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Direct determination of small cations in proteinaceous samples using a flow injection–capillary electrophoresis system

Petr Kuban, Olga Oldhoff, Bo Karlberg*

Department of Analytical Chemistry, Stockholm University, S-106 91 Stockholm, Sweden

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Abstract

A method is described for the direct determination of small inorganic cations in samples containing large amounts of proteins, such as milk or blood plasma. The method is based on electrokinetic injection in a flow injection analysis–capillary electrophoresis (CE) system. The selected CE-electrolyte, containing 5 m*M* 4-aminopyridine and 7 μ*M* cetyltrimethylammonium bromide at pH 4.5, prevents detrimental protein adsorption on the capillary walls. Therefore, no sample pretreatment, except for dilution, is required. Up to 30 repeated injections in one electrophoretic run can be performed, yielding RSD values of the migration time of less than 1 and 2.5% ($n=30$) for milk and blood plasma samples, respectively.

Keywords: Milk; Inorganic cations; Proteins

1. Introduction

Since the introduction of capillary electrophoresis by Jorgensson and Lukacs [1,2], it has been known that proteins adsorb on the walls of fused-silica capillaries. It is believed that the adsorption occurs due to the interaction between the silanol groups, $Si–O^-$, on the capillary wall (at pH>2) and proteins possessing substantial charge at pH values different from their isoelectric points (p*I*). Efforts have been made to deactivate the wall charge of the fused-silica capillaries. The use of coated capillaries has been recommended [3–21], although some of the coatings can be used only within a limited pH range. Further drawbacks are the limited time stability, the tedious preparation and the irreproducible behaviour. Various other solutions to prevent protein adsorption on the walls of uncoated capillaries have been proposed and impressive results with respect to speed of analysis, resolution and efficiency have been achieved. For example, the addition of inorganic salts [22], surfactants [23,24] and zwitterions [25] have been reported as well as the use of electrolytes with either high or low pH values [26,27].

The determination of small ions in proteinaceous samples, i.e., samples containing up to 10% of proteins of various kinds, is an analytical challenge. In milk, for instance, the distribution of small ions between the liquid and colloidal phases has an impact on the coagulation and is therefore important in the cheese-making process [28]. In clinical analysis, the determination of the ion content in samples such as blood or blood plasma is routinely performed [29]. When capillary electrophoresis (CE) is applied

*Corresponding author. Tel.: +46-8-921-184; fax: +46-8-156-391.

E-mail address: bo.karlberg@anchem.su.se (B. Karlberg)

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to the determination of small ions in these samples, protein adsorption becomes a serious problem. Adsorbed proteins alter the rate of the electroosmotic flow (EOF) and, therefore, the amounts of sample introduced can vary. The separation efficiency decreases and the peak shapes of small ions deteriorate. Several solutions to this problem have been suggested. The most straightforward approach is to rinse the capillary between runs [29] and a 0.1-*M* solution of NaOH has been found to effectively remove the proteins. However, when strong alkaline solutions are used, subsequent capillary conditioning with the electrolyte becomes mandatory so that stable electrophoretic conditions are achieved. Another approach involves the physical separation of the proteins by centrifugation for 15 min before sample injection into the capillary [28]. In the case of milk samples, physical separation of caseins can only be achieved by using ultracentrifugation at very high speeds and, indeed, this certainly alters the mineral balance, since the colloidal calcium phosphate precipitates with casein micelles. During the centrifugation process, the liquid–colloidal balance might vary between the solid pellet and the liquid bulk of the sample, and a difference in ion concentration can be obtained. It is evident that both methods are time-consuming and might lead to erroneous results.

In the present paper, we describe suitable electrolytes and experimental conditions permitting the direct determination of small cations in protein-containing samples such as milk and blood plasma.

