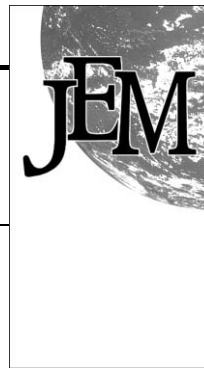


Assessment of relative accuracy in the determination of organic matter concentrations in aquatic systems†



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Accurate determinations of total (TOC), dissolved (DOC) and particulate (POC) organic carbon concentrations are critical for understanding the geochemical, environmental, and ecological roles of aquatic organic matter. Of particular significance for the drinking water industry, TOC measurements are the basis for compliance with US EPA regulations. The results of an interlaboratory comparison designed to identify problems associated with the determination of organic matter concentrations in drinking water supplies are presented. The study involved 31 laboratories and a variety of commercially available analytical instruments. All participating laboratories performed well on samples of potassium hydrogen phthalate (KHP), a compound commonly used as a standard in carbon analysis. However, problems associated with the oxidation of difficult to oxidize compounds, such as dodecylbenzene sulfonic acid and caffeine, were noted. Humic substances posed fewer problems for analysts. Particulate organic matter (POM) in the form of polystyrene beads, freeze-dried bacteria and pulverized leaf material were the most difficult for all analysts, with a wide range of performances reported. The POM results indicate that the methods surveyed in this study are inappropriate for the accurate determination of POC and TOC concentration. Finally, several analysts had difficulty in efficiently separating inorganic carbon from KHP solutions, thereby biasing DOC results.

Introduction

Accurate determinations of total (TOC) and dissolved (DOC) organic carbon concentrations are critical for understanding the geochemical, environmental, and ecological roles of aquatic organic matter. Issues associated with the analysis of organic matter have long been of concern among aquatic scientists, primarily the oceanographic community.¹ With the introduction of wet oxidation methods for the analysis of DOC in seawater,² a number of papers have been published focusing on different technologies and problems inherent in the measurement of DOC.³ Presently, the most common methods for determining DOC concentrations involve the oxidation of organic matter to CO₂. These methods, which include high-temperature combustion, persulfate oxidation, and ultraviolet photo-oxidation, have been thoroughly reviewed by Urbansky.⁴

Problems in the analysis of DOC and TOC arise, in part, because the samples themselves are composed of complex mixtures of materials with a range of molecular sizes, weights, and reactivities. Other problems related to the methods of analysis include the efficiency of oxidation, the possibility of sample contamination, and the difficulty of obtaining reasonable values for blanks.^{1,5} The methods using wet oxidation with persulfate, for instance, are dependent on the efficiency of oxidation, which may not be the same for all compound classes or in all sample matrices.⁶ Other factors, such as thermal degradation of persulfate at elevated temperature, also affect the reaction.⁷

With the recent promulgation of the disinfectant/disinfection

byproduct (D/DBP) rule by the US EPA, TOC has taken on a new significance for the drinking water industry, as TOC measurements are now a basis for compliance with these regulations. As part of the Information Collection Rule (ICR), the US EPA certified laboratories based on performance (certification range of $\pm 20\%$) with a calibration standard, potassium hydrogen phthalate (KHP), dissolved in deionized water. In the case of D/DBP compliance, however, measurements are being made on unfiltered samples with complex organic and inorganic matrices. To address uncertainties about the analytical accuracy of DOC and TOC measurements in freshwater, we assessed the capabilities of 31 laboratories that use a broad array of commercially available analytical instruments. A total of 34 instruments were used in this study. Participating laboratories were sent samples of a variety of standards prepared in deionized water. The methods used to oxidize organic carbon included catalysis, heat, chemical oxidation, ultraviolet radiation, or a combination of these methods.

Methods

The interlaboratory comparison was coordinated through the Stroud Water Research Center. All standards were prepared at the Stroud Center and distributed to the participating laboratories. Thirty-one participating laboratories were sent standards three times during the project. The participating laboratories included drinking water utilities, academic laboratories, government laboratories and TOC instrument manufacturers. Each shipment consisted of a series of eight standards, each supplied in triplicate in 40 mL vials. The instruments used in this study are given in Table 1. All of the instruments used nondispersive infrared detectors, with the

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Table 1 TOC analyzers represented in this study

