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Kinetic speciation of mercury-humic acid complexes in aqueous solutions by using competing ligand exchange method

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Abstract

Competing ligand exchange method (CLEM) in conjunction with direct mercury analyzer (DMA) was found to be a useful method for kinetic speciation study of (mercury-humic acid) Hg-HA complexes in aqueous systems. Ambersep-GT74 (containing a thiol functional group) was used as a suitable competing ligand for this study. The CLEM/DMA was used to estimate lability and dissociation rate constant of Hg-HA complexes in aquatic systems. Influences of pH, metal loading and varying major cation (Ca^{2+}) concentrations on Hg-HA complexes were investigated. Concentration of dynamic Hg-HA complexes gradually increased with increasing Hg loading in the system. The dissociation rate constant (k_{d1}) of labile Hg-HA complexes were found vary from $4.3 \times 10^{-3} \text{ s}^{-1}$ to $9.2 \times 10^{-3} \text{ s}^{-1}$ with increasing Hg loading from $1.0 \times 10^{-8} \text{ M}$ to $1.42 \times 10^{-7} \text{ M}$. The concentrations of Hg-HA complexes with low thermodynamic stability were found to be similar at pH 4 and 5 (~59% and 58% of the total Hg with k_{d1} values of $\sim 2 \times 10^{-3}$ and $1.7 \times 10^{-3} \text{ s}^{-1}$ respectively). The complexation of Hg with HA was found to increase with increasing pH from 4 to 7. The concentration of inert complexes (c_2) with dissociation rate constant (k_{d2}) gradually increased with increasing pH. Increasing concentration of Ca^{2+} in the model solutions increased the concentrations of Hg-HA dynamic complexes in the system. This finding suggests that Hg-HA bond is predominately ionic in nature. It suggests that Ca^{2+} ion screen out Hg^{2+} ions from undergoing complexation reaction with HA.

Key words:

Mercury speciation, competing ligand exchange method, dynamic complexes, dissociation rate constant, Ambersep GT74, Dissolved Organic Carbon, and Direct Mercury Analyzer,

1. Introduction

Mercury,(Hg) one of the most studied elements, has been considered as a high priority pollutant because of its persistence in environments and high toxicity to organisms [1–3]. Toxicity of Hg in fresh, estuarine and sea water mainly depends on its speciation[4]. Humic substances, a major component of dissolved organic carbon (DOC), modify the speciation of Hg in natural/marine systems and influence the capacity of natural/marine waters to sustain life even after Hg pollution events [5,6]. This Hg-DOC interaction plays an important role in controlling the reactivity, bioavailability and transport of Hg in natural/marine environments [7]. Hg undergoes mainly two different types of reactions with different functional groups of DOC: (i) Hg^{2+} ion undergoes strong complexation reaction with S, N containing functional groups, (ii) Hg^{2+} acts as an electron acceptor from electron rich DOC in aquatic system and form elemental Hg (Hg^0). It is extremely challenging to understand Hg-DOC interactions and Hg speciation in aquatic systems. There are various experimental methods/techniques have being used for metal speciation study in aquatic systems and these approaches include batch techniques[8–10], flow techniques[9,11] kinetic extraction methods[12–17], Diffusive Gradients in Thin Films technique[8,18,19]. However, the kinetic extraction method has been found to be a simple and sensitive technique [12,20], This method is capable of determining kinetically distinguishable (labile and inert) metal complexes (in water column/soil/sediment) and their corresponding dissociation rate constants in aquatic environment. However, no information is available in literature on kinetic speciation study of Hg in aquatic environment. It has been extremely difficult to find a suitable competing ligand for kinetic speciation studies of Hg in aquatic systems. It is well known that thiol functional group has higher affinity towards Hg than carboxylic/phenolic-OH groups. Both Hg and sulfur are 'soft' in their ionic forms. According to Pauling's concept of Hard Soft Acid Base theory (HSAB) evidences that hard acids prefer to combine with hard bases, and soft acids prefer to combine with soft bases, to maximize covalency. A new commercially available ion exchange resin Ambersep GT74 with thiol functional groups has been used as a chelator of Hg for kinetic speciation in aquatic system[21]. It has been reported that inorganic – Hg^{2+} , methylmercury – CH_3Hg^+ , ethylmercury – $\text{C}_2\text{H}_5\text{Hg}^+$ and phenylmercury – $\text{C}_6\text{H}_5\text{Hg}^+$ can be easily bound to Ambersep-GT74 resin[22].

In this study CLEM in conjunction with DMA was used to study Hg-DOC interaction in aquatic environment. Kinetic speciation study was performed to estimate dissociation rate constants of Hg-DOC complexes in aquatic system. An effort was also made to realize the effects of varying pH, Hg loading, and major cation competition on Hg speciation in aquatic system.

2. Materials and methods

2.1 Chemicals and materials

A stock solution ($1,000 \text{ mg dm}^{-3}$, NIST Hg (NO_3)₂ in 10 % HNO_3 CertiPUR) of Hg was purchased from Merck Chemicals, Germany. Standard solutions of Hg were prepared daily by dilution of the stock solution with ultrapure water (of resistivity $18.2 \text{ M}\Omega \text{ cm}^{-1}$) acidified to contain 1 % (v/v) ultrapure HNO_3 (Merck, Germany). Stock solutions of humic acid (HA) was prepared using HA from Fluka (commercialized by Sigma-Aldrich, cat. No. 53680, Sigma-Aldrich, Inc, Milwaukee, WI, USA). This HA was used as the representative of humic substances.

The elemental composition of the HA has been reported to contain 48.36 % of C, 26.91 % of O, 4.24 % of H, 0.78 % of N and 0.78 % of S[23]. The atomic ratios reported in the literature are 1.04 (H/C), 0.42 (O/C), and 0.012 (N/C) which are within the values reported for soil HAs, with the exception of the N/C ratio. This indicates that the HA used in this study was a soil humic acid. The molar ratio of protons on saturated and unsaturated carbon atoms is approximately 2.1:1 in the studied HA[23]. Thus, Fluka HA may not be a good representative of aquatic humic substances. It is also well known that soil humic substances (HS) that leach out from soil usually become an integral part of dissolved organic carbon in river or coastal waters via runoff and drainage. The purpose of using Fluka HA (soil HA) in this study was to understand the distribution of Hg in soil HA in aqueous medium. It is also necessary to mention that Fluka HA was inexpensive and easily available.

