

Preconcentration of phosphate ion onto ion-imprinted polymer

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Abstract

In this study, selective separation and preconcentration of phosphate ions on the phosphate-imprinted chitosan-succinate beads have investigated. Chitosan-succinate, phosphate, epichlorohydrin were used as the complexing monomer, template and crosslinking agent, respectively. In the first step, chitosan was modified with succinic anhydrides and complex formation occurred between carboxylic acid functional groups and iron(III) ions. Secondly, Fe(III)-chitosan-succinate particles were reacted with phosphate ions. Afterwards, particles were crosslinked with epichlorohydrin and the template (phosphate ions) was removed using 1 M KOH solution. Selective cavity for the phosphate ion was obtained in the phosphate-imprinted metal-chelate polymer. These phosphate-imprinted metal-chelate polymer was used in the adsorption-desorption process. The adsorption process was fast and equilibrium was reached around 30 min. The adsorption behaviour of this system was described approximately by the Langmuir equation. Percent extraction, distribution ratio and selectivity coefficients of phosphate and other ions using non-imprinted and phosphate-imprinted polymer were also determined and comparison of these data was reported.

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1. Introduction

Phosphorus is an important element making a major contribution to agricultural and industrial development. However, its release to surface waters in agricultural runoff and wastewaters has legislation, such as the European Union Urban Wastewaters Directive (commission of European Communities, 1991), designed to remove phosphorus from domestic and industrial wastewater [1]. The concentration of phosphate in environmental water usually ranges from ten to several tens ng mL^{-1} [2]. Several alternative methods have also been developed for measuring low concentration of phosphate in a matrix, including ion exchange chromatography [3,4], gas chromatography with the phosphine generation system [5,6], amperometric detection [7], fluorometry [8,9] and enzymatic reaction [10,11], molybdenum blue methods [12] and flow injection [13].

The determination of low levels of analytes relies generally on a suitable separation/preconcentration process. The scientist attempts have been made at the preconcentration of phosphate [14–16]. The solvent extraction [9,17] and solid phase extraction with an ion associate complex [8] have used and several reagents have been reported, such as Malachite Green [18], Crystal violet [19], Rhodamine 6G [8,9,17] and Rhodamine B [20] for the preconcentration of phosphate. Some carriers have used in solid phase extraction, such as an organic soluble membrane filter [8].

Molecular imprinting is a technique for preparing tailor-made fashioned synthetic polymers capable of molecular recognition for given molecules [21–23]. In molecular imprinting process, a template molecule or ionic species associates with one or more functional monomers to form a complex. This complex is then polymerized with a matrix-forming monomer (crosslinker) to produce a resin. Upon removal of the template species, cavities may then be used to selectively rebind the template from a mixture of chemical species [24,25]. Ion-imprinted polymers (IIPs) are similar to MIPs, but templated resins have prepared using polyvalent metal ions [26–30]. There are some

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publications, recently, for the removal of anions by using molecular imprinting technique [31–33].

This work presents the preparation of very selective phosphate-imprinted polymeric particles for the removal and preconcentration of phosphate ion. For this purpose, Fe(III)-chitosan-succinate was synthesized, reacted with $\text{Na}_3\text{PO}_4 \cdot 12\text{H}_2\text{O}$ and crosslinked with epichlorohydrin. Then the template (phosphate) was removed from the phosphate-imprinted metal-chelate polymer. The interaction among the metal ion, ligand and template ion offer a route in order to prepare a receptor polymer for highly specific recognition and separation of phosphate ion.

2. Experimental

2.1. Materials

Chitosan, succinic anhydride and epichlorohydrin were supplied from Aldrich Chemical (USA). All other chemicals were analytical reagent grade and purchased from Merck (Darmstadt, Germany).

2.2. Instrumentation

Unicam UV–vis scanning spectrophotometer was used for the determination of phosphate ion. For the colorimetric determination of phosphate by ascorbic acid method, phosphate absorbance measurements were made at 880 nm [34].

Jenway 3100 pH-meter was used to measure pH values.

