A Science Based Performance Comparison of On-Line TOC Analyzers

Sievers 500 RL
On-Line TOC Analyzer

imagination at work

Background

The majority of pharmaceutical companies in the United States and Europe use laboratory Total Organic Carbon (TOC) analyzers for the US and EP compendial TOC release of water to manufacturing. Many companies have interests, plans, or are actively converting from laboratory TOC analysis to automated water release using on-line TOC analyzers. The motivation is to improve quality and lower the costs of producing purified water. The FDA documents, *Pharmaceutical cGMPs for the 21st Century–A Risk-Based Approach*[1] and *PAT—A Framework for innovative Pharmaceutical Development, Manufacturing, and Quality Assurance,*[2] encourage the application of scientifically and risk-based approaches to the development of automated process control systems in the pharmaceutical industry. The basis of a properly designed and qualified on-line TOC based real-time release (RTR) system is to identify and understand the critical parameters for success. These include an assessment of the current knowledge and experience, the capabilities of the available analyzers, an evaluation of risks, and an accurate investment analysis. The focus of this study is on the performance capabilities of pharmaceutical on-line TOC analyzers.

We began the investigation with an "on-line TOC" pharmaceutical survey to find out what the industry had already learned.[3,4] From the responses of 200 on-line TOC users, 18 indicated they were releasing water in real-time using an on-line TOC analyzer. We found that most were releasing water to manufacturing using laboratory TOC or a combination of on-line and laboratory TOC analyzers, and most wanted to automate the process at some time in the future. The survey gave us contacts for companies already doing release with on-line TOC. Our group became directly involved with three sites that were actively in the process of converting to on-line TOC RTR systems. Many on-line TOC release projects were working well, but some of the projects were stopped or delayed for various reasons. We contacted the principals involved to learn from their experiences. It became clear that some had experienced difficulties with their on-line TOC analyzers producing false positive TOC measurements, results that could not be confirmed on laboratory TOC analyzers. One of these companies recently experienced a severe situation resulting from the simultaneous malfunction of all the on-line TOC equipment. They were using one on-line analyzer as the instrument of record for releasing water to manufacturing. During the TOC upset, all the on-line TOC analyzers were reporting TOC values in excess of 1000 ppb C, while grab samples of the same water measured by laboratory TOC analyzers reported only 70 ppb C. Eventually, these analyzers all started producing over-range errors and completely failed to report any TOC results. Since they had successfully used these instruments for over five years, the designer of the water system requested that we study how and why this could happen. To better understand this situation, we designed a ½ factorial experiment to evaluate the comparative performance of commercial on-line analyzers. To our knowledge, there are no other published works on this topic. We expect others will be able to reproduce and improve on this work and that it will be a useful reference tool for future pharmaceutical on-line TOC users.

Comparison Study Test Plan

Properly validated on-line TOC analyzers should not provide incorrect TOC results. It is important to understand how to properly validate an on-line TOC analyzer and the TOC method it uses. For guidance we looked to the international standard *ICH Q7A Good Manufacturing Practice Guidance for Active Pharmaceutical Ingredients,*[5] Validation of Analytical Methods (12.8) section:

> Analytical methods should be validated unless the method employed is included in the relevant pharmacopoeia or other recognized standard reference. The suitability of all testing methods used should nonetheless be verified under actual conditions of use and documented. These requirements are also found in the cGMP regulations 21 CFR 211.194a. Methods should be validated to include consideration of characteristics included within the ICH (Q2B)[6] guidance's on validation of analytical methods. The degree of analytical validation performed should reflect the purpose of the analysis and the stage of the API production process. Appropriate qualification of analytical equipment should be considered before initiating validation of analytical methods.

In addition, the guidance document *Qualification of Analytical Instruments for Use in the Pharmaceutical Industry: A Scientific Approach*[7] was developed during a collaborative workshop between the FDA, drug manufacturers, pharmaceutical scientists, and equipment vendors. This effort was created to address a lack of clear and authoritative guidance for the validation/qualification of analytical instruments and was sponsored by the American Association of Pharmaceutical Scientists (AAPS), co-sponsored by the International Pharmaceutical

Federation (IPF) and International Society for Pharmaceutical Engineering (ISPE). Finally, the USP Chapter <1225> "Validation of Compendial Methods" attempts to harmonize the USP with the cGMP, ICH, and AAPS analyzer validation approaches.

There are multiple on-line TOC methods commercially available, but both the USP and EP TOC chapters do not reference any particular method. USP <643> states "This Chapter does not limit or prevent alternative technologies from being used, but provides guidance on how to qualify these analytical technologies for use." Unfortunately, this statement can be misunderstood to mean that if the TOC analyzer passes the requirements of Chapter <643>, then it is qualified for use in pharmaceutical waters, but USP <643> does not address analytical instrument method validation requirements, nor does it reference USP<1225>. Based on 21 CFR 211.194a, ICH Q7A, ICH Q2B and the AAPS guidance, on-line TOC methodologies should be validated as part of their qualification for use. In addition, Q7A indicates that on-line TOC methods should be validated and proven suitable under the actual conditions of the pharmaceutical application.

In spite of the USP <643> and EP <2.2.44> Chapters' suggestion that simply passing the system suitability test is sufficient for TOC qualification, the other guidances clearly state otherwise. To validate the different TOC methodologies based on this input, we decided to introduce known concentrations of compendial organics and likely real-world organics directly into pharmaceutical-grade water. These controlled solutions were then introduced into the different on-line TOC analyzers to determine their suitability for use. We implemented a ½ factorial design of experiment (DOE) where we varied the on-line TOC methodology, the organic compound, the concentration of the organic compound, the concentration of the dissolved oxygen, and the concentration of dissolved CO_2 in the sample water to determine the critical factors affecting on-line TOC performance.

Apparatus and Test Equipment

We used an ultrapure water (UPW) system that produced extremely low levels of TOC, oxygen, and ions (less than 5 ppb C, 4 ppb O_2, and deionized, respectively). The primary water purification system consisted of an activated carbon bed, water softener bed, single stage reverse osmosis unit, ultra low organic removal bed, ultraviolet light (UV) reactor operating at 184 nm and 254 nm wavelengths, and a mixed ion-exchange bed. This water was injected into a storage tank and then recircu-

lated through a second, larger UV reactor, two larger mixed bed ion-exchange beds, to the distribution loop, and back into the main storage tank. There were multiple points of use connected to the main distribution loop, one of which was used for our testing station.

Most pharmaceutical water systems use storage tanks that are vented to the air through microbial blocking vent filters, thereby adding dissolved CO_2 and O_2 to the water. To our primary water sample drop stream we added equipment to introduce controlled levels of dissolved carbon dioxide and dissolved oxygen to simulate this situation. The dissolved CO_2 was used to vary the water conductivity from 0.055 µS/cm (deionized) to 1.2 µS/cm. This upper conductivity level matches the USP Chapter <645> conductivity limit for Water for Injection (WFI) at 25 °C. Since the inlet water was deionized and degassed, we used an on-line conductivity sensor[8] to measure the CO_2 level and an on-line dissolved oxygen (DO) sensor[9] to measure the DO of the water. These gases were diffused into the UPW using a commercially available hollow fiber membrane device[10] designed to add or remove gases from water.

We previously developed a method to accurately add organics to a water stream.[11] With this method, the test water flow was measured with a (NIST-traceable) turbine flow meter[12] and then introduced to an injection tee. Organics were injected into the tee from a syringe at micro-liter-per-minute flow rates. A computer monitored the turbine meter to dynamically control the syringe's injection rate of organics to achieve a fixed dilution ratio. With this system, we were able to introduce controlled organic compound concentrations into the test water stream. Using propagation of errors analysis, we estimated a solution concentration accuracy of ± 3% of the target organic concentration. The system allowed us to easily inject different organics at levels from 0.25 ppb C to 600 ppb C into the test stream. This resulting solution of organic, CO_2, and DO enters into the analyzer manifold and into the on-line TOC analyzers. An overall schematic of the test apparatus is shown in **Figure 1**.

Organic Compounds

By definition, a typical TOC analyzer oxidizes all the covalently bonded organic carbon in solution to CO_2, then measures the CO_2 as carbon mass per solution volume. Common units of measure are µg of carbon/liter or parts per billion as carbon (ppb C). There are millions of organic compounds. For understanding the performance of on-line TOC analyzers, the organics we selected are not completely definitive, but rather they are considered to

be indicators of actual performance. For this study, we selected organic compounds that are used or proposed for use in the USP, EP, and JP TOC compendia and those actual organic compounds that are known to pass through the various water purification units found in real pharmaceutical water systems.

