Determination of aluminum in beverages by automated non-segmented continuous flow analysis with fluorescent detection of the lumogallion complex

The Analyst

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A method for the automated determination of aluminum in a variety of beverages is described. The method utilizes lumogallion as a complexing agent in a buffer solution. The system is very similar to flow-injection analysis (FIA), however, the tubing id is larger than that typically used in FIA. Therefore, the system is best described as non-segmented continuous flow analysis using fluorescence spectroscopy detection. The method is extremely simple, requiring virtually no sample preparation and only one reagent. The instrument detection limit for aluminum is 0.012 μg ml^{-1} and calibration is linear to 3 μg ml^{-1}. Results from a variety of beverage matrices are discussed and compared with the frequently used 8-hydroxyquinolone method utilizing a chloroform extraction and fluorescence spectroscopy detection.

Introduction

Aluminum levels in beverage products are very important to the beer, beverage, and packaging industries. Beer and beverage manufacturers are concerned about aluminum pick-up because of the potential unfavorable flavor it may cause in their products at certain levels; furthermore, aluminum can cause some beverage products to lose their color. For example, fruit type soft drinks containing azo dyes may fade in color with aluminum pick-up. The packaging industry uses aluminum content as an inverse measure of the inside coating performance of beverage cans. Beer, beverage, and can manufacturers conduct product test packs, a major part of which is aluminum pick-up. This is done to test new inside coatings, to evaluate new can designs, and to see if other manufacturing process and secondary packaging changes affect can performance.

The methodology that was being used for this aluminum assay utilized 8-hydroxyquinolone as a complexing agent and a chloroform extraction. The chloroform extract containing the aluminum–quinolone complex was then analyzed by fluorescence spectroscopy. This method is ubiquitous, working for most beverage products. This was the method of choice because many of the interferences associated with analyzing beverages for aluminum, such as the presence of citrate, are overcome by this methodology due to the high partition coefficient for the aluminum–quinolone complex in the chloroform. However, this method is very labor intensive and less desirable to work with due to the large volumes of chloroform currently required by this procedure. This is a modification of the original method first proposed by Goon *et al*.[1] Since the Goon method is so labor intensive and because of the safety issues associated with the use of chloroform, a new method was sought for the determination of aluminum in beverages.

Many methods for the assay of aluminum exist including AA, ICP, and others. However, AA and ICP are expensive and require skilled operators. Also, because many beverages have high dissolved solids (> 10%), analyzing a large number of these types of samples using one of these techniques requires at most a digestion or at least dilution before introducing the sample into the instrument. Other methods utilizing ion chromatography, capillary electrophoresis, and flow-injection analysis (FIA) exist. These use various complexing agents and fluorometric and spectrophotometric modes of detection.[2–8] However, the high levels of potential interferents found in many beverage products can cause problems for these methods. Some of these methods do not operate at a sufficient speed for the workload mentioned above.

Because of these drawbacks, a method was sought that would be reasonably fast and mostly interference free, involve minimal sample preparation, eliminate the use of chloroform, utilize the high sensitivity of fluorescent detection, and that could be automated. Sutheimer and Cabaniss[9] showed that lumogallion was virtually interference free when used to analyze aluminum in natural waters. The lumogallion method also met all of the conditions above. Because of these facts, the use of lumogallion was investigated as a means to analyze aluminum in beverages.

Experimental

FIA lumogallion method

Apparatus. See Fig. 1 for a diagram of the flow-injection set-up. All tubing in the analytical flow system is natural FEP Teflon, 1.59 mm od × 0.76 mm id, from Upchurch Scientific (Oak Harbor, WA, USA) (with the exception of the debubbler tubing and the autosampler probe tubing which are 0.50 mm id). Flangeless ETFE ferrules with (¼–28 thread) Delrin nuts, also from Upchurch Scientific, are used to connect the tubing to the valve and peristaltic tubing adapters. The reaction coil is 8.9 cm diameter, and the mixing and dilution coil is three wraps at 7.6 cm diameter. The main pump is a low pulsation peristaltic pump from Ismatec (purchased from Cole-Parmer, Vernon Hills, IL, USA; model 78001-22), and the sampling pump is of in-house design. The main pump tubing for the reagent is 1.52 mm id. The main pump tubing for the debubbler is 0.25 mm id. The sampling probe pump tubing is 0.50 mm id, and the pump tubing for the dilution water is 1.85 mm id. All of these pump tubes are made of Tygon. The injection valve is a VICI (Houston, TX, USA) model C22-3186EH and is fitted with a 50 μl sample loop. A glass T connector from Bran & Luebbe (Buffalo Grove, IL, USA), part number 116 0202 01, is utilized

