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An HPLC Method with UV Detection, pH Control, and Reductive Ascorbic Acid for Cyanuric Acid Analysis in Water

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Every year over 250 million pounds of cyanuric acid (CA) and chlorinated isocyanurates are produced industrially. These compounds are standard ingredients in formulations for household bleaches, industrial cleansers, dishwasher compounds, general sanitizers, and chlorine stabilizers. The method developed for CA using high-performance liquid chromatography (HPLC) with UV detection simplifies and optimizes certain parameters of previous methodologies by effective pH control of the eluent (95% phosphate buffer: 5% methanol, v/v) to the narrow pH range of 7.2−7.4. UV detection was set at the optimum wavelength of 213 nm where the cyanuric ion absorbs strongly. Analysis at the lower pH range of 6.8−7.1 proved inadequate due to CA keto−enol tautomerism, while at pHs of <6.8 there were substantial losses in analytical sensitivity. In contrast, pHs of >7.4 proved more sensitive but their use was rejected because of CA elution at the chromatographic void volume and due to chemical interferences. The complex equilibria of chlorinated isocyanurates and associated species were suppressed by using reductive ascorbic acid to restrict the products to CA. UV, HPLC−UV, and electrospray ionization mass spectrometry techniques were combined to monitor the reactive chlorinated isocyanurates and to support the use of ascorbic acid. The resulting method is reproducible and measures CA in the 0.5−125 mg/L linear concentration range with a method detection limit of 0.05 mg/L in water.

Cyanuric acid (CA) and chlorinated isocyanurates are added as standard ingredients in formulations for household bleaches, institutional and industrial cleansers, automatic dishwasher compounds, and chlorine stabilizers, which are very well known for preventing the total photolytic decomposition of chlorine disinfectants.[1−5] Most of the CA produced in industry is converted chemically into its chlorinated derivatives.[1] In addition, the chlorinated isocyanurates are registered for use as disinfectants, sanitizers, algaecides, fungicides, fungistats, bactericides, bacteriostats, microbiocides, and microbistats.[6,7] The worldwide production of chlorinated isocyanurates in 1996 was estimated to be 292 million pounds (U.S. 195,[8] Japan 52,[8] and Western Europe 45[8]). These wide applications and large chemical production volume demand practical methodology to ensure these compounds are used without posing undue hazard to human health or the environment.

Cyanuric acid occurs as a mixture of keto (2,4,6-trioxo-*s*-triazine) and enol (2,4,6-trihydroxy-*s*-triazine) tautomers in solution (Figure 1, structures **1** and **2**, respectively).[10−12] In alkaline solution, cyanuric acid exists in the enolic form, while in acidic solutions, the keto form is more stable.[12] The keto tautomer **1** is usually called isocyanuric acid. Cyanuric acid can undergo reaction at either *O*- or *N*-triazine substituents depending upon its environment.[13] The dichloro (DCCA, **4**) and trichloro (TCCA, **5**) isocyanurates are produced by stoichiometric reaction of CA, NaOH, and chlorine gas in solution. Monochloroisocyanurate (**3**) is not readily available because of its rapid disproportionation to CA and DCCA. A considerable number of techniques such as titrimetry,[4,14] turbidimetry,[15] colorimetry,[16,17] pulse polarography,[18] gas chromatography (GC),[19] high-performance liquid chromatography (HPLC),[15,20−33] and commercial devices[16,17,34] have been developed

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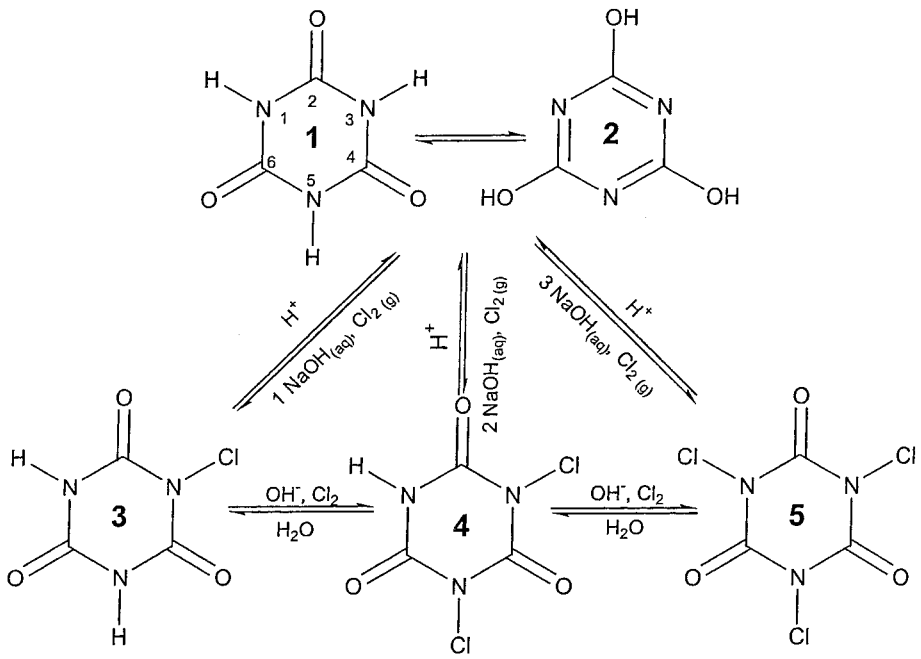

Figure 1. Chemical structures of keto (**1**) and enol (**2**) tautomers of cyanuric acid and synthetic routes to their monochloro- (**3**), dichloro- (**4**), and trichloro- (**5**) isocyanurate derivatives.

for CA analysis. Titration of CA as a monobasic acid is restrictive because of interferences from other weak acids during end point determinations. Application of potentiometry, amperometry, and pulse polarographic techniques is frequently difficult, requiring complex electrochemical methods with special electrode arrangements, cell designs, supporting electrolytes, and organic solvent requirements. Turbidimetry suffers from coprecipitation of interferences such as ammelide, ammeline, and melamine. GC methods have proved functional, but the derivatization and extraction steps require extra time for the total analysis. Finally, HPLC methods with UV detection have been the most popular because of their operational selectivity and sensitivity.

