STUDIA UNIVERSITATIS BABEŞ-BOLYAI, CHEMIA, LV, 1, 2010

ANTIOXIDANT CAPACITY DETERMINATION BY THE BRIGGS-RAUSCHER OSCILLATING REACTION IN A FLOW SYSTEM

An application of functional dynamics in analytical chemistry

NORBERT MUNTEAN[a], IOAN BÂLDEA[a], GABRIELLA SZABÓ[a], ZOLTÁN NOSZTICZIUS[b]

ABSTRACT. A new method to determinate the antioxidant capacity by means of Briggs-Rauscher system under flow conditions is being described. The basis of this method is a continuous perturbation of an oscillating system performed in a continuously fed stirred tank reactor (CSTR) by an inlet flow of an antioxidant solution, namely resorcinol. Under proper experimental conditions the amplitude and the time period of the oscillations will be strongly affected by the concentration of the antioxidant inflow. In our experiments the amplitude and the frequency of the oscillations followed a supercritical Hopf bifurcation scenario where the resorcinol concentration plays the role of the bifurcation parameter. Switching between pure water and resorcinol solutions can be performed many times, applying standard solutions of the antioxidant or the analyte providing data for the calibration curve or for the actual measurement.

Keywords: *Briggs-Rauscher oscillating reaction, inhibitory effect, CSTR, analytical method, resorcinol*

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. 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 [1].

[a] *Department of Physical Chemistry, "Babeş-Bolyai" University, Cluj-Napoca,11 Arany Janos Str. Romania, RO-400028. E-mail: gszabo@chem.ubbcluj.ro*

[b] *Center for Complex and Nonlinear Systems and the Department of Physics, Budapest University of Technology and Economics, H-1521 Budapest, Hungary*

These non-linear phenomena, known as oscillating chemical reactions, include regular oscillations, period doubling, quasi-periodicity and deterministic chaos, among others [2-5]. These phenomena usually depend strongly on certain parameters of the system. Functional dynamics applies that parameter dependence to measure the actual values of the parameters after a calibration procedure.

Oscillating chemical reactions are complex systems involving a large number of chemical species as reactants, products, and intermediates that interact via unusual mechanisms. The Briggs-Rauscher (BR) reaction is one of the most spectacular oscillating reactions, which is a hybrid of the well-known Belousov-Zhabotinsky and the Bray-Liebhafsky reactions. The net chemical transformation of the BR reaction is the oxidation and iodination of malonic acid by hydrogen-peroxide and iodate ion, catalyzed by manganous ion in acidic media [6].

An oscillating reaction is very sensitive to small perturbation and this behavior is used in several analytical determinations, like the antioxidant capacity [7] of various chemical compound or some plants extract. The basis of this analytical method is that various antioxidants, for example resorcinol, change the dynamics of the BR reaction fundamentally by suppressing the oscillations even in a surprisingly low concentration (usually in a few micromoles/ liter) [8-12].

The BR reaction method is based on the inhibitory effects by antioxidant scavengers of free radicals on the oscillations of the BR reaction. Free radicals can play an important role in the BR reaction, e.g. the strong CO and CO_2 evolution discovered recently in the reaction [9] has also a radical mechanism [13]. The generated hydroperoxyl radicals (HOO·) are among the main intermediates of the BR system. The mechanism of the action of antioxidants against HOO· radicals in the BR system has recently been described [10, 14]. When antioxidant scavengers of free radicals are added to an active oscillating BR mixture there is an immediate quenching of the oscillations, an inhibition time that linearly depends on the concentration of the antioxidant. The inhibition time is defined as the time elapsed between the end of the addition of the antioxidant and the first regenerated oscillation.

Based on these results a new analytical method was developed for the determination of the antioxidant activity of free radical scavengers.

It is a disadvantage of the method that a re-start of the oscillating regime is necessary each time before a new determination if a chemical oscillator like the BR is applied in a batch reactor.

The analyte pulse perturbation (APP) technique is performed in a continuous-flow stirred tank reactor (CSTR). Injecting a small amount of an analyte into the reactor causes a transient modification of the oscillator properties (affecting its amplitude and/or the period). It was first proposed by Jimenez-Prieto et al [15-16].

