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Potentiometric, Kinetic, and Thermodynamic Investigations into Cu^{2+} Ion Binding Properties of Vinyl Imidazole Containing IMAC Adsorbent

Tülay Çam, Bilgen Osman, Ali Kara, Emel Demirbel, Necati Beşirli, Gazi Irez
Department of Chemistry, Uludag University, Bursa, Turkey
Correspondence to: B. Osman (E-mail: bilgeno@uludag.edu.tr)

ABSTRACT: In this study, the use of the potentiometric method for the determination of the protonation constant of vinyl imidazole (VIM) and the stability constant of the Cu^{2+} ion complex of VIM used in the immobilized metal ion affinity chromatography (IMAC) was investigated. For this purpose, poly(ethylene glycol dimethacrylate-*n*-vinyl imidazole) [poly(EGDMA–VIM)] microspheres (average diameter 150–200 μm) were prepared. The microspheres were characterized by elemental analysis, N_2 adsorption/desorption isotherms, elemental analysis, energy dispersive spectroscopy (EDS). Protonation constants of vinyl imidazole and the metal-ligand stability constant of vinyl imidazole with Cu^{2+} ions have been determined potentiometrically in 0.1*M* NaCl aqueous solution at 298, 318, and 338 K, respectively. The corresponding thermodynamic parameters of protonation and complexation processes (ΔG, ΔH, and ΔS) were derived and discussed. The formation kinetics of Cu^{2+}-vinyl imidazole complex were also investigated, and the process obeyed the pseudo-second-order kinetic model. © 2013 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2014**, *131*, 39751.

KEYWORDS: copolymers; crosslinking; functionalization of polymers

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INTRODUCTION

Immobilized metal affinity chromatography (IMAC) is a separation technique that uses covalently bound chelating compounds on solid chromatographic supports to entrap metal ions, which serve as affinity ligands for various proteins, making use of the coordinative binding of some amino acid residues that are exposed on the surface. Most commonly used are the transition-metal ions Cu^{2+}, Ni^{2+}, Zn^{2+}, Co^{2+}, and Fe^{3+}, which are electron-pair acceptors and can be considered to be Lewis acids. Iminodiacetic acid (IDA), nitrilotriacetic acid (NTA), carboxymethylated aspartic acid (CM-Asp), and triscarboxymethyl ethylene diamine (TED) are used as chelating ligands. A number of other chelating ligands,[1–12] have also been synthesized and used successfully for the separation of proteins.

In addition, 1-vinyl imidazole is a monodentate ligand that forms complexes with Cu^{2+} ions. Up to four imidazoles bind to one Cu^{2+}-ion; the log K (where K is association constant) for each imidazole ligand is decreasing from log $K_1 = 3.76$ for binding the first imidazole ligand to log $K_4 = 2.66$ for binding the fourth imidazole ligand.[13] The binding of a single imidazole ligand to the Cu^{2+} ion in a solution is much weaker compared with the binding of tridentate IDA (log $K = 11$),[14] Galaev et al.[15] reports that the imidazole ligands are not used for IMA chromatography when coupled with solid matrices because imidazole ligands are spatially separated due to a predominantly 1 : 1 complex formation, and the proper orientation of the ligands to form a complex with the same Cu^{2+}-ion is unlikely. The authors used water-soluble copolymers of vinyl imidazole (VIM) for affinity precipitation of the Kunitz soy-bean trypsin inhibitor. They explained the successful use of imidazole ligands in metal-affinity precipitation contrary to IMA chromatography via the flexibility of the water-soluble polymer as compared with the rigidity of the IMA chromatography matrix. Several imidazole ligands on a polymer molecule in a solution can come close enough to interacting with the same Cu^{2+}-ion and thus provide sufficient strength of polymer-Cu^{2+} interactions.

To overcome the rigidity of the insoluble support and to allow several imidazole ligands to interact with the same Cu^{2+}-ion, a silica surface was covered with the flexible polymer, poly(vinyl imidazole). Silica-poly-vinyl imidazole-Cu^{2+} supports bind bovine serum albumin (BSA), which is eluted by increasing the imidazole concentration.[16] This support was used for the fractionation of the three main genetic variants of desialylated human o~l-acid glycoprotein.[9] Another way to tackle the problem is to immobilize on the support of a prearranged structure containing several adjacent imidazole ligands—for instance peptide (Gly-His-His-Pro-His)*n*-Gly, where $n = 1$–3. The

Additional Supporting Information may be found in the online version of this article.

immobilized 11-residue peptide was loaded with Cu^{2+}-ions and used to demonstrate selective adsorption and isolation of proteins from human plasma.[17,18]

In our previous studies, we developed a novel approach for the preparation of a metal-chelating matrix containing vinyl imidazole, which can be used in IMA chromatography.[19] In this approach, the comonomer vinyl imidazole (VIM) is polymerized in the presence of a crosslinker ethylene glycol dimethacrylate (EGDMA), and poly(ethylene glycol dimethacrylate-*n*-vinyl imidazole) [poly(EGDMA-VIM)] hydrogel microspheres were prepared. They do not dissolve in aqueous medium but do swell depending on the degree of crosslinking.

The most important advantage of this approach over conventional techniques used for metal-chelating matrix preparation is that no need to activate the matrix for the chelating-ligand immobilization exists because the comonomer VIM acted as the metal-chelating ligand. A Cu^{2+}–poly(EGDMA-VIM) chelate matrix was prepared by adding poly(EGDMA-VIM) adsorbent to the aqueous solution of Cu^{2+} ions. The prepared Cu^{2+}–poly (EGDMA-VIM) chelate adsorbent was used for protein adsorption.[19,20] Cu^{2+} ions coordinate with the vinyl imidazole chelating-ligand, and the protein binds the polymer via the chelated-Cu^{2+} ions. This approach enables the use of an imidazole ligand in IMA chromatography when coupled with a solid matrix. This is because the several imidazole ligands on a polymer molecule can come close enough to interacting with the same Cu^{2+}-ion and thus provide sufficient strength of polymer-Cu^{2+} interactions for protein binding.

