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A Chromatographic Technique To Investigate the Lability of Copper Complexes under Steady-State Conditions Using High Specific Activity ^{64}Cu

Jacco van Doornmalen,* Johannes T. van Elteren, and Jeroen J. M. de Goeij

Department of Radiochemistry, Interfaculty Reactor Institute, Delft University of Technology, Mekelweg 15, 2629 JB Delft, The Netherlands

A novel chromatographic technique is described which has prospects for studying the lability of a metal complex in an aqueous system. It is based on interactions of metal species with an ion-exchange column under steady-state conditions. For this purpose, the column is equilibrated with the sample itself by using it as the mobile phase. With the aid of a high specific activity radiotracer, the characteristics of the metal species interaction with the ion-exchange column can be visualized in a radiochromatogram. Under particular experimental conditions, information regarding the dissociation kinetics of the metal complex can be extracted from this radiochromatogram. Erroneous results due to undesirable interactions of metal species with the chromatographic system are prevented, since the system remains in constant chemical equilibrium with the sample to be analyzed. The potential of this technique was investigated with copper complexes, using high specific activity ^{64}Cu for labeling. Preliminary results obtained with four complexes (Cu−EDTA, Cu−NTA, Cu−citrate, and Cu−glycine) are discussed. For three complexes, dissociation rate constants could be determined: $(6.2 \pm 0.3) \times 10^{-3}$ s^{-1} for Cu−EDTA, $(1.0 \pm 0.04) \times 10^{-2}$ s^{-1} for Cu−NTA, and $(3.1 \pm 0.2) \times 10^{-2}$ s^{-1} for Cu−citrate. No dissociation rate constant could be determined for Cu−glycine owing to incompatible experimental conditions.

To predict the behavior (e.g., mobility, bioavailability, and toxicity) of a trace metal in an aqueous system, it is essential to have proper knowledge of its chemical speciation, i.e., the distribution of its species resulting from the interaction with its direct environment. Speciation calculations are usually based on thermodynamic parameters and predict the occurrence of possible species for a system in total equilibrium. However, these calculations cannot provide sufficient information to describe the behavior of a trace metal, since the time scales in which species are formed or interconverted (determined by kinetic parameters) are not taken into account. It is well-known, for instance, that the reactivity of a trace metal toward an organism is not always directly related to the amount of free metal ions in equilibrium. The presence of metal complexes with high rates of dissociation and/or low rates of association may have a considerable influence on toxicity as well.[1−3]

The term "lability" is often used to describe kinetic parameters of metal complexes. This refers to the ability of a metal complex to dissociate into the free metal ion and the ligand to which it was initially bound. It is important to note that lability is not an absolute concept, since it has to be related to a time scale of interest. Consequently, the best way to describe the lability of a metal complex unambiguously is with its dissociation rate constant, k_d.

Several techniques have been developed to determine the dissociation rate constants of metal complexes in (natural) aqueous systems. They are based on a time-dependent separation of the free metal ions from metal complexes, where the duration of separation and the k_d value determine the degree of dissociation.

Most techniques use ion-exchange materials to separate free metal ions from metal complexes. The dissociation kinetics of individual[4] as well as groups of metal complexes[5−7] have been determined in this way. Voltammetry can also be used as a separation technique, for instance, by using anodic stripping voltammetry (ASV) combined with different ion-exchange methods[8] or by determining the rate of metal accumulation on a chemically modified electrode in ASV.[9] The voltammetric time scale itself is sometimes used as a measure of lability, since it is considered as fairly comparable to the time scales of biouptake reactions.[10,11]

* Corresponding author. E-mail: j.vandoornmalen@iri.tudelft.nl. Fax: ++31-15-278-3906.

