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Green detection of trace cyanuric acid and free chlorine together via ion chromatography

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**HIGHLIGHTS**
- An IC method is proposed to analyze cyanurate (CYA) and free chlorine (FC) simultaneously.
- IC is greener and more robust than conventional methods in detecting trace CYA and FC.
- The method’s detection limits of CYA and FC reached 3.6 and 9.0 μg/L respectively.
- Hypochlorite ion can be analyzed with UV detector after separation from other anions.

**GRAPHICAL ABSTRACT**

**ABSTRACT**
Chlorinated cyanurates (CCAs) are a type of disinfectants currently used worldwide for fight of Coronavirus. However, CCAs upon dosed into water can release not only free chlorine (FC), a strong disinfectant, but also cyanurate (CYA), a persistent compound potentially harmful to human and environment. Therefore, detecting CYA and FC in water are very important not only for ensuring sufficient disinfection but also for monitoring the impacts of FC and CYA on receiving watershed. However, conventional analytical methods for them are mostly based on colorimetric methods, which have high method detection limits (MDLs) and rely on chemical reactions that are likely sensitive to coexisting chemicals. To overcome these issues, we herein proposed a facile and reaction-free method to detect CYA and FC together in just one run by ion chromatography (IC) equipped with both conductivity and ultraviolet absorbance detectors. The method features obvious advantages over colorimetric methods in being lower MDLs (3.6 μg/L for CYA and 9.0 μg/L for FC), environmental-friendly (i.e., no organic solvent involved), and more resistant to alkaline solution. With this method, trace levels of CYA (i.e., 34–44 μg/L), which were nondetectable by conventional method, were found in two river water samples, implying that the local environment was already polluted by CCAs during the pandemic period. Overall, this study demonstrates a robust tool that may assist better understanding and monitoring the fate and transport of trace CCA derivatives in water.

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1. Introduction

During the fight for Coronavirus Disease 2019 (COVID-19) pandemic, many disinfectants are intensively used around the globe. Among disinfectants, chlorinated cyanurates (CCAs) are relatively persistent under sunlight exposure and low temperature conditions (Canelli, 1974; O'Brien et al., 1974; Downes et al., 1984); therefore, many CCA compounds like trichloroisocyanuric acid (TCCA) and sodium dichloroisocyanurate (NaDCCA) are widely applied. For example, the China Center for Disease Control and Prevention has promulgated the uses of two cryogenic disinfectants (one at -18°C and another at -40°C) in February 2021, and both contain NaDCCA intended for alpine region and cold-chain package disinfection (China Center for Disease Control and Prevention, 2021). In addition, CCAs have been accepted and used abundantly by swimming pool users traditionally (Wahman, 2018). Even in drinking water industry, CCAs have also been approved in July 2001 for drinking water disinfection under the United States (US) Federal Insecticide, Fungicide, and Rodenticide Act, and so far many makers have obtained the NSF 60 certificates to sell CCAs (Wahman, 2018) although CCAs are not routinely used in drinking water treatment plants now. Therefore, people should pay close attention to the uses and potential risks of CCAs and relevant derivatives in water.

| Method                           | Principle(s)                                                                                  | LCR       | MDL       | Ref.                        |
|----------------------------------|-----------------------------------------------------------------------------------------------|-----------|-----------|---------------------------|
| Colorimetry                      | CYA reacts with a melamine reagent to produce some turbidity in water that can be measured as a visible wavelength of 420 nm. | 5–50 mg/L | 1.5 mg/L  | Downes et al. (1984)       |
| Electrochemical Methods          | CYA sample introduced into an electrochemical analyzer without any previous treatment is used for differential pulse polarography and cyclic voltammetry measurements. | 0.06–3.5 mg/L | 0.02 mg/L | Yilmaz and Yazar (2010)   |
| Mass Spectrometry Complex        | CYA sample is introduced into a polarographic analyzer and measurement is conducted using a static mercury drop electrode in three-electrode cell and an Ag/AgCl reference electrode. | 1.29–129 mg/L | 1 mg/L     | Struys and Wolfs (1987)   |
| Liquid Chromatography HPLC-UV    | CYA is extracted from water through a microscale liquid-liquid extraction, and then injected into an electro spray mass spectrometer through a mass-selective stable association complex for quantification. | 0–40 mg/L  | 0.13 mg/L | Magnuson et al. (2001)     |
| Ion Chromatography               | The method used an AEC, a carbonate buffer as eluent, and a CM detector for the determination of CYA and anions (fluoride, formate, nitrite, hydrogen phosphate, sulfate) in milk powder. | 0.1–100 mg/L | 0.083 mg/L | Wang et al. (2010)         |

