Polymeric Hydrogels Modified with Ornithine and Lysine: Sorption and Release of Metal Cations and Amino Acids

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ABSTRACT: A novel, convenient synthesis, using copper ions, is described for the multigram-scale preparation of acryloyl and methacryloyl ornithine and lysine without the need to use protecting groups and chromatographic purifications. Three methods of removing the copper ions from the amino acid derivatives were examined. The obtained acryloyl and methacryloyl ornithine and lysine were copolymerized with *N*-isopropylacrylamide and *N,N′*-methylenebisacrylamide as crosslinking agents, resulting in a series of hydrogels with varying incorporated amino acid content. The relative content of a given amino acid was estimated from the ^{1}H NMR data and compared with its molar fraction used in the polymerization process. We investigated the influence of the amount of amino acid groups incorporated into the polymer network on the swelling behavior of the gels in the presence of metal ions of different ability to form complexes (Cu^{2+}, Co^{2+}, and Ca^{2+}) with α-amino acid groups and the sorption of copper ions. Next, the presence of α-amino acid groups attached to the polymer network was used to bond the compounds which can cocomplex metal ions. Phenylalanine was selected for examination of its cocomplexation of Cu^{2+} with the polymer-network amino acids and its consecutive release from the gel after appropriate change of pH. © 2011 Wiley Periodicals, Inc. J Polym Sci Part A: Polym Chem 50: 542–550, 2012

KEYWORDS: adsorption; amino acids; drug delivery systems; hydrogels; metal complexes; polyamides; release; sorption; synthesis

INTRODUCTION Amino acids are biologically important compounds and play an invaluable role as chiral auxiliaries and building blocks in organic synthesis. Scientists working in various areas have focused their research on stimuli-responsive amino acid-based polymer networks. Stimuli-responsive polymers have moreover gained much attention because of their wide range of potential applications, including in drug delivery systems, artificial muscles, separation techniques, enzyme immobilization matrixes, sensor construction, swing absorbers, and molecular recognition.[1–18] The presence of amino acid moieties may give gels new properties such as catalytic activity, sorption properties, sensitivity to pH, ionic strength, and the presence of specific ions. The temperature of the volume phase transition of thermosensitive gels based on *N*-isopropylacrylamide (NIPA) and the corresponding volume change may increase or decrease, and the volume phase transition may switch from discontinuous to continuous.[19–25]

Most of the polymers designed for biomedical and industrial use contain amino acid residues in the main chain and are expected to exhibit biocompatibility and biodegradability similar to polypeptides. The groups we used for modification of the polymeric chains were appropriately modified α-amino acids.[21–23,26–30] The advantage of using amino acids in preparing functional polymers is that that amino acids are biologically active and should behave similarly in the polymeric network. However, the amino groups of those α-amino acids are usually bound due to the way the polymeric chains are built and, therefore, lose their typical properties.

We have recently reported the synthesis of new polymeric gels with free α-amino acid groups based on modified L-ornithine and L-lysine and optionally NIPA. The presence of α-amino acid in those polymers makes it possible to control the charge sign and the excess of charge in the polymeric network by changing pH. The synthesized gels showed an interesting swelling behavior in response to changes in temperature, pH, and concentration of divalent metal ions.[24,25] Moreover, the gels based on poly(*N*-δ-acryloyl ornithine) lightly crosslinked with *N,N′*-methylenebisacrylamide (BIS) are characterized by relatively high sorption capacity, high uptake capacity in a wide range of pH, and short time of reaching equilibrium toward copper ions.[19]

In this study, we examined a few methods for the preparation of acryloyl and methacryloyl lysine and ornithine. To avoid using the protecting group methodology, the copper complex method was chosen to prepare side-chain derivatives of ornithine and lysine. In this method, a stable

copper–amino acid complex is formed. In the next step, the appropriate acid chloride reacts with the side chain of the functional group, because the α-amino group and the carboxylic group are bound to the copper ion. Finally, the copper ion is removed and the desired functionalized amino acid is obtained. There are several methods for removing copper from the amino acid–copper complexes, but some of them fail when an additional functional group is present. In our investigation, we prepared copper complexes of acryloyl and methacryloyl L-lysine and L-ornithine and compared the usability of three methods [ethylenediaminetetraacetic acid (EDTA), $NaBH_4$, and thioacetamide] for the removal of copper from the amino acids complexes.

We used the L-lysine and L-ornithine derivatives as monomers in the free-radical copolymerization with NIPA and BIS to prepare a series of hydrogels, obtaining gels with varying amounts of free α-amino acid groups. We examined the influence of the amount of amino acid groups incorporated into the polymer network on the swelling behavior of the gels immersed into pure water and aqueous solutions of Cu^{2+}, Co^{2+}, and Ca^{2+} and the sorption properties toward Cu^{2+}. The presence of free amino acid groups attached to the polymer network opens up the possibility of further modification of the gel using the compounds that contain an amino acid group capable of cocomplexing the metal ions with the polymer network of the gel and creation of the complexes polymer network–metal ion–compound. The compounds used to modify the gel could then be released from the gel by altering, for instance, the pH of the environment. The amount and mechanism of phenylalanine release as a function of pH were investigated.

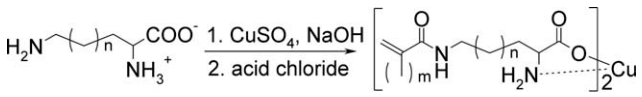
RESULTS AND DISCUSSION

Synthesis of Amino Acid-Based Monomers

We began our study by seeking an appropriate method of synthesis for both acryloyl and methacryloyl derivatives of two amino acids lysine (**1**) and ornithine (**2**). We decided to use a method that allowed for the avoidance of the protecting group methodology, chromatographic purification, while being easy to use in multigram scale.

