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Impact of environmental air particle numbers on the accumulation of organic carbon in WFI during sampling

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Abstract

Total organic carbon (TOC) presents an essential quality parameter for purified water (AP) and water for injection (WFI). For the monitoring of pharmaceutical water systems, the analysis of TOC occurs online and offline. However, monitoring data collected throughout the industry readily indicates little comparability between available online and offline measurement systems and outlier values are a common occurrence in offline samples while online devices display results with high stability. Using a recently implemented and heavily controlled WFI-system with stable online TOC values of < 4 ppb we analysed the impact of environmental air particle numbers in controlled production and technical areas on offline TOC analyses. The detected correlation strongly links environmental air particle numbers to the accumulation of organic carbon in water samples indicating outlier values do not necessarily represent a loss of quality within the generation or distribution system but rather an environmental impact or hygienic changes in the surrounding area. Our data highlights the importance of comparative and redundant offline and online analyses using various parameters to distinguish systematic and local valve contaminations from the displayed impact via the sampling environment when monitoring and evaluating complex systems.

Keywords: TOC, WFI, Environment, Air particle numbers, Sampling

1. Introduction

Total organic carbon (TOC) is an essential quality parameter in water monitoring serving as a potent indicator for microbial or organochemical contaminations in water generation and distribution systems (Matsuda et al. 1987; Husted et al., 1996). As such, TOC measuring technology is applied in a variety of industries but is especially focussed by producers of pharmaceutical products and the semiconductor industry as both require purified water (AP) or water for injection (WFI) of highest quality. Microelectronics producers use pure water mostly as a potent solvent during the production and cleaning/rinsing processes of wafers to ensure product quality and essentially requiring minimum conductivity, carbon levels and microbial burden (Kulakov et al. 2002). To some degree, detecting a loss of quality from material degradation and release of leachables and extractables may also be complemented by TOC-systems (Jenke et al. 2012; Jenke et al. 2014; Menzel et al. 2022a, 2022b) utilizing measuring technology that efficiently distinguishes between inorganic and organic carbon during the process. For pharmaceutical producers of parenteral medicinal products WFI purity is additionally indispensable to protect production facilities, clean room areas and most importantly patients from contamination with potentially pathogenic bacteria (Röder & Sandle 2022; Kulakov et al. 2002), secondary metabolites (Klaus et al. 2020; Ramírez-Rendon et al. 2022) or endotoxins from water-borne Gram-negative bacteria (Hawer et al. 2024). Hence, pharmacopeia's have harmonized specifications for TOC levels in AP and WFI to a maximum of 500 ppb with modern systems undergoing substantial qualification and validation to consistently generate WFI containing TOC concentrations of < 5 ppb when analysed in a qualified and calibrated online measuring device. Yet, to assure full compliance, offline testing at critical positions in the system as well as all points of use (POU) should be conducted at defined frequencies and is recommended daily at POU for compounding of parenteral drug products. In comparison to online technology, offline TOC measurements are known to produce less reliable data, more outlier values and significantly higher results. Such differences are commonly attributed to the exposure of the sample to the surrounding environment during sampling or in the laboratory during analysis, or to the differences in measuring technology, equipment and material. However, in contrast to common belief, no study has distinctively shown the impact of environmental factors or cleanroom classification on offline TOC measurements in WFI and presented respectable controls.

We therefore comprehensively analysed offline TOC in WFI samples from a controlled generation and distribution system to assess the impact of the sampling environment using air particle numbers as an indicator for room and hygiene status. Furthermore, we deployed a statistical analysis to display intercorrelations between environmental air particle numbers and the accumulation of organic carbon in WFI during sample exposure times. Our data adds an important perspective to the evaluation of offline TOC results in the pharmaceutical industry, specifically in technical areas, and highlights the importance of comparative and redundant offline and online analyses.