as a debubbler between the reaction coil and the spectrometer.

Detection, sampling and control. The luminesence spectrometer is a Perkin-Elmer (Norwalk, CT, USA) LS 30. The 76 place Dionex (Sunnyvale, CA, USA) autosampler, Model SHLR, is driven by a PC with a ADC/DIO board and software written in Labview, National Instruments (Austin, TX, USA). This sampler has a continuous rinse station that the sampling probe returns to between samples. The feed and drain on this rinse station are pumped using 2.03 mm id Viton® peristaltic pump tubing. An HP (Palo Alto, CA, USA) 3396A integrator is used for integration. The 4 l water bath is heated with a 400 W quartz immersion rod heater from Electrothermal Engineering (Gillette, NJ, USA). The temperature is controlled to ±0.1 °C by a Pt RTD probe and a Love series 1600 controller with fuzzy logic option from Dwyer Instruments (Michigan City, IN, USA). The bath level is maintained with a Velleman (purchased from Jameco, Belmont, CA, USA) liquid level controller that operates a peristaltic pump of in-house design. The bath water is circulated using a 1/200 hp centrifugal pump from Little Giant Pump Company (Oklahoma City, OK, USA) that outputs 9.8 l min^{-1}.

Procedure. *Reagents, standards and samples.* All reagents and calibration stocks and standards are made using deionized water. The grades (and supplier where appropriate) of each reagent and standard material are: lumogallion, Pfaltz and Bauer (Waterbury, CT, USA); sodium acetate trihydrate, reagent grade; glacial acetic acid, reagent grade; aluminum standard, 10 000 μg ml^{-1} high purity standard; Triton X-100, J. T. Baker Chemical Company (Phillipsburgh, NJ, USA).

A buffer solution is prepared of 0.1 mol dm^{-3} sodium acetate trihydrate adjusted to pH 4.0 with glacial acetic acid. 100 ml of a 0.0018 mol dm^{-3} lumogallion solution is prepared using the buffer solution described above (the lumogallion is dissolved into the buffer solution with gentle warming, not allowing the solution to boil). This lumogallion buffer solution is brought to a final volume of 1 l with buffer and three drops of Triton X-100 are added to improve the solution's wetting characteristics. Finally, this reagent is degassed under 635 mm of vacuum and sonicated for at least 20 min. The 10 000 μg ml^{-1} aluminum standard is used to make 100 and 200 μg ml^{-1} stock solutions, which are then used to make calibration standards and to spike samples. All calibration stock solutions and standards are made in dilute nitric acid (1 + 40). Calibration standards are prepared at concentrations of 0.1, 0.5, 1, 2, and 3 μg ml^{-1} and are stored in high density poly(ethylene) bottles. The carbonated beverage samples may either be sonicated to degas or allowed to degas in a refrigerator prior to analysis. This is necessary for bubble free filling of the sample loop. One sample of each different beverage type is spiked with 2 μg ml^{-1} of aluminum to demonstrate recoveries.

