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A comparative analysis of two different analysers used for determination of the Total Organic Carbon in pharmaceutical grade water

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Total Organic Carbon (TOC) is a routine test for pharmaceutical grade water. Several manufacturers supply equipment of different designs but there is a dearth of published, peer-reviewed, information evaluating the various analysers. In this study, we compared two TOC analysers, both validated to the same pharmacopoeial criteria, but with different oxidation and detection methods. The results in this paper show that there were no unexplained out-of-specification results and that both analysers operated equivalently in terms of the pharmacopoeial 500ppb pass/fail limits. However, significant differences between the TOC levels reported from paired samples were observed, two paired samples recorded a pass/fail conflict (albeit flagged with an overestimation warning), as well as differences in analyser responses between spiked samples that contained low levels of nitro- and chloro-carbon compounds.

Key words: WFI, purified water, pH, nitrogen, halide, conductivity.

Introduction

Total Organic Carbon (TOC) is a routine test for water purity in the pharmaceutical, electronics and engineering industries. Introduced into the United States Pharmacopeia in 1996 as an alternative to the oxidisable substances test, it became mandatory in 1998, with the European Pharmacopoeia following suit in 2000[1,2]. The level of TOC in a water sample depends upon the purification process and any subsequent water 'holding' systems: organic contamination may originate from natural materials, such as soil or humus, or from man-made materials, such as detergents or ion exchange resins. In a pharmaceutical setting, where purified water (PW) or water for injection (WFI) is generated by steam distillation and held in a heated loop, organic contaminants are normally microbiologically derived, such as endotoxins, planktonic microorganisms and extracellular components from adherent biofilm. Biofilms produce amino acids and alcohols as waste products[3].

There are no specific pharmacopoeial requirements for the design and operation of TOC measurement equipment, only that the apparatus should be able to measure, with reasonable precision, sucrose and benzoquinoline standards. Most TOC analysers for high purity water use a two-step assay process: oxidation of organic carbon to CO_2 and subsequent CO_2 measurement. Oxidation can be performed in a variety of ways (catalytic oxidation, photo-oxidation, thermo-chemical oxidation and photo-chemical oxidation) and detection is either done by non-dispersive infra-red detection, which provides a true reading of CO_2, or by measuring water conductivity. Conductivity measurements rely on the relationship between dissolved CO_2 levels and electrical resistance and can be performed directly, or after filtration of the solution through a CO_2 selective membrane. The use of membranes is intended to remove some of the ionic interferences (for example, hydrogen, halide and other inorganic anionic species) that increase the conductivity measured and elevate the 'reported' TOC content.

There are very few comparative studies of TOC analysers available and the reports that have been published are usually conference papers and originate from instrument suppliers[3–5]. This may be due to the complexities of the experimentation and costs involved. The references used in this manuscript were available online (some under 'Google Scholar'), but the authors could find no information from traditional peer-review sources.

Godec published an extensive study with five different instruments from different suppliers at five different sites on

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two different continents, looking at TOC response level (at sub-5ppb levels) in standards solutions made up from different classes of organic compounds[3]. This work confirmed that, in analysers where CO_2 quantification was based upon direct conductivity, the presence of chlorinated compounds generates chloride ions, elevating conductivity and producing higher than expected TOC responses. It was also found that nitrogen-containing compounds generated lower than expected responses in some instruments due to difficulties in oxidising this class of compounds. In another report, using similar instrumentation, Godec's findings were confirmed at higher TOC levels (between 5 and 500ppb)[4].

In this study, we report a comparison of TOC results using two different analysers on 245 samples over a 7-month period. In addition, we tested purified water samples spiked with glycine, DNA, synthetic low-density lipoprotein (sLDL) and chloroform. The first three were chosen to act as suitable mimics for the (microbiologically-derived) proteins, nucleic acids and cell membrane components found in pharmaceutical water systems. Chloroform was selected as a suitable halogenated compound.