(1) Hudson, R. J. M. *Sci. Total Environ.* **1998**, *219*, 95−115.
(2) Campbell, P. G. C. In *Metal Speciation and Bioavailability in Aquatic Systems*; Tessier, A., Turner, D. R., Eds.; John Wiley: Chichester, U.K., 1995; pp 45−103.
(3) Ma, H.; Don Kim, S.; Cha, D. K.; Allen, H. E. *Environ. Toxicol. Chem.* **1999**, *18*, 828−837.
(4) Procopio, J. R.; Del Mar Ortiz Viana, M.; Hernandez, L. H. *Environ. Sci. Technol.* **1997**, *31*, 3081−3085.
(5) Lu, Y.; Chakrabarti, C. L.; Back, M. H.; Grégoire, D. C.; Schroeder, W. H. *Anal. Chim. Acta* **1994**, *293*, 95−108.
(6) Rocha, J. C.; Toscano, I. A. S.; Burba, P. *Talanta* **1997**, *44*, 69−74.
(7) Chakrabarti, C. L.; Lu, Y.; Grégoire, D. C.; Back, M. H.; Schroeder, W. H. *Environ. Sci. Technol.* **1994**, *28*, 1957−1967.
(8) Figura, P.; McDuffie, B. *Anal. Chem.* **1980**, *52*, 1433−1439.
(9) Labuda, J.; Buckova, M.; Halamova, L. *Electroanalysis* **1997**, *9*, 1129−1131.
(10) Jansen, R. A. G.; van Leeuwen, H. P.; Cleven, R. F. M. J.; van den Hoop, M. A. G. T. *Environ. Sci. Technol.* **1998**, *32*, 3882−3886.
(11) Whitfield, M.; Turner, D. R. In *Chemical modeling in aqueous systems*; Jenne, E. A., Ed.; ACS Symposium Series 93; American Chemical Society: Washington, DC, 1979; pp 657−680.

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It is required that these techniques be able to achieve a rapid and distinct separation of free metal ions from metal complexes. For this purpose, the separation systems have to possess a high affinity for free metal ions with virtually no affinity for metal complexes and exhibit fast kinetics of free metal ion binding. This is especially true when the metal complexes have high rates of dissociation. If these conditions are not met, measurements of lability will result in erroneous k_d values.

We have developed a chromatographic technique that may provide insight into the lability of metal complexes in aqueous systems under steady-state conditions. The chromatographic system is first brought into equilibrium with the sample to be analyzed. Then an aliquot of the same sample spiked with a radiotracer is introduced, which does not change the equilibrium attained. Thus, undesirable interactions of metal species with the analytical column are avoided. The potential of this technique was investigated with four copper complexes having different kinetic as well as chemical properties: Cu−EDTA, Cu−NTA, Cu−citrate, and Cu−glycine. The first two were expected to possess lower rates of dissociation relative to the latter two. Cu−glycine was a positively charged complex and the others were negatively charged under the conditions used.

GENERAL CONCEPT

The simplest form of interaction between a free copper ion and a ligand in an aqueous solution is illustrated by the chemical equilibrium

$$Cu^{2+} + L^n \underset{k_d}{\overset{k_a}{\rightleftharpoons}} [Cu{-}L]^{2+n} \tag{1}$$

where Cu^{2+} is the free copper ion, L the ligand, n the charge of the ligand, $[Cu{-}L]^{2+n}$ the copper complex with charge $2 + n$, k_a the association rate constant (in $L{\cdot}mol^{-1}{\cdot}s^{-1}$), and k_d the dissociation rate constant (in s^{-1}).

In equilibrium, the chemical form of a copper atom is constantly changing from "free ionic" (Cu^{2+}) to "complexed" (Cu−L) and back again, according to eq 1. Assuming first-order reactions, the rate at which a copper atom changes from one form into another depends on two parameters—the concentration of all species taking part in the equilibrium and the rate constants involved:

$$F_a = k_a[Cu^{2+}][L] \tag{2}$$

$$F_d = k_d[Cu{-}L] \tag{3}$$

Here F_a is the rate of copper complex formation (in $mol{\cdot}L^{-1}{\cdot}s^{-1}$), F_d is the rate of copper complex dissociation (in $mol{\cdot}L^{-1}{\cdot}s^{-1}$), and $[Cu^{2+}]$, [L], and [Cu−L] are the concentrations (in $mol{\cdot}L^{-1}$) of the free copper ion, the ligand, and the copper complex, respectively.

Under steady-state conditions, the rates of copper complex formation and dissociation are equal:

$$F_a \equiv F_d \tag{4}$$

The equilibrium concentration of all species can be calculated using the ratio of the association and dissociation rate constants:

$$\frac{k_a}{k_d} = \frac{[Cu{-}L]}{[Cu^{2+}][L]} = K \tag{5}$$

where K is the thermodynamic stability constant of the equilibrium.

A continuous separation of free copper ions from the copper complex prevents association reactions and causes the copper complex to dissociate according to:

$$[Cu{-}L]_t = [Cu{-}L]_0 e^{-k_d t} \tag{6}$$

where $[Cu{-}L]_t$ is the copper complex concentration (in $mol{\cdot}L^{-1}$) remaining after time t (in s) and $[Cu{-}L]_0$ is the initial copper complex concentration (in $mol{\cdot}L^{-1}$).

