Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography A, 1072 (2005) 267–272
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Determination of rate constants and activation energy of 3-chloro-1,2-propanediol hydrolysis by capillary electrophoresis with electrochemical detection

Xiaoping Xing [a,b], Yuhua Cao [a,*], Licong Wang [a]

[a] *School of Chemical and Material Engineering, Southern Yangze University, Wuxi 214036, China*
[b] *Department of Science and Industry, Yancheng Institute of Technology, Yancheng 224003, China*

Received 20 December 2004; received in revised form 8 March 2005; accepted 10 March 2005

Abstract

A method based on capillary electrophoresis with electrochemical detection (CE–ED) to calculate the rate constants and activation energy of 3-chloro-1,2-propanediol (3-MCPD) hydrolysis was described. Effects of several factors, such as the pH value and the concentration of the running buffer, separation voltage, injection time and the potential applied to the working electrode, were investigated to find the optimum conditions. With a 50 cm length of 25 μm diameter fused-silica capillary at a separation of 10 kV, well-defined separation of 3-chloro-1,2-propanediol from glycerol was achieved in 30 mmol/l borax (pH 9.24) within 13 min. Operated in a wall-jet configuration, a 328 μm copper-disk electrode used as the working electrode exhibits good response at 0.65 V (versus SCE) for 3-MCPD and glycerol. The rate constants of 3-MCPD hydrolysis at different temperatures were determined by monitoring the concentration changes of 3-MCPD. At 80, 85 and 90 °C, the measured rate constants of 3-MCPD hydrolysis were 3.8×10^{-3} min^{-1}, 7.1×10^{-3} min^{-1} and 11.5×10^{-3} min^{-1}, respectively. The activation energy for 3-MCPD hydrolysis was calculated to be 118.1 kJ/mol, which is in good agreement with the value in the literature.

Keywords: Rate constants; Activation energy; 3-Chloro-1,2-propanediol; Capillary electrophoresis; Electrochemical detection

1. Introduction

3-Chloro-1,2-propanediol is a known carcinogen [1,2]. The risk assessment for 3-chloro-1,2-propanediol (3-MCPD) has been evaluated by the EU Scientific Committee on Food [3] and by the Joint FAO/WHO Expert Committee on Food Additives [4]. Toxicological studies showed that 3-MCPD is a potential human carcinogen [5] and a maximum tolerable daily intake of 2 μg/kg body weight has been established [6]. In many countries, the food industries have been requested [7] to take all steps to reduce concentrations of 3-MCPD to lowest achievable level in foods and food ingredients.

The highest level of 3-MCPD has been found in hydrolyzed vegetable proteins (HVP) and related sauce products. This is produced by treating protein-rich plant extracts, e.g. from soya beans, with concentrated hydrochloric acid at high temperatures. Recently, 3-MCPD has also been found in other foods [8–10], especially in cereal products, which have been heated such as baking, roasting or toasting. Some experiment [11] has been simulated to investigate the influence of the composition, the moisture, the pH value and the temperature on the formation kinetics of chloropropanediols in wheat flour dough. Though the formation mechanism of 3-MCPD has been studied in several laboratories [12], it has not been fully elucidated and used in practice.

On the other hand, 3-MCPD is easily hydrolyzed in basic solution. The resultant is glycerol, a nontoxic natural compound. If the HVP used in foods were processed with the appropriate pH value and temperature, the content of 3-MCPD would be decreased significantly to even lower than the determination limit with the present method. Therefore, it is very important to investigate the reaction of 3-MCPD hydrolysis

* Corresponding author. Tel.: +86 510 581 1348; fax: +86 510 586 5424.
E-mail address: yuhuacao@yahoo.com.cn (Y. Cao).

0021-9673/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2005.03.043

for evaluating the half life, selecting the optimum hydrolysis condition.