Materials and methods

Pharmaceutical grade water was generated using a Prima 90 reverse osmosis (RO) purification (Elga, Marlow, Bucks, UK), followed by steam distillation and storage in a WFI circulation loop maintained at above 80˚C (custom-made equipment designed and built by Freestead, Bristol, UK and Telstar, Barcelona, Spain). The equipment functions continually within the Cancer Research UK Formulation Unit manufacturing GMP suite.

TOC levels were measured in water samples taken from one RO sample point and five WFI sample points between February and August 2011. Samples (245) were split after sampling and sent for both internal and external analysis. The remaining water tests (bioburden, endotoxin, nitrates, conductivity and periodic heavy metals) were consistently within the pharmacopoeial specifications.

Samples for internal analysis were measured by using a PAT700 (Hach Lange, Manchester, UK) operating in our licensed EU GMP facility. This instrument (referred to as UVOx/DirCon in the discussion below) oxidises organic carbon by ultraviolet (UV) light to CO_2, which is subsequently measured by direct conductivity. This instrument reports the TOC results as well as a 'P-curve', the latter depending upon the shape of the time–conductivity oxidation plot.

Samples for external analysis were measured by using a Sievers 900 operated by International Laboratory Services (Derbyshire, UK) a UKAS accredited facility operating to EU GMP. This instrument (referred to as ChemOx/MemCon in the discussion below) oxidises organic carbon using acidified ammonium persulphate and UV light before the CO_2 is transferred through a membrane and conductivity measured. These results are just reported as a single TOC value.

Chloroform, glycine and calf thymus DNA were reagents of commerce, and sLDL was prepared using a previously published method[5]. Spiked samples of these substances were diluted in purified water to 50ppb TOC for chloroform and 250ppb for the remaining analytes. The preparation of all solutions was done with consideration of the substance's water solubility. No correction was made for blank water TOC values, or the salt/lyophilised water content of the samples.

Statistical analysis of the results was performed using Graph Pad Prism, Version 4, Graph Pad Software running on a Mac OS X 10.6 computer.

Results

For the RO and WFI samples the UVOx/DirCon analyser was found to produce two classes of P-curve with the water samples: P3 and P5. According to the manufacturers literature, P5 curves originate from samples containing carboxylic acids where the conductivity of the sample before oxidation is higher than that afterwards. In our practical experience, P3 curves are typically found for

