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MAGNETITE MOLECULARLY IMPRINTED NANOPARTICLES FOR SELECTIVE DETECTION OF PHENOL IN WASTEWATER SAMPLES FOLLOWED BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

In this work a novel, environmental friendly , fast and simple extraction method based on magnetite molecularly imprinted polymer nanoparticles (MMIPNP$_S$) as a sorbent for measurement of phenol was investigated. The synthesized silica-coated magnetite modified with polymer by using of methacrylic acid (MAA) as a functional monomer and ethylene glycol dimethacrylate (EGDMA) as across-liker and phenol as the template. Main factors influencing the extraction efficiency including pH, amount of sorbent, time of extraction and type of desorption solvent were optimized. Then for analysis of phenol from extraction solvent, high performance liquid chromatography (HPLC) with ultraviolet (UV) detection on reversed-phase octadecyl silica (C$_{18}$) column using 0.05 M phosphat buffer– methanol as the mobile phase has been used. Method validations such as linear range, detection limit, precision and extraction recovery were evaluated. Phenol was separated, preconcentrated and determined successfully in spiked sample of wastewaters.

KEYWORDS: Phenol, Molecularly Imprinting Polymer, Magnetic Nanoparticles, Wastewater, High Performance Liquid Chromatography

Phenol as an aromatic semi volatile hydrocarbon is highly toxic (even at low concentrations) that presents in wastewaters of most industries such as chemical, petrochemical and pharmaceutical industries [1,2]. Phenol has a cute and chronic effects on human health and as priority pollutants it can change blood pressure and can cause liver, nervous system and kidney damage (Environmental Protection Agency). Phenol may be remain in air, water and soil for much longer times if it is continually or consistently released to these media from mention sources [3]. For removal and determination of phenol, different methods such as ion exchange [4], electrochemical oxidation [5], photo-catalytic degradation [6], adsorption [7], HPLC [8], Gas Chromatography-Mass Spectrometry (GC-MS) [9], spectrochemical method [10] and GC [11] have been used . For detection of trace amounts of phenol in real sample before analysis, preconcentration is require, one of the important techniques for sample preconcentration is solid phase extraction (SPE) that this method reported perviously as a simple, rapid and accurate for determining phenols at low concentrations in real samples [12,13].

In recent years, magnetic nanoparticles (MNP$_S$) have attracted great interest for removal of toxic target compounds for their unique properties [14-17]. MNP$_S$ as an adsorbent for SPE have higher surface area-to-volume ratio and a short diffusion route. For these prominences, adsorption capacity of target molecules can improve and preconcentration process are fast by using of a magnetic field , due to the magnetically assisted separation of these particles from the sample solution [18,19]. For selective extraction of target compounds, modification of these particles is necessary [20-22], also the MNP$_S$ as solid support substrate for magnetite molecularly imprinted polymer nanoparticles (MMIPNP$_S$) is utilizable [23]. Molecularly imprinted polymers (MIP$_S$) formed in the attend of analyte and elimination of it done by suitable solvents such as methanol and acetonitrile to formation of specific ditches for analyte in shape, size and functional groups, therefore high selectivity and affinity for target have been attained such as pharmaceutical analysis [24,25], food analysis [26,27] and environmental analysis [28-30]. For modification of MMIPNP$_S$, the surface of these particles modified with different materials [31-36].

In this work, the MIP was coated on magnetic nanoparticle and the prominence of this study was to investigate the selective and effective sorbent (MMIPNP$_S$) for preconcentration and removal low level of phenol as an organic pollutant from waste waters, then the selectivity of the MMIPNP$_S$ were compared with magnetic non imprinted polymer nanoparticles (MNIPNP$_S$) that this method has not been employed perviously.

