Evaluating and Improving the Reliability of the UV-Persulfate Method for the Determination of TOC/DOC in Surface Waters

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Abstract

The UV-persulfate oxidation method is widely used for determining the total organic carbon concentration of aqueous samples (denoted for convenience as UVP-TOC). However, for some surface water samples, the measurement of TOC by this method can be unreliable, deviating significantly from the true carbon content. In this study, the performance of the UVP-TOC method has been investigated by comparing the results from the analysis of a variety of aqueous samples that included two kinds of surface water samples and related surface water model substances: bovine serum albumin

(BSA), sodium alginate (SA), humic acid (HA), tannic acid (TA), benzoic acid (BA) and citric acid (CA), with those from a high-temperature combustion method (elemental analysis); the latter providing the true carbon content value. By comparing the above data, it was found that the UVP-TOC method significantly underestimated the TOC value of the surface water samples, and it was also found that the model components BSA (protein) and HA (humic substances, HS) had a substantial influence on the TOC underestimation, while the SA (polysaccharide), TA (complex organic molecule) and CA/BA (small molecules) had little effect. The results showed that the agglomeration within and between BSA and HA molecules was an important reason for the inaccurate UVP-TOC values of BSA and HA. A further limitation was that for BSA, surfactants (e.g. sodium dodecylbenzene sulfonate, SDBS) and other surfactant-like substances, foam was formed during the CO₂ removal purging process by N₂ that seriously interfered with the determination of TOC. The study provides new information and insight into the causes of inaccuracies in the UVP-TOC analysis of surface waters and possible approaches to improve the accuracy.

Keywords: TOC; UV-persulfate oxidation method; NOM; Surface water; Incomplete oxidation.

1 Introduction

The total organic carbon (TOC) concentration is one of the most important and widely used indicators of the quality of natural and anthropogenic water samples,

representing the total content (as carbon) of organic substances in solution (Aiken 1992). Compared with spectral measurements, such as from UV-visible and fluorescence spectrometry, the TOC measurement can more authentically represent the total organic content as the signal intensity from UV-visible or fluorescence analyzers can be greatly influenced by many factors (Johnson et al. 2002, McKay et al. 2018, Sharpless and McGown 1999). For some substances like polysaccharides, which have poor UV-visible and fluorescent sensitivities, the determination of TOC is one of the few methods of characterizing them (Ci et al. 1999). As a consequence of these advantages, and the speed of TOC measurement and simple sample preparation, TOC analyzers have been widely applied in the characterization and monitoring of water quality in practice and related research in recent years (Ahmad et al. 2019, Kopáček et al. 2018, Yu et al. 2018, Zhang et al. 2015). The currently accepted and widely used standard method for TOC analysis is given in EPA 5310C (Corwin and Summers 2011, Ikehata et al. 2018, Pepich et al. 2004).

The two most commonly used TOC analyzers for aqueous samples can be distinguished by their different oxidation methods, involving either high-temperature combustion or photo-chemical UV-persulfate oxidation (Bisutti et al. 2004a). For the high-temperature combustion TOC (HTC-TOC) method, the samples are completely oxidized at high temperature ($> 600\text{ }^{\circ}\text{C}$) in the presence of Cu, Pt, or other catalysts; for the UV-persulfate oxidation TOC (UVP-TOC) method, the samples are oxidized by persulfate radicals in a photocatalytic reaction (Avramidis et al. 2015, Huang et al. 2017, Schumacher 2002). In comparison to the HTC-TOC method, the UVP-TOC method

can measure TOC in samples containing high salt concentrations (except the halide salts), such as surface waters and wastewaters (Peyton 1993, Visco et al. 2005). However, the HTC-TOC method may contaminate the catalyst with substances generated from the S or N elements in the above samples, which may lead to catalyst deactivation or catalyst hardening (Visco et al. 2005).

The EPA 5310C standard indicates that because the UVP-TOC has a slow mineralization rate for substances with large molecular weight and complex structure, such as humic acid, the HTC-TOC method is more suitable for samples at higher concentrations of TOC and/or samples that contain chemically refractory organic substances, than the UVP-TOC method (Avramidis et al. 2015, Bisutti et al. 2004b, Schumacher 2002, Sharp 1973, Urbansky 2001, Wallace et al. 2002). However, there has been some previous research also showing that UVP-TOC was able to obtain accurate detection results for natural organic matters (NOM, including humic acid) (Benner and Hedges 1993, Kaplan 1992, Wallace et al. 2002). Also, some research has reported that the HTC-TOC method cannot accurately determine the TOC value of a part of organic substances (Spyres et al. 2000). Furthermore, the EPA 5310C standard also mentions that the two methods are both suitable for the TOC range of 1-50 mg/L. Therefore, many studies have used the UVP-TOC method to measure the TOC of NOM and similar complex organic samples (Avramidis et al. 2015, Laszakovits et al. 2020). However, the literature contains some previous studies, including research by the authors, which have demonstrated that in some cases the TOC value of NOM obtained by the UVP-TOC method was lower than the true carbon content of the samples

(Laszakovits et al. 2020). After eliminating the possibility of Cl^- ion interference, this phenomenon was attributed to the incomplete oxidation of the NOM (Table S1, Figure S1a). The extent and reasons for deviations between the UVP-TOC value and the true carbon content value in surface water samples are of general importance to the characterization of water quality and evaluation of treatment performance and ensuring the validity of TOC data obtained by the use of UVP-TOC analyzers. Thus, the possible inaccuracy of UVP-TOC in NOM analysis is a very important question for researchers.