In principle, if a TOC analyzer recovers all organics equally well in the actual conditions of use, then one could select the most difficult-to-oxidize compound and test its recovery at the compendial TOC limit. This would establish the ability of the TOC analyzer to measure all the TOC. However, we have observed that the selection of the most difficult-to-oxidize organic compounds depends on the TOC methodology, sample pre-treatment, oxidation system stability, and water sample components such as DO, temperature, pH, and conductivity. Laboratory TOC analyzers are the least susceptible to variations in water sample conditions, but compared to on-line TOC analyzers they require additional maintenance to ensure good performance. For a combustion oxidation based TOC, the compounds that are considered thermally refractory are considered difficult-to-oxidize compounds. In a short wave UV light-persulfate oxidation-based TOC system, the organics that are resistant to hydroxyl radical or sulfate ion radical attach are considered difficult to oxidize. A reagentless on-line TOC analyzer using only short wave UV light will have difficulty oxidizing organics that are optically non-absorptive or that require a lot of oxygen in order to be completely oxidized. The latter factor also depends on the presence of enough available DO in solution to meet the stoichiometric requirements for complete oxidation to CO_2.

To determine the likely organics present in purified pharmaceutical waters, we reviewed water purification organic removal articles from the *Ultrapure Water Journal* archive files.[13,14,15,16] In addition, Dr. Stephan Huber of DOC-Labor has analyzed pharmaceutical waters using his TOC/liquid chromatographic analyzer.[17,18,19] His analysis system is able to analyze general classes of organic compounds in drinking water and narrower classes or specific compounds in purified pharmaceutical water.

Based on these references, we chose to test the organics and test concentrations listed in **Table 1**. Sucrose and 1,4-benzoquinone at 500 ppb C are the current compounds used for determination of TOC analyzer system suitability in the USP and EP. Nicotinamide is considered a difficult-to-oxidize organic and is being studied in Japan as an alternative to 1,4-benzoquinone.

Alcohols are natural biological decomposition products found in surface waters. They can also be introduced into water from industrial waste streams. Methanol and isopropanol are simple, small, highly soluble, aziotropic during distillation, non-ionic, and used as solvents in chemical and pharmaceutical manufacturing. All these qualities make them difficult to remove using the typical water purification equipment used in the pharmaceutical industry.
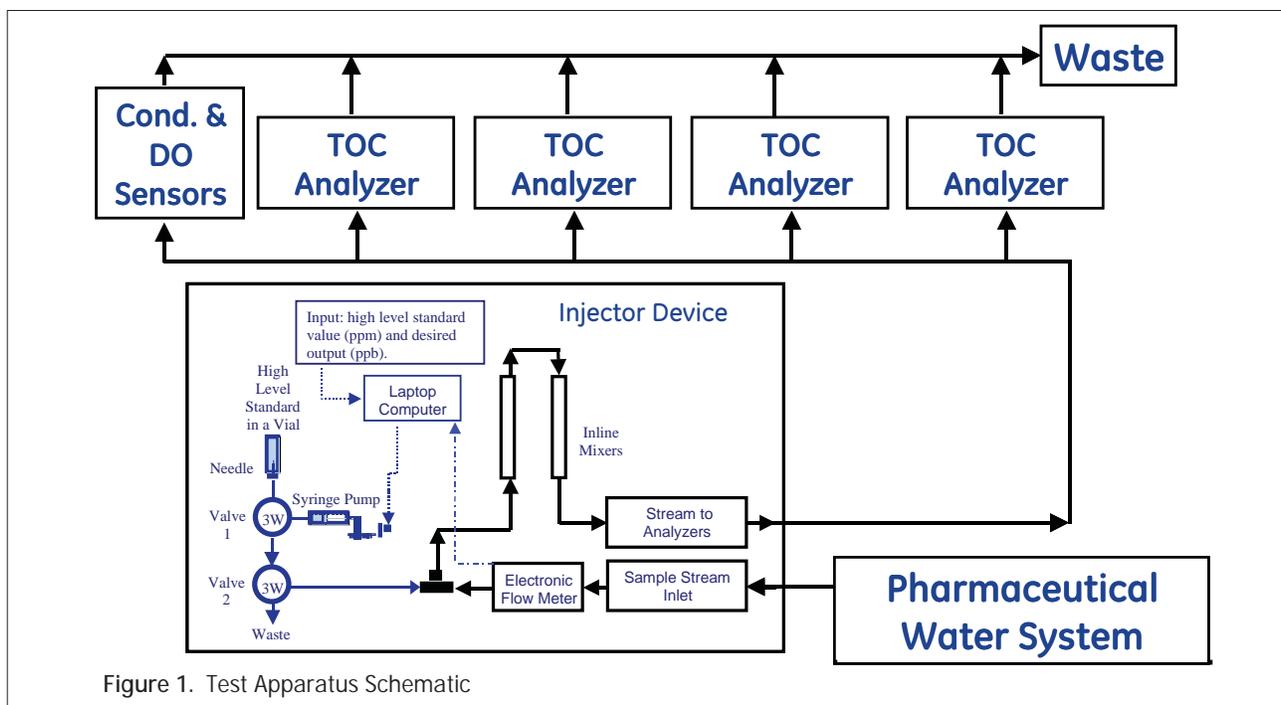

Figure 1. Test Apparatus Schematic

The weak organic acids (acetic and formic) and base (trimethylamine) are also products of the natural decay of biological matter. In their non-ionized state they are small, highly soluble, volatile, and used in chemical and pharmaceutical manufacturing. Contrary to common belief, they may not be well removed by ionic removal processes. Both are partly non-ionic and are not well retained on ion-exchange resins. They are the first components to leak from deionization resin beds as they deplete. Many older pharmaceutical water systems use only water softeners and distillation, both allowing organic acids and bases to pass. Even though these organic compounds are ionic, they are allowable in both purified-water (PW) and water-for-injection (WFI) because the compendial conductivity limits are relatively high. Dr. Huber has confirmed their presence in pharmaceutical waters. The newer continuous electro-deionization systems have difficulty removing weak acids and weak bases, especially if they were designed to just meet the compendial conductivity limits. The weak base trimethylamine was chosen because it is a component of the strong base ion-exchange resin and has frequently been measured as a water system extractable organic in pharmaceutical and purified water.[20]

Chloroform is a synthetic organic compound formed when water is chlorinated or chloramined for sanitary reasons. It is one of the disinfection by-products in the trihalomethane (THM) group that has been regulated by the USEPA to a level of less than 80 ppb as com-pound. Chloroform concentrations can vary by large amounts in drinking water. In response to large rainstorms or during spring run-off, municipalities often increase the chlorination level to ensure sufficient disinfection of the water, and the result is unusually high levels of chloroform. Chloroform is poorly retained by activated carbon, ion exchange, reverse osmosis (RO), and distillation purification processes. It is volatile and can be gas stripped or vacuum degassed from source waters. We know it is not readily removed by RO because we measured about 20 ppb as chloroform ($CHCl_3$) in both the inlet and outlet of our properly operating single-stage RO unit.

On-line TOC Analyzers Tested

We acquired the latest versions of three reagentless on-line TOC analyzers commercially available for pharmaceutical use. The three analyzers use different TOC methodologies. The first methodology is direct conductometric detection with partial oxidation of the organics. We will refer to it as the "DC/UV Partial" method.[21] The second methodology uses direct conductometric detection with complete oxidation. We will refer to this methodology as the "DC/UV" method.[22] The third methodology uses membrane-conductometric (MC) detection with complete oxidation. We will refer to it as the MC method. We used two versions of this methodology. The first version is the reagentless membrane conductometric and UV-only oxidation system, and we will refer to it as "MC/UV" method.[23] The second version is membrane conductometric and UV with persulfate analyzer that we will refer to as the "MC/UV-Persulfate" method.[24] We used the "MC/UV-Persulfate" analyzer as a reference TOC analyzer. It is a popular laboratory TOC analyzer in the industry and is the most common TOC analyzer of record used to meet the TOC compendial test requirements. This analyzer was configured for on-line operation and used acid and oxidizing reagents for operation. Before we began our testing, we required that all tested analyzers have factory-issued calibration certificates and new UV lamps, and that each analyzer had successfully passed the USP <643> TOC recommended system suitability test.

