DIONEX

Application Update 153

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Fast Determinations of Phosphate and Citrate in Carbonated Beverages Using On-Line Degassing with the Carbonate Removal Device (CRD) and a Reagent-Free™ Ion Chromatography System

INTRODUCTION

Phosphoric and citric acids are critical additives to colas for flavor and preservation. Carbon dioxide is added for flavor or effervescence and also acts as a preservative. When samples are analyzed by ion chromatography (IC) with hydroxide and tetraborate eluents, carbonate in the sample can sometimes coelute and interfere with the quantification of an anion of interest. The gas bubbles from the carbonation also cause variability in the amount injected, resulting in poor peak area reproducibility. Carbonation must be removed to achieve precise and accurate phosphate and citrate determinations.

In Application Note 169[1], we showed that an ICS-2000 Reagent-Free ion chromatography (RFIC™) system outfitted with an IonPac® Fast Anion III column set was a fast and rugged solution for phosphate and citrate determinations in colas. Phosphate and citrate were accurately and precisely determined in 5 min. AN169 used off-line sample degassing for 20 min in an ultrasonic bath. To eliminate this labor, we incorporated a carbonate removal device (CRD) into the RFIC method. The CRD is typically used for removing carbonate from a sample after it is separated and has passed through a suppressor[2], but it can remove carbonate from acidic samples prior to injection. In this application update, we show that phosphate and citrate determinations of samples with carbonate removed on-line with the CRD are equivalent to determinations of samples degassed off-line.

EXPERIMENTAL

Equipment

Dionex ICS-2000 Reagent-Free (RFIC) fully integrated IC system

AS40 Autosampler with 5 mL PolyVial™ vials and plain caps (P/N 039532)

Chromeleon® 6.7 data management system

Filter unit, 0.2-µm nylon (Nalgene® Media-Plus with 90-mm filter, Nalge Nunc International, P/N 164-0020) or equivalent nylon filter

Vacuum pump

PEEK tubing

- Black (0.25-mm or 0.01-in. i.d., P/N 052306 for 5 ft) for connecting columns and ASRS ULTRA II backpressure loops.
- Orange (0.51-mm or 0.02-in. i.d., P/N 052309 for 5 ft) to make the injection line from AS40 to CRD to injection valve
- Red (0.127-mm or 0.005-in. i.d., P/N 052310 for 5 ft) to make a 1.2-µL loop
- Yellow (0.076-mm or 0.003-in. i.d., P/N 052301 for 5 ft) for system backpressure loop

Low-pressure Teflon® (E.I. du Pont de Nemours) tubing (1.6-mm or 0.063-in. i.d., P/N 014157) tubing for the CRD and degas waste lines

Micropipettor and tips for preparing samples, standards, and pipetting samples into vials

Reagents and Standards

Deionized water, Type 1 reagent-grade, 18.2 MΩ cm resistivity or better, freshly degassed by vacuum filtration

Use only ACS reagent grade chemicals for all reagents and standards.

Sodium phosphate, dibasic anhydrous (JT Baker, BioReagent, ultrapure, P/N JT4062-1)

Trisodium citrate, dihydrate (Sigma, P/N S4641)

Samples

Regular colas 1 and 2

Reduced sugar cola 1

Diet colas 1–4, diet colas 5–6 with flavoring

Conditions

Columns:	IonPac® Fast Anion III, 3 × 250 mm (P/N 062982) and Guard, 3 × 50 mm (P/N 062981)
Flow Rate:	1.0 mL/min
Eluent:	EluGen KOH, 20 mM Potassium hydroxide
Temperature:	30 ºC
Inj. Volume:	1.2 µL
Sample Prep:	4-mm CRD (P/N 062983) installed between autosampler and injection port
Detection:	Suppressed conductivity, in recycle mode, 70 mA
Background:	<1 µS
Backpressure:	~2100 psi
Typical Noise:	<1.6 nS
Run Time:	5 min

PREPARATION OF SOLUTIONS AND REAGENTS

Eluent Preparation

It is essential to use high-quality, Type 1 water, >18.2 MΩ–cm and it should contain as little dissolved carbon dioxide as possible. Degas the deionized water before eluent preparation.

Standard Preparation

To prepare separate stock solutions of 10,000 mg/L of phosphate and citrate, weigh the amount of reagent grade, dibasic sodium phosphate (FW 142.0 g/mol) and reagent grade, tri-sodium citrate dihydrate (FW 294.1 g/mol) respectively, into separate 125-mL polypropylene bottles (Table 1). Add degassed deionized water to a total weight of 100.0 g. Shake thoroughly to dissolve the solids.

