*Int. J. Electrochem. Sci.*, 6 **(2011)** 1760 - 1771

International Journal of
ELECTROCHEMICAL
SCIENCE
www.electrochemsci.org

# Determination of L-cysteine Based on its Potentiometric Effect on a Belousov-Zhabotinskii Oscillating Chemical Reaction

*Jahan-Bakhsh Raoof* [1,*], *Abolfazl Kiani*[2], *Reza Ojani* [1], *Farideh Sakhavi* [1], *Akramsadat hamidi*[1]

[1] Electroanalytical Chemistry Research Laboratory, Department of Chemistry, Faculty of Chemistry, University of Mazandaran, Babolsar, Iran
[2] Department of Chemistry, faculty of science, University of Isfahan, Isfahan, Iran
[*]E-mail: j.raoof@umz.ac.ir

*Received:* 29 August 2010 / *Accepted:* 11 March 2011 / *Published:* 1 June 2011

---

Here, a simple and convenient method with suitable selectivity and high sensitivity for the determination of *L*-cysteine based on Belousov-Zhabotinskii (B-Z) oscillating chemical reaction is presented. The addition of *L*-cysteine to the oscillating system increased the oscillating period. This increasing in oscillating period is used as the analytical signal for determination of *L*-cysteine. The optimum conditions for the determination of *L*-cysteine were also investigated through a three-level Box-Behnken design. The analytical signal was linearly proportional to the logarithm of the *L*-cysteine concentration over the range of $4.0 \times 10^{-7}$ to $3.0 \times 10^{-5}$ M under optimum conditions. The detection limit was estimated $4.0 \times 10^{-7}$ M and relative standard deviation (RSD) for five measurements of $5.0 \times 10^{-6}$ M *L*-cysteine was 3.4٪.

---

**Keywords:** *L*-cysteine, Belousov-Zhabotinskii (BZ) reaction, oscillating chemical reaction, potentiometric, statistical experimental design, Box-Behnken design

## 1. INTRODUCTION

The best known and most widely studied oscillating chemical system is based on the Belousov-Zhabotinskii (BZ) reaction [1]. Oscillating reactions are always complex and involve a number of chemical species, which can be ranked as reactants, products or intermediates interact via unusual mechanisms [2]. During an oscillating chemical reaction, the concentrations of reactants decrease and the concentration of intermediates or catalyst species exhibit oscillations [3]. This reaction involves the oxidation of an organic species such as malonic acid by an acidified bromate solution in the presence of a metal ion catalyst like $Ce^{III/IV}$ and $[Fe(II)(phen)]^{2+}/[Fe(III)(phen)]^{3+}$ couples which are two of the most widely used catalysts. The color of the solution alternates between yellow and colorless (for $Ce^{III/IV}$ couple).

In 1972, Zhabotinskii [4] attained a linear correlation between the reactant concentration and the period of the BZ reaction and proposed the use of this phenomenon for chemical analysis. The BZ reaction was interesting for a few analytical chemists who published the first paper concerning the use of regular chemical oscillation for the determination of trace amount of ruthenium(III) ions was published in 1978 [5]. Then, applicability of BZ reaction in analytical studies was investigated [6-9]. Several other researchers improved the idea with addition of different catalysts or inhibitors to the reaction for determination of $Fe\ (CN)_6^{3-}$ [10-11] and Mn(II) [12].

It is known that *L*-cysteine [(R)-2 amino-3-mercaptopropanoic acid] is an important amino acid duo to its determinant roles in biological systems. For example, it can be used as a valid radiation protector and cancer indicator. Deficiency in *L*-cysteine is associated with a number of clinical situations such as liver damage, skin lesions, slowed growth, and AIDS [13-19]. This amino acid participates in a complex series of glutathione. Also, *L*-cysteine is widely used in food industry as an antioxidant. In pharmaceutical industry it is used severely in drug formulation and as a biomarker [20]. Therefore, measuring cysteine is highly valuable in many biological, medical, and clinical studies, where much effort has been made to develop sensitive methods for its detection. Till now, numerous chemical and instrumental techniques for *L*-cysteine detection have been reported [21-27]. However, most of them suffer from difficulties in sample preparation, the need for derivatization or the lack of sufficient sensitivity, all of which limit their utility [28]. In this direction, we have previously reported the construction of some chemically modified carbon paste electrodes for electrocatalytic determination of *L*-cysteine [29-32], but there is not any report about potentiometric determination of *L*-cysteine based on BZ oscillating system. On the other hand, to the best of our knowledge, there are currently no reports on optimization of the oscillating system by statistical experimental design (RSM). Therefore, in the present paper, we have investigated the effect of *L*-cysteine on the oscillating properties of the "$KBrO_3$-KBr-malonic acid (MA)-$H_2SO_4$-Ce (IV)" BZ oscillatory system as a matrix in order to determine its concentration in a closed system. For this purpose, the effect of different variables on *L*-cysteine perturbation was optimized using statistical design for the BZ oscillating system in the first step. Finally, a logarithmic calibration curve was obtained for *L*-cysteine.

## 2. EXPERIMENTAL PART

### *2.1. Regents and Material*

All reagents were analytical grade (Fluka) without further purification. All stock solutions of $KBrO_3$, malonic acid, KBr, $H_2SO_4$, Ce(IV) salt and *L*-cysteine were separately prepared in distilled water.

