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UV detection of derivatized carbonyl compounds in rain samples in capillary electrophoresis using sample stacking and a Z-shaped flow cell

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Abstract

The feasibility of trace analysis of carbonyl compounds in size classified rain samples by capillary electrophoresis (CE) is demonstrated. Limits of detection (LOD) in the range of 170–300 nmol/l were reached after derivatization of aldehydes with 5-(dimethylamino)-naphthalene-1-sulfon-hydrazide (dansylhydrazine, DNSH) and using both a Z-shaped flow cell (ZFC) and sample stacking. Advantages and disadvantages of the detection using a ZFC providing an optical path length of 3 mm were examined. The improvement of LOD by factors of 20 and a loss of resolution for example from 4.7 (on-column detection) to 2.5 (ZFC) is due to the extended light path. Furthermore an enrichment of the analytes is possible by sample stacking. Problems according to the higher susceptibility of the detection system to disturbances in the electrolyte system could be solved using a special sample injection technique.

Keywords: Rain water; Water analysis; Stacking; Flow cell; Environmental analysis; Derivatization, electrophoresis; Carbonyl compounds

1. Introduction

Analysis of carbonyl compounds is usually carried out by gas or liquid chromatography due to the very low limits of detection (LOD) down to 20 n*M* [1–3]. If separation of carbonyl compounds is needed in aqueous microvolumina of about 1 μl (when no enrichment by extraction is possible) CE tends to be the method of choice.

Since carbonyl compounds are important pollutants of the atmosphere their determination in atmospheric samples is a main part of environmental research [4–7]. To determine the importance of rain as a cleaning mechanism for the atmosphere it is necessary to analyze the concentration of pollutants in relation to dropsize. According to a described procedure [8] the raindroplets are frozen in liquid nitrogen and then separated on sieves owing to their size. So, it is possible to analyze single raindroplets [9] or size classes. For the determination of size classified rain samples only a few μl of an aqueous sample are available. For this purpose the present method was developed.

Since carbonyl compounds have no mobility in the electric field and no optical detectability in the required concentration range they have to be derivatized. The most applied derivatization reagents for determination of carbonyl compounds in liquid chro-

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matography are 2,4-dinitrophenylhydrazine (DNPH) [10,11] and 5-(dimethylamino)-naphthalene-1-sulfonhydrazide (dansylhydrazine, DNSH) [3]. As already reported the derivatization for analysis by CE is carried out by DNSH [12]. The obtained hydrazones provide sufficient sensitivity for both UV and fluorescence detection. On the other side a deprotonation of the derivatives and therefore a separation by CE as organic anions is possible.

An improvement of the LOD can be achieved using sample stacking as already shown by Chien and Burgi [13–16]. However, the hydrazones have a much lower mobility than the EOF and would not reach the detector using negative polarity during separation, so that they have to be separated at positive polarity. For stacking of the analytes the stacking procedure including removal of the sample matrix has to be carried out before starting the run at negative polarity. As calculations show there are strong limitations in maximum sample volume to be stacked. Using the electrophoretic mobility of the analytes and the mobility of the EOF it is possible to calculate the maximum filled length of the capillary without any loss of analytes [13]. Depending on this fact only a certain part of the capillary can be filled with sample. To obtain a significant improvement of the LOD stacking alone is not sufficient, and other methods as e.g., improvement of the detection mode have to be used simultaneously.

UV-absorbance detection is the most universal detection method in CE. However, the optical path length in on-column detection is given by the capillary inner diameter, which is normally 50–75 μm. Thus, relative limits of detection in CE are not sufficient especially in trace analysis. Several attempts have been made to enhance the sensitivity by extending the light path through the capillary [17–20].

Tsuda et al. have shown that the use of a rectangular capillary with an optical path length of 1 mm has some advantages over normal on-column detection [17]. It shows an increase in sensitivity and reduces optical distortion and light scattering due to the flat capillary wall. Using a multireflection cell Wang et al. [18] increased the effective detection path length. Furthermore the so called bubble cell developed by Hewlett-Packard is a possibility to improve the LOD by extending the light path through the capillary [19].