2. Experimental

2.1. Electrolyte, standard and sample solutions

All electrolyte and standard solutions were prepared by dissolving reagent-grade chemicals in deionized water from an Elgastat UHQII (Elga, High Wycombe, UK). The electrolyte was prepared daily from the following stock solutions: 50 m*M* *p*-aminopyridine (PAP), 30 m*M* cetyltrimethylammonium bromide (CTAB, 5% acetonitrile in water), 0.1 *M* H_2SO_4 and 0.1 *M* HCl. The pH of the electrolyte was first adjusted with H_2SO_4 to about five and, subsequently, with HCl to a final value of 4.5. The resulting mixture was then degassed and filtered (if necessary). Stock solutions of the cations, 10 000 ppm, were prepared from their chloride salts. Working standard solutions containing the cations being investigated were prepared by mixing the corresponding cation stock solutions and diluting with deionized water to the desired final concentrations.

The real samples, i.e., the milk and blood plasma samples, were introduced without any pretreatment other than dilution, which was 1:10 (v/v) for the milk samples and 1:20 (v/v) for the blood samples. The milk samples had a fat content in the range of 0.5 to 3.0%.

2.2. Instrumentation

Fused-silica capillaries, 375 μm O.D.×50 μm I.D. (Polymicro Technologies, Phoenix, AZ, USA), having a total length of 70 cm, were utilised. The detection window was burnt at a distance of 20 cm from the capillary end. When a capillary was used for the first time, it was preconditioned with 0.1 *M* NaOH for 20 min, then with distilled water for 10 min and with air for 5 min, followed by flushing with electrolyte solution for 20 min. The daily closing-down procedure consisted of rinsing the capillary with distilled water for 5 min followed by flushing with air for 5 min. Flow injection analysis (FIA)–CE systems with electrokinetic [30] and hydrodynamic [31] injection were used for the direct introduction of samples and standard solutions.

3. Results and discussion

3.1. Choice of the injection mode

Two injection modes, electrokinetic (EK) and hydrodynamic (HD) using FIA–CE systems [30,31], were initially tested to assess their suitability for direct injection of proteinaceous samples. An electrolyte composition of 5 m*M* PAP and 7 μ*M* CTAB at pH 4.5 was used. The results are shown in Table 1, indicating that the EK injection mode was superior with respect to sensitivity and the magnitude of the matrix effects. Furthermore, the discrimination of large, less charged, components in the sample, for instance proteins, seems to be reproducible for this injection technique. There are two possible explana-

Table 1
Comparison of the repeatability (RSD,%) of EK and HD injection modes for milk samples (n=10)

	K^+		Na^+		Ca^{2+}		Mg^{2+}	
	t_M[a]	PA[b]	t_M	PA	t_M	PA	t_M	PA
EK injection	0.61	1.76	0.81	2.53	0.88	2.74	0.98	4.96
HD injection	0.38	5.62	0.32	6.51	0.52	7.67	0.53	9.22

[a] t_M=migration time.
[b] PA=peak area.

tions for the imprecision observed for the HD injection technique. First, the hydrodynamic flow into the capillary may be altered between injections due to memory effects caused by capillary wall interactions with fat and colloids in the sample. Second, the HD injection is less sensitive, i.e., less sample is introduced in comparison with EK injection. When less sample is introduced, the imprecision increases. Consequently, the EK injection mode in the FIA–CE system was used in all subsequent experiments.

3.2. Choice of electrolyte

PAP has been shown to provide high separation efficiency and sensitivity when used as a background electrolyte in the determination of small inorganic cations and one obvious advantage is its applicability over a large pH range, from three to ten [32]. In preliminary experiments, the separation of the inorganic cations K^+, Na^+, Ca^{2+}, Mg^{2+} and Li^+ was carried out in a 5-m*M* PAP electrolyte solution of pH values of three to five. Large variations in the migration times of these ions were observed in milk and plasma matrices when these samples were consecutively injected. A systematic study was carried out in order to evaluate the effect of pH, and of the addition of CTAB to the running electrolyte, on the rate and magnitude of protein adsorption. It was found that a decrease in pH and the addition of CTAB improved the run-to-run repeatability of the analysis. An electrolyte composition of 5 m*M* PAP and 7 μ*M* CTAB at pH 4.5 seems to be a suitable compromise when it comes to avoiding adsorption of proteins on the capillary wall. In Fig. 1, electropherograms of inorganic cations in milk and blood plasma samples are depicted. By increasing the CTAB concentration further, protein adsorption could be totally circumvented, however, the EOF then decreased and the migration times for cations became unacceptably long. The RSD values of the migration times are within 1% for milk and 2.5% for plasma samples (Table 2) when an electrolyte composition of 5 m*M* PAP and 7 μ*M* CTAB, pH 4.5, is