### *2.2. Instrumentation*

The experimental set-up used to accomplish the oscillating reaction was consisted of a glass reaction vessel furnished with a thermostated jacket connected to a “polystat CCl” thermostat (Huber Co.) and a magnetic stirrer (Jank & Kunkel). A pH meter 780 coupled to a Pentium III personal computer was used to record the potential changes. A platinum electrode monitored the oscillating reaction and the reference electrode was a simple glass electrode.

### *2.3. Procedure*

The water-jacketed glass vessel was loaded with 10 ml of 0.35 M $KBrO_3$, 10 ml of 0.6 M malonic acid in 0.125 M potassium bromide. After reaching thermal equilibrium (25 °C ± 0.1), the electrodes were inserted into the solution, which was continuously and steadily stirring. Then, 10 ml of Ce (IV) $6.0 \times 10^{-3}$ M in 7.5 M $H_2SO_4$ was added into the system as at the last species. The total volume of the reaction mixture was 30 ml in all experiments. After the system reached a steady state, its oscillation was perturbed by injecting 0.5 ml of *L*-cysteine solution with different concentrations.

## 3. RESULTS AND DISCUSSION:

In the Belousov-Zhabotinskii oscillating system the net reactions are [33]:

$$\underset{\text{Malonic acid}}{CH_2(CO_2H)_2} + \underset{\text{Ceric}}{6Ce^{4+}} + H_2O \rightarrow 2CO_2 + HCO_2H + 6Ce^{3+} + 6H^+ \quad (1)$$

$$\underset{\text{Cerous}}{10Ce^{3+}} + \underset{\text{Bromate}}{2BrO_3^-} + 12H^+ \rightarrow 10Ce^{4+} + Br_2 + 6H_2O \quad (2)$$

Based on the Field, Koros and Noyes (FKN) mechanism, the oscillatory concentration of $Br^-$ and Ce(IV) led to variations in medium potential. These variations in the concentration and potential appeared as an oscillating system that could be recorded with a Pt electrode using the potentiometry method. On the other hand, the potential due to a redox couple (Ce(IV)/(Ce(III) could be measured on the Pt electrode versus a reference electrode. Therefore, the oscillations were potentiometrically recorded upon addition of Ce(IV) in $H_2SO_4$ solution. Variations in the oscillation period -as a consequence of perturbation- were used as an analytical signal, for depicting a calibration plot. For this purpose, we measured the difference between the first oscillation period after analyte injection (P) and the last oscillation period before injection ($P^0$), and then used it as an analytical signal, as follows:

$$\Delta P = P^0 - P \quad (3)$$

The conventional method for response optimization is one factor-at-a-time approach, which is time consuming, and unmanageable when large number of variables has to be investigated. Also this method is unable to depict the combined effect of multiple factors involved. Optimizing the parameters by statistical experimental design can eliminate the limitations of single factor optimization process [34]. A three-level Box- Behnken design was used in this study. This design is suitable for exploration of quadratic response surfaces and construction of a second order polynomial model [35]. This design allows estimating and interpreting the interaction between various variables at a time during an optimization process [36].

**Table 1.** Factors and levels used in the Box-Behnken design.

| Independent variable | Coded symbol | Coded level -1 | 0 | 1 |
|---|---|---|---|---|
| KBr | $X_1$ | 0.07 | 0.11 | 0.15 |
| $KBrO_3$ | $X_2$ | 0.20 | 0.30 | 0.40 |
| Ce(IV) | $X_3$ | 0.0034 | 0.0187 | 0.034 |
| $H_2 SO_4$ | $X_4$ | 3.00 | 5.25 | 7.50 |
| MA | $X_5$ | 0.30 | 1.65 | 3.00 |

Table 1 lists the five studied variables, while $X_1$, $X_2$, $X_3$, $X_4$ and $X_5$ are KBr, $KBrO_3$, Ce(IV), $H_2SO_4$ and MA, respectively. The parameters of highest confidence levels were prescribed into three levels, coded -1, 0, and +1 for low, middle and high values, respectively. The concentrations of all salts were adjusted as mol per liter. Responses to the input variables were evaluated as a function of the change in oscillation period, which is indicated by Y. A total of 46 experimental runs of response surface methodology (RSM) were designed and carried out as per the design (Table 2) and second-degree polynomials (equation 4) were calculated with the statistical package (Minitab, Version 14) to estimate the response of the dependent variables.

$$Y = \beta_0 + \sum \beta_i X_i + \sum \beta_{ii} X_i^2 + \sum \beta_{ij} X_i X_j \qquad (4)$$

While Y is the predicted response, $x_i$, $x_j$ are input variables which influence the response variable Y; $ß_0$ is the offset term; $ß_i$ is the $i^{th}$ linear coefficient; $ß_{ii}$ is the $i^{th}$ quadratic coefficient and $ß_{ij}$ is the $ij^{th}$ interaction coefficient.

The statistical significance of the predicted models was evaluated by the analysis of variance (ANOVA) and the least squares techniques. The ANOVA analysis determines which of the factors significantly affects the response variable in the study, using a Fisher's statistical test (*F*-test).

