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Exploiting a Simple Water Extract of a Flower as a Natural Reagent for Acidity Assay Using a Lab-on-Chip☆

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Abstract

A simple water extract of a flower was exploited as a natural reagent for acidity assay. The natural reagent from Gomphrena globosa was obtained by simple extraction and introduced into a simple lab-on-chip (LOC). The natural reagent adjusted to pH 9 was loaded into the vertical channel of the LOC, and then acetic acid standard/sample was injected into the horizonal channel of the LOC. The injected acetic acid solution migrated downward and merged with the natural reagent. The absorbance spectrum was monitored at the detection point. A liner calibration curve for acetic acid was obtained in the range of 3–7 % (w/w). The proposed method was applied to the determination of acetic acid in vinegar samples.

Keywords Natural reagent, lab-on-chip, acetic acid

1. Introduction

In chemical analysis, many chemicals are used and wasted. In some cases toxic chemicals are used. That may cause contamination of the environment. From the point of view of green chemistry, it is desirable that these wastes are reduced.

Manz *et al.* [1] proposed the concept of micro total analysis system (μTAS) in 1990. In μTAS called on lab-on-a-chip, all devices such as a pump, a valve and a detector are integrated on a small chip, and therefore, it has great advantage in the reduction of reagent consumption, simplicity of the analysis system and rapidity of the analysis. The field of μTAS has been growing rapidly and the trend of this field was reviewed [2–4]. Recently, Grudpan *et al.* [5] developed a simple and economical lab-on-chip (LOC) with time-based detection. Four chemical reactions were introduced into the LOC and it was applied to the determination of ascorbic acid, acetic acid and iron in real samples. But the colored reaction zone was detected visually by naked eyes and using a stopwatch.

The use of reagents from natural materials like plants is one of the environment-friendly methods. Uchiyama *et al.* [6,7] developed an amperometric flow injection (FI) analysis system using cucumber juice as a carrier solution for the measurement of ascorbic acid. Ascorbate oxidase in the cucumber catalyzes the oxidation of ascorbic acid. The oxygen consumed in this reaction was detected by an oxygen electrode. A guava leaf extract was exploited for FI determination of iron [8]. The guava leaf extract formed a colored complex with iron and it was detected by a spectrometer, and a normal FI system, a reverse FI system and a column FI system were tested for the determination of iron at ppm levels. A green tea extract was also used for quantification of iron [9].

Some plants contain water-soluble pigments like anthocyanin or betacyanin compounds [10,11]. The chemical structures of those pigments depend on pH. Therefore, the extract containing anthocyanins or betacyanins can be used as a pH indicator. Gomphrena globosa contains betacyanins in its petal [12–15]. The extract from it changes from yellow in basic solution to reddish purple in acidic solution.

In the present study, a simple water extract of a flower was exploited as natural reagent for acidity assay. Acid-base neutralization reaction with the natural reagent was introduced into a simple LOC, and the color change was detected with a reflection/backscattering fiber optic probe coupled to a spectrometer. The natural reagent from Gomphrena globosa was obtained by simple extraction and its spectral characteristic was studied. The proposed method was applied to the determination of acetic acid in vinegar samples.

2. Experimental

2.1. Reagents

2.1.1. Natural reagent

A 0.7 g portion of dry petal of Gomphrena globosa was put into 50 mL of hot water at 60–70°C and kept for an hour. Boiling water is not suitable for extraction because the structure of the dye might change or decompose by the high temperature. After extraction, petals were removed by filtering the extract through filter paper. The extract could be kept at least for three days in the refrigerator. Before loading it into the LOC system, it was adjusted to pH 9 using 1 M sodium hydroxide solution.

2.1.2. Standard acetic acid solution

A 20 % (w/w) stock solution of acetic acid was prepared by diluting glacial acetic acid (≧99%, Analytical reagent grade, LAB-SCAN, Ireland) with water. The standardization of the stock standard solution was carried out by the titration method using sodium hydroxide. Working solutions of acetic acid were daily prepared by dilution of the stock solution with water.

2.2. Instrumentation

The diagram of the LOC system used in this work is shown in Fig. 1. This system consists of a chip unit (Fig. 2), two solenoid pumps, two solenoid valves and a detector. The LOC was made

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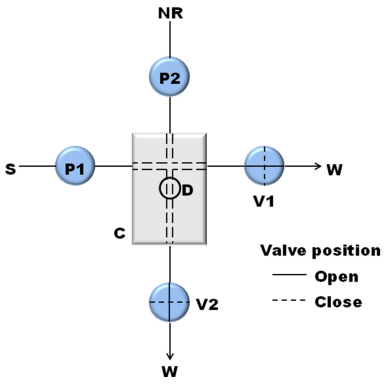