They applied the H_2O_2-NaSCN-$CuSO_4$ system in an alkaline medium which is an oscillatory system in CSTR as it was discovered by Orban [17]. The CSTR technique allows a system to be kept oscillating for a long time (as long as we have reactants, usually for several hours) and thus be employed as an inexhaustible indicator system for successively added samples and/or standards. Up to now, the usefulness of the APP technique has been demonstrated with various oscillating reaction for the determination of several chemical substances, for example heavy metal ions in aqueous solution, reduced glutathione, gallic acid [18-23].

No similar technique was developed, however, until now to determinate the antioxidant capacity with BR reactions in a CSTR. The aim of this paper is to present such a new determination possibility of antioxidant capacity using an active BR mixture in CSTR. To this end a mini-CSTR with a low flow rate was developed to minimize the consumption of chemicals needed for an experiment and a new perturbation technique different from the APP method was applied switching between a continuous inflow of either pure water, and the aqueous solution of the perturbing antioxidant.

RESULTS AND DISCUSSION

Perturbation of a BR system in its oscillatory regime with resorcinol causes a change in the oscillation amplitude as well as in their period see Figure 1.

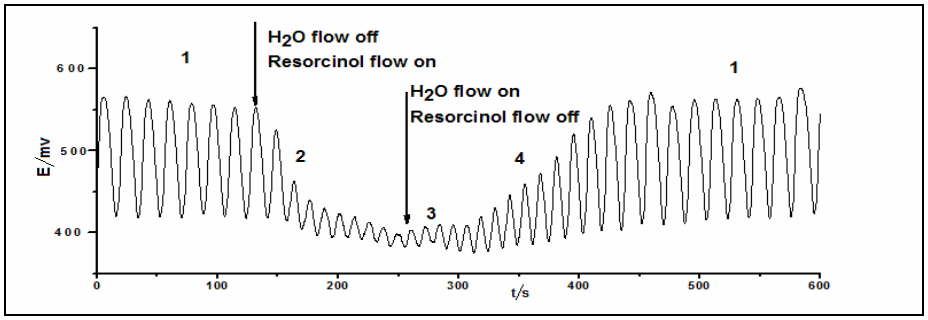


Figure 1. Evolution of an active BR mixture's oscillations in presence of resorcinol added with a 0.25mL/min flow rate for 120 s The arrows indicate the moment when feeding of 100 μM resorcinol solution was started or stopped.

State 1 represents the normal behavior of the unperturbed BR system, showing the regular oscillations of the active mixture.

The arrows indicate moments of switching from pure water to the resorcinol solution, or back. In the presence of resorcinol the amplitude and period decrease with time, and when the concentration of resorcinol reaches its maximum value in the mixture, the amplitude reaches a minimum.

Now, switching the resorcinol flow to that of distilled water, the amplitude and period increases slowly again, and finally returns to the unperturbed state. The variations of these parameters are presented in Figure 2.

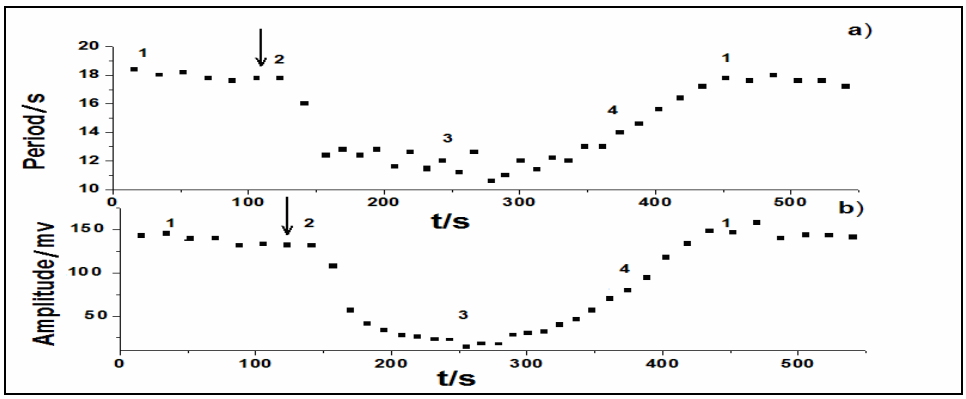


Figure 2. Variation of the period (a) and the amplitude (b) of the oscillations caused by the addition (indicated with arrow) of resorcinol.

