

## Reagent-free analytical flow methods for the soft drink industry: Efforts for environmentally friendly chemical analysis\*

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**Abstract:** The evolution of an entirely green analytical system for industrial quality control of carbonated drinks is described. The developed flow system is capable of providing analytical data of the dissolved CO<sub>2</sub>, sucrose, and color of a sample consecutively in real-time. The system has been carefully designed on the basis of “reagent-free”, meaning that no added chemicals are required for the analysis. The system first vaporizes CO<sub>2</sub> from the soft drink in a gas–liquid separation chamber, with a channel for a flow of pure water as the CO<sub>2</sub> acceptor. The dissolved CO<sub>2</sub> alters the conductivity of the water stream, which is directly related to the concentration of CO<sub>2</sub> in the soft drink. The sucrose content is measured based on the “schlieren effect”, the sample plug flows out of the vaporization chamber into a colorimeter with a near-infrared/light-emitting diode (NIR/LED) as light source. The schlieren effect arises at the boundary of pure water and soft drink with refraction of light in proportion to the sugar concentration. The system also measures the absorbance of the sample using an RGB-LED. The related principles and preliminary experiments as proof of concept are described as well as the construction of the flow system for this completely reagent-free analyzer. A simple flow injection system using the schlieren effect was also developed for rapid quantitative analysis of sugar in noncarbonated soft drinks.

**Keywords:** capacitively coupled contactless conductivity detector (C4D); flow analysis; green analytical chemistry; membraneless vaporization unit; reagent-free; sequential injection; soft drinks.

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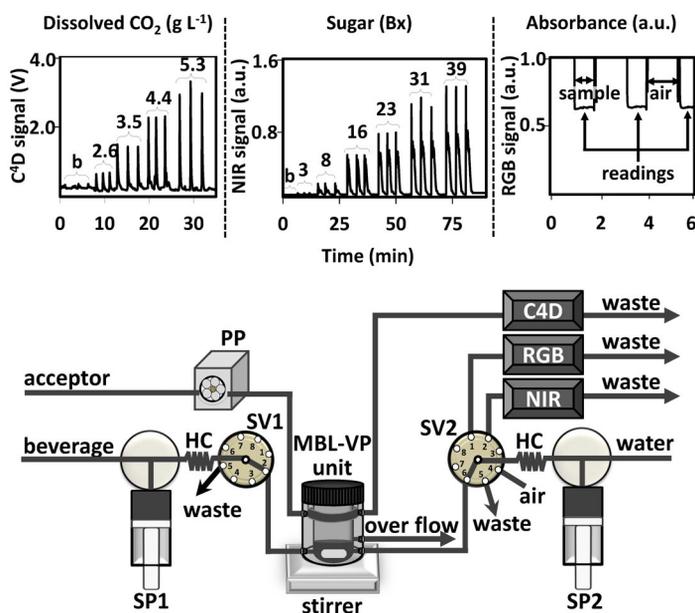
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## INTRODUCTION

There has been an increasing concern about the state of the environment such as global warming and climate change. Nevertheless, there are still economic needs for industrial activities that generate toxic chemical wastes. In order to encourage reduction of the use and generation of toxic substances, the philosophy of “green chemistry” was proposed in early 1990 [1]. Green chemistry, or so-called “sustainable chemistry”, includes the design, development, and implementation of chemical products and processes to reduce or eliminate the use and generation of substances hazardous to human health and the environment. In 1998, John Warner and Paul Anastas set out the “12 principles” of green chemistry in their book [2], which is accepted worldwide as guidelines by the chemical community.

The philosophy of green chemistry can be narrowed down to specific but important activity such as “green analytical chemistry” (GAC). Since analytical chemistry is particularly employed to measure the emission of toxic chemicals or to detect pollutants in the environment, this branch of chemistry should play a leading role in developing new methodologies, which are more environmentally friendly than conventional procedure. Recently, Marek Tobiszewski and his group have proposed [3] that there are four priorities within the 12 principles in relation to GAC. These include (i) elimination or, at least, significant reduction of reagent in analytical procedures; (ii) reduced emissions of vapors and gases in analytical laboratories; (iii) elimination of highly toxic and/or ecotoxic reagents from analytical procedures; and (iv) reduced labor and energy consumption of the analytical procedure (per single analysis). Development of alternative analytical methods that fulfill at least one of the four aforementioned priorities is necessary. There have been a number of useful reviews of clean and alternative analytical methodologies [3–6].

