

Flow Injection Analysis-Spectrophotometry for Rapid Determination of Total Polyphenols in Tea Extracts

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Abstract

A simple flow injection analysis-spectrophotometric system for the rapid determination of total polyphenols (TP) in tea extracts was developed. The method is based on Folin-Ciocalteu (FC) reaction, which involves the oxidation/reduction of polyphenolic compounds by FC reagent in alkaline medium, producing a blue molybdenum-tungsten complex. The linear range of the calibration graph was 10 – 500 mg gallic acid equivalent (GAE)/l. The relative standard deviation (*RSD*) (*n*=5) of the calibration graph was 2.93%. The precisions (%*RSD*, *n*=10) of the determination at low and high concentrations were 2.74% for the 10 mg GAE/l and 3.02% for the 500 mg GAE/l, respectively. The detection limit was 7 mg/l. The sample throughput was 30 per hour. The TP contents in tea extracts determined by the developed system were compared with those determined by the standard batch method and no significant difference was found (*t* test, at 95% confidence level).

Keywords Flow injection, total polyphenols, tea extracts, Folin-Ciocalteu method, spectrophotometry

1. Introduction

Polyphenols in tea, one of the most popular beverages brewed from leaves of tea plant (*Camellia sinensis* L.), have been known for their antioxidant properties. They play an important role in the prevention of various diseases such as cancers, cardiovascular, and neurodegenerative diseases [1]. There are various kinds of polyphenols present in tea, e.g. phenolic acids, flavonoids, tannins and other phenolics [2]. Catechins, a group belongs to flavonoids, have been reported to be the most effective antioxidants in tea [3,4]. However, polyphenol content is varied with many factors, i.e., tea varieties and origins, plantation area, tea types, and infusion conditions [1,5,6].

In Thailand, tea plant is mostly grown in the North, especially in Chiang Rai, 687 km from the capital Bangkok [7]. Thai tea products are produced by local factories, run by villagers. Assessment of tea quality, such as determination of total polyphenols, cannot be performed at the sites due to the lack of scientific instruments, high-cost chemicals and laboratory skills. Thus, it is often not to be done. Tea Institute at Mae Fah Luang University was then established since 2005 with the purpose to study and enhance the quality of Chiang Rai tea, and to support the tea factories in improving tea products. Large numbers of tea samples are expected to be analyzed for active ingredients, including the determination of TP, by the Tea Institute.

Spectrophotometric methods have been widely employed for the determination of TP, generally based on a reaction between phenolic compounds and a reagent, producing a colored complex to be measured. Reactions, such as Folin-Ciocalteu, Prussian blue and o-phenantroline, and Folin-Denis reactions have been used [8,9]. Among those reactions, Folin-Ciocalteu (FC) method is the most used and well established [10].

In the FC method, polyphenols are determined colorimetrically using FC reagent. The reagent contains phospho-tungstic acids as oxidizing agents, which on reduction by readily oxidized phenolic hydroxyl groups in alkaline medium yield a blue color with a broad maximum absorption at 730-770 nm. This is due to

the formation of tungsten and molybdenum blues. Although it was established as the standard method for the determination of TP in tea samples, ones can find some drawbacks due to long equilibration time (up to 1 hour) and the need of high amount of reagents. Flow injection analysis (FIA) can be developed in order to overcome these drawbacks. That is because FIA is based on injecting a small, fixed volume of sample/standard to flowing stream(s) of carriers and/or reagents with constant flow rates in microtubings, and, thus, does not rely on complete equilibrium of reaction. The amount of the analyte can be determined comparatively because standard and sample are dispersed and processed in the same way [11-13].

There have been some researches developing FI method for the determination of polyphenols in wine and tea samples. Methods of detection employed in those FI systems included capillary electrophoresis [14], atomic absorption spectrophotometry [15], biosensor [4,16], and visible spectrophotometry [17,18]. Among these detection techniques, visible spectrophotometry is the simplest and visible spectrophotometers are readily available in most laboratories. Reduction/oxidation reaction of polyphenols is employed and a colored complex product is monitored. However, most FI-spectrophotometric systems were developed for the determination of polyphenols in wine samples, not yet applied to tea samples.

