Comparison between stability constants of cadmium and lead complexes with humic substances of different molecular weight isolated from Baltic Sea water*

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Waldemar Grzybowski
Institute of Oceanography,
University of Gdańsk,
al. Marszałka J. Piłsudskiego 46, PL–81–378 Gdynia, Poland;
e-mail: grzyb@panda.bg.univ.gda.pl

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Abstract

The complexation of cadmium and lead with humic substances was studied using differential pulse anodic stripping voltammetry and a standard addition technique. The titration was done for humic substances of different molecular weight that had been isolated from seawater and subsequently redissolved in organic-free seawater. The different molecular weight fractions were obtained by ultrafiltration using 1000 D (Dalton), 5000 D and 10 000 D pore size filters. Comparison of the calculated stability constants suggests that the strengths of lead complexes in the analysed fractions are similar and that cadmium is complexed by the fraction smaller than 1000 D.

1. Introduction

The so-called passive uptake of metals by organisms (initial adsorption onto the surface followed by diffusion into the cell) is a function of the strength of the bond between a metal and a ligand. Since metals are hydrophilic and cell membranes hydrophobic, transport across a membrane is promoted by complexation with an organic ligand; within the cell, exchange of one ligands for a more strongly complexing one may prevent

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back diffusion. Another suggested type of uptake is passage from the aqueous environment to the interior of the cell along a sequence of ligands of increasing strength (Simkiss *et al.* 1982). Thus, the degree and nature of complexation are important factors in determining and predicting the ecological significance of heavy metals. The experimental parameter describing the strength of metal complexation is the stability constant. The purpose of the present research was to analyse the binding of lead and cadmium by marine humic substances, the most important organic components of seawater capable of complexing metal ions. These substances are operationally defined (Thurman & Malcolm 1981) class of macromolecular, refractory organics responsible, among other things, for the colour of seawater. The intention was to compare the strength of metal binding by substances of different molecular weight under conditions closely resembling those in seawater. The Baltic Sea humic substances were separated into fractions of different molecular weight by high-pressure ultrafiltration.

2. Material and methods

Humic substances were isolated from Gulf of Gdańsk surface water by sorption on Amberlite XAD resin. The details are given in Grzybowski & Pempkowiak (1991). The solutions thus obtained were separated into different molecular weight fractions by ultrafiltration in a pressurised, stirred ultrafiltration cell (Amicon 202). The concentration factor (volume ratio of ultrafiltrate to concentrate) did not exceed 5. Prior to filtration the filters were extensively flushed with redistilled water until UV absorbance of the ultrafiltrate was negligible.

The ultrafiltrates of the humic substances were then vacuum-distilled and the concentration of the fractions determined gravimetrically. As the quantity of the $>10\,000$ D fraction was insufficient to prepare a large enough solution for analysis, the experiment was limited to the fractions (f) $f < 1000$ D, $1000 \text{ D} < f < 5000$ D and $5000 \text{ D} < f < 10\,000$ D. The different molecular weight fractions were redissolved in organic-free seawater at pH ~8 to a concentration of 1 mg dm^{-3}. This concentration is close to that encountered in Baltic Sea water (Pempkowiak 1989) and is sufficiently low to avoid interference from the reduction of organic substances at the working electrode (Varney *et al.* 1984). Organic substances were removed from the seawater by irradiation with a 1 kW medium-pressure Hg lamp.

The metal concentrations were measured by Anodic Stripping Voltammetry (ASV) (Florence 1971) with a rotating, pre-deposited mercury-film electrode. The titrations were done separately for lead and cadmium. The voltammetric measurement consisted of the following steps:

- deoxygenation by a 10 min purge with nitrogen,
- reduction of metal ions (potential of –0.8 V versus Ag/AgCl electrode) during a deposition time of up to 30 min,
- cessation of mercury-film electrode rotation; after a 20 s rest time, the deposition potential was scanned positively to –0.1 V in the differential pulse mode at a pulse height of 0.5 mV.

The oxidation current was recorded as a function of the potential using an X–Y recorder. The free metal concentration was calculated from the height of the oxidation current peak by the standard addition method. The coefficient of variation did not exceed 20%.

3. Results and discussion

In order to obtain environmentally significant data, the research was performed at realistically low concentrations of both metal ions and humic substances, and the complexation was measured in a solution containing the full inorganic seawater matrix at its natural pH. The experimental technique used was anodic stripping voltammetry (ASV), a technique commonly used for determining metal concentrations and investigating complexation processes. It is sufficiently sensitive to monitor metal concentrations in unpolluted waters without the need for their extraction or concentration.