To prepare combined phosphate and citrate working standards from the 10,000 mg/L stock standards (Table 2), pipette both 10,000 mg/L stock solutions into 125-mL polypropylene bottles. Dilute these working standards with degassed deionized water to 100.0 g total weight. The stock solutions are stable for more than a month when refrigerated. The working standards should be prepared weekly.

Table 1. Amount of Compound Used to Prepare 100.0 g of Individual 10,000 mg/L Stock Solutions

Anion	Compound	Mass (g)
Citrate	Trisodium citrate, dihydrate ($NaOOCCH_2C(OH)(COONa)(CH_2COONa{\cdot}2H_2O)$)	1.555
Phosphate	Sodium phosphate dibasic, anhydrous (Na_2HPO_4)	1.495

Sample Preparation

Table 2. Amount of 10,000 mg/L Stock Solutions Used to Prepare 100.0 g of Combined Working Standards

Standard	Citrate Stock Solution (µL)	Phosphate Stock Solution (µL)
50 mg/L citrate, 200 mg/L phosphate	500	2000
100 mg/L citrate, 300 mg/L phosphate	1000	3000
150 mg/L citrate, 400 mg/L phosphate	1500	4000
200 mg/L citrate, 500 mg/L phosphate	2000	5000

Some cola samples were degassed for 5 min with ultrasonic agitation and then 15 min with ultrasonic agitation and applied vacuum. These were control samples to compare to the same colas analyzed without off-line degassing using the method in this application update.

SYSTEM SETUP

To install the EluGen II potassium hydroxide cartridge, CR-ATC, columns, ASRS® ULTRA II suppressor, and backpressure loops for the suppressor and the eluent generator, refer to the Installation section of the ICS-2000 Operator's Manual.[3] Hydrate the CR-ATC and ASRS ULTRA II suppressor according to the ICS-2000 Installation and Quickstart[4] instructions. Install both the EluGen II KOH cartridge and the CR-ATC in the ICS-2000 cartridge holder and condition the EluGen II KOH cartridge. Install the columns after the injection valve and heat exchanger according to the IonPac Fast Anion III Product Manual[5] and the ICS-2000 Installation section. Install the suppressor, in recycle mode, between the columns and the conductivity cell. Install the backpressure loops after the cell and before the suppressor. After the installations are completed, check the total system pressure. The total system pressure should be >2000 psi for the eluent generator with an optimum operating pressure of 2300 psi. If the system pressure is <2000 psi, refer to the Installation section and install yellow (0.076-mm or 0.003-in. i.d.) PEEK tubing between the degas module and the injection valve to increase the system pressure to ~2300 psi. Do not allow the system pressure to exceed 3000 psi, as this could damage the degas module.

Preparation of a 1.2-µL Sample Loop

To prepare a 1.2-µL sample loop, cut a 10-cm length of red PEEK tubing (0.127-mm or 0.005-in. i.d.). The sample loop volume must be calibrated by weight using an analytical balance because the tubing inside diameter can vary by as much as 20%. The sample loop volume is the difference between the empty loop and the loop filled with deionized water. (See Dionex Application Note 166 for an example of this calculation.)[6] The loop used for the work reported in this application update had a calibrated volume of 1.1 µL.

CRD Installation

Typically, the CRD (for theory and operation, see the CRD manual and Technical Note 62)[2] is used to remove the carbonate peak from the sample just prior to detection, and it would be installed after the suppressor and before the detector. In this application update, the CRD is used as a sample preparation device to remove carbonate from the colas during injection; therefore it is installed between the AS40 Autosampler and the injection valve. Hydrate the 4-mm CRD according to the QuickStart Instructions.[7] Because the CRD is a membrane-based device, high pressure can irreversibly damage it. Always remove all plugs from the CRD before hydrating it or installing any tubing. Replace the PEEK injection tubing on the bleed valve of the AS40 Autosampler with a ~50-cm (18-in.) piece of orange PEEK (0.51-mm or 0.02-in. i.d.) tubing. Install the free end into the "Eluent In" port of the 4-mm CRD. Cut another ~20-cm (8-in.) piece of orange PEEK and install one end into the "Eluent Out" port of the CRD and the other end into Port 5 of the injection valve. Connect the regenerant waste line (1.6-mm or 0.063-in. i.d. Teflon) from the degas module to the "Regen In" port of the CRD. Connect one end of another length of Teflon tubing into the "Regen Out" port of the CRD and direct the other end to waste. The CRD is designed to slip over the top of the suppressor. The CRD can be positioned either outside the ICS-2000 system or secured on the ASRS ULTRA II suppressor according to Figure 1.[8]

Figure 1. Carbonate Removal Device.

