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Kinetic Studies of Fast Equilibrium by High-performance Liquid Chromatography I. Ternary Complex Formation of *N,N*-Disubstituted Dithiocarbamate Chelates of Ni(II) and Cu(II)

Masataka Moriyasu* and Yohei Hashimoto

Kobe Women's College of Pharmacy, Motoyamakita-machi, Higashinada-ku, Kobe 658

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The equilibrium of labile ternary complex formation of metal dialkyldithiocarbamate chelates ($MA_2 + MB_2 \underset{k-}{\overset{k}{\rightleftharpoons}} 2MAB$, $K=[MAB]^2/[MA_2][MB_2]$) has been investigated by high-performance liquid chromatography, the separation process of which is so fast that kinetically unstable ternary complexes can be eluted with no disproportionation during the course of chromatography. High-performance liquid chromatography makes it possible to halt the progress of considerably fast reactions at the moment when the solution of reaction system is injected into the column. Thus, chromatograms obtained indicate the concentration of each species before chromatography, facilitating the determination of equilibrium constant K of Ni(II) and Cu(II) chelates. The equilibrium constants obtained are always 4.0, suggesting that ternary complex formation is controlled by a statistical factor. Disproportionation takes place by collision of two ternary metal chelates, the rate constants being of the order 10^1—10^2 M^{-1} s^{-1} (Ni(II) chelates) and 10^3 M^{-1} s^{-1} (Cu(II) chelates). The activation parameters of ternary complex formation have been obtained.

Study of fast reactions in solution has drawn the attention of investigators, the flow and relaxation methods being utilized. The conventional method, in which two reactants are mixed and then the change of concentration of reactants or products is measured, was found to be ineffective because of the lack of appropriate means of rapid analysis. High-performance liquid chromatography (HPLC) developed in recent years has been successfully applied to microdetermination of various substances. The merit of rapid separation in HPLC might be hopeful for determining kinetic characteristics of considerably fast reactions in solution.

Since the first report on metal acetylacetonates by Huber and Kraak,[1] only a few reports have appeared on HPLC separation of metal chelates, many of them being on HPLC of metal diethyldithiocarbamates.[2-9] We carried out quantitative determination of various metal diethyldithiocarbamates.[7] This article deals with the applicability of HPLC to kinetical investigation of relatively fast equilibrium of labile ternary complex formation.

Experimental

Reagents. Commercial sodium salts of diethyldithiocarbamic acid and dibenzyldithiocarbamic acid were used. Sodium salts of other *N,N*-disubstituted dithiocarbamic acid were prepared by the usual procedure from dialkylamine, carbon disulfide and sodium hydroxide. They were recrystallized from either chloroform–methanol or chloroform–hexane. Standard solutions of Ni(II) and Cu(II) were prepared by dissolving $NiCl_2 \cdot 6H_2O$ and $CuSO_4 \cdot 5H_2O$ in water. The concentrations of these metal ions were determined by colorimetry as diethyldithiocarbamate chelates.

Apparatus. An HPLC apparatus we constructed was used.[7] The two plunger reciprocal pump (Model KHD-W-294, Kyowa Seimitsu Co. Ltd.), damper, sample injector (5 μl or variable) and column were combined . A single-beamed spectrometer (Model Spectra 20, Toshiba Beckmann Co. Ltd., 210—700 nm) equipped with a flow cell was used as a detector. Since the detector is not designed for HPLC use, its sensitivity is not so high. The upper limit of rate constants determined by the present method is restricted by the sensitivity of the detector. Silica gel packings (LiChrosorb SI 100, 5 μ, E. Merck Darmstadt and Wako gel LC-5H, 5 μ, Wako Pure Chemicals Co. Ltd.) were packed into stainless steel columns (diameter 2 mm, length 25 cm) by a balanced slurry packing technique.[10] A pre-packed silica gel column (Shodex silipak, 5 μ, diameter 4 mm, length 15 cm, Showa Denko Co. Ltd.) was used. Absorption spectra of metal chelates were observed with a double-beamed spectrometer (Model 124, Hitachi Co. Ltd.).

