ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Flow Injection Spectrophotometric System for Determination of Flavonoids in Tea Using Modified Dowd Assay

Piyanete Chantiratikul* and Kwanyuen Leamsomrong

Department of Chemistry and Center of Excellence for Innovation in Chemistry (PERCH-CIC), Faculty of Science, Mahasarakham University, Kantarawichai, Maha Sarakham,44150 Thailand

ABSTRACT

A simple, fast and reliable FI-system for the determination of flavonoids in tea was investigated. The proposed method was based on the reaction of sodium nitrite and aluminum chloride in alkaline solution, with spectrophotometric detection at 500 nm. The experimental parameters were optimized. Linear calibration graph was obtained in the range of 0.5-50.0 mg L^{-1} (as catechin). The relative standard deviations (n=20) were in the range 1.41-2.19%, with detection limit (S/N=3) was 0.03 mg L^{-1}. The sample throughput was 32 samples h^{-1}. The results revealed no significant interference. According to paired sample t-test, there was no significant difference between Dowd's standard spectrophotometric method and the proposed method. The method was successfully applied to flavonoids analysis in tea samples.

Keywords: Flow injection; Spectrophotometry; Flavonoids; Dowd assay; Tea

**Corresponding author*

ISSN: 0975-8585

INTRODUCTION

Teas are used as beverages worldwide and widely consumed in China and Japan. The two main types of tea are green and black. Most phenolic compounds found in tea are polyphenols which consisting of more than one benzene ring with each containing at least one hydroxyl group (-OH). Health benefits of tea are believed to be widely due to the presence of high levels of polyphenols, mainly flavonoids. Flavonoids are phenol derivatives that are distributed in plants and known to exhibit higher antioxidative activities. The main polyphenols present in tea are the flavonoids. The most subclass of flavonoids in tea is the flavanols (primarily catechins) [1]. The flavonoids are largely responsible for the distinctive taste and color of tea [2]. A number of studies suggest that tea consumption may reduce the risk for cardiovascular disease [3], decrease carotid atherosclerosis [4], blood pressure [5] and chloresterol [6].

There are several methods for determining flavonoids in tea such as LC-MS [7], RP-HPLC with UV detection [8-9], NIR reflectance spectroscopy [10], capillary electrophoresis [11,12] and flow injection with adsorptive stripping voltammetry [13,14] and amperometry [15-16].

This study was aimed to establish a simple, fast and reliable flow injection system based on the reaction of sodium nitrite and aluminum chloride in alkaline solution with visible detection at 500 nm for the determination of flavonoids contents in tea infusion. The optimizations of experimental parameters and validation of the proposed method were also investigated.

MATERIALS AND METHODS

Reagents

All reagents used were of analytical reagent grade and all solutions were prepared in deionized water (18.2 MΩ cm) from a Milli-Q water purification system (Millipore Co., USA). $AlCl_3$, $NaNO_2$, NaOH, fructose, sucrose were purchased from BDH, England. Catechin and NaCl was purchased from Fluka, Switzerland. The calibration curve was prepared by diluting catechin solutions from 0.5 to 50.0 mg L^{-1} from stock solutions.

Preparation of Samples

Tea sample solutions were prepared by infusing 1 g of tea powder samples (purchased from a local market in Maha Sarakham province) in hot double-distilled water for about 10 min. Then, the tea solutions were filtrated and adjusted to a 100 ml volumetric flask prior to injection into the proposed FI-system.

Analytical Procedure

The content of flavonoids was determined by spectrophotometric method described previously [17] and modified in our laboratory. Briefly, aliquots of standard catechin solution or appropriate dilution of samples solution were reacted with 5.0% w/v $NaNO_2$ solution. Then, a flavonoid-aluminum complex was occurred using aluminum trichloride. The pink color product was measured at 500 nm after standing at room temperature for 5 min and compared to that of catechin standard.

A schematic diagram for the proposed flow injection analysis system is presented in Fig.1. A peristaltic pump (Perkin Elmer, FIAS-300, U.S.A.) was used for propelling the carrier solution (CS) and reagent (RS1, RS2). Solinoid injection valve, I, with a sample loop (200 μl) was used for introducing catechin as standard solutions, as well as sample solutions into carrier stream. PTFE tubing (i.d.= 0.89 mm) was used for flow lines. The absorbance was measured with UV-Visible spectrophometer (Perkin Elmer Lambda Bio-40, U.S.A.). A personal computer with FIA monitor data processing software (Perkin Elmer, U.S.A.) was used for controlling the apparatus and recording data.