Although this phenomenon was mentioned in many studies, even in the EPA 5310C standard, there has been no systematic research work to explain the mechanisms of this phenomenon (Avramidis et al. 2015, Bisutti et al. 2004b, Schumacher 2002, Sharp 1973, Urbansky 2001, Wallace et al. 2002). To address this deficiency, in this study we have employed substances that may have a major impact on UVP-TOC measurement, including bovine serum albumin (BSA), sodium alginate (SA) and humic acid (HA, sodium salt) to represent protein-like substances, polysaccharides and humic substances (HS) in surface water, respectively. In addition, tannic acid (TA), citric acid (CA), and benzoic acid (BA), were used respectively to represent complex organic macromolecules (TA) and small molecular organic acids (CA/BA), widely found in surface water. The true carbon content values of these samples were measured by an elemental analyzer to establish the true TOC value and compared to the corresponding values from the UVP-TOC method. Subsequently, model substances were used to investigate the mechanisms responsible for the deviation between the true TOC and UVP-TOC values, and the conclusions were verified by using two surface water samples. Finally,

some approaches for improving the accuracy of the UVP-TOC method are proposed, such as sample pre-oxidation with hydrogen peroxide, pretreatment with acid, and addition of defoamer, which may enhance the reliability of the UV-persulfate oxidation method in future water treatment research and water industry applications.

2 Materials and methods

2.1 Surface water samples and NOM model substances

The surface water samples were collected from the lake of the Olympic Park (OL) and Qing River (QR), north of Beijing. In both cases, the samples were collected at a depth of 20 cm below the water surface in October and November 2019. The samples were filtered by 0.45 μm membrane to eliminate the effects of the particulate organic carbon (POC) on the TOC value (Wu et al. 2020, Yin et al. 2019). Each filtered sample was divided into three sub-samples. One sub-sample was freeze-dried after adjusting to pH 2 (0.1 M HCl solution) for Fourier transform infrared (FTIR) analysis; another sub-sample was freeze-dried directly before carbon content analysis by the elemental analyzer and re-dissolved in deionized (DI) water (by Millipore water machine, 18.25 M Ω /cm, TOC<5 ppb) to obtain the corresponding UVP-TOC values and their linearity with concentration. The remaining sub-samples were stored for other analyses in this study.

HA was used to represent the HS in surface water. The HA stock solution was prepared by dissolving 1.00 g sodium humic acid (H16752, Sigma Aldrich, USA) and 0.10 g NaOH into 100 mL DI water with strong stirring. The different HA samples were

prepared by diluting the stock solution with DI water to the target concentration and adjusting the pH to 7.0 by adding 0.1 M HCl or 0.1 M NaOH solution.

BSA (Sinopharm Chemical Reagent Co., Ltd., Shanghai, China), SA (180947, Sigma Aldrich, USA), tannic acid (TA, J&K Scientific Ltd., China), citric acid monohydrate (CA, Sinopharm Chemical Reagent Co., Ltd., Shanghai, China) and benzoic acid (BA, Sinopharm Chemical Reagent Co., Ltd., Shanghai, China) test solutions were prepared by dissolving 100 mg BSA or SA in 1 L DI water and then diluting them to the target concentrations.

All of the stock solutions and samples were stored in the dark at 4 °C, and allowed to recover to room temperature (25±1 °C) before use.

2.2 Test instruments and methods

The UVP-TOC analyzer (TOC-Vwp, Shimadzu, Japan, with ASI-V autosampler) was set to the non-purgeable organic carbon (NPOC) test mode. High purity N₂ gas (99.999%) was used as the carrier gas. All aspects of the acidic reagent (H₃PO₄, ~3 M) and UV-oxidizing reagent (Na₂S₂O₈, ~0.5 M, contained ~0.5 M H₃PO₄) preparation and settings followed the instrument manual recommendations. The principle and components of the UVP-TOC method are shown in Scheme 1. The method involved the following steps: H₃PO₄ was added to remove the inorganic carbon (HCO₃⁻ and CO₃²⁻) in the solution by N₂ purging (of CO₂) and provide an acidic environment for the subsequent radical reaction; the sodium persulfate was used as the oxidation reagent to generate radicals under UV light at 80 °C, thereby mineralizing the organic matter in the sample to CO₂; the integral curves of CO₂ signal can reflect the reaction status of

the mineralization, and the UVP-TOC values were calculated from the integration of CO₂ signal. This method is a modified EPA 5310C method, which uses UV light to assist the oxidation processes at 80 °C (not 90-100 °C in EPA 5310C, and is fixed).

The carbon content values were detected by the elemental analyzer (Vario EL III, Elementar, German), and a range of other instruments, including a UV-visible spectrophotometer (UV-2600, SHIMADZU, Japan), FTIR spectrometer (Spectrum Two, PerkinElmer, USA, with the diamond ATR accessory), excitation-emission matrix (EEM) spectrophotometer (F-4600, HITACHI, Japan) and zeta potential/particle size analyzer (Nano ZS90, Malvern, UK), were used to analyze the changes in the samples in terms of concentration, functional groups, structures, and particle sizes. A thermogravimetric analyzer (TGA, TG209 F3, NETZSCH, Germany) was used to measure the H₂O content of SA.

