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An enhanced cerium(IV)–rhodamine 6G chemiluminescence system using guest–host interactions in a lab-on-a-chip platform for estimating the total phenolic content in food samples

Buthaina Al Haddabi, Haider A.J. Al Lawati *, FakhrEldin O. Suliman

Department of Chemistry, College of Science, Sultan Qaboos University, Box 36, Al-Khod 123, Oman

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ABSTRACT

Two chemiluminescence-microfluidic (CL-MF) systems, e.g., Ce(IV)–rhodamine B (RB) and Ce(IV)–rhodamine 6G (R6G), for the determination of the total phenolic content in teas and some sweeteners were evaluated. The results indicated that the Ce(IV)–R6G system was more sensitive in comparison to the Ce(IV)–RB CL system. Therefore, a simple (CL-MF) method based on the CL of Ce(IV)–R6G was developed, and the sensitivity, selectivity and stability of this system were evaluated. Selected phenolic compounds (PCs), such as quercetin (QRC), catechin (CAT), rutin (RUT), gallic acid (GA), caffeic acid (CA) and syringic acid (SA), produced analytically useful chemiluminescence signals with low detection limits ranging from 0.35 nmol L^{-1} for QRC to 11.31 nmol L^{-1} for SA. The mixing sequence and the chip design were crucial, as the sensitivity and reproducibility could be substantially affected by these two factors. In addition, the anionic surfactant (i.e., sodium dodecyl sulfate (SDS)) can significantly enhance the CL signal intensity by as much as 300% for the QRC solution. Spectroscopic studies indicated that the enhancement was due to a strong guest–host interaction between the cationic R6G molecules and the anionic amphiphilic environment. Other parameters that could affect the CL intensities of the PCs were carefully optimized. Finally, the method was successfully applied to tea and sweetener samples. Six different tea samples exhibited total phenolic/antioxidant levels from 7.32 to 13.5 g per 100 g of sample with respect to GA. Four different sweetener samples were also analyzed and exhibited total phenolic/antioxidant levels from 500.9 to 3422.9 mg kg^{-1} with respect to GA. The method was selective, rapid and sensitive when used to estimate the total phenolic/antioxidant level, and the results were in good agreement with those reported for honey and tea samples.

1. Introduction

A number of studies have investigated the behavior of phenolic acids and polyphenols using a Ce(IV) and rhodamine 6G (R6G) or rhodamine B (RB) chemiluminescence (CL) system. The effects of 32 phenolic compounds (PCs), including phenols, polyphenols, hydroxycinnamic acids, and flavonoids, on Ce(IV)–R6G CL were investigated using a flow injection (FIA) procedure. Most of the PCs produced a CL signal with the Ce(IV)–R6G CL system because of the presence of phenolic hydroxyl groups, with signal magnitudes corresponding to the positions of the substituents on the benzene ring. However, some of these compounds also quenched the CL signal [1]. An HPLC method was also developed using Ce(IV)–R6G CL for the determination of 20 PC. The method was successfully applied for the determination of PCs in red wine samples [2]. Yao et al. investigated the behaviors of some flavonoids using the Ce(IV)–RB CL system and determined that the proposed CL system was fast and suitable for use in a flow injection system. In addition, CL detection could potentially be used for the determination of flavonoid compounds in food, as well as in biological and environmental samples [3]. However, in either study, the methods were not used or evaluated for the determination of the total phenolic content in food samples. In another study, the determination of the flavonoid levels in *Cirsium oleraceum* and *Cirsium rivulare* extracts was carried out using Ce(IV)–R6G CL detection. However, the extracts are known to primarily contain two types of flavonoids, e.g., apigenin and linarin derivatives [4]. Common phenolic acids and polyphenols were not evaluated in the study. Recently, a study on the selection of a CL system for an FIA procedure to determine the total polyphenol index in plant-derived foods was carried out. However, a number of CL-FIA methods were evaluated in this study, and the authors concluded that the sensitivity of the Ce(IV) and R6G or RB CL system was considerably worse compared to that of the other CL

* Corresponding author.
E-mail address: haiderl@squ.edu.om (H.A.J. Al Lawati).

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systems. Therefore, these systems were excluded from further investigation in that study [5]. Clearly, rhodamine systems require more careful investigation to allow appropriate comparison among all the CL systems used to evaluate the PCs contents in food samples.

Therefore, our aim was to compare the Ce(IV)–RB and Ce(IV)–R6G CL systems separately for the determination of the PCs in food samples and to unravel why these systems were abandoned in previous studies. The study was conducted using a novel microfluidics platform. In contrast to most FIA-based methods, planar microchannels were employed instead of tubing, which facilitated planar integration of CL detection components to create a fully portable system [6]. In addition, reagent consumption is significantly reduced by using this miniaturized platform [7]. Nevertheless, several challenges needed to be resolved before this technique could be utilized. The sample volume is typically small in microfluidics compared to that in the FIA systems. Therefore, it is more difficult to obtain the lower limit of detection. In addition, the mixing occurs at a much lower flow rate in microfluidics than in the FIA systems, which may significantly affect the CL signal, especially with the very short residence time that is typically encountered in microfluidics [8]. Therefore, careful optimization of the chip geometry, volume of the detection chip, and other experimental factors is required to overcome these challenges.

The effects of several surfactants (e.g., cetyltrimethylammonium bromide (CTAB), Tween 20, Brij 35, Triton X-100, and sodium dodecyl sulfate (SDS)) on both the CL systems (i.e., Ce(IV)–R6G and Ce(IV)–RB) were also investigated. Several reports have indicated that some of these surfactants were capable of improving the sensitivity of various CL systems [9].