In the experimental Box-Behnken procedure, the influences of the five factors (see Table 1) on the response were determined (Table 2). Box-Behnken design was applied to analyze the interactions between parameters and also to determine the optimum condition for response electrode. Data represented were analyzed using linear multiple regression and the following equation were obtained:

$$Y = 2.666 - 0.1250\,X_1 - 4.037\,X_2 - 2.250\,X_3 - 3.525\,X_4 + 1.812\,X_5 - 0.720\,X_1^2 + 3.162\,X_2^2 + 2.945\,X_3^2 + 1.745\,X_4^2 + 1.862\,X_5^2 - 0.5000\,X_1X_2 - 1.250\,X_1X_3 + 0.750\,X_1X_4 - 1.500\,X_1X_5 + 2.750\,X_2X_3 + 0.600\,X_2X_4 - 2.500\,X_2X_5 + 1.750\,X_3X_4 - 1.250\,X_3X_5 - 1.000\,X_4X_5 \qquad (5)$$

while Y is the predicted value of oscillation period after injection of *L*-cysteine, $X_1$, $X_2$, $X_3$, $X_4$ and $X_5$ are the coded values for KBr, $KBrO_3$, Ce(IV), $H_2SO_4$ and MA, respectively.

**Table 2.** Box-Behnken design matrix.

| Run | KBr | $KbrO_3$ | Ce(IV) | $H_2SO_4$ | MA | Response |
|---|---|---|---|---|---|---|
| 1 | 0.11 | 0.4 | 0.0187 | 7.50 | 1.65 | 1 |
| 2 | 0.11 | 0.3 | 0.0034 | 7.50 | 1.65 | 3 |
| 3 | 0.11 | 0.3 | 0.0034 | 3.00 | 1.65 | 15 |
| 4 | 0.15 | 0.2 | 0.0187 | 5.25 | 1.65 | 10 |
| 5 | 0.11 | 0.3 | 0.0187 | 5.25 | 1.65 | 3 |
| 6 | 0.07 | 0.3 | 0.0187 | 5.25 | 3.00 | 7 |
| 7 | 0.11 | 0.3 | 0.0187 | 3.00 | 0.30 | 6 |
| 8 | 0.07 | 0.3 | 0.0034 | 5.25 | 1.65 | 5 |
| 9 | 0.11 | 0.3 | 0.0187 | 7.50 | 0.30 | 1 |
| 10 | 0.11 | 0.4 | 0.0187 | 5.25 | 0.30 | 4 |
| 11 | 0.11 | 0.4 | 0.0340 | 5.25 | 1.65 | 5 |
| 12 | 0.15 | 0.3 | 0.0187 | 5.25 | 3.00 | 4 |
| 13 | 0.15 | 0.3 | 0.0187 | 5.25 | 0.30 | 4 |
| 14 | 0.11 | 0.3 | 0.0187 | 5.25 | 1.65 | 2 |
| 15 | 0.11 | 0.2 | 0.0187 | 3.00 | 1.65 | 15 |
| 16 | 0.11 | 0.2 | 0.0187 | 5.25 | 0.30 | 7 |
| 17 | 0.11 | 0.3 | 0.0187 | 7.50 | 3.00 | 5 |
| 18 | 0.07 | 0.3 | 0.0340 | 5.25 | 1.65 | 3 |
| 19 | 0.11 | 0.4 | 0.0187 | 3.00 | 1.65 | 7 |
| 20 | 0.11 | 0.3 | 0.0187 | 5.25 | 1.65 | 2 |

Effects with less than 95% of significance, or higher *p*-value than 0.05, were discarded and pooled into the error term and a new analysis of variance was performed for the reduced model as shown in Fig. 1, it is evident, that the interactions between KBr, $KBrO_3$, Ce(IV), $H_2SO_4$ and MA was significant. *P*-values presented in Table 3 can also prove this significance.

To validate the regression coefficient, analysis of variance (ANOVA) was performed for responses as shown in Table 3. The *P*-values were used as a tool to investigate the significance of each variable, also to indicate the strength of interactions between each two independent variables [37]. The values of Model F and Model $P > F$ were found to be 37.15 and 0.0000, respectively, which implies that the model was significant. The *p*-values of lack of fit F and lack of fit $P > F$ were found to be 1.95 and 0.236, respectively, which implies that the lack of fit was no significant.

Non-significant lack of fit made the model fit. Goodness-of-fit for this model was also evaluated by the coefficient of determination [38]. Here, this value (R) is calculated 0.9670 indicating a good agreement between experimental and predicted values of oscillation period. The residuals also had to be examined for normal distribution.

