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# Analysis of individual raindrops by capillary zone electrophoresis

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## Abstract

A new technique for the analysis of individual raindrops by CZE was developed. This system is at present the only method which facilitates a simultaneous determination of concentration of trace elements in individual raindrops and the size of individual raindrops. The use of this method allows in future works the determination of the concentration of trace compounds as a function of the radius of individual raindrops. This paper presents the development of an injection technique and the development of a method to store and to handle small raindrops (i.e., volumes in the nanoliter range). The storing method makes it possible to handle drops in the nanoliter range without any contamination. The injection occurred using a new developed microinjection system which allows a direct introduction of individual raindrops into the separation capillary. Furthermore different methods to determine the volume of single raindrops were investigated. To employ this new system for the analysis of real samples calibration graphs for inorganic cations were made. The absolute limits of detection for the cations  $\text{NH}_4^+$ ,  $\text{K}^+$ ,  $\text{Na}^+$ ,  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$  and  $\text{Zn}^{2+}$  ranged from 40–80 fmol using indirect UV detection. The applicability of the new developed method to real rain samples was demonstrated.

*Keywords:* Water analysis; Environmental analysis; Rain water; Air analysis; Inorganic cations

## 1. Introduction

In environmental research it is the main intention to develop models which allow to predict anthropogenic influences on natural processes. For these studies processes in the atmosphere are of great importance. There are three main points which influence the atmosphere: (1) anthropogenic and natural emissions of substances; (2) chemical reactions; (3) dry and wet deposition.

Dry and wet deposition are the natural cleaning mechanisms of the atmosphere. In particular physical and chemical parameters which influence wet deposition are not well understood because wet deposition is a complex multiphase system. Developing model

calculations [1,2] and simulations in the laboratory it is hardly possible to take all parameters (e.g., condensation nuclei, gas scavenging, aerosol scavenging, evaporation, sunlight, collision, coalescence and break up) into account. Field measurements give suitable data which serve as a basis to estimate these parameters.

With this aim in view analysis of rain is usually made in the bulk phase. The analysis of rain as bulk phase will lead to a loss of detailed information, because the chemical content of raindrops depends on the drop size. This was first investigated by Turner [3], Georgii and Wötzel [4] and later on proved by Bächmann et al. [5–12].

Today it is possible to collect individual and size-classified raindrops [5,8,9] and it is also possible to analyse individual raindrops [5,6,9,11], but this

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method of analysis is not competent to determine simultaneously the drop size. That is why distributions of concentrations for individual raindrops with a definite radius can not be made. Such distributions could possibly give further information of parameters that influence wet deposition (e.g., coalescence and break-up [1]).

The aim of this article is to present an analytical method to determine simultaneously the chemical content and the size of individual raindrops in the nanoliter range. For the analysis CZE is used. This method is predestined for the analysis of ultrasmall volumes because of high resolution and little sample requirements (fL–nL range). The applicability of CZE for the analysis of small volumes was shown by analysing single cells [13–23] (fL–pL) and nL volumes handled in chip-based microvials [24,25]. In this work the main inorganic cations occurring in rain are analysed by CZE with indirect UV detection.

## 2. Experimental

### 2.1. Instrumentation

We used a laboratory-built CZE system. In order to observe the injection of the raindrop by a microscope (Leica, Bensheim, Germany) the capillary was fixed in an angle of 30°. The injection of the drop occurred by applying a vacuum with a syringe at the outlet vessel. The separations were carried out using conventional fused-silica capillaries (75 cm×50 µm I.D.) from CS-Chromatographie Service GmbH (Langerwehe, Germany). Indirect UV detection was accomplished using a capillary on-column detector (Dionex, Idstein, Germany). The high voltage from a 30 kV high-voltage power supply (F. u. G. Elektronik, Rosenheim, Germany) was used in the positive voltage mode. The electropherograms were recorded by an A/D board from ERC (Altglofsheim, Germany) using APEX chromatography software (Autochrom, Milford, MA, USA).

### 2.2. Chemicals

The used solutions were prepared daily using ultra-pure water purified with a Milli-Q system (Millipore, Eschborn, Germany). All reagents were

analytical-reagent grade. The electrolyte consisted of 4 mM 4-N-methylaminophenol (Fluka, Buchs, Switzerland) and 4 mM 18-crown-6 (Merck, Darmstadt, Germany) and was degassed under vacuum for 15 min. Stock standard solutions (10 mM) were prepared from their chloride and nitrate salts (Merck). *n*-Pentadecane (Aldrich, Steinheim, Germany) and *n*-heptane (Fluka) were purified by distillation. Paraffin oil, Uvasol (Merck) was purified extracting ten times with water.

