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Short communication

New derivatization method for carboxylic acids in aqueous solution for analysis by capillary electrophoresis and laser-induced fluorescence detection

M. Kibler, K. Bächmann*

Darmstadt University of Technology, Petersenstrasse 18, 64287 Darmstadt, Germany

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Abstract

A new derivatization method for carboxylic acids in aqueous solution for analysis with capillary electrophoresis and laser-induced fluorescence detection was developed. The derivatization is based on a phase-transfer reaction with 4-aminofluorescein as fluorophore and dicyclohexylcarbodiimide as activating agent. This reaction procedure includes an enrichment factor of the analyte up to 10-fold. The limits of detection of carboxylic acids are in the range of 3–150 nmol/l. An advantage of this system is the indifference against inorganic matrices like carbonate, chloride, etc., up to the mmol/l range. Using this technique for the first time, the measurement of diurnal profiles of carboxylic acids (C_5–C_9) in ambient air with a time resolution of 1 h is possible.

Keywords: Air analysis; Derivatization, electrophoresis; Environmental analysis; Aminofluorescein; Carboxylic acids; Dicyclohexylcarbodiimide

1. Introduction

The investigation of oxidation processes and the formation of ozone in the atmospheric gas phase is an important subject of current atmospheric research [1,2]. In order to obtain a better understanding of these processes, short-chain carboxylic acids (C_2–C_9) as stable intermediates in the oxidation processes of alkenes [3–7], were determined in ambient air. Therefore, sampling was performed by a misting chamber (scrubber) [8–10] containing a 1 mmol/l sodium hydroxide solution. For this method the achieved collection yield depends on the flow-rate of the air.

The analyte concentration of the scrubber solution depends on the solution volume and the sampling time. The concentrations of the carboxylic acids in the sample are in the low nanomolar range, and due to the 1 mmol/l sodium hydroxide solution in the scrubber, a millimolar carbonate matrix occurs. For this kind of sample no application for capillary electrophoresis (CE) exists (direct-, indirect UV detection [11–14] or conductivity detection [15]) with adequate limit of detection (LOD) and sufficient matrix digestibility, and it is therefore necessary to develop a new method for capillary electrophoresis and laser-induced fluorescent detection (CE-LIF).

*Corresponding author. Fax: +49-6151-163673.

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As aliphatic carboxylic acids lack any suitable chromophores or fluorophores, the sensitive detection of these compounds requires a derivatization. Moreover, the derivatization of a particular functional group of an analyte class provides a high selectivity and the possibility of enrichment of analytes during the derivatization procedure. Labelling of the analytes with a fluorophore is preferable due to the possible high sensitive detection with laser-induced fluorescence.

However, the derivatization of short-chain carboxylic acids in aqueous solution is most challenging because of the low reactivity of the carboxylic acid function in water. Coenen et al. [16] derivatized monocarboxylic acids with 2-nitrophenylhydrazine (NPH) and 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC) in aqueous solution for determination with HPLC. Schneede et al. [17] investigated the derivatization of short-chain dicarboxylic acids with pyrenyldiazomethane (PDAM) in aqueous solution and separation with CE. Yamaguchi et al. [18] developed a procedure for labelling fatty acids with 6,7-dimethoxy-1-methyl-2(1H)-quinoxalinone-3-propionylcarboxylic acid hydrazide (DMEQ-PAH) in aqueous solution after activation with EDC for HPLC. Mechref and El Rassi [19] derivatized carboxylated carbohydrates with 7-aminonaphthalene-1,3-disulfonic acid (ANDSA) and EDC in aqueous solution for CE. Up to now no analytical derivatization procedures for short-chain monocarboxylic acids in aqueous solution and a separation in CE have been reported.

A potential suitable fluorophore has to provide different properties in terms of LIF detection, separation in CE and derivatization of carboxylic acids. The fluorophore needs an excitation in the range of available laser lines and a high quantum yield for a sensitive detection. For the derivatization and separation by CE it has to be soluble in aqueous solution and possess a charged group. At last it needs a functional group for the reaction with carboxylic acids.

4-Aminofluorescein meets all these requirements. The fluorescence of this molecule (excitation, 491 nm; emission, 520 nm) is very intensive, it possesses a carboxylic group for the charge and an amino function that can form stable carboxylic acid amides with the analytes.

