950 and 0.9960, respectively. From the linear regression equation of MWNTs-MIPs, the values of K and q_m were 0.043 and 135.15 mg g^{-1} , respectively. The adsorption process of tartrazine onto tartrazine -MIP could be considered monolayer adsorption.

Table 1

3.3. Specific recognition of MWNTs-MIPs

Competitive adsorption studies were tested with tratarzine, sunset yellow and carmine. The selectivity of these materials could be frequently evaluated by the distribution coefficient (k_d), selectivity coefficient (k), and relative selectivity coefficient (k') obtained from competitive binding experiments.[24] $k_d = \frac{c_i - c_f}{c_f} \times \frac{v}{m}$, $k = \frac{k_{d1}}{k_{d2}}$, $k' = \frac{k_{imprinted}}{k_{nonimprinted}}$, where C_i and C_f was initial and final concentrations, respectively. The results were listed in **Table 2.** The k_d of tratarzine in the MWNTs-MIPs was 14.72, which was the four times of sunset yellow and carmine, these data demonstrated that MWNTs-MIPs high recognition toward tratarzine in comparison with sunset yellow and carmine. The k_d of tratarzine, sunset yellow, carmine in MWNTs-NIPs or raw MWNTs was almost same, propably because after the polymerization of no template, crosslinking agent and functional monomers, a dense and less porous NIP film on the MWNTs surface emerged, this NIP layer did not improve the specific surface area and the pore size distributions of MWNTs. And they cannot exhibit obvious difference in the rebinding capacities toward tartrazine, sunset yellow and carmine, which demonstrated that their nonspecific adsorption.

Table 2

3.4. Optimized of MWNTs-MIPs/SPE

Successful selective adsorption due to hydrophobic interactions and sterically complementary to the analytes is possible in aqueous solutions by optimizing washing, and eluting conditions. In order to evaluate the effect of washing solvent in MWNTs-MIPs/SPE procedure, considering tartrazine has good polarity that it is easy to dissolve in water, 10.0 mL of different washing solvents involving methanol/water (9:1, 7:3, 5:5, 3:7, 1:9, v/v) were investigated. The experimental results showed that excess methanol could result in the loss of tartrazine, in view of the recoveries obtained and the ability to remove matrix of polar compounds, the ratio of methanol and water at 1:9 is the optimized volume of washing solution.

5.0 mL different ratios of methanol containing 10-70% ammonia were used to elute tartrazine from the MWNTs-MIPs/SPE column. As results were shown in **Fig. 6**, 5.0mL of methanol/ammonia (7:3, v/v) mixture solution was employed as the eluting solution which could elute most of tartrazine.

Fig. 6

3.5. repeatability testing of MWNTs-MIPs-SPE

The regenerated SPE was assessed for rebinding the templates (tartrazine). According to optimized MIP-SPE procedure, tartrazine standard solutions (20.00 mg

L^{-1}) were passed through the same MWNTs-MIPs-SPE column at different time points for seven times interday or interweek, tartrazine recoveries in **Table 3** displayed that the binding capacity of tartrazine kept a high recovery over 96.0% within 1 day or 1 week, which illustrated that the recognized sites were stable and the material could be reusable after a regeneration process. Few researches about the repeatability of MWNTs-MIPs-SPE were reported, so the characteristics of the sorbents were superior to other MIPs materials.

Table 3

3.6. Application for real sample analysis

3.6.1. Recovery Test

The method was applied to extract and determine tartrazine in five different drink samples. The chromatograms of standard tratarzine solutions and five drink samples before and after with the MWNTs-MIPs/SPE column were displayed in **Fig. 7**. While injecting sample solution directly into HPLC without pretreatment of MWNTs-MIPs, the peak of tartrazine could hardly be observed from the chromatogram of samples, after the MWNTs-MIPs/SPE enrichment, the peak of tartrazine appeared distinctly at 6.87 min. Fixed amounts of tratarzine standards were added in the samples before sample extraction can be used to assess the recovery of tartrazine in drink samples. The results were summarized in **Table 4** and good recoveries in all samples were achieved, It was found that the recoveries were in the range of 88.6-102.4%, the relative standard deviation (RSD, n=3) values obtained were ≤ 5.0% in all cases, and the RSDs were ranged from 1.0 to 4.6%. It could be concluded that a reliable analytical method based on the MWNTs-MIPs/SPE coupled with HPLC was developed for highly selective detection and separation of tratarzine from the drink samples.