High-performance liquid size exclusion chromatography (HPSEC) was used to detect the molecular weight (MW) distribution of organic matter by a Waters HPLC system (1525 Binary Pump, Model 1500 Column Heater, 2998 Photodiode Array Detector, 2707 Autosampler) with SEC column (Phenomenex, BioSep 5 µm, SEC-s3000, 290 Å) and SecurityGuard™ Cartridges (Phenomenex, KJ0-4282, with AJ0-4488 filter element). The mobile phase was 10 mM sodium acetate (CG grade, Sigma Aldrich, USA) with a flow rate of 1 mL/min; the column temperature was set at 25 °C, and the detection wavelength was 254 nm; the injection volume was 100 µL. All HPSEC samples were filtered by 0.22 µm filter to remove particles before use.

Scheme 1.

2.3 The measurement of carbon content and selection of variables

Firstly, all of the samples were dried under vacuum at 40 °C for 48 h to remove adsorbed water and then their carbon content was determined by the elemental analyzer, representing the true TOC value in this study.

The parameters which can be set in the UVP-TOC analyzer control software, and which may affect the UVP-TOC values, were the H₃PO₄ addition (0, 0.1, 0.2, 0.3, 0.4, and 0.5 M) and the volume of sodium persulfate solution (0, 0.5, 1.0, 1.5, 2.0, 2.5 mL). To study the effects of acidification and pre-oxidation on UVP-TOC values, different dosages of H₃PO₄ (0, 0.1, 0.2, 0.3, 0.4, and 0.5 M) and H₂O₂ (based on the samples) were added into the corresponding samples to be tested in advance and allowed to react for 6 h. In contrast, other parameters such as the number of washes, sample inject volume and sample dilution factor were considered as not influencing the TOC determination and were fixed in this study.

The default settings of the UVP-TOC analyzer were as follows: the sampling volume was 300 µL; the concentration of H₃PO₄ was 0.2 M; the volume of sodium persulfate solution was 3.0 mL; the integral time was automatically determined by the TOC analyzer (maximum is 20 min); the concentration of added H₂O₂ was 0 mM; system cleaning was 3 times after each sample testing; determinations were repeated at least 2 times for single samples to ensure the standard deviation (SD) was less than 2%. According to the requirements in the EPA 5310C Standard, the CA samples (100 mg/L)

with/without NaHCO_3 (100 mg/L) were used to test and compare the UVP-TOC values with 3 min purging time, and the results indicated that 3 min purging time was enough to eliminate the inorganic carbon in samples (Figure S1b). The above method conformed to the EPA 5310A standard (Method C). The TOC analyzer was installed, commissioned and accepted by Shimadzu engineers. The instrument was calibrated by using potassium hydrogen phthalate ($\text{C}_8\text{H}_5\text{KO}_4$, AR grade, Shimadzu, Japan) before use. The blank UVP-TOC values of sodium persulfate, phosphoric acid, and hydrogen peroxide used in this study are listed in Table S1.

The difference between the true carbon content and the UVP-TOC values of the surface water samples, BSA, SA, HA, CA, and BA solutions were determined by the UVP-TOC analyzer with the default settings. The two surface water samples were diluted to 0.1-1.0 times the original water concentration. The concentration ranges of the re-dissolved QR and OL samples were 20-200 mg/L and 50-500 mg/L, respectively. The concentration range of the BSA, SA, HA, CA, and BA samples was 1-100 mg/L.

In this work, the carbon content values, which were calculated based on the molecular formula of the samples, were considered as theoretical organic carbon content values (TC). Similarly, the elemental analyzer (combustion method) determined carbon content values were taken as the experimental organic carbon content values (EC), which was considered to be equal to the true carbon content value. The TC and EC of all samples and reagents are listed in the Supporting Information (SI, Table S2).

2.4 Methods for data testing

The student (t) test method was used to test whether there was a significant difference between the UVP-TOC values/true carbon content values and between the UVP-TOC values that were detected in different test conditions. The confidence of the test method was selected as 95%.

3 Results

3.1 Carbon content measurement by elemental analysis

The EC of the OL and QR samples were low (1.00 ± 0.03 % and 0.51 ± 0.01 %, respectively), which was attributed to the high concentration of salts in the surface waters (SI, Table S3). For the BSA, the EC and TC were in close agreement (53.33 ± 0.09 % and 52.68%), while for the SA, the EC (36.37%) was slightly higher than TC ($31.91\% \pm 0.08$ %), which may have been caused by moisture absorption during weighing and the bound water of SA. These inferences were based on the thermogravimetric analysis (TGA) results (Figure S1c). The EC of HA was 40.45 ± 0.16 %, which was close to the reported value (regarded as the TC). For the specific organic acids, the EC of CA (36.72 ± 0.02 %) was the same as the TC, while the EC of the BA (72.71 ± 0.12 %) was slightly greater than the TC (68.84 %), but similar to the TC of benzoic acid anhydride (BAA, 74.32 %), which suggested that a proportion of the BA molecules transformed to BAA in storage (Baigorri et al. 2007, Trelu et al. 2016, Yu et al. 2018).