Table 1 summarizes HPLC methods for CA. It reveals a wide variety of separation modes, columns, mobile-phase compositions, pHs (1−10), and settings for UV detection (195−226 nm). The lack of agreement in these parameters warrants further examination of the conditions in these methods to extract their useful properties and to define operational conditions for the measurement of CA. Table 1 also includes various detectable levels for comparative purposes.

The present work provides the necessary background regarding existing methodology and presents significant improvements and results for CA measurement. It is composed of five parts. In part I, judicious criteria for selection of the separation phase are applied based upon method preference, rapidity, toxicity, and practicality. In part II, the UV spectrum of CA is discussed with special emphasis on the restrictive keto−enol tautomerism affecting the HPLC analysis of CA. In part III, the UV and HPLC−UV techniques are combined to monitor the sensitivity and retention of CA in the pH ranges of 1.94−7.41 (acidic) and 7.44−10.06 (basic). The sensitivity trends are established by comparison of the UV absorption peaks and integrated peak areas from the HPLC. The retention trends are established with measured capacity factors, *k*'s. Parts II and III delineate optimum conditions for CA analysis. In part IV, the method is expanded to include DCCA and TCCA whose reactivities in water were monitored using HPLC−UV and electrospray ionization mass spectrometry (ESI MS) techniques. Cyanuric acid calibration curves were prepared for quantitative analyses and the method detection limit (MDL) was calculated. Finally, part V examines several reductive dechlorinators in regard to their spectroscopic and separation compatibility to CA.

The HPLC method developed has been adapted to demonstrate its ruggedness in the analysis of real-world water samples during field studies[9] and extended to the analysis of CA in biological body fluids for exposure studies.[9] Common matrix interferences in water were found to be strong absorbing inorganic anions such as nitrates, phosphates, sulfates, chlorates, etc., while body fluids contained additional interferences such as proteins and nonpolar

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Table 1. HPLC Methods for Cyanuric Acid Analysis

mode	column	mobile-phase composition (v/v, %)	pH	UV	detectability (mg/L)	refs
normal phase	amino	78/22 $CH_3CN/KH_2PO_4-Na_2HPO_4$	6.7	214	0.02	27
reversed phase	octadecyl	99/1 H_2O/H_3PO_4	1	195	0.25	31
		acidic H_2O	3	205	4, 0.005	32,33
		95/5 Na_2HPO_4/CH_3OH	7.0	213	0.1	28
		95/5 Na_2HPO_4/CH_3OH	7.0	225	0.1	20−22, 29
		95/5 H_2O/CH_3CN	n/a	226	5	25
	graphite	70/30 sodium phosphate/CH_3OH	7	220	0.003	32−33
		$KH_2PO_4/H_3PO_4/CH_3OH$	7.7	215	10	23
ion pair	octadecyl	didodecyldimethylammonium bromide in phosphate buffer	7.7	220	n/a[a]	26
		didodecyldimethylammonium bromide in 50/50 H_2O/CH_3OH		n/a	13	30
ion exchange	strong anion exchange	tris(hydroxymethyl) methylamine sulfate	7.8	215	5	15
		K_2HPO_4/KOH	10	210	40[b]	24

[a] n/a, not available. [b] Obtained from HPLC and thermal gravimetric analysis, after heating 100.0 g of urea initially present.

compounds. The effective application of the method and sample cleanup procedures will be described elsewhere.[9]

EXPERIMENTAL SECTION

Reagents. Cyanuric acid and chlorinated isocyanurate salts were purchased from Aldrich Chemical (Milwaukee, WI). Stock solutions of 530 mg/L CA (98%), 520 mg/L DCCA, sodium salt (96%), and 535 mg/L TCCA (97%) were prepared by dissolving the solid in deionized water with stirring and gentle heating. All of the solutions were passed through a 0.45-μm cellulose filter. The standard solutions were prepared in the 0.5−125.0 mg/L concentration range in phosphate buffer. Their concentrations were corrected to reagent purity specifications. The phosphate buffers contained 0.005 M monobasic potassium phosphate (KH_2PO_4) and 0.0125 M dibasic potassium phosphate (K_2HPO_4). A minimum of either acid or conjugate base solid was added to achieve the exact pH within 1 pH unit of the buffer capacity. Gradual changes in pH to acidic or alkaline conditions were carried out by dropwise addition of either 1 M H_2SO_4 or 1 N NaOH. The pH was measured using a pH Field System (Thomas Scientific, Swedesboro, NJ) with a 0.01 pH resolution over the pH range 0−14. Ascorbic acid (99+%) and sodium thiosulfate (99%) were obtained from Aldrich Chemical and sodium sulfite (98.9%) was obtained from Fisher Scientific (Pittsburgh, PA).

HPLC System and Conditions. The HPLC was performed with a model 200B liquid chromatograph (Bioanalytical Systems, W. Lafayette, IN) equipped with a Rheodyne (Cotati, CA) model 7125 injector employing a 20-μL sample loop and a built-in single-channel UV−visible variable-wavelength detector. The UV detector was set at the wavelength of 213 nm.

A 25 cm × 0.46 cm C_{18} Luna column end capped (Phenomenex, Torrance, CA) with 5-μm particle size was used. The stability of the column was monitored by measuring the number of plates (N) of the CA peak over a 2-month period. Initial N were 11 600 ± 500 and subsequent usages after 2 months yielded N = 8200 ± 600. The plates were calculated from $N = 5.55\ t_r^2/w^2_{1/2}$, where t_r is retention time of CA and $w_{1/2}$ is the width at half-height of the CA peak.

The HPLC was carried out under isocratic elution conditions. Other parameters included a flow rate of 1 mL/min and a data acquisition rate of 150 points/min. The solvents were purged using high-purity helium gas (99.9%, Murray Hill, NJ). Twenty column volumes were passed through the column for equilibration. Each sample was injected three to five times to monitor reproducibility. Peak areas from the chromatograms were computed using Chromgraph Report version 1.2 (BAS). Final figures were displayed using Igor pro software version 3.14 (WaveMetrics, Inc., Portland, OR).

