

## Macromolecular Sorbent Materials for Urea Capture

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**ABSTRACT:** Three types of chitosan–glutaraldehyde (Chi–Glu) crosslinked copolymer materials were prepared at various Chi–Glu weight ratios (i.e., 1 : 0.0835, 1 : 0.334, and 1 : 0.585) and variable reaction times. The corresponding Chi–Glu copolymer materials were imbibed in CuSO<sub>4</sub> solution to yield impregnated materials in the form of copolymer/Cu(II) complexes. The copolymer materials were characterized using FTIR spectroscopy and thermogravimetry analysis. Urea sorption isotherms were obtained in aqueous solution at 295 K and pH 7 with pristine chitosan, Chi–Glu copolymers (i.e., 1 : 0.0835 and 1 : 0.585), and the corresponding Chi–Glu/Cu(II) complexes. The concentration of unbound urea was monitored indirectly using a colorimetric method with *p*-dimethylamino-benzaldehyde. The equilibrium adsorption data were analyzed using the Sips isotherm model. The uptake of urea with pristine chitosan was 4.7% w/w, whereas Chi–Glu copolymers display increased sorption ( $Q_m = 10.6\text{--}17.1\%$  w/w) with increasing glutaraldehyde content. Urea sorption is further enhanced ( $Q_m = 16.3\text{--}26.4\%$  w/w) for copolymer Chi–Glu/Cu(II) complexes. The preparation of Chi–Glu copolymers at various conditions illustrates that the sorption capacity and molecular recognition of urea can be systematically tuned via crosslinking and the formation of copolymer/Cu(II) complexes, and these results are related to a previously reported study (Shimizu and Fujishige, *J. Biomed. Mater. Res.* 1983, 17, 597). © 2012 Wiley Periodicals, Inc. *J. Appl. Polym. Sci.* 000: 000–000, 2012

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### INTRODUCTION

Urea capture is important for environmental remediation and in hemodialysis applications.<sup>1–4</sup> Urea is widely used in inorganic fertilizers as a convenient source of nitrogen, and large quantities are often found in aquatic environments after precipitation runoff events in agricultural regions. The transport and build-up of urea in aquatic environments represents a serious concern and potentially threatens the health of aquatic environments and ecosystems. Based on current fertilizer production and agricultural consumption statistics, it is estimated that urea represents more than 50% of the global source of nitrogen, and the demand is projected to continually increase over the next 20 years.<sup>1</sup> The potential effects of runoff and leaching represent major causes of eutrophication.<sup>2,5</sup> Thus, there is a need to develop materials and methods for the capture of urea to address issues related to its uptake and transport in aquatic environments. Sorbent materials for the controlled uptake of urea have important applications in biomedical devices for hemodialysis to cure uremia.<sup>3,4</sup> Natural biopolymers such as cellulose, modified cellulose, and synthetic polymers such as poly(acrylonitrile), poly(methyl methacrylate), and poly(sulfone) are suitable as membrane materials for hemodialysis in biomedical devices.<sup>3,4</sup>

Previous studies indicate a range of urea uptake values for modified and synthetic sorbents,<sup>5–10</sup> as follows: activated carbon (9.0 mg g<sup>-1</sup>),<sup>1</sup> poly(ethylenepolyamine)/Cu(II) complex (75.2 mg g<sup>-1</sup>),<sup>2</sup> chitosan/Cu(II) complex (78.8 mg g<sup>-1</sup>),<sup>3</sup> oxidized crosslinked  $\beta$ -cyclodextrin (50.6 mg g<sup>-1</sup>),<sup>3</sup> oxystarch (6–8.2 mg g<sup>-1</sup>),<sup>4</sup> oxycellulose (13 mg g<sup>-1</sup>),<sup>5</sup> and chitosan coated with dialdehyde cellulose (90.4 mg g<sup>-1</sup>).<sup>6</sup> Chitin and chitosan have unique structural features, which enable the formation of strong H-bonds (cf. Figures 4 and 5 in Ref. 11) between adjacent chains.<sup>11,12</sup> Crosslinked chitosan with variable crosslink density represents a potential macromolecular platform for the design of tunable sorbent materials for urea sorption.<sup>11</sup> We hypothesize that glutaraldehyde crosslinked chitosan materials in the presence and absence of Cu(II) will display favorable sorption and molecular recognition properties toward urea.<sup>13–15</sup> Covalent (e.g., glutaraldehyde) and coordinate [e.g., Cu(II)] crosslinking of chitosan will disrupt the H-bonding between adjacent polymer units, thereby creating favorable urea binding sites (e.g., see Figure 5 in Ref. 14). The objectives of this study were to prepare chitosan copolymers and their Cu(II) complexes at variable crosslink density and to characterize the equilibrium sorption properties toward urea in aqueous solution at 295 K.

## EXPERIMENTAL METHODS

### Materials

Acetic acid, hydrochloric acid, sulfuric acid, acetone, methanol, absolute ethanol, and NaOH were obtained from EMD (Edmonton, Canada). High-molecular-weight (HMW) chitosan (150–375 kDa;  $\geq 75\%$  deacetylation) and low-molecular-weight (LMW) chitosan were obtained from Sigma-Aldrich (Oakville, ON, Canada) with 75–80% deacetylation, Brookfield viscosity 20 cps, and a polydisperse molecular weight range (50–190 kDa). Glutaraldehyde (50% w/w in water),  $\text{CuSO}_4$ , urea, and *p*-dimethylaminobenzaldehyde (PAB) were obtained from Sigma-Aldrich. KBr was obtained from BDH Chemicals (Halifax, NS, Canada). All materials were used as received unless specified otherwise.

