

Easy and Rapid Application for Residual Chlorine Analysis in Water Samples

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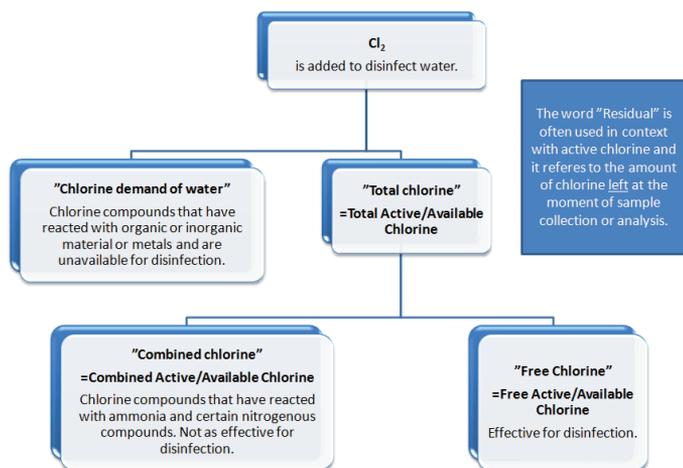
Introduction

Chlorine is commonly used for disinfection in a wide range of applications. For sanitizing the water in swimming pools, chlorine is added in the form of sodium or potassium hypochlorite or chlorine gas.

Immediately after its addition to water, a part of the chlorine reacts with manganese, sulfides, or other organic compounds and is unavailable for disinfection. This part is called the "chlorine demand of water" as shown in Figure 1.

The portion remaining is called "active chlorine" or "total active chlorine." Part of this total chlorine can react with certain nitrogenous compounds, mostly from sweat and urine, forming ammonium chlorides. This group of compounds is called "combined chlorine" and it is not as effective a disinfectant as the "free chlorine" fraction, which consists of hypochlorite ions, hypochlorous acid, and chlorine gas.

FIGURE 1. Chemical reactions in water chlorination.



The intended purpose of chlorination is to kill the microorganisms and bacteria that are harmful to people. However, it may also create some undesired byproducts, such as carcinogenic trichloromethane (chloroform), mono-, di- and tri-chloroacetic acids, or trichloramines, and highly toxic cyanogen chloride which are formed when chlorine is combined with uric acid.

Combined chlorine in the water of swimming pools is often a symptom of an inadequate amount of free chlorine. This may cause unwanted effects, such as skin irritation, breathing difficulties, and a strong odor. Therefore, it is preferred that the amount of combined chlorine is as low as possible.

To achieve sufficient disinfection and to minimize any undesired byproducts, it is essential to monitor both free and combined residual chlorine concentrations in the water.

Rapid Analysis Using the Gallery Analyzer STAT-Protocol

The unstable nature of residual chlorine in water samples requires a rapid analysis method. Thermo Scientific™ Gallery™ discrete analyzer offers a STAT-Protocol that minimizes the exposure of samples to the atmosphere. In many water laboratories the discrete analyzer runs non-stop, performing multiple analyses for various types of water samples simultaneously. When analyzing residual chlorine, it is recommended that the time a sample is in contact with the atmosphere is minimized. When using a Gallery discrete analyzer, a free residual chlorine analysis can be requested using the STAT-Protocol. As a result, the analysis will be performed immediately after inserting the sample into the analyzer.

This paper compares the Residual Chlorine method used by the Gallery analyzer to the manual method based on EPA 330.5 and SFS-EN ISO 7393-2:2000.

Materials and Methods

Method Principle

Free residual chlorine (FRC) reacts instantly with the N,N-diphenyl-p-phenylene diamine (DPD) indicator to produce a red color in a pH range of 6.2 to 6.5. Absorbance is measured at a wavelength of 510 nm.

In the total residual chlorine (TRC) method, chlorine liberates iodine from potassium iodide when an excess amount of iodine is present. The liberated iodine reacts with DPI to produce a red-colored solution. Absorbance is measured at a wavelength of 510 nm.