Fig. 7

Table 4

3.6.2. Validation and Application of the method

Detection limits (DL) were determined using progressively lower concentrations of tartrazine at a peak area when the peak height/noise ratio was 3: 1 (S/N = 3), and with an executed volume of 50.0 mL. Also, quantification limit (QL) was calculated at a peak area when the peak height/noise ratio was 10:1. The DL and QL correspond to 8.33×10^{-5} and 2.78×10^{-5} ng L^{-1}, respectively. And the RSDs were ranged from 2.9-5.6%. At the same time, the five drink samples were determined by the China standard method of GB/T 5009.35-2003 to verify this method. The results of the comparison were presented in **Table 5**. The results displayed that t values were in range of 0.28-1.86, when P=0.95 and f = 5, $t_{0.05,5}$=2.58, $t<t_{0.05,5}$, So the method have no significant difference with China standard method. In China and European Union, the permitted maximum limit of tratarzine additive in drink is 0.1g kg^{-1} (GB2760-2011, Directive of the European Parliament and of the council 94/36/EC),[25] the contents of tratarzine in these five drinks are under this level.

Table 5

4. Conclusions

In this study, a composite material based on MWNTs polymerized with a layer of bifunctional monomers molecularly imprinted tratarzine was synthesized. The resultant MWNTs-MIPs possess some attractive characteristics, such as great adsorption amount and good selectivity. The imprinted material was applied as sorbets for the enrichment and determination of tratarzine in real drink samples by SPE–HPLC. It exhibited excellent extraction characteristics for tratarzine. The MIPs grafting method also provides a significant reference for other radical polymerization reactions based on MWNTs.

Acknowledgement: This work was financially supported by the Shaanxi Provincial Science and Development Projects (2012k08-14).

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Table 1 Adsoption model

The type of polymer	Freundlich model	K_f	R^2	Langmuir model	K	R^2
MWNTs-MIPs	$Logq_e=0.32LogC_e\pm1.19$	1.19	0.8223	$1/q_e=0.17/C_e\pm0.0074$	0.043	0.9950
MWNTs-NIPs	$Logq_e=0.24LogC_e\pm1.41$	1.41	0.8254	$1/q_e=0.20/C_e\pm0.0088$	0.044	0.9960

Table 2 Competitive adsorption test

Parameter	Adsorbent	MWNTs-MIPs	MWNTs-NIPs	raw MWNTs
The adsorption capacity (mg g^{-1})	tartrazine	85.44±2.37	27.77±1.32	29.19±1.75
	sunset yellow	21.26±1.25	22.17±1.67	39.01±2.09
	carmine	15.62±0.84	34.62±1.96	31.35±2.18
K_d	tartrazine	14.72±0.29	4.78±0.15	5.02±0.18
	sunset yellow	3.38±0.18	3.52±0.20	6.20±0.20
	carmine	3.42±0.13	7.58±0.23	6.86±0.24
K	sunset yellow	4.35±0.09	1.36±0.03	0.81±0.02
	carmine	4.29±0.09	0.63±0.02	0.73±0.03
K’	sunset yellow	—	3.20±0.08	5.37±0.11
	carmine	—	6.81±0.11	5.88±0.12

Table 3 Repeatability testing results of the same MWNTs-MIPs-SPE column

Item	Spiked level (mg L^{-1})	Found level (mg L^{-1})	Recoveries (%)	RSD (%) (n=7)
Inter-day	20.00	19.75	98.8	1.9
Inter-week	20.00	19.20	96.0	3.2

Table 4 Recovery test of tratarzine for some tested drink samples(n=3)

Drink type	Mean level (mg kg^{-1})	Amount of added (mg kg^{-1})	Amount of found (mg kg^{-1})	Recovery (%)	RSD (n=3) (%)
Drink 1	16.4	50.0	67.6±2.4	102.4	3.5
Drink 2	11.5	50.0	60.3±0.6	97.5	1.0
Drink 3	6.1	50.0	51.9±2.4	91.7	4.6
Drink 4	39.2	50.0	83.5±1.9	88.6	2.3
Drink 5	2.5	50.0	50.0±0.5	95.1	1.0

