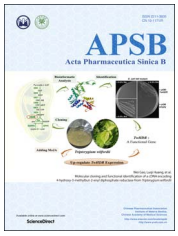
Acta Pharmaceutica Sinica B ▮▮▮▮;▮(▮):▮▮▮–▮▮▮

Chinese Pharmaceutical Association
Institute of Materia Medica, Chinese Academy of Medical Sciences

Acta Pharmaceutica Sinica B

www.elsevier.com/locate/apsb
www.sciencedirect.com



SHORT COMMUNICATION

Scanometry as microplate reader for high throughput method based on DPPH dry reagent for antioxidant assay

Mochammad Amrun Hidayat, Aulia Fitri, Bambang Kuswandi*

Chemo and Biosensor Group, Faculty of Pharmacy, University of Jember, Jember 68121, Indonesia

Received 10 November 2016; revised 2 January 2017; accepted 27 January 2017

KEY WORDS

Scanometry;
High throughput;
DPPH;
Optical sensor;
Antioxidant

Abstract The stable chromogenic radical 1,1′-diphenyl-2-picrylhydrazyl (DPPH) solution was immobilized on the microwell plate as dry reagent to construct a simple antioxidant sensor. Then, a regular flatbed scanner was used as microplate reader to obtain analytical parameters for antioxidant assay using one-shot optical sensors as scanometry technique. Variables affecting the acquisition of the images were optimized and the analytical parameters are obtained from an area of the sensing zone inside microwell using the average luminosity of the sensing zone captured as the mean of red, green, and blue (RGB) value using ImageJ® program. By using this RGB value as sensor response, it is possible to determine antioxidant capacity in the range 1–25 ppm as gallic acid equivalent (GAE) with the response time of 9 min. The reproducibility of sensor was good (RSD < 1%) with recovery at 93%–96%. The antioxidant sensor was applied to the plant extracts, such as sappan wood and Turmeric Rhizome. The results are good when compared to the same procedure using a UV/Vis spectrophotometer.

*Corresponding author. Tel./fax: +62 331324736.
E-mail address: b_kuswandi.farmasi@unej.ac.id (Bambang Kuswandi).

Peer review under responsibility of Institute of Materia Medica, Chinese Academy of Medical Sciences and Chinese Pharmaceutical Association.

http://dx.doi.org/10.1016/j.apsb.2017.02.001

1. Introduction

Antioxidant capacity is a broadly used term as a parameter to characterize different substances and food samples with the ability of scavenging or neutralizing free radicals. This capacity is associated to the presence of compounds capable of protecting a biological system against harmful oxidation[1]. There are several synthetic radicals, such as 2,2′-azinobis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS), 1,1′-diphenyl-2-picrylhydrazyl (DPPH), and *N*,*N*-dimethyl-*p*-phenylendiamine (DMPD), employed for determining antioxidant capacity of various samples[2–5]. The DPPH method is one of the most frequently used to assess the ability of compounds as free radical scavengers or hydrogen donors, and to evaluate the antioxidant capacity of food samples. The method was introduced by Blois[5], and improved by some authors for measuring antioxidant activity of numerous substances and determining antioxidant capacity of various food and plant samples[3,6,7]. In brief, this method is based on the reduction of the chromogenic DPPH radical by an antioxidant, such as in a plant extract, which causes the radical to change color, and this change can be monitored and quantified using spectrophotometer at 515–520 nm[8]. The radical DPPH is stable and does not have to be generated for hours before the analysis, as in other radical scavenging assays[9].

In conventional spectrophotometric method, a large volume (1.0–5.0 mL) of freshly prepared DPPH solution in a cuvette is required[10–15]. In order to reduce large amount of DPPH solution, a microwell plate can be used in the assay, as it was done by Lee et al.[16]. Lately, it was known that no significant different parameters (repeatability, reproducibility, percentage recovery) were observed between microwell and cuvette-based method within intra-laboratory validation[9]. The DPPH microwell-based method is continuously used then by some authors as high throughput screening for antioxidant capacity[17–20]. To make the assay simpler and faster as high throughput screening, the microwell was used as solid support for DPPH in dry reagent format as described elsewhere[19]. By adding methanol or ethanol into the wells, the system can be used for high throughput antioxidant screening of various samples (banana, green tea, pink guava, and honey dew), and it was shown that the results were in good agreement with that of conventional DPPH-microwell platform. However, this method is not suitable for field analysis since specialized, cost-expensive instrument such as microplate reader (spectrophotometer) is required to conduct the assay. To overcome this limitation, flatbed scanner can be used as a microplate reader to obtain digital color image which can be further analyzed quantitatively.