AS40 Setup

To connect the AS40 Autosampler to the ICS-2000 system, refer to the AS40 Autosampler Operator's manual[9] and the ICS-2000 Operator's Manual.[10] After installing the AS40, configure it by selecting Concentrate, Bleed On, 3 samples per vial, and Proportional. The AS40 in Concentrate mode injects the sample at a slower flow rate, and thus applies less pressure to the CRD. The "bleed on" function bleeds off excess liquid or air to waste. Always verify after any start-up or power-up that the AS40 is in Concentrate mode.

Configuring the AS40 in the Chromeleon Timebase and Program

To configure the timebase with only the ICS-2000 system, open the Chromeleon configure program, create a new timebase, and add the ICS-2000 system, according to the instructions in the ICS-2000 Operator's Manual. Select the TTL Input tab and select "normal edge" for TTL Input Mode and "TTL Input 1" for the Load and Inject valve. Although the AS40 Autosampler cannot be added during Server Configuration, Chromeleon 6.7 will automatically detect it and add it to the Panel.

Creating a program with the Chromeleon Program Wizard is thoroughly discussed in the ICS-2000 Operator's Manual. Enter the loading and injecting commands for the AS40 autosampler in the Injection Options tab (Table 3).

Table 3. Entries in Chromeleon Program Wizard for the AS40 Autosampler

Injection Options	Entry
Trigger Load Operations	Pump_ECD_TTL_1
Load Sample, Before Injection	2.3 min
Inject Duration	5 s

It is helpful to ensure that the injection valve is in the load position at the start of the program. It is also helpful to shorten the duration commands. In the program review mode, insert on the first line of the program, "Load Position" and "Pump_ECD_TTL_1.5". Change the "Duration = 138" at -2.3 min to "Duration = 5" and delete the "Duration = 5" at 0 min.

Verifying that the CRD is Operational

If peak reproducibility is poor, the CRD may be leaking and has failed. To test if the CRD is leaking, use the procedure described in the CRD Product Manual instructions.[11] There is also a quick pH test on the sample leaving the CRD that will identify whether the CRD is leaking. To test for a leak, remove the PEEK tubing from Injection Valve Port 2 (opposite end from Eluent Out on the CRD) and test a few drops on pH paper. If the pH is basic, then the CRD is leaking and has failed.

RESULTS AND DISCUSSION

First, four colas were analyzed (two regular and two diet colas) with off-line degassing using ultrasonic agitation to establish the expected values for citrate and phosphate. Three of these colas were previously analyzed in AN169. The phosphate and citrate concentrations were consistent with those determined in AN169. The CRD was then installed and the same colas analyzed without off-line degassing. Figures 2 and 3 show that the retention times of phosphate and citrate remained the same for both sample preparation methods and both regular and diet colas. Phosphate had retention times of 2.78 to 2.79 min (Table 4). Citrate for Cola 2 and both diet colas had retention times ranging from 3.73 to 3.77 min. Cola 1 did not contain citrate. The reproducibilities for both retention time and concentration of phosphate and citrate were good, <0.3 %RSD (n = 20). The phosphate and citrate concentrations were comparable to those degassed off-line, 100.2–102.8%.

To verify the linearity of phosphate and citrate with on-line sample carbonate removal, four standards (six replicates each, from 200 to 600 mg/L and 50 mg/L to 200 mg/L, of phosphate and citrate, respectively) were analyzed. The noise over 60-min runs of five water injections was also determined by measuring the noise in 1-min intervals, from 5 to 60 min of each run. The results were comparable to the original method. The linearity for both anions was ≥0.999 and the average noise was 1.6 ± 0.5 nS (n = 5).

