Critical Review

Approaching the use of oscillating reactions for analytical monitoring

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Summary of contents

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Dolores Pérez-Bendito has been Professor of the Analytical Chemistry Department of the University of Córdoba, Spain since 1981. She has been head of this Department for 12 years. Her research interests include trace analysis, molecular spectroscopy, kinetic methods of analysis (with special emphasis on multicomponent analysis, stopped-flow methodology, kinetometrics, chemiluminescence spectroscopy, immunoassay and micellar catalysis) and environmental analysis. She has published extensively on these topics and is co-author of a monograph on *Kinetic Methods in Analytical Chemistry* and the biannual reviews of the ACS on this subject, as well as of several textbooks.

Introduction

Kinetic methods of analysis are currently regarded as highly effective tools in analytical chemistry for both the kinetic determination of a single species and the simultaneous kinetic determination of several species in a mixture with no prior separation.[1,2] The dynamic regime of the chemical process involved in these methods is mainly monotonic; however, various types of dynamic regime have been explored in recent years in order to characterize non-linear chemical phenomena in the context of theoretical and experimental chemical kinetics.[3–7] These non-linear phenomena, known as 'oscillating chemical reactions', include regular oscillations, period doubling, quasi-periodicity and deterministic chaos, among others.

Oscillating chemical reactions are complex systems that have so far been primarily examined in physico-chemical terms with a view to elucidating the intricate underlying mechanisms of the oscillations. Basically, an oscillating chemical reaction is one where some species (usually a reaction intermediate) exhibits fluctuations in its concentration; such fluctuations are usually simple (*i.e.*, periodic)—non-periodicity or even chaos is also possible, however, under specific reaction conditions. The fluctuations are reflected in colour changes (if the oscillating species exhibit different colours), pH changes (variations in the H^+ or OH^- concentration in the medium), redox potential changes, *etc.* For a chemical reaction to be the source of an auto-oscillating system, at least the following requirements must be met:[8] (a) the system should be far from thermodynamic equilibrium, *i.e.*, its Gibbs free energy difference (ΔG) should be large and negative; (b) there should be at least one autocatalytic step or, alternatively, cross-catalysis between two steps of the reaction mechanism; and (c) the system should possess at least two steady states under the initial conditions.

Although oscillations are most readily apparent in open systems, closed systems also may exhibit them for a limited time (until thermodynamic equilibrium is achieved).[9–11] The open system most widely used to ensure that the reaction will be permanently far from thermodynamic equilibrium is a continuously stirred tank reactor (CSTR); other types of reactors have also been successfully used for this purpose.[12] A CSTR can be considered a homogeneous, well stirred system where mass and energy are continuously exchanged with the surrounding environment. Therefore, the mass balance for each initial reactant should include a positive term accounting for the amount of reactant contributed by the feed stream and two negative terms corresponding to reactant consumption by chemical reaction and the removal of unreacted reagent in the reactor's outgoing stream, *i.e.*,

$$\left(\frac{d[A]}{dt}\right)_{CSTR} = \left(\frac{d[A]}{dt}\right)_{input} - \left(\frac{d[A]}{dt}\right)_{reaction} - \left(\frac{d[A]}{dt}\right)_{output}$$

When the variation of the reactant concentration with time is zero, *i.e.*, $d[A]/dt = 0$, the steady state is reached. Provided the external conditions do not change, such a state will persist indefinitely and the system will oscillate with constant amplitude and period. In the absence of an external reactant supply,

the mass balance will only include the term for reactant consumption by chemical reaction and the oscillations will be damped. This relaxation process is also interesting since transient oscillations can result from slow relaxation.

The reaction can be monitored by fitting an appropriate detection system (*e.g.*, a potentiometer, spectrometer or fluorimeter) to the CSTR. The signal thus recorded can be monitored with a microcomputer equipped with an analog-to-digital converter or a suitable peripheral (*e.g.*, a recorder or plotter). Fig. 1 depicts a typical flow reactor for studying oscillating chemical reactions.

Fig. 2 shows the typical oscillation profiles involved in different dynamic regimes (regular oscillation, period doubling and chaos). As shown below, uses of the oscillating reactions for analytical monitoring are based mainly on these non-linear phenomena. A detailed description of these and related aspects including limit cycles, bifurcation point, monostability, bistability, Fourier power spectra and Lyapounov exponents is beyond the scope of this review; interested readers are referred to pertinent books[3–5] and papers.[7,8]

The first paper that considered the use of regular chemical oscillations for analytical monitoring was published by Tikhonova *et al.*[13] in 1978. Since then, little additional research into the analytical potential of oscillating chemical reactions has been carried out.[14] Not only regular chemical oscillations have been used for analytical purposes; for example, the potential of chaotic chemical systems was recently evaluated.[15]

In general, the bridge between the theoretical (physical chemistry) and practical aspects (analytical chemistry) of
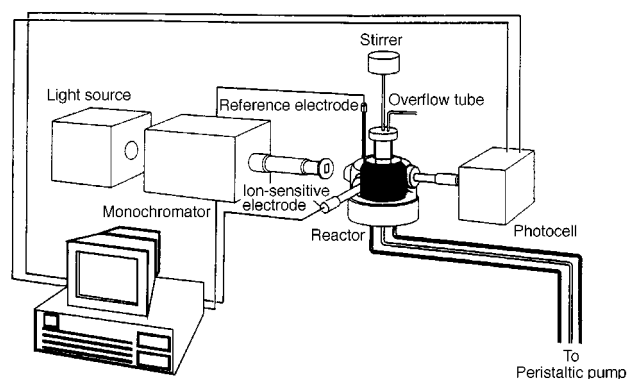