Oxidizing agents interfere with the method and these are usually present in very low concentrations compared to the residual chlorine concentrations. However, sunlight, exposure to air, and agitation of the sample may critically effect the outcome. Therefore a quick analysis of the free residual chlorine is essential. Turbidity and color may also interfere with colorimetric analysis and this can be avoided by application design. A high concentration of monochloramine can interfere with free chlorine determination. With these samples; the application can be redesigned to avoid this by stopping the reaction with arsenite or thioacetamide.

Equipment

A Thermo Scientific Gallery, Gallery Plus, or Aquakem™ analyzer can be used. The method in this paper was performed with the Gallery analyzer measured at a wavelength of 510 nm.

Gallery Application

Gallery applications are based on EPA 330.5 for the TRC measurement. This method is similar to SFS-EN ISO 7393-2:2000 for both TRC and FRC. Differences in the official methods and the Gallery applications are shown in Table 1.

Table 1. Comparison of residual chlorine methods.

| | EPA 330.5 | SFS-EN ISO 7393-2:2000 | Gallery Application | Chlorine Ref (TFS R&D) |
|----------------------------|---|--|---|--|
| Calibrator | potassium permanganate | potassium iodate + KI | potassium permanganate | potassium permanganate |
| Calibration method | no KI, no incubation, calibration for TRC | KI in calibrator, incubation 2 min, same calibration for FRC and TRC | FRC: No KI, no incubation TRC: KI, 1 min (37°C) incubation | No KI, no incubation, same calibration for FRC and TRC |
| Wavelength | 515 nm | 510 nm | 510 nm | 510 nm |
| KI (%) in reaction mixture | 1.00 % | 1.00 % | 0.83 % | 0.83 % |

In the Gallery TRC applications (TRC Low (0.1 to 0.5 mg/L) and TRC High (0.5 to 3 mg/L)), 6 µL of the phosphate buffer (RC R1) and 6 µL of the DPD indicator reagent (RC R2) were automatically added to a disposable cuvette. This was followed by an automatic addition of 120 µL of sample and potassium iodide solution (RC R3). In the TRC Low application, the sample was introduced first and RC R3 next. In the TRC High application, this was done in the reverse order. An incubation for 60 seconds at 37°C occurred after the last reaction component was added. Due to the higher temperature utilized, the incubation time is shorter compared to the standard methods. Finally, absorbance of the reaction mixture was measured at 510 nm.

FRC applications (FRC Low and FRC High) are similar to TRC applications, but without the addition of potassium iodide and the incubation step.

Combined residual chlorine (CRC) applications (CRC Low and CRC High) are applications that subtract the FRC results from the TRC results. These calculations can be automatically performed with the Gallery analyzer.

Contamination from other sample matrices or reagents was not studied, therefore analyzing chlorine samples using the STAT-Protocol or by inserting samples one at a time is recommended. A laboratory can also validate the method for mixed runs.

A hypochlorite wash is usually done as part of the discrete analyzer's daily routine. If this protocol is used, re-flushing the tubing is recommended. A zero concentration sample can also be measured before sample analysis to ensure that all residual analyte is fully removed from the instrument.

Reagent Preparation for Gallery Analyzer

Reagents RC R1, RC R2, and RC R3 were inserted into the analyzer approximately 30 minutes before the required analysis to ensure adequate stabilization of the reagents. Reagents in the analyzer vials were replaced each day. Reagents RC R1 and RC R2 were refrigerated, and the reagent RC R2 was protected from light. Reagent RC R2 showed no visible sign of aging (a pink color) during the two weeks of storage. Reagent RC R3 was prepared daily.

Preliminary tests using frozen reagents showed identical results when compared to refrigerated reagents.

0.02 M KMnO₄ Calibrator Stock Solution

The 0.02 M potassium permanganate (KMnO₄) calibrator stock solution is 3547 mg/L as equivalent to Chlorine. The solution was prepared from a commercial concentrate.