### Preparation of Chitosan–Glutaraldehyde Copolymers

**Short Reaction Time.** The preparation of chitosan–glutaraldehyde (Chi–Glu) copolymers was adapted from a published procedure reported by Monteiro and Airoidi.<sup>16</sup> Chitosan (1 g) was placed in a 100-mL round-bottom flask and dissolved with stirring overnight with 50 mL 2% v/v acetic acid solution. Upon dissolution of chitosan in aqueous acetic acid, a desired quantity of glutaraldehyde was added rapidly at room temperature at several weight ratios of Chi : Glu (i.e., 1 : 0.0835, 1 : 0.334, and 1 : 0.585). The Chi–Glu copolymers are represented by the following notation: CP-1, -2, and -3, respectively, and indicate an incremental amount of glutaraldehyde (i.e., -1, -2, and -3) per gram of chitosan. The mixture was allowed to stir until gelation and then allowed to react for an additional 3 h without stirring before neutralization to approximately pH 6 with a 0.2M NaOH solution, with additional stirring for 3 h. The orange-yellow product was washed with several portions (50 mL) of cold Millipore water and 10 mL of cold acetone, with filtration through a Whatman filter paper under vacuum. The material was crushed and air dried for 2 days with subsequent grinding in a mortar and pestle. The powdered product was passed through a 40-mesh sieve and washed with methanol in a Soxhlet extractor under reflux conditions for 12 h. The material was finally dried in a vacuum oven at 50°C overnight and stored in a desiccator.

**Long Reaction Time.** The protocol for the above procedure was adapted by allowing the neutralization process to continue with stirring for 48 h instead of 3 h. In keeping with the above nomenclature for the copolymers, the Chi : Glu copolymer (1 : 0.0835; CP-1) is referred to as CP-1 (long), in reference to the longer reaction time.

### Preparation of Chi–Glu Copolymer/Cu(II) Complexes

A  $\text{CuSO}_4$  solution (10% w/w) was added (50 mL) to the neutralized mixture and equilibrated while stirring overnight. After filtration, the copolymer product was washed with 500 mL of cold Millipore water and cold acetone with vacuum filtration. All of the same workup procedures were followed, as described above.

### Characterization of the Copolymer Materials

**FTIR Spectroscopy.** IR spectra were obtained with a Bio-RAD FTS-40 (Cambridge, MA) instrument, and samples were analyzed in reflectance mode. Solid samples were prepared by mix-

ing copolymers ( $\sim 5$  mg) with pure spectroscopic grade KBr ( $\sim 50$  mg) with grinding in a mortar and pestle. The diffuse reflectance infrared Fourier transform spectra were recorded at room temperature with a resolution of  $4\text{ cm}^{-1}$  operating in the range of  $400\text{--}4000\text{ cm}^{-1}$  and recorded in reflectance mode (Kubelka–Munk intensity units). Sixteen scans were averaged and corrected against a background spectrum of pure KBr.

**Thermogravimetry Analysis.** Thermogravimetry analysis (TGA; TA Instruments) results were obtained using a TGA Q50 (New Castle, DE) with a heating rate of  $5^\circ\text{C min}^{-1}$  to a maximum temperature of  $500^\circ\text{C}$  under a  $\text{N}_2$  (carrier gas) atmosphere. This thermal method was used to provide composition of the respective components for the copolymer materials. The thermal stability was evaluated using derivative plots of derivative weight ( $\%/^\circ\text{C}$ ) vs. temperature ( $^\circ\text{C}$ ).<sup>17,18</sup> The uptake of Cu(%) was determined semiquantitatively using TGA by estimation of the residual weight  $\sim 500^\circ\text{C}$  relative to the initial sample weights for samples with and without Cu(II), respectively. As well, similar measurements were performed for bulk samples imbibed with Cu(II) and drying to constant weight  $\sim 60^\circ\text{C}$  *in vacuo*.

### Water Swelling Properties of Copolymer Materials

Approximately 50 mg of material was equilibrated in 2 mL of Millipore water for 24 h. The weight of hydrated polymer ( $W_s$ ) was tamped dry with filter paper, weighed, and dried in an oven at  $60^\circ\text{C}$  for 12 h to a constant dry weight ( $W_d$ ). The swelling ratio was calculated using Eq. (1).

$$S_w = \frac{W_s - W_d}{W_d} \times 100\% \quad (1)$$

### Copolymer Sorption Study

Variable concentrations (1–30 mM) of urea solutions were prepared in 100-mL volumetric flasks with Millipore water at pH 6 (without buffer). The sorbents were weighed and added to 4-dram glass vials with 7 mL of urea solution. The vials were further sealed with parafilm between the cap and the glass bottle, and the samples were incubated in a horizontal shaker for 12 h. After equilibration, samples were centrifuged with a Precision Micro-Semi Micro Centrifuge, Precision Scientific (Chicago, IL) at 1550 rpm. The absorbance of the urea-containing supernatant was measured using a Varian CARY 100 double beam spectrophotometer (Mississauga, Canada) at room temperature ( $22^\circ\text{C} \pm 0.5^\circ\text{C}$ ) at  $\lambda = 431\text{ nm}$ . The pH of aqueous solutions did not vary significantly ( $< 0.1$  pH units) before and after sorption. The quantification of urea was estimated indirectly through the formation of a colored complex (i.e., PAB/urea) through the addition of 2 mL of supernatant urea solution to a solution (0.5 mL) containing 4% (w/v) of PAB and 4% (v/v) of sulfuric acid in absolute ethanol.<sup>19</sup> The absorbance values of samples were measured relative to a blank after 10 min of color development using a calibration curve of standard solutions according to the Beer–Lambert law.<sup>19,20</sup> The formation of the yellow PAB/urea complex ( $\epsilon = 203 \pm 9\text{ M}^{-1}\text{ cm}^{-1}$ ) was reported previously.<sup>19</sup> The linear region ( $R^2 > 0.99$ ) of the Beer–Lambert calibration plot occurs for urea concentrations up to 5 mM. In cases where the urea concentration exceeded 5

**Table I.** Synthetic Yields for Chi–Glu Copolymers at Variable Mole Ratios

Copolymer	Weight (g)	Yield (%)
CP-1	1.1177	95.9
CP-1 (long)	0.7538	68.5
CP-2	1.0724	88.4
CP-3	1.2103	89.3

The yield of the copolymer is calculated according to the mass of chitosan and glutaraldehyde with a starting amount of 1 g of chitosan.

mM, the supernatant was diluted accordingly to enable analysis using the Beer–Lambert calibration results.