Fig. 1 presents the results of the ultrafiltration of humic substances isolated from Gdańsk Deep water as a percentage of different molecular weight fractions in the bulk material.

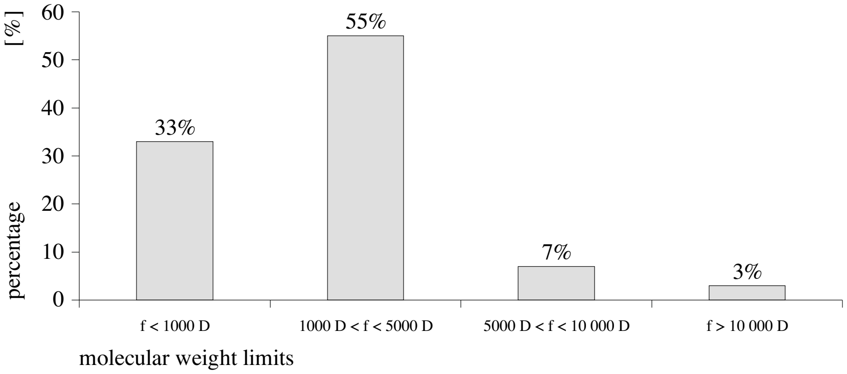

Fig. 1. Distribution of different molecular weight fractions of humic substances isolated from Gulf of Gdańsk surface water

As can be seen in Fig. 1, the efficiency of separation was high, with only ~2% lost due to irreversible sorption. Almost all of the substances isolated fall in the range $< 10\,000$ D. The distribution did not differ significantly from that obtained by Makinen *et al.* (1994). However, there is a report on the molecular size of Baltic humic substances (Plechanov *et al.* 1983) where no substances > 5000 D were detected. It should be stressed that the pattern of molecular weight distribution obtained in the present work may differ from that in seawater due to artefacts resulting from the isolation procedure.

The AAS analysis of humic substances did not detect the presence of Pb or Cd. The respective initial concentrations of lead and cadmium in organic-free seawater that served as solute were 0.11 and 0.06 μg dm^{-3}. In order to improve the sensitivity of complexation detection, the metal levels were lowered by the long-term storage of the UV-irradiated seawater (at natural pH) in acid-cleaned glass vessels. The decrease in ASV-detectable metal ions during storage can be ascribed to irreversible sorption on the glass (Davison 1987). This phenomenon was not observed in the PTFE analytical cell.

Monitoring the complexation process involved titrating the solution with an added metal and plotting the signal (the oxidation peak height) as a function of the added metal. It is assumed that the response of the apparatus (the increase in peak height) is related to the concentration of non-complexed, 'free' metal species. After the complexing capacity of humic substances has been used up, all of the added metal is detected as non-complexed where the titration curve becomes steeper.

The calculation of the stability constants demanded discrimination between the complexing and non-complexing sections of the titration slope. To do this, use was made of the fact that the equilibration time between metal and ligand in seawater is longer than the measurement period, very probably because of the presence of Ca and Mg (Sugai & Healy 1978). If complexation takes place, there is a gradual decrease in the sequentially recorded peak height. Fig. 2 illustrates the average range of the changes (error bars represent 1 S.D.).

All the DPASV measurements were made in duplicate: immediately after standard addition and after 40–60 min. If no statistically significant differences were detected, it was assumed that the complexing capacity had been used up. Determination of stability constants was based on data from the initial sections of the titration plots where complexation occurred. Fig. 3 shows an example of a cadmium titration plot obtained in a solution of < 1000 D humic substances. The data points represent values recorded after the equilibration period.

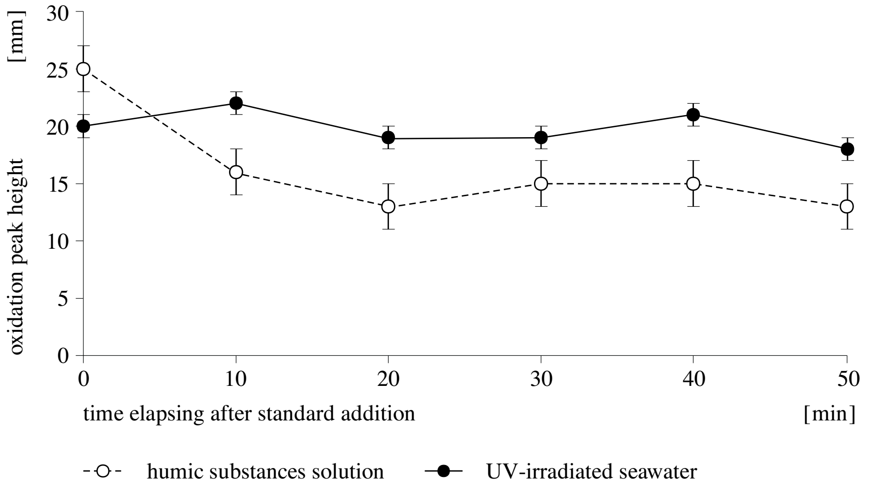

Fig. 2. Change in the lead oxidation peak height measured sequentially after a single standard addition