3.2 Comparison between UVP-TOC and true carbon content values

Firstly, two surface water samples were used to compare the difference between the

UVP-TOC value and the true carbon content value. HPSEC was used to analyze the MW distribution of the different organic compositions in surface water samples. In this study, the NOM in surface water was classified into biopolymers (100-10 kDa, composed of protein-like matter and polysaccharides), HS (10-1 kDa), and small molecular organic acids (<1 kDa). It was found that the OL sample had more biopolymers but fewer HS and small molecular organic acids than the QR sample (SI, Figure S2a and S2b).

The UVP-TOC values of the OL and QR re-dissolved samples and the raw (filtered only) samples were analyzed with different dilution proportions (Figures 1a, 1b, and SI, Figures S2c, S2d). The dotted lines in Figures 1a and 1b were the EC results determined by the elemental analyzer (V_E), and the data points were the UVP-TOC values determined by the UVP-TOC analyzer (T_E). The student (t) test was used to detect significant differences between data in this study. Formula (1), as shown below, is the student (t) test formula, where $\bar{x}$ is the average T_E , μ is the V_E , s is the standard deviation of T_E , and n is the number of tests (3 in this work). Formula (2) is derived from Formula (1), which is used to calculate the confidence interval of T_E . The s in Formula (1) is replaced by σ (σ is calculated from Formula (3)), and t is replaced by the $t_{0.05,3}=3.18$. Thus, the calculated μ' is the confidence interval of the measured value when the confidence is 95%. If V_E is in the range of $T_E \pm \mu'$, it indicates that the T_E has no significant difference from the V_E , otherwise the T_E has a significant difference from the V_E .

$$t = \left| \frac{\bar{x} - \mu}{s/\sqrt{n}} \right| \quad (1)$$

$$\mu' = \bar{x} \pm \frac{\sigma \times t_{0.05,3}}{\sqrt{n}} \quad (2)$$

$$\sigma = \sqrt{\frac{\sum_{i=1}^n (s_i)^2}{(n-1)}} \quad (3)$$

In Figure 1a and 1b, it can be seen that the slope of T_{E-OL} (k_{OL}) was only 64.2% of the slope of V_{E-OL} (k_{OL}^0), and the slope of T_{E-QR} (k_{QR}) was only 70.4% of the slope of V_{E-QR} (k_{QR}^0). The differences between the T_E and V_E values were statistically different, which indicated that the UVP-TOC analyzer underestimated the true carbon content value for both surface water samples. Also, the integral curves of the CO_2 signal showed that the tailing peak area increased with the increase of sample concentration, which indicated there was some recalcitrant (difficult-to-oxidize) matter in the surface water samples (SI, Figures S3a and S3b).

BSA is one of the most commonly used model substances for studying protein-like substances in surface water (Yu et al. 2017). From Figure 1c, it can be seen that the substantial deviation between V_E and T_E increased with the increase of BSA concentration (see Figure inset, $C_{BSA} > 20$ mg/L). The integral curves of BSA samples also had a long trailing peak at high concentrations (SI, Figure S3c).

SA is one of the most representative and widely used polysaccharides in surface water (Katsoufidou et al. 2007). In Figure 1d, it can be seen that the UVP-TOC underestimated the true TOC, and although the deviation was small as indicated by the closeness of the T_{E-SA} and V_{E-SA} , the difference between the T_E and V_E was statistically significant. However, the integral curves of SA samples did not show peak tailing even at the highest concentration (SI, Figure S3d), which indicates that some other factors

may cause measurement errors, rather than the incomplete oxidation of SA.

Humic substances (HS) are a major part of NOM in surface waters and represent a large contribution to the TOC value (Yu et al. 2018). In this study, Aldrich HA was used to simulate the fraction of HS which have the most aromatic and highest oxidation state in surface water (Aeschbacher et al. 2012, Kozyatnyk et al. 2018, Schellekens et al. 2017). It was found that the UVP-TOC values substantially underestimated the true TOC. Specifically, in the concentration range between 0 to 10 mg/L, the ratio of T_{E-HA} slope (k_{HA}) to V_{E-HA} slope (k_{HA}^0) was 55.5%, while in the range of 10 to 100 mg/L, the ratio was 69.9% (Figure 1e). The reason for the greater slope in the higher concentration range is possibly from the photocatalytic properties of HA itself increasing the efficiency of the UV-persulfate reaction (Sharpless et al. 2014, Tsui and McNeill 2018). This speculation may be supported by the results showing that the ratio of the trailing peak area to the total area in the integral curves increased with the HA concentration (SI, Figure S3e). Therefore, to avoid such effects, the working concentration of HA was set to 5 mg/L in the subsequent investigation of the UVP-TOC variables.

TA is a complex organic macro-molecule that is listed in the EPA 5310C standard method, and it is difficult to be measured accurately by the UVP-TOC method. However, Figure 1g showed that T_{E-TA} was not statistically different from the V_{E-TA} , which means the TA can be accurately measured by the TOC analyzer and the methods used in this study. The integral curves of TA samples also indicated that TA was completely oxidized (Figure S3f).