First, we examined the reaction of hydrochlorides of ornithine (**1**) and lysine (**2**) with copper sulfate in alkaline solutions, followed by treatment of the reaction mixtures with acryloyl or methacryloyl chlorides, which leads to the formation of copper complexes of ornithine (**3** and **4**) and lysine (**5** and **6**) (Scheme 1). The complexes of copper with lysine and ornithine are water soluble and their α-amino functions are blocked what allowed selective side-chain functionalization, here acylation of δ- and ε-amino groups of ornithine (**1**) and lysine (**2**), respectively.

We found that the modified copper complexes **3**–**6** are insoluble in most common organic solvents as well as in water. This facilitated the isolation and purification of the derivatives. The precipitated products were filtered and washed several times. The yield was in the range of 62–84%.



1 n=1
2 n=2

3 n=1, m=0
4 n=1, m=1
5 n=2, m=0
6 n=2, m=1

SCHEME 1 Synthesis of copper complexes of amino acids.

Next, for producing free monomers, we examined the methods of removing copper from the synthesized complexes. There are several procedures based on treatment with EDTA, thioacetamide, hydrochloric and hydrobromic acids, sodium borohydride, potassium cyanide, ion exchange resin, and 8-quinolinol.[25,31–38] We selected just three of them for further examination: EDTA, sodium borohydride, and thioacetamide. All reactions were carried out in water (see Scheme 2).

The EDTA method is most widely used but seemed inappropriate in our case because both copper–EDTA complexes formed and liberated amino acid derivatives are water soluble, which causes difficulties in the separation and purification of the desired products. The separation of the copper–EDTA complex from the obtained amino acid derivative can be performed using chromatography.

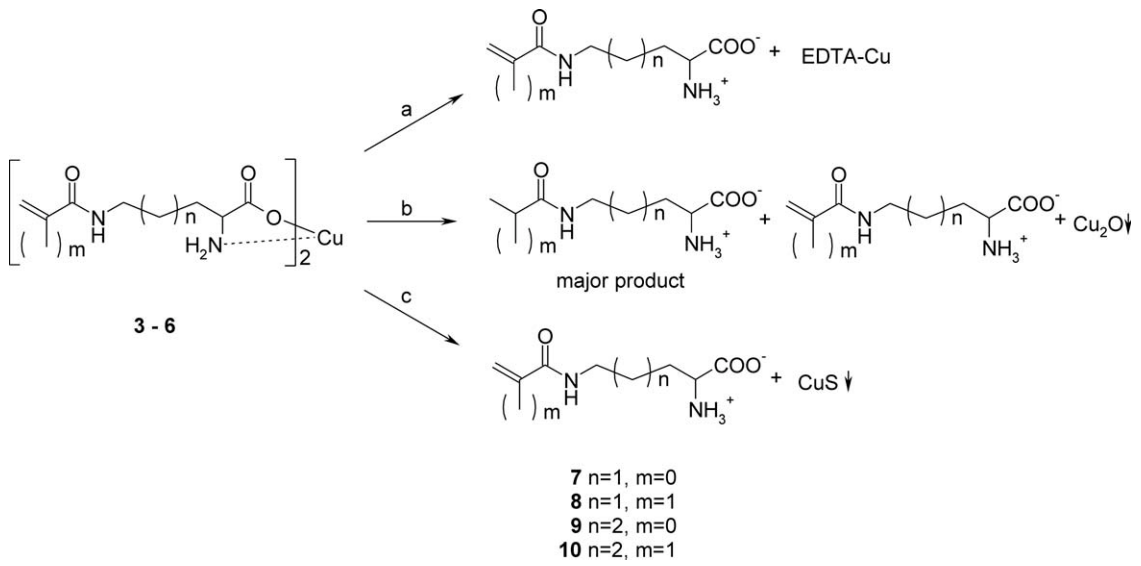
Sodium borohydride, in turn, was successfully applied to the preparation of mono-protected derivatives of basic amino acids using copper complexes methodology. The copper ion in the amino acid derivative complex is reduced to insoluble copper oxide, so it is easy to separate it. Unfortunately, in our cases, the acrylic and methacrylic function is reduced by sodium borohydride in the presence of copper ions.

The last of the selected methods, based on thioacetamide, proved to be useful. The method is cheap and fast (the reaction usually taking up to 30 min). As a result of this reaction, copper sulfide precipitates and can be removed by filtration. The filtrate contains exclusively liberated an amino acid derivatives **7**–**10** that can be finally purified by simple crystallization.

Swelling Behavior

The synthesized compounds were then used as comonomers in the free-radical polymerization reaction with NIPA in the presence of BIS as the linker to prepare a series of hydrogels with varying molar compositions of amino acid moieties.

We next characterized the volume change of gels with varying amounts of incorporated α-amino acid groups in the presence of a series of metal ions. For this investigation, NIPA-AcOrn gels of various composition ($y = 0$, 2, 5, 10, and 20%) were selected. The gels obtained in 7.7-mm-diameter glass pipes were cut into uniform pieces of $\sim$ 4 mm in diameter. Then, the gel samples were immersed into pure water and 50 mL of Cu^{2+}, Co^{2+}, and Ca^{2+} 5 mM solutions. After a few days, the gel samples were separated and changes in the volumes were analyzed (Fig. 1). The volume of gels immersed into pure water increased as the amino acid group



SCHEME 2 Procedures for removing copper from amino acid–copper complexes using: (a) EDTA, (b) sodium borohydride, and (c) thioacetamide.

content increased. This is a simple consequence of introducing ionizable groups into the polymeric network, increasing the hydrophilicity of the gels. Upon exposure to solution of Cu^{2+} and Co^{2+} ions, the gels exhibited a reduction in volume and became colored. Those changes became more visible after the amount of amino acid groups in the network was increased. In the case of gel series immersed in Ca^{2+} solution, the changes in volume are very similar to those observed for gel series immersed in pure water. The observed behavior of the gels could be explained in terms of the formation of complexes between metal ions and amino acid groups attached to the polymeric chains of the gel networks. It is known that amino acids can form stable complexes with some metal cations. Three complexes of stoichiometry 1:1, 1:2, and 1:3 can be formed:[39–41]

$$\mathrm{Me}^{m}(\mathrm{aq}) + \mathrm{RCH_2NH_3^+COO^-} \rightarrow \mathrm{Me(RCH_2NH_2COO)}^{(m-1)} + \mathrm{H^+} \quad \text{(I)}$$

$$\mathrm{Me(RCH_2NH_2COO)}^{(m-1)} + \mathrm{RCH_2NH_3^+COO^-} \rightarrow \mathrm{Me(RCH_2NH_2COO)_2}^{(m-2)} + \mathrm{H^+} \quad \text{(II)}$$

$$\mathrm{Me(RCH_2NH_2COO)_2}^{(m-2)} + \mathrm{RCH_2NH_3^+COO^-} \rightarrow \mathrm{Me(RCH_2NH_2COO)_3}^{(m-3)} + \mathrm{H^+} \quad \text{(III)}$$