A liquid chromatography system with an ion-exchange column is used here to investigate the lability of a copper complex. To avoid undesired interactions of copper species with the column material, the system is operated in an unconventional way: the sample itself is used as the mobile phase. This ensures that the actual measurements will be performed under steady-state conditions; i.e., no net mass transport of copper species to and from the stationary phase will occur. However, copper species will still interact with the stationary phase owing to exchange mechanisms:

$$S + S'{-}R \leftrightarrow S' + S{-}R \tag{7}$$

where S and S′ denote two different atoms/molecules of the same copper species that interact with a functional group R on the stationary phase of the analytical column. Exchange mechanisms cause each copper species to have a characteristic retention time in the column, without disturbing the equilibrium concentrations of all species present (steady-state condition).

Before an aliquot of the sample to be analyzed is introduced into the column, it has to be labeled with a tracer to distinguish its copper species from the ones present in the mobile phase. The sample should remain chemically identical to the mobile phase to ensure steady-state conditions during analysis. This means not only that the tracer should have the same chemical properties as the copper species under study but also that its introduction to the aliquot should not disturb initial speciation. To accomplish this, the sample is spiked with a high specific activity ^{64}Cu radiotracer, so that a negligible mass of copper is added.

It has to be realized that this configuration of the liquid chromatography system does not perform a physical separation of free copper ions and copper complexes. At each moment, the same amounts of copper species enter and leave the analytical column and, moreover, copper atoms still change their chemical form from "free ionic" to "complexed" during analysis, according to eqs 2−4. Nevertheless, the retention behavior of the copper species present in the spiked sample will be revealed in a radiochromatogram.

To obtain information regarding the lability of a copper complex from a radiochromatogram, the experimental conditions (column size, flow velocity, column material, etc.) must be chosen such that free ionic copper (Cu^{2+}) has a retention behavior different from that of the copper complex. This will accomplish a

separation between radioactive free copper ions and radioactive copper complexes. When both of the latter species are visible in a radiochromatogram as two separate peaks, the peak belonging to the copper complex represents the amount of copper complex that did not dissociate during the time it was present in the column. Using the thermodynamic stability constant K, the initial species distribution, including $[Cu-L]_0$, can be calculated and compared to the species distribution in the radiochromatogram, including $[Cu-L]_t$. Hence, knowing the ratio $[Cu-L]_t/[Cu-L]_0$ and the time t during which the complex is allowed to dissociate, one can determine the dissociation rate constant k_d via eq 6.

When the copper complex dissociates completely during the time of analysis or has a retention behavior similar to that of the free metal ion, only one peak will appear in the radiochromatogram. In this case, no information regarding the lability of the copper complex can be collected. It is therefore important to choose the experimental conditions such that both species can be seen separately in the radiochromatogram.

EXPERIMENTAL SECTION

Reagents and Standards. All chemicals were at least of analytical reagent grade. Milli-Q-Plus (MQ) water (Millipore-Waters, Milford, MA) was used for all solution preparations. A copper AAS standard solution (1000 mg·L^{-1} Cu in 5% HNO_3) was purchased from Johnson Matthey GmbH (Karlsruhe, Germany). Ligands for complexation were EDTA (ethylenediaminetetraacetic acid, disodium salt, Merck, Darmstadt, Germany), NTA (nitrilotriacetic acid, trisodium salt, Sigma, St. Louis, MO), citrate (trisodium citrate dihydrate, Fluka Chemie AG, Buchs, Switzerland), and glycine (J. T. Baker, Deventer, The Netherlands). Ionic strengths and pHs of the solutions were adjusted with sodium chloride (J. T. Baker, Deventer, The Netherlands), MES buffer (β-morpholinoethanesulfonic acid monohydrate, Sigma-Aldrich, Steinheim, Germany), and sodium hydroxide (J. T. Baker, Deventer, The Netherlands). High specific activity $^{64}Cu^{2+}$ ($t_{1/2} = 12.7$ h; γ 0.511 MeV) was prepared according to the method described by van Elteren et al.[12] via the $^{64}Zn(n,p)^{64}Cu$ reaction. A high-purity ZnO target (Grade A1 (99.9995%), Johnson Matthey, Materials Technology, U.K.) was irradiated in the reactor facility of the Interfaculty Reactor Institute (Delft, The Netherlands), followed by a chemical separation of Cu from Zn, yielding a 500 μL aqueous solution with a specific activity of $>8 \times 10^{15}$ Bq·g^{-1} Cu. This solution was tested for the presence of traces of Zn, which could compete with the ligands and thus bias results. However, both radiometric and voltammetric analyses could not detect any trace of Zn.