2.3. Preparation of chitosan-succinate

Chitosan-succinate was synthesized according to the procedure reported in the Aiedeh and Taha's work [35]. Chitosan (1.00 g, corresponding to approximately 6.20 mmols glucoseamine) was dissolved in the acetic acid aqueous solution (1%, 100 mL) at ambient temperature, and a solution of the succinic anhydride (6.25 mmol) in pyridine (5 mL) was added dropwise with vigorous stirring. The reaction pH was maintained at 7.0 by the dropwise addition of NaOH solution (1.0 M). After 40 min the reaction was terminated by the addition of NaCl aqueous solution (20%, 200 mL). The resulting precipitate was filtered, washed with acetone and diethyl ether, and desiccated to give chitosan-succinate conjugates.

2.4. Preparation of phosphate-imprinted metal-chelate polymer

Chitosan-succinate (1 g) was dissolved in the acetic acid (5%) and $\text{Fe}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$ (2 g) was added slowly to this solution with continuous stirring at room temperature. Fe(III) ions were complexed with the amide groups of chitosan-succinate, and $\text{Na}_3\text{PO}_4 \cdot 12\text{H}_2\text{O}$ (8.86 g) was added to Fe(III)-chitosan-succinate mixture. This mixture was slowly dropped into 150 mL of 1 M aqueous sodium hydroxide solution. The suspended solu-

tion was stirred at 200 rev min^{-1} for 12 h. After filtering this yellow suspended solution and drying in vacuum, the obtained phosphate-complexed chitosan-succinate was crosslinked using 5 mL of epichlorohydrin in 250 mL acetic acid (5%) solution under refluxing conditions in an oil bath (ca. 110°C) for 1 h. Afterwards, 250 mL of 0.1 M aqueous sodium hydroxide solution was added to complete the crosslinking reaction. After vacuum filtration, the clearly orange product of crosslinked phosphate-complexed chitosan-succinate was washed with 1 M KOH and deionized water several times. The reddish-brown product was obtained by the removing of phosphate ions from the phosphate-complexed chitosan-succinate using 1 M KOH.

2.5. Adsorption/desorption/reuse

2.5.1. Adsorption studies

Effects of the pH and the initial concentrations of $\text{Na}_3\text{PO}_4 \cdot 12\text{H}_2\text{O}$ on the adsorption rate and capacity were investigated in batch adsorption-equilibrium experiments. The effect of pH on the adsorption rate of the phosphate-imprinted polymer was investigated in the pH range 2.0–7.0 at 25°C . The maximum adsorption was observed at pH 2.0 and after that, this pH was applied for all experiments. The suspensions were brought to the desired pH by adding NaOH and HNO_3 solution. The pH was maintained in a range of ± 0.1 U until equilibrium was attained. In all experiments, polymer concentration was kept constant at 25 mg/25 mL.

The effect of the initial ion concentration on the adsorption was investigated at pH 2.0 as described above and the concentration of phosphate ions in the adsorption medium was varied between 50 and 15,000 mg L^{-1} .

Competitive adsorption of phosphate/flouride and phosphate/thiocyanate from their mixture was also investigated in a batch system. A solution (25 mL) containing 25 mg L^{-1} from each anions was treated with the phosphate-imprinted microparticles at a pH of 2.0 at room temperature. After the adsorption equilibrium, the concentration of anions in the remaining solution was measured by a UV spectrometer.

The amount of adsorbed ions was obtained using the following expression.

$$Q = \frac{[(C_0 - C)V]}{M} \quad (1)$$

where Q is the amount of ions adsorbed onto the unit amount of the polymer (mg g^{-1}); C_0 and C are the concentrations of the ions in the initial solution and in the aqueous phase after adsorption, respectively (mg L^{-1}); V is the volume of the aqueous phase (mL); M is the amount of polymer (g).

2.5.2. Desorption and reusability

25 mg of the imprinted polymer was placed in a 25 mL solution containing 25 ppm of phosphate ion for the adsorption process. Then adsorbed phosphate anions were desorbed by the treatment of the imprinted polymer with 1 M KOH solution stirring at 600 rpm for 30 min. Desorption ratio was calculated from

the following expression:

$$\text{Desorption ratio} = \frac{\text{amount of ions desorbed to the elution medium}}{\text{amount of ions adsorbed onto the sorbent}} \times 100 \quad (2)$$

Adsorption–desorption cycle was repeated seven times by using the same sorbent for the reusability of the phosphate-imprinted polymers. The imprinted polymer was washed several times with 1 M KOH solution and demineralized water after each use and then reloaded.