Investigations into complex stability constants and coordination numbers between metal ions with adsorbents containing different chelating agents have generally been disregarded, although these factors indicate strong and stable complexes. Only limited studies have concentrated on the acid-base and metal binding properties of new and traditional chelating adsorbents and on changes in the physicochemical properties of immobilized metal chelate complexes.[21–24] Because of the weak interaction between vinyl imidazole and Cu^{2+} ions in the solid IMA chromatography matrixes, no data exist on complex stability and complex stoichiometry in literatures. Until now, the strength of the Cu^{2+}-vinyl imidazole complex has been determined only in the water-soluble linear poly (1-vinyl imidazole) systems.[13,25,26] To our knowledge, this is the first report on the potentiometric, kinetic, and thermodynamic investigations into the Cu^{2+} ion binding properties of a solid IMA chromatography adsorbent containing vinyl imidazole as a chelating agent.

In this study, potentiometric approach was developed for determination of metal-ligand interactions in IMAC adsorbents by investigating the interaction between Cu^{2+} ions and VIM groups in the poly(EGDMA-VIM) hydrogel. The potentiometric titration method was used for the determination of the protonation constants of VIM and the stability constants of the Cu^{2+}-VIM complexes at 25, 45, and 65°C. The protonation and stability constants were determined from potentiometric titration data by using the BEST microcomputer program at three different temperatures. Kinetic studies were also conducted to clarify the Cu^{2+}-VIM complex formation process. The potentiometric approach presented in this article is helpful to researchers working on the field of protein chromatography, especially affinity chromatography, investigation of metal-ligand interactions in polymeric systems containing newly synthesized ligands. Because the stability of metal–ligand interactions is important for the protein binding, this approach will give valuable information that will lead to protein–metal interactions.

EXPERIMENTAL

Materials

Ethylene glycol dimethacrylate (EGDMA) was obtained from Merck (Darmstadt, Germany), purified by passing through active alumina, and stored at 4°C until its use. *N*-vinyl imidazole (VIM, Aldrich, Steinheim, Germany) was distilled under a vacuum (74–76°C, 10 mm Hg). Also, 2,2′-Azobisisobutyronitrile (AIBN) was obtained from Fluka A.G. (Buchs, Switzerland). Poly(vinyl alcohol) (PVAL; M_w: 100.000, 98% hydrolyzed) was supplied by Aldrich Chem (United States of America [USA]). All other chemicals (copper chloride, [Merck 99.%], disodium salt of ethylenediaminetetraacetic acid, [Merck 99%], sodium hydroxyde, and hydrochloric acid) were of an analytical grade and used without further purification. A stock solution of Cu^{2+} was prepared by dissolving the proper amount of $CuCl_2$ in a small amount of HCl (Merck 37% purity) to prevent hydrolysis. The concentration of free acid in the stock solution of Cu^{2+} was checked by potentiometric titration. The stock solution of Cu^{2+} was standardized complexometrically by ethylenediaminetetraacetic acid (EDTA) titration using the method of Schwarzenbach.[27] All water used in the binding experiments was purified using a Barnstead (Dubuque, Iowa, USA) ROpure LPw reverse osmosis unit with a high-flow cellulose acetate membrane (Barnstead D2731) followed by a Barnstead D3804 NANOpurew organic/colloid removal and ion exchange packed-bed system. All glassware were extensively washed using diluted nitric acid before being used.

Synthesis and Characterization of the Poly(EGDMA-VIM) Microspheres

The poly(EGDMA-VIM) adsorbent was selected as the metal-chelate affinity adsorbent and produced by suspension polymerization technique as described in our previous article.[19]

To evaluate the degree of VIM incorporation, the synthesized poly(EGDMA-VIM) microspheres were subjected to elemental analysis using a Leco Elemental Analyzer (Model CHNS-932, USA).

The average size and size distribution of the poly(EGDMA-VIM) adsorbent were determined via screen analysis performed using standard sieves (Model AS200, Retsch Gmb & Co., KG, Haan, Germany).

To SEM visualization, energy dispersive spectroscopy (EDS) was used to determine the elements (atoms) of which the surface of the sample is composed (Vega 3 TESCAN, Kohoutovice, Česká Republika).

The specific surface area of the adsorbent in a dry state was determined using a multipoint Brunauer-Emmett-Teller (BET) apparatus (Quantachrome Corporation, Autosorb-6, USA).

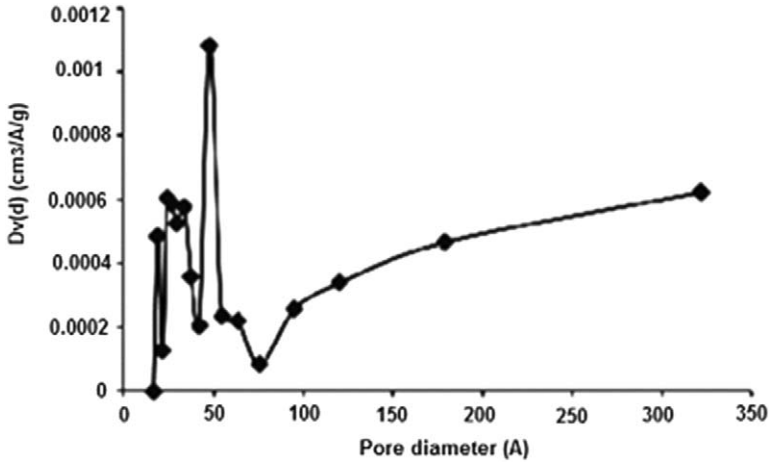

Figure 1. Dv (d) (differential pore size distribution) according to average pore diameter for the poly(EGDMA-VIM) microspheres.

