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Contributed Paper

Flow Injection Potentiometric Method Based on Ce(IV)/Ce(III) Redox Reaction for Determination of Total Antioxidant Capacity

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ABSTRACT

A simple and low-cost method for determination of total antioxidative capacity (TAC) was developed using a flow injection potentiometric method. The reaction involved the measurement of electrical potential change caused by the disturbance of Ce(IV)/Ce(III) ratio of a redox-reagent solution caused by the reduction of Ce(IV) to Ce(III) by the antioxidant. The optimum condition is as follow: reagent solution contained 0.06 mmol L^{-1} Ce(IV), 0.01 mmol L^{-1} Ce(III), and 0.5 mol L^{-1} H_2SO_4, and flow rate of solution of 0.8 mL min^{-1}. A linear calibration graph was 0.08-1.00 mmol L^{-1} of ascorbic acid equivalent. The system provided good precision, i.e., %RSD (n=11) of 6.0, 1.4, and 4.1 for 0.08, 0.40 and 1.00 mmol L^{-1} ascorbic acid, respectively. The method was applied to evaluating TAC of tea infusion samples compared with the batch ceric reducing antioxidant capacity (CERAC) method and the batch ferric reducing antioxidant power (FRAP) method. Good correlation of the results between these methods was observed. The developed method is low cost, low reagent consumption, and low waste production. Moreover, it did not suffer from colored and colloidal substances presented in the samples.

Keywords: flow injection analysis, potentiometry, antioxidant capacity, Ceric reducing antioxidant capacity

1. INTRODUCTION

Oxidation processes, including cellular respiration and metabolism in the human body, can produce free radicals, which can damage the cells and cause many diseases

such heart disease, cardiovascular diseases and cancers. The oxidation reaction can be accelerated by stress, cigarette smoking, alcohol, sunlight, pollution, and other factors. The intake of many fruits, vegetables and tea are well known as the prevention of the diseases. They contribute antioxidants such as ascorbic acid, selenium, carotenoid, and polyphenols which can neutralize the free radicals [1, 2]. The new analytical methods for screening of the antioxidant capacity of food and drink are required to increase sensitivity, selectivity, and simplicity, and reduction of cost, time, and consumption of reagent and sample in the analysis [3].

The evaluation of antioxidant capacity involves the properties of antioxidant including reducing power, free radical scavenging, and metal chelating activities. Therefore, no single antioxidative assay can evaluate the real antioxidant activity. Chromatographic methods are widely used for the identification and qualification of compounds that contributed to the TAC. These techniques combine separation step with gas chromatography (GC) or high-performance liquid chromatography (HPLC) and add post column antioxidant detection. An on-line HPLC with post-column reaction provides high sensitivity, selectivity, and quick identification of antioxidant compounds in fruits, foods and plant extracts [4-8]. However, these methods involved expensive instrument and sample preparation are complicated.

The spectrophotometric methods are extensively used for the determination of the TAC. The most common methods are 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2,2′-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) assays. The stable DPPH radical in a methanol solution is presented a strong absorption at a wavelength of 515 nm. The ABTS radical has a maximum absorbance at 734 nm. The scavenging of free radicals by antioxidants leads to a decrease in absorbance at the characteristic wavelength. Other spectrophotometric protocols based on reducing power of the antioxidant are also widely used. Ferric reducing antioxidant power (FRAP) assay is based on the reaction of antioxidant and a ferric-tripyridyltriazine complex to produce the ferrous form. The resulted intense blue color product has an absorption maximum at 593 nm. Copper reducing antioxidant capacity (CUPRAC) method utilizes the Cu(II)-neocuproine reagent as the chromogenic oxidizing agent. Cu(I)-neocuproine chelate formed by the reaction with antioxidant has a maximum absorption at 450 nm [9-14]. Ceric reducing antioxidant capacity (CERAC) assay used Ce(IV) to oxidize the antioxidants, and the absorbance of the excess Ce(IV) is measured at 320 nm [15]. The spectrophotometric methods are simple, low cost, employing simple instrument, giving good sensitivity, and having diverse applications. However, they have serious interferences from the pigment and colloidal substances presented in the samples.