2.2 Model solutions

Series of model solutions were prepared, containing HA (5.0 mg dm^{-3}) and varying concentrations of Hg (ranging from 1.0×10^{-8} to $1.42 \times 10^{-7} \text{ M}$) in ultrapure water, to understand the impact of varying Hg loading on kinetic speciation of Hg-HA complexes. Teflon containers were used to minimize Hg adsorption on the wall of the containers. The concentrations of Hg used in this study were relatively higher than those of environmental concentration levels. However, Hg concentrations in the coastal surface water, near the industrial cities in India has been reported to be high [24–27]. Thus, the concentrations of Hg used in this study were environmentally relevant. It was also extremely difficult to understand the complexation of Hg with DOC interactions at environmentally relevant concentrations of Hg due to heterogeneity and complexity of DOC. Use of relatively higher concentrations of Hg helped to clearly study the distribution and speciation of Hg in the aquatic medium.

A series of model solutions were prepared to study the impact of varying pH, on Hg speciation. The concentrations of Hg ($6.1 \times 10^{-8} \text{M}$) and HA (5.0 mg dm^{-3}) were kept constant in all the solutions. The pHs of the solutions were varied from 4 to 8. The pH was adjusted using 2 M ultrapure HNO_3 from Merck and/or 2 M purified NaOH from Merck. WTW series inoLab pH/Conductivity meter 720 (WTW, Germany) was used to check and adjust the pH of the solutions. The ionic strength of the model solutions [containing Hg ($6.1 \times 10^{-8} \text{M}$) and HA (5.0 mg dm^{-3})] was adjusted (from 10.0 to 0.1 mM) by varying the concentrations of ultrapure NaNO_3 (99.995 % trace metals basis, Aldrich, US) solution. The experiments were carried out with a background electrolyte, (NaNO_3) to minimize the effect of varying Hg^{2+} loading on the ionic strength of the medium. Ionic strength modifies the structural feature of HA in the solutions.

2.3 Kinetic Extraction Procedure

Kinetic extraction experiments were conducted in model solutions containing HA and varying concentration of Hg. The solutions were equilibrated for 24 hrs. Teflon reactor of 400 ml (containing Hg and HA solution) was continually stirred with a Teflon coated magnetic stirring bar throughout experiment. A definite (2.5g) amount of Ambersep-GT 74 was added in the reactor. 2ml of suspensions were filtered through a 0.2 syringe filter (millex Millipore) at set time intervals (0min, 2min, 4min, 6min, 8min, 10min, 15min, 20min, 25min, 30min, 45min, 60min, 90min, 120min, and 180min). The initial time for kinetic measurement (i.e. $t = 0$) was taken the time before the Ambersep GT74 was added to the model solution.

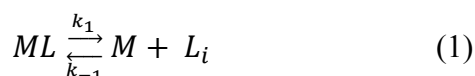
2.4 Ambersep GT-74

Ambersep GT74 has one or many thiol functional groups (ionic form) in solid beads. The structure of this resin is shown in **Fig.1**. The thiol group is an excellent ligand for Hg [28]. Ambersep-GT74 and it is a weakly acidic cation exchange resin with very pronounced selectivity for Hg. It has been mainly used to remove Hg from different solution and gaseous streams. This resin can be regenerated very efficiently with hydrochloric acid. The selectivity sequence is $\text{Hg} > \text{Ag} > \text{Cu} > \text{Pb} > \text{Cd} > \text{Ni} > \text{Co} > \text{Fe} > \text{Ca} > \text{Na}$ [29]

3. THEORY:

3.1 Competing Ligand Exchange Method (CLEM)

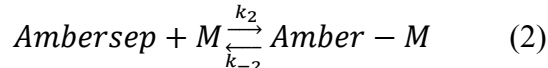
CLEM was used for the determination of dissociation kinetics of Hg-humate complexes in aquatic solution. Ambersep GT74 cation exchange resin was used as a solid-phase competing ligand. The kinetic model proposed by Olson & Shuman[30] was used to study the dissociation kinetics of metal complexes, ML_i , where M is a metal ion and L_i is the i th binding site on a polyfunctional, macromolecular, organic complexant, such as HA. For simplicity, the charges on M and L_i have been omitted.



Consider an aqueous mixture of n components in which each component, designated ML_i , undergoes a first-order or pseudo-first-order reaction and exists in equilibrium with its dissociation products:

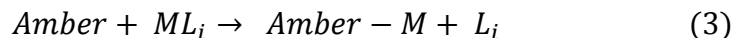
Where k_1 and k_{-1} are the rate coefficients for the forward and the backward reactions, respectively

Ambersep GT-74 reacts with M as follows:



Where Ambersep represents Ambersep GT-74 cation exchange resin, and k_2 and k_{-2} are first-order (or pseudo-first-order) rate coefficients for the forward and the backward reaction, respectively.

The model is based on two assumptions: (i) that reaction (2) is much faster than reaction (1); and (ii) that $[Ambersep] \gg [M]$. As $[Ambersep]$ is large and $[Ambersep] \gg [M]$, $[Ambersep]$ can be considered as constant and reaction (2) as pseudo-first-order. Since k_2 is large, and Ambersep is in large excess, the condition $k_2 [Ambersep] \gg k_{-1} [L_i]$ holds and the overall reaction is given by.



Overall the reaction is assumed to be irreversible, and the rate of dissociation of ML_i is

$$\frac{d[Ambersep-M]}{dt} = -\frac{d[ML_i]}{dt} = k_1 [ML_i] \quad (4)$$

The two assumptions made above were verified,

If each complex, MLi undergoes independently and simultaneously, a first-order or pseudo-first-order dissociation reaction the sum, $C(t)$, of the concentrations of all components remaining undissipated in the test solution at time t can be described as

$$C(t) = \sum_{i=1}^n C_i^0 \exp(-k_i t) \quad (5)$$

Where C_i^0 is the initial concentration of MLi, the i th component. $C(t)$ is determined by DMA-80, by measuring the decrease in the amount of metal ions in the test solution due to their binding by the Ambersep GT74, as a function of time.

4.1 Impact of varying Hg loading on kinetic speciation of Hg-HA complexes in synthetic model solutions.

Competing ligand exchange method in conjunction with direct mercury analyzer (DMA-80) was used to understand the impact of varying concentrations of Hg on kinetic speciation of Hg-HA complexes in aquatic system. The kinetically distinguishable Hg-HA complexes are shown in **Fig 3**. There are two distinguishable parts in each curve. The steep slope at the beginning of each curve can be attributed to rapidly dissociating Hg-organic complexes (c_1) with fast dissociation rate constant (k_{d1}). The later part of the curves, which run parallel to x-axis, can be attributed to the Stronger Hg-humate complexes (c_2) with slow dissociation rate constants (k_{d2}). The experimental curves were fitted to a two component model because it was the simplest model (i.e., minimum number of components) that gave an adequate statistical and visual fit to the data (i.e., good representation of the system). Although the use of more components in the model often statistically fit the data better, the additional components were often of negligible concentration or had a high degree of uncertainty. The percentage of dynamic and inert complexes of Hg and their corresponding dissociation rate coefficients are given below in **Table 1**.