Another approach solving this problem is the use of a Z-shaped flow cell (ZFC) where the detection is carried out longitudinal to the capillary axis [20]. The design of the detection cell provides an optical path length of 3 mm. Compared with detection in capillaries having inner diameters (I.D.) of 50 μm and 75 μm a theoretical improvement of the LOD by factors of 60 and 40, respectively can be calculated. Since the improvement does not only depend on the optical path length but also on the detection cell volume, different noise levels in the compared capillaries, different light scattering at the walls and other parameters the theoretically possible factors will not be reached.

2. Experimental

2.1. CZE system

Using a laboratory-made CZE system consisting of a programmable absorbance detector type ABI 785A (Applied Biosystems, Weiterstadt, Germany) and a high voltage power supply (F.u.G. Electronic, Rosenheim, Germany) as main parts, the separations were carried out with conventional untreated fused-silica capillaries (50 μm I.D.) from CS-Chromatographie Service (Langerwehe, Germany). A laboratory-made safety box was used holding vials, electrodes and the capillary. The Z-shaped flow cell is commercially available from LC-Packings (LC-Packings International, Amsterdam, Netherlands). Data were processed by APEX chromatography software (Autochrom, Milford, USA). Therefore the output of the signal is in the unit μV (1 V=1 AU).

2.2. Chemicals

All solutions electrolytes and standards were prepared using ultra pure water from a Milli-Q supply (Millipore, Eschborn, Germany) distilled according to a described procedure [2]. Aldehydes and ketones were purchased from Fluka (Fluka-Chemie, Neu-Ulm, Germany). The other reagents in analytical reagent grade and the used isopropanol (SLSI-selectipure) were obtained from Merck (Darmstadt, Germany).

The electrolyte consists of 5 m*M* Na_3PO_4, 10 m*M*

$Na_2B_4O_7$ and 20% acetonitrile. The pH was adjusted to 8 with H_2SO_4.

2.3. Sample preparation

For the derivatization of the carbonyl compounds a reagent solution of 2 m*M* DNSH in isopropanol was prepared. Using isopropanol the obtained blank values of formaldehyde and acetaldehyde are at the detection limit. Only acetone showed increased values. The choice of the solvent depends on the analytical requirement determining which blank value can be tolerated. This solution can, if stored in a refrigerator, be maintained for at least one week. The derivatization of carbonyl compounds is carried out according to a described procedure [12] by mixing of the aqueous sample solution and the reagent solution at a ratio of 1:1 and heating the mixture for 1 h in a water bath at 50°C. For hydrostatic injection the sample was only cooled down to room temperature. If sample stacking was used the pH of the sample had to be adjusted to 9 using tris(hydroxymethyl)aminomethane (Tris) providing a complete deprotonation and thereby a complete stacking of all analytes.

3. Results and discussion

3.1. Enrichment and separation of the analytes

The obtained hydrazones deprotonated at pH values above 7 are mobile in the electric field and can be separated by CE (see Fig. 1) [12]. The employed electrolyte contains 20% of acetonitrile. This modifier acts as slowing agent for the EOF on the one hand, and supports the solubility of the hydrazones in aqueous media on the other hand. As the analytes are anions in the used pH range they can be enriched using sample stacking. However, as they have a lower mobility as the EOF they have to be separated and detected in the same direction as the EOF. The stacking procedure carried out at negative polarity has to be stopped to switch the polarity for separation [13]. To guarantee complete stacking of the analytes the pH of the sample has to be adjusted by addition of Tris to a value of 9. The low conductivity of Tris ensures the proper enrichment of the analytes because the sample zone has a significantly lower conductivity than the electrolyte. At pH 9 the hydrazones are completely deprotonated having their highest mobility which is, however, only one tenth of the mobility of the EOF. According to Chien and Burgi [13] only one tenth of the capillary could be filled with sample without any loss of analytes during stacking. To diminish laminar flow profiles at the boundary during stacking —owing to the different velocities of the EOF in both zones— the concentration of organic modifier acetonitrile in the electrolyte was optimized to 20% decelerating the EOF to a value comparable to the velocity of the EOF in the sample compartment. The progress of matrix removal is controlled by monitoring the current.