Fig. 1 Schematic representation of a typical flow reactor system for studying oscillating chemical reactions.
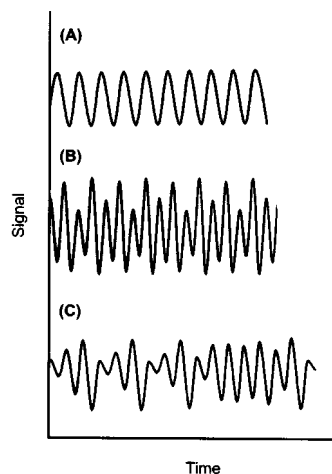

Fig. 2 Typical oscillations exhibited by oscillators used for analytical monitoring: (A) regular oscillations; (B) period doubling; and (C) chaotic regimes.

oscillating chemical reactions is their response to external perturbations. Thus, trace amounts of some substances have been shown to alter the complex dynamics of oscillating chemical reactions; also, the relationship of the oscillation attributes (period, amplitude, Fourier spectrum, largest Lyapunov exponent, *etc.*) to the external perturbation concentration can be used to construct calibration graphs. In order to improve the analytical features of oscillating reaction-based determinations, a good physico-chemical knowledge of oscillating chemical reactions is needed. In other words, constructing a realistic kinetic scheme for the whole process and performing a subsequent simulation study will be of help in understanding the role of a compound introduced into the reaction. These results are of great interest with a view to evaluating the sensitivity, precision and selectivity of an oscillating chemical reaction in relation to different compounds.

Physico-chemical features of oscillating chemical reactions involved in chemical analysis

This section deals with oscillating chemical reactions used so far for analytical monitoring purposes. The description is not exhaustive, and only the most relevant physico-chemical aspects related to their analytical potential (*e.g.*, the role of various catalytic species, the effect of experimental variables on the oscillation attributes and the dynamic regimes involved) are considered. Thus, the Belousov–Zabotinskii reaction and the most widely studied copper oscillator developed by Orbán, Epstein and co-workers are dealt with in detail since these underlying oscillating chemical reactions have been used as analytical tools for developing the oscillating reaction-based determinations described in the next section. The peroxidase–oxidase biochemical oscillator is also commented on because its mechanism and the experimental parameters that affect it have been studied in depth despite the fact that this oscillator is a specially complex chemical system. Although its analytical potential has also been evaluated from theoretical data, this biochemical oscillator is a highly promising tool for quantitative enzyme analysis.

Belousov–Zhabotinskii reaction

One of the best known and most thoroughly investigated oscillating chemical reactions is the Belousov–Zhabotinskii (BZ) reaction, which involves the oxidation of an organic compound (usually malonic acid) by bromate ion in concentrated sulfuric acid. This reaction is catalysed by traces of transition metal ions that possess two oxidation states differing in a single electron, whether in free form [*e.g.*, Ce^{III}–Ce^{IV},[6,16,17] Mn^{II}–Mn^{III}[18]] or as complexes [*e.g.*, $Ru(bpy)_3^{2+}$–$Ru(bpy)_3^{3+}$,[19–22] ferroin–ferriin[15]]. During the reaction, the autocatalytic species $HBrO_2$ is formed.

The BZ reaction can be monitored *via* changes in the potential of a Pt electrode against a reference electrode, which result from variations in the concentrations of the oxidized and reduced forms of the metal catalyst or other reaction ingredients,[6,15,20] from changes in colour between the oxidized and reduced forms of the catalyst if the two absorb at different wavelengths[6,17] and from changes in the chemiluminescence intensity when the reaction is catalysed by the $Ru(bpy)_3^{2+}$–$Ru(bpy)_3^{3+}$ couple.[16,18,19,21]

One interesting aspect of the BZ reaction is the search for new species that have a catalytic effect on it. Recent examples are tetraazacopper(II) (and nickel) complexes.[23] The oscillating reaction essentially involves the oxidation of the unsaturated Cu^{II} macrocyclic complex by bromate ions in a phosphoric acid medium. Song *et al.*[24–26] examined various oscillating systems involving complexes of Cu^{II} with a 14-membered tetraaza macrocyclic ligand. In the $KBrO_3$–$H_2C(COOH)_2$–H_2SO_4–

CuL(ClO$_4$)$_2$ system, where L is the tetraaza ligand, the complex acts as the catalyst for the BZ reaction;[24] a non-macrocyclic ligand in phosphoric rather than sulfuric acid has also been found to result in oscillations, however.[27] A ferroin complex such as Fe^{II}–5-nitro-*o*-phenanthroline has also been found to catalyse the BZ reaction in a sulfuric acid medium[28] and to have a marked effect on the initial oscillating period and amplitude, and also on the period and duration of oscillations relative to other Fe^{II} complexes. Finally, one special modification of the BZ reaction uses the aniline–bromate–sulfuric acid system as an oscillator.[29,30] The reaction has also been implemented in a flow injection system.[17]

The goal of much of the research on the BZ reaction has been to elucidate the mechanism of the reaction and to design model equations for accurately predicting the oscillatory behaviour of the system. Virtually all possible dynamic regimes may be experimentally observed in the BZ reaction. Each regime exists over a given range of control parameters, bifurcation points being where one regime changes to another.

