Microchim. Acta 143, 39–44 (2003)
DOI 10.1007/s00604-003-0026-0

Microchimica Acta
Printed in Austria

Original Paper

Spectrofluorimetric Determination of Piroxicam in Pharmaceutical Preparations and Spiked Human Serum Using Micellar Media

Jamshid L. Manzoori* and **Mohammad Amjadi**

Department of Analytical Chemistry, Faculty of Chemistry, Tabriz University, Tabriz, Iran

Received November 24, 2002; accepted April 13, 2003; published online August 8, 2003

Abstract. The fluorescence properties of piroxicam in various micellar media were investigated. It was found that the presence of 0.05 M sodium dodecyl sulfate (SDS) surfactant (pH 1.5–2, nitric acid) causes an approximately 5-fold enhancement in the fluorescence of this drug. An experimental design approach based on central composite design was used to investigate the influence of the main variables (pH, SDS concentration and temperature) on the fluorescence signal. Based on the obtained results, a micelle-enhanced fluorescence method was developed for the determination of piroxicam in pharmaceuticals and also in spiked human serum (after extraction with diethyl ether). The linear calibration ranges of the methods were 0.05–1.5 and 0.2–10 $\mu g\,mL^{-1}$ for aqueous solution and serum samples, respectively. The detection limits were 0.015 and 0.10 $\mu g\,mL^{-1}$ in aqueous and serum samples, respectively.

Key words: Piroxicam; spectrofluorimetry; micelles; central composite design.

Piroxicam, 4-hydroxy-2-methyl-N-2-pyridinyl-2H-1,2-benzothiazine-3-carboxamide 1,1-dioxide, is a non-steridal anti-inflammatory agent which is widely used for treating rheumatoid arthritis, osteoarthritis and other inflammatory diseases [1]. Several analytical methods have been developed for the determination of piroxicam in pharmaceutical preparations and biological fluids, including UV and visible spectrophotometry [2, 3], derivative spectrophotometry [4, 5], high performance liquid chromatography [6–10], capillary electrophoresis [11] and voltammetry [12]. Recently, direct spectrofluorimetry was used by Damiani et al. [13] for the determination of this drug in a pharmaceutical preparation. In this case, piroxicam was dissolved in alkaline medium and the solutions were strongly acidified before fluorescence measurement. Furthermore, Escandar [14] determined piroxicam in capsules by spectrofluorimetry after its complexation with β-cyclodextrin.

Micelles are dynamic aggregates of amphiphilic molecules (surfactants) formed at surfactant concentrations above the critical micelle concentration (cmc). These assemblies have been extensively used in various areas of analytical chemistry to increase selectivity and sensitivity [15].

It is well known that the use of micellar microheteregeneous systems in fluorescence spectrometry presents several advantages over conventional homogenous solutions including increased sensitivity, reduced interference and enhanced experimental convenience [16]. Several reports on the application of micelles to improve the performance of spectrofluorimetric methods have been published [17–20].

This paper investigates the fluorescence properties of piroxicam in various micellar media. It is shown

* Author for correspondence. E-mail: manzoori@tabrizu.ac.ir

that the fluorescence intensity of piroxicam markedly increases in SDS micellar solution. Therefore a simple and sensitive analytical method is proposed for the determination of piroxicam in pharmaceutical formulations and spiked human serum. An experimental design approach based on central composite design has been used to investigate the influence of the main variables on the fluorescence intensity of piroxicam. The chemometric methodology allows simultaneous variation of all experimental factors and distinguishing any interacting among them, which is not detectable with the classical experimental method. This approach also reduces the number of experiments required [21].

Experimental

Apparatus

Fluorescence spectra and intensity measurements were made on a Shimadzu RF-540 spectrofluorimeter (Kyoto, Japan) equipped with a 150W xenon lamp, using a 1.0 cm quartz cell. The slit widths of both monochromators were set at 5 nm. All measurements were performed at $20 \pm 0.1\,^{\circ}C$ by means of a thermostated cell holder and a thermostatically controlled water bath (Tokyo, Rikakika LTD, UA-1). Absorbance measurements were carried out using a Shimadzu UV-265FW spectrophotometer (Kyoto, Japan). A Metrohm model 654 pH meter (Herisau, Switzerland) was used for pH measurements.

Reagents

All reagents used were of analytical reagent grade. Triply distilled water was used throughout.