Fig 4a shows that increasing concentration of Hg in a system (i.e., increasing [Hg]/ [HA] ratio) increased the concentration of dynamic Hg-HA complexes in the aquatic system. At low Hg loading, strong binding sites of HA remained available to undergo strong complexation with Hg. Thus, less concentration of Hg-HA labile complexes (with slow dissociation rate constant) were found. However, with increasing Hg loading the strong binding sites became saturated and the excess Hg then bound to the weaker sites, and thus, more weak Hg-HA complexes (with faster dissociation rate constant(k_{d2})) were formed. These weaker complexes (with fast dissociation rate constant) were considered as labile/dynamic complexes.

The fast dissociation rate constant of Hg-HA complexes was found vary from $4.3 \times 10^{-3} \text{ s}^{-1}$ to $9.2 \times 10^{-3} \text{ s}^{-1}$ with increasing Hg loading from $1.0 \times 10^{-8} \text{ M}$ to $1.42 \times 10^{-7} \text{ M}$. **Table 1** shows that the concentration of dynamic Hg-HA complexes gradually increased with increasing Hg loading in the system. Fig. 4b shows that the dissociation rate constants (k_{d1} , dissociation rate constant of labile Hg–HA complexes) increased with the increasing Hg/HA ratio. This is because of the fact that at low Hg/HA ratio, Hg tends to undergo strong complexation with the strong binding sites of HA and form thermodynamically stable Hg–HA complexes. However, increased in Hg^{2+} loading increased liable complexes as Hg^{2+} and thus, k_d value gradually increased.

4.2 Kinetic speciation of Hg-HA complexes at different pH in synthetic model solution

The effect of pH on kinetic speciation of Hg-HA complexes in syntactic model solutions was examined at room temperature by varying the pH of the medium from 4.0 to 8.0. The experimental data are shown in **Table 2**, and the experimental curves also showed in Fig 5. The first part of all curves in fig 5 is almost indistinguishable from one another, suggesting that they represent dissociation of one or more Hg complexes having very similar fast dissociation rate constants. They are all of Hg-HA complexes with low thermodynamic stability and were dynamic in nature (within the time scale of the measurement). The concentration of labile Hg-HA complexes were found to vary from $\approx 46\%$ to 60% of the total Hg content in the system with varying pH from 4.0 to 6.0 of synthetic solution. The dissociation rate constants and dynamic Hg-HA complexes were found to vary from $1.9 \times 10^{-3} \text{ s}^{-1}$ to $4.3 \times 10^{-3} \text{ s}^{-1}$. The data are presented in **Table 2**. The concentrations of Hg-HA complexes (c_1) with low thermodynamic stability were found to be similar at pH 4 and 5 ($\sim 59\%$ and 58% of the total Hg with k_{d1} values of $\sim 2 \times 10^{-3}$ and $1.7 \times 10^{-3} \text{ s}^{-1}$ respectively) (Table 2).

Fig 6a shows the variation of dynamic and stable Hg-HA complexes at varying pH of the aqueous solution. The complexation of Hg with HA was found to increase with increasing pH from 4 to 7. The concentration of inert complexes (c_2) with dissociation rate constant (k_{d2}) gradually increased with increasing pH. This indicates that at lower pH there was a competition between H^+ and Hg^{+2} ions for the same binding sites in HA present in the solution. It is well documented that cationic binding with dissolved organic carbon is pH dependent (presumably through competition between H^+ and metal cations for binding sites) [31]. Thus, one can conclude that increasing H^+ competition increases labile Hg-HA complexes in aquatic system at lower pH.

It has been reported at low pH and high ionic strength HA may form relatively large aggregates by H-bonding and van der Waal's attraction[32]. Aggregation/ disaggregation property of HA depends on pH of the medium. These aggregates are able to disaggregate/reaggregate, depending on the nature and concentration of HA, pH and ionic strength of the medium[27,33,34]. Disaggregation property of HA may also increase Hg complexation at higher pH [27,33,34].

Fig. 6b shows that the variation of dissociation rate constants (k_{d1} , and k_{d2} of Hg–HA complexes). It was found that k_{d2} value decreased in solution as a function of pH of the medium. This is because of the fact that at low pH there was competition between H^+ and Hg^{2+} ion for complexation with the same binding sites of HA. Hg^{2+} ion along with small dynamic complexes predominated in the solution at lower pH, Hg was found to undergo strong complexation with the strong binding sites of HA and form thermodynamically stable Hg–HA complexes with slow dissociation rate constant (k_{d2}) at higher pH. At lower pH, stronger sites of HA get occupied by H^+ ions and Hg^{2+} moves towards the weaker sites of HA to form thermodynamically weaker complexes with faster dissociation rate constant. However, with increasing pH, the H^+ competition to Hg for HA binding sites will be reduced and there is a chance for Hg^{2+} to complex with stronger sites of HA and form thermodynamically stable complexes. This is clearly shown in 6b that k_{d2} decreases with the increasing pH.

4.3 Major cation (Ca^{2+}) effect on mercury kinetic speciation synthetic model solution

Competition of Ca^{2+} with Hg^{2+} ions for binding sites of a well-characterized humic acid (HA) in synthetic solutions at constant pH and ionic strength was investigated by using CLEM. In this study, the contribution of Columbic interactions between the negatively charged surfaces of HA and the major cation Ca^{2+} and in the total bonding of the target trace metal ion, Hg^{2+} , HA were considered.

Fig 7 shows the effect of varying concentrations of Ca^{2+} on the release of Hg^{2+} from Hg-HA complexes. In the absence of Ca^{2+} , the strong binding sites of the HA were occupied by Hg^{2+} , forming strong complexes that are inert (top curve). However, increasing concentrations of Ca^{2+} ($1 \times 10^{-4}M$ to $2 \times 10^{-3} M$) (each curve from top to bottom) had dramatic effect on the lability of Hg -HA complexes in the solution. High concentrations of Ca^{2+} resulted in formation of weak Hg -HA complexes that were labile. Increasing in Ca^{2+} ion concentration was found to influence the lability of Hg-HA complex. Several studies showed that ionic strength can facilitate the aggregation of Ca-HA form a highly compacted structure (squeezing-out effect), thus suppressing the complexation of Hg with HA.