For the CA and BA, as can be seen in Figures 1f and 1g, the V_{E-CA} , and V_{E-BA} values

were almost equal to the T_{E-CA} and T_{E-BA} , which means that the UVP-TOC values were accurate for the measurement of these small molecular weight organic acids. Also, the integral curves of CA samples and BA samples were similar to that of the SA samples (Figure S3g and S3h).

The above results indicated that the organic matter which affected the accuracy of the UVP-TOC measurement was not the hard-to-oxidize material in the conventional sense, but the substances with a large molecular weight and complex structure, such as protein-like substances and HS. Thus, this study changed its focus away from further investigation of TA, BA, and CA, to consider in more detail the oxidation mechanism of BSA and HA that have a large molecular weight and complex structure.

Figure 1.

3.3 Influence of H_3PO_4 concentration, sodium persulfate dosage and H_2O_2 pre-treatment concentration on the UVP-TOC measurement

For convenience, in the following discussion, the cases where the UVP-TOC value increased as the variable increased, is described as a "positive effect", and the cases where the UVP-TOC value decreased as the variable increases, is described as a "negative effect". The UVP-TOC values of each sample under the conditions of different H_3PO_4 concentrations, sodium persulfate dosage and H_2O_2 pre-treatment concentration, are given in Figure S4-6. The red marked data were the UVP-TOC values that were detected by the default method. In these tests, it was evident that an

increase in sodium persulfate dosage can improve the oxidation degree of samples (Figure S5). For the addition of H_2O_2 , it was used to pre-oxidize the samples to make the samples more completely oxidized by the UVP reaction (Criquet and Leitner 2009, Liang and Su 2009).

To establish whether the changes of various factors have a significant impact on the TOC measurement, the student test method was used to analyze the data statistically. To do this, the confidence level was set to 95% ($t_{0.05, 3} = 3.18$), according to the calculation result of Formula (1). For the case of $|t| < t_{0.05, 3}$, this means that the factor has no significant effect on the UVP-TOC measurement, otherwise, the factor is considered to have a significant effect on the UVP-TOC measurement. The calculated results are shown in Table S5.

It was found that for the OL samples, only H_2O_2 had a positive effect on the UVP-TOC values. The H_2O_2 and persulfate had a positive effect on the UVP-TOC values of the QR samples, and H_3PO_4 had a negative effect. The results for HA were similar to the QR samples, with a positive effect with H_2O_2 and persulfate, and a negative effect with H_3PO_4 , which corresponded to a reduced mineralization rate and a slightly decreased $V_{\text{T-HA}}$. For BSA samples, all three factors had a positive effect, while for SA samples, only the persulfate had a positive effect, the H_3PO_4 had no effect and the H_2O_2 had a negative effect.

From these results, and considering the integral curve of SA, it can be inferred that the oxidation mechanism of SA was different from the other samples. As has been shown previously, SA is prone to agglomeration in water and changes in pH will affect

its viscosity (Hecht and Srebnik 2016, Yang et al. 2009). As no trailing peak appeared in the SA integration curve, it can be speculated that the addition of H_3PO_4 in the process of CO_2 purging may cause SA to agglomerate or adhere to the tube walls, which may result in a decrease in UVP-TOC value. The addition of sodium persulfate can improve the mineralization efficiency of NOM, as expected from its oxidation properties, and hence the accuracy of the measured TOC value. The decrease of UVP-TOC value after adding H_2O_2 may be caused by the mineralization of SA by H_2O_2 before SA entered the reactor. The results also indicated that to improve the accuracy of the UVP-TOC result of NOM, the concentration of H_2O_2 should be controlled to lie in an optimal range, which depends on the chemical properties and concentration of NOM in the sample. Excessively high H_2O_2 concentrations will lead to NOM being mineralized before entering the reaction, while too low an H_2O_2 concentration will not effectively mineralize NOM in the reactor, result in the TOC test results being far lower than the real TOC value.

3.4 Mechanism of H_3PO_4 influence on the UVP-TOC measurement

In Figure 2a, it can be seen that while pre-acidification increased the UVP-TOC value of the BSA sample, subsequent filtration by 0.22 μm microfilter (MF, PTFE, 2.5 cm diameter, ANPEL laboratory Technology (Shanghai) Inc.) had no significant effect on the UVP-TOC value. In contrast, for HA samples (Figure 2b), MF filtration of acidified samples reduced the UV-TOC value, indicating the removal of particulate matter, which was consistent with previous studies that have shown that at low pH ($pH < 1$), the HA may agglomerate into tight, large micelles (Suteerapataranon et al. 2006, Thurman and

Malcolm 1981).

In addition, the effect of H_3PO_4 on the surface charge of BSA and HA samples was studied by determining the zeta potential. The concentration of H_3PO_4 for the BSA and HA samples was 0.1 M in both cases, which was selected as the minimum that was found to affect the UVP-TOC value. In Figure 2c, the zeta potential measurements indicated that the pH of the BSA sample with 0.1 M H_3PO_4 was far from the protein isoelectric point (IP), which meant that the BSA was unlikely to precipitate (Peng et al. 2004). For the HA samples (Figure 2d), it can be seen that the initial zeta potentials of HA were -23.6 mV and -21.4 mV before and after filtration, while after acidification, the zeta potential decreased to -12.6 mV, and further decreased to -4.81 mV after filtration. The results indicate that the acidified HA sample might have formed compact micelles larger than the MF pores (220 nm) from the decrease of zeta potential (less electrostatic repulsion). Furthermore, the corresponding EEM spectra (SI, Figure S7) also revealed the occurrence of HA agglomeration by the acidification of the sample, as molecular agglomeration has been shown to lead to fluorescence quenching (Wang et al. 2015).