Analyzer	Inorganic carbon removal	Oxidation	Number of analyzers
Anatel A-2000	Internal sparge	UV-promoted wet oxidation	1
Astro 2001	Internal sparge	UV-promoted wet oxidation	2
Dohrmann Apollo 9000	External sparge	High temperature combustion	1
Dohrmann DC-80	External sparge	UV-promoted wet oxidation	1
Dohrmann DC-180	External sparge	UV-promoted wet oxidation	5
Dohrmann Phoenix 8000	External sparge	UV-promoted wet oxidation	6
OI 700	Internal sparge	Pt-catalyzed wet oxidation	3
OI 1010	Internal sparge	Pt-catalyzed wet oxidation	2
OI 1020	External sparge	High temperature combustion	1
Shimadzu 5000A	Shimadzu 5000A	High temperature combustion	8
Shimadzu 5050	External sparge	High temperature combustion	2
Sievers 800	Internal sparge	UV-promoted wet oxidation	2

exception of the Sievers 800, which uses conductivity detectors. Standards prepared in deionized water were the focus of the first round of analyses, followed by a focus on inorganic carbon and mineral salt matrix modifications for subsequent sets. The concentration range for dissolved standards was 2–4 mg C L⁻¹. Each participant received a data sheet for reporting and was asked to analyze and return the data within a 4–6 week period. Reported data were normally distributed with the exception of the polystyrene beads and P-17 bacteria. Overall relative accuracies, defined as the ratio of the measured value to actual value multiplied by 100, were calculated for all standards.

The standards included KHP, caffeine, dodecylbenzene sulfonic acid, humic acid, fulvic acid, leaf powder, polystyrene microbeads, and freeze-dried bacteria. Caffeine and KHP were reagent grade. Dodecylbenzene sulfonic acid was only available in technical grade. The organic carbon content of each standard was verified by elemental analysis (Huffman Laboratories, Golden, CO). As a further check on the reproducibility and stability of the standards, concentrations in 60 vials from each lot were measured at the Stroud Center. Three TOC analyzers, representing each oxidation type, were used to analyze 10 vials each prior to shipment. These measurements were repeated after the last data set was received from participants measuring standards from that lot. The fulvic and humic acid samples, isolated from the Suwannee River, were obtained from the International Humic Substances Society. Leaf powder was prepared from red maple leaves that were collected after abscission, which were air dried, and ground in a Wiley mill with a Monel metal sieve of 60 mesh. Polystyrene microspheres (1 µm diameter) were obtained from Polysciences, Inc. Freeze-dried bacteria were obtained by first inoculating 0.1% tryptone yeast extract with *Pseudomonas fluorescens* (strain P-17, American Type Culture Collection 49642) and growing the bacteria to the late log phase on a shaker table at room temperature. The cells were harvested by centrifugation and the resulting pellets resuspended in sterile phosphate buffer (pH 7). Three washes were performed to eliminate any tryptone yeast extract. The supernatant fluid from the final wash was decanted to waste, the pellet resuspended in cold deionized water, and the suspension freeze dried. Subsamples of all particles were sent to Huffman Laboratories for the determination of inorganic carbon and organic carbon by elemental analysis.⁸ The organic carbon content of the particles was used to prepare standards of suspensions with concentrations of approximately 2 mg C L⁻¹. Stock solutions of suspensions were thoroughly mixed to obtain representative subsamples to send to participants in the study.

Standards were amended with inorganic carbon (IC) from sodium carbonate (30 mg IC L⁻¹) and mineral salts

(conductivity of 512 µS) to assess the influence of sample matrix on TOC/DOC measurements. Sodium carbonate was combusted at 550 °C for 6 h to remove potential organic residues and then tested for inorganic and organic carbon content. The sodium bicarbonate was found to contain 0.075% w/w organic carbon. Mineral salts consisted of 10 mg L⁻¹ CaCl₂·2H₂O, 50 mg L⁻¹ MgSO₄·7H₂O, 10 mg L⁻¹ KH₂PO₄, 10 mg Ca(NO₃)₂·4H₂O, 1 mg L⁻¹ Al₂(SO₄)₃·18H₂O, and 10 mg L⁻¹ NH₄Cl. Each salt was tested individually for the presence of organic carbon, which was found to be negligible in all cases.