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EXPERIMENTAL

Reagents and Apparatus

Total reagents were of analytical grade and all aqueous solutions were prepared by using of doubly distilled deionized water. The water was purified on a Milli-Q-Ultrapure purification system purchased from Millipore (Millford, MA, USA). Ferric chloride ($FeCl_3.6H_2O$), ferrous chloride ($FeCl_2.4H_2O$), methanol, acetonitrile, ethanol, aceton, potassium dihydrogen phosphate , sodium hydroxide, tetraethyl orthosilicate (TEOS) , 3 – (trimethoxysily) propyl methacrylate (MPS) , methacrylic acid (MAA) and 2 , 2 – azo bis iso butyronitrile (AIBN) were purchased from merck (Darmstadt , Germany), Ethylene glycol dimethacrylate (EGDMA) was obtained from Sigma – Aldrich , Standard of phenol and wastewater obtained from Daroupaksh Drug Company , Fourier Transform Infrared Spectrometer (FT-IR) was from PerkinElmer (USA). For magnetic separations a strong magnet of NdFeB (10 × 5 × 4 cm) was used. A pH meter model-713 from (Metrohm, Swiss) was used. The Vortex from IKA (USA) , Ultrasonic was from Bandeline Sonorex (USA), Transmission electron microscopy(TEM) was from Philips EM208 (Voltage100 KV) and a Centrifuge model Celements GS-200 (USA) were used.

HPLC Analysis

HPLC (Waters, USA) system equipped with a 515 (Waters, USA) pump and UV detector was used and the sample injection volume was set to 20µl and the stationary-phase column was $C_{18,}$ (4 μm-150×3.9mm) from Nova-pak from waters (USA). A mixture of 0.05 M phosphate buffer– methanol has been used as the mobile phase and its flow rate was set at 1.0 mL. min^{-1}. Oven temperature was set at 25^oC and the detection was made at the wavelength of 254nm.

Synthesis of Fe_3O_4 @ SiO_2

Fe_3O_4 @ SiO_2 were prepared by co-precipitation technique [31]. Briefly, 9.13 g $FeCl_{3.}$ $6H_2O$ and 3.42 g $FeCl_2$. $4H_2O$ under nitrogen gas and stirring (1000 rpm) were dissolved in 160 mL double distilled water. Then 20 mL of ammonia solution was added fall in drop to this mixture under nitrogen gas and stirring for 2h at 85˚C. The black magnetic nano-inorganic oxide particles were collected by an external magnet, then $Fe_3O_4MNP_S$ washed 3 times by double distilled water that degassed perviously and dried under vaccum in desiccator, then 900 mg of Fe_3O_4 nanoparticles were dispersed and ultrasonicated in 300 mL of ethanol and double distilled water solution, after than 15 mL of ammonia solution and 2.1 mL of TEOS were added to this mixture continuosly. The solution was stirred for 12h at (40˚C) and finally Fe_3O_4 @ SiO_2 product was collected by applying of magnetic field.

Modification of Fe_3O_4 @ SiO_2

For modification of Fe_3O_4 @ $SiO_{2,}$ 250 mg of Fe_3O_4 @ SiO_2 MNP_S was added to mixture of 50 mL toluene and 5 mL MPS and the solution was reacted for 24h at (70˚C) under nitrogen gas. Modified Fe_3O_4 / SiO_2 were separated by the application of a magnet outside the container , then these particles washed by water and dried in a vaccum oven, the products (Fe_3O_4 @ SiO_2 – C = C) were obtained.

Preparation of $MMIPNP_S$ and $MNIPNP_S$

0.3 mmol standard of phenol and 1 mmol of MAA were dissolved in 50 mL acetonitrile, the solution was shaken in water bath (25˚C) for 12h [37]. In order to prepare $MMIPNP_{S,}$ 200 mg Fe_3O_4 @ SiO_2 – C = C was added to above mixture and stirred for 2h.

Finally, 5 mmol of EGDMA and 16mg of AIBN were added into the mixture and the solution was ultrasonicated in water bath (60˚C) under nitrogen gas for 24h. Then, the synthesized $MMIPNP_S$ -phenol were separated by an external magnetic field and for removal of phenol (as the template) the $MMIPNP_S$ -phenol elueted by a mixture of acetonitrile - acetic acid and for investigation the selectivity of $MMIPNP_S$, the magnetite non-molecular imprinted polymer nanoparticles ($MNIPNP_S$) were prepared under the same conditions without phenol [31].