Studies on the rate constants of 3-MCPD by reversed phase high performance liquid chromatography with refractive index detector (HPLC-RI) [13,14] has been reported, in which 3-MCPD was found to degrade according to first-order kinetics and the degradation reaction obeyed Arrhenius equation with a constant activation energy (E_a) of 119.2 kJ/mol over the pH range of 5–9. The 3-MCPD and glycerol are in general difficult to separate and detect due to the similar molecular structure and lack of chromophores. At present, direct detection with refractive index and conductivity has been used, but is limited because of the poor sensitivity. On the other hand, it is found that 3-MCPD can be oxidized at copper electrode in basic solution. So we first present an amperometric detection method for 3-MCPD in this paper. In addition, Capillary electrophoresis (CE) is an important separation technology for its speed, efficiency, reproducibility and minimal consumption of solvent. With electrochemical detection (ED), CE–ED offers high sensitivity and good selectivity for electroactive analytes. CE–ED is mostly used in the quantitative analysis. Recently, CE has also been applied as a new important technology to measure the dissociation constant (pK_a) [15–24] and other chemical physics constants. But CE–ED used in the determination of reaction rate constants was not fully explored [25–27]. In this work, we first used CE–ED to develop a simple and rapid method for determination of the reaction rate constants and activation energy of 3-MCPD hydrolysis.

2. Materials and methods

2.1. Materials

3-Chloro-1, 2-propanediol was purchased from Unitech Research Inc. (Tianjin China), glycerol were obtained from Shanghai Chemical Reagent Factory (Shanghai, China). Stock solutions of 1.3×10^{-4} g/ml 3-MCPD and 1.2×10^{-4} g/ml glycerol were prepared with deionized water. 0.1 mol/l citric acid mixed with 0.2 mol/l disodium hydrogen phosphate with the pH value of 6.8 was used as the buffer solution for the hydrolysis reaction medium. 30 mmol/l borax with the pH value of 9.24 was used as the running buffer for separation medium in CE. All reagents were analytical grade.

2.2. Apparatus

Hydrolysis reaction of 3-MCPD was carried out in a 501 constant temperature water bath (Shanghai Experiment Instrument Factory, Shanghai, China).

A laboratory-built [28] CE–ED system was used in this work. A 30 kV high voltage power supply (Shanghai Institute of Nuclear Research, China) provided a voltage between the ends of the capillary. The inlet end of the capillary was held at a positive potential and the outlet end was maintained at ground. The inlet cell was filled with the running buffer while the outlet end was laid in the detection cell filled with 0.05 mol/l sodium hydroxide solution. A 50 cm length of 25 μm i.d. fused-silica capillary (Hebai Yongnian Factory, China) was used for the separation.

The design of CE–ED detector was based on the end-column approach in which the working electrode was simply placed at the outlet of the separation capillary and detection was carried out in the outlet reservoir. A copper electrode with 328 μm diameter was employed as the working electrode and positioned carefully opposite to the capillary outlet with the aid of micropositioner (Shanghai Lianyi Instrument Factory, China). A three-electrode cell system consisting of a copper-working electrode, a platinum auxiliary electrode and a saturated calomel electrode (SCE) reference electrode was used in combination with a BAS LC–3D amperometric detector (Biochemical System, West Lafayette, IN, USA). The electropherograms were recorded and processed with HW-2000 chromatography workstation (Jiteng Trading Pte Ltd., Shanghai, China).

2.3. Hydrolysis procedures

The constant temperature water bath was adjusted to the needed temperatures at which the hydrolysis was carried out (80, 85 and 90 °C). Both 3-MCPD solution of 1.3×10^{-4} g/ml and the buffer solution of pH 6.8 were preheated to the same temperature for 15 min in the constant temperature water bath. Then 5 ml 3-MCPD and 5 ml buffer solution were mixed in a 10 ml metric flask by shaking the flask. The solution was immediately put in the same water bath and the initial time of hydrolysis was recorded simultaneously. An accurate volume of 100 μl hydrolysates was taken out at the hydrolysis time of 60, 90 120, 150 and 180 min, respectively, and diluted with 1.4 ml of 30 mmol/l borax. Thus the solution can be immediately injected electrokinetically for analysis with CE.