3. Result and discussion

3.1. Characterization of polymer

The Fourier transform infrared spectroscopy (FTIR) spectra of chitosan-succinate, Fe(III)-chitosan-succinate and phosphate-imprinted chitosan-succinate were obtained through the use of a FTIR spectrophotometer (Perkin-Elmer Model 100).

The IR spectra of the chitosan-succinate showed amide carbonyl band at 1650 cm^{-1} , carboxylic acid carbonyl band at the 1735 cm^{-1} , indicating the formation of amide links with succinate [35].

The IR spectrum of the Fe(III)-chitosan-succinate polymeric matrix showed principal complex absorption bands at around 1640 and 1565 cm^{-1} , in contrast, the IR spectrum of chitosan-succinate gave a carboxyl carbonyl band at 1735 cm^{-1} and an amide carbonyl band at 1650 cm^{-1} . Accordingly, one can conclude that both carboxyl and amide carbonyls were shifted to lower frequencies upon complexation with iron, i.e., from 1650 to 1565 cm^{-1} for amide carbonyls and from 1735 to 1640 cm^{-1} for carboxylic carbonyls [36].

The IR spectra of the phosphate-imprinted chitosan-succinate showed amide carbonyl band at 1632 cm^{-1} , carboxylic carbonyl band at the 1652 cm^{-1} and phosphate band at the 564 cm^{-1} . This result was indicated that phosphate was connected to Fe(III)-chitosan-succinate. The phosphate band at the 564 cm^{-1} was not seen in the IR spectra of phosphate-imprinted polymer because of the removing of phosphate. Thus, it is understood that selective cavities for phosphate ions in the phosphate-imprinted polymer have been formed.

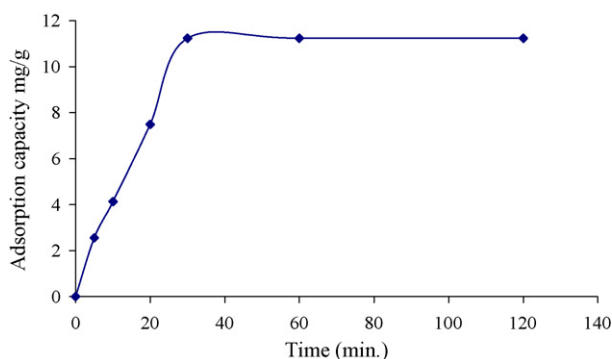


Fig. 1. Adsorption rates of phosphate ions on the phosphate-imprinted polymer: pH, 2.0; T , 25°C .

3.2. Adsorption capacity of phosphate-imprinted polymer

3.2.1. Adsorption rate

Fig. 1 shows adsorption rates of phosphate ions onto the phosphate-imprinted polymer from aqueous solutions containing 25 mg L^{-1} of phosphate ions at a constant pH of 2.0. Note that the ordinate values were calculated by using Eq. (1). As seen here, high adsorption rates were observed at the beginning and then plateau values (i.e., adsorption equilibrium) were gradually reached within 30 min.

Several experimental data on the adsorption of various ions by chitosan have shown a wide range of adsorption rates. For example, Inukai et al. have investigated adsorption of germanium(IV) on the 2,3-dihydroxypropyl chitosan resin and beads and reported 5 h equilibrium adsorption time [37]. Guo et al. have used molecularly imprinted chitosan beads for the separation of hemoglobin and found 10 h as an equilibrium time [38].

3.2.2. Adsorption capacity

Fig. 2 shows the effect of initial concentration of phosphate ions on the adsorption capacity of the phosphate-imprinted polymer at pH 2.0. For this purpose, the experiment was applied within the range 0 – $15,000 \text{ mg L}^{-1}$ initial concentration. As seen from figure, the amount of phosphate ions adsorbed per unit mass of the polymer (i.e., adsorption capacity) increased with the initial concentration of phosphate ions. The maximum adsorption for $12,500 \text{ mg L}^{-1}$ phosphate ion initial concentration was found to be $11.5 \text{ mg phosphate g}^{-1}$ 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 phosphate adsorption by imprinted polymer. Fig. 3 shows the dependence of the equilibrium concentration on the adsorbed amount of phosphate onto the phosphate-imprinted polymer. Langmuir adsorption model assumes that the molecules are adsorbed at a fixed number of well-defined

