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| Institute for Sanitary Engineering, Water Quality and Solid Waste Management ● Bandtäle 2 ● 70569 Stuttgart  **Editorial Board**  **Journal of Visualized Experiments**  **Phillip Steindel, Ph.D.** | D-70569 Stuttgart (Büsnau)  Bandtäle 2  Phone +49 711 685 – 60497  Fax +49 711 685 – 63729  E-Mail: eduard.rott@iswa.uni-stuttgart.de  Date  February 2nd 2018 | |

Subject: Response to reviewers

On behalf of the authors, I like to thank the reviewers for their suggestions increasing the quality of the article. My co-authors and I have revised the manuscript according to the recommendations of the reviewers and the editors and hope that this was done to their full satisfaction. We also responded to some requests for change with a rebuttal in the case we did not agree with the recommendation.

**Editorial comments:**

**General:  
1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues.**

We have proofread the manuscript (see trackings).  
  
**Protocol:  
1. Please and align all text to the left margin to ensure the length of the protocol can be properly measured.**

Done.

**2. For each protocol step, please ensure you answer the “how” question, i.e., how is the step performed?**

We do not think there is a more detailed way each step can be described.  
  
**Specific Protocol steps:  
1. 1. This information should be moved to the Introduction.**

The mentioned information was moved.

**2. 2.1.2.3: What does ‘sufficiently cooled down’ mean?**

We added the information (max. 40 °C):

“Take the 3 L beaker carefully out of the bucket as soon as it is sufficiently cooled down (max. 40 °C) and transfer its content into a 2 L glass bottle.” (lines 245 and 257).

**3. 2.3.: Do you mean that the 32% HCl listed in the Table of Materials should be used as the 10.2 M HCl?**

We changed the sentence to “Use 32% HCl (w/w) as 10.2 M HCl.”  
  
**Discussion:  
1. Please move extensive discussion of the results to the Representative Results section.**

We moved all paragraphs regarding „Influence of buffer on phosphonate adsorption and required buffer concentration”, “Calibration of ISOmini method and compliance with ISO” and “Plausibility and buffer-dependent dosage quantities of ISOmini method” to the Representative Results section.

**2. As we are a methods journal, please revise the Discussion to explicitly cover the following in detail in 3–6 paragraphs with citations:  
a) Critical steps within the protocol**

**b) Any modifications and troubleshooting of the technique**

**c) Any limitations of the technique**

**d) The significance with respect to existing methods**

**e) Any future applications of the technique**We changed the total Discussion section and added new texts:

“The increasing significance of phosphonates requires research for reliable methods of removing these compounds from wastewater to protect wastewater treatment plants or receiving water bodies. At present, very few studies have been carried out on the removal of phosphonates from industrial wastewater5, 11-14, 16. The procedure presented here shows that investigations regarding the elimination of phosphonates by adsorption on polar iron oxide containing materials, in particular granular ferric hydroxide, can be carried out quickly and reliably when in accordance with the given protocol.

The decisive point in conducting adsorption studies is the maintenance of the pH value. This cannot be done in rotating centrifuge tubes without using a buffer. In this article it was shown that Good buffers allow an acceptable pH adjustment only at a concentration of 0.01 M and even at this concentration have no significant influence on the adsorption of phosphonates onto GFH. The application of Good buffers is also the reason why the procedure presented here cannot be used for studies on adsorption of phosphonates onto rather non-polar materials such as activated carbon. Good buffers would compete with phosphonates for free adsorption sites.

Since the direct analysis of phosphonates by means of HPLC22 or IC-ICP-MS21 is very complex and expensive, the presented method suggests that the phosphonate after contact with the adsorbent should be measured indirectly via the determination of the total P. A standardized method (ISO 687828) is generally used for the total P determination, in which a digestion is carried out by means of H2SO4 and K2S2O8 on a hotplate, the pH value is then set to 3–10 by means of NaOH and a blue color complex (the color intensity of which is linearly proportional to the phosphate concentration) is formed with the aid of ascorbic acid and molybdate solution. This standardized method is very labor and time consuming, which is why a faster variant of the ISO method (ISOmini) was developed. The ISOmini method reduces the total volume to one-fifth. The digestion takes place comfortably in a thermostat and the NaOH dosage after digestion is fixed. This method enables a large number of phosphorus determinations to be carried out within a very short time and does not compromise accuracy in comparison to the ISO method.

Each buffer has a different COD. In addition, the relatively high necessary buffer concentration of 0.01 M means that, in order to ensure sufficient digestion of the sample constituents, considerably higher amounts of oxidizing agent have to be dosed than it is stipulated in the ISO method. If the K2S2O8 dosage is too low or too high, incorrect measurement results do occur. In the ISOmini method, this K2S2O8 dosage is thus matched to each buffer individually. Another critical point is the dosage of NaOH. As a rule, regeneration solutions have NaOH concentrations of > 0.1 M. In order to avoid that the [H+]:[Mo] ratio required for the formation of the color complex25-26 is not adhered to, a proper adjustment of the H2SO4 quantity prior to digestion is therefore necessary. The problem arises when the regeneration solution is reused several times, thereby changing its pH value and COD. Since a reliable and simple pH measurement is not possible in screw cap vials and an appropriate pH adjustment is not provided, the ISOmini method presented here, thus, reaches its limits for samples with very high pH values. For regeneration solutions it is therefore recommended to use the ISO method.”