Electrochemical methods are particularly appealing for the screening of the antioxidant properties of the sample including voltammetric, amperometric, and potentiometric methods. Cyclic voltammetry measures electron donation ability of the antioxidants related to their redox potential. Antioxidant capacities can be analyzed by three parameters, i.e., anodic peak current, oxidation peak potential, and the area under anodic peak current [16]. The parameters involve biological oxidation potential of the sample and the concentration of components in the sample [17]. The amperometric method can be used for measuring the current of remaining electroactive reagent after reacting with the antioxidant such as DPPH [18].

Trend to develop the electrochemical methods for determination of antioxidant capacity is an electrode modification and using of electroactive reagent. Modification of a working electrode is proposed for improving the sensitivity of measurement such as carbon nanotubes, enzyme or polymer modified on a glassy carbon or other metal electrodes [19-22]. Although these developments provide high sensitivity and selectivity, the electrode modification processes are high-cost, non-renewability, and complicated. The potentiometric titration is widely use for determination of various redox species. The potential changes due to redox reaction between analyte and redox couple reagent. The widely used redox reagent couples in the potentiometric method are ferri-ferrocyanide, Fe(III)/Fe(II), iodate-periodate, bromine-bromide and ceric-cerate couples [23-25]. The latter is a strong oxidizing agent, stable in acidic medium, and can react with wide ranges of antioxidant compounds. The initial potential is very stable in the redox couple solution. The potentiometric methods offer a relatively simple, low-cost instrument, and does not suffer from colored and colloidal substances. Flow injection system can improve the performance of the analytical methods such as it can detect intermediate, since a reaction time was controlled in the flow system and reduce the risk of the operator to directly contact to the chemicals.

Because of the interesting of this concept, this work aims to develop a simple flow injection potentiometric method for determination of total antioxidant capacity based on reducing power of antioxidant. The measurement of a change in electrical potential at a platinum wire indicating electrode due to the change of a Ce(IV)/Ce(III) ratio when the antioxidant reacted with Ce(IV) was employed. The method has been applied to determine the total antioxidative capacity of tea infusion samples. The obtained results were in good correlation to those obtained by the batch-Ce(III)/Ce(IV) potentiometric method and the batch-FRAP based on Fe(III)-phenanthroline method.

2. MATERIALS AND METHODS

2.1 Chemicals and Samples

Deionized water obtained from the Millipore system (Sweden) was used for the preparation of all solutions. The analytical reagent grade of ascorbic acid, ceric sulfate, and cerium sulfate were obtained from Merck (Merck, Germany). Sulfuric acid was obtained from QRec (QRec, New Zealand). Fifteen of tea infusion samples were purchased from a local supermarket in Chiang Mai, Thailand.

2.2 Sample Preparation

A portion of 1.00 g of tea sample was placed in 50 mL hot water 80°C for 30 min [26]. Then, the solution was filtered through a Whatman filter paper No. 1 and adjusted the volume of sample to 50 mL in a volumetric flask. The samples were kept in a refrigerator before analysis. The solution was diluted with deionized water in the appropriate range for the analysis.

2.3 Flow Injection Potentiometric System

The proposed flow injection potentiometric system (Figure 1) consisted of a peristaltic pump (Ismatec, Switzerland) with Tygon pump tubing of diameter 1.00 mm, a six-port injection valve (Upchurch, USA) with injection loop of 200 µL volume, a mixing coil (PTFE, i.d. 0.5 mm), a home-made flow-through cell, and a home-made potentiometer. The system was

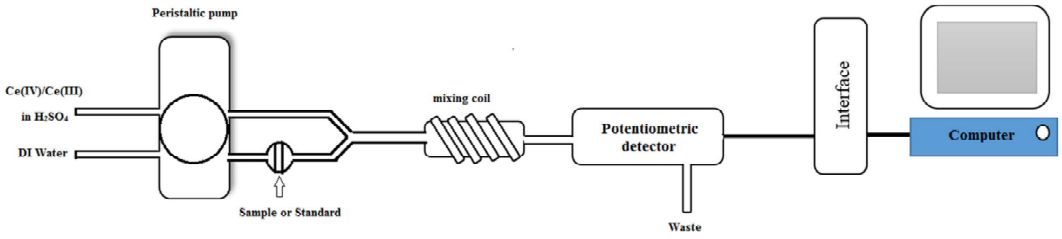

Figure 1. Manifold of a flow injection potentiometric method based on Ce(IV)/Ce(III) redox-reagent for determination of TAC. Concentration of Ce(IV), Ce(III), and H_2SO_4 are 0.06 mmol L^{-1} , 0.01 mmol L^{-1}, and 0.5 mol L^{-1}, respectively.

controlled by a personal computer using a software program written in-house.

