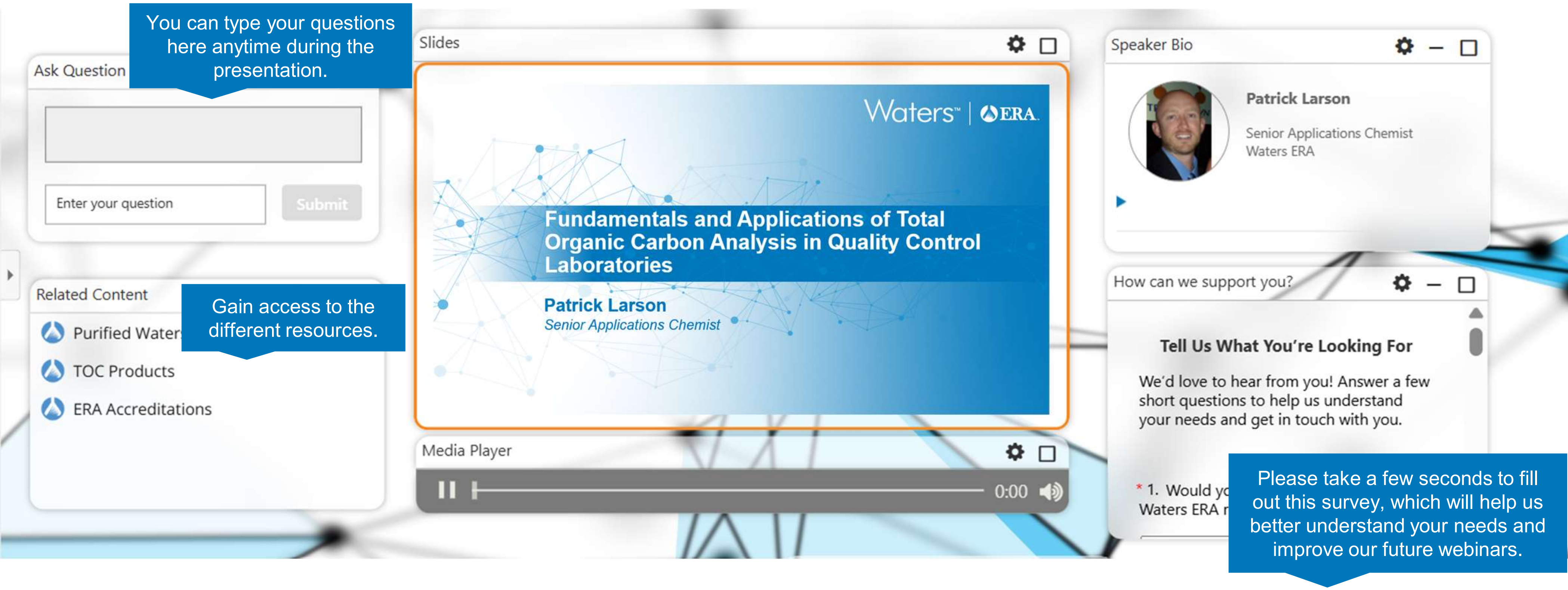
Fundamentals and Applications of Total Organic Carbon Analysis in Quality Control Laboratories

Patrick Larson
Senior Applications Chemist

Webinar survey – your feedback is appreciated!



Waters ERA – Who we are

Waters™ | ERA

- Founded in 1977
- First PTs offered in 1992
- Acquired by Waters Corporation in 2006
  - Acquired APG in 2008
- Manufacturing in Golden, CO USA and Wexford, Ireland
- Leading environmental PT and CRM provider
  - 50 PT studies per year in 8 matrices
  - ~9,000 labs per year, over 80 countries
  - ~66,000 PT reports processed annually
  - ~480,000 data points analyzed annually
  - ~270,000 products shipped annually
- https://info.eraqc.com/hubfs/videos/food-environmental-video.mp4
- Provider of Purified Water Consumables
  - TOC CRMs, turbidity, conductivity supplies
- https://info.eraqc.com/hubfs/videos/life-sciences-video.mp4

Mastering TOC Analysis

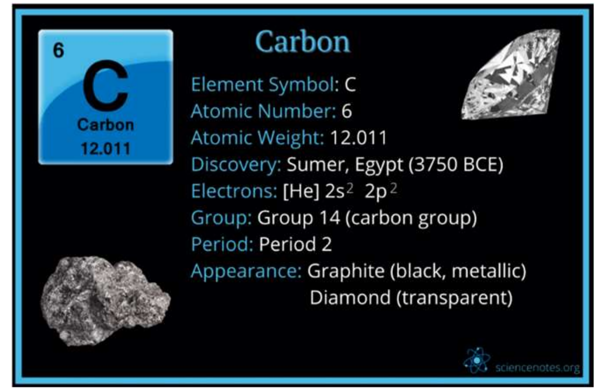
- Key Learning Topics
  - Fundamentals of TOC
  - Apply best practices for implementing TOC analysis
  - Ensure product integrity with TOC
- Speaker – Patrick Larson
  - Senior Applications Chemist