2.4. Determination of 3-MCPD and glycerol

3-MCPD and glycerol were determined with CE–ED. The samples were injected electrokinetically for 8 s and separated at the separation voltage of 10 kV in the running buffer of 30 mmol borax with the pH value of 9.24. Before use, the surface of the copper electrode was polished with emery sand paper, and sonicated in deionized water. The potential applied to the working electrode was 0.65 V (versus SCE), at which the currents of the analytes were detected.

3. Results and discussion

3.1. Effect of the potential applied to the working electrode

Copper electrode can catalyze the oxidation of the carbohydrates, sugar acids and alditol in basic media [29] and

glycerol has been determined by CE–ED with copper electrode [30]. Compared with molecular structure of glycerol, 3-MCPD replaces a hydroxyl group by a chlorine group. Because 3-MCPD has vicinal dihydroxy groups, it could be oxidized at copper electrode in alkaline solution.

Based on the above assumption, electrochemical detection at copper electrode in 0.05 mol/l sodium hydroxide solution for 3-MCPD, as well as glycerol, was studied in this experiment. The potential applied to the working electrode directly affects the sensitivity and the detection limit of this method. To obtain best detection results hydrodynamic voltammetry was investigated to find the optimum potential. As shown in Fig. 1, the applied potential was more than +0.50 V (versus SCE), 3-MCPD indeed can be oxidized at the copper electrode in basic media, though it need higher oxidation potential than glycerol. When the applied potential was more than +0.55 V (versus SCE), the oxidation currents of the analytes increase rapidly with increasing applied potential. However, when the applied potential is more than +0.68 V (versus SCE), both the baseline noise and the background current increase very strongly, which are obviously disadvantage for sensitive and stable detection. Therefore the applied potential to the working electrode was maintained at +0.65 V (versus SCE), where the background current is not too high, and the S/N ratio is the highest. Moreover, the working electrode showed good stability and high reproducibility at this optimum potential.

3.2. Effects of the pH value and concentration of the running buffer

At first, 0.05 mol/l Sodium hydroxide solution was considered as the running buffer to accord with the buffer in detection cell. Unfortunately, 3-MCPD cannot be separated from glycerol completely. Moreover, 3-MCPD is not stable in the sodium hydroxide solution, because a substitution reaction is easy to happen for 3-MCPD in a strong basic medium. So sodium hydroxide solution cannot be used as the running buffer.

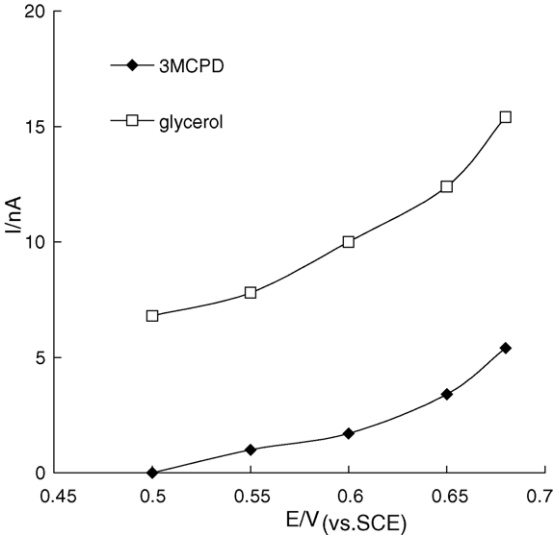

Fig. 1. Effect of potential applied to the working electrode on the peak current. Working electrode: 328 μm copper electrode; the running buffer: 30 mmol/l borax (pH 9.24); the concentration of two analystes: 1.3×10^{-5} g/ml and 1.2×10^{-5} g/ml for 3-MCPD and glycerol, respectively; inject time: 8 s, separation voltage: 10 kV.