Table 5 t-texts (n=6)

Drink type	The measured value (mg kg^{-1})	GB measured value (mg kg^{-1})	F	S	t test value
Drink 1	16.42±2.1	17.26±1.0	4.41	1.0	1.46
Drink 2	11.55±1.1	12.14±0.6	3.20	0.6	1.70
Drink 3	6.11±0.9	6.97±0.8	1.26	0.8	1.86
Drink 4	39.15±1.8	38.39±1.5	2.25	1.5	0.28
Drink 5	2.49±0.5	2.71±0.3	2.28	0.3	1.26

Figure captions

Fig. 1 Chemical structures of carmine; sunset yellow; tartrazine.

Fig. 2 FTIR spectra of (a) MWNTs-MIPs; (b) MWNTs-β-CD-MMA; (c) raw MWNTs.

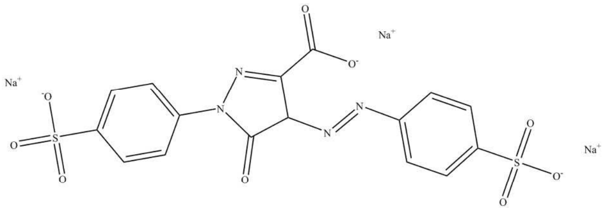
Fig. 3 Schematic representation of the preparation of the MWNTs–MIPs.

Fig. 4 TEM images of the raw MWNTs (a) and MWNTs–MIPs (b).

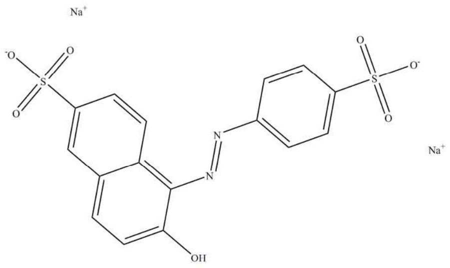
Fig. 5 Adsorption isotherm curves of raw MWNTs, MWNTs-MIPs and MWNTs-NIPs toward tartrazine, Amount of polymers, 50.0 mg; volume, 25.0 mL; initial concentration of tartrazine, 0.1 mmol L^{-1}.

Fig. 6 The recovery of tartrazine in different ratio of 5mL elution solution (methanol/ammonia, v/v=9:1; 7:3; 5:5; 3;7).

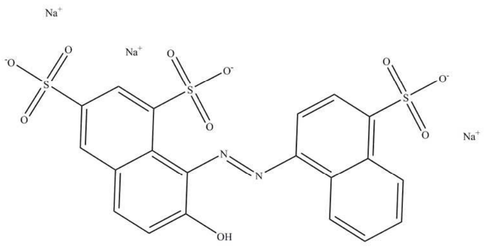
Fig. 7 Chromatograms: A tratarzine solution (a) Drink 1 untreated with MWNTs-MIPs; (b) Drink 1 extracted by MWNTs-MIPs; (c) Drink 2 untreated with MWNTs-MIPs; (d) Drink 2 extracted by MWNTs-MIPs; (e) Drink 3 untreated with MWNTs-MIPs; (f) Drink 3 extracted by MWNTs-MIPs; (g) Drink 4 untreated with MWNTs-MIPs; (h) Drink 4 extracted by MWNTs-MIPs; (i) Drink 5 untreated with MWNTs-MIPs; (j) Drink 5 extracted by MWNTs-MIPs.