In this work, we present development of a green analytical system with its related instrumentation that fulfills all the four priorities of GAC. The system (Fig. 1) is for an in-line simultaneous mon-



**Fig. 1** The system for consecutive monitoring of dissolved  $\text{CO}_2$ , sugar, and color in soft drinks with examples of signal profiles. SP1 and SP2: syringe pumps; PP: peristaltic pump; HC: holding coil 127 cm; SV1 and SV2: selection valves; NIR: portable NIR photometer; RGB: portable LED-photometer; C4D: capacitively coupled contactless conductivity detector; GLS unit: gas–liquid separation unit [8]. Analytical equation for dissolved  $\text{CO}_2$ : Height,  $V_{\text{dc}} = (1.11 \pm 0.02) [\text{CO}_2, \text{g L}^{-1}] - (2.35 \pm 0.09)$ ,  $r^2 = 0.999$ . Analytical equation for sugar: Height, a.u. =  $(0.026 \pm 0.001) [\text{sucrose, Bx}] - (0.097 \pm 0.018)$ ,  $r^2 = 0.998$ .

itoring of dissolved CO<sub>2</sub>, sugar, and color of carbonated drink using the methodology of sequential-injection analysis (SIA) [7]. The system does not generate any chemical waste because the single liquid compound required is pure water. This reagent-free system was first reported in ref. [8]. In this paper, fundamental concepts are described along with supplementary results. In addition to the system for multiple analyses, as shown in Fig. 1, a simple and rapid flow-injection analysis (FIA) system suitable for determination of sugar in beverages is also presented.

## DETERMINATION OF SUGAR

Cane sugar or sucrose is the major form of sugar used in Thai food industries, including beverages and carbonated soft drinks. Sugar content in water is normally reported in Brix (Bx) unit. A solution with a value of 10 Bx contains 10 g of sugar and 90 g of water [9]. Refractometry is the traditional method for measuring sucrose [10]. A refractometer measures the amount of an analyte based on refraction of light when passing through the media [11]. Refractometry is in fact a measurement of the total dissolved solid in a solution, and therefore the technique is not specific to sucrose. However, it is an accepted industrial practice to use refractometry to determine sucrose content in soft drinks. Refractometers are available as both hand-held and bench-top instruments. However, most of the low-cost refractometers are not flow-through instruments and therefore are not suitable for use as an in-line detector in the manufacturing process.

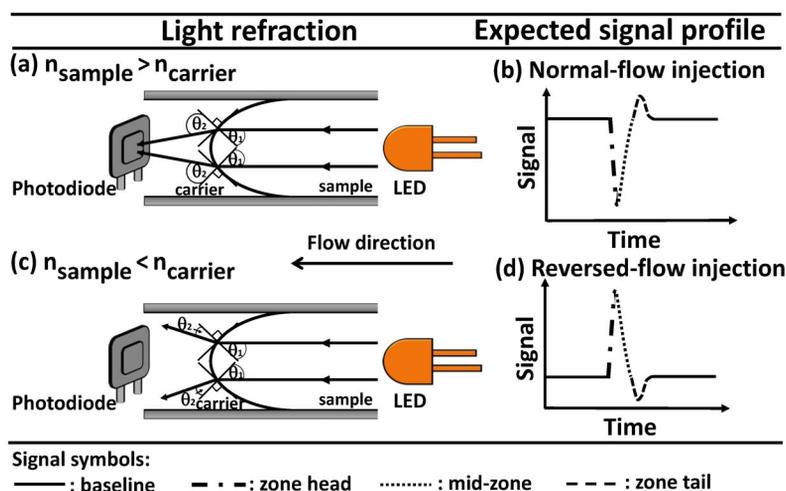
### Schlieren effect in flow analysis

Herein, we report employment of a phenomenon of light refraction, known as the “schlieren effect”, which occurs in liquid flow system, as an in-line detection method. The word “schlieren” is often used in physics to describe optical inhomogeneities in transparent material not visible to the human eye. These inhomogeneities are localized differences in optical path length that cause light deviation [12]. Characterization of the inhomogeneities can be done using refractive index (RI) measurements [13]. In FIA [14] and its related techniques, the schlieren effect is observed when injecting a medium with high RI into a medium with lower RI or vice versa. Such actions result in a concentration gradient that can cause undesirable signals with optical detectors due to the refraction of light as it passes through this gradient [15–17]. The schlieren effect in FIA was first mentioned by Krug et al. in 1977 [18].