Potentiometric Measurements

Potentiometric titrations were performed on a Schott Titroline Alpha Plus automatic titrator with a combined pH electrode (Schott), which was connected to a computer. All titrations were carried out in a double-walled glass cell. The temperature was kept constant inside the cell at 25.0°C ± 0.1°C, 45.0°C ± 0.1°C, and 65.0°C ± 0.1°C by circulating water from an external thermostat (VWR, precision ±0.1°C). The pH-meter was calibrated daily using standard buffer solutions (Mettler-Toledo). The combined glass electrode calibration was carried out daily from the titration of a strong acid (HCl, 0.1*M*) with a strong base (NaOH, 0.1*M*) at the same ionic strength before each titration, as was previously done. The ionic strength of the solutions was adjusted to 0.1*M* by NaCl, and a total volume of 50 mL was used for each titration. The data for the potentiometric titrations were treated using the microcomputer program BEST, as previously described.[28,29] The BEST software was used to minimize the standard deviation of the fit (σ_{fit}) between the observed and calculated pH values for the overall titration data. The species distribution diagram was obtained using the SPE program.[30] Potentiometric titrations were carried out using three different Cu^{2+} concentrations (4×10^{-3} *M*; 3×10^{-3} *M*; 2×10^{-3} *M*).

The methods used in potentiometric titrations for the determination of protonation and stability constants can be summarized as follows:

a. 5 mL 0.1*M* HCl + 5 mL 1 M NaCl (for cell calibration)
b. 5 mL 0.1*M* HCl + na mmol vinyl imidazole containing poly[(EGDMA-***H***VIM)]$^{n+}$ adsorbent + 5 mL 1*M* NaCl (for the determination of the protonation constant of VIM) [$n = 1$ to 4].
c. Solution b + a mmol Cu^{2+} ions (for the determination of the stability constant of Cu^{2+}-VIM complex) [$a = 0.01$ to 0.02].

Determination of Protonation Constants. For the determination of protonation constants (log K) of VIM, potentiometric titrations were performed at 25, 45, and 65°C. First, the VIM content of the poly(EGDMA-VIM) adsorbent that can form a complex with Cu^{2+} ions was determined to be mmol protons (H^+) / g adsorbent by potentiometric titration. The amount of proton is equal to the amount of VIM in the poly(EGDMA-VIM) structure due to the one protonable amine group in the imidazole ring. For this purpose, [poly(EGDMA-***H***VIM)]$^{n+}$ adsorbent was prepared from poly(EGDMA-VIM) adsorbent by protonation of the VIM groups in the poly(EGDMA-VIM) structure according to the following eq. (1):

$$\text{Poly (EGDMA} - \text{VIM)} + \text{n H}^+ \rightleftharpoons [\text{Poly (EGDMA -}\boldsymbol{H}\text{VIM)}]^{n+} \quad (1)$$

Briefly, the poly(EGDMA-VIM) adsorbent (1 g) was transferred in an HCl solution (0.1*M*, 100 mL), and the medium was incubated in a shaking water-bath at 100 rpm for 5 h at room temperature. Following this period, the [poly(EGDMA-***H***VIM)]$^{n+}$ microspheres were filtered and washed with an excess amount of water. Then, the microspheres were dried and used for the potentiometric titration experiments. The mmol protons (H^+) per gram [poly(EGDMA-***H***VIM)]$^{n+}$ adsorbent were determined via potentiometirc titration of protons (H^+) with a 0.1*M* NaOH solution in a 0.1*M* NaCl medium at 25, 45, and 65°C. The protonation constants of VIM for three different temperatures were calculated from potentiometric titration data using

Table I. Elemental Analysis of Poly(EGDMA-VIM) Microspheres

Sample	Elemental analysis (experimental value)			Mole fractions in copolymer (%)	
	N (wt %)	C (wt %)	H (wt %)	EGDMA	VIM
Poly(EGDMA-VIM)	10.22	51.25	10.71	47.1	52.9

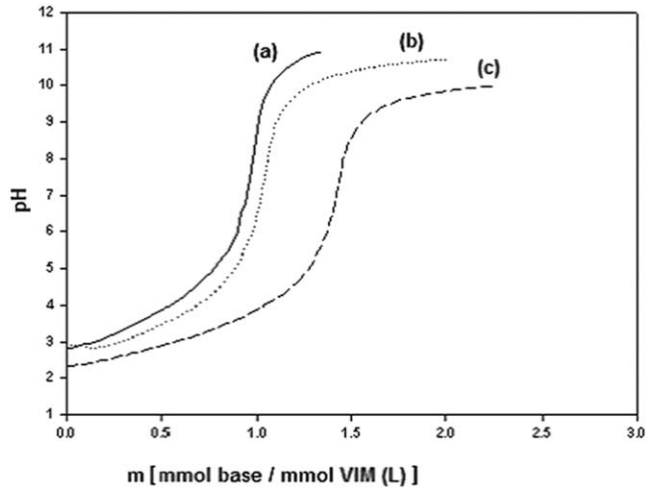

Figure 2. Titration curves of [poly(EGDMA-***H***VIM)]$^{n+}$ adsorbent in a 0.1*M* NaCl medium at (a) 25°C, (b) 45°C, and (c) 65°C.