Safety. The radionuclide ^{64}Cu (half-life 12.7 h) emits β^+ and β^- particles as well as 511 keV γ-radiation (due to annihilation of β^+ particles). Therefore, all work was performed in a specialized laboratory equipped for handling radioactive solutions. Standard procedures were followed, including shielding and prevention of contamination with radioactivity. The amount of radioactivity per experiment was kept to a minimum, viz. 0.16 MBq (equivalent to 4.3 μCi) of ^{64}Cu. Without any shielding, this would give a dose rate of 6×10^{-5} mSv·h^{-1} at a distance of 30 cm. Application of shielding reduced the dose rate at this distance by a factor of at least 100.

Preparation of Samples. Aqueous solutions (500 mL) were prepared, all containing 0.1 mol·L^{-1} NaCl, 0.01 mol·L^{-1} MES buffer, and an initial Cu^{2+} concentration of 10^{-6} mol·L^{-1}. The pHs of these solutions were adjusted to 6.0 using a concentrated NaOH solution. One of the ligands, EDTA, NTA, citrate, or glycine, was added to three of these solutions in concentrations of 5×10^{-7}, 1×10^{-6}, and 2×10^{-6} mol·L^{-1}. No ligand was added to the fourth solution, which was to be used as a blank.

System Setup. Analyses were performed with a Class-LC10 HPLC system (Shimadzu Corp., Kyoto, Japan) coupled to a custom-made flow-through γ-counting detector. All parts of the system (pump heads, tubing, injection valve, etc.) were constructed of inert materials (PEEK, Tefzel) to avoid adsorption of copper as much as possible. A Sep-Pak Light Silica cartridge (Waters Corp., Milford, MA) was used as an analytical column. This column possesses cation-exchange properties. The ^{64}Cu present in the effluent was detected in a 200 μL loop (PEEK Flexconnect, Alltech, Deerfield, IL) contained in a well-type NaI detector (3 in. × 3 in.). A Tracor Northern series 7200 multichannel analyzer (Tracor Northern Instruments, Middleton, WI) was coupled to a rate meter (Canberra Industries, Meriden, CT) as an interface between the detector and the data acquisition hardware. The response function was determined by injecting 20 μL of a radioactive Cu solution directly into the measurement system.

Analysis and Data Acquisition. A 5 mL aliquot was withdrawn from the sample prepared and spiked with $\sim 1.6 \times 10^5$ Bq ^{64}Cu ($\sim 2 \times 10^{-11}$ g of Cu). The spiked sample was allowed to equilibrate for approximately 1 h. This action did not alter the sample's initial chemical characteristics significantly because only 10 μL of the tracer solution was added. The cation-exchange column was equilibrated with the mobile phase (0.5 mL·min^{-1}) for at least 1 h prior to injection (20 μL) of the radioactive sample solution. A radiochromatogram was recorded for 20 min. Each sample was analyzed in triplicate, and the average of these radiochromatograms was taken for further interpretation. The resulting radiochromatograms were corrected for decay, background radiation, and differences in total radioactivity. Peak retention times were determined with the chromatography software.

Radioactive copper species that have a negligible interaction with the column material will elute in the void volume. This corresponds to an elution in the time interval 0−2 min under the experimental conditions used. The peak area in the 0−2 min time interval was compared with the one obtained in the 2−20 min time interval for calculation of the dissociation rate constant.

Species distributions were calculated with MINEQL+, version 3.01.[13] This widely used computer program uses thermodynamic stability constants of all possible species in an aqueous phase as a basis for equilibrium speciation calculations. All thermodynamic stability constants originate from recognized databases (EPA or comparable).

RESULTS AND DISCUSSION

Table 1 shows the theoretical copper species distribution of the experimental sample solutions in the absence of a ligand and

(12) van Elteren, J. T.; Kroon, K. J.; Woroniecka, U. D.; de Goeij, J. J. M. *Appl. Radiat. Isot.* **1999**, *51*, 15−19.

(13) Schecher, W. D.; McAvoy, D. C. *Comput., Environ. Urban Syst.* **1992**, *16*, 65−76.

