Pergamon

Talanta, Vol. 41, No. 11, pp. 1865–1871, 1994

0039-9140/94 $7.00 + 0.00

SEQUENTIAL OPTIMIZATION OF A FLOW INJECTION SPECTROPHOTOMETRIC METHOD FOR THE ASSAY OF CHLORPROMAZINE IN PHARMACEUTICAL PREPARATIONS

Fakhr Eldin O. Suliman and Salah M. Sultan

Chemistry Department, King Fahd University, KFUPM Box 2026, Dhahran 31261, Saudi Arabia

(*Received* 29 *January* 1994. *Revised* 25 *April* 1994. *Accepted* 26 *April* 1994)

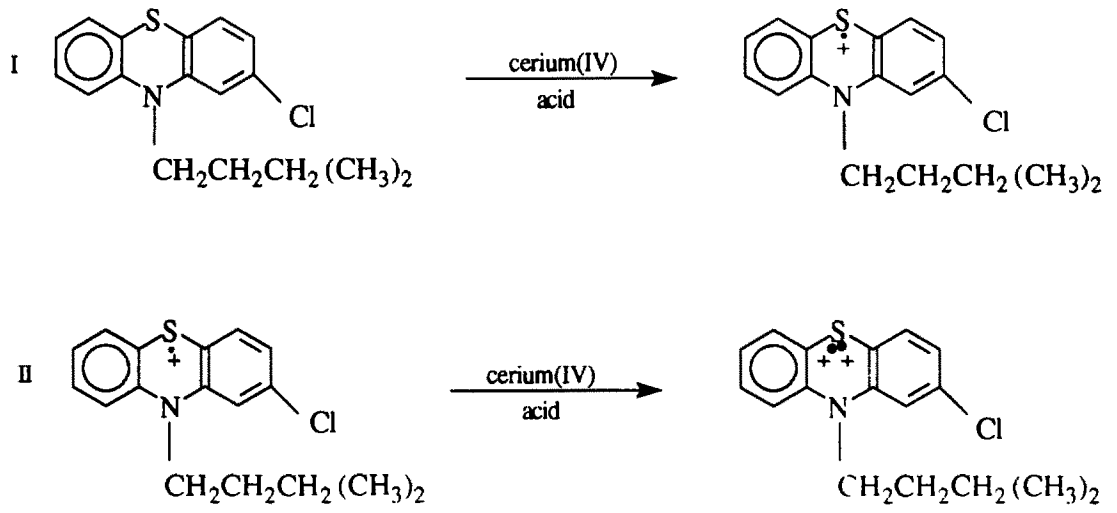
Summary—A new simple flow injection spectrophotometric method for the assay of chlorpromazine using cerium(IV) in sulfuric acid media was developed. The oxidized form of the drug was monitored at the maximum absorbance of 526 nm. The optimum conditions were 0.035*M* sulfuric acid, $3.80 \times 10^{-3}M$ cerium(IV), flow rate 4.85 ml/min, coil length 45 cm and sample size 110 mm^3. Optimization was carried out by the modified simplex method. Response surface methodology was employed to investigate the ruggedness of the method. A sampling frequency of 120 hr^{-1} was attained. Relative standard deviations for standard sample were usually less than 0.75. The method was applied to the determination of chlorpromazine in proprietary drugs and results were statistically compared with the official British Pharmacopoeia (BP) method.

Chlorpromazine hydrochloride is [3-(2-chlorophenothiazine-10-yl) propyl] dimethylamine hydrochloride, and is a member of a family of drugs commonly known as neuroleptic tranquilizers, used as sedatives, antihistamines, antiemetics and anaesthetics. A variety of methods have been reported for the determination of chloropromazine and have been recently reviewed.[1] Few FI methods have been proposed for the determination of chlorpromazine, including spectrofluorometric determination after photochemical derivitization[2,3] and a volumetric method.[4] In the British pharmacopoeia[5] (BP) monograph, chlorpromazine, in syrup or in tablet form, is treated by long extraction procedures and measurement of the resultant solution spectrophotometrically in the UV region.

The recent FI methods adopted for the assay of chlorpromazine and parent compounds[1,6,7] demonstrated the complexity of their reaction kinetics, mechanism and unstability of products. However, the availability of useful computerized programs applicable to a variety of analytical methodologies led to the development of the present FI method well established by such chemometrical applications for the adoption of confident experimental conditions reliable for precise determination of this compound.