The phenomena of the schlieren effect in flow injection can be described using Fig. 2a for the injection of a non-light-absorbing sample into a carrier stream (e.g., injection of a NaCl solution into water carrier). The interface of the carrier and sample forms a lens. Here, we only draw two rays of light as an example. We also draw imaginary normal lines perpendicular to the tangent of interface. In this case, where  $n_{\text{sample}} > n_{\text{carrier}}$  ( $n = \text{RI}$ ), we may expect a significant population of light rays to bend toward the photodiode. As a result, the absorbance signal decreases from the baseline (1<sup>st</sup> (-ve) peak) as the zone head enters the flow cell (Fig. 2b). When the zone head has moved past, the mid-zone now fills the flow cell, and we may expect the signal to return to baseline. If there is an appreciable inhomogeneity within the concentration gradient, we can see a small positive signal when the zone tail travels through the light path.

Figures 2c and d illustrate contrary phenomena to the abovementioned case, where now  $n_{\text{sample}} < n_{\text{carrier}}$ . In this case, a sample zone with a lower RI is injected into the flowing stream of a liquid with higher RI (e.g., injecting water into a flowing stream of a NaCl solution). The light refraction leads to fewer photons reaching the photodiode. The absorbance signal, therefore, increases as the zone head enters the flow cell. The signal returns to baseline as the mid-zone flows through the flow-cell. A noticeable negative signal may appear at the end as the zone tail passes through the flow-cell.

Researchers working in flow-based systems have found that the schlieren effect is undesirable, and they had to arrange their flow systems and/or design detection cells to avoid this effect. Several methods have been presented to minimize the schlieren effect [19–22]. Nonetheless, the schlieren effect



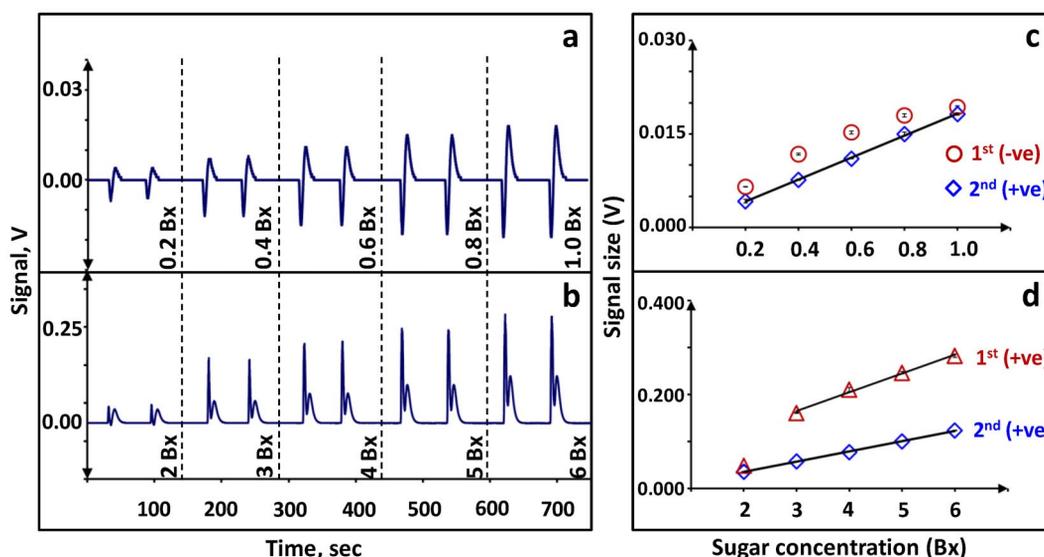
**Fig. 2** Illustration of the refraction of lights (as one of the sources of the schlieren effect) and expected signal profiles for normal-flow and reversed-flow injection [14].

can be useful [23]. In 1978, Betteridge et al. [24] exploited this effect to measure refractive indices of liquids using a home-made light-emitting diode (LED) flow-cell. Later, Pawliszyn made use of the schlieren effect as concentration gradient detectors with laser light source for separation techniques [24,25]. The same author included some results of application for sucrose measurement in FIA [25,26].