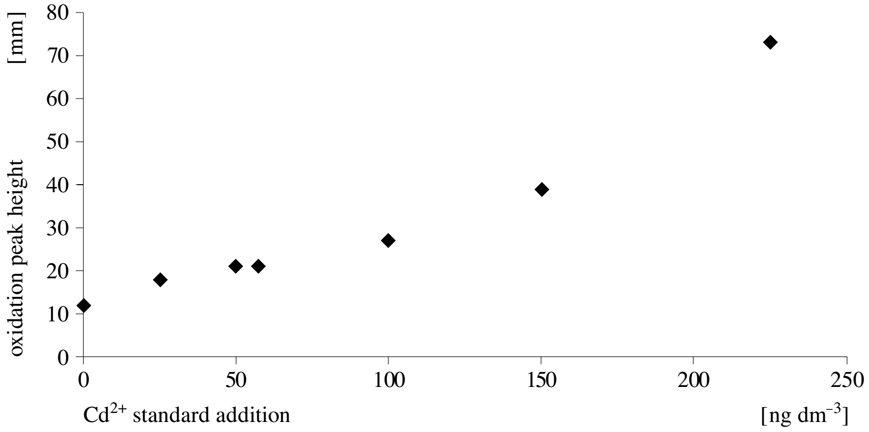

Fig. 3. Cadmium titration curve of a 1 mg dm^{-3} solution of humic substances of molecular weight < 1000 D in organic-free Baltic Sea water

Assuming 1:1 stoichiometry, the equilibrium between metal and ligand is expressed by eq. (1):

$$[\mathrm{Me{-}L}] \leftrightarrow [\mathrm{Me_{free}}]\ [\mathrm{L}], \qquad (1)$$

where

[Me–L] – complex concentration,
$[\mathrm{Me_{free}}]$ – free metal concentration,
[L] – free ligand concentration.

The stability constant K is defined as follows:

$$K = \frac{[\mathrm{Me{-}L}]}{[\mathrm{Me_{free}}]\,[\mathrm{L}]} = \frac{[\mathrm{Me_{tot}}] - [\mathrm{Me_{free}}]}{[\mathrm{Me_{free}}]([\mathrm{L_{tot}}] - [\mathrm{Me_{tot}}] + [\mathrm{Me_{free}}])}, \tag{2}$$

where

$[\mathrm{Me_{tot}}]$ – concentration of the total metal (metal ions present in the sample before titration plus the sum of standard additions),
$[\mathrm{Me_{free}}]$ – concentration of the 'free' metal (detected by DPASV),
$[\mathrm{L_{tot}}]$ – total concentration of humic substances.

The typical method of calculating the stability constant is based on the linearisation of the titration curve according to the equations of Ruzic (1982). The calculated concentration represents not chemically defined moieties but binding sites available to the metal. That method of determining stability constants is usually applied to seawater containing undefined organic substances of unknown molar concentration (Bruland 1992, Muller 1996).

In this work, the applicability of the Ruzic method to the titration curves was limited owing to problems with obtaining dependable linearised plots. Apart from their poor repeatability, the calculated Ruzic plots were not linear, possibly because the analysed substances contained more than one class of complexing ligands (Ruzic 1982). Values of K were therefore calculated using the assumed molar concentration of humic substances based on the nominal cut-off of the ultrafiltration filters. The weak points of this procedure are that, owing to the impossibility of establishing the actual molecular weight distribution, the set of molecular weights is chosen in arbitrary fashion, and that the apparent value of K is a sum resulting from different ligands present in the analysed fraction. Thus, comparison of the values in Table 1 with those calculated using the Ruzic (1982) method is basically qualitative. Moreover, the problem of the limited comparability of published values is also due to the diversity of analytical

Table 1. Logarithms of the conditional stability constants of lead and cadmium complexes with marine humic substances determined in an inorganic seawater matrix at natural pH

Limits of molecular size fractions	Logarithm of stability constants	
	cadmium	lead
f < 1000 D	6.1	6.4
1000 D < f < 5000 D	–	6.6
5000 D < f < 10 000 D	–	7.4

techniques used to detect 'free' metal ions–see the review by Mantoura (1981). Nevertheless, though absolute values are of limited applicability, they do allow an assessment of the mutual relationship between different molecular weight fractions and metals, and they can be used in comparisons with other marine organic substances with a fixed molar concentration.

