Electrochemical Determination of Meloxicam in Pharmaceutical Preparation and Biological Fluids Using Oxidized Glassy Carbon Electrodes

Khalil FARHADI* and Ahmad KARIMPOUR

Department of Chemistry, Faculty of Science, Urmia University; Urmia, Iran.
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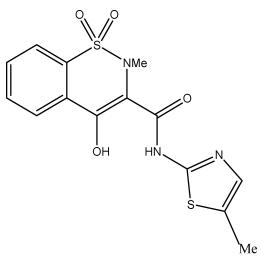
The adsorptive and electrochemical behaviors of meloxicam (MLC) was investigated on a glassy carbon electrode that was electrochemically treated by anodic oxidation at +1.8 V, following potential cycling in the potential range from −0.8 to 1.0 V *vs.* Ag/AgCl reference electrode. The resulting electrode showed a good activity to improve the electrochemical response of the drug. MLC was accumulated at an electrochemically activated glassy carbon electrode (phosphate buffer pH 6) in a certain time and then determined by linear sweep voltammetry. The oxidative peak currents showed a linear function in the concentration ranges of 0.02 to 10 μM using a 240 s preconcentration time. The preconcentration medium-exchange approach was utilized for the selective determination of the drug in spiked urine and plasma samples with satisfactory results. The recovery values of the proposed method obtained 105% (RSD 2.5%) and 100% (RSD 1.8%) for urine and plasma samples, respectively. Also, the proposed method has been successfully used for determination of MLC in tablets.

Key words meloxicam; electrochemical determination; stripping; biological fluids

Meloxicam (MLC), 4-hydroxy-2-methyl-*N*-(5-methyl-2-thiazolyl)-2*H*-1,2-benzo thiazine-3-carboxamide-1,1-dioxide, is a highly potent non-steroidal anti-inflammatory drug (NSAID) of the enolic acid class of oxicam derivatives.[1,2] This drug is indicated for the treatment of rheumatoid arthritis, osteoarthritis, and other joint diseases.[2] Due to the vital importance of MLC determination in pharmaceutical preparations and in biological fluids, many analytical techniques have been reported in literature. MLC has been determined in pharmaceutical preparations using spectroscopic,[3—7] HPLC[8—13] and electrochemical[14—18] methods. Studies of the therapeutic and toxic effects of drugs require a sensitive method for determination of them at a trace level. All reported spectroscopic methods suffer from low sensitivity. On the other hand, high performance liquid chromatographic methods, while having the advantage of requiring minimal sample preparation, are relatively slow and expensive, require filtration, degassing and expensive grades of reagents, eluents and equipment. In addition, the most of the reported electrochemical methods are based on the reduction of MLC on dropping or hanging mercury electrodes. Radi *et al.* have developed an electrochemical oxidation method for determination of MLC in tablet dosage form using a carbon paste electrode.[16] According to this report, MLC is irreversibly oxidized from amide and enol functions over pH range 2—11.5 in buffer media. Adsorptive stripping voltammetry has been demonstrated as a sensitive analytical method for a wide range of pharmaceutical compounds adsorbing on the electrode surface.[19] As a result, a wide variety of substances possessing surface-active properties are easily measurable at very low concentration levels.[20] The adsorptive properties of a glassy carbon electrode can be changed with the electrochemical pretreatment procedure. Electrode surface modifications and pretreatments have been widely used to improve the electrochemical responses of biological compounds and to construct electrochemical detectors for liquid chromatography involving the detection of analytes that oxidize only at extreme positive potentials.[21—23] The behavior of carbon electrodes at high positive potentials has received considerable interest owing to a desire to improve both the activity and reproducibility of carbon electrodes. The objective of the present work was to develop an adsorptive stripping voltammetric procedure for the determination of MLC, with higher sensitivity than the reported ones. It has been found that MLC could be adsorbed on an electrochemically pretreated glassy carbon electrode (EPGCE). Using this phenomenon and by accumulating this compound at the electrode surface prior to a differential pulse voltammetry and linear sweep voltammetry measurements, a higher sensitivity has been readily achieved. This work deals with the application of the proposed voltammetric methods to assay MLC in biological samples and pharmaceutical dosage form.