Figure 2.

To investigate the mechanism further, FTIR was used to analyze the changes in the groups and molecular structures of BSA and HA samples after acidification. Figure 3a showed that after acidification of the BSA samples, the peaks of Amide I-III (1700-

1600 cm^{-1} , 1600-1500 cm^{-1} , and 1450-1300 cm^{-1}) decreased, and the peaks of -COOH (1720 cm^{-1} and 1450 cm^{-1}) and -C-N (1235 cm^{-1}) increased, which indicated that the protein structures of BSA had been hydrolyzed (Lessing et al. 2012, Militello et al. 2004, Murayama and Tomida 2004). For the HA (Figure 3b), it can be seen that the FTIR spectra of the HA samples were substantially changed after acidification, which was caused by protonation (Hecht and Srebnik 2016). However, taking together the UVP-TOC results and existing research, it is believed that acidification mostly caused molecular agglomeration but did not cause hydrolysis.

The UV-visible spectra of BSA samples (Figure 3c) showed that there were no significant changes in the UV-visible intensity of BSA samples after H_3PO_4 acidification, but there was a light blue shift after acidification which could have been caused by the degradation of tryptophan and tyrosine residues in the BSA molecule (Malfoy and Reynaud 1980, Roeser et al. 2010). The EEM spectra further confirmed these observations (SI, Figure S8). Similar to the BSA samples, H_3PO_4 acidification did not affect the UV-visible intensity of HA samples, but there was no red or blue shift, and the intensity of HA samples after filtration decreased substantially, which indicated that acidification produced filterable (size > 0.22 μm) molecular agglomeration (Figure 3d).

HPSEC analysis was also used to verify whether H_3PO_4 addition caused hydrolysis or agglomeration of BSA and HA molecules. From Figure 3e, it was evident that the MW peak of BSA gradually decreased and shifted to the smaller molecular weight range with increasing H_3PO_4 concentration. Overall, the results supported the

hypothesis that acidification hydrolyzes BSA into smaller molecules, which could affect the measurement of UVP-TOC. For the HA samples (Figure 3f), it can be seen that with increasing H_3PO_4 concentration, the width of peaks was increased and the integral area decreased, which was consistent with the formation of agglomerated HA molecules that were removed by the 0.22 μm membrane filtration.

Equivalent diameter, as measured by dynamic light scattering (DLS), can indicate the size of the micelles of molecules in the solution. It was found that the equivalent diameter of BSA doubled when the H_3PO_4 concentration was 0.1 M, compared to the absence of H_3PO_4 , but decreased with increasing H_3PO_4 concentration (Figure 3g). As the equivalent diameter of the BSA measured by the DLS method includes the hydrated layer and the intramolecular void volume, it can be speculated that in the acidic H_3PO_4 solution, the BSA initially unfolded, and then gradually hydrolyzed into small molecules, which were responsible for the increase of the -COOH and -C-N groups evident in the FTIR spectra. For the HA samples (Figure 3h), the equivalent diameter of HA systematically increased with H_3PO_4 concentration up to 0.3 M and remained unchanged at higher H_3PO_4 concentrations. These results supported those described earlier that indicated HA agglomeration under acidic conditions has a deleterious effect on the accuracy of the UVP-TOC analysis (Figure 2b).

Figure 3.

3.5 Mechanism of H_2O_2 pre-treatment on the UVP-TOC measurement

In Figure 4a, the FTIR spectra of BSA samples showed that the peaks of Amide I and Amide II significantly decreased with the increase of H_2O_2 , and the peaks of $-\text{COC}-$ (1080 cm^{-1}) and $>\text{SO}_2$ (525 cm^{-1}) increased (Guler et al. 2016, Joy and Lewis 1991). These changes indicated that the oxidation of $-\text{S-S}-$ and $-\text{SH}$ groups occurred, as well as the epoxidation reaction on the side chains which generated $-\text{COC}-$ (Lessing et al. 2012). For the HA samples (Figure 4b), it was seen that with increasing H_2O_2 concentration, the peaks of $-\text{CH}_2/-\text{CH}_3$ ($3000\text{-}2700\text{ cm}^{-1}$) progressively decreased, and the peaks of $-\text{COOH}$ (1725 cm^{-1}), C-O-C (1230 cm^{-1}), and $-\text{OH}$ ($3300\text{-}3100\text{ cm}^{-1}$ and 1220 cm^{-1}) increased (Deng and Bai 2003, Lumsdon and Fraser 2005). The results indicated that alkane-type groups ($-\text{CH}_2/-\text{CH}_3$) in HA were oxidized to generate carboxyl groups by H_2O_2 .