2.4 Analytical Procedure

Sample or standard solution was injected into the carrier stream and mixed in-line with the reagent solution while they were flowing through a mixing coil to the potentiometric flow cell. The antioxidants reacted with Ce(IV) to produce Ce(III) leading to the electrical potential change of Ce(IV)/Ce(III) redox couple which could be detected by the potentiometer with a Pt electrode as an indicating electrode. The signal was recorded as an FIA peak. The TAC (as ascorbic acid equivalent) was calculated from a calibration equation, plotting between the peak height obtained and the logarithm of the concentration of ascorbic acid.

2.5 Batch-Ce(III)/Ce(IV) Potentiometric Method

An aliquot of 200 µL of the sample or standard solution was added to the reagent solution Ce(IV)/Ce(III) redox reagent (0.06:0.01 mmol L^{-1}). The antioxidants reacted with Ce(IV) to produced Ce(III) causing the change in electrical potential which could be measured by using a Pt and Ag/AgCl (3 mol L^{-1} KCl) electrode as an indicating and a reference electrodes, respectively. The TAC (as ascorbic acid equivalent) was calculated from a calibration equation, plotting between the potential and logarithm of the concentration of ascorbic acid (mmol L^{-1}).

2.6 Batch-FRAP Method Based on Fe(III)-phenanthroline Complex

The reagent of Fe(III)-1, 10-phenanthroline (Fe(III)-phen) solution was prepared by dissolving 0.0720 g of ammonium iron(III) sulfate in DI water, and 2.00 mL of 1.0 mol L^{-1} hydrochloric acid was added. Then, mixed the solution with a 5.00 mL of 10% w v^{-1} 1, 10-phenanthroline solution and adjusting the volume to 100.00 mL. Sample or standard solution (1.00 mL) was added to the reagent solution (4.00 mL). Antioxidants react with Fe (III) to form a red Fe(II)-phen complex. The colorimetric detection of Fe(II)-phen complex at 510 nm was carried out. TAC (as ascorbic acid equivalent) was calculated from a calibration equation, plotting between the absorbance and concentration of the ascorbic acid (mmol L^{-1}) [27].

3. RESULTS AND DISCUSSION

3.1 Optimization of Flow Injection Potentiometric-ceric Reducing Antioxidant Capacity Method (FIP-CERAC)

The potential of a potentiometric detection is given by the Nernst equation [28]

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$$E = E^0 + (\frac{RT}{nF}) \ln \frac{a_{OX}}{a_{red}}$$

$$E = E^0 + (\frac{RT}{nF}) \ln \frac{[Ce(IV]}{[Ce(III)]} \quad (1)$$

Where E^o is the standard reduction potential, R is the gas constant, T is the temperature in Kelvins, n is the number of electrons in the redox reaction, F is Faraday's constant, and a is the chemical activity of the relevant species. From the equation, the potential depends on the component of ions in solution. From the above equation, the electrical potential is directly related to the logarithm of the activity (or concentration) of the redox species. Firstly, the initial potential of the redox couples reagent is stable as shown as a constant baseline of the FIAgram. When antioxidant standard or sample reacts with reagent, the potential of the redox electrode is changed due to the change in the composition of the Ce(IV)/Ce(III) redox couple which is measured as a peak of the FIAgram.

Parameters including concentrations of Ce(IV), Ce(III), sulfuric acid solutions, and total flow rate which affected analytical performance of the system were optimized. The sensitivity was considered from the slope of calibration graph as described above.