In regular oscillations, the oscillating period and amplitude depend strongly not only on the concentrations of the reaction ingredients but also on other factors, including temperature and the flow rates of reactants and products entering and leaving the CSTR, respectively. Thus, the oscillating amplitude generally ranges from about 30 to 100 mV with potentiometric measurements and from 0.3 to 0.5 absorbance with spectrophotometric detection.[6,17,31] Also, the oscillating period typically ranges from 30 s to a few minutes, depending on the particular conditions.[6,17,31] It should be pointed out that the accuracy of the parameters involved is a result of oscillations that take place over a limited range of phase space.[31]

The BZ reaction exhibits an induction period and, similarly to the above-mentioned oscillation attributes, its length depends on the specific reaction conditions but is usually several minutes.[6,17,18] The time interval between oscillations (oscillation period) or the time to the onset of oscillations (induction period) was first used as an analytical parameter of interest by Zhabotinskii.[32]

One other highly influential experimental factor in this context is the presence of concomitant species in the reaction medium. In fact, changes in the oxygen concentration in the medium can alter the oscillating attributes amplitude and period. Thus, Saigusa[21] has shown that when an oxygen perturbation is applied to the BZ reaction in a closed system and is then removed, the reaction exhibits phase transitions between perturbed and unperturbed oscillatory states, the latter exhibiting longer oscillation periods. The results of the perturbation experiments have been expressed in the form of phase response curves. In summary, although the effect of oxygen on the BZ reaction has been extensively studied,[4,6,33] its mechanism remains controversial.

Chaos has been one of the most exciting topics in chemistry over the past decade.[34,35] Chaotic dynamics are built up by the expanding and folding processes of chaotic orbits and may be described in terms of the local expansion rate of nearby orbits.[36,37] Chaos exhibits exponential divergence from adjacent starts. If the initial conditions of a chaotic system are known only with some margin of error, then the final outcome becomes unpredictable. This sensitivity to initial conditions or divergence is used as an indicator of chaos; the most usual measure of the divergence is called the Lyapunov exponent.[36–38] Under specific initial conditions, the BZ reaction may proceed into chaotic regimes, which has been exploited for analytical purposes.[15] The methodology is based on the high sensitivity of some types of chaotic regimes of the BZ reaction to small perturbations of the initial conditions introduced by low concentrations of some metal ions. Recently, the influence of temperature on the chaotic regime near its generation threshold was investigated for the ferroin-catalysed BZ reaction in a

CSTR.[39] Temperature changes were found to result in a non-equilibrium transient phase in the reactor that was concomitant with an abrupt change in the reciprocal induction period. Oscillations occurred according to a probabilistic pattern near the phase-transition temperature.

Copper oscillators

Copper(II) takes part as a catalyst in a major group of oscillating systems. For example, the reactions of H$_2$O$_2$ with Na$_2$S$_2$O$_3$ and KSCN have been found to exhibit oscillations in pH and redox potential at Pt and selective copper electrodes, oscillations in the redox potential, pH and O$_2$ concentration in the K$_2$S$_2$O$_8$–Na$_2$S$_2$O$_3$ system and oscillations in the redox potential for the ClO$^-$$_2$–S^{2-} system, the Cu^{II}–Cu^I redox couple being the catalyst in all cases.[40–43]
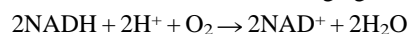
The most widely studied among Cu^{II} oscillators is the reaction developed by Orbán based on the oxidation of KSCN by H$_2$O$_2$ in a strongly alkaline medium, which is catalysed by traces of copper.[44] The reaction takes place in both open and closed systems; in the latter, however, oscillations are gradually damped and eventually disappear altogether. It can be monitored photometrically or potentiometrically. The system oscillates with an amplitude of 15–35 mV and a period from 30 s or less to several minutes.

As in the BZ reaction, the system is strongly influenced by experimental variables. Thus, an increase in the Cu^{II} or H$_2$O$_2$ concentration decreases the oscillating amplitude and period, whereas an increase in the SCN$^-$ concentration has little effect on the amplitude but increases the period. The temperature has a marked effect on the kinetics; thus, an increase of only 5 °C decreases the oscillating period by up to 15% without significantly altering the amplitude.[45] The inclusion of foreign species, such as luminol, in the reaction medium gives rise to similar oscillating systems, exhibiting oscillations in the chemiluminescence at 424 nm.[46,47]

The mechanism for this oscillating reaction was established[48] from previous investigations of Luo *et al.*[49] and Wilson and Harris,[50,51] on the H$_2$O$_2$–CuSO$_4$ and H$_2$O$_2$–KSCN sub-systems. The model involves 30 kinetic equations and 26 independent variables. The reaction involves the decomposition of H$_2$O$_2$, catalysed by traces of Cu^{II}, which is much more favourable in a strongly alkaline than in an acidic medium. Also, the formation of a yellow superoxide–copper(I) complex [HO$_2$–Cu^I], which exists only above pH 9, plays a crucial role in determining the pathway of copper catalysis under different conditions of pH;[49] thus, at a high enough pH and appropriate reactant concentrations, a yellow colour is observed to appear and disappear and the potential of the Pt electrode changes with a regular periodicity. Oscillations stop below pH 9, where the formation of the HO$_2$–Cu^I complex is hindered.

Peroxidase–oxidase biochemical oscillators

Peroxidases are a class of enzymes which usually use hydrogen peroxide to oxidize a variety of organic compounds. Some peroxidases, however, can catalyse oxidation using molecular oxygen instead of hydrogen peroxide. This type of reaction is called a peroxidase–oxidase reaction. One such reaction, which oxidizes the common biochemical reducing agent NADH is

$$2NADH + 2H^+ + O_2 \rightarrow 2NAD^+ + 2H_2O$$

where NAD$^+$ is the oxidized form of NADH (β-nicotinamide adenine dinucleotide). This oscillating reaction is a very complex system. Since its discovery by Yamazaki *et al.*[52] in 1965, a substantial number of papers on the models developed for reproducing many of the involved non-linear phenomena (*e.g.*, bistability,[53] mixed-mode and quasi-periodic oscillations[54,55] and period doubling and chaotic behaviour[56–60]) have been published.