The experimental sorption results were evaluated using an isotherm represented as a plot of the amount of urea removed from aqueous solution per mass of copolymer ( $Q_e$ , mg/g) versus the residual equilibrium concentration ( $C_e$ ) of urea in bulk solution. Equation (2) defines  $Q_e$  in terms of experimental variables, where  $C_0$  is the initial stock concentration (mM) of urea,  $V$  is the volume of solution (mL), and  $m$  is the mass of sorbent (mg).

$$Q_e = \frac{(C_0 - C_e) \times V}{m} \quad (2)$$

The Sips isotherm [Eq. (3)] model was used to analyze the equilibrium sorption data.<sup>21</sup> The heterogeneity of the sorbent surface was estimated using the exponent term ( $n$ ), where a value that deviates from unity indicates heterogeneity. The Sips isotherm model is a general utility isotherm that accounts for Langmuir or Freundlich isotherm behavior, in accordance with the magnitude of the adjustable parameters.<sup>22</sup> The monolayer sorption capacity of urea onto the copolymer framework is given by  $Q_m$ . The equilibrium constant is represented as  $K$ . The criterion of “best fit” between the experimental data and the Sips isotherm was obtained by minimizing the sum of square of errors (SSE) [cf. Eq. (4)].  $Q_{e,i}$  is the experimental sorption value,  $Q_{f,i}$  is the simulated value according to the Sips isotherm [cf. Eq. (3)], and  $N$  is the number of experimental data points.

$$Q_e = \frac{Q_m(KC_e)^n}{1 + (KC_e)^n} \quad (3)$$

$$SSE = \sum \sqrt{\frac{(Q_{e,i} - Q_{f,i})^2}{N}} \quad (4)$$

## RESULTS AND DISCUSSION

### Synthesis of Chi–Glu Copolymer Materials

Chi–Glu copolymers were prepared with variable mass ratios (1 : 0.0835, 1 : 0.334, and 1 : 0.585) and denoted as CP-1, -2, and -3, respectively. The incremental mass ratio of glutaraldehyde per gram of chitosan corresponds to variable crosslinking of chitosan, according to the Schiff base mechanism.<sup>16</sup> The mass ratios for CP-1, -2, and -3 correspond to variable levels of crosslinking for each copolymer are as follows: CP-1 (~36%), CP-2 (~100%), and CP-3 (~100%, excess glutaraldehyde). The yields for the preparation of Chi–Glu copolymers are favorable, as listed in

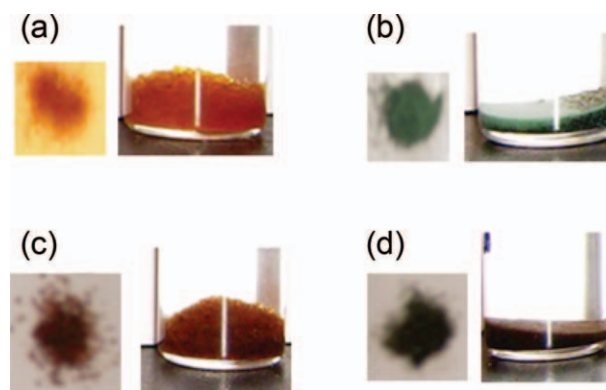
Table I. The mechanism of crosslinking between chitosan and glutaraldehyde was reported previously,<sup>16,23</sup> where an imine bond is formed between glutaraldehyde and the glucosamine nitrogen of chitosan (cf. Scheme 3 in Ref. 24).

### Synthesis of Chi–Glu Copolymer with Different Reaction Times

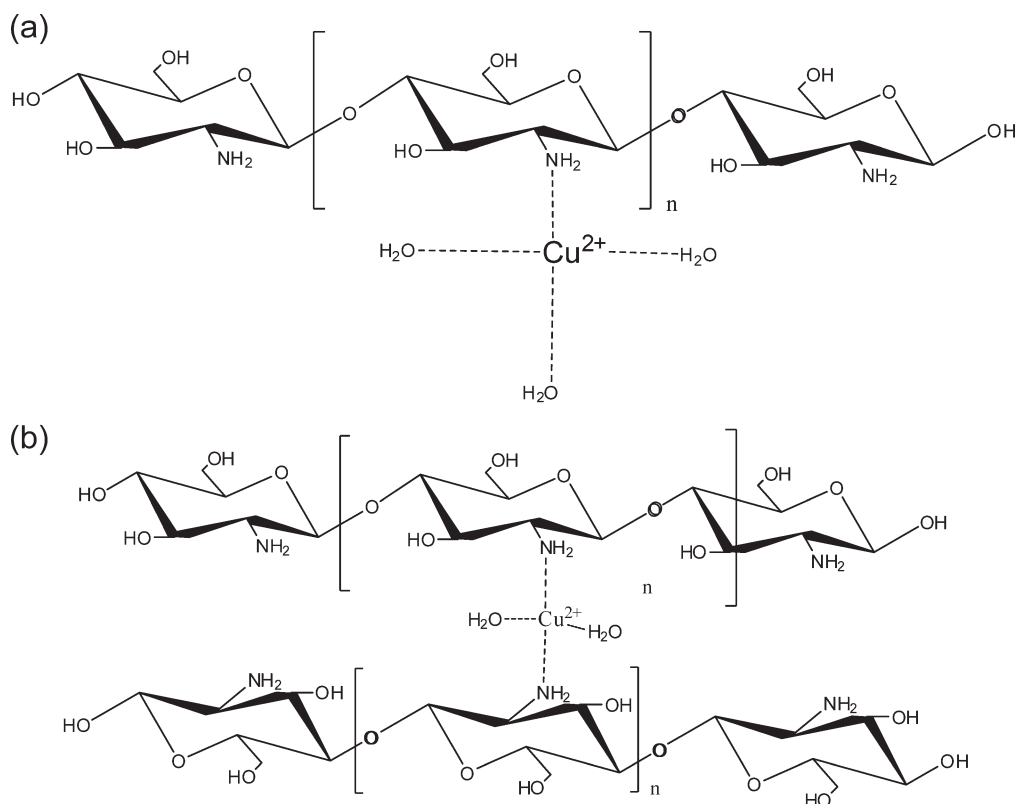
As outlined above, Chi–Glu copolymers were prepared at variable reaction times according to the duration of the neutralization step (3 vs. 48 h). Above pH 5.6, proton transfer occurs, which facilitates the first step of the aldol condensation reaction.<sup>23</sup> In the case of the CP-1 copolymer, the color of the final product appeared darker as the neutralization time increased, as shown in Figure 1(a,c). Products obtained at short versus long neutralization time vary from light versus dark in their coloration,<sup>22</sup> respectively. The dark-colored copolymer material correlates with an increased level of crosslinking, in agreement with a previous report<sup>25</sup> of chitosan aerogels by Chang et al. Images of the copolymers prepared at different reaction times in the presence and absence of Cu(II) are also shown in Figure 1(b,d). After imbibing with CuSO<sub>4</sub>, the product with short reaction time shows the appearance of two different phases according to the sample coloration, a blue-green and a dark brown phase. However, the material prepared at longer neutralization time appears more uniform in appearance, as evidenced by a homogenous dark green phase. The addition of CuSO<sub>4</sub> serves as an indicator stain, which may reveal copolymer domains with framework heterogeneities resulting from incomplete crosslinking. The variable staining of CuSO<sub>4</sub> within the copolymer can be understood in terms of chitosan domains containing variable levels of available glucosamine ligands for coordination to Cu(II). The green coloration [cf. Figure 1(b,d)] of the products provide support of the formation of copolymer/Cu(II) complexes, in agreement with previously published reports (cf. Table I in Ref. 24 and Figure 1 in Ref. 26).