Table 1. Copper Species Distribution in the Absence and Presence of Ligands L in a Range of Concentrations ($[Cu]_{tot} = 10^{-6}$ mol·L^{-1}; pH = 6.0; [NaCl] = 0.1 mol·L^{-1})

		Cu distribution (% of total)			
ligand L	species	[L] = 0	[L] = 5×10^{-7} mol·L^{-1}	[L] = 10^{-6} mol·L^{-1}	[L] = 2×10^{-6} mol·L^{-1}
	Cu^{2+}	76.1			
	$Cu(OH)_2$	1.6			
	$CuCl_2$	1.1			
	$CuCl^+$	20.5			
EDTA	Cu^{2+}		38		
	$CuCl^+$		10.2		
	$Cu-EDTA^{2-}$		50	100	100
NTA	Cu^{2+}		38.2	3.9	
	$CuCl^+$		10.3	1.1	
	$Cu-NTA^-$		49.3	94.1	98.9
citrate	Cu^{2+}		48.3	29	12.7
	$Cu(OH)_2$		1		
	$CuCl^+$		13	7.8	3.4
	$Cu-citrate^-$		36.5	61.9	83.3
glycine	Cu^{2+}		60.1	51.2	41.4
	$Cu(OH)_2$		1.3	1.1	
	$CuCl^+$		16.2	13.8	11.1
	$Cu_2-(glycine)_3^+$		20.5	31.6	43.8
	$Cu-glycine^+$				1.7

in the presence of the ligands EDTA, NTA, citrate, and glycine. It can be seen that, in the absence of a ligand, ca. 97% of the copper is present as positively charged Cu (Cu^{2+} and $CuCl^+$). In the presence of a ligand, the fraction of positively charged copper species is lower and the only negatively charged copper species are the complexes under study. Only in the case of glycine are no negatively charged copper species formed.

The retention behavior of the ^{64}Cu−labeled copper complexes on the preequilibrated cation-exchange column depends mainly on their charge. It is expected that positively charged copper species will undergo a considerable retention compared to negatively charged species. In theory, this can give rise to the appearance of two peaks in a radiochromatogram, the first one belonging to negatively charged copper species and the second one belonging to positively charged species. When dissociation of the negatively charged copper complex is considerable on the time scale of analysis, the relative area of the first peak will be smaller than expected from the calculated copper species distribution given in Table 1 or the peak will not be visible at all.

The radiochromatograms of spiked samples containing the ligands EDTA and NTA are shown in Figure 1, and those for spiked samples containing the ligands citrate and glycine are shown in Figure 2. For comparison, a radiochromatogram of the sample without a ligand is given as well in these figures. Relative areas of the first peaks extracted from these radiochromatograms are summarized in Table 2. They are given as percentages of the total area.

There is a noticeable difference between the radiochromatograms of the samples containing Cu−EDTA and Cu−NTA, on one hand, and Cu−citrate and Cu−glycine, on the other hand. The former two clearly show that the copper complexes can be separated from the free copper, whereas the latter two show mainly one combined peak with a retention time similar to that for the free copper ion. This implies that the dissociation rate of Cu−citrate is much higher than those of Cu−EDTA and Cu−NTA. For Cu−glycine, no unambiguous explanation can be given for the behavior observed in the column. The fact that Cu−glycine is positively charged means that it could have a retention behavior similar to that of the free copper ion and may not be separated from it at all. However, even if Cu−glycine had negligible interactions with the stationary phase, its dissociation rate would probably be too high to produce a peak in the first time interval (0−2 min). From its dissociation rate constant reported elsewhere (2.34 s^{-1}),[4] it can be inferred that Cu−glycine is dissociated completely after 1 min. Thus, if Cu−glycine did show the same behavior as the other copper complexes and elute from the column after approximately 1 min, its high dissociation rate would still give rise to one peak in the radiochromatogram.

For Cu−EDTA and Cu−NTA, it is observed that the copper complex peak increases with the total ligand concentration at the cost of the free copper ion peak. When the relative peak areas of the copper complex peaks are compared with those of the theoretical species distribution (Table 1), it is clear that less copper complex is detected than thermodynamically expected. For instance, 5×10^{-7} mol·L^{-1} EDTA should cause 50% of the total amount of copper to appear as Cu−EDTA, while the relative peak area of Cu−EDTA in the radiochromatogram is only 33%. This implies that a fraction of the labeled copper complexes entering the analytical column dissociates during the analytical procedure.

Furthermore, the relative peak areas of Cu−EDTA and Cu−NTA reach a maximum value (∼97%) when an excess (2×10^{-6} mol·L^{-1}) of ligand is used. Apparently, the presence of free ligands prevents the retention of free copper ions originating from the dissociation of the copper complex. This suggests a high rate of complex formation. In this case, it is impossible to determine the fraction of the copper complex that dissociates during analysis.