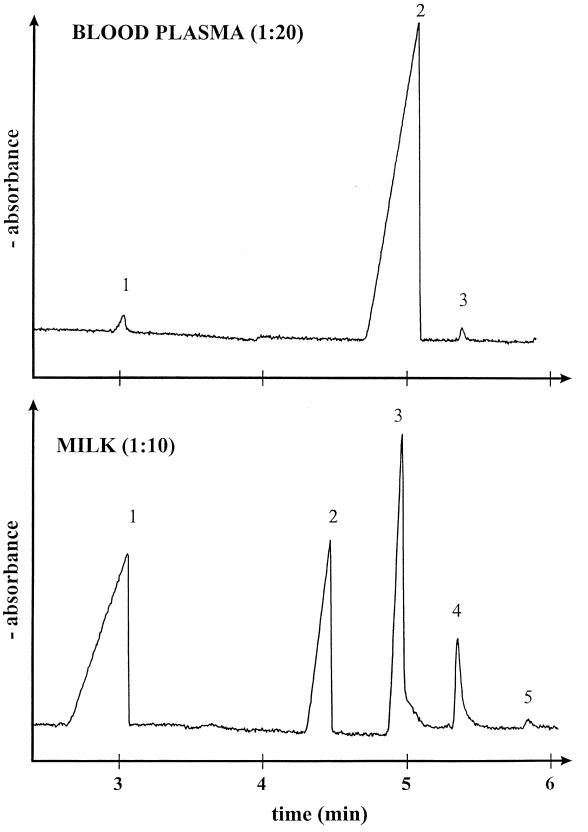

Fig. 1. Electropherograms of diluted (1:10, v/v) milk (A), and diluted (1:20, v/v) blood plasma (B) samples using the electrolyte composition 5 m*M* PAP+7 μ*M* CTAB, pH 4.5. Conditions: FIA–CE system with EK injection; sample loop, 100 μl; sample flow-rate, 3 ml/min; HV, 25 kV; indirect UV detection, 262 nm. Peaks: 1, K^+; 2, Na^+; 3,Ca^{2+}; 4, Mg^{2+}; 5, unidentified.

Table 2
Repeatability (RSD) of migration times for some cations in milk and blood plasma samples (n=30)

	K^+	Na^+	Ca^{2+}	Mg^{2+}
Milk	0.58	0.80	0.91	1.00
Plasma	2.07	2.42	2.47	–

used. This difference is probably due to the presence of proteins in the plasma samples for which CTAB is a less suitable flow modifier, but further investigations are required. While complete elimination of protein wall adsorption has been achieved for the milk samples, the proteins in the blood plasma samples still tend to be partially adsorbed (see Fig. 2). Electropherograms of 20 consecutive direct injections of diluted milk and blood plasma samples are shown in Fig. 3. Table 3 shows the results of the quantitative analysis and reference values. Li^+ was used as an internal standard. A previously described internal standard method [33] was applied.

4. Conclusions

It has been shown that the FIA–CE system with electrokinetic injection can be successfully used for the direct analysis of proteinaceous samples. The combination of this injection mode and the proposed electrolyte composition improves the run-to-run repeatability of migration times of the selected inorganic cations significantly, due to the selective injection discrimination and decreased protein–wall