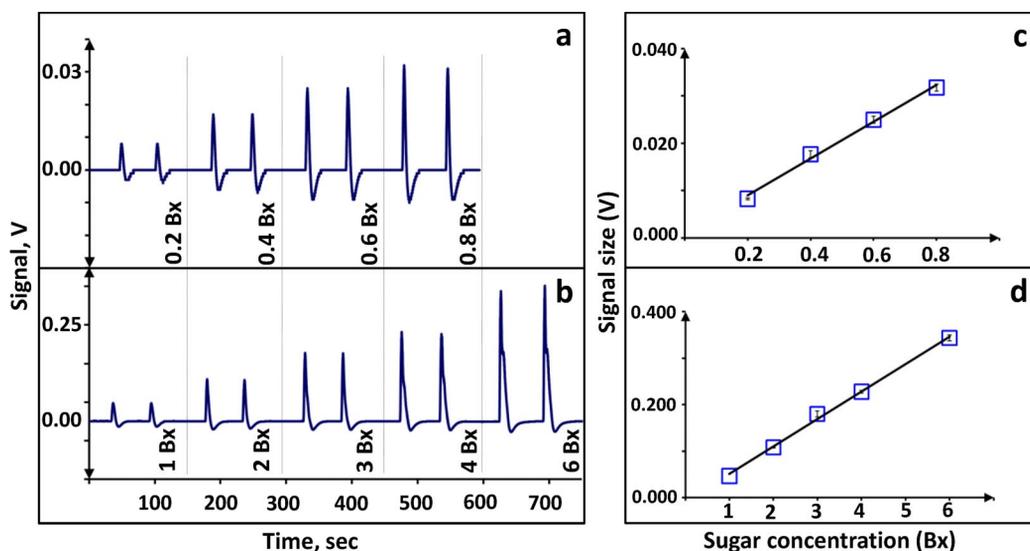
### Normal- and reversed-flow injection for Brix analysis

We initially employed a single-line normal-flow injection system (Fig. 1, supplement) to test the use of the schlieren effect for quantitative analysis of sugar in soft drinks. We simply used a flow-through cuvette cell (Quartz SUPRASIL, 10-mm light path), and a spectrometer (Lambda 25, Perkin Elmer, USA). Solutions of standard sucrose (Mitr Phol, Thailand) were injected into the system. Signal profiles obtained for 0.2 to 1.0 Bx (Fig. 3a) are similar to that predicted in Fig. 2b, indicating that the liquid boundary at the zone head was regular. However, the pattern of the signal profile changed when the concentration of sucrose was greater than 1 Bx. There may be distortion of the zone due to increased viscosity as the concentration of sugar is raised. Viscosity can play a vital role in influencing the dispersion coefficient as well as the shape of zone, and therefore giving rise to the different profiles in Fig. 3b. Under this experimental condition (Fig. 1a, supplement), the plot between the 1<sup>st</sup> (-ve) signal and concentration at low concentration range (0.2–1.0 Bx) is not linear (Fig. 3c). However, for higher concentrations, linear analytical curves could be obtained for both the 1<sup>st</sup> (+ve) and 2<sup>nd</sup> (+ve) signals.

We also tested injection of water into the carrier stream of sugar (reversed-flow injection in Fig. 1b, supplement). The profiles shown in Figs. 4a and b are similar to the predicted profile of Fig. 2d. Linear analytical curves were obtained for both concentration ranges, 0.2–0.8 and 1–6 Bx, respectively. Nevertheless, the reversed-flow configuration is also suitable for a low concentration range of sucrose. The signal becomes irreproducible when working with concentrations greater than 6 Bx, as the viscosity begins to affect the shape of the zone (data not shown). Therefore, the configuration of normal-flow injection is preferable to the reversed-flow injection for the analysis of sugar. An optimization was carried out for the normal-flow injection. Figure 2a (supplement) shows that the sensitivity increased when increasing the injection volume from 100 to 300  $\mu\text{L}$ . The sensitivity did not change much with larger volumes. Therefore, an appropriate condition was chosen at 300  $\mu\text{L}$  of injection volume. With this volume, we can conveniently use the 1<sup>st</sup> (+ve) peaks to plot the calibration of the normal-flow injection



**Fig. 3** Profiles obtained from the normal-flow injection system with plug injections of sugar solutions (a and b) and the plots of the signal size against sugar concentrations (c and d). Analytical equation of 2<sup>nd</sup> (+ve) signals for 0.2–1.0 Bx:  $y = [(1.76 \pm 0.03) \times 10^{-2}]x + [(6.60 \pm 1.93) \times 10^{-3}]$ ,  $r^2 = 0.999$ . Analytical equation of the 1<sup>st</sup> (+ve) for 3–6 Bx:  $y = [(3.99 \pm 0.24) \times 10^{-2}]x - [(4.55 \pm 1.11) \times 10^{-2}]$ ,  $r^2 = 0.993$ . Analytical equation of the 2<sup>nd</sup> (+ve) for 2–6 Bx:  $y = [(2.18 \pm 0.04) \times 10^{-2}]x - [(8.20 \pm 1.59) \times 10^{-3}]$ ,  $r^2 = 0.999$ .