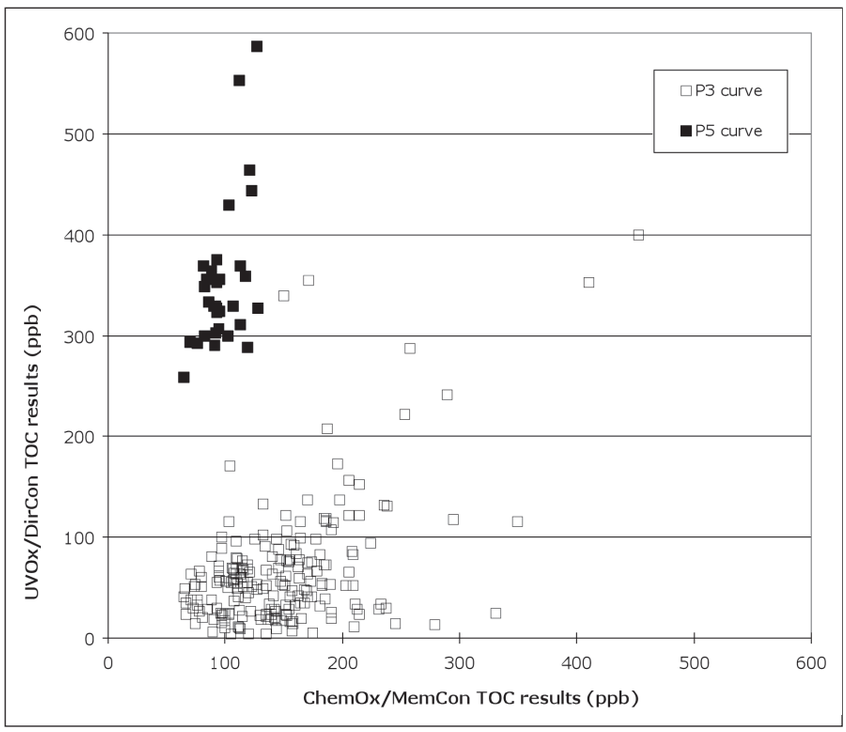

Figure 1. Comparison of the measured TOC levels in 245 PW/WFI sample points as reported by internal, UVOx/DirCon, and external, ChemOx/MemCon, analysis.

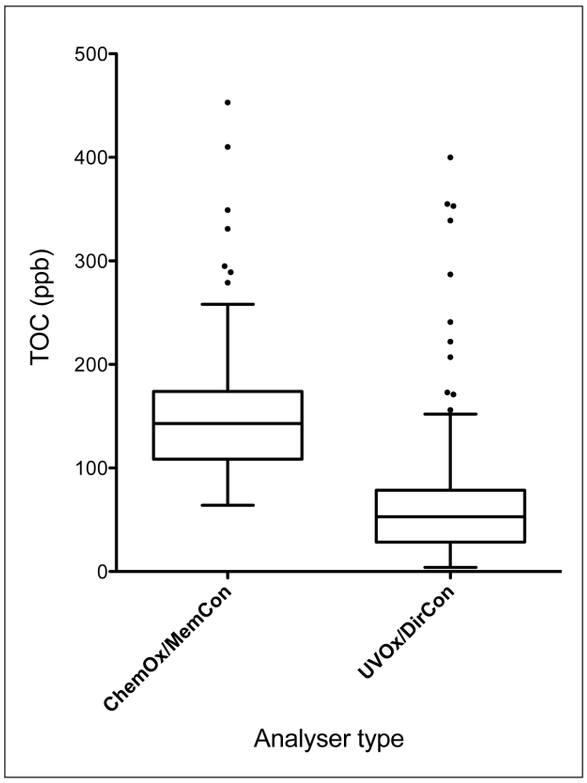

Figure 2. Box plot diagram (with Tukey whiskers) of P3 sample data from the two different types of analyser.

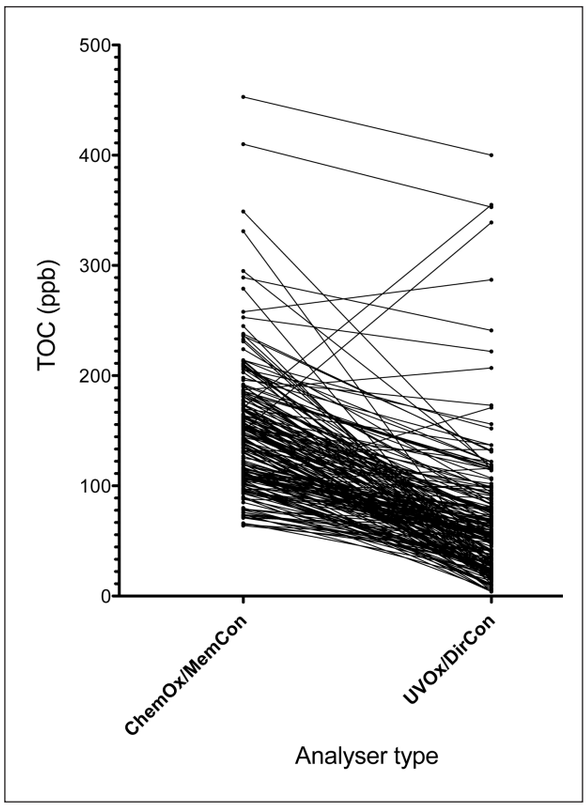

Figure 3. Comparison line diagram of the paired P3 curve samples from the two different types of analyser.

routine water samples as well as samples where known organics are added (including the alcohols used in cleaning sprays, sucrose and benzoquinone calibrants).

