Talanta 77 (2009) 1559–1566

Contents lists available at ScienceDirect

Talanta

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

Review

Flow injection based methods for fast screening of antioxidant capacity

Luís M. Magalhães, Miguel Santos, Marcela A. Segundo*, Salette Reis, José L.F.C. Lima

REQUIMTE, Serviço de Química-Física, Faculdade de Farmácia, Universidade do Porto, Rua Aníbal Cunha, 164, 4099-030 Porto, Portugal

ARTICLE INFO

Article history:
Received 31 July 2008
Received in revised form 19 October 2008
Accepted 24 October 2008
Available online 31 October 2008

Keywords:
Antioxidant capacity
Flow injection analysis
Automatic methods
DPPH
ABTS
Folin-Ciocalteu
Electrochemical methods

ABSTRACT

The role and importance of antioxidants in different fields, ranging from physiology to food technology, have become evident in the past years, requiring adequate analytical methodologies. Therefore, the determination of antioxidant capacity as a routine or screening analysis fosters its automation. In this context, several flow injection methods based on scavenging of 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulphonate) radical cation ($ABTS^{\bullet+}$) or 2,2-diphenyl-1-picrylhydrazyl radical ($DDPH^{\bullet}$) or based on the determination of total reducing capacity have been proposed. The objective of the present review is to critically compare the different approaches, regarding their degree of automation, their performance *vs.* the respective batch procedure and its applicability to real samples.

Contents

1. Introduction

In the past years, the importance of antioxidants in the protection of organisms or tissues, or of nonliving systems against oxidative stress has become evident. This statement is supported by studies performed in a variety of areas, including physiology [1,2], pharmacology [3,4], nutrition [5–7] and even food processing [8,9]. In all these areas of research fast, reliable methods for antioxidant assessment are needed [10,11]. Generally, the ideal method for determination of antioxidant properties should assess the effect of a compound/sample in reaction conditions that mimic those found when oxidative stress is induced in vivo by reactive nitrogen species (RNS) and reactive oxygen species (ROS). However, this kind of assessment may be considered exaggerated for screening purposes, considering the individual testing against numerous ROS/RNS (e.g. H_2O_2, $O_2^{\bullet-}$, $HO^{\bullet}$, HOCl, $^1O_2$, $NO^{\bullet}$, and $ONOO^-$) and the conditions of assay in vivo (e.g. use of cultured cell lines or lab animals). In this scenario, in vitro methods to determine "total antioxidant capacity" are ideal as an exploratory screening step prior to characterization or isolation of bioactive compounds [12].

Along the past two decades, several methods, including the $ABTS^{\bullet+}$ (2,2′-azinobis-(3-ethylbenzothiazoline-6-sulphonate) radical cation) assay [13,14], the $DPPH^{\bullet}$ (2,2-diphenyl-1-picrylhydrazyl radical) assay [15], the ferric reducing antioxidant power (FRAP) assay [16], and the electrochemical estimation of total reducing capacity [17,18] have been proposed for assessment of antioxidant capacity. Considering that these methods are routinely used for screening purposes, their automation is relevant. In this respect, automation using flow injection based methods can offer several advantages, besides the enhancement of sample throughput, when

* Corresponding author. Tel.: +351 222078994; fax: +351 222078961.
E-mail address: msegundo@mail.ff.up.pt (M.A. Segundo).

0039-9140/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.talanta.2008.10.034

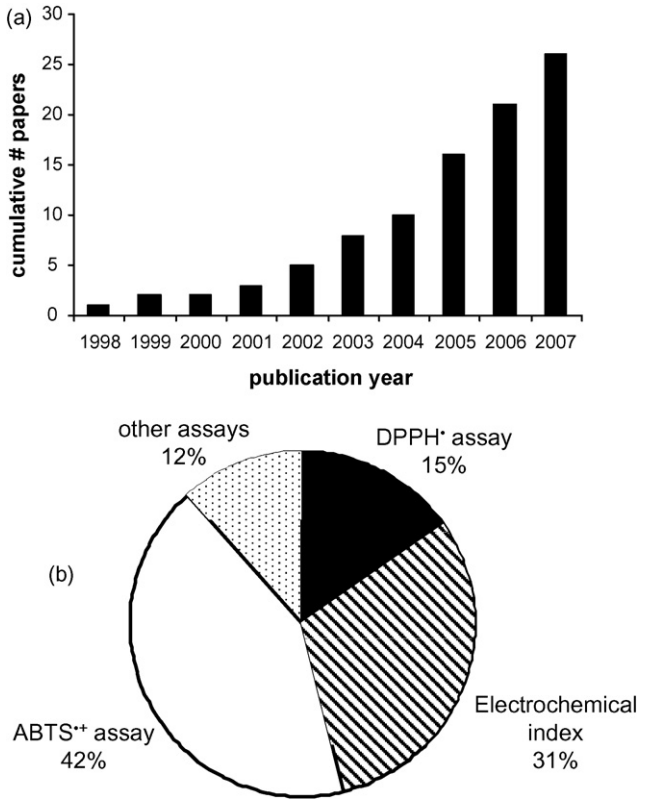

Fig. 1. (a) Cumulative distribution of papers dealing with automatic flow based determination of total antioxidant capacity per publication year. (b) Distribution of the same papers regarding assay type. Papers from 2008 are not included.