In this paper Ce(IV) was used as an oxidant for a FIA spectrophotometric method for the determination of chlorpromazine in sulfuric acid media. The reaction, as suggested earlier using a different inorganic oxidant,[1] proceeds via formation of the monocation radical in one step and to the dication radical in the second step thus leading to unstable products and could be similarly suggested with cerium(IV) as shown in Scheme 1. The modified simplex method was used to select the optimum operating conditions, which were further investigated by response surface methodology (RSM) by using a 3^2 factorial design in order to check for the ruggedness of the method.

The response surface methodology (RSM) is a collection of mathematical and statistical techniques useful for analyzing relationships between several experimental variables and one or more responses.[8,9] These techniques involve the design of experiments, fitting of empirical or theoretical models to the experimental data, and interpretation of the fitted response. Application of a properly designed experiment and adequate multifactor models may lead to better understanding of the response surface and safer prediction of the optimum operating conditions.



Scheme 1.

EXPERIMENTAL

Apparatus

The flow injection apparatus used was previously described.[1] It consisted of a four-channel peristaltic pump, a Rheodyne model 5041 injector, a PTFE reactor module, a spectronic mini 20 spectrophotometer (Milton Roy, U.S.A.) equipped with a 20-μl ultra-micro flow through cell (Unovic, NY, U.S.A.) of path length 1.0 mm and connected to a single channel detector strip-chart recorder model 0555 (Cole Parmer, U.S.A.).

A Lambda 5 UV-visible spectrometer (Perkin–Elmer) together with 10.00 mm cells was used for analysis by the spectrophotometric original method for statistical comparative study.

Computer programs

The simplex program (chemometrical optimization by simplex, *i.e.* COPS) was supplied by Elsevier Scientific Co. (Amsterdam, The Netherlands).

Statgraphics statistical graphics system by Statistical Graphics Corporation (U.S.A.) was used.

Reagents

Standard drug solutions: a stock solution of 1000 ppm was prepared by dissolving the pure analytical grade (May and Baker, supplied by Rhône–Poulenc, batch No. E99) in water. Working solutions were prepared by proper dilutions.

Tablets: solutions with a concentration of 500 ppm were prepared by dissolving an amount of crushed and powdered tablets equivalent to the required amount of chlorpromazine in water, heating in water bath for 20 min, filtering, washing and the filtrate made up to volume with water after cooling to room temperature. Working solutions were prepared by appropriate dilutions.

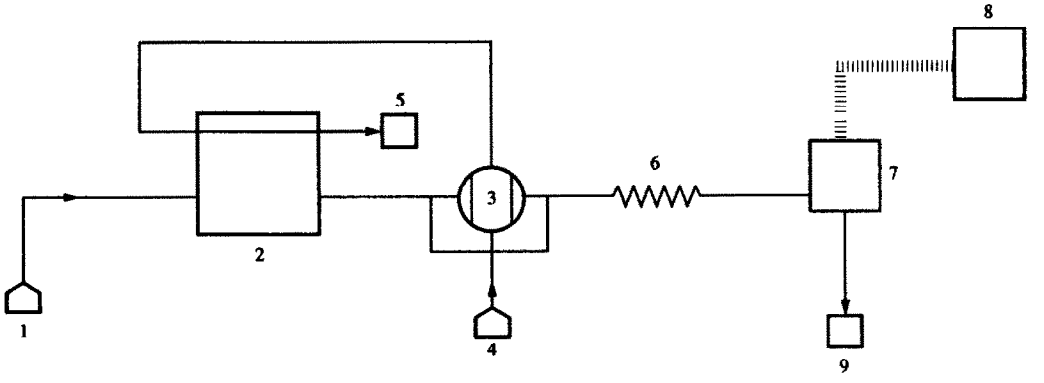


Fig. 1. Single-line manifold comprised: 1, $3.80 \times 10^{-3}M$ cerium(IV) in $0.035M$ H_2SO_4 solution carrier; 2, peristaltic pump; 3, injector of 110 μl loop size; 4, drug samples injected; 5, drug sample waste; 6, 45 cm coil length; 7, Spectronic Mini 20 spectrophotometer; 8, XY recorder and 9, products waste.