Procedure. In adsorption chromatography, adjustment of column is important as regards the mode of elution and reproducibility, and the water content on silica gel was controlled as follows. *Ca.* 100 cm^3 of water containing acetone (3%) was pumped through the column. At least 200 cm^3 of water saturated hexane flowed out, the activity of silica gel column being reduced. Reproducible results were obtained when water saturated eluent was used. Before use each organic solvent was saturated with water by shaking the mixture of the solvent and water vigorously, followed by standing for 1 d. Since it is desirable in some cases to perform chromatography at low temperature, column temperature was kept low by plunging solvent reservoir and column into an ice bath when necessary.

Metal chelates were prepared by mixing the corresponding metal ion and sodium dialkyldithiocarbamate. The metal chelates produced were extracted with chloroform under conditions of complete extraction (at pH 4.0 in acetate buffer). In order to avoid any effects of residual free ligands in chloroform, the extract was washed with water four times. The standard solutions of each metal chelate (0.1 to 2 mM) thus prepared were used for HPLC after being diluted to proper concentration. Measurements of rate constants were carried out as follows. Two dilute solutions of binary complexes were mixed under thermostated conditions. After the lapse of a certain time, a portion of the mixture was supplied to HPLC. All the measurements were carried out three times.

Theoretical

Possible chromatogram patterns of a mixture of two

different binary complexes, MA_2 and MB_2, will be considered first. It is assumed that no decomposition of each chelate takes place during the course of chromatography. If no interaction exists between the two binary complexes, two peaks corresponding to each complex should appear. However, the chromatogram patterns would be complicated if a mixed ligand complex is formed in the mixture as follows.

$$MA_2 + MB_2 \underset{k_-}{\overset{k}{\rightleftharpoons}} 2MAB \quad (1)$$

$$K = [MAB]^2/[MA_2][MB_2] \quad (2)$$

Equilibrium is hardly attained when M is a metal ion which forms very inert complexes. In such a case, a ternary complex will be formed when the mixture is left to stand for a long time or brought to elevated temperature. The ternary complex formed can be separated by the conventional method without disproportionation. If M is a metal ion which forms very labile complexes, the ternary complex undergoes disproportion into two binary complexes, as soon as it is separated from binary complexes. Studies have been carried out on the equilibrium by mixing two solutions of MA_2 and MB_2 and observing the resulting remarkable change of physico-chemical properties such as UV absorption. The separation process of HPLC is so fast that it might compete with the progress of disproportionation in the column. Possible chromatogram

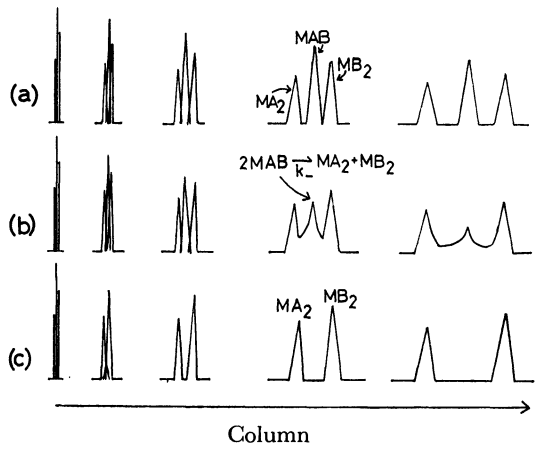

Fig. 1. Possible chromatogram patterns of the system of labile ternary complex formation.

patterns are shown in Fig. 1. When the separation process is so fast that disproportionation of the ternary complex during chromatography can be neglected, three peaks corresponding to MA_2, MB_2, and MAB would appear on the chromatograms (Fig. 1(a)), the chromatograms obtained directly indicating the concentration of each species before chromatography was carried out. When disproportionation is much faster than the separation process, peaks of only two binary complexes would appear on the chromatograms (Fig. 1(c)). In case of the intermediate of these two extremes, the peak of a ternary complex would appear accompanied by disproportionation in the column (Fig. 1(b)). In this case disproportionation is retarded with increase in flow rate, bringing about a change of chromatogram patterns. Thus, if the peak height of the ternary complex remains unchanged with flow rate, disproportionation is negligible.

