Cyclic Flow Injection Analysis with Spectrophotometric Detection for Assay of Acidity in Fruit Juices and Soft Drinks

Michio Zenki, Masako Kojima and Takashi Yokoyama

Department of Chemistry, Faculty of Science, Okayama University of Science,

1-1, Ridai-cho, Okayama, 700-0005, Japan

Abstract

A single-line flow system, through which a reagent carrier stream is circulated, is proposed for the spectrophotometric determination of acidity (expressed as citric acid content) in fruit juices and soft drinks. The reagent carrier solution consists of pH indicator (Methyl Orange, MO) and sugar (sucrose) in an acetate buffer solution. The method is based on the acid-base neutralization detecting the color change of MO. The resulting solution, after passing through a flow through cell, returns to the reservoir and reused repeatedly. Sucrose is added in the reagent carrier solution to compensate both the differences in refractive index and viscosity between the reagent carrier solution and the sample solution, which make the sample injection directly without any dilution process. Several parameters were optimized, resulting in a simple, fast, and ready constructed acidity analyzer. Fruits juices and soft drinks could be analyzed without any prior chemical treatment.

Keywords Acidity, citric acid, cyclic flow injection analysis, fruit juices and soft drinks

1. Introduction

Among several organic acids, citric acid is the major acid in fruit juices. It is frequently added to the industrial drinking derivatives to keep quality and flavor of products constant. The acidity is determined routinely during the processing of drinking products. It is expressed usually as citric acid content. Commonly used techniques for this analysis is titration with the standard base[1]. Normally all batch titrations involve quite high volume of reagents consumption and manually operations which are subjected to time consuming and personal error. Therefore, an on-line, near real time acidity analyzer is very desirable. Flow injection analysis (FIA) is widely accepted as an analytical technique capable to perform rapid analysis, and offers many advantages over batch titration [2-4]. For the assay of acidity in fruit juices, several procedures [5-7] based on the flow injection method have been proposed employing mixing chamber or variation in reagent flow rate, using spectrophotometry or potentiometry as detection techniques.

Imato and Ishibashi reported the spectrophotometric FIA determination of strong acids and bases using a pH indicator and buffer solution [8,9]. This method is excellent because the estimation of end point is not necessary. The calibration graph can be constructed from the peak heights (absorbances) of the standard solutions of various concentrations. We developed this method into a cyclic FIA for the determination of strong acids and bases, where the reagent carrier solution was circulated and reused repeatedly [10]. Recently we found this cyclic FIA was applicable to citric acid determination in a similar manner as above.

In quality control, actual sample analysis without any prior chemical treatment should be desirable in order to decrease sample handling steps while also saving time of analysis. These facilities can be provided by exploiting the direct determination with the simplest manifold. This paper therefore describes the modifications and improvements made to the spectrophotometric FIA determination of citric acid, estimating the direct injection of sample solution without any dilution procedures, thereby facilitating the routine determination of acidity in fruit juices and soft drinks.
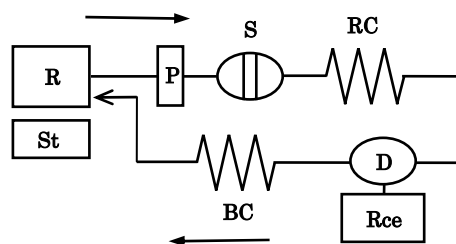

Fig. 1 Schematic diagram of cyclic flow injection system. R: reservoir, P: pump, S: sample injector, RC: reaction coil, D: detector, Rec: recorder, BC: backpressure coil, St: stirrer.

2. Experimental

2.1. Apparatus

A schematic diagram of cyclic FIA used in this work is shown in Fig. 1 [10-12]. It consisted of a single line manifold; an one-channel peristaltic pump (CRP-01, Kurahashi Giken, Japan), a rotary injection valve with 5μl sampling loop (9725, Rheodyne, USA), a visible spectrophotometer (S-3250, Soma Kougaku, Japan) equipped with a flow through cell (10 mm light pass, 18 μl inner volume), and a strip chart recorder (U-228, Nippon Denshi, Japan). Flow lines were made of PTFE tubing (0.5 mm i.d.) and connectors.

2.2. Reagent and sample

All chemicals were of analytical reagent grade and de-ionized water was used for the preparation of all reagents. Methyl Orange (MO), citric acid and sucrose were purchased from Wako Pure Chemical Industries (Osaka, Japan).

The series of standard citric acid solutions was prepared in 12% (w/v) sucrose solution to match the sample matrix.

Commercially available apple, grape and orange juices and soft drinks were filtered through a membrane filter (0.45 μm) to

remove solids. These samples are injected directly without any manual dilution.