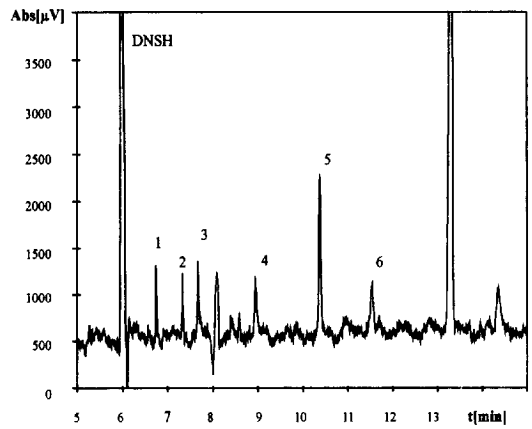

Fig. 1. Separation of DNSH derivatives of carbonyl compounds. Capillary: total length 80 cm, length to the detector 60 cm; electrolyte: 5 m*M* Na_3PO_4, 10 m*M* $Na_2B_4O_7$, 20% acetonitrile, pH=8.0; detection: 218 nm; high voltage: 25 kV; injection: hydrostatic 1 min, 10 cm. 1=Acetone, 2=acetaldehyde, 3=propionaldehyde, 4=benzaldehyde, 5=formaldehyde, 6=methylglyoxal each 40 μ*M*.

With hydrostatic injection only a volume of 20 nl is injected into the capillary whereas sample stacking allows an enrichment of the 10-fold volume. In Fig. 2 two separations with and without stacking are shown. As pointed out in Table 1 the LOD can be improved using stacking only by a factor of 10 due to the enriched sample volume resulting from the fact that the analytes are very slow anions. A disadvantage is the simultaneous stacking of the decomposition products of the derivatizing agent DNSH. The products have a higher mobility than the analytes and are stacked in front of the sample zone.

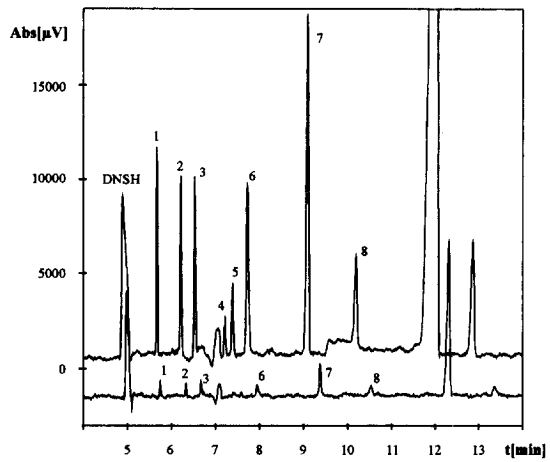

Fig. 2. Comparison between hydrostatic injection (bottom) and stacking (top). For separation conditions see Fig. 1. Injection for stacking: 10 cm, 10 min. 1=Acetone, 2, 4=acetaldehyde, 3, 5=propionaldehyde, 6=benzaldehyde, 7=formaldehyde, 8=methylglyoxal each 40 μ*M*.

The occurrence of matrix effects in the sample zone created by stacking can lead to baseline disturbances.

3.2. Use of a Z-shaped flow cell

The LOD can further be improved using a ZFC. The design of this cell provides an optical path length of 3 mm. Compared to the optical path length in a capillary having an inner diameter of 50 μm a theoretical improvement of the LOD by a factor of 60 can be calculated. We obtained only values of 20 (see Table 1). These results are not in accordance with the theoretical predictions. However, they are significantly higher as reported values in literature [20]. Chervet et al. found only an improvement of the signal to noise ratios by factors of 4–6 [20]. These various values are mainly the result of two different facts: the cell volume in on-column detection and the influence of the zone width on the sensitivity in extended light path detection.

