

Full Paper

## **Design and fabrication of a micro-volume autotitrator with potentiometric end-point detection for the determination of acidity of some fruit juices**

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**Abstract:** A miniaturised micro-volume autotitrator with potentiometric end-point detection was designed and fabricated for the determination of acidity of some Thai fruit juices. The method was based on on-line potentiometric titration of the organic acids with sodium hydroxide. The conditions such as volume of fruit juice sample, volume and concentration of potassium chloride used as supporting electrolyte, and flow rate of the titrant were optimised by using univariate optimisation. A sample throughput of 83 samples/hr at the titrant flow rate of 0.28 mL/min was achieved with satisfactory results. The results obtained by the proposed method agreed with those obtained by the classical titration method.

**Keywords:** micro-volume autotitrator, potentiometric end-point detection, acidity, fruit juice

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### **Introduction**

Acidity serves numerous purposes in modern food processing in addition to its major role of rendering foods more palatable and stimulating. Various functions of acidity include flavouring agent, buffer, preservative, synergist, viscosity modifier, melting modifier and meat curing agent [1]. The acidity of a fruit juice is due to the presence of several organic acids such as citric, malic, fumaric, acetic, ascorbic, and galacturonic acids. Measuring organic acid levels in foods and beverages is important from the standpoint of monitoring of fermentation process, checking of product stability,

validating of authenticity of juices and concentrates, and studying of the organoleptic properties of fermented products such as wines [2]. The most common acid in soft drinks and citrus and other fruit juices is citric acid, which can be obtained either in anhydrous or monohydrate form [3]. Citric acid (2-hydroxy-1,2,3-propanetricarboxylic acid), unlike other hydroxy acids, is tribasic with a major advantage of high solubility in water. Several fresh fruits such as lemon and lime owe their tangy taste to the presence of citrate ions [1].

Acidity can be determined by a large number of methods such as ion chromatography [4], gas chromatography [5], high performance liquid chromatography [6], capillary electrophoresis [7], and flow injection analysis [8]. Although some methods can selectively determine different acids, the acidity is usually reported in terms of the citric acid content. Titration is used as the classical method for the determination of citric acid in fruit juices [9-10]. The disadvantage of titration is that it is time and reagent consuming. Potentiometry is also alternatively used to determine the total acid content in fruit juices in comparison with classical titration [11]. In this present work, we have designed and fabricated a micro-volume autotitrator with potentiometric end-point detection for the determination of acidity of the juices of eight Thai fruits collected in the northern area of Thailand. The method is based on an on-line potentiometric titration. Variables affecting the proposed method, viz. volume of fruit juice sample, volume and concentration of potassium chloride used as supporting electrolyte, and flow rate of titrant were evaluated and optimised.

## **Materials and Methods**

### *Materials*

Sodium hydroxide, potassium hydrogen phthalate (KHP), phenolphthalein, and potassium chloride were obtained from Merck (Germany). All chemicals were of analytical reagent grade.

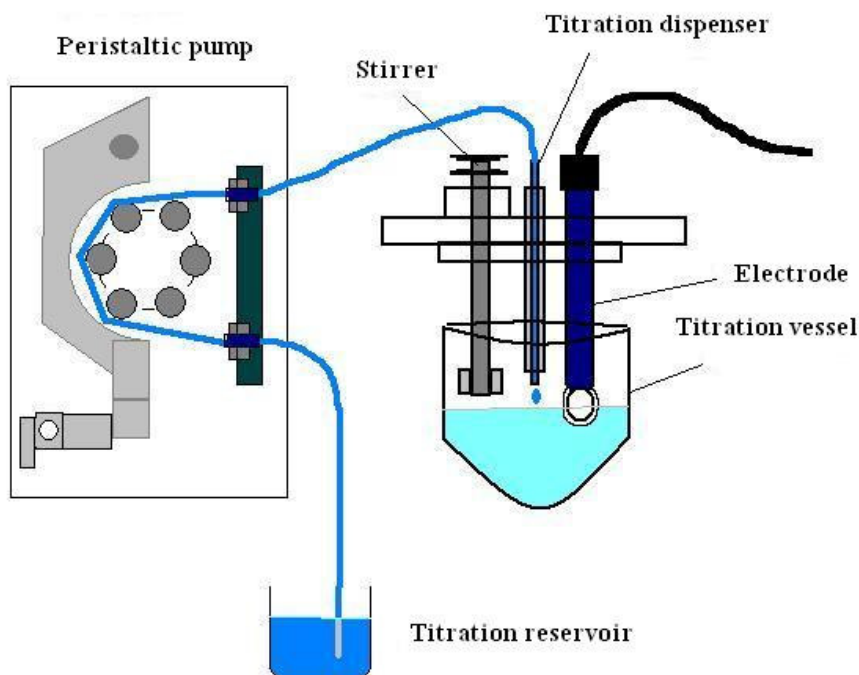
Eight fruit samples, viz. lime, pomelo, tangerine, red and green grape, red and green apple, and pineapple were collected from the northern area of Thailand by random sampling.