Piroxicam (Esteve, Spain) was kindly provided by Razak Laboratory (Tehran, Iran). A $200\,\mu g\,mL^{-1}$ stock standard solution of this drug was prepared by dissolving 20.0 mg of piroxicam in methanol and diluting to the mark in a 100 mL volumetric flask. Sodium dodecyl sulfate (SDS), Polyoxyethelene lauryl ether (Brij-35) and N-cetyl-N,N,N-trimethylamonium bromide (CTAB), diethyl ether, hydrochloric acid and nitric acid were purchased from Merck (Darmstadt, Germany). A 0.05 M stock solution of SDS was prepared in triply distilled water.

General Procedure

Aliquots of working standard solution containing $50\,\mu g\,mL^{-1}$ piroxicam in methanol were transferred into 10 mL volumetric flasks to produce solutions with concentrations in the range of 0.05–$1.5\,\mu g\,mL^{-1}$. Then 3.0 mL of 0.05 M SDS solution and 0.2 mL of 1 M HNO_3 solution were added. The solutions were thermostated at $20 \pm 0.1\,^{\circ}C$, and fluorescence was measured at 460 nm using an excitation wavelength of 340 nm.

Procedure for Capsules

Ten capsules of piroxicam were weighed in order to find the average mass of each capsule. Then the contents were powdered and mixed. A portion of 10.0 mg of this powder was accurately weighed and dissolved in about 10 mL methanol and filtered into a 25 mL volumetric flask. The residue was washed several times with methanol, and the solution was diluted to the mark with the same solvent. A suitable aliquot of this solution was taken for the determination of piroxicam according to the general procedure and using a calibration graph.

Procedure for Gels

About 0.5 g of gel was accurately weighed and dissolved in 10 mL methanol while stirring. The resulting solution was centrifuged at 3500 rpm for 10 min. The clear supernatant solution was transferred to a 25 mL volumetric flask and diluted to the mark with methanol. A suitable aliquot of this solution was taken for the determination of piroxicam according to the general procedure and using a calibration graph.

Procedure for Serum

Serum standard solutions for calibration graph were prepared by adding appropriate volumes from the working standard solution of piroxicam to a series of 15 mL screw cap tubes with Teflon linings. After the solvents were evaporated, 1.0 mL of drug-free serum was added to each tube. Serum solutions had piroxicam concentrations in the range of $0.2–10\,\mu g\,mL^{-1}$. A procedure similar to that of Klopas et al. [5] was used for extraction of piroxicam from serum samples. The samples were acidified with 0.3 mL of 1 M HCl, and 5 mL of pure diethyl ether were added. The solutions were shaken for two minutes, and the organic layers were separated by centrifugation at 3500 rpm for 10 min. The separated organic layers were evaporated to dryness in a water bath of $50\,^{\circ}C$. The residues were reconstructed in 1.5 mL 0.05 M SDS and 0.1 mL 1 M HNO_3. The fluorescence of the resulting solutions was measured at 460 nm using an excitation wavelength of 340 nm.

Experimental Design

Solutions of piroxicam ($1\,\mu g\,mL^{-1}$) were prepared in the desired conditions, and their relative fluorescence intensity was measured at predetermined conditions for each experiment. The pH of the solutions was adjusted by HNO_3 solutions of various concentrations. The data analysis was performed using the statistical package Statgraphics Plus 5.1.

Results and Discussion

Fluorescence Properties of Piroxicam in Micellar Media

In order to obtain a better analytical performance for the determination of piroxicam, the fluorescence properties of piroxicam in various micellar media were studied using anionic (SDS), cationic (CTAB) and non-ionic (Brij-35 and Triton X-100) surfactants. It was observed that the fluorescence spectra are not significantly modified by the effect of cationic or non-ionic surfactants. On the other hand, it has been verified that the addition of SDS causes a net increase in piroxicam fluorescence of about 500% (Fig. 1).