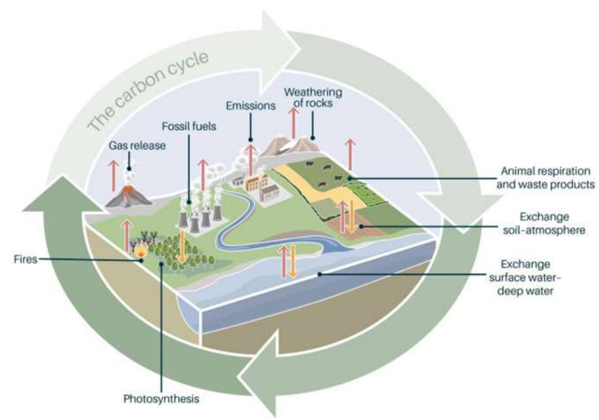
Outline

- Fundamentals of Carbon
- History
- Importance of TOC Analysis
- The Basics
  - Oxidation Methods
  - Detection
- TOC Applications in the Pharma Industry
  - USP <643>
  - Cleaning Validation
- General Troubleshooting

FUNDAMENTALS of CARBON – Fun Facts

- 4th most abundant element behind hydrogen, helium and nitrogen.
- Carbon is a nonmetal element with the symbol C and atomic number 6, known as the "backbone of life" because it forms the basis of all living organisms.
- Its ability to form four covalent bonds allows it to create millions of complex molecules, including proteins, DNA, and lipids.
- It exists in various forms, or allotropes, such as diamond and graphite, and is also a key component of many non-living materials, including fossil fuels and carbon dioxide.
- Carbon-14 (^{14}C) is a radioactive isotope with a half-life of about 5,730 years, which is used to date organic materials.
- The amount of carbon we have on Earth doesn't change. It is the same now as it was millions of years ago when the dinosaurs roamed the Earth.
- Most carbon is stored in reservoirs, or sinks, such as rocks and sediments, while the rest is stored in the atmosphere, oceans, and living organisms. Carbon is released back into to the atmosphere through respiration, volcanic eruptions and by burning materials such as wood, oil and gas. This is called the carbon cycle.





Brief History of TOC Analysis

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1756

Joseph Black found a way to measure carbon dioxide by Loss on Ignition. In this method, samples were heated and when heated the samples loss mass. From this difference in mass, the amount of carbon could be determined.

1924

T.D. Yensen laid steel samples into a 1000°C furnace along with oxygen, which combusted the carbon and then collected the CO_2 cryogenically

1948

American Cyanamid patented the first infrared (IR) analyzer, which was a precursor to modern TOC analyzers.

1967

Dow Chemical patented, "Method and Apparatus for Determination of Total Carbon Content in Aqueous Systems." This system manually injected aqueous samples directly into a gas stream of oxygen in a 700-900 °C furnace. The CO_2 produced during this process was measured with infrared absorbance. This was the first combustion TOC instrument produced.

1974

The Safe Drinking Water Act (SDWA) was originally passed by Congress in 1974 to protect public health by regulating the nation's public drinking water supply.

1976

RCRA was signed into law on October 21, 1976 to address the increasing problems the nation faced from our growing volume of municipal and industrial waste.

1984

G. Cauwet is credited for improvements in methods for the determination of dissolved organic carbon (DOC) in seawater, particularly at sub-ppm (parts per million) range

1997

TOC test introduced to USP. Replaced several other tests and ensured TOC instruments used would be suitable for analysis of pharmaceutical grade waters. (most recent version became official in May 2021)

Importance of TOC

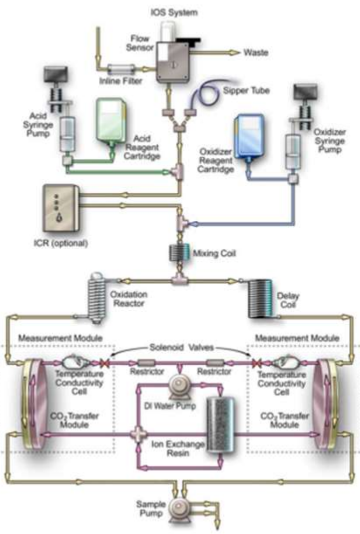
- Total Organic Carbon (TOC) analysis is often used as an “indirect”, “non-specific” indicator of water quality.
  - “Indirect” in that the analysis does not directly measure organic carbon but rather the quantification of CO_2 produced as a result of the oxidation process.
  - In addition, TOC analysis is also “Non-specific” in that it doesn't identify individual organic compounds.
  - Provides general screening, monitoring and acceptance criteria for various industry applications and processes.
    - Wastewater treatment
    - Drinking water quality
    - Pharmaceutical grade waters
    - Semiconductor processes
  - While organic carbon isn’t necessarily toxic, high concentrations can significantly impact ecosystems, water treatment processes and various manufacturing processes that rely on clean water.

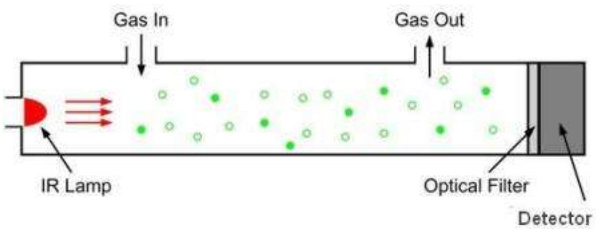
Compliance and Industry

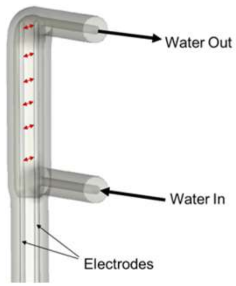
- Wastewater and Drinking Water Compliance
  - Environmental (EPA)
    - 40 CFR Parts 141 and 142
    - Safe Drinking Water Act (SDWA) – Disinfection Byproducts Rule
- FDA
  - Bottled Water
  - Pharmaceutical
    - USP <643>, EP 2.2.44, JP 2.59
  - Cleaning Validation
- Process Monitoring
  - Semi-Conductor
  - Power
  - Pharmaceutical (water system)