As Figure 2 shows the system has four different dynamical states: two asymptotic (1, 3) and two transient ones (2, 4).

State1 (asymptotic state after all transients died out):

- only H_2O is fed in the fourth channel. Stable high amplitude oscillations can be observed;

State 2 (transient between the asymptotic states 1 and 3):

- antioxidant solution is fed in the fourth channel with the same flow rate as H_2O was fed in state 1. The amplitude and the time period decrease in time;

State 3 (asymptotic state after all transients died out)

- still the antioxidant solution is fed to the reactor but the small amplitude oscillations are already stabilized;
- when the concentration of resorcinol reaches its maximum, the oscillations became monotone and stable (stage 3);

State 4 (transient between states 3 and 1):

- Feeding pure H_2O in the fourth channel is resumed. The oscillation parameters slowly reach their initial values.

It is interesting to observe that the period and amplitude changes in state 4 is more gradual than in state 2 and in a wide time interval within state 4 the period and the amplitude increase in a close to linear fashion with time.

Experiments were repeated with the same resorcinol solution, the same flow rate and identical addition time of the analyte. Results are presented in Figure 3.

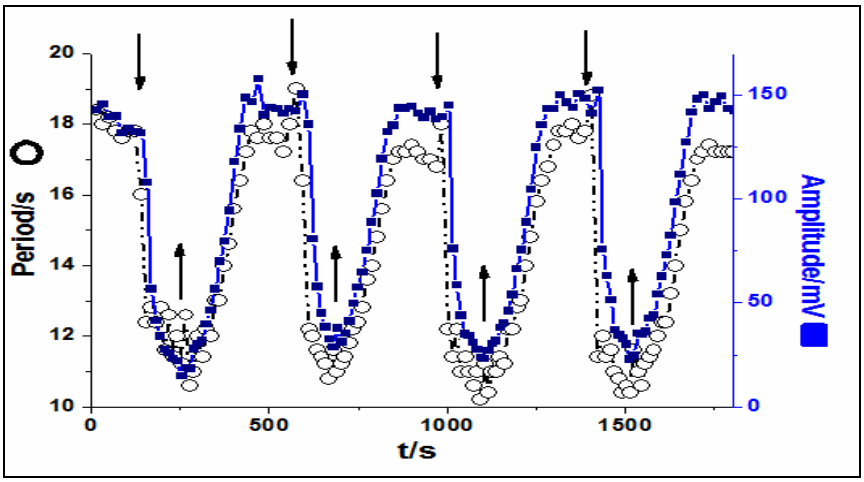


Figure 3. BR system's oscillations period and amplitude evolution caused by resorcinol inflow. The arrows pointing down indicate the moment when feeding of 100 μM resorcinol solution was started with the usual 0.25mL/min flow rate. After 120 s the inflow of the channel was switched to pure H_2O (arrows pointing up) with the same 0.25mL/min flow rate but for 4 minutes (time equal to mean residence time). Then the whole cycle was repeated.

The figure reveals the same pattern of oscillations during the perturbation of BR system with resorcinol solution and the repetitiveness of the behavior.

The oscillation amplitude and time period are plotted as a function of the analyte concentration of the resorcinol in Figure 4.

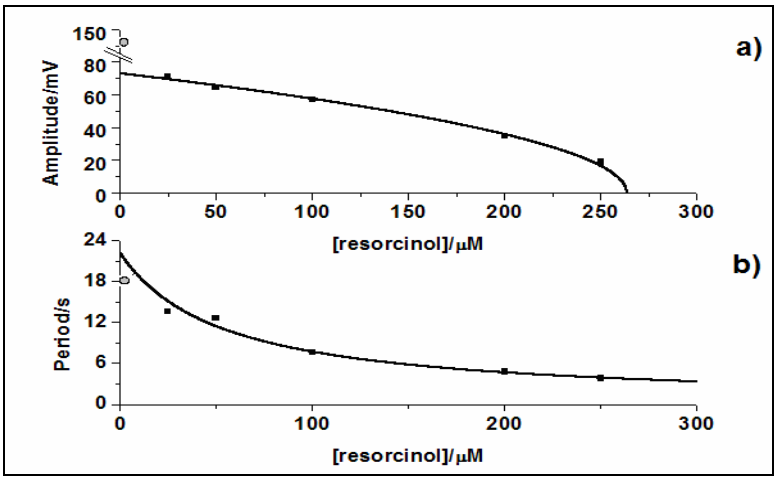


Figure 4. Plot of the amplitude a) or the period b) in state 3 (indicated with ■) vs. the concentration of resorcinol; the average period and amplitude in state 1 were indicated with (○)