**Table 3.** Analysis of variance for suggested second-order model.

| Source | DF | Seq SS | Adj SS | Adj MS | F | P |
|---|---|---|---|---|---|---|
| Regression | 20 | 873.059 | 873.059 | 43.653 | 37.15 | 0.000 |
| Linear | 5 | 593.445 | 593.445 | 118.689 | 100.99 | 0.000 |
| Square | 5 | 181.924 | 181.924 | 36.385 | 30.96 | 0.000 |
| Interaction | 10 | 97.690 | 97.690 | 9.769 | 8.31 | 0.000 |
| Residual Error | 25 | 29.380 | 29.380 | 1.175 | | |
| Lack-of-Fit | 20 | 26.047 | 26.047 | 1.302 | 1.95 | 0.236 |
| Pure Error | 5 | 3.333 | 3.333 | 0.667 | | |
| Total | 45 | 902.439 | | | | |

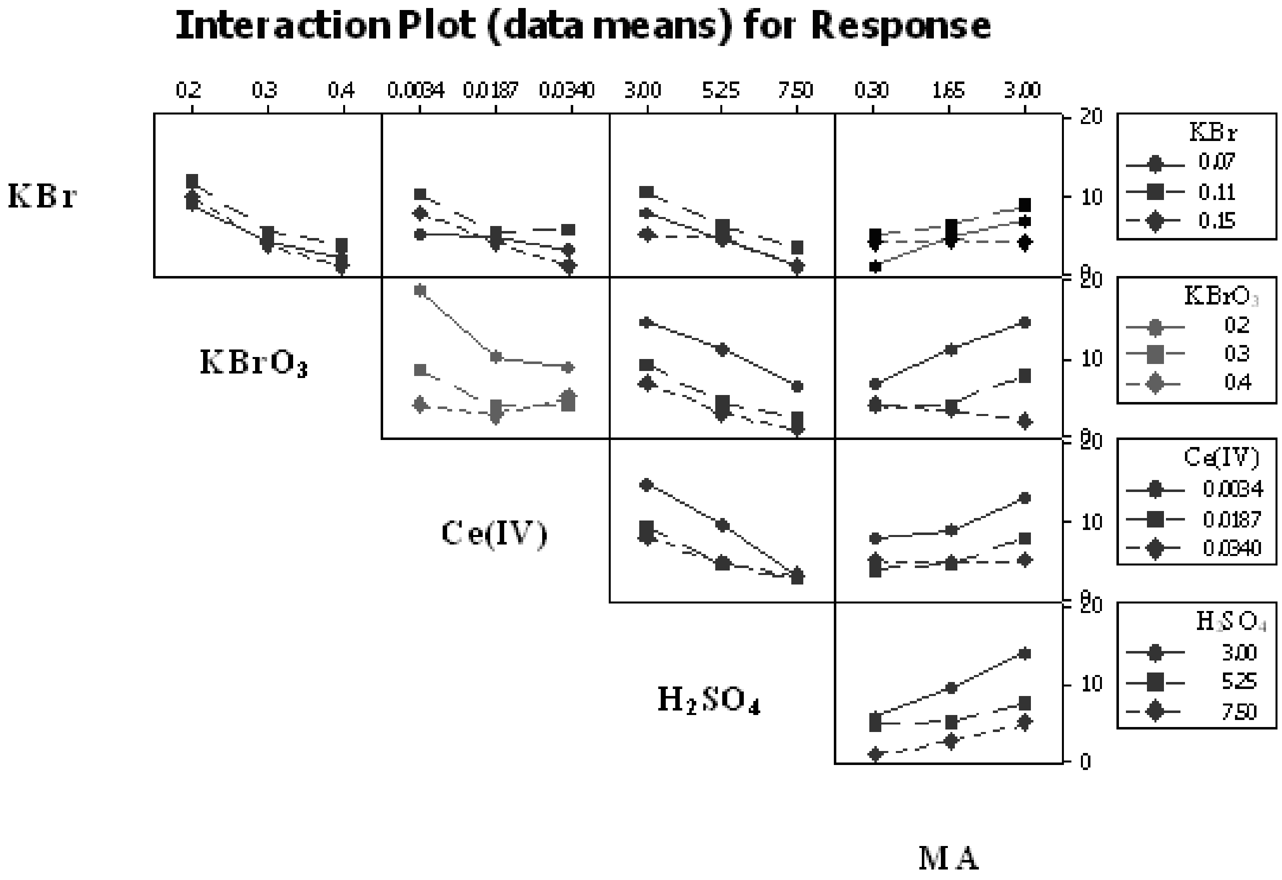


**Figure 1.** The interaction interactive effects plot. The unit of the parameters is as the main effect plot.

### *3.1. The surface response and selection of optimum conditions*

Three dimensional surface plots were depicted to illustrate the main and interactive effects of the independent variables on the response of electrode.

Figure 2 shows some of response surface plots which indicate 3D plots relationship between two variables and period (s) while other variables were kept in center levels. Figure 3 shows the main effects plots. The optimum $\Delta$P (19 s) was obtained when the KBr, $KBrO_3$, Ce(IV), $H_2SO_4$ and MA, Ce(IV) and *L*-cysteine were 0.05 M, 0.066 M, 0.0023 M, 1.0 M, 0.36 M and 3 × $10^{-5}$ M, respectively