In this work, a FI-spectrophotometric system was developed for the determination of total polyphenols in tea extracts. Effects of alkaline concentration (2.5×10^{-3} , 1, 5, and 10%w/v NaOH), FC concentration (1, 5, 7.5, and 10%v/v), and flow rate (0.5, 1.0, 1.2, and 1.5 ml/min) were studied. The TP contents obtained by the developed FI method were compared with those obtained by the standard batch FC method using *t* test.

2. Experimental

2.1. Reagents

FC reagent (Merck, Germany) was used as the oxidizing agent. Alkaline solution used was sodium hydroxide (Merck, Germany).

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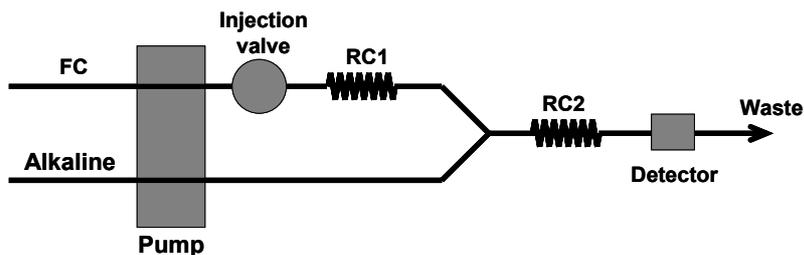


Fig. 1 Schematic diagram of the FI system. RC1 and RC2: reaction coil 1 and 2 (100 cm × 0.076 cm i.d.).

Gallic acid (Fluka, Switzerland) was used as the standard solution. A stock standard (1000 mg/l) was prepared and kept at 4°C. Working solutions were freshly prepared by diluting the stock standard. Methanol (Fluka, Switzerland) (70%v/v) was used for extracting phenolic compounds from tea samples.

2.2. Tea samples

Two types of tea samples were employed in this work; (i) leaf tea products and (ii) tea plants. Leaf tea products (6 samples) were purchased from a local manufacturer. They are different in product forms (loose leaves, roll leaves and bags) and varieties (*C. sinensis* var. *sinensis* and *C. sinensis* var. *assamica*). Tea plant samples (4 plants; two of *C. sinensis* var. *sinensis* and two of *C. sinensis* var. *assamica*) were kindly provided by the Tea Institute, Mae Fah Luang University. The age of the tea plants were 7 months. The plants were divided into 5 parts: buds, leaves, branches, stems and roots. Total polyphenols in each tea sample were extracted following the standard method for determination of substances; characteristic of green and black tea (method no.: ISO14502-1:2005(E)) [10].

2.3. Folin-Ciocalteu method

The FC reagent reacts with a wide range of polyphenol compounds. Although the response can vary with the individual components, the standard method for determination of substances; characteristic of green and black tea (method no.: ISO14502-1:2005(E)) uses gallic acid as a standard, enabling useful total polyphenol data to be obtained [10,19]. An aliquot (1 ml) of tea extract solution or gallic acid working standard solution was mixed with 5 ml of 10%v/v FC reagent. After 3-8 min, 4 ml of alkaline solution was added and mixed thoroughly. The mixture was allowed to stand at room temperature for 1 hour. Finally, absorbance of the colored solution was measured. TP content is calculated as gallic acid equivalent (GAE).

2.4. FI setup

Schematic diagram of the FI setup are shown in Fig. 1. It is a simple double line FI system consisting of a peristaltic pump (REGLO Analog MS-2/6, Ismatec, Switzerland), a 6-port injection valve (V-450, UpChurch Scientific, Washington, USA) with a 50- μ l sample loop, two reaction coils (PTFE tubings, 0.076 cm i.d., 100 cm long) and a spectrophotometer (Spekol 1200, Analytik Jena AG, Germany). The FC reagent and the alkaline solution were pumped through the system at the same flow rate, making the final flow rate double of the initial one. A sample or standard solution was introduced into the FC stream via the injection valve and then mixed thoroughly in the RC1. The mixture zone was propelled further to mix with the alkaline

stream in the RC2. The product zone was finally detected spectrophotometrically at the wavelength of 730 nm.