tartrazine

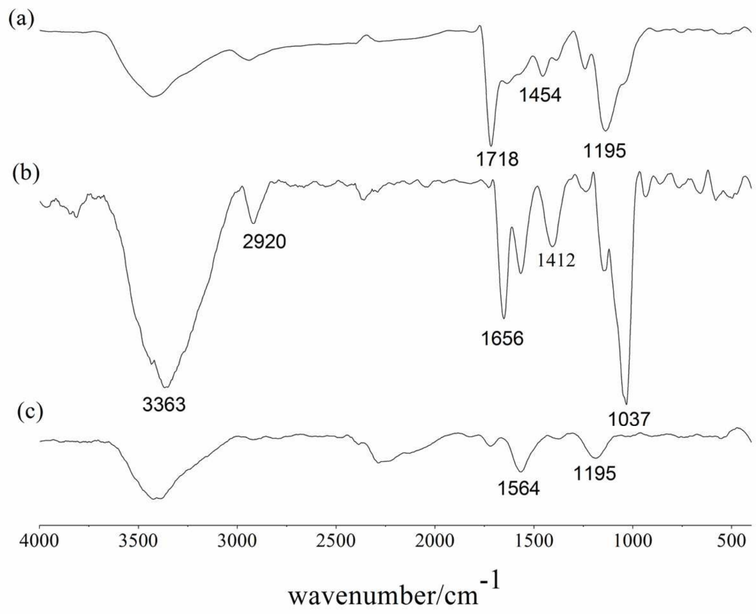


sunset yellow

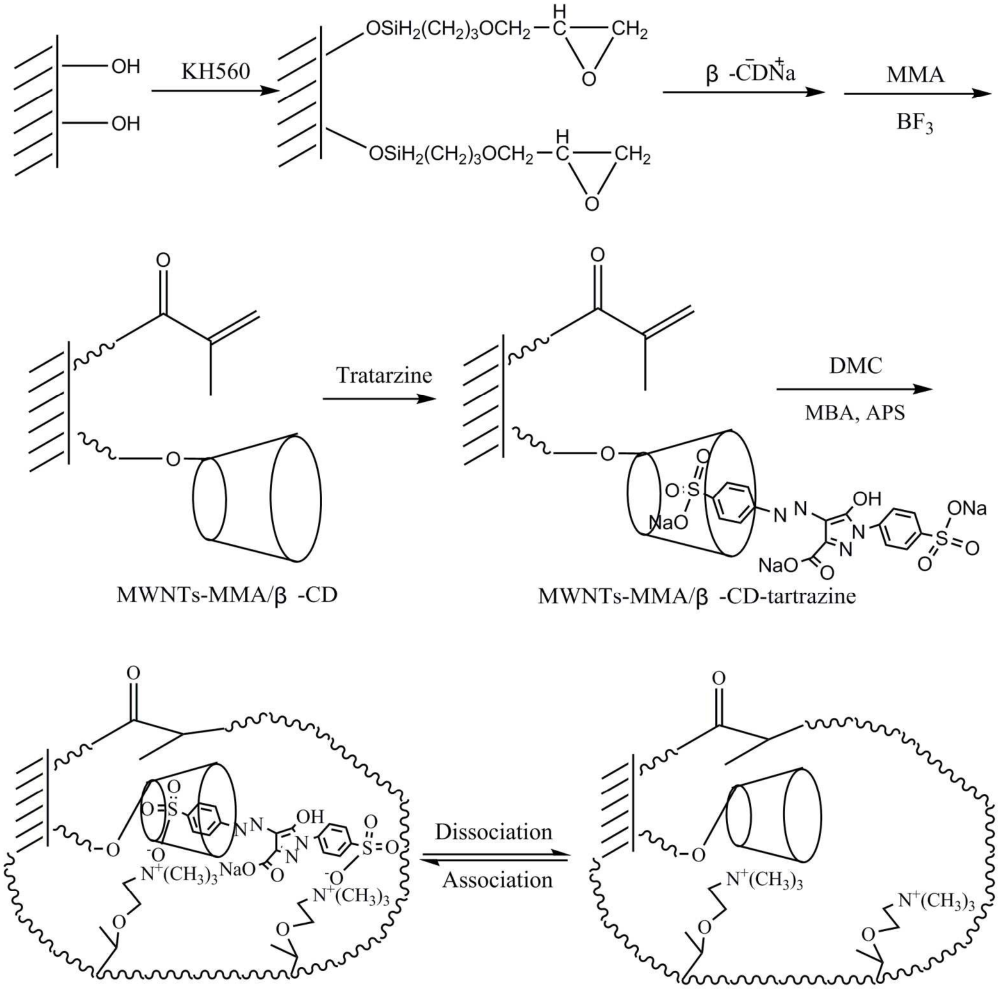


carmine

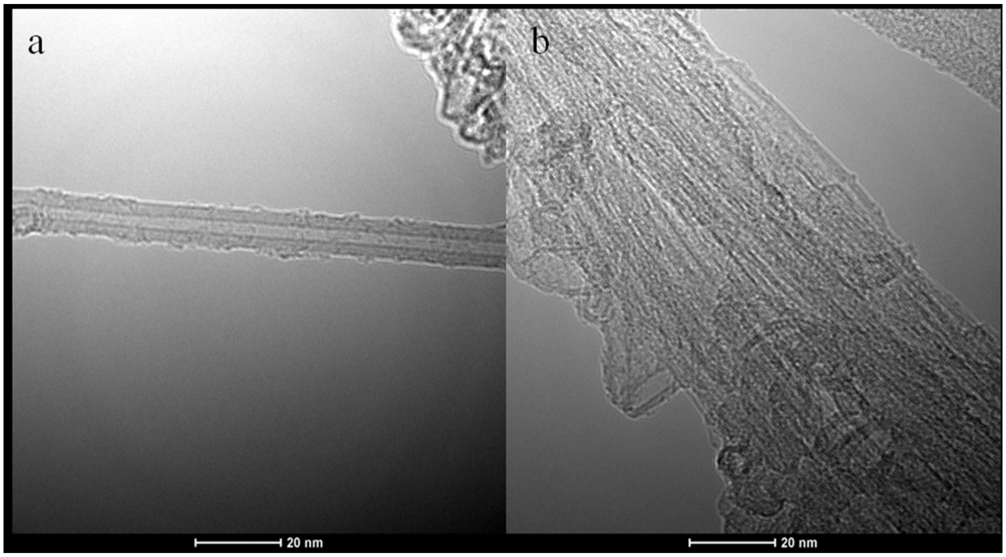