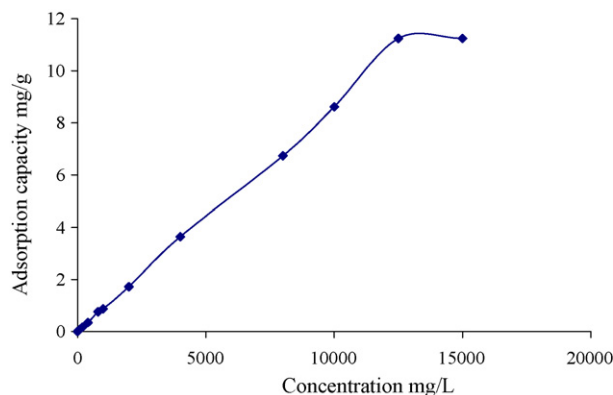


Fig. 2. Adsorption capacity of phosphate ions on the phosphate-imprinted polymer: pH, 2.0; T , 25°C .

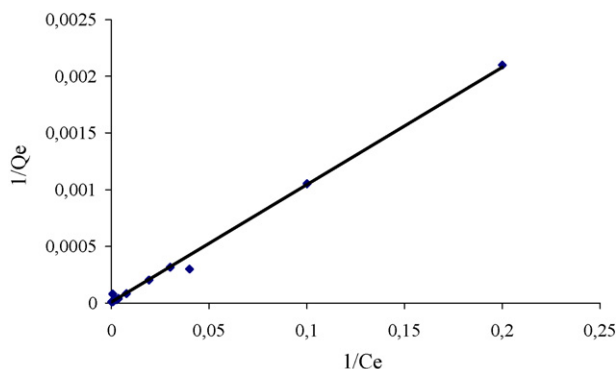


Fig. 3. Adsorption isotherm of phosphate ions on the phosphate-imprinted polymer: pH, 2.0; T , 25 °C.

sites, each of which can only hold one molecule. These sites are also assumed to be energetically equivalent, and distant to each other so that there are no interactions between molecules adsorbed to adjacent sites [39]. The corresponding transformations of the equilibrium data for phosphate ions gave rise to a linear plot, indicating that the Langmuir model could be applied in these systems and described by the equation:

$$Q = \frac{Q_{\max} b C_e}{1 + b C_e} \quad (3)$$

where Q is the concentration of bound phosphate ions on the adsorbent ($\mu\text{mol g}^{-1}$), C_e the equilibrium phosphate ions concentration in solution ($\mu\text{mol L}^{-1}$), b the Langmuir constant ($\text{g } \mu\text{mol}^{-1}$) and Q_{\max} is the maximum adsorption capacity (mol g^{-1}). This equation can be linearized.

The maximum adsorption capacity (Q_{\max}) data for the adsorption of phosphate ions was obtained from the experimental data and was found to be 11.87 mg g^{-1} . The correlation coefficient (R^2) was 0.9943. The Langmuir adsorption model can be applied in this affinity adsorbent system.

The pseudo-first- and second-order kinetic models were used to test the mechanism of adsorption process [40]. A comparison of the experimental adsorption capacity and the theoretical values are presented in Table 1. The theoretical Q value estimated from pseudo-first-order kinetic model is more close to the experimental value and the correlation coefficient. Thus, obtained results suggested that the pseudo-first-order adsorption mechanism is predominant for this phosphate-imprinted adsorbent system and that overall rate of the phosphate adsorption process appeared to be controlled by chemical reaction.

The correlation coefficient for the linear plot of t/Q_t versus t for the pseudo-second-order equation was 0.97592. Since the theoretical Q_e value was slightly more different from the exper-

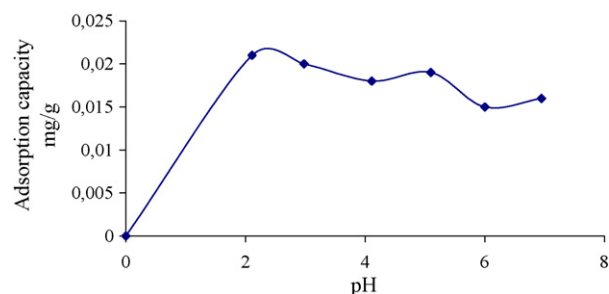


Fig. 4. Effects of pH on phosphate adsorption: phosphate initial concentration: 25 ppm; T , 25 °C.

imental value, this adsorbent system was not so well described by the pseudo-second-order kinetic model.