The comparative TOC data from both P3 and P5 classes are shown in the scatter plot in **Figure 1**. It can be seen that for the majority of 'P3' results the ChemOx/MemCon analysis reports a higher level of carbon than the UVOx/DirCon results, whereas for the 'P5' results the opposite occurs. We examined the possibility that the differences between the two sets of results could be due to other variables, however, we found no indication of such bias when the results were classified by weekday of sampling (a surrogate for the length of time the samples were stored before external analysis), sample date or water system sample point. Boxplots and paired line diagrams for the P3 results are shown in **Figures 2** and **3**.

The comparative results of the spike samples are shown on **Figure 4**. All samples reported a P3 curve from the UVOx/DirCon analyser, except glycine, which produced a P2 curve.

One instance occurred where an out-of-specification result was reported from both analysers. This was investigated and found to be alcohol spray contamination (data not shown).

Figure 4. Comparison line diagram of spiked samples (CHCl3 at 50ppb, DNA, glycine and sLDL at 250ppb)

Discussion

Before considering the differences between the PW/WFI samples, it is more beneficial to discuss the spiked samples (see **Figure 4**). The increased response of the UVOx/DirCon to chlorinated organics when compared to the ChemOx/MemCon is obvious and concordant with the available literature[3,4]. While not previously commented upon, this phenomena may be predicted for other halogenated compounds based upon conductivity values alone. The limiting ionic molar conductivity for Cl- is 76.3Λ while for F-, Br- and I- are 55.4, 78.1 and 76.8Λ, respectively[6].

Perhaps of more relevance are the spiked samples used to mimic microbiologically derived contaminants: glycine, DNA and sLDL. It has been suggested that UVOx/DirCon systems significantly underestimate TOC levels from nitrogen-containing compounds due to 'under-oxidation'[3,4,7]. This would be of particular interest for WFI loops and storage systems where proteins and nucleic acids could be contaminants. Our analysis shows that, while both systems overestimate the TOC levels in the glycine and DNA spiked samples, the UVOx/DirCon values are, unexpectedly, higher than the ChemOx/MemCon results. There is no precedent in the literature for this observation, but this overestimation of TOC levels by the direct conductivity system may have the same cause as the elevation effect found with chlorinated compounds: oxidation of nitrogen from the covalent to an ionic form (NOx, or NH4+) will increase the conductivity and subsequently the reported TOC level. The oxidation of nitrogen (albeit by a chemical and catalysed UV irradiation system) is used to measure