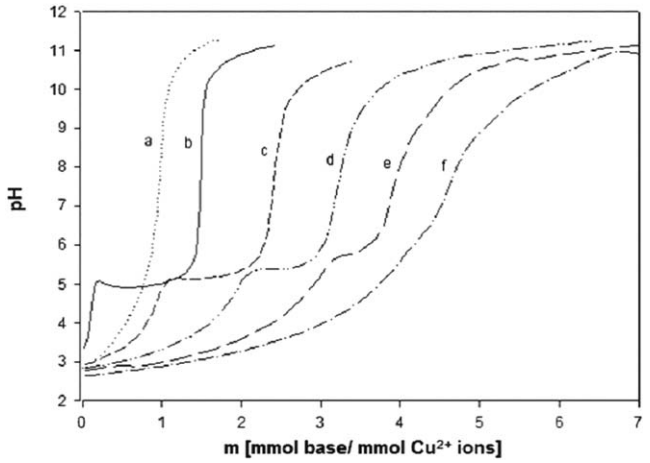

Figure 3. The titration curves of (a) [poly(EGDMA-***H***VIM)]$^{n+}$ adsorbent; (b) Cu^{2+} ions; (c) (1 : 1); (d) (1 : 2); (e) (1 : 3); and (f) (1 : 4) Cu^{2+}:[poly(EGDMA-***H***VIM)]$^{n+}$ systems at 25°C.

the BEST program. The protonation constant (log *K*) values were used to calculate the thermodynamic parameters (ΔG, ΔH, and ΔS) of the protonation process.

Determination of Stability Constants. To determine the stability constant (log β) of the Cu^{2+}-vinyl imidazole complex at 25, 45, and 65°C, potentiometric titrations of Cu^{2+} : [poly(EGDMA-***H***VIM)]$^{n+}$ systems prepared at different Cu^{2+} : VIM mole ratios were performed. The four different Cu^{2+} : [poly(EGDMA-***H***VIM)]$^{n+}$ systems for which the Cu^{2+} : VIM mole ratios are (1 : 1), (1 : 2), (1 : 3), and (1 : 4) were prepared by taking into consideration the VIM group content of the [poly(EGDMA-***H***VIM)]$^{n+}$ adsorbent. Potentiometric titrations were conducted as described previously, and the stability constant (log β) values of the Cu^{2+} : VIM complex were calculated at 25, 45, and 65°C by using the BEST microcomputer program. The thermodynamic parameters (ΔG, ΔH, and ΔS) of the complex formation process were determined.

Batch Studies

For the determination of Cu^{2+} : VIM complex stoichiometry, Cu^{2+}-chelated poly(EGDMA-***H***VIM)]$^{n+}$ adsorbents for which Cu^{2+} : VIM ratios are (1 : 1), (1 : 2), (1 : 3), and (1 : 4) were also prepared in a batch system. A total of 0.25 g of the adsorbent was mixed with 25 mL of aqueous solutions containing Cu^{2+} ions at different concentrations, at a constant pH of 5.0 (adjusted using HCl and NaOH), which was the optimum pH for Cu^{2+} chelate formation at room temperature. The flasks were stirred magnetically at 100 rpm for 3 h (sufficient to reach equilibrium), and the microspheres were filtered. The concentration of the Cu^{2+} ions in the resulting solution was determined with a graphite furnace atomic absorption spectrometer (Analyst 800/Perkin–Elmer, USA). The results were used for the determination of the mmol Cu^{2+} ions per mmol VIM in the poly(EGDMA-VIM) chain.

Kinetic Studies for Complex Formation

Kinetic studies of Cu^{2+}-VIM complex formation were investigated by using the potentiometric method. Firstly, 20 mL of an aqueous solution containing Cu^{2+} ions (0.01*M*) was prepared at a pH of 5.0. Then, [poly(EGDMA-***H***VIM)]$^{n+}$ adsorbent was added to a Cu^{2+} ions-containing solution, and the pH of the solution was measured at 30-s time intervals. The obtained data were used to evaluate the fitting of kinetic models.

RESULTS AND DISCUSSION

Properties of Poly(EGDMA-VIM) Microspheres

The poly(EGDMA-VIM) adsorbent was prepared as previously described[19] in the spherical form in the size range of 150–200 μm. The specific surface area of the poly(EGDMA-VIM) adsorbent was found to be 66.3 $m^2\ g^{-1}$, which is relatively high due to the roughness of the bead surfaces. The ratio of EGDMA and VIM in the poly(EGDMA–VIM), as calculated from the nitrogen stoichiometry based on the elemental analysis data, is shown in Table I.

The elemental analysis results suggested that mole fractions of EGDMA and VIM in the copolymer structure are 47.1 and 52.9%, respectively. The ratio of EGDMA to VIM was 1 : 1. On the other hand, the mole fractions of EGDMA and VIM are

Table II. Protonation Constants (log K ± σ^a) and Thermodynamic Functions for the Protonation of Vinyl Imidazole in 0.1*M* NaCl Ionic Medium at 25°C, 45°C, and 65°C

Ligand	Temperature (K)	Protonation constants (log K ± σ^a)	Gibbs energy change ΔG^0 (KJ mol^{-1})	Enthalpy change ΔH^0 (KJ mol^{-1})	Entrophy change ΔS^0 (J mol^{-1} K^{-1})
Vinyl imidazole	298	3.61 ± 0.05	−20.60 ± 0.28	−45.54 ± 0.39	−82.63 ± 0.39
	318	3.28 ± 0.06	−19.97 ± 0.37		
	338	2.66 ± 0.08	−17.52 ± 0.52		