Table 1 Organic Compounds Tested		
Compound	Concentrations	Class
Sucrose	500 ppb C	Compendial (USP/EP)
1,4 Benzoquinone	500 ppb C	Compendial (USP/EP)
Nicotinamide	500 ppb C	Difficult to Oxidize
Methanol	50 and 500 ppb C	Simple Alcohol
Isopropanol (IPA)	50 and 500 ppb C	Simple Alcohol
Acetic Acid	19 ppb C (0.3 uS/cm)	Weak Organic Acid
Formic Acid	9 ppb C (0.3 uS/cm)	Weak Organic Acid
TMA (Trimethylamine)	15 & 60 ppb C	Weak Organic Acid
$CHCl_3$ (Chloroform)	12, 13, 15 ppb C	Interference Organic

On-line TOC Methodologies

Organic Carbon (OC) is defined as the concentration of all covalently bonded carbon in organic molecules in the water. Inorganic carbon (IC) is defined as the sum of the concentrations of CO_2, bicarbonate, and carbonate in water. CO_2 is very soluble in pure water and reacts with water to form carbonic acid (H_2CO_3) that partially ionizes to bicarbonate (HCO_3^{-1}) and carbonate (CO_3^{-2}). The sum of OC and IC is defined as total carbon (TC). Total Organic Carbon (TOC) is equal to the TC concentration minus the IC concentration. Inorganic carbon (IC) produces a background in the TOC analysis and must be accounted for by either removing it or measuring and subtracting it from the TC concentration.

Organic Oxidation Methods

Oxidation of the covalently bonded carbon in the organics can be achieved by wet chemical or combustion means. Wet chemical oxidation without the addition of reagents is the most commonly used oxidation method for on-line TOC analyzers. It relies on the generation of hydroxyl radicals and the subsequent reaction of these radicals with the organic compounds to completely oxidize the covalently bonded carbon to CO_2. Table 2 indicates the hydroxyl radical has one of the highest oxidation potentials of oxidative species. Hydroxyl radicals in water can be produced by the direct irradiation of water with short wave UV light (less than 190 nanometers in wavelength), by the thermal or optical activation of an oxidizing chemical in water such as persulfate, and by the photochemical production of surface-attached hydroxyl radicals on titanium oxide.

The soluble hydroxyl radicals react homogeneously with organics, while surface-attached hydroxyl radicals react heterogeneously with organic compounds. The reagentless wet chemical oxidation methods are the UV-only and the UV/TiO_2 approaches. Our studies have shown the oxidation efficacy of these methods requires the presence of at least 1.5 ppm dissolved oxygen to oxidize 500 ppmC of a difficult-to-oxidize organic compound.

CO_2 Measurement Methods

The background compounds CO_2, HCO_3^{-1}, and CO_3^{-2} are defined as inorganic carbon (IC). The CO_2 produced from the oxidation process is organic sourced CO_2. The inorganic and organic sourced CO_2 in the water sample is measured using various methods that include direct-conductivity, membrane-conductometric, and non-dispersive infrared (NDIR) absorption. Each sensing method has its advantages and disadvantages. The conductometric methods are simpler to implement than the NDIR methods in that the CO_2 is measured in the water and does not need to be stripped from the sample into a carrier gas and then sent to a gas phase detector (NDIR) for analysis. This accounts for the popularity of conductometric methods for on-line TOC. However, separation of the CO_2 from the water improves selectivity and helps prevent interferences, two common problems with the direct conductometric methods.[25]

Table 2 Oxidation Potential of the Hydroxyl Free Radical			
TOC Oxidation Method: UV or UV/Persulfate: Hydroxyl Free Radicals	**Relative Oxidation Power of Various Oxidizing Species**		
• Effective wet chemical oxidation reactions depend on the generation of OH• and other radicals to completely oxidize organics to CO_2 • UV light < 190 nm will react with water to generate OH• • Oxidizing chemicals can be converted to OH• with UV light < 280 nm or heat • Persulfate + UV: (S_2O_8•- + H_2O → OH•) • OH• reacts with all known organics	Species	Oxidation Potential (Volts)	Relative Oxidation (Power*)
	Fluorine	3.06	2.25
	Hydroxyl free radical	2.80	2.05
	Atomic oxygen	2.42	1.78
	Ozone	2.07	1.52
	Hydrogen peroxide	1.77	1.30
	Perhydroxyl free radical	1.70	1.25
	Hypochlorous acid	1.49	1.10
	Chlorine	1.36	1.00
	*Based on chlorine as reference (= 1.00) Mittelman and Geesey, (1987)		

The conductometric measurement of CO_2 in water is based on its reaction with water to form the HCO_3^{-1} and CO_3^{-2} ions that result in an increase of water conductivity. The ionization of the ultrapure deionized water by the addition of CO_2 can be described mathematically. **Figure 2** shows the chemometric equations used to describe these reactions. They are based on mass balances, charge balances, ionization (dissociation) equilibriums (both carbonic acid and water), and the effect of each ion's specific conductance and concentration on the total conductivity of the CO_2 and H_2O solution. These equations describe the relationship between temperature, conductivity, and CO_2 concentration in solution when isolated from the atmosphere.
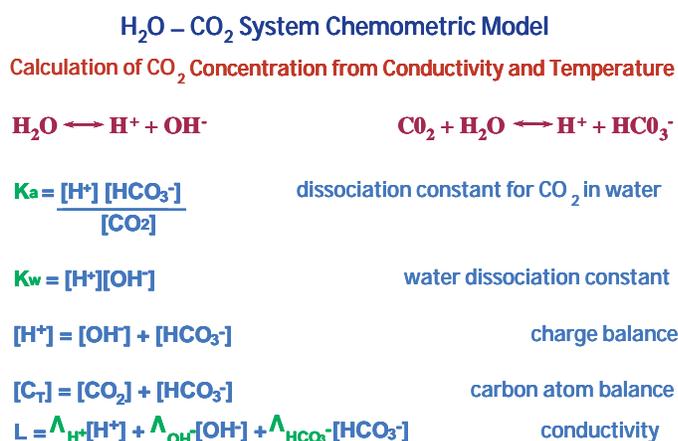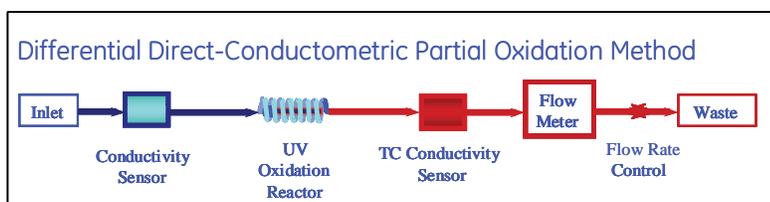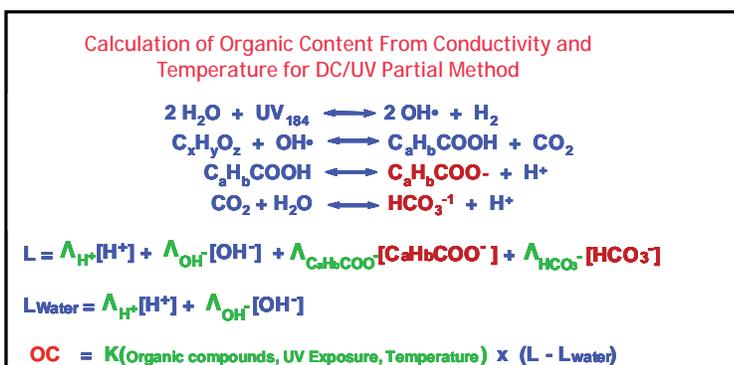
The terms $[H^+]$, $[HCO_3^{-1}]$, $[CO_2]$, and $[OH^{-1}]$ are the concentrations of each respective compound. $[C_T]$ represents the total carbon concentration in the solution. Ka and Kw are the temperature-dependent dissociation constants for CO_2 and water, respectively. L is the conductivity equation and it is the sum of each ionic species' specific conductance multiplied by its respective concentrations. Each ionic species' specific conductance is a function of the water temperature. If the water is free of other ions and only CO_2 is present, then this model is accurate. As other ions increase in concentration, the model becomes less accurate and eventually fails. The NDIR method relies on the absorption of infrared light by gas phase CO_2. In this approach, the CO_2 must be gas stripped from the water sample and then passed through the NDIR. NDIR measurement of CO_2 is very selective to CO_2 when the carrier and CO_2 gases are dried and the optimum CO_2 infrared light frequency is used.

Direct Conductometric Partial UV Oxidation Method (DC/UV Partial)

The first on-line TOC methodology is defined to be the direct-conductometric partial oxidation method ("DC/UV Partial"). This method was first used in the US 4,344,918 patent for an organic detection device shown in **Figure 3**. This methodology measures the incoming conductivity of the flowing water, passes the water through a UV oxidation reactor to oxidize the organics, and measures the resultant conductivity change. The delta conductivity change is related to the presence of organics in the water. This relationship is dependent on a number of factors, including water pH, dissolved oxygen, completion level of organic oxidation, background ions, and temperature.

The "DC/UV Partial" patented method was based on the observation that when organics are partially oxidized, organic acids are often formed as intermediate oxidation products. These organic acids ionize to a greater extent than carbonic acid on a per carbon basis or, in other words, they exert a stronger conductivity in the water than an equivalent carbon content of CO_2. This is shown mathematically in the chemometric equations in **Figure 4**.

