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Simple Lab-on-chip with Light Scattering Measurement for Determination of Sweetness in Grape Juices

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Abstract

A simple lab-on-chip with reflective optical fiber system was developed for determination of sweetness in grape juice samples. The detection principle is based on the measurement of light scattering due to Schlieren effect that occurs when a sample solution containing sugar merges with another liquid (DI water) of different refractive indices. The degree of Schlieren effect and thus, the intensity of scattered light, relates to the concentration of sugar in the sample. The angle of the LOC at 65° with respect to the horizontal plane was found to be suitable to provide a well-defined response peak shape for easy peak area integration. The system was evaluated at detection wavelengths 700, 750, and 800 nm. The linear range of the sugar contents of 7-20 degree Brix (°Bx), and RSD of less than 5% (n=5), were achieved for the three detection wavelengths. The results obtained from the red and white grape juice samples agree well with the reading from the refractometer.

Keywords Lab-on-chip, sweetness, light scattering, Schlieren effect, grape juice

1. Introduction

A simple lab-on-chip (LOC) made of low cost acrylic plastic pieces was introduced by Grudpan *et al.* in 2009 and its initial usages were conducted with naked eye-time based detection [1,2]. Since then, the LOC has been coupled with other detectors such as web cam and optical fibers to improve the detection capability and expand the applications of the system. The LOC has also been applied in conjunction with some low cost reagents and software. Various unique green analytical chemistry methodologies have been demonstrated including acidity assay from %RGB change of phenolphthalein [3], acidity assay using natural extract from flowers [4,5], and iron assay using tea solution as chromogenic reagents [4].

Schlieren effect, a common phenomenon encountered in spectrophotometric flow based analysis method, was also proposed for determination of sugar content in syrup using the simple LOC with both naked eye and optical fiber detection [6]. Schlieren effect is often considered as a limitation in flow based analysis when coupling with spectrophotometric detection. When liquids of different densities or refractive indices are merged, Schlieren effect will cause the change in spectrophotometric response that may interfere with the measurement of the analyte or reaction of interest. However, Ardnaree *et al.* made use of this phenomenon for sugar content analysis by measuring timing (migration time) of the sample zone to an observing point. The migration time depends on viscosity and concentration of sugar solution. In this time-based system, the angle of the light source and the detector (fiber-optical spectrophotometer) was set at 180°. Despite the satisfactory results reported for syrup samples with high sugar contents, the method was not applicable for samples with low sugar contents e.g. less than 50 °Bx. This is because it was difficult to differentiate the migration times based on Schlieren effect of solutions with low sugar concentrations, even though optical fibers were used to aid in the signal recording step.

Therefore, this current work aims to improve the simple LOC-optical fiber system for the measurement of sweetness of samples with low sugar content by measuring light scattering intensity directly rather than measurement of the migration time. The reflective fiber optic bundle unit usually used for light reflection and back scattering techniques, in which the measurement is conducted at angles other than 180°, was employed. This optical fiber is designed to detect scattered light from more than one angle. This is more suitable to the nature of light scattering in which light reflection occurs at various angles. The system was applied to determine the sweetness of red and white grape juice samples with sugar contents of less than 20 °Bx, to demonstrate the detection capability of the system.

2. Materials and methods

2.1 Standard and sample preparation

Stock standard sucrose solution was prepared by weighing 66.67 g sucrose, adding DI water to obtain the final total weight of 120 g, and stirring until sucrose was completely dissolved. As sugar content in the unit of °Bx is defined as grams of sugar in 100 grams of solution, the concentration of this stock solution was 55.56 °Bx. Working sucrose standard solutions were prepared by diluting the stock standard solution with DI water to the desired concentrations.

Grape juice samples (both red and white), taken from a local market, were filtered with 0.45 µm nylon membrane filter (13 mm diameter: Filtrex, USA) prior to being injected into the LOC, without dilution. This is to remove particulates in the samples which may cause false positive in the measurement of light scattering.