The peroxidase–oxidase oscillator has a high potential for quantitative enzyme analysis; in fact, because it uses NADH (a common reducing agent in many biochemical reactions), any enzyme reaction that consumes or produces NADH should in theory lend itself to analysis using this oscillator. The potential of this oscillator for analytical monitoring was examined in two interesting papers by Olson and Scheeline.[12,61] In the earlier one,[61] the theoretical basis for quantitative enzyme determinations was developed from a modification of the Olsen model[58] including a competing (analyte) reaction. Using period doubling, a theoretical calibration graph can be constructed on account of the sensitive dependence of the oscillatory oxygen transient on the analyte enzyme concentration. Interesting conclusions on the precision, sensitivity and approaches to the calculation of limit of detection were drawn. Although the limits of detection achieved are not particularly low, they can be improved by using appropriate experimental conditions. In the later study, Olson and Scheeline[12] developed and characterized the analytical tools (15 variables were recognized and described) needed to study this particularly complex chemical system. Variables were studied in depth, which ensured good reproducibility of the oscillations by using controlled experimental parameters. It is worth noting that the effect of structurally different peroxidases (*viz.*, horseradish peroxidase, lactoperoxidase, soybean peroxidase and coprinus peroxidase) was recently investigated by using NADH as substrate.[62,63] These new data are of great interest with a view to increasing the analytical potential of this biochemical oscillator.

Oscillating reactions in analytical determinations

As stated above, studies on oscillating chemical reactions have preferentially been approached from the physico-chemical standpoint with a view to elucidating the intricate non-linear behaviours observed in the experimental system. In fact, few analytical applications have so far been reported; however, an appreciable number of papers on this topic have been published in the last few years. The growing interest can be ascribed to two factors, namely: (1) the physico-chemical background of oscillating chemical reactions is becoming better known every day—today, the chemist uses or develops appropriate tools to explain the complex behaviour of these reactions; and (2) the good results recently achieved in the reported analytical determinations have encouraged others to work on this exciting analytical chemical topic.

In general, determinations rely on the interaction of the species to be determined with an oscillating reaction; changes in some characteristic of the oscillator (whether the induction period, oscillating amplitude or oscillating period in regular oscillations or even in chaotic regimes) in the presence and absence of the analyte are correlated with its concentration. The response of the system in the absence of analyte is taken as a reference for establishing the quantitative relationships needed to determine the species of interest. Until fairly recently, these reactions were implemented batchwise. Analytical determinations entailed preparing a fresh oscillating system in each; however, fill reactors,[12] flow injection[17] and the recent inception of the analyte pulse perturbation (APP) technique[45] have introduced substantial practical advantages derived from the ability to operate in a continuous manner.

The use of oscillating chemical reactions for analytical monitoring has concentrated on two oscillators, namely the BZ reaction and the copper oscillator described in detail above.
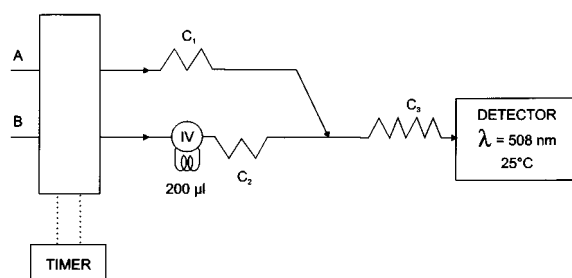
Analytical use of the Belousov–Zhabotinskii reaction

Determination of metal ions

As stated above, the catalytic determination of ruthenium based on a self-oscillating chemical reaction developed by Tikhonova

et al.[13] in 1978 was the first attempt at using oscillating chemical reactions for analytical monitoring. In this system, the sulfates of both Ru^{III} and Ru^{IV} increase the frequency of the oscillations (monitored through the absorbance at 360 nm) in the Ce^{III}-catalysed BZ reaction. A linear relationship was observed between the decrease in the period of a single oscillation and the ruthenium concentration over the range 7–330 ng ml^{-1}. In addition to high sensitivity, the method exhibits appreciable precision (the RSD is $<2.8\%$ for about 0.25–1 μg of ruthenium) and selectivity for platinum and rhodium; however, palladium interferes at levels below that of ruthenium in the sample. Karavaev *et al.*[16] used this reaction to determine ruthenium on the basis that traces of $Ru(bpy)_3^{2+}$ enhance the chemiluminescence (CL) intensity in the Ce^{III}-catalysed BZ reaction. Despite the increased sensitivity of CL measurements, the proposed method is less sensitive than the photometric method, but has a wider dynamic range; from log–log calibration, ruthenium can be determined at levels between 0.2 nM and 0.1 mM (μg ml^{-1} level), although the limit of quantification can be lowered by measuring the combined CL of several oscillations.