2.6. Adsorption experiments

To 10 mL of aqueous solution, various amount of MMIPNPs or MNIPNPs was added (20 and 50 mg) respectively. The solution was shaken for 12h (25˚C) and then MMIPNPs or MNIPNPs were isolated by applying of magnet, and the residual of phenol in solution was determined by HPLC-UV. Adsorption capacity (Q) was calculated from equation (1).

$$Q = \frac{(C_o - C_e)V}{M} \qquad (1)$$

Q is adsorption capacity, C_o , C_e (mg. L^{-1}) are the initial and equilibrium concentration of phenol, respectively. V (mL) is the volume of initial solution and M (g) is the mass of MMIPNPs and MNIPNPs.

Procedure for determination of phenol

To 10 mL of spiked water samples, 20 mg of MMIPNPs or MNIPNPs was added and then this mixture was stirred at (25˚C) for 3h. MMIPNPs were collected by applying an external magnetic field and for desorption of phenol from sorbent, MMIPNPs or MNIPNPs eluted by 500 μL acetonitrile containing of 0.1% acetic acid. Finally desorption solvent was injected into the HPLC for analysis. The extraction recovery (ER) and preconcentration factor (PF) of the phenol were calculated by the following equations:

$$\mathrm{ER}\ \% = \left(\frac{C_{E,\mathrm{final}} \times V_{E,\mathrm{final}}}{C_{S,\mathrm{initial}} \times V_{S,\mathrm{initial}}}\right) \times 100 \qquad (2)$$

$$\mathrm{PF} = \frac{V_{S,\mathrm{initial}}}{V_{E,\mathrm{final}}} \times \mathrm{ER}\ \% \qquad (3)$$

where $C_{E,\mathrm{final}}$ and $C_{S,\mathrm{initial}}$ are the final and initial concentrations of phenol in eluent and sample, respectively. $C_{E,\mathrm{final}}$ of the extracted phenol was calculated from the calibration curve. V_S and V_E are the volumes of the sample solution and eluent, respectively. The extraction recoveries were higher than 97% and preconcentration factor > 19 was obtained by enriching 10 mL of aqueous solution to 0.5 mL of desorption solvent (ER% > 97).

Thus the results indicated that the proposed method has high preconcentration factor with good recoveries.

RESULTS AND DISCUSSION

Characterization of MMIPNPs

The size of magnetite imprinting polymers were studied by TEM and shown in Fig 1. The diameter of these paraticles were less than 100 nm. The FT – IR spectrogram of Fe_3O_4 @ SiO_2 and modified Fe_3O_4 @ SiO_2 shown in Fig 2. The characteristic peak around 580 cm^{-1} can be attributed to the Fe-O stretching vibration and a strong peak at 1100 cm^{-1} is related to the Si – O – Si vibration and C – H stretching vibration peak at around 2960 cm^{-1} described to C – H was modified onto the surface of Fe_3O_4 @ SiO_2 and peak at around 3400 cm^{-1} corresponding to adsorbed water on the silica or the silanol groups of the silica .

Figure 1: The TEM image of the synthesized MNP_s, magnification160000

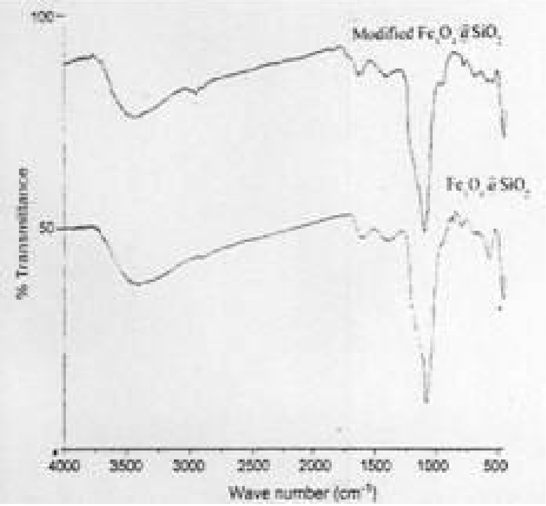

Figure 2: FT-IR spectrogram of (a) Fe_3O_4 @ SiO_2 (b) modified Fe_3O_4 @ SiO_2

Comparison experiment

In same conditions the adsorption capacity of MMIPNPs and MNIPNPs were compared, the results showed that the response of phenol extracted by MMIPNPs was higher than MNIPNPs (Fig .3) , for adsorption of analyte into the imprinted cavities, more adsorption of phenol occurred.