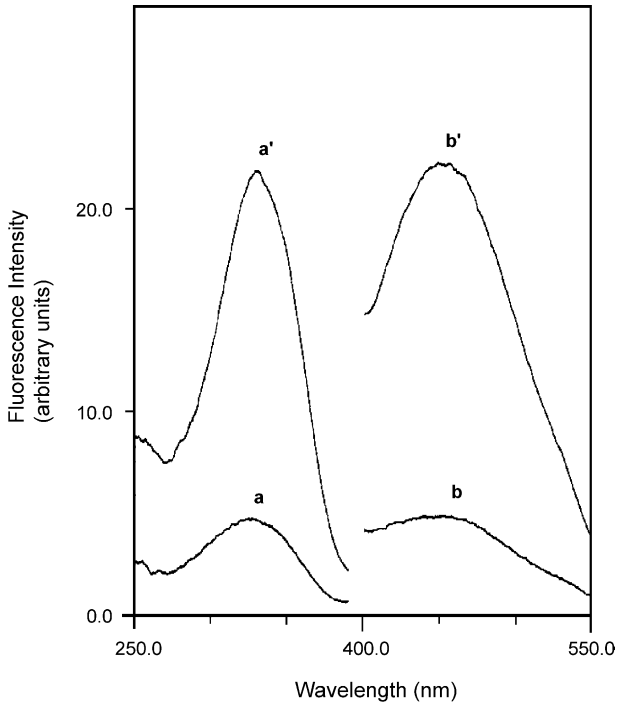

Fig. 1. Fluorescence excitation (a, a') and emission (b, b') spectra of piroxicam (1 μg mL^{-1}) in the absence (a, b) and presence (a', b') of 0.015 M SDS. pH = 1.5, $\lambda_{ex} = 340$ nm, $\lambda_{em} = 460$ nm

No significant shift in excitation or emission spectra was observed.

In the presence of 0.05 SDS, the intensity of the absorption band of piroxicam at 338 nm increases with respect to the surfactant-free solution, and a blue shift appears in the spectrum. The molar absorptivity of piroxicam in the presence of SDS was determined at 338 nm. The $\varepsilon_{micellar}/\varepsilon_{water}$ ratio is approximately 1.3, which indicates that the increase in sensitivity is mainly due to an increase in the fluorescence quantum yield of piroxicam by protection of the lowest excited singlet state in the micellar microenvironment.

Since the piroxicam stock solution was prepared in methanol, the effect of this solvent on the fluorescence intensity of piroxicam in micellar media was investigated. The results show that a methanol concentration of up to 5% (v/v) has no significant effect on fluorescence, however, larger amounts lead to a decrease in fluorescence intensity. Since the methanol content of calibration solutions of piroxicam does not exceed 3%, there is no need for adjusting its concentration.

Optimization of Parameters

A central composite design was implemented in order to determine the optimum conditions for the micelle-enhanced spectrofluorimetric method. The variables considered in the optimization process were pH, concentration of SDS, and temperature. This model allows direct evaluation of the variables and also the first and second order interaction terms.

The central composite design consisting of $2^k + 2k + n$ runs, where k is the number of studied factors (3 in this paper), 2^k are the points from the factorial experiments carried out at the corners of the cube, and 2k are the points carried out on the axes at a distance of $\pm\alpha$ from the center. The distance α is calculated so as to obtain rotatability, and a three variable central composite design is rotatable if $\alpha = 1.68$ [21]. The number of experiments carried out at the center of experimental domain, n, was fixed at 3. Table 1 gives the design matrix for these experiments and the relative fluorescence signal obtained in each run.

The estimated effects and interactions as well as the evaluation of their statistical significance by ANOVA are presented in Table 2. The corresponding Pareto chart is shown in Fig. 2. As can be seen, six effects have a P-value of less than 0.05, indicating that they are significantly different from zero at 95% confidence level. The concentration of SDS is affected most of all by a positive sign. pH and temperature are also statistically significant. They are affected by

Table 1. Design matrix and relative fluorescence intensity values in central composite design for three variables: pH, SDS concentration (M) and temperature (°C)

Runs no.	pH	[SDS]	T
1	−1	−1	−1
2	1	−1	−1
3	−1	1	−1
4	1	1	−1
5	−1	−1	1
6	1	−1	1
7	−1	1	1
8	1	1	1
9	−1.68	0	0
10	1.68	0	0
11	0	−1.68	0
12	0	1.68	0
13	0	0	−1.68
14	0	0	1.68
15	0	0	0
16	0	0	0
17	0	0	0
Coded value −1.68	1.3	0.0016	13
Coded value −1	2	0.005	20
Coded value 0	3	0.01	30
Coded value 1	4	0.015	40
Coded value 1.68	4.7	0.0185	47

