Appendix 3

METHODS FOR MEASURING TRANSPARENT EXOPOLYMER PARTICLES AND THEIR PRECURSORS IN SEAWATER

Loreen O. Villacorte[1,2], Jan C. Schippers[1] and Maria D. Kennedy[1]

[1]UNESCO-IHE Delft Institute for Water Education, Delft, The Netherlands
[2]GRUNDFOS Holding A/S, Bjerringbro, Denmark (current affiliation)

1 INTRODUCTION

Transparent exopolymer particles (TEP) and their precursors produced by phyto-/bacterio-plankton in fresh and marine aquatic environments are increasingly considered as a major cause of organic/particulate fouling in MF/UF membranes and organic/particulate and biological fouling in SWRO membranes. The following sections comprise detailed descriptions of two methods for measuring transparent exopolymer particles in seawater, namely $TEP_{0.4\mu m}$ and TEP_{10kDa}. The $TEP_{0.4\mu m}$ method measures transparent exopolymer particles retained by membrane filters having pores of 0.4 µm and conventionally known as TEP (Passow and Alldredge, 1995). The TEP_{10kDa} method covers transparent exopolymer particles retained by membrane filters with molecular weight cut-off of 10 kDa. Consequently, this method covers both TEP and most (if not all) of their colloidal precursors. $TEP_{0.4\mu m}$ is a more rapid method than TEP_{10kDa} and is recommended for routine TEP monitoring in untreated seawater. The TEP_{10kDa} method is more time consuming, however, it gives much more information because it covers both TEP and their colloidal precursors.

2 $TEP_{0.4\mu M}$ METHOD

The most widely used method for TEP, referred to here as $TEP_{0.4\mu m}$, was developed in the mid-90s (Passow and Alldredge 1995). Recently, it was found that this method may overestimate TEP in seawater due to the reaction of Alcian blue (AB) with residual saline moisture on the filter (Villacorte 2014). To minimize this effect, Villacorte et al. (2015) proposed that the membrane with the retained TEPs be pre-rinsed with ultrapure water (UPW) before staining with AB. The modified procedure for measuring $TEP_{0.4\mu m}$ is illustrated in Figure 1 and is briefly described as follows:

Preparation of Alcian blue dye solution

1. The stock dye solution is prepared by dissolving 250 mg/L of Alcian blue in ultrapure water spiked with acetic acid to lower pH to 2.5. The prepared stock solution is stirred until most of the Alcian blue powder is dissolved (up to 18 hours). The working solution is prepared on the same day of analysis by filtering 20 mL of the stock solution through 0.05 µm polycarbonate (PC) membranes before staining. The remaining stock solution is stored in the dark at 4°C and a new stock solution should be prepared after 4 weeks.