2.3. Procedure

A typical circulating reagent solution was made up of 100-ml containing 2.5×10^{-4} M MO, 12% (w/v) sucrose in 0.01 M acetate buffer (pH 4.5). This solution in the reservoir was constantly stirred with the help of a magnetic stirrer. The sample solution was injected into the reagent carrier stream with a rotary injection valve, and the color change of MO was measured immediately (at 543 nm). The concentrations of citric acid were calculated from their peak-heights. Then the reagent carrier stream was carried back to the reservoir *via* a back pressure coil (0.5 mm i.d., 5 m long) to reuse repeatedly. A flow rate of circulating solution was 0.9 ml min^{-1}.

2.4. Principle of circulation

When a sample solution is inserted in the flow system, it mixes with and partially neutralizes the reagent carrier solution, causing the discoloration of an indicator (detected as a peak height), which is proportional to the acidity of sample solution. After passing through the flow through cell, subsequent dispersion and mixing occur in the back-pressure coil. Since the circulating reagent solution is buffered with acetate to resist the changes in pH upon the addition of acids, the color of MO returns to the original, which makes the system reversible. The resulting solution then comes back to the reservoir and reuses repeatedly.

3. Results and discussion

3.1. Circulating reagent carrier solution

Preliminary tests have revealed that MO in acetate buffer (pH 4.5) is preferable for the spectrophotometric reagent to determine citric acid as well as strong acids and bases [10]. The most critical experimental parameter of the proposed flow system was the concentration of the buffer solution. A low concentration could be not enough to keep the baseline constant for the repetitive determination, whereas a high concentration causes a decrease in the detection sensitivity. The optimum concentration of acetate buffer was chosen as 0.01 M as a compromise between the sensitivity and the number of sample injections.

Normally, the reservoir volume in a cyclic FIA should be as small as possible to reuse the reagent effectively and to minimize the waste. At the same time, the reservoir volume is dictated mainly by the number of samples to be determined and the approximate content. In this work, the reservoir volume was decided to be 100 ml, taking into account an effective reuse of the reagents and minimizing the waste.

3.2. FIA optimization

The pH dependent spectra of MO exhibit a broad absorption maximum around 510 nm for the red mono-cation and an absorption maximum around 440 nm for the yellow molecule. The absorbance at 543 nm due to mono-cation form is monitored considering the sensitivity and the lower back ground absorption.

The length of the reaction coil determined the time that the neutralization and dispersion proceed. The signals decreased

Table 1 Optimum conditions for the assay of acidity in fruit juices and soft drinks

Parameter	Optimal value
Reservoir volume	100 ml
Sucrose	12% (w/v)
Methyl Orange	2.5×10^{-4} M
Acetate buffer	0.01 M
pH	4.5
Sample volume	5 μl
Sample frequency	30 h^{-1}
Reaction coil length	65 cm
Backpressure coil length	500 cm
Flow rate	0.9 ml min^{-1}
Wavelength	543 nm

with increasing the reaction coil length, therefore, the outlet of the sample injection valve was directly connected to the flow cell inlet. The total length between the two devices was 65 cm. The flow rates of the circulating reagent solution from 0.5 to 1.8 ml/min. were investigated. No differences were observed because the distance from the sample injector to flow through cell was too short to affect the dispersion and mixing.

Other FI variables were optimized by maximizing the obtained peak heights. Optimum conditions estimated are summarized in Table 1.

3.3. Sample matrix

Fruit juices normally contain various organic matters and the most common one is sugar which is added or is naturally present in fruit juices and soft drinks. When a sample was injected directly into the reagent carrier stream, consisting of MO and acetate buffer, a set of negative and positive background signals were observed. This might be due to the light refraction of high density and high viscosity of sugar in a sample solution. We have noted the necessity of exactly matching the concentration of sugar in the samples and the reagent carrier stream to eliminate the blank peaks. A series of standard citric acid solutions in which added up to 20% (w/v) of sucrose was prepared and the effect of the sugar on the color reaction of MO was investigated. It should be noted that sugar has no significant influence on the signal intensity of MO for the determination of citric acid. Since sugar contents in commercially available juices are from 10 to 15% [5-7], 12% sucrose was added to the reagent carrier solution to avoid interferences due to differences in the refractive index of the reagent carrier and sample solutions.

By matching the matrix in the reagent carrier and sample solutions, it makes possible to inject a sample solution into the FIA system directly without any dilution procedure. Therefore, the system operation is very simple, which can be considered an additional advantage. The cyclic flow system is designed such that the use of sucrose as well as MO and acetate is minimized, thus reducing its consumption. In this manner, any refilling of the reagent carrier solution is not necessary.

3.4. Analytical characteristics

The calibration graph was made with the standard citric acid solution to which sucrose (12% (w/v)) was added in the same concentration as in juices. A linear relationship was obtained between the absorbance and citric acid concentration in the range

Table 2 Assay for the acidities (expressed as citric acid content) in commercially available fruit juices and soft drinks

Sample	Citric acid found (% (w/v))	
	Proposed cyclic FIA[a]	Titration[b]
A (apple)	0.19 ± 0.02	0.21 ± 0.05
B (grape)	0.72 ± 0.17	0.78 ± 0.02
C (orange)	0.28 ± 0.02	0.56 ± 0.02
D (sport drink)	0.10 ± 0.00	0.15 ± 0.03
E (sport drink)	0.11 ± 0.00	0.12 ± 0.01

a) Average of five determinations.

b) Average of three determinations.

of 0-1.5 % (w/v) with a correlation coefficient, r=0.9964. The detection limit was 0.01% (w/v).