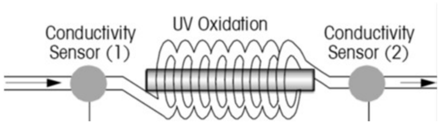
Fundamentals of TOC Analysis

- TOC analyzers must do 3 things:
  - Oxidize organic carbon compounds to CO_2
  - Detect CO_2
  - Differentiate between Inorganic & Organic Carbon









Fundamentals of TOC Analysis

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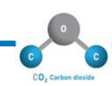
Sample Introduction

- Autosampler
- Vials/Bottles
- On-Line

Oxidation

- Combustion
- Heated Persulfate
- UV/Persulfate
- UV

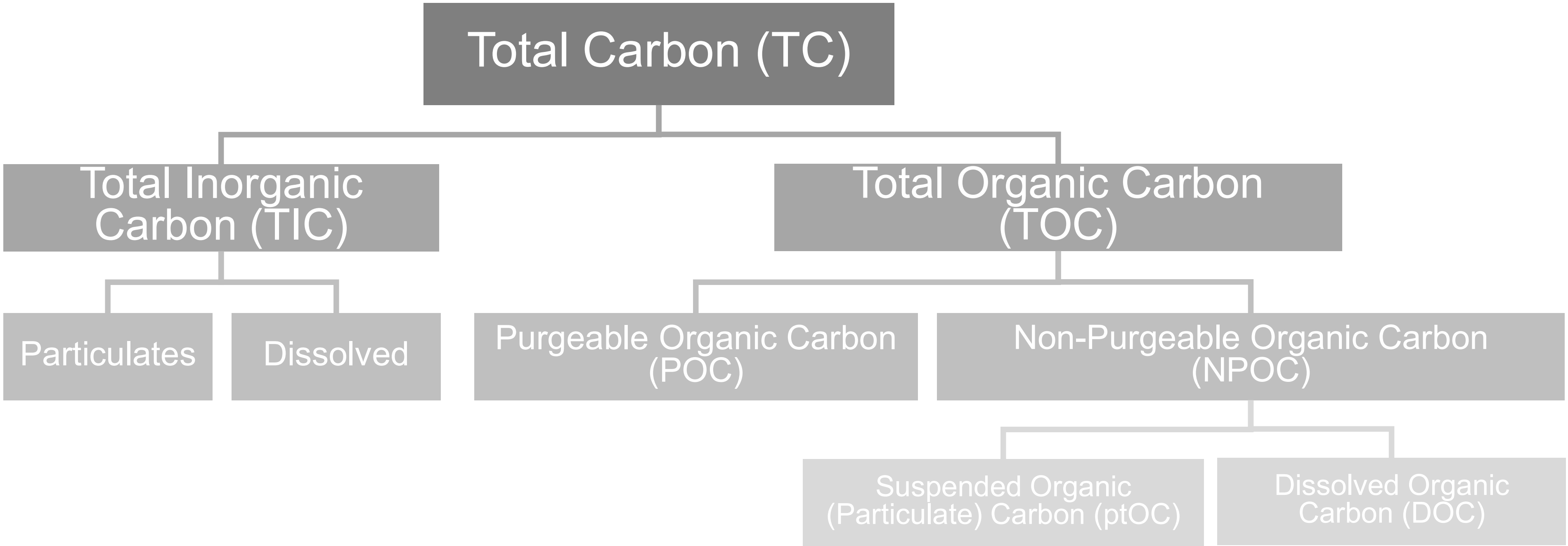


Detection

- Non-Dispersive Infrared (NDIR)
- Membrane Selective Conductivity
- Direct Conductivity

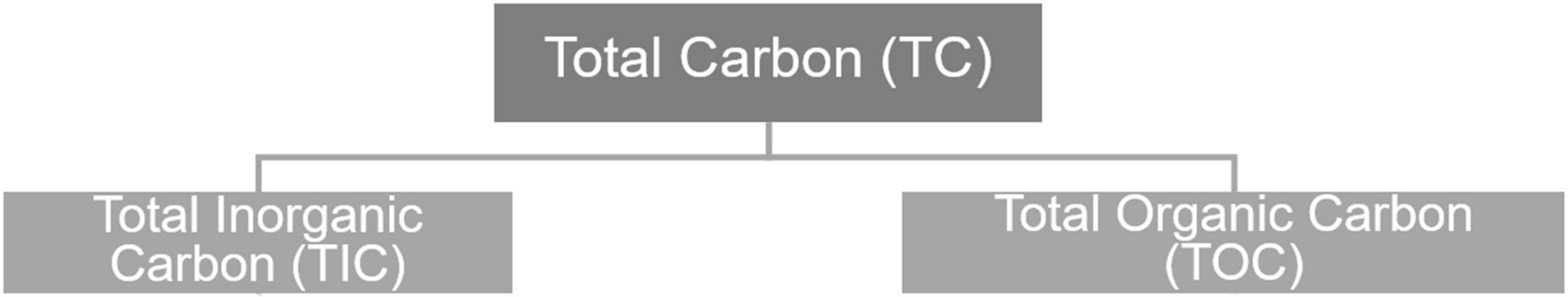
Results

Fundamentals of TOC Analysis

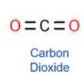


Fundamentals of TOC Analysis

- Carbon can be classified into 2 categories:



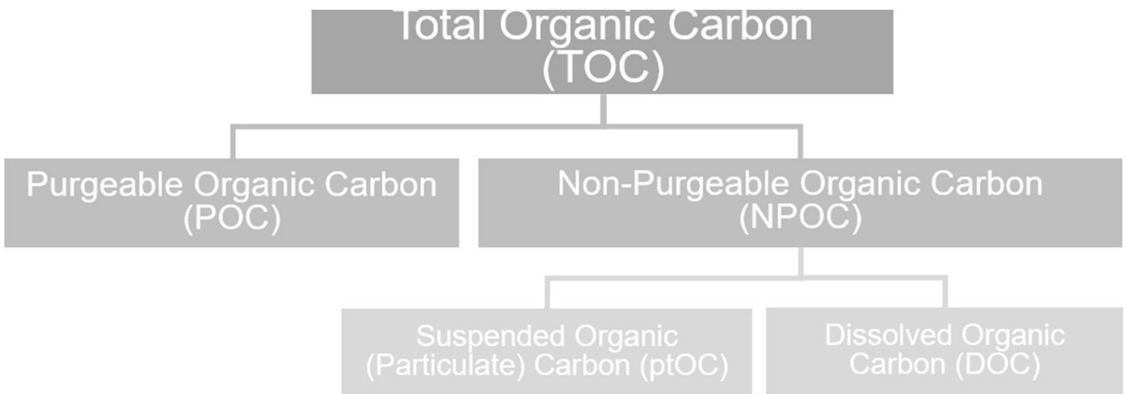
- Inorganic Carbon:
  - From non-living organisms
  - Found in minerals, ores, the atmosphere (think carbon dioxide – CO2)
  - Simple compounds such as carbonates, carbon monoxide
  - May be dissolved or particulate
  - Can be removed by lowering pH



- Organic Carbon
  - Originating from living organisms
  - Simple to complex compounds
  - C – H bonds
  - Examples; hydrocarbons such as methane, or proteins, lipids…etc.

Fundamentals of TOC Analysis

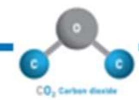
- Organic Carbon (or TOC) can further be classified as:



- Purgeable Organic Carbon (POC)
  - Can be removed from by purging with an inert gas.
  - May also be referred to as Volatile Organic Compounds (VOC) and usually determined by Purge and Trap (P&T) Gas Chromatography.
  - Examples: benzene, toluene, chloroform
- Non-Purgeable Organic Carbon (NPOC):
  - Fraction of organic carbon that remains after purging
  - Examples: detergents, pesticides, urea, humic & fulvic acid
    - Suspended (Particulate) Organic Carbon (ptOC) – vegetation, bacteria
    - Dissolved Organic Carbon (DOC) – KHP, detergents, fertilizers

Oxidation Techniques

Oxidation

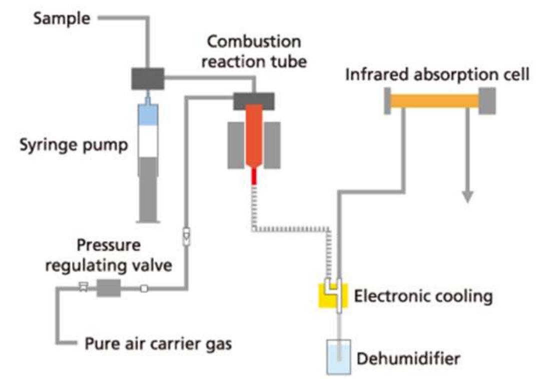


- Combustion
- Heated Persulfate
- UV/Persulfate
- UV

Oxidation Techniques

- High Temperature Catalytic Combustion
  - High-temperature catalytic combustion oxidizes organic carbon by using a catalyst (typically platinum) at high temperatures, typically between 750 - 800°C, in an oxygen-rich environment to convert the organic carbon into carbon dioxide (CO_2) and water vapor.
  - The generated CO_2 is transferred to the detector by a carrier gas.
  - The reaction can be generalized as follows:

$$\text{Organic Carbon} + O_2 \xrightarrow{\text{Heat, Catalyst (Pt)}} CO_2 + H_2O$$



- Reasons to chose Combustion
  - Complete Oxidation: complete oxidation of all organic compounds, including complex or particulate matter.
  - Wide Applicability: suitable for a broad range of samples across various industries.
  - Interferences: high salt content samples, carbon memory effects leading to high blanks
  - Operating Ranges: 0.5 mg/L up to 20,000 mg/L

Oxidation Techniques

- **Heated Persulfate**
  - Heated persulfate oxidation is an advanced oxidation process (AOP) that uses heat to activate persulfate ($S_2O_8^{2-}$), generating reactive sulfate radicals (SO_4^{-}) to break down organic compounds.

$$S_2O_8^{2-} + activator\ (heat) \rightarrow 2\mathrm{SO}_4^{\cdot -}$$

$$\mathrm{SO}_4^{\cdot -} + Organic\ Carbon\ (C) \rightarrow \mathrm{CO}_2 + \mathrm{H}_2O + \mathrm{SO}_4^{2-}$$

  - The sample is heated $(80 - 100°C)$. The heat activates the persulfate (SO_4^{-}), the sulfate radicals which act as a strong oxidizing agent.
- **Reasons to chose Heated Persulfate**
  - Suitable for labs wanting to avoid the higher system background and potential carbon memory effects of high-temperature combustion systems.
  - Operating Range: 0.01mg/L – 50 mg/L
    - Typically, this method is used for water with TOC concentrations below 1.0 mg/L and samples with moderately complex organic compounds.