Table 1 A summary of CYA analytical methods reported in literatures.
In terms of disinfection mechanism, the disinfection ability of CCAs essentially lies in that they can release a known disinfectant, free chlorine (FC). However, an undesirable side effect of using CCAs is that they also liberate cyanuric acid (CYA, or namely cyanurate), which is a chemically and biologically refractory compound that might harm the environment and human (Canelli, 1974; Hammond et al., 1986; Suchy et al., 2009). For example, when CYA reacts with melamine (MEL), it can induce high nephrotoxicity and aggravate renal toxicity (Wang et al., 2012; Chang et al., 2014; Zhu and Kannan, 2018, 2019c, b). In addition, when abundant CYA was present in water, the disinfection efficiency of FC became lower toward Cryptosporidium oocysts (Murphy et al., 2015) and Giardia (Falk et al., 2019). In fact, CYA has been detected frequently in human urine samples (Sathyaranayana et al., 2019; Zhu and Kannan, 2019a). As a result, the maximum contamination levels of CYA have been regulated to be 30 and 100 mg/L respectively in China’s indoor and outdoor swimming pool water (CJ/T 244–2016). Similar regulation was applied in USA rule too (ANSI/ASPF-11 2009).

To comply these rules, some analytical methods have been established to detect CYA in water, including titration (O’Brien et al., 1974), colorimetry (or namely turbidimetry) (Downes et al., 1984), electrochemistry (Struyys and Wolfs, 1987; Yilmaz and Zayar, 2010), mass spectrometry (Magnuson et al., 2001), liquid chromatography (LC) (Briggle et al., 1981; Jessee et al., 1981; Downes et al., 1984; Cantù et al., 2000, 2001; Patel and Jones, 2007; Panuwet et al., 2010), and gas chromatography (GC) (Fiamegos et al., 2003; Tang et al., 2009). The principles and application scopes of typical CYA analytical methods are summarized in Table 1. Likewise, due to the concerns to disinfection ability, many methods have been developed to measure FC, including chemiluminescent (Song et al., 2013), colorimetric (or namely spectrometric) (Pak et al., 2018; Wang et al., 2019), electrochemical (Sol-datkin et al., 1997; Wang, 2002), and chromatographic methods (Nejdli et al., 2013; Zhang et al., 2018). Of them, colorimetric methods are commonly used due to the convenience of use and low cost. However, these methods are usually high in method detection limits (MDLs) and highly reliant upon chemical reactions between specific chemical reagents with CYA or FC, which are likely to be interfered by coexisting compounds in water (Downes et al., 1984). In addition, previous studies detected CYA and FC separately, which are laborious, and there is no facile method reported before that can measure CYA and FC together in just one process.

Specifically, the MDL of colorimetry for CYA detection is as high as 1.5 mg/L (Downes et al., 1984), making it unable to detect trace (i.e., μg/L) level of CYA. While LC method may fill the gap (Briggle et al., 1981; Jessee et al., 1981; Downes et al., 1984; Cantù et al., 2000, 2001; Patel and Jones, 2007; Panuwet et al., 2010), LC inevitably uses organic solvents (e.g., methanol or acetonitrile) as eluents (or say mobile phase). Unlike common LC which equips with CM detector only, this study is novel in employing UV detector in IC too because it is able to achieve sensitive detection of CYA and FC. In addition to calibration curves, MDLs, relative standard deviations (RSDs, a symbol of sensitivity), and recoveries of CYA and FC from a set of synthetic and real samples, we also assessed the impacts of a series of separation conditions. In addition, a comparison of the present method and conventional methods towards some real samples were made. Finally, the achieved results were also compared with the data predicted by a water chemistry model.

2. Materials and methods

2.1. Reagents and solutions

CYA and TCCA were procured from Aladdin Inc., China. The stock solutions, including 500 mg/L CYA (98%) and 500 mg/L TCCA (97%), were prepared by dissolving solid chemicals into ultrapure water. Sodium hypochlorite (5.9% as ClO−) was calibrated before use. Other chemical reagents such as sodium acetate, fluoride, chloride, bromide, sulfate, nitrate, sulfite, hydrochloric acid, and KOH were purchased from Aladdin Inc., China. All stock solutions were stored in a refrigerator (4°C). Standard solutions were prepared by dilution of stock solution with ultrapure water (18.2 MΩ cm) produced by a Millipore water generator (Direct-Q3).

