

Biogeosciences Discuss., author comment AC3
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Reply on CC1

Loes J. A. Gerringa et al.

Author comment on "Comparing CLE-AdCSV applications using SA and TAC to determine the Fe-binding characteristics of model ligands in seawater" by Loes J. A. Gerringa et al., Biogeosciences Discuss., <https://doi.org/10.5194/bg-2021-134-AC3>, 2021

Below the remarks of the reviewer (**Reviewer**), followed by our reactions (**Answer**)

Reviewer: This is an interesting manuscript and a welcome addition to this field, however in reading through its conclusions regarding the underestimation of the model ligand concentrations it is apparent that some key aspects have been overlooked in the analysis to date.

Answer: Thank you Peter Croot.

Reviewer:

- Independent determination of ligand concentration

For many of the siderophore ligands the purity of commercial sources is not 100% and for desferrioxamine B it is typically 90-95% depending on the manufacturer and lot/batch number. Earlier works also suggested that some siderophores were not stable in solution as they were easily degraded, though other studies have shown that solutions can be stable for days to weeks (Hayes et al., 1994). It is important then to obtain an independent determination of the model ligand concentration, rather than simply assume 100% and using the mass weighed out initially. This is frequently done using ASV in clean KCl or NaCl solutions and titrating with Cu, as then there should be no interferences. In the current work there is no information on how the ligand concentration was assayed prior to analysis.

Answer: This is an interesting point raised by the reviewer. We did not estimate the purity of the siderophores. A deviation from 100% purity of the siderophores can explain the deviation of [L] from the added amount. However, it does not change the differences between the applications and the discussion on this point. We added in the text that we did not do any research on purity of the siderophores:

At the methods section second line of 3.1.4 lines 238-240: "No tests were undertaken to check the purity of the siderophores. The solutions were used within two weeks after preparation, and kept in the refrigerator in the dark at 4°C, which should at least for DFOB be short enough to prevent degradation (Hayes et al., 1994)".

In the discussion we added at lines 525-528 (accepted version): "It is possible that the siderophores used are not of 100 % purity, which would result in a systematic underestimation of L. However, whilst it is interesting to note absolute values, our research focusses on differences between the three applications, which is should not be impacted by any impurities."

Reviewer

- Composition of seawater used in this study:

The paper states that a range of leftover samples were used in this study, though there is no information on their salinity or potential for having other metals which may complex the model ligands under the experimental conditions. For example Cu and V may also be present in seawater at significant concentrations to chelate DTPA, siderophores and fulvic/humic acids thus resulting in lower than expected ligand concentrations when titrated with iron. The question then is, which metals could be present under these conditions to outcompete iron for the model ligands tested? This also is a reminder that all measurements done in natural waters are conditional measurements and this applies to the ligand concentration as well as the stability constant.

Answer:

The reviewer is right, we should have added information on this point. All water used was from the Atlantic Ocean, surface waters were not used. The same water was used per experiment for the three applications, thus the competition between metals for the model ligand should have been equivalent across all three methods. However other metals could have influenced the results. This should have been discussed. As you will see below in our answer, we used part of your text in the addition to the manuscript.

For your information below some info on the samples taken for ligands at depths>100m. We do not give this info in the manuscript, since it might be misleading, we do not know which samples were used, above all they were mixed and for every treatment the three applications received sample from the same UV irradiated mixture:

Parameter	average	stdev	unit	N
pH	7.93	0.08		370
DFe	0.64	0.41	nM	434
DMn	0.23	0.18	nM	434
Salinity	35.09	0.61		434

We added in the method section at fourth line of section 3 Methods (line 186), after:"Consequently, one batch differs from others with respect to DFe content":

", and also potentially in other constituents, such as other trace metals. Since surface samples were not used we do not expect large differences in salinity, the average salinity

was 35.09 ± 0.61 (N=434), obtained as average of all samples >100 m depth taken for the ligand analysis in Gerringa et al. (2015)."

We added in the discussion section at 4.2 Titrations Line 424: "Differences due to variations in sample materials are assumed to be small. However, a variance in the content of metals that could compete with Fe for ligand sites can have influenced the results and might have caused an underestimation of the model ligand concentration and indirectly also have influenced the value of β . This could not have influenced the comparison between the applications, since always the same mixed sample was used per experiment for the three applications. We again emphasize that CLE-AdCSV titrations in natural waters result in the derivation of conditional parameters and this applies to the ligand concentration as well as the stability constant"

Reviewer:

- Phytic acid is not a strong iron chelator under seawater conditions:

While the earlier study on Phytic acid by Witter et al. (2000) suggested that this ligand was capable of chelating Fe(III) in seawater, subsequent work suggests this isn't the case. Indeed calculations based on thermodynamic data (Crea et al., 2008; Torres et al., 2005) suggest that no significant complexes would be formed under seawater conditions. Voltammetric studies (Marolt and Pihlar, 2015) do indicate that both Fe(III) and Fe(II) complexes are formed however though they are very weak. Ultrafiltration studies (Schlosser and Croot, 2008) also indicate that the conditional binding constants in seawater for Phytic acid are significantly lower than that reported in the kinetic titrations of Witter et al. (2000). While Purawatt et al. (2007) using FFF found that Phytic acid reacts with Fe(III) to form colloidal material. These results suggest that Phytic acid is not a strong iron chelator (Luther et al., 2021) and the results reported in the current manuscript should be reinterpreted along those lines.

Answer:

Thank you for this useful information, we indeed should have elaborated on this, since our estimations of β by all methods (except one duplicate obtained with TAC and this value has a large error) are lower than the values of 22.3 (with respect to Fe^{3+}) given by Witter et al., 2000.

We added at section 4.2, line 516:

Reviewer:

Lastly a recent paper (Sanvito and Monticelli, 2021) has suggested that pH buffering is not required for measurements such as this though despite earlier works indicating that it is a critical parameter. One aspect where all speciation work could be improved, and the current work should be no exception, is to include the relevant information on the pH scale (Dickson et al., 2016) being used (NBS, total, seawater, free) to describe the system, along with temperature and salinity (ionic strength) to fully describe the experimental system.

Answer:

We are also convinced that pH is a critical parameter. This applies for the actual measurement as well as the natural conditions (Avendano et al., 2016; Gledhill et al., 2015; Ye et al., 2020; Zhu et al., 2021). We added the pH scale we used, thank you for pointing out this omission. We used the NBS scale added now at the third line of the section 3.2 AL calibration.

author Luis Laglera gave a comment on the paper of Sanvito and Monticelli:

What Sanvito and Monticelli do is leave the pH drift exclusively at the limit layer of the electrode (microns thick) exclusively during the potential scan. In this period H₂O₂ and OH⁻ are formed at the electrode surface as a result of the oxygen reduction reaction (half wave potential about -0.1 to -0.2 V). Their solution bulk pH is controlled by the natural carbonate buffer since they do not purge, this buffer is not affected. What they claim is that this substantial increase of pH during the few seconds of the voltammetric scan increases the sensitivity. This is something Luis checked personally.

Since the amount of OH⁻ formed is so small, the pH of the limit layer can go to 9 (which can be determined by the drift of the peak potential) but the pH of the bulk of the solution remains constant leaving the sample unaffected (checked with a pH electrode inserted in the cell) (Laglera et al 2016). So, the pH is stable up to the quiescence period and then only the tiny percentage of complexes adsorbed onto the electrode which complexed iron is going to be reduced experience a rise in pH for a few seconds.

Answer references:

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Reviewer:

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