Oxidation Techniques

- UV Persulfate
  - UV persulfate oxidation is a process used to measure total organic carbon (TOC) in water by using UV light to break down persulfate ($S_2O_8^{2-}$) into highly reactive sulfate radicals ($SO_4^{\cdot-}$) which then oxidize organic matter to carbon dioxide (CO_2).

$$S_2O_8^{2-} + UV\,\text{light} \rightarrow 2SO_4^{\bullet-}$$

  - The UV light assists in the oxidation of organics by generating hydroxyl radicals or through direct photodissociation of organic molecules.

$$SO_4^{\bullet-} + \text{Organic Carbon (C)} \rightarrow \text{Intermediates} \rightarrow CO_2 + H_2O + SO_4^{2-}$$

  - This CO_2 is then detected and quantified.
- Reasons to chose UV Persulfate
  - Suitable for labs analyzing low-level and ultra-pure water.
  - Not recommended for seawater analysis or samples with particulate matter.
  - Operating Range: 0.01mg/L – 50 mg/L

Oxidation Techniques

- UV
  - UV light oxidizes organic carbon through a process called UV-oxidation, primarily by generating highly reactive species like **hydroxyl radicals** (OH^-) or through the direct **photodissociation** of organic molecules. This process breaks down organic compounds into carbon dioxide (CO_2) and water (H_2O).

$$H_2O + UV(185nm) \rightarrow H + OH$$

$$OH + Organic - C \rightarrow CO_2 + H_2O$$

- Reasons to chose UV
  - Suitable for labs analyzing low-level and ultra-pure water generally at or below 0.5 mg/L
  - Operating Range: in the 0.01mg/L – 1 mg/L range
  - Simple to maintain, low operating costs
  - Commonly used for on-line analysis allowing for real-time and continual monitoring of water quality

Detection

Sample Introduction

- Autosampler
- Vials/Bottles
- On-Line

Oxidation

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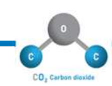
- UV/Persulfate
- UV



Detection

- Non-Dispersive Infrared (NDIR)
- Membrane Selective Conductivity
- Direct Conductivity

Results

Detection

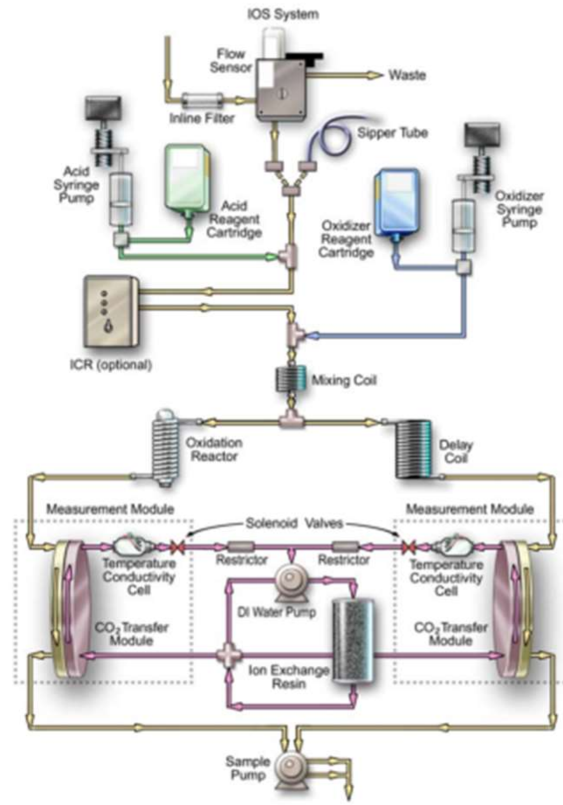
- Non-Dispersive Infrared (NDIR)
  - One of the most commonly used detection method to detect CO_2 in TOC analyzers is the NDIR technique (non-dispersive infrared).
  - An NDIR detector consists of three main important components:
    - The light source that emits IR light
    - The measuring cell through which the CO_2 and carrier gas flows
    - The measuring sensor or detector
  - Process Flow
    - The CO_2 from the sample is swept into the measuring cell.
    - IR light Source: An IR lamp emits a broad spectrum of infrared light
    - Detector: measures the amount of light absorbed by the CO_2 gas
    - Calculation: The amount of light absorbed is directly proportional to the concentration of CO_2 present. This measurement is then used to calculate the total organic carbon concentration in the sample.

Detection

- Membrane Conductivity (CO_2 Selective)
  - This technique uses a selective membrane to separate the CO_2 gas from the water sample.
  - CO_2 is not inherently conductive.
    - CO_2 reacts with water to form carbonic acid which dissociates into hydrogen and bicarbonate ions

$$H_2O + CO_2 \rightleftharpoons H_2CO_3 \rightleftharpoons H^+ + HCO_3^-$$

    - The resulting carbonic acid, hydrogen and bicarbonate ions affect conductivity
  - The change in conductivity is then correlated to the initial TOC concentration.
  - Note: conductivity must be temperature corrected.