The values for kinetic parameters of the Hg-HA complexes in the presence of varying concentrations of Ca^{2+} are presented in **Table 3**. The presence of the relatively high concentration of Ca^{2+} (2×10^{-3} M), increases the dissociation rate coefficient (k) of Hg-HA complexes. **Fig 8a** shows that the concentrations of Hg-HA dynamic complexes gradually increased with the increasing concentration of major cation in the model solutions. This finding suggests that Hg-HA bond is predominately ionic in nature. It suggests that Ca^{2+} ion screen out Hg^{2+} ions from undergoing complexation reaction with HA.

Fig. 8b shows that the dissociation rate constants (k_{d2} , dissociation rate constant of thermodynamically stable Hg–humate complexes) decreased with the increasing Ca^{2+} concentration. This is because of the fact that at low Ca^{2+} concentration Hg tends to undergo strong complexation with the strong binding sites of HA and form thermodynamically stable Hg–HA complexes with slow dissociation rate constant. However, with the increasing Ca^{2+} concentration, strong sites of HA get occupied by Ca^{2+} and screen out Hg form undergoing complexation reaction with HA and allowed to form thermodynamically weaker complexes with faster dissociation rate constant. (**Fig 8b**). The variation of k_{d1} (dissociation rate constant of labile Hg–Humate complexes) with Ca^{2+} is not appreciable.

5. Conclusions

This study explores the kinetic speciation of Hg-HA complexes in aquatic system and it reveals the impact of varying pH, Hg loading, and major cation (Ca^{2+}) concentrations on kinetic speciation of Hg-HA complexes in the system. It was observed that

1. Concentrations of dynamic Hg-HA complexes gradually increased with increasing Hg loading in the system, and dissociation rate constants (k_{d1} , dissociation rate constant of labile Hg–HA complexes) increased with the increasing Hg/HA ratio.
2. The complexation of Hg with HA was found to increase with increasing pH from 4 to 7. The concentration of inert complexes (c_2) with dissociation rate constant (k_{d2}) gradually increased with increasing pH.
3. Increasing Ca^{2+} concentration, strong sites of HA get occupied by Ca^{2+} and screen out Hg form undergoing complexation reaction with HA and allowed to form thermodynamically weaker complexes with faster dissociation rate constant. It also suggested that Hg-HA bond is predominately ionic in nature.

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Captions and Legends for Figures

Fig 1: Selection of suitable competing ligands for Hg. **Fig 2:** optimization competing ligand concentration.

Fig 3: Percentage of Hg(II) released from Hg(II)–DOC complexes in model solution, determined by CLEM/ DMA-80. Ambersep GT-74 was the competing ligand. (●), 1.0×10^{-8} M of Hg solution; (○), 6.1×10^{-8} M of Hg solution; (▼), 9.3×10^{-8} M of Hg solution; (Δ), 1.4×10^{-7} M of Hg solution. Solid lines represent non-linear curve fitting

Fig 4a: Kinetic speciation parameters Hg-DOC Complexes with function pH.

Fig 4b: Variation of dissociation rate constants of thermodynamically weak (kd1) and strong (kd2) Hg-complexes with varying [Hg]/[HA] ratio.

Fig 5: Percentage of Hg(II) released from Hg(II)–DOC complexes in model solution, determined by CLEM/ DMA-80. Ambersep GT-74 was the competing ligand. In function of pH (●), pH 4 Hg solution; (○), pH 5 Hg solution; (▼), pH 6 Hg solution; (Δ), pH 7 Hg solution; (■), pH 8 Hg solution. Solid lines represent non-linear curve fitting.

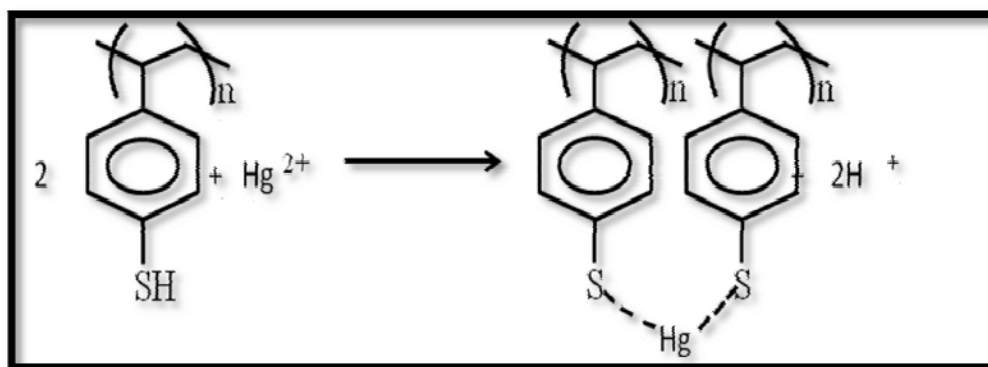
Fig 6a: Kinetic speciation parameters

Fig 6b: Variation of dissociation rate constants of thermodynamically weak (kd1) and strong (kd2) Hg-complexes with varying pH

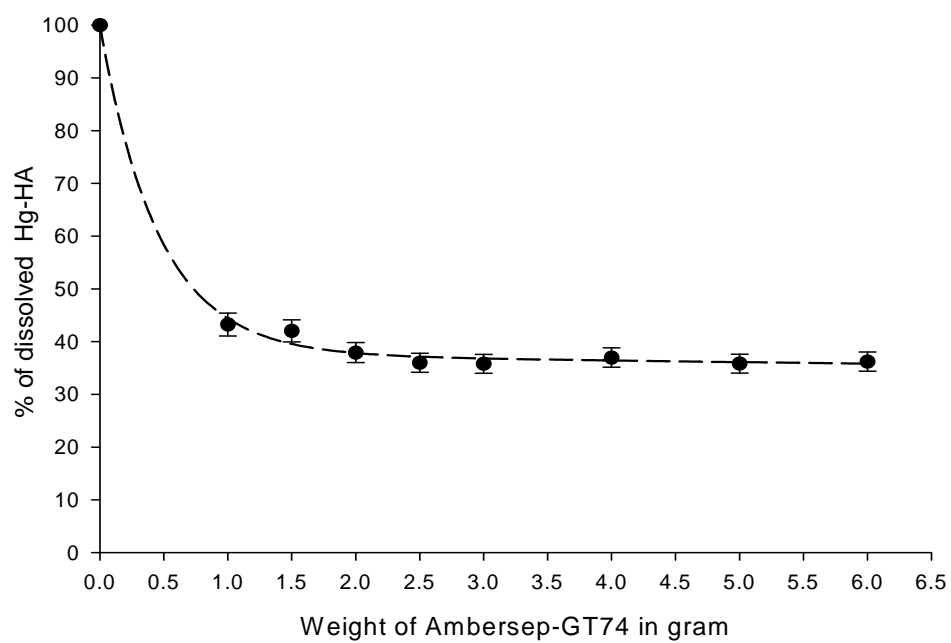
Fig 7: Percentage of Hg(II) released from Hg(II)–DOC complexes in model solution, determined by CLEM/ DMA-80. Ambersep GT-74 was the competing ligand. In function of major cation (Ca) (●), 2.0mM of Ca solution; (○), 1.0mM of Ca solution; (▼), 0.5mM of Ca solution; (Δ), 0.1mM of Ca solution; (■), 0.0mM of Ca solution. Solid lines represent non-linear curve fitting