Effect of the Ce(IV) concentration was studied in the range of 0.01-0.10 mmol L^{-1} Ce(IV) solution. The ascorbic acid standard solution was injected into the FI system, and it reacted with Ce(IV) causing a change in Ce(IV)/Ce(III) ratio, thus leading to the potential change from the baseline. It was found that the sensitivity increased with the increase of the Ce(IV) concentration up to 0.06 mmol L^{-1}. From the equation (1), at low concentration of Ce(IV) in the reagent, antioxidant reacted with low Ce(IV) which made the ratio of redox pairs change a little leading to a small potential change from the initial. Therefore, it resulted in a small observed signal from the baseline, and hence low sensitivity. However, at too high concentration of the Ce(IV) in the reagent, when the antioxidant reacted with Ce(IV), the concentration of the Ce(IV) did not change much, which resulted in a low sensitivity too as shown in Figure 2a.

The effect of an initial concentration of Ce(III) was studied. As shown in Figure 2b, the sensitivity decreased when increasing the concentration of Ce(III) in the reagent because it has a high ratio of the product of the reagent couple. But when Ce(III) concentration was lower than 0.00001 mol L^{-1}, there was no difference in the signal of various concentration of antioxidant. Ce(III) concentration of 0.00001 mol L^{-1} was selected.

Sulfuric acid is used to dissolve and stabilize Ce(IV)/Ce(III) redox solution. Ce(IV) sulfate solution in a sulfuric acid medium is known to be stable over prolonged periods [29]. The concentration of sulfuric acid was studied in the range of 0.1-3.0 mol L^{-1}. Sulfuric acid at 0.5 mol L^{-1} gave the highest sensitivity as shown in Figure 2c.

As shown in Figure 1, the FIA system consisted of two lines which have equal flow rates. The effect of total flow rate of the system is shown in Figure 2d. The sensitivity decreased when increasing the total flow rate because the reaction between antioxidant and reagent requires time to take place. The lower flow rate provided the longer residence time in a mixing coil leading to the higher sensitivity. The result indicated that 0.8 mL min^{-1} total flow rate gave the highest sensitivity and stable baseline.

The optimum conditions for operation of the proposed flow injection potentiometric system are summarized in Table 1.