Meloxicam (MLC)

Experimental

Materials and Reagents Stock Solutions of MLC (1×10^{-2} M) were prepared in dimethylformamide (DMF). Standard solutions were prepared daily by diluting the stock solutions with 0.2 M phosphate buffer (pH 6). All of the reagents were of analytical grade (Merck) and solutions were prepared using doubly distilled water.

Apparatus A potentiostat-galvanostat Autolab with PGSTAT30 multipurpose instruments equipped with a Metrohm Stand model 663 VA was used to record the voltammograms. Three-electrode systems with a GC wire counter electrode, Ag/AgCl, 3 M KCl reference electrode, and a solid state working glassy carbon electrode (GC) with diameter of 2 mm, were purchased from Metrohm. The working electrode were polished with alumina powder (0.05 μm) for 1 min and then washed with water before using. The pH of the buffered solution was measured with a Metrohm digital pH-meter using a combined glass electrode.

* To whom correspondence should be addressed. e-mail: kh.farhadi@mail.urmia.ac.ir

Procedure. Pretreatment of the Glassy Carbon Electrode[24] A glassy carbon electrode was polished with 0.05 μm alumina powder on a polishing cloth until a mirror-like finish was obtained. The electrode was then washed with double-distilled water; an electrochemical pretreatment of the glassy carbon electrode was performed by anodic oxidation at 1.8 V for 4 min in 0.2 M phosphate buffer at pH 6.0. The electrode was then cycled between −0.8 and +1.0 V at a scan rate of 100 mV s^{-1} until a stable current–voltage profile was obtained.

Measurement Procedure Three electrodes were immersed in a solution containing MLC and 0.2 M phosphate buffer at pH 6.0. Since dissolved oxygen did not interfere with the anodic voltammetry, no deaeration was performed. MLC was accumulated to the surface of electrochemically pretreated glassy carbon electrode for a selected time while the solution was being stirred (open circuit). After a rest period of 10 s, linear sweep voltammetry technique (with a scan rate of 100 mV/s) initiated in the anodic direction up to the final potential of 0.7 V, was performed. The electrode cleaning procedures (see text) were carried out after each experiment.

Procedure for Determination of MLC in Tablets An accurately weighed portion of the finely powdered MLC tablet samples (equivalent to about 30 mg of the drug) were transferred into a 50 ml beaker containing 8 ml of DMF and vigorously shaken for about 15 min. These solutions were filtered into a 10 ml volumetric flask, washed with small portions of DMF, and diluted to the mark with DMF. An accurate microliter volume of this solution was pipetted into a 10 ml volumetric flask, diluted to the mark with a 0.2 M phosphate buffer (pH 6) solution and then transferred into the voltammetric cell. The accumulation of MLC at the surface of electrochemically pretreated glassy carbon electrode was carried out by stirring during 240 s. The stripping voltammograms were recorded between 0.3 and 0.7 V (*vs.* Ag/AgCl), finally.

Procedure for Determination of MLC in Urine A quantity of the MLC was added to the urine and diluted the spiked urine sample with 0.2 M phosphate buffer (pH 6) solution of the appropriate concentration, an aliquot of 0.1 ml was taken and diluted up to 10 ml with phosphate buffer (pH 6.0), and the resulting drug urine solutions were used for voltammetric analysis. Stirring carried out the accumulation of drugs at the electrochemically pretreated glassy carbon electrode during 240 s. The electrode was then rinsed with water and placed in a measurement cell containing 10 ml of phosphate buffer, and the stripping voltammogram was recorded between 0.3 and 0.7 V. Quantitative amount of MLC was obtained by means of the working-curve method.

Procedure for Determination of MLC in Plasma The deproteinizations were accomplished using a sodium tungstate solution (11 g of sodium tungstate+1.8 ml sulfuric acid conc. in 1 l water). Two milliliters of this solution were added to the 1 ml of serum samples containing an aliquots of standard solutions of the drugs, after centrifugation at 3500 rpm in 15 min, 0.1 ml of the protein-free supernatant solution was transferred into 10 ml volumetric flask and diluted to mark with 0.2 M phosphate buffer (pH 6), then the proposed voltammetric procedure was performed similar to urine.