The HPSEC results of BSA samples showed that the MW distribution of BSA samples shifted to the large molecular weight range with increasing H_2O_2 concentration, particularly for $[\text{H}_2\text{O}_2] \geq 5\text{ mM}$, and some small particles appeared at high H_2O_2 concentration (Figure 4c). These results might indicate the aggregation of BSA molecules under the action of H_2O_2 , and also that there was no obvious hydrolysis (Yu et al. 2017). The corresponding EEM spectra showed that tryptophan and tyrosine residues of BSA were oxidized (SI, Figure S9). For HA samples, the MW profiles, illustrated in Figure 4d, showed that the area of the peaks of HA slightly increased with the increase of H_2O_2 , which was possibly due to the oxidation increasing the UV absorbance of HA fractions at 254 nm . From the corresponding EEM spectra of HA (SI, Figure S10), it was found that the characteristic peaks were not significantly altered

after HA reaction with H_2O_2 , and HA decomposition products did not appear (Wu et al. 2014). Therefore, the results indicated that H_2O_2 did not appear to disrupt the aromatic structures of HA.

Figure 4.

The equivalent diameter results of BSA showed that the diameter increased with the H_2O_2 concentration, up to a maximum at 5 mM H_2O_2 (Figure 5a). The reason for this behavior was speculated to be the result of changes to the molecular structure and possibly micro-flocculation of the BSA at $[\text{H}_2\text{O}_2] \leq 5$ mM, which lead to the equivalent diameter increasing, while at $[\text{H}_2\text{O}_2] \geq 8$ mM the BSA molecules were gradually oxidized into smaller fractions and mineralized. Overall, the results indicated that the H_2O_2 improved the mineralization efficiency by degrading the protein structures of BSA, which lead to an increase of UVP-TOC values of the BSA samples. For HA (Figure 5b), the effect of H_2O_2 was similar to BSA, where an H_2O_2 concentration of ~5 mM gave a maximum TOC value and maximum equivalent diameter. Based on the results obtained, it can be inferred that H_2O_2 did not cause the agglomeration of HA but increased the amount of carboxyl, hydroxyl, and C-O-C groups by oxidation, which thereby reduced the inner cohesive force of the molecules and unfolded the structure of the HA molecules to expose more reaction sites; as a consequence, the overall oxidation efficiency of HA was greatly increased.

These observations concerning the influence of H_2O_2 on BSA and HA were also

supported by the decrease of tailing peak area in the integral curves (Figures 5c and 5d). It was evident from the integral curves that the addition of H_2O_2 significantly advanced the signal appearance time and effectively improved the symmetry of the integral curves (Figures S11a and S11b), which indicated that the addition of H_2O_2 increased the oxidation rate of NOM in the surface water samples.

Figure 5.

3.6 Effects of defoamer (DF) on the UVP-TOC measurement

In this study, it was found that BSA samples formed a large amount of foam during the purging process (Figure 6a and supporting video S1), which was believed to be one reason for the inaccuracies of the UVP-TOC value of the BSA samples. It was similar to the foam (bubble) enrichment of surfactants that lead to the BSA enrichment and adhesion on the walls of the purging tube, which resulted in a decrease of the UVP-TOC value of BSA samples, as shown in Scheme 2 (Chingin et al. 2018). It was found that the addition of H_3PO_4 had little effect on reducing the foam generation (Figure 6b and supporting video S2), but the addition of a defoamer (DF, silicone emulsion) effectively eliminated the foam (Figure 6c and supporting video S3). By comparing the UVP-TOC values of samples of BSA raw, BSA with 0.1 M H_3PO_4 , and BSA with 10 $\mu\text{g/L}$ DF, it was clear that the addition of the DF increased the UVP-TOC value of BSA by eliminating the foam (Figure 6d). To verify this, the same experiment was conducted using sodium dodecylbenzene sulfonate (SDBS) as a representative small

molecular weight surfactant, and one of the most difficult compounds to oxidize, as listed in EPA 5310C. It was found that the UVP-TOC value of SDBS was only one-sixth of the theoretical value without the addition of defoamer, but almost the same as the theoretical value with the defoamer (Figure 6e). For the effect of acidification, the results showed that the sample containing more protein-like matter, the OL water, had a greater increase in UVP-TOC value caused by acidification than the QR water (Figure 6f).

Scheme 2.

Figure 6.

4 Conclusions

In this research, it was found that the TOC-value detection by the UVP-TOC method was inaccurate for the surface water samples which contained the humic substances, proteins, and polysaccharides. Contrary to the viewpoints and conclusions of previous research, this study found that the inaccuracy in the TOC determination of the above-mentioned substances was caused by intramolecular and intermolecular agglomeration but not by the refractory structures in molecules such as the aromatic structures. Destroying the agglomerate structure of the above-mentioned substances by hydrolysis or oxidation in advance can effectively reduce the difference between the measured value and the true value. Furthermore, surfactants and surface-active substances (such

as BSA, etc.) also cannot be accurately detected for the reason of foaming during the purging processes. The foam enrichment effect caused the decrease of the substance concentration in the solution. This study has conducted in-depth research of the mechanisms causing inaccuracy during the UVP-TOC analysis of surface water samples, which has significantly enhanced understanding of the UVP-TOC analytical method. As such, it has helped explain the reasons for previously reported anomalies in TOC determinations, and will be of considerable value to future studies employing UVP-TOC analysis to quantify organic carbon concentrations in solution.

