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# Flow-Injection Spectrophotometric System for Sequential Determination of Sugar and Orthophosphate in Soft Drinks and Sugarcane Juice

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**ABSTRACT**

This work describes the development of a flow injection (FI) system for sequential analysis of sucrose and orthophosphate in soft drinks and sugarcane juice. A simple sample preparation was firstly employed in order to avoid disturbance from sample matrix such as color of the sample, $CO_2$ gas in soft drink and high turbidity of sugarcane juice. Detection of sucrose was carried out by utilizing the schlieren or lens effect, a well-known phenomenon of light refraction in the visible spectrophotometric FI system. The difference in refractive index at the liquid interfaces between sample plug and water carrier causes light refraction, resulting in a peak signal of sucrose in the sample. Subsequently, the same sample plug was analyzed for the orthophosphate content based on the molybdenum blue method. This FI system only used one visible detector at 880 nm for detecting both the sucrose and orthophosphate signals. Under the optimum condition, linear working range for sucrose and orthophosphate were obtained from 1-50 degree Brix and 5-300 mg $P_2O_5$ $L^{-1}$, respectively, with the determination of coefficient ($r^2$) more than 0.990. The developed system provides rapid analysis, within 3.5 min per sample, of the two analytes. The system was successfully applied to determine sucrose and orthophosphate in soft drinks products and sugarcane juice.

**Keywords:** Flow injection; Molybdenum blue method; Orthophosphate; Schlieren effect; Sucrose

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## 1. Introduction

Sucrose is the sugar that is most used in household and industry. It is produced from sugarcane or beetroot mostly. The main sugar constituent in sugarcane juice is sucrose which represents over 88% of the total sugar [1-3]. The typical level of sucrose in sugarcane juice is in range of 10 to 15 degree Brix (°Bx) [4]. The level of sucrose determines the quality and price of sugarcane which specify the grower's revenue. Moreover, the sucrose contents are being monitored several times during the sugar manufacturing processes. Besides sucrose, a small amount of orthophosphate in the sugarcane juice is also an important constituent that needed to be controlled for assuring sugar quality. Orthophosphate content in sugarcane juice plays an important role in clarification process such as clarity of juice, colloid elimination and rapid settling [3-4]. The suitable amount of orthophosphate in sugarcane juice is 300-400 mg $P_2O_5$ $L^{-1}$ [2, 5]. Thus, both sucrose and orthophosphate are important elements that need to be monitored during the production of sugar.

The carbonated soft drink industry is one of the largest industries in the world. The main ingredients of a carbonated soft drink are water, sweetener, carbon dioxide and acidulants [6]. In general, sucrose is the principal sweetener ingredient except for the sugar-free type. Moreover, in some carbonated soft drinks, particularly in cola drinks, phosphoric acid was added in order to create a sharper flavor [6]. Hence a carbonated soft drink is another case in which the quantity of sucrose and orthophosphate are crucial for the quality of the product.

There are several methods that have been presented for determination of sucrose in sugarcane juice or soft drinks: the Lane-Eynon titration [2], flow-injection spectrophotometry by oxidation with some reagents [7-9], enzymatic analysis [10], infrared spectroscopy [11-13], chromatography [14-16], polarimetry [17] and refractometry [2]. Recently, a green analytical method based on schlieren effect in flow-based technique has been proposed for analysis of sucrose in beverages [18-20] or sugarcane juice [21]. Using a light refraction effect occurring from a refractive index gradient at the liquid interface between the sample zone and the water carrier, the amount of sucrose can be determined without any chemical usage. This method presents an attractive feature for sucrose analysis.

On the other hand, the publications on determination of orthophosphate or phosphoric acid in sugarcane juice or soft drinks can be summarized as follows: using molybdenum blue reaction with spectrophotometric detection [2, 20-24], acid-base titration [25], infrared spectroscopic detection [26], turbidity detection [20] and ion chromatography [27].

Since sucrose and orthophosphate are two key ingredients in quality control of the product in soft drink and refined sugar manufacturing industries, it is important to have a simple, fast, and reliable method for the determination of sucrose and orthophosphate in sugarcane juice and soft drinks. Thus, the goal of this work was to develop a versatile flow injection (FI) system with visible spectrophotometric detection for routine multi analysis. The sucrose analysis was carried out based on the schlieren effect (or lens effect) in flow analysis which is considered a reagent-free method. In the same FI system, orthophosphate in samples will be analyzed subsequently based on the molybdenum blue method which is reliable, sensitive and selective to orthophosphate. Furthermore, a convenient semi-automatic method of sample preparation was also proposed for supporting a large number of sugarcane juice samples.

## 2. Materials and Methods

### 2.1 Chemicals and reagents

All chemicals used were of analytical reagent grade except for sucrose standard, which was commercial food grade (Mitr Phol, Thailand). Deionized-distilled (DI)

water (ElgastatUHQPS Elga, England) was employed for preparations of standard and reagent solutions, and carrier stream.

A stock solution of sucrose standard (60 °Bx) was prepared as described in the previous literature [18]. The stock solution was stored at 4°C in a refrigerator and used within one week. A stock solution of orthophosphate standard (1000 mg $P_2O_5$ $L^{-1}$) was prepared by dissolving 0.1916 g of potassium dihydrogen phosphate ($KH_2PO_4$, Carlo Erba, Italy) in 100.00 mL DI water. Working standard solution, mixed of sucrose and orthophosphate, for calibration plot were prepared in DI water by appropriate dilution from the stock solutions.