3.2.3. Effects of pH

The effect of pH on the phosphate ions adsorption using phosphate-chitosan-succinate particles is shown in Fig. 4. As can be seen in Fig. 4, the phosphate-imprinted polymers exhibited a high affinity in acidic conditions (pH 2.0) for phosphate ions.

Fujiwara et al. have investigated the adsorption behavior and preparation of condensed-phosphate templated resin and reported optimum pH 2.5–3 for this resin [31].

3.3. Selectivity studies

Competitive adsorption of phosphate/flouride and phosphate/thiocyanate from their couple mixture was also investigated in a batch system. Adsorption capacities of the phosphate-imprinted and non-imprinted particles for each anion (25 mg L^{-1} for each anion) under competitive conditions are given in Table 2.

The phosphate adsorption capacity of the phosphate-imprinted particles was higher than that of other ions. The competitive adsorption capacity of the phosphate-imprinted particles for phosphate ions was also higher than that of non-imprinted particles. When they exist in the same medium, a competition starts for the same attachment sites. It can be concluded that the phosphate-imprinted particles showed the following metal ion affinity order under competitive conditions: phosphate > flouride > thiocyanate. It should be noted that the imprinted microparticles was showed excellent selectivity for the target molecule (phosphate ions) due to molecular geometry.

3.3.1. Constants [41,42]

The constants such as percent extraction ($E\%$), distribution ratio (D) and selectivity coefficient ($S_{\text{Phosphate}/X}$) were determined using ascorbic acid method.

Table 1
Kinetic constants for the phosphate-imprinted polymer

Initial concentration (mg L^{-1})	Experimental Q_e (mg g^{-1})	First-order kinetic			Second-order kinetic		
		k_1	Q_e	R^2	k_2	Q_e	R^2
12,500	11.25	0.0566	11.75	0.9856	0.0047	12.5	0.97592

Table 2
Percent extraction, distribution ratio and selectivity coefficients of non-imprinted polymer/imprinted polymer

Ion	Non-imprinted polymer			Phosphate-imprinted polymer		
	$E\%$	D	$S_{\text{PO}_4/\text{X}}$	$E\%$	D	$S_{\text{PO}_4/\text{X}}$
PO_4^{3-}	91.04	10.16	–	96.84	30.65	–
SCN^-	58.68	1.42	7.15	70.88	2.43	12.61
PO_4^{3-}	89.04	8.12	–	95.84	23.03	–
F^-	55.04	1.22	6.65	56	1.31	17.58

The extraction percent ($E\%$) of phosphate and other selected anions is given by

$$\text{Extraction } (\%) = \left(\frac{A_2}{A_T} \right) \times 100 \quad (4)$$

where A_2 is the amount of anion enriched by non-imprinted polymer/imprinted polymer at equilibrium and A_T is the total amount of anion utilized in extraction.

The distribution ratio (D) was also determined using A_2 and A_1 .

$$D = \frac{A_2}{A_1} \quad (5)$$

where A_1 is the amount of anion in aqueous solution at equilibrium and A_2 is the amount of anion enriched on non-imprinted polymer/imprinted polymer at equilibrium.

Selectivity coefficient ($S_{\text{Phosphate}/\text{X}}$) is defined as

$$S_{\text{Phosphate}/\text{X}} = \frac{D_{\text{Phosphate}}}{D_{\text{X}}} \quad (\text{X} = \text{SCN}^-, \text{F}^-) \quad (6)$$

where $D_{\text{Phosphate}}$ is the distribution ratio of phosphate ion with non-imprinted polymer/imprinted polymer and D_{X} is the distribution ratio of anion with non-imprinted polymer/imprinted polymer.

The extraction percentage distribution ratio of anions and the selectivity coefficients of the phosphate ions with respect to flouride and thiocyanate ions using non-imprinted polymer/imprinted polymer are summarized in Table 2. By comparing phosphate-imprinted polymer and non-imprinted polymer data, it can be seen clearly that $E\%$ and D increased for phosphate and decreased for flouride and thiocyanate ions. Non-imprinted polymer showed minimal selectivity for phosphate over thiocyanate and flouride. On imprinting, selectivity coefficient ($S_{\text{Phosphate}/\text{thiocyanate}}$ and $S_{\text{Phosphate}/\text{flouride}}$) increased from 7.15 to 12.61 and 6.65 to 17.58, indicating reasonable separation of phosphate when it presents together with other anions.