### Preparation of the Copper-Imbided Materials

Copper-imbided materials were prepared by equilibrating the copolymers in a solution containing an excess amount of CuSO<sub>4</sub>. The structure of the copper-imbided Chi–Glu copolymer



**Figure 1.** Images of copolymers in the presence and absence of Cu(II) at room temperature in aqueous solution at 295 K: (a) CP-1, (b) CP-1/Cu(II), (c) CP-1 (long), and (d) CP-1/Cu(II) (long). [Color figure can be viewed in the online issue, which is available at [wileyonlinelibrary.com](http://wileyonlinelibrary.com).]



**Scheme 1.** Different coordination models for chitosan/Cu(II) complexes: (A) the “pendant model” and (B) the “bridge model.”

is poorly understood, but it can be interpreted in terms of the bridge and pendant models<sup>27,28</sup> of copper-imbibed chitosan biopolymers, as shown in Scheme 1.<sup>28</sup> The formation of variable copolymer morphology is related to the pH conditions of the reaction.<sup>23</sup> According to Table II, the relative uptake of Cu(II) is similar for the various copolymers containing variable weight ratios of glutaraldehyde. CP-1 is predicted to have the greatest number of free amine groups, in accordance with the predicted level of crosslinking (~36%). However, the similar uptake values do not correlate with the predicted levels of crosslinking. The grafting of glutaraldehyde groups onto chitosan and inefficient crosslinking of glutaraldehyde monomers may result in the presence of aldehyde groups on the copolymer surface. The presence of such ligands may contribute to coordination of Cu(II), in addition to the noncrosslinked glucosamine monomer units of

chitosan. The potential self-condensation of glutaraldehyde monomers has been previously reported<sup>16,23</sup> and may also contribute to the variable Cu(II) uptake observed in Table II.

#### Characterization of the Copolymer Materials and Copper-Imbibed Materials

The FTIR spectra of chitosan, CP-1, -2, and -3 copolymers are shown in Figure 2(A,B). The broad band appearing at ~3000–3500  $\text{cm}^{-1}$  is associated with the stretching of OH groups of chitosan. The shoulder above 1700  $\text{cm}^{-1}$  (cf. expanded region of Figure 2) corresponds to the vibrational band of aldehyde groups (cf. Scheme 3 in Ref. <sup>23</sup>), because chitosan is ~20–25% acetylated. The vibrational band at ~1660  $\text{cm}^{-1}$  indicates the carbonyl group signature of an acetyl group. The peak around 1650  $\text{cm}^{-1}$  (i.e., amide I band) overlaps with the imine group

**Table II.** Experimental Cu(II) Uptake (%) for Chi–Glu Copolymers and Theoretical Uptake (%) According to Two Types of Coordination Models

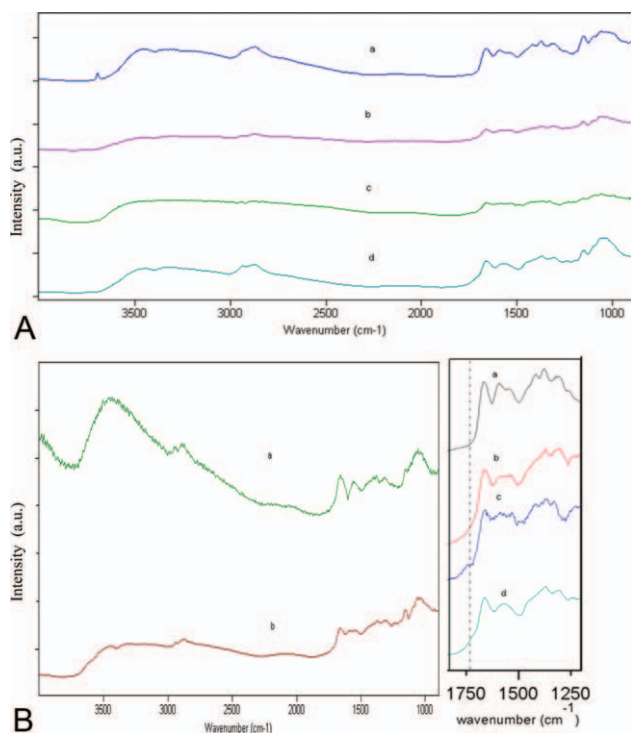
Copolymer (Chi–Glu)	Calculated Cu(II) uptake (%)	Theoretical <sup>a</sup> uptake (%) “bridge model”	Theoretical <sup>a</sup> uptake (%) “pendant model”	TGA estimates Cu(II) uptake <sup>b</sup>
CP-1	34.8 ± 2.0	18.0	36.0	11.3 ± 2.3
CP-1 (long)	29.4 ± 1.7	18.0	36.0	8.30 ± 1.7
CP-2	24.3 ± 1.4	10.0	20.0	6.30 ± 1.3
CP-3	29.5 ± 1.7	1.3	2.6	12.8 ± 2.6

(Long) refers to long reaction time, as outlined in the ‘Experimental Methods’ section.

The Cu(%) uptake does not explicitly account for Cu(II) complexes with imine or aldehyde groups.