Acknowledgment

This work was also supported by the National Natural Science Foundation of China (Grants 51108444), and the Key Research and Development Plan of the Ministry of Science and Technology (2019YFD1100104).

Appendix A. Supplementary data

The Supporting Information contains 4 Tables, 11 Figures, and 3 Videos. The Tables provide the background TOC values of the reagents (Table S1), the carbon content results of the samples (Table S2), the main water quality characteristics of the samples (Table S3), and the student test results of UVP-TOC values (Table S4) in this study. The Figures provide the following: the HPSEC results of the surface water samples and the UVP-TOC values of surface water samples with different proportions of the original samples and the influence of Cl^- to the UVP-TOC value (Figure S1); the integral curves

of the samples (Figure S2); the UVP-TOC values of samples with different H_3PO_4 concentrations, persulfate dosages and H_2O_2 concentrations (Figure S3-S6); the EEM spectra of HA samples with acidification treatments (Figure S7); the EEM spectra of BSA samples with acidification treatments (Figure S8); the EEM spectra of BSA samples with different concentrations of H_2O_2 (Figure S9); the EEM spectra of HA samples with different concentrations of H_2O_2 (Figure S10); the integral curves of the surface water samples before and after pre-oxidation by H_2O_2 (Figure S11). The Videos show the occurrence of foaming during the UVP-TOC reaction stage for the BSA sample (Video S1), the BSA sample after the addition of H_3PO_4 (Video S2), and the BSA sample after the addition of defoamer (Video S3).

Author Contributions

Hankun Yang and Wenzheng Yu designed the experiments; Hankun Yang completed the main experiments and wrote the draft; Wenyu Wang and Mengjie Liu were involved in some experiments; Nigel J. D. Graham, Mengjie Liu, and Wenzheng Yu made the final revision to the manuscript.

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Figure captions

Scheme 1. The principle and components of the UV-persulfate oxidation method (UVP-TOC).

Scheme 2. The concentrating effect of N₂ bubbles (foam) in purging processes of UVP-TOC detection of BSA.

Figure 1. Variation of UVP-TOC value with the concentration of the “re-dissolved” OL samples (a) and QR samples (b), BSA (c), SA (d), HA (e), TA (f), CA (g), and BA (h). (The UVP-TOC measurement settings were as default).

Figure 2. Influence of H₃PO₄ concentration on the analysis of BSA and HA samples: UVP-TOC values of BSA samples (a) and HA samples (b); zeta potential of BSA samples (c) and HA samples (d); All of the samples with added H₃PO₄ (0.2 M, a-h) were reacted for 8 h before detection. The BSA and HA concentrations were 10 mg/L and 5 mg/L, respectively.

Figure 3. The FTIR spectra of BSA samples (e) and HA samples (f); UV-visible spectra of BSA samples (g) and HA samples (h); variation of HPSEC MW profiles of BSA samples (i) and HA samples (j); equivalent diameter of BSA samples (k) and HA samples (l). The BSA and HA concentrations were 10 mg/L and 5 mg/L, respectively. The UVP-TOC measurement settings were as default. The diameter of the MF filter pores was 0.22 μm . The mobile phase for the HPSEC was 10 mM sodium acetate, the flow rate was 1 mL/min, the column temperature was 25 $^{\circ}\text{C}$ and the detection wavelength was 254 nm, and the samples were pre-filtered with 0.22 μm filter).

Figure 4. Influence of pre-dosed H_2O_2 concentration on the analysis of BSA and HA samples: FTIR spectra of BSA samples (a) and HA samples (b); HPSEC MW profiles of BSA samples (c) and HA samples (d). The FTIR analysis samples were prepared by freeze-drying after 0.2 % (v/v) HCl addition; the mobile phase for the HPSEC MW analysis was 10 mM sodium acetate, the flow rate was 1 mL/min, the column temperature was 25 $^{\circ}\text{C}$ and the detection wavelength was 254 nm.

Figure 5. Influence of pre-dosed H_2O_2 concentration on the analysis of BSA and HA samples: equivalent diameter of BSA samples (a) and HA samples (b); integral curves (raw data) of BSA samples (c) and HA samples (d) without, and with 8 mM, H_2O_2 .

Figure 6. Influence of defoamer (DF) addition: example photographs of BSA raw (a),

BSA with 0.1 M H_3PO_4 for 6 h (b) and BSA with 0.2 $\mu\text{g/L}$ DF (c) samples; UVP-TOC values of BSA raw, BSA with 0.1 M H_3PO_4 for 6 h, BSA with 10 $\mu\text{g/L}$ DF (DF), BSA with 0.1 M H_3PO_4 (for 6 h) and 10 $\mu\text{g/L}$ DF, and 10 $\mu\text{g/L}$ DF background (DF BG) samples (d); theoretical UVP-TOC values of SDBS, SDBS with 10 $\mu\text{g/L}$ DF (DF), SDBS raw and 10 $\mu\text{g/L}$ DF background (DF BG) samples (e); UVP-TOC values of OL and QR samples before and after acidification, DF addition, acidification and DF addition (f). The concentrations of BSA and SDBS were both 10 mg/L.

Scheme 1. The principle and components of the UV-persulfate oxidation method (UVP-TOC).

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