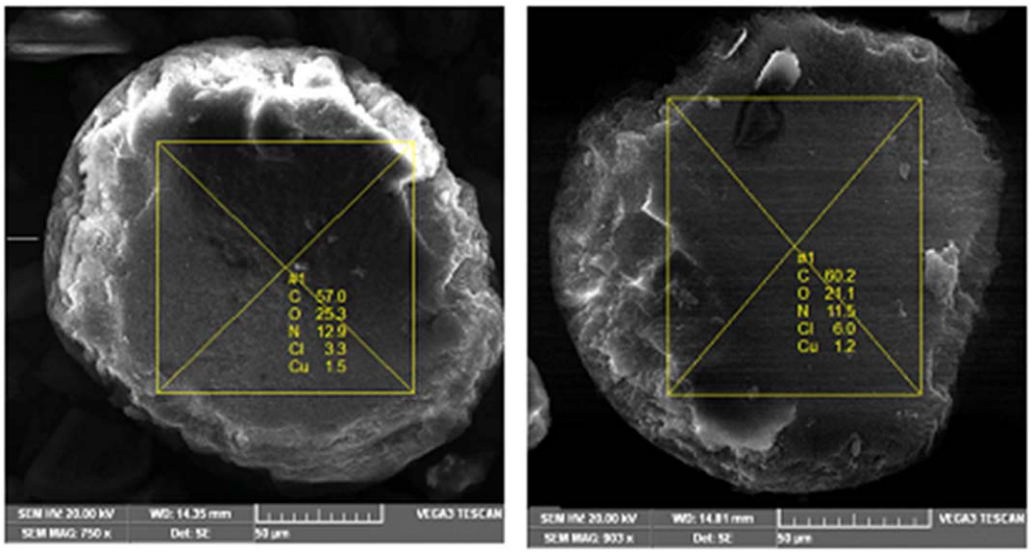

Figure 4. SEM/EDX analysis of Cu^{2+}-chelated poly(EGDMA-VIM) adsorbent prepared at (a) 1 : 1 and (b) 1 : 4 Cu^{2+}:[poly(EGDMA-***H***VIM)]$^{n+}$ systems. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

calculated using VIM group content of the poly(EGDMA-VIM) structure obtained from potentiometric data. The ratio of mmol EGDMA to VIM was calculated as 5. This result shows that repetitive units were formed from 5 mol EGDMA to 1 mol VIM. From this result, the mole fractions of EGDMA and VIM were determined to be 83.3 and 16.7%, respectively. When compared with elemental analysis results, the decrease in the mole ratio of VIM shows that many vinyl imidazole groups in the poly(EGDMA-VIM) chain can be inaccessible in an aqueous solution. Therefore, the chelation of Cu^{2+} ions can occur mainly on the surface of the adsorbent and in the pores that the hydrated Cu^{2+} ions can diffuse.

To obtain information on pore size of the poly(EGDMA-VIM) microspheres, the N_2 adsorption/desorption isotherm was also evaluated. The isotherm and corresponding pore size distribution curve for the poly(EGDMA-VIM) microspheres are shown in Figure 1. The BET surface area (S_{BET}), pore volume (V_P), and pore size are given in Supporting Information Table SI1.

The average pore size of the poly(EGDMA-VIM) microspheres was determined to be 67.74 angstrom by using the N_2 adsorption/desorption isotherm data. The ionic radius of a hydrated Cu^{2+} ion (four coordinated) is 0.71 Å in an aqueous solution. When the ionic radius of the hydrated Cu^{2+} ion is considered, it can be clearly said that the hydrated Cu^{2+} ions can diffuse into the pores near the surface of the adsorbent and form a chelate with accessible VIM groups.

Determination of the Protonation Constant and Thermodynamic Parameters

The protonation constant (log *K*) is a value that shows the basicity of the ligand. Log *K* values of VIM have been determined potentiometrically in a 0.1*M* NaCl medium at 25, 45, and 65°C. In Figure 2, the titration curves of [poly(EGDMA-***H***VIM)]$^{n+}$ in a 0.1*M* NaCl medium at 25, 45, and 65°C were depicted. From the potentiometric titration data of [poly(EGDMA-***H***VIM)]$^{n+}$ adsorbent at 25°C, the mmol VIM was determined to be 0.868 mmol per g poly(EGDMA-VIM) adsorbent.

Because of the fact that change in temperature affects the activities of the ions as well as the liquid-junction potentials, shifts in the m values of the titration curves occur with increasing temperature. In the titration curve of the [poly(EGDMA-***H***VIM)]$^{n+}$ adsorbent, only a single inflection point exists because the VIM groups in the poly(EGDMA-VIM) chain have one protonable amine group. Log *K* values of the VIM ligand (L) were calculated from potentiometric data using BEST software at 25, 45, and 65°C and are provided in Table II.

Protonation process of VIM ligand can be described by the following equation:

$$L + H^+ \overset{K}{\rightleftharpoons} [LH]^+ \quad K = [LH^+]/[H^+][L] \tag{2}$$

where L and $[LH]^+$ symbolize VIM and protonated-VIM in the poly(EGDMA-VIM) chain, respectively. *K* is the equilibrium constant of the protonation reaction.

Thermodynamic parameters—the enthalpy change (ΔH), the Gibbs energy change (ΔG), and the enthalpy change (ΔH) of the protonation processes—were also calculated from potentiometric titration data. The ΔG and ΔH values, which one can use to deduce the entropy changes (ΔS), were also calculated for the protonation process of vinyl imidazole.

The thermodynamic parameters of the protonation process of VIM were also recorded in Table II. From these results, the following conclusions can be made:

a. The log *K* values decrease with increasing temperature, i.e. the acidity of the vinyl imidazole ligand (L) increases.
b. A negative value of ΔH indicates that the process is exothermic.

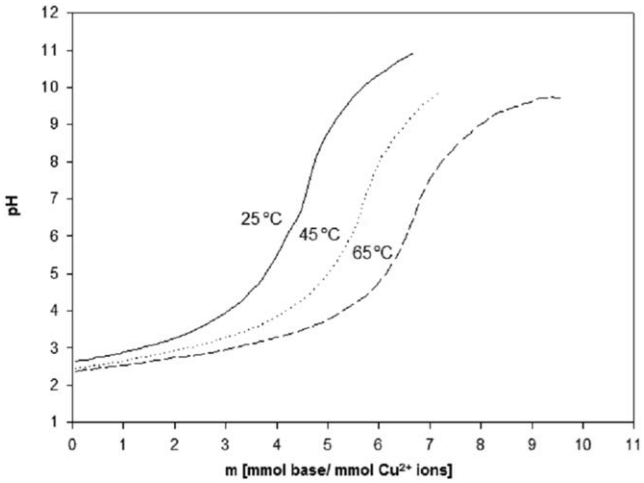

Figure 5. The titration curves of the (1 : 4) Cu^{2+}:[poly(EGDMA-*H*VIM)]$^{n+}$ system at 25, 45, and 65°C.