Borate has a complexation reaction with 3-MCPD and glycerol, which increases the difference of ratios of charge to mass between the two analytes, hence, borate buffer was chosen as the running buffer in this work. The concentration of the running buffer varying from 10 to 40 mmol/l was studied in this experiment. In the 10 and 20 mmol/l running buffers, the 3-MCPD and glycerol cannot be separated completely. While in the 40 mmol/l running buffers, 3-MCPD and glycerol can be separated but the analysis time is longer and the peak currents decrease severely. At the concentration of 30 mmol/l, there is a good separation between the two analytes and short analysis time.

The pH value of the running buffer affects the separation and detection of the analytes. Hence, the effect of pH value was investigated in the range of 8.5–9.5. When the pH value is less than 8.98, the copper electrode has not current response at all. Maybe the pH value is too low to generate oxidation current at copper electrode. While the pH value is more than 9.50, 3-MCPD and glycerol were not separated completely. At the pH value of 9.24, a good separation of the two analytes can be achieved. So the 30 mmol/l borate with the pH value of 9.24 is selected as the optimum running buffer.

3.3. Effect of the separation voltage and the inject time

Separation voltage affects the velocity of electro-osmotic flow and the migration time of the analytes. So the influence of the separation voltage on the migration time of the analytes was also studied in this experiment. It can be concluded that higher separation voltage gives shorter migration times for all the analytes. While the voltage is above 12 kV, the separation of 3-MCPD from glycerol is poor and the baseline noise increases larger. The optimum separation voltage was selected at 10 kV, at which good separation and short analysis time can be obtained for all the analytes within 13 min.

Injection time determines the sampling amount, and affects both peak current and peak width. The effect of injection time on peak current was studied by varying the injection time from 4 to 10 s at 10 kV. As shown in Fig. 2, when the injection time increases, the peak currents increase simultaneously. When the injection time is longer than 8 s, peak currents increase slowly but peak broadening becomes severe, which result in a poor separation between 3-MCPD and glycerol. In this experiment, 8 s (at 10 kV) was selected as the optimum injection time.

3.4. Reproducibility, linearity, detection limits

The reproducibility of the peak currents was estimated by making repetitive injections of a standard mixture solution

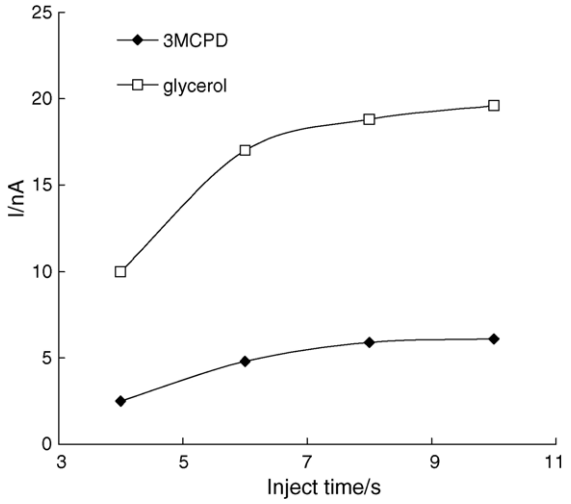

Fig. 2. Effect of injection time on the peak current. Working potential is 0.65 V (vs. SCE). Other conditions are the same as in Fig. 1.

(1.3×10^{-5} g/ml and 1.2×10^{-4} g/ml for 3-MCPD and glycerol, respectively) under the optimum conditions. The relative standard derivations (RSDs) of the peak current were 2.45 and 1.32% for 3-MCPD and glycerol ($n = 7$), respectively.

A series of standard mixture solutions from 1.0×10^{-4} g/ml to 6.6×10^{-8} g/ml were tested to determine the linearity of the current response on concentration for 3-MCPD and glycerol. The calibration curve exhibits excellent linearity. The results of regression analysis and detection limits were shown in Table 1.

