Talanta 186 (2018) 286–292

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Programmable electrochemical flow system for high throughput determination of total antioxidant capacity

Kornautchaya Veenuttranon, Loc Thai Nguyen*

Department of Food, Agriculture and Bioresources, School of Environment, Resources and Development, Asian Institute of Technology, 58 Moo 9, Km. 42, Paholyothin Highway, Klong Luang, Pathumthani 12120, Thailand

ARTICLE INFO

Keywords:
Programmable flow system
Electrochemical
Amperometric
DPPH•
Total antioxidant capacity

ABSTRACT

The aim of this work was to develop a programmable flow system for rapid assessment of total antioxidant capacity (TAC). Novel features of the prototype include a single pressure-driven source, versatile manipulation of fluid flows, ability to adapt to different TAC assays, and compatibility with microfluidic design. Antioxidant activity was determined by electrochemical measurement of residual 2,2-diphenyl-2-picrylhydrayl hydrate (DPPH•) free radicals in the solution. The overall performance of the device was validated by the spectrophotometric method. The results indicated that dosing of reagents and samples could be controlled by pressure (R^2 = 0.992) and time (R^2 = 0.999) with high accuracy, and the mixing uniformity of the device was equivalent to that of the batch mixing (R^2 = 0.994). TAC assays of a standard antioxidant, ascorbic acid, as well as selected samples such as orange and pomegranate juices, white wine, and green tea by the device were comparable to traditional measurement techniques. Due to the short incubation time, the approach may be more suitable for fast, rather than slow reacting antioxidant compounds. The developed system could be used for rapid TAC screening of food and biological samples.

1. Introduction

The importance of antioxidants for biological systems has been widely recognized [1]. Antioxidants maintain the nutritional quality of foods, and lessen oxidative damage caused by reactive oxygen species (ROS) in the human body [2]. Various studies have shown that antioxidative compounds have the potential to prevent neurodegenerative diseases, cancers, cardiovascular diseases, inflammation, and aging [3]. Therefore, antioxidants are of great interest to the food industry, biologists, and clinicians [2]. The composition of food and biological samples are complex; further made so by the interactions between antioxidants. Analysis of total antioxidant capacity (TAC), an estimation of antioxidant protective activity, is therefore a useful metric for clinical and public health applications [4]. Current methods of TAC measurement include quantification of free radicals in solution, such as by 2,2-diphenyl-1-pycrylhydrazyl radical (DPPH•) or 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulphonate) radical cation ($ABTS^{\bullet+}$). Other methods include the ferric reducing antioxidant power (FRAP) or Folin-Ciocalteu reducing capacity assays. The analysis of antioxidant capacity by DPPH• or $ABTS^{\bullet+}$ mainly relies on the quenching of these radicals by antioxidant compounds. The residual free radicals can be quantified by spectrophotometric or electrochemical measurement [3]. Unlike optical methods, electrochemistry-based assays are useful for turbid and dark-colored samples [5]. Many authors have successfully applied electrochemical techniques, such as cyclic voltammetry and amperometry to determine antioxidant capacity of biological samples [6–10]. Despite their high applicability, these assays are time-consuming, laborious, and costly, especially for routine analysis or rapid, high-throughput screening [11,12]. Therefore, it is desirable to develop automatable methods for determining antioxidant capacity; this would allow high-throughput analysis, lessen operator intervention, reduce consumption of reagents and samples, decrease residues, and improve reproducibility [13]. Significant advancement in automation of antioxidant capacity assays has been reported in recent years [14,15]. Techniques such as flow injection analysis (FIA) [16,17], sequential injection analysis (SIA) [18–20] and high-performance liquid chromatography-flow injection analysis HPLC-FIA [21–24] were investigated. However, the versatility and efficiency components including fluid manipulation, system control, and integration of these systems need continued enhancement. For example, using the EC_{50} assay on plant-based extracts requires multi-step operations, such as serial dilutions of the sample, mixing with reagents, pumping, measurement, and washing [25]. In assays which involve several reagents, such as ABTS or FRAP, simultaneous or sequential mixing steps are required [6,26,27]. In addition, the

* Corresponding author.
E-mail address: locnguyen@ait.ac.th (L.T. Nguyen).

https://doi.org/10.1016/j.talanta.2018.04.073
Received 25 January 2018; Received in revised form 22 April 2018; Accepted 23 April 2018
Available online 25 April 2018
0039-9140/

programmable control of the device should be flexible so that the same system can be used for different TAC analyses (DPPH, ABTS, FRAP or Folin–Ciocalteu)[28].