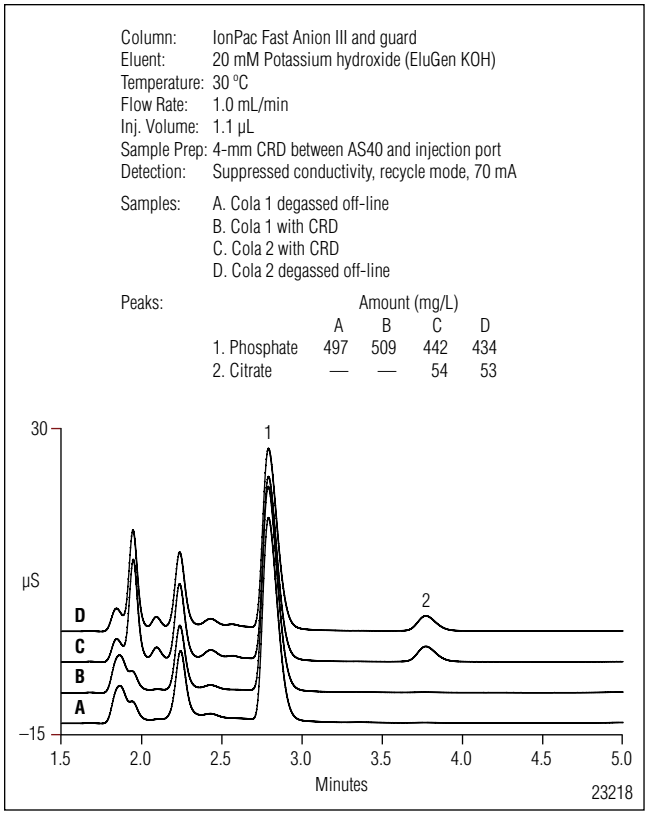

Figure 2. Colas with carbonate removed on-line and off-line.

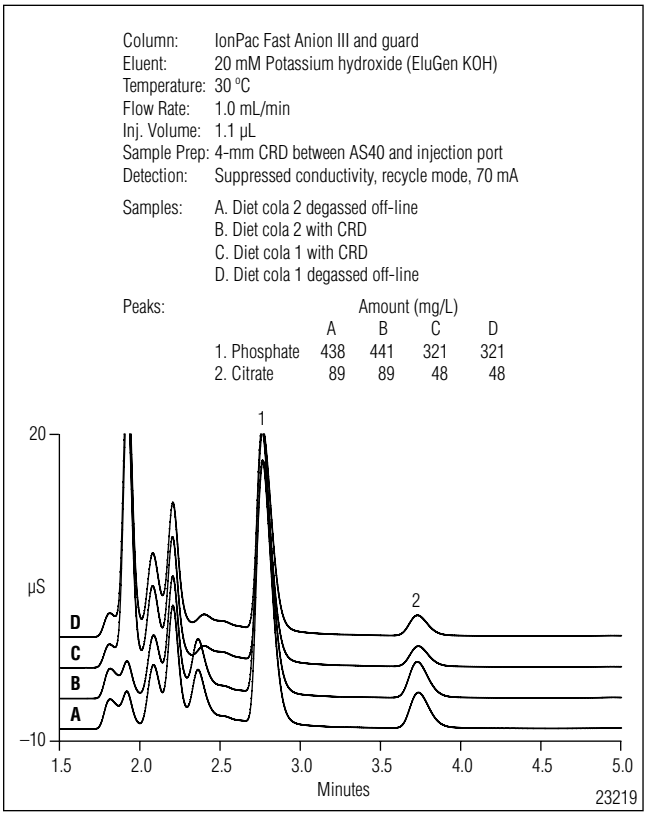

Figure 3. Diet colas with carbonate removed on-line and off-line.

Table 4. Comparison of Cola Analyses Using On-Line and Off-Line Carbonate Removal

		Phosphate				Citrate			
	Carbonate Removal	**Retention Time (min)**	**RSD**	**Peak Area (µS-min)**	**RSD**	**Retention Time (min)**	**RSD**	**Peak Area (µS-min)**	**RSD**
Cola 1	CRD	2.79 ± 0.00	0.05	3.30 ± 0.01	0.22	N.A.[b]	N.A.	N.A.	N.A.
Cola 1	Off-line	2.79 ± 0.00	0.00	3.23 ± 0.01	0.20	N.A.	N.A.	N.A.	N.A.
Cola 2	CRD	2.79 ± 0.00	0.06	2.87 ± 0.00	0.13	3.77 ± 0.00	0.04	0.31 ± 0.00	0.08
Cola 2	Off-line	2.79 ± 0.00	0.05	2.82 ± 0.01	0.20	3.77 ± 0.00	0.05	0.30 ± 0.00	0.27
Diet cola 1	CRD	2.77 ± 0.00	0.06	2.08 ± 0.00	0.12	3.74 ± 0.00	0.04	0.28 ± 0.00	0.16
Diet cola 1	Off-line	2.77 ± 0.00	0.04	2.08 ± 0.00	0.13	3.73 ± 0.00	0.04	0.28 ± 0.00	0.24
Diet cola 2	CRD	2.76 ± 0.00	0.05	2.87 ± 0.00	0.11	3.73 ± 0.00	0.06	0.51 ± 0.00	0.22
Diet cola 2	Off-line	2.76 ± 0.00	0.00	2.85 ± 0.01	0.22	3.73 ± 0.00	0.03	0.50 ± 0.00	0.25

[a] N.A. Not applicable.