2. Experimental

2.1. Chemicals

The carboxylic acids, diethyl ether, dicyclohexylcarbodiimide (DCC), 4-aminofluorescein and other chemicals were purchased from Fluka (Neu Ulm, Germany) or Sigma (Deisenhofen, Germany). All chemicals used were of analytical grade. Deionized water (18 MΩ) was performed by a Milli-Q purification system (Millipore, Eschborn, Germany). The reaction vials were from Eppendorf (Hamburg, Germany).

2.2. Apparatus

The experiments were carried out on a Spectrophoresis 100 (TSP, Egelsbach, Germany) with a KF-2 LIF detector system (Sopra, Büttelborn, Germany) or on a modular laboratory-built CE-system with a KF-1 laser-induced fluorescence detection (Sopra, Büttelborn, Germany), equipped with a He-Cd laser, 442-nm excitation (50 mW) and a 470-nm cut-off filter. Untreated fused-silica capillaries with 50 μm I.D. were employed (Chromatographie Service, Langerwehe, Germany). The total length of the capillaries was 63 cm for the Spectrophoresis 100 and 79 cm for the modular system, the length to the detection window was 55 cm in both cases. The measurements with ind. UV detection were made with a Spectraphoresis 1000 (TSP, Egelsbach, Germany). For the mixing of the reaction solution a REAX 2000 (Heidolph, Kelheim, Germany) was used.

2.3. Derivatization of carboxylic acids

The carboxylic acid standard was prepared in an aqueous 1 mmol/l sodium hydroxide solution. As reaction vial, a capped 2-ml micro test tube (save lock vial) was used. A total of 1.5 ml of the sample or standard solution were used. The pH value of 2 was adjusted with 1 mol/l hydrochloric acid using a pH meter. Ten μl of a 100 μmol/l caprinic acid solution was added as internal standard. A total of 375 μl of a solution of 250 mmol/l DCC in diethyl ether were added to 1.5 ml of this standard. The two

phases of the reaction solution were mixed for 10 min at ambient temperature in a vortex mixer (6000 rpm) and then the organic phase was separated. The procedure was repeated with another 375 μl of DCC in ether. The two ether phases were combined and 10 μl of 50 mmol/l 4-aminofluorescein in dimethylformamide were added. The mixture was vortexed (6000 rpm) at ambient temperature for 1 h. The organic phase was extracted three times with 50 μl of 1 mmol/l sodium hydroxide solution. A portion of the resulting 150 μl sodium hydroxide solution was injected into the CE.

3. Results and discussion

3.1. Optimization of derivatization conditions

The derivatization of carboxylic acids with 4-aminofluorescein is a two-step reaction described in Fig. 1. First DCC and the acid form an isourea derivative with a reactivity comparable to a carboxylic acid anhydride. As the formed product is nonpolar, a phase transfer is involved from the aqueous to the organic phase. In the separated organic phase the fluorophore reacts with the isourea derivative to the corresponding carboxylic acid amide and an urea derivative. In the last step the labelled acids are reextracted from the organic phase into an aqueous sodium hydroxide solution, by changing the configuration from the lactoide to the chinoide form. In order to achieve the highest possible yield, the reaction was optimized in terms of pH value of the aqueous solution, concentration of the DCC, reaction time for the activation with DCC, repetition of the DCC reaction, concentration of 4-aminofluorescein, reaction time with the fluorophore, extraction time, and repetition of the extraction. The optimum conditions found are described in Section 2.3. The resulting yields for the reaction are between 63% for pelargonic acid and 9% for acetic acid (see Table 1) which was calculated by the peak area of the derivatives of the acids with a quantum yield for the acetaminofluorescein of 0.48 and the peak area of 4-aminofluorescein. This quantum yield was determined by a comparison of the signals of 4-aminofluorescein (quantum yield, 0.02 [20]) and 4-acetaminofluorescein measured in one sample with adsorption and fluorescence detection. To validate the fluorescence measurements the known quantum yield of 4-aminofluorescein was determined by correlation of the peak areas of fluorescein and 4-aminofluorescein. The result of 0.02 agrees with the literature [20].

A further activation of the isourea derivative formed between DCC and the carboxylic acids with hydroxybenzotriazol (HOBT) according to a literature procedure [21,22] even decreased the observed reaction yield, and was thus not included in the derivatization here described.

Using this procedure the derivatization of mono- and dicarboxylic acids in aqueous solution was achieved.