3. Results and discussion

3.1. Optimization of the FI method

3.1.1. Effect of alkaline concentration

In the FC reaction, the FC reagent oxidizes phenolates and blue molybdenum-tungsten complex is produced. However, phenolates are present only in alkaline medium. Sodium hydroxide was used instead of sodium carbonate which is suggested in the ISO standard method to prevent the bubbles due to carbon dioxide.

Concentration of the alkaline affected the production of the blue Mo-W complex. Using 2.5×10^{-3} %w/v NaOH, the blue product could not be measured colorimetrically. At higher concentrations (1, 5 and 10%w/v), the blue solution was produced but white precipitates were observed in the flow-cell when 5 and 10%w/v were used. This could be explained by the precipitation of phenolic compounds in very strong base. Therefore, 1%w/v NaOH was selected.

3.1.2. Effect of FC concentration and sample volume

The concentration of the FC reagent used in the batchwise method is 10%v/v. To reduce the consumption of the reagent, its concentration (1, 5, 7.5 and 10%v/v) was varied. Effect of sample volume (50 and 100 μ l) was also studied. Linear range of 10-50 mg/l was obtained when low FC reagent concentration (1%) and 100- μ l sample loop were used (see Table 1). It was found that the higher the FC reagent concentration and the less the sample volume, the better the linearity and the wider the dynamic range. Comparing using 7.5 and 10%FC when employed 50- μ l sample volume, identical sensitivities and linearity were provided, indicating the sufficient phospho-molybdic acid to oxidize up to 500 mg GAE/l of polyphenolic compounds. Hence, 50- μ l sample volume and 7.5%FC were used for further study.

3.1.3. Effect of flow rate

The flow rates of the FC reagent and alkaline streams were equal. Initial flow rate of each line (0.5, 1.0, 1.2 and 1.5 ml/min) was varied. For the range of 10-500 mg GAE/l, less sensitivity was obtained when slow flow rate (0.5 ml/min) was used (Table 2). Higher flow rate increased the sensitivity (slope) and linearity (R^2) of calibration graphs due to the increase in the degree of dispersion and mixing. To compromise the analysis time and the reagent consumption, the flow rate of 1.2 ml/min was chosen.

Table 1 Effect of sample volume and FC concentration on calibration graph

Sample Volume / μl	FC Concentration / %v/v	Linear Range / mg GAE/l	Equation	R^2
50	1.0	10-50	$y=0.0042x-0.0226$	0.9993
	5.0	10-100	$y=0.0041x+0.0038$	0.9996
	7.5	10-500	$y=0.0044x-0.0142$	0.9998
	10	10-500	$y=0.0042x-0.0312$	0.9989
100	1.0	10-50	$y=0.0062x-0.0097$	0.9863
	5.0	10-100	$y=0.0091x-0.0206$	0.9968
	7.5	10-250	$y=0.0442x+0.2317$	0.9959
	10	10-500	$y=0.0113x-0.1159$	0.9981

Table 2 Effect of flow rate on calibration graph in the concentration range of 10-500 mg GAE/l

Flow rate / ml/min	Equation	R^2
0.5	$y=0.0032x-0.005$	0.9973
1.0	$y=0.0043x-0.0312$	0.9977
1.2	$y=0.0042x-0.005$	1
1.5	$y=0.0044x-0.0312$	0.9996

Table 3 Percentage relative standard deviation (%RSD) of the measurements for standard gallic acid using the developed FI method

Standard concentration / mg GAE/l	%RSD (n=10)
10	2.74
25	1.84
50	4.51
100	3.55
250	3.40
500	3.02

3.2. Characteristics of the FI method

3.2.1. Optimum conditions

The conditions of the proposed FI method for the determination of TP were obtained. The alkaline (NaOH) and FC reagent concentrations were 1%w/v and 7.5%v/v, respectively. The flow rates were 1.2 ml/min. The sample volume was 50 μl . The lengths of the reaction coils were 100 cm. The detection wavelength was 730 nm. Using these conditions, the sample throughput was 30 per hour and the reagent consumption was less than 80 ml/h. Although the developed FI method did not accelerated the redox reaction time of polyphenols with the FC reagent, it could reduce analysis time because, employing the exact time of all events in FIA, it does not need to wait until the reaction equilibrium is reached.