Finally, the Ce(IV)–R6G CL system was used to determine the total phenolic content in various green, black and herbal (ginger root) tea samples. We also compared the PCs of certain local honey samples, which are known as *Sumer*, with those of pomegranate molasses, sugarcane molasses and date molasses. These results were compared to those obtained using the Folin–Ciocalteu assay (FC) and the 2,2-diphenyl-1-picrylhydrazyl radical scavenging assay (DPPH$^{\bullet}$).

2. Materials and methods

2.1. Chemicals and reagents

All of the reagents were of analytical grade, and the dilutions were performed using deionized water (Millipore, MilliQ water system, France). SDS was purchased from Kanto (Japan). Cetyltrimethylammonium bromide (CTAB) was purchased from Merck (Germany). Tween 20, Brij 35 and Triton X-100 were purchased from Calbiochem (US and Canada). The Folin–Ciocalteu reagent and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were purchased from Sigma (USA). Ammonium cerium (IV) nitrate, rhodamine B and rhodamine 6G were purchased from Sigma (UK). GA, CA, CAT, RUT, SA, QRC, β-D-(+)-glucose, sucrose, D-(−)-fructose, leucine, N-acetyl-L-cysteine, glycine, L-valine, DL-alanine, DL-tryptophan, hydrated magnesium sulfate, sodium sulfate, potassium chloride, aluminum sulfate hydrate, copper chloride, manganese sulfate hexahydrate, ferrous chloride, caffeine, folic acid, thiamine (vitamin B_1) and nicotinamide (vitamin B_3) were purchased from BDH (Poole, England). Sulfuric acid and sodium hydroxide were purchased from Sigma Aldrich (Germany).

2.1.1. Reagents

R6G solution (0.04 mmol L^{-1}) was prepared by dissolving approximately 2.0 mg of R6G in 100 mL of SDS (0.04%). Ammonium cerium(IV) nitrate solution (10 mmol L^{-1}) was prepared by dissolving 55.0 mg in 10 mL of 0.03 mol L^{-1} sulfuric acid. Stock solutions (100 mg L^{-1}) of the antioxidants (i.e., CA, GA, SA, QRC, CAT and RUT) were prepared daily by dissolving 10 mg of each antioxidant in 100 mL of a 0.5 mmol L^{-1} NaOH solution. Further dilutions were made using deionized water.

2.1.2. Samples

Eight commercially available sweetener samples were purchased, e.g., two honey samples, two pomegranate molasses samples, two date molasses samples, and two sugarcane molasses samples. These samples were prepared by dissolving 2.5 g of each sweetener in 100 mL of a boiling 0.5 mmol L^{-1} NaOH solution and stirring the sample. Six commercially available tea samples were purchased, e.g., two green tea samples, two black tea samples, and two herbal tea samples (ginger root) from Annique Health and Beauty (South Africa). These tea samples were prepared by dissolving 200 mg of each sample in 100 mL of boiling water and filtering prior to use.

2.2. Instrumentation

2.2.1. Determination of the total phenolic content using the Folin–Ciocalteu assay

The total phenolic content was determined as previously described, with some modifications [10,11]. In contrast to the described method, the sweetener samples were used without further dilution, whereas dilutions were required for tea samples. A 0.5 mL aliquot of each standard (10–100 mg L^{-1}) or sample solution was added to 2.5 mL of water and 0.5 mL of the Folin–Ciocalteu reagent, followed by incubation for 5 min. Next, 2 mL of a 20% (w/v) sodium carbonate solution was added. The reaction mixture was allowed to stand at room temperature for 1 h prior to measuring the absorbance at 785 nm. The total phenolic content was determined based on the absorbance obtained with respect to GA as the standard.

2.2.2. DPPH radical scavenging method

The radical scavenging activities of the honey samples were tested based on the scavenging of the DPPH free radical. Each sample (0.5 mL of 500 mg mL^{-1} in methanol) was mixed with 3.5 mL of DPPH (6×10^{-5} mol L^{-1} in methanol) in a test tube. The tube was incubated at room temperature in the dark for 2 h, followed by centrifugation at 4200 rpm for 10 min. The absorbance of the DPPH control was noted and subtracted from that of the sample to obtain the scavenging activity of the honey sample using the following equation: $((Abs_{control} - Abs_{sample})/Abs_{control}) \times 100\%$.

In each experiment, the honey sample was analyzed three times, and the average of the three analyses was used in further calculations.

2.2.3. Microfluidics setup for determination of TPC

Chip A (Fig. 1) was a serpentine and teardrop chip (depth: 150 μm; width: 200 μm; total volume: 15 μL, with 13 μL for the serpentine part and 2 μL for the teardrop part). Chip B (Fig. 1) was a spiral with 32 flow splits (depth: 150 μm; width: 200 μm; volume: 3 μL). Chip C (Fig. 1) was a serpentine (depth: 150 μm; width: 200 μm; volume: 6 μL) and a spiral with 32 flow splits (depth: 150 μm; width: 200 μm; volume: 6 μL). Fluidic Connect 4515 chip holders and fused silica capillaries were obtained from Micronit (Netherlands), and the syringe pumps were obtained from Basi Bee (USA). The detector was a Hamamatsu H7155-2 photomultiplier tube (PMT) (Japan). It consisted of a photomultiplier tube with a high speed counting circuit and a high voltage power supply, which allowed photon counting measurement by connecting to a +5 V supply. Using fluidic connect 4515