2.2. Collection and treatment of real samples

Several real samples were collected, including tap water samples from the laboratory faucet and river waters, lake waters, rain waters, wastewater effluent, and swimming pool waters from Shenzhen, by using high-density polyethylene bottles (5 L). Prior to IC injection, real samples were preconditioned by KOH solution with the pH similar to IC eluent (i.e., 12.3 ± 0.1) and then filtered by 0.45 and 0.22 μm cellulose filters in sequence to remove insoluble particulates. This procedure was applied to eliminate possible blockage of AEC caused by impurities present or formed in the samples. The samples were stored in refrigerator at 4°C prior to use. All samples were measured within three days after collection.
2.3. Apparatus and operation procedures

The IC system used in this study consists of a host engine (IC-2010, Tosoh Inc., Japan), a KOH EDG (EDG-100, Minghao Corp., China), an eluent suppressor (WLK-8A, Reepo Corp., China), a CM detector, and a UV detector (SPD 20A, Shimadzu, Japan). In addition, the IC host engine contains an autosampler, a pump, a degasser, an injection valve with loop of 500 μL, a column oven (set at 30 °C), and an AEC (either AS-15 or AS-19, 4.0 mm i.d. × 250 mm length, Thermofisher, US). The eluent was run in isocratic elution mode.

The conventional colorimetric method for CYA measurement involves the use of a MEL reagent (Shenzhen Sinsche Technology Co. Ltd., China) that reacts with CYA to produce a solid detectable at the wavelength of 420 nm. The DPD methods for FC and total chlorine (TOTCl) measurements involve the use of DPD reagents purchased from Hach, US (FC: 14,070-28, TOTCl: 14,064-28) which react with FC or TOTCl to produce a color detectable at the wavelength of 530 nm after 1 min reaction for FC and 3 min reaction for TOTCl (Hach, 2009). The UV absorbance was measured by a UV–visible spectrophotometer (DR3900, Hach, US) using a 1 cm quartz tube. The pH of tested water was determined using a pH meter (Thermofisher model: 8103BN).

3. Results and discussion

3.1. Method demonstration and comparison

3.1.1. Separation of coexisting anions

Theoretically, this method is insensitive to coexisting ions and organics provided that they do not react with CYA and FC rapidly (in fact no detection method is reliable if rapid reaction occurs). So, as shown in Fig. 1, anions like fluoride, acetate, bromide, nitrate, and sulfate at selected levels exerted no significant impacts on the detection of CYA because they have completely different retention times. Compared to previous CYA detection methods, which also employed IC columns (Debowski and Gerber, 1993; Koebel and Elsener, 1995; Wang et al., 2010), this study detected not only CYA but also FC as well as other anions, therefore enabling reliable detection of both CYA and FC.

However, the elution time of chloride was identical to that of FC and they were coeluted under the separation conditions tested here. Fortunately, the present method uses two detectors and these two analytes can be distinguished according to their optical absorption differences. Specifically, FC has both UV and CM signals whereas chloride has only CM response, thus FC can be measured by UV detector, while the levels of chloride can be indirectly obtained by the differences between FC and the sum of chloride and FC using CM signals. Figure S2 shows the calibration curves of FC and chloride with correlation coefficient of determination ($R^2$) over 0.97, indicating that both FC and chloride can be measured with high confidence. Compared to earlier literatures, although IC was used to analyze CYA too before (Koebel and Elsener, 1995; Wang et al., 2010), this study is the first study in reporting detections of CYA, FC, and chloride together in just one run.

3.1.2. Impact of water pH

The effects of water pH value on the CYA measurement in the present method and conventional one were investigated by varying the pH values in the range of 1.8–12.4. As depicted in Fig. 2, the signals did not change significantly for the IC method except for a slight decrease from pH 11.8 to 12.4, which was likely caused by insufficient suppression of the KOH eluent and consequently elevated baseline noise. In contrast, the detection signals at pH 11.8 or above measured by the colorimetric method were much lower than those measured at low pH values, suggesting that the MEL reagent is unstable to be used in highly alkaline solution. Thus, the proposed IC method outperformed the conventional colorimetric method in CYA detection under strongly alkaline condition.

3.2. Method assessment

3.2.1. Eluent flowrate and concentration

In order to better understand the method, we evaluated the effects of typical influencing factors on the elution time, peak width, height, and area of CYA, FC, and nitrate, where nitrate was used as a reference compound. As shown in Figure S3a, the peak areas of CYA and FC generally increased whereas the peak heights remained constant when eluent flowrate decreased from 1.0 to 0.6 mL/min, suggesting that a lower MDL might be obtained at lower eluent flowrates. However, lower eluent flowrates led to longer elution time and lower sample throughput.