In this study, we propose a novel, automated, pressure-controlled flow system for determining TAC in food samples. The prototype was adapted from microfluidic systems and was cut with a low-cost laser cutting machine. The prototype was used to assess TAC by amperometric measurement of DPPH• free radical. The system can automate a complete, multistep TAC assay and was applied to determine TAC of both standard antioxidants and real food samples. The prototype can be further developed into miniaturized devices in the future.

2. Materials and methods

2.1. Chemicals and sample preparation

2,2-diphenyl-1-picrylhydrazyl (DPPH•) and gallic acid (GA) (98.0%) were purchased from Sisco research laboratories (Maharashtra, India). Sodium dihydrogen phosphate (NaH_2PO_4) (99.0%) and disodium hydrogen phosphate (Na_2HPO_4) (99.0%) were purchased from Daejung (Seoul, South Korea). All reagents were analytical grade. Phosphate buffered saline (PBS) (0.03 mM, pH 6.0) was prepared as previously reported [18]. DPPH• stock solution (0.5 mM) was prepared in ethanolic phosphate buffer (EPBS), which contained 1:1 mixture of PBS and absolute ethanol. The standard GA stock solution (1.0 mM) was prepared in deionized water.

The green tea infusion was prepared by brewing 1 g of dry green tea leaves (Lao Chen Company, China) in 50 ml of deionized water at 60 °C for 20 min. The infusion was filtered and allowed to equilibrate to ambient temperature prior to analysis. Juices, freshly extracted from oranges and pomegranates, were passed through filter paper (1.0 μm) to remove the pulp. The wine sample (China Agricultural University, China) was analyzed in its original condition. All samples were stored in dark containers prior to experiments.

2.2. System design and operations

Fig. 1 shows a schematic diagram of the prototype device. There are three main components: the flow cell, the electrochemical detection cell, and the flow control system. The complete system, including the computer control component, flow cell, and electrochemical detection unit, is shown in Fig. S1, S2 and S3.

The flow cell (Fig. 1B) was designed by SolidWork software (version 2012, SolidWorks Corp., USA) and cut from an acrylic block (200 × 154 × 8 mm) using a laser cutting machine. The channel depth was fixed at 1 mm. The flow cell served as the central reactor for mixing samples and reagents. Both simultaneous and sequential mixing were possible with suitable programming. The flow cell, with a total volume of 1500 μL, housed two separate chambers for sample injection and mixing, respectively. The electrochemical detection unit had an inner volume of 120 μL and contained a screen-printed carbon electrode with 3.0 mm diameter working electrode (Quasense, Thailand). The components of the device were connected by Tygon® tubing (ID 1/16″) using luer adapters. To avoid backflow of liquid from ports $P_{1–5}$, J_2, and J_4, luer check valves were installed in the corresponding lines. Liquid flow in the system was driven by compressed nitrogen gas, and was regulated by solenoid valves at ports $P_{1–5}$, J_1 and F_1 (Fig. 1A).