Fig 8a: Kinetic speciation parameters of Hg-DOC Complexes with function of major cation (Ca)

Fig 8b: Variation of dissociation rate constants of thermodynamically weak (kd1) and strong (kd2) Hg-complexes with varying conc of Ca^{2+}

Figures**Fig.1**

Optimization of Ambersep-GT74 weight

**Fig.2**

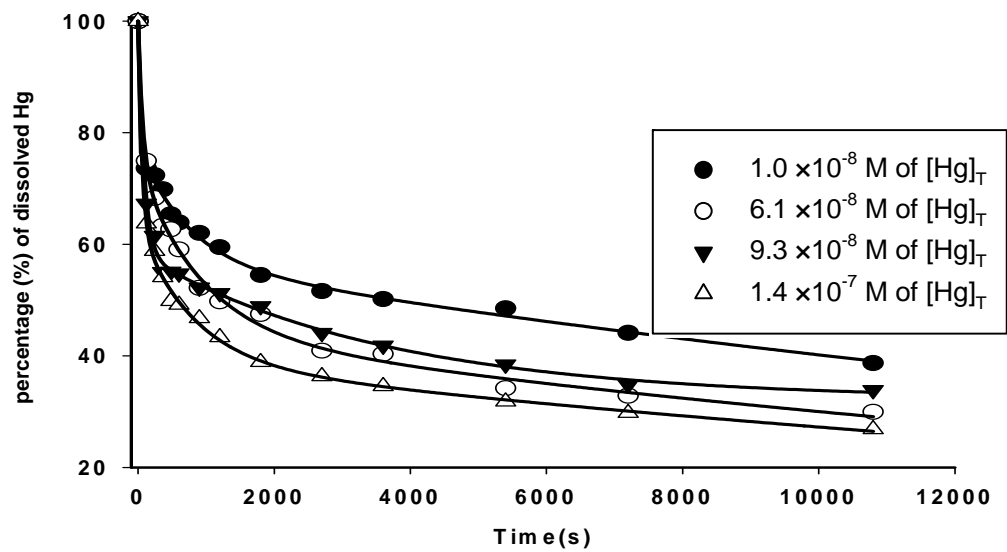


Fig 3

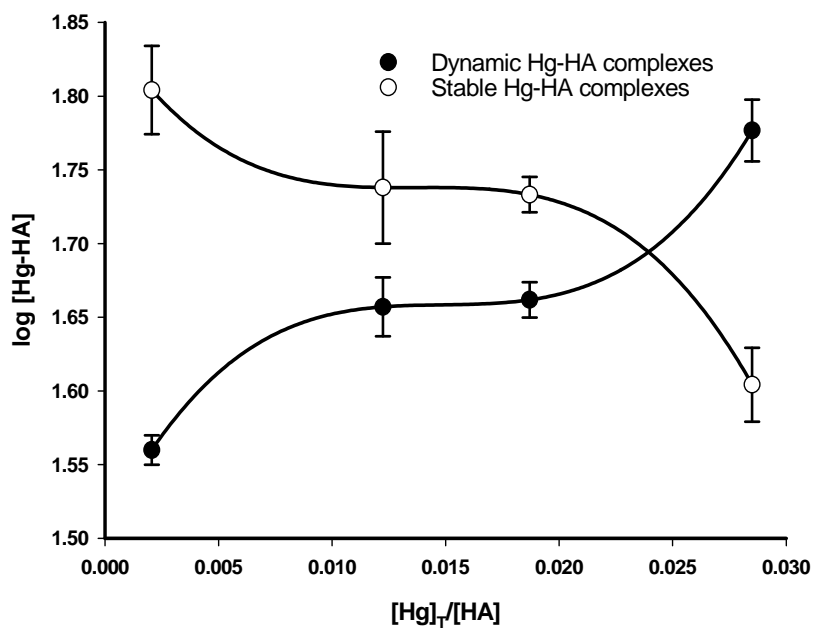


Fig 4a

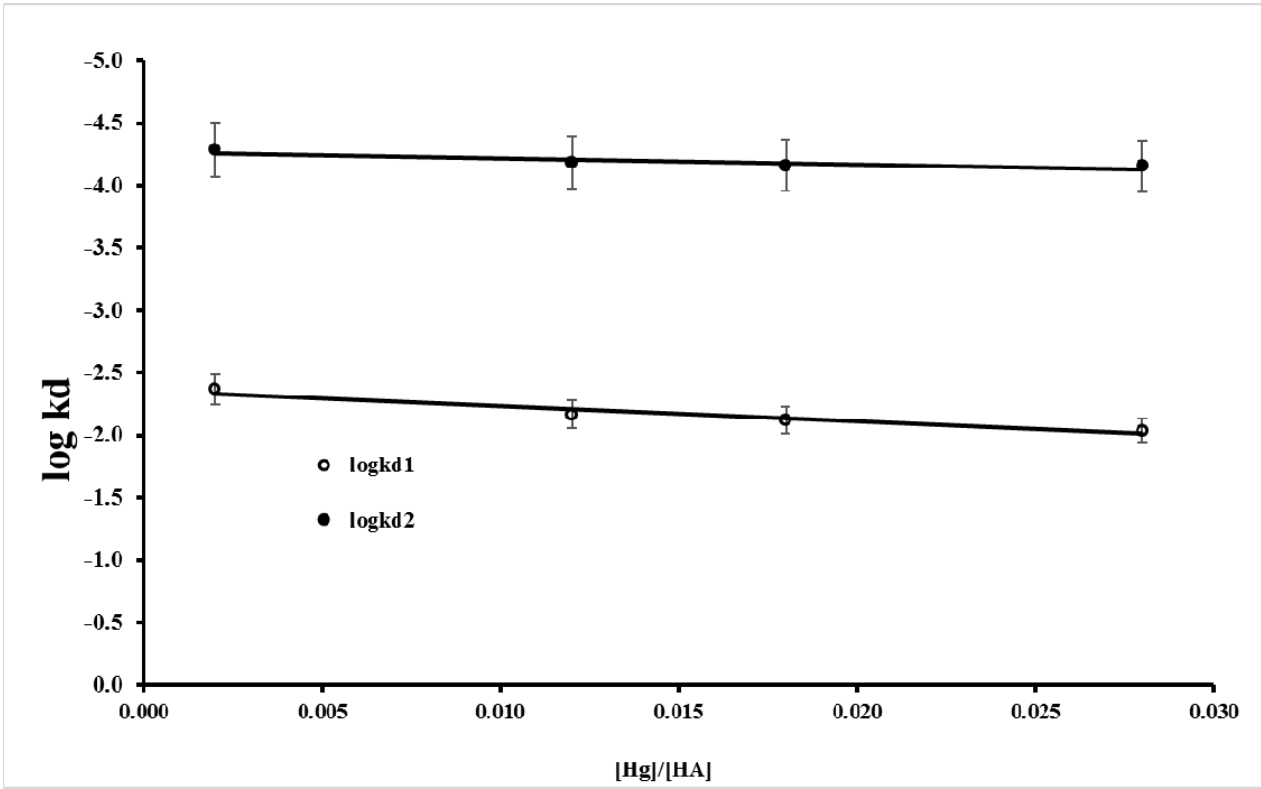


Fig 4b

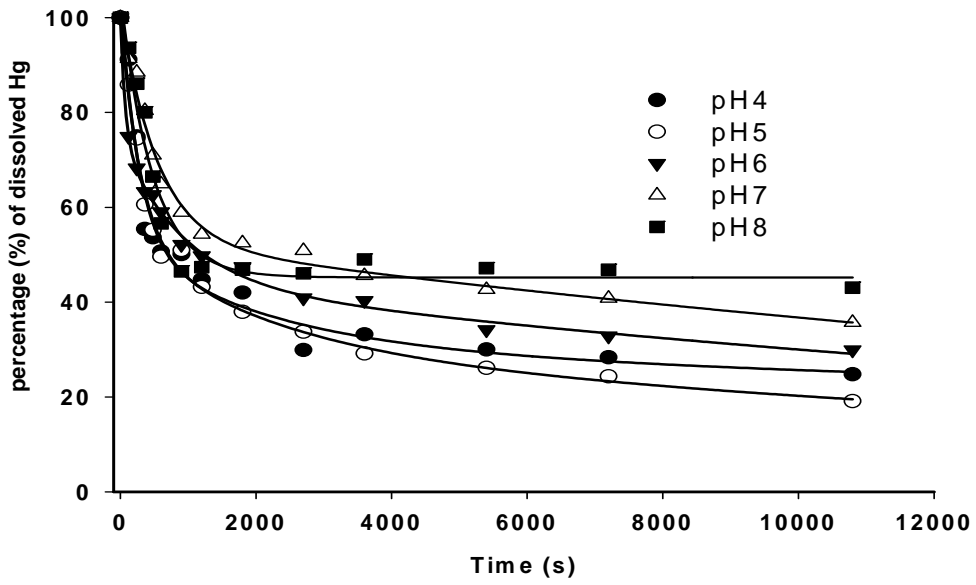


Fig 5

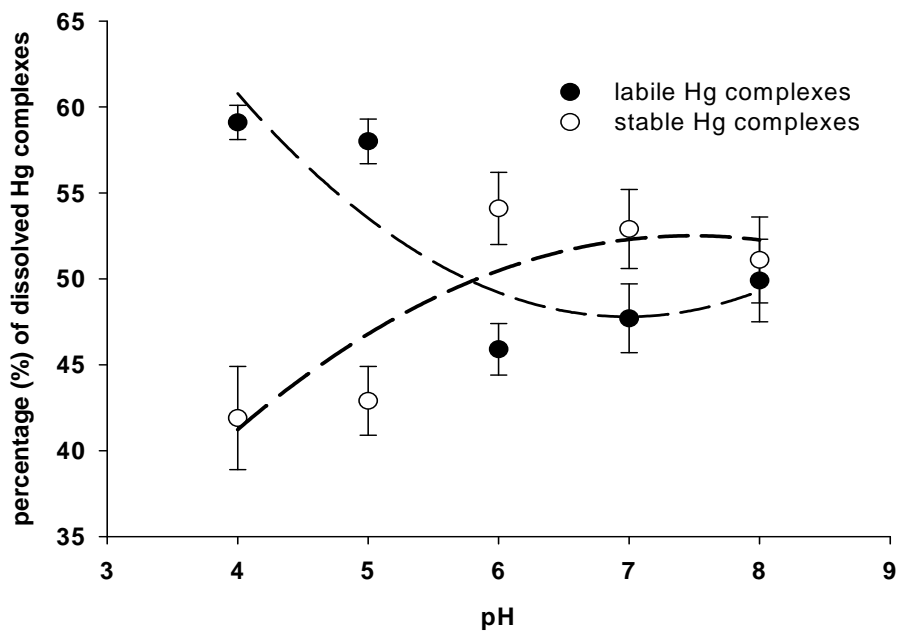


Fig 6a

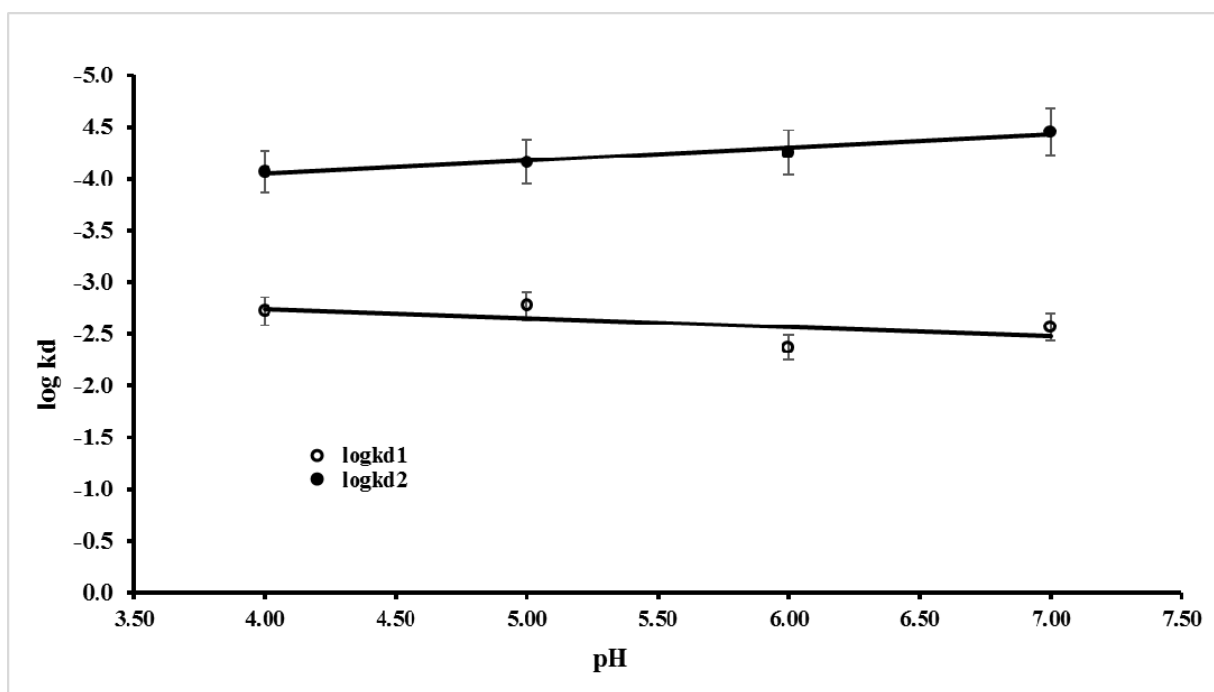


Fig 6b

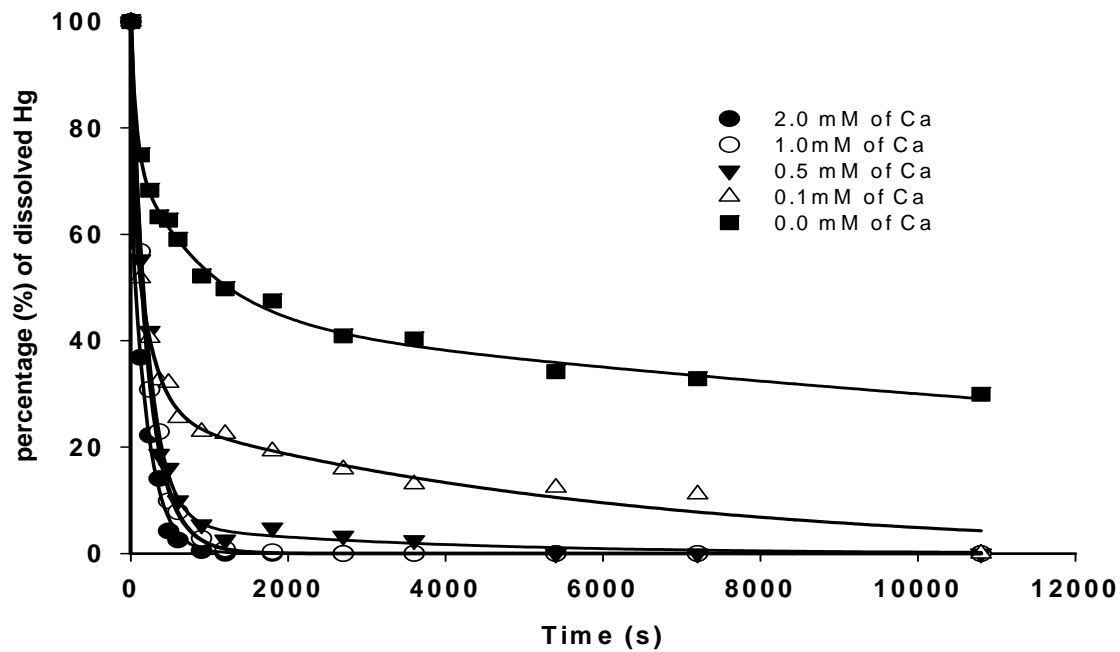


Fig 7

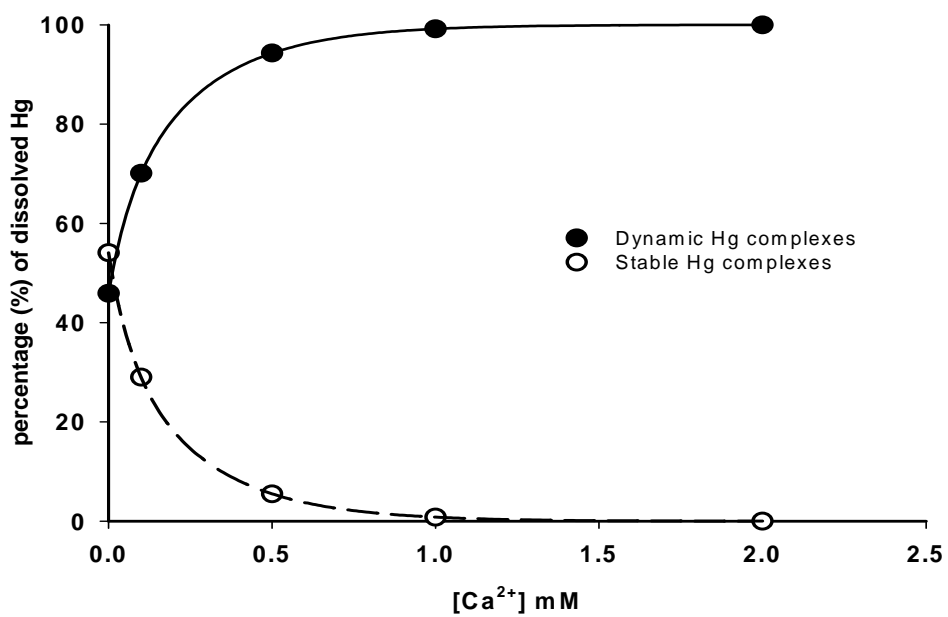


Fig 8a

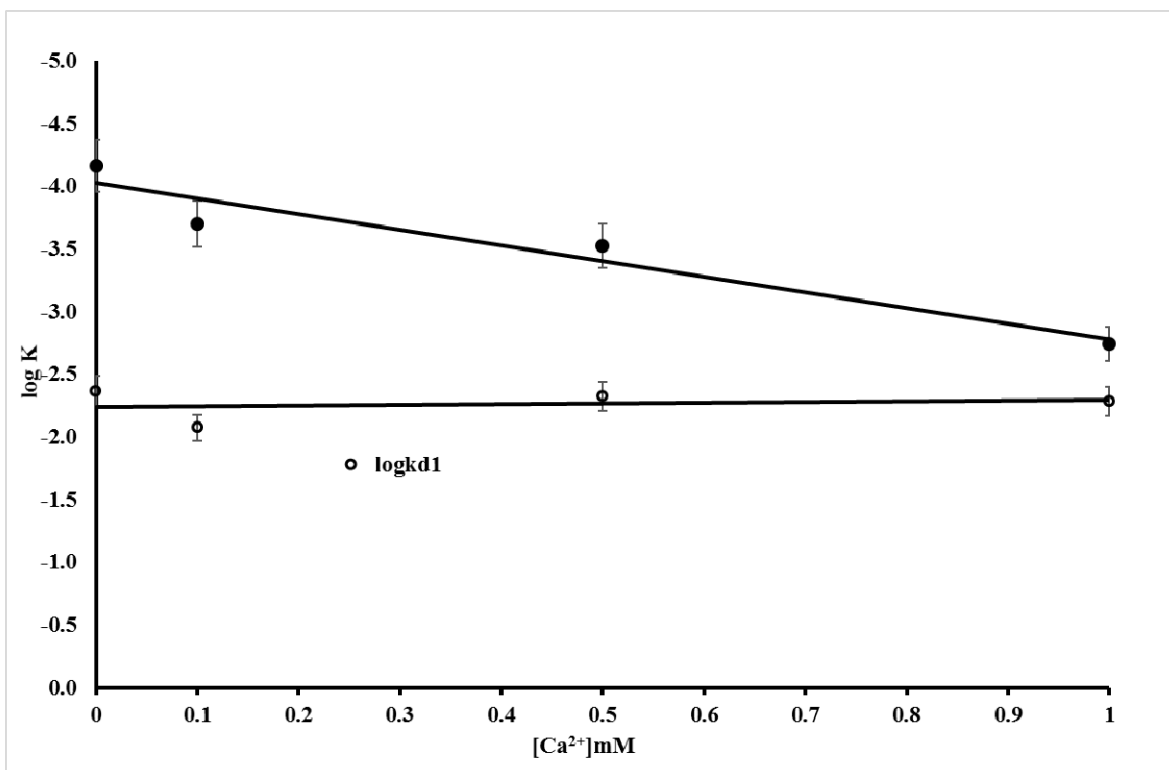


Fig 8b

Table1. Kinetically distinguishable components of Hg-HA complexes at varying concentrations of $[\text{Hg}]_{\text{T}}$ in the synthetic solutions, determined by CLEM/DMA at $T=23\pm 2^{\circ}\text{C}$, $\text{pH } 6.0\pm 0.1$; ionic strength, $3\times 10^{-2} \text{ mol.dm}^{-3}$