Methods

Production of WFI

The WFI-generation system utilized double prefiltered (5 µm) drinking water from a drinking water system in the north of Germany with a well described microbiome (Hawer et al. 2024). Two ion-exchange units generated deionised water that was filtered (5 µm) again before entering a two-step reverse osmosis process with intermediate membrane degassing and subsequent ultrafiltration with a 6000 Dalton molecular weight cut off. The resulting water quality was WFI in accordance with the European Pharmacopeia. The system was constructed in regard to innovative hygienic designs and materials and comprehensively validated and qualified using an integrated risk-based approach (Klöber-Koch et al. 2018). The quality of the medium was shown to be consistently high over an extended period. The adjacent distribution system feeds a variety of consumers in different production and technical areas. Continuous process verifications and contamination control were established adjacent to the respective guidelines and include a diverse sanitisation strategy, online and offline monitoring as well as regular trend analyses.

Sampling of WFI

Sampling occurred in randomized intervals over a period of 6 months and at independent sampling points fed from the same WFI generation and distribution system. A minimum of 10 litres of water was discarded before sampling to rinse valves. Samples were taken in glass sampling tubes to avoid contamination with softeners from polypropylene beakers. Valves were disinfected with 70% Isopropanol only after sampling to avoid contamination of TOC samples with alcohol-containing aerosols.

TOC analysis

Offline TOC measurements were conducted on qualified Multi N/C pharma UV devices from Jena Analytics and by determination of non-purgeable organic carbon (NPOC) or measurement by difference of total carbon (TC) and inorganic carbon (IC) (Matsuda et al. 1987, Yoon et al. 2018; Schäfer et al. 2022). All utilized systems were qualified and validated to produce comparable results. Samples taken in the controlled production area were also analysed in this area to reduce negative impact of the laboratory environment.

Online TOC measurements were conducted with qualified and calibrated stationary ACCURA H systems from T&C technical that employ differential flowrate, temperature and conductivity measurements to assess the organic carbon content in WFI samples.

Measurement of air particle numbers

For measurements of air particle numbers, a CI-1052x particle counter from CRT Cleanroom-Technology GmbH was used. The Equipment was qualified and calibrated. The device was placed at three different positions around each sampling valve and for each position a total of three individual measurements were performed. Airflow of 100 l/min was used to detect particles of > 0.5 µm in size over a laser diode and to calculate particle numbers per cm³.

Statistical analyses

To check for normal distribution of the generated data and subsequently choose a suitable correlation measure, we first conducted a Shapiro-Wilk test (see Figure 1C). Thereafter, a correlation measure that does not require normally distributed data was preferred. Spearman correlation assesses how well the relationship between two variables can be described by a monotonic function (Zar 1972). This method does not require the data to follow a specific distribution, such as normality, and is therefore more robust in the presence of non-normal distributions and outliers compared to other correlation measures such as Pearson (Akoglu 2018). Thus, Spearman correlation was applied as it can capture the strength and direction of the association without being influenced by the original distribution of the data.

Results and discussion

Offline-TOC values in WFI diverge with room classification of sampling area

We monitored the described WFI system using continuous online TOC measurements in the generation system and the return of the distribution loop throughout the entire study. During this time, organic carbon content of the distributed WFI did not exceed 4 ppb with an average of 2 ppb detected (data not shown). In accordance with regulations, valves at the start and return of the distribution loop located in a technical area present critical quality sampling points and were monitored comprehensively during the qualification period and subsequently remained part of the routine monitoring strategy. Similarly, all POU or consumers in controlled production areas were monitored extensively to decrease any risk of local contaminations. Overall, the entire WFI-system was heavily controlled before and during data collection for this study.

To analyse the impact of the sampling environments on the offline TOC measurements, we initially identified three individual environments that samples were exposed to with each comprising defined hygiene properties and risks during sample exposure to the adjacent room: the technical area of the generation system that also includes the start and return of the distribution system (technical area), the laboratories of the quality control department (laboratory rooms) and rooms of the production area (controlled rooms with specific hygiene concepts). Out of the three, the last presents the most controlled environment with air pressure, temperature, hygiene sluice and clothing concept, air locks, and particle numbers highly regulated and controlled.