H_2O – CO_2 System Chemometric Model

Calculation of CO_2 Concentration from Conductivity and Temperature

$$H_2O \longleftrightarrow H^+ + OH^- \qquad CO_2 + H_2O \longleftrightarrow H^+ + HCO_3^-$$

$$Ka = \frac{[H^+][HCO_3^-]}{[CO_2]} \qquad \text{dissociation constant for } CO_2 \text{ in water}$$

$$Kw = [H^+][OH^-] \qquad \text{water dissociation constant}$$

$$[H^+] = [OH^-] + [HCO_3^-] \qquad \text{charge balance}$$

$$[C_T] = [CO_2] + [HCO_3^-] \qquad \text{carbon atom balance}$$

$$L = \Lambda_{H^+}[H^+] + \Lambda_{OH^-}[OH^-] + \Lambda_{HCO_3^-}[HCO_3^-] \qquad \text{conductivity}$$

Figure 2. H_2O and CO_2 System Chemometric Model

Differential Direct-Conductometric Partial Oxidation Method

Inlet | Conductivity Sensor | UV Oxidation Reactor | TC Conductivity Sensor | Flow Meter | Flow Rate Control | Waste

Figure 3. Direct-Conductometric/UV Partial Oxidation (On-line DC/UV-Partial) Method

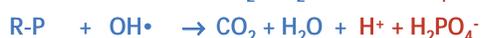**Calculation of Organic Content From Conductivity and Temperature for DC/UV Partial Method**

$$2 H_2O + UV_{184} \longleftrightarrow 2 OH\bullet + H_2$$
$$C_xH_yO_z + OH\bullet \longleftrightarrow C_aH_bCOOH + CO_2$$
$$C_aH_bCOOH \longleftrightarrow C_aH_bCOO^- + H^+$$
$$CO_2 + H_2O \longleftrightarrow HCO_3^{-1} + H^+$$

$$L = \Lambda_{H^+}[H^+] + \Lambda_{OH^-}[OH^-] + \Lambda_{C_aH_bCOO^-}[C_aH_bCOO^-] + \Lambda_{HCO_3^-}[HCO_3^-]$$

$$L_{Water} = \Lambda_{H^+}[H^+] + \Lambda_{OH^-}[OH^-]$$

$$OC = K_{(Organic\ compounds,\ UV\ Exposure,\ Temperature)} \times (L - L_{water})$$

Figure 4. Chemometric Equations Describing the DC/UV-Partial Method

This method can be summarized in a series of equations where the UV light is absorbed by water, and hydroxyl radicals are formed that react with the organics to produce organic acids (C_aH_bCOOH) and CO_2. Since the method is continuous and because it does not oxidize organics completely to CO_2, it excels in producing rapid organic concentration-related measurements. If the conductivity and temperature sensors used are stable and very sensitive, it can result in a high sensitivity to organics. This method can be implemented very simply and reliably in hardware and is useful for general monitoring for organics in semiconductor-grade final high-purity water.

One weakness of this method is that different organics oxidize at different rates and therefore small changes in oxidation time can cause big changes in the final result. Changes in the type of or mix of organics can also affect the final result. An additional possibility is that some of the organics in the water may contain atomic chlorine, nitrogen, sulfur, or phosphorous. **Figure 5** shows chlorinated organics will oxidize to form a strong mineral acid (HCl) and cause an artificial-

Oxidation of Organic Heteroatoms produces acids

Organic (R) + OH• $\rightarrow$ CO_2 + H_2O

R-Cl + OH• $\rightarrow$ CO_2 + H_2O + H^+ + Cl^-
R-Br + OH• $\rightarrow$ CO_2 + H_2O + H^+ + Br^-
R-N   + OH• $\rightarrow$ CO_2 + H_2O + H^+ + NO_3^-
R-S   + OH• $\rightarrow$ CO_2 + H_2O + H^+ + SO_4^{-2}
R-P   + OH• $\rightarrow$ CO_2 + H_2O + H^+ + $H_2PO_4^-$

Added "acid conductivity" produces high TOC readings

Figure 5. Direct Conductometric Interferences

Direct Conductometric UV: Complete Oxidation US Patent 4,626,413

UV Photochemical Batch Reactor
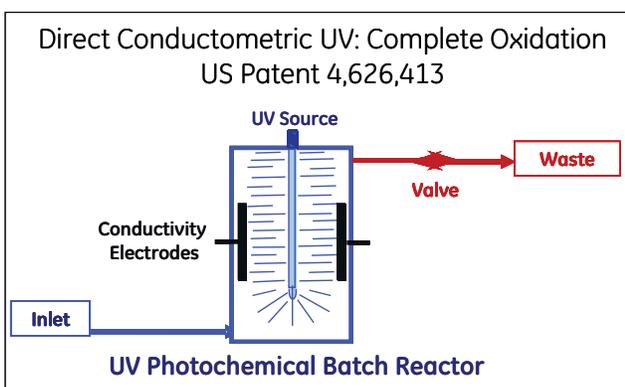
Figure 6. Schematic of the On-line DC/UV Complete Oxidation Method

ly high response. It has been reported in the scientific literature that chloroform sourced in the initial feed water of pharmaceutical water purification systems can be present in high enough concentrations in PW or WFI pharmaceutical water to affect the accuracy of direct conductometric CO_2 sensors.[26,27]

Direct Conductometric—Complete UV Oxidation Method (DC/UV)

A second commercial implementation of the direct-conductivity CO_2 detection method is based on the US 4,626,413 patent. The apparatus is shown in **Figure 6**. In this method, the sample is introduced into a photochemical reactor, the conductivity and temperature is measured, UV light is introduced, the conductivity is monitored until it stops changing, the final conductivity and temperature is determined, and a delta CO_2 is calculated to arrive at a TOC value. Assuming enough dissolved oxygen (DO) is available, this method effectively oxidizes the organics completely to CO_2 and the partial oxidation limitations of the "DC/UV Partial" method are overcome. With the UV light turned off, the initial temperature and conductivity measurement is used to calculate the IC by assuming the water sample contains only water and carbon dioxide in solution.

The same chemometric equations that model the H_2O-CO_2 system are used for this calculation and solved for C_T. Turning on the UV light, stopping the flow, and tracking the conductivity and temperature as a function of time are used to monitor completion of the oxidation. The method assumes only non-ionic organics, H_2O and CO_2 are present in the pre-oxidation state and only H_2O and CO_2 are present in the post-oxidation state. For both IC and TC, the amount of CO_2 present is calculated using the chemometric model for the H_2O-CO_2 system. The advantage of this approach is high sensitivity to TOC changes and given enough DO it achieves a good equal molar carbon response for non-ionic organics. This method is simple and can be implemented with a minimum of components, producing a reliable on-line analyzer.

A weakness of this direct-conductometric approach is that it uses the H_2O-CO_2 model system in calculation of the IC and TC and does not account for other ions in the initial water or after oxidation for organics that may have chlorine or other mineral-acid-forming atoms in their structure. Because the oxidation is effectively complete it is easier to predict the effect of compounds such as chloroform on the TOC result. Small amounts of chloroform can produce a significant interference.
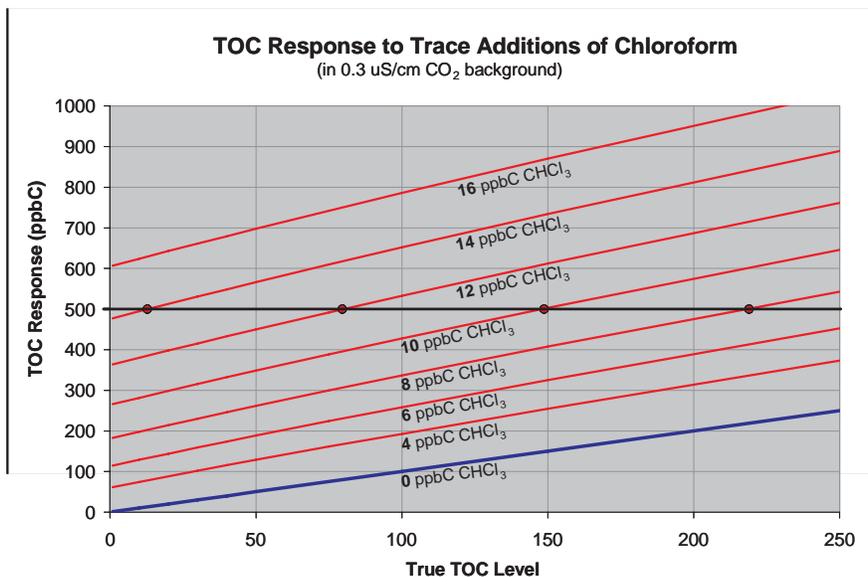
Figure 7. Predicted DC/UV Method Response to Chloroform

Figure 8. Schematic of Membrane-Conductometric Method

Figure 9. Membrane-Conductometric CO_2 Equilibrium Considerations

By assuming that chloroform is oxidized completely to CO_2 and HCl and using the H_2O-CO_2 system model, the effect of the additional conductivity from the ionized HCl on the TOC response can be calculated and is shown in **Figure 7**. The left axis is the predicted "DC/UV" TOC response. The bottom axis is the amount of total TOC in the water. For example, if there were 140 ppb C of TOC in the water and 10 ppb C of chloroform is added, the new total TOC is 150 ppb C. Lining up 150 ppb C from the bottom axis with the 10 ppb C diagonal Chloroform line would result in a predicted "DC/UV" method TOC response of 500 ppb C. This chart shows as little as 14 ppb C chloroform with no other TOC in solution can result in a "DC/UV" method TOC response of over 500 ppb C. We will test the accuracy of this model when we inject chloroform into the "DC/UV" analyzer.