compared to conventional batch methods. For this particular application, the features of flow injection analysis (FIA) [19] systems provide a strict control of reaction conditions in both space and time, which are essential for determination of species that are sensitive to environmental conditions (light, temperature, presence of O_2, for instance). Furthermore, the evolution of FIA to sequential injection analysis [20] (SIA), described as a mechanically simpler alternative to FIA, and to other strategies based on the flow network concept [21] expanded the benefits of automation. This last type of flow systems includes multi-commutation [22,23], multisyringe flow injection analysis (MSFIA) [24,25] and multipumping [26], where the manifold channels are connected to computer-controlled devices (solenoid valves or micro-pumps) that enable the flexible access to reagent(s), sample and carrier in any software-defined combination.

The aim of this review is to establish a critical comparison between the different automatic flow based systems developed until the present moment for fast screening of antioxidant capacity and to highlight the advantages of automatic methods toward the corresponding batch procedure. As depicted in Fig. 1a, the number of publications devoted to this subject has grown significantly, especially in the past 3 years. Moreover, different assays have been automated (Fig. 1b). In fact, more than half of the proposed applications are based on the utilization of colored, radical species ($ABTS^{\bullet+}$ or $DPPH^{\bullet}$) that mimic the ROS/RNS found in vivo. Other methods aimed the determination of "total reducing capacity", for which the amperometric determination of an "electrochemical index" accounts for about 31% of the flow systems reported. Considering this division, an overview is presented in the next sections.

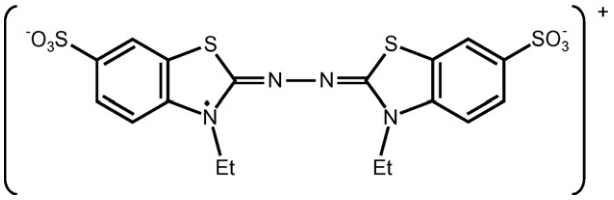

Fig. 2. $ABTS^{\bullet+}$ chemical structure.

2. Flow methods based on scavenging of 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulphonate) radical cation ($ABTS^{\bullet+}$)

One of the most common methods for assessing the antioxidant capacity is the $ABTS^{\bullet+}$ or TEAC (trolox equivalent antioxidant capacity) assay based on the scavenging of 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulphonate) radical cation (Fig. 2) [13]. Generally, the sample to be tested is added to a solution containing a certain amount of $ABTS^{\bullet+}$. After a period of time that may vary between 1 and 30 min, the concentration of the remaining $ABTS^{\bullet+}$ is determined spectrophotometrically [14]. ABTS is commercially available but the $ABTS^{\bullet+}$ radical cation must be formed prior to determination. Several strategies have been described for performing this step, based on (i) chemical reaction using manganese dioxide [27], or 2,2′-azobis(2-amidinopropane) dihydrochloride [28], or potassium persulfate [14]; (ii) enzymatic reaction using metmyoglobin [13] or horseradish peroxidase [29]; (iii) electrochemical generation [30].

The automation of $ABTS^{\bullet+}$ assay (Table 1) was first implemented by Pellegrini et al. [31] using a single channel flow injection system similar to that presented in Fig. 3a. Therefore, after injecting a sample containing antioxidant compound(s), a negative peak representing the decolorization of $ABTS^{\bullet+}$ was obtained (Fig. 4a), whose area was proportional to the concentration of $ABTS^{\bullet+}$ that was reduced. The TEAC value corresponds to the trolox concentration providing a discoloration of $ABTS^{\bullet+}$ equal to that caused by the sample. The proposed flow injection system was applied for the evaluation of antioxidant capacity of pure compounds (ascorbic acid, caffeic acid, ferulic acid, gallic acid, naringenin, quercetin, α-tocopherol, and vanillic acid) and results were compared to the batch assay. In general, the two set of results were in good agreement, with exception of naringenin. The applicability of the technique was tested by measuring the antioxidant capacity of several common beverages (beer, coffee, cola, fruit juices, and tea) and results were not statistically different from the batch assay, using trolox as standard compound.

Bompadre et al. [32] reported that the previous FIA-$ABTS^{\bullet+}$ assay partially failed when more complex biological samples, such as plasma, were analyzed. Hence, they proposed minor changes (sample volume, reaction coil configuration) in the flow manifold and also introduced temperature control. Therefore, the temperature and time/way of exposure of the active compounds present in the samples with $ABTS^{\bullet+}$ were strictly controlled. Using these experimental conditions, the authors showed that the temperature was a critical aspect in the measurement of plasma antioxidant capacity whilst its influence was less important in the assay of non-complex biological samples (mouthrinse, white wines). Hence, the temperature of the reaction coil was fixed at 35 °C whilst the reaction time was defined as 1.3 min. The improved FIA-$ABTS^{\bullet+}$ method was useful to screen rapidly, without dilution, and with high repeatability the antioxidant capacity of both non-complex biological mixtures and plasma samples. The same flow injection system was later applied to determine the antioxidant capacity of enriched toothpastes [33].