Biogeosciences Discussions

Biogeosciences Discuss., author comment AC2
https://doi.org/10.5194/bg-2021-134-AC2, 2021

Reply on RC1

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-AC2, 2021

- General comment

Comment: In this manuscript, three CLE-ACSV methods (TAC, SA5, SA25) for analyzing the concentration and conditional stability constants of organic Fe-binding ligands using several model ligands (DTPA, phytic acid, desferrioxamine B, ferrichrome, ferrioxamine E, vibriobactin, FA and HA). It is notable that not only comparing the analysis results from the CLE-ACSV titrations, but also this manuscript investigates and discusses the differences in the characteristics of the competing ligands SA and TAC and differences in analytical instruments. Although these points have been pointed out as possibilities for some time, there are few studies that discuss their effects based on the actual measured value. In the Conclusion, it is suggested that future studies of organic Fe-binding ligands in seawater requires alternatives to the current CLE-ACSV method, and I agree. Although the model ligands used in this manuscript are just "model" and can be considered different from the natural ligands in the ocean, understanding the characteristics of each CLE-ACSV method was exactly what was needed for research in this area. In addition to this, I am interested in the influence of the results on the multiple analytical windows method. In recent years, a multiple analytical windows technique with SA has been applied to determine the multiple classes of Fe-binding ligands in seawater (e.g., Bundy et al., 2014, which has been refereed in this manuscript). How do you think about the influence of these results on the evaluation of multiple analytical window method?

Answer: We thank the reviewer for her/his comments

The question about the multiple window approach is very interesting. We think the multiple analytical window approach is a very good step forward to obtain more details on metal speciation, but since it estimates more parameters one has to consider carefully the degrees of freedom in the estimation.

First, this approach will only work if distinct groups of organic metal binding ligands do exist and are not shielded by other organic ligands which have a continuum of ligand sites (thinking of humics and the work of Buffle). Continuing in this line of thinking, this approach has more chance of success with Cu because the difference in alpha factor for Cu-binding organic ligands is larger and concentrations are larger, making distinguishing more than one ligand group easier.

Second, by applying the multiple window approach the added ligand concentration [AL]

varies. From the work of Abualhaija and also from our experiments we know that probably the formed metal-added ligand (Me_x-AL_y) species varies with the [AL]. Thus, the range of formed species and whether they are yes of no electro active must be known, together with the conditional binding strengths per Me_x-AL_y species. It surely complicates matters. For example with SA, with increasing [AL], the non-electro-active species will increase (according to Abualhaija), it might even be at cost of the electro-active species, resulting in a decrease instead of the expected increase of the signal.

Third, the kinetic problem we discuss in our manuscript in the 25µMSA application will interfere with the multiple window approach. When a short equilibration is used the slow kinetics of the exchange between iron and natural organic ligands will probably be influenced differently at different [AL]. We think that overnight equilibration will largely overcome this problem.

Fourth, we suggest the possibility that formation of $FeSA_2$ is irreversible. We cannot prove this with the experiments we did. However, suppose we are right then this would interfere with the multiple window approach.

Comment: Overall, this manuscript is organized and well-written. I believe that this manuscript is going to have a strong influence on the development of research in this area. After responding to the following minor comments, I think this manuscript can be accepted.

Answer: We thank the reviewer for the interesting comment above and the suggested improvements below.

Minor comments

Comment: Line 17. Fe3+ should be Fe^{3+}

Answer: Thank you

Comment: Line 125~160. The "Langmuir isotherm assumption" is often mentioned in this manuscript and the outline of the assumption itself is explained. However, as shown in Line 163(…assumption 2 or 6), for example, it is not clear from this manuscript alone what each assumption number refers to, so please indicate it in the text after Line 132.

Answer: Indeed, this is awkward, we added 1-6 in lines 125-160.

Comment: Line 185~. Please indicate the temperature conditions of the samples during equilibration period for each experiment in the main text.

Answer: We added at line 207: "Equilibration between the samples and AL was attained at room temperature."

Comment: Line 227. Please show the reference information of the UV irradiation for the past experiments related to Co and Cu ligand analyses.

Answer: Rapp, et al. (2017) and Wuttig, et al. (2019) assessed the influence of quartz and FEP vessels on UV-digestion efficiency and contamination. No difference between a FEP bottle and a quartz cuvette was observed with regards to the efficiency of UV-digestion using either vessel material.

We added in the text the references: Rapp et al., 2017; Wuttig et al., 2019.

Comment: Line 331~. The explanations of the In-cell experiments and bottle experiments are complicated to understand for people outside the field, so I thought it would be nice to have a schematic diagram as a supplementary figure.

Answer: We understand that it is somewhat complicated, we added a scheme in the Supplementary information as Figure S4

Comment: Line 385. "Using Eqs. (1) and (3) give"

Answer: Indeed, thank you, we changed the text as suggested.

Comment: Figure 1. Please add [A] – [H] in each figure.

In figure [H], the HA concentration is 1 mg and 2 mg in the legend, so please correct it.

Answer: Apologies, this was the wrong figure, we replaced it with the correct one. Thank you for spotting this. Moreover, the complete figure is new, since we changed the colors (see comment at figure 2).

Comment: Line 434. Since the standard deviation can be shown with 3 or more data. If the number of data is 2, strictly speaking, the difference from the average value is the correct notation.

Answer: Thank you, we changed the text as suggested.

Comment: Figure 2. I sometimes suffered from distinguishing the color patterns in these figures. Could you change the color so that it can be easily distinguished in each series?

Answer: We chose these colors to make it easier for persons suffering from color blindness, but we agree that the result is not very good, we made new figures 1 and 2 with other colors.

Comment: Line 449-450. 2A and C?

Answer: Thank you, this is a mistake, we changed it as suggested

Comment: K^cond can be written as K^{cond}

Answer: The reviewer is correct.

Comment: Line 461. sweater: Seawater?

Answer: Yes! Thank you.

Comment: Line 475. Please insert the unit for [L].

Answer: We added nM Eq

Comment: Line 481. There are 3 significant digits and 4 significant digits of log K, so it is better to unify them (only the 0 in the 4th digit disappears?). I think this can be said for the entire manuscript and tables. Or is it due to a difference in method (TAC or SA)?

Answer: Yes we agree and changed all $\log K^{cond}$ values into values with one digit.

Comment: Line 489. Buck et al. (2007)?

Answer: 2010. Thank you for pointing out this mistake, the 2010 is in the reference list.

Comment: Figure 6. The resolution of the figures is rough.

Answer: We improved the resolution.