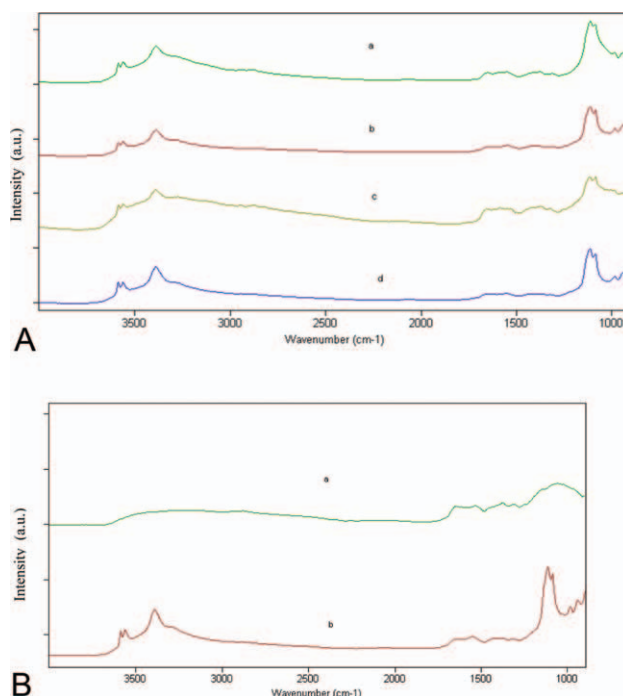
<sup>a</sup>See Ref. 19, where the models assume the following Cu(II): amine stoichiometry: bridge models (1 : 2) and pendant (1 : 1).

<sup>b</sup>Chitosan/Cu(II) TGA estimate for Cu(II) uptake was ~ 20.0%.



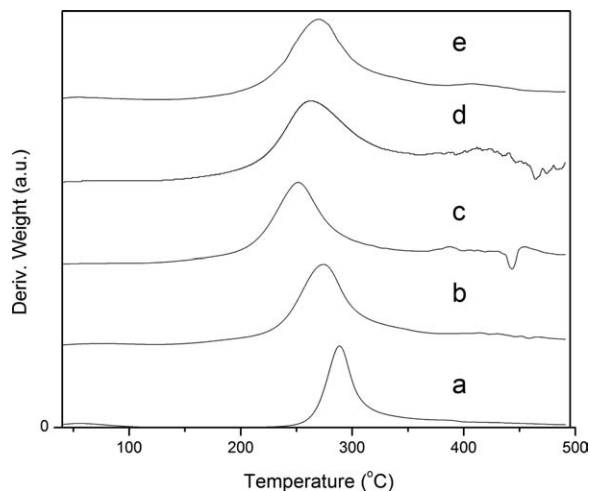
**Figure 2.** (A) FTIR spectra of chitosan and its copolymers: (a) chitosan, (b) CP-1, (c) CP-1, and (d) CP-3 Chi-Glu. The accompanying expansion for the 1250–1750  $\text{cm}^{-1}$  region is shown below where the spectra are identified as above. (B) FTIR spectra of CP-1 copolymer materials prepared at different reaction times: (a) CP-1 (long) and (b) CP-1. [Color figure can be viewed in the online issue, which is available at [wileyonlinelibrary.com](http://www.wileyonlinelibrary.com).]

( $-\text{C}=\text{N}-$ ) and provides strong support that crosslinking occurs between chitosan and glutaraldehyde, as described above. With increasing glutaraldehyde content of the copolymer, the band at  $\sim 1650 \text{ cm}^{-1}$  for the imine group becomes broader, as evidenced in the expanded region of Figure 2(B). The band at  $1591 \text{ cm}^{-1}$  corresponds to the N–H bending vibration of chitosan. Figure 2(B) illustrates the IR spectra for CP-1 obtained at variable reaction times. The IR spectra are relatively similar over the entire spectral region; however, CP-1 (long) shows a pronounced imine vibrational band at  $\sim 1650 \text{ cm}^{-1}$ . The latter may be attributed to increased levels of crosslinking for these conditions. Figure 3(A) illustrates the FTIR spectra of copper-imbibed copolymers (i.e., CP-1, -2, and -3) where the two sharp peaks above  $3500 \text{ cm}^{-1}$  represent contributions due to intermolecular bonded  $-\text{NH}$  groups coordinated to Cu(II).<sup>27</sup> The coordination of Cu(II) with chitosan according to the “bridge” model (cf. Scheme 1) is supported by an enhancement of the C–O band at  $\sim 1150 \text{ cm}^{-1}$ . As well, the amine group of chitosan is involved because the N–H bending of chitosan shifts from  $1591$  to  $1560 \text{ cm}^{-1}$ , providing additional support of the formation of Cu(II) complexes. Figure 3(B) illustrates the FTIR spectra of the Cu(II)-imbibed CP-1 (long) copolymer. The IR bands above  $3500 \text{ cm}^{-1}$  for CP-1 (long) are not observed for the Cu(II)-imbibed materials. The results suggest that Cu(II) has fewer available amine/aldehyde groups relative to the

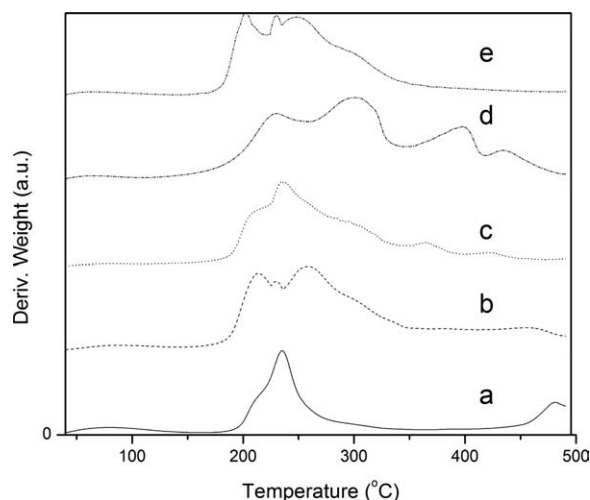


**Figure 3.** (A) FTIR spectra of copper-imbibed sorbent materials: (a) chitosan/Cu(II), (b) CP-1/Cu(II), (c) CP-2/Cu(II), and (d) CP-3/Cu(II) complexes. (B) FTIR spectra of CP-1/Cu(II) complexes prepared at different reaction times: (a) CP-1/Cu(II) (long) and (b) CP-1/Cu(II). [Color figure can be viewed in the online issue, which is available at [wileyonlinelibrary.com](http://www.wileyonlinelibrary.com).]

imbibed copolymer material prepared at shorter reaction time. The reduction in free aldehyde groups is consistent with the conversion of grafted glutaraldehyde copolymers to crosslinked products as the reaction time increases.<sup>12,16</sup> According to Figures 2 and 3, it is less likely that Cu(II) binds to the imine groups of the Chi-Glu copolymers and rather coordinates to the free amine groups of chitosan. This is further supported by independent sorption studies of Cu(II) with chitosan and its



**Figure 4.** TGA of chitosan and copolymers, where a = chitosan, b = CP-1, c = CP-2, d = CP-3, and e = CP-1 (long).



**Figure 5.** TGA of chitosan/ and copolymer/Cu(II) complexes, where a = chitosan/Cu(II), b = CP-1/Cu(II), c = CP-2/Cu(II), d = CP-3/Cu(II), and e = CP-1/Cu(II) (long).

copolymers (cf. Figure 5 in Ref. 29). The similar Cu(II) uptake values for the copolymers are related to the relative availability of amine groups on chitosan and its copolymers, according to the crosslink density of the copolymers.