The presence of complexes in the gel may influence its swelling ratio. The first complex (1:1) should expand the polymer network by introducing an excessive positive charge to the polymeric chains, which leads to an increase in the osmotic pressure between the solution and the gel. The second (1:2) and third (1:3) complexes increase the overall crosslink density of the gels and lead to the shrinking of the polymer network. Among the metal ions investigated, copper showed the highest stability constants. The average values for complexes with unmodified glycine are as follows: log $\beta_{ML} = 8.1$ and log $\beta_{ML_2} = 15.3$. However, as Figure 1 shows, the strongest influence on the swelling ratio is observed for cobalt ions. In spite of smaller stability constants (log $\beta_{ML} = 5.0$ and log $\beta_{ML_2} = 8.0$), cobalt ions show a distinct tendency for the creation of complexes with stoichiometry 1:3 (log $\beta_{ML_3} = 11.5$). It should be noted that copper and cobalt have relatively high overall stability constants, and, moreover, the complexes with stoichiometry 1:3 are more efficiently crosslinked than complexes with stoichiometry 1:2 and should lead to a more shrunken state of the gel. The weak influence of calcium ions on swelling ratio, on the other hand, could be explained in terms of the formation of very weak complexes only with stoichiometry 1:1 between the calcium and α-amino acid groups (log

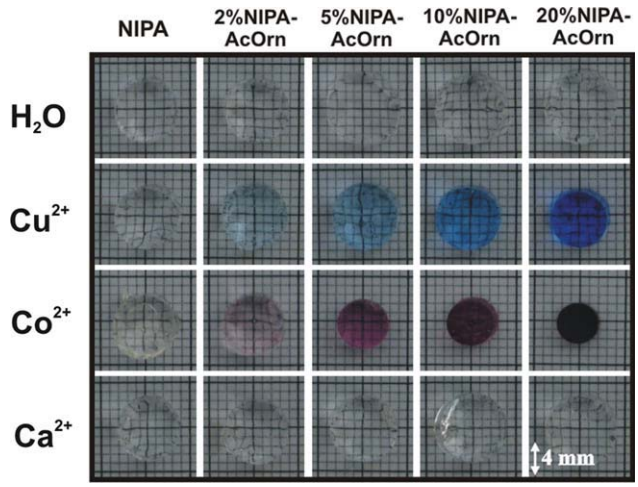


FIGURE 1 Response of NIPA-AcOrn gels with differing amounts of incorporated α-amino acid groups to aqueous solution of metal ions.

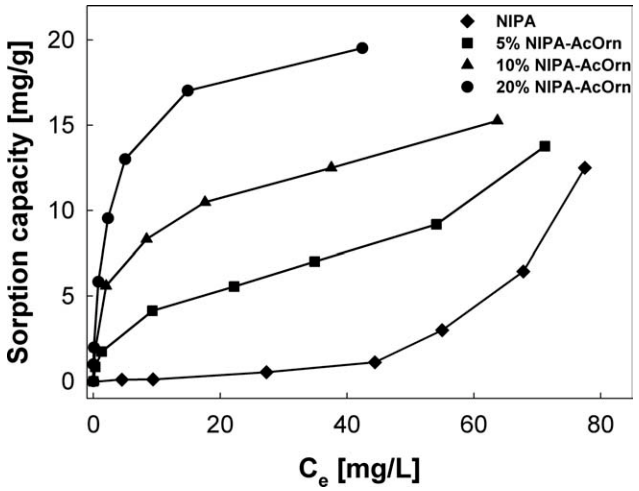


FIGURE 2 Sorption isotherm of Cu^{2+} by gels with differing amounts of incorporated AcOrn. Experimental conditions: C_0 = 5–140 mg/L; pH = 5; sorbent dose: 50 mg/10 mL.

$\beta_{ML} = 1.4$). All average values of stability constants between metal ions and glycine were taken from ref. 40.

Sorption Process

Sorption capacity is an important factor because it determines the amount of sorbent, which is required for the sufficient enrichment of the analyte (present at trace level) from the examined solution. NIPA-AcOrn gels with various composition (y = 0, 5, 10, and 20%) were investigated. Figure 2 shows the dependence between the metal-ion equilibrium concentration and the amount of the adsorbed Cu^{2+} onto the employed gels. The sorption capacity of the NIPA gel increases slightly up to an initial concentration of Cu^{2+} of $\sim$ 50 mg/L and then increases rapidly to the end of the investigated concentration range, for example, 140 mg/L. The shape of this dependence curve does not fit the typical adsorption isotherms used to describe sorption properties polymeric gels, such as the Langmuir, Freundlich, Temkin, Reundlich-Peterson, and Sips isotherms.[42–46] This suggests that the interactions between copper ions and the polymeric network are very weak. For the gels that contain α-amino acid groups, sorption capacity increases rapidly with concentration in the low range of copper ion concentration and then, for higher concentrations, the increase is not that strong.