Electronic Spectra. Using a Beckman DU-640 spectrophotometer, the absorption spectra of solution samples were collected with a special 1-cm quartz cell. The blank solution and sample solutions were adjusted to the same pH. The resolution of the diode array detector was set at 0.1 nm. Multiple scans (3−15) were averaged to improve the signal-to-noise ratio.

FIA-ESI MS. A Finnigan LCQ DECA (San Jose, CA) ion trap mass spectrometer was calibrated in the positive ion ESI mode using an Ultramark 1621MRFA/caffeine/mixture and used in the negative ion ESI mode. The heated capillary (350 °C), nitrogen sheath gas (80 arbitrary units, AU), and nitrogen auxiliary gas (30 AU) were optimized, in the negative ion ESI mode, using a solution of 20 mg/L benzoic acid infused at 0.8 mL/min in 95:5 water/methanol (%, v/v). A Hewlett-Packard 1090 liquid chromatograph (Agilent Technology, Wilmington, DE) was used for the flow injection analysis (FIA). In ESI MS, the instrument was scanned from 110 to 300 amu with ESI needle voltage at −4 kV. The samples at pH 7.3 were analyzed by FIA, using a 50-μL injection loop into a 0.8 mL/min flow of 95:5 water/methanol (%, v/v).

RESULTS AND DISCUSSION

HPLC Method Selection and Preliminary Experiments. Careful analysis of Table 1 reveals a preference for reversed-phase separation mode in the HPLC analysis of CA. The high polarity and hydrophilic characteristics of CA[32] have been advantageous in obtaining its rapid detection in reversed-phase liquid chromatography (RPLC). Weak hydrophobic interaction between the nonpolar octadecyl silica and the CA chromophore results in its early elution from the analytical column. Elution at the void volume has been prevented by maximizing water in the composition of the mobile phase. The combination of the octadecyl silica column and high water content mobile phases promotes maximum retention in RPLC. Also, the analysis is relatively nontoxic, using ≥95% phosphate buffer and ≤5% methanol (v/v), which are not a serious concern in analytical laboratories.

Normal-phase liquid chromatography is not widely used due to the limited solubility of CA in organic solvents. Cyanuric acid exhibits high solubility in solvents such as dimethyl sulfoxide, dimethyl formamide, and 1,4-dioxane. However, the first two solvents can be ruled out for CA analysis because of their high UV cutoffs of 268 nm[35] and toxicity. Even though dioxane has a UV cutoff of 215 nm,[35] it is highly toxic and it is not a good solvent choice versus the popular aqueous buffers used in RPLC.

Although ion pair chromatography appears appealing due to rapid column equilibration with terbutylamine,[36] its toxicity is very high.[37] Additionally, the pH of the eluent cannot be adjusted above the pH of 8 because tertiary butylamine can undergo base-catalyzed oxidation to the corresponding *N*-oxide.[36] Alternative and less toxic ion pair reagents consisting of long-chain tetraalkylammonium salts require long column equilibration times of several hours.[26] Ion exchange chromatography is not widely used because it can give complicated chromatograms since the ionic CA has to compete with other aqueous anions such as halides, chlorides, hydroxyl, and nitrate. This is important because the nitrate ion may be present in potable water at concentrations of up to 50 ppm.[38] After reviewing Table 1, RPLC was chosen as the separation technique for developing the method for CA.

Figure 2a shows the chromatogram of a 10 mg/L CA standard solution. The initial HPLC conditions employed an octadecyl column and a 95:5 phosphate buffer/methanol (%,v/v) eluent.

The pH of the buffer was ~6.94 while the detector was fixed at 213 nm. A theoretical acid−ion pair distribution diagram was constructed using CA's first acid dissociation constant, $pK_{a1} = 6.88$,[1,4,39] and the equilibrium expression $K_{a1} = [H^+][CA^{-1}]/[CA]$. It is shown in Figure 2c and proved useful in this work. The use of a high ionic strength buffer is assumed to give a constant ionic medium, minimizing any influences from the activity coefficients. The chromatogram in Figure 2a shows a striking splitting of the CA peak, not previously reported in any of the methods from Table 1. This scenario is certainly a serious limitation for the qualitative and quantitative analyses of CA where single peak identification and symmetric peak area calculations are needed for accuracy. As will be shown, this limitation was suppressed by effectively controlling the pH of the mobile phase. However, prior to establishing the effective pH(s), the pair of electronic transitions (Figure 2b) exhibited by CA in solution were analyzed and the most sensitive was chosen for UV detection in HPLC.

Electronic Properties of Cyanuric Acid and Their Implication in Sensitivity and Retention in HPLC. Figure 2b shows a typical UV spectrum of a CA solution at the pH ~7. The spectrum consist mainly of two absorption peaks, one near 200 nm, while the other one is at 213 nm. The intensity of the first absorption peak has been explained from $\pi-\pi^*$ transitions which originate through the p overlap of C═O groups (keto tautomer) via the nonbonding electrons of nitrogen,[10] while the intensity of the second absorption peak has been assigned to $n-\pi^*$ transitions