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2.2 Lab-on-chip set up

The simple chip was made of an acrylic piece (20 mm x 30 mm x 12 mm WLH, painted black). The channels (1.2 mm ID, total internal volume 50 µL) were drilled through the chip in both vertical and horizontal directions for reagent/sample interaction at the crossing point. The reacted mixture passed through the detection point located 10 mm from the crossing point. One of the 2 open ends of the vertical channels was connected to a solenoid pump, (Pump A, Biochem Valve, USA) where a carrier was introduced. One of the 2 open ends of the horizontal channels was for inserting a syringe to inject standard/sample solution. The remaining 2 open ends were connected to solenoid valves (Valve A and Valve B, Takasago, Japan) where waste was delivered out of the system. Another piece of black acrylic is attached to the chip as a protective layer to prevent light from outside during detection step. These components were installed onto an adjustable base in which the angle of the chip with respect to the horizontal ground level could be adjusted as desired. Figure 1 (a) shows the picture of the chip and other components, and the diagrams in Figure 1 (b) and (c) illustrate the system for an easy viewing.

The operational step was started by opening valve A, then filling the vertical line with DI water using pump A. Then valve A was closed and the recorder was started using the program Ocean Optic Spectra Suite. After that, valve B was opened for injection of 0.3 mL standard/sample solution into the horizontal line using a 1.0 mL syringe, and then the valve was closed. The standard/sample solution was carried from the crossing point to the detection point where the reflective absorption fiber optic probe (R200-7-UV-VIS, Ocean Optics Inc., USA) was installed. Another end of the probe was connected to a light source (360-2000 nm, LS-1 Tungsten Halogen Light Source, Ocean Optics Inc., USA) and a detector (200-1100 nm, Ocean Optics USB4000 Spectrometer, Ocean Optics Inc., USA). Finally, after 100s, the recorder was stopped and valve A and valve B were opened to let waste out. The operations of pump A, valve A and B were controlled with a control box through a 12 volt DC power supply that gave electrical current to the control switches of the pump and valves. This system provides a more automatic analysis as compared to the previous LOC version [6]. By doing this, signal due to the light scattering intensity can be continuously recorded when the procedure proceeds and signal (relative light intensity) *vs* signal accumulation time can be plotted (Figure 3). The chip was washed with DI water before starting the next injection and each standard/sample was analyzed with 3 replicates. The chip was reused for months.

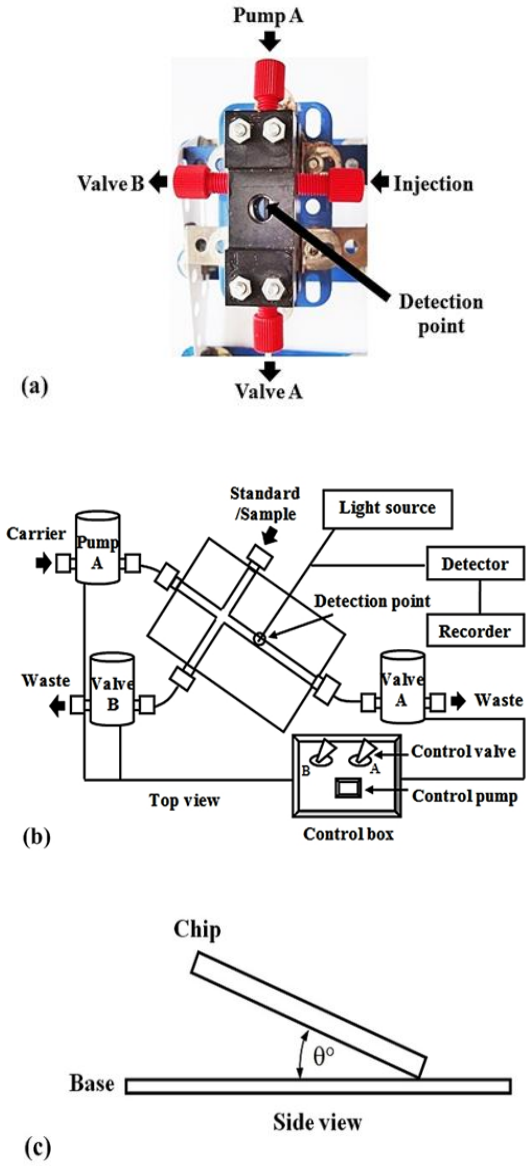

Figure 1. (a) Components of the simple LOC, (b) the diagram of the overall system showing the LOC connected to pumps, valves and a control box, and (c) the side view diagram showing the angle of the LOC with respect to the horizontal plane.