Table 1. Experimental ranges of variables used in optimization and optimum conditions obtained

Variable	Range	Optimum
Flow rate	0–6.0 *ml/min*	4.85 *ml/min*
Coil length	45–200 *cm*	45 *cm*
Sample volume	50–250 *μl*	110 *μl*
$[H_2SO_4]$	0.01–0.5*M*	0.035*M*
[Ce(IV)]	1.0×10^{-4}–$1.0 \times 10^{-2}M$	$3.8 \times 10^{-3}M$

Cerium(IV) solution: 0.01*M* solution of cerium(IV) was prepared by dissolving 6.3200 g of $(NH_4)_4[Ce(SO_4)_4] \cdot 2H_2O$ in 1 l. of 0.05*M* sulfuric acid solution.

Manifold and procedure

The configuration used in this work was a single line FIA shown in Fig. 1. The carrier pump tubing was a PVC type of 1.3 mm I.D. A 110 μl sample volume size was used to inject a sample containing a definite amount calculated as ppm into the carrier stream of cerium(IV) solution dissolved in sulfuric acid through the rotary valve. The carrier stream passed through a reaction coil, 45 cm long and 0.5 mm I.D., where the chlorpromazine was oxidized and a color developed and measured in the flow cell of the spectrophotometer at λ_{max} 525 nm.

Experimental design

Simplex optimization. The simplex method[10–12] is a powerful technique of optimization; it investigates different combinations of factor levels by moving from regions of poor response toward regions of improved response. Since the simplex is a geometric figure whose vertices are $n + 1$, where n is the number of variables a simplex in two dimensions is a triangle. In this study two factors were selected for optimization by simplex; these are the sulfuric acid concentration and cerium(IV) concentration.

Factorial design. A 3^2 factorial design with replication[8,9] was used to study the behavior of the response and to verify the optimum obtained by the simplex. The factorial study involved nine treatment combinations (18 experiments) and was located around the optimum region obtained by the simplex. The results from those experiments can be used to fit a full second-order polynomial model by means of multiple regression analysis.[8,13]

RESULTS AND DISCUSSION

Optimization

The influence of the most critical variables on the magnitude of the peak absorbance and reproducibility of the results was studied carefully. The variables were divided into two groups, chemical variables [sulfuric acid concentration and cerium(IV) concentration] and system variables (flow rate, coil length and sample loop size). From preliminary investigations and previous experience,[6,7,14] the system variables were fixed to the optimum values shown in Table 1. The simplex method was then used to optimize the chemical variables separately. The first point conditions and response were fed into

Table 2. Simplex optimization of chemical variables

Experiment No.	$[H_2SO_4](M)$	$Ce(IV) \times 10^{-4}(M)$	Peak absorbance
1	0.050	2.000	0.209
2	0.168	8.40	0.449
3	0.082	25.9	0.668
4	0.200	32.2	0.697
5	0.184	29.2	0.675
6	0.113	49.8	0.684
7	0.127	39.6	0.696
8	0.245	46.0	0.638
9	0.140	32.9	0.702
10	0.212	25.7	0.657
11	0.152	35.6	0.708
12	0.090	36.2	0.717
13	0.035	38.0	0.744
14	0.048	40.8	0.726
15	0.064	39.4	0.736