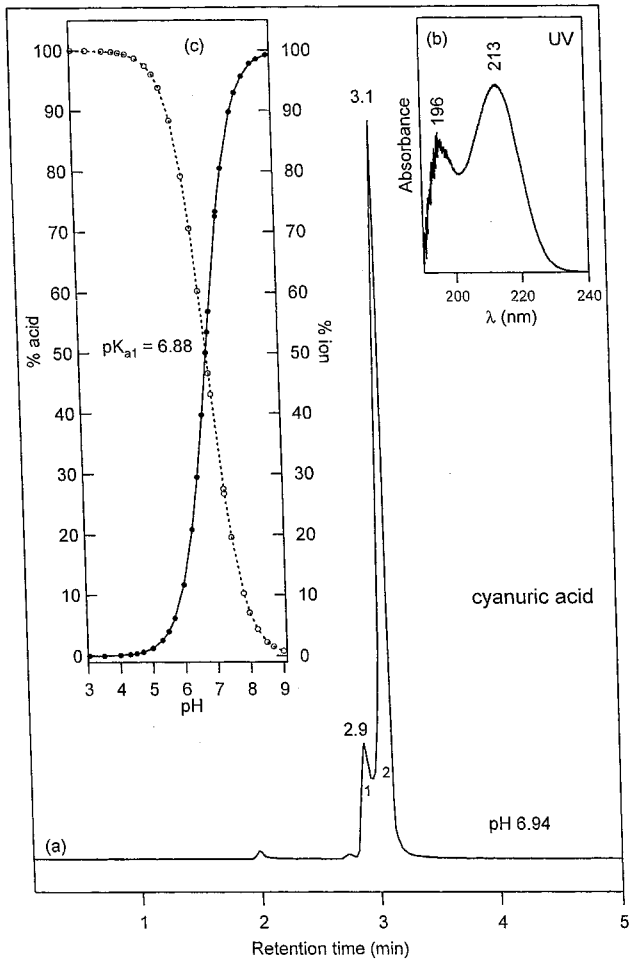

Figure 2. (a) HPLC, (b) UV spectrum, and (c) ion distribution diagram of cyanuric acid in solution.

of nonbonding electrons residing on the triazine nitrogens (enol form).[10] The molar absorptivity coefficient (ϵ) measures the strength of the chromophore and it is directly responsible for the observed sensitivity using a UV−visible detector.[40] O'Brien[4,39] measured ϵ values for the neutral CA (keto form) and ionic CA (enol form) which showed that the ionic species is a stronger absorber of UV light, as evidenced by its molar absorptivity of 8.80×10^3 M^{-1} cm^{-1} at $\lambda_{213(max)}$ compared to 1.83×10^3 M^{-1} cm^{-1} at $\lambda_{196(max)}$ for the acidic keto tautomer. Assignment of the ionic CA (peak 1) and the nondissociated CA (peak 2) in Figure 2a does not agree with the ϵ's using retention arguments in RPLC.[10−13] There appears to be some uncertainty as to which form predominates in solution to represent the correct structure of CA at the pH ~7. The coexistence of both keto and enol tautomers in solution has been suggested.[4,10−13]

Ten of the 12 methods in Table 1 have chosen $\lambda \geq 210$ nm to enhance the sensitivity of CA derived from the free resonance conjugation of the enolate form since $\epsilon_{213} \gg \epsilon_{196}$. However, Schöler and co-workers[31] selected UV detection at 195 nm using a buffer at the pH ~1. They reported additional retention of the CA peak near 5 min compared to the tautomeric peak near 3 min shown earlier in Figure 2a. A very acidic pH of the eluent can implicate a drastic shift in the equilibrium of CA in solution. The equilibrium

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Table 2. pH Control Experiments and Cyanuric Acid Peak Characteristics Using HPLC−UV[a]

acidic pH	k'	peak area (%)	basic pH	k'	peak area (%)
6.88 ± 0.01[b]	0.346 ± 0.002[b]	40325 ± 2[b]	7.13 ± 0.01	0.284 ± 0.020	50871 ± 6
6.03 ± 0.01	0.408 ± 0.004	14315 ± 2	7.41 ± 0.01	0.217 ± 0.001	56957 ± 2
4.83 ± 0.01	0.471 ± 0.003	2921 ± 12	7.44 ± 0.01	0.223 ± 0.003	60245 ± 2
3.88 ± 0.01	0.468 ± 0.002	1344 ± 11	8.07 ± 0.01	0.134 ± 0.005	69449 ± 3
2.97 ± 0.01	0.473 ± 0.008	1045 ± 14	9.08 ± 0.01	0.097 ± 0.001	76336 ± 5
1.94 ± 0.01	0.478[c]	1123 ± 12	10.06 ± 0.01	0.057 ± 0.000	75559 ± 2

[a] Data collected from HPLC with a 10 mg/L cyanuric acid solution. [b] Standard deviation for pH ($n = 5$), k's ($n = 3$), and peak areas ($n = 5$) with UV detection at 213 nm. [c] K' from UV detection at 196 nm due to severe sloping.

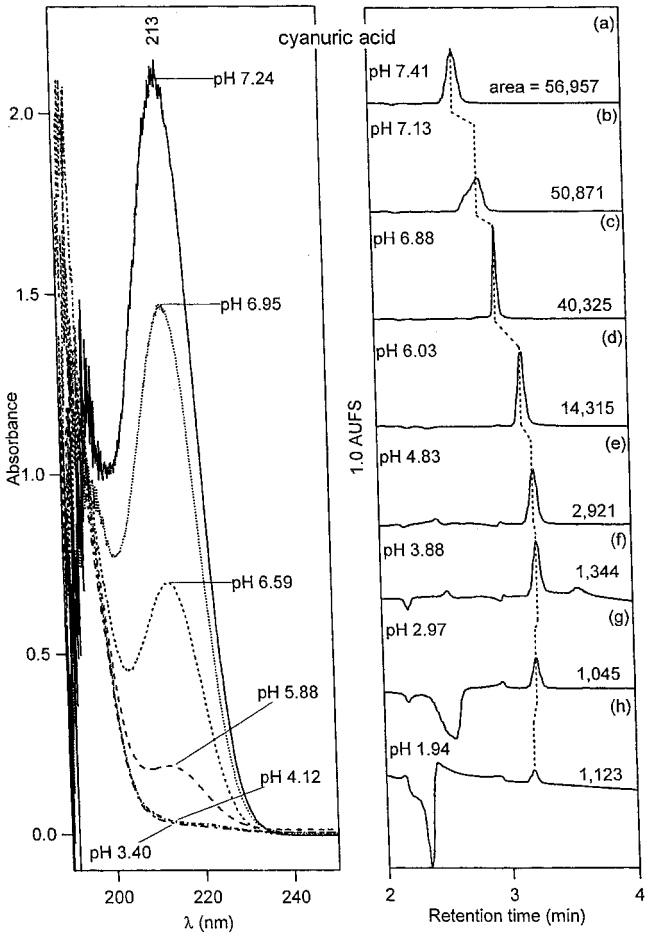

Figure 3. Acidic influences on the UV spectrum of a 40 mg/L cyanuric acid solution (left panel). Acidic effects on the HPLC of a 10 mg/L cyanuric acid solution (right panel).