The TGA results for Chi–Glu copolymers and their Cu(II) complexes are shown in Figures 4 and 5. The thermal decomposition of both materials occur in the 200–400°C range. The TGA results for pure chitosan display a thermal event at ~300°C.<sup>30</sup> Crosslinking between chitosan and tripolyphosphate illustrates a thermal event at lower temperature relative to pure chitosan for crosslinked chitosan.<sup>31</sup> Beppu et al.<sup>32</sup> have reported TGA results for chitosan crosslinked with glutaraldehyde and a transition is observed (<300°C), which is significantly broadened relative to pristine chitosan (cf. Figure 4 in Ref. 31). The reduced temperature onset observed for the copolymers may be attributed to the effects of crosslinking because it is anticipated to reduce the thermal stability due to attenuation of the intermolecular H-bonding between adjacent chitosan polymer units. The TGA results for chitosan and its Cu(II) complex reveal a similar lowering of the temperature onset, as observed in the TGA profile for the chitosan/Cu(II) complexes (cf. Figure 5). Similar effects

were observed by Ng et al.<sup>33</sup> where the DSC transition (cf. Figure 4 in Ref. 32) for the Cu(II) complex was attenuated by ~100°C relative to chitosan, further supporting that reduced intermolecular H-bonding occurs between adjacent polymer chains, and attenuates the heat capacity of these crosslinked copolymers. The greater thermal stability of crosslinked chitosan is related to the presence of Cu(II) complexes, according to the observed temperature effects and activation energy of the degradation process.<sup>34</sup> Additional thermal events are observed for copolymers and their complexes with Cu(II) appear in the 200–300°C range (cf. Figure 5), indicating that Cu(II) may be bound at multiple coordination sites of chitosan.<sup>15,33</sup> The presence of multiple sites provides support that ligation of free aldehyde groups along with the amine groups of chitosan. Thermal events in the 300–500°C range are attributed to the glutaraldehyde crosslinking because of the greater thermal stability of such copolymers, as reported previously.<sup>32,33,35</sup>

The TGA results for the CP-1 copolymer/Cu(II) complex at short versus long neutralization times are shown in Figure 5. The major difference in the TGA results for the products prepared at these conditions is seen in the 300–500°C region. Figure 5 shows that CP-1 (long) exhibits a different thermal profile relative to the respective copolymers (i.e., CP-1, -2, and -3) prepared at shorter reaction times. In Figure 5, the broadened thermal transition between 300 and 500°C is reduced because of the effects of crosslinking.<sup>32,33,35</sup> The earlier onset of thermal events at lower temperatures is evident for copolymer/Cu(II) complexes prepared at longer reaction time versus the shorter reaction time. These results indicate that greater crosslinking occurs when the reaction time is increased. As well, self-condensation of glutaraldehyde may occur in cases when excess weight ratios of glutaraldehyde are used (i.e., CP-3), as described above.

### Water Swelling Properties

The water swelling results [ $S_w$ ; Eq. (1)] for chitosan, Chi–Glu copolymers, and their Cu(II) complexes are shown in Table III. Selected literature values for the swelling of polysaccharide-based materials are given as follows: cellulose (cotton) 49.9%<sup>37</sup> and Chi–Glu copolymer 213%.<sup>36</sup> According to Table III, the  $S_w$  values for the copolymers decrease with greater crosslinking, in agreement with a previous report.<sup>36</sup> Swelling is further reduced for copolymer/Cu(II) complexes relative to the nonimbibed

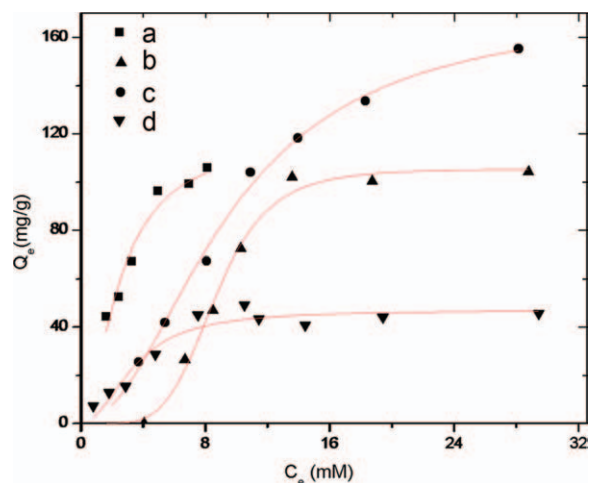
**Table III.** Swelling Ratios ( $S_w$ ) in Water and Sips Isotherm<sup>a</sup> Parameters for the Sorption of Urea at 295 K and Ambient pH Conditions

Copolymer	$Q_m$ (mg g <sup>-1</sup> )	$K_{Sips}$	$n_s$	$S_w$ (%)
HMW chitosan <sup>b</sup>	47.1 ± 3.4	0.291 ± 0.044	2.1 ± 0.6	322 <sup>c</sup>
CP-1	118.0 ± 20	0.407 ± 0.100	1.7 ± 0.6	332 ± 25
CP-1 (long)	105.5 ± 3.6	0.116 ± 0.003	5.1 ± 0.7	277 ± 21
CP-3	171.2 ± 9.0	0.106 ± 0.007	2.0 ± 0.2	257 ± 19
CP-1/Cu(II)	163.2 ± 15.7	0.283 ± 0.060	1.2 ± 0.2	139 ± 14
CP-1/Cu(II) (long)	233.5 ± 12.1	0.087 ± 0.05	2.6 ± 0.2	81 ± 8
CP-3/Cu(II)	264.7 ± 6.5	0.223 ± 0.013	1.6 ± 0.1	72 ± 7

<sup>a</sup>According to the Sips isotherm [cf. Eq. (3)].