Table 2. Estimated effects and ANOVA results for central composite design

Term	Estimated effect	Sum of squares	d.f.	F-ratio	P-value
A: pH	− 6.595	148.485	1	99.94	0.0000
B: [SDS]	7.645	199.554	1	134.31	0.0000
C: T	− 6.004	123.087	1	82.84	0.0000
AA	− 2.044	11.7761	1	7.93	0.0259
AB	− 0.525	0.55125	1	0.37	0.5617
AC	2.175	9.46125	1	6.37	0.0396
BB	− 3.599	36.5207	1	24.58	0.0016
BC	− 1.075	2.31125	1	1.56	0.2524
CC	− 0.241	0.163669	1	0.11	0.7497
Total error		10.4006	7		

R-squared = 98.06, R-squared (adjusted for d.f.) = 95.57.

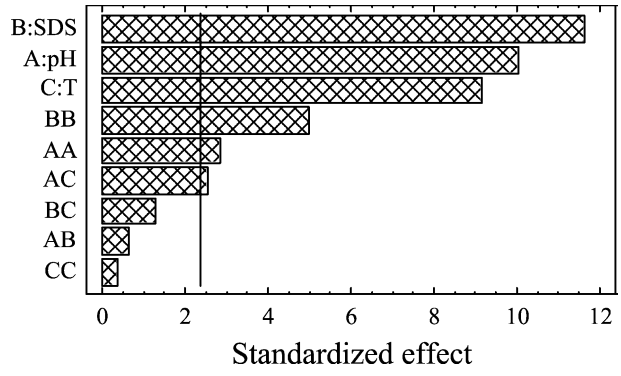

Fig. 2. Pareto chart for the standardized effects in the central composite design including two-factor interactions

a negative sign. Also, the interaction between pH and temperature is significant and positive. The R-squared statistic indicates that the model as fitted explains 98.06% of variability in response (RFI).

Three-dimensional response surface plots keeping one of the variables at the central point value are presented in Fig. 3. As can be seen, fluorescence intensity reaches a maximum when the pH and SDS concentration are near the highest values. In addition, the fluorescence signal is inversely proportional to temperature, so that better experimental responses are obtained at lower temperatures.

In summary, after evaluation of the main factors and their interactions, the following conditions were chosen for micelle-enhanced spectrofluorimetric determination of piroxicam: pH 1.5–2, an SDS concentration of 0.015 M and a temperature of 20 °C.

Analytical Figures of Merit

For micelle-enhanced spectrofluorimetric determination of piroxicam in aqueous solution, a series of 9 standard solutions (three replicate for each) of piroxicam were measured by following the procedures described under *Experimental*. The calibration graph was found to be linear in the range of 0.05–1.5 $\mu g\,mL^{-1}$. The equation for calibration graph is $F = 3.29\ (\pm 0.18) + 18.03\ (\pm 0.25)C$ $(r = 0.9993)$, where F is the fluorescence intensity (in arbitrary units) and C is the concentration of piroxicam expressed in $\mu g\,mL^{-1}$. The detection and quantification limits as defined by IUPAC [22], $C_{LOD} = 3\,S_b/m$ and $C_{LOQ} = 10\,S_b/m$ (where S_b is the standard deviation of the blank and m is the slope of the calibration graph) were found to be 0.015 and 0.05 $\mu g\,mL^{-1}$, respectively. The slope of the calibration graph (m) is the calibration sensitivity according to IUPAC definition. Table 3 compares the limit of detection and the analytical range of the proposed method with other comparable methods for the determination of piroxicam.

In order to study the precision of the method, two series of 9 solutions containing 0.2 and 1.2 $\mu g\,mL^{-1}$ piroxicam were measured on the same day. Applying the IUPAC definition, the relative errors are 0.6% and 0.7%, respectively. The relative standard deviations for 9 replicate analyses were 1.6% and 1.3%, respectively.

Determination of Piroxicam in Pharmaceutical Preparations

Piroxicam was satisfactorily determined in two pharmaceutical products (Piroxicam capsules with a nominal content of 10 mg and piroxicam gels with a nominal content of 0.5%) by using the micelle-enhanced spectrofluorimetric method. Table 4 shows the results for three replicate analyses. Statistical analysis of the assay results produced satisfactory precision of both the proposed methods with no significant differences between certified and experimental results.