### *Micro-volume autotitrator*

The schematic diagram of the designed autotitration system is shown in Figure 1. A peristaltic propeller was used as a device to aspirate accurately and continuously the amount of titrant in the range of 0.02 to 0.24 mL/min (depending on the tube resolution and rotor speed). The peristaltic pump was controlled by an AT89C4051 microcontroller which generated a pulse-width modulator (PWM) signal to drive the pump motor, which could be commanded by a software via a RS232 port. An aspiration volume calibration of the pump was carried out by water-weighting method.

A combined glass electrode (Horiba, USA) was used as a proton sensor. It was connected to an analog-to-digital converter device namely "UT60D" multimeter (Uni-Trend International Limited). The output of ASCII mV reading was sent to the PC via RS232C port with the baud rate of 2,400 bps no parity 7 data bit and 2 stop bits. A PC recorded the change and directly plotted the graph which could be saved for further calculation or exported in many formats.

A Microsoft Visual Basic 6.0 was used as a developed tool to control the peristaltic pump and receive the signal from the UT60D multimeter. It employed Microsoft Communication Control 6.0 ActiveX to communicate with the control board in ASCII mode.



**Figure 1.** Schematic diagram of the designed autotitration system

### *Sample preparation*

Eight kinds of fresh fruits, viz. lime, pomelo, tangerine, red grape, green apple, green grape, pineapple, and red apple were separately squeezed with a juice extractor (Comfort HR 182I, Philips, Netherland). A 1.0 mL aliquot of each fresh juice was put in a 25 mL beaker containing 5.0 mL of 10 mM KCl as supporting electrolyte and 15.0 mL of deionised water. A micro-volume autotitrator as shown in Figure 1 was operated by propelling 0.1 M NaOH at the flow rate of 0.28 mL/min. A titration curve and the first derivative curve could be recorded as shown in Figure 2 for each titration batch.

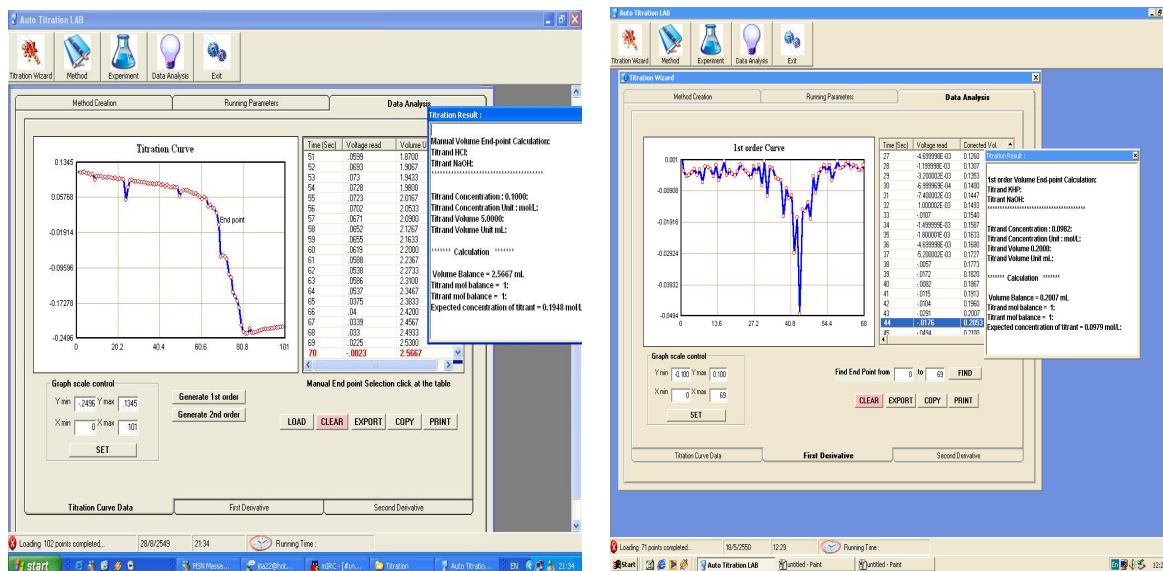


Figure 2. Titration curve and first derivative curve obtained from the designed autotitration