3. Results and discussion

Light scattering was measured covering most the area where Schlieren effect took place rather than just for the parts that involving the migration time. This was done by applying the reflective optical fiber bundle (R200-7-UV-VIS) that is usually employed in light reflection and back scattering techniques. The efficiency of light scattering measurement would depend on the measurement angle (detector *vs* light source) which should be an angle that is free from interferences from the light source itself. This concept has been used for quantitative analysis of turbid samples, such as in nephelometry. For the sample that has intense color or highly turbid, multiple scattering and absorption of scattered light may cause inaccurate measurement. The detector design so called "surface scatter" is used to measure reflective light from the surface of the sample by focusing the light beam on the sample surface and generates scattered light toward a photodetector that is also located above the sample surface. The light beam penetrates less into the sample of high turbidity. The response due to short light path compensates for interference from multiple scattering [7].

Although the angle of scattered light from Schlieren effect is unpredictable from run to run because the migration profile of one liquid into another liquid cannot be completely controlled, but it should be consistent for the same sets of liquids under the same conditions of the run. In this work, the commercial reflection optical fiber bundle was employed and was put for detecting scattered light from different angles surrounding the center area where scattering originates and for reducing interference from the light source as well as minimizing the effect from dark colored samples. Details and diagram of this fiber optic unit can be found on the manufacturer's website [8].

Briefly, the light source illuminates the sample through a bundle of 6 small optical fibers. The incident light beams exit from each of the 6 fibers as 19° cones and shine onto the sample. The size of area, where reflections and/or scattering (Schlieren effect) occur, relates to the sugar content in the migrated sample solution. By using a bundle of optical fibers rather than a single fiber to deliver light from the light source, a wider detection area can be illuminated. Reflection and/or scattering of light from different angles can be detected by a single receptor fiber located in the middle of the fiber bundle. This helps to reduce error as compared to the measurement of scattered light from only one angle of 180° as in some other optical fiber designs.

3.1 Detection wavelengths

Although measurement of the scattered light can be carried out at any wavelength, to minimize error due to light absorption by the samples, the detection should be done outside the absorption range. To find a suitable detection scattering wavelength, absorption spectra of red and white grape juices (40 folds dilution with DI water) were scanned with a UV-Vis spectrophotometer (model 1800, Shimadzu Co., Japan) in the range of 200-800 nm. Figure 2 indicates that grape juice absorbs light in the range of 200-650 nm. Therefore, to avoid error due to light absorption, higher detection wavelengths at 700, 750, and 800 nm were selected for measuring light scattering.

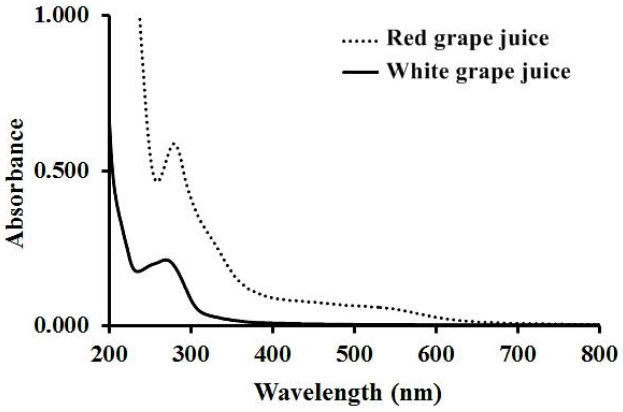

Figure 2. Absorption spectra of red and white grape juices (with 40 folds dilution).

3.2 Detection angle of the chip

Angle of the chip with respect to the ground plane significantly affects the migration process of the liquid sample and the degree of Schlieren effect which in turns affects the peak shape. Ardnaree *et al.* [6] reported the angle of 60^{o} for determination of high sugar content in syrups by using the migration time from the injection point to detection point. However, in this current work, we aim to apply the system using samples with low sugar contents of less than 20 °Bx by choosing a more suitable angle of the chip that will create a high degree of Schlieren effect. This chip angle should yield the best response signal shape that enables easy identification of the peak base and facilitates accurate integration of the peak area.