**Fig. 4** Profiles obtained from the reversed-flow injection system with plug injections of water into carrier streams of sugar solution from 0.2 to 6.0 Bx (a and b) and the plots of the signal size against sugar concentrations (c and d). Analytical equation obtained from the 1<sup>st</sup> (+ve) signal in the range of 0.2–0.8 Bx (c) and of 1.0–6.0 Bx (d) were  $y = [(3.89 \pm 0.21) \times 10^{-2}]x + [(1.18 \pm 1.17) \times 10^{-3}]$ ,  $r^2 = 0.994$  and  $y = [(5.91 \pm 0.18) \times 10^{-2}]x + [(8.02 \pm 6.65) \times 10^{-3}]$ ,  $r^2 = 0.997$ , respectively.

system. We also examined flow rates from 1 to 4 mL min<sup>-1</sup> (Fig. 2b, supplement). However, we chose to work at 2 mL min<sup>-1</sup>. At the carrier flow rate of 2 mL min<sup>-1</sup>, the sample throughput is satisfactorily large up to 120 samples h<sup>-1</sup>. Linear analytical curve could be achieved for 3–47 Bx.

### Application of normal-flow injection for Brix analysis

Our method employing the normal-flow injection system (Fig. 1a, supplement), was validated against conventional refractometric method. A bench-top refractometer was employed to analyze the sugar content of five samples of noncarbonated soft drinks. Our flow system was applied to the same set of samples (Table 1). The results agree statistically well with the results obtained from the refractometer ( $t_{\text{stat}} = 2.19$ ,  $t_{\text{crit}} = 2.77$ ,  $P = 0.05$ ). This good agreement indicated that the normal-flow injection system that exploits the schlieren effect is applicable to real samples. The color of the soft drinks did not interfere when the schlieren effect was observed in the near-infrared (NIR) region (890 nm).

**Table 1** Brix analysis in five non-carbonated beverages by using normal-flow injection and conventional refractometer.

Sample (color)	Sucrose content (Bx)	
	Normal-flow injection	Refractometry <sup>a</sup>
Amino drink (pink)	9.7 ± 0.2	8.2 ± 0.1
Green tea (yellowish green)	10.8 ± 0.2	9.7 ± 0.0
Palm sugar syrup (brownish yellow)	13.5 ± 0.2	13.9 ± 0.1
Sweet syrup 1 (colorless)	90.7 ± 2.4	89.4 ± 4.1
Sweet syrup 2 (orange)	86.9 ± 1.8	83.2 ± 0.6

<sup>a</sup>Bausch & Lomb, USA.

### DEVELOPMENT OF THE SEQUENTIAL-INJECTION FLOW SYSTEM FOR IN-LINE QUALITY CONTROL

Sequential injection is the second-generation flow injection system [27], which employs computer-controlled pumps for precise liquid handling. A basic sequential-injection system consists of four major components comprising a syringe pump, a selection valve, a holding coil, and an appropriate detector. The flow line is formed by connecting tubes (i.d. 0.75–1.0 mm) between these components. The selection valve and syringe pump is used to select connection between the various reagents and the flow path. In this work, we employed a sequential-injection mode to construct a flow system for an in-line simultaneous determination of Brix value and dissolved CO<sub>2</sub> in carbonated soft drinks. The system also monitors the consistency of the color of the soft drink by measurement of absorbance of the sample [8].

### Utilization of schlieren effect in the sequential-injection flow system

The results, obtained from the normal-flow injection system, clearly demonstrate the application of the schlieren effect in flow analysis to determine Brix value. The validation results in Table 1 also show that the wavelength of 890 nm had no absorbance from the food colorant. The schlieren method is attractive since it is a reagent-free method for sugar analysis. The concept is thus suitable for further application for in-line monitoring of sugar by sequential injection. In order to make the system more com-

fact than that shown in Fig. 1a (supplement) a portable photometer (Bangkok High Lab Co., Ltd., Thailand), was employed. The photometer in the sequential-injection system is equipped with an NIR light source [8].

