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Determination of gallic acid by an oscillating chemical reaction using the analyte pulse perturbation technique

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Abstract

A new analytical method for the determination of gallic acid by the sequential perturbation caused by different amounts of acid on the oscillating chemical system involving the Cu(II)-catalysed reaction between hydrogen peroxide and sodium thiocyanate in an alkaline medium is proposed. The method relies on the linear relationship between the changes in the oscillation amplitude of the chemical system and the concentration of gallic acid, which is in this work exposed for the first time. The calibration curve is linear over the range 0.075–2.00 μmol of gallic acid. The method features good precision (rsd 0.67%) and excellent throughput (14 samples h^{-1}) thanks to the expeditiousness with which the steady state is regained after each perturbation. Some aspects of the interaction of gallic acid with the oscillating chemical system are discussed. This is the first reported determination of gallic acid based on an oscillating reaction.

Keywords: Oscillating chemical reaction; Analyte pulse perturbation; Gallic acid

1. Introduction

Some far-from-equilibrium chemical systems exhibit an oscillating behaviour as a result of their complex mechanisms including and autocatalytic step [1]; such systems are usually referred to as 'oscillating reactions'. Oscillating reactions can take place both in the liquid phase [2–4] and in the gaseous phase from heterogeneous catalytic systems [5]. There are also several references to naturally occurring oscillating reactions such as those resulting in periodic changes in the calcium concentration in a variety of cell types, which are highly significant to protein phosphorylation [6]. Most of such systems have been primarily studied from a physico-chemical point of view in order to elucidate the complex reaction mechanisms behind oscillations, and to develop theoretical models accounting for the experimental results [7]. The most widely known and studied oscillating chemical systems are those based on the Belousov–Zhabotinskii [8–10] and Bray–Liebhafsky reactions [11–13]. More recently, oscillating systems involving no halogen compound (particularly the $CuSO_4$-catalysed reaction of hydrogen peroxide with thiocyanate ion [14]) have aroused increasing attention. For oscillations to last long enough, the reagent inflow and product outflow must be carefully maintained constant in order to ensure that the reactor is kept under steady concentration conditions [15,16]; this is usually accomplished by using a continuous-flow stirred-tank reactor (CSTR).

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Oscillating reactions have scarcely been explored for analytical purposes with the exception of some discrete determinations including those of hexacyanoferrates [17] and chloride in human serum [18] by use of the Belousov–Zhabotinskii reaction, or that of Cu(II) at the microgram-per-millilitre level with the H_2O_2/malonic acid/Mn(II) system [19]. Also, the Belousov–Zhabotinskii reaction has been implemented in a flow injection system [20]. Recently, our group developed the so-called 'analyte perturbation technique' as a tool for analytical determinations in far-from-equilibrium dynamic systems, where the oscillating system is sequentially perturbed by the analyte, which is determined in a continuous manner; the system regains the steady state after each perturbation, which allows several determinations to be carried out simultaneously on the same system [21].

The proposed method widens the scope of oscillating chemical reactions in quantitative analytical determinations. It relies on the analytical potential of the copper sulphate-catalysed reaction of hydrogen peroxide with thiocyanate ion in an alkaline medium to determine gallic acid with the aid of a CSTR. The chemical system is perturbed with variable amounts of gallic acid (3,4,5-trihydroxybenzoic acid), which results in substantial changes in the oscillation amplitude that are proportional to the acid concentration.