Sucrose solutions of concentrations in the range of 7-20 °Bx were injected into the chip that was adjusted to various angles with respect to the ground plane. It was found that when the chip was set at lower than 60°, there was no difference between signals of standard sucrose solutions and DI water. Experiments carried out in the chip at higher angles (60, 65, and 70°) resulted in signals involving light scattering due to Schlieren effect as shown in Figure 3. From the graphs (Figure 3), total relative light scattering intensity signal of each run could be obtained. The signal of light scattering intensity was recorded by the spectrophotometer in the absorbance mode, and therefore shows higher negative signal with higher light scattering. The angle of 65° yielded the best well defined peak shape (narrower and sharper peaks). From signals shown, the peak of interest can be obtained during the recording step as short as 80s after sample injection.

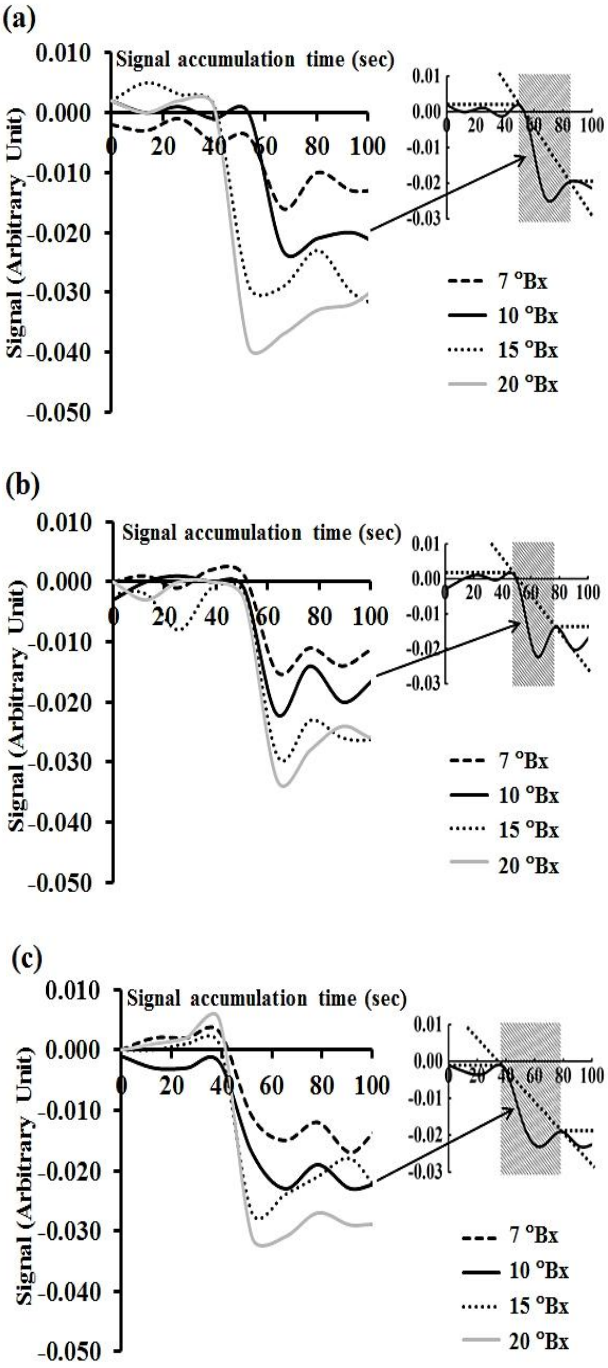

Figure 3. Response signals from standard sucrose solutions (7-20 °Bx) obtained at 700 nm, with data acquisition frequency of 1 point per sec, using various chip angles (a) 60, (b) 65, and (c) 70 degrees. The inset shows an example of peak integration of the light scattering signal due to 10 °Bx solution.

3.3 Performance of the system

From the graphs (Figure 3), total relative light scattering intensity signal of each run could be obtained by integrating peak area after smoothing the recorded data, to get better base line, as demonstrated in the insets in Figure 3. This was done by using the software Origin Pro 8.0. The total relative light intensity (peak area) was used to plot against concentration to yield a calibration graph.