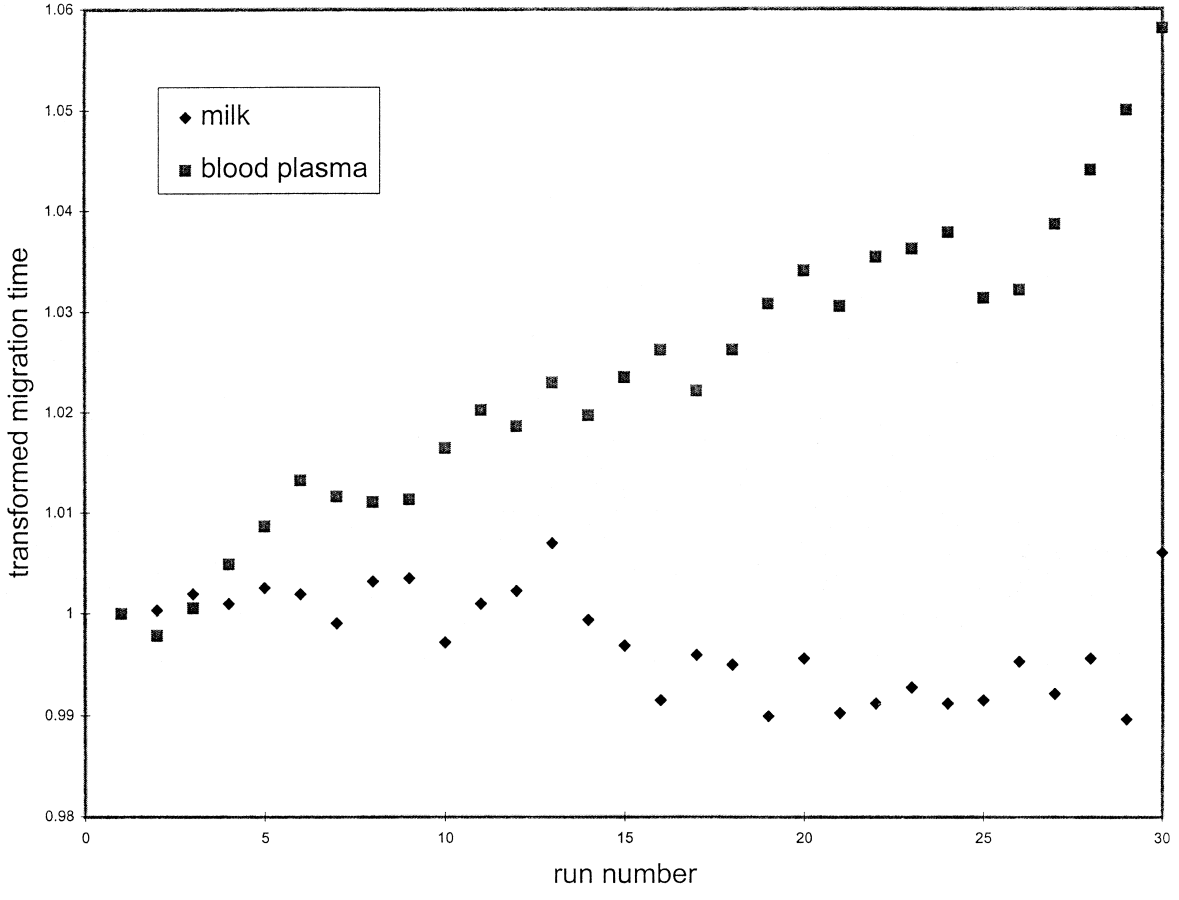

Fig. 2. Change in migration times for 30 consecutive injections of milk and blood plasma samples. Transformed migration times (t_{Mi}/t_{M1}) are plotted against the injection number. Conditions were the same as in Fig. 1.