can be driven from the enolic tautomer (most stable at neutral and alkaline pHs) to the keto tautomer (stabilized in acidic pH). This results in the less polar keto isocyanuric acid receiving more retention benefits than the more polar enol cyanuric acid using the nonpolar octadecyl column. Although this strategy proves more effective in retaining CA, it is predicted to lack sensitivity since $\epsilon_{213} \gg \epsilon_{196}$. Also, the strong interference of molecular oxygen near 200 nm can occur at their detection wavelength (Figure 3, left panel). Low sensitivity can also occur from using a wavelength distant enough from the optimum 213 nm. For example, Ghiorghis and Talebian[25] carried out HPLC with UV detection at 226 nm and reported a detection limit of 5 mg/L.

Electronic Spectra of Cyanuric Acid with pH Control Experiments in HPLC. *Electronic Spectra and Acid Influences in HPLC.* Figure 3 (left panel) shows the UV spectrum of a 40 mg/L CA solution at pH 7.24 (solid line) and the effects of lowering solution pH on the intensity of the 213-nm absorption peak. As the pH is lowered from 7.24 to 6.95, there is the first drastic loss in intensity of this absorption attributed to the reduction of ion content (aromatic nature) in solution (69 to 55%). Further acidification to pH 6.59 perpetuated this trend as the ion content dropped to 33%. Adjustments to pH 5.88 (9%) and 4.12 (0.2%) resulted in additional intensity reduction. At pH 3.40 (0.03%), the isocyanuric ion peak has been nearly depleted. These UV solution experiments proved useful for predicting the sensitivity when the HPLC−UV technique was used; however, they are inconclusive in explaining the tautomeric splitting of CA near pH 7 in HPLC (Figure 2a). Assignment of structures to these CA peaks is complicated by the existence of the strong resonance of both keto and enol tautomers. CA (keto form) with a six-membered cyclic nonaromatic ring can be converted to cyanuric ion (enol form) with alternative resonating double bond aromatic structures. This electromeric polarization is induced in alkaline solution at pHs greater than 7. Experiments are in progress to explain this behavior.

The HPLC of a 10 mg/L CA standard solution is shown in Figure 3 (right panel). The UV detection was set at 213 nm and the pH of the mobile phase ranged from 1.94 to 7.41. The k's and integrated peak areas with their relative standard deviations are included in Table 2. A pH of 7.41 yielded good sensitivity (Figure 3a, area 56 957) but it provided the lowest retention ($k' = 0.217$). As the pH was lowered to 7.13 (Figure 3b), the peak symmetry became complicated, possibly from keto−enol CA tautomerism. These influences are suppressed to a larger extent in Figure 3c where the first peak is nearly gone. Single peak detection is observed at pH 6.03 (Figure 3d) and other acidic conditions (Figure 3e−h). A pH of 4.83 exhibits additional retention gains with $k' = 0.471$ but with the continual loss of peak area (Figure 3e, area 2921). The chromatogram at pH 3.88 (Figure 3f) begins to display void volume features, which become very dominant at pH 2.97 (Figure 3g) and 1.94 (Figure 3h). None of these lower pHs demonstrated any more retention as the k's were all in the range of 0.468−0.478 with very low sensitivity. The sensitivity of CA (Figure 3h, area 1123) is lowered drastically at the benefit of increased retention ($k' = 0.408$). The lack of reproducibility in peak areas should be noted by their relative standard deviations that are above 10% at pHs of <5 (Table 2).

Electronic Spectra and Basic Influences in HPLC. Figure 4 (left panel) shows a UV spectrum of a 25 mg/L CA solution at pH 6.98 (bottom, dotted line) and the effects of increasing solution

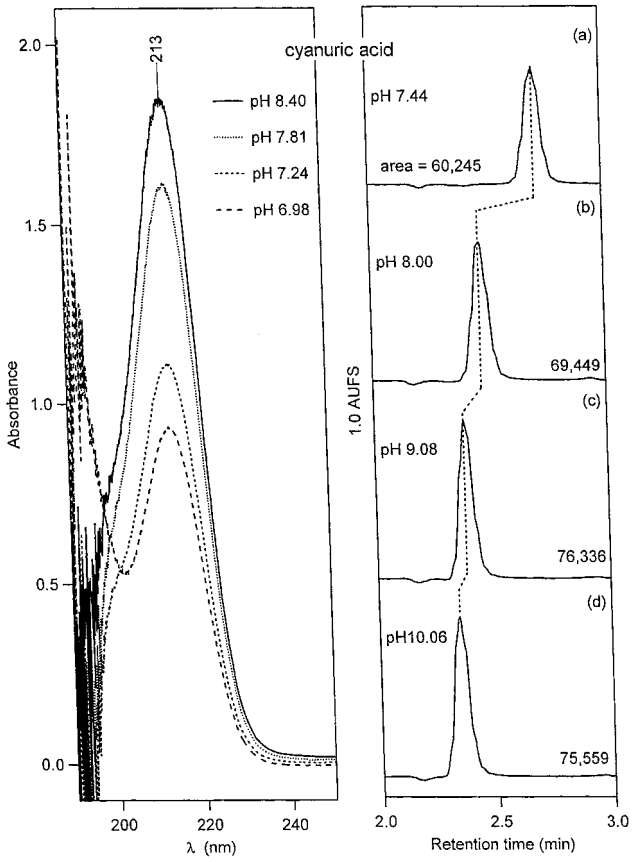

Figure 4. Basic influences on the UV spectrum of a 25 mg/L cyanuric acid solution (left panel). Basic effects on the HPLC of a 10 mg/L cyanuric acid solution (right panel).

