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A New Method for the Determination of Partition Coefficients of Air Sensitive Copper(I) Complexes

Hans-Dieter Gaisser[1], Henk van der Goot[1], and Henk Timmerman[1,2]

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Abstract: Determinations of log P values of copper complexes in oil/water were performed in a new, totally closed apparatus connected with a filter-probe extractor. The results indicate that the system may be generally suitable for the determination of log P values of oxidizable and nonoxidizable metal complexes. In the case of the copper (I) complexes spectrophotometric analysis was not feasible, since 1-octanol was extracted by these complexes into the aqueous phase, resulting in a change in the extinction coefficient. To establish accurately the concentration in each phase, copper was determined by atomic absorption spectrometry.

Recently, Pijper (1) performed a Hansch analysis on the antimycoplasmal activity of compounds structurally related to 2,2'-bipyridyl. Using Σf, Taft E_S and Hammet σ an excellent correlation was found ($n = 33$, $r = 0.976$). In this relationship lipophilicity is the parameter with the strongest contribution. However, these compounds only show their activity in the presence of copper sulfate levels that by themselves are nontoxic. From studies on the mode of action, Antic et al. (2) and Smit et al. (3) assumed, that a copper(I) complex enters the cell. Therefore, it is important to test whether there exists a linear relationship between the lipophilicity of a ligand and that of the corresponding copper(I) complex. For this purpose the lipophilicity of these complexes had to be measured. As some of these are air sensitive, a procedure in which the presence of air is excluded, has to be used. Shake-flask experiments are most common, but it would be difficult to avoid oxidation by air. In a few experiments with reversed-phase TLC we observed strong tailing, so that this method was abandoned. In experiments with reversed-phase HPLC using RP-2, RP-6, RP-18 columns and oxygen free solvents, no peaks from the copper complexes could be detected.

Recently new methods for the separation of oil/water mixtures have been published. With the Segsplit (4) approach segmented flow is used, while the filter-probe extractor (5) based on the work of Mohammed and Cantwell (6) separates the two phases. We developed an apparatus using the filter-probe extractor, which allows the determination of the partition coefficient in a totally closed, oxygen free system. Using this system the results obtained with air sensitive copper(I) complexes are described.

Materials and Methods

Chemicals

2,9-Dimethyl-1,10-phenanthroline (**1**) was purchased from Aldrich Europe. 1-Amino-3-(2-pyridyl)isoquinoline (**2**), 1-amino-3-(6-methyl-2-pyridyl)isoquinoline (**3**), 1-amino-6-methyl-3-(2-pyridyl)isoquinoline (**4**), 1-amino-8-methyl-3-(2-pyridyl)isoquinoline (**5**), 3-(2-pyridyl)isoquinoline (**6**) and 1-chloro-3-(2-pyridyl)isoquinoline (**7**) were from laboratory stock (7). Bis [2,9-dimethyl-1,10-phenanthroline]copper(I) nitrate (**8**), bis[1-amino-3-(2-pyridyl)isoquinoline]copper(I) nitrate (**9**), bis[1-amino-3-(6-methyl-2-pyridyl)isoquinoline]copper(I) nitrate (**10**), bis [1-amino-6-methyl-3-(2-pyridyl)isoquinoline] copper(I) nitrate (**11**), bis[1-amino-3-(2-pyridyl)isoquinoline]copper(I) nitrate (**12**), bis [3-(2-pyridyl)isoquinoline]copper(I) nitrate (**13**) and bis[1-chloro-3-(2-pyridyl)isoquinoline]copper(I) nitrate (**14**) were prepared as described (8). 2,4-Pentanedione was obtained commercially. Bis [2,4-pentanedione]copper(II) was prepared according to Adams and Hauser (9). All other chemicals were of analytical grade (J. T. Baker) and were used without further purification.

