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CALORIMETRIC INVESTIGATION OF COMPLEX FORMATION Of SOME HUMIC COMPOUNDS

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Abstract

In the present paper we studied the complexation capacity towards copper ion of fulvic acids extracted from Arno River and Lake Como sediments, as well as Antarctic Sea water at different pHs in order to evaluate the interaction of different complexing groups of fulvic acids with copper ions. The binding capacity studies were carried out by means of titration with a copper-selective electrode and calorimetric measurements. For the same purpose, the heat of reaction in the complexation of copper ions with small molecules containing functional groups similar to fulvic acid was also determined. Titration results indicated that the fraction of bound metal increases with increasing pH (from 5 to 7). This may be accounted for by the increase in the ionisation of the fulvic acid molecule. Results obtained from calorimetric measurements show that the quantity of total heat involved in the metal ion-fulvate interactions determines a decreasing exothermic response with increasing pH values.

Keywords: calorimetry, complexing capacity, fulvic acid,

Introduction

Aquatic humic substances include fulvic acids, humic acids and humin, all of which share the characteristics of being heterogeneous biomolecules which are yellow to brown or black in colour, and have high to moderate molecular mass and are biologically refractory. Fulvic acids are organic acids that are soluble at any pH; humic acids are soluble above pH 2; humin is insoluble over the entire pH range [1].

Aquatic humic substances occur in both dissolved and solid phases, with molecular masses ranging from about 500 D for dissolved fulvic acids to up to more than 100000 D for humic acids in sediments. Humic compounds are truly ubiquitous; however, their abundance with regard to the major ion and mineral phases varies greatly in the various aquatic ecosystems. In the water column, dissolved fulvic acids are much more abundant than dissolved humic acids. Within suspended particulate material, humic and fulvic acids can be equally abundant. In lacustrine and marine sediments, humic acids are more abundant than fulvic acids. They can be derived from allochthonous sources of organic

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matter and also produced within the aquatic ecosystem (autochthonous source) [2, 3]. Their structures contain a wide variety of functional groups, such as carboxyl (COOH), carbonyl (C=O), and hydroxyl (OH), which can react with metal ions to form complexes or chelates. Moreover, humic acids are also characterised by the presence of sulphur- and nitrogen-containing groups, prevalently S=C, SH and amide functions, which are able to bind the metals. Though the pathways for the uptake of the metal ion into living systems and their toxicological mechanisms are still unclear, many toxic effects are attributed to the free metal ions. Therefore, the strong binding capacity of humic substances towards metals gives these molecules a fundamental role in the reduction of the toxic potential of metals.

In the present paper we studied the complexation capacity towards copper ion of fulvic acids extracted from Arno River and Lake Como sediments, as well as from Antarctic Sea water. Complexation capacity values were obtained using an ion-selective electrode (ISE) titration procedure at different pHs in order to show up the pH effect on metal ion-humic compound interactions.

At the same time calorimetric measurements were performed at different pH values in order to study the possibility of interaction of different complexing groups of fulvic acids with copper ions. Moreover, the heat of reaction in the complexation of copper ions with small molecules containing functional groups similar to fulvic acid was also determined.

Experimental

Humic substances

Fulvic acids (FA) were extracted from Arno River and Lake Como sediments according to Rashid and King's method [4]. After acidification (pH 2) of the solution, the sedimentary fulvic acids extracted by basic solution were purified by passing through an XAD-8 resin column that retained the fulvic acids, following Thurman and Malcolm's procedure for water samples [5]. The FA recovery by column was carried out with 0.5 M NaOH and, after neutralisation, the FA solution was diafiltered using an ultrafiltration procedure (Amicon stirred ultrafiltration cell, model 8400, capacity 400 mL, using a 500 Dalton membrane disc (diameter 76 mm)). The cell was connected to a reservoir (5 L) containing diafiltrate solution (deionized water) and pressurised in a nitrogen atmosphere (4 atm). This method allowed the large amount of inorganic salts remaining as sample impurities after recovery by XAD-8 resin column to be eliminated. After purification, the fulvic acids were lyophilised.

The procedure proposed by Thurman and Malcolm [5] was used for FA recovery from Antarctic seawater. Briefly, after acidification to pH 2, the water sample (30 L) was passed through the Amberlite XAD-8 resin column (36 mL) [6]. FA was recovered from the columns with 0.1 M NaOH. FA solution was diafiltered using the ultrafiltration procedure mentioned above and then lyophilised.

The water sample (30 L) was not filtered so as to allow the simultaneous recovery of dissolved fulvic acids sorbed onto the particulate material.

Standard solutions

Water purified using a Milli-Q system (Millipore, resistance 18 M) was used to prepare all solutions. A standard Cu(II) solution (0.1 M Cupric Activity Standard solution Orion Cat. N°942906) was used to prepare dilute solutions (10^{-5} 10^{-3} M) for electrode calibration. 0.5 N NaOH and 0.5 N HNO_3 solutions were used to obtain pHs of 5.0, 6.0 and 7.0, and to maintain constant pH values during titration. Titrations were performed under constant ionic strength conditions, which were obtained with an ionic strength adjuster (ISA) (5 M $NaNO_3$).

