

Ocean Sci. Discuss., referee comment RC1
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Comment on os-2020-120

Jens Daniel Müller (Referee)

Referee comment on "Technical note: Stability of tris pH buffer in artificial seawater stored in bags" by Wiley H. Wolfe et al., Ocean Sci. Discuss.,
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General comments

Short summary: This technical note introduces the use of gas tight bags for the storage of pH buffer solutions, as an alternative to the storage in bottles. The authors demonstrate - through comparison pH measurements with the spectrophotometric method - that the decrease of the buffer pH can be limited to <0.01 over a period of one year and under laboratory conditions. This is an important finding indicating the potential suitability of such storage bags for repeated calibration measurements of pH instruments during field deployments. The observed pH decrease is attributed to the accumulation of CO₂ and likely sources of CO₂ are discussed.

Overall quality: The technical note is well structured and clearly written. Appropriate references are included and the most important information to ensure reproducibility of the findings are given. This manuscript certainly presents an important contribution to the field and is well placed as a technical note in the Ocean Science journal. I would like to thank the authors for their effort to perform, evaluate and present this study.

Despite the overall high quality of this study, following main aspects could profit from major revisions:

(1) Spectrophotometric pH measurements performed on Certified Reference Materials reveal a spread that is larger than the repeatability that could be achieved with the instrument used (Carter et al., 2013). I wonder why this is the case and if it has an implication for the interpretation of the measurements on the buffer solutions.

(2) It should be emphasized that the most pronounced pH changes occurred in a damaged

bag. As this happened even under laboratory conditions, it appears likely that the structural integrity of the bags will represent a major challenge for successful field deployments of the bags, in particular in the sometimes harsh conditions of the coastal zone. This aspect is mentioned in the discussion, but I think it could be emphasized a bit more, in order to avoid that the approach is considered “ready-to-go” without further testing.

(3) Although the essential information is already covered throughout the manuscript, I would like to encourage the authors to summarize specific instructions for potential users of this approach in a dedicated “Recommendations” section. This section could be guided along three questions: What are the core requirements (material, fittings, etc) of gas tight bags to be used for pH buffer storage? What needs to be considered for proper handling of the bags (cleaning, filling, storage, etc)? Which measures could be taken to ensure that the bags perform well under in situ conditions?

Specific comments

L. 11: Usually, the buffers are prepared as “equimolal” not “equimolar” solutions (DeValls and Dickson, 1998). Please check and - if applicable - correct throughout ms.

L. 13: I wouldn’t consider the tris batches “experimental conditions”, but rather replicates of the same experiment. Consider removing it from the list here.

L. 15: It appears equally (or even more) important to inform the reader about the range rather than the mean of pH changes that the buffers are likely to experience. This information is indicated by the CI ($\pm 0.0008 \text{ yr}^{-1}$), but surprisingly this CI seems to disagree with the range given in the caption of Figure 3 where the upper and lower bounds are given as -0.0028 and -0.0091 pH per year. Can you clarify how the CI of the slope relates to the upper and lower bounds, and include the upper and lower bounds in the abstract?

L. 33: Uncertainty thresholds are listed in the wrong order. It should be 0.02 for the weather and 0.003 for the climate goal (Newton et al., 2015).

L. 37: The expression “roughly once per decade throughout most of the ocean” sounds to me as if only one major ocean cruise can be conducted per decade. Maybe change to “decadal reoccupations of a few major sections per ocean basin”.

L. 81ff.: When I understood correctly, bag type 1 was custom made and type 2 is commercially available. Is this correct and can it be clarified early in chapter 2?

L. 99: When I understand it correctly, the unit error affected only the HCl concentration but not the TRIS concentration. If this is the case, then the TRIS/TRISH⁺ ratio was not exactly 1:1. Please clarify this, and also revise the use of the term "equimolar" accordingly.

L. 100: Please include the degree of purity of the reagents, where possible. (See also comment on l. 223 below).

L. 107-122: The information in text and Table 1 appear redundant. Maybe the text could be restricted to general explanations of procedures, whereas the table could cover the specific routines for each test.

L. 118: The description of the CT measurements would profit from more technical details. Did you need to adjust the method to measure the comparably low CT concentrations in the buffer solutions? Were CRM measured along with the buffer samples? If yes, please indicate the batch.

L. 133: The temperature dependence of the TRIS buffer applied here strictly refers to the equimolar buffer composition, whereas some deviations need to be expected for the buffer composition used in this study. Can you estimate how large this difference might be? Does the dependence of $pH_{spec,Tc}$ on temperature agree with the expected temperature dependence of the buffer solutions?

L. 136: The time series of CRM measurements does not show a systematic drift, but a spread (~ 0.03 pH units) that is about an order of magnitude higher than the precision that can be achieved with the instrument used, and also larger than the good agreement between measured and calculated TRIS pH in this study would suggest (compare l. 185 - 192). Measured pH values appear clustered by CRM batch and/or measurement time. Would you have an explanation for this and an idea how this would impact your findings?