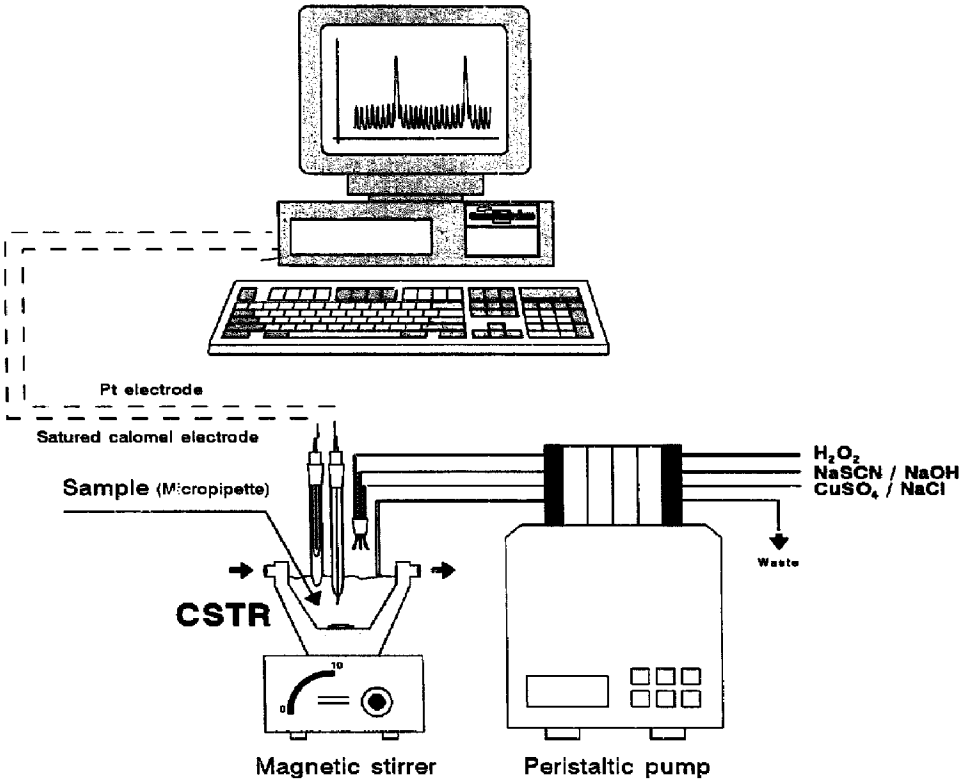

Fig. 1. Experimental set-up for the implementation of oscillating reactions using the analyte pulse perturbation technique.

In addition, the oscillating system regains the steady state shortly after each perturbation.

2. Experimental

2.1. Reagents

All chemicals used were of analytical reagent grade (Merck). Gallic acid, sodium thiocyanate, copper sulphate pentahydrate, hydrogen peroxide, sodium hydroxide and sodium chloride solutions were all prepared in bidistilled water.

2.2. Apparatus

The experimental set-up used to implement the oscillating reaction (Fig. 1) consisted of a 10 ml glass reaction vessel (CSTR) furnished with a thermostated jacket connected to a Selecta 6000383 thermostat and an Eyela RC-2 magnetic stirrer for homogenization. Potential changes were recorded by means of a Metrohm EA202 Pt electrode and an Amel 303 SCE interfaced to a PC-AT 12 MHz compatible computer via a PC-Multilab PCL-812PG 12-bit analog-to-digital converter (ADC). Samples were injected in the analyte pulse perturbation mode by means of a Nichiryo 5000 micropipette. The reactor was kept under steady state conditions by using a Gilson Minipuls-3 four-channel peristaltic pump; three of the channels were used to deliver the reactants and the fourth to keep the volume of the reaction mixture in the CSTR constant. Poly(vinyl chloride) pumping tubes and PTFE tubing for the manifold were used throughout.