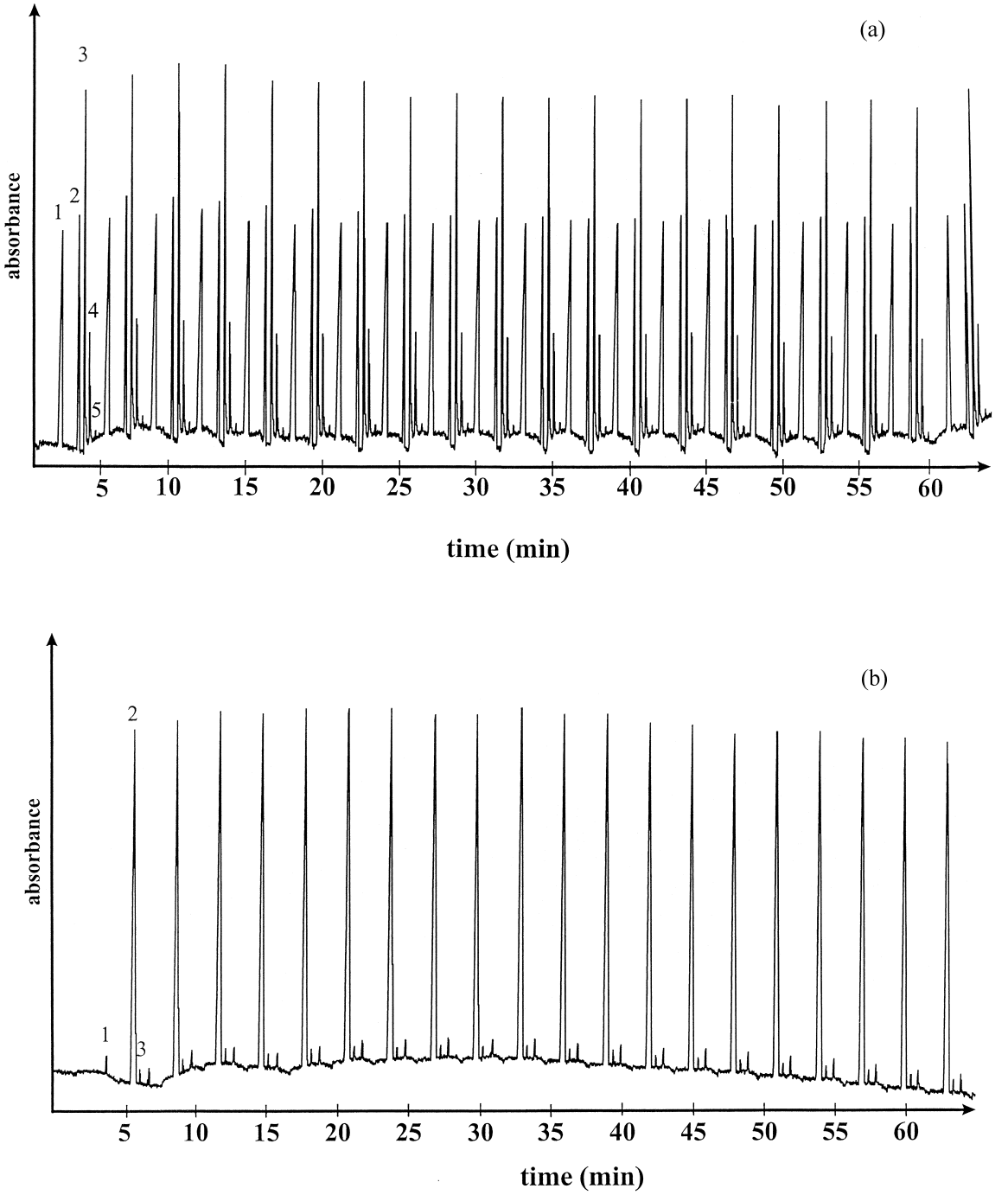

Fig. 3. Electropherograms of 20 consecutive injections of milk (a) and blood plasma (b) samples. Conditions and peaks are the same as in Fig. 1.

Table 3
Measured concentrations (mg/l) of inorganic cations in milk and blood plasma samples

	K^+	Na^+	Ca^{2+}	Mg^{2+}
Milk	3231 (1400)[a]	775 (400)[a]	751 (1200)[a]	84.0 (120)[a]
Plasma	104 (156)[a]	3817 (3300)[a]	24.5 (100)[a]	ND[b] (22)[a]

[a] Reference values.
[b] Not detected.

adsorption. The performance of the FIA–CE system was superior to that observed for commercial CE systems. The evaluation of similar electrolyte systems, additives and other electrolyte parameters that can be used for fast and direct analysis of samples with high protein concentration is in progress.