For orthophosphate analysis, the reagent solutions, R1 and R2, were prepared for molybdenum blue method. The mixed reagent solution (R1) was a solution of 0.016 mol $L^{-1}$ ammonium molybdate tetrahydrate ($(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$, Carlo Erba, Italy), 0.6 mol $L^{-1}$ sulfuric acid ($H_2SO_4$, RCI Labscan, Thailand) and 0.016 mol $L^{-1}$ tartaric acid ($C_4H_6O_6$, Carlo Erba, Italy). The reducing reagent (R2) was 0.1 mol $L^{-1}$ ascorbic acid ($C_6H_8O_6$, Carlo Erba, Italy).

The following chemicals were used for the interference study: glucose ($C_6H_{12}O_6$, Carlo Erba, Italy), fructose ($C_6H_{12}O_6$, Carlo Erba, Italy), and sodium metasilicate anhydrous ($Na_2SiO_3$, Fluka, Switzerland).

## 2.2 Sample preparations

Sample preparation is an essential procedure when analyzing samples with high turbidity and intense color, particularly if the analysis is based on spectrophotometry. Even though most of the sample preparations are tedious, laborious and might require certain skill, our sample preparation is plain and can be adjusted accordingly.

### *2.2.1 Soft drinks*

Commercial soft drinks with different brands and colors were purchased from Thai supermarkets. All samples were degassed in an ultrasonic bath (SONOREX SUPER RK 156, Bandelin, Germany) for 15 min to remove dissolved $CO_2$ gas. Root beer and cola drink samples were diluted with DI water in the ratio of 1:4 to avoid disturbance from caramel color of samples prior analysis.

### *2.2.2 Sugarcane juice*

Fresh sugarcane juice samples were randomly obtained from the local markets in Bangkok and Pathumthani, Thailand. The colors of the samples were ranged from yellow to jade green with high turbidity; therefore, a sample preparation scheme was necessary. In this work, a home-made preparative column adapted from a 3-mL plastic syringe (Nipro, Thailand) was used as shown in the dotted area of Fig. 1. The column was made by using cotton as a column plug to prevent charcoal from flowing through column and then packed with 0.28 g of charcoal (particle size 40 µm, Fluka, Switzerland) before being covered with a cotton layer. To prevent clogging, the sugarcane juice was diluted with DI water (1:4) before loading into the column (Fig. 1). The purpose of the diluting step was to extend the column usage to 3 extractions before changing the column. In practice, the home-made column can be arranged as a set of columns to support large numbers of samples. The diluted sugarcane juice samples were simultaneously passed through columns and were collected for 5 mL simultaneously. Finally, each collected solution was then filtered by syringe filter through a 0.45-µm cellulose acetate membrane (Sartorius Stedim Biotech GmbH, Germany) to remove colloid particles and prepare for analysis. These sample preparation procedures are simple and cost effective but if laboring is the main trepidation, a high-speed centrifuge (capable of at least 8000 rpm) might be an alternative method.

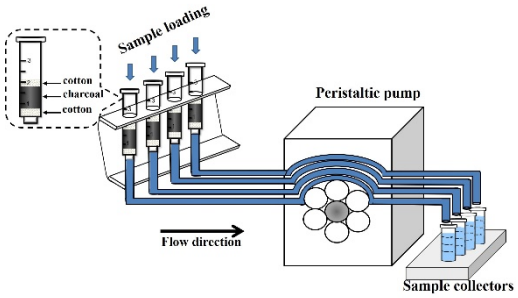


**Fig. 1.** Schematic diagram of a set of home-made charcoal columns for preparation of sugarcane juice.

## 2.3 FI system and its operating procedure

### *2.3.1 The FI scheme*

The schematic diagram of the FI manifold for the determination of sucrose and orthophosphate is shown in Fig. 2. A peristaltic pump (ISM827B, Ismatec, Switzerland) was used to propel the solutions throughout the FI system. Tygon$^{TM}$ pumping tubes (Ismatec, Switzerland), with 2.06 mm i.d. for carrier and 0.89 mm i.d. for reagents, total of 4 channels were fitted with cassettes. PTFE tubes with 0.75 mm i.d. (VICI, Switzerland) were used throughout the flow system. A 6-way injection valve (1106, Omnifit, USA) equipped with a 100 µL PTFE loop was used to introduce the standard/sample solution into the flow system. A 3-way switching valve and a 4-way switching valve (Upchurch, USA) were used to switch the flow direction of the sample plug for sequential monitoring of sucrose and orthophosphate. A Lambda 35 UV-Vis spectrophotometer (Perkin-Elmer, USA), equipped with a flow-through cell (178.010-QS, Hellma Germany) of 80 µL volume (10 mm pathlength), was used for detecting the schlieren signal of sucrose and the absorbance of phosphomolybdenum blue complex (PMB). The detection wavelength was set at 880 nm which is suitable for sucrose [20] and orthophosphate [28, 29] measurements. At 880 nm, not only did it deliver the maximum absorption of PMB but also the absorbance of color matrix was minimized.