3.2. Analytical conditions

The optimum conditions for the buffer system are 50 mmol/l lithium borate buffer at pH 10 (prepared by titration of a boric acid solution with lithium hydroxide), containing 15% (v/v) methanol. Lithium was used as coion because its mobility is 20% smaller than sodium. This lower contribution to the electrolyte conductivity causes an increase of the plate numbers.

The samples were injected at the anode hydrostatically or hydrodynamically. A volume of approximately 9 nl was injected. The applied field strength for the separation was 450 V/cm with a current of 26 μA. Fig. 2 shows an electropherogram of a 5 μmol/l standard solution of mono- and dicarboxylic acids.

3.3. Calibration, reproducibility and limits of detection

The acids were calibrated from 10 μmol/l down to 100 nmol/l. The regression coefficients R^2 are in the range of 0.998–0.999 for each compound ($n=5$). The reproducibility of the migration times was found to be <1% R.S.D. and for the peak area between 16 and 6% R.S.D. (see Table 1). The calculated limits of detection for the monocarboxylic acids are between 3 nmol/l for caprylic acid and 150 nmol/l for acetic acid. The measurement of the reaction blank shows only contaminations of acetic acid (75–90

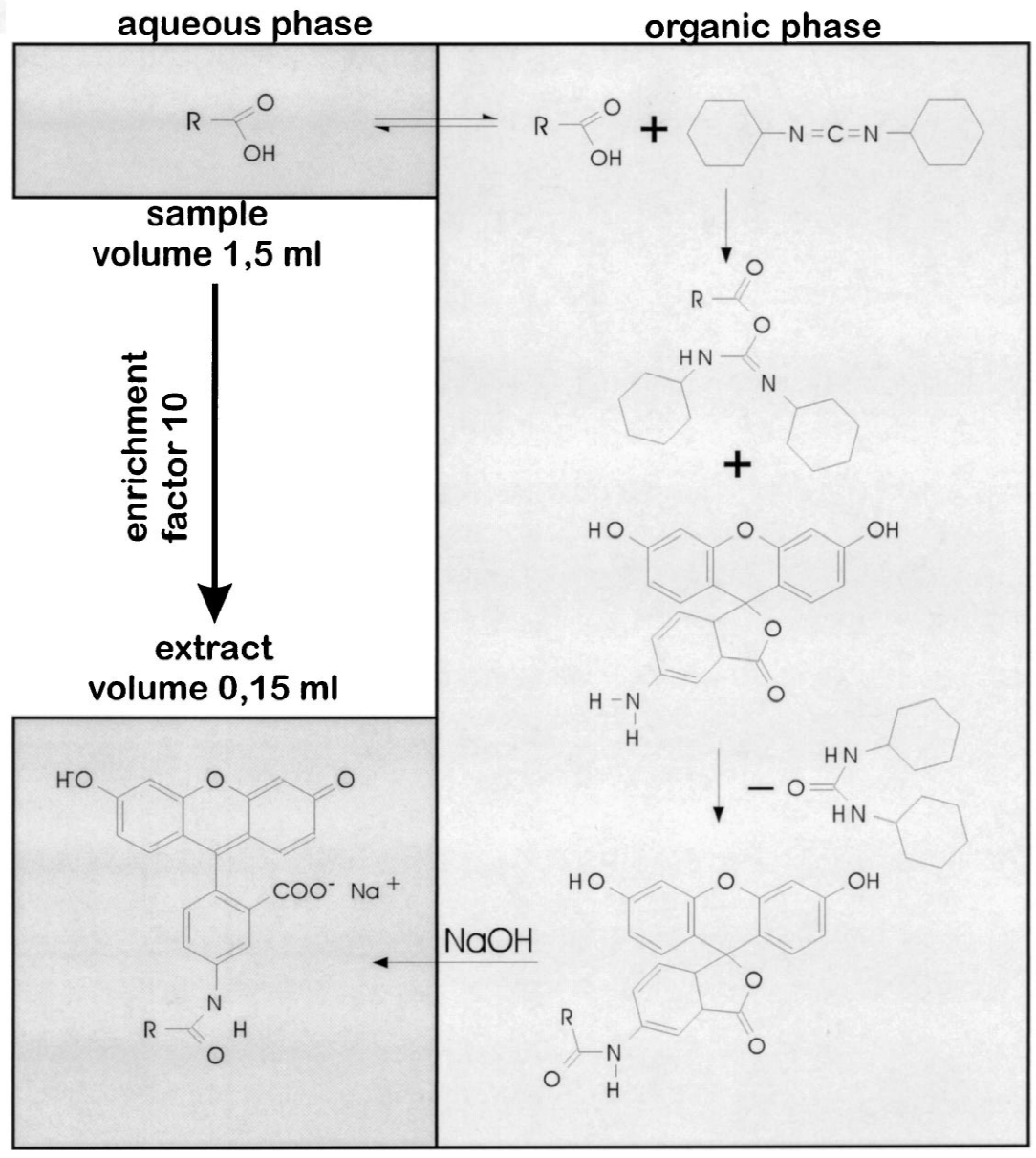

Fig. 1. Derivatization of carboxylic acids with DCC and 4-aminofluorescein using phase transfer reaction.

nmol/l) and propionic acid (40–55 nmol/l). Even lower detection limits should be possible, but the practically obtained values are limited by the purity of the employed reagents and by potential side reactions during the derivatization and not by the detection system.

3.4. Determination of monocarboxylic acids in the atmospheric gasphase

Ambient atmospheric air was sampled with a misting chamber (scrubber) for 1 h, giving an air volume of 400 l. The scrubber contained a 1 mmol/l

Table 1
Reproducibility, reaction yield and limits of detection for the derivatization with 4-aminofluorescein[a]

	RSD (n=6) migration-time	RSD (n=6) corr. peak area	Reaction yield (%)	LOD (nmol/l)
Pelargonic acid	0.9	6	63	9
Caprylic acid	0.9	8	69	3
Oenathic acid	0.9	8	58	30
Capronic acid	0.9	12	38	45
Valeric acid	0.9	13	29	50
Butyric acid	0.9	12	23	70
Propionic acid	0.9	13	13	130
Acetic acid	0.9	16	9	150

[a] The reaction yield is calculated by a measured quantum yield of 0.48 for acetaminofluorescein.