Fig. 1 Schematic diagram of the LOC system

C: Chip, D: Detection point, P1 and P2: Solenoid pumps, V1 and V2: Solenoid valves, NR: Natural reagent, S: Sample/Standard, W: Waste

as described in a previous report [5]. The chip is an acrylic piece (2 cm × 3 cm × 1 cm) with two channels (1 mm i.d.) crossed perpendicularly. As shown in Fig. 2, the chip was tilted at 20° angle with respect to the horizontal in order to give it gravimetric force. The threads were made at each channel opening to fit a 1/4 inch nut. The chip was put between two acrylics. The surface of the chip unit was colored black to avoid interference from outside light. A spectrophotometer (USB 2000, Ocean Optics Inc., USA) with a reflection/backscattering fiber optic probe (R200-7-UV-VIS, Ocean Optics Inc., USA) and a tungsten halogen light source (LS-1-LL, Ocean Optics Inc., USA) was used as the detector. To place the fiber optic head at the detection point on the chip, the top acrylic of the chip unit was drilled to provide a hole. The detection point was set about 5 mm down from the junction of the vertical channel and the horizontal channel. Two solenoid pumps (Biochem valve, USA) were used to load the natural reagent and the acetic acid standard/sample into each channel. Both pumps were connected to inlets of the channels. Two solenoid valves (Takasago, Japan) were connected to outlets of the channels. These pumps and valves were controlled by switches [16]. FIAlab software was used to record absorbance spectra at the detection point.

2.3. Operating procedure

First, V1 was opened and V2 was closed. An acetic acid standard/sample was loaded into the horizontal channel by P1. After that, V1 was closed and V2 was opened. The natural reagent adjusted to pH 9 was pumped into the vertical channel by P2. After loading the reagent, V2 was closed and V1 was opened again. P1 was switched once to load the standard/sample. At the same time, the program for measurement of absorbance spectra at the detection point was started. Afterward, V1 was closed immediately. The loaded acetic acid standard/sample migrated toward the detection point and merged with the natural reagent. The migration occurred from the difference in concentration of the reagent and sample zones which was helped by capillary action and gravimetric force [5]. This resulted in the change in pH and the color of the natural reagent was changed.

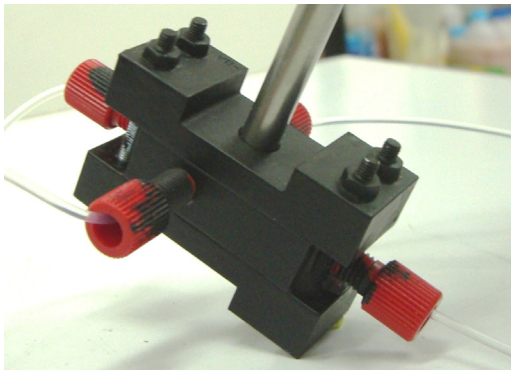

Fig .2 Photograph of the chip unit

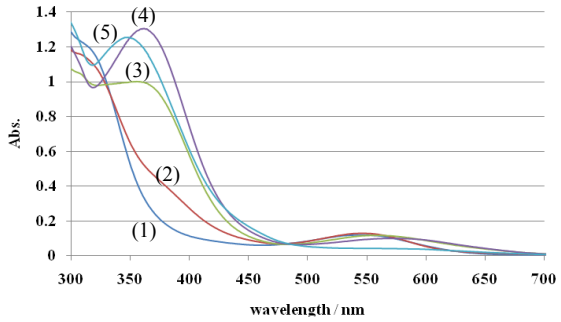

Fig.3 Absorbance spectra of the extract from Gomphrena globosa at various pH value

(1): pH 1, (2): pH 8, (3): pH 9, (4): pH 12, (5): pH 13

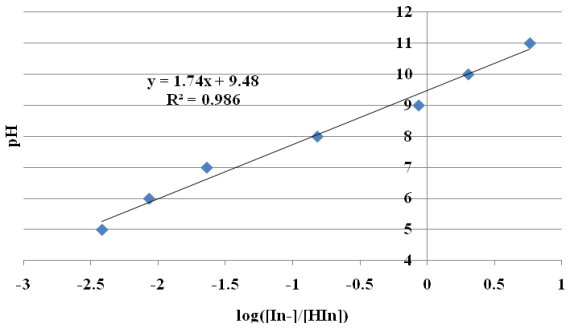

Fig. 4 A plot of pH as a function of log([In$^-$]/[HIn]).