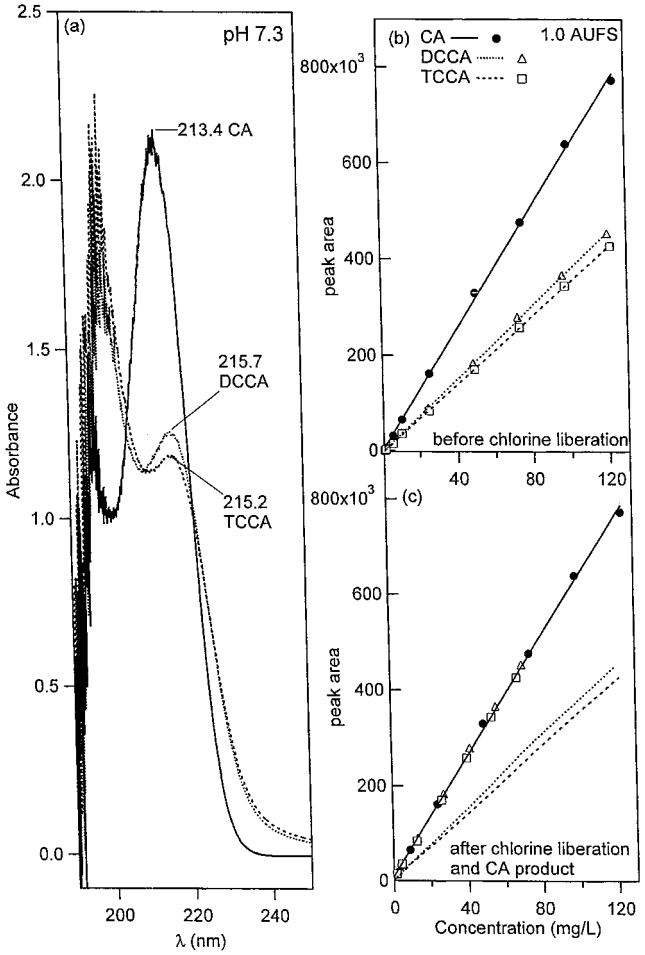

Figure 5. (a) UV solution spectra of 40 mg/L standard solutions of cyanuric acid, dichloro-, and trichloroisocyanurates in water. Standard calibration curves (b) before and (c) after release of residual chlorine.

pH to alkaline conditions on the intensity of the 213-nm electronic band. The CA peak at pH 6.98 corresponds to 65% ionized isocyanurate in solution and exhibits increased intensity as the pH is changed slightly to 7.24, promoting further ionization (79%). Enhancement is maximized by promoting additional ionization of CA when the pH is adjusted to 7.81 (90%) and 8.40 (97%) as judged by the intensity gain of the absorption peak. Note, four methods in Table 1 used alkaline eluents at pH 7.7 and 7.8, which correspond to 87 and 89% CA ionization, respectively (Figure 2c). Three of these methods involved ion-exchange separation mechanisms while the fourth was carried out in RPLC using a graphitic column (Table 1). UV detection was in the 215−220-nm range. Wavelengths above the optimum 213 nm were possibly chosen to avoid matrix influences or in anticipation of the second acid dissociation of CA, which has been reported to produce a bathochromic shift of the CA peak.[39] In the absence of interferences, the UV spectrum of CA in phosphate buffer revealed no such influences up to pH 8.40 (Figure 4, left panel). However, the CA peak shifted to 215 nm in the presence of hypochlorite ion (discussed in section Reactivity of Dichloro- and Trichloroisocyanuric Acids and shown in Figure 5a).

The pHs of 7.7 and 7.8 delineate the limit for ion-exchange methods for CA analysis because more alkaline eluents can catalyze ion pair reagents[36] and interfere with UV detection by the presence of the strong absorption of the hydroxyl ion. Careful examination of Table 1 shows only one method with the mobile phase at the pH of 10. When solutions of NaOH were analyzed using UV detection, a strong absorption at 192 nm for the hydroxyl ion was observed at pH 10. This absorption became more pronounced and shifted to give a broad and asymmetric peak at 195, 205, and 213 nm at pHs 11, 12, and 13, respectively (not shown). HPLC was difficult using eluents at these pHs. Such high-alkaline pHs are to be avoided because alkylation of CA can take place to form *O*-alkyl derivatives[13] and ring rupture of the triazine structure has been reported to occur under alkaline conditions.[41,42] Also, hypochlorite ion, commonly found in bleach, is known to cleave the triazine ring forming N_2 and HCO_3^{1-}.[43,44] Therefore, the useful pH range is 7.2−9.9. A pH of 7.2 is chosen as the lower limit because of CA tautomerism at 6.8−7.1 (Figures 2a and 3b,c) and losses in analytical sensitivity at pHs of <6.8 resulting from the reduction of ion content in solution.

The HPLC of a 10 mg/L CA standard solution is shown in Figure 4 (right panel). The measurements were conducted with UV detection at 213 nm and the pH of the eluent in the range of 7.44−10.06. The *k*'s and peak areas are included in Table 2. The peak area of CA is increased by adjusting the pH of the eluent from 7.44 (Figure 4a, area 60 245) to the more alkaline pHs of

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Table 3. Optimum HPLC Conditions for Cyanuric Acid Analysis in Water

mode	reversed-phase HPLC
stationary phase	octadecyl C-18 (5-μm particle size)
column dimensions	25 cm (l) × 0.46 cm (w)
mobile phase	95/5 KH_2PO_4−K_2HPO_4 buffer: CH_3OH (%, v/v)
eluent pH range	7.2−7.4
flow rate	1.0 mL/min
temperature	35 °C
UV	213 nm
recommended dechlorinator	ascorbic acid
overall method advantages	rapid practical sensitivity, intermediate retention, reproducibility and low ring structure reactivity