Fig. 1. A depiction of IC chromatograms for the detection of CYA, FC, and coexisting anions in one sample by (a) UV detector and (b) CM detector.

Fig. 2. A comparison of measured CYA concentrations between IC and colorimetry methods under varying pH conditions.
3.2.2. UV detection wavelength

In literatures, four UV wavelengths (i.e., 190, 205, 213, and 225 nm) have been used before for CYA detection in either IC or LC methods (Downes et al., 1984; Guenn and Hennion, 1994; Koebel and Elsener, 1995; Cantú et al., 2000). In order to identify the optimal detection wavelength for the present method, we compared the molar absorptivities of H\textsubscript{2}Cy at various wavelengths from 200 to 260 nm (Figure S1a). The choice of H\textsubscript{2}Cy is because HCy\textsuperscript{−} becomes H\textsubscript{2}Cy after eluent suppression. Given that the molar absorptivities of H\textsubscript{2}Cy generally decrease with increasing wavelength (Sancier et al., 1964), a shorter wavelength is expected to yield stronger UV signals. However, the baseline noise increases at shorter UV wavelength as well. E.g., the signal to noise ratio (S/N) for 0.01 mg/L CYA at 213 nm is 52, while it reduces to be 30 at 205 nm. So, 213 nm was preferred for detecting sub-μg/L level of CYA although the absolute signal is smaller at this wavelength. Meanwhile, Figure S1b shows the molar absorptivities of HOCl. Although HOCl has a characteristic wavelength at 235 nm (i.e., 82 L mol\textsuperscript{−}1 cm\textsuperscript{−}1) is lower than that at 213 nm (i.e., 87 L mol\textsuperscript{−}1 cm\textsuperscript{−}1). Therefore, based upon these trade-offs, we finally chose 213 nm as the detection wavelength to detect both CYA and FC.

3.2.3. The stability of FC

To clarify a potential concern toward the vulnerability of FC in strong alkaline solution at room temperature (i.e., about 25°C), Figure S4a shows the spectra of a solution dosed with 10 mg/L of sodium hypochlorite over time under a pH of 12.3. Although the initial peak of ClO\textsuperscript{−} at its characteristic wavelength 292 nm (Furman and Margerum, 1998) increased from pH 9.4 to 12.3, which is likely due to the extra absorbance of UV caused by elevated hydroxide ion, the absorbance maintained consistent (< 5%) within 40 min. This confirms that ClO\textsuperscript{−} is stable under pH of 12.3. In addition, the concentrations of FC released from TCCA (10 mg/L) in pH 12.3 solution were also measured by conventional DPD method, which exhibited little changes too within 40 min (Figure S4b); so, it confirms that FC is stable under alkaline condition.

Therefore, IC can measure CYA and FC together without concerning about their susceptibility in alkaline system.

3.2.4. Calibration curves

Herein we developed some calibration curves for CYA and FC across three orders of magnitude (Fig. 3). In general, good calibrations (R\textsuperscript{2} > 0.986 for CYA and R\textsuperscript{2} > 0.998 for FC) were obtained for a wide concentration range (0.01–10 mg/L) with the overall slope being 400,105 for CYA and 35,451 for FC. However, when the concentrations were divided into three sections, the slopes for CYA decreased gradually from 1,026,052 at 0.01–0.1 mg/L range to 682,103 at 0.1–1 mg/L and to 354,711 at 1–10 mg/L range. The decreasing trend suggests that there is a risk of overestimation of samples containing trace levels of CYA if a calibration curve developed for high level of CYA is used. Likewise, the slopes of FC decreased with increasing concentrations of FC too. Therefore, it is recommended to establish specific calibration curves for each of concentration range.

3.2.5. MDLs

According to the USEPA method (USEPA, 2016), the MDLs of CYA and FC were determined to be 3.6 μg/L (S/N = 5.2) and 9.0 μg/L (S/N = 6.6), respectively. In principle, the MDLs were calculated by the product of the standard deviation of at least seven replicate samples and a statistical coefficient. Figure S5a and Figure S5b demonstrates the chromatograms of 6 μg/L CYA and 20 μg/L FC, respectively, which have peaks clearly higher than the eluent baseline noises, confirming that their MDLs are indeed lower than these concentrations. The MDL of CYA is three orders of magnitude lower than the MDLs reported by conventional colorimetry method (i.e., 1.5 mg/L (Downes et al., 1984)) and one order of magnitude smaller than that reported by previous IC methods (i.e., ≥ 0.083 mg/L (Debowksi and Gerber, 1993; Koebel and Elsener, 1995; Wang et al., 2010)). The MDL of FC is around one order of magnitude lower than that of DPD method (i.e., 0.1 mg/L). Therefore, this method features a clear advantage in detecting lower levels of CYA and FC, which may help better understand and monitor the fate and transport of them in the environment.