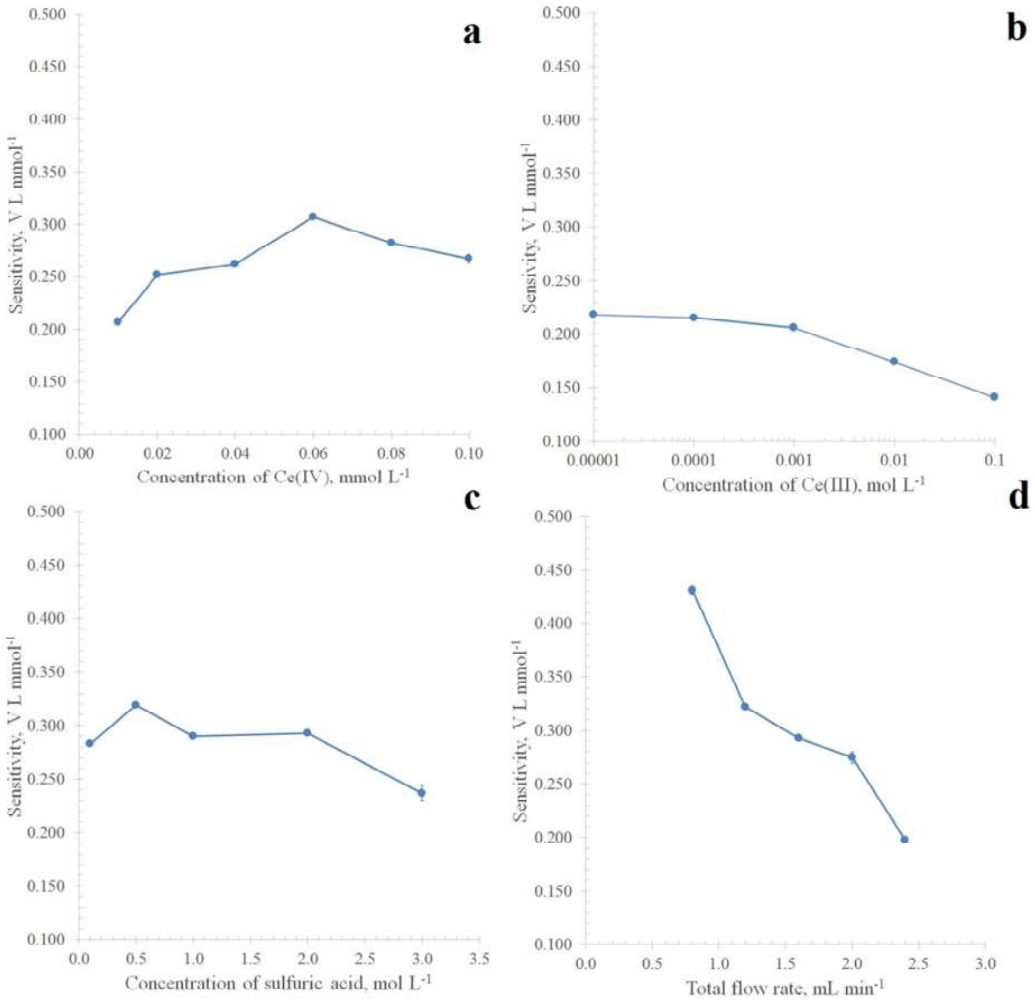

Figure 2. Effect of some parameters on the sensitivity of the method. a) concentration of Ce(IV), b) concentration of Ce(III), c) concentration of sulfuric acid, and d) total flow rate.

Table 1. Optimum condition of the flow injection potentiometric method for determination of antioxidant capacity.

Parameter	Studied range	Selected condition
Ce(IV) concentration (mmol L^{-1})	0.01-0.1	0.06
Ce(III) concentration (mol L^{-1})	0.00001-0.1	0.00001
Sulfuric acid (mol L^{-1})	0.1-3.0	0.5
Total flow rate (mL min^{-1})	0.4-2.4	0.8
Sample volume (µL)	-	200
Mixing coil (cm)	-	50

3.2 Calibration, Precision and Interferences Study

The CERAC method based on spectrophotometric detection is well known for determining of TAC. Various compounds can be used as a standard for determination of TAC such as quercetin, trolox, catechin, sinapic acid, and ascorbic acid [15, 29-32]. In this work, ascorbic acid is selected as an antioxidant standard because ascorbic acid is a well-known antioxidant which found in food, plant and dietary supplement. In addition, ascorbic acid is a common chemical which is readily available in most laboratory and cheaper than other antioxidant standards.

Under the optimum condition as shown in Table 1, the FI responses for the injection of various concentrations of ascorbic acid were obtained as illustrated in Figure 3a. A calibration graph was constructed by plotting peak height versus the logarithm of the concentration of ascorbic acid. The linear calibration graph was obtained in the range of 0.08-1.00 mmol L^{-1} of ascorbic acid ($y = 0.1883\ln(x) + 0.5071$, $R^2 = 0.9965$) as shown in Figure 3b.

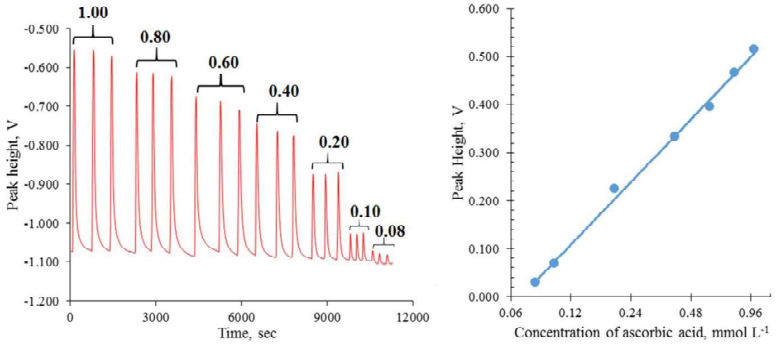

Figure 3. Calibration graph study; a) FIA profiles obtained from the injection of various concentrations of the ascorbic acid (mmol L^{-1}), and b) The linear calibration graph plotting between peak height and the concentration of ascorbic acid (logarithmic scale).