At first, theoretical calculations of the improvement factors can not be made considering only the optical path length but the cell volume, since path length given by the capillary inner diameter is only achieved if the UV beam cuts the center of the capillary. The width of the detection cell lit by the UV beam cannot be neglected. To avoid band broadening the detector cell width should not exceed one tenth of the zone width. Within these limits an increase in sensitivity can be achieved by a slightly increased detector window length. The quite different designs of cells in commercially available UV detectors can lead to quite different cell volumes and therefore to significant differences in the factors obtained in comparison to the ZFC. In our investigation we used a detection cell with a focusing lens at the capillary surface providing a very small detector window. For this reason the high enhancement factors compared to the literature are explainable.

The second point that can lead to insufficient enhancement factors is the zone width. Peak widths in CZE may be smaller than 3 mm and so the path length and the cell volume in extended light path detection is not completely used for detection. Therefore, the maximum improvement factors depend strongly on the peak width. In on-column detection the zone width can be estimated from the migration time and the observed peak width if peak broadening due to the cell width is neglected. Despite sample stacking the used hydrazones show a zone width of several millimeters (5 mm) so that a complete use of the cell volume of the ZFC is guaranteed.

We have observed the phenomenon that the noise

Table 1
LOD (3σ) of different detection and injection methods

Component	LOD (hydrostatic injection, 50 μm I.D.) (μ*M*)	LOD (stacking, 50 μm I.D.) (μ*M*)	LOD (hydrostatic injection, Z-shaped flow cell) (μ*M*)	LOD (stacking, Z-shaped flow cell (n*M*)
Formaldehyde	30	3.5	1.5	170
Acetaldehyde	39	4.5	2	250
Propionaldehyde	30	4	1.5	180
n-Valeraldehyde	27	3.5	1.5	200
Benzaldehyde	54	6.5	2.5	300

detected in the ZFC is only half the noise in normal on-column detection. A possible reason for this fact can be the longitudinal lighting of the capillary. In this way less stray light reaches the photocell whereas no light within the capillary is lost due to light diffraction or refraction.

Taking into account these facts the use of a ZFC can lead to a much more sensitive detection in the direct UV-mode as is demonstrated by the obtained very low LOD (see Table 1). The observed decreased resolution is due to the extended light path. In Table 2 the resolution for two chosen pairs of analytes is shown. It is obvious that resolution in on-column detection has to be excellent to ensure sufficient resolution using extended light path detection.

3.3. Depletion of the disturbing reagent

In Fig. 3 the improved sensitivity using a ZFC is shown. This figure shows an extreme increase in the level of the baseline which can not be seen in on-column detection. The explanation for this effect is the continuous decomposition of the derivatizing reagent during the separation which proceeds very rapidly in aqueous media. As could be demonstrated this phenomenon is due to the concentration of DNSH in the sample. By this reaction species with a high mobility in the electric field (e.g., sulfonic acids) are produced. These species are separated from the EOF which transports the uncharged DNSH and can be detected at the same wavelength as the analytes. The continuous production of these species leads to an increase of the baseline (not detectable in normal on-column detection due to the lower LOD). It is difficult to solve this problem because DNSH has to be added in excess to the sample and the stacking process is stopped at a point where a certain amount of the sample containing the DNSH is present in the capillary. A possible solution to this problem is the depletion of the DNSH during the stacking process of the analytes. Fig. 4 shows

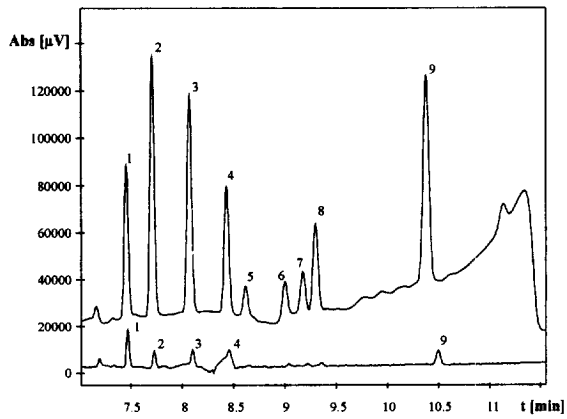

Fig. 3. Comparison between on-column detection (bottom) and Z-shaped flow cell (top). For separation conditions see Fig. 1. Injection: stacking. 1=Acetone, 2, 5=*n*-valeraldehyde, 3, 6= propionaldehyde, 4, 7=acetaldehyde, 8=benzaldehyde, 9= formaldehyde.