| $[\text{Hg}]_{\text{T}}$ | HA mg. dm^{-3} | $[\text{Hg}]_{\text{T}}/[\text{HA}]$ | c_1 (%) | k_{d1} (s^{-1}) | c_2 (%) | k_{d2} (s^{-1}) |
|-------------------------------|----------------------------|--------------------------------------|---------------|------------------------------|---------------|------------------------------|
| $1.0\times 10^{-8} \text{ M}$ | 5.0 | 0.002 | 36.3 ± 0.7 | $(4.3\pm 0.8)\times 10^{-3}$ | 63.7 ± 1.2 | $(5.2\pm 0.3)\times 10^{-5}$ |
| $6.1\times 10^{-8} \text{ M}$ | 5.0 | 0.12 | 45.9 ± 0.4 | $(6.8\pm 0.1)\times 10^{-3}$ | 54.6 ± 1.1 | $(5.6\pm 0.3)\times 10^{-5}$ |
| $9.3\times 10^{-8} \text{ M}$ | 5.0 | 0.018 | 45.9 ± 0.4 | $(7.6\pm 0.3)\times 10^{-3}$ | 54.1 ± 1.1 | $(6.9\pm 0.4)\times 10^{-5}$ |
| $1.4\times 10^{-7} \text{ M}$ | 5.0 | 0.028 | 59.8 ± 1.1 | $(9.2\pm 0.4)\times 10^{-3}$ | 40.2 ± 0.8 | $(7.0\pm 0.4)\times 10^{-5}$ |