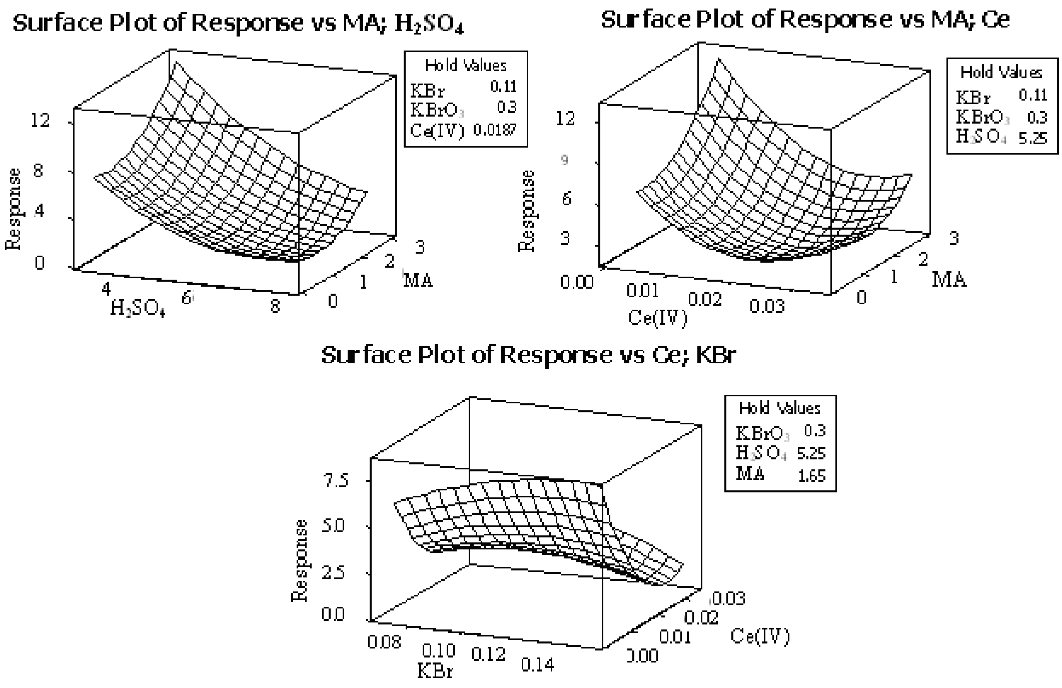


**Figure 2.** The response surface plots.

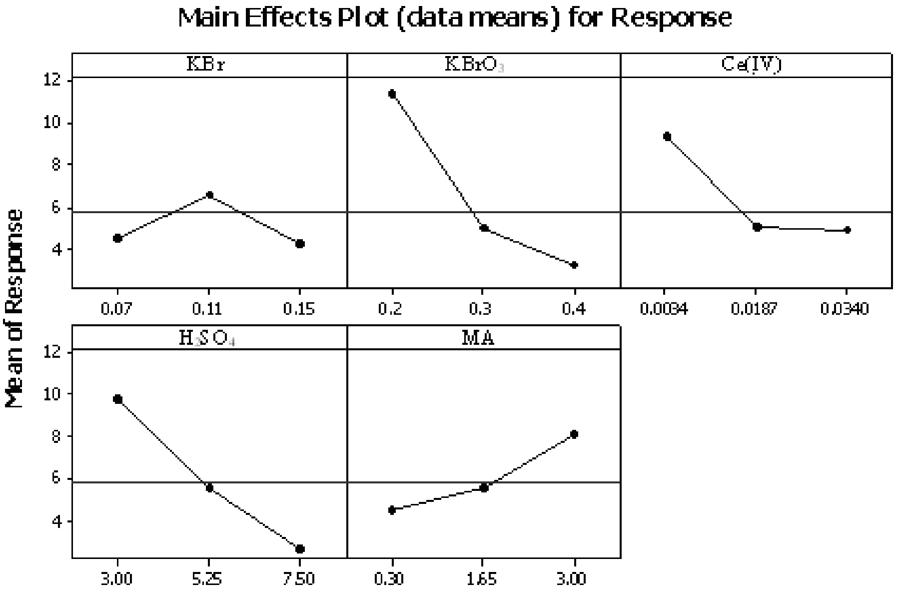


**Figure 3.** The main effects plot.

### 3.2. Validation of the models

The statistical model was validated with respect to all five variables within the design space.

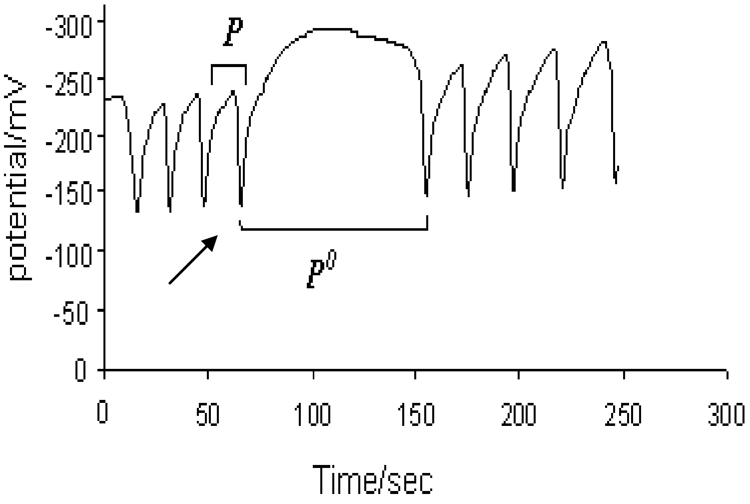


**Figure 4.** Typical profile for the Ce(IV)-$H_2SO_4$-MA-KBr-$KBrO_3$ oscillating system after injection of *L*-cysteine ($5.0 \times 10^{-4}$ M). The solution condition were 0.036M KBr, 0.06 M $KBrO_3$, 1 M malonic acid, $6.2 \times 10^{-3}$ M Ce(IV), 1.75 M $H_2SO_4$ at 25 °C.