Using eq 6 and the data in Tables 1 and 2, we calculate the dissociation rate constants of Cu−EDTA and Cu−NTA to be (6.2 ± 0.3) $\times 10^{-3}$ and (1.0 ± 0.04) $\times 10^{-2}$ s^{-1}, respectively. These values are the averages of the dissociation rate constants calculated from the second and third radiochromatogram rows.

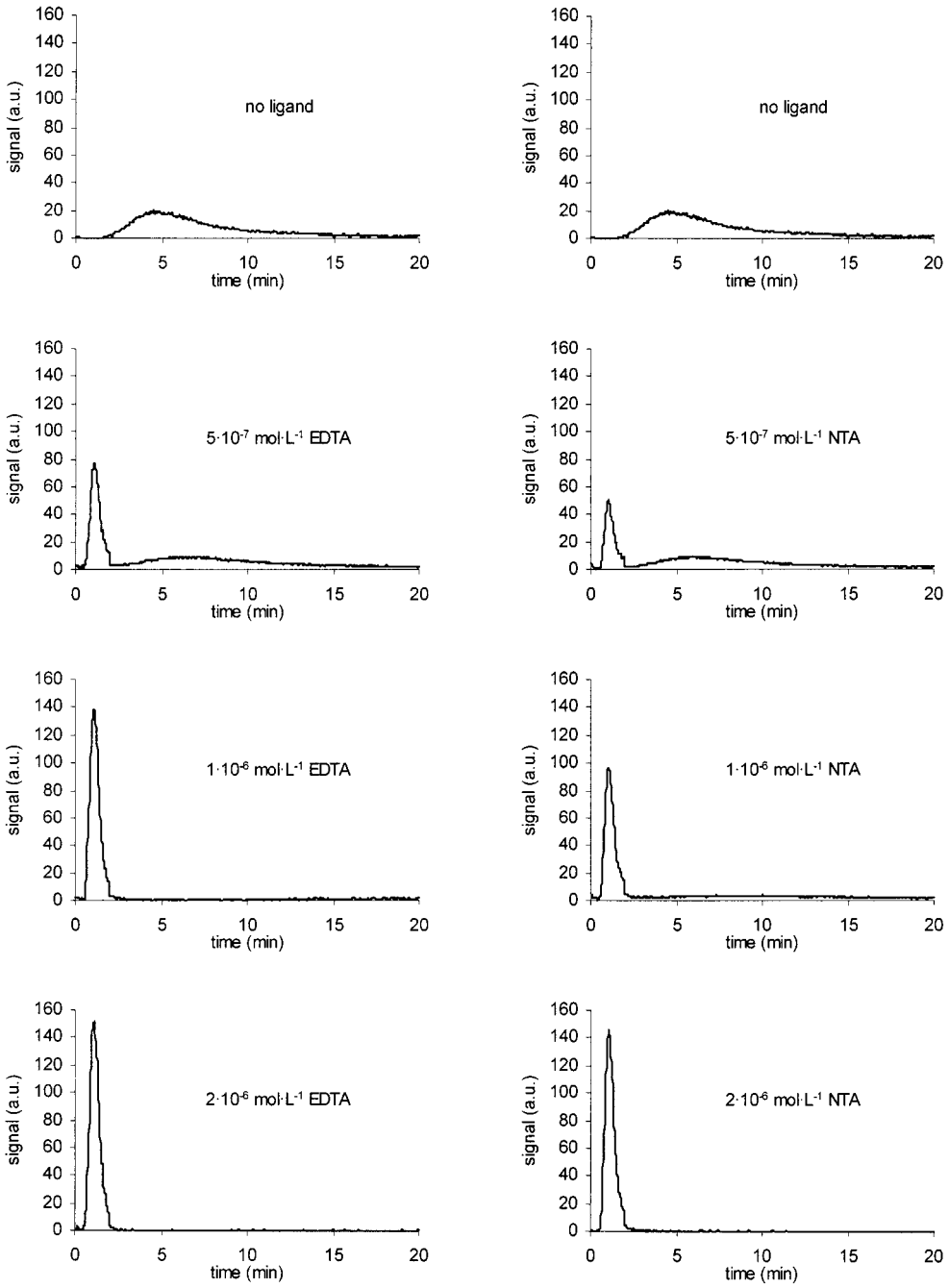

Figure 1. Radiochromatograms obtained for various Cu/CuL ratios. EDTA (left column) and NTA (right column); in all cases, $[Cu]_{tot} = 10^{-6}$ mol·L^{-1}, pH = 6.0; [NaCl] = 0.1 mol·L^{-1}.