Both of the direct-conductivity-based methods have found use as on-line carbon analyzers in the pharmaceutical industry. They are mechanically simple and robust. For both of these methods, the measured pharmaceutical water should be substantially free of chloroform to prevent positive interferences. Neither of these methods is used in the laboratory due to the relativity high purity water matrix requirements required for proper analysis.

Membrane-Conductometric (MC)—Complete Oxidation Method

The next class of conductometric-based CO_2 measurement TOC methods is the membrane-conductometric method as described in the patent US 5,132,094. This method uses a CO_2 permeable membrane between the sample water chamber and a deionized water acceptor chamber to separate the CO_2 measurement from the

sample matrix, substantially eliminating the organic heteroatom interference limitations and allowing use in non-CO_2-based ionic water samples. It uses the same H_2O-CO_2 system chemometric model as before to determine the concentration of CO_2 in the acceptor chamber. As illustrated in **Figure 8**, the ionic components in the sample water do not pass through the membrane and are not measured in the acceptor side DI water, and therefore they do not show up as interferences. The membrane is a homogeneous non-porous Teflon PFA that only passes gases. It is important to know or control the pH on the sample side of the membrane-conductivity module. This can be done by addition of acid to make the sample side a low pH and converting all the IC to dissolved CO_2, or by knowing the sample side only contains DI water and CO_2.

Figure 9 shows the equilibrium considerations between the reagentless and reagent versions of this methodology. If sample is acidified, then the CO_2 equilibrium shifts to form only dissolved CO_2 gas, which then diffuses through the CO_2 selective membrane into the DI-water side of the gas transfer module. The CO_2 that has transferred is calculated by solving for the CO_2 (aq) term in the CO_2-H_2O model. In the reagentless configuration of this method, the sample-water side of the transfer module contains CO_2 and DI water. In this case, deionized water and CO_2 are on both sides of the membrane and the CO_2 is in both ionized and un-ionized forms; the amount of diffused CO_2 is calculated by solving for the total carbon (C_T) term in the CO_2-H_2O model.

As stated, there are two versions of this method commercially available; the first one is reagentless and is labeled "MC/UV." The second one uses acid and, if needed, an oxidizing reagent, and is labeled "MC/UV-Persulfate." The reagentless version is shown in the schematic in **Figure 10**. The sample is drawn into the analyzer and passes though a temperature and conductivity sensor for the sample water conductivity measurement and then is split into two streams. Stream one flows into a long-residence UV reactor, and into the sample side of a membrane gas transfer module. Sufficient time is allowed in the oxidation reactor to ensure complete oxidation of the organics to CO_2. The new organic sourced CO_2 produced from the oxidation of the organics is available to diffuse through the membrane. The second stream passes into a delay coil and into the sample side of a gas

transfer membrane module to allow the inorganic CO_2 to diffuse across the membrane.

In both cases the DI water receiving sides of the membrane gas transfer modules collect the diffused CO_2 and then periodically flow into separate conductivity and temperature sensors for the TC and IC channels. The conductivity and temperature sensor data is applied to the H_2O-CO_2 system chemometric model to determine the CO_2 concentrations in the IC and TC streams. Because the organic sourced CO_2 produced in the oxidation reactor is passed through a membrane and any mineral acids that may have also formed are blocked, there is little interference from chlorinated organics such as chloroform. This method works well in water that is deionized or deionized with CO_2 present and, as a result, is generally confined to on-line pharmaceutical water applications. This method also allows the measurement of weak acids and weak bases, which is not possible with the direct conductometric methods.
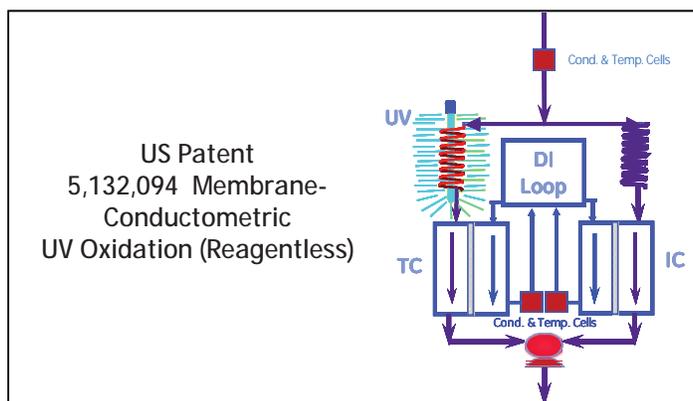

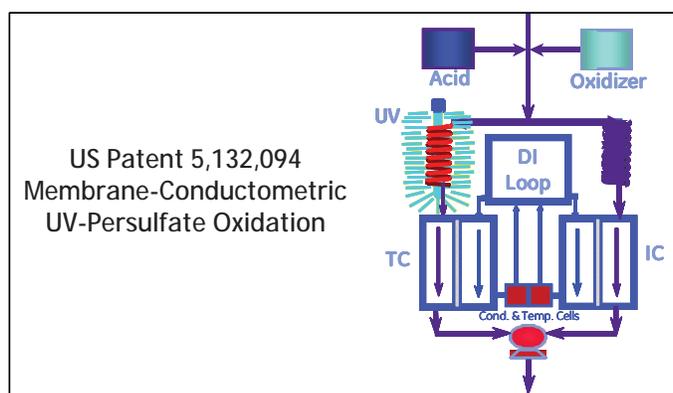Figure 10. Reagentless Membrane-Conductometric Method (MC/UV)

Figure 11. Reagent Version of Membrane-Conductometric Method (MC/UV-Persulfate)

The second version of the membrane-conductometric method uses acid and possibly oxidation reagents (MC/UV-Persulfate) and is shown in the schematic in **Figure 11**. We used a version of "MC/UV-Persulfate" that was configured for on-line TOC analysis. It was used in this study as a laboratory type TOC reference for comparison to the reagentless on-line TOC analyzers. As in the reagentless version, this version measures both the IC and TC channels with a membrane-conductometric CO_2 sensor, the H_2O-CO_2 system chemometric model, and uses its own source of DI water on the acceptor side of the membrane. Because acid is added to the sample side of the membrane and the acceptor side of the membrane is using DI water, it is possible to use this method in water samples that contains other ions than those allowed in the H_2O-CO_2 system chemometric model. It is possible to measure organics at various sample water pH levels and various conductivities without producing errors in the TOC measurement. The result is an analytically robust method that has the high sensitivity and stability of the other conductometric methods, but does not suffer from the analytical deficiencies of those simpler methods.

This method is commonly used to measure TOC in drinking waters and for pharmaceutical cleaning validation samples. The improved analytical capability is counterbalanced by the increased mechanical complexity and cost. This method allows the addition of oxidation agents such as persulfate ions which, combined with the UV reactor, produce a much greater concentration of hydroxyl radicals than can be achieved with just UV alone. The oxidation reagent provides the additional source of oxygen that is needed to complete the oxidation of high levels of organics to CO_2. This is important in the situation where the TOC is higher than the available amount of dissolved oxygen. This reagent version of the membrane-conductometric method is the most popular TOC in pharmaceutical laboratories and is the primary laboratory instrument of record for meeting the USP and EP compendial requirements.