Recently, using scanner as scanometric technique gained its popularity due to its application in various chemical and biochemical assays. The scanometric technique which relies on either a light scattering instrument or flatbed scanner coupled with various probes or sensors can be used for the detection of bacteria, dopamine, magnesium ions, lead ions, thrombin, and mercury ions[21–26]. Being subclass of colorimetry, scanometry uses a gray scale as opposed to the various color space. The gray intensity, typically a result of silver enhancement is the measured signal in scanometry[27]. Scanometry was used to characterize optical feature of various dyes, such as disperse orange 3, methyl orange, fluorescein, eosin Y, rhodamine B, trypan blue, prussian blue, malachite green, methylene blue, chlorophyll b, and DPPH, in a microwell plate[28]. As the color intensity in red, green, and blue (RGB) value was mathematically converted to RGB-resolved absorbance, it was shown that flatbed scanner was comparable with spectrophotometer.

Here, we propose a scanometric technique for conducting DPPH assay. In this work, DPPH solution was immobilized on a 96 microwell as dry reagent to construct antioxidant sensor. Then, a regular flatbed scanner was used as microplate reader to evaluate antioxidant capacity of several plant extracts. When it was compared with other DPPH-based sensors[29,30], the proposed sensor is simpler, since in other optical sensors the DPPH solution has to be immobilized in polymer (e.g., PVA, PVC) in a long time chemical synthetic reaction to construct the sensor. Moreover, the sensors have to be transferred into a cuvette prior to antioxidant assay in UV/Vis spectrophotometer which made the afore mentioned methods[29,30] need longer procedure than the proposed method. As regular flatbed scanner was employed for obtaining sensor response, the developed method is extremely cheaper than microplate reader (ELISA reader) or UV/Vis spectrophotometer. In the microplate reader, the absorbance of DPPH after antioxidant addition was measured, while in the proposed scanometric technique, the color intensity (mean RGB) of DPPH after antioxidant addition has been measured for determining antioxidant capacity. Hence, it is obvious that, using scanometric technique, no need the samples to be transparence, as in our previous work[19]. In this paper, even non-transparence or opaque sample can be used, since the different analytical response and different instrument were used as a reader for antioxidant sensor response. In addition, we also used less reagent concentration (125 μg/L) compared to the previous one (150 μg/mL)[19]. Thus, it make reagent used more efficient, as it is used in one shot measurement. Furthermore, the developed method can be suitable for field analysis and/or in remote area, where medicinal plant extracts can be screened for their antioxidant capacity on site.

2. Materials and methods

2.1. Chemicals

Gallic acid (GA) and DPPH were obtained from Sigma–Aldrich (USA). Methanol was purchased from Merck (Germany). All chemicals were of analytical reagent grade.

2.2. Herbal samples

Herbal samples used in this work, *i.e*., Sappan wood (*Caesalpinia sappan* L.), and Turmeric Rhizome (*Curcuma domestica* Val.), were purchased from local market of Jember, East Java. All herbal samples were authenticated and deposited at Pharmacognosy Laboratory, Faculty of Pharmacy, University of Jember, Indonesia. Herbal samples were air dried and powdered until their particle size freely passed through sieve 100 mesh.

2.3. Sensor fabrication

Sensor fabrication was done as in our previous work[19] with slight modification. A solution of DPPH in methanol at various concentrations (50, 100, 125, and 150 ppm) were transferred (200 μL) into 96 microwell plate as matrix sensor. The solvent was then evaporated under mild condition at room temperature to construct antioxidant sensor based on DPPH. Afterward, the

Please cite this article as: Hidayat MochammadAmrun, et al. Scanometry as microplate reader for high throughput method based on DPPH dry reagent for antioxidant assay. *Acta Pharmaceutica Sinica B* (2017), http://dx.doi.org/10.1016/j.apsb.2017.02.001