In HPLC there are some favorable factors to retard disproportionation in the column. (1) Each species is diluted rapidly during the course of chromatography, suppressing disproportionation which is anticipated to occur by the bimolecular collisional process of two ternary complexes. Actual concentration in the liquid phase will be low since a considerable part of each species is retained on the column, collision of two ternary complexes adsorbed on silica gel surface being unlikely to occur. (2) The rate of disproportionation can be controlled by a suitable choice of initial concentrations of MA_2 and MB_2, disproportionation being slow when initial concentrations are low. (3) Control of column temperature is useful, disproportionation being retarded when column temperature is kept low. Thus a combination of high speed separation, rapid dilution, suitable choice of initial concentrations and low temperature, similar to the case of the rapid quenching method,[11] might halt the progress of the reaction in the column. If these conditions are fulfilled, the equilibrium constant K will be determined by direct measurements of peak heights appearing on chromatograms.

Rate constants k and k_- are determined as follows. Two dilute solutions of MA_2 and MB_2 are mixed. A ternary complex is produced gradually and the equilibrium Eq. 1 will be attained. After the lapse of a certain time a portion of the mixed solution is supplied to HPLC. The progress of the reaction ceases when the solution is injected into the column. Thus, concentrations of each species at a definite time after mixing can be determined by HPLC. The rates of formation of ternary complex and decrease of binary complexes are expressed as follows.

$$\frac{d[MA_2]}{dt} = \frac{d[MB_2]}{dt} = k_-[MAB]^2 - k[MA_2][MB_2] \quad (3a)$$

$$\frac{d[MAB]}{dt} = k[MA_2][MB_2] - k_-[MAB]^2 \quad (3b)$$

Assuming that the initial concentrations of MA_2 and MB_2 are the same ($=a_0$), we have

$$\frac{dx}{dt} = 4k_-(a_0 - x)^2 - kx^2$$

$$= (4/K - 1)kx^2 - 8a_0kx/K + 4a_0^2k/K \quad (4a)$$

$$\frac{dy}{dt} = k(a_0 - y/2)^2 - k_-y^2$$

$$= -(ky^2/4)(4/K - 1) - a_0ky + a_0^2k, \quad (4b)$$

where x and y are concentrations of MA_2 (or MB_2) and MAB, respectively. Rate constants k and k_- are determined by means of Eq. 4a or 4b. When $K=4.0$, we have the following equations by integrating Eqs. 4a and 4b.

$$-\ln(1 - 2x/a_0) = ka_0t \quad (5a)$$

$$-\ln(1 - y/a_0) = ka_0t \quad (5b)$$

When $K \neq 4.0$, k and k_- are determined by means of the following equation.[12]

$$k = (2/mt)\left[\ln\left(\frac{\left(\frac{2a_0-m}{1-4/K}\right)-y}{\left(\frac{2a_0+m}{1-4/K}\right)-y}\right)+\left(\ln\frac{(2a_0-m)}{(2a_0+m)}\right)\right] \quad (6)$$