In addition, a calibration graph of gallic acid was studied under the same condition as ascorbic acid. A calibration graph was constructed by plotting peak height versus the logarithm of the concentration of gallic acid. The linear calibration graph was obtained in the range of 0.16-1.00 mmol L^{-1} of gallic acid ($y = 0.1581\ln(x) + 0.6862$, $R^2 = 0.9951$) as shown in Figure 4.

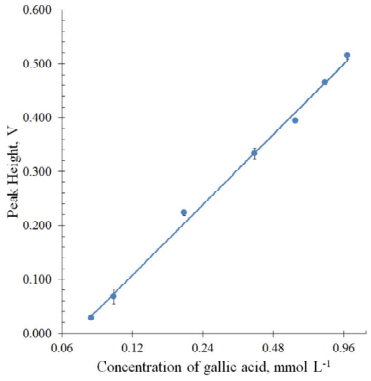

Figure 4. The linear calibration graph plotting between peak height and the concentration of gallic acid (logarithmic scale).

Recovery of the method was examined by spiking standard ascorbic acid solution 0.05-0.20 mmol L^{-1} into some selected tea infusion samples. The recovery percentages were calculated from the results obtained from the calibration graph as compared to the expected spiking values. The TAC (expressed as ascorbic acid equivalent) obtained from the standard addition graph and the calibration graph are close to each other (t-calc = 0.17 and t-table 3.182). The recovery percentages are in the ranges of 95-122%. The results are summarized in Table 2-3.

Table 2. Percentage recovery study calculated from the results obtained from the calibration curve as compared to the expected values.

Sample	Added conc. of ascorbic acid (mmol L^{-1})	Obtained signal (V)	Expected Signal (V)	% Recovery
A	0.00	0.0655	-	-
	0.05	0.1007	0.0825	122
	0.10	0.1684	0.1635	103
	0.15	0.2236	0.1935	115
	0.20	0.2625	0.2752	95
B	0.00	0.0878	-	-
	0.05	0.1273	0.1048	121
	0.10	0.1968	0.1858	105
	0.15	0.2486	0.2158	115
	0.20	0.2821	0.2974	95
C	0.00	0.0959	-	-
	0.05	0.1277	0.1129	113
	0.10	0.1944	0.1939	100
	0.15	0.2530	0.2239	113
	0.20	0.2955	0.3055	97

Table 3. Comparisons of TAC (ascorbic acid equivalent) between calculated from standard addition graph and calibration graph.

Sample	calibration graph (mol L^{-1})	standard addition graph (mol L^{-1})
A	9 ± 1	6 ± 1
B	10 ± 1	9 ± 1
C	10 ± 1	10 ± 1

The relative standard deviations obtained from 11 injections of 0.08, 0.40, and 1.00 mM of ascorbic acid were 6.0, 1.4, and 4.1%, respectively, indicating good reproducibility of the method. The method offered sample throughput of 5 h^{-1}.

Simple sugars and organic compounds widely found in food plants, e.g., glucose, sucrose, citric acid, and tartaric acid did not appreciably change the recovery of the system at 1000 times concentration level higher than that of the analyte. Common cations and anions, e.g., Na^{+}, Ca^{2+}, Al^{3+}, Cl^{-}, NO_3^{-}, SO_4^{2-} and PO_4^{3-} did not interfere. The percentages of recovery are 86.3-113.1% which was close to 100%. The results are shown in Table 4.

Table 4. Recovery of interference study (Compared with ascorbic acid 0.50 mmol L^{-1}).