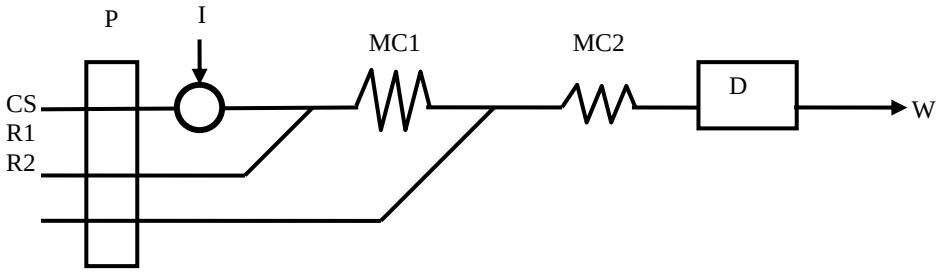

Figure 1: Schematic manifold of FIA spectrophotometric system for determination of flavonoid. CS: carrier (deionized water); R1: AlCl3 solution mixed with NaNO2 solution; R2: NaOH solution; P: peristaltic pump; I: injection valve; MC1 and MC2: mixing coil 1 and 2; D: spectrophotometric detector; W: waste.

A standard/sample solution was injected into a carrier stream of deionized water and flowed to merged with a stream of RS1 ($AlCl_3$ solution mixed with $NaNO_2$ solution) and RS2 (NaOH solution), respectively. The complex formation was take place inline in mixing coil (MC1 and MC2) and was then monitored by measuring absorbance change at wavelength 500 nm. A calibration graph was plotted between absorbance and catechin concentration in standard solution. It was employed for the determination of flavonoids in tea samples.

RESULTS AND DISCUSSION

Selection of detection wavelength

Catechin solution as standard solution was prepared according to the standard procedure and the absorption spectra was obtained in the range from 400 to 800 by a

spectrophotometer. The maximum absorption wavelength of the pink color product was 500 nm (Fig. 2).

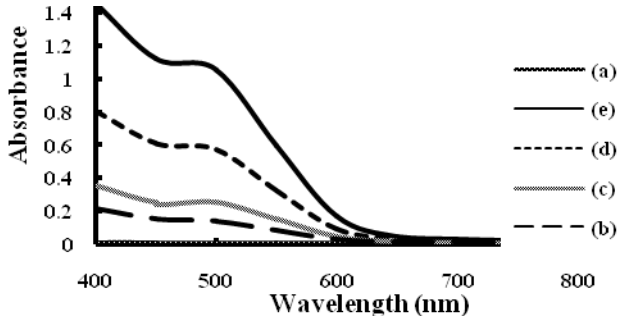

Figure 2: The UV-vis absorption spectra of complexes at different catechin concentrations, (a) blank; (b) 5 mg L-1 catechin; (c) 10 mg L-1 catechin; (d) 25 mg L-1 catechin; (e) 50 mg L-1 catechin.

Optimization of experimental variable for FI-system

The effect of a sample injection volume in the range of 50 to 500 μl was investigated. It could be seen that the absorbance increased with an increase of sample volume up to 200 μl, beyond which the highest absorption remained constant. A sample injection volume of more than 200 μl caused the broad peak and used analysis time. Thus, a sample injection volume of 200 μl was selected for further study.

The effect of flow rate of carrier (double-distilled water, CS) and reagent solutions, which was $AlCl_3$ solution mixed with $NaNO_2$ solution (RS1) and NaOH solution (RS2), was studied in the range of 0.8 to 2.4 ml min^{-1}. The results indicated that the sensitivity slightly increased with an increase of flow rate. However, too low flow rates caused board peak and could lead to poor reproducibility and sample throughput. Thus, the signals decreased when the flow rate increased. Therefore, 1.7, 1.4 and 1.1 ml min^{-1} were selected as the optimum flow rate of carrier, RS1 and RS2, respectively.