Detection

- Direct Conductivity
  - A direct conductivity measurement for total organic carbon (TOC) involves two steps:
    - measuring the sample's initial conductivity, oxidizing organic compounds (via UV Oxidation) to convert them to carbon dioxide (CO_2), and then measuring the final conductivity.
  - The difference between the two conductivity measurements is proportional to the amount of dissolved CO_2, which is used to determine the TOC concentration.
  - There are 2 configurations that can accomplish this and involve 1 or 2 conductivity sensors:

1 Conductivity Sensor	2 Conductivity Sensor
– Sample water is trapped – Initial conductivity/temp. measured (= TIC) – Trapped sample oxidized (UV) – Conductivity/Temp. measured (= TC)	– Continuous Flow – Conductivity/temp. measured pre UV (=TIC) – Sample flows through UV reactor coil – Conductivity/temp. measured post UV (=TC)

Detection

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Sample Introduction

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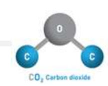

Detection

- Non-Dispersive Infrared (NDIR)
- Membrane Selective Conductivity
- Direct Conductivity

Results

Results - Calculating TOC

TOC by Subtraction

$$TC – TIC = TOC$$

- Measure the Total Inorganic Carbon (TIC) in the sample by:
  - Acidifying the sample to convert carbonates to CO_2 which is then measured or,
  - Measure the sample conductivity prior to oxidation attributing the signal to TIC levels
- Measure the Total Carbon (TC) in the sample.
- Subtract the TIC value from the TC value to find the TOC.

TOC by Sparging

$$TOC = NPOC$$

- Acidify the sample to remove the inorganic carbon as CO_2.
- Spage or purge the sample with an inert gas to remove the CO_2.
- Measure the remaining organic carbon, which is the Non-Purgeable Organic Carbon (NPOC), and this value is considered the TOC.
- In this technique TOC is equal to NPOC so long as <u>POC is negligible</u>.

Choosing a Suitable TOC Analysis Method

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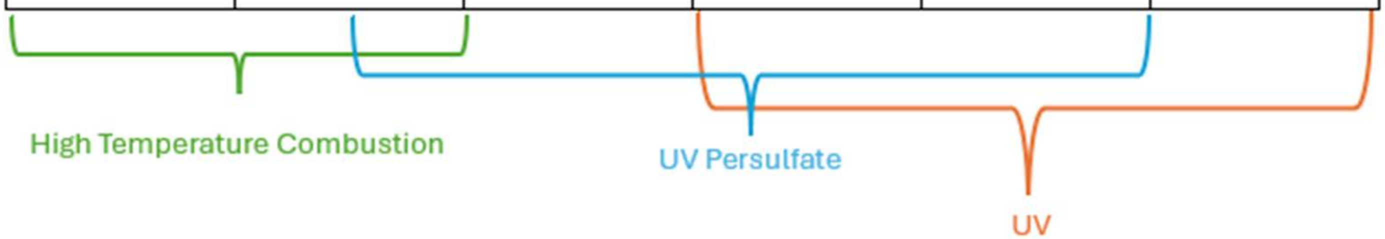
Difficult to Oxidize ⟷ **Easy to Oxidize**

Industrial Wastewater	Surface Water	Drinking Water	Purified Water	Water for Injection	Highly Purified Water
100,000ppm	<50ppm	<2ppm	<500ppb	<500ppb	<0.1ppb



TOC Applications in Industry

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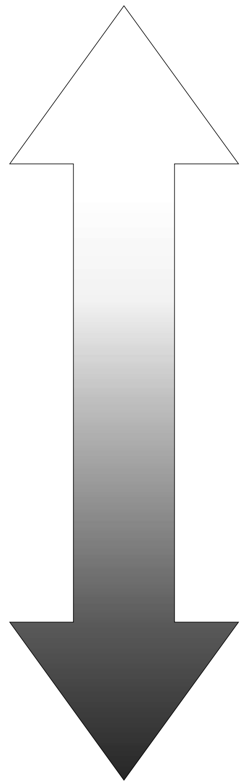
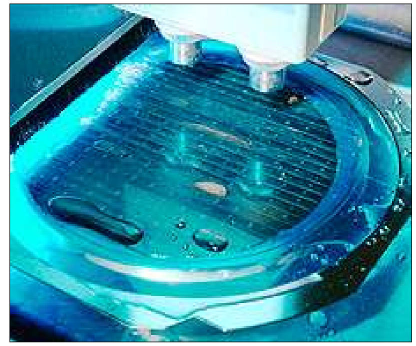
- Semiconductor
- **Pharma**
- Power
- Oil & Gas, Chem/Petrochem
- Drinking Water
- Food & Beverage (Dairy)
- Airport De-icing
- Research/Academia
- Wastewater
  - (Municipal and Industrial)

Very Clean
< 1 ppb



Very Dirty
$> 50,000$ ppm



Types of water used in Pharmaceutical Industry

- Non-potable for cooling
- Potable (drinkable) water
- **USP purified water**
- **USP water for injection (WFI)**
- **USP sterile water for injection**
- USP sterile water for inhalation
- USP bacteriostatic water for injection (contains an anti-bacterial agent)
- USP sterile water for irrigation - irrigation in medicine means the washing of a body cavity or wound by a stream of water