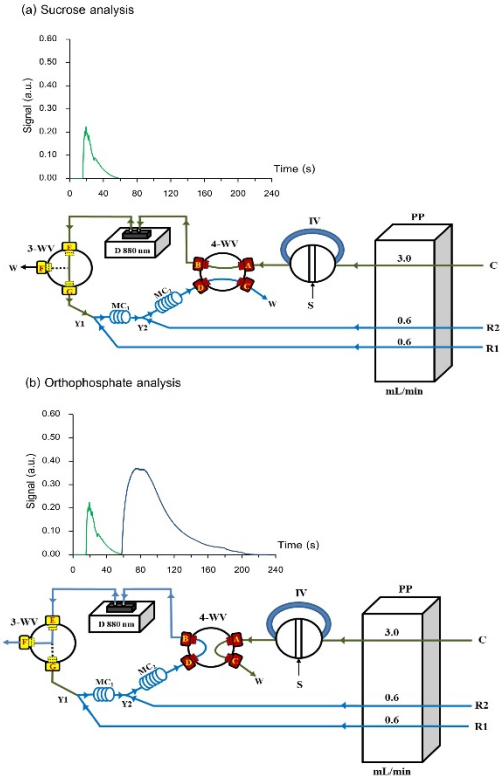


**Fig. 2.** The FI system developed for sequential detection of sucrose (a) and orthophosphate (b) in soft drink and sugarcane juice. PP: peristaltic pump, IV: injection valve, 4-WV: 4-way switching valve, 3-WV: 3-way switching valve, D: spectrophotometer, MC1 and MC2: mixing coils (0.75 mm i.d. x 60 cm length and 0.75 mm i.d. x 120 cm length), Y1 and Y2: merging points, S: sample, C: water carrier, R1: acidic ammonium molybdate solution with tartaric acid, R2: ascorbic acid solution, W: waste.

### *2.3.2 Operating procedure*

The FI system (Fig. 2) was operated using continuous flow mode, the procedures were described in Table 1. The water carrier (C) was passed through the spectrophotometer for setting the baseline while the reagent solutions (R1 and R2) were flowed through the mixing coils (MC1 and MC2) before starting the analysis. At the beginning of operating sequences, sample solution (S) of 100 µL was loaded into the

sample loop via the injection valve (IV) and was then injected into the carrier stream. The plug of sample was carried through the detector by water carrier for recoding the schlieren signal of sucrose (Fig. 2(a) and step III of Table 1). The sample plug was continuously flowed out of the detector and reacted with the chemical reagents (R1 and R2) at confluence points (Y1 and Y2) to produce PMB (step III of Table 1). Once the sucrose signal returned to the baseline, the 3-WV and then 4-WV were switched to the position that directed PMB to flow back to the detector for spectrophotometric detection (Fig. 2(b) and step IV of Table 1). Sucrose and orthophosphate contents were sequentially detected at 880 nm; hence, a single wavelength spectrophotometer is sufficient for our proposed method.

**Table 1.** The operational steps for the FI system for sequential analysis of sucrose and orthophosphate contents in soft drink and sugarcane juice.

| Step | Operation time | Position of valve for flow direction | | | Duration time | Process | Type of analysis |
|---|---|---|---|---|---|---|---|
| | | IV | 4-WV | 3-WV | | | |
| I | at 8 s | Load | from A to B and from D to C | from E to G | 0–9 s | •100 µL of sample is loaded into injection loop via the injection valve. | Sucrose (maximum signal occurs at 20 s) |
| II | at 10 s | Inject | from A to B and from D to C | from E to G | 10–24 s | •Sample is injected into the carrier stream and passed through the detector. • The plug of sample is continuously flowed out the detector and is then mixed with reagents (R1 and R2) at merging points (Y1 and Y2). | |
| III | at 25 s | Load | from A to B and from D to C | from E to G | 25–49 s | • The injection valve is switched to load position after getting the maximum schlieren signal of sucrose. • The PMB is progressively produced throughout the sample plug. | |
| IV | at 50 s | Load | | from E to H | 50–51 s | •3-WV and then 4-WV are switched respectively to deliver the PMB through the detector. Absorbance of PMB is monitored for orthophosphate analysis | Orthophos-phate (maximum absorbance occurs at 79 s) |
| | at 52 s | | from D to B and from A to C | | 52–185 s | | |
| V | at 186 s | Load | from A to B and from D to C | | 186–187 s | • 4- WV and 3- WV are turned to the starting position, respectively. | |
| | at 188 s | | | from E to G | 188–210 s | Absorbance of PMB reaches to baseline within 210 s | |

## 3. Results and Discussion

### 3.1 Home-made column for sample preparation

In this work, a semi-automatic method was developed for pretreatment of sugarcane juice samples by coupling continuous-flow system with columns (Fig. 1). A set of columns was employed to increase speed of sample preparation. Charcoal and membrane filters were utilized to obtain clarified samples. Charcoal was for decolorization, whereas the membrane filter was for colloidal elimination; hence, no absorbance signal from color and colloid at 880 nm. The optimization was to study two major parameters of the manifold in Fig. 1, the amount of charcoal and pump flow rate. The amount of charcoal was first investigated (n

= 3) at 0.14, 0.28, 0.56 and 1.30 g by fixing the pump flow rate of the diluted sample at 2.6 mL $min^{-1}$. Results were observed that with an excessive amount of charcoal (0.56 and 1.30 g); with flow rate of 2.6 mL $min^{-1}$, it was very difficult to power the sample passing through the column. On the other hand, use of a lesser amount of charcoal (0.14 g) did not yield enough efficiency to decolorize the sample (absorbance ± SD = 0.0389 ± 0.0598). Therefore, 0.28 g of charcoal weight was chosen by balancing the extraction time and the decolorizing efficiency (absorbance ± SD = 0.0002 ± 0.0001). The preparation time was 5 min for collection of 5 mL in each sample. The number of samples that can be prepared simultaneously depends on the amount of tubing that can be equipped to the pump system.