Results and discussion

Dissolved performance standards

All participating laboratories performed well on samples of KHP, a compound commonly used as a standard in carbon analysis (Table 2). The overall average relative accuracy for the 34 instruments used to measure KHP was 103.2 ± 7.2%, with a range of 82–165.6%. All but five of the 97 values reported over the course of the study were within the earlier US EPA guidelines of ±20% accuracy. Seventy-five of the reported values were within 10% of the theoretical value, with 46 of them within 5% or better. These results are not surprising given that KHP is a relatively easily oxidized compound routinely used to calibrate instruments for TOC and DOC analyses.⁹ Hedges *et al.*¹⁰ reported similar results for a comparison of four different commercial instruments using glucose, another easily oxidized compound. In that study, good precision and accuracy were obtained for freshwater and seawater samples amended with glucose, however, the authors correctly noted that the results obtained with easily oxidized materials do not suggest that all properly handled DOC analyzers would perform equally well on solutions containing other organic compounds.

Reported data were normally distributed with the exception of the polystyrene beads and P-17 bacteria. Statistical analysis (ANOVA test) indicated significant differences ($P = 0.05$) between the recoveries obtained with solutions of KHP and those obtained with caffeine, dodecylbenzene sulfonate and the humic substances. Dodecylbenzene sulfonic acid (Fig. 1) was the most difficult of the dissolved standards to oxidize with an average overall relative accuracy of 80.2% (±19.9%). Forty of the 97 reported values were less than 80% of the actual value, with the relative accuracies ranging from 22.3% to 115.6%. The range of relative accuracies obtained by analysts using identical instruments was 41.7–108.8% for the six Dohrmann Phoenix 8000 instruments, 47.8–91.7% for the six Dohrmann DC-180 instruments, and 22.3–115.6% for the eight high-temperature Shimadzu 5000A instruments used. The range of results obtained with dodecylbenzene sulfonic acid by different analysts using the same model of instrument indicate that, while differences may exist in the performances of individual instruments, operator error is an important factor.

Better results were obtained for caffeine, with an overall relative accuracy of 89.9% (±14.4%). However, in this case, 20

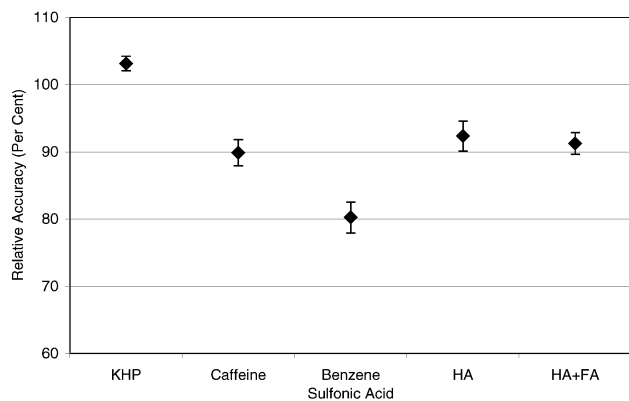
‡The use of trade, product or firm names is for descriptive purposes only and does not imply endorsement by the U.S. Government.

Table 2 Relative accuracies for analyses of standards

Standard	Number of data reports	Range of recoveries (%)	Overall relative accuracy (%)	Standard deviation (%)
<i>Dissolved materials:—</i>				
KHP	97	82–165.6	103.2	± 7.2
Caffeine	64	56–119.6	89.9	± 14.4
Dodecylbenzene sulfonic acid	97	22.3–115.6	80.2	± 19.9
Humic acid	32	70.6–116.6	92.4	± 12.5
Humic and fulvic acid	32	65.2–109	91.3	± 9.3
KHP plus NaHCO ₃	63	60–257	115.3	± 20.8
Caffeine plus NaHCO ₃	32	75.3–160.5	103.9	± 18.5
Humic–fulvic acid plus NaHCO ₃	32	71.1–416.1	109.8	± 57.7
KHP plus salts	35	89.1–118.9	101.9	± 6.2
Fulvic acid plus salts	33	77.1–115	96.1	± 9.0
<i>Particulate materials:—</i>				
Polystyrene beads	63	1.4–115.8	43.3	± 34.9
Dried bacteria	64	9.4–132.5	51.8	± 32.0
Leaf powder	33	53.6–99.8	70.3	± 9.6
Polystyrene beads plus salts	31	2.4–129.8	38.2	± 34.9

of the 64 reported results, about a third, were not within the 20% certification range. Relative accuracies ranged from 56% to 119.6%. On average, the high temperature systems overestimated the concentrations of caffeine, while the UV–persulfate and the Pt–persulfate systems underestimated the caffeine concentrations. Ranges for operators of the same model instruments were smaller than those for dodecylbenzene sulfonic acid, including 56.1–83.6% for the six Phoenix 8000 instruments, 62–111.9% for the six Dohrmann DC-180 instruments, and 90.3–119.6% for the eight Shimadzu 5000A instruments. In almost all cases, however, the overall relative accuracies for dodecylbenzene sulfonic acid and caffeine were lower than those obtained for KHP, indicating less than 100% oxidation efficiency for these compounds. Both dodecylbenzene sulfonic acid and caffeine revealed analytical problems not observed for KHP, demonstrating the usefulness of these compounds for assessing both instrument and operator performance.