Figure 4 shows that already 25 μM resorcinol in the inflow causes a substantial change in the dynamics as the amplitude of the oscillations is halved. On that basis one would expect that the effect of the resorcinol in the interval between 0 and 25 μM (e.g. at 12.5 μM) would be still considerable. This is not the case, however, according to our experiments. We found that the resorcinol concentration should reach a threshold level (somewhere below but close to 25 μM) before it can affect the dynamics considerably. Unfortunately, the details of that strongly nonlinear behavior close to the threshold, was difficult to study experimentally. On the other hand the dynamics was more reproducible above 25 μM thus we calibrated the system applying such resorcinol concentrations. In that region the amplitude decreases to zero smoothly with the increasing resorcinol concentration.

The parabolic type decrease of the amplitude, depicted in Figure 4 and the decreasing but finite time period of the disappearing oscillations suggested a Hopf bifurcation. To check this the square of the amplitude (the average amplitude of state 3) and the frequency (inverse of the average period of state 3) was plotted against the resorcinol concentration as a bifurcation parameter (see Figure 5).

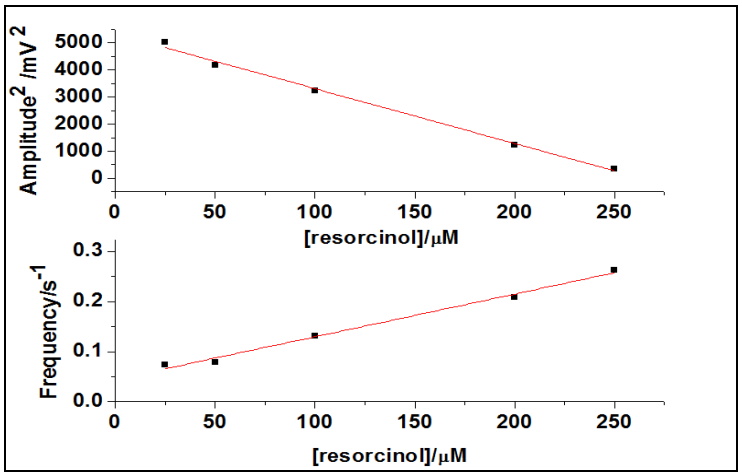


Figure 5. Plot of the analytical signal vs concentration of resorcinol

The equations of calibration curve are :

$Amplitude^2=5323.38(\pm125.4)-20.19(\pm0.82)[resorcinol]$ $R^2=0.993$

$Frequency=0.045(\pm0.0068)+8.51*10^{-4}$ $(\pm4.2*10^{-5})[resorcinol]$ $R^2=0.99$

CONCLUSIONS AND OUTLOOK

The BR system's oscillations were perturbed by continuous inflow of resorcinol into the reactor. In the 25 μM -250 μM region the square of amplitude and the frequency of the oscillations were found to be proportional to the concentration of the resorcinol in the inflow. On the basis of this relationship a new analytical method for determination of the antioxidant capacity [7] can be developed for various mono- and polyphenolic compounds

This technique is automatic, fast, relatively inexpensive, and provides two analytical signals at the same time. The approximately linear variation of the period and amplitude with the slowly decrease of resorcinol concentration in tube indicates the sensitivity of the method.

Under the selected experimental conditions, the system oscillated in the CSTR for at least 3 h, during which time as many perturbations as required to implement quantitative analytical determinations could be applied without oscillations being stopped.

Based on the time needed for the system to recover after each perturbation, the throughput can be estimated to be 8 sample/h, which is quite acceptable, taking into account how slow most oscillating reactions are. This sample throughput clearly better those of determinations based on Briggs Rauscher reaction operated in batch modem.

In such determinations, the analyte is added jointly with the reagents to the oscillating system from the start, which needs preparing a fresh sample for each analysis; this obviously reduces throughput to less than 2 sample/h.