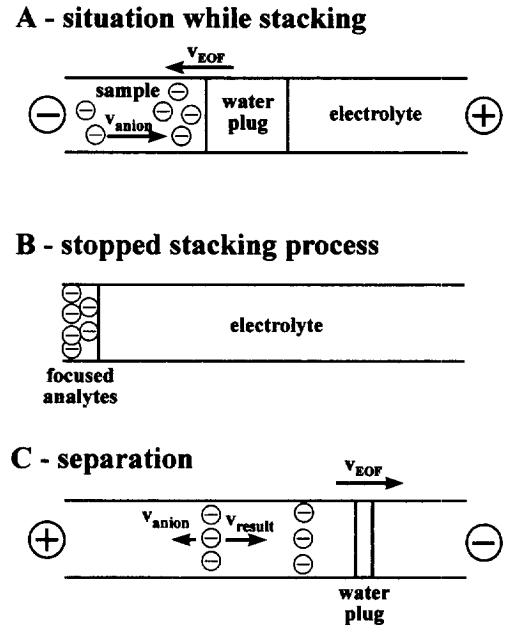

Fig. 4. Scheme of the DNSH depletion procedure.

Table 2
Comparison of resolution in on-column detection and in detection using a ZFC

Resolution (R_s) of:	R_s (50 µm I.D.)	R_s (Z-shaped flow cell)
n-Valeraldehyde/propionaldehyde	4.7	2.5
Acetaldehyde (2nd isomer)/benzaldehyde	1.4	1.0

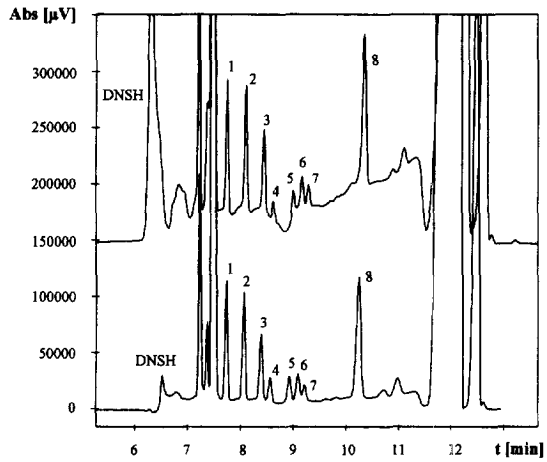

Fig. 5. Comparison between separations carried out with (bottom) and without (top) depletion of DNSH. For separation conditions see Fig. 1. 1, 4=*n*-Valeraldehyde, 2, 5=propionaldehyde, 3, 6=acetaldehyde, 7=benzaldehyde, 8=formaldehyde.

schematically the procedure for DNSH depletion. Before injection of the sample a water plug (pH=9 to obtain high mobility of the analytes in this zone) is injected into the capillary (see Fig. 4A). The sample plug containing the excess reagent can be removed completely from the capillary during stacking at negative polarity. The analytes stack at the boundary between the water plug and the electrolyte. If the current reaches 90% of the separation current the process is stopped manually (Fig. 4B). The polarity is switched again and separation occurs (Fig. 4C). As can be seen in Fig. 5 the DNSH reagent peak is decreased significantly. The level of the baseline is also decreased and the quantification of the peaks is facilitated.