Table2. Kinetically distinguishable components of Hg-HA complexes at varying pH in the synthetic solutions, determined by CLEM/DMA at T=23±2°C, [Hg]_T=62.0nM; ionic strength, 3×10⁻² mol.dm⁻³

| pH | HA mg. dm ⁻³ | [Hg] _T /[HA] | c ₁ (%) | k _{d1} (s ⁻¹) | c ₂ (%) | k _{d2} (s ⁻¹) |
|-----------|----------------------------|-------------------------|--------------------|------------------------------------|--------------------|------------------------------------|
| 4.00±0.05 | 5.0 | 0.012 | 59.1±3.0 | (1.9±0.1) × 10 ⁻³ | 41.1±2.1 | (8.5±0.4) × 10 ⁻⁵ |
| 5.00±0.05 | 5.0 | 0.012 | 58.0±2.3 | (1.7±0.1) × 10 ⁻³ | 52.2±2.6 | (6.9±0.3) × 10 ⁻⁵ |
| 6.00±0.05 | 5.0 | 0.012 | 45.9±2.3 | (6.8±0.3) × 10 ⁻³ | 54.6±2.4 | (5.6±0.3) × 10 ⁻⁵ |
| 7.00±0.05 | 5.0 | 0.012 | 47.7±2.1 | (2.7±0.2) × 10 ⁻³ | 52.9±2.6 | (3.5±0.2) × 10 ⁻⁵ |
| 8.00±0.05 | 5.0 | 0.012 | 49.9±2.5 | (2.7±0.2) × 10 ⁻³ | 51.1±1.9 | (<1±0.2) × 10 ⁻⁶ |