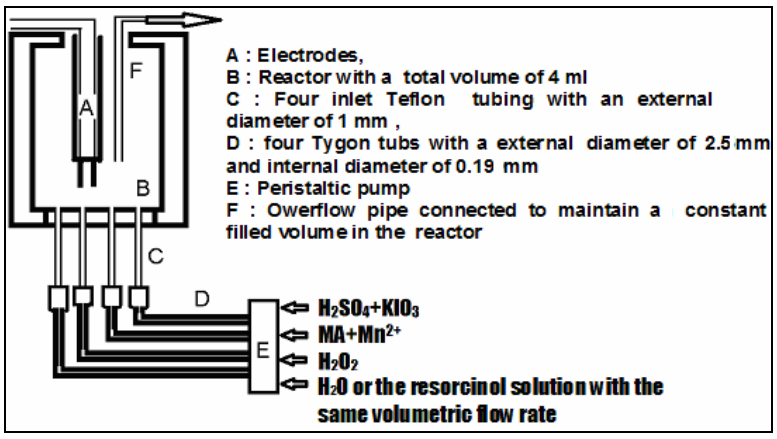
To increase further the sensitivity of the method shorter residence times are needed. To this end higher flow rates or an even smaller CSTR is necessary, the research being in progress in our laboratoty.

EXPERIMENTAL SECTION

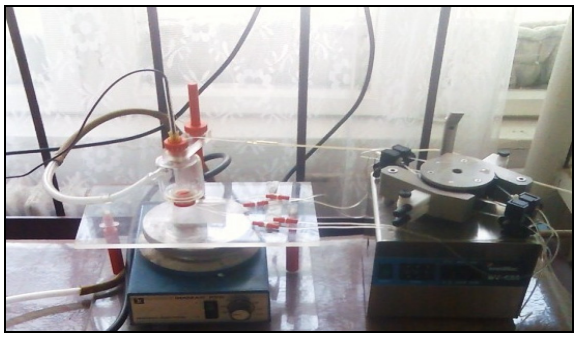
Apparatus. The instrumental set-up used to implement the proposed method consisted of a double walled glass vessel of 4 mL capacity, fitted with auxiliary equipment necessary to fed this CSTR with reactant solutions and an overflow pipe situated near the top of the vessel to maintain a constant volume. Connection to a FALC FA 90 thermostat ensures a constant temperature by water circulation through the temperature jacket. We have chosen a value of 25°C. Continuous and thorough stirring was provided by a FALC 60 magnetic stirrer. Reactants were pumped into the reactor by means of a peristaltic pump, through four Tygon tubes with a 0.25 mL/ min volumetric flow rate each. These Tygon tubes were connected to inlet Teflon tubes and the reagents were introduced to the reaction vessel through them. Low volumetric flow rate is necessary to reduce the volume of the processed

solution and, consequently, the cost of the experiments. The overflow outlet tube was connected to a vacuum pump and maintains a constant level of the mixture in the reactor (Figure1). Oscillations were monitored with a Pt electrode and an Ag/AgI indicator electrode, both handmade. In the BR reaction both the Platinum and the Ag/AgI electrodes are so called “indicator “ electrodes i.e. their potential oscillate with respect to a reference electrode. Such a reference electrode should be connected to the system via a double junction salt bridge. To fit a double junction salt bridge into the reactor, however, increases the reactor volume considerably. Moreover the liquid–liquid junction of a salt bridges always a source of contamination and “memories”. To keep the reactor volume at a minimum and to avoid memory effects we applied two indicator electrodes. The voltage between these electrodes in the BR reaction was found to be still oscillatory thus the dynamic state of the reactor can be followed by recording that voltage. Potentiometric traces recorded this way were quite reproducible.

They were connected to a PC through a PCI 6036 E data-acquisition interface.



a)



b)

Figure 6 a) Experimental setup for implementation of oscillating reactions in a CSTR; **b)** picture of the experimental setup.

