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Determining the levels of tannin in tea by amperometry of ferricyanide pre-reaction with a sample in a flow-injection system

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Abstract

An amperometric flow-injection assay was proposed for measuring the levels of tannin in tea. Electrode fouling owing to electro-oxidation of tannin could be efficiently avoided by utilizing ferricyanide as an oxidant to pre-oxidize tannin for indirect quantification without electrode modification. Appropriate concentrations of ferricyanide were pre-mixed with tannic acid and then measured by a platinum electrode in an electrochemical flow cell. The effects of working potential and flow rate on amperometric response were investigated, and the optimal condition was obtained when the working potential was −0.1 V (versus Ag/AgCl) and the flow rate was 2.28 mL min^{-1} in this work. The linear detection range for tannic acid could be effectively adjusted according with application demand, and the results of this study indicated two ranges of 10–50 μg mL^{-1} and 100–500 μg mL^{-1} (CV < 5%, $n = 3$). Beverage samples of tea with various fermentation degrees were measured (15 samples h^{-1}) and verified with Folin–Ciocalteau method ($r = 0.9794$) as well as the ferrous tartrate method ($r = 0.9918$).

Keywords: Tannic acid; Amperometry; Flow-injection; Ferricyanide

1. Introduction

Tannin is defined as water-soluble phenolic compound with a molecular weight ranging between 500 and 3000 [1]. It is the main secondary metabolite of plants [2] and is abundant in numerous plant-based beverages, such as tea, red wine and beer. Tannin can bind and/or precipitate water-soluble proteins, and astringency, a key organoleptic property of tea [3], results from the non-covalent interactions between proline-rich proteins in saliva and tannin in the beverage [4]. Moreover, the ability of tannin to serve as a biological antioxidant has been reported [5,6]. Because of quality control and health issues, tannin content is a key index in tea classification and processing. Therefore, a reliable and convenient method of on-line tannin determination is urgently needed in industrial processing of tea beverages.

Numerous methods such as spectrometry [7,8], chemiluminescent [9] and fluorescent [10] have been proposed for determining the levels of tannins. Regarding spectrometric methods, Folin–Ciocalteau [11] and ferrous tartrate methods are adopted as the official methods in the USA and Japan, respectively. The Folin–Ciocalteau (or Folin–Denis) method based on redox chemistry is one of the most widely used methods. Owing to complex-formation with tannin, the ferrous tartrate method is not affected by the co-existing ascorbate [12]. Nonetheless, trivial preparation of reagents, time-consuming incubation and heat processing for colorful complex-formation of spectrometric methods make measurement inconvenient and on-line application difficult.

Cyclic voltammetry, an electrochemical method, used to determine tannic acid has previously been reported [13]. However, when sufficient positive potential was applied for electrochemical oxidation of tannins, phenol and phenolic compounds were reported to form an inhibitory layer on surfaces of Pt, Au and glass carbon electrode [14–16]. Redox reaction at the electrode is gradually blocked by the formatted layers, which have high molecular weight and comprise oxidized mate-

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rials [14]. Polishing performed between every measurement for surface cleaning was reported to eliminate this drawback of electrode fouling [17]. Although electrochemistry has the advantages of simplicity and convenience for constructing an on-line and real-time sensing system, further methods of improvement will be required in the future.

Prussian blue assay, which is also a spectrometry, came to our notice owing to its potential for creating a new strategy for establishing an electrochemical on-line system for sensing tannins. Amperometry with a pre-oxidation process was utilized for determining tannin. Eqs. (1) and (2) show the reaction mechanism:

$$\text{tannin} + \text{Fe(CN)}_6^{3-} \rightarrow \text{oxidized tannin} + \text{Fe(CN)}_6^{4-} + \text{residue of Fe(CN)}_6^{3-} \quad (1)$$

$$\text{residue of Fe(CN)}_6^{3-} + e^- \xrightarrow{-0.1\,\text{V versus Ag/AgCl}} \text{Fe(CN)}_6^{4-} \quad (2)$$

Ferricyanide serves as an oxidant for tannin pre-oxidation, and tannin content is quantified by the reduction current of residual ferricyanide, which is proportional to the remaining quantity of ferricyanide. The proposed method thus could avoid fouling of the working electrode owing to electrochemical oxidation of tannins without the need for electrode modifications. Furthermore, on-line measurement using the proposed method is developed with a flow-injection analytical system.