3.5. Kinetics equations

3-MCPD can be easily hydrolyzed to produce glycerol in basic solution. But in foods, the alkaline condition is little used. So we select the pH value of 6.8, a neutral condition, to investigate the hydrolysis reaction of 3-MCPD. The temperature and the pH value affect the hydrolysis rate constants. According to the kinetics theory, we could get the kinetics equations of 3-MCPD hydrolysis, which were shown as follows:

$$\ln c = -kt + \ln c_0 \quad (1)$$

$$\ln k = -\frac{E_a}{RT} + \ln A \quad (2)$$

where c is the concentration of 3-MCPD at the hydrolysis time t, c_0 is the initial concentration of 3-MCPD, and k stands for the hydrolysis rate constant. E_a is activation energy of the reaction, R is the mole gas constant of 8.314 J/K mol, and A is called frequency factor. From the slope of the Eq. (2), the activation energy value of the hydrolysis could be calculated.

3.6. Determination of rate constants for hydrolysis of 3-MCPD

Eletropherograms for the standard mixture solution and the hydrolysis solution of 3-MCPD were shown in Fig. 3(A–E), in which the hydrolysis progress could be intuitively illustrated.

The regression equations for $\ln c - t$, correlation coefficients and k values determined experimentally at different temperatures were presented in Table 2, which shows that the rate constants increase simultaneous with the increasing temperature. And from the relationship between $\ln c$ and t, it can be confirmed that the hydrolysis progress of 3-MCPD is a first-order reaction. The regressing equation of the Arrhenius plot of $\ln k$ against T^{-1} is $\ln k = -14209\,T^{-1} + 34.683$ with the correlation coefficient of 0.9978. The activation energy value of the hydrolysis reaction of 3-MCPD could be calculated as 118.1 k J/mol, which is in good agreement with the value in the literature [14].

This study develops a new rapid method to investigate the characteristics of 3-MCPD. It is valuable to use this method to research other toxicants in foods.

Table 1
The results of regression analysis of 3-MCPD and glycerol

Compound	Regression equation[a]	Correlation coefficient	Linear range ($\times 10^{-4}$ g/ml)	Dectection[b] limits (g/ml)
3-MCPD	$y = 1.273 \times 10^{10}x + 1.211 \times 10^{4}$	0.9993	0.066–2.0	2.2×10^{-7}
Glycerol	$y = 6.475 \times 10^{10}x + 9.219 \times 10^{4}$	0.9989	0.008–1.8	5.3×10^{-8}

Working potential is 0.65 V (vs. SCE). Other conditions are the same as in Fig. 1.
[a] y is the peak area; x is the concentration of the analytes (g/ml).
[b] The detection limits corresponding to concentrations of signal to noise ratio of 3.

Table 2
The results of regression analysis of 3-MCPD hydrolysis at different temperatures

Temperature (K)	Regression equation[a]	Correlation coefficient	Rate constant (10^{-3}/min)
353.15	$y = -0.0038x - 9.6933$	0.9993	3.8
358.15	$y = -0.0071x - 9.8197$	0.9982	7.1
363.15	$y = -0.0115x - 9.9640$	0.9997	11.5

Working potential is 0.65 V (vs. SCE). Other conditions are the same as in Fig. 1.
[a] y is the logarithm of the concentration of 3-MCPD $\ln c$, x is the hydrolysis time t.