TOC Applications in Pharma Industry

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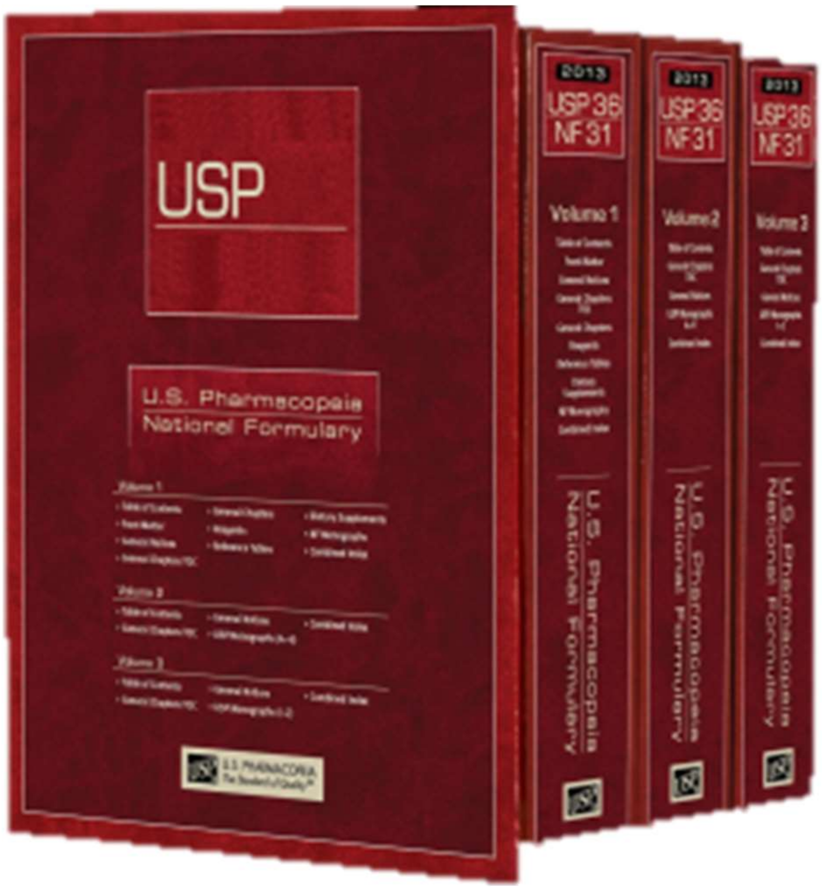
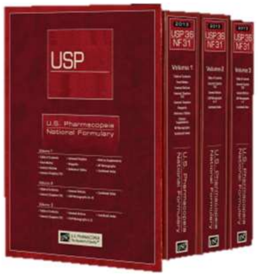


- Regulations
  - <USP> 643 – Total Organic Carbon
    - History of USP 643 and changes
  - EP 2.2.44
  - JP 2.59
- Scope:
  - Verify suitability of the TOC instrument
  - Establish the TOC Limit Response (r_L) for bulk water
  - Verify packaged sterile water meets container specifications
- No mandated oxidation method or instrument but must be able to distinguish between organic and inorganic carbon
- Specifies instrument detection limit requirements.

TOC Applications in Pharma Industry



Bulk Water

- Bulk Pharmaceutical Waters have a limit of 0.500mg/L TOC
  - System Suitability tests establish the Limit Response (r_L) value and is derived from the 0.500mg/L Sucrose (r_s) standard – Blank(r_w)
- System Suitability Test also required to ensure relative response (RE) of TOC analyzer for Sucrose and 1,4-Benzoquinone recovery
- System Suitability Kit consists of 3 standards:
  - Sucrose (Rs) being a relatively easy organic carbon to oxidize.
  - 1,4-Benzoquinone (Rss) being a relatively difficult organic carbon to oxidize.
  - Rw = Reagent Water (same water source used to make Rs and Rss Standards)
  - RE Formula: $\% \, Response\ Efficiency\ (RE) = 100 \times \frac{r_{ss} - r_w}{r_s - r_w}$

RE must be 85 – 115%

- USP <643> / EP 2.2.44, have essentially the same requirements
- JP 2.59 (use of SDBS for system suitability test as a “difficult” organic molecule to oxidize). Recovery must be at least 90% (or 0.450mg/L)

TOC Applications in Pharma Industry

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USP <643>: **Bulk** Pharmaceutical Waters

Apparatus Requirements: This test method is performed either as an on-line test or as an off-line laboratory test using a calibrated instrument. The suitability of the apparatus must be periodically demonstrated as described below. In addition, it must have a manufacturer's specified limit of detection of 0.05 mg/L (0.05 ppm) or lower of carbon.

Standard Solution: Unless otherwise directed in the individual monograph, dissolve in the *Reagent Water* an accurately weighed quantity of USP Sucrose RS to obtain a solution having a concentration of 1.19 mg/L of sucrose (0.50 mg/L of carbon).

System Suitability Solution: Dissolve in *Reagent Water* an accurately weighed quantity of USP 1,4-Benzoquinone RS to obtain a solution having a concentration of 0.75 mg/L (0.50 mg/L of carbon).

Reagent Water Control: Use a suitable quantity of *Reagent Water* obtained at the same time as that used in the preparation of the *Standard Solution* and the *System Suitability Solution.*

Water Sample: Obtain an on-line or off-line sample that suitably reflects the quality of water used.

