# Thiol groups controls on arsenite binding by organic matter: New experimental and modeling evidence

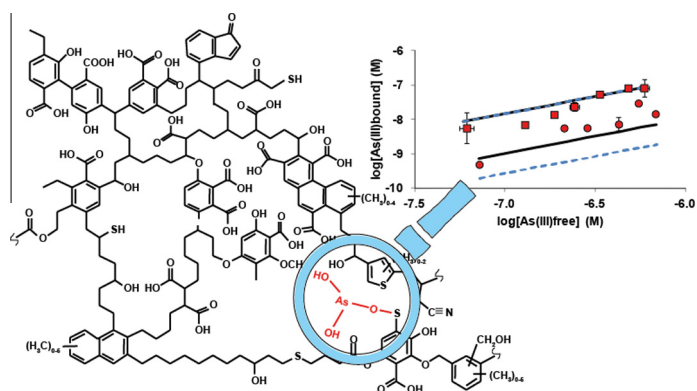Charlotte Catrouillet [a,*], Mélanie Davranche [a], Aline Dia [a], Martine Bouhnik-Le Coz [a], Mathieu Pédrot [a], Rémi Marsac [b], Gérard Gruau [a]

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## GRAPHICAL ABSTRACT



## ARTICLE INFO

## ABSTRACT

Although it has been suggested that several mechanisms can describe the direct binding of As(III) to organic matter (OM), more recently, the thiol functional group of humic acid (HA) was shown to be an important potential binding site for As(III). Isotherm experiments on As(III) sorption to HAs, that have either been grafted with thiol or not, were thus conducted to investigate the preferential As(III) binding sites. There was a low level of binding of As(III) to HA, which was strongly dependent on the abundance of the thiols. Experimental datasets were used to develop a new model (the modified PHREEQC-Model VI), which defines HA as a group of discrete carboxylic, phenolic and thiol sites. Protonation/deprotonation constants were determined for each group of sites ($pK_A = 4.28 \pm 0.03$; $\Delta pK_A = 2.13 \pm 0.10$; $pK_B = 7.11 \pm 0.26$; $\Delta pK_B = 3.52 \pm 0.49$; $pK_S = 5.82 \pm 0.052$; $\Delta pK_S = 6.12 \pm 0.12$ for the carboxylic, phenolic and thiols sites, respectively) from HAs that were either grafted with thiol or not. The $pK_S$ value corresponds to that of single thiol-containing organic ligands. Two binding models were tested: the Mono model, which considered that As(III) is bound to the HA thiol site as monodentate complexes, and the Tri model, which considered that As(III) is bound as tridentate complexes. A simulation of the available literature datasets was used to validate the Mono model, with $\log K_{MS} = 2.91 \pm 0.04$, i.e. the monodentate hypothesis. This study highlighted the importance of thiol groups in OM reactivity and, notably, determined the As(III) concentration bound to OM (considering that Fe is lacking or at least negligible) and was used to develop a model that is able to determine the As(III) concentrations bound to OM.

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# 1. Introduction

According to the World Health Organization (WHO), arsenic (As) is known to be a major poison in the world. Even at low concentrations, As causes serious damage to human health such as cutaneous lesions (black foot disease), cancers (skin, lung, bladder, etc.), cardiovascular diseases, respiratory problems.... The main contamination process occurs through the consumption of As-contaminated water and the ingestion of contaminated crops (such as rice). Arsenic-contaminated water affects millions of people in Argentina, Bangladesh, Chili, China, United States, India, Mexico, etc. The abundance of As in the soil and water primarily depends on the geology and human activity (historic or current). As(III) is the most toxic inorganic form. Many studies have been performed to understand the mechanisms responsible for the contamination of water by As [1–5]. Wetlands and floodplains have been highlighted as a source of As for the surrounding environments [6–8]. Arsenic-rich sediments, in which As is bound to Fe-oxyhydroxides, are deposited in riparian wetlands and floodplains during flooding events. In the anoxic, organic-rich environments that characterize these zones, Fe-oxyhydroxides are reductively dissolved and the associated As is released into the soil solutions, and are then available for transfer in the underlying aquifers. However, there is a lack of information regarding the fate of As in this type of Fe(II), OM-rich solution. In such environments, OM is often discussed as a source of carbon for the heterotrophic bacteria able to reductively dissolve Fe-oxyhydroxides and solubilize the associated elements, such as As, or to directly reduce As(V) to As(III) in their detoxification metabolism [9–13]. Organic matter is also shown to be a competitor of anions, such as arsenite, for their binding to the functional sites of Fe-oxyhydroxides [4,14–16]. Several studies present OM as a possible ligand for As(III), but no consensus exists about the nature of the exact direct or indirect mechanisms involved. Thanabalasingam and Pickering and Warwick et al. proposed that As(III) is bound to humic acid (HA) through cationic bridges involving Al, Fe and Ca impurities occurring in HA or by direct binding through HA amino groups [17,18]. Using Suwannee River HA (SRHA), Buschmann et al. and Lenoble et al. hypothesized that As(III) could be bound to HA through its carboxyl and phenolic functional groups [19,20]. Alcohols are able to bind As(III) by losing an $OH^-$ [21], suggesting that As(III) could be bound to phenolic groups of OM. Regarding the weaker and more abundant carboxylic groups, Buschmann et al. suggested that binding could occur through the formation of H-bridges between the $OH^-$ group of $As(OH)_3$ and the $=O$ part of the group without any OH loss. In their study, these authors also proposed that Fe might act as a bridge between As(III) and HA [19]. Liu and Cai and Fakour and Lin, through experimental and modeling approaches, hypothesized that As(III) was bound to HA by two kinds of binding sites, one strong and one weak [22,23].