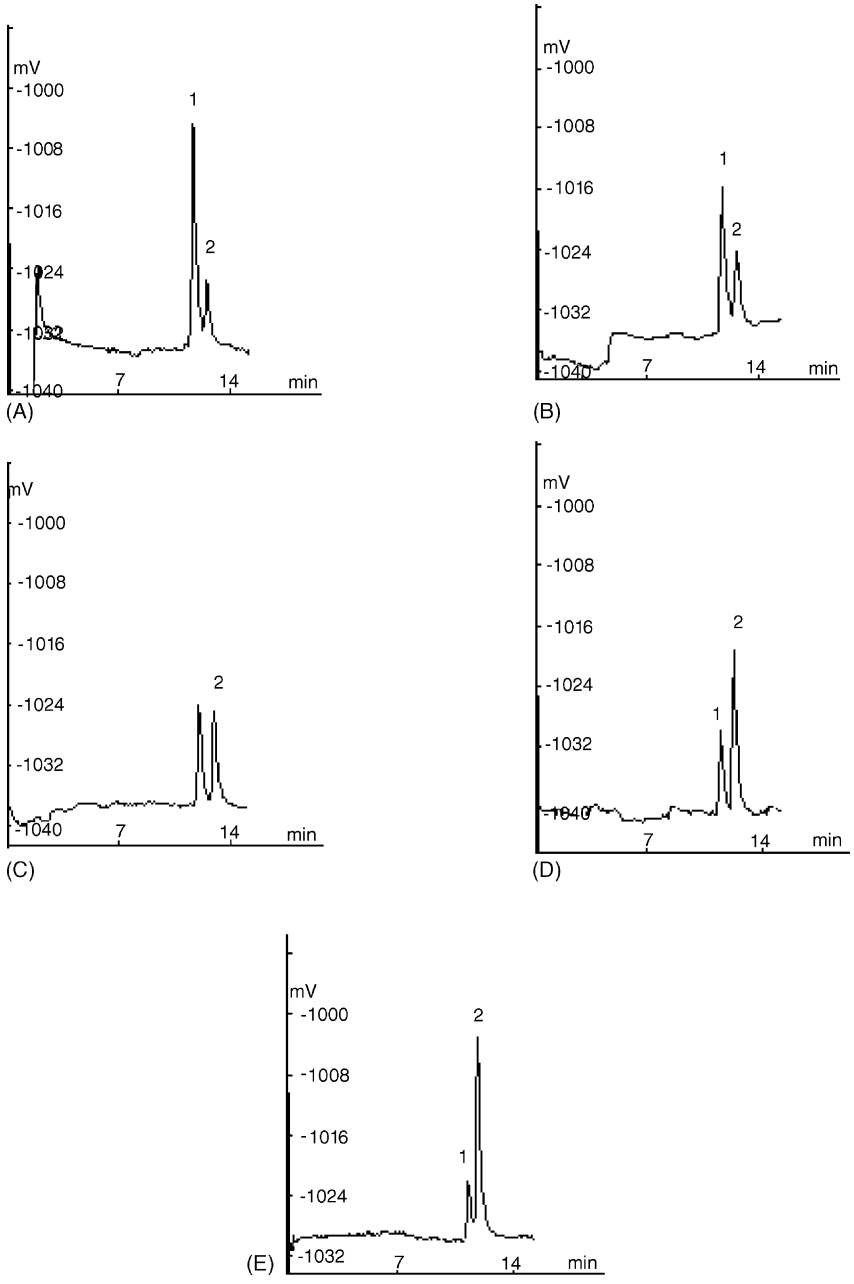

Fig. 3. (A–E) Eletropherograms for the standard mixture solution and the 3-MCPD solution hydrolyzed during different times at 85 °C. (A) 60 min, (B) 90 min, (C) 120 min, (D) 180 min, (E) the standard mixture solution (1.3×10^{-5} g/ml and 1.2×10^{-5} g/ml for 3-MCPD and glycerol, respectively). Peak identification: 1, 3-MCPD; 2, glycerol. Working potential is 0.65 V (vs. SCE). Other conditions are the same as in Fig. 1.

Acknowledgement

The project are partial financial supported by School of Chemical and Material Engineering, Southern Yangze University.

References

[1] G. Sunhara, I. Perrin, M. Marchessini, Report No. RE-SR93003, Nestec Ltd., Research and Development, Switzerland, 1993.