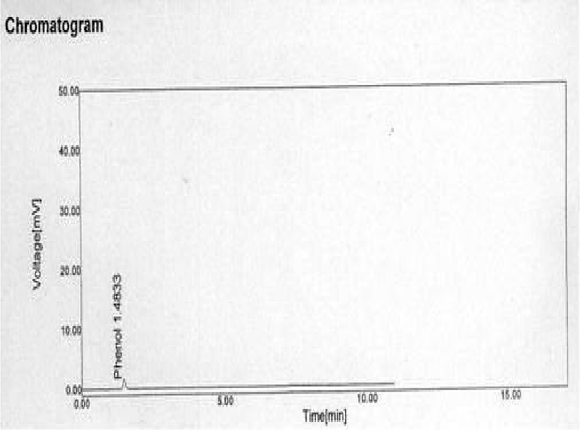

Figure-3A

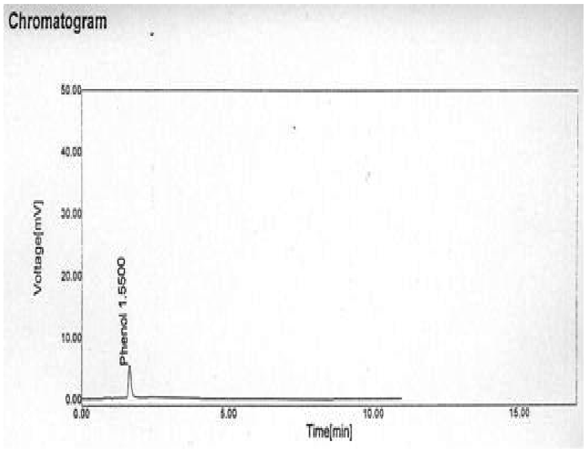

Figure-3B

Figure 3: The chromatograms for (A) Adsorption capacity of MMIPNP$_s$ (B) Adsorption capacity of MNIPNP$_s$

Optimization of conditions

Different conditions including amount of adsorbent, pH, extraction time and desorption solvent were evaluated. Various amount of MMIPNPs were investigated in range of 10 to 70 mg and results showed that the best recovery was obtained by 20 mg sorbent, thus 20mg was selected for next experiments. Phenol (pKa around 10) and MMIPNPs contain carboxy groups that attend in anionic or neutral from in aqueous solution therefore the extraction recoveries of phenol depend on pH values. The pH values in range of 3-8 was investigated (Fig.4) that the highest extraction efficiency obtained at pH 3-5.

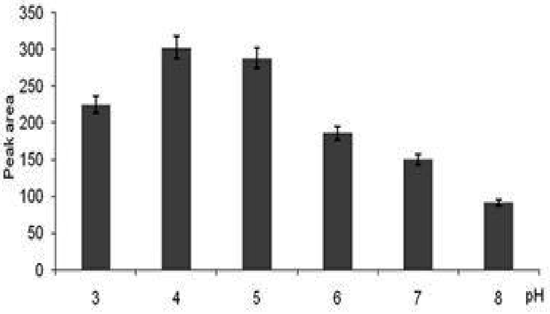

Figure 4: Adsorption efficiency of MMIPNP$_s$ as a function of samples pH

Under this conditions, phenol and MMIPNPs existed in neutral forms thus affinity and hydrophobic interaction between polymer backbone and phenol occurred. To attain higher extraction recoveries, the pH was adjusted to 4.0 for the next experiments. Extraction time investigated in range of 1 to 20 min, due to thin layer of molecularly imprinting polymer on Fe_3O_4 / SiO_2 surface, time had no significant effect on the extraction recoveries and results showed that 1 min was sufficient time to desorption of phenol from the magnetite sorbent. The desorption solvent was evaluated by different solvents that showed in Fig.5 , results indicated that 1mL of acetonitrile containing 0.1% acetic acid was the best solvent for removal of phenol from MMIPNPs .