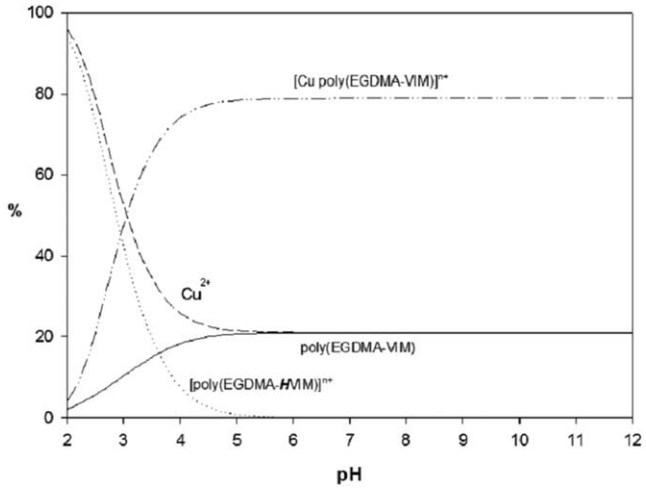

Figure 6. The distribution curves of Cu^{2+} and their coordination species at the Cu^{2+}:[poly(EGDMA-***H***VIM)]$^{n+}$ system (metal-to-ligand ratio of 1 : 4).

c. A negative value of ΔG indicates that the process is spontaneous.
d. The protonation process for vinyl imidazole (L) has a negative value of ΔS due to the increased order.

Analysis of Cu^{2+}-VIM Complex Formation

Potentiometric Titrations of Cu^{2+}:[poly(EGDMA-HVIM)]$^{n+}$ System. To clarify the Cu^{2+}-VIM complex formation, potentiometric titrations of Cu^{2+}:[poly(EGDMA-***H***VIM)]$^{n+}$ systems were performed in a 0.1*M* NaCl medium. The temperature was kept in 25°C. The aqueous solutions containing [poly(EGDMA-***H***VIM)]$^{n+}$ adsorbent and Cu^{2+} ions were also separately titrated with a 0.1*M* NaOH solution in a 0.1*M* NaCl medium. The titration curves were provided in Figure 3.

Two inflection points were observed in the titration curves of Cu^{2+}:[poly(EGDMA-***H***VIM)]$^{n+}$ systems [Figure 3(c–f)]. While the first inflection points belong to Cu^{2+}-VIM complexes, the second inflection points result from the hydrolysis of Cu^{2+} ions that do not participate in complex formation. When compared with the titration curve of [poly(EGDMA-***H***VIM)]$^{n+}$ adsorbent [Figure 3(a)], the shifts in *m* values that occurred with the first inflections and the decreases in the initial pH values of the titration curves of Cu^{2+}:[poly(EGDMA-***H***VIM)]$^{n+}$systems demonstrate the Cu^{2+}-VIM complex formation for all of the investigated Cu^{2+}:[poly(EGDMA-***H***VIM)]$^{n+}$ systems. When the titration curves regarding (1 : 1), (1 : 2), (1 : 3), and (1 : 4) Cu^{2+}:[poly(EGDMA-***H***VIM)]$^{n+}$ systems were compared with that of Cu^{2+} ions [Figure 3(b)], it can be said that residual Cu^{2+} ions exist and the hydrolysis of Cu^{2+} ions occurs above a pH of 5.0. The amount of residual Cu^{2+} ions decreased from (1 : 1) to (1 : 4) Cu^{2+}:[poly(EGDMA-***H***VIM)]$^{n+}$ systems because the ratio of Cu^{2+} to VIM decreased. The presence of residual Cu^{2+} ions at a (1 : 1) Cu^{2+}:[poly(EGDMA-***H***VIM)]$^{n+}$ system proves that the stoichiometry of the complexes that formed between Cu^{2+} ions and VIM ligands in the polymeric chain is not 1 : 1 (Cu^{2+} : VIM). This is because the Cu^{2+} ions in the medium are sufficient to form complexes with all available imidazole groups in the [poly(EGDMA-***H***VIM)]$^{n+}$ structure.

Batch Studies of Cu^{2+}:[poly(EGDMA-HVIM)]$^{n+}$ System. For the determination of complex stoichiometry, Cu^{2+}:[poly(EGDMA-***H***VIM)]$^{n+}$ systems were also studied in batch systems. The Cu^{2+}-chelated poly(EGDMA-VIM) adsorbent was prepared by incubating the poly(EGDMA-***H***VIM)]$^{n+}$ adsorbent in the Cu^{2+} ion solutions. The concentration of the Cu^{2+} ions in the resulting solutions were determined and used for the calculation of Cu^{2+}-VIM complex stoichiometry. It was shown that 2.13, 2.18, 2.03, and 2.08 vinyl imidazole ligand binds per one Cu^{2+} ion on Cu^{2+}-chelated poly(EGDMA-VIM) adsorbent prepared at (1 : 1), (1 : 2), (1 : 3), and (1 : 4) Cu^{2+}:[poly(EGDMA-***H***VIM)]$^{n+}$ systems, respectively. These results show that two vinyl imidazole ligands in the polymeric chain are bound to the one Cu^{2+} ion. In other words, two

Table III. Stability Constants (log $\beta \pm \sigma^a$) and Thermodynamic Functions for the CuL_2 Type Complex Formation in 0.1*M* NaCl Ionic Medium at 25°C, 45°C, and 65°C