**References:  
1. Please ensure references have a consistent format.**The only thing we found that could have been not consistent with the template was we had added “doi:” in front of the doi link. However, the template is confusing here. In the example this “doi:” is missing while later it says “DOI (if available), preceded by a comma and listed as “doi:” then the number.” However, we deleted all “doi:”. Furthermore, the template only describes the citation style for scientific papers. Website links, laws and ISOs are not described, so we kept it the way we think it is ok.

**Table of Materials:  
1. Please ensure the Table of Materials has information on all materials and equipment used, especially those mentioned in the Protocol.**We did not add glass materials such as flasks, beakers or cylinders in that list since it is common laboratory equipment. Materials that have to be bought especially for the protocol are contained in the materials table.

**Reviewers' comments:**  
  
**Reviewer #1: Manuscript Summary:  
A very clearly written protocol on determining phosphonates and their adsorption in model and real system. The only additions I could think of are a specification of the quality of the chemicals to be used in the process, and an indication of the eventual accuracies and sensitivities to be expected for a few examples.**

“Specification of the quality of the chemicals”: The information about suppliers, chemicals and instruments is put in the materials excel table. Maybe the reviewers did not get the materials list? To ensure that the reader is aware of this aspect, we have inserted the following sentence at the beginning of the protocol: “The required degree of purity of chemicals can be found in the attached material list.”

“indication of the eventual accuracies and sensitivities”: We added a new paragraph (lines 736–740) to address this aspect: “All dark green absorbance values in Figure 7 (n=12), converted into the total P concentration according to the calibration line in Figure 6, give an average value of 1.013 mg/L. The standard deviation is 0.014 mg/L. The typical deviation from the target value (1.000 mg/L) is therefore only 0.11–2.67% ((1.013 – 0.014 – 1.000) / 1.000 × 100% = 0.11%; (1.013 + 0.014 – 1.000) / 1.000 × 100% = 2.67%). This shows a high accuracy of the ISOmini method.”

**Reviewer #2: Manuscript Summary:  
In this manuscript the authors reported the procedure to investigate the adsorption of phosphonates onto iron-containing GFH filter materials. Some good steps are obtained. The results seem to new support for constructing new self-assembly nanomaterials with good steps and details. I think it can be accepted after minor revisions.**

**Q1. Pages 1, Abstract part, the sentence of "the total P is measured using a determination method (ISOmini) that is a modification and simplification of the ISO 6878 method:….." should be modified as clear sentences; please definite P, etc. with the first time;**

We separated the sentence into two sentences: “Subsequently, after membrane filtration (0.45 µm pore size), the total phosphorus (total P) concentration is measured using a specifically developed determination method (ISOmini). This method is a modification and simplification of the ISO 6878 method: …”. Although we think that “P” is a common chemical element symbol for phosphorus, we defined “total P” with the first use.

**Q2. Introduction part, some important and relative reports about self-assembled nanocomposites should be added to show clear background, such as the following literatures;  
ACS Sustainable Chem. Eng., 2017, 5(6), 4948-4956.  
ACS Sustainable Chem Eng, 2018, DOI: 10.1021/acssuschemeng.7b03635  
Nanoscale Research Letters 2017, 12: 99**

This is a very subjective opinion. In addition, all of the articles mentioned deal with materials that have not been examined in the context of our article. It is therefore not certain whether the process presented by us can be used with such materials. Own investigations with similar self-assembled nanocomposites showed that buffers in high concentrations contributed to strong influences on the adsorption behavior of phosphonates. The mention of self-assembled nanocomposites would thus possibly mislead the reader into thinking that the method we are proposing could be used with these materials. That is not the point of the article.

**Q3. Materials part, some chemical and instrument suppliers should be listed;**

The template does not include a materials part in the manuscript. The information about suppliers, chemicals and instruments is put in the materials excel table. Maybe the reviewers did not get the materials list? To ensure that the reader is aware of this aspect, we have inserted the following sentence at the beginning of the protocol: “The required degree of purity of chemicals can be found in the attached material list.”

**Q4. Fig.6 the color plot line should be improved to show clear results;**

In Figure 6, there is no colored line. Furthermore, the reviewer is not specific enough, so we cannot understand what is unclear in Figure 6. We think the figure is very clear and simple.

**Q5. Some minor Language error should be modified;**

We used the opportunity of revising the manuscript to thoroughly proofread the article.

**Q6. Page 20-22, The references styles should be well corrected.**

Without examples we do not know what the reviewer is referring to here. However, please see our comments above regarding the reference style.

With kind regards

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Dr. Eduard Rott