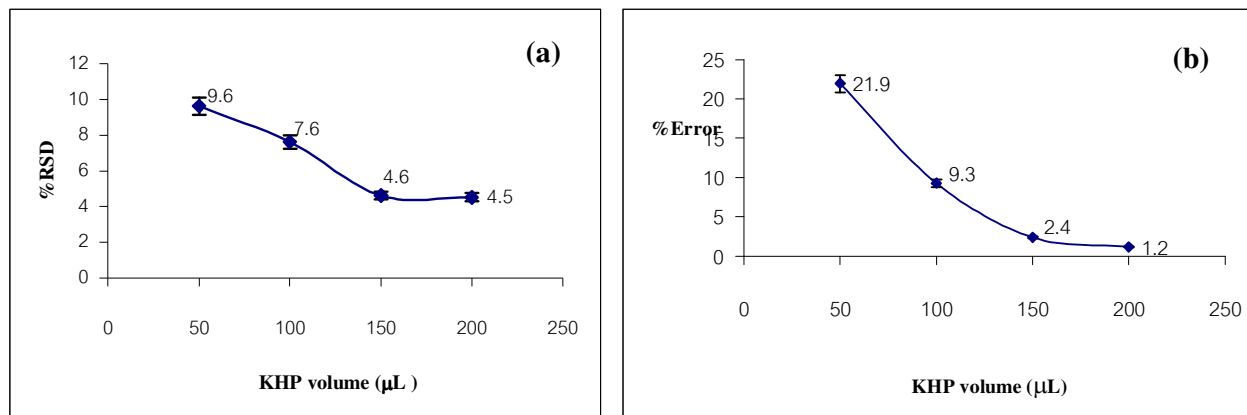
## Results and Discussion

### Optimisation for the determination of citric acid

In this experiment, the volume of KHP used as titrand, the volume and concentration of KCl used as supporting electrolyte, and the flow rate of titrant (NaOH) were optimised using the designed autotitration system as shown in Figure 1. The method for optimisation is the univariation, in which one parameter is varied while the others are kept constant. After optimisation of each parameter, the new optimised value is used for the optimisation of the next parameter. In the following optimisation, the reasonable %RSD and % Error were used for selecting the optimal value of each parameter. %RSD was calculated from 11 replicates ( $n = 11$ ) and %Error from comparing the result from the proposed technique with that from the standard classical acid-base titration method.

### Effect of KHP volume

The experiment was carried out by using 0.1000 M KHP (titrand) to titrate with 0.1000 M NaOH (titrant). Various volumes of KHP (50, 100, 150 and 200  $\mu\text{L}$ ) were pipetted into a 25 mL beaker containing 15.0 mL of deionised water and 5.0 mL of 10 mM KCl (supporting electrolyte). Then the flow of titrant was generated at 0.20 mL/min. Titration curves and first derivative curves were obtained and the results were mathematically compared with those obtained with the classical acid-base titration. The influence of KHP volume on the percentage of relative standard deviation (%RSD) and error (%Error) is exhibited in Figure 3. The KHP volume of 200  $\mu\text{L}$  showed lowest %RSD and %Error, therefore it was chosen for the next experiment. (Although a little lower %RSD and %Error might be obtained when KHP volume was increased to more than 200  $\mu\text{L}$ , it was not used on the reason of waste reduction.)



**Figure 3.** Influence of KHP volume on %RSD (a) and %Error (b) in acidity determination by the designed micro-volume autotitrator