More recently, spectroscopic and experimental studies have suggested that thiol ($SH^-$) could play an important role in As(III) binding to peat and HA [24,25]. These authors demonstrated that As(III) is bound to tridentate or monodentate complexes via thiol groups, depending on the selected peat and HA. By spiking HA and peat with bisulfides, Hoffmann et al. showed that As(III) binding increased with increasing bisulfide concentrations [25]. Using EXAFS, they provided evidence of the formation of a monodentate thiol-As(III) complex on S(-II)-spiked HA and peat. Conversely, Langner et al. used EXAFS data to show that As(III) is bound to three S in peat from Gola di Lago (Switzerland) [24]. The different complexes might be explained by differences in the origin of the organic matter (OM). In the Gola di Lago peatland, peat was formed in an As-enriched environmental context. Arsenic was absorbed by plants and/or microorganisms, which were the precursors of the peat. In these precursors, As was bound to proteins, enzymes, etc., mainly as tridentate complexes with the $SH^-$ group of cysteine; a configuration that seems to be conserved in the peat structure [25]. The binding of As(III) with thiol is not surprising considering that As(III) is bound to dithiol and trithiol sites in many proteins and peptides [26–33], either completely or partly inhibiting their specific actions in the body.

Here, we present a new contribution to evaluate the role of thiol sites in the binding of As(III) to OM. More specifically, considering the recent spectroscopic studies, we tried to determine the mechanisms of complexation between As(III) and the thiol groups in HA (the formation of mono- or tridentate complexes), using experimental and modeling approaches. Arsenic(III) was reacted with three samples of HA containing different concentrations of thiol sites. The experimental dataset was subsequently used to test the hypothesis of As(III)–HA binding through mono or tridentate complexes via HA thiol groups, using a combination of the PHREE-PLOT (fitting program) and PHREEQC-Model VI programs. No model is currently available in the literature to describe the interactions between As(III) and organic matter. In a first step, the thiol groups had to be described and their binding parameter was introduced in PHREEQC-Model VI. Finally, the extrapolated binding parameters from the hypothesis of mono- or tridentate As(III)–thiol (HA) complexes were tested using the whole datasets available in the literature to identify the more valuable binding mechanisms. The goal of the model developed in the present study is to determine the speciation of As(III) in OM-rich water.

# 2. Experimental, analytical and modeling methods

## 2.1. Reagents and materials

All aqueous solutions were prepared with analytical grade Milli-Q water (Millipore). The As(III) solutions were prepared with sodium arsenite ($NaAsO_2$) from Sigma Aldrich. The S(-II) solution was prepared with sodium sulfide nonahydrate ($Na_2S\cdot9H_2O$) from Sigma Aldrich. NaOH, HCl and $HNO_3$, all sub-boiling ultrapure grade, came from Fisher Chemical, Merck and VWR, respectively.

Humic acids corresponded to the standard HA purified Leonardite from the International Humic Substance Society (IHSS) and the Aldrich HA (AHA) from Sigma Aldrich, which have different concentrations of S. To remove humins from the humic and fulvic acids, AHA was purified (PAHA) using the method described by Vermeer et al. [34]. Prior to the experiments, molecules <10 kDa were removed using a Labscale TFF system equipped with a Pellicon XL membrane (PGCGC10, Millipore™) for the two standard solutions used (Leonardite and AHA).

All materials were soaked in 10% $HNO_3$ and then rinsed with deionized water twice overnight.

## 2.2. Experimental setup

### 2.2.1. Thiol grafting experiment

To obtain HA with different concentrations of thiol groups (S(-II)), S(-II) were sorbed to Leonardite using a dialysis bag (pore size = 12–14 kDa) at a ratio [S(-II)]/[DOC] ≈ 6 mmolS/molC, as was done previously in a precedent study [25]. No grafting was performed for PAHA because of its natural high concentration in S. Humic acids and the S(-II) solutions were placed inside the membrane, whereas S(-II) was outside. The pH was maintained at 6 and the ionic strength (IS) was fixed at 0.05 M with NaCl. The Leonardite grafted with thiol groups will be noted S(-II)-Leonardite hereafter. To prevent any oxidation of S(-II), the experiments were performed in a Jacomex isolator glove box. The