A random set of six experimental combinations were used for validation of the statistical model.

**Table 4.** Model validation experiments.

| Run No. | $\Delta$P (sec) | $\Delta$P (sec) |
|---|---|---|
| | Observed value | Predicted value |
| 8 | 5 | 5.9 |
| 22 | 8 | 6.07 |
| 25 | 6 | 9.37 |
| 18 | 3 | 3.97 |
| 33 | 7 | 7.85 |
| 35 | 1 | 0.08 |

The results of analysis (Table 4) indicated that there was a good agreement between experimental values and predicted ones. Figure 4 shows the oscillation profiles for the proposed oscillating chemical system in the presence of *L*-cysteine perturbation under experimental conditions described previously. In order to obtain larger effects after *L*-cysteine perturbation, it is not necessary to inject the sample at maximum or minimum potential.

### *3.3. Determination of L-cysteine*

The oscillating system was perturbed with various concentrations of *L*-cysteine under the previously-described optimum conditions. When *L*-cysteine was added to the system, the oscillation period increased (Fig. 4). A typical oscillation which was carried out under the optimum experimental conditions, in the presence of increasing concentrations of *L*-cysteine is plotted in Fig. 5. Several repeated experiments indicated that there was a good linear relationship between the changes in the oscillation period ($\Delta P$) and the logarithm of *L*-cysteine concentration in the range of $4.0 \times 10^{-7}$ to $3.0 \times 10^{-5}$ M (Fig. 6). If the *L*-cysteine concentration was below $4.0 \times 10^{-7}$ M, the effect could hardly be measured, and if the concentration of *L*-cysteine exceeded from $3.0 \times 10^{-5}$ M, the increase of oscillation period was not a linear relationship with the logarithmic concentration. Therefore, the calibration data obeys the following logarithmic regression equation:

$$P = 2.6762 \, \mathrm{Ln}[L\text{-cysteine}] + 45.811 \qquad (6)$$

The detection limit was found $4.0 \times 10^{-7}$ M (according to the IUPAC method) [39]. Table 5 shows our results versus other *L*-cysteine determinations.