Then, the pump flow rate was also varied in the range of 1.3 to 3.2 mL $min^{-1}$. The flow rate of 2.6 mL $min^{-1}$ was selected since this flow rate yielded the short extraction time (within 5 min for 5 mL in each sample collection) and the extracted samples were colorless.

The optimum conditions for sample preparation are summarized in Table 2.

**Table 2.** Recommended conditions for sample preparation.

| Parameter | Soft drink | | Sugarcane juice | |
|---|---|---|---|---|
| | Studied | Selected | Studied | Selected |
| Degassed sample | Required | | Not applicable | |
| Dilution (sample:water) | 1:0, 1:4 | 1:4 | 1:0, 1:4, 1:10 | 1:4 |
| Column size (mL) | Not applicable | | - | 3.0 |
| Charcoal content (g) | Not applicable | | 0.14 – 1.30 | 0.28 |
| Flow rate of sample loading (mL $min^{-1}$) | Not applicable | | 1.3-3.2 | 2.6 |
| Use of membrane filter | Not applicable | | Yes/No | Yes |

### 3.2 Optimization of the FI system

This work was to design the FI system which sequentially monitored sucrose and orthophosphate by using single wavelength detection. There are 2 parts for system optimization: analysis of sucrose by schlieren effect (no chemical reaction) and analysis of orthophosphate by molybdenum blue reaction. The various parameters and the selected values, for the FI system, are given in Table 3.

#### *3.2.1 Optimization study for sucrose analysis*

The sucrose analysis in the proposed method is reagent free and depends on measuring of the schlieren signal. Dispersion of the sample zone can decrease the magnitude of the schlieren signal. Thus, the first consideration of designated FI system was for sucrose analysis. The sample volume and flow rate were optimized.

To investigate how the schlieren signal and peak shape change with sample volume, repetitive injections of 10 °Bx standard sucrose solution ($n$ = 3) were carried out at various injection volumes ranging from 100 to 1000 µL. The results showed no significant difference of the signal height from various injection volumes. However, using high sample volume will create a large sample zone (longer length) that would result in longer analysis time than using low sample volume. Therefore, 100 µL was preferred as the optimum injection volume.

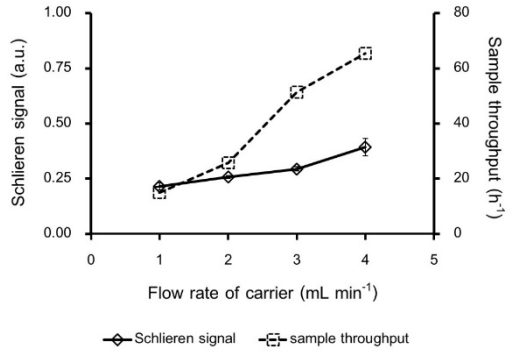


**Fig. 3.** Effect of carrier flow rate on the schlieren signal and throughput obtained from 10 °Bx of sucrose solution.

The effect of carrier flow rate on peak height was studied over the range of 1.0 – 4.0 mL $min^{-1}$. Increasing the carrier flow rate slightly enhanced the schlieren signal (Fig. 3). However, at 4.0 mL $min^{-1}$ flow rate, even increasing the schlieren signal yielded poor reproducibility which was probably due to the turbulent flow. Therefore, in this study, the flow rate of 3.0 mL $min^{-1}$ was chosen.

### *3.2.2 Optimization study for orthophosphate analysis*

In the molybdenum blue method, orthophosphate reacts with molybdate ions in acid solution (R1) to form 12-molybdophosphoric acid (12-MPA), which is later reduced to the PMB by ascorbic acid (R2) [28]. Therefore, the effect of the chemical reagents, R1 and R2, on sensitivity of PMB formation was studied.

The concentration of ammonium molybdate was studied from 0.004 to 0.024 mol $L^{-1}$. Results in Fig. 4(a) indicated sensitivity was increased when increasing concentration of ammonium molybdate solution from 0.004 to 0.016 mol $L^{-1}$ then steady after that. Thus, the concentration of ammonium molybdate at 0.016 mol $L^{-1}$ was chosen in this work.