Sampling sequence, system parameters and analysis. The main pump speed is set such that lumogallion reagent flow is 1 ml min^{-1}. The sampling pump speed is set such that the dilution deionized water flow is 5 ml min^{-1}. The sampling sequence is as follows. The sample is drawn through the system for 74 s. After a 2 s delay, the valve is switched to the inject position. The valve stays in this position for 15 s before switching back to the load position. The probe then returns to the rinse station for 144 s. Lastly, there is a 5 s delay before the entire cycle starts again. All of these timing functions are fully adjustable, even while the system is operational. A sample is injected into the system every 4 min. A 1 μg ml^{-1} quality control check sample is analyzed after every 12 samples. Spectrometer parameters are set as follows: excitation wavelength, 500 nm; emission wavelength, 595 nm; response, 4; pmt voltage, 600 V; attenuation factor, 4096. This response level is the spectrometer's second highest level of data smoothing and the attenuation factor is the second least sensitive. This level of sensitivity is used to keep the working linear range to a maximum. The integrator parameters are set as follows: chart speed, 0.1 cm min^{-1}; attenuation, 10 (this corresponds to 1 V full scale); peak width, 1.0 min; area rejection, 500 000; and threshold, 9. The results for the samples are determined by comparing the area from the unknowns to the calibration established by the areas from the standards. This is accomplished using a standard spreadsheet program.

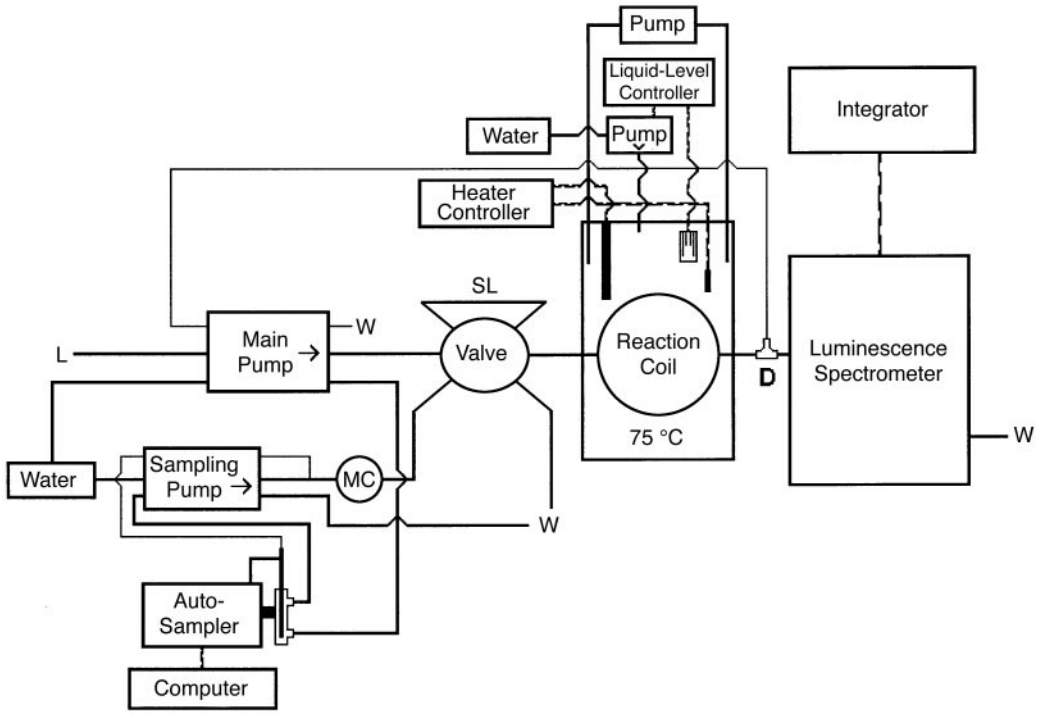

Fig. 1 Schematic diagram of flow-injection set-up: L, buffer solution with lumogallion reagent; W, waste; SL, sample loop; D, debubbler; MC, mixing/dilution coil.

8-Hydroxyquinolone method

Apparatus. Most of the equipment used in this method is ordinarily found in an analytical laboratory. The samples are prepared in 250 ml separatory funnels. A separatory funnel shaker is used for agitation. All reagents are stored in amber bottles with dispensers. Samples are added to funnels using a pipetter with disposable tips. Aliquots of the chloroform extracts are placed into 15 ml glass vials for analysis. The same spectrometer used in the lumogallion method is used for detection in this method.