Thallium(I) and mercury(II) have also been determined with good selectivity and sensitivity on the basis of their complexes with Br$^-$, a critical species for the behaviour of the reaction.[64] The determination of these metal ions is influenced by the temperature, so thallium(I) can be quantified at concentrations between 0.08 and 10 μg ml^{-1} at 26 °C, whereas mercury can be measured over the same range at 35 °C. Oscillations are monitored by using potentiometric measurements with a Pt or Br ion-selective indicator electrode and an SCE reference electrode. Recently, both metal ions were determined by using the BZ reaction in a double-line flow injection (FI) manifold[17] (see Fig. 3). The manifold includes a peristaltic pump, a six-port pneumatic injection valve, a 30 μl flow cell and a UV/VIS spectrophotometer. The FI system operates in the stopped-flow mode and reproducible oscillations (0.6–2.2%) can be monitored for several minutes before the oscillatory behaviour becomes damped or gas bubbles (carbon dioxide) interfere with reaction monitoring. Spatial non-homogeneity (the BZ reaction is unstirred) turns the flow-cell dimensions into the key to the oscillator's performance. With the FI system, mercury(II) and thallium(I) can be determined by the increase in the induction period of the BZ reaction in the presence of the metal ions in the reaction medium (the induction period is 35 s shorter than that for the reaction in the presence of 400 ng ml^{-1} of mercury). The method exhibits a determination level similar to that of the above method and poor precision (very small variations in the initial conditions lead to larger experimental errors). In spite of these results, this is an interesting contribution to the use of oscillating chemical reactions for analytical monitoring (the analytical potencial of the procedure is clear).

Fig. 3 Flow injection system for mercury(II) and thallium(I) determination based on their perturbations on the BZ reaction. C_1, C_2 and C_3 are 90, 90 and 200 cm long mixing coils, respectively; IV, injection valve. Solutions A (malonic acid–cerium–ferroin) and B (bromate) and the detector flow cell (30 μl) are thermostated at 25 °C. The timer controls the delay time between injection and stopping of the pump. Adapted from ref. 17.

It is also worth noting the determination of copper(II)[65] based on the Brigg–Rauscher reaction (a combination of the BZ and Bray–Liebhafsky reactions). In this reaction, a mixture of hydrogen peroxide and iodate in sulfuric acid is used as the oxidizing agent, manganese(II) as the catalyst and malonic acid as the organic reaction substrate.[66] Because the oscillation times are increased by increasing concentrations of copper(II), this metal ion can be determined with a limit of detection of 0.1 μg ml^{-1} and an RSD of 2.7% for 0.5 μg ml^{-1} of copper.

Determination of inorganic anions

In addition to trace amounts of metal ions, anions have been also determined using the BZ reaction. Thus, hexacyanoferrates [$Fe(CN)_6^{3-}$ or $Fe(CN)_6^{4-}$] can significantly decrease the amplitude of this oscillator while the oscillation frequency remains almost unchanged.[67] On the basis of potentiometric measurements made at 25 °C, the decrease in the amplitude is linearly proportional to the concentration of hexacyanoferrates over the range 7.0×10^{-8}–5.0×10^{-6} M (the RSD is 2.7% at 1.0×10^{-6} M). The method provides good selectivity: of the 40 foreign ions tested, only mercury(II), thallium(I) and manganese(II) interfere, although their tolerated limits are favourable [the maximum tolerated mole ratio for these ions relative to $Fe(CN)_6^{3-}$ is 10]. A study was carried out by using cyclic voltammetry to elucidate the interaction of hexacyanoferrates with the BZ reaction. The results revealed that cyanide, which results from the decomposition of hexacyanoferrate, is the main active species. The system was applied to the determination of the analytes in silver plating and photographic solutions. As can be seen from Table 1, the method is a good alternative to the determination of these compounds in this type of sample judging by the consistency between the experimental results and certified values.

The inhibitory effect of chloride and iodide on this reaction have also been exploited to develop analytical methods. Chloride ion prevents the regeneration of the cerium(IV) catalyst, thus causing a decrease in the oscillation amplitude and an increase in the period.[68] The decrease in the recorded voltage peaks (amplitude) measured with a bromide electrode is rectilinearly related to the chloride content from 1.0×10^{-6} to 1×10^{-4} M. One interesting application of the method is the determination of trace amounts of chloride in human serum. On the other hand, iodide is a strong inhibitor of the reaction.[69] Thus, after the induction period produced by the addition of iodide, the decrease in amplitude of the first oscillation period is linearly related to the iodide concentration over the range 1.0×10^{-5}–1.0×10^{-3} M.

Determinations based on chaotic regimes

As stated above, chaotic regimes in the BZ reaction have also been exploited for analytical purposes.[15] Thus, trace amounts of manganese(II) were determined by their perturbating effect on some types of chaotic regimes in the ferroin-catalysed BZ reaction. The Lyapunov exponent, λ_L, calculated by using Borland Turbo Pascal software based on the Wolf algorithm,[70] and the time interval T_i from the starting point to the first maximum of the potential–time graph [Fig. 4(A)] were calculated, and the product of λ_L and T_i was plotted against the logarithm of the manganese(II) concentration. The calibration graph thus obtained was linear from 1.0×10^{-11} to 1.0×10^{-5} M manganese [Fig. 4(B)]. As can be seen, the analytical sensitivity, expressed as the slope of the calibration plot, was very low [in other words, the variation of the product of λ_L and T_i was very small relative to the wide range of manganese(II) concentrations tested]. As can be seen in Table 2, this considerably reduces the reproducibility of the method by the effect of the high sensitivity of the chaotic regimes to the experimental conditions and the inherent irreproducibility. The authors proposed the following expression for the limit of detection:

$$\lambda_L T_i = \alpha \log(C_{Mn}/C^*_{Mn})$$

where α and C^*_{Mn} can be calculated by extrapolation of the linear portions of the $\lambda_L T_i$–log C_{Mn} plots. The limit of detection thus calculated for manganese(II) was about 10^{-15} M (3 pg ml^{-1}) much lower than those typically provided by traditional catalytic kinetic methods (about 100 pg ml^{-1}). The limit of detection is closely related to the starting ferroin
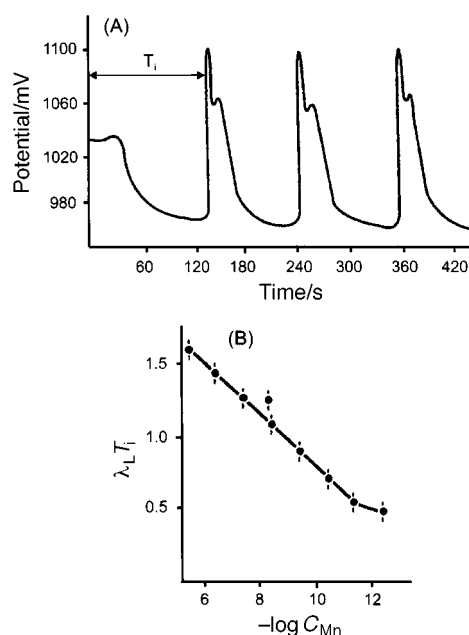
Fig. 4 (A) Oscillations in the BZ chaotic reaction used for the determination of manganese(II). The time elapsed between the starting point and the first maximum on the Pt electrode potential *versus* time plot is shown. (B) Calibration plot for the determination of manganese using this oscillator. Adapted from ref. 15.

Table 1 Determination of hexacyanoferrates in silver plating baths and photographic solutions using the BZ reaction. Adapted from ref. 65

Sample	Certified value*/ mg ml^{-1}	Found†/ mg ml^{-1}	Error (%)
Silver plating bath solution 1‡	53.22	53.6 ± 0.32	0.71
Silver plating bath solution 2‡	71.30	72.3 ± 0.28	1.40
Photographic solution 1	4.24	4.2 ± 0.08	−0.94
Photographic solution 2	3.08	3.0 ± 0.08	−2.67

* Hexacyanoferrates as hexacyanoferrate(II) and -(III) in silver plating baths and photographic solutions, respectively. † Means of five determinations ± s. ‡ Small amounts of potassium bromide were added to precipitate silver(I) before the determination.

Table 2 Determination of manganese(II) by its perturbation on the chaotic BZ chemical system using a $\lambda_L T_i$ *versus* log C_{Mn} calibration plot. Adapted from ref. 15

Manganese added/g ml^{-1}	Manganese found/g ml^{-1}	Error (%)
7.5×10^{-3}	$(7.5 \pm 1) \times 10^{-3}$	0.0
1.1×10^{-5}	$(9 \pm 2) \times 10^{-5}$	718
4.3×10^{-6}	$(4 \pm 2) \times 10^{-6}$	−7.5
3.0×10^{-7}	$(5 \pm 4) \times 10^{-7}$	67

(catalyst) concentration in this oscillating chemical reaction. In summary, although this method for maganese(II) is complicated and cumbersome, it is highly analytically significant because it demonstrates for the first time that some types of chemical chaos lend themselves to analytical applications.

Typical oscillation attributes such as the oscillation period and amplitude were recently used to evaluate their use in chemical analysis by using this chaotic system.[71] Again, manganese(II), and also vanadium(IV), were perturbing species; their analytical concentrations were related to the oscillation period. In fact, the chaotic BZ chemical system is very sensitive to low concentrations of these metal ions [$< 10^{-9}$ M for manganese(II) and $< 10^{-8}$ M for vanadium(IV)], although in different ways: the periods between oscillations decrease in the presence of vanadium(IV) and increase in the presence of manganese(II). The calibration graphs (period *versus* log $C_{\text{metal ion}}$) are complicated; in any case, vanadium(IV) can be determined over the range 10^{-8}–10^{-5} M, where it exhibits a virtually linear relationship.

Analyte pulse perturbation technique

The recently introduced APP technique[45] uses a CSTR and relies on the sequential perturbation of an oscillating reaction by successive additions of analytes (or standards) after the regular oscillations are restored. Maintaining optimum experimental conditions, the system remains in an oscillating state for at least 8 h, acting as a continuous indicator system. This provides a rapid, simple method for performing many determinations on the same oscillating system. This operating mode offers obvious advantages over discrete systems and endows the APP technique with high practical potential.

As can be seen from Fig. 5, the experimental set-up for implementation of the APP technique is very simple. It consists of a CSTR furnished with a thermostating jacket, a Pt indicator electrode and a reference electrode connected to an analog-to-digital converter in a microcomputer for monitoring oscillations and their perturbation (by potentiometry), a peristaltic pump for delivery of the reaction ingredients intended to ensure attainment of the steady state in the CSTR (one of the pump lines is used to replenish the reactor) and an autoburette or micropipette for dispensing a small volume (in the microlitre region) of the analyte solution in order to perturb the oscillating system.

The analytical performance of the APP technique has been evaluated in the determination of $Na_2S_2O_3$ from the decrease in the oscillating period and amplitude relative to their values before the system was perturbed by injecting the analyte. The decrease is quantitatively correlated with the analyte concentration.[45] The oscillating reaction used for this purpose was the above-described oxidation of KSCN by alkaline hydrogen peroxide, catalysed by traces of copper(II). Fig. 6 shows the response of the oscillating system to $Na_2S_2O_3$ perturbations, and also those of other analytes (*e.g.*, an increase in the oscillating amplitude by effect of a perturbation with gallic acid[72] or an increase in the oscillating period by virtue of the perturbation with a given amount of reduced glutathione,[73] which resulted in first- and second-order correlations, respectively, between the responses and the injected analyte concentration). As can be seen from Table 3, the determinations are sensitive (the dynamic linear range is typically from a few nanomoles to a few micromoles) and highly precise (the RSD is usually about 1%). Of special practical significance in these determinations is the fairly high throughput achieved by using the APP technique. In fact, up to 10 samples h^{-1} can often be processed, which is acceptable for an oscillating reaction and much better than the typical sample throughput of discrete approaches (1–2 h^{-1} at the most).