Results and Discussion

Optimization Conditions for the Preparation of EPGCE The anodization of the glassy carbon electrode at a high positive potential resulted in stable peak currents. Electrochemical activation of the carbon surface resulted in an oxidized film containing functional groups, especially of the carbon-oxygen type.[23] These functional groups increased the density of the active sites at the electrode surface and improved the electron transfer of the reaction. In addition, after such a treatment, the porous film modified the electrode and the effective surface area was increased. Therefore, in order to improve the electrochemical response of the studied drugs, the electrode was pretreated in phosphate buffer at pH 6.0 by anodic oxidation and continuous cycling until a stable voltammogram was obtained. Cyclic voltammograms for the oxidation of 4 μM MLC at unmodified and electrochemically activated glassy carbon electrodes with 5 min accumulation time in the same solution are shown in Fig. 1. The appearance of two peaks in anodic scan and no cathodic peak indicates that the MLC is irreversibly oxidized. As seen, under

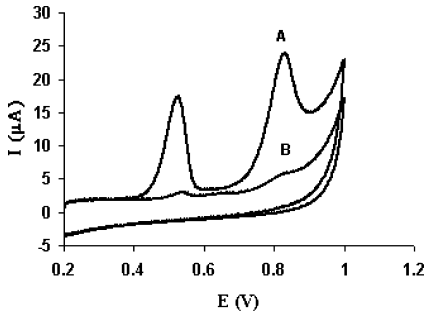


Fig. 1. (A) Cyclic Voltammogram of 4 μM MLC in Phosphate Buffer (pH 6) on Activated GC Electrode with 5 min Accumulation Time and (B) Cyclic Voltammogram of Same Solution at Unmodified GC Electrode

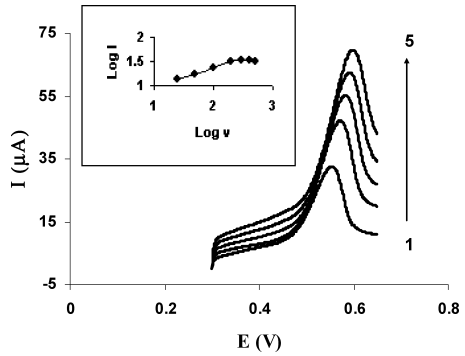


Fig. 2. Linear Sweep Voltammograms of 4 μM MLC in Phosphate Buffer (pH 6) on Activated GC Electrode with 5 min Accumulation Time at Different Scan Rates: 1) 100; 2) 200; 3) 300; 4) 400; 5) 500 mV s^{-1}

The inset shows the plot of log I_p (μA) *vs.* log ν (mV s^{-1}).

these conditions, while no measurable signal is obtained for MLC on unmodified GC electrode (Fig. 1B), a pronounced oxidation signal is appeared on EPGCE (Fig. 1A). The peak currents decrease to a constant value with succeeding potential scans, suggesting an adsorbed species formation on the electrode surface (fouling). Linear sweep voltammograms at low scan rates provided a linear relation between log I_p and log ν and confirmed an adsorption-controlled process (Fig. 2). The effect of pH and nature of buffer used in the electrooxidation of MLC was investigated. For this purpose, solutions of MLC in different pHs (2—8) prepared in Robinson buffer and acetate buffer. In all tested solutions, the obtained signals were decreased or omitted. In addition, for further studies, the electrooxidation of MLC in phosphate buffer solution (pH 6), and in KCl solution (pH 6) as supporting electrolyte was investigated (Fig. 3). As it is obvious from Fig. 3, MLC is easily oxidized with high sensitivity in phosphate buffer solution (Fig. 3A) in comparison to KCl solution (Fig. 3B).

Adsorptive Stripping Behavior The adsorption of MLC at an electrochemically activated electrode surface was used as an effective preconcentration step prior to voltammetric quantitation of the drug. Figure 4 shows linear sweep voltammograms related to first oxidation step for a 4 μM solution of MLC without accumulation, and after a 60 s accumulation time step. An approximately twice enhancement of the peak current was observed over that attained without accumulation. This finding suggested that a considerable enhancement in sensitivity could be provided by applying ad-

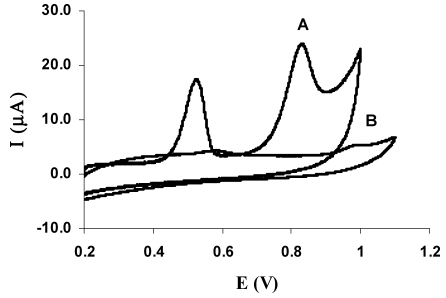


Fig. 3. (A) Cyclic Voltammogram of 4 μM MLC in Phosphate Buffer (pH 6) on Activated GC Electrode with 5 min Accumulation Time and (B) Cyclic Voltammogram of 4 μM Meloxicam in 0.1 M KCl (pH 6) on Activated GC Electrode