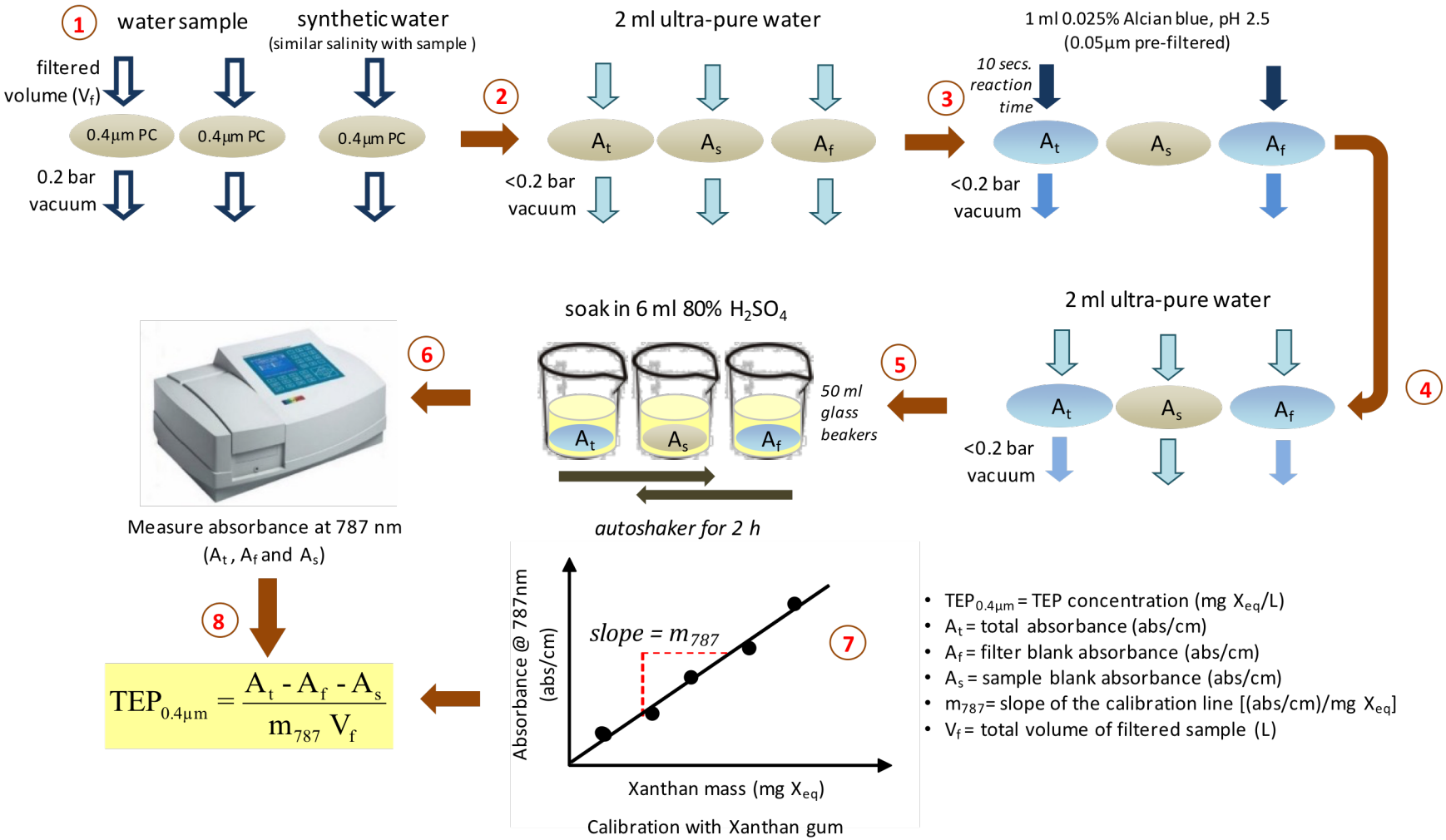

Figure 1. Procedural diagram for measuring $TEP_{0.4\mu m}$ (adapted from Villacorte et al. 2015)

Sample filtration and staining

2. First, the water sample (typically >20 mL) is filtered through a 47 mm diameter polycarbonate filter (0.4 µm pore size) by applying a vacuum of 0.2 bar. Then filter (<0.2 bar vacuum) 2 mL of UPW through the filter-retained TEP to wash the remaining sample moisture through the filter.
3. Subsequently, 1 mL of the working AB dye solution is applied over the filter, allowed to react with TEP for 10 seconds, and then the unreacted dye is flushed through by vacuum filtration (<0.2 bar). To remove the remaining unreacted dye, a rinsing step is performed by filtering 2 mL of UPW.
4. The rinsed filter is transferred to a 50 mL glass beaker and soaked in 6 mL of 80% sulfuric acid solution. The beaker is covered with parafilm and gently mixed on a shaker for 2 hours.
5. The acid solution is then transferred to a 1cm cuvette and the absorbance (A_t) is measured at 787 nm wavelength - the wavelength of maximum absorbance of Alcian blue when dissolved in sulfuric acid.

Blank measurement

6. A filter blank (A_f) is measured in the same way (steps 2-5), but filtering TEP-free blank samples (e.g., synthetic water with similar ion concentration as the water sample) instead of actual water samples. For sample correction (A_s), water samples are filtered in the same way as for determination of the total absorbance, but skipping the AB staining procedure.

Concentration calculation (without calibration)

7. The concentration of $TEP_{0.4\mu m}$ in terms of abs/cm/L is calculated as follows in equation 1:

$$TEP_{0.4\mu m} = \frac{A_t - A_f - A_s}{V_f} \quad (1)$$

where (A_t) is the total absorbance of the dye that reacted with TEP and the filter (abs/cm); (A_f) is the absorbance of the dye adsorbed to the filter (abs/cm); (A_s) is the absorbance of unstained sample (abs/cm) and V_f is the volume of sample filtered (L).