	Ratio [Interference] : [Ascorbic acid]	Average signal (n=3) (V)	% Recovery
Glucose	0 : 1	0.3574	-
	1 : 1	0.3597	101
	50 : 1	0.3542	99.1
	100 : 1	0.3555	99.5
	500 :1	0.3526	98.7
	1000 : 1	0.3566	99.8
Sucrose	0 : 1	0.3277	-
	1:1	0.3493	107
	50 : 1	0.3512	107
	100 : 1	0.3620	110
	500 :1	0.3506	107
	1000 : 1	0.3527	108
Citric acid	0 : 1	0.3518	-
	1 : 1	0.3611	103
	50 : 1	0.3620	103
	100 : 1	0.3633	103
	500 :1	0.3553	101
	1000 : 1	0.3412	97.0
Tartaric acid	0 : 1	0.3280	-
	1:1	0.3616	110
	50 : 1	0.3708	113
	100 : 1	0.3668	112
	500 :1	0.3622	110
	1000 : 1	0.3425	104
Na^+	0 : 1	0.3015	-
	1 : 1	0.2852	94.6
	50 : 1	0.2824	93.7
	100 : 1	0.2953	97.9
	500 :1	0.2646	87.7
	1000 : 1	0.2753	91.3
Ca^{2+}	0 : 1	0.3523	-
	1:1	0.3489	99.0
	50 : 1	0.3449	97.9
	100 : 1	0.3647	104
	500 :1	0.3513	99.7
	1000 : 1	0.3048	86.3
Al^{3+}	0 : 1	0.3517	-
	1:1	0.3593	102
	50 : 1	0.3408	96.9
	100 : 1	0.3498	99.5
	500 :1	0.3533	100
	1000 : 1	0.3226	91.7

3.3 Samples Analysis

The developed method was applied to the analysis of 15 tea infusion samples obtained from a local supermarket in Chiang Mai, Thailand. The preparation of samples is assumed the brewing of tea infusion for drinking as described above. The results for determination of TAC by the proposed method and the two batch methods are summarized in Table 5. The calibration graph was used for quantification in all the methods. Although the results from all the methods are not equal, they have good correlation. Sample no. 4 gave the highest and sample no. 8 gave the lowest antioxidant capacity. The correlation plots of the results obtained from the proposed method versus the batch-Ce(III)/Ce(IV) potentiometric method and the batch-FRAP are shown in Figures 5a and 5b, respectively. It was found that the results were in good correlation (R^2 of 0.901 and 0.802). From the results, the batch FRAP method gave higher antioxidant capacity than CERAC method indicating that the antioxidant effectively reacted with Fe(III) than Ce(IV). The batch CERAC method provided higher antioxidant activity than the FIP-CERAC method which may result from the longer reaction time between sample and reagent in the batch method. However, the FI method provided higher sample throughput, lower reagent consumption, and more convenient operation than the batch method. It is common that the TAC values obtained from different methods are not equal, because there are many compounds in the sample that contributing to the antioxidant activity. Those compounds may react with different reagents to different extent. Therefore, the correlation between the methods is normally used for validation. The methods should have the same trend or good correlation of the results so that they can be used for comparing the antioxidant capacity of various samples [3].

Table 5. Comparative results of the determination of antioxidant capacity by the proposed method and the batchwise methods.

Sample number	FIA-Ce(III)/Ce(IV) Potentiometric method μg g^{-1} sample	Batch-Ce(III)/Ce(IV) potentiometric method μg g^{-1} sample	Batch - FRAP based on Fe(III)-phenanthroline complex method μg g^{-1} sample
1	4.08 ± 0.01	6.90 ± 0.01	35140 ± 220
2	4.58 ± 0.01	8.50 ± 0.01	42560 ± 240
3	3.87 ± 0.01	7.90 ± 0.01	28310 ± 240
4	5.32 ± 0.01	11.51 ± 0.01	44090 ± 180
5	4.77 ± 0.01	8.70 ± 0.01	32820 ± 220
6	0.12 ± 0.01	0.87 ± 0.01	15470 ± 230
7	0.13 ± 0.01	0.63 ± 0.01	15110 ± 240
8	0.14 ± 0.01	0.37 ± 0.01	13530 ± 240
9	0. 93 ± 0.01	3.42 ± 0.01	19000 ± 220
10	1.59 ± 0.01	4.83 ± 0.01	27420 ± 210
11	1.50 ± 0.01	2.89 ± 0.01	30260 ± 220
12	0.13 ± 0.01	1.40 ± 0.01	17200 ± 220
13	0.14 ± 0.01	3.42 ± 0.01	23280 ± 220
14	0.73 ± 0.01	3.02 ± 0.01	25240 ± 140
15	2.20 ± 0.01	3.33 ± 0.01	30300 ± 200