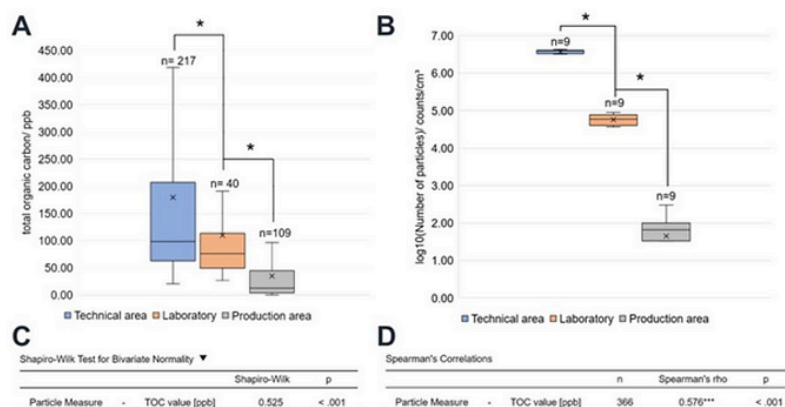


Figure 1: Correlation of offline TOC results and the number of environmental air particles present during sample processing. A: Box plot of offline TOC analyses of WFI from a controlled distribution loop. During processing, samples were exposed either to a technical area (blue), a laboratory (orange) or a production area D (grey). Significance was determined using two-tailed Mann-Whitney tests (* $P < 0.01$). B: Box plot of environmental particle number measurement detecting particles $> 0.5 \mu\text{m}$ in a technical area (blue), a laboratory (orange) and a production area (grey). Significance was determined using two-tailed Mann-Whitney tests (* $P < 0.01$). C: Shapiro-Wilk Test for Bivariate Normality. D Spearman's Correlation between the results of the offline TOC analyses and the particle numbers detected in the individual areas determined by using one-tailed tests for positive correlation (*** $P < 0.001$).

Intriguingly, offline TOC results displayed significant difference when comparing samples from the three tested environments with samples from the technical area presenting the highest mean value of 179 ppb, median of 98 ppb and interquartile range between 62 ppb and 207 ppb. Samples exposed to laboratory environment conferred a TOC mean value of 109 ppb, a median of 76 ppb and interquartile range of 49-114 ppb. Lastly, analysis of samples that underwent exposure to the controlled production area presented the lowest TOC results with a mean value of 35 ppb, a median of 12 ppb and an interquartile range of 4 ppb and 45 ppb (see Figure 1A). Previously, we did not specifically compare sample groups by classification of their respective environments and hence, we were satisfied to immediately find such profound/significant differences. As all online measurements were unaffected by the differential offline results displayed in Figure 1A or any outlier values detected during offline analyses, we investigated other root-causes that potentially impact offline-TOC-measurements. During a comprehensive investigation we furthermore systematically excluded issues with sampling containers, the analytical method and sample handling as root causes essentially leading to the hypothesis of an environmental impact potentially related to hygiene status and control of the respective areas.

Environmental particle numbers correlate with the accumulation of TOC in WFI

In general, particle-attached and free-floating airborne bacteria are ubiquitous and therefore represent reservoirs of organic carbon (Hu et al. 2020). Although transfer of large numbers of bacterial cells and diverse microbiomes requires large particle sizes $> 5\text{-}10 \mu\text{m}$ or amounts (Klejnowski et al. 2012; Liu et al. 2018), recent research shows that smaller air particles ($\leq 2.5 \mu\text{m}$) suffice to readily carry cell constituents such as rRNA (Liu et al. 2018; Acuña et al. 2022) and therefore present potent sources of environmental organic carbon. Hence, environmental air particles were considered as potential root causes to account for the observed effects on offline TOC measurements (Figure 1A). We therefore chose air particle measurements as a representative parameter and indicator for classification of the surrounding sampling area. In line with the expectations, we identified significant differences regarding the number of air particles ($> 0.5 \mu\text{m}$) in the technical area (MV $3.8 \times 10^6/\text{cm}^3$), the laboratory environment (MV $6.1 \times 10^4/\text{cm}^3$) and the controlled production area (MV $85/\text{cm}^3$) with all three sampling pools conferring modest variation and interquartile range (Figure 1B). Considering nanoparticle concentration in our outdoor environment may vary between 102 and $107/\text{cm}^3$, with an additional number of micro- and macroparticles in the range of $104\text{-}105/\text{cm}^3$ (Held et al. 2008; Klejnowski et al. 2013), our analyses present reasonable particle numbers within the technical areas and significant and gradual reduction in laboratory and production environments.