159x102mm (300 x 300 DPI)



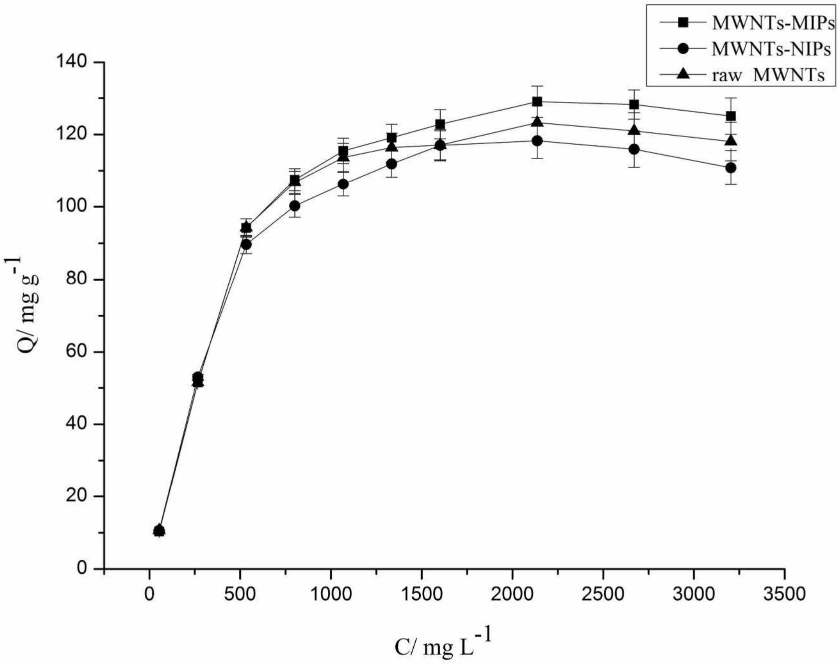
143x101mm (300 x 300 DPI)



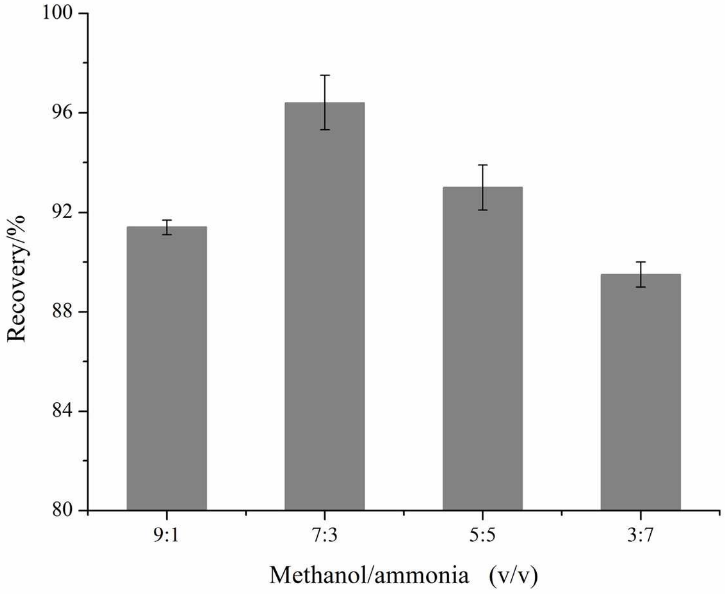
179x178mm (300 x 300 DPI)