$$m = 4a_0\sqrt{1/K}$$

Results and Discussion

Determination of Equilibrium Constant. By use of a well deactivated silica gel-column, various metal chelates of *N,N*-disubstituted dithiocarbamic acid gave chromatograms with no decomposition of chelates. Metal chelates of Cu(II), Hg(II), Ni(II), Pd(II), and Co(III) *etc.* gave linear calibration graphs (detection limit, few μg-few ng). A mixed solution of two different *N,N*-disubstituted dithiocarbamate chelates of Ni(II) always gave three peaks[13,14] (Fig. 2(c)). Peak heights of these three peaks remained unchanged in the flow rate range of the pump 3.0—0.4 cm³/min (Figs. 2(c)—2(f)). This indicates that the conditions shown in Fig. 1(a) are satisfied. When the initial concentrations of two binary complexes were chosen to be the same ($=a_0$), the equilibrium concentrations of the chelates were both $a_0/2$. Thus the equilibrium concentration of ternary complex was equal to a_0. Exactly half of the initial binary complexes was transformed into a ternary complex, the equilibrium constant K being 4.0. Formation of the ternary complex is controlled by a statistical factor. In all the systems of a mixture of two binary complexes K was always equal to 4.0, suggesting that the lack of the factor either stabilizes of destabilizes the ternary complex.[15]

Cu(II) chelates showed different chromatogram

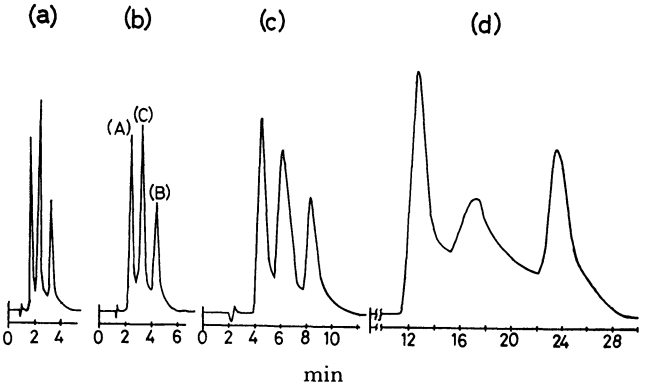

Fig. 3. Chromatogram patterns of two different dithiocarbamate chelates of copper at relatively higher concentrations.
Column: LiChrosorb SI 100 (2 mm×25 cm), eluent: hexane : cyclohexane : isopropyl acetate=50 : 50 : 4 (water saturated), flow rate: (a) 1.3 cm³/min; (b) 0.9 cm³/min; (c) 0.5 cm³/min; (d) 0.2 cm³/min, detector: 440 nm, sample: 1 mM $Cu[(CH_3CH_2CH_2)_2NCSS]_2$+1 mM $Cu[(CH_3CH_2)_2NCSS]_2$ in $CHCl_3$, sample size: 5 μl.
(A): $Cu[(CH_3CH_2CH_2)_2NCSS]_2$, (B): $Cu[(CH_3CH_2)_2NCSS]_2$, (C): $Cu[(CH_3CH_2CH_2)_2NCSS][(CH_3CH_2)_2NCSS]$. The broadending of this peak (C) is due to disproportionation of this ternary chelate in the column.