Organic Injection Test Results

Figure 12 shows the graphical results of the response from the four test analyzers for an injection of 500 ppb as carbon of methanol. The TOC response was determined from the stabilized peak values with a minimum of 10-30 data points. The baseline value is subtracted from the peak value. The baseline was selected from the analyzer's results prior to or after the injection or both, depending on the stability and if there has been sufficient time to rinse down to the water purification system's TOC level. **Figure 12** also illustrates the different TOC reporting times from each of the TOC analyzers. The "DC/UV Partial" analyzer reports a result every one minute, the "MC/UV Persulfate" analyzer every four minutes, the "MC/UV" analyzer every six minutes, and the "DC/UV" analyzer has variable reporting times from three to twenty minutes. This injection was done with the dissolved oxygen level greater than 4.9 ppm as O_2. This level provides enough dissolved oxygen to exceed the stoichiometric requirements for complete oxidation of the organic to CO_2, without the need to add additional oxygen. The typical inorganic carbon (IC) level was about 70 ppb as carbon, or about 0.3 µS/cm of conductivity. This is the typical conductivity level we often see in pharmaceutical water systems that have purified water storage tanks that are vented to air.

Organic Percent Recovery Results

The ½ factorial DOE showed a strong correlation between the TOC methodology and the organic compound. **Figure 13** summarizes the response of the on-line TOC analyzers to the different concentrations of the various organics studied. It shows the results of
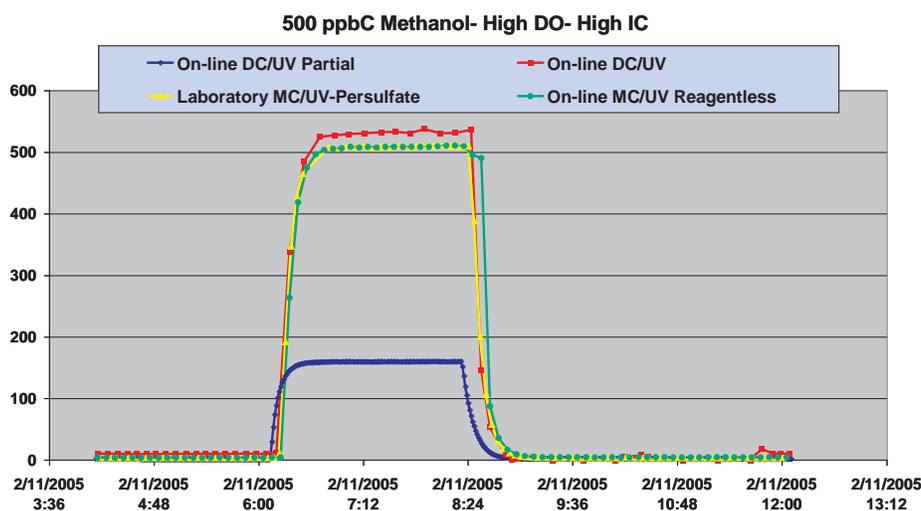

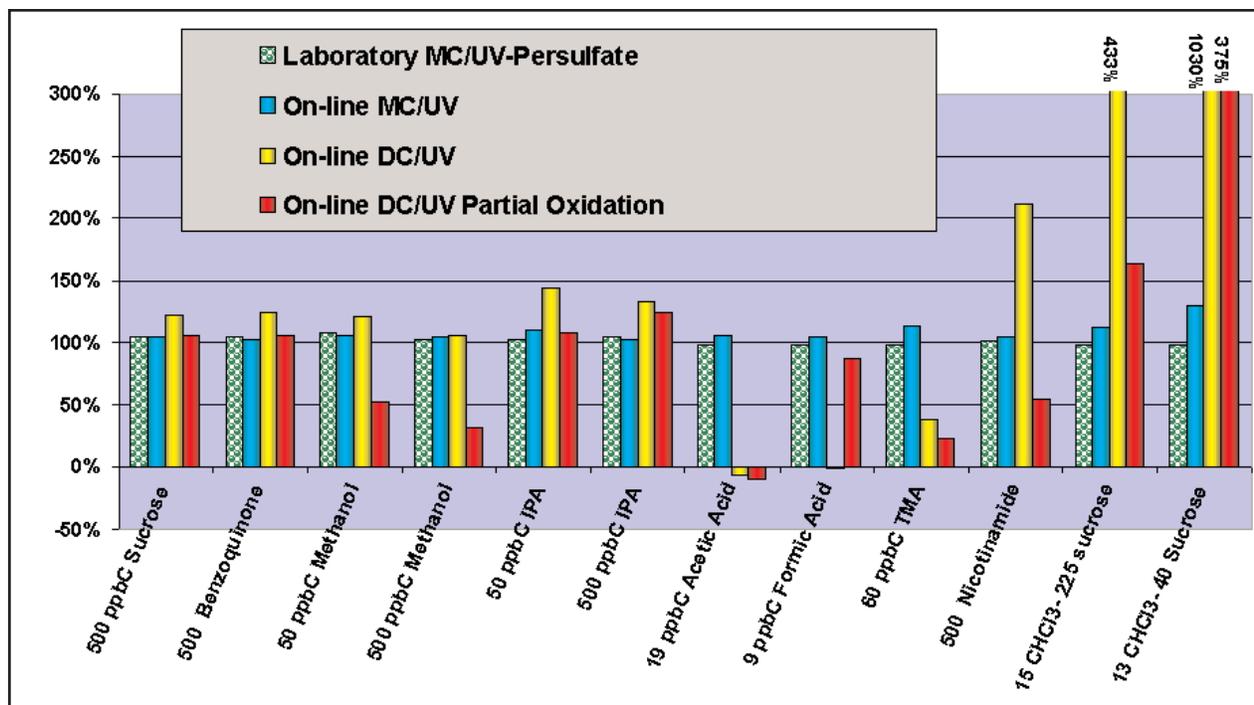**Figure 12.** Typical Data from Organic Injection Run

Figure 13. Response of the Different TOC Analyzers in High DO

the injections of compendial and real-world organics into the on-line TOC analyzers. All the tested TOC analyzers recovered 500 ppb or more of the USP and EP system suitability standards, sucrose and 1,4-benzoquinone, and all analyzers would have passed a USP and EP system suitability test.

The response of the analyzers to the two alcohols showed good percent recovery for all TOC methods, except for the direct conductometric partial oxidation method, which showed very low recovery at both concentrations of methanol. This was surprising, as methanol is one of the smallest and simplest organics. This result may reflect methanol's low UV absorption and the short sample residence time in the UV light oxidation reactor. Additionally, the percent recovery is not the same for either methanol or isopropanol (IPA), suggesting a non-linear response for these compounds.

Both types of the direct conductometric TOC analyzers showed no or low recovery of the weak acids and bases, with the exception of the "DC/UV Partial" method, which recovered about 80% of the formic acid. This can be explained by the fact that the tested weak acids and base were more conductive on a per carbon basis than the resulting oxidation product. The analyzer using the "DC/UV" method is designed to completely oxidize the organics to CO_2 and then measure the resulting conduc-

tivity of the ionized equilibrium portion of the CO_2. The "DC/UV" method analyzer assumes the initial incoming water conductivity is fundamentally from dissolved and ionized CO_2. In the case of conductive weak acids and bases, the initial conductivity is greater than the final conductivity. The analyzer concludes there was no organic carbon present in the sample and reports the very low percent recovery. The "DC/UV Partial" method does not oxidize the organic completely to CO_2, so its response is more unpredictable. In the case of the acetic acid, it shows a similar response as the "DC/UV" method of no recovery, but for formic acid it shows an 80% recovery. The only explanation is that the formic acid is not oxidized to CO_2, but rather it is oxidized to another intermediate conductive species that is more conductive than formic acid on a per carbon basis.

It is important to remember that both WFI and PW conductivity limits are higher than DI water conductivity and therefore allow the presence of these conductive organic compounds. Weak acids and bases have been measured in purified pharmaceutical waters. Historically, both the DC method analyzers were first developed for use in completely DI semiconductor manufacturing water. Neither of these methodologies can correctly determine the TOC in conductive waters if the ions are not in the H_2O-CO_2 chemometric model. The assumption that this conductivity is from CO_2 may be

reasonable for the general case, but in special cases it is not. If the water contains weak acids or bases, the TOC measurement will be in error for these methodologies.

The difficult-to-oxidize nicotinamide read low on the "DC/UV Partial" method, but high on the "DC/UV" method. This organic contains organically bound nitrogen that, when completely oxidized, will product nitric acid in the resulting solution. The analyzer using the "DC/UV" method completely oxidizes this organic to nitric acid, which increases the final solution

Figure 14. Chloroform Addition

conductivity, and the analyzer reports a higher percent recovery than it should. The "DC/UV Partial" analyzer result can be explained by the partial oxidation of the compound and the resulting lower percent recovery.