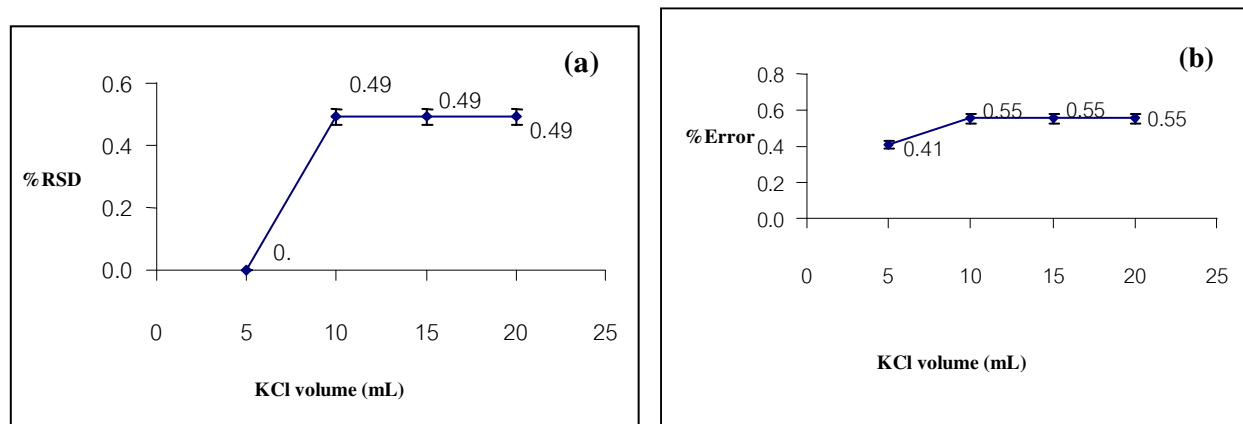
#### *Effect of KCl volume*

Supporting electrolytes are required in controlled-potential experiments to decrease the resistance of the solution, to eliminate electromigration effects, and to maintain a constant ionic strength (i.e. to “swamping out” the effect of variable amounts of naturally occurring electrolytes) [12]. The inert supporting electrolyte may be an inorganic salt, a mineral acid, or a buffer. Potassium chloride or nitrate, ammonium chloride, sodium hydroxide, or hydrochloric acid are widely used as electrolyte in aqueous solution. The usual electrolyte concentration range is 0.1-1.0 M, i.e. in large excess of the concentration of all electroactive species.

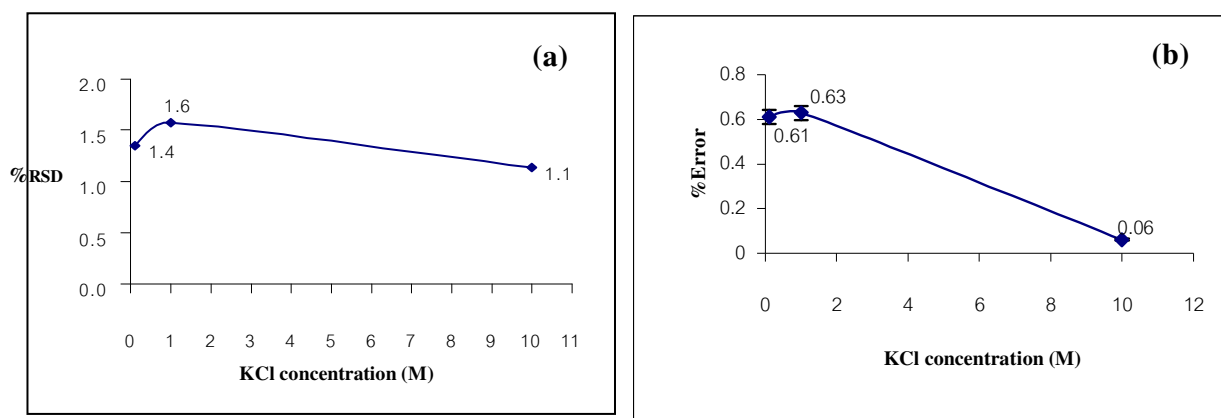
A similar experiment to that for studying the effect of KHP volume was carried out but the volume of KHP and the flow rate of titrant were fixed at 200 μL and 0.20 mL/min respectively. Various volumes of 10 mM KCl used as supporting electrolyte (5, 10, 15 and 20 mL) were each pipetted into a 25 mL beaker containing 200 μL of KHP and 15.0 mL of deionised water. The results are shown in Figure 4. It was observed that at 5 mL of 10 mM KCl the results from 11 replicates showed no difference in each value of the results so %RSD was calculated to be zero. Comparing the results with those obtained from the standard acid-base titration, the %Error at 5 mL of 10 mM KCl was lowest. Therefore, it was selected for the next experiment.

#### *Effect of KCl concentration*

The experiment was carried out by varying KCl concentration between 0.1-10 mM at fixed KHP volume (200 μL), flow rate (0.20 mL/min), and KCl volume (5 mL). From Figure 5, it is found that 10 mM KCl gave lowest %RSD and %Error. Therefore, it was chosen for the next experiment.