A KMnO₄ solution is often used in calibrating the residual chlorine method because it is more stable than an active chlorine solution. The permanganate ion oxidizes the DPD molecule in the same way as any active chlorine compound.

This solution is stable for up to 12 months if tightly sealed and refrigerated in an amber bottle in the dark. KMnO₄ is light sensitive and degrades over time (especially at low concentrations).

The calibration may be performed by separate calibrators when the dilution is done manually, or by serial calibration when the dilution series is done automatically by the Gallery or Aquakem analyzer.

Working KMnO₄-solutions

A 12 mg/L equivalent Chlorine standard was prepared for High Range calibration (0.5 to 3 mg/L) and a 1.5 mg/L equivalent Chlorine standard for Low Range (0.1 to 0.5 mg/L) calibration.

Sample Analysis and Sample types

Samples were taken from several public swimming pools. Sample concentrations were from 0.12 to 0.58 mg/L for FRC and 0.11 to 0.89 mg/L for TRC. To cover the whole concentration range of the calibration (0.1 to 3.0 mg/L), spike samples were prepared. Small volumes of potassium hypochlorite solution were added to a series of pool water samples and the concentrations of the spiked samples were analyzed in the method comparison study. Spiked sample concentrations included 0.12 to 2.94 mg/L for FRC and 0.11 to 2.89 mg/L for TRC.

Quality control samples for this study were prepared from sodium hypochlorite solution (free chlorine), which was verified the same day by sodium thiosulphate titration.

Reference Method Used for the Study

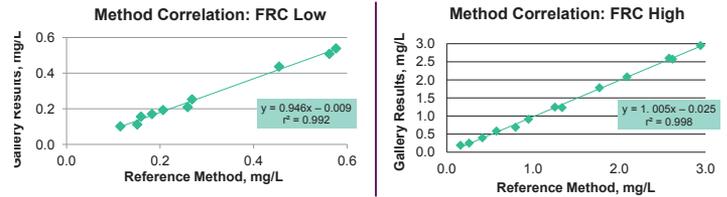
Evaluation of the Residual Chlorine methods was done in the Thermo Fisher Scientific Finland R&D laboratory using the Gallery discrete analyzer. In this evaluation, a series of samples were measured against a manual DPD-spectrophotometric method (Chlorine Ref) based on EPA 330.5 TRC measurement. The major difference is the EPA method does not measure free chlorine. This manual reference method is similar to SFS-EN ISO 7393-2:2000 which determines both total and free residual chlorine. A Thermo Scientific Multiskan™ GO Spectrophotometer was used for the manual measurements at a wavelength of 510 nm.

Results and Discussion

Method Comparison Studies

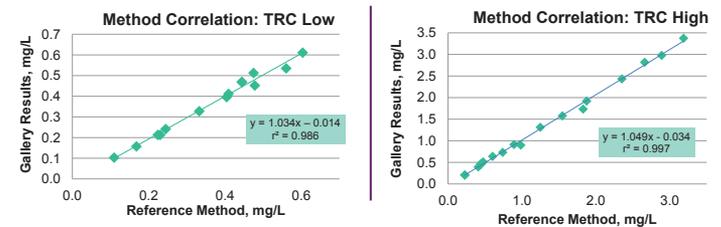
The Gallery analyzer FRC low and high concentration applications were compared to the similar manual spectrophotometric analysis applications and the results are shown below in Figures 2 and 3.

FIGURES 2-3. Method correlation of Gallery analyzer vs. manual DPD-photometric methods using FRC Low and FRC High applications.



The Gallery analyzer TRC low and high concentration applications were compared to the similar manual spectrophotometric analysis applications and the results are shown below in Figures 4 and 5.

FIGURES 4-5. Method correlation of Gallery analyzer vs. manual DPD-photometric methods using TRC Low and TRC High applications.



According to these method comparison studies, all TRC methods correlated well with each other with no significant systematic error as shown in Figures 4 and 5. The average bias in this evaluation was 2.1 % for the lower concentration area (0.1 to 0.5 mg/L) and 4.9 % for the higher concentration area (0.5 to 3.0 mg/L).