Measurement of the Partition Coefficient of the Copper (I) Complex

Mixing chamber: The mixing chamber (Fig. 1) consisted of a 300 ml water jacket vessel with a special lid in which appropriately located holes allowed the insertion of a nitrogen inlet and outlet tube; a buret inlet connected to an autoburet (Mettler DV10) with a water jacket reservoir (250 ml) equipped with a nitrogen inlet and outlet tube; an outlet for the filter probe (with a polytef film, Mitex LC 10 mcm with 68 %

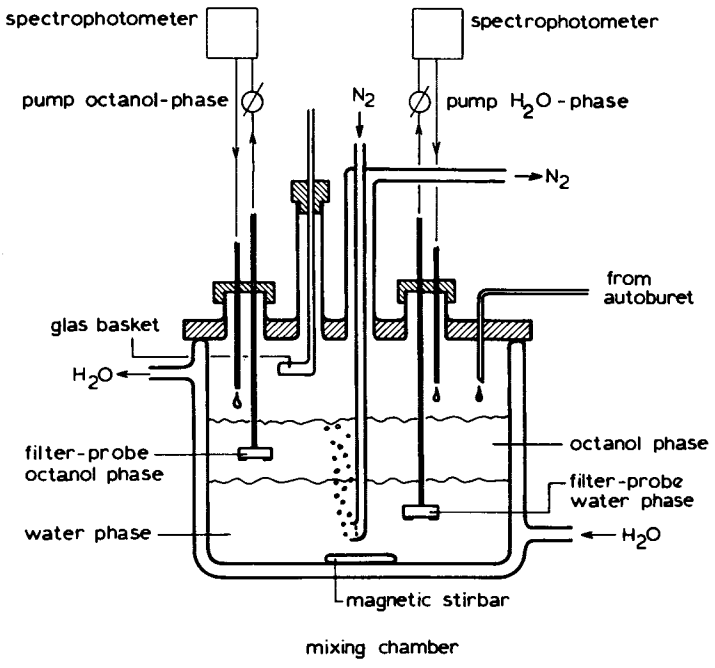
[1] Department of Pharmacochemistry, Vrije Universiteit, De Boelelaan 1083, 1081 HV Amsterdam, The Netherlands
[2] Correspondence

porosity, Millipore) for probing the oil phase and an inlet for the pumped oil; an outlet for the filter-probe (with a cellulose filter paper, Ederol No. 15 J. C. Binzer, Hatzfeld/Eder, GFR) for probing the aqueous phase, and an inlet for the pumped water and a small "glass basket" supported by a glass rod passing through an airtight gland. Water of 25° C was circulated through the jacket vessel and reservoir.

Connections: All connecting tubes (Fig. 1) together with the pump membrane were made of stainless steel. The filter-probe was connected to a high performance pump (Orlita TW 1515). The pump outlet was connected to a threeway tap. One outlet was connected to a spectrophotometer fitted with an 80 µl flow-through cell thermostated at 25° C; the other side was fitted with a short tube to sample for the AAS measurements. The outlet of the spectrophotometer was connected to the inlet of the pumped phase. In this manner the connection for the oil and aqueous phase were made.

Oxygen removal: Ultrapure nitrogen, necessary to remove oxygen from solutions, was obtained by passing commercially available nitrogen containing approximately 5 ppm oxygen through a column filled with pyrophoric catalyst (R3-11, BASF (GFR)) and kept at 100° C. This purified gas, with an oxygen content of less than 0.02 ppm, was bubbled through the solutions in the mixing chamber and the reservoir of the autoburet. It appeared that after 2 h a minimum of 0.035 ppm O_2 (measured with an oxygen sensitive electrode connected to a Beckman oxygen analyzer) was reached. Under these conditions during a period of 36 h conversion of the easily oxidizable copper (I) complex **9** to the corresponding copper (II) complex could not be detected potentiometrically (platinum electrode vs saturated calomel electrode).

Determination of the partition coefficient: The mixing chamber was filled with water saturated 1-octanol (100 ml). Then a complex was placed in the "glass basket", which was adjusted above the surface of the liquid. The vessel was gastight closed and the solution magnetically stirred. The reservoir of the autoburet was filled with water saturated with 1-octanol. Purified nitrogen was then bubbled through the solution in the mixing chamber and the reservoir. After 3 h the "glass basket" was lowered to dissolve the complex. After complete dissolution either the absorption was measured at an appropriate wavelength or a sample (1 ml) was taken at the tap for determination of copper with AAS. Then 30 ml of the aqueous phase were added, followed by stirring. After equilibrium was reached, the absorption at the same wavelength was measured or a sample was taken at the short tube. After each subsequent addition of 30 ml or 40 ml of the aqueous phase, the whole procedure was repeated. From the concentrations in the organic phase and the aqueous phase the log P was calculated.