To investigate the reproducibility and repeatability, cyclic analysis of 100 repetitive determinations for 0.3% citric acid was carried out under the optimized conditions. The flow signals are shown in Fig. 2. The precision of this measurement (as relative standard deviation) was also checked and found to be 1.5%. This indicates that the proposed method has good precision.

The interference effects of several substances on the determination of citric acid were examined. One per cent of NaCl, 0.8% of sorbitol and 0.07% of ascorbic acid, in considerable amount, had no significant influence on the signal intensity, whereas 0.05% malic and 0.06% lactic acids interfered with the determination.

3.5. Analysis of fruit juice samples

Commercially available fruit juices and soft drinks were analyzed by the proposed methods. A comparison of the results obtained by this method and by the standard titration method is shown in Table 2. The results are good agreement with the reference method except orange juice. Orange juices were usually colored, and interfered, to a certain extent, with the measurements.

The stability of response and the reproducibility of the peak height were tested by 50 consecutive injections of the sample solutions. The flow signals obtained with apple and grape juices are shown in Fig. 3 (A) and (B). Although a slight gradual increase in the baseline shift could not be avoided, the strengths of peaks were unchanged during the measurements, showing good repeatability of this method. Thus it allowed the continuous monitoring of acidity in fruit juices by the periodical injection of sample solution into the flow system. The proposed procedure could replace more complex and time consuming methods for the quality control in food industries.

4. Conclusion

The cyclic FIA represents a simple, fast and repetitive method for the determination of acidity in fruit juices and soft drinks. It can be practically applied to the continuous monitoring of acidity in fruit juices and soft drinks by direct introduction of a sample solution to the flow system.
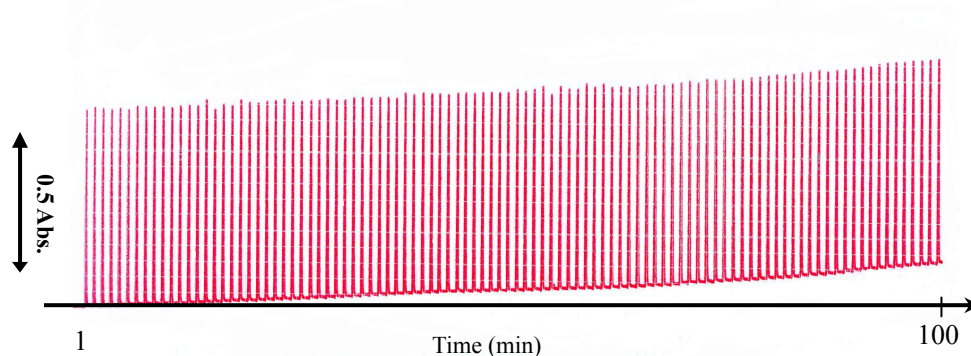

Fig. 2   Flow signals for 100 repetitive determinations of standard citric acid solution.
Citric acid concentration: 0.3% (w/v); reservoir volume: 100 ml;   methyl orange: 1.0×10^{-5} M; acetate buffer(pH 5.0): 0.01 M; sample frequency: 30 h^{-1} ;
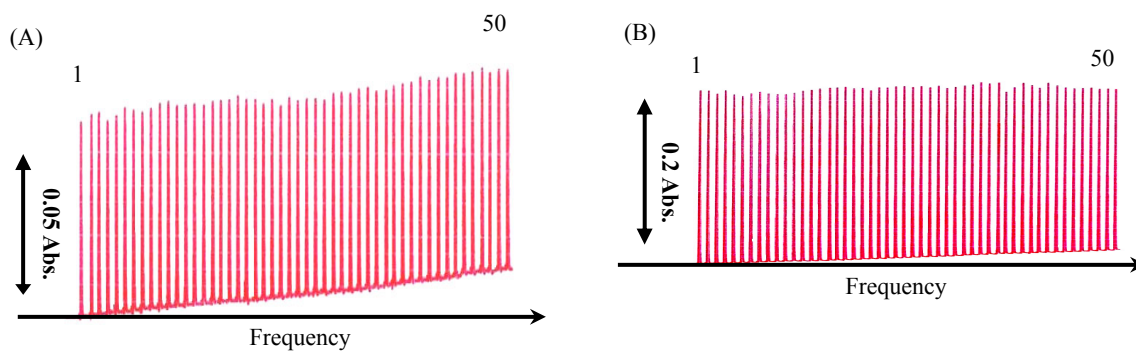
Fig. 3　Flow signals of fruit juices obtained by 50 repetitive injections.
(A): apple juice; (B): grape juice.

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