2.3. Electrochemical measurements

Electrochemical measurements were performed by a portable potentiostat (DY2116B, Digi-Ivy, USA). The electrochemical behaviors of DPPH and GA were studied by cyclic voltammetry over potential range − 0.1–0.6 V and scan rate of 0.05 V/s. DPPH scavenging activity of

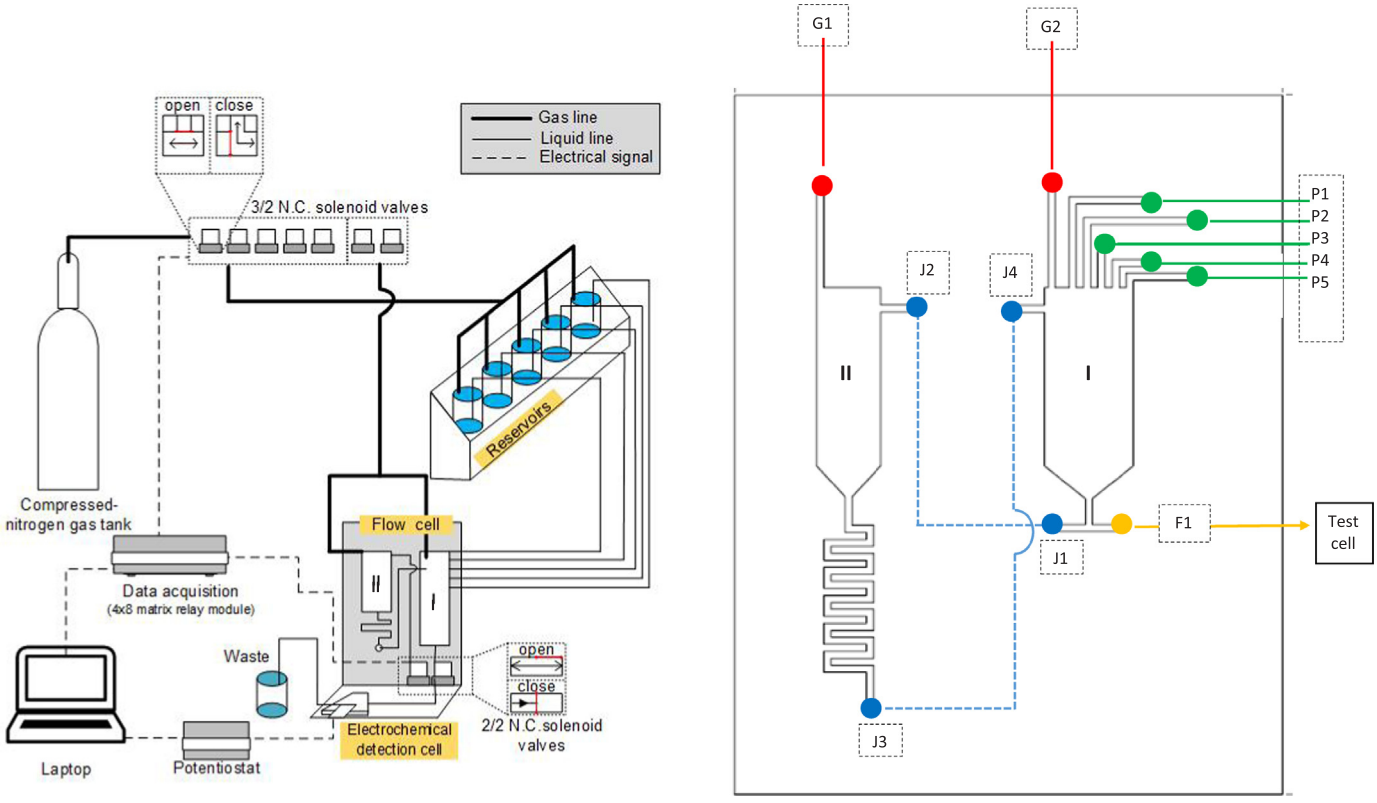

Fig. 1. (A) Schematic diagram of the system. Major components including 1) flow cell, 2) electrochemical detection cell and 3) flow control system (compressed N_2 gas, sample and reagent reservoirs, solenoid valves, matrix relay module, computer). (B) Flow cell: I) feeding channel, II) mixing channel. Port connections: (G1, G2) - compressed nitrogen gas, (P1) - PBS reservoir, (P2) - distilled water reservoir, (P3) - DPPH solution reservoir, (P4) - sample reservoir, (P5) - EPBS reservoir, (J1-J4) - connecting junctions, and (F1) -electrochemical detection cell.