8.00 (Figure 4b, area 69 449), and 9.08 (Figure 4c, area 76 336). At this last pH, 99% of CA has been ionized (Figure 2b for reference) and no further gain in peak area is observed even at higher pHs, e.g., pH 10 (Figure 4d, area 75559). Although a more alkaline eluent at pH 9 favors sensitivity, it has low retention ($k' = 0.097$). This is a disadvantage because many ionic interferences have low k's on reversed-phase columns. Additional retention is gained at pH 8 ($k' = 0.134$) and nearly doubled at pH 7.4 ($k' = 0.223$), providing more retention. An eluent prepared at pH 7.4 is favored over the more alkaline pH of 8 or 9 because of potential dissolution of the silica support and collapse of the column bed over time.[35] The alternative graphitic carbon column is more effective in retaining CA; however, it lacks the higher efficiency (high number of plates) that silica-based columns possess. The carbon column requires longer equilibration time and suffers from higher column back pressures. The conditions for the rapid and simplified HPLC method for the analysis of CA in water are summarized in Table 3.

Reactivity of Dichloro- and Trichloroisocyanuric Acids. Figure 5a shows the UV spectra of 40 mg/L standard solutions of CA, DCCA, and TCCA. The spectra are similar in peak position with only 2-nm red shifts in the DCCA and TCCA. The major difference is peak intensity, which is ∼50% lower for the chlorinated derivatives. HPLC−UV experiments yielded the calibration plots as those shown in Figure 5b. The calibration curves for quantitative analyses were $Y = 6319X + 4031$ (CA), $Y = 3791X + 386$ (DCCA), and $Y = 3527X - 51$ (TCCA) with correlation coefficients $r^2 \geq 0.9999$. The MDL of CA was determined to be 0.05 mg/L according to the equation MDL $= t_{(n-1,1-\alpha=0.99)}$ (S), where $t_{(n-1,1-\alpha=0.99)}$ = the Student's t value appropriate for a 99% confidence level and a standard deviation estimate with $n - 1$ degrees of freedom and S is the standard deviation of seven replicate analyses.[45] Other detectable levels of CA are included in Table 1 for comparative purposes. When either DCCA or TCCA was injected, a peak was observed with the same retention time as CA with no clear indication of unique peak identity, even when eluents at different pHs were used (not shown). Reaction of TCCA and water to produce CA has been suggested previously.[20,21,46] The assumption of stoichiometric reactions of TCCA and DCCA in water implies production of CA and the release of residual chlorine. Figure 5c shows good agreement for the assumed reaction by the good fit the former calibration points from the DCCA and TCCA regressions have on the original CA plot. The new calibration plots were $Y = 6460X + 385$ (former DCCA) and $Y = 6350X - 50$ (former TCCA) with $r^2 \geq 0.9999$, and the same CA plot.

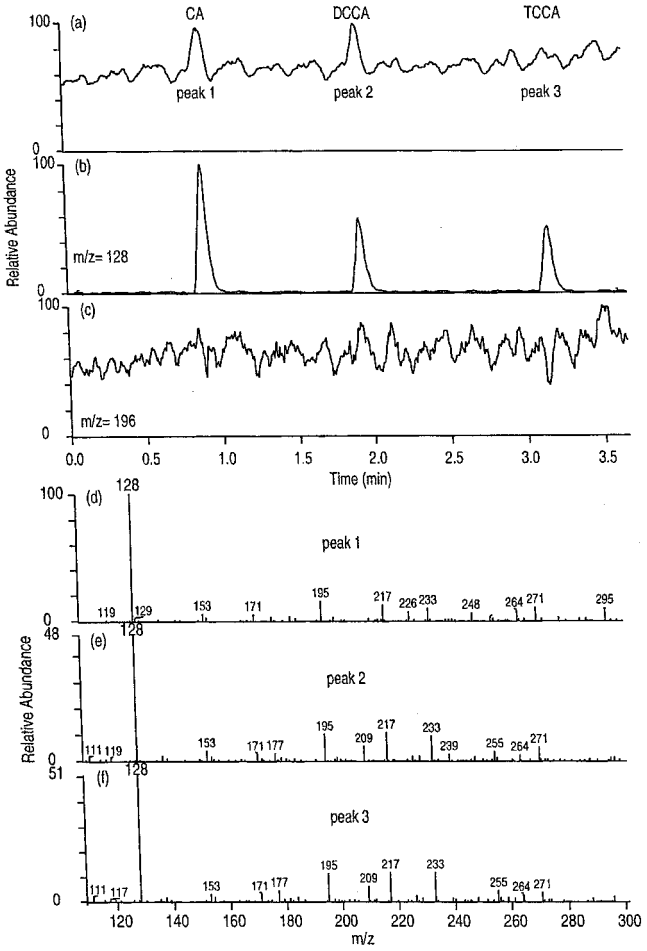

Figure 6. (a) TIC of 5 mg/L solution samples of cyanuric acid (peak 1) and reacted dichloro- (peak 2) and trichloro- (peak 3) isocyanurates in water after 5 min. (b) Selected ion monitoring (SIM) for cyanuric acid ion at m/z 128 and (b) SIM for dichloro- and trichloroisocyanuric ions at m/z 196. (d−f) Mass spectra of peaks 1−3 from TIC.

To add proof to these suggestions, FIA-ESI MS analyses were conducted using solutions of 5 mg/L CA, DCCA, and TCCA. The ESI mass spectra (not shown) of CA (MW 129) and DCCA (monoisotopic MW 197) produced $[M - H]^-$ ions at m/z 128 and 196, respectively. The DCCA also fragmented to give a base peak at m/z 162 $[M - Cl]^-$. In the case of TCCA (monoisotopic MW 231), ESI MS produced a spectrum (not shown) identical to DCCA except that m/z 196 $[M - Cl]^-$is the base peak instead of m/z 162 $[M - 2Cl + H]^-$. Figure 6a shows the total ion chromatogram (TIC) of sequential injections of 5 mg/L solutions of CA, DCCA, and TCCA reacted in water for 30 min at room temperature. Monitoring m/z 128 (CA) in Figure 6b and m/z 196 (DCCA and TCCA) in Figure 6c indicates DCCA and TCCA were converted to CA. The mass spectra of the three peaks, shown in Figure 6d−f, confirm the presence of CA and the absence of DCCA and TCCA.