The APP technique has been applied not only to individual determinations but also to the resolution of binary mixtures of species that elicit differential responses from an oscillating
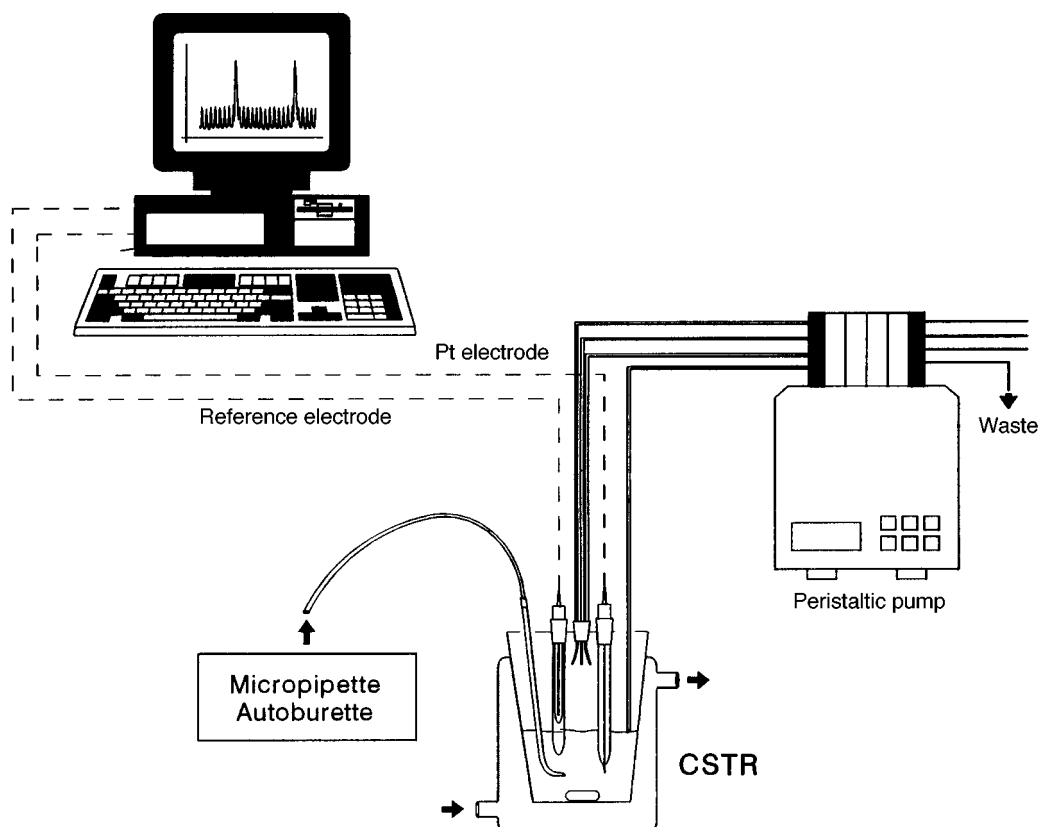

Fig. 5 Manifold for implementing of the analyte pulse perturbation technique by use of a CSTR.

system. Thus, gallic acid and resorcinol can be determined by the increasing oscillating amplitude and period produced by the former and latter, respectively, in relation to the system in the steady state.[74] The ensuing method allows both species to be determined at the micromole level with gallic acid-to-resorcinol ratios from 1 : 6 to 1 : 35, even in the presence of a synergistic effect, with acceptable precision (RSD 4.42 and 3.58%, respectively).

The above determinations use potentiometric detection to monitor oscillations. Two different detection systems (the above-described potentiometric detector and a chemiluminescent system based on the use of luminol as one of the reaction
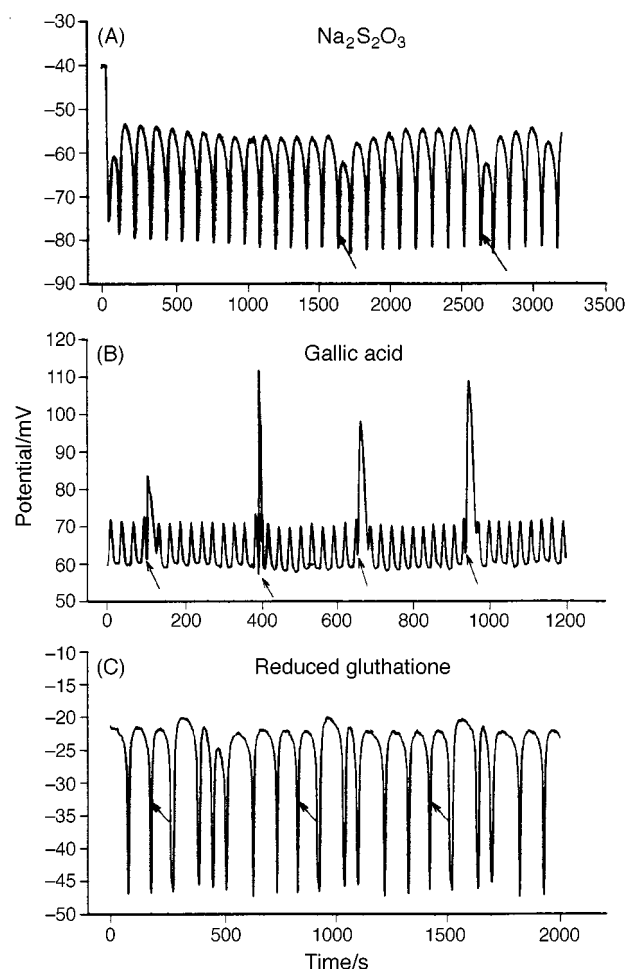
Fig. 6 Typical profiles for the H_2O_2–NaSCN–NaOH–$CuSO_4$ oscillating system in the absence and presence of (A) sodium thiosulfate, (B) gallic acid and (C) reduced gluthathione. Arrowheads indicate the times at which oscillations were perturbed.