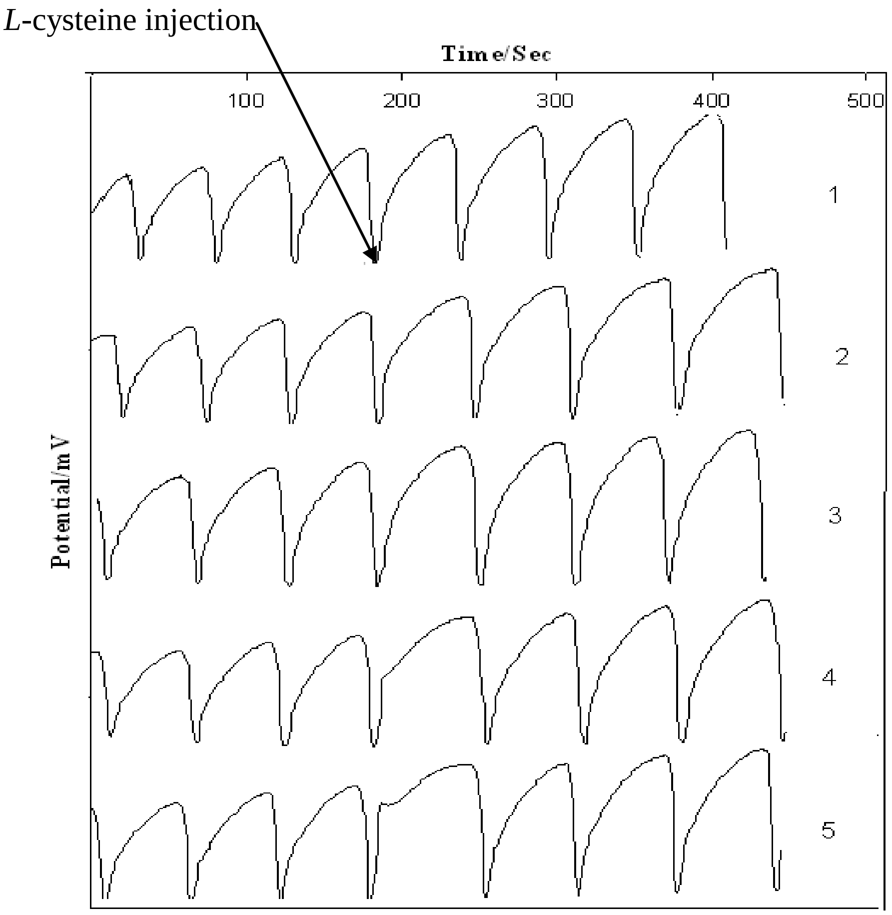


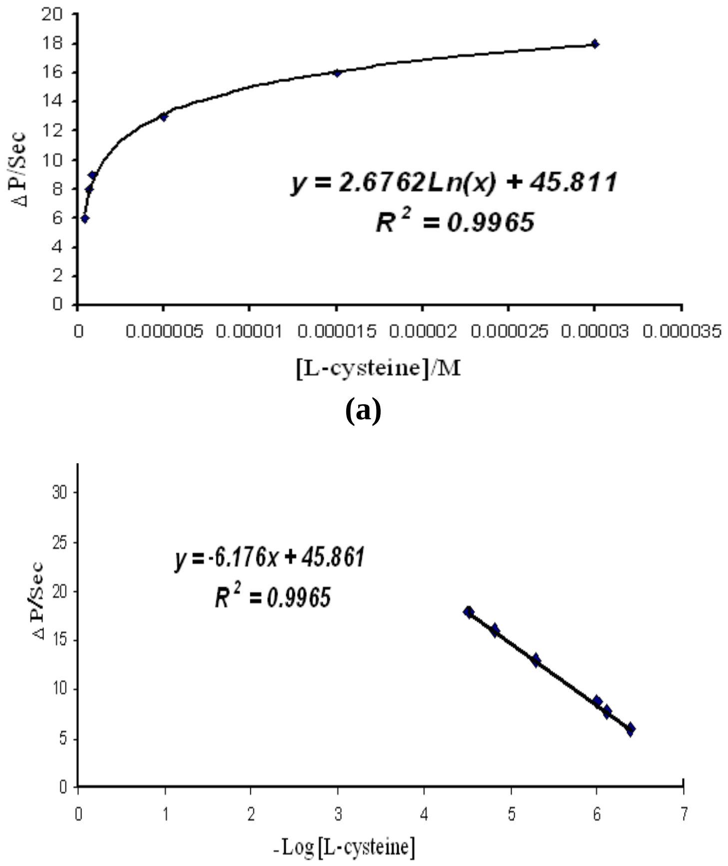
**Figure 5.** Typical oscillation profiles of BZ system in the presence of increasing concentration of *L*-cysteine 1) 4 µM, 2)7 µM, 3)9 µM, 4) 150 µM and 5) 300 µM in optimum condition.

**Table 5.** Comparison of some analytical methods used for the determination of *L*-cysteine.

| Method | Detection Limit (μ M) | Dynamic Range (μ M) | Reference |
|---|---|---|---|
| Amperometry | 2 | 2-200 | 40 |
| Spectrofluorimetric | | 2-15 | 41 |
| FTIR Spectrometric | 170 | 500-24700 | 42 |
| Potentiometric biosensor | 1 | 1.7- 1250 | 43 |
| Flow injection voltammetry | 0.9 | 1-200 | 44 |
| Square wave voltammetry | 1.4 | up to 560 | 45 |
| Oscillating chemical reaction | 3 | 4-300 | present work |

### *3.4. Interferences study*

We also investigated the effect of five foreign species that may exist with *L*-cysteine in real samples for their possible influence on the determination of *L*-cysteine concentration with the proposed method under optimum conditions (Table 6).



(a)

(b)

**Figure 6.** Calibration curve for determination of *L*-cysteine. The variation of Δ P vs. concentration of *L*-cysteine (a) and logarithmic concentration of *L*- cysteine (b).

**Table 6.** The Influence of foreign species on the determination of *L*-cysteine ($10^{-7}$ M).

| Maximum of | |
|---|---|
| species | tolerated molar ratio |
| Ascorbic acid and Glycine | 300 |
| Triptophane | 800 |
| L-alanine | 200 |
| Thereionine | 400 |

## 4. CONCLUSION

As described above, kinetic methods of analysis, especially chemical reaction, are currently regarded as highly effective tools in analytical chemistry. Thus, we attempted to determine *L*-cysteine using its perturbation effect on the classic BZ reaction. Therefore, Box-Behnken design was selected as an useful method to investigate the effects of parameters that support the oscillating system. As expected, the injection of *L*-cysteine solution changed the oscillating period. These variations had a linear relationship with the logarithm of *L*-cysteine concentration in the range of $4.0 \times 10^{-7}$ M to $3.0 \times 10^{-5}$ M. The LOD and RSD were obtained as $4.0 \times 10^{-7}$ and 3.4%, respectively. The addition of *L*-cysteine to the oscillating system increased the oscillating period. Finally, the greatest advantage of this method is simplicity in technique and instrumental set-up.