Concentration calculation (with calibration)

8. $TEP_{0.4\mu m}$ can be further calibrated and expressed in terms of equivalent weight of standard acid polysaccharide - Xanthan gum – as mg X_{eq}/L:

$$TEP_{0.4\mu m} = \frac{A_t - A_f - A_s}{m_{787} V_f} \quad (2)$$

where m_{787} is the slope of the calibration line [(abs/cm)/mg X_{eq}] which is determined by plotting the mass of the standard (Xanthan gum) against the corresponding absorbance of AB that reacted to it (see Passow and Alldredge 1995).

Note: The standard calibration procedure in step 8 involves dry weight measurements to determine the mass of Xanthan gum retained on polycarbonate filters. This is prone to several inaccuracies during drying (dust contamination) and weighing (electrostatic force

interference) at very low quantities (5-50 µg) of Xanthan. It is also very challenging to prepare a homogeneous and artifact-free solution of Xanthan for the calibration experiment. An option to avoid such difficulties is to skip the calibration step entirely (follow until step 6 only), whereby concentrations of TEP are expressed in terms of abs/cm/L. It is only feasible, however, when comparing results using the same batch of AB staining solutions. Moreover, absorbance results are not directly comparable with TEP_{10kDa} due to differences in wavelength at which the absorbance were measured. For standardized results, an alternative calibration procedure was introduced by Villacorte (2014) as described in steps 9-13.

Alternative calibration method

9. Homogenized standard solutions (4 mL) containing different concentrations (0, 1, 2, 3, 4 and 5 mg/L) of Xanthan gum are prepared from a stock solution (40 mg/L). The pH is adjusted to pH 2.5 by adding 0.05 mL acetic acid to each solution and then briefly agitated.
10. The solution is then stained by adding 1 mL of pre-filtered AB staining solution, mixed for 10 s and incubated for 10 min.
11. Filter 4 mL of the resulting solution through a 0.1 µm PC membrane by vacuum filtration (0.2 bar).
12. The PC membrane used to filter AB-stained standard solution is carefully transferred to a 50 mL beaker. Six mL of 80% sulfuric acid solution is added, covered with Parafilm and mixed on an auto-shaker for 2 hours. The acid solution is transferred to a 1cm cuvette and absorbance was measured at 787 nm.
13. To determine the calibration slope (m_{787}), the mass of Xanthan gum retained on the PC membrane is calculated by multiplying the volume filtered (4 mL) by the concentration of Xanthan in the stained standard solution. The calculated mass is then plotted against the corresponding AB absorbance measured at 787 nm wavelength, whereby the average linear slope is the m_{787}. $TEP_{0.4\mu m}$ is then calculated using equation 2.

3 TEP_{10KDA} METHOD

The TEP_{10kDa} is a method intended to measure TEP and their precursors (down to 10 kDa). This method was developed by Villacorte et al. (2015), which is partially based on the principles developed by Thornton et al. (2007) for measuring acidic polysaccharides. Figure 2 illustrates the procedure for measuring TEP_{10kDa} and is described as follows:

Sample filtration and TEP extraction

1. The water sample is filtered constant flux (60 $L/m^2/h$) through a 10 kDa MWCO regenerated cellulose membrane (25 mm diameter) using a syringe pump.
2. After filtering 10-100 mL of sample, the syringe is replaced with a clean syringe containing about 10 mL of air. Air is then injected to the filter holder (60 $L/m^2/h$) until all the remaining water in the feed side of the membrane holder has passed through the membrane. The total filtered sample volume is then measured after collecting the filtrate
3. To rinse out residual saline moisture, 5 mL of UPW is injected to the filter holder at 60 $L/m^2/h$. Air is again injected until all the rinse water on the feed side of the membrane holder has passed through the membrane.
4. The membrane is then carefully removed from the filter holder and placed feed side down, in a clean disposable plastic container filled with 10 mL of UPW. The sample is covered, vortexed for 10 s and sonicated for 60 min.