The effect of reaction coil length (RC1) was examined in the range of 100 to 1600 mm. The results showed that the absorbance was almost identical when the 750-1300 mm reaction coil length was used. Longer reaction coils gave a longer residence time, but the dispersion of the sample zone became larger, and the output peaks were broadened. The reaction coil length of 800 mm was chosen for rapid analysis measurement.

The effect of reaction coil length, RC2 was investigated in the range of 200 to 1000 m. It can be seen that the absorbance increased with an increase of the length of the reaction coil up to 300 mm. At longer than 300 nm, too large dispersion was occurred. Therefore, 300 mm was used for further study.

The effect of concentration of sodium nitrite solution was examined in a range from 0.1 to 1.0% w/v. It was found that the sensitivity increased with increasing of sodium nitrite concentration. At the concentration of sodium nitrite solution was more than 0.5%, air bubble was occurred. In further experiments, 0.5% sodium nitrite solution was selected.

The effect of concentration of aluminum chloride solution was studied from the range of 0.5 to 2.0% w/v. The sensitivity increased with increasing concentration of aluminum chloride solution. However, a concentration of aluminum chloride was higher than 1% caused precipitation and high baseline. The suitable concentration of aluminum chloride solution was 1% for optimum FIA system.

The effect of concentration of sodium hydroxide solution was studied from the range of 0.1 to 4.0% w/v. The sensitivity increased with increasing concentration of aluminum chloride solution and remained constant when sodium hydroxide concentration was higher than 1.0%. In order to reduce reagent consumption, the suitable concentration of sodium hydroxide solution was 1% for further study.

The studied parameters for flavonoids determination using down assay and their optimum value were summarized in Table 1.

Table 1: The studied parameters and their optimum value of FI system for determination of flavonoids

Analytical characteristics	Studied range	Optimum value
Wavelength (nm)	400-800	500
Sample injection volume (µl)	50-500	200
Flow rate of carrier (ml min^{-1})	0.8-5.4	1.7
Flow rate of sodium nitrite (R1) (ml min^{-1})	0.8-5.4	1.4
Flow rate of sodium hydroxide (R2) (ml min^{-1})	0.8-5.4	1.1
Length of mixing coil 1 (mm)	100-1600	800
Length of mixing coil 2 (mm)	100-1000	300
Concentration of sodium hydroxide (M)	0.1-4.0	1.0
Concentration of sodium nitrite (%w/v)	0.1-1.0	0.5
Concentration of aluminium trichloride (%w/v)	0.5-2.0	1.0

Validation of the proposed FI-system

In order to investigate the linearity response, the catechin standard solutions were used for preparing the calibration curve at the concentration range from 0.5 to 50.0 mg L^{-1}. The equation of calibration graph was expressed as Y = 0.0065X + 0.0089, where Y and X were absorbance and catechin concentration, respectively, with a correlation coefficient of 0.9913. The relative standard deviation (RSD) was 2.14% for 12.55 mg L-1 (n=20). The limit of detection (LOD) was 0.0288 mg L^{-1} (calculated from 3S/N). Thirty-two samples per hour were obtained for sample throughput. The investigation interference species was conducted with regard to possible chemical interferences. The tolerable concentration is defined as the concentration of species causing less than ± 5% relative error. The results revealed no significant interference from citric acid, fructose, sucrose and sodium chloride. These data demonstrated that the

proposed FI-system is sensitive and adequate to determine flavonoids contents at low concentrations.

Application of the proposed FI-system to real samples

The proposed FI-system was applied to the determination of flavonoids in 9 tea powder samples and compared with the original spectrophotometric method (Table 2). It was found that the proposed and original spectrophotometric assay [17] were in good agreement with a correlation coefficient of 0.999 as shown in Fig. 3. Due to the ability to operate continuously, it is possible to analyze about 32 samples h^{-1}, making the method for fast determination of for flavonoids contents in tea.