To better characterize the sorption process on amino acid groups of the gels, sorption capacities per one mole of NIPA unit in polymer network were estimated for appropriate C_e, and then the isotherms for 5, 10, and 20% NIPA-AcOrn were appropriately corrected. The obtained isotherms for sorption on amino acid groups, presented in Figure 3, are of similar shape. The sorption capacities of the polymeric gel used increase gradually with Cu^{2+} concentration and reach a plateau, which represents the maximum sorption capacity. Isotherms were analyzed in terms of the Langmuir model (the polymer chains can be covered by only a monolayer of adsorbate, and there is no subsequent interaction between the adsorbed and dissolved molecules). The expression for the Langmuir isotherm is as follows:

$$q = q_m \frac{K_L C_e}{1 + K_L C_e}, \quad (1)$$

where q is the amount of Cu^{2+} adsorbed per gram of adsorbent (mg/g); C_e denotes the equilibrium concentration of the metal ions in the solution (mg/L); q_m is the theoretical saturation capacity of the monolayer (mg/g); and K_L represents the Langmuir constant (L/mg) that is related to the affinity of the binding sites. The values of q_m and K_L could be calculated from the intercept and slope of the linear plot of C_e/q versus C_e.

The analyzed data fit the Langmuir isotherm model very well, as the R^2 value for all of C_e/q versus C_e plots was better than 0.995. The theoretical saturation capacities (q_m) determined from this model for 5, 10, and 20% NIPA-AcOrn gels are 6.65, 12.15, and 19.34 mg/g, respectively.

Assuming that the relative content of the amino acid incorporated into the polymeric network of the gels is equal to its mole ratio in the pregelation solution, and that all α-amino acid groups are involved in 1:2 complexes with the copper ions, the calculated maximum sorption capacities for 5, 10, and 20% NIPA-AcOrn gels are 13.56, 26.30, and 49.61 mg/g, respectively.

The obtained values of saturation capacity from the Langmuir isotherm model constitute 49.0, 46.2, and 39.0% of the calculated maximum sorption capacity. Additionally, as has been reported,[20] the gel based on *N*-δ-acryloyl ornithine and the crosslinking agent BIS could achieve only 11% of theoretical maximum sorption capacity. This could be explained in terms of steric reasons that make many amino acid groups inaccessible to the metal ions. In other words, the formation of complexes with copper ions increases the crosslink density and makes the polymeric gels collapse. The internal structure of the gel becomes denser, which makes the penetration of the gel by subsequent ions harder, and limits

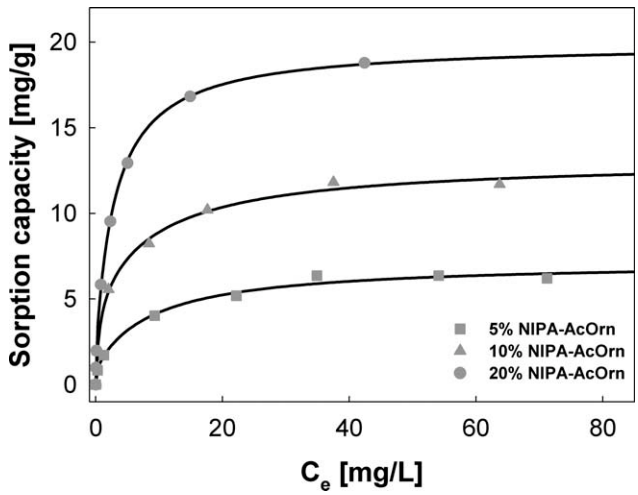


FIGURE 3 Sorption isotherm of Cu^{2+} on amino acid units in gels with various amount of incorporated AcOrn. Experimental conditions: C_0 = 5–140 mg/L; pH = 5; sorbent dose: 50 mg/10 mL.

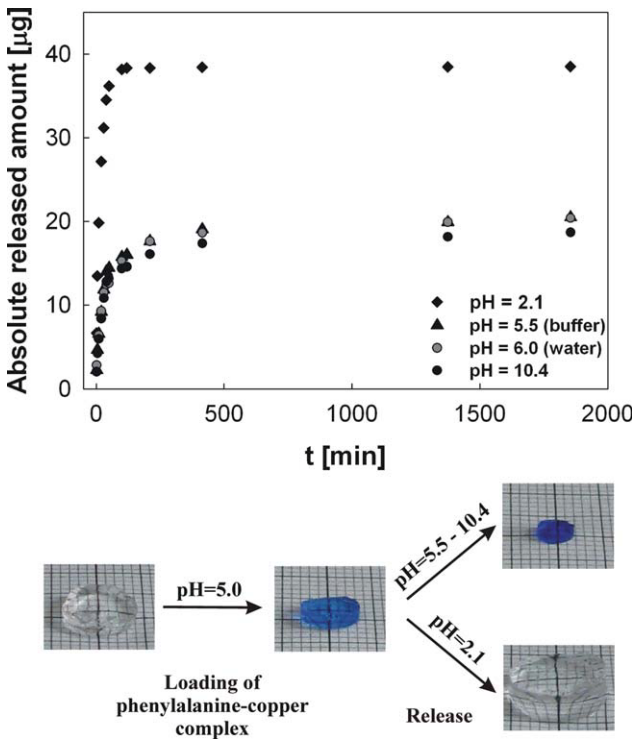


FIGURE 4 Effect of pH on release of phenylalanine from 20% NIPA-AcOrn gel. $T = 20$ °C.

the spatial orientation of the amino acid groups suitable for the formation of complexes of 1:2 stoichiometry. The sorption capacities obtained by us are higher than those reported recently for carbon nanotubes[47] as well as for various chelate-functionalized materials such as iron-based magnetic nanoparticles with polyacrylic acid[48] and imprinted polymethacrylic microbeads with 4-(2-pyridylazo) resorcinol.[49] They are comparable with those reported recently for polymeric hydrogels containing the chelating groups.[50–53]

The selectivity of the gels was not investigated. However, based on the investigation of the swelling behavior of the gels in the presence of metal ions (Cu^{2+}, Co^{2+}, and Ca^{2+}) and the overall stability constants of metal complexes with α-amino acids,[40,54] some conclusions on the selectivity aspects could be made. The alkaline earth metals have very low overall stability constants (log $\beta \approx 1.5$), whereas for other metal cations, important from the environmental pollution point of view (Pb^{2+}, Cd^{2+}, Hg^{2+}, Cu^{2+}, Fe^{3+}, and Mn^{2+}), log β are usually higher than 10. More detailed analysis of the values of the overall stability constants showed that selectivity of investigated gels decreases in the order: $Hg^{2+} \gg Cu^{2+} \gg Cd^{2+} \geq Pb^{2+} \geq Fe^{3+} \gg Mn^{2+} \gg Mg^{2+} \geq Ca^{2+}$.