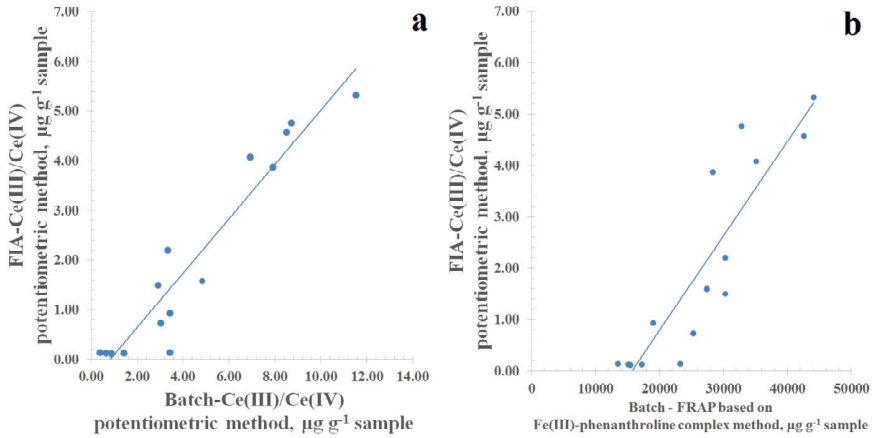

Figure 5. Correlation graph of the total antioxidant capacities determined by the proposed method and the comparative methods: a) versus batch-Ce(III)/Ce(IV) potentiometric method (Linear equation: y = 0.553x - 0.490, correlation coefficient (R^2) = 0.910) and b) versus batch-FRAP based on Fe(III)-phen complex method. (Linear equation: 0.185x - 2.922, correlation coefficient (R^2) = 0.802).

Ce(IV)/Ce(III) is selected as a redox couple reagent because it is more stable than other metal-based reducing power methods such as Fe(III) that can be oxidized by air and unstable in the light. In addition, Ce(IV)/Ce(III) is common and available in general laboratory. The proposed method was applicable to real sample analysis and provided a sample throughput of 5 sample h^{-1} while the batchwise methods gave 2 samples h^{-1}, respectively. The developed method also consumed lower amounts of sample and lower waste production. In the previous studies, spectrophotometric detection and other FI-potentiometric detection use commercial instruments which need expensive spectrophotometer and potentiometer, while the developed method uses a cheap home-made flow-through cell and a home-made potentiometer. Moreover, the proposed method is a semiautomatic operation; it can reduce human error. Table 6 summarized the performance of the developed method and the batchwise methods.

Table 6. Comparison between the developed method and batchwise methods.

Catagories	Developed method	Batch-Ce(III)/Ce(IV) potentiometric method	Batch-FRAP based on Fe(III)-phenanthroline complex method
Detection	Potentiometry	Potentiometry	Spectrophotometry
Reagent for assay	Ce(IV)/Ce(III)	Ce(IV)/Ce(III)	Fe(III)-phenanthroline
Sample volume (µL)	200	200	1000
Waste production/ sample (mL)	1	25	5
Sample throughput (h^{-1})	5	2	2
Interference from colloidal and colored substances	low	low	high
Operation	Semiautomatic	Manual	Manual

4. CONCLUSION

In this work, a potentiometric method combined with flow injection system based on redox reaction of Ce(IV)/Ce(III) reagent solution has been developed for the determination of the total antioxidant capacity (TAC) (expressed as ascorbic acid equivalent). Antioxidants react with Ce(IV) to produce Ce(III) leading to the change in the redox potential of the reagent which is directly proportional to the logarithm of TAC. The proposed method is simple, low-cost, low reagent consumption, and convenient operation. It shows good precision, low interference and gave results which correlated well with those obtained by the batch-Ce(III)/Ce(IV) potentiometric method and the batch-FRAP based on Fe(III)-phen. Moreover, the proposed FIP-CERAC did not suffer from the colored and colloidal substances of samples which can affect the spectrophotometric detection. The developed method should be suitable for screening TAC of some natural products.

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