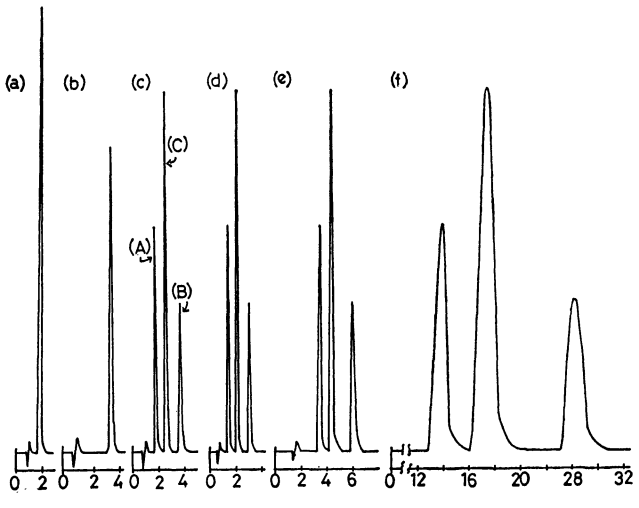

Fig. 2. Chromatogram patterns of two different dithiocarbamate chelates of nickel.
Column: Shodex silipak (4 mm×15 cm), eluent: hexane : ethyl acetate=100 : 7 (water saturated), flow rate: (a)—(c) 2.5 cm³/min; (d) 3.0 cm³/min; (e) 1.5 cm³/min; (f) 0.4 cm³/min, detector: 325 nm, sample: (a) 0.4 mM $Ni[(CH_3CH_2CH_2)_2NCSS]_2$ in $CHCl_3$; (b) 0.4 mM $Ni[(CH_3CH_2)_2NCSS]_2$ in $CHCl_3$; (c) 0.4 mM $Ni[(CH_3CH_2CH_2)_2NCSS]_2$ +0.4 mM $Ni[(CH_3CH_2)_2NCSS]_2$ in $CHCl_3$, sample size: 5 μl.
(A): $Ni[(CH_3CH_2CH_2)_2NCSS]_2$, (B): $Ni[(CH_3CH_2)_2NCSS]_2$, (C): $Ni[(CH_3CH_2CH_2)_2NCSS][(CH_3CH_2)_2NCSS]$.

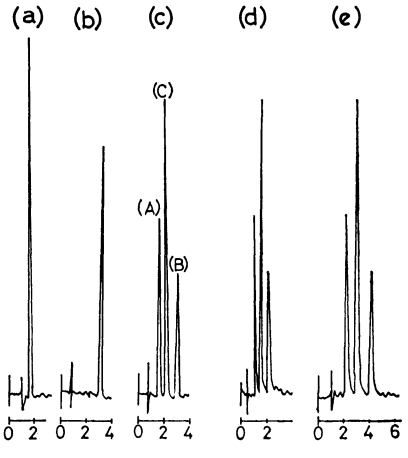

Fig. 4. Chromatogram patterns of two different dithiocarbamate chelates of copper at low concentrations.
Column: Shodex silipak (4 mm×15 cm), eluent: hexane : ethyl acetate=100 : 15 (water saturated), flow rate: (a)—(c) 2.5 cm³/min; (d) 3.0 cm³/min; (e) 1.5 cm³/min, detector: 280 nm, sample: (a) 4.0×10^{-6} M $Cu[(CH_3CH_2)_2NCSS]_2$; (b) 4.0×10^{-6} M $Cu[(CH_3)_2NCSS]_2$; (c) 4.0×10^{-6} M $Cu[(CH_3CH_2)_2NCSS]_2$+4.0×10^{-6} $Cu[(CH_3)_2NCSS]_2$; these samples are solutions in hexane : chloroform : ethyl acetate=8 : 1 : 1, sample size: 50 μl.
(A): $Cu[(CH_3CH_2)_2NCSS]_2$, (B): $Cu[(CH_3)_2NCSS]_2$, (C): $Cu[(CH_3CH_2)_2NCSS][(CH_3)_2NCSS]$.
No disproportionation was observed in this case.