3.2.6. Recoveries

The method’s recoveries were examined by using a set of waters spiked with varying amounts of CYA and FC. Table 2 shows that the IC method obtained 88–109% recoveries of CYA and 87–112% recoveries of FC from tested waters spiked with 0.08, 0.1, 0.5, or 1.0 mg/L CYA and...
3.3. Real sample analysis and comparison

3.3.1. Tap water

According to the Chinese National Standard of drinking water (GB 5749-2006), the level of FC in finished drinking water at treatment plant discharging point should be maintained between 0.3 and 4.0 mg/L and meanwhile the FC in the end of distribution system (i.e., tap of user) should be kept ≥ 0.05 mg/L. The requirement is designed to avoid pathogens proliferation in pipeline and to reduce disinfection byproduct formation (Yang et al., 2018) and skin and mucous membrane irritation which might cause respiratory diseases (Slaughter et al., 2019). Herein, a few faucet samples were chosen to test the levels of FC for methods comparison (Table 2). The results indicate that the selected two tap waters have FC around 0.15 mg/L, which meets the rules well. The FC levels detected by IC were similar to the results measured by colorimetry method (0.14 mg/L), indicating that the two methods have comparable analytical accuracy. However, in terms of analytical precision, the IC method (RSD = 7.8%) was better than the colorimetric method (RSD = 10.6%).

3.3.2. Swimming pool water

CCAs are widely used in swimming pools; so, we selected some samples from four swimming pools for method comparison. Results show that both analytical methods identified similar levels of CYA and FC, with the differences between them being less than 5.0% (Table 2), because the amount of CYA and FC are all above their MDLs.
3.3.3. Wastewater effluent

Given that swimming pool water is ultimately discharged to wastewater treatment plant (WWTP), we also measured a few samples in a discharging point of a WWTP. The new analytical method found that the plant effluent has around 0.23 mg/L of CYA but little FC, while the chromatographic analysis also detect CYA more intensively and extensively in the environment. In this study, we did not find any CYA in two natural lake water and two rainwater samples, meaning that they are not polluted by anthropogenic activities. However, the study detected 44 and 34 μg/L of CYA in two sampling points of a local river (Table 2), suggesting that this watershed has been polluted somehow by human activities as a result of using CCAs. The IC chromatogram of the real river water sample containing CYA is presented in Figure S7, and it can be seen that the chromatographic peak of CYA was well separated from other coexisting compounds. As conventional colorimetric cannot capture such phenomenon whereas IC method can, it proves the superiority of the new method.

3.3.4. Environmental samples

In case CCAs are released into the environment, CYA is likely to persist in environmental samples. In this study, we did not find any CYA in two natural lake water and two rainwater samples, meaning that they are not polluted by anthropogenic activities. However, the study detected 44 and 34 μg/L of CYA in two sampling points of a local river (Table 2), suggesting that this watershed has been polluted somehow by human activities as a result of using CCAs. The IC chromatogram of the real river water sample containing CYA is presented in Figure S7, and it can be seen that the chromatographic peak of CYA was well separated from other coexisting compounds. As conventional colorimetric cannot capture such phenomenon whereas IC method can, it proves the superiority of the new method.

4. Conclusions

Overall, this study proposed and verified a robust and reaction-free method for detection of CYA and FC together in water. The method showed much lower MDLs (3.6 μg/L for CYA) than conventional ones. The recoveries from synthetic and natural water samples ranged from 88 to 109% for CYA and 87–112% for FC, with the average RSDs (9.8%) being lower than colorimetric methods (10.6%). In addition, one clear advantage of IC method over conventional colorimetric method is its resistance to high pH, thus allowing more reliable measurement of CYA, FC, and other CCA derivatives. With the new method, this study found 34–44 μg/L of CYA in a local river water, suggesting that the uses of CCAs as disinfectants in fight of COVID-19 have accidently released some undesirable chemicals into the environment. Future researches may employ this method to survey the occurrence, fate, and transport of CYA more intensively and extensively in the environment.

Author contributions statement

Yiya Wei: Writing – original draft, Formal analysis, Investigation, Data curation, Visualization. Yang Yang: Data curation, Validation. Baiyang Chen: Conceptualization, Writing – reviewing and editing, Funding acquisition, Supervision. Bingcheng Yang: Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chemosphere.2021.133378.

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