Determination of Piroxicam in Human Serum Samples

For the determination of piroxicam in serum samples, a series of 10 standard solutions (three replicate for each) of piroxicam were measured. The calibration graph was found to be linear in the range of 0.2–10 $\mu g\,mL^{-1}$, and its corresponding equation is $F = 7.51\ (\pm 0.18) + 2.84\ (\pm 0.039)C$ $(r = 0.9991)$. The detection and quantification limits were 0.10 and 0.30 $\mu g\,mL^{-1}$, respectively.

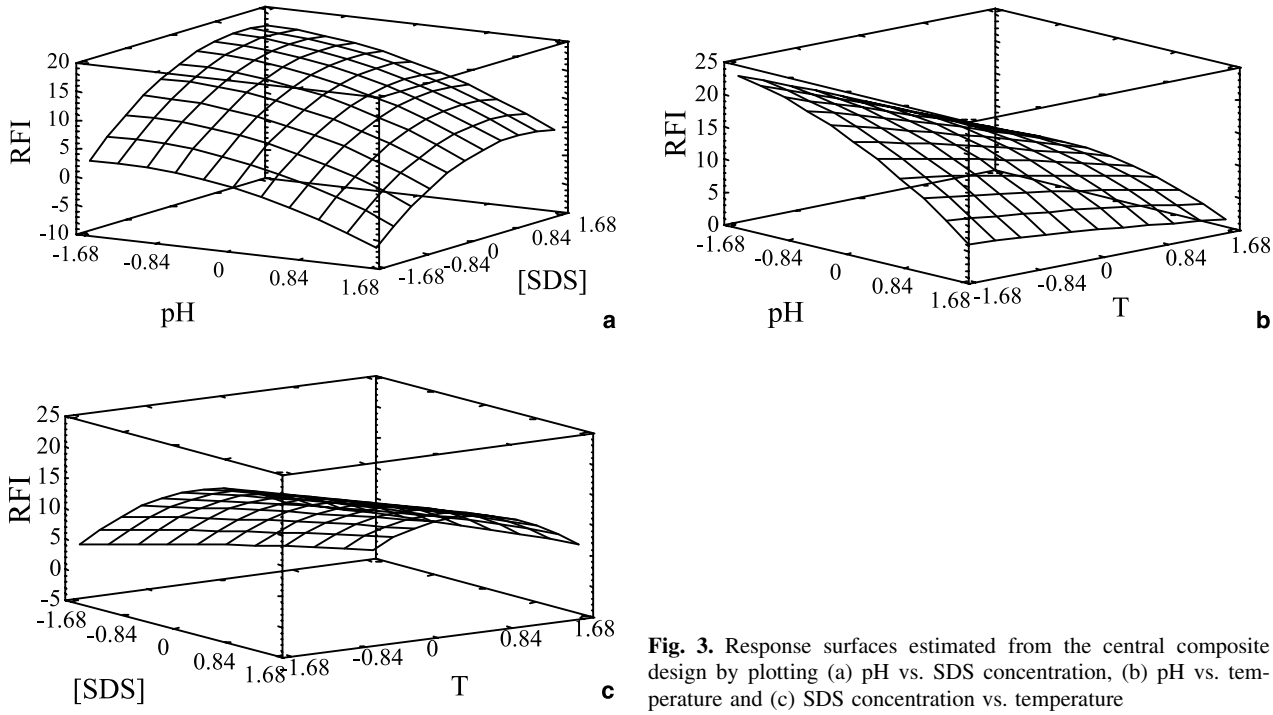

Fig. 3. Response surfaces estimated from the central composite design by plotting (a) pH vs. SDS concentration, (b) pH vs. temperature and (c) SDS concentration vs. temperature

Table 3. Figures of merits of comparable methods for determination of piroxicam

Method [ref.]	Limit of detection/μg mL^{-1}	Analytical range/μg mL^{-1}	Comments
This method	0.015	0.05–1.5	
Spectrofluorimetry [14]	0.5	0.5–1.6	in 1% v/v dioxane-water
Spectrophotometry [3]	–	0.2–6.5	

Table 4. Determination of piroxicam in pharmaceutical preparations

Product	Nominal content	Found ± SD[a]	Recovery (%)[b]
Piroxicam capsule	10 (mg per capsule)	9.9 ± 0.2	99 ± 2
Piroxicam gel	0.50 (g per 100 g)	0.52 ± 0.02	104 ± 3

[a] Standard deviation (average of three determinations).
[b] Recovery is calculated from the content reported by the laboratory.