- No mention of frequency for System Suitability Test (SST) – this is up to each company to decide.
- Risk Based Approach
  - Longer time between SST = GREATER RISK!
- Some companies are doing SST daily or weekly in their QC labs!
- Stability of System Suitability Standards
  - use before expiration date
  - if prepared in-house make fresh or perform stability studies to validate shelf-life

TOC Applications in Pharma Industry

Packaged **Sterile** Water

- Prior to May 2021 packaged sterile water had a standard limit of 8mg/L TOC
- USP recognizes Packaged Sterile Water may contain higher levels of TOC introduced primarily from the packaging material.
- Therefore, allowable levels of TOC in Packaged Sterile Water varies based on the size of the container the water is packaged:

Nominal Container Volume	Limit 1 (L1) mg/L of Carbon	Limit 2 (L2) mg/L of Carbon
≤5mL	32	48
>5mL and ≤100mL	24	36
>100mL	8	12

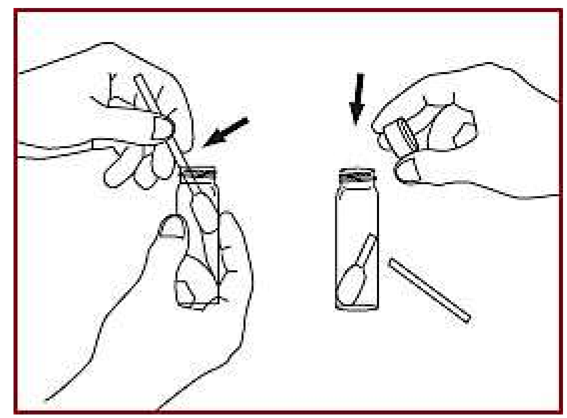
- Samples from packaged sterile water tested and compared to the Limit 1 and 2 per nominal container volume
  - If lower than Limit 1: no action
  - If greater than Limit 1 but less than Limit 2: identify organic compound(s). If individual contaminants are ≤0.2mg/L no action, if >0.2mg/L determine patient safety impact.
  - If greater than Limit 2 – Packaged Sterile Water fails

TOC Applications: Cleaning Validation (CV)

- Types of CV Testing
  - Production CV Testing
  - Development CV Testing
- Methods of CV Testing
  - Rinse Water
  - Swabs w/ water extraction
  - Swabs – Direct combustion
- Analytical Methods for CV Testing
  - Specific (HPLC)
  - **Non-specific (TOC)**

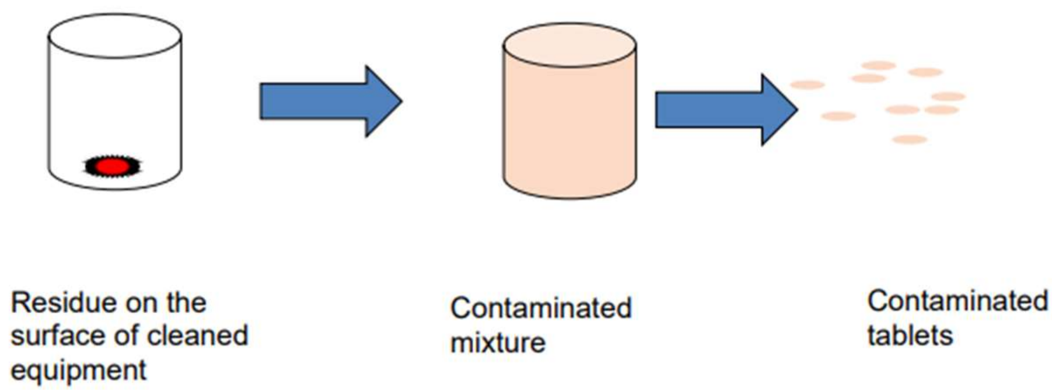
Rinse Water

Swab Testing



TOC Applications: Cleaning Validation (CV)

- Steps and Considerations
  - Risk Assessment
  - Cleaning Agent
  - Acceptance Criteria
  - Analytical Methods
  - Dirty Hold Time Studies
  - Clean Hold Time Studies
  - Sample Collection
  - Execution and Documentation
  - Revalidation



- Why is it Important
  - Safety
  - Compliance
  - Consistency

Troubleshooting Common TOC Analysis Issues

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Low or High Recovery

- Reagent Flow Rate
- Age of Reagent
- UV Lamp
- Sample Contamination
- Carryover
- Incomplete or Improper Oxidation

System Suitability Failure

- 1,4-Benzoquinone – Is Light Sensitive
- Microbial Contamination
- Reagent Water
- Instrument

Repeatability

- Instrument Maintenance
- Fluid Path Obstruction or Leak
- Inhomogeneous Sample
- Instrument Technology
- Air bubbles
- TOC / IC ratio

Sample Carryover

- Increase Flushes/Cleanup
- Modify Repetitions and Rejects to allow for flush and priming of the lines.
- SDBS is a “sticky” molecule and generally requires extra rinses following analysis

Troubleshooting Common TOC Analysis Issues

Waters™ | ERA

Sample Collection and Handling

- Contaminants
  - IPA
  - Personnel
- Interferents
  - Salts
  - Water Vapor
  - Inorganic Ions (Direct Conductivity)

Transportation and Storage

- Cold Chain Transportation and Storage
- Stability
- Shipping Conditions
- Shipping Delays

Instrument Maintenance

- PM Schedule
- Replacement Parts
- Filters
- Reagents

Negative TOC

- Temperature and Conductivity
- Instrument Maintenance/Autozero

Live Q&A Session: Fundamentals and Applications of Total Organic Carbon Analysis in Quality Control Laboratories

Please submit your questions into the Q&A Chatbox

https://www.eraqc.com