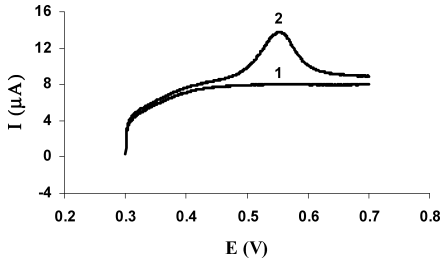


Fig. 4. Linear Sweep Voltammograms for a 4 μM Solution of MLC 1) without Accumulation, and 2) after a 60 s Accumulation Time Step

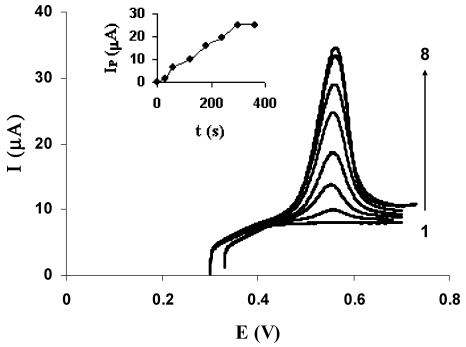


Fig. 5. Linear Sweep Voltammograms for a 4 μM Solution of MLC at Different Preconcentration Times 1) 0 s; 2) 30 s; 3) 60 s; 4) 120 s; 5) 180 s; 6) 240 s; 7) 300 s; 8) 360 s

Table 1. Analytical Parameters of the Calibration Plot for the Determination of MLC

Accumulation time (s)	240
Linearity range (μM)	0.02—10
Slope (μA/μM)	6.2447
Intercept (μA)	−0.2794
Correlation coefficient	0.9997
Detection limit (nM)	7.0

sorptive stripping voltammetry to the determination of MLC. The optimum conditions for the maximum adsorption should be utilized during the accumulation step to achieve the maximum sensitivity by the adsorptive stripping voltammetric method. The electrochemical signal for MLC was optimized according to the following parameters, as the adsorption process depends on many variables. It should be noted that all studies were focused on the first oxidation process, due to low reproducibility of second oxidation step.

The influence of the pH on the voltammetric response was examined between pH 4 and 8. From preliminary studies, it was found that the value of the peak current without adsorption increased in the pH range of 4—6, and then decreased at alkaline pH conditions. The pH of the solution also had an effect on the preconcentration step, which exhibited varying degrees of accumulation. The response preceded by adsorption suggested that the best accumulation could be attained in a phosphate buffer at pH 6.0. This pH value was selected for the accumulation step.

The effect of the accumulation potential on the peak intensity was also evaluated for a 40 μM MLC solution following a 60 s preconcentration time over the range of 0.2 to 0.4 V. No enhancement in the sensitivity of the proposed method was obtained using the tested accumulation potential. Therefore, it was suggested that the stripping analyses was done without applying first accumulation potential and performed with open circuit method.

The accumulation time was investigated in order to optimize the previous accumulation process of the substance on the electrode surface for its following anodic stripping. Figure 5 shows the dependence of the adsorptive peak current on the preconcentration time at concentration level, 4 μM of MLC. As seen an increase in the analytical signal intensity was observed as the accumulation time increased, indicating an enhancement of the MLC concentration at the electrode surface. The linear relationship between the amount of accumulated compound and the deposition time at low concentrations pointed out a constant adsorption. For longer accumulation times, the peak current was stabilized, showing the equilibrium between the concentration of the substance in solution and that on the surface of the electrode. However, the saturation coverage of the electrode area was reached and decreasing peak intensity occurred at high concentrations. This result was taken into consideration when constructing calibration plots. The choice of the preconcentration time depended on the concentration range studied.

Because fouling the surface of electrode, after each scan the surface of electrode refreshed with alumina powder (0.05 μm) then pretreated that described above and analyses was carried out with linear sweep voltammetry method. The best peak definition was found when using 1 mV step potential and a 100 mV s^{-1} scan rate.

The electrooxidation of MLC on unmodified Pt, Au, and GC electrodes in phosphate buffer (pH 6) showed no considerable signal in comparison to activated GC electrode. Therefore, appearing high sensitive with excellent reproducible signals encourages the application of activated GC electrode in electrochemical determination of MLC.