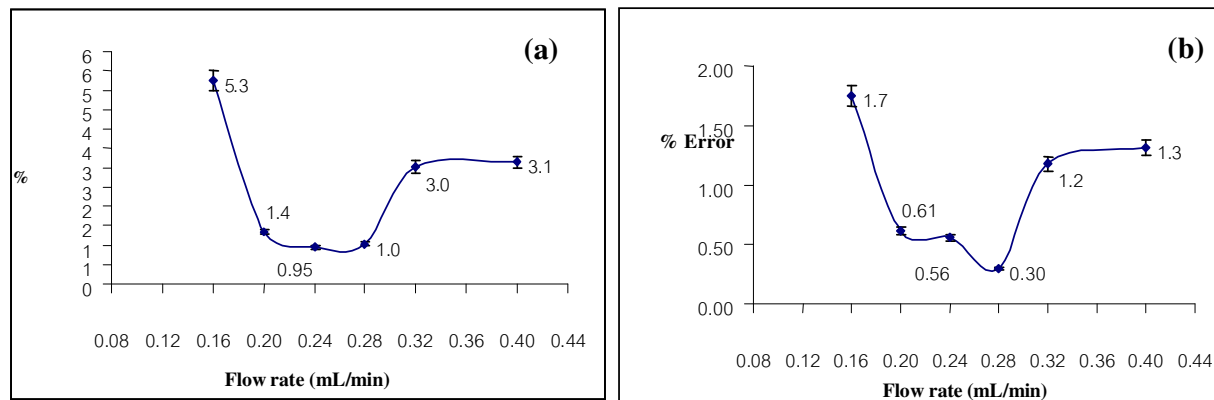
**Figure 4.** Influence of KCl volume on %RSD (a) and %Error (b) in acidity determination by the designed micro-volume autotitrator



**Figure 5.** Influence of KCl concentration on %RSD (a) and %Error (b) in acidity determination by the designed micro-volume autotitrator

#### *Effect of flow rate*

By fixing the volumes of KHP and KCl at 200  $\mu\text{L}$  and 5 mL respectively, and the concentration of KCl at 10 mM, the flow rate of NaOH used as titrant was varied between 0.16-0.40 mL/min. From Figure 6, although the flow rate at 0.24 mL/min gave the lowest %RSD, it provided higher %Error than that at 0.28 mL/min. Moreover, the sample throughput was increased from 72 samples/hr to 83 samples/hr when the flow rate was changed from 0.24 to 0.28 mL/min. Therefore, the flow rate of 0.28 mL/min was chosen. The summary of the optimum conditions is shown in Table 1.



**Figure 6.** Influence of flow rate of NaOH (titrant) on %RSD (a) and %Error (b) in acidity determination by the designed micro-volume autotitrator

**Table 1.** Optimum values of variables for the on-line potentiometric titration using micro-volume autotitrator

Variable	Range studied	Optimum value
KHP volume ( $\mu\text{L}$ )	50-200	200
KCl volume (mL)	5-20	5
KCl concentration (mM)	0.1-10	10
Flow rate (mL/min)	0.16-0.40	0.28

#### *Application to real samples*

Though organic acids in Thai fruits are varied, a common component seems to be citric acid (which has three active protons). The organic acid content (calculated as citric acid) in the fruit juice from each kind of fruits determined by classical titration and on-line potentiometric titration is shown in Table 2. A paired t-test was used to compare the results obtained by both techniques. They were in excellent agreement at 95% confidential limit.

**Table 2.** Organic acid (calculated as citric acid) content in various Thai fruit juices

Fruit	Organic acid content obtained by classical titration (n=11) <sup>*</sup> (x10 <sup>-3</sup> M) (%RSD)	Organic acid content obtained by on-line potentiometric titration (n=11) <sup>*</sup> (x10 <sup>-3</sup> M) (%RSD)
Lime	5.26 (0.23)	5.42 (2.98)
Pomelo	3.76 (0.33)	3.77 (6.72)
Tangerine	3.24 (0.37)	3.29 (15.4)
Red grape	3.00 (0.40)	3.04 (7.33)
Green apple	2.89 (0.38)	2.88 (10.2)
Green grape	2.73 (1.05)	2.72 (6.92)
Pineapple	1.76 (0.63)	1.77 (9.30)
Red apple	1.49 (1.49)	1.51 (3.80)

<sup>\*</sup> 11 replicates

## Conclusions

Determination of organic acid content in fruit juices by the micro-volume autotitrator fabricated in our laboratory is fast and convenient with the sample throughput of 83 samples/hr at the titrant flow rate of 0.28 mL/min. It can be used in routine analysis of acid content. The titrant and titrand used are in the level of a few mL, much less than those used in the classical titration technique, thus contributing to the green and home-made technology.

## Acknowledgements

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