### RGB-LED for quality control of color

Color is an important factor in appearance of foods and drinks. The level of color must be carefully controlled to maintain the quality of the product and satisfaction of customers. For soft drinks, there are various coloring agents. Outside standard methods for color measurements, monitoring of the absorbance of food colorant is an alternative method. Absorbance reading at a wavelength specific for a food coloring compound reflects the concentration level of the colorant. Measuring the absorbance of beverage can be easily carried out by attaching a portable colorimeter with a flow-through cuvette cell to the process line. In our system (Fig. 1), we employed an RGB-LED photometer to monitor the absorbance. The RGB light source gives a broad range of wavelengths, making it applicable to all color ranges of soft drinks.

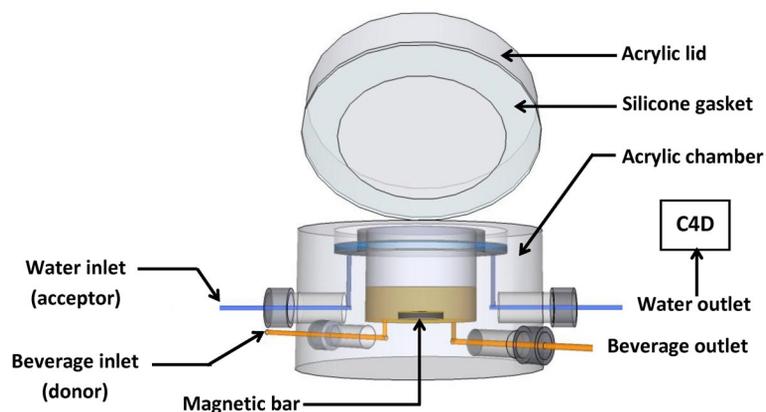
### Gas–liquid separation technique: A possible in-line method for determination of dissolved CO<sub>2</sub>

For carbonated soft drinks, control of the amount of CO<sub>2</sub> dissolved in the drink is very important. The taste will be flat if the concentration of CO<sub>2</sub> is too low. It also does not taste good if the level of CO<sub>2</sub> exceeds a certain limit. The most common method for determination of CO<sub>2</sub> in beverage is to measure total pressure and temperature of a sample. This method, based on Henry's law, can overestimate the amount of CO<sub>2</sub> due to the presence of other gases [28]. Techniques for direct measurement using IR spectroscopy have been presented to solve this error [29]. Gas-diffusion (GD) flow injection has been utilized for determination of total CO<sub>2</sub> in soft drinks [30]. Bromocresol purple was added into the acceptor stream flowing on one side of the GD membrane for monitoring of the color change, which corresponds to the amount of diffused CO<sub>2</sub>. A commercial in-line system with a selective membrane sensor is also available [31].

### Membraneless vaporization unit and C4D detection

In this work, we proposed to use gas–liquid separation technique to determine dissolved CO<sub>2</sub> in carbonated soft drinks. Previously, our group developed a flow-through device called a “membraneless vaporization unit” (MBL-VP unit) [32,33] for vaporization of CO<sub>2</sub> for direct determination of carbonate in calcium tablet [32] and cement [33]. In this work, this device was modified and adapted for separation of gaseous CO<sub>2</sub> from carbonated soft drinks, prior to the measurement. Figure 5 shows a schematic diagram of the MBL-VP unit with the lid opened. Formerly, we only had a vial containing the sample placed in the unit. This current unit was modified by drilling two more holes in the base of the unit. These holes are the inlet and outlet of the beverage sample. Sample vial is no longer used in our current unit. Inside this unit, there is another open channel located just above the donor. This channel is called an “acceptor channel” and deionized–distilled water flows through this channel constantly. In the operation, the MBL-VP unit degases CO<sub>2</sub> from the carbonated soft drink via constant stirring with a magnetic bar (400 rpm) to eliminate the bubbles, which can interfere with photometric detections at the NIR (sugar) and the RGB (color). During the degassing process, volatilized CO<sub>2</sub> partially dissolves into the stream of pure water in the acceptor channel. The dissolved CO<sub>2</sub> results in the increase in the conductivity of the water carrier. This small change in conductivity can be detected at the capacitively coupled contactless conductivity detector (C4D) [34] located next to the acceptor outlet (Fig. 5).

