Analytica Chimica Acta 764 (2013) 70–77

Contents lists available at SciVerse ScienceDirect

Analytica Chimica Acta

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

Development of a generic assay for the determination of total trihydroxybenzoate derivatives based on gold-luminol chemiluminescence

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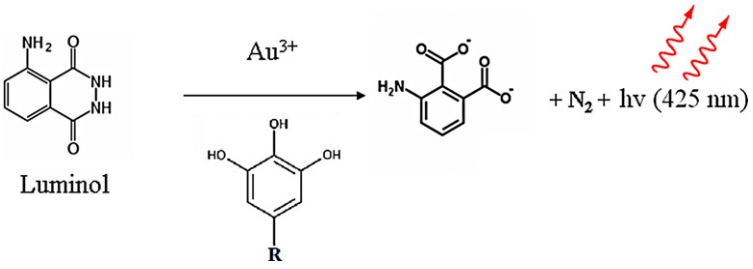
HIGHLIGHTS

- Gold is used as an alternative oxidant to luminol under mild conditions.
- Trihydroxybenzoates are selectively determined in complex mixtures.
- The method is straightforward without any interim steps.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 25 September 2012
Received in revised form 7 December 2012
Accepted 14 December 2012
Available online 22 December 2012

Keywords:
Gallic acid derivatives
Trihydroxybenzoates
Gold
Luminol
Flow injection analysis

ABSTRACT

A selective assay for the determination of one of the most important class of phenolic compounds, namely trihydroxybenzoates (monomeric and polymeric compounds having at least one gallate moiety) based on their enhancing effect on the chemiluminogenic reaction between gold ions and luminol is described for the first time. In the presence of trihydroxybenzoate derivatives, the light emission generated when alkaline luminol is oxidized by gold ions is amplified several orders of magnitude compared to other common phenolic compounds which exhibit minor reactivity or no reactivity at all (e.g. hydroxycinnamates, flavonols, benzenediols). Based on this property, the experimental conditions were optimized in order to enable the determination of total trihydroxybenzoates in complex mixtures without resorting to separation techniques. The method was applied to samples of different composition (teas, herbal infusions and wines) with satisfactory analytical features yielding detection limits at the 10^{-7} mol L^{-1} level, intra-day precision of 3.1%, inter-day precision less than 10% and recoveries between 88.7 and 97.6%. The strengths and weaknesses of the method were identified and discussed in relation to its application in real samples.

1. Introduction

Food analysis greatly relies on universal detection methods that determine the total concentration of a specific class of compounds (e.g. phenolics, flavonoids) or a specific property of the sample (antioxidant activity, radical scavenging activity, etc.) [1,2]. Although successfully employed for more than two decades, a major issue regarding these methods is selectivity as different classes of compounds react simultaneously. Even when combined with appropriate extraction or reaction steps (e.g. co-precipitation of flavonoids by formaldehyde, reaction of catechins with vannilin) [3,4], the majority of these methods yield a generic but non-specific response that provide a macroscopic representation of the overall sample properties and composition [1–6]. Another issue of concern,

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http://dx.doi.org/10.1016/j.aca.2012.12.025

is that many of these methods require prolonged incubation times or accurate timing in order to yield reproducible results due to the different reaction kinetics with each compound in the sample. Thus, strict regulation of the experimental conditions needs to be exercised in order to ensure the accuracy and reproducibility of the measurements [1,2,5,6]. However, as the diversity of samples increases, there is a growing interest in the development of selective analytical procedures that enable the determination and comparison of specific sample components, the assessment of sample authenticity and its compliance to regulated standards [1,2,5]. At the same time, the large number of samples that need to be analyzed, especially commercialized products, necessitates the development of fast, simple and reliable methods that afford high sample throughput [1,2].

Simplicity, high degree of automation along with high sensitivity and reproducibility are inherent attributes of chemiluminescence (CL) detection. Up to date the CL technique has been extensively used in food analysis and detailed reviews have appeared on the topic [7,8]. By far the most studied chemiluminogenic reactions are those of luminol with various oxidants and a variety of applications have been described for the determination of free-radicals scavenging activity [9], peroxide value [10], total antioxidant capacity [11], oil antioxidant stability, detection of authenticity and adulteration, food additives, etc. [7,8,12,13]. In the arsenal of oxidants used to generate the chemiluminogenic emission of luminol, gold ions have recently been recognized [14,15]. Their use, however, has been limited in chemiluminescence immunoassays (CLIAS) and hybridization assays (CLHAs) which employ appropriately modified gold nanoparticles (AuNPs) as scaffolds to accomplish specific interactions with certain analytes. In these studies, gold ions are generated by oxidative dissolution of AuNPs, a procedure that yields a large flux of metal ionic species (i.e. gold) that oxidize luminol, emitting light. Although successful, this strategy is limited to the determination of Au^{3+} ions only, while the dissolution process is time consuming and takes place under unfavourably acidic conditions which vary with the nature of the metal nanoparticle [14,15]. As a result, studies except CLIAs and CLHAs, concerning the analytical utility of Au^{3+} ions as an oxidant for luminol CL reactions have not been reported to date.