Calibration Curves Under optimum analytical conditions, the linearity of the linear sweep stripping peak current in different concentrations of MLC was evaluated. Table 1 summarizes the characteristics of the calibration plot. The obtained results show a wide linear dynamic range from 0.02 to 10 μM. Samples of linear sweep voltammograms and related calibration curves are shown in Fig. 6.

Using an accumulation time of 300 s, the limit of detection is reduced to 7 nM. In addition, a lower detection limit was achieved and a wide range of concentrations could be used for analytical purposes.

Table 2. Results of Determination of MLC in Pharmaceutical Sample

Pharmaceutical form	Declared	Proposed method	Official method[25)]	RSD%[a)]
Tablet	15 mg per tablet	14.99±0.49 mg	15.32±0.08 mg	3.3

a) Average of four replicate measurements.

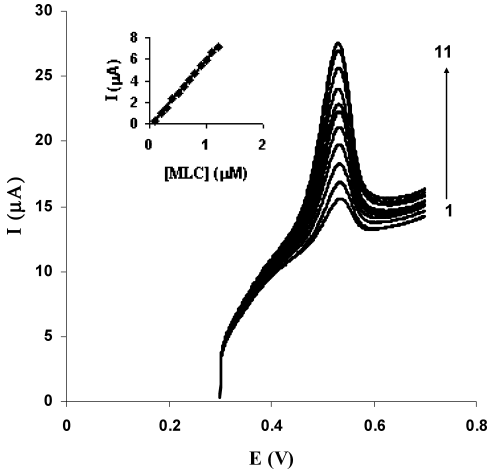


Fig. 6. Linear Sweep Voltammograms Obtained for Varying Concentration of MLC in Phosphate Buffer (pH 6)

The MLC concentration (μM) is: 1) 0.6; 2) 0.8; 3) 1; 4) 1.2; 5) 1.4; 6) 1.6; 7) 1.8; 8) 2; 9) 2.2; 10) 2.4; 11) 2.6. The inset shows calibration graph.

Using the adsorptive stripping voltammetric conditions described above, the reproducibility of the assay was investigated. Adequate precision in the voltammetric signal could not be obtained unless the electrode surface was cleaned and pretreated by an activation procedure before each measurement. The repeatability of the peak current at new surfaces, expressed by the relative standard deviation, was 1.45% (n=5) for the concentration of 4 μM MLC.

Determination of MLC in Pharmaceutical Samples Assay of MLC content in pharmaceutical formulations was carried out using the proposed stripping voltammetric procedure. The content of MLC in the tablets was determined by the working-curve method. The mean concentration obtained was 14.99 mg per tablets with a relative standard deviation of 3.3%, indicating adequate precision and accuracy of the proposed method (Table 2). The obtained results were compared to official method.[25)] As seen, no significant different observed between results using two methods.

Determination of MLC in Biological Samples. Urine The proposed method was used to analyze MLC added to human urine. The samples were spiked and the drug was determined by means of the working-curve method. The generally poor selectivity of voltammetric techniques can cause difficulties in the analysis of biological fluids, which contain oxidizable substances. It was possible to avoid this interference by doing a medium-exchange procedure. The working electrode with the extracted drug was transferred from the complex sample to an electrolytic blank solution between the adsorption and stripping steps. This procedure could avoid the need for a lengthy cleanup pretreatment of the urine, and could keep the analysis time to a minimum. However, after a medium exchange experiment, a peak at +0.42 V was recorded. It was probably due to uric acid, which is fairly readily oxidizable.[15)] The peak observed did not increase with increasing the accumulation time, and was well differentiated from that of MLC. The calibration plots obtained by spiking the cell electrolyte containing blank urine with standard MLC were found to be linear in the concentration range of 10—35 μg ml^{-1}. Good recovery of MLC was achieved from this type of matrix (Table 3). Following this procedure, the estimated detection limit, which was defined as three-times to noise, was 2 μg ml^{-1} in spiked urine samples.

Table 3. Results of Determination of MLC in Biological Samples

Sample	Spiked	Found	%recovery	RSD%[a)]
Urine	15.0 μg ml^{-1}	15.7 μg ml^{-1}	105	2.5
Plasma	20.0 μg ml^{-1}	20.0 μg ml^{-1}	100	1.8

a) Average of four replicate measurements.