The Orion model 94-29 Cupric Electrode was used to measure free cupric ions during titration. It was connected to a Crison model 2002 pH-meter. The ion-selective electrode was conditioned for 24 h with solutions of increasing concentration of copper (10^{-7}÷10^{-3} M). The Orion model 90-02 Double Junction Reference Electrode was also connected to the pH-meter. The reference electrode was a sleeve-type Ag/AgCl with an outer chamber filled with 10% KNO_3 solution which isolated the inner reference element and filling solution (saturated AgCl solution) from the sample. pH values were measured with a Crison combination pH electrode connected to a model HI8014 Hanna pH-meter. Before each experiment a calibration curve was constructed to test the ion-selective electrode slope, which is influenced by temperature (at 25°C the slope is 29.58); all experiments were performed under constant light conditions because the Cu(II) ISE is photosensitive.

Binding capacity determination

Binding capacity of fulvic acids was measured by adding different volumes of standard Cu (II) solution (10^{-3} M) to 50 mL of fulvic acid solution (20 mg L^{-1}) at pH 5.0, 6.0 and 7.0 (I=0.1 M $NaNO_3$). NaOH 1.0 M was added to adjust the pH value after adding Cu (II) solution [7]. The measurements were made after 24h with an ISE. The complexation capacity curves were obtained by plotting free metal (mmol L^{-1}) *vs.* total added metal (mmol L^{-1}). The plot pattern presented a slope change with a final portion; the slope, approximately equal to 1.0, indicated that the complexing capacity had been reached. At least three titrations were performed for each sample at each pH.

Calorimetric method

Calorimetric measurements were made using an LKB 2107 batch microcalorimeter of the heat conduction type [8], equipped with two gold vessels of about 7 mL volume (consisting of a chamber divided by an interior wall into two compartments), a multi-temperature cooling circulator (LKB model 2209), a control unit (LKB model 2107-350) and a potentiometric recorder (LKB model 2210). The reactants are introduced into the two compartments separately; when the experiment starts the calorimetric drum rotates, thereby the reactants are mixed and the reaction takes place.

All the equipment was housed in a thermostatic room at 25±1°C and all measurements were made at 25.00±0.01°C. The calorimetric accuracy was checked by measuring the sucrose dilution heat and the results were in agreement with the literature values within 0.5 % [9].

Measurements were made in a differential way by filling the calorimetric vessel as follows: 2 mL of the copper (II) solution 10^{-3} M at pH 5 were introduced into the 2.5 mL compartment and 4 mL of the fulvic acid solution (40 mg L^{-1}) at the various pH values, into the other compartment (measuring vessel). 2 mL of the same copper (II) solution as before were placed in the reference vessel, together with 4 mL of water at the same pH values as the FA solution.

In order to measure any possible heat effect due to the FA dilution or neutralisation, some preliminary measurements were made by filling the measuring vessel with 2 mL of water at pH 5 and 4 mL of FA solution at various different pH values, the reference vessel was filled with 2 mL of water at pH 5 and 4 mL of water at pH values as FA solutions. No heat effects were found in any of these cases. All the solutions were prepared in 0.1 M $NaNO_3$ to maintain the ionic strength constant.

Results

For fulvic acids the complexing capacity with copper (II) ion was determined at pH 5, 6 and 7 (Table 1). The fraction of metal bound generally increased with increasing pH values.

For fulvic acids from the Arno river and Lake Como the increase between pH 5 and pH 6 was small or non-existent. Conversely, large differences were observed between pH 6 and 7. For the Antarctic sample the increase was practically the same in all three pH values.

Table 1 Complexation capacity of Fulvic Acids at different pH values

	Complexation capacity/μg g^{-1}		
pH	Antarctica	Como	Arno
5	14.2±0.8	53.1±1.3	44.5±0.9
6	25.1±0.3	60.2±1.6	40.7±0.7
7	34.1±1.1	136.9±3.1	97.7±1.3

The results were attributed to an increase in the ionization of the fulvic acid molecules. However it must be borne in mind that humic compounds from different sources have functional groups that differ both in number and in nature.

Calorimetric measurements show that the quantity of heat involved in the metal ion-fulvate interactions determines a decreasing exothermic response with increasing pH values. This behaviour is mainly evident in the fulvic acids extracted from the Arno River, which even give rise to an inversion of heat sign (endothermic response) at pH 7 (Fig. 1).