With the above in mind, this work explores for the first time, the use of Au^{3+} as an alternative oxidant to luminol CL reactions under mild conditions typical to flow analysis. The system's response to various compounds was used to develop a detection method for the selective determination of trihydroxylbenzoate compounds, namely gallic acid and its monomeric and polymeric derivatives, which constitute some the most important phenolic species in real samples such as teas and wines. The principle of the method lies on the significant amplification of the chemiluminescence signal generated when trihydroxylbenzoates are oxidized by gold ions, yielding a flux of reactive oxygen species (ROS) that increase the CL emission. To the best of our knowledge, this is the first study reporting the selective determination of compounds having the gallate moiety without sample separation in real mixtures.

2. Experimental

2.1. Reagents and materials

All reagents were of analytical grade unless otherwise stated. Luminol, Folin–Ciocalteu reagent, potassium dihydrogen phosphate and L-ascorbic acid were obtained by Merck. Gold chloride trihydrate, tannic acid, resorcinol, pyrocatechol, o-coumaric acid, L-cysteine, folic acid, alginic acid, glutathione, cinnammic acid, ferulic acid, trolox, (+)-catechin, caffeic acid, vanillic acid, gallic acid monohydrate and epigallocatechin gallate, pyrogallol, chloranilic acid and quercetin dihydrate were purchased from Sigma–Aldrich. Butyl-hydroxy-anisole (BHA), butyl-hydroxyl-toluene (BHT), propyl gallate and ter-butylhydroquinone (TBHQ) were obtained from Acros Organics. 2-Propanol, acetonitrile, ethanol, and methanol were purchased from Panreac.

Phosphate buffer (pH 8.00) was prepared by appropriate dissolution of potassium dihydrogen phosphate in distilled water and the pH was adjusted by the addition of 0.100 mol L^{-1} NaOH (Titrisol, Merck). Luminol (1.00×10^{-2} mol L^{-1} in 0.100 mol L^{-1} NaOH) and Au^{3+} (1.00×10^{-2} mol L^{-1}) stock solutions were prepared in doubly distilled water and stored in dark containers at 4 °C. Working solutions were prepared after dilution of the stock solutions in appropriate buffer medium (0.100 mol L^{-1} NaOH for luminol and Na_2HPO_4–NaOH, pH = 8.00 for Au^{3+}). Solutions of inorganic anions (F^-, SO_3^{2-}, HCO_3^-, PO_4^{3-}, Br^-, Cl^-, NO_3^-, MoO_4^{2-}) were prepared by their respective sodium or potassium salts while inorganic cations (Cr^{3+}, Fe^{3+}, Sb^{3+}, Zn^{2+}, Pb^{2+}, Al^{3+}, Mg^{2+}, Cd^{2+}, Cu^{2+}) were prepared by dilution of spectroscopic grade stock standard solutions (BDH, Poole,UK).

Commercially available teas (mainly red, black, white teas and mixtures of green teas with the former species) and herbal infusions (fruit teas, herbal teas, chamomile and mixtures of them) as well as red and white wines were purchased from local stores. Portions of dried tea and herbal samples were accurately weighted (1.3–3.0 g) and extracted in 250 mL of boiling water (to simulate realistic application and use). The extracts were diluted as necessary depending on the method applied: 100-fold for CL analysis, 10-fold for the Folin–Ciocalteu's assay and no dilution for HPLC analysis. Red wines were diluted 2000-fold for CL analysis, 40-fold for the Folin–Ciocalteu's assay and 2-fold for HPLC analysis; white wines were diluted 200-fold for CL analysis, 25-fold for the Folin–Ciocalteu's assay and no dilution for HPLC analysis.

2.2. Instrumentation

Flow experiments were performed in a FIA manifold comprised of a peristaltic pump (Gilson, Miniplus 3) equipped with a six-port valve and a flow-cell CL-detector consisting of a red sensitive photomultiplier (Thorn, EMI 9865 B) powered by 1200–1400 kV. The recorded signals were processed in peak height mode. The experimental setup is demonstrated in Fig. S1 (Supplementary Information). Chemiluminescence spectra were recorded on a JASCO FP-777 Spectrofluorimeter with the excitation source off, employing wide slits (20 nm) and scan speed 2000 nm min^{-1}. A pH metre (WTW 552 Model) was employed to measure the pH values. UV–vis absorption measurements were performed in a Jenway 6405 UV/vis spectrophotometer using matched quartz cells of 1 cm path length. Liquid chromatographic analysis was performed in a Shimadzu LC-10AD high-pressure solvent delivery pump, with a 20-μl Rheodyne manual sample loop injector and a Shimadzu SPD-M20A UV/diode-array detector. The analytical column was an Eclipse XDB-C18, with 5 μm particles (15 cm × 4.6 mm I.D.) Water (3% acetic acid)/methanol were used for the gradient elution of the analytes, according to a previous protocol [16]. Peak areas were recorded at 275 nm.

2.3. Analytical procedures

2.3.1. Luminol-Au^{3+} FIA-CL assay

On-line CL measurements were performed by injecting the samples through a 100 μL sampling valve in a stream of Au^{3+} working solution (1.00×10^{-4} mol L^{-1}) which was used as a carrier, and merged with the luminol working solution (1.00×10^{-4} mol L^{-1}) in a Y-confluence just in front of the CL cell. The total flow was 4.0 mL min^{-1} (2.0 mL min^{-1} for each stream) (Fig. S1-Supplementary Information). The presence of trihydroxybenzoate