Analysis of low concentration FRC methods in this study was fairly good as shown in Figures 2 and 3. The average bias was -5.4 % for the low range and 0.5 % for the high range. However, some of the individual results deviated significantly, as much as -26 % from the reference results. This may be due to the nature of the analyte or delays in the analysis of samples. Therefore, inserting the FRC samples one at a time or using the STAT-Protocol is recommended.

During the analysis of free residual chlorine, it was noticed that the highest evaporation (%) was detected from low concentration samples. Therefore, for future analysis, analyte evaporation from the samples should be minimized by using 10 mL test tubes which are filled to 1/3 of their full volume.

Quality Control Samples

Each application had low and high quality control (QC) samples. QC samples were measured by both the Gallery analyzer Residual Chlorine applications and the manual spectrophotometric reference method (Chlorine Ref). Results are shown in Table 2.

Table 2. QC sample results with Gallery applications and Chlorine Ref 2.

| Sample\ | Analyte | Gallery Applications | | | Chlorine Ref | | | Bias (Methods) % |
|-------------|----------|----------------------|------------|-----------|--------------|------------|-----------|------------------|
| | | Result, Avg. mg/L | Recovery % | Bias mg/L | Result mg/L | Recovery % | Bias mg/L | |
| QC 0.1 mg/l | FRC Low | 0.08 | 82 % | -0.02 | 0.08 | 82 % | -0.02 | 1 % |
| QC 0.5 mg/l | FRC Low | 0.46 | 92 % | -0.04 | 0.51 | 102 % | 0.01 | -9 % |
| QC 0.1 mg/l | TRC Low | 0.09 | 92 % | -0.01 | 0.09 | 90 % | -0.01 | 1 % |
| QC 0.5 mg/l | TRC Low | 0.48 | 97 % | -0.02 | 0.49 | 99 % | -0.01 | -2 % |
| QC 0.5 mg/l | FRC High | 0.46 | 93 % | -0.04 | 0.51 | 102 % | 0.01 | -9 % |
| QC 1.0 mg/l | FRC High | 0.97 | 97 % | -0.03 | 1.01 | 101 % | 0.01 | -5 % |
| QC 2.5 mg/l | FRC High | 2.46 | 98 % | -0.04 | 2.68 | 107 % | 0.18 | -8 % |
| QC 1.0 mg/l | TRC High | 0.98 | 98 % | -0.02 | 1.05 | 105 % | 0.05 | -6 % |
| QC 2.5 mg/l | TRC High | 2.65 | 106 % | 0.15 | 2.70 | 108 % | 0.20 | -2 % |

Both methods showed fairly good to good recoveries from the QC samples between 0.5 to 2.5 mg/L. Recoveries were from 92 to 98% for FRC and from 97 to 106% for TRC. The smallest QC (0.1 mg/L) was close to the quantitation limit and showed recoveries of 82% for FRC and 92% for TRC. Results for the reference method were 82% and 90%, respectively. Low recoveries are thought to be the result of evaporation of the analyte.

In general, Gallery analyzer applications showed lower results than the reference method, Chlorine Ref.

Conclusion

This study shows that TRC can be measured using the discrete analyzer, and the Gallery method correlates very well with the EPA 330.5 and SFS-EN ISO 7393-2:2000 manual methods. Quality control sample recoveries varied from 92 to 106%. The water samples tested originated from several swimming pools and their concentrations were between 0.11 to 2.89 mg/L, including the spiked samples. Gallery applications are designed for concentration areas of 0.1 to 0.5 mg/L (low range) and 0.5 to 3.0 mg/L (high range).

The FRC method also correlates well with SFS-EN ISO 7393-2:2000 manual methods. Tested samples varied in concentration from 0.12 to 2.94 mg/L, including the spiked samples. However the unstable nature of FRC offers a challenge which was demonstrated in the low concentration area. From the QC sample of 0.1 mg/L, an 82% recovery was also shown with the reference method. Other QC samples showed good recoveries ranging from 92% to 98%.

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