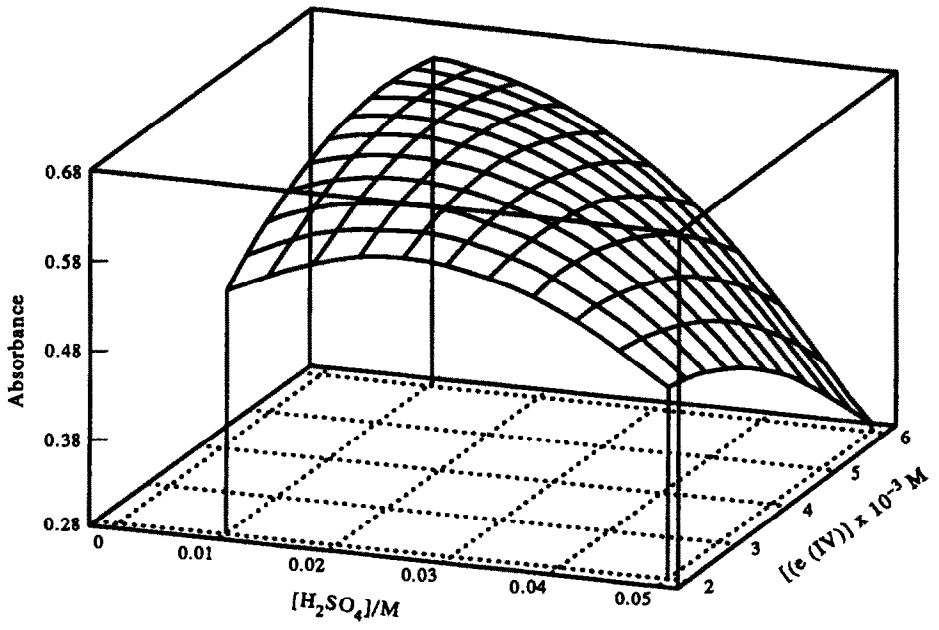


Fig. 2. Response surface plot of peak absorbance *vs.* cerium(IV) and sulfuric acid concentrations.

the computer and the program automatically calculated vertices of the starting simplex as in Table 2. The simplex rapidly located the optimum region, and the optimization was halted after 15 experiments, since no further substantial improvement was expected. The optimum chemical conditions for the assay of chlorpromazine were sulfuric acid concentration $0.035M$ and Ce(IV) concentration $3.8 \times 10^{-3}M$. The results obtained by the simplex manifested a successful application to the system with a low number of steps required to attain maxima. From step 3 onwards, all points fall within the level of optimum conditions and for this reason, the phenomenon exhibited in Fig. 2 for the decrease of absorbance at higher Ce(IV) concentrations was not identified, simply due to the limited range of Ce(IV) concentration which is below the critical concentration that causes the decrease in absorbance. In this region the cerium(IV) effect is eliminated and it is only the sulfuric acid that plays a major role in the unstability of the product, and this is considered to be another development achieved.

Response surface modeling

A three level, two factor, full factorial design (with replications) study was carried out in the region of the simplex optimum. The analysis of variance (ANOVA) results are presented in Table 3 indicating that both factors, sulfuric acid concentration and Ce(IV) concentration, show significant interaction. The peak absorbance was then fitted to a full second order polynomial model of the form:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{12} X_1 X_2, \quad (1)$$

where Y represents the estimated response, β_0 is an intercept parameter, β_1 and β_2 are linear parameters; β_{11} and β_{22} are quadratic parameters, β_{12} is an interaction parameter, X_1 and X_2 represent sulfuric acid and cerium (IV) concentrations, respectively. Table 4 gives the

Table 3. Analysis of variance (ANOVA)

Source of variation	Sum of squares	Degrees of freedom	Mean square	*F*-ratio*
$[H_2SO_4]$	0.155125	2	0.077563	443.8
[Ce(IV)]	0.016514	2	0.008257	47.2
Interaction	0.094551	4	0.023647	135.2
Residual	0.001573	9	1.74778×10^{-4}	
Total	0.267762	17		

*Level of significance = 99.9%.

Table 4. Regression analysis

Variable	Parameter*	Estimate	Level of significance (%)†
Intercept	β_0	0.26299	99.9
Sulfuric acid	β_1	14.8333	98.0
	β_{11}	−207.71	97.5
Cerium(IV)	β_2	120.437	90.0
	β_{22}	−9833.33	85.0
Interaction	β_{12}	−1881.25	99.5

*See Equation (1).
†Level at which null hypothesis can be rejected.

parameter estimates obtained by matrix least squares.[8,13] The level of significance shown in Table 4 is the level at which the null hypothesis for each parameter (H_0, $\beta = 0$) can be rejected; Fig. 2 is a pseudo-three dimensional plot of the response surface for the fitted model in the region of the optimum. It shows a diagonal ridge that decreases as both sulfuric acid and Ce(IV) concentrations were increased. Decreasing both reagents also resulted in a decrease in the peak absorbance. Both variables, sulfuric acid and Ce(IV), showed a broad optimum at the intermediate level. Intermediate levels of 0.030M sulfuric acid and $4.0 \times 10^{-3}M$ Ce(IV) depicted from the simplex optimum conditions clearly exhibited broad leveling in the pseudo-three dimensional plot, which correlates very well with the results obtained by the simplex, thus confirming rigidity and tolerance of the small changes of variables of the simplex which is an indication of the successful location of optimum.