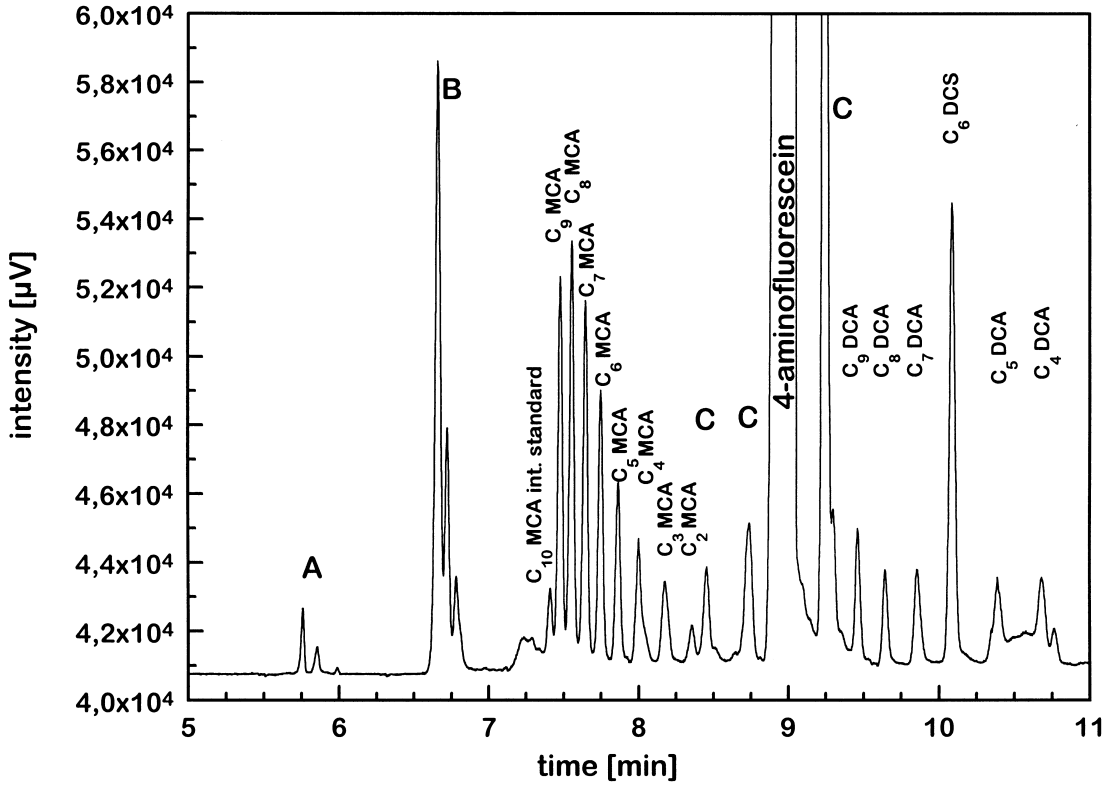

Fig. 2. CE separation with LIF detection of 5 μmol/l mono- and dicarboxylic acids (MCA, DCA) derivatized with 4-aminofluorescein. The concentration of caprinic acid (C_{10} MCA) as internal standard is 660 nmol/l. Peaks A are from the derivatization, peaks B are the di-derivatives from C_9 to C_7 DCA, peaks C are impurities from 4-aminofluorescein. Conditions of derivatization are described in Section 2.3. The volume of the sample is 1.5 ml, and 0.15 ml after derivatization. Electrolyte: 50 mmol/l lithium