Table 3 Analytical figures of merit of oscillating reaction-based determinations based on the APP technique

Analyte	Dynamic linear range/μmol	RSD (%)	Ref.
$Na_2S_2O_3$	1–18	0.71	45
Gallic acid	0.075–2.0	0.67	72
Reduced glutathione	0.1–0.7	0.67	73
Resorcinol	1.5–12	1.53	74
Vanillin	1–30	0.78	76
Paracetamol	0.5–6.0	0.61	76
Ascorbic acid	0.5–5.0	4.65	76
Vitamin B_6	0.5–20	3.04	75

ingredients) have been compared with a view to increasing their sensitivity.[75] The reaction was found to be analytically useful with both types of detection system and, as expected, somewhat more sensitive with chemiluminescence detection.

Validation in the analysis of real samples

The true measure of the actual potential of an analytical technique can only be obtained by applying it to real samples. In this respect, the APP technique has been found to permit the use of oscillating reactions for routine analyses. Thus, a method for the rapid, straightforward determination for vanillin, paracetamol and ascorbic acid,[76] and another for vitamin B_6,[75] all in food and pharmaceutical samples, have been developed. As can be seen from Table 3, the methods possess favourable analytical features and allow the determination of the above-mentioned species in real samples (Tables 4 and 5).

Conclusions

Oscillating chemical reactions as dynamic systems have traditionally aroused interest in the context of kinetic methods of analysis. However, they have largely been the subject only of academic investigations owing to their little practical interest. In the last few years, several studies have been published that show the potential of these reactions in analytical-based determinations. From these papers, the following conclusions can be drawn:

(1) The key to the analytical use of oscillating chemical reactions seems to be based in their response upon perturbation with different species (analytes), the BZ reaction being one of the most often used.

(2) Different non-linear regimes of the BZ reaction perturbed by both metal ions and anions have been used for analytical purposes. In general, lower limits of detection are achieved in the determination of metal ions, especially in chaotic chemical regimes. The understanding of the physico-chemical aspects of the oscillator and those of the subsequent interaction of the

Table 4 Use of the analyte pulse perturbation technique in food analysis. Adapted from ref. 76

Analyte	Sample	Standard method[*]/ mg g^{-1}	Found/ mg g^{-1}	Error (%)
Vanillin	Vanilla sugar 1	78.6 ± 5.2	83.7 ± 4.3	6.43
	Vanilla sugar 2	3.6 ± 0.3	3.3 ± 0.2	−8.61
	Vanilla sugar 3	82.1 ± 2.7	85 ± 12	4.41
	Stick vanilla 1	15.6 ± 1.1	15.1 ± 0.3	−3.39
	Stick vanilla 2	13.8 ± 2.3	14.5 ± 2.3	4.56
Ascorbic acid	Orange juice	0.563 ± 0.002	0.527 ± 0.007	−6.23

[*] AOAC and NBS methods for vanillin and ascorbic acid, respectively.

Table 5 Use of the analyte pulse perturbation technique in pharmaceutical analysis. Adapted from refs. 75 and 76

Analyte	Trade name	Nominal content	Found	Error (%)
Paracetamol	Termalgin	500 mg/tablet	522 ± 57	4.50
	Efferalgan	500 mg/tablet	519 ± 35	3.86
	Saldeva	300 mg/tablet	307 ± 15	2.43
	Cortafriol	500 mg/tablet	516 ± 46	3.30
	Melabon	350 mg/tablet	364 ± 14	4.03
	Propalgina plus	500 mg/bag	496 ± 25	−0.82
Vitamin B_6	Pacium	33.3 mg g^{-1}	33 ± 8	−1.1
	Vertigum	275.1 mg g^{-1}	247 ± 13	10.1
	Taurobetina	112.4 mg g^{-1}	104 ± 5	−7.4
	Trofimilina	387.1 mg g^{-1}	370 ± 30	−4.5

analyte with it allows the development of methods with good analytical figures of merits. On the other hand, the type of detection system used to monitor the oscillating reaction is not decisive in this context.

(3) The inception of the analyte pulse perturbation technique (which has been reported by using a system other than the BZ reaction) has improved the use of oscillating chemical reactions for analytical monitoring. The fact that an analyte may perturb oscillations (*viz.*, alter their amplitude and/or period) and the ability to develop a continuous system in permanent oscillation that regains its regular state after each analyte (or standard) perturbation have opened up new avenues for practical applications of oscillating reactions, especially for organic analytes, which have not been determined using the BZ reaction. In summary, this technique has enormously facilitated the use of oscillating reactions, formerly of purely theoretical interest, for routine analytical monitoring practices, as shown in this review of their existing applications to real samples.

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