For Cu−citrate, a higher dissociation rate is observed, since hardly any copper complex peak is seen in the radiochromatogram. The dissociation rate constant calculated from the results is $(3.1 \pm 0.2) \times 10^{-2}$ s^{-1}. However, the increase of the peak area belonging to Cu−citrate is not consistent with the increase in total citrate concentration. This suggests that the current experimental conditions are not ideal for determining the dissociation rate constant of Cu−citrate accurately.

There are a few properties of our system that may introduce uncertainties into the calculation of the dissociation rate constants. These are discussed below.

The peaks belonging to copper complexes have a wide shape and display a tail to the right. This is due not to processes on the column but to the response function of the detector itself. The size of the measuring cell and the integration time cause the performance of the detector to be slow compared to that of conventional detectors. The shapes of the copper complex peaks in the radiochromatograms are not different from the response function measured when the column was bypassed. We feel that the top of each copper complex peak represents reasonably well the time t available for dissociation. Nevertheless, the definition of t may be improved by development of a detector that produces narrowed peaks in its radiochromatograms.

Another source of uncertainty is an incomplete separation of the copper complex peak from the free copper peak in the radiochromatogram. In addition, it is conceivable that free copper

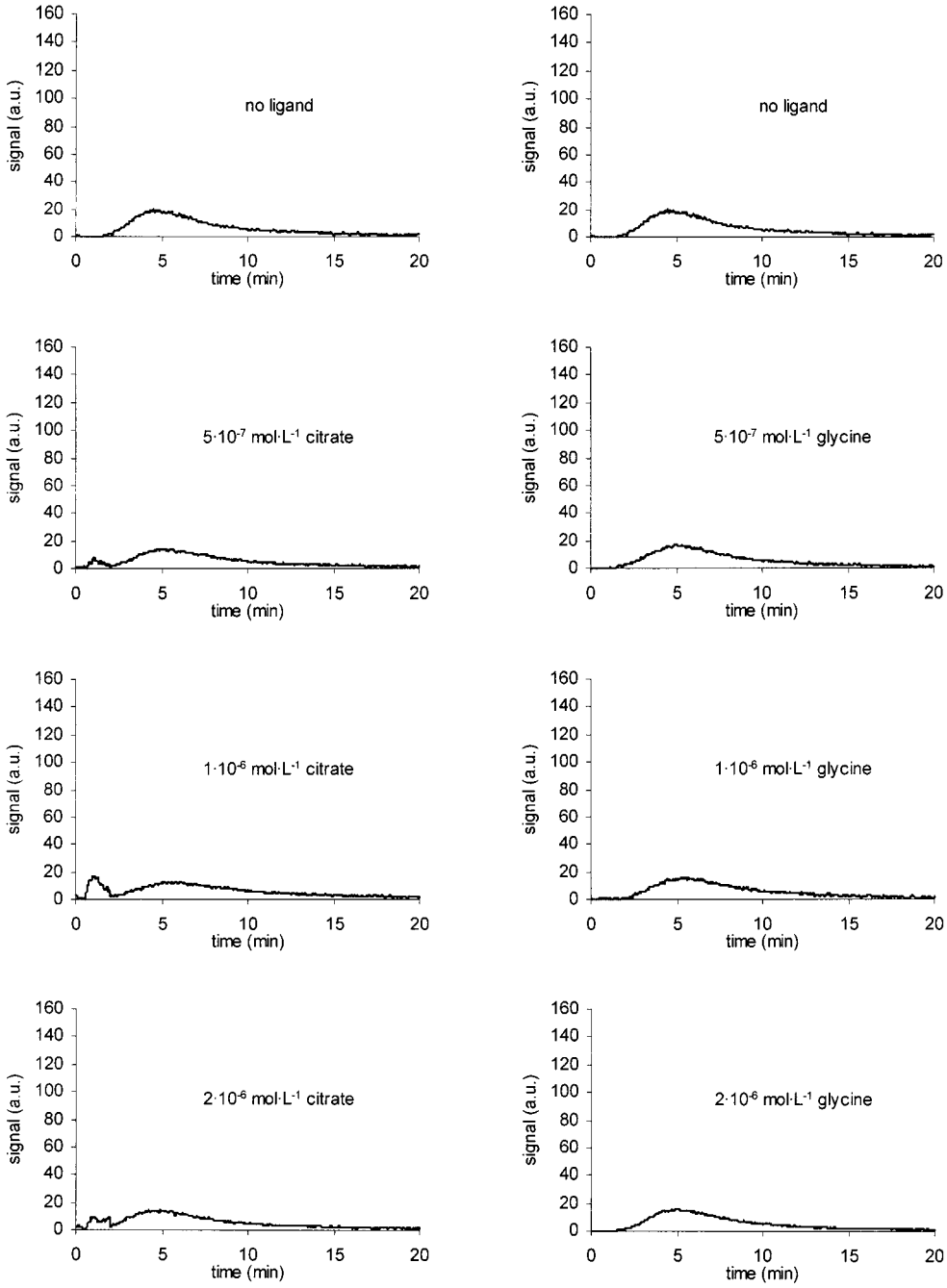

Figure 2. Radiochromatograms obtained for various Cu/CuL ratios. Citrate (left column) and glycine (right column); in all cases, $[Cu]_{tot} = 10^{-6}$ mol·L^{-1}, pH = 6.0, and [NaCl] = 0.1 mol·L^{-1}.

formed just at the end of the column may be eluted almost simultaneously with the copper complex and thereby may show up in the peak attributed to the nondissociated copper complex. However, if this is the case, it is expected that a range of retardations would appear in the radiochromatogram between the appearance of the copper complex and that of the free copper, generated over the full length of the column. When the second and third radiochromatogram rows in Figure 1 are compared to the first row, no shift of the free copper peak appears under the copper complex peak. Actually, the baseline is almost reached again after the copper complex peak. Comparison of the shapes of the copper complex peaks in the second radiochromatogram rows with the corresponding ones in the third and fourth rows (with less or almost no free copper) did not reveal any bias with respect to the right-hand tail of the copper complex peaks. The maximum error due to the presence of free copper under