borate; pH 10; 15% (v/v) methanol; +30 kV, 26 μA. Capillary: length, 79 cm, 55 cm to detector, 75 μm I.D. Injection: hydrostatically, 15 cm, 30 s (9 nl).

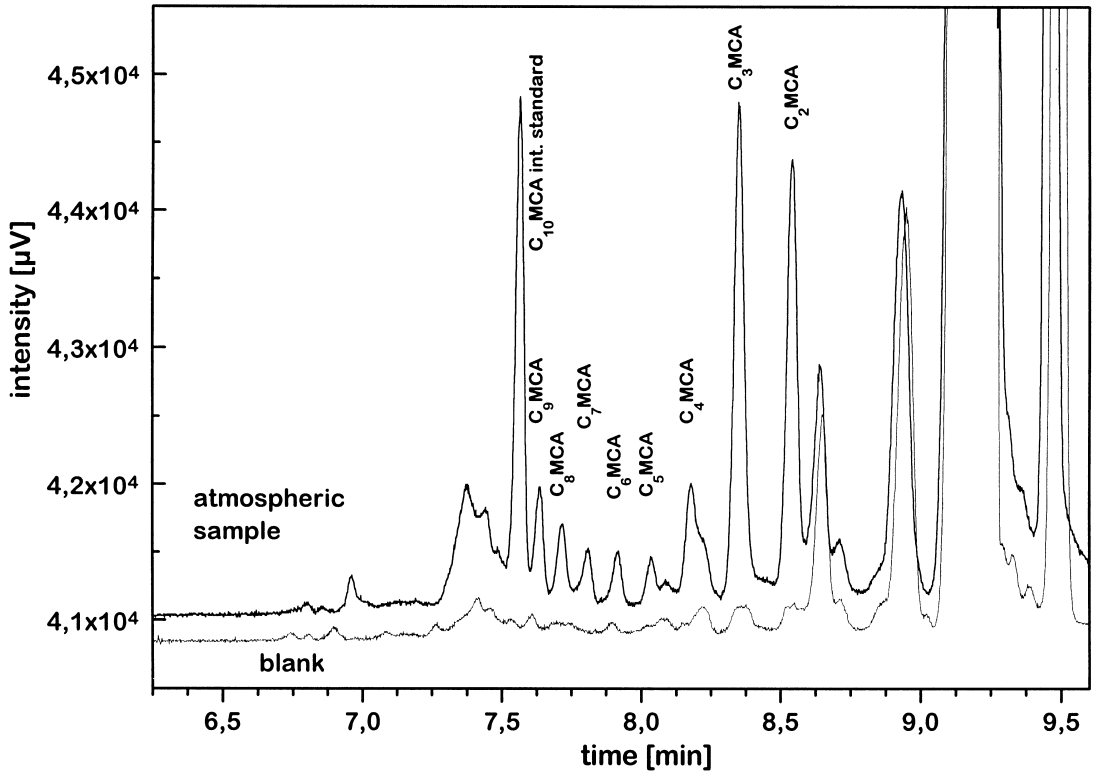

Fig. 3. Determination of an atmospheric air sample and a reaction blank by CE-LIF. The concentrations of the acids were 9 μmol/l for acetic, 3.5 μmol/l for propionic, 530 nmol/l for butyric, 150 nmol/l for valeric, 170 nmol/l for capronic and oenanthic, 20 nmol/l for caprylic and 90 nmol/l for pelargonic acids, respectively. Conditions are described in Fig. 2