Procedure. *Reagents, standards and samples.* All reagents and calibration stocks and standards are made using deionized water. The grades (and supplier where appropriate) of each reagent and standard material are: 8-hydoxyquinolone, reagent grade (GFS Chemical Company, Powell, OH, USA); ammonium acetate, practical grade; ammonium hydroxide, 30% solution, reagent grade; glacial acetic acid, reagent grade; chloroform, reagent grade; aluminum standard, 10 000 μg ml^{-1} high purity standard.

In a 1 l volumetric flask, a 0.14 mol dm^{-3} 8-hydroxyquinolone solution is prepared that also contains 60 ml of glacial acetic acid. In a 2 l volumetric flask, a 2.59 mol dm^{-3} ammonium acetate buffer solution is prepared that is pH adjusted with 140 ml of ammonium hydroxide. A 100 μg ml^{-1} stock aluminum solution in dilute nitric acid (1 + 40) is prepared from the 10 000 μg ml^{-1} standard. From this, a 2 μg ml^{-1} aluminum solution is prepared for the calibration standard. Blank, standard, and samples are then prepared as follows. Deionized water (100 ml) is added to each 250 ml separatory funnel. Then, 2 ml of standard or degassed sample, 2 ml of the 8-hydroxyquinolone solution, 5 ml of the acetate buffer solution, and 50 ml of chloroform are also added. The separatory funnels are placed on the shaker at moderate speed for five minutes. The flasks are placed in a rack after shaking. Most of the sample preparations phase separate immediately; however, some will form an emulsion that may take five to ten minutes to separate. A small amount of chloroform is drained out into a beaker to rinse the separatory funnel tip. Then about 10 ml of the chloroform extract is filtered through a small piece of cotton in a 3 ml funnel and into a vial. The cotton is used to trap any droplets of water from entering the vial.

Analysis and instrument parameters. The chloroform extracts are then analyzed by fluorescence spectroscopy. The extracts are drawn into the flow cell by the instrument's pump. The instrument is calibrated with a blank and the 2 μg ml^{-1} standard. One sample of each different beverage type is spiked with 2 μg ml^{-1} of aluminum prior to being prepared to show recoveries. Spectrometer parameters are as follows: excitation wavelength, 385 nm; emission wavelength, 510 nm; response, 4; pmt voltage, 750 V; attenuation factor, set for autocalibration; and 99% attenuation filter in place. The samples' concentrations are determined and printed automatically by the instrument by comparing with the blank and standard responses. Every 12 samples, the instrument calibration and baseline are checked by analyzing the standard and blank.

Note: all portions of this procedure involving chloroform are performed in a fume hood and appropriate personal protective equipment is utilized.

Results and discussion

Initially, the chemistry and FIA apparatus proposed by Sutheimer and Cabaniss[9] were attempted for the analysis of aluminum in beverages. Poor recoveries were obtained for all beverage types. Beverage samples are often viscous, so recoveries were improved by the use of a larger id reaction coil (0.76 mm), which encouraged some dispersion and a better reaction with the lumogallion. However, even with the larger id reaction coil, poor recoveries were still obtained for many beverages. All of these challenging samples were similar in that they were fruit type soft drink beverages and all contained high levels of citric acid. Citric acid (pK_1 = 3.128, pK_2 = 4.761, and pK_3 = 6.396) forms stable complexes with many metal ions and is often used as a sequestering agent to remove trace metals in various industries. To overcome the interference from citric acid, different pH levels below the 5.2 used by Sutheimer and Cabaniss were tried. Lower pH would force the equilibrium of citric acid closer to its molecular form and favor the release of the Al^{3+}. At pH = 4, the overall best response, recoveries, and reaction time were achieved. A water bath was heated to 75 °C to speed the reaction and improve recoveries. In addition to a larger id, a single reaction coil was used with a 3 ml volume, and the coils were wound at a larger diameter to encourage dispersion. Lastly, all calibration standards and samples were diluted in-line by a factor of 10.