Efficiency of Release of Amino Acids

The presence of free α-amino acid groups attached to the polymer network also opens up the possibility of further modification of the gel by introducing compounds that contain an amino acid groups and can cocomplex metal ions with the polymer network. The compounds used to modify the gel could then be released from the gel by altering the pH of the environment. The influence of pH on phenylalanine release from 20% NIPA-AcLys is shown in Figure 4. The amount of the released amino acid from a moderately acidified medium is approximately double that for pH range 5.5–10.4. Additionally, the complete release of phenylalanine is achieved after 100 min at pH 2.1 and after 500 min for pH in the range of 5.5–10.4. Photographs of the gel sample taken after the release processes were completed (Fig. 4), indicating that the mechanism of release is different in the two cases. At pH 2.1, the gel samples lose their blue color and become more swelled; in a more basic medium, gel samples shrunk and the blue color become darker. The observed results could be explained in terms of the influence of pH on the complexation reactions between copper ions and amino acid groups attached to the polymeric network on one side and to phenylalanine on the other side. The amino acid groups are known to exist in three forms: as a cation [protonated amino group ($^{+}H_2NCHRCOOH$)], as a neutral form, a zwitterion ($^{+}H_2NCHRCOO^{-}$), and as an anion [dissociated carboxylic group ($H_2NCHRCOO^{-}$)]. In a more acidic medium, the equilibrium of the complex formation reaction is shifted to the left side (reactions I–III). Free metal ions are formed and uncomplexed phenylalanine can be released from the gel. Additionally, at pH 2.1, functional groups of the polymeric network of the gel are protonated and these ionized groups create an osmotic pressure in the network and, therefore, prompt the swelling process. Also, the charges on the ionized groups generate electrostatic repulsive forces between the polymer chains, which lead to further swelling of the network. In the case of a more basic medium, pH 5.5–10.4, the formation of complexes with amino acid groups is preferred. Shrinking of gel sample suggests that some copper ions again formed 1:2 complexes with the amino acid groups attached to the polymeric network of the gel. The presence of the 1:2 complexes increases the overall crosslink density of the gels and leads to the shrinking of the polymer network. The formation of 1:2 complexes with the amino acid groups attached directly to the polymeric network means that the phenylalanine ligand is freed and released to the solution.

The mechanism of release was also analyzed in terms of the Higuchi model[55] extended by Peppas and Ritger:[56]

$$\frac{M_t}{M_\infty} = kt^n, \tag{2}$$

where M_t and M_∞ are the masses of drug released at time t and ∞, respectively, k is a constant incorporating characteristics of the macromolecular network system and drug, and n is the diffusional exponent. Information about the release mechanism can be gained by fitting the release data (for the first 60% of fractional release) and comparing the values of n to the semiempirical values for various geometries.[56] For a cylindrical geometry, values of n up to 0.45 correspond to a purely Fickian diffusion mechanism. Values of n greater than 0.89 indicate a relaxation controlled-release mechanism, and n values between 0.45 and 0.89 indicate an anomalous

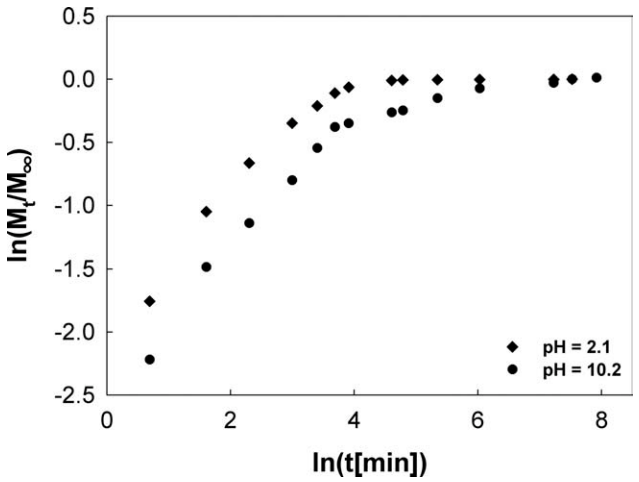


FIGURE 5 The relationship $\ln(M_t/M_\infty)$ versus $\ln(t)$ for 20% NIPA-AcLys hydrogel.

release mechanism. The relationships between $\ln(M_t/M_\infty)$ and $\ln(t)$ for two values of pH are shown in Figure 5. The values for diffusional exponent n value for pH 2.1 and 10.4 are 0.68 and 0.61, respectively. The obtained values of n indicate an anomalous release mechanism that is in line with the proposed above release mechanisms.

EXPERIMENTAL

Instrumentation

NMR spectra were recorded with a Varian Unity Plus 200 MHz spectrometer for D_2O solutions using residual H_2O as the internal reference. Mass spectra were recorded with an ESI/MS Mariner (PerSeptive Biosystem) mass spectrometer. Reactions were monitored by TLC (silica gel 60 F_{254}, 0.2 mm, Merck), and the visualization was effected with UV. Column chromatography was performed on silica gel (Merck, 230–400 mesh). HPLC was performed using a LaChrom Merck instrument equipped with a Metachem (Varian) Polaris C-18A column (5 μm, 250 mm $\times$ 4.6 mm) and C-18 Phenomenex precolumn; Diode Array Detector: (190–400 nm), integration of the signal at 215 nm; isocratic elution, 1.0 mL/min of water: acetonitrile (90:10). Infrared spectra were obtained with a Perkin-Elmer 2000 FTIR spectrometer.