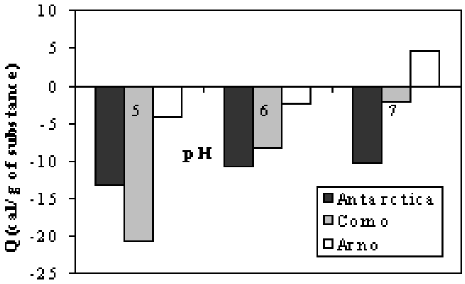


Fig. 1 Heat quantities relative to the complex interactions of the Fulvic Acids (FA) of different origin with copper(II) ions, obtained at different pH values

These results may be accounted for by considering that fulvic acids are a heterogeneous ligand, and that the binding process consequently involves several binding sites [10, 11]. Metal ions are bound both with and without proton release and pH changes can only act on binding sites involving proton release.

To gain further insight into the complexation interactions of FA with copper ions, similar calorimetric measurements were carried out on small organic molecules, and the relative heats of reaction evaluated. These molecules are characterised by functional groups that may be different or similar to that of fulvic acids.

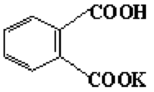
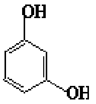
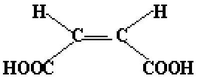
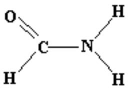
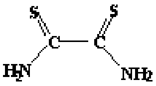
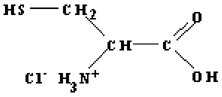
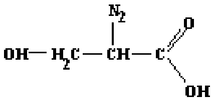
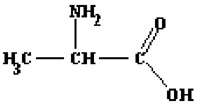
The organic compounds and the respective heat of reaction found at different pH values are listed in Table 2.

It is apparent that only the formation of the Cu(II)-dithiooxamide complex gives rise to an exothermic response, which is not dependent on the pH value. Moreover, the amount of heat involved in the reaction is the highest in absolute terms. The other compounds instead give an endothermic response, which generally increases with increasing pH value. The particular position of the sulphur groups in the dithiooxamide molecule could be responsible for the calorimetric results obtained. Consequently, the presence of sulphur atoms having a similar disposition in the fulvic acid molecule could justify the results at pH 5. At this pH value only a small portion of the protonated groups are present in deprotonated form and their quantity depends on the nature of the single group. In this situation the actual amount of metal bound in the complexing process is relatively unimportant. The pH increase produces a further deprotonation of acidic groups which are able to bind copper ions. The complexation process due to these functional groups seems to be characterised by an endothermic response, which could balance the exothermic effect due to other groups that coordinate copper ions without proton release.

It must be underlined that acidic groups are present in fulvic molecules in greater amounts than non acidic groups.

The elemental analysis (Table 3) of the fulvic acids examined shows the presence of sulphur atoms in all three samples; although significant, this presence is not sufficient because the structural arrangement of the element in the molecules, which is of fundamental importance, is not known.

Table 2 Heat reaction of binding between copper(II) ion and small organic molecules, found at different pH values

Substances		pH	Q (cal/g of substance)
Potassium phthalate monobasic		5	+12.19±0.31
		7	+21.89±0.63
Resorcinol		5	+3.38±0.13
		7	+3.89± 0.19
Maleic acid		5	+8.94± 0.31
		7	+33.75±0.13
Formamide		5	+3.06± 0.38
		7	-0.94±0.19
Dithiooxamide		5	-134.69±0.31
		7	-136.94±0.25
Cysteine chlorhydrate		5	+18.81±0.29
		6	+21.94±0.03
		7	+16.19±0.14
DL-Serine		5	+15.50±0.38
		6	+16.63± 0.13
		7	+38.81±0.15
DL-Alanine		5	+16.13±0.24
		6	+13.19±0.31
		7	+7.63±0.21

We can only make assumptions based on the knowledge of the place of origin.

It is possible that in the Antarctic sample a significant part of the sulphur is present as cysteine because of the nature of its parent material; conversely, in the Como sample, the sulphur is probably present in a reduced form because the sample came from an anthropogenic environment.

Conclusions

Our study indicated that calorimetric analysis is a technique that can provide information about the kind of functional groups taking part in complexing processes between heavy metals and humic compounds. This technique must be supported by a targeted structural characterization.

Table 3 Element analysis (free-ashes) of fulvic acids

	N/	C/	H/	S/	O*/
			%		
Antarctica	9.23	37.05	6.55	4.40	42.77
Como	7.74	50.04	6.30	0.48	35.44
Arno	8.86	51.74	7.09	0.86	31.45

Relative standard deviations are less than 3%
*calculated by difference w.r.t. 100

For small organic molecules in particular we found that the sulphur-containing groups (S=C) gave a strong exothermic response and that the endothermic response of the carboxylic groups was not so strong in absolute terms. In fulvic acids the number of sulphur-containing complexing sites is less than for acid groups, and consequently, at pH values 5 and 6, the exothermic response may be associated with the presence of C=S groups. Increasing the pH value increases both the deprotonization process and the amount of metal bound to carboxylic and phenolic groups. This process is characterized by an endothermic response.

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