CHEMICALS AND PROCEDURE

All chemicals were of analytical grade and were used without further purification. Stock solutions with the following concentration were made: $[H_2SO_4]_0$=0.078 M, $[KIO_3]_0$= 0.200 M, $[MA]_0$=0.150 M, $[MnSO_4]_0$=0,027 M, $[H_2O_2]_0$=0.023 M by using twice distilled water

CSTR was fed with reactant solutions and water through four inlet tubings as Figure 1 shows. The volumetric feeding rate was 0.25 mL for all channels producing a 4 min residence time in the reactor. After the oscillation amplitude and period had stabilized the H_2O flow was interrupted and started the resorcinol addition to the system. After 120 s the flow of antioxidant was interrupted and replaced with water for 240 s. This procedure has been repeated several times.

APPENDIX: *Hopf bifurcation*

Oscillations of the state variables can occur in different dynamical systems in Physics, Biology, and in Chemistry. The same system can be both oscillatory and nonoscillatory depending on the given experimental conditions called as parameters of the system. By changing one of its parameters (denoted by μ, for example) an oscillating system can be driven from its oscillatory state to a nonoscillatory one back and forth. That qualitative change in the asymptotic behavior of the dynamical system occurring at a critical parameter value μ_c is called bifurcation. Transitions between limit cycle oscillations and stable steady states can proceed via various bifurcation scenarios which can be observed in chemical systems as well [24]. One possibility is the so called supercritical Hopf (or Poincaré-Andronov-Hopf [25]) bifurcation where oscillations are born (or disappear) with a small amplitude and with a finite time period. For dynamical systems described by ordinary differential equations the general form of the Hopf bifurcation in polar coordinates [25, 26]:

$$\frac{dr}{dt} = d\mu r + ar^3; \qquad \frac{d\theta}{dt} = \omega + c\mu + br^2$$

Here *r* and θ are the polar coordinates in the phase plane *a, b, c, d* are fixed constants and μ is the bifurcation parameter. (The above formula is a good approximation of general nonlinear oscillators usually in the neighborhood of the bifurcation point only, when μ and *r* values are relatively small and higher order terms of the Taylor expansion used to derive the above formula can be neglected.) A special case - the so called normal form of the supercritical Hopf bifurcation - can be obtained by choosing *d*=1, and *a* = -1:

$$\frac{dr}{dt} = \mu r - r^3; \qquad \frac{d\theta}{dt} = \omega + c\mu + br^2$$

The above system has one fixed point which is in the origin (r=0). By changing the parameter μ the following bifurcation scenario can be observed. When μ <0 the fixed point is a stable focus but when μ >0 it becomes an unstable one and a small stable limit cycle appears surrounding the unstable focus.

Thus in the normal form the supercritical Hopf bifurcation takes place at μ_c =0. The amplitude r_{LC} of the limit cycle oscillations can be calculated as

$$r_{LC} = \sqrt{\mu},$$

because limit cycle oscillation is an asymptotic state where:

$$\left(\frac{dr}{dt}\right)_{LC} = 0$$

The frequency ω_{LC} of the limit cycle oscillations can be obtained by substituting r_{LC} into the second differential equation:

$$\omega_{LC} = \left(\frac{d\theta}{dt}\right)_{LC} = \omega + f\mu \quad \text{where} \quad f = (c + b)$$

Thus in the case of a supercritical Hopf bifurcation when plotting the frequency or the square of the amplitude of the limit cycle oscillations vs. μ the bifurcation parameter linear graphs will be obtained as:

$$r_{LC}^2 = \mu \quad \text{and} \quad \omega_{LC} = \omega + f\mu$$

In our experiments the bifurcation parameter should be proportional with the resorcinol concentration. An increase of this parameter, however, causes not an increase but a decrease of the oscillation amplitude in our experiments. Another deviation from the simple normal form is that the critical parameter value, (where the oscillations disappear) is not zero but occurs at a finite resorcinol concentration. Thus, to be compatible with the above normal form picture the bifurcation parameter can be chosen as:

$$\mu \propto (c_c - c)$$

Where c_c is the critical resorcinol concentration where the oscillations disappear. (In these linear graphs the factor of proportionality does not play a role thus it is not denoted.) This transformation of the experimental parameter c to the theoretical bifurcation parameter is not necessary, however, to evaluate the experimental data, as linear plots of the frequency vs. c and (amplitude)^2 vs. c can be obtained without such a transformation.

ACKNOWLEDGMENTS

This work was partially supported by OTKA grants K-60867 and K-77908 and by the foundation Domus Hungarica Scientiarum et Artium. N. M. thanks the ESF Programme Functional Dynamics for supporting his visits to Z. Noszticzius's lab.