2.3. Procedure

To the CSTR, thermostated at 30°C, were added in the following sequence: 2.0 ml of 0.7 M sodium chloride, 1.0 ml of 0.245 M sodium thiocyanate, 1.0 ml of 0.42 M sodium hydroxide, 0.23 ml of 33% (m/v) hydrogen peroxide and bidistilled water up to a final volume of 6.0 ml, the mixture being homogenized by magnetic stirring. The oscillating reaction was started by adding 1.0 ml of 1.75×10^{-3} M Cu(II) and, without delay, the peristaltic pump delivered the three reactant streams — the overall feed stream was 0.64 M in H_2O_2, 7×10^{-2} M in NaSCN, 0.120 M in NaOH, 2.5×10^{-4} M in $CuSO_4$ and 0.2 M in NaCl — at a constant flow-rate of 7 ml min^{-1}. The system evolved gradually to constant oscillating

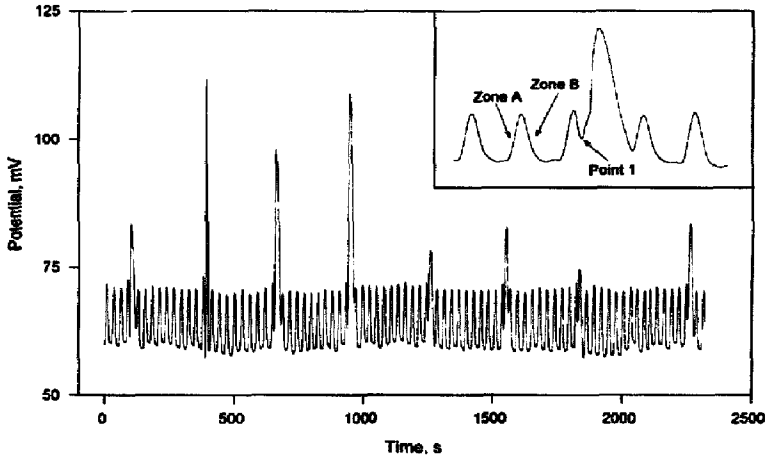

Fig. 2. Typical oscillation profiles obtained in the absence and presence of variable amounts of gallic acid under selected experimental conditions. The inset illustrates the selection of the injection point. The meaning of zones A and B, and point 1, is explained in the text.

amplitude and period (a steady state). As soon as the signal and time increments for the oscillations levelled off, variable volumes (a few microlitres) of sample or standard containing also variable amounts of gallic acid from 75 to 2000 nmol were sequentially injected. Data were acquired at a rate of 1.35 point/s using software developed by the authors in Microsoft QuickBasic v. 4.0; the oscillating amplitude obtained in the presence of gallic acid was used as the measured parameter to construct the calibration plot.

3. Results and discussion

Oscillating chemical systems exhibit periodic changes in the concentration of some species that reflect in cyclic colour, pH or potential changes, depending on the particular system [22,23]. As a rule, oscillating systems are highly vulnerable to external perturbations such as temperature changes [24] or variations of the O_2 concentration in the medium [25]. The Cu(II)-catalysed oxidation of SCN^- ion by H_2O_2 in a strongly alkaline medium involves successive oscillations in the concentrations of the oxidized and reduced form of the metal ion which give rise to the formation and subsequent disappearance of a yellow superoxide–cuprous complex (H_2O–Cu(I)) that governs the rate of the process and is responsible for the colour changes observed [26]. On interaction with this oscillating system, gallic acid causes changes in the oscillation amplitude and period, thereby perturbing the steady state; however, the system gradually regains the steady state after the perturbation ceases. Because the above-described changes are proportional to low gallic acid concentrations and the system recovers from the perturbation quite rapidly, we explored the possibility of exploiting this behaviour for the determination of gallic acid.

Fig. 2 shows typical oscillation profiles for the proposed system in the presence and absence of gallic acid under the above-described working conditions. The inset shows the oscillating region where the perturbation must be introduced. Thus, if a normal oscillation cycle is assumed to raise the potential (zone A) to a maximum, from which it subsequently drops (zone B) to the starting conditions, the sample containing the analyte should be injected once recovery has started (point 1) since this is the zone where the system will respond maximally to the gallic acid perturbation. Injections result in a sharper rise in the potential, which reaches a maximum and then falls to normal oscillation levels. The system regains the oscillating conditions prevailing before the perturbation is introduced within 4–5 min after injection. Once that time has elapsed, a new determination can be started.