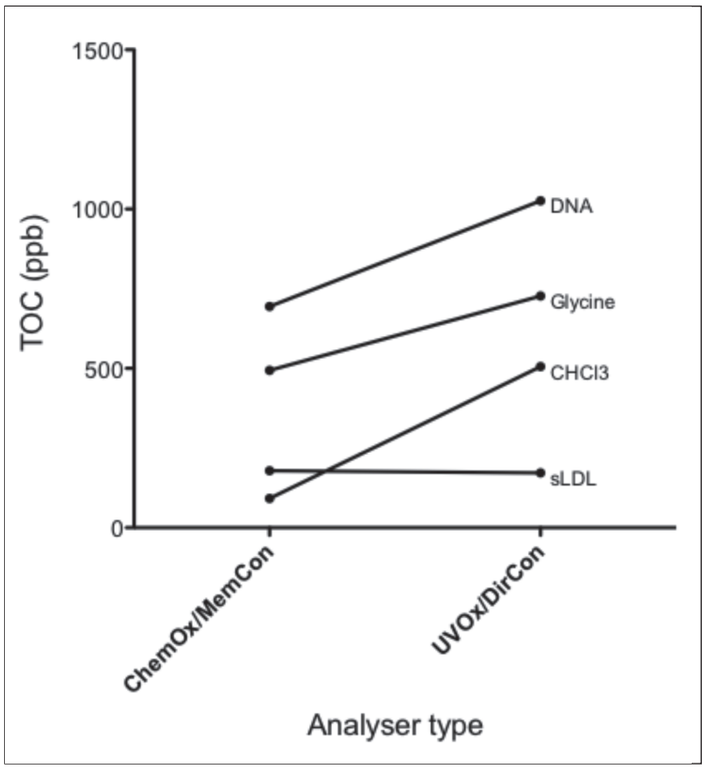

Figure 4. Comparison line diagram of spiked samples (CHCl3 at 50ppb, DNA, glycine and sLDL at 250ppb)

nitrogen levels in water in a similar way to the TOC analysis8. NO3- and NH4+ species have limiting ionic molar conductivity of 76.8 and 73.5Λ, respectively, which is significantly higher than the HCO3- value of 44.5Λ 6.

The nature of the conductivity–TOC relationship is worth examining. Dissolved CO2 exists in equilibrium as shown in **Figure 5a**. However, as a weak acid, the carbonic acid will also be a pH-dependent dissociation equilibrium, shown in **Figure 5b** together with the conductivity of the ionic species[6]. It is not clear from any of the previous literature how the final pH of the oxidised solution affects the overall 'TOC value'. It seems likely that for the carbonic anions, as with the other halide and nitrogen-containing anions being discussed here, the overall conductivity being measured will be mediated through the H+ and OH- ions since these last two ionic species, with limiting ionic molar conductivity of 349.8 and 198.3Λ for H+ and OH-, respectively, have conductivities several times any of the other species being considered here. While it could be argued that the low level of contaminants found in high purity water are unlikely to alter pH, we have observed pH variation in spiked samples of between 5.5 and 7.5. Furthermore, it is worthwhile noting that in a 50ppb (as carbon) CHCl3 sample the amount of residual halide after oxidation would be 1.2 x 10-5M, assuming this is as H+Cl- (aq), the pH of the associated solution (assuming no other interferences) would be 4.9, significantly below the first pKa of carbonic acid. Our attempts to measure pH of the discharged post-analysis sample solution from the UVOx/DirCon system were hampered by the additional purge volumes used during the instruments analysis cycle.

Figure 5. (a) CO2–carbonic acid equilibrium; (b) Ionic equilibrium of carbonic acid (the area of the textbox represents the proportional values of the intrinsic conductivity of each species).

In our results, where a P3 curve is reported, the ChemOx/MemCon system reports a higher value by, on average, 15%. Initially, we considered that this may be due to 'under-oxidation' of nitrogen-containing compounds, however, the elevated TOC levels measured by the UVOx/DirCon on the glycine and DNA spiked samples suggests otherwise.

The reported TOC levels of sLDL were very similar, and close to the estimated TOC value. This observation, combined with the required accuracy of the pharmacopoeial standards, sucrose and benzoquinone, suggests that these compounds – made up largely of carbon, hydrogen and oxygen – can be accurately estimated by both systems.

The P3 curve results shown in **Figure 1** are plotted as boxplots and pair line diagrams in **Figures 2** and **3**, respectively. The box diagram indicates that the data is not normally distributed. A Wilcoxon matched pairs test results in a P value <0.0001: the two analytical systems produced statistically different results. **Figure 3** shows clearly the general (though not exclusive) trend for the UVOx/DirCon results to be lower than the equivalent results from the ChemOx /MemCon system.