Materials

NIPA (97%), BIS (99%), ammonium persulfate (APS, 99.99%), *N,N,N′,N′*-tetramethylethylenediamine (TEMED, 99.5%), L-ornithine monohydrochloride salt (99%), L-lysine monohydrochloride salt (98%), acryloyl chloride (96%), methacryloyl chloride (96%), and sodium borohydride (98%) were purchased from Aldrich and Fluka. Ethylenediaminetetraacetic acid disodium salt dihydrate (EDTA, 99%), sodium hydroxide (NaOH, 99%), copper (II) sulfate pentahydrate ($CuSO_4.5H_2O$, 98%), and thioacetamide (98%) were purchased from POCh (Poland). All chemicals were used as received except for NIPA, which was recrystallized twice from the benzene/hexane mixture (90:10 v/v). All solutions were prepared using high-purity water obtained from a Milli-Q Plus/Millipore purification system (water conductivity: 0.056 μS/cm).

Synthesis of Copper Complexes of Acryloyl and Methacryloyl Derivatives of Ornithine and Lysine

To a solution of NaOH (2.35 g, 58.7 mmol, in 55 mL of water), amino acid monohydrochloride (29.6 mmol) was added. Next, a solution of $CuSO_4{\cdot}5H_2O$ (3.75 g, 15 mmol, in 55 mL of water) was added and the resulting deep blue solution was cooled to 10 °C. Acryloyl or methacryloyl chloride (37.5 mmol) and 2 M NaOH (15 mL) were simultaneously added dropwise, maintaining the pH at 9–10. After the addition was complete (ca. 30 min), the reaction mixture was allowed to reach room temperature and was stirred overnight. The blue precipitate was filtered and washed successively with water (50 mL $\times$ 2), ethanol (50 mL $\times$ 2), and diethyl ether (50 mL). The precipitate was air dried for 24 h.

N-δ-*Acryloyl Ornithine Copper Complex (3)*

Yield 62% (blue solid), IR (KBr): 3278, 3077, 2934, 2878, 1655, 1615, 1552, 1482, 1455, 1398, 1338, 1313, 1279, 1252, 1237, 1141, 1092, 993, 961, 808, 784, 675, 582, 430.

N-δ-*Methacryloyl Ornithine Copper Complex (4)*

Yield 76% (blue solid), IR (KBr): 3291, 3257, 3059, 2930, 2878, 1652 1615, 1537, 1483, 1453, 1395, 1325, 1276, 1213, 1141, 1088, 925, 806, 792, 754, 707, 651, 579, 431.

N-ε-*Acryloyl Lysine Copper Complex (5)*

Yield 84% (blue solid), IR (KBr): 3283, 3152, 3065, 296, 2868, 1655, 1619, 1541, 1474, 1462, 1402, 1383, 1319, 1308, 1259, 1250, 1237, 1147, 1099, 985, 954, 921, 806, 779, 729, 667, 589, 538, 440.

N-ε-*Methacryloyl Lysine Copper Complex (6)*

Yield 76% (blue solid), IR (KBr): 3274, 3153, 2945, 2935, 2868, 1652, 1618, 1529, 1473, 1461, 1401, 1379, 1312, 1280, 1259, 1215, 1146, 1096, 1010, 917, 871, 80, 779, 729, 666, 587, 491, 447, 424.

Synthesis of Acryloyl and Methacryloyl Derivatives of Ornithine and Lysine

To a stirred suspension of well-powdered amino acid–copper complex (2.3 mmol) in water (25 mL), thioacetamide (2.5 mmol) was added. After stirring for 20 min, the mixture was adjusted to pH 9 and the copper sulfide precipitate formed was filtered through a celite pad giving colorless filtrate. The water was evaporated, and the residue was dissolved in $MeOH/CF_3COOH$ (2% CF_3COOH in MeOH) and precipitated with Et_2O. The crude product was recrystallized from $MeOH/Et_2O$.

N-δ-*Acryloyl Ornithine (7)*

Yield 82% (white solid), ^{1}H NMR (500 MHz, D_2O): δ 6.19–6.33 (m; 2H), 5.79 (dd; J = 10 Hz, J = 1.5 Hz; 1H), 3.79 (t; J = 6.25 Hz; 1H), 3.30–3.40 (m; 2H), 1.86–1.98 (m; 2H), 1.59–1.75 (m, 2H). ^{13}C NMR (50 MHz, D_2O): δ177.20, 171.25, 132.60, 129.86, 57.11, 41.40, 30.53, 26.91. IR (KBr): 3302, 3083, 2945, 2872, 2629, 2123, 1656, 1626, 1608, 1583, 1556, 1516, 1461, 1448, 1408, 1382, 1358, 1328, 1243, 1204, 1135, 987, 954, 837, 803, 750, 723, 706, 671, 575, 547, 438, 412. High-resolution mass calcd for $C_8H_{14}N_2O_3Na$ $[M + Na]^+$: 209.0902. Found: 209.0894.

N-δ-*Methacryloyl Ornithine (8)*
Yield 83% (white solid), ^{1}H NMR (200 MHz, D_2O): δ 5.66 (s; 1H), 5.42 (s, 1H), 3.74 (t; J = 5.9 Hz; 1H), 3.28 (t; J = 6.7 Hz, 2H), 1.90 (s; 3H), 1.75–1.95 (m; 2H), 1.55–1.70 (m; 2H). ^{13}C NMR (50 MHz, D_2O): δ174.52, 172.08, 139.20, 121.05, 54.50, 38.95, 27.91, 24.39, 17.76. IR (KBr): 3299, 2947, 2662, 2107, 1656, 1618, 1582, 1537, 1465, 1449, 1416, 1352, 1328, 1280, 1222, 1173, 1153, 920, 825, 743, 721, 700, 663, 562, 535, 466, 431. High-resolution mass calcd for $C_9H_{16}N_2O_3Na$ $[M + Na]^+$: 223.1059. Found: 223.1064.