2. Experimental

2.1. Chemicals and reagents

Potassium ferricyanide, potassium dihydrogen phosphate, disodium phosphate, sodium carbonate, and ammonium ferrous sulfate hexahydrate were obtained from Nacalai Tesque, Inc. (Kyoto, Japan). Moreover, tannic acid and potassium sodium(+)-tartrate tetrahydrate were purchased from Wako Pure Chemical Industries, Ltd. Finally, Folin–Ciocalteau reagent was obtained from Sigma Chemical Co. (St. Louis, MO, USA). All chemicals were of analytical-reagent grade and used as received without further purification.

2.2. Flow-injection manifold

The system (Fig. 1) was assembled by conventional flow-injection tubing (silicon tubes with 1 mm i.d.) and polypropylene connectors. The carrier and samples were driven using peristaltic pumps (SMP-23S, Tokyo Rikakikai Co., Japan). Samples loaded in a sampling loop (200 μL) were injected and delivered through a homemade flow cell with an Ag/AgCl/3M KCl (RE-6, BAS) as the referenced electrode, a stainless tube as the counter electrode (1 mm i.d.) and a platinum wire (1 mm diameter) as the working electrode for three-electrode amperometric measurement [18]. Amperometric signals were monitored and recorded with a digital potentiostat/galvanostat (PGSTA-30, Autolab) and GPES 4.9 software (Eco Chemie BV, The Netherlands).

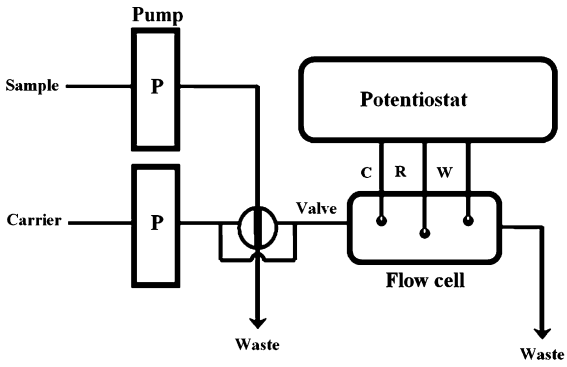

Fig. 1. Schematic representation of the proposed flow-injection analysis (FIA) manifold. P: peristaltic pump; valve: 20 μL of injection valve; W: platinum working electrode (1 mm diameter).

2.3. Methods for determining tannins

4.5 mL of tannic acid solution (100–500 μg mL^{-1}) as a standard was adjusted to pH 7 using 0.5 mL of 1 M phosphate buffer and mixed with 5 mL of 20 mM ferricyanide solution for pre-oxidation. The mixture was injected and driven (2.28 mL min^{-1}) by the carrier (pH 7 phosphate buffer, 0.1 M) to the manifold for amperometric measurement (−0.1 V versus Ag/AgCl). For a calibration range of 10–50 μg mL^{-1}, 20 mM ferricyanide solution was replaced by 2 mM ferricyanide solution. Tea infusion samples were appropriately diluted and treated as the tannic acid solution.

2.3.1. Folin–Ciocalteau method [19]

One milliliter of sample (20–100 μg mL^{-1}) was added to 5 mL of 10-fold diluted Folin–Ciocalteau reagent. The solution was incubated at ambient temperature for 5 min and mixed with 75 g L^{-1} sodium carbonate solution. The absorbance at 765 nm was measured by UV–vis spectrometry (V-530, Jasco) following incubation for 2 h at ambient temperature.

2.3.2. The ferrous tartrate method (Japanese official method) [20]

0.2 mL of sample (50–300 μg mL^{-1}), 0.5 mL of ferrous tartrate solution comprising 0.1 g of ammonium ferrous sulfate hexahydrate plus 0.5 g of potassium sodium (+)-tartrate tetrahydrate dissolved in 100 mL of deionized water and 2.5 mL phosphate buffer (0.1 M pH 8) were mixed sequentially. The absorbance at 540 nm was measured after 20 min of mixing at ambient temperature.

2.4. Preparation of tea infusions

Four varieties of tea leaves, including Pi-lo-chun green tea (non-fermented tea), Wenshan Pouchong tea light partially fermented tea, Formosa Oolong tea (heavy partially fermented tea) and Taiwanese No. 18 black tea (fermented tea), were assessed. For each variety of tea 3 g of tea leaves were infused in 150 mL hot water (83 °C) for 30 min. The infusions were filtered through standard filter paper (Whatman, 11 μm of diameter) and then cooled to 25 °C via a water bath. The tea infu-