For the 'P3 curve' samples, it is not clear what mechanism underlies the differences in reported TOC values between the two analysers or which apparatus provides better accuracy. One possibility is that the delay between sampling and external analysis results in an increase in TOC due to unexpected leaching or microbiological growth, however, the fact that sampling date and day had no effect on the ratio between the two sets of experimental results would not support this

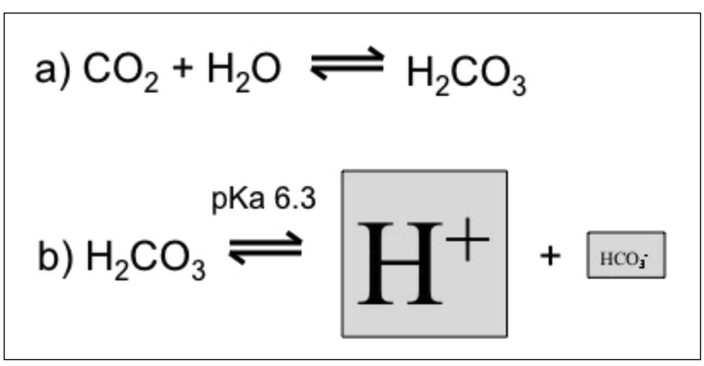

Figure 5. (a) CO2–carbonic acid equilibrium; (b) Ionic equilibrium of carbonic acid (the area of the textbox represents the proportional values of the intrinsic conductivity of each species).

hypothesis. (Additionally, the sampled water is known to be sterile.) Inter-equipment variability was observed by Godec[3] even in studies carefully set up on the same site using sampling systems configured to ensure sample consistency.

The elevated TOC response, as compared to the ChemOx/MemCon, from the UVOx/DirCon analyser when the latter reports a P5 curve system is consistent with the supplier's assertion that the reported value is an overestimate of the actual TOC level. It should be noted that two paired samples reported ChemOx/MemCon values less than the pharmacopoeial limit, but UVOx/DirCon values, with a P5 curve, above 500ppb. This data supports the proposal that when a P5 curve is reported, the TOC value will be an overestimate. However, in the event of a PW/WFI sample failing TOC limits with a P5 curve on a UVOx/DirCon analyser, an alternative sampling or analysis strategy would need to be pursued.

Conclusions

The measurement of TOC is an important part of the required quality control checks for pharmaceutical water systems, however, there is little literature available for the cross-comparison of TOC analysis equipment and what information exists originates from equipment manufacturers. The observations and conclusions reported in this paper have been generated within an academic laboratory (operating to EU GMP) with no affiliation to commercial suppliers.

Our results on chlorinated organics confirm the previously published data[3–5] that direct conductivity TOC analysers overestimate the actual result. However, in our experiments, the under-oxidation of nitrogen-containing compounds (reported in references 3, 4 and 7) was not observed, and, in fact, we report an opposite effect: glycine and DNA showed higher TOC values in the direct conductivity analyser than was reported in the membrane conductivity system, possibly due to ionic forms of nitrogen increasing the final conductivity.

Both analysers tested showed equivalent results when testing compounds consisting of carbon, hydrogen and oxygen, but this is not the case with species of more complicated compositions containing nitrogen and halogens. It appears that the pharmacopoeial suitability test is only quantitatively appropriate where the analysers are responding to PW/WFI contaminants with similar atomic compositions to the pharmacopoeial calibrants (that is carbon, hydrogen and oxygen), and while these perhaps represent important carbon-containing analytes within a pharmaceutical water system, other moieties, particularly nitrogen-containing proteins, amino acids and DNA, may well be overestimated.

In our data, based upon a 7-month study with over 240 samples measured in duplicate, no unexplained out-of-specification results were reported. While both analysers operate on different principles, they are both validated to the same pharmacopoeial criteria and are considered suitable for TOC measurement of pharmaceutical grade water; however, each analyser produces different results both with actual PW/WFI samples and when tested with solublised compounds of different elemental compositions. While we have not isolated the cause of the variation, our results do suggest that changing a TOC analyser may adversely affect data trending procedures. Our observations concur with previous workers[3] that if two analysers are available on a regular basis then the differences between the reported TOC values can be used diagnostically to determine water chemistry.

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Conflict of interest

The authors are not funded by, or associated with, either of the analyser manufacturing companies indicated in this article.

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