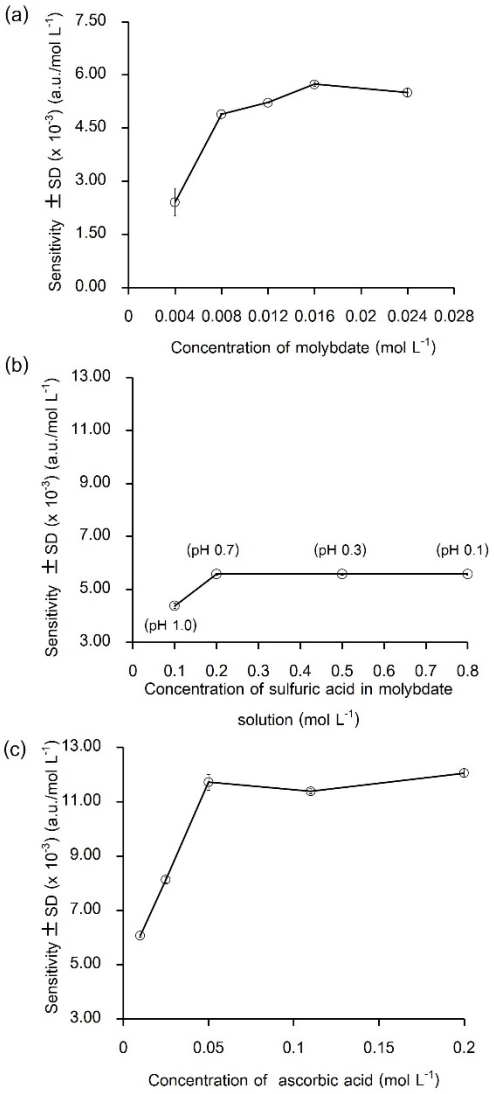


**Fig. 4.** Effect of chemical reagents on sensitivity of PMB formation by molybdenum blue method

Acidity in molybdate solution is one of the crucial parameters for PMB formation. There are reports on suitable pH for PMB formation reaction that it should not greater than 0.9 to prevent auto-reduction of molybdenum in the absence of orthophosphate [28,29]. Moreover, it has been reported that silicate strongly interferes with the molybdenum blue reaction, particularly when pH is more than 0.8 [28]. In this work, acidity of ammonium molybdate solution was adjusted by utilizing

sulfuric acid concentration from 0.1 to 0.8 mol $L^{-1}$ (corresponding to pH of 1.0 to 0.1). Results (Fig. 4(b)) revealed that the sensitivity when acid concentration was from 0.2 to 0.8 mol $L^{-1}$ were comparable. Therefore, 0.8 mol $L^{-1}$ sulfuric acid concentration was selected to ensure acidity of the solution (pH < 1).

The concentration of ascorbic acid can affect the reduction of 12-MPA to PMB. Therefore, the concentration of ascorbic acid was investigated from 0.01 to 0.2 mol $L^{-1}$. Results (Fig. 4(c)) shows that the absorbance increased abruptly when increasing the ascorbic acid concentration up to 0.05 mol $L^{-1}$. For this work, 0.1 mol $L^{-1}$ was selected because it is in the middle range where plateau of the sensitivity was observed so the amount of ascorbic acid was certainly sufficient.

### 3.3 Effect of the MC2 length on peak signals of orthophosphate

From the FI manifold in Fig. 2, the length of MC2 must accomplish two important tasks which are to avoid overlapping of the sucrose and orthophosphate signals, and to obtain the sufficient distance for PMB formation in the system (relating to the step III and IV of Table 1).

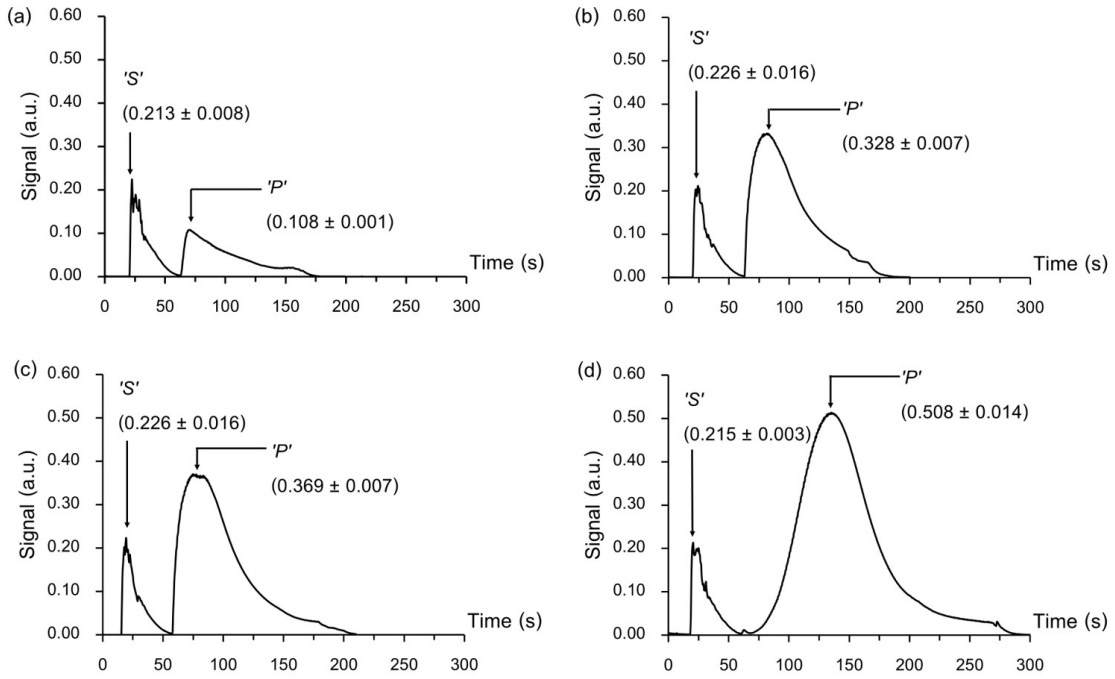


**Fig. 5.** Example of signal profiles to study the effect of MC2 length on absorbance signals of orthophosphate at 0 cm (a), 60 cm (b), 120 cm (c), and 240 cm (d). 'S' and 'P' represents signal obtained from mixed standards, 10 °Bx sucrose and 100 mg $P_2O_5$ $L^{-1}$ orthophosphate.

Suitable length of the MC2 was investigated by evaluating the absorbance signal of PMB when using various coil lengths (Fig. 5). A series of mixed standard sucrose and orthophosphate solutions (10 °Bx and 100 mg $P_2O_5$ $L^{-1}$) were injected into the FI system for optimization of the MC2 length study. The results show that at 0 cm (without MC2), the absorbance signal of orthophosphate was very low because of the small amount of PMB formation before reaching the detector. Increasing the length of MC2 can increase the absorbance signal because it allowed R2 and 12-MPA to

completely mix and form PMB before arriving at the detector. However, the results in Fig. 5 show that increasing the MC2 length also increases the analysis time. Thus, the MC2 length of 120 cm was chosen because it produced a good signal, reasonable analysis time and no signal overlapping between sucrose and orthophosphate. The MC2 length of 240 cm produced a higher absorbance signal for orthophosphate analysis than 120 cm but the analysis time was increased to more than 5 min. In this study, the length of 120 cm provided adequate sensitivity for soft drink and sugarcane juice samples analysis.