3.4. Desorption and repeated use

The regeneration of the adsorbent is likely to be a key factor in improving process economics. Desorption of the adsorbed phosphate ions from the imprinted particles was also studied in a batch experimental set up. Fig. 5 shows the adsorption–desorption cycle of phosphate-imprinted polymer.

When KOH is used as a desorption agent, the ions were released from the phosphate templates into desorption medium.

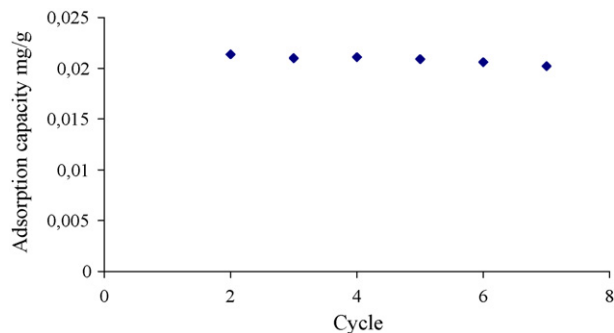


Fig. 5. Adsorption–desorption cycle of phosphate-imprinted polymer.

In order to show the reusability of the phosphate-imprinted particles, adsorption–desorption cycle was repeated seven times by using the same imprinted particles. The results showed that the phosphate-imprinted microparticles can be used repeatedly without losing their adsorption capacities significantly. Adsorption capacity of phosphate-imprinted polymer was decreased only 1% after seven repeated adsorption–desorption cycle.

3.5. Preconcentration of phosphate ion in aqueous sample

For the preconcentration of phosphate ion, 100 mL of the aqueous solution containing 1.0 ng mL^{-1} of phosphate ions was treated with 25 mg of phosphate-imprinted particles at pH 2 for 30 min. Finally, polymeric particles were separated from the adsorption media and 10 mL of 0.1 M KOH was added and stirred at 350 rpm. Imprinted particles were removed from the desorption medium and concentration of phosphate ions in the desorption medium was determined by UV spectrometer.

It is observed that preconcentration was performed more than 147.9-fold. In this manner, detection limit of 0.04 ng mL^{-1} was reached.

3.6. Analytical performance of the methods

The characteristic performance data for the off-line preconcentration procedure are given in Table 3.

The precision of method for a standard, which was evaluated as the relative standard deviation (R.S.D., $n=7$) was 0.38 ng mL^{-1} phosphate ions. The detection and determination limits defined as the concentration of analyte giving signals equivalent to 3 and 10 times, respectively, the standard devi-

Table 3
Performance characteristics of the preconcentration procedure

Parameter	Phosphate
Precision (R.S.D.)	0.38
Detection limit (3s)	0.04 ng mL^{-1}
Linear calibration range	$0.2\text{--}1.6 \text{ ng mL}^{-1}$
Regression equation (after preconcentration)	$y = 0.0343 \text{ phosphate} + 0.0022$
Conventional regression equation	$y = 0.2318 \text{ phosphate} + 0.029$
Enrichment factor	$(0.0343 \times 1000)/0.2318 = 147.9$

ation of the blank plus the net blank intensity for 100 mL of sample volume, were 0.04 and 0.31 ng mL⁻¹. The preconcentration procedure showed a linear curve within the concentration range from 0.2 to 1.6 ng mL⁻¹.

4. Conclusions

Molecular imprinted materials have been demonstrated to possess a very high degree of selectivity towards targeted substances. In this study, phosphate-imprinted metal-chelate polymer was prepared by a molecular imprinting technique. High adsorption rates are observed at the beginning of adsorption process, and then plateau values (i.e., adsorption equilibrium) are gradually reached in about 30 min. Maximum phosphate adsorption capacity of phosphate-imprinted metal-chelate polymer was found to be very high (11.25 mg g⁻¹). Adsorption values increased with increasing concentration of phosphate ions, and a saturation value is achieved at ion concentration of 12,500 mg L⁻¹, which represents saturation of active binding cavities on the phosphate-imprinted metal-chelate polymer. The phosphate-imprinted polymer showed higher selectivity towards phosphate over thiocyanate and fluoride in a couple mixture of these ions. The phosphate-imprinted metal-chelate polymer can be used many times without decreasing their adsorption capacity significantly. It can be concluded that the phosphate-imprinted metal-chelate polymer with high adsorption rates and capacities may effectively be used for specific removal of phosphate ions from aqueous solutions.

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