3. Results and discussion

3.1. Spectral characteristic of the extract

Absorption spectra of the extract at various pH values were measured by a batchwise method. The extract was adjusted to pH from 1 to 13 using hydrochloric acid solution (for pH 1–3), acetic acid solution (for pH 4–6) and sodium hydroxide solution (for pH 7–13). The extract was diluted four folds through the pH adjustment. The absorption spectrum of the solution was

Table 1 Correlation coefficient values of calibration curves for acetic acid and repeatability at various conditions

Waiting	400 nm		545 nm		580 nm		600 nm	
time / s	r^2 [a]	RSD[b], %	r^2 [a]	RSD[b], %	r^2 [a]	RSD[b], %	r^2 [a]	RSD[b], %
10	0.668	18.1	0.902	3.27	0.922	4.18	0.800	10.6
15	0.910	21.0	0.932	3.11	0.999	2.55	0.957	9.63
20	0.742	14.7	0.948	4.25	0.862	5.11	0.706	10.5
25	0.584	10.7	0.957	4.11	0.896	4.10	0.780	8.83

a. Correlation coefficient values of calibration curves for 1–7% (w/w) acetic acid.
b. Relative standard deviation values of ten determinations of 5% (w/w) acetic acid.

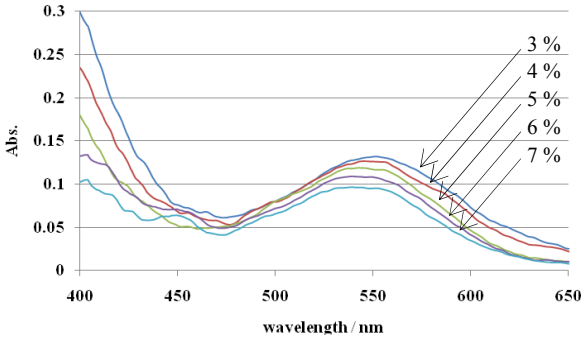

Fig. 5 Absorbance spectra at the detection point (the spectra were measured exactly 15 s after the loading acetic acid solution)

Table 2 Determination of acetic acid in vinegar samples

Sample	Concentration of acetic acid / % (w/w)[a]	
	Proposed method	Titration method
1	4.55±0.17	4.37±0.04
2	5.19±0.21	5.04±0.01
3	5.24±0.21	5.41±0.03
4	5.20±0.13	5.13±0.03
5	5.66±0.14	5.37±0.01

a. Average value and standard deviation for three determinations.

measured by a spectrophotometer (SHIMADZU, UV-1800, Japan). As can be seen in Fig. 3, at lower pH, the extract has maximum absorption at 545 nm, and a red shift occurred with an increase in pH. On the other hand, the maximum absorption was observed at 360 nm in the basic solution. At pH 13, a blue shift was observed.

The pK_a value of this extract was estimated from the intercept of the plot of pH as a function of log([In^-]/[HIn]). Results are shown in Fig. 4. The estimated pK_a was 9.48.

3.2. Starting pH of the natural reagent

The starting pH of the natural reagent was chosen based on the characteristic spectra. A pH at or above 9 is suitable for acidity assay, as a change of the absorbance is observed at lower pH than pH 9. But the absorbance of the extract was unstable at pH above 11. Taking into consideration the spectral characteristics and the stability of the absorbance of the extract, we chose pH 9 as the starting pH.

3.3. Selection of the waiting time before measurement and wavelength

Capillary action and gravimetric force caused the penetration of a loaded acetic acid standard solution into a natural reagent. Therefore, it was necessary to figure out the suitable waiting time which a sample zone passed through the detection point. We examined the effect of waiting time before the measurement of the absorption spectrum. The absorption spectra were measured at exactly 10, 15, 20 and 25 s after the loading of acetic acid standard solutions in the range of 1–7% (w/w). Based on the results, we prepared calibration curves for acetic acid with different wavelengths (400–600 nm). Linearity and repeatability of the calibration curves at various conditions are shown in Table 1. The better calibration curve was obtained at 15 s for the waiting time and 580 nm for the wavelength. The data source of the calibration curve is shown in Fig. 5. As can be seen in Fig. 5, the absorbance at 580 nm decreased proportionally to the concentration of acetic acid.

3.4. Calibration curve for acetic acid

A liner calibration curve for acetic acid was obtained in the range of 3 to 7 % (w/w). The equation was $Y = -0.0110X + 0.137$ with a correlation coefficient of 0.999, where Y is the absorbance at 580 nm and X is the concentration of acetic acid in % (w/w). The relative standard deviation was 2.55 % for ten determinations of 5 % acetic acid standard solution.

3.5. Application

This LOC system using natural reagent was applied to determination of acetic acid in vinegar samples. All samples were purchased from a store and labeled as 5% (w/w). The analytical values were obtained by the calibration curve method. Results are summarized in Table 2. A paired t-test was performed on the data obtained by the proposed method and the titration method. The experimental t-value was 1.350 which is less than the critical t-value at the 95% confidence level for four degrees of freedom (2.776). The statistical analysis revealed that there is no significant difference between the two methods.

4. Conclusion

The acidity assay using the simple LOC with the natural reagent was demonstrated. The proposed method was applied to the acetic acid determination in vinegar samples. This method is a cost-effective and environment-friendly analysis. The use of the natural reagent is expected to be applied to other chemical analysis.

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