The C4D was made from the polyetherether ketone (PEEK) tube (1 mm i.d. and 1.6 mm o.d.) with total length of about 150 mm. The two electrodes (length: 10 mm each) were made by painting the



**Fig. 5** The modified MBL-VP unit for in-line CO<sub>2</sub> separation and for determination of dissolved CO<sub>2</sub> in beverages.

PEEK tube with silver paint vanish. We have investigated the fabricated detector (Fig. 3, supplement) and selected the input voltage of 30 V and frequency at 100 kHz (high sensitivity condition). With a set of amplifier-rectifier and I/V converter (Bangkok High Lab Co., Ltd., Thailand), we could use the C4D cell to monitor the increase of electrical conductivity of the water stream due to the dissolved CO<sub>2</sub> the acceptor stream.

### Design and sequence of analysis

In Fig. 1, the beverage is drawn to the flow system via selection valve SV1, using the syringe pump SP1. A part of the beverage zone is degassed inside the MBL-VP unit (see ref. [8] for details). The degassed sample is first sent to measure the schlieren signal for Brix analysis at the NIR-LED photometer via aspiration and path selection using syringe pump SP2 and selection valve SV2. The same set of SP2 and SV2 is used to transfer the latter part of the zone for absorbance measurement at the RGB photometer for quality control of the amount of coloring agent.

In addition, the flow system in Fig. 1 also monitors the change in conductivity at C4D. The output from this detector corresponds to the concentration of dissolved CO<sub>2</sub> in the soft drink. Continuous stirring of beverage to degas the sample accelerate vaporization of CO<sub>2(g)</sub>. Dissolution of CO<sub>2(g)</sub> into the acceptor stream of pure water leads to an increase in its conductivity. Therefore, we can determine dissolved CO<sub>2</sub> by calibrating the signal height of C4D and the concentration of CO<sub>2(aq)</sub>.

### Analytical performance and its applications

Table 2 is the summary of the analytical performance of the developed sequential-injection flow system in determination of dissolved CO<sub>2</sub> and sugar. The analytical feature for monitoring the absorbance with the RGB-LED photometer is also included. Total analysis time for all three parameters per sample is very short (6.5 min). The developed system in Fig. 1 was applied to some commercial samples including carbonated and noncarbonated soft drinks. Determination of dissolved CO<sub>2</sub> was compared with a flow injection method with a GD unit [35]. The sugar analysis was compared with the conventional refractometric analysis. The measurement of absorbance was compared with normal routine measurement in a cuvette using a spectrophotometer set at a wavelength in the same region as the light emission from the RGB source.

**Table 2** Analytical features of the sequential-injection flow system for simultaneous determination of sugar and dissolved CO<sub>2</sub> in soft drinks as well as for the monitoring of the absorbance to control color.

Feature	Dissolved CO <sub>2</sub>	Sugar	Absorbance
1. Working range	2.64 – 5.28 g L <sup>-1</sup>	3.10 – 46.50 Bx	0.0 – 2.0 a.u.
2. Linear calibration, <i>r</i> <sup>2</sup>	(peak height, <i>V</i> <sub>dc</sub> ) = (1.11 ± 0.02)[CO <sub>2</sub> , g L <sup>-1</sup> ] – (2.35 ± 0.09), <i>r</i> <sup>2</sup> = 0.999	(peak height, a.u.) = (0.026 ± 0.001)[sugar, Bx] – (0.097 ± 0.018), <i>r</i> <sup>2</sup> = 0.998	–
3. Reproducibility (% RSD)	6.3 (3.5 g L <sup>-1</sup> ; <i>n</i> = 15)	3.2 (16 Bx; <i>n</i> = 10)	1.7 (λ = 640 nm; <i>n</i> = 15)
4. LOD	2.23 g L <sup>-1</sup> (3SD)	2.79 Bx (3S/N)	–
5. LOQ	2.53 g L <sup>-1</sup> (10SD)	2.88 Bx (10S/N)	–
6. Analysis time/sample	← 6.5 min →		

From the results in Table 3, the levels of CO<sub>2</sub> in carbonated drinks are somewhat lower than the designated levels in the cans or bottles. Since the analysis was carried out after opening the container, some of the CO<sub>2</sub> would have vaporized into the atmosphere. The results of sucrose analysis from our method are comparable to the results by the refractometric method. For the control of the color, the absorbance measured by the RGB photometer furnished with a flow-through cell are similar to the absorbance measured by the spectrophotometer using a regular cuvette.