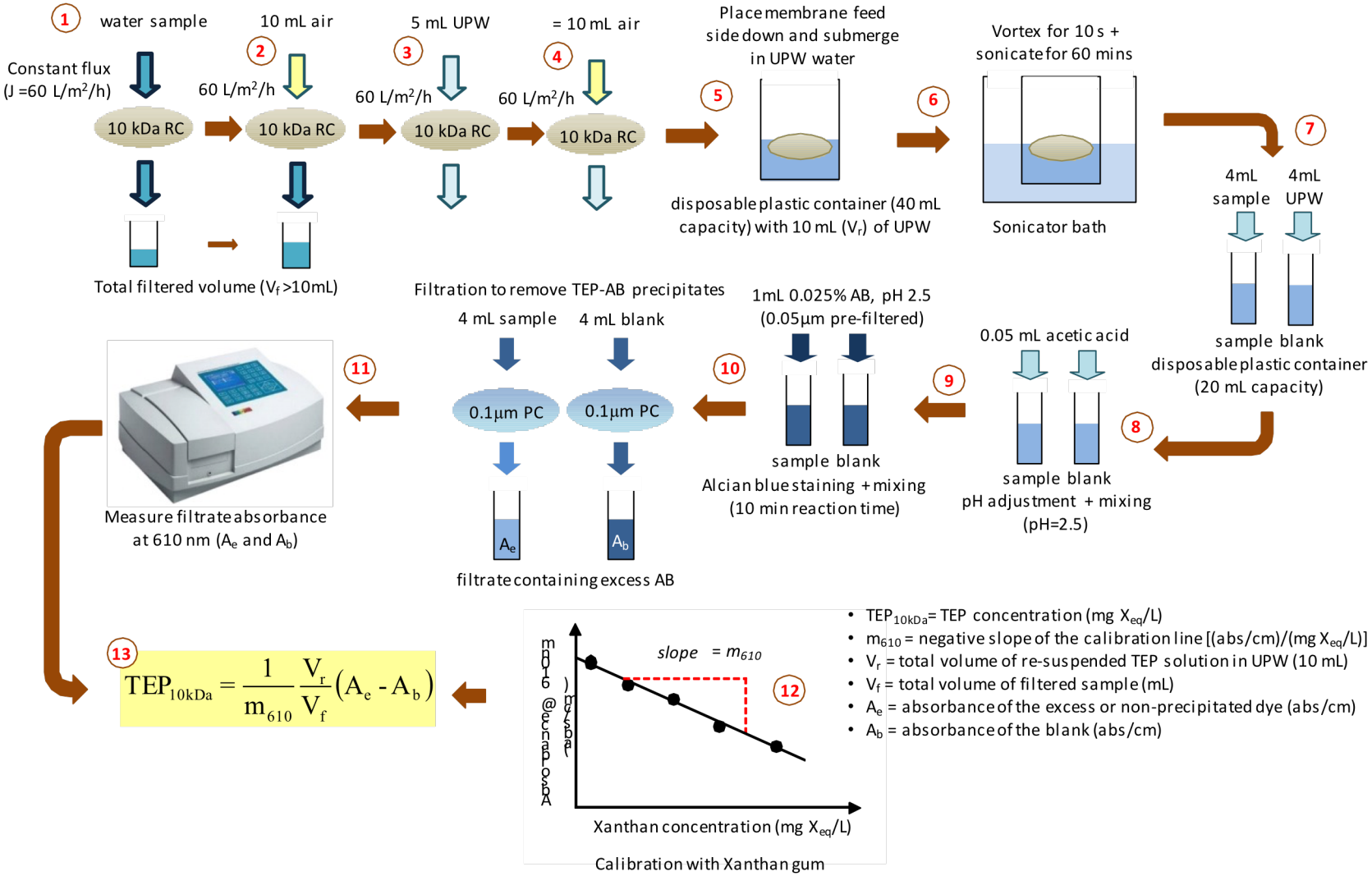

Figure 2. Procedural diagram for measuring TEP_{10kDa} (adapted from Villacorte et al. 2015).