L. 137: I'm a bit sceptical about the approach to correct for dye impurities. First, I'm wondering if this correction is required at all, as the aim is to track pH changes (or better pH stability) over time and therefore the pH-dependent impact of dye impurities should be almost identical for all buffer measurements. More importantly, the comparison measurement of pure vs impure dye made on seawater solutions should also be affected by the pH perturbation of the dye addition. This pH perturbation is related to the pH of the stock solution and can be different for the two stock solutions used here. Did you minimize this pH perturbation by adjusting the stock solution pH to the sample pH, or correct for it by extrapolating your measurements to zero dye concentration? If not, I'm afraid your

correction term in Eq. (2) might be impacted. Please revise this approach.

L. 145: What do you mean with “normal practices”? Is this a standard operating procedure, or a threshold defined in your lab? Can you provide a reference?

L. 198: The estimates of the upper and lower bounds should be given more weight. From a users perspective and for the application of this storage solution without regular pH test measurements, the likely range of pH changes seems even more important the average rate of change! Please explain in the main text, how these bounds must be interpreted in contrast to the 95% CI of the slope.

L. 204: The consequence of bag damage deserves more attention, in particular with respect to the use of bags under in situ conditions. How can this damage happen even under laboratory conditions, and more importantly, how can it be avoided?

L. 214: Could your interpretation “that the drift in tris pH was primarily driven by an increase in CO₂” also be supported by the change of CT over time? Did you see a consistent increase of CT? I assume this should be the case, due to the fact that pH is decreasing over time and CT and pH seem to be correlated. However, an explicit statement about this would not hurt.

L. 216: Can you please describe in more detail what you mean with an “ad hoc acid–base equilibrium model of seawater including tris in addition to the CO₂ and other minor acid–base systems”? I’ve an idea what you mean but it is not entirely clear to me.

L. 223: Respiration of organic matter is proposed as one potential source for the accumulation of CT. Could you try to relate the amount of accumulated CT to the size of potential sources? Would it be possible to give a conservative estimate of how much organic material could cover the inner wall of the bags? Which quantity of organic matter must be expected to be contained in the reagents used to produce the buffer solutions? Is tris itself - which is also routinely used in biological experiments to stabilize pH - likely to be respired? I think a bit more detailed discussion to this end would help to identify how the accumulation of CT can be prevented in the future.

L. 280: I was not able to access the data at UC San Diego Library Digital Collections through the doi, nor through a keyword search. Please make sure that the data are correctly uploaded and accessible.

Technical corrections

L. 13: I think the wording "flexible bag" is a pleonasm. The word "flexible" can be removed here and throughout the ms.

L. 17: The explicit drift rate can be removed here in order to avoid repetition of the same number within the abstract.

L. 18 Consider replacing "value" by "potential", as in situ applicability has not yet been demonstrated.

L. 33: A second edition of this document was made available by Newton et al. (2015). Please update the reference.

L. 56: Rephrase "deep, comparatively stable ocean" to "deep ocean with comparatively stable pH" or similar

L. 62: Replace ", one or more times" by "repeatedly"

L. 65: Include reference Papadimitriou et al., (2016)

L. 78: Replace "for CO₂" with "for oceanic CO₂ measurements"

L. 91: Introduce abbreviation HDPE

L. 153: Data availability statement can be removed here, as it is given in a separate section below.

L. 156: To my impression, the term "drift" is more frequently used to describe the change of a measured value due to changes in instrument performance, i.e. instrument drift. Here, you are referring to real pH changes of the solution. Please consider rephrasing to "A near-linear decrease of pH was ..." or similar.

L. 158: Replace "is" by "was"

L. 158: Does Table 2 report measured values at $t = 0$ when those are available, or always the intercept of the fitted regression model? Text and table caption read contradictory in this respect.

Fig. 2: Showing one type of symbol and the corresponding legend per panel appears redundant. I recommend to use either the same symbols and color in all panels and keep only the descriptive label in each panel, or replace the individual legends by three joined legends indicating what the symbols, color and fill represent. Overall, axis labels and text appear small in this figure. Please try to increase text size and - if necessary - make use of the full page height to plot the panels. Consider starting the caption with "Individual time series of measured pH in tris buffer solutions ..."

Fig. 3: Consider starting the caption with "Combined time series of measured pH in tris buffer solutions ..."

L. 201-202: The sentences "By definition ... small magnitude" could be removed.

L. 220: Consider replacing "has been designed to" with "is known to"

L. 250: Please revise placement of "the bag" in "studies successfully used bag type 2 submerged the bag in seawater for less time"

L. 260: For consistency, remove "purportedly" here, or also include it in the abstract.

Supplementary materials: According to the manuscript preparation guidelines, the supplementary figures of this study should be placed in appendices. (Copied from the Ocean Science website: "Additional figures, tables, as well as technical and theoretical developments which are not critical to support the conclusion of the paper, but which provide extra detail and/or support useful for experts in the field and whose inclusion in the main text would disrupt the flow of descriptions or demonstrations may be presented as appendices." and "Supplementary material is reserved for items that cannot reasonably be included in the main text or as appendices. These may include short videos, very large images, maps, CIF files, as well as short computer codes such as matlab or python script.")

References used in this review

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Papadimitriou, S., Loucaides, S., Rérolle, V., Achterberg, E. P., Dickson, A. G., Mowlem, M., and Kennedy, H.: The measurement of pH in saline and hypersaline media at sub-zero temperatures: Characterization of Tris buffers, *Mar. Chem.*, 184, 11–20, <https://doi.org/10.1016/j.marchem.2016.06.002>, 2016.