Using the selected conditions, calibration graphs were prepared from peak areas of standard sucrose solutions of concentrations 7-20 °Bx obtained using the detection wavelengths at 700, 750, and 800 nm. Table 1 summarizes linear range, linear regression equations and R^2. All three detection wavelengths yielded $R^2 > 0.99$. Precision of the system was investigated by injecting 5 replicates each of standard sucrose solutions at the concentrations of 7 and 15 °Bx. Percent RSDs of the light scattering intensities of each run of each concentration, as peak areas, were less than 5% at all the three detection wavelengths, as summarized in Table 2. It can be concluded that the results from all the three detection wavelengths are not significantly different and that any of these detection wavelengths can be used.

Table 1. Summarization of linear calibrations obtained at different detection wavelengths

Wavelength (nm)	Linear range (°Bx)	Linear regression equation	R^2
700	7.0 - 20.0	Y = 0.049X - 0.152	0.993
750	7.0 - 20.0	Y = 0.058X - 0.161	0.994
800	7.0 - 20.0	Y = 0.058X - 0.201	0.994

Table 2. Precision of peak areas (5 replicates) of standard sucrose solutions (7 and 15 °Bx)

Run No.	Total relative light scattering intensity (peak area; Arbitrary Unit.sec)					
	7 °Bx			15 °Bx		
	700 nm	750 nm	800 nm	700 nm	750 nm	800 nm
1	0.196	0.252	0.233	0.593	0.643	0.620
2	0.199	0.244	0.219	0.643	0.648	0.647
3	0.208	0.242	0.245	0.604	0.706	0.686
4	0.202	0.253	0.235	0.586	0.656	0.645
5	0.217	0.232	0.235	0.638	0.661	0.625
Mean	0.204	0.245	0.233	0.613	0.663	0.645
SD	0.008	0.009	0.009	0.026	0.025	0.026
%RSD	3.92	3.67	3.86	4.24	3.77	4.03

3.4 Analysis of sucrose contents in real samples

Sweetness of grape juice samples was investigated through sugar content analysis. Eleven samples, 9 red grape juice (RG) and 2 white grape juice (WG), purchased from local supermarkets in Thailand were analyzed with both the proposed simple LOC-optical fiber light scattering system and the direct reading of a refractometer. The results in Table 3 show that the two methods are not significantly different at 95% confidence as compared using paired t-test.

Table 3. Comparison of sucrose contents found in the grape juice samples using the Lab-on-chip at different detection wavelengths and using the refractometer (triplicate results)

Sample No.	[Sucrose] °Bx			
	Lab-on-chip			Refractometer
	700 nm	750 nm	800 nm	
RG1	12.5±0.2	12.6±0.2	12.8±0.1	13.0±0.1
RG2	13.2±0.3	12.9±0.3	13.3±0.4	13.7±0.1
RG3	12.5±0.2	13.0±0.0	13.0±0.0	13.5±0.0
RG4	13.1±0.3	13.1±0.5	13.6±0.2	14.0±0.1
RG5	11.8±0.2	11.8±0.5	11.9±0.2	12.5±0.0
RG6	15.4±0.2	15.1±0.4	15.1±0.4	15.8±0.1
RG7	12.3±0.2	12.7±0.2	12.6±0.4	13.0±0.1
RG8	16.3±0.2	17.1±0.2	16.5±0.2	16.9±0.1
RG9	13.6±0.3	13.6±0.3	13.1±0.2	14.4±0.1
WG1	17.5±0.5	17.2±0.3	16.6±0.1	16.3±0.1
WG2	15.7±0.1	15.7±0.3	16.1±0.3	15.3±0.1

4. Conclusion

This report demonstrates the coupling of the simple LOC for determination of sweetness via the measuring of "total light scattering" due to the Schlieren effect, which is different from the previous report [6] that made use of Schlieren effect to measure the "migration time" of the sample zone. This current system enables the analysis of samples with lower sugar content (less than 20 °Bx) which may be a model for the development of LOC-light scattering systems for other applications.

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