[2] P. Olsen, Chloropropanols in JECFA, Toxicological evaluation of certain food additives and contaminants, in: Proceedings of the 41st Meeting of WHO Food Additives Series. World Health Organization, Geneva, 1993, p. 267.

[3] European Commission, Opinion of the Scientific Committee on Food on 3-Monochloro-propane-1,2-diol (3MCPD) Updating the SCF Opinion of 1994(adopted on 30 May 2001), SCF/CS/CNTM/OTH/17Final, 2001.

[4] J. Schlatter, A.J. Baars, M. DiNovi, S. Lawrie, R. Lorentzen, WHO Food Addit. Ser. 48 (2002) 401.

[5] B.S. Lynch, D.W. Bryant, G.J. Hook, E.R. Nestmann, I.C. Munro, Int. Toxicol. 17 (1998) 47.

[6] Joint FAO/WHO Expert Committee on Food Additives, Summary of the 57th Meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), Rome, 5–14 June 2001, p. 20.
[7] Food Advisory Committee,108th Meeting of the FAC 26th October 2000, FAC press release 8/00, 2000.
[8] C.M. Breitling-Utzmann, H. Koebler, D. Herbolzheimer, A. Maier, Deut. Lebensm. Rundsch. 99 (2003) 280.
[9] C.G. Hamlet, S.M. Jayaratne, W. Matthews, Food Addit. Contam. 19 (2002) 15.
[10] C. Crews, P. Brereton, A. Da vies, Food Addit. Contam. 18 (2001) 271.
[11] C.G. Hamlet, P.A. Sadd, D.A. Gray, Eur. Food Res. Technol. 216 (2003) 122.
[12] P. Calta, J. Vel-Dek, M. Dole-al, S. Hasnip, C. Crews, Z. R-blov, Eur. Food Res. Technol. 218 (2004) 501.
[13] M. Doležal, J. Velíšek, Chem. React. Foods 11 (1992) 297.
[14] C.G. Hamlet, P.A. Sadd, Eur. Food Res. Technol. 215 (2002) 46.
[15] J. Cleveland, C. Martin, S. Gluck, J. Chromatogr. A 679 (1994) 167.
[16] J. Cleveland, S. Gluck, J. Chromatogr. A 680 (1994) 43.
[17] J. Cleveland, S. Gluck, J. Chromatogr. A 680 (1994) 49.
[18] S. Bellini, M. Uhrová, Z. Deyl, J. Chromatogr. A 772 (1997) 91.
[19] R. Jankowsky, M. Friebe, B. Johannsen, J. Chromatogr. A 833 (1999) 83.
[20] S. Mendonsa, R. Hurtubise, J. Chromatogr. A 841 (1999) 43.
[21] E. Jiménes-Lozano, I. Marqués, D. Barrón, J. Barbosa, Anal. Chim. Acta 464 (2002) 37.
[22] G.A. Caliaro, C.A. Herbots, J. Pharm. Biomed. Anal. 26 (2001) 427.
[23] D. Wang, G. Yang, X. Song, Electrophoresis 22 (2001) 464.
[24] Y. Ishihama, M. Nakamura, T. Kajima, N. Asakawa, J. Pharm. Sci. 91 (2002) 933.
[25] J.W. Rhim, R.V. Nunes, V.A. Jones, J. Food. Sci. 54 (1989) 22.
[26] J. Ye, X. Zhao, Q. Sun, Mikrochim. Acta 128 (1998) 119.
[27] Y. Cao, Y. Wang, J. Anal. Sci. 20 (2004) 187.
[28] J. Ye, R.P. Baldwin, Anal. Chem. 65 (1993) 3525.
[29] J. Ye, R.P. Baldwin, J. Chromatogr. A 687 (1994) 141.
[30] X. Fang, Z. Xie, J. Ye, Y. Fang, Chin. J. Chromatogr. 14 (1996) 467.