To evaluate the observed causality between offline TOC results and the number of environmental air particles in the surrounding sampling area, we performed a Shapiro-Wilk test for bivariate normality (Vetter 2017) which displayed non-normal data distribution (Figure 1C). Hence, to assess the significance of the correlation at hand we utilized Spearman's Correlation coefficient (Akoglu 2018) to test for positive correlation using one-tailed tests (Figure 1D). The resulting analysis suggests a strong correlation between the offline TOC results and the number of environmental air particles (*** $P < 0.001$ and Spearman's rho of 0.576).

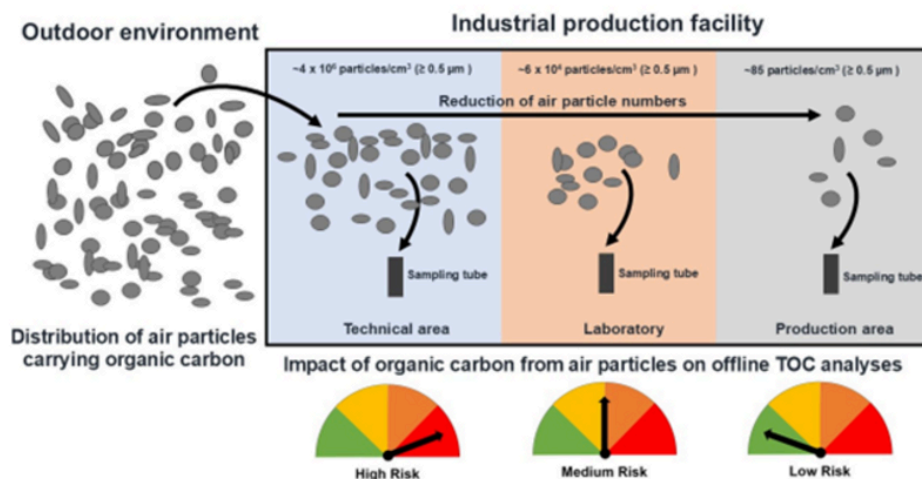


Figure 2: Impact of organic carbon from air particles on offline TOC analyses. Air particles can be distributed from various outdoor sources and may accumulate in industrial production facilities. In turn, reduction of air particles significantly reduces the risk of contaminating offline TOC samples.

As organic carbon-containing matter is established as a constituent of air particles (López-Caravaca et al. 2023; Finlayson-Pitts et al. 2020; Liu et al. 2018; Acuña et al. 2022; Klejnowski et al. 2012), our data readily provides a rational and significant insight into the accumulation of organic carbon in WFI samples even during the limited period of environmental exposure during sampling, which may be as short as 5-10 seconds. The latter timeframe therefore appears to be sufficient for air particles to enter the sampling tubes or directly into the WFI during filling from valves to the sampling containers.

We therefore propose a model in which any reduction of environmental air particles will stabilise offline TOC trends by reducing the risk of organic contaminations during sampling (Figure 2). Overall, our findings highlight the importance of qualified heating ventilation and air conditioning (HVAC) systems as well as usage and control of air filter and pressure systems in pharmaceutical production facilities. In addition, our work underlines recent advances to foster indoor air particle studies regarding airborne microbiomes and respective influence factors such as particle origin and composition, hygienic room designs, cleaning protocols, equipment, process controls and ventilation (Duarte & Duarte 2021; Meadow et al. 2014).

Conclusion

Monitoring total organic carbon in modern water systems represents an essential quality control to continuously assure production of purified waters of the highest quality. Especially online-systems have proven to be useful and reliable tools that offer data output with high stability. However, local valve contaminations confer a high risk especially at POU valves in production areas which are often located far from online TOC analysers and may therefore not be detected timely. Complementary offline analyses are therefore required at POU to prevent the emergence and carryover of local microbial hot spots. As the number of air particles profoundly impacts sample purity, our study provides valuable insights on the stability of the respective offline-method and into the evaluation of singular outlier results during offline TOC monitoring, especially for samples taken from uncontrolled or technical areas. To reduce this effect during monitoring and to protect any water generation and distribution system, all areas with sampling or POU valves should be part of the risk based and superordinated hygiene, cleaning and contamination control strategy.

Conflict of interest

The Authors declare there is no conflict of interest.

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