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Table 4. Long-Term Reproducibility and Stability of Cyanuric Acid in Distilled Water at 9.80 mg/L[a]

analysis date	replicate no. (n)	mean ± SD[b] (mg/L)	% RSD[b]
day 1	5	9.75 ± 0.13	1.3
day 8	5	9.44 ± 0.05	0.5
day 13	3	9.98 ± 0.11	2.0
day 43	5	9.81 ± 0.31	3.2

[a] The standard solution was kept in a cold room at 5 °C at all times except for 12−24 h time intervals when HPLC analyses were conducted and the sample was allowed to equilibrate to room temperature. [b] (R)SD, (relative) standard deviation.

Finally, the reproducibility of the method and the stability of CA proved functional as the relative standard deviations (RSDs) over 43 days for the analysis of a standard solution containing 9.80 mg/L of CA were ≤3.2%, indicating good long-term reproducibility and sample stability (Table 4). The reproducibility is comparable to other methodologies[20] including the method developed by Briggle and co-workers[29] (7.61% RSD) or Ishiwata and others (1.8% RSD).[27]

Dechlorination of Isocyanurates Derivatives. The reactivity of the DCCA and TCCA to become CA was shown in the section Reactivity of Dichloro- and Trichloroisocyanuric Acids. However, water samples containing CA and excess chlorine are ideal for CA and its chlorinated derivatives to act as chlorine reservoirs giving rise to 10 potential species in solution.[5,47−49] This scenario adds complexity to the HPLC analysis because the samples need to be dechlorinated first so the multiple chlorinated isocyanurates are converted to CA. Failure to do this can possibly lead to lack of reproducibility affecting the peak shape and the retention time stability of CA.[31] The dechlorination mechanism must (1) reduce the free available chlorine in the water sample, (2) react with all chlorinated isocyanurates to produce CA and to release additional residual chlorine, and (3) further reduce the excess chlorine formed after reaction. Figure 7 shows the UV spectra of several dechlorinators used in the EPA drinking water methodology.[50] The amount of dechlorination agent should be in excess to ensure adequate dechlorination of any and all aqueous samples. Since the proposed method employs UV detection at 213 nm, it is necessary to employ a dechlorinator having minimum spectral interference with CA. Sodium sulfite with absorption at 198 nm exhibits a significant spectral overlap with the CA peak. Also, the use of sodium sulfite resulted in baseline drift in the chromatogram and gave rise to a number of unidentified peaks (not shown). Sodium thiosulfate is also inadequate due to the spectral matching to the UV absorption of CA. A fresh solution of ascorbic acid shows an absorption peak at 265 nm with minimal spectral interference and is chromatographically compatible with CA (Figure 7b). Old solutions of ascorbic acid are to be avoided because of its spontaneous oxidation to dehydroascorbate.[51]

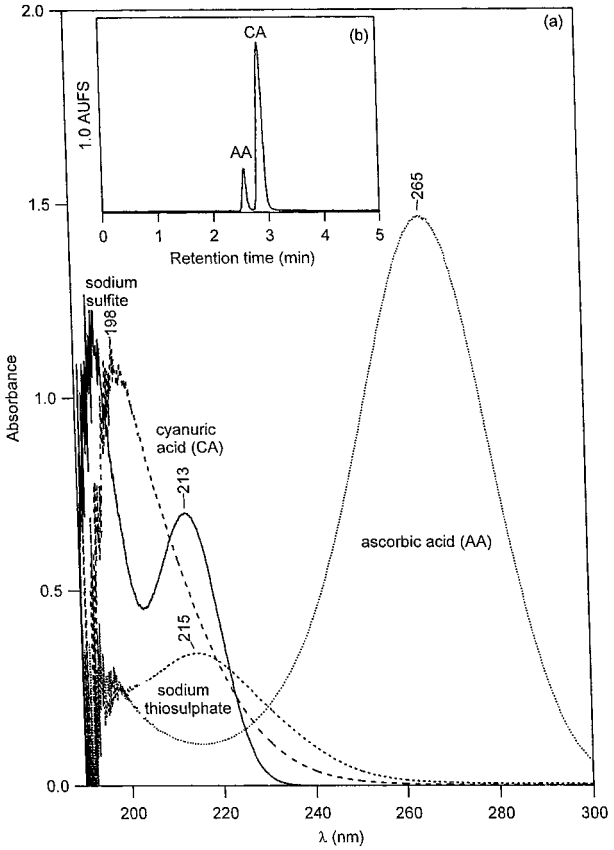

Figure 7. (a) Spectral compatibility of reductive dechlorinators and cyanuric acid. (b) Separation of cyanuric acid from ascorbic acid in HPLC.

CONCLUSION

Overall HPLC conditions for CA analysis in water were established based upon method preference, rapidity, low toxicity, and suppression of multiple isocyanurate species with the aid of the reductive ascorbic acid. The simplified method developed employs RPLC, which was the most popular separation phase of the existing methodology. The analysis is rapid with the CA peak eluting in 3 min. The optimum pH for the mobile phase and UV detection wavelength were found at the pH range of 7.2−7.4 and detection at 213 nm, respectively. This pH range is effective in suppressing CA tautomeric behavior at 6.8−7.1 and avoids the low sensitivity at pHs of <6.8. It was shown that eluents at pHs of >7.4 are more sensitive; however, they are limited because of the lack of retention of CA in the octadecyl column. The analysis is relatively nontoxic using the popular phosphate buffers. The method proved reproducible, and the samples were shown to be stable over 43 days. Finally, the CA method was expanded to encompass DCCA and TCCA. DCCA and TCCA reacted in water to give the CA product using the reductive ascorbic acid, which was found to be spectral and chromatographic compatible to CA. The method measured CA in water in the 0.5−125 mg/L range with an MDL of 0.05 mg/L.

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