<sup>b</sup>Denotes the HMW chitosan.

<sup>c</sup>See Ref. 36.



**Figure 6.** Sorption isotherm of chitosan and Chi-Glu copolymers with urea in aqueous solution at pH 7, where a = CP-1, b = CP-1 (long), c = CP-3, and d = HMW chitosan. The solid line refers to the best fit according to the Sips isotherm. [Color figure can be viewed in the online issue, which is available at [wileyonlinelibrary.com](http://wileyonlinelibrary.com).]

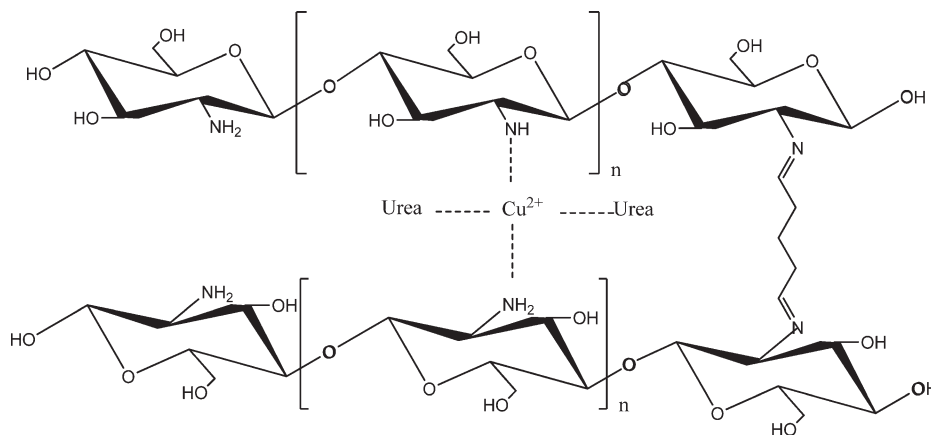
copolymers. The coordination between the amine groups of chitosan or surface-bound aldehyde groups with Cu(II) appears to attenuate the water swelling behavior, in a similar manner to crosslinked Chi-Glu copolymers.<sup>16,25,35</sup>

### Sorption Studies

Figure 6 illustrates the sorption results of urea with chitosan and Chi-Glu copolymers at 295 K and pH 6 in aqueous solution. In the case of chitosan, the value of  $Q_e$  increases monotonically as  $C_e$  increases up to  $\sim 9$  mM. The concentration dependence of  $Q_e$  is more pronounced for the Chi-Glu copolymers as the glutaraldehyde content increases, as evidenced by the greater  $Q_m$  values (cf. Table III). The CP-3 copolymer displays the greatest overall sorption with urea with saturation of the sorption sites occurring at  $C_e$  values  $>30$  mM. In contrast, the CP-1 copolymer exhibits reduced sorption and levels off at  $\sim 12$  mM. The solid lines through the experimental data in Figure 6 represent the best-fit curve according to the Sips isotherm [Eq. (3)]. The sorption parameters ( $Q_m$ ,  $K$ , and  $n$ ) are

listed in Table III where the relative ordering of the sorption capacity ( $Q_m$ ) is as follows: CP-3  $>$  CP-1  $>$  chitosan. The heterogeneity parameter ( $n$ ) for each sorbent is greater than unity, indicating that the sorbent surface is heterogeneous in nature, as anticipated for such crosslinked copolymer materials. The variation in sorption observed for the copolymers appears to be inversely correlated to the extent of swelling in aqueous solution (cf. Table III). Changes to the sorbent surface area (SA) and the sorption properties in aqueous solution occur due to swelling of such “soft materials.” Similar effects were observed for cross-linked urethane copolymers reported by Wilson et al.<sup>38</sup> H-bonding interactions between urea and the polar functional groups ( $-\text{OH}$  and  $-\text{NH}_2$ ) of chitosan and its copolymers are anticipated to contribute to the sorption phenomena observed in Figure 6. The variation in the sorption and swelling behavior is related to the relative hydrophile-lipophile balance of the sorbent, consonant with the level of crosslinking density.<sup>32</sup> Urea is a hydrophilic guest molecule that competes with solvent for sorption sites; thus, H-bonding interactions are deemed to play an important role.<sup>12</sup> In contrast, polysaccharide sorbents such as oxystarch and oxycellulose have attenuated sorption with urea, as compared with the chitosan-based sorbents produced herein (cf. Table IV). Crosslinking at variable levels is anticipated to alter the sorbent SA and the formation of micropore sites, which create additional binding domains for urea in the copolymer framework, as shown in Scheme 2. The literature values for the various sorbent materials in Table V cover a range of urea uptake values ranging from 0.9 to 9.0% (w/w). The experimental  $Q_m$  values in Table III for chitosan (4.7% w/w), Chi-Glu copolymers (12–17% w/w), and copolymer/Cu(II) complexes (16–26% w/w) have a wider range of sorption values compared with those in Table IV.

Figure 7 illustrates the sorption results for urea and copolymer/Cu(II) complexes at 295 K and pH 6 in aqueous solution. The solid lines through the experimental data in Figure 7 represent the best-fit according to the Sips isotherm [Eq. (3)], and the sorption parameters are listed in Table III. The relative ordering of the sorption capacity for each copolymer/Cu(II) complex is as follows: CP-3/Cu(II)  $>$  CP-1 Chi-Glu/Cu(II) (long)  $>$  CP-1/Cu(II). Comparable heterogeneity factors are observed for the copolymer/Cu(II) complexes relative to the nonimbibed Chi-