References

[1] J.W. Jorgenson, K.D. Lukacs, Anal. Chem. 53 (1981) 1298.
[2] J.W. Jorgensson, K.D. Lukacs, Science 222 (1983) 266.
[3] S. Hjerten, J. Chromatogr. 347 (1985) 191.
[4] G.J.M. Bruin, J.P. Chang, R.H. Kulman, K. Zegers, J.C. Kraak, H. Poppe, J. Chromatogr. 471 (1989) 429.
[5] J.K. Towns, F.E. Regnier, J. Chromatogr. 516 (1990) 69.
[6] D. Josic, R. Zeilinger, W. Reutter, A. Bottcher, G. Schmitz, J. Chromatogr. 516 (1990) 89.
[7] K.A. Cobb, V. Dolnik, M. Novotny, Anal. Chem. 62 (1990) 2478.
[8] W. Nashabeh, Z. El. Rassi, J. Chromatogr. 559 (1991) 367.
[9] Y.F. Maa, K.J. Hyver, S.A. Sweedberg, J. High Resolut. Chromatogr. 14 (1991) 65.
[10] M. Huang, W.P. Vorkink, M.L. Lee, J. Microcol. Sep. 4 (1992) 233.
[11] Z.X. Zhao, A. Malik, M.L. Lee, Anal. Chem. 65 (1993) 2747.
[12] D. Schmalzing, C.A. Piggee, F. Foret, E. Carrilho, B.L. Karger, J. Chromatogr. A 652 (1993) 149.
[13] A. Cifuentes, J.M. Santos, M. de Frutos, J.C. Diez-Masa, J. Chromatogr. A 652 (1993) 161.
[14] C.L. Ng, H.K. Lee, S.F.Y. Li, J. Chromatogr. A 659 (1994) 427.
[15] M. Gilges, M.H. Kleemiss, G. Schomburg, Anal. Chem. 66 (1994) 2038.
[16] M. Huang, J. Plocek, M.V. Novotny, Electrophoresis 16 (1995) 396.
[17] M.H.A. Busch, J.C. Kraak, H. Poppe, J. Chromatogr. A 695 (1995) 287.
[18] R.W. Chiu, J.C. Jiminez, C.A. Monnig, Anal. Chim. Acta 307 (1995) 193.
[19] J.-L. Liao, J. Abramson, S. Hjerten, J. Cap. Electrophoresis 2 (1995) 191.
[20] M. Chiari, N. Dell'Orto, A. Gelain, Anal. Chem. 68 (1996) 2731.
[21] G. Kleindienst, C.G. Huber, D.T. Gjerde, L. Yengoyan, G.K. Bonn, Electrophoresis 19 (1998) 262.
[22] J.S. Green, J.W. Jorgenson, J. Chromatogr. 478 (1989) 63.
[23] A. Cifuentes, M.A. Rodriguez, F.J. Garcia-Montelongo, J. Chromatogr. A 742 (1996) 257.
[24] Å. Emmer, M. Jansson, J. Roeraade, J. Chromatogr. 547 (1991) 544.
[25] M.M. Bushney, J.W. Jorgenson, J. Chromatogr. 480 (1989) 301.
[26] H.H. Lauer, D. McManigill, Anal. Chem. 58 (1986) 166.
[27] R.M. McCormick, Anal. Chem. 60 (1988) 2322.
[28] M. Schmitt, F. Saulnier, J. Chromatogr. 640 (1993) 419.
[29] W. Buchberger, K. Winna, M. Turner, J. Chromatogr. A 671 (1994) 375.
[30] P. Kuban, A. Engström, J.C. Olsson, G. Thorsen, R. Tryzell, B. Karlberg, Anal. Chim. Acta 337 (1997) 117.
[31] P. Kuban, R. Pirmohammadi, B. Karlberg, Anal. Chim. Acta 378 (1999) 55.
[32] W. Beck, H. Engelhardt, Fresenius' J. Anal. Chem. 346 (1993) 618.
[33] P. Kuban, K. Tennberg, R. Tryzell, B. Karlberg, J. Chromatogr. A 808 (1998) 219.