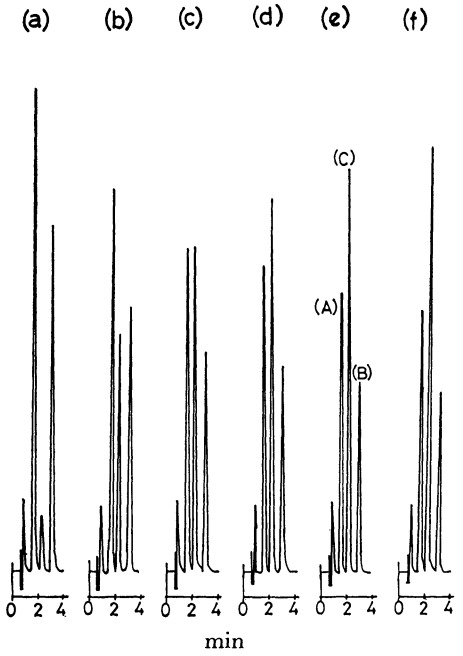

Fig. 5. Change of chromatogram patterns of the mixture of two different dithiocarbamate chelates of nickel.
Column: Shodex silipak (4 mm × 15 cm), eluent: hexane : ethyl acetate = 100 : 20 (water saturated), flow rate: 2.5 cm³/min, detector: 325 nm, sample: 2.5×10^{-5} M $Ni[(CH_3CH_2)_2NCSS]_2$ + 2.5×10^{-5} M $Ni[(CH_3)_2NCSS]_2$ (a) After mixing 0.5 min; (b) 4.0 min; (c) 7.5 min; (d) 11.0 min; (e) 18.0 min; (f) 60.0 min (equilibrium); these samples are solutions in hexane : chloroform: ethyl acetate = 3 : 1 : 1 at 25 °C, sample size: 50 μl.
(A): $Ni[(CH_3CH_2)_2NCSS]_2$, (B): $Ni[(CH_3)_2NCSS]_2$, (C): $Ni[(CH_3CH_2)_2NCSS][(CH_3)_2NCSS]$.

patterns. When a relatively high a_0 was chosen ($a_0 = 1.0\times10^{-3}$ M), chromatogram patterns were seriously affected by the flow rate, suggesting the disproportionation of the ternary complex in the column. On the other hand, when a very low a_0 was chosen ($a_0 = 4.0\times10^{-6}$ M), they were unaffected by the flow rate (from 1.5 to 3.0 cm³/min) (Fig. 4). In order to compensate the decrease of concentration of the sample, it was necessary to inject a large volume of sample. The composition of the solvent of the injected sample should be similar to that of the eluent, otherwise serious broadening of each peak takes place. About 200 μl of sample volume could be injected into the column without remarkable broadening of each peak. For Cu(II) chelates the chromatogram patterns were also sensitive to column temperature. At ambient temperature, when a_0 was chosen 4.0×10^{-5} M, chromatogram petterns were affected slightly by flow rate. However, when column temperature was low, no disproportionation in the column was observed. The values K were also always equal to 4.0 for all the systems of Cu(II) chelates.

Determination of Rate Constants. As shown in Fig. 5, a trace peak of the ternary complex appeared on chromatograms immediately after mixing of two solutions. With the lapse of time the peak of the ternary complex rose while the peaks of binary complexes fell. The rate constants k and k_- were determined by means of Eq. 5b. The results are summarized in Tables 1 and 2 (for Ni(II) and Cu(II) chelates, respectively). The values of k and k_- were sensitive to temperature and composition of solvent of the mixture. Since k and k_- were affected by the water content of the mixed solution, experimental conditions were so chosen that each solution of MA_2 and MB_2 was saturated with water before mixing. Disproportionation was slow when solvent was not saturated with water. Column temperature and composition of eluent play insignificant role. By use of HPLC we can promptly separate various species including kinetically unstable ones and elute them. Varying chromatographic conditions such as composition of eluent, column temperature and flow rate does not change the values of k and k_- if disproportionation can be neglected during the course of chromatography.

The rate constants k of Ni(II) chelates are of the order 10^1—10^2 M^{-1} s^{-1}. Since Cu(II) chelates are more labile than Ni(II) chelates, values of k of Cu(II) chelates are larger and of the order 10^3 M^{-1} s^{-1}. Values of k seems to be small when molecular weights of two

Table 1. Rate constants of formation and disproportionation of labile ternary dithiocarbamate chelates of Ni(II)

$$NiA_2 + NiB_2 \underset{k_-}{\overset{k}{\rightleftharpoons}} 2NiAB, \qquad K = [NiAB]^2/[NiA_2][NiB_2] = k/k_- = 4.0$$