**Table 3.** Optimum condition for the FI-spectrophotometric system.

| Parameter | Sucrose analysis | | Orthophosphate analysis | |
|---|---|---|---|---|
| | Studied | Selected | Studied | Selected |
| 1. Sample volume per analysis (μL) | 100-1000 | 100 | - | 100 |
| 2. Flow rate of water carrier (mL $min^{-1}$) | 1.0-4.0 | 3.0 | - | 3.00 |
| 3. Flow rate of reagents (mL $min^{-1}$) | Not applicable | | 0.4-1.4 | 0.6 |
| 4. Length of mixing coil 1 (cm) | Not applicable | | - | 60 |
| 5. Length of mixing coil 2 (cm) | Not applicable | | 0-240 | 120 |
| 6. Concentration of ammonium molybdate (mol $L^{-1}$) | Not applicable | | 0.004-0.024 | 0.016 |
| 7. Concentration of sulfuric acid (mol $L^{-1}$) | Not applicable | | 0.1-0.8 | 0.8 |
| 8. Concentration of ascorbic acid (mol $L^{-1}$) | Not applicable | | 0.01-0.20 | 0.10 |
| 9. Masking reagent | Not applicable | | - | Tartaric acid[a] |

[a]applicable to sugarcane extracts

### 3.4 Effect of cross-interference between sucrose and orthophosphate

We further evaluated the applicability of the proposed FI method (Fig. 2 and Table 1), as interference between sucrose and orthophosphate was a concern. In this study, comparison between single standard and mixed standard was investigated by 1) sucrose concentration at 10 °Bx with orthophosphate concentration from 50-200 mg $P_2O_5$ $L^{-1}$, and 2) orthophosphate concentration of 100 mg $P_2O_5$ $L^{-1}$ with sucrose concentration from 5-20 °Bx. All results (Table 4) show no significant difference between single standard and mixed standard by using paired *t*-test at 95% confidence [30]. Therefore, this could assure no cross-interference between sucrose and orthophosphate.

**Table 4.** Interference between sucrose and orthophosphate measurement ($n$ = 3).

| Type of standard solution | Signal ± SD (a.u.) | Paired *t*-test at 95% confidence |
|---|---|---|
| Single standard of 10°Bx sucrose | 0.226 ± 0.018 | $t_{stat}$ = 0.28, |
| Mixed standard 1 | 0.236 ± 0.018 | $t_{crit}$ = 3.18 |
| Single standard of 100 mg $P_2O_5$ $L^{-1}$ phosphate | 0.364 ± 0.011 | $t_{stat}$ = 0.39, |
| Mixed standard 2 | 0.368 ± 0.009 | $t_{crit}$ = 3.18 |

Mixed standard 1: the concentration of sucrose was fixed at 10 °Bx whereas the concentrations of orthophosphate were varied from 50 to 200 mg $P_2O_5$ $L^{-1}$.
Mixed standard 2: the concentration of orthophosphate was fixed at 100 mg $P_2O_5$ $L^{-1}$ whereas the concentrations of sucrose were varied from 5 to 20 °Bx.
Each concentration was carried out in triplicated injections

### 3.5 Interference from other sugar and silicate

The interference study was focused only the sugarcane juice samples. There are two possible types of interference monosaccharide (glucose and fructose) and silicate ion. In order to determine the tolerance limit, various concentrations of glucose or fructose standards and silicon standard were added to sucrose standard and orthophosphate standard, respectively. The tolerance limits for the interferences are defined as the change in ± 3SD of signal of each pure standard solution (sucrose or orthophosphate alone).

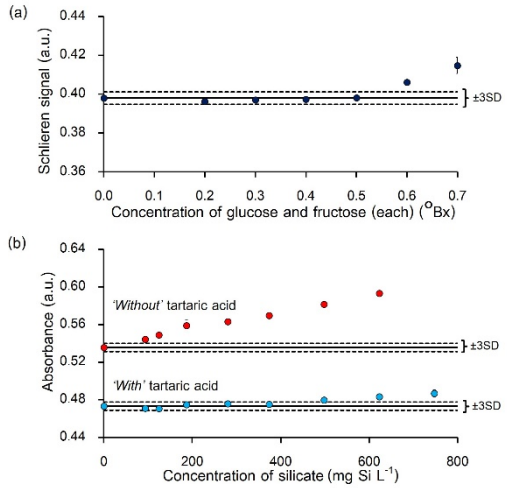


**Fig. 6.** Interference study (a) effect of glucose or fructose in 12 °Bx standard sucrose (b) effect of silicate ion in 75 mg $P_2O_5$ $L^{-1}$ with addition of tartaric acid and without tartaric acid.

Sucrose is the main sugar in sugarcane juice (92% of total sugars contains 88% of sucrose and approximate 4% of glucose and fructose) [1]. The effect of glucose or fructose on single 12 °Bx sucrose standard was studied in the range of 0.2 to 0.7 °Bx. The results in Fig. 6(a) indicate that our method can tolerate interference from glucose and fructose up to 0.5 °Bx (4% of total sugars).