Table 3. Kinetically distinguishable components of Hg-HA complexes at varying concentrations of [Ca]_T in the synthetic solutions, determined by CLEM/DMA at T=23±2°C, pH 6.0±0.1; ionic strength, 3×10⁻² mol.dm⁻³

| [Ca] _T | HA mg. dm ⁻³ | [Hg] _T /[HA] | c ₁ (%) | k _{d1} (s ⁻¹) | c ₂ (%) | k _{d2} (s ⁻¹) |
|-------------------|----------------------------|-------------------------|--------------------|------------------------------------|--------------------|------------------------------------|
| 0.0mM | 5.0 | 0.012 | 45.9±3.9 | (4.3±0.8) × 10 ⁻³ | 54.1±3.9 | (6.9±1.1) × 10 ⁻⁵ |
| 0.1mM | 5.0 | 0.012 | 70.1±3.3 | (8.4±0.9) × 10 ⁻³ | 29.8±2.0 | (2.0±0.2) × 10 ⁻⁴ |
| 0.5mM | 5.0 | 0.012 | 94.3±4.2 | (4.7±0.4) × 10 ⁻³ | 5.6±0.8 | (3.0±0.3) × 10 ⁻⁴ |
| 1.0mM | 5.0 | 0.012 | 99.2±2.3 | (5.2±0.6) × 10 ⁻³ | 0.8±0.2 | (1.8±0.2) × 10 ⁻³ |
| 2.0mM | 5.0 | 0.012 | 99.3±1.2 | (8.8±1.1) × 10 ⁻³ | 0 | -- |