Complex type	Temperature (K)	Stability constants (log $\beta \pm \sigma^a$)	Gibbs energy change ΔG^O (KJ mol^{-1})	Enthalpy change ΔH^O (KJ mol^{-1})	Entrophy change ΔS^O (J mol^{-1} K^{-1})
CuL_2	298	6.71 ± 0.20	−38.29 ± 1.14	−88.78 ± 1.11	−167.6 ± 1.11
	318	5.85 ± 0.23	−36.65 ± 1.40		
	338	4.86 ± 0.12	−31.45 ± 0.78		

Table IV. Kinetic Parameters for the Complex Formation Between Cu^{2+} Ions and VIM Ligands

		Pseudo-first-order kinetic model $\log(q_e - q_t) = \log q_e - \frac{k_1 t}{2.303}$			Pseudo-second-order kinetic model $\frac{t}{q_t} = \frac{1}{k_2 q_e^2} + \frac{1}{q_e} t$			Ritchie's-second-order kinetic model $\frac{1}{q_t} = \frac{1}{k_R q_{eq} t} + \frac{1}{q_e}$			Intraparticle diffusion model $q_1 = k_i t^{1/2}$	
Parameters Temperature (K)	Experimental q_e (mg g^{-1})	$k_1 \times 10^{-1}$ (1/min)	q_{eq} (mg g^{-1})	R^2	h ((mg g^{-1}) min^{-1})	k_2 ((g mg^{-1}) min^{-1})	q_{eq} (mg g^{-1})	R^2	k_R (1/min)	q_{eq} (mg g^{-1})	R^2	$k_i \times 10^{-1}$ ((mg g^{-1}) $min^{-0.5}$)	R^2
298	2.394	3.984	0.3720	0.8538	22.42	3.817	2.409	0.9999	22.23	2.380	0.7515	2.497	0.3240
318	1.709	3.952	0.3170	0.8420	12.33	4.141	1.723	0.9999	16.93	1.697	0.9416	1.872	0.3570
338	1.229	3.429	0.2243	0.8315	5.894	5.220	1.240	0.9999	12.04	1.221	0.9550	1.392	0.3844

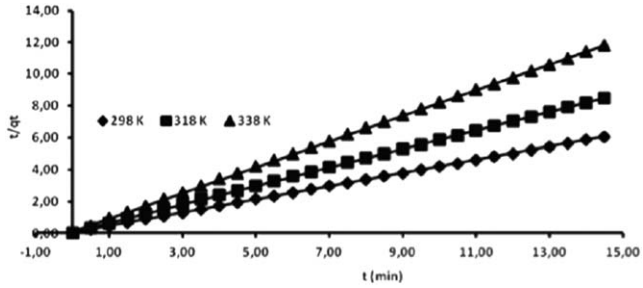

Figure 7. Formation kinetics of the Cu^{2+}-VIM complex at different temperatures: pseudo-second-order kinetic model.

imidazole groups are close enough to form a complex with the same Cu^{2+} ion, thus providing significant strength of an interaction in the poly(EGDMA-VIM) structure. Kumar et al. prepared Cu^{2+} chelated, the water-soluble copolymer of *N*-isopropylacrylamide (NIPAM), with 1-vinylimidazole (VIM) and used this matrix for purifying an α-amylase inhibitor from wheat meal. The spectrophotometric method was used for Cu^{2+} determination in the copolymer structure. The authors also reported that one Cu^{2+} ion is bound to about two imidazole groups in the copolymers.[31] These results show that potentiometric approach can be used for investigation of metal-ligand interactions in insoluble polymers.

SEM/EDX Analysis of Cu^{2+}-Chelated Adsorbent. In conjunction with generating SEM images, the electrons generate X-rays from the surface of the materials in the sample. The X-rays emitted from the sample can be interpreted using EDX to determine the elements (atoms) of which the surface of the sample is composed as well as the elemental composition of the features on the sample. To get information on the surface composition of the Cu^{2+}-chelated poly(EGDMA-VIM) adsorbent, SEM/EDX analysis was conducted for the adsorbent prepared at (1 : 1) and (1 : 4) Cu^{2+}:[poly(EGDMA-***H***VIM)]$^{n+}$ systems. The SEM photographs coupled with surface elemental compositions are given in Figure 4. As can be seen from the figure, surface composition is not changed with a decrease in the Cu^{2+} : VIM ratio. The findings support that a particular complexation manner in the Cu^{2+}-VIM complex exists in the poly(EGDMA-VIM) chain.

Determination of the Stability Constant and Thermodynamic Parameters. Until now, a limited number of studies have concentrated on Cu^{2+}-vinyl imidazole complex formation.[25,26,31,32] Moreover, none of them were related to Cu^{2+}-to-vinyl imidazole interaction in solid IMA chromatography material. The poly (EGDMA-VIM) polymer is the first chromatographic adsorbent for which vinyl imidazole can be used as a chelating ligand in the immobilized metal ion affinity. Despite the fact that the experiments were not conducted for the determination of stability constants, Kumar et al. predicted that one could expect the binding strength of log β 5.5–6.0 when about two imidazole ligands bound to the Cu^{2+} ion.[31] The stability constants (log β) of the CuL_2 (L is VIM) type complex formation at 25, 45, and 65°C were calculated using the BEST computer program using the potentiometric titration data from the (1 : 4) Cu^{2+}:[poly (EGDMA-***H***VIM)]$^{n+}$ system. The mole ratio of (1 : 4) was used for the stability constants formation because the amount of

Table V. Linear Equations ($y = ax + b$) for all Kinetic Models with Standard Deviations

Temperature (K)	Pseudo-first-order kinetic model	Pseudo-second-order kinetic model	Ritchie's-second-order kinetic model	Intraparticle diffusion model
298	Y = 0,173 ± 0.241x+ 0,429 ± 0.706	Y = 0,4150 ± 0.00069x+ 0,0446 ± 0.00592	Y = 0,0189 ± 0.198x+ 0,4202 ± 0.136	Y = 0,2497 ± 0.0792x+ 1,6458 ± 0.217
318	Y = 0,171 ± 0.266x+ 0,499 ± 0.780	Y = 0,5750 ± 0.00218x+ 0,0811 ± 0.0187	Y = 0,0348 ± 0.235x+ 0,5893 ± 0.161	Y = 0,1872 ± 0.170x+ 1,1451 ± 0.464
338	Y = 0,148 ± 0.259x+ 0,649 ± 0.760	Y = 0,8065 ± 0.00169x+ 0,1246 ± 0.0145	Y = 0,0680 ± 0.276x+ 0,8187 ± 0.190	Y = 0,1392 ± 0.267x+ 0,8060 ± 0.731

residual Cu^{2+} ions is minimal. The titration curves of the (1 : 4) system at three different temperatures are given in Figure 5. The log β values and thermodynamic parameters for the CuL_2-type complex formation are provided in Table III.