Silicate was considered an interference in the molybdenum blue method. The interference from silicate can be suppressed by adding organic acids such as tartaric acid to the acidic ammonium molybdate solution [28]. Therefore, in this work, tartaric acid was added to R1 and the tolerance limit of silicate was verified (Fig. 6(b)). Without tartaric acid, even small amounts of silicate can interfere with the analysis, and the orthophosphate signals are increased significantly. Adding tartaric acid into the procedures, on the other hand, gave the tolerance limit of silicate 5 times of the orthophosphate concentration (400 mg Si $L^{-1}$ in 75 mg $P_2O_5$ $L^{-1}$ standard orthophosphate). Normally silicate content in samples is approximately just 2 times higher than orthophosphate content [2]. So, silicate interference can be resolved by adding tartaric acid in R1.

### 3.5 Analytical features

Under the selected conditions and operation for the FI-spectrophotometric system in Fig. 2, the final analytical feature of the developed method was examined. Calibration curves for both sucrose and orthophosphate were done in the linear working range of 1 to 50 °Bx sucrose ((peak height, a.u) = (0.032 ± 0.002)[sucrose, °Bx] – (0.071 ± 0.027): $r^2 = 0.993$) and of 5 to 300 mg $P_2O_5$ $L^{-1}$ ((peak height, a.u.) = ((0.033 ± 0.001) x$10^{-1}$)[mg $P_2O_5$ $L^{-1}$] – (0.039 ± 0.010): $r^2$ = 0.999). Mixed standard of 10 °Bx sucrose and 100 mg $P_2O_5$ $L^{-1}$ were replicate injections (n = 9) to obtain system precision of 2.66% and 2.94% (RSD). The limit of detection (3S/N) of 1 °Bx and 1.25 mg $P_2O_5$ $L^{-1}$ was obtained for sucrose and orthophosphate, respectively. Recovery tests of sucrose and orthophosphate in samples ranged from 104 to 106%. Simultaneous measurement of two analytes in sample was carried out within 3.5 min in an analysis cycle.

A comparison of sucrose and orthophosphate detection methods is shown in Table 5. The proposed methods are capable of sucrose and orthophosphate analysis in two different sample matrices (soft drinks and sugarcane juices). The in-house preparative column can be equipped to an automated system which could accelerate

**Table 5.** Comparison of sucrose or orthophosphate detection methods.

| Ref. | Sample matrices: preparation method | Analyte | Analysis system | Detection system | Sample volume | Linearity range (LOD) | Analysis time (Through-put) |
|---|---|---|---|---|---|---|---|
| [10] | ***Sugarcane juice***: Not applicable | Sucrose | BIA | Sensing enzyme thermistor | 25 µL | 0.1–0.5 M (Not reported) | 2-3 min (20-30 $h^{-1}$) |
| [23] | ***Sugarcane juice***: 20-fold dilution ***Fertilizer***: Dissolution and filtration ***Detergent***: Dry ashing, wet digestion, and filtration ***Water***: Filtration and hydrolysis | Phosphate | Batch | UV-VIS spectrophotometer for monitoring absorbance of PMB at 840 nm. | 1000 µL | 0.1-11 ppm (Not reported) | 45 min (~1.3 $h^{-1}$) |
| [18] | ***Soft drinks***: On-line degasser | Sucrose | SIA | 1.) NIR-LED photometer for monitoring schlieren signal at 890±40 nm. 2.) RGB-LED photometer for monitoring absorbance 3.) $C^4D$ for monitoring conductivity | 300 µL | 3.10-46.50°Bx (2.79 °Bx) | 6.5 min (~9 $h^{-1}$) |
| | | Color | | | 800 µL | Not reported (Not reported) | |
| | | Dissolved $CO_2$ | | | 1100 µL | 2.64-5.28 g $L^{-1}$ (2.23 g $L^{-1}$) | |
| [20] | ***Cola drinks***: Degas and one-fold dilution | Sucrose | SIA[a] | PEDD for sequential monitoring schlieren signal (sucrose analysis) and scattering signal (phosphate analysis) at 890 nm. | 1750 µL | 1-7 °Bx (0.5 °Bx) | 171 s (~21 $h^{-1}$) |
| | | Phosphate | | | 500 µL | 50-200 mg $PO_4^{3-}$ $L^{-1}$ (20 mg $PO_4^{3-}$ $L^{-1}$) | |
| [21] | ***Sugarcane juice***: four-fold dilution, color removal, centrifugation, and filtration | Sucrose | SIA[a] | 1.) DAD for dual wavelength detections to measure schlieren signal at 1000 nm (sucrose analysis) and to measure absorbance of PMB at 800 nm and 1000 nm (for orthophosphate analysis). 2.) ISFET | 20 µL | 0.5-5 °Bx (0.2 °Bx) | 5 min (12 $h^{-1}$) |
| | | Orthophos-phate | | | 20 µL | 20-200 mg $P_2O_5$ $L^{-1}$ (5.4 mg $P_2O_5$ $L^{-1}$) | |
| | | pH | | | 1500 µL | 0-14 pH (Not available) | |
| This work | ***Soft drinks***: Degas and four-fold dilution ***Sugarcane juice***: Four-fold dilution and home-made preparation column[b], and filtration | Sucrose | FIA | UV-VIS spectrophotometer for single wavelength detection to measure schlieren signal and absorbance of PMB at 880 nm (for sucrose and orthophosphate). | 100 µL | 1-50 °Bx (1 °Bx) | 3.5 min (~17 $h^{-1}$) |
| | | Orthophos-phate | | | | 5-300 mg $P_2O_5$ $L^{-1}$ (1.25 mg $P_2O_5$ $L^{-1}$) | |

[a]Requires the washing step for cleaning the flow path; [b]Capable of developing an automated system. BIA: Batch injection analysis; SIA: Sequential injection analysis; FIA: Flow injection analysis; $C^4D$: Capacitively coupled contactless conductivity detector; PEDD: Paired emitter-detector diode; DAD: Photodiode-array detector; ISFET: Ion-selective field effect transistor.

the sample preparation, especially for routine analysis. The sample volume, limit of detection and analysis time of the developed method were on average with the others. The linearity range was the dominant analytical feature of the proposed method.