3.1. Influence of experimental variables

The effect of experimental variables on the proposed oscillating reaction was studied in order to establish the optimal working conditions for determining the species via which the perturbation was introduced: gallic acid. In order to ensure the maximum possible sensitivity and precision in the determination, as well as the ability to perform large series of analyses, conditions were optimized with two primary objectives in mind, namely: (a) to achieve the maximum possible stability in the oscil-

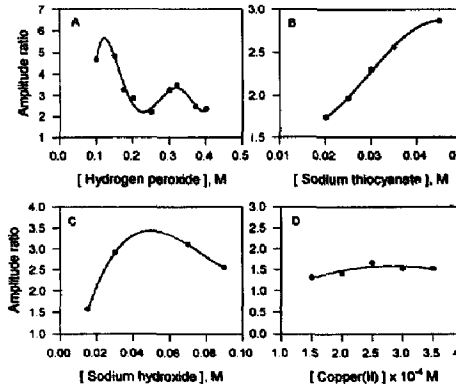

Fig. 3. Influence of the concentration of (A) hydrogen peroxide, (B) sodium thiocyanate, (C) sodium hydroxide and (D) copper(II) on the gallic acid-perturbed oscillating reaction. Conditions: (A) 560 nmol of gallic acid, 2.5×10^{-2} M NaSCN, 2.5×10^{-2} M NaOH and 1.5×10^{-4} M Cu(II); (B) 280 nmol of gallic acid, 0.32 M H_2O_2, 2.5×10^{-2} M NaOH and 1.5×10^{-4} M Cu(II); (C) 225 nmol of gallic acid, 0.32 M H_2O_2, 3.5×10^{-2} M NaSCN and 1.5×10^{-4} M Cu(II); (D) 168 nmol of gallic acid, 0.32 M H_2O_2, 3.5×10^{-2} M NaSCN and 6.0×10^{-2} NaOH.

lating system over time; and (b) to ensure that the oscillation amplitude allowed the effect of gallic acid on it to be accurately determined. Thus, the influence of selected experimental variables was studied in the presence and absence of gallic acid using the amplitude ratio obtained under both types of conditions as the measured parameter.

The effect of the hydrogen peroxide concentration was studied over the range from 0.1 to 0.4 M. Low H_2O_2 concentrations were found to adversely affect the oscillating system (induction periods exceeding 10 min were observed at the beginning of oscillations that resulted in the loss of the oscillating state by effect of minimal perturbations and eventually led to a chaotic state). As the hydrogen peroxide concentration was increased, both the oscillation amplitude and period decreased, and the induction period eventually disappeared altogether. On the other hand, the perturbation with gallic acid elicited a response that fitted a curve such as that of Fig. 3A. As can be seen, the curve fell as the H_2O_2 concentration was increased; it reached a minimum and then rose up to a maximum at a concentration of 0.32 M, which was adopted as optimal — while the response to the perturbation was somewhat small relative to lower H_2O_2 concentrations, the system oscillated more uniformly and was subject to no induction period.

The sodium thiocyanate concentration in the CSTR was changed between 2.0 and 4.5×10^{-2} M. As the concentration was increased, the oscillating period for the chemical system lengthened while the amplitude remained fairly constant. The effect of the gallic acid perturbation was such that it increased the response with increase in the sodium thiocyanate concentration (Fig. 3B). An NaSCN concentration of 3.5×10^{-2} M was chosen as a compromise between maximum sensitivity (amplitude) and minimum analysis time (period of the oscillating system).

The influence of the NaOH concentration was investigated over the range $(1.5–9.0) \times 10^{-2}$ M; concentrations below 2.5×10^{-2} M resulted in a marked drift in the potential zone where oscillations took place. Raising the pH suppressed the drift and caused the system to oscillate more uniformly without significantly affecting the oscillation amplitude or period. The effect of the NaOH concentration in the presence of the gallic acid is illustrated in Fig. 3C. An NaOH concentration of 6.0×10^{-2} M was finally chosen in order to maximize the system response to the gallic acid perturbation.