As expected from the literature, both of the direct conductometric methods showed very high responses to chloroform, as shown in **Figure 14**. The oxidation product of chloroform includes hydrochloric acid and with both the DC methods this additional conductivity is confused with the assumed ionized CO_2 product. The "DC/UV Partial" method did not show as high an interference from the chloroform as the "DC/UV" method. The prior partial oxidation examples support the idea that this "DC/UV Partial" analyzer is truly only partially oxidizing the chloroform to produce a lower interference. If this analyzer were to be modified to completely oxidize the organics, then we would expect the interference to be the same as the "DC/UV" analyzer that completely oxidizes the organics. As our model predicted, the "DC/UV" analyzer exceeded 500 ppb C with the solutions of 15 ppb C chloroform only, 13 ppb C chloroform and 40 ppb C TOC, and 12 ppb C of chloroform and 85 ppb C TOC. This result confirms the false positive response of the direct conductometric on-line TOC analyzer to the chloroform contamination.

The membrane-conductometric (MC) methodologies performed well for all the organics tested. The reagentless MC version produced the same results as the laboratory MC version using reagents under the test conditions.
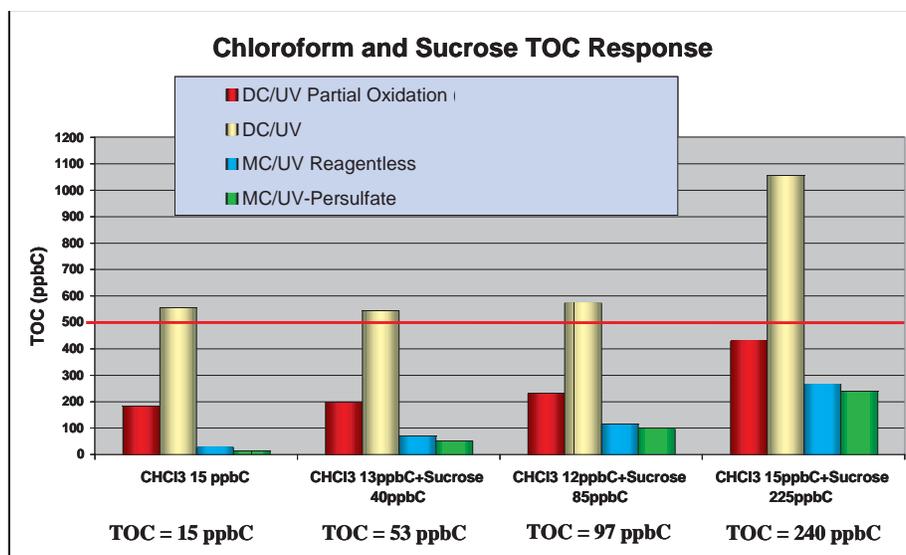
The experimental data can also be expressed as a probability determination of the likelihood the TOC method will incorrectly measure the TOC value within ± 15% of the true value at a 95% confidence level. **Figure 15** shows the normal distributions of the TOC response to all the compounds tested as a function of the TOC methodology. The data for these charts were collected at the typical pharmaceutical water conductivity level of 0.3 µS/cm (from dissolved CO_2) and DO level of 4.9-5.2 ppm. The dissolved oxygen level was enough to ensure ample available oxygen for complete organic oxidation to CO_2, assuming the oxidation is given enough time. A capable system when plotted in this way will show the normal distribution of the responses centered well between the lower specification limit (LSL) and the upper specification limit (USL). Responses outside of these limits are considered to be defects.

The "MC/UV-Persulfate" analyzer responses are well within the limits, and at a 95% confidence level only 0.00003% of the responses are expected to be defective. The reagentless on-line "MC/UV" analyzer has a probability of generating 0.17% defective results. However, the "DC/UV Partial" analyzer results are mostly outside the USL and LSL and result in a high probability of 81% defective measurements.

The "DC/UV" analyzer shows over 91% of the TOC results will be outside the ± 15% TOC accuracy limits. The "DC/UV" analyzer showed a higher than expected response to the injected organics, and this was in contrast to the addition of organics during the system
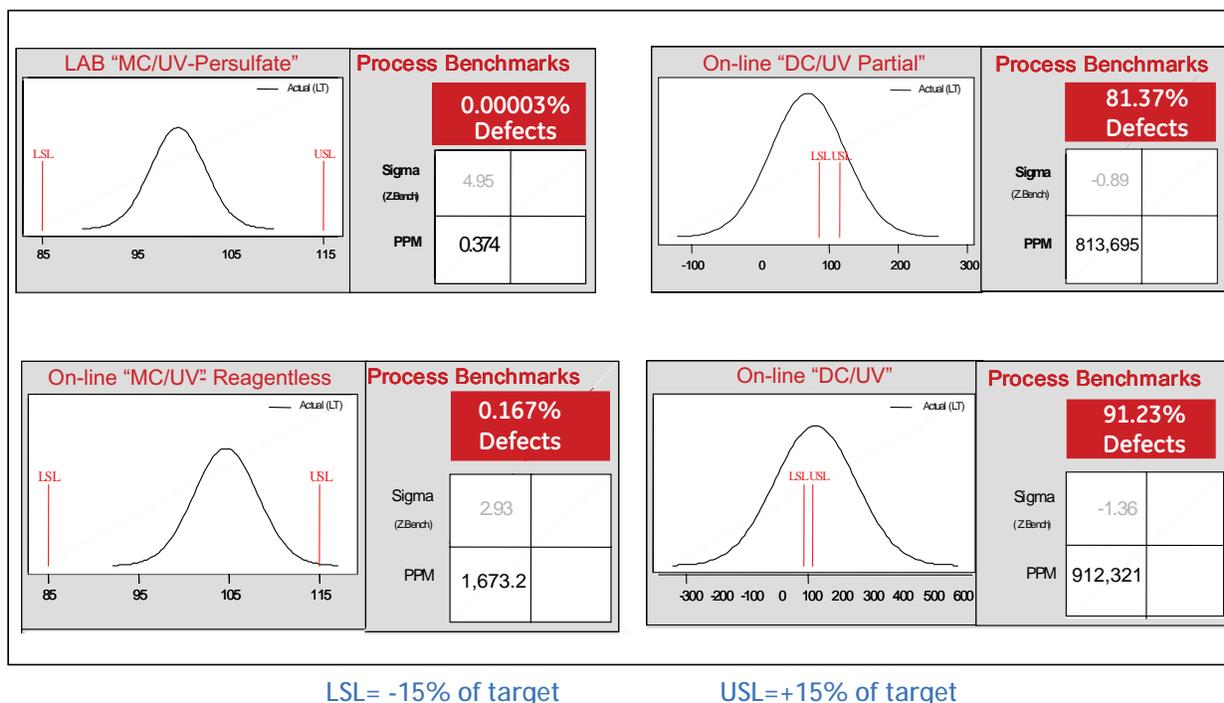
LSL= -15% of target   USL=+15% of target

Figure 15. "Real Organics" Probability of Incorrect TOC Result (95% Confidence)

suitability tests or during the calibration of the analyzer. Typically, we calibrated the analyzer on a 500 ppb C sucrose calibration standard, but when we ran a 500 ppb C sucrose injection solution into the analyzer it would respond at about 610 ppb C. We do not understand why this is so, but it was reproducible. We even sent the analyzer back to the factory for a calibration certificate and the returned analyzer performed the same way. The USP <643> and EP <2.2.44> TOC Chapters define the system efficiency as:

System Efficiency (SE) = 100 x [(Rss-Rw)/(Rs-Rw)]

Where:

Rss = analyzer response to 500 ppb C 1,4-benzoquinone
Rs = analyzer response to 500 ppb C sucrose
Rw = analyzer response to the water used to make RSS
    and Rs

And the system is suitable if: 85% ≤ SE ≤ 115%

Because both the 500 ppb C sucrose and benzoquinone standards responded equally high, the USP and EP system suitability test would pass.

Alternatively, the probability of successfully measuring the system suitability SE ratio, the sucrose and benzoquinone TOC response, and the real world organic TOC response correctly within ± 15% of the target value is shown in **Figure 16**. The % probability on the chart can be thought of as the system capability of the TOC methodology to measure the correct value. It is clear that all the TOC methods are able to pass the USP System Suitability test. The probability of a correct measurement is 100% for the membrane conductometric (MC) methods and 99% to 94% for the direct conductometric (DC) methods. The MC methods measure the sucrose and 1,4-benzoquinone (BQ) correctly 99.9% of the time. The "DC/UV Partial" oxidation method will measure the sucrose and BQ correctly 96.4% of the time and the "DC/UV" method will measure the sucrose and BQ correctly only 59.5 % of the time. The last result is due to the "DC/UV" TOC analyzer reading all the injected USP compounds high even though it had a factory calibration certification and it was calibrated prior to all the organic injections. The most interesting result is that the TOC analyzers based on the MC methods will measure the real world organics correctly 99.8% of the time, but the DC methods will only measure them correctly at 18.7% and 8.8% of the time.