**Table 6.** Analysis of soft drinks and sugarcane juice for sucrose and orthophosphate using the FI-spectrophotometric system as compared with conventional methods.

| Sample | Sucrose (°Bx) | | Orthophosphate (ppm) | |
|---|---|---|---|---|
| | Our method | Refractometry[a] | Our method | Batch method |
| Regular cola1 | 10.60 ± 0.00 | 10.60 ± 0.00 | 112.30 ± 0.01[b] | 106.70 ± 0.00[b] |
| Regular cola2 | 10.50 ± 0.01 | 10.90 ± 0.00 | 114.70 ± 0.00[b] | 102.10 ± 0.01[b] |
| Regular cola3 | 11.20 ± 0.01 | 10.90 ± 0.00 | 98.60 ± 0.01[b] | 101.80 ± 0.00[b] |
| Regular cola4 | 17.71 ± 0.01 | 17.03 ± 0.00 | 112.50 ± 0.00[b] | 112.23 ± 0.00[b] |
| Sugar-free cola1 | n.d. | 0.63 ± 0.00 | 115.60 ± 0.00[b] | 103.30 ± 0.01[b] |
| Sugar-free cola2 | n.d. | 0.65 ± 0.00 | 123.00 ± 0.01[b] | 115.30 ± 0.00[b] |
| Root beer | 11.40 ± 0.01 | 13.30 ± 0.00 | n.d. | n.d. |
| Clear soda1 | 13.50 ± 0.02 | 13.50 ± 0.00 | n.d. | n.d. |
| Clear soda2 | 11.72 ± 0.00 | 12.71 ± 0.00 | n.d. | n.d. |
| Strawberry1 | 13.60 ± 0.01 | 13.30 ± 0.00 | n.d. | n.d. |
| Strawberry2 | 12.57 ± 0.00 | 13.75 ± 0.00 | n.d. | n.d. |
| Orange | 13.12 ± 0.00 | 12.85 ± 0.00 | n.d. | n.d. |
| Sugarcane Juice1 | 15.28 ± 0.10 | 15.57 ± 0.00 | 57.90 ± 0.01[c] | 58.10 ± 0.01[c] |
| Sugarcane Juice2 | 15.61 ± 0.10 | 16.37 ± 0.01 | 61.29 ± 0.01[c] | 63.22 ± 0.01[c] |
| Sugarcane Juice3 | 15.00 ± 0.00 | 15.04 ± 0.00 | 56.21 ± 0.00[c] | 56.45 ± 0.00[c] |
| Sugarcane Juice4 | 15.90 ± 0.00 | 15.31 ± 0.01 | 61.78 ± 0.01[c] | 60.99 ± 0.01[c] |
| Sugarcane Juice5 | 16.41 ± 0.10 | 15.17 ± 0.00 | 62.19 ± 0.00[c] | 57.34 ± 0.01[c] |
| Sugarcane Juice6 | 14.97 ± 0.00 | 15.17 ± 0.00 | 62.42 ± 0.01[c] | 55.07 ± 0.00[c] |

n.d.: non-detectable (< LOD 1°Bx). [a]Refractometer 30PX/GS, Mettler Toledo, USA. [b]in the unit of mg $H_3PO_4$ $L^{-1}$. [c]in the unit of mg $P_2O_5$ $L^{-1}$.

### 3.6 Application and validation

The developed method was applied to soft drinks and sugarcane juice. Validation of the method was done by analyzing sucrose and orthophosphate contents using the proposed FI method against a commercial refractometer and a conventional batch method, respectively. For sucrose analysis, since the proposed method utilized the schlieren effect for detection which has the same principle as a refractometer, the results from both methods were quite similar. As for the orthophosphate, our method and the batch method both employed the molybdenum blue method. However, the experimental procedures were somewhat dissimilar. If interference, such as organic acids, silicate, sulfate, iron and chloride [3,31] is present in the sample, the results from both methods probably diverge, sugarcane juice 5 and 6. In this case quantification by standard addition method is suggested. According to the results in Table 6, the Analysis of Variance (ANOVA: single factor) [30] shows that the results of both analytes are not significantly different to the conventional methods at 95% confidence limit ($F_{stat}$ = 0.022, $F_{crit}$ = 4.026) which indicated that the accuracy and reliability of the developed method is well acceptable.

## 4. Conclusion

This work presents an alternate method which can be effectively implemented for routine analysis of sucrose and orthophosphate in soft drinks and sugarcane juice for quality assessment. With this system, two analytes were both measured within 3.5 min per analysis cycle. The proposed FI system also provides sufficient sensitivity, adequate accuracy and precision. In addition, only a small amount of sample (100 µL) was used for analysis.

Besides the monitoring system, we have also proposed a simple sample preparation method. The method involves sample dilution before pretreatment of turbid and intense color of samples by homemade

charcoal column which can be arranged for single sample or multiple samples at a time.

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