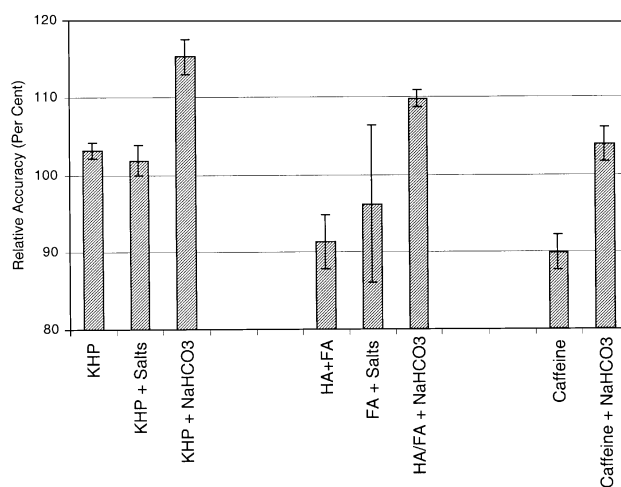
Naturally occurring humic substances posed fewer problems for analysts (Table 2, Fig. 1). Average relative accuracies of 92.4% (± 12.5%) for humic acid samples and 91.3% (± 9.3%) for humic–fulvic mixtures were reported. Six of the 32 results for the humic acid sample and two of 32 values reported for the humic–fulvic mixture were not within the 20% certification range. The high-temperature and the UV–persulfate instruments had the most difficulty with the humic and fulvic acids. The range of accuracies for both standards with the eight Shimadzu 5000A instruments was 65.2–116.6%, 82.2–95.8% with the six Phoenix 8000 instruments, and 70.6–96.7% with the six Dohrmann DC-180 instruments. These ranges again indicate the importance of operator error in the analysis of

**Fig. 1** Comparison of overall relative accuracies for analyses of dissolved standards. Error bars represent standard errors.

DOC concentration. Humic substances are a major fraction of the dissolved organic matter pool in most natural waters, accounting for 20–80% of the DOC.¹¹ Our data suggest that the refractory organic compounds that comprise these materials are more difficult to oxidize than compounds such as KHP and glucose.

Matrix effects

Inorganic sample matrices differ between samples collected from different environmental settings and can play significant roles in the analysis of DOC. There was little influence of the added mineral salts (conductivity adjusted to 512 µS) on the analysis of KHP, as 92% of the instruments operated within 10% or better of the theoretical value (Fig. 2). Similarly, no effect of the mineral salts was noted for the analysis of caffeine. While the conditions used in the study were realistic for many freshwater environments, especially those used for drinking water, the influence of inorganic constituents other than carbonate can increase with increasing concentration. For instance, Aiken⁶ reported that the presence of Cl⁻ in concentrations greater than 0.02 M interferes with the analysis of aqueous DOC concentrations by the Pt-catalyzed wet oxidation method. Two reasons were noted for this interference. First, Cl⁻ can be oxidized to a higher oxidation state and compete with the organic matter for oxidant, thus lowering the overall reaction efficiency for the oxidation of organic

**Fig. 2** The effects of a salt matrix and NaHCO₃ on the overall relative accuracies for analyses of KHP, humic substances and caffeine. Error bars represent standard errors.

matter. This is a situation that can arise with any substance that can be oxidized that is in the system. Second, the resulting HOCl from the oxidation of Cl^- in water is a powerful oxidizing agent that can react with organic matter to produce significant amounts of chlorinated intermediate compounds in addition to CO_2 .