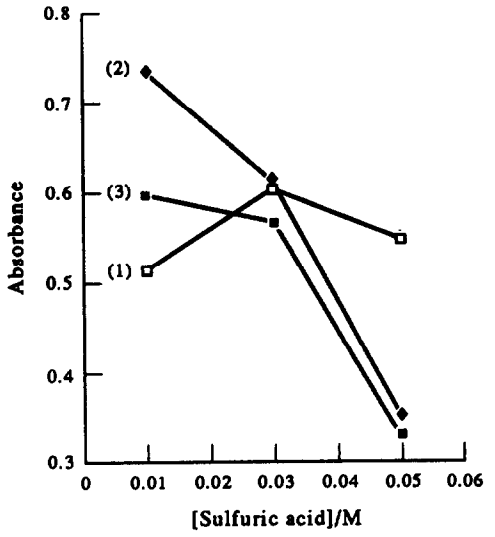


Fig. 3. Cell mean plot showing the effect of sulfuric acid concentration at different levels of cerium(IV) concentrations of 0.002M, 0.004M, 0.006M.

Cell mean plots

The cell mean plot of a factor is a graph of the measured response of each level of that factor averaged over all levels of the other factors. The relative importance of each factor can be obtained from this plot without fitting a model to the data. The interaction between two factors can be inferred from the cell mean plots of one factor at the different levels of the second factor.[15,16] The main effect cell mean plots are greatly dependent on the significance of interaction between factors. If the interaction between factors is insignificant, then increasing the level of a factor will result in the same behavior at all levels of other factors.

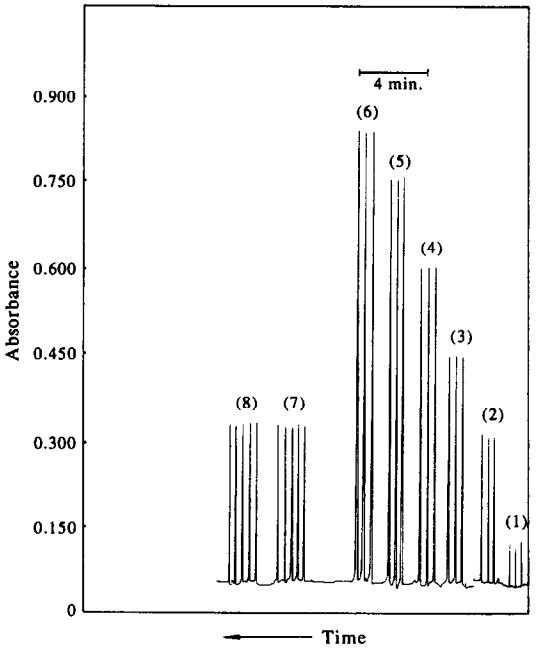


Fig. 4. Recorder tracing for FI measurements for series of standard chlorpromazine solutions of 1, 50; 2, 95; 3, 120; 4, 150; 5, 180; 6, 200; 7, 100 (Largactil tablet 100 mg); and 100 ppm chlorpromazine (Largactil tablet 25 mg).

Table 5. Results obtained by the FIA method and the BP[5] method for the analysis of chlorpromazine in proprietary drugs

Drug	Supplier	Contents (*mg*)	Mean recovery + SD(%)* FIA method	BP method	*t*†
Largactil (tablet)	Specia (France)	Chlorpromazine 100	100.1 ± 0.80	101.2 ± 0.70	1.6
Largactil (tablet)	Specia (France)	Chlorpromazine 25	99.6 ± 0.86	100.5 ± 0.50	1.8

*$n = 5$.

†Theoretical value = 2.31, $n = 5$.

Figure 3 shows the effect of sulfuric acid concentration at different Ce(IV) concentration on the peak absorbance. At low level of Ce(IV), increasing the sulfuric acid concentration caused the peak absorbance to pass through an optimum at the middle level of the factor. At intermediate and high levels of Ce(IV), the peak absorbance decreased with increasing sulfuric acid concentration.