N-ε-*Acryloyl Lysine (9)*
Yield 65% (white solid), ^{1}H NMR (200 MHz, D_2O): δ 6.10–6.35 (m; 2H), 5.72 (dd; J = 9.2 Hz, J = 2.8 Hz, 1H), 3.73 (t; J = 6.1 Hz, 1H), 3.26 (t; J = 6.8 Hz, 2H), 1.75–2.00 (m; 2H), 1.50–1.70 (m; 2H), 1.25–1.50 (m; 2H). ^{13}C NMR (50 MHz, D_2O): δ174.67, 168.58, 130.11, 127.16, 54.61, 39.04, 30.10, 28.04, 21.86. IR (KBr): 3302, 3073, 2944, 2867, 1656, 1627, 1605, 1582, 1521, 1443, 1410, 1360, 1325, 1247, 1202, 1134, 982, 836, 800, 722, 670, 536, 444, 419. High-resolution mass calcd for $C_9H_{16}N_2O_3Na$ $[M + Na]^+$: 223.1059. Found: 223.1031.

N-ε-*Methacryloyl Lysine (10)*
Yield 62% (white solid), ^{1}H NMR (200 MHz, D_2O): δ 5.65 (s; 1H), 5.41 (s; 1H), 3.70 (t; J = 6.0 Hz, 1H), 3.24 (t; J = 6.3 Hz, 2H), 1.89 (s; 3H), 1.75–2.00 (m; 2H), 1.50–1.70 (m; 2H), 1.30–1.50 (m; 2H). ^{13}C NMR (50 MHz, D_2O): δ 174.82, 171.97, 139.27, 120.90, 54.74, 39.18, 30.19, 28.16, 21.89, 17.78. IR (KBr): 3322, 2947, 2926, 2864, 2606, 2146, 1654, 1619, 1581, 1519, 1446, 1415, 1359, 1351, 1325, 1217, 1204, 1162, 1135, 980, 917, 863, 812, 799, 736, 721, 666, 532, 442, 416. High-resolution mass calcd for $C_{10}H_{18}N_2O_3Na$ $[M + Na]^+$: 237.1215. Found: 237.1198.

Synthesis of Gels Containing Lysine and Ornithine Units
The amino acid groups, which can interact with heavy metals, were introduced into poly(*N*-isopropylacrylamide) network by simple free-radical solution copolymerization. The total concentration of NIPA, derivatives of amino acid, and BIS was kept constant at 700 mM. The pregel solutions were degassed, and the polymerization was initiated and accelerated by APS (1.88 mM) and TEMED (32 mM) and carried out at 5 °C for 20 h. The gels were synthesized with the mole fraction of the amino acid derivative (Ac/MAcLys/Orn) equal to $Y_{solution}$ in the pregel solution. $Y_{solution}$ is defined as:

$$Y_{solution} = \frac{n_{Ac/MAcLys/Orn}}{n_{NIPA} + n_{Ac/MAcLys/Orn} + n_{BIS}} 100\% \qquad (3)$$

In this article, $Y_{solution}$ = 2, 5, 10, 20, and 0%. The mole fraction of BIS was kept constant at 1%. The gels were synthesized in a glass tube of inner diameter, d_o, 7.7 mm. After synthesis, the gel samples were immersed into pure water to remove excess reagents and finally dried.

NIPA-AcOrn
Yield 91% (white solid), IR (KBr): 3439, 3075, 2974, 2936, 2877, 2115, 1659, 1550, 1460, 1388, 1368, 1278, 1172, 1131, 977, 928, 883, 839, 647, 534.

NIPA-MAcOrn
Yield 77% (white solid), IR (KBr): 3439, 3074, 2974 2937, 2877, 2119, 1658, 1554, 1460, 1388, 1367, 1277, 1236, 1172, 1131, 974, 898, 883, 838,646, 537.

NIPA-AcLys
Yield 76% (white solid), IR (KBr): 3440, 3074, 2974, 2936, 2877, 2125, 1657, 1548, 1460, 1388, 1368, 1276, 1173, 1131, 976, 927, 883, 838, 654, 532.

NIPA-MAcLys
Yield 72% (white solid), IR (KBr): 3440, 3074, 2974, 2936, 2876, 2111, 1651, 1548, 1460, 1388, 1367, 1276, 1232, 1172, 1131, 971, 927, 883, 839, 655, 537.

Determination of Amount of Amino Acid Incorporated into Polymeric Network
The amount of amino acid incorporated into the polymeric network of the gels was estimated from the NMR spectra. The gel samples for the NMR experiments were prepared by swelling the pieces of dry polymeric network in D_2O in NMR tubes. The ^{1}H NMR spectra were recorded after 1 day. In each case of ^{1}H NMR spectra of polymeric networks containing lysine or ornithine units, the region between 2.5 and 4.5 ppm was used for the calculations. The distinguishable signal at 3.2 ppm (representing the δ-protons or ε-protons of ornithine and lysine, respectively) and overlapped signals at 3.75–4.1 ppm (representing α-protons from amino acid, protons from the isopropyl group, or methylene protons from the linker, respectively) were used for estimating the amino acid loading content into the network.

$$Y_{gel} = \frac{\frac{1}{2} < b >}{< a, c, d >} 100\%, \qquad (4)$$

where $<b>$ denotes the area of the signal at 3.2 ppm and $<a, c, d>$ is the area between 3.75 and 4.10 ppm, respectively.

To make the equation more precise, the area of the methylene protons from the linker should be divided by 2 to reflect the presence of two protons in this residue; however, the corresponding proton signals are not sufficiently well separated. Fortunately, the error is very small, as the mole fraction of the linker was kept at a low level. The estimated percentages of incorporated amino acids are in good accordance with the molar fraction of monomers used in the polymerization process. Data for NIPA-MAcOrn gels are shown in Table 1.

Typical spectra of polymer networks based on methacryloyl L-ornithine with differing amounts of incorporated ornithine moieties are shown in Figure 6. With an increase in ornithine loading, both the signal at 3.2 ppm and the overlapped signals at 3.75 ÷ 4.10 ppm increase.