When one Cu^{2+} ion binds to two imidazole ligands, the stability constant (log β) of the CuL_2-type complex was determined to be 6.71 ± 0.20 at 25°C in a 0.1*N* NaCl ionic medium. Complexation equilibria of Cu^{2+} ions with linear water-soluble poly(vinyl imidazole) has been also investigated potentiometrically. The stability constant (log β_2) of the Cu^{2+} ion complex was reported to be 7.66 for two vinyl imidazole ligands binding to one Cu^{2+} ion.[26] The stability constant value of the CuL_2-type complex in a poly(EGDMA-VIM) chain is lower than that of the linear poly(VIM). This result shows that the existence of crosslinker EGDMA in the polymeric structure makes the formation of the complex more difficult. However, the stability of the Cu^{2+}-VIM complex in the poly(EGDMA-VIM) chain is sufficient for a strong interaction between vinyl imidazole ligands and Cu^{2+} ions as well as a Cu^{2+} ions and protein interaction.

The thermodynamic parameters of the complexation process of vinyl imidazole were calculated and recorded in Table III. Examination of these values shows that:

a. The stability constants for adsorbent-linked vinyl imidazole complexes decrease with increasing temperature, which means that the stability of the complexes decreases at higher temperatures.
b. The negative value of ΔG for the complexation process suggests the spontaneous nature of such a process.
c. The Δ*H* values are negative, meaning that these processes are exothermic and unfavorable at higher temperatures.
d. The Δ*S* values for the ligand complexes are negative, confirming that the complex formation is entropically unfavorable.

The distribution curves of Cu2+ and their coordination species that form at the Cu2+:[poly(EGDMA-***H***VIM)]$^{n+}$ system (1 : 4) were prepared by SPE system and are shown in Figure 6. As can be seen from the distribution curves, 80% of the Cu^{2+} ions participate in the complex formation. The complex formation occurs at a pH of 5.0, and 80% of the [poly(EGDMA-***H***VIM)]$^{n+}$ take part in the complex formation.

Kinetic Analysis of Complex Formation

For testing the dynamic complex formation experimental data, the pseudo-first-order kinetic model,[33] the pseudo-second-order kinetic model,[34] the modified Ritchie's-second-order kinetic model[35] and the intraparticle diffusion model[36] were used at the initial concentration—0.01*M*, of Cu^{2+} ions and three temperatures (298, 308, and 318 K) at a pH of 5.0.

$$\text{Pseudo-first-order } \log(q_e - q_t) = \log q_e - k_1 t/2.303 \quad (3)$$

$$\text{Pseudo-second-order } \frac{t}{q_t} = \frac{1}{k_2 q_e^2} + \frac{1}{q} t \quad (4)$$

$$\text{Ritch-second-order } \frac{1}{q_t} = \frac{1}{k_R q_e t} + \frac{1}{q_e} \quad (5)$$

$$\text{Intraparticle diffusion } q_t = k_i t^{1/2} \quad (6)$$

where k_1(1/min), k_2((g mg^{-1}) min^{-1}) and k_R(1/min) are kinetic constants for pseudo-first-order, pseudo-second-order and Ritch-second order kinetic models, respectively. k_i ((mg g^{-1})/min$^{-1/2}$) is the intraparticle diffusion rate constant. q_e and q_t (mg g^{-1}) are the amounts of the Cu^{2+} adsorbed at equilibrium and at time (min), respectively.

The values of constants in eqs. (3–6) can be obtained from the slopes and intercepts of the fitted curves, and the results are shown in Table IV. The highest correlation coefficient values (0.9999) of the pseudo-second-order model for all of the studied temperatures and the closest q_e (experimental) to q_e (calculated) indicated the second-order nature of the present complexation process (Figure 7). The results of calculation performed according to the all models were presented in Table V in the form of a linear equation: $y = ax + b$. The standard deviations were also included in Table V.

CONCLUSIONS

In this study, the Cu^{2+} binding properties of the vinyl imidazole containing solid IMA chromatography material were investigated. Poly(EGDMA-VIM) microspheres were synthesized by suspension polymerization and characterized using N_2 adsorption/desorption isotherms, elemental analysis, EDS analysis. The protonation constants of VIM and the thermodynamic parameters of the protonation process were calculated at three different temperatures. The values of the protonation constants decreased with increasing temperature. Δ*G*, Δ*H*, and Δ*S* values showed that the protonation process is spontaneous, exothermic, and entropically unfavorable. The stoichiometry of the Cu^{2+}-VIM complex was also determined. In the poly(EGDMA-VIM) chain,

two vinyl imidazole groups bind to one Cu^{2+} ion. The stability constants and thermodynamic parameters (ΔG, ΔH, and ΔS) of the CuL_2-type complex formation were calculated first in the literature. The results show that the complex formation occurs spontaneously and that the formation process is exothermic and entropically unfavorable. The complex formation process could be best described by the pseudo-second-order kinetic model. Overall, the findings presented in this study are first in the literature for vinyl imidazole containing a solid IMA chromatography matrix and will offer insight into protein-binding studies of vinyl imidazole containing solid matrix.

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