From a theoretical point of view the above observations can be interpreted as follows. The redox potential of Ce(IV) increases with increasing the acid concentration resulting initially in an increase in the amount of the oxidation product and hence in the peak absorbance. The dication radical is believed to be the species responsible for the signal at the chosen wavelength rather than the monocation radical as in the case when using stronger oxidants such as the molybdate and dichromate.[1,6,7] However, the rate of degradation of this compound increases with increasing acid and oxidant concentrations resulting in a decrease in peak absorbance. This is clearly manifested in Fig. 3, with a sharp decrease of peak absorbance at high sulfuric acid and Ce(IV) concentration. At this level, and with respect to the kinetics of the first step reaction, an increase in cerium(IV) and sulfuric acid concentrations would result in an appreciable increase in the production of the monocation radical intermediate which in turn acts as a reactant for the second step in the presence of excess Ce(IV) and sulfuric acid, thus rendering the monocation radical a highly unstable intermediate leading to a sharp decrease in response. This clearly demonstrates that the choice of Ce(IV) as a milder oxidant than molybdate and dichromate used for the assay of this drug in the previous methods[1,6,7] is a great advantage and a significant development reflected in higher precision and more acceptable reproducibility. The RSD calculated was found to be less than 0.7% in the present method and > 1.8% in the molybdate and dichromate methods. A RSD of 5% was earlier reported.[12,16] Therefore, one should carefully adjust the concentration of both reagents to maximize the amount of the oxidation product and to minimize degradation of this product.

Analytical appraisal

Series of standard solutions containing chlorpromazine in the range 50–200 ppm were injected in triplicate in a typical run as shown in Fig. 4. A peak height with an average relative standard deviation of 0.70% for five repeated injections indicates high reproducibility. The plot of absorbance (A) *vs.* chlorpromazine concentration (C) was linear over the concentration range 50–200 ppm. A weighted regression line was plotted, which resulted in the following calibration equation:

$$A = -0.01725 + 4.8327 \times 10^{-3}C$$

with a correlation coefficient (r) of 0.999, where A is absorbance and C is concentration in ppm.

A sample frequency of 120 hr^{-1} could be obtained, and this was determined by measuring the peak width at the baseline, which was found to be 30.0 and 3.25 sec at 60% of the peak height indicating minimal dispersion.

The method was applied to the determination of chlorpromazine in proprietary drugs. Largactil tablets with typical runs are represented by peaks (7) and (8) in Fig. 4. The same batches were analyzed by the BP[5] method and the results were statistically compared as shown in Table 5. The present method showed almost the same degree as accuracy of the BP method, and the results obtained indicated no interference from excipients added in dosage forms.

CONCLUSION

The sequential optimization of variables validated the flow injection method with better confidence and reliability of the results obtained, indicated by higher precision, excellent reproducibility and precision with higher sensitivity and wider range of drug concentration when compared to previously reported methods. The

applicability of the method to chlorpromazine in tablet formulations is another advantage.

Acknowledgement—The first author acknowledges KFOPM for the Ph.D. Scholarship.

REFERENCES

1. S. M. Sultan, *Analyst (London)*, 1991, **116**, 177.
2. D. Chen, A. Rios, M. D. Lugue de Castro and M. Valcarcel, *Talanta*, 1991, **38**, 1227.
3. J. Martinez Calatayud and C. Gomez Benito, *Anal. Chim. Acta*, 1992, **256**, 105.
4. J. Wang and B. A. Fereiha, *Anal. Chem.*, 1983, **55**, 1285.
5. British Pharmacopeia, 5th Edn. H.M. Stationery Office, London, 1988.
6. S. M. Sultan, *Talanta*, 1993, **401**, 681.
7. S. M. Sultan, *Microchem. J.*, 1991, **44**, 304.
8. D. C. Montgomery, *Design and Analysis of Experiments*, 2nd Edn. Wiley, New York, 1984.
9. R. H. Mvers, *Response Surface Methodology*. Allyn and Bacon, Boston, 1971.
10. J. A. Nelder and R. Mead, *Comput. J.*, 1965, **7**, 308.
11. W. Spendley, G. R. Hext and F. R. Himsworth, *Technometrics*, 1962, 441.
12. S. L. Morgan and S. N. Deming, *Anal. Chem.*, 1974, **46**, 1170.
13. N. R. Draper and H. Smith, *Applied Regression Analysis*. Wiley, New York, 1966.
14. S. M. Sultan and F. O. Suliman, *Analyst*, 1993, **118**, 573.
15. L. R. Parker Jr, S. L. Morgan and S. N. Deming, *Appl. Spectrosc.*, 1975, **29**, 429.
16. A. S. Olansky and S. N. Deming, *Anal. Chim. Acta*, 1976, **83**, 241.