B	k (M^{-1} s^{-1})	k_- (M^{-1} s^{-1})	Retention time (min)			Eluent
			MA_2	MAB	MB_2	
$(CH_3)_2NCSS$	$(1.3\pm0.1)\times10^2$	$(3.3\pm0.4)\times10^1$	1.2	2.2	3.1	A
$(CH_3CH_2CH_2)_2NCSS$	$(2.4\pm0.2)\times10^2$	$(6.0\pm0.6)\times10^1$	3.9	2.7	1.8	C
$(CH_2)_4NCSS$	$(1.1\pm0.07)\times10^2$	$(2.8\pm0.2)\times10^1$	3.1	4.2	5.9	B
$(CH_2)_5NCSS$	$(9.7\pm0.5)\times10^1$	$(2.4\pm0.15)\times10^1$	4.4	5.1	5.7	D
$(C_6H_5CH_2)_2NCSS$	$(1.9\pm0.2)\times10^2$	$(4.8\pm0.5)\times10^1$	3.9	2.8	1.8	C
$(CH_2CH_2OCH_2CH_2)NCSS$	$(7.3\pm0.5)\times10^1$	$(1.8\pm0.1)\times10^1$	1.2	2.4	4.1	A

A: $(CH_3CH_2)_2NCSS$, $a_0 = 2.5\times10^{-5}$M. Chromatographic conditions: column: Shodex silipak (4 mm × 15 cm), eluent: hexane: ethyl acetate (water saturated) = 100: 20 (A), 100 : 8 (B), 100 : 6 (C), 100 : 5 (D), flow rate: 2.5 cm³/min, detector: 325 nm, sample size: 50 μl, column temperature: ambient, Equal amounts of NiA_2 and NiB_2 dissolved in the mixed solvent system, chloroform : ethyl acetate : hexane = 3 : 1 : 1 (water saturated), were mixed at 25 °C.

Table 2. Rate constants of formation and disproportionation of labile ternary dithiocarbamate chelates of Cu(II)

$$CuA_2 + CuB_2 \underset{k_-}{\overset{k}{\rightleftharpoons}} 2CuAB, \qquad K = [CuAB]^2/[CuA_2][CuB_2] = k/k_- = 4.0$$

B	k ($M^{-1}s^{-1}$)	k_- ($M^{-1}s^{-1}$)	Retention time (min) MA_2	MAB	MB_2	Eluent
$(CH_3)_2NCSS$	$(3.5\pm0.4)\times10^3$	$(8.8\pm1.0)\times10^2$	1.5	2.0	2.8	A
$(CH_3CH_2CH_2)_2NCSS$	$(6.4\pm0.8)\times10^3$	$(1.6\pm0.2)\times10^3$	3.0	2.1	1.5	B
$(CH_2)_5NCSS$	$(1.2\pm0.05)\times10^3$	$(3.0\pm0.15)\times10^2$	3.7	4.3	5.1	C
$(C_6H_5CH_2)_2NCSS$	$(3.4\pm0.3)\times10^3$	$(8.5\pm0.8)\times10^2$	3.0	2.5	1.8	B

A: $(CH_3CH_2)_2NCSS$, $a_0=4.0\times10^{-6}$M. Chromatographic conditions: column: Shodex silipak (4 mm × 15 cm), eluent: hexane : ethyl acetate (water saturated) = 100 : 15 (A), 100 : 4 (B), 100 : 3(C), flow rate: 2.5 cm³/min, detector: 270 nm, sample size: 50 μl, column temperature: 0 °C (Column and solvent reservoir were plunged into an ice bath). Equal amounts of CuA_2 and CuB_2 dissolved in the mixed solvent system, chloroform : ethyl acetate : hexane = 8 : 1 : 1 (water saturated), were mixed at 25 °C.