Surprisingly, a significant effect was noted for the addition of inorganic carbon in the form of Na_2CO_3 . For all the methods used in this study, IC, usually in the form of hydrogen carbonate (HCO_3^-), must be removed before analysis of organic carbon. This is commonly accomplished by acidifying the sample with phosphoric acid ($\text{pH} < 3$) followed by a gas purge to remove CO_2 from the sample. This step in the analysis of organic matter in water is critical since almost all waters with $\text{pH} > 4$ have inorganic carbon from atmospheric and geologic sources.¹² Several analysts had difficulty in efficiently separating an IC amendment from the KHP standard when a 30 mg IC L^{-1} spike was added to the 2.5 mg C L^{-1} standard. Seventeen of the 65 reported values overestimated the actual KHP concentration by 20% or greater (failing US EPA certification), indicating incomplete removal of inorganic carbon. In addition to KHP, two other standards, caffeine and a humic–fulvic mix were amended with inorganic carbon. In all three cases, statistical analyses (*t*-tests) indicated significant differences between the results obtained in the absence and presence of NaHCO_3 . While the reported data were closer to the actual values for caffeine and the humic–fulvic mix, these data are misleading in that the average values in the presence of NaHCO_3 were significantly greater than those measured in its absence.

Problems with the removal of IC are largely analyst related. Some of the instruments used in this study were designed to separate inorganic from organic carbon as part of the analysis. Other instruments require an external purge of IC while still others measure organic carbon as the difference between total carbon and IC. Analysis of the data for individual instruments indicates that several were operated improperly to adequately remove IC from KHP, caffeine, and the humic–fulvic mix. The need to remove IC from samples for organic carbon determination has long been recognized. As early as 1965, van Hall *et al.*¹³ demonstrated that purging samples adjusted to pH values below pH 4 for 3–5 min was sufficient to remove essentially all the carbonate in solutions containing $200 \text{ mg L}^{-1} \text{HCO}_3^-$. Hydrogen carbonate is present in almost all natural waters. The spike used in our study (30 mg IC L^{-1}) is less than the average global concentration for HCO_3^- for many rivers and lakes of 58.4 mg L^{-1} , with a range of $0 \rightarrow 1000 \text{ mg L}^{-1}$ reported for surface waters.¹⁴ Less than complete removal of the IC in a sample can result in significant errors. Our results suggest that careful attention should be paid to the effectiveness of IC removal regardless of the instrument used.

Particulate materials

Particulate organic matter (POM) samples in the form of polystyrene beads, freeze-dried bacteria, and pulverized leaf material were the most difficult for all analysts, with a wide range of performances reported (Table 2). Average relative accuracies and ranges were 70.3% (± 9.6) (range 53.6–99.8%) for the leaf powder, 51.8% (± 32) (range 9.4–132.5%) for freeze-dried bacteria, and 43.3% (± 34.9) (range 1.4–115.8%) for the polystyrene polybeads (Fig. 3). On average, the high-temperature instruments were superior to the other oxidation technologies with the particles, however, there were very broad ranges of operator performance with the same model analyzer. The range of accuracies for the high-temperature instruments was 58–99.8% with the leaf powder, 15–99% with the freeze-dried bacteria and 67–99% with the polystyrene polybeads. In a previous study, Gershey *et al.*¹⁵ also reported that high-temperature oxidation was more effective than the wet

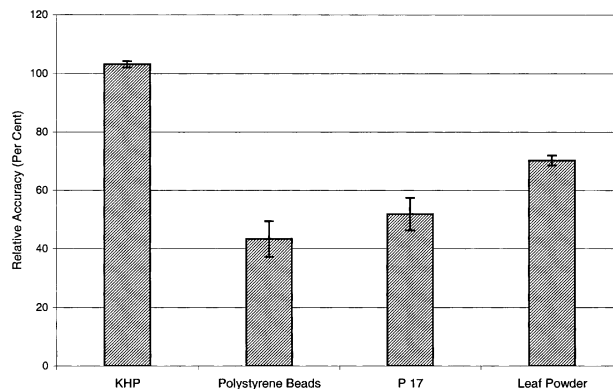


Fig. 3 Comparison of the overall relative accuracies for analyses of KHP, polystyrene beads, bacteria (P-17) and leaf powder. Error bars represent standard errors.

chemical oxidation methods for the determination of particulate materials in seawater.

The overall performances for dissolved and particulate materials for each instrument type represented in this study are presented in Fig. 4. It should be noted that the number of each instrument type represented in Fig. 4 is variable (Table 1), and in some cases the data were generated using a single instrument. Averaged over all instruments and analysts, standards that were solutions were analyzed with 91.4% relative accuracy, while standards that were particles were analyzed with 55.1% relative accuracy. The range for the individual analysts with standards that were solutions was 22.3–165.6%, while the range with particles was 1.4–132.5%. These results indicate that a well-calibrated instrument does not guarantee accurate results with organic solutions. Furthermore, the POM results indicate that the methods surveyed in this study are inappropriate for the accurate determination of TOC concentrations and raise concern about the quality of TOC data reported in the literature.