Plasma The procedure for spiked plasma is described in Experimental. The calibration plots obtained by spiking the cell electrolyte containing blank plasma with standard MLC were found to be linear in the concentration range of 15—45 μg ml^{-1}. Good recovery of MLC was achieved from this type of matrix (Table 3). Following this procedure, the estimated detection limit, which was defined as three-times to noise, was 6 μg ml^{-1} in spiked plasma samples.

Conclusion

The developed adsorptive stripping analysis of MLC at an electrochemically pretreated glassy carbon electrode significantly improved the sensitivity of method. In addition, a lower detection limit was achieved, and a wide range of concentrations could be used for analytical purposes. The method was used to determine this drug in pharmaceuticals without interference. It has also been successfully applied for the determination of them in biological samples. The proposed method is an attractive method due to the low cost of the instrumentation and the short time required for the analysis.

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References

1) Noble S., Balfour J. A., *Drugs*, **51**, 424—430 (1996).
2) Türck D., Roth W., Busch U., *Brit. J. Rheumatol.*, **35**, 13—16 (1996).
3) Zawilla N. H., Mohammad M. A. A., El-Kousy N. M., El-Moghazy Aly S. M., *J. Pharm. Biomed. Anal.*, **32**,1135—1144 (2003).
4) Garcia M. S., Sanchez-Pedreno C., Albero M. I., Marti J., *Eur. J. Pharm. Sci.*, **9**, 311—316 (2000).
5) Kuchekar B. S., Lata S. G., Shingavi A. A., Shinde D. B., *Indian Drugs*, **37**, 596—600 (2000).
6) Sane R. T., Surve V., Francis M., *Indian Drugs*, **37**, 390—394 (2000).
7) Hassan E. M., *J. Pharm. Biomed. Anal.*, **27**, 771—777 (2002).
8) Sane R. T., Surve V., Francis M., *Indian Drugs*, **37**, 251—254 (2000).
9) Abd Elbary A., Foda N., Elkhateeb M., *Anal. Lett.*, **34**, 1175—1187 (2001).

10) Dasandi B., Prakash S., Saroj H., Bhat K. M., *J. Pharm. Biomed. Anal.*, **28**, 999—1004 (2002).
11) Wiesner J. L., Jager A. D., Sutherland F. C. W., Hundt H. K. L., Swart K. J., Hundt A. F., Els J., *J. Chromatogr. B*, **785**, 115—122 (2003).
12) Baeyens W. R. G., Van der Weken G., D'haeninck E., García-Campaña A. M., Vankeirsbilck T., Vercauteren A., Deprez P., *J. Pharm. Biomed. Anal.*, **32**, 839—846 (2003).
13) Nemutlu E., Kir S., *J. Pharm. Biomed. Anal.*, **31**, 393—400 (2003).
14) Radi A., Ghoneim M., Beltagi A., *Chem. Pharm. Bull.*, **49**, 1257—1260 (2001).
15) Altiokka G., Atkosar Z., Tuncel M., *Pharmazie*, **56**, 184—185 (2001).
16) Radi A., El-Ries M. A., El-Anwar F., El-Sherif Z., *Anal. Lett.*, **34**, 739—748 (2001).
17) Beltagi A. M., Ghoneim M. M., Radi A., *J. Pharm. Biomed. Anal.*, **27**, 795—801 (2002).
18) Altinoz S., Nemutlu E., Kir S., *Farmaco*, **57**, 463—468 (2002).
19) Wang J., "Analytical Electrochemistry," VCH Publisher Inc., New York, 1994, p. 147.
20) Wang J., "Stripping Analysis, Principles, Instrumentation and Applications," VCH Publishers, Deerfield Beach, Florida, 1985, p. 128.
21) Engstrom R. C., *Anal. Chem.*, **54**, 2310—2314 (1982).
22) Bowers M. L., Yenser B. A., *Anal. Chim. Acta*, **243**, 43—48 (1991).
23) Cabaniss G. E., Diamantis A. A., Murphy W. R., Linton T. W., Mayer T. J., *J. Am. Chem. Soc.*, **107**, 1845—1854 (1985).
24) Nigovic B., *Anal. Sci.*, **20**, 639—643 (2004).
25) "British Pharmacopeia," Her Majesty's Stationary Office, London, 1998, p. 170.