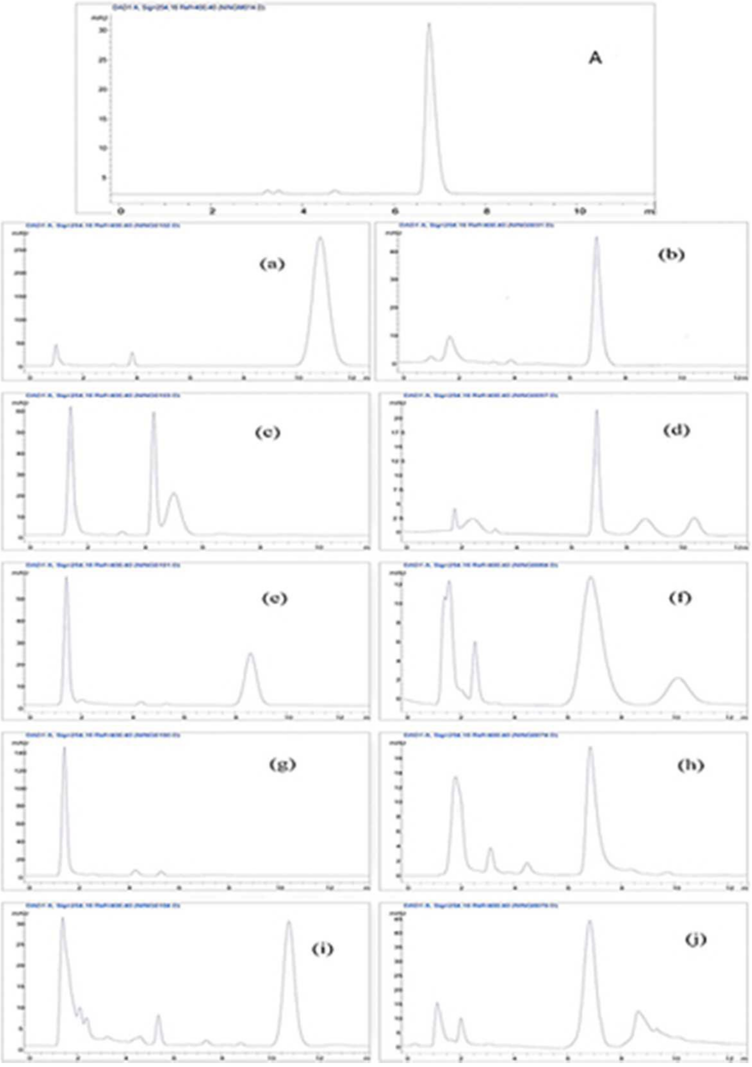
165x91mm (150 x 150 DPI)



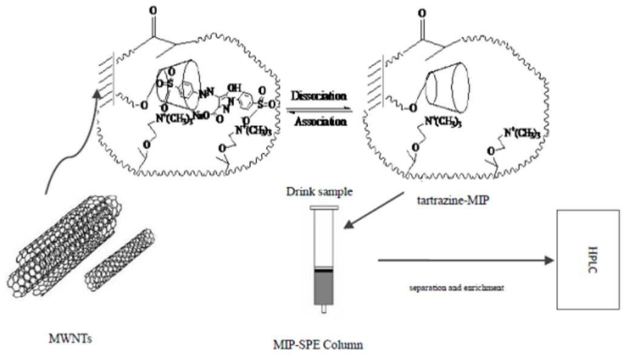
210x148mm (300 x 300 DPI)



143x101mm (300 x 300 DPI)



32x45mm (300 x 300 DPI)



Novel bifunctional monomers MIP with an improved selectivity for tartrazine on the surface of MWNTs was synthesised