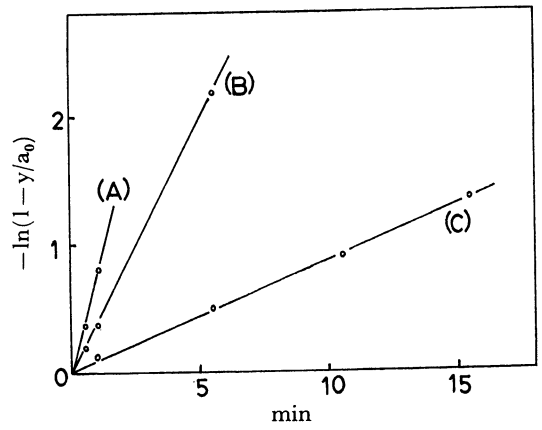

Fig. 6. Plot of Eq. 5a at different a_0.
Chromatographic conditions: column: Shodex silipak (4 mm × 15 cm), eluent: hexane : ethyl acetate = 100 : 7 (water saturated), flow rate: 2.5 cm³/min, detector: 325 nm, sample: the mixed solution of Ni-$[(CH_3CH_2CH_2)_2NCSS]_2$ and $Ni[(CH_3CH_2)_2NCSS]_2$ in hexane : chloroform : ethyl acetate = 3 : 1 : 1 were subjected to HPLC after mixing a definite time at 25 °C, (A): $a_0=5.0\times10^{-5}$ M, (B) $a_0=2.5\times10^{-5}$ M, (C): $a_0=6.25\times10^{-6}$ M.

Table 3. Activation parameters of ternary complex formation of *N,N*-disubstituted dithiocarbamate chelates

$$M[(CH_3CH_2)_2NCSS]_2 + M[(CH_3CH_2CH_2)_2NCSS]_2 \rightleftharpoons M[(CH_3CH_2)_2NCSS]\,[(CH_3CH_2CH_2)_2NCSS]$$

M	$H^{\neq}$ (kJ mol⁻¹)	$G^{\neq}$ (25 °C) (kJ mol⁻¹)	$S^{\neq}$ (kJ K⁻¹ mol⁻¹)
Ni(II)[a]	65±5	60±3	$(1.7\pm2.7)\times10^{-2}$
Cu(II)[b]	24±5	51±4	$(9.1\pm3.0)\times10^{-2}$

a) In chloroform : ethyl acetate : hexane = 1 : 1 : 3 (water saturated). b) In chloroform : ethyl acetate : hexane = 1 : 1 : 8 (water saturated).

dithiocarbamates are close to each other. Validity of the bimolecular collisional process of disproportionation was confirmed by changing a_0 (Fig. 6). When a_0 exceeds this range, the rate of disproportionation does not increase in proportion to a_0.

The activation parameters of disproportionation was determined by measuring k values at various temperatures. This was carried out by mixing two solutions of MA_2 and MB_2 under thermostated conditions at various temperatures. Results of the system consisting of diethyldithiocarbamic acid, dipropyldithiocarbamic acid and metal ion are given in Table 3. The activation enthalpy of Cu(II) chelates is smaller than that of Ni(II) chelates.

Conclusion

Fast bimolecular reactions can be traced by HPLC. Chromatographic conditions should be so chosen that no decomposition of species occurs during the course of chromatography. This can be done to a certain extent by choice of column packings and solvent systems. The upper limit of the rate constants is restricted by the sensitivity of the detector. Absorption of 0.05 units could be extended to a full scale without serious noise with the spectrometer we used. It seems that rate constants of the order $10^4\ M^{-1}s^{-1}$ can be determined. Since the sensitivity of a commercial UV detector for HPLC is about ten times larger than that of the present one, fast reactions with rate constants of the order $10^5\ M^{-1}s^{-1}$ might be traced by HPLC if absorption coefficient of the detected species is large ($\log\varepsilon>4$). The present method will also be useful for investigation of physicochemical properties of kinetically unstable species in solution.

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