Measurement of the partition coefficient of the ligand: Mixtures of water (50 mM borate buffer of pH 9.0 to prevent protonation of the ligands) saturated with 1-octanol and a solution of the ligand (conc. $\pm 1\times10^{-2}$ M or $\pm 2.5\times10^{-2}$ M) in 1-octanol saturated with water were shaken for 1 h and 1.5 h, respectively, in water-jacketed bottles at a temperature of 25° C. The mixture was centrifuged for 15 min at 2000 rpm, the two phases separated and the ligand concentration in the aqueous phase determined spectrophotometrically.

Analytical Procedures

Calibration curves for the UV-VIS spectrophotometric (Pye Unicam SP 8) determination were made using the filter-probe extractor containing the appropriate phase.

Water content of 1-octanol was determined using the modified Karl-Fischer titration method of Verhoef and Barendrecht (10).

1-Octanol content of the aqueous phase was determined by GLC using a Tenax 60–80 column (ID 2 mm, length 1.5 m, T (column) = 180° C, Hewlett Packard 5750 G).

Determination of copper was carried out by flame atomic absorption spectrometry (AAS) (Perkin-Elmer 4000 atom absorption spectrophotometer) using an acetylene/air mixture (11).

Results and Discussion

To determine the lipophilicity of copper (I) complexes in 1-octanol/water we made use of the filter-probe extractor (5) and developed the apparatus described in the experimental section. In this apparatus an oxygen content of 0.035 ppm was reached after bubbling nitrogen through the solution for 2 h. No evaporation of the phases was detected over this time period. By measuring the redox potential of the air sensitive copper (I) complex (**9**) for 36 h, no oxidation could be detected. Thus such an oxygen content can be regarded to be sufficiently low to avoid oxidation of the copper (I) complexes. The calibration lines obtained from the experimental data of all copper (I) complexes, gave statistically acceptable results ($r>0.995$).

During the determination of the lipophilicity of bis[2,9-dimethyl-1,10-phenanthroline]copper (I) nitrate (**8**), some unusual phenomena were observed. After adding more aqueous phase the

Table I. Initial Results from Partitioning Experiments and Spectrophotometric Determination of Cu(I) $(DMP)_2$.

Exp.	Vol.ratio oct./H_2O	E_{454}-H_2O	E_{454}-oct.	log P	Recovery[a]
I	120/0	—	0.917	—	—
	120/30	0.098	0.831	0.92	29 %
	120/60	0.118	0.794	0.82	49 %
	120/100	0.128	0.742	0.76	62 %
II	120/0	—	0.920	—	—
	120/30	0.102	0.859	0.92	45 %
	120/60	0.138	0.811	0.76	64 %
	120/100	0.191	0.779	0.60	117 %
III	120/0	—	0.901	—	—
	120/30	0.105	0.840	0.89	46 %
	120/60	0.104	0.840	0.90	91 %
	120/100	0.126	0.787	0.79	98 %

[a] recovery = $\frac{\text{mol } (H_2O\text{-phase})}{\Delta\text{mol (octanol-phase)}} \times 100$

absorption measured at the specific wavelength of the copper (I) complex in the aqueous phase increased, and in the organic phase the absorption decreased. The effect was always observable, but quantitatively not reproducible (Table I). The mass balance of the complex showed a significant loss, but improved when more aqueous phase was added. Similar results were observed for complex **9**.

Oxidation cannot be an explanation for these results, because the corresponding copper(II) complex has no absorption at the wavelength employed. On the other hand a decrease in dissociation of the complex cannot account for the higher absorption as dissociation increases on dilution. Partitioning experiments with the filter-probe apparatus using the neutral complex bis[2,4-pentanedione]copper(II) gave a correct mass balance and a log P value of 0.237±0.0069 (n=11), which indicates that the apparatus works adequately.

In analytical chemistry **1** is known as a reagent for the determination of copper. The procedure includes the reduction of Cu(II) to Cu(I), and extraction of **8** to an organic layer followed by spectrophotometric determination.

We compared the filter-probe experiments of **8** with shake-flask experiments, and the two methods gave similar results. Examination of the absorption spectra of **8** before and after equilibrium showed no change in peak location and shape.

Another possible explanation for the higher absorption in the aqueous phase after dilution may be a change in the composition of this phase during the experiment (12, 13). Therefore the dependence of the extinction coefficient of **8** (λ = 454 nm) on the 1-octanol concentration in water was investigated. The results in Table II show that the extinction coefficient tends to increase with the 1-octanol concentration in water, although regression analysis indicates that the molar extinction coefficient may not be linearly related to the 1-octanol concentration. No studies of this type of interaction have been described in the literature.