Table 2: Flavonoids contents in tea infusion samples obtained by FI system and spectrophotometric method

Sample No	FI system (mg g^{-1})	Dowd's method (mg g^{-1})	FIA/Dowd
1	3.35±0.08	3.39±0.06	0.98
2	0.32±0.01	0.32±0.01	1.00
3	3.96±0.10	4.39±0.33	0.90
4	13.69±0.32	14.98±0.33	0.91
5	8.06±0.20	8.71±0.13	0.92
6	0.45 ± 0.01	0.46± 0.01	0.97
7	0.034±0.02	0.042±0.00	0.80
8	5.93±0.06	7.06±0.13	0.83
9	13.40±0.21	14.63±0.01	0.91

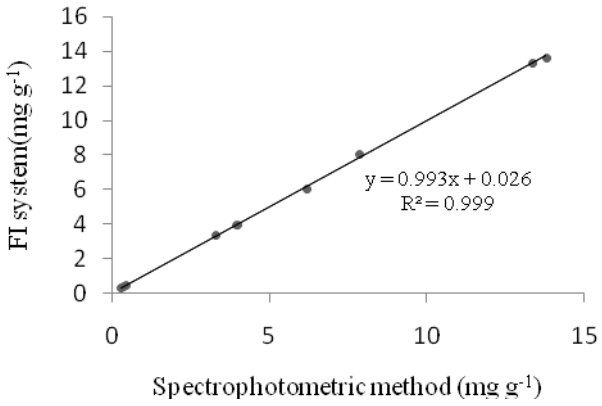

Figure 3: Correlation relation between the proposed FI system and original spectrophotometric method.

CONCLUSION

A simple, fast and reliable FI-system based on the reaction of $NaNO_2$ and $AlCl_3$ in alkaline solution for the determination of flavonoids in tea was developed. The complex product has maximum absorption wavelength of 500 nm. This system could be used for the determination of flavonoids in the range 0.5-50.0 mg L^{-1} (as catechin). It showed no significant difference with the original spectrophotometric method. This proposed method is sensitive, fast, reliable and

adequate to determine flavonoids contents and can be directly applied to the determination of flavonoids in tea samples.

ACKNOWLEDGEMENTS

The authors are grateful for the financial support from Mahasarakham University Budget fiscal year 2011 and Center of Excellence for Innovation in Chemistry (PERCH-CIC), Commission on Higher Education, Ministry of Education.

REFERENCES

[1] Hodgson JM, Croft KD. Mol Aspects Med 2010; 31: 495-502.
[2] Peterson J, Dwyer J, Bhagwat S, Haytowitz D, Holden J, Eldridge AL, Beecher G, Aladesanmi J. J Food Comp Anal 2005; 18: 487-501.
[3] Iwai N, Ohshiro H, Kurozawa Y, Hosoda T, Morita H, Fumakawa K, Okamoto M, Nose T. J Epidemiol 2002; 12: 191-198.
[4] Mursu J, Nurmi T, Tuomainen TP, Ruusunen A, Salonen JT, Voutilainen S. J Nutr 2007; 98: 814-818.
[5] Hodgson JM, Croft KD. J Sci Food Agric 2006; 86: 2492-2498.
[6] Hodgson JM. Asia Pacific J Clin Nutr 2008; 17: 288-290.
[7] Justesen U, Knuthsen P, Leth T. Cancer Lett 1997; 114: 165-167.
[8] Hertog MGL, Hollman PCH, van de Putte B. J Agric Food Chem 1993; 41: 1242-1246.
[9] Bramati L, Aquilano F, Pietta P. J Agric Food Chem 2003; 51: 7472-7474.
[10] Schulz H, Engelhardt UH, Wegent A, Drews H, Lapczynski S. J Agric Food Chem 1999; 47: 5064-5067.
[11] Kreft S, Knapp M, Kreft I. J Agric Food Chem 1999; 47: 4649-4652.
[12] Ozyurt D, Demirata B, Apak R. Talanta 2007; 71: 1155-1165.
[13] Volikakis GJ, Efstathiou CE, Talanta 2000; 51: 775-785.
[14] Volikakis GJ, Efstathiou CE. Anal Chim Acta 2005; 551: 124-131.
[15] Pedrosa VA, Malagutti AR, Mazo LH, Avaca LA. Anal Lett 2006; 39: 2737.
[16] Yashin AY. Russ J Gen Chem 2008; 78: 2566-2571.
[17] Adom KK, Liu RH. J Agric Food Chem 2002; 50: 6182-6187.