Examination of Amino Acid Release
The drug release properties were evaluated using the 20% NIPA-AcLys gel and phenylalanine. The gels synthesized in a 7.7-mm-diameter glass pipe were cut to obtain uniform pieces ~ 4 mm in diameter (mass of the dry polymer was ~ 2 mg). The gel samples were immersed in a 10-mL solution of phenylalanine and copper–ion complexes of stoichiometry 1:1.

TABLE 1 Content of Amino Acid in the Pregel Solutions and of Amino Acid Incorporated into Polymeric Network of the Gels (Estimated from ^{1}H NMR Spectra)

	2% NIPA-MAcOrn	5% NIPA-MAcOrn	10% NIPA-MacOrn	20% NIPA-MAcOrn
$Y_{solution}$	2.0%	5.0%	10.0%	20.0%
Y_{gel}	2.6%	6.4%	11.1%	21.0%

The solution of copper complexes with phenylalanine was prepared by mixing 2 mM solution of copper with 2 mM solution of phenylalanine. Both solutions were prepared in acetate buffer of pH 5.0. To decrease the amount of free copper ions (aqua complexes) in the final solution, a 10% excess of phenylalanine was used in the mixing process. Then, the solution was separated from the precipitated blue crystals (1:2 complexes) and used to introduce the amino acid to the gel. The loading process was considered to be completed after 2 h; then, the entire gel samples turned a uniform dark blue color. Next, the gel samples were immersed three times into 10 mL of pure water to remove unbound species and placed in a 3-mL solution of various pH (from 2.1 to 10.4) where the release process was investigated.

The amount of released phenylalanine was determined by chromatography. The working calibration plot used in the determination of phenylalanine was as follows: $y = 4.05 \times 10^8 c - 567$, $R = 0.9999$.

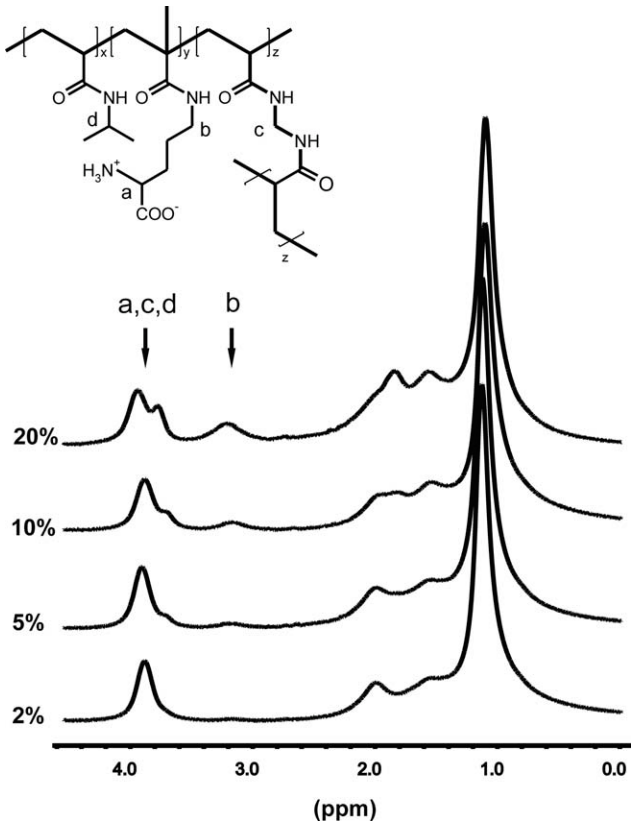


FIGURE 6 ^{1}H NMR spectra of methacryloyl L-ornithine-based hydrogels.

Examination of Copper Sorption

For the examination of sorption properties, the gels with 0, 5, 10, and 20% content of AcOrn were selected. The synthesized gels were dried gently, at ~ 60 °C, to constant mass and then ground. To estimate the sorption capacity, 50 mg of dry polymer was mixed with 10 mL of metal ion solution (pH 5.0, concentration range 5–140 mg/L). The mixtures were shaken for 2 h; then, the metal concentration in the aqueous solutions was determined by atomic absorption spectrophotometry. The sorption capacity, q_e (mg/g), was estimated as follows: $q_e = [(C_o - C_e)V]/m$, where C_o and C_e are the initial and equilibrium concentrations (mg/L) of metal ion in the aqueous solution, respectively, V is the volume of the metal ion solution, and m is the weight of the sorbent.

The regression equation of copper solution working curve was $y = 0.0224x + 0.345$, $R = 0.9982$.

CONCLUSIONS

We have shown that hydrogels containing free α-amino acid groups can be easily prepared by free-radical polymerization of acryloyl and methacryloyl derivative of ornithine and lysine. Copper-complex formation followed by thioacetamide reaction were found to give a convenient and scalable procedure for preparation of amino acid derivatives (acryloyl and methacryloyl amino acids). The volume of the gel depends on the amount of amino acid moieties incorporated into the polymer network and on the presence of metal ions. Metal ions that have the ability to create complexes with α-amino acid groups lead to the shrinking of the gels. Investigation of the sorption of Cu^{2+} by the α-amino acid groups revealed that the adsorption process proceeds in accordance with the Langmuir isotherm. The shrinking of the polymeric network due to sorption of some metal ions leads to better mechanical properties of the gel, so it could be useful in the removal of the gel from the solution, for instance, for further regeneration. Nevertheless, the obtained results show that the equilibrium adsorption capacities are relatively high. Additionally, the presence of free amino acid groups attached to the polymer network offers a possibility of loading the gel with compounds which can cocomplex the metal ions. These compounds could be released from the gel by appropriate alteration of the environmental pH. For phenylalanine, we have shown that the mechanism and the amount of released amino acid strongly depend on pH. The amount of released amino acid at medium pH = 2.1 is approximately double that for pH in the range of 5.5–10.4. Additionally, at pH 2.1, the gel samples lose their dark blue color and become more swelled, whereas in a more basic medium the samples shrink and the blue color becomes darker. Because some

molecules important from the medical, pharmaceutical, or therapeutic points of view have free amino acid groups (e.g., glutathione), such gels with free amino acid groups are also interesting from the standpoint of drug delivery systems.

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