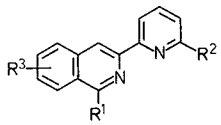
For further elucidation of the apparently incorrect mass balance of **8** (Table I), we determined the total copper concentration in both phases with atomic absorption spectrometry (AAS). In this way a good mass balance for copper was found. The log P of each complex calculated from the copper concentration in each phase did not depend on the volume ratio octanol/water (Table III).

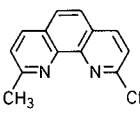
Table II. Extinction Coefficient of bis[2,9-dimethyl-1,10-phenanthroline] Copper(I) Nitrate in Relation to the 1-Octanol Concentration in Water.

Concentration of 1-octanol in water	Mol. extinction coefficient (λ=454 nm)
0.00×10^{-4} M	6498
0.41×10^{-4} M	6606
1.12×10^{-4} M	6389
2.25×10^{-4} M	6606
3.38×10^{-4} M	6768
4.09×10^{-4} M	6877
4.50×10^{-4} M	7418

Each sample was prepared from a stock solution of 1.70×10^{-5} M Cu(I) $(DMP)_2$ in pure water and a stock solution 1.70×10^{-5} M Cu(I) $(DMP)_2$ in water saturated with 1-octanol (in duplicate).
Linear regression analysis gave the following statistics: r = 0.814; F = 9.821; S = 216.84.

Table III. Log P Values of Copper(I) Complexes and their Corresponding Ligands.



		R^1	R^2	R^3	log P (complexes)	log P (ligand)
2	ligand	NH_2	H	H		2.74(±)0.046 n=10
9	complex				1.33(±)0.061 n=6	
3	ligand	NH_2	CH_3	H		3.18(±)0.022 n=6
10	complex				2.60(±)0.105 n=8	
4	ligand	NH_2	H	6-CH_3		3.04(±)0.053 n=10
11	complex				1.35(±)0.100 n=6	
5	ligand	NH_2	H	8-CH_3		3.05(±)0.085 n=6
12	complex				0.85(±)0.26 n=6	
6	ligand	H	H	H		3.05(±)0.027 n=10
13	complex				0.68(±)0.097 n=7	
7	ligand	Cl	H	H		a
14	complex				1.22(±)0.104 n=7	
1						2.46(±)0.076 n=10
8	complex				1.50(±)0.079 n=8	

a. Concentration in water not measurable.

Nevertheless, one has to realize that the AAS analysis does not discriminate between different copper species, but instead gives the total copper concentrations. For this reason there are some complications possible. One of them, the occurrence of oxidation, has already been ruled out. Another complication may be dissociation of the complex. Complete dissociation of the complex can be ruled out, as the color of the complex remains constant during the partitioning experiment. However, if the complex partly dissociates, which is unlikely according to the dilution experiments described above, the hydrophilic copper will accumulate in the aqueous phase. On dilution this gives rise to a variation in the ratio of total copper in the aqueous phase and 1-octanol phase. As this is not the case we conclude that the partitioning of copper in the octanol/water system is equivalent to the partitioning of the copper (I) complex between these phases. Moreover, preliminary experiments on the stability constants of the copper (I) complexes indicate that dissociation in water occurs to an extent of less than 0.1 % of the concentrations used.

The log P values of the ligands in the 1-octanol/water system (Table III) were determined using the shake-flask method as described in the experimental part. Unfortunately, 1-chloro-3-(2-pyridyl) isoquinoline did not dissolve to a measurable extent in water, so that it was not possible to obtain a calibration curve to calculate a log P value.

A statistical analysis of the results in Table III suggested that there is no linear relationship between the log P values of the copper (I) complexes and their corresponding ligands ($r=0.12$). Thus, the lipophilic properties of the substituents of the ligands do not change uniformly when the ligands coordinate the tetrahedral Cu(I).

Conclusions

The described method may be generally suitable for the determination of partition coefficients of sufficiently thermodynamically stable, oxidizable and non-oxidizable metal complexes. With respect to the copper (I) complexes that were used in this study, the log P values could be calculated via the total copper content in the 1-octanol and aqueous phases, whereas spectrophotometric assay of the copper (I) complexes was not possible, at least in the aqueous phase. Furthermore, our results show that there is no linear relationship between the log P values of the copper (I) complexes and the log P values of the corresponding ligands. However, the excellent quantitative structure-antimycoplasmal activity relation described by Pijper (1) includes the lipophilicity of the free ligands. Thus, our results may indicate that the ligands have there own role in the mode of action.

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