Dissolved Oxygen Limitations

The ½ factorial DOE also showed a significant correlation between the TOC methodologies, the organic, and the DO level (DO). TOC analyzers showed the following effects on recovery of 500 ppb C 1,4-benzoquinone when the dissolved oxygen was lowered from normal oxygen water equilibrium conditions to 0.67 ppm DO.

This is a situation that can happen if the water purification system has a degassing unit process. Degassing units are installed after RO systems to lower the amount of CO_2 gas that passes through the RO. This prolongs the lifetime of the ion-exchange beds. Some of the new continuous electronic deionization systems also require inlet degassing to improve their output conductivity levels. **Figure 17** shows the effect on 500 ppb C BQ when the DO was lowered. It is clear that as the DO decreased the response dropped off for the MC/UV analyzer and the "DC/UV Partial" analyzer. The "DC/UV" analyzer response increased as the DO decreased. It is likely due to the formation of organic acids intermediate oxidation compounds created as a result of an insignificant DO concentration to allow complete oxidation to CO_2. If the DO were to be decreased further, it is expected the "DC/UV" analyzer would finally show lower recovery. The reference MC analyzer that used reagents showed no changes with the decreasing DO level. This is because this analyzer uses an oxidizing agent for low oxygen situations. A future experiment would be to decrease the DO to 1 ppb O_2 to observe the effect of low oxygen on the oxidation of this compound in each analyzer.

Figure 16. Process Capability (Probability of Correct TOC ± 15%)
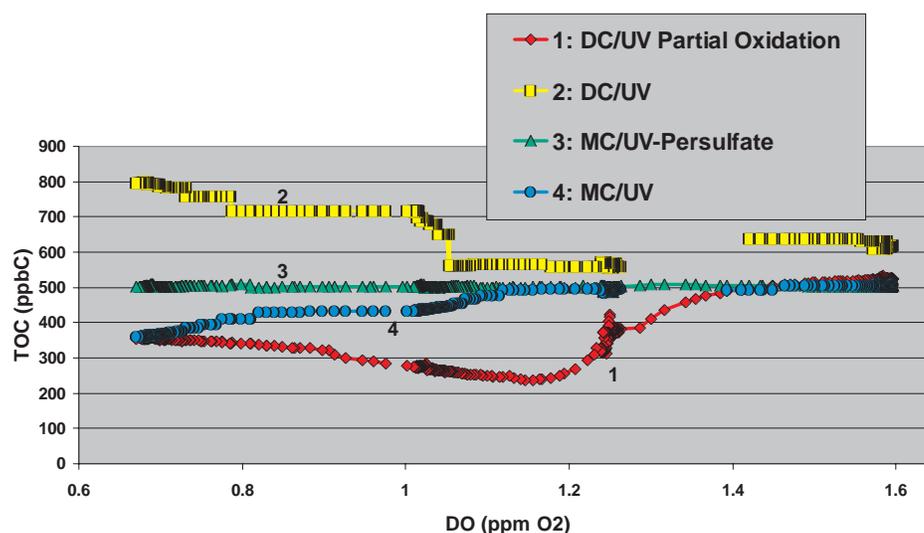
Figure 17. 500 ppb C Benzoquinone at DO Levels between 0.67-1.6 ppm O2

Conclusions

The experiment showed a major correlation between TOC methodology and organic compound and a strong correlation between DO and TOC methodology.

Consider the "DC/UV Partial" methodology with the partial oxidation system. The consequences of not completely oxidizing the organics include a lack of equal molar carbon response, a lack of linear response to a given organic, and an unpredictable recovery of ionic organics. The regulatory ramifications of this are that this analyzer could pass the USP/EP compendial limit test but produce erroneous results when measuring sample water, resulting in TOC exceeding the regulatory limit when in fact TOC is well below the limit. Perhaps worse, this method could fail to indicate a TOC event that could contaminate the product. This would be possible with weak organic acids or bases and methanol-type compounds. When the true methanol TOC was over 500 ppb C the "DC/UV Partial" analyzer only measured 150 ppb C. If a methanol contamination were to affect the water system, this analyzer would not exceed the TOC 500 ppb response limit until the actual real methanol concentration exceeded 1666 ppb C, well above the compendial limit. Methanol is one of the simplest organics known, leading us to wonder
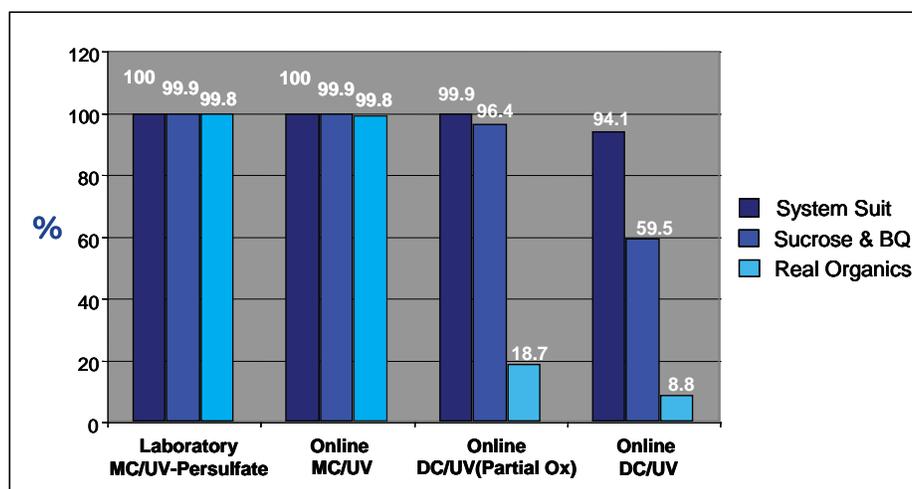
what other real-world organics would behave similarly.

False negative TOC results are not acceptable for the analyzer of record in a regulated pharmaceutical environment. The observed false positive TOC results, as was the case with the chloroform, can cause unnecessary out-of-specification problems for the pharmaceutical company. Because this analyzer does not respond correctly to so many of the tested organics on an equal molar carbon basis, we cannot have confidence in the TOC result it reports. We believe that the "DC/UV Partial" TOC methodology is not a valid TOC methodology, as defined by the USP <643>, USP <1225>, ICH Q2B, ICH Q7A, cGMP's 21-CFR-211.194a, and the FDA's guidance documents. Based on our testing, we conclude it is unsuitable for use on pharmaceutical water systems.

The "DC/UV" methodology with complete oxidation is more predictable than the partial oxidation analyzer. We have shown the "DC/UV" suffers from false negative results when tested on weak organic acids and bases. We have demonstrated it also suffers from false positive results with organics containing organically bound chlorine and nitrogen atoms. Implementing a real time TOC release system using this on-line TOC methodology with pharmaceutical water containing these organic organics or chlorinated organics would be difficult and is inherently risky. The confidence needed to complete the system qualification would not be there. Because we demonstrated the lack of recovery for the ionic organics and chlorinated organics, this methodology would be unsuitable for use with pharmaceutical waters, unless these compounds could be shown to not exist in the water under any conditions. The removal of THM's from the water system would be a great help for the "DC/UV" method and is technically possible. THM removal processes usually require a gas stripping or degassing operation. The method used should not remove too much DO or the on-line TOC ana-lyzers may likely report false negative TOC results. The ionic organics could also be removed if the method to remove them were assured to work under worse case conditions. Without the assured lack of presence or the removal of the problem organic compounds, the experimental data suggest that the "DC/UV" TOC method would not successfully pass the method validation requirements as defined by the USP <1225>, ICH Q2B, ICH Q7A, cGMP's 21-CFR-211.194a, and the FDA's guidance documents.

The lowest risk method is the membrane-conductometric methodologies with complete oxidation as used in both the "MC/UV" and the "MC/UV-Persulfate." Under the conditions of the test, the "MC/UV" performed equivalent to the MC/UV-Persulfate version in all cases, except when the DO was less than 1.3 ppb O_2. The lack of wildly high responses to THM compounds combined with high accuracy for all the tested compounds show both versions to be good TOC analyzers. Using the method validation criteria stated in USP <1225>, ICH Q2B, ICH Q7A, cGMP's 21-CFR-211.194a, and the FDA's guidance documents, the tested membrane-conductometric TOC methods are acceptable for use in the pharmaceutical industry.

Finally, this on-line TOC study suggests that, contrary to the suggestive wording of the currently approved "USP <643> TOC" and "EP <2.2.44> TOC" compendial requirements, passing the system suitability tests or other requirements therein are not sufficient to guarantee the suitability of an on-line TOC analyzer for use on pharmaceutical water or for real-time TOC release applications.

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Please see the next page.

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