The effect of the Cu(II) concentration in the reactor was studied over the range $(1.5–3.5) \times 10^{-4}$ M; the oscillation period was found to decrease with increasing copper concentration without significantly altering the oscillation amplitude. As can be seen in Fig. 3D, the effect of the gallic acid perturbation was similar at every Cu(II) concentration tested. A copper(II) concentration was thus chosen as optimal since it caused the system to oscillate highly uniformly prior to the perturbation and responded quite predictably to it.

Changes in the ionic strength of the reaction medium were also found to affect the determination; raising ionic strength by adding increasing concentrations of NaCl up to 1.0 M increased the response to the gallic acid perturbation, with no appreciable change in the oscillation period or amplitude. Also, in qualitative terms, an increase in the NaCl concentration was found to inhibit the passivation of the Pt electrode by precipitated copper hydroxide, so the measured potential was subject to no increasing background noise as time elapsed. An NaCl concentration of 0.2 M was thus adopted in order to exploit all the above-mentioned advantages.

The temperature also had a strong influence of the oscillating system: raising it from 25 to 45°C dramatically decreased the oscillation period (by about 70%), with virtually no change in the oscillation amplitude. However, the system response to the gallic acid perturbation was not altered by temperature changes. A temperature of 30°C was therefore chosen as optimal as it ensured correct, reproducible oscillations.

Finally, the effect of the overall reactant flow-rate fed to the CSTR was studied; this variable influenced the ease with which the steady state was regained after each perturbation and hence the reproducibility and the system resistance to the perturbations. Decreasing the ratio of the system volume (7 ml) to the overall reactant flow-rate — which was changed between 1 and 7 ml min^{-1} — was found to result in an increasingly reproducible response to the gallic acid perturbation. A unity ratio (i.e. an overall reactant flow-rate of 7 ml min^{-1}, obtained with one of ca. 2.3 ml min^{-1} for each reactant) was adopted as optimal.

3.2. Determination of gallic acid

The oscillating system was perturbed with variable amounts of gallic acid under the above-described optimum conditions and the response obtained was analysed in two ways, namely: (a) by measuring the amplitude on each perturbation; and (b) from the potential measured at the peak of each perturbation. Fig. 4 shows the maximum potentials obtained for all the oscillations (both perturbed and unperturbed) in a typical determination sequence. As can be seen, there is a well-defined baseline defined by the potentials for the system oscillations in the steady state and a series of points lying off the line that represent the perturbations. A similar recording can be obtained by using oscillation amplitudes rather than potentials.

A plot of amplitude or maximum potential for each perturbation as a function of the injected analyte concentration was linear in both cases. This allows one to address the determination of gallic acid by using an amplitude or a maximum potential method. Table 1 summarizes the figures of merit of the calibration graphs. As can be seen, the precision and sensitivity achieved in both types of determination were quite good; in any case, the potential method was somewhat better on account of its higher precision. We finally determined the throughput; under the above-described experimental conditions, the chemical system was allowed to oscillate for at least 4 h, time during which the oscillating reaction acted by way of a detection system (it produced signals on injection of micro amounts of gallic acid). From the time taken by the system to regain the steady state after each perturbation, the throughput was estimated to be about 14 samples h^{-1}.

Table 1
Regression data and analytical figures of merit for the determination of gallic acid

Parameter	Determination method	
	Amplitude	Maximum potential
Linear range, μmol	0.075 – -2.0	0.075 – -2.0
Slope, mV μmol^{-1}	28.80 ± 0.73	35.62 ± 0.68
Intercept, mV	8.50 ± 0.50	63.1 ± 1.0
Standard error of estimate	1.28	1.75
Regression coefficient ($n = 9$)	0.9977	0.9973
Detection limit [a], nmol	22	20
Precision (rsd) [b], %	2.05	0.67

[a] Calculated according to IUPAC's recommendations [27].
[b] From eleven determinations of 0.3 μmol gallic acid.