sodium hydroxide solution. The sample solution was derivatized according to Section 2.3 and analyzed with the buffer system described in Section 3.3. Fig. 3. shows an electropherogram of a reaction blank typical and an air sample collected by a scrubber. Besides caprinic acid as internal standard the homologous from acetic to pelargonic acid was determined. The new developed analytical technique in combination with the scrubber allows the measurement of diurnal profiles of monocarboxylic acids (C_5–C_9) in ambient air with a time resolution of 1 h for the first time.

4. Conclusion

A new derivatization in aqueous solution for the determination of carboxylic acids using CE with LIF detection in the atmospheric gasphase is shown. The labelling of the acids is based on the reaction of 4-aminofluorescein activated with dicyclohexylcarbodiimide (DCC). The derivatization procedure involves a phase transfer and reextraction process. An up to 10-fold enrichment of the analytes is achieved. The obtained detection limits range between 3 nmol/l for caprylic acid and 150 nmol/l for acetic acid. The calibration is linear from 100 nmol/l to 10 μmol/l with regression coefficients R^2 between 0.998 and 0.999 for each compound. The reproducibility of migration times is $<1\%$ R.S.D. Using the misting chamber as sampling system this new method allows the measurement of diurnal profiles of monocarboxylic acids (C_5–C_9) in ambient air with a time resolution of 1 h for the first time.

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References

[1] M.E. Jenkin, Atmos. Environ. 31 (1997) 81–104.
[2] R.G. Derwent, M.E. Jenkin, S.M. Saunders, Atmos. Environ. 30 (1996) 181–199.
[3] R. Atkinson, Atmos. Environ. 23 (1990) 911–920.
[4] W.P.L. Carter, Atmos. Environ. 24A (1990) 481–518.
[5] D. Grosjean, E. Grosjean, E.L. Williams II, Environ. Sci. Technol. 28 (1994) 186–196.
[6] S. Hatakema, T. Tanonaka, J. Weng, H. Bandow, H. Takagi, H. Akimoto, Environ. Sci. Technol. 19 (1985) 935–942.
[7] S. Hatakema, M. Ohno, J. Weng, H. Takagi, H. Akimoto, Environ. Sci. Technol. 21 (1987) 52–57.
[8] W.R. Cofer, V.G. Collins, R.W. Talbot, Environ. Sci. Technol. 19 (1985) 557–560.
[9] R.W. Talbot, K.M. Beecher, R.C. Harriss, W.R. Coffer III, J. Geophys. Res. 93 (1988) 1638–1652.
[10] W.R. Hartmann, M.O. Andreae, G. Helas, Atmos. Environ. 23 (1989) 1531–1533.
[11] H. Angel, Clin. Chem. 42 (1996) 477–478.
[12] D. Vogller, A. Zemann, G.K. Bonn, M.J. Antal, J. Chromatogr. A 758 (1997) 263–276.
[13] A. Mainka, P. Ebert, M. Kibler, T. Prokop, B. Tenberken, K. Bächmann, Chromatographia 45 (1997) 158–162.
[14] A. Röder, K. Bächmann, J. Chromatogr. A 689 (1995) 305–311.
[15] B.L. De Backer, L.J. Nageles, Anal. Chem. 68 (1996) 4441–4445.
[16] A.J.J.M. Coenen, M.J.G. Kerkhoff, R.M. Heringa, S.J. van der Wal, J. Chromatogr. 593 (1992) 243–253.
[17] J. Schneede, J.H. Mortensen, G. Kvalheim, P.M. Ueland, J. Chromatogr. A 669 (1994) 185–193.
[18] M. Yamaguchi, T. Iwata, K. Inoue, S. Hara, M. Nakamura, Analyst 115 (1990) 1363–1366.
[19] J. Mechref, Z. El Rassi, Electrophoresis 15 (1994) 627–634.
[20] R.P. Haugland, Handbook of Fluorescent Probes and Research Chemicals, 6th ed, 1996.
[21] W. König, R. Geiger, Chem. Ber. 103 (1970) 788–798.
[22] M. Jung, W.C. Brumley, J. Chromatogr. A 717 (1995) 299–308.