the copper complex peak was estimated as follows. A tangent was drawn starting from the origin and ending in the minimum appearing just after the copper complex peak. The area under this line was taken as the maximum error. This amounted to 6.2% and 6.9% of the copper complex peaks for EDTA and NTA, respectively, at a ligand concentration of 5×10^{-7} mol·L^{-1}. A better separation of the copper complex and free copper responses may be helpful in improving the accuracy.

Table 2. Relative Peak Areas of the 0−2 min Time Interval Obtained Experimentally for Various Ligands L in a Range of Concentrations ($[Cu]_{tot} = 10^{-6}$ mol·L^{-1}; pH = 6.0; [NaCl] = 0.1 mol·L^{-1})

	relative peak area (SD) (%)			
ligand L	[L] = 0	[L] = 5×10^{-7} mol·L^{-1}	[L] = 1×10^{-6} mol·L^{-1}	[L] = 2×10^{-6} mol·L^{-1}
EDTA	0.5 (±0.1)	33.0 (±1.1)	67.4 (±0.7)	96.9 (±4.1)
NTA	0.5 (±0.1)	26.7 (±1.0)	48.1 (±1.4)	96.7 (±1.5)
citrate	0.5 (±0.1)	5.3 (±1.6)	12.0 (±0.8)	8.2 (±0.9)
glycine	0.5 (±0.1)	0.9 (±0.2)	0.6 (±0.2)	0.7 (±0.2)

It is stressed that uncertainties in the outcomes of the species calculations play a role in the overall uncertainty budget as well. However, these errors are difficult to assess experimentally.

CONCLUSIONS

With the technique presented here, the lability of a metal complex in an aqueous system can be studied under equilibrium conditions. Prerequisite for this approach is that the mass of radiotracer added be so small that the chemical equilibrium is not disturbed. In this study the mass of ^{64}Cu added was only 6.3 $\times 10^{-3}$% of the mass of copper already present in the sample. The addition of the radiotracer led to a volume increase of only 0.2%.

The choice of the experimental conditions is critical for the performance of the system. Flow rate, column length, and column type determine a finite time frame in which a metal complex can undergo dissociation. The corresponding dissociation rate constant can be calculated easily when complex dissociation is roughly between 10 and 90% in this time frame.

The technique requires exact knowledge of the equilibrium concentration of the metal complex, to be calculated with a model. Furthermore, the metal-to-ligand ratio must be chosen such that a fraction of the metal is present in the free ionic form. With an excess of ligand, the metal may be completely shielded from the stationary phase, which would prevent the separation process. Metal complexes with the same sign of charge as the free metal ion, such as Cu−glycine, may be difficult to study with this method, since the separation procedure is based on charge. With the cation-exchange column used here, we were able to study only negatively charged copper complexes, in this case Cu−EDTA, Cu−NTA, and Cu−citrate.

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