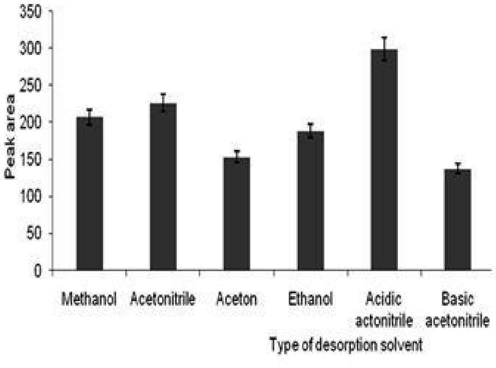

Figure 5: Adsorption efficiency of MMIPNP$_s$ as a function of type of desorption solvent

Analytical features

The linearity, limit of detection (LOD), quantification limit (LOQ) and relative standard deviation (RSD) were investigated for prepared sorbent . A good linearity in the range of (0.1–20) µg. L^{-1} was obtained (with six concentration and three replications), with correlation coefficient (R^2) of 0.9961. The values of LOD based on signal to noise ratio of 3 and LOQ based on signal to noise ratio of 10 were 0.03 and 0.08 µg .L^{-1} respectively. For investigation of the accuracy of the method, aqueous samples spiked with phenol at 5, 10 and 15 µg .L^{-1} concentrations. The results for wastewater of pharmaceutical company extracted by synthesized MMIPNPs and MNIPNPs were showed in table 1.

The chromatogram of wastewater samples that spiked with 10µg .L^{-1} phenol are shown in Fig 6.

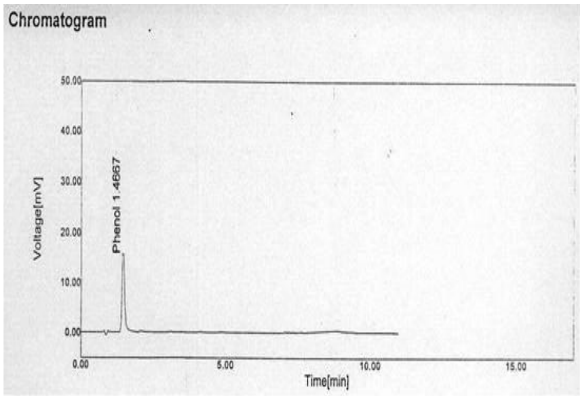

Figure 6: The chromatogram of wastewater sample that spiked with 10µg .L^{-1} phenol

Table 1: Phenol determination in waste water samples by MMIPNPs and MNIPNPs (n=5)

		MMIPNPs				MNIPNPs			
Sample	Spiked (µg.L^{-1})	Detected (µg.L^{-1})	Recovery (%)	RSD (%)	Preconcentration Factor	Detected (µg.L^{-1})	Recovery (%)	RSD (%)	Preconcenration Factor
Waste water	0	N.D[a]	-	-	-	N.D	-	-	-
	5	4.86	97.20	4.13	19.44	1.32	26.40	3.07	5.28
	10	9.83	98.30	2.76	19.66	2.76	27.60	3.11	5.52
	15	14.75	98.33	3.08	19.66	5.86	25.59	3.39	5.12

[a] Not detected

CONCLUSION

A selective and effective magnetic surface molecularly imprinted polymer nanoparticles (MMIPNPs) was successfully synthesized by copolymerization of functional groups containing monomers and cross liker around of silica nanoparticles in the presence of phenol as the template molecule. A thin layer of imprinted shell around of magnetic nanoparticles could be collected by applying of magnet rapidly. Furthermore , MMIPNPs as a new sorbent in solid phase extraction were studied for removal low level of phenol as toxic compound from wastewater sample and incomparison of MMIPNPs , extraction recovery and preconcentration factor was higher , that selectivity and sensitivity of HPLC makes this technique as a robust methodology for this purpose.

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