Typically, calibration correlation coefficients are better than 0.999. Fig. 2 displays actual responses from a calibration and typical samples. Under the conditions described here, the response from solutions slightly over 3 μg ml^{-1} saturates the instrument's detector. As Table 1 indicates, the recoveries for most spikes are well over 80%. Most of the samples in this table represent challenging samples because of the presence of high amounts of citrate. Most cola type samples give very near to 100% recoveries. Statistics for multiple analyses are well within acceptable values, as demonstrated in Table 2. Table 1 indicates good correlation between the two methods discussed here.

Lumogallion is an acceptable reagent to determine aluminum concentration in many types of beverage samples. The method described here is easily automated, and acceptable recoveries are obtained for most beverage types. The method is inexpensive due to the small amounts of the reagents that are used. Also, the chemicals used are safe to work with compared with other techniques. An additional benefit is that the lumogallion reagent stays soluble in the buffer solution much longer at the lower pH. The method is robust, in that it is not sensitive to

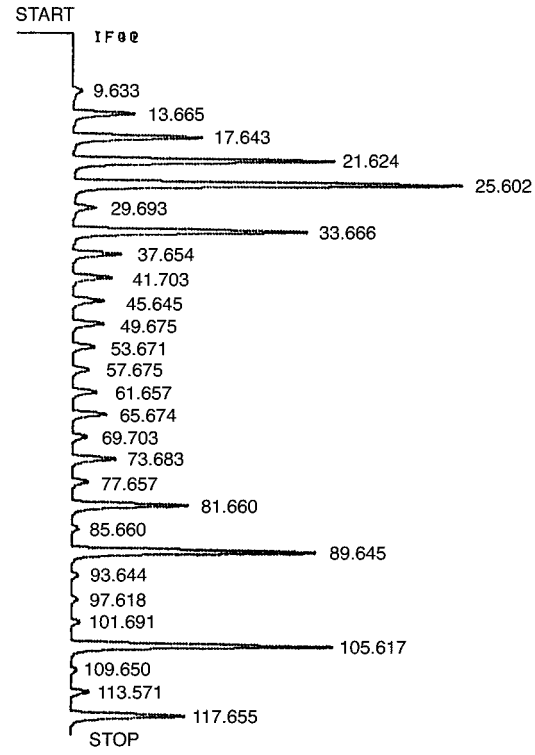

Fig. 2 Detector responses from a calibration and typical samples.

slight deviations in the way the lumogallion reagent is made. The work here shows that lumogallion can be successfully used at pH values below 5.2. Under normal circumstances, between 15 and 20 samples can be analyzed per hour, depending on aluminum concentration. This makes the method slow by FIA standards, but much faster than the modification of the Goon method described.

Table 1 Method results comparison[a]

Sample	L result/ μg ml^{-1}	Spike recovery (%)	Q result/ μg ml^{-1}	Spike recovery (%)
Cola 1	0.42	99.0	0.33	91.9
Cola 2	0.23	100.0	0.19	93.4
Lemon lime 1	0.15	90.4	0.16	93.4
Lemon lime 2	0.83	88.4	0.87	95.5
Lemon lime 3	0.46	67.7	0.54	84.3
Diet lemon lime	0.21	84.3	0.22	95.0
Grape	0.30	88.9	0.29	92.4
Orange	0.13	88.9	0.12	95.0
Watermelon	0.44	85.4	0.45	88.9
Fruit punch	0.96	83.3	1.01	92.4
Raspberry	0.40	88.9	0.33	84.3
Average recoveries		87.7		91.5

[a] L, result from lumogallion method; Q, result from quinolone method; all results, aluminum in μg ml^{-1}; spikes were performed at 2 μg ml^{-1}.

Table 2 Aluminum (μg ml^{-1}) in beverages, statistical analysis

Sample	First analysis	Second analysis	Third analysis	Average	SD	RSD (%)
Cola 1	0.38	0.39	0.39	0.39	0.006	1.49
Cola 2	0.27	0.24	0.23	0.25	0.021	8.44
Lemon lime	0.12	0.13	0.13	0.13	0.006	4.56
Diet lemon lime	0.18	0.17	0.17	0.17	0.006	3.33
Grape	0.43	0.36	0.45	0.41	0.047	11.43
Orange	0.11	0.13	0.11	0.12	0.012	9.90

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