3.2.2. Precision

The precision was assessed by percentage relative standard deviation (%RSD) of 10 repeated measurements of various concentrations of standard solutions, and the slopes and coefficients of determination (R^2) of calibration graphs. Table 3 summarizes the precision data. Using the optimized conditions, good precision (less than 5%RSD) was obtained over the standard concentration range. The repeatability of the method was also good (%RSD_{n=5} of slope = 2.93% and %RSD_{n=5} of R^2 = 0.003%).

Table 4 Comparison of the determination of TP using the proposed FI and the batchwise standard method

Sample	Total polyphenol content / mg GAE/g tea ^a	
	Proposed FI method	Batchwise method
1	139.8 \pm 7.7	131.4 \pm 2.0
2	101.5 \pm 3.4	110.5 \pm 4.6
3	130.5 \pm 9.2	146.7 \pm 3.6
4	104.6 \pm 1.6	91.7 \pm 1.5
5	200.0 \pm 4.2	184.2 \pm 2.7
6	124.0 \pm 3.0	131.0 \pm 4.4

^a Average value \pm standard deviation for $n=3$.

3.3. Applications to the tea extracts

3.3.1. Leaf tea products

Leaf-tea product samples (6 samples) were analyzed for TP using the FI method compared with the batch standard method. The result data (Table 4) were treated statistically with F test and t test methods. The F test results (at 95% confidence level) showed that the precision of the FI method and the standard method were not significantly difference. The t test at 95% confidence level illustrated that there was no significant difference between the two methods. Therefore, the proposed FI method can be used for the determination of TP in tea extract samples, providing advantages over the batch standard method, such as less time and reagent consuming.

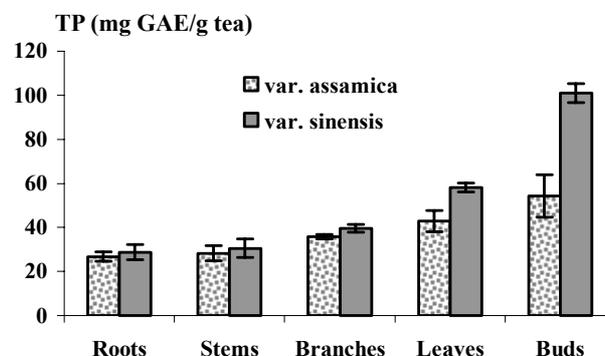


Fig. 2 Total polyphenols (TP) content in parts of tea plants

3.3.2. Tea plant extracts

Extracts of five parts; i.e., buds, leaves, branches, stems and

roots, from tea plants (*C. sinensis* var. *sinensis* and *C. sinensis* var. *assamica*) were analyzed using the FI method. The highest polyphenol content was found in the buds while the less amounts were found in the leaves, branches, stems and roots, respectively (Fig. 2). The results agreed with literatures [3,20]. Biosynthesis of tea polyphenols was researched, and it was found that polyphenols are present in all parts of tea plant and, however, their formation is prolific in the buds and upper leaves [3]. In addition, the results indicated that the *C. sinensis* var. *sinensis* plant contained higher polyphenols than the *C. sinensis* var. *assamica* plant. However, it was contradictory to literature reporting that the variety *assamica* had higher polyphenols than the variety *sinensis* [1,21]. This was due to the less maturity [16] of the *C. sinensis* var. *assamica* plant used in this work. Although the ages of the plants were the same, the heights and number of leaves and buds in the *C. sinensis* var. *assamica* plants were less than those in the *C. sinensis* var. *sinensis* ones.

4. Conclusion

The proposed FI method is a simple and rapid method for determination of TP in tea extract samples. It overcomes the drawbacks found in using the batch standard method by providing higher sample throughput and less reagent consumption with high precision over the wide concentration range, good sensitivity and accuracy. Nevertheless, the proposed method was repeatable over several weeks. However, FC reagent solution is needed to be prepared freshly.

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