Note: A standard deviation value of 0.00 represents a standard deviation less than the last significant figure for the experiment.

To determine whether these results were typical of on-line sample carbonate removal, we analyzed cola samples with three CRDs. Each CRD was used continuously for at least one week. During the course of these experiments, three lots of Cola 1 and two lots of Diet Cola 2 were tested. The results show excellent reproducibility and agreement between CRDs (Table 5) and cola lots. Cola 1 and Diet Cola 2 contained 509.2–513.9 mg/L and 448.7–454.7 mg/L of phosphate and 509.2–513.9 mg/L and 448.7–454.7 mg/L of citrate with an RSD of <0.15 %RSD (n = 20) for each analyte in each sample. The phosphate and citrate retention time reproducibility (2.74 ± 0.12 and 3.61 ± 0.00 min, n = 1317, respectively) and peak area reproducibility (<0.3 %RSD) for 950–2660 injections were as good as the original method.

Having established this method, it was applied to a wider range of samples, one reduced sugar cola and four diet colas (Table 6). For these five samples, the retention times for phosphate and citrate were 2.72–2.77 min and 3.56–3.70 min, respectively. The phosphate and citrate concentrations varied with product, ranging from 221–523 mg/L and 43–172 mg/L, respectively.

CONCLUSION

Using the CRD to remove cola carbonation adds ~2.5 min to the total analysis time while retaining the precision (<0.15 %RSD for retention time), linearity ($r^2 > 0.999$), and reproducibility (<0.3 %RSD) of the original method. This eliminated the extra sample handling and the 20 min required for off-line degassing. Although only colas were analyzed, this sample preparation method can be used with other acidic carbonated samples.

Table 5. Phosphate and Citrate Concentrations in Cola and Diet Cola Using Different CRDs

	Phosphate (mg/L)						Citrate (mg/L)					
	CRD #1	RSD	CRD #2	RSD	CRD #3	RSD	CRD #1	RSD	CRD #2	RSD	CRD #3	RSD
Cola 1[a]	509.2 ± 0.7	0.14	513.4 ± 0.6	0.12	513.9 ± 0.4	0.07	N.D.[b]		N.D.[b]		N.D.[b]	
Diet cola 1[a]	454.7 ± 0.7	0.14	454.3 ± 0.4	0.08	448.7 ± 0.3	0.06	86.1 ± 0.2	0.18	86.5 ± 0.2	0.23	85.6 ± 0.1	0.09

n = 20

[a] We tested three lots each of Diet cola 1 and Cola 1 during the course of the experiments. Not all three lots were tested on every CRD.

[b] N.D. None Detected

Table 6. Product Analysis Using On-Line Carbonate Removal

	Phosphate				Citrate			
	Retention Time (min)	RSD	Concentration (mg/L)	RSD	Retention Time (min)	RSD	Concentration (mg/L)	RSD
Reduced sugar cola 1	2.76 ± 0.00	0.05	523.3 ± 0.7	0.13	3.70 ± 0.00	0.05	90.0 ± 0.1	0.09
Diet cola 3	2.77 ± 0.00	0.05	221.3 ± 0.1	0.07	3.66 ± 0.00	0.07	172.1 ± 0.2	0.14
Diet cola 4 with flavoring	2.72 ± 0.00	0.06	259.2 ± 0.1	0.04	3.56 ± 0.00	0.04	162.0 ± 0.2	0.12
Diet cola 5 with flavoring	2.72 ± 0.00	0.06	258.8 ± 0.3	0.10	3.57 ± 0.00	0.05	162.8 ± 0.2	0.09
Diet cola 6	2.76 ± 0.00	0.06	397.9 ± 0.5	0.12	3.69 ± 0.00	0.04	42.9 ± 0.1	0.26

n = 10

Note: A standard deviation value of 0.00 represents a standard deviation less than the last significant figure for the experiment.

Precautions

The AS40 injections induce pressure changes on the CRD, and over time these pressure changes will eventually cause the CRD to leak and fail. If a failure is suspected, test the CRD for leaks as discussed earlier in this document. Of the three CRDs tested for this application, only one was tested to failure. It failed after ~2660 injections. The other two CRDs were each subjected to ~950 injections.

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