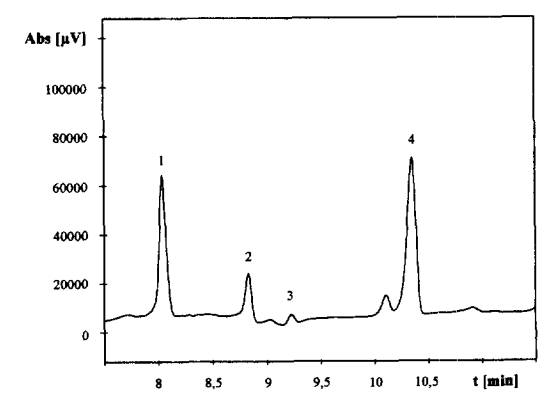

Fig. 6. Electropherogram of a size class of a rain sample. For separation conditions see Fig. 1. Injection: stacking; average diameter of the droplets: 450 µm. 1, 2=acetaldehyde, 3=benzaldehyde, 4=formaldehyde.

Based on this concept it is possible to determine carbonyl compounds in size classified rain samples. Fig. 6 shows the electropherogram of a rain sample using stacking and the ZFC. Derivatization is carried out as described above down to 1 µl sample volume. Using the combination of the two enhancement techniques the identification of more species in rain compared to on-column detection is possible.

4. Conclusion

The applicability of a Z-shaped flow cell for trace analysis in CE is shown. To reach lowest LOD using this detector cell the cell volume should not exceed the peak volume. Therefore peaks should have a peak width at least comparable to the used detector path length. Taking into account these facts an improvement of the LOD by a factor of 20 is reached using a ZFC. Including stacking the LOD of the method is decreased by a factor of 200 compared to hydrostatic injection and on-column detection. The applicability of this method for the determination of carbonyl compounds in aqueous microvolumina as size classified rain is demonstrated.

References

[1] W.H. Glaze, M. Koga and D. Cancilla, Environ. Sci. Technol., 23 (1989) 838.
[2] D.A. Cancilla, C.-C. Chou, R. Barthel and S.S. Que Hee, J. AOAC Int., 75 (1992) 842.
[3] W. Schmied, M. Przewosnik and K. Bächmann, Fresenius Z. Anal. Chem., 335 (1989) 464.
[4] A. Vairavamurthy, J.M. Roberts and L. Newman, Atmos. Environ., 26A (1992) 1965.
[5] D. Grosjean, Atmos. Environ., 22 (1988) 1637.
[6] S. Steinberg and I.R. Kaplan, Int. J. Environ. Anal. Chem., 18 (1984) 253.
[7] D.J. Munger, B.C. Jacob and L.W. Horowitz, J. Geophys. Res., 100 (1995) 9325.
[8] K. Bächmann, I. Haag and A. Röder, Atmos. Environ., 27A (1993) 1951.
[9] B. Tenberken and K. Bächmann, J. Chromatogr. A, 755 (1996) 121.

[10] J.W. Birks et al., Chromatographia, 32 (1991) 33.
[11] J. Lehotay and K. Hromulakova, J. Liq. Chromatogr., 17 (1994) 579.
[12] K. Bächmann, I. Haag, K.Y. Han and R.Q. Schmitzer, Fresenius J. Anal. Chem., 346 (1993) 786.
[13] R.-L. Chien and D.S. Burgi, Anal. Chem., 64 (1992) 1046.
[14] R.-L. Chien and D.S. Burgi, Anal. Chem., 64 (1992) 489.
[15] R.-L. Chien and D.S. Burgi, J. Chromatogr., 559 (1991) 141.
[16] R.-L. Chien and D.S. Burgi, J. Chromatogr., 559 (1991) 153.
[17] T. Tsuda, J.V. Sweedler and R.N. Zare, Anal. Chem., 62 (1990) 2149.
[18] T. Wang, J.H. Aiken, C.W. Huie and R.A. Hartwick, Anal. Chem., 63 (1991) 1372.
[19] D.N. Neiger, P. Kaltenbach and H.-J.P. Sievert, Electrophoresis, 15 (1994) 1234.
[20] J.P. Chervet, R.E.J. van Soest and M. Ursem, J. Chromatogr., 543 (1991) 439.