Because the apparent molar concentration of humic substances was at least two orders of magnitude higher than that of the metals, eq. (2) could be approximated as follows:

$$K = \frac{[\mathrm{Me_{tot}}] - [\mathrm{Me_{free}}]}{[\mathrm{Me_{free}}]([\mathrm{L_{tot}}] - [\mathrm{Me_{tot}}])}. \tag{3}$$

$[\mathrm{Me_{free}}]$ was obtained from the titration plot according to

$$[\mathrm{Me_{free}}] = h\ S, \tag{4}$$

where

h – height of the oxidation peak,

S – sensitivity of the ASV technique obtained from the slope (metal addition/Δh) of that section of the titration curve where the complexation capacity was used up.

The values of log K in Table 1 were calculated using the points of the titration curve from the section where complexation occurred, with an assumed molecular weight at the mid-point between the fraction limits. Since the pH and inorganic constituents of the solution both strongly affect metal allocation (Davison 1987, Hering & Morel 1988), the measured values are conditional, *i.e.* influenced by the seawater matrix. Since the analysed solutions had the same salinity, that typical pH (∼8.1) of the Baltic Sea, one can assume that the results give some insight into natural processes, and that the observed differences result from the inherent features of the analysed fractions of humic substances.

The absence of cadmium complexation in the >1000 D fractions does not necessarily mean that the metal is not organically complexed. This could be because the measurement sensitivity was low, the complexing capacity was consumed by cadmium originally present in seawater, or the metal-humus binding was too weak (Raspor *et al.* 1984). However, the above result concurs with reports on cadmium complexation detected only in a low-molecular fraction of humic substances isolated from a lake (Giesy 1983) and estuarine water (Haekel 1984). The stability constant obtained for cadmium is higher than that reported by Mantoura *et al.* (1978) for oceanic water. This discrepancy may be due to the different salinity of the analysed solutions–investigations on soil humic substances have shown that log K values decrease with ionic strength (Stevenson 1976).

The stability constants of lead complexes increase with molecular weight. This relationship, however, is very weak and may be an artefact resulting from the assumed ligand concentration. The stability constants of lead within the range obtained had been reported for freshwater humic substances by Buffle *et al.* (1977).

The stronger complexation of lead has been detected in experiments with artificial ligands (EDTA) in seawater (the values however were 3–4 orders of magnitude higher) (Raspor *et al.* 1980, Nürnberg & Valenta 1983). The complexation capacity of lead was also found to be higher in an experiment with non-fractionated humic substances (Pinheiro *et al.* 1994).

The published data on metal complexation in seawater obtained with the Ruzic method (1982) in general agree qualitatively with the data presented here: cadmium complexes were detected mainly in the <1000 D fraction (Wells *et al.* 1998, Muller 1999) or their amount was ∼3 times higher than that in the 1000 D–10 000 D fraction (Wen *et al.* 1999).

The absolute values reported in this paper are lower than those determined in experiments with solutions of glutamic acid and glycine (Stumm & Brauner 1975). Assuming they are comparable to some extent, one can conclude that migration across a cell membrane (passive uptake) of cadmium and lead in the marine environment is unidirectional (*i.e.* from humic complexes towards amino acid complexes within the cell plasma).

The fact that cadmium binding was observed only in the low-molecular-weight humus substances may also be of environmental significance: the small organic molecules are more easily transferred via the cell membrane than larger molecules. In addition, the removal of organic substances by flocculation in areas where salt- and fresh-water mix, affects mainly the high-molecular fraction (Sholkowitz 1976). The influence of that process on cadmium concentration was examined by Powell *et al.* (1996), who demonstrated that small cadmium complexes are resistant to removal.

In summary, the measured stability constants of humic substances of different molecular weight with lead did not differ significantly. This suggests that the analysed fractions possessed binding sites of comparable efficiency. A different pattern was observed for cadmium, where complexation was detected solely in the low-molecular-weight fraction. This fraction complexed cadmium and lead with comparable strength. Even though experimental bias cannot be excluded, this finding may indicate that discrimination between these metals occurs in fractions larger than 1000 D.

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