Recovery experiments on human serum samples spiked with different amounts of piroxicam were carried out. The results are given in Table 5, indicating that the proposed method has sufficient precision and accuracy for the determination of piroxicam in human serum.

Table 5. Recovery of piroxicam added to human serum

Amount added/ μg mL^{-1}	Amount found/ μg mL^{-1}	Recovery (%)
0.50	0.48 ± 0.02	97 ± 4
2.0	2.02 ± 0.06	101 ± 3
6.0	5.8 ± 0.1	97 ± 2
10.0	9.9 ± 0.3	99 ± 3

Conclusions

A micelle-enhanced spectrofluorimetric method for the determination of piroxicam was developed. It was found that the presence of SDS micelles enhances the fluorescence of this drug by a factor of about 5, which leads to a corresponding improvement in the sensitivity of the fluorimetric method. The proposed method is more sensitive and much simpler than other fluorimetric methods [13, 14]. The method

was satisfactorily applied to the determination of piroxicam in spiked human serum and also in pharmaceutical formulations.

References

[1] Paulus H. E.; Furst D. E.; Dromgoole S. H. (1987) Drugs for rheumatic disease. Churchill Livingstone, New york, pp. 389–398

[2] Amin A. S. *J. Pharm. Biomed. Anal.* **2002**, *29*, 72

[3] Nagaralli B. S.; Seetharamappa J.; Melwanki M. B. *J. Pharm. Biomed. Anal.* **2002**, *29*, 859

[4] Ródenas V.; Soledad García M.; Sánchez-Pedreño C.; Isabel Albero M. *Analyst* **1998**, *123*, 1749

[5] Klopas A.; Panderi I.; Parissi-Poulou M. *J. Pharm. Biomed. Anal.* **1998**, *17*, 515

[6] Dadashzadeh S.; Vali A. M.; Rezagholi N. *J. Pharm. Biomed. Anal.* **2002**, *28*, 1201

[7] Yrita M.; Parra P.; Fernández J. M.; Barbanoj J. M. *J. Chromatogr. A* **1999**, *846*, 199

[8] de Jager A. D.; Ellis H.; Hundt H. K. L.; Swart K. J.; Hundt A. F. *J. Chromatogr. B* **1999**, *729*, 183

[9] Maya M. T.; Pais J. P.; Morais J. A. *J. Pharm. Biomed. Anal.* **1995**, *13*, 319

[10] Avgerinos A.; Axarlis S.; Dragatsis J.; Karidas Th.; Malamataris S. *J. Chromatogr. B* **1995**, *673*, 142

[11] Donato M. G.; Baeyens W.; Van den Bossche W.; Sandra P. *J. Pharm. Biomed. Anal.* **1994**, *12*, 21

[12] Acuna J. A.; de la Fuente C.; Vazquez M. D.; Tascon M. L. *Talanta* **1993**, *40*, 1637

[13] Damiani P. C.; Bearzotti M.; Cabezón M.; Olivieri A. C. *J. Pharm. Biomed. Anal.* **1998**, *17*, 233

[14] Escandar G. M. *Analyst* **1999**, *124*, 587

[15] MacIntire G. L. *Crit. Rew. Anal. Chem.* **1990**, *21*, 257

[16] Hinze W. L.; Singh H. N.; Baba Y.; Harvey N. G. *Trends Anal. Chem.* **1984**, *3*, 193

[17] Sbai M.; Ait Lyazidi S.; Lerner D. A.; del Castillo B.; Martin M. A. *J. Pharm. Biomed. Anal.* **1996**, *41*, 959

[18] Sabry S. M. *Anal. Chim. Acta* **1998**, *367*, 41

[19] García-Campaña A. M.; Aaron J. J.; Bosque-Sendra J. M. *Talanta* **2001**, *55*, 531

[20] Manzoori J. L.; Amjadi M. *J. Pharm. Biomed. Anal.* **2003**, *31*, 507

[21] Morgan E. (1995) Chemometrics: experimental design. Wiley, Chichester, pp. 237–259

[22] Winefordner J. D.; Long G. L. *Anal. Chem.* **1983**, *55*, 712A