Summary and conclusions

In general, all the instruments performed reasonably well on dissolved samples, especially KHP, while the high-temperature instruments performed better on the particulates. The strong performances of all the participating laboratories on the analyses of KHP solutions raise questions about the effectiveness of using KHP as a certification standard for organic carbon analysis. The analysis of KHP and other easily oxidized compounds, such as glucose, provide little challenge for commercially available instruments operated by reasonably competent analysts. In addition, KHP is used by many analysts as a calibration standard, which, of itself, should obviate its use as a certification standard. The need exists, therefore, for a more challenging compound to serve as a certification standard. The results reported for both dodecylbenzene sulfonate and caffeine were generally found to be the lowest of the dissolved compounds with the largest range of results. These readily available compounds may be, therefore, good candidates to test overall analytical performance when measuring DOC, TOC, or POC concentrations in drinking water sources.

The data presented in this paper confirm the importance of the individual operator in providing accurate data. Operators using the same instrument reported significantly different results for analyses on the same solutions, suggesting that differences in reported results are more dependent on operator skill than on the instruments used for the analyses. While it was not our goal to compare the effectiveness of different carbon analyzers, operator-related variability in the data preclude a critical evaluation of the strengths and weaknesses of the

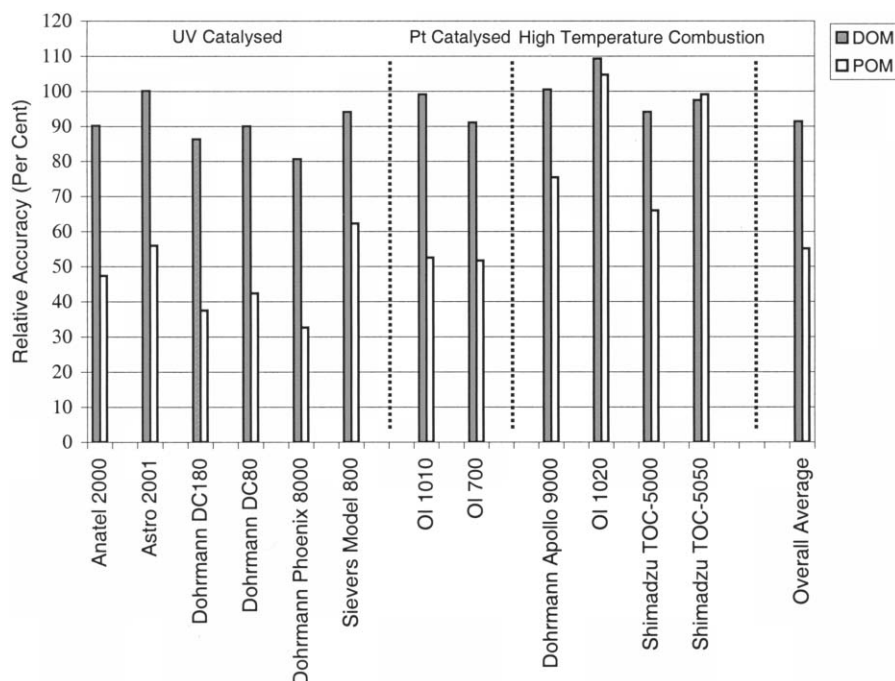


Fig. 4 Comparison of the average relative accuracies between dissolved compounds and suspended particles for each instrument type.

different methods used in this study. Of particular significance were the results reported for samples amended with NaHCO_3 . The removal of inorganic carbon is an important, albeit, relatively simple task. The large variability and positive bias in results reported for solutions of standards containing a small amount of IC compared with most natural waters indicate that greater care should be given to this part of the analysis. It is recommended that certification standard solutions contain IC.

Finally, the results obtained with particulate materials raise serious concern about the ability to adequately measure POC concentrations by the methods used in this study. Traditionally, POC concentrations have been determined by directly measuring organic matter retained on a filter by rigorous wet chemical or high-temperature combustion techniques. Since TOC data are not as useful as DOC and POC data for understanding processes, environmental chemists and geochemists have not given much attention to problems associated with TOC analysis. If reliable TOC data are to be determined, problems associated with oxidation efficiency must be addressed. Furthermore, suitable particulate materials should be identified to serve as performance standards.

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