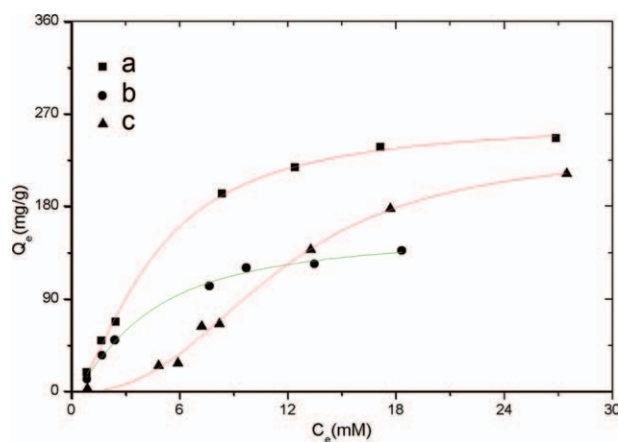


**Scheme 2.** An illustration of Cu(II) coordination in Chi-Glu copolymers.

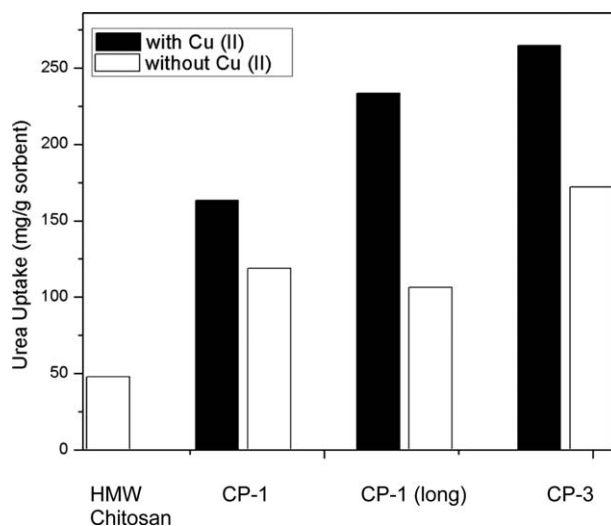
**Table IV.** Urea Uptake of Different Polymeric Sorbents at Various Experimental Conditions

Sorbent material	$Q_m$ (mg g <sup>-1</sup> )	Experimental conditions
Activated carbon <sup>37</sup>	9.0	Ambient conditions
Oxystarch <sup>8</sup>	12.0	pH 7.2–7.4 buffer and 310 K
Oxycellulose <sup>9</sup>	13.0	pH 7.2 buffer and 310 K
Chitosan-coated oxycellulose <sup>10</sup>	90.4	pH 7.2 buffer and 310 K
Oxidized $\beta$ -cyclodextrin/epichlorohydrin copolymer <sup>7</sup>	50.6	pH 7.4 buffer and 310 K
Chitosan/Cu(II) complex <sup>3</sup>	78.8	Buffered at pH 7.0 and 295 K
Chitosan/Cu(II) film <sup>39</sup>	22.0	Buffered at pH 7.4 and 310 K

Glu copolymers. The sorption results in Figure 7 are comparable to those in Figure 6; however, the uptake of urea for the copolymer/Cu(II) complexes is approximately doubled, as shown in the bar graph of Figure 8. A comparison of the sorption capacity of the various sorbents in Table IV ( $Q_m \sim 9\text{--}79$  mg g<sup>-1</sup>) with the results obtained herein for the copolymers and their complexes with Cu(II) (cf. Figure 8;  $Q_m \sim 106\text{--}265$  mg g<sup>-1</sup>) reveals some important differences. In Table IV, chitosan-coated oxycellulose, oxidized  $\beta$ -cyclodextrin/epichlorohydrin, and Cu(II) chitosan complexes display the greatest overall uptake of urea. The chitosan copolymers prepared in this work have significantly greater uptake than the sorbent materials reported in the literature, and this is attributed to the improved synthetic design strategy described herein.



**Figure 7.** Sorption isotherm of Chi-Glu copolymer/Cu(II) with urea in aqueous solution at pH 7, where a = CP-3, b = CP-1, and c = CP-1 (long). The solid line refers to the best fit according to the Sips isotherm. [Color figure can be viewed in the online issue, which is available at [wileyonlinelibrary.com](http://wileyonlinelibrary.com).]



**Figure 8.** Equilibrium uptake of urea (mg g<sup>-1</sup> sorbent) for chitosan and Chi-Glu copolymers in the presence and absence of Cu(II) in aqueous solution at pH 7 and 295 K.

The greater urea sorption observed for the copolymer/Cu(II) complexes is related to the presence of favorable coordination sites for urea because of the presence of Cu(II).<sup>40</sup> Glucosamine monomers form stable complexes with Cu(II),<sup>41</sup> and chitosan similarly forms stable Cu(II) complexes ( $pK_1 = 5.47$  and  $pK_2 = 2.67$ ; Ref. 27). In addition to the hydroxyl and amine groups within the copolymer framework, the incorporation of Cu(II) introduces favorable Lewis acid coordination sites for urea ligands,<sup>42</sup> as illustrated in Scheme 2, and further supported by the results in Figure 8. The variation in the sorption properties of Chi-Glu copolymers and their Cu(II) complexes is consistent with the changes in SA and pore structure of these sorbent materials. The Chi-Glu copolymer sorbents described herein illustrate that a rational materials design approach that enables tuning of the sorption capacity and molecular recognition of urea. By comparison with chitosan as a sorbent material, its sorption properties were substantially improved through incremental crosslinking with glutaraldehyde and the formation of copolymer/Cu(II) complexes.

## CONCLUSIONS

Chitosan copolymers containing glutaraldehyde were prepared from the reaction of chitosan and glutaraldehyde at various mole ratios and variable reaction times. The copolymer materials were imbibed in aqueous CuSO<sub>4</sub> to form copolymer/Cu(II) complexes which were characterized using FTIR and TGA. Variable thermal stability of the copolymers was observed according to the glutaraldehyde content and the occurrence of coordinative crosslinking with Cu(II).

Glutaraldehyde crosslinked chitosan and the corresponding copolymer/Cu(II) complexes display favorable sorption properties toward urea in aqueous solution at 295 K and pH 7. The Sips isotherm model provides a good description of the experimental data. The copolymers display increased sorption ( $Q_m = 10.6\text{--}17.1\%$  w/w) as the content of glutaraldehyde increases (i.e., CP-



1, -2, and -3). Sorption is further enhanced ( $Q_m = 16.3\text{--}26.4\%$  w/w) for copolymer Chi-Glu/Cu(II) complexes. Chi-Glu copolymers and their Cu(II) complexes display substantially improved sorption and molecular recognition properties toward urea. The enhanced sorption properties for copolymer/Cu(II) complexes illustrate that copolymers containing macromolecular units are tunable for sorption-based processes involving the sequestration and immobilization of urea in aquatic environments and hemodialysis applications.

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