## References

1. R.J. Field, M. Burger, Oscillations and Trarvelling Waves in Chemical System, Wiley, New York, 1985.
2. R.J. Field, F.W. Schnieder. *J. Chem. Edu.*, 66 (1989) 195.
3. S. Anic, L.Z. Kolar-Anic, *J. Chem. Soc. Faraday Trans*, 48 (1988) 3413.
4. A.M. Zhabotinskii, *Zh Anal. Khim.*, 27 (1972) 437.
5. L.P. Tichonava, L.N. Zakrevskaya, K.B. Yatsmirikii, *Zh. Anal. Khim.*, 33 (1978) 1991.
6. R. Jimenez-Prieto, M. Silva, D. Perez-Bendito, *Anal. Chem*., 67 (1995) 729.
7. Z. Ke, M. Wanhong, C. Raxiu, L. Zhixin, G. Nanqin, *Anl. Chim. Acta*, 413 (2000) 115.
8. P.E. Strizhak, O.Z. Didenko, T.S. Ivascenko, *Anal. Chim. Acta*, 428 (2001) 15.
9. J. Gao, J. Ren, W. Yang, X. Liu, H. Yang, Q. Li, H. Deng, *J. Electroanal. Chem.*, 520 (2002) 157.
10. M. Jiang, Y. Li, X. Zhou, Z. Zhao, J. Wang, J. Mo, *Anal. Chim. Acta*, 236 (1990) 411.
11. G. Nanqin, A. Congjiun, L. Yi, C. Ruxiu, *Analyst*, 123 (1998) 2395.
12. K.B. Yatsimirskii, P.E. Strizhak, T.S. Ivaschenko, *Talanta*, 40 (1993) 1227.
13. H.L. Chen, M.S. Li, structure and function of biomacromolecules, Shanghai science press, shanghai, China, 1999.
14. J. Kulys, A. Drungiliene, *Anal. Chim. Acta*, 243 (1991) 287.
15. A.Townshend, encyclopedia of Analytical Sience, Academic Press, London, 1995.
16. B. Filanovsky, *Anal. Chim. Acta*, 394 (1999) 91.
17. W. DrÖge, H.P. Eck, S. Mihm, *Immunol. Today*, 13 (1992) 211.
18. S. Shahrokhian, Anal. *Chem.*, 73 (2001) 5972.

19. W-H. Wang, O. Rusin, X.Y. Xu, K.K. Kim, J.O. Escobedo, S.O. Fakayode, K.A. Fletcher, M. Lowry, C.M. Schowalter, C.M. Lawrence, F.R. Fronczek, I.M. Warner, R.M. Strongin, *J. Am. Chem. Soc.*, 127 (2005) 15949.
20. P.C. White, N.S. Lawrence, J. Davis, R.G. Compton, Anal. *Chim. Acta*, 447 (2001) 1.
21. D. Rhodes, A.C. Myers, G. Jamieson, *Plant Physiol*, 68 (1981) 1197.
22. J. Dumonceaux, C. Goujon, V. Joliot, P. Briand, U. Hazan, *J. Virol.*, 75 (2001) 5425.
23. P. Schrynemackers-Pitance, S. Schoos-Berbette, *Clin. Chim. Acta*, 166 (1987) 91.
24. C.V. Krishnan, M. Garnett, B. Chu, *Int. J. Electrochem. Sci.*, 3 (2008) 873.
25. A.Samadi-Maybodi, K. Abolfazli, *Int. J. Electrochem. Sci.*, 4 (2009) 684.
26. S. Shahrokhian, R.-S. Saberi, *Int. J. Electrochem. Sci.*, 2 (2007) 158.
27. J.B. Raoof, R. Ojani, Z. Mohammadpour, *Anal. Bioanal. Electrochem.*, 2 (2010) 24.
28. G. Chwatko, E. Bold, *Talanta*, 52 (2000) 509.
29. J.B. Raoof, R. Ojani, M. Kolbadinezhad, *Electroanalysis*, 17 (2005) 2043.
30. J.B. Raoof, R. Ojani, H. Beitollahi, R. Hossienzadeh, *Anal. Sci.*, 12 (2006) 1213.
31. J.B. Raoof, R. Ojani, H. Beitollahi, *Electroanalysis,* 19 (2007) 1822.
32. R. Ojani, J.B. Raoof, E. Zarei, *J. Electroanal. Chem*., 638 (2010) 241.
33. R.J. Field, *J. Phys. Chem. Edu*., 49 (1972) 308.
34. Y. Han, Z. Li, X. Miao, F. Zhang, *Process Biochem.*, 43 (2008) 1088.
35. S. Cansee, J. Uriyapongson, C. Watyotha, *Amer. J. Appl. Sci*., (11): 5 (2008) 1535.
36. R. Kammoun, B. Naili, S. Bejar, *Bioresour. Technol*., 99 (2008) 5602.
37. M.A. Palukurty, N.K. Telgana, H.S.R. Bora, Sh.N. Mulampaka, *Afr. J. Microbiol. Res.*, 2 (2008) 087.
38. J. Zolgharnein, A. Shahmoradi, M.R. Sangi, *Talanta*, 76 (2008) 528.
39. E. Lindner, P.R. Buuck, *Anal. Chem*., 72 (2000) 336A.
40. Y.D. Zhao, W.D. Zhang, H. Chen, Q.M. Luo, *Sens. Actuat*., 92 (2003) 279.
41. C. Yanhong, C. Ruxiu, *Spectrochim. Acta*, 59 (2003) 3357.
42. K. Kargosha, SH. Ahmadi, M. Zeeb, S.R. Moeinossadat, *Talanta*, 74 (2008) 753.
43. Saad S.M. Hassan, Ashraf F.EL-Baz, Hisham S.M. Add. Rabboh. *Anal. Chim. Acta*, 602 (2007) 108.
44. D.A. Tryk, A. Fujishima, *Anal. Chem*., 73 (2001) 514.
45. J.M. Zen, A.S. Kumar, J-C. Chen, *Anal. Chem*., 73 (2001) 1169.