Staining and absorbance measurement

5. Transfer 4 mL of the re-suspended TEP solution to a clean 20 mL disposable plastic container. To adjust the sample pH to 2.5, 0.05 mL of acetic acid solution is added to the solution.
6. Add 1 mL of the working AB dye solution (for dye preparation see step 1 of $TEP_{0.4\mu m}$ method) to the sample, mix vigorously and leave to react for 10 min.
7. Filter 4 mL sample of the TEP-AB solution through a 0.1 μm PC filter by vacuum filtration (<0.2 bar).
8. The filtrate is collected in a plastic container (10 mL), transferred to a 1-cm cuvette and absorbance (A_e) at 610 nm wavelength - the wavelength of maximum absorbance (visible light range) of AB when dissolved in acetic acid solution - is measured with a spectrophotometer.

Blank measurement

9. The blank absorbance (A_b) is measured to correct for the amount of stain adsorbed by the polycarbonate filter (0.1μm). This is performed following steps 5-8 but replacing the sample with UPW.

Concentration calculation (without calibration)

10. The TEP_{10kDa} concentration in absorbance per cm per liter of filter water (abs/cm/L) is calculated as follows:

$$\mathrm{TEP_{10kDa}} = \frac{\mathrm{A_b} - \mathrm{A_e}}{\mathrm{V_f}} \tag{3}$$

where A_b is the absorbance of filtered blank (abs/cm), A_e is the absorbance of the excess or un-reacted dye (abs/cm) and V_f is the volume of filtered sample (mL).

Concentration calculation (with calibration)

11. Alternatively, the TEP_{10kDa} concentration can be calibrated and expressed in terms of mg Xanthan equivalent per litre (mg X_{eq}/L):

$$\mathrm{TEP_{10kDa}} = \frac{1}{m_{610}} \frac{\mathrm{V_r}}{\mathrm{V_f}} (\mathrm{A_e} - \mathrm{A_b}) \tag{4}$$

where m_{610} is the slope of the calibration curve [(abs/cm)/(mg X_{eq}/L)] and Vr is the total volume of the re-suspended TEP sample solution (i.e., 10 mL).

12. For the calibration, standard solutions (4 mL) containing different concentrations (0, 1, 2, 3, 4 and 5 mg/L) of Xanthan gum are prepared from a homogenized stock solution. To adjust sample pH to 2.5, 0.05 mL acetic acid is added to each solution and then briefly agitated. The solution is then stained by adding 1 mL of pre-filtered AB staining solution, mixed for 10 seconds and incubated for 10 min.
13. Filter 4 mL of the resulting solution through a 0.1 μm PC membrane by vacuum filtration (0.2 bar). The filtrate is collected, transferred to 1cm cuvette and absorbance is measured at 610 nm.
14. The Xanthan concentration of the stained dye is plotted against the measured absorbance (excess dye absorbance) and the average linear slope is the m_{610}. Since

concentration is inversely proportional to the excess dye absorbance, the calibration slope (m_{610}) is always a negative value.

Note: The TEP_{10kDa} concentration calculated using Eqn. 4 can be directly compared with $TEP_{0.4\mu m}$ calculated based on Eqn. 2 as both are calibrated with Xanthan gum standard. However, results for TEP_{10kDa} calculated based on Eqn. 3 and $TEP_{0.4\mu m}$ calculated based on Eqn. 1 are not comparable as the absorbance values were measured at different wavelengths - 610nm and 787nm, respectively.

4 STORING WATER SAMPLES

Storing samples for a period of time before analysis may lead to significant disparity between the measured and in-situ TEP concentration as a consequence of coagulation, bacterial release, bacterial degradation, adsorption on walls of sample bottles or a combination thereof. Bottle tests at 4^{o}C temperature revealed that $TEP_{0.4um}$ concentration may increase over a period of time either due to coagulation of TEP precursors or through bacterial TEP release (Villacorte 2014). On the other hand, TEP_{10kDa} concentration can rapidly decrease by up to 45% within the 3 days of storage resulting from either bacterial degradation or adsorption to walls of sample bottles (Villacorte 2014). Further investigations are still necessary to fully understand the mechanisms involved in the TEP loss or increase as well as to develop reliable measures to preserve TEP samples (e.g., sample bottle, freezing, preservative addition). It is therefore important that samples be analyzed immediately (within 24 hours) after sampling to obtain reliable TEP concentrations. For TEP_{10kDa}, it is advisable to filter and then flush the sample immediately after sampling so the membrane can be kept at 4^{o}C until analysis.

5 REFERENCES

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