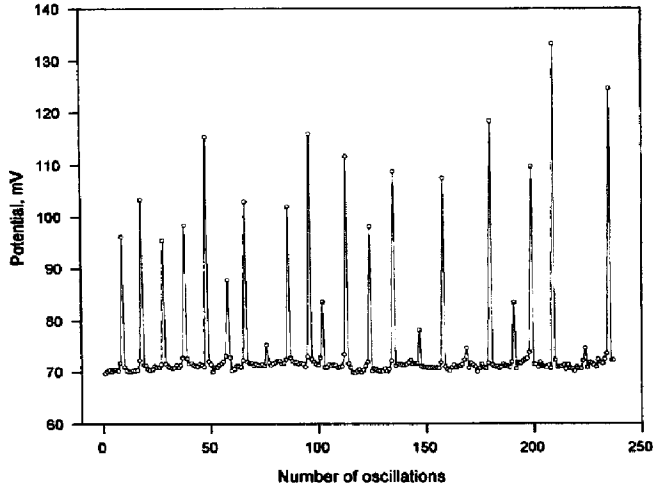

Fig. 4. Effect of variable perturbations of gallic acid on the maximum potential for the oscillating reaction. For gallic acid concentrations, linear dynamic ranges and other conditions, see under Experimental.

Table 2
Influence of chemically related species on the determination of 0.4 μmol gallic acid

Foreign species	Tolerated ratio
Benzoic acid, phenylacetic acid, salicylic acid, 2-chlorophenol	60
Resorcinol	25
Phenol, 4-acetamidophenol, 4-hydroxybenzaldehyde	10
4-Hydroxyphenylacetic acid, vanillin	6
3-Dimethylaminophenol	2
4-Aminophenol	0.6
Pyrogallol	0.2

Oscillating systems are reportedly very easily altered by the presence of foreign species in the reaction medium. Consequently, we investigated the effect of some potential interferences from substances with chemical structures resembling that of gallic acid. For this purpose, the oscillating system was perturbed with a sample containing a fixed amount of gallic acid (0.4 μmol) and variable amounts of interferents (less than 24 μmol in order to avoid solubility problems). The results obtained are shown in Table 2. As can be seen, the proposed method is acceptably selective, which can be attributed to the special features of the analyte pulse perturbation technique used to monitor the oscillating reaction. We should note the strong interference of pyrogallol, which is structurally highly similar to gallic acid, and that of 4-aminophenol — the oscillating system is readily altered by the presence of primary amino groups in the reaction medium [14]. As a rule, the reducing character of the species studied had a strong influence on the extent to which they were tolerated: the more reductant, the more interfering.

3.3. Mechanism of action of gallic acid on the oscillating system

The mechanisms of oscillating reactions are usually rather complex; while some authors have attempted to elucidate them, the inferences are mere approximations that necessitate some refining. According to Epstein et al. [26], the oscillating reaction used in this work consists of 30 kinetic steps that involve 26 independent variables; also, the centrepieces of the mechanism are positive and negative feedback loops by which a yellow superoxide–copper(I) complex is formed and destroyed, respectively, and on which the autocatalytic process relies. In order to elucidate the interaction of gallic acid, the oscillating system was monitored photometrically; no effect was observed as a result of the gallic acid perturbation, however. This allows one to infer that, at least, gallic acid does not take part in the appearance or disappearance of the yellow superoxide–Cu(I) complex, whether or not it is involved in any other step of the oscillating mechanism. Further batch assays in which binary, ternary and quaternary mixtures of the ingredients of the oscillating reaction where supplied with a small amount of gallic acid, revealed a significant increase in the potential in the presence of Cu(II), whether in the aqueous or in the alkaline medium, but always in the absence of hydrogen peroxide. From these results, the gallic acid may be assumed to interact with copper owing to its reducing character.

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