**Table 3** Analysis of carbonated and noncarbonated soft drinks by using our sequential injection compared with other methods.

Beverage	Parameters					
	Dissolved CO <sub>2</sub> (g L <sup>-1</sup> )		Sugar (Bx)		Absorbance (a.u.)	
	Our method	FIA-GD method <sup>a</sup>	Our method	Refractometric method <sup>d</sup>	Our method	Spectrometric method
<i>Carbonated:</i>						
1. Regular cola	3.30 ± 0.08	3.16 ± 0.01	8.34 ± 0.17	8.61 ± 0.00	0.322 ± 0.004 <sup>b</sup>	0.313 ± 0.001
2. Sugar-free cola	2.76 ± 0.20	2.93 ± 0.05	n.d.	n.d.	0.327 ± 0.002 <sup>b</sup>	0.319 ± 0.000
3. Orange	2.39 ± 0.11	2.37 ± 0.03	12.33 ± 0.15	11.78 ± 0.00	0.045 ± 0.001 <sup>b</sup>	0.043 ± 0.000
4. Mixed fruit	3.05 ± 0.17	3.04 ± 0.04	11.65 ± 0.13	11.09 ± 0.03	0.450 ± 0.001 <sup>b</sup>	0.443 ± 0.003
5. Strawberry	2.77 ± 0.13	2.80 ± 0.01	12.26 ± 0.02	11.54 ± 0.03	1.927 ± 0.000 <sup>c</sup>	1.943 ± 0.003
<i>Non-carbonated:</i>						
6. Chrysanthemum tea	n.d.	n.d.	7.15 ± 0.03	6.78 ± 0.00	0.232 ± 0.001 <sup>c</sup>	0.232 ± 0.001
7. Black tea	n.d.	n.d.	8.48 ± 0.08	8.56 ± 0.01	0.392 ± 0.002 <sup>c</sup>	0.393 ± 0.001
8. Coconut syrup	n.d.	n.d.	10.59 ± 0.12	10.56 ± 0.01	0.439 ± 0.002 <sup>c</sup>	0.436 ± 0.001
9. Energy drink	n.d.	n.d.	15.27 ± 0.11	15.00 ± 0.01	0.117 ± 0.002 <sup>c</sup>	0.119 ± 0.004

<sup>a</sup>See ref. [35].

<sup>b</sup>With the LED set at 640 ± 20 nm.

<sup>c</sup>With the LED set at 470 ± 25 nm.

<sup>d</sup>Refracto 30PX/GS. Mettler Toledo, USA.

n.d.: non-detectable

Paired-t test results (*P* = 0.05): dissolved CO<sub>2</sub> (*t*<sub>stat</sub> = -0.130, *t*<sub>crit</sub> = 2.776), sugar (*t*<sub>stat</sub> = 0.680, *t*<sub>crit</sub> = 2.262), and color (*t*<sub>stat</sub> = 0.458, *t*<sub>crit</sub> = 2.262).

## CONCLUSIONS

Here we present the concept and development of a flow system for an in-line analytical device for the soft drink industry. Within 6.5 min, the device offers simultaneous measurements of dissolved CO<sub>2</sub> and sugar content, together with monitoring of consistency of the color. The MBL-VP unit was developed

and employed for separation of CO<sub>2</sub> from soft drinks and for eliminating bubbles prior to further analyses of color and sugar. A C4D was employed in determination of the dissolved CO<sub>2</sub>.

The schlieren effect was effectively employed for determination of sugar. We were able to demonstrate the use of a common flow-through cuvette cell for quantitative determination of sugar. Low-cost NIR-LED is adequately suitable as a light source to observe the schlieren signal. We also employed an RGB-LED detector in the system for monitoring of the color.

The objective of this work is to develop a completely reagent-free system. The methodologies have been pragmatically designed to fulfill all four priorities in GAC, i.e., (i) the system does not require use of chemicals and therefore is a reagent-free system; (ii) there is insignificant emission of vapor from our system; (iii) there are no toxic reagents in the analytical procedure; and (iv) the analyses are rapid, thus there is reduction in labor and energy use.

In addition to the in-line system, a simple and rapid flow-injection method suitable as an off-line method for determination of sugar content in beverages was also obtained. The method was also based on the observation of the schlieren profile. The sample throughput of this method is 120 samples h<sup>-1</sup>.

## SUPPLEMENTARY INFORMATION

Supplementary figures are available online (doi:10.1351/PAC-CON-12-02-06).

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