REFERENCES

1. R. Jimenez Prieto, M. Silva, D. Perez Bendito, *Analyst,* **1998**, *123,* 1R-8R.
2. S. K.Scott, “Oscillations waves and chaos in chemical kinetics”, Oxford, **1998**.
3. I. R. Epstein, J. A. Pojman, “An introduction to nonlinear chemical dynamics”, Oxford, **1998**.
4. S. K. Scott, B. R. Johnson, A. F. Taylor, M. R. Tinsley, *Chemical Engineering Science*, **2000**, *55,*209.
5. Z. Noszticzius., W.D McCormick, H. L. Swinney, *J. Phys. Chem.,* **1989**, *93*, 2796.
6. E. Briggs, A. Rauscher, *J. Chem. Educ.,* **1973**, *50,* 496.
7. R. A. Moyer, K. E. Hummer, C. E. Finn, B. Frei, Wrolstad, *J. Agric Food Chem.,* **2002**, *50,* 519.
8. R. Cervellati, N. Crespi-Perellino, S. D. Furrow, M. Anacleto, *Helv. Chim. Acta*, **2000**, *83: 12,* 3179.
9. R. Cervellati, K. Hoener, S. D. Furrow, C. Neddens, S. Costa, *Helv. Chim. Acta,* **2001**, *84(12),* 3533**.**
10. R. Cervellati, C. Renzulli, M. C. Guerra, E. Speroni, *J. Agric. Food Chem*., **2002**, *50, 7*504.
11. L. Onel, G. Bourceanu, M. Wittmann, Z. Noszticzius, G. Szabó, *J. Phys. Chem. A*., **2008**, *112,* 11649*.*
12. T. Lawson, J. Fülöp, M. Wittmann, Z. Noszticzius, N. Muntean, G. Szabó, L. Onel, *J. Phys. Chem. A*., **2009**,*113,* 14095.
13. N. Muntean, G. Szabó, M. Wittmann, T. Lawson, Z. Noszticzius, J. Fülöp, L. Onel, *J. Phys. Chem. A.,* **2009**, *113*, 9102.
14. R Cervellati, K. Hoener, S. R. Furrow, F. Mazzanti, *Helv. Chim. Acta,* **2002***, 85: (8),* 2523.
15. R. Jimenez-Prieto, M. Silva, D. Perez Bendito, *Analyst,* **1997**, *122,* 287.
16. R. Jahan-Bakhsh, O. Reza, Abolfazl, *Analytical Sciences,* **2004**, *20,* 883.
17. M. Orbán, *J. Am. Chem. Soc.*, **1986**, 108, 6893.
18. L. Minuti, R. Pellegrino, *J. Chromatogr. A,* **2008**, *1185,* 23**.**
19. Jinzhang Gao, Jie Ren, Wu Yang, Xiuhui Liu, Hua Yang, Qizhi Li, Hualing Deng, *J. Electroanal. Chem.*, **2002**, *520,* 157.
20. Jinzhang Gao, Hua Yang, Xiuhui Liu, Jie Ren, Xiaoquan Lu, Jingguo Hou, Jingwan Kang, *Talanta*, **2001**, *55,* 99.
21. Peter E. Strizhak, Olga Z. Didenko, Tatyana S. Ivashchenko*, Anal. Chim. Acta*, **2001**, *428,* 15.
21. H. Chen, W. Yang, H. Xia Dai, X. Xia Wei, J. Qu, J. Zhang Gao, *Chin. Chem. Letters,* **2006**, *17(9),* 1221.
23. J.Gao, H. Yang, X. Liu, J. Ren, L. Qizhi, J. Kang, *Talanta*, **2007**, *57,* 105.

24. Z. Noszticzius, P. Stirling, M. Wittmann, *J. Phys. Chem.,* **1985**, *89*, 4914.
25. S. Wiggins, “Introduction to Applied Nonlinear Dynamical systems and Chaos”, Springer, New York, **1990**, p. 272.
26. J. Guckenheimer, P. Holmes, “Nonlinear Oscillations, Dynamical Systems, and Bifurcations of Vector Fields”, Springer, New York, **1986**, p. 151.