### 2.3. Collection of rain drops

Individual raindrops and size-classified raindrops were collected using the “Guttalgor method”, described in detail elsewhere [5,8,9]. The Guttalgor [gutta (lat.)=drop; algor (lat.)=frost] mainly consists of a dewar vessel filled with liquid nitrogen. Raindrops falling into the liquid nitrogen freeze in less than 1 s and remain at the surface of the gas film. Reaching the evaporation temperature of liquid nitrogen the drops sink to ground because of their higher density. The raindrops keep their spherical shape during the freezing process, so it is possible to separate raindrops of different sizes using polyethylene sieves (Büttner, Wasserburg, Germany) of different mesh widths in the range 0.1–1.4 mm. The whole system is surrounded by an inert gas box to avoid contamination and condensation of water vapour. This collection method allows the analysis of rain as size fraction and as individual raindrops. Experimental investigations with synthetic rainwater produced by a sprayer system show that the error based on the collection procedure is 10%.

### 2.4. Handling and injection of single raindrops

Owing to the small volumes of single raindrops (volumes range from 10<sup>-5</sup>–10<sup>-9</sup> L), there are difficulties as to sampling and handling the drops, especially for small raindrops (nanoliter range) because of evaporation and contamination. The concentration of the main inorganic cations and organic and inorganic anions in rain are in the µM range [5–12]. Therefore low absolute amounts of ions have to be analysed. A further problem is the determination of the volume without destroying the sample.

Excluding evaporation the droplets have to be

stored and handled under a matrix immiscible with water. This matrix should be transparent, easy to purify, viscous, not damaging to health and it should have a low vapour pressure. *n*-Pentadecane, *n*-heptane [27] and paraffin oil were tested. To test these substances water droplets were produced using a sprayer system and inserted in *n*-pentadecane, *n*-heptane and paraffin oil in a petri dish of polystyrol. The drops were fixed at the surface of the petri dish but do not moisten the surface. Consequently they remain an ideal spherical shape.

The separation capillary was inserted directly in the petri dish and positioned in front of the drop. Moving the petri dish the drop was connected to the capillary tip and by applying a vacuum at the outlet vessel the drop was injected into the separation capillary. This process is demonstrated in Fig. 1 photographically. The petri dish was then exchanged by an electrolyte vessel and CZE was started (requirements see Fig. 4 [26]).

*n*-Heptane and *n*-pentadecane turned out to be no suitable matrices for storing and handling individual raindrops. There still remained blank readings of  $\text{NH}_4^+$ ,  $\text{K}^+$ ,  $\text{Na}^+$ ,  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$  and  $\text{Zn}^{2+}$  in the  $\mu\text{M}$  range in drops stored under *n*-heptane and *n*-pentadecane although these solvents were purified by means of distillation.

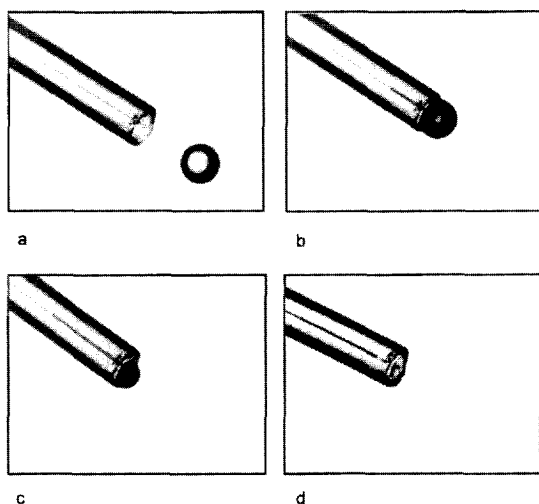


Fig. 1. Photographs of the injection of a raindrop. The drops are handled under paraffin oil: (a) sighting of the drop; (b) connection of drop and capillary; (c) injection of the drop; (d) completely injected drop.

Paraffin oil, a mixture of high-boiling [b.p. (1000 hPa)=300°C] hydrocarbons, proved to be the best matrix. Drops are fixed well because of a high viscosity [ $\eta$  (20°C)  $\geq$  110 mPa s]. Paraffin oil was purified extracting ten times with water. There were no detectable blank readings of the main cations occurring in rain. Consequently paraffin oil was chosen as matrix to store and to handle single raindrops.

### 3. Results and discussion

#### 3.1. Determination of the volume of single raindrops

For the analysis of real raindrops it is necessary to determine the exact volume to get a precise determination of the concentrations of trace compounds. Three methods were investigated to determine the volume of a drop.

##### 3.1.1. Determination of the volume using the electroosmotic flow

This method allows the determination of the volume directly from the electropherogram. Drops of definite volumes were produced by a 1- $\mu\text{L}$  syringe using different standard solutions (concentrations in the range of 10–100  $\mu\text{M}$ ) of  $\text{NH}_4^+$ ,  $\text{K}^+$ ,  $\text{Na}^+$ ,  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$  and  $\text{Zn}^{2+}$  and were introduced in paraffin oil. The volumes of the produced drops ranged from 10–140 nL. After injecting a drop into the capillary separation was started (requirements see Fig. 4). In order to obtain a relation between the volume of a drop and the electropherogram a coefficient  $X$  of half width of the EOF signal  $w_{1/2\text{eof}}$  and the retention time  $t_{\text{eof}}$  was defined:

$$X = \frac{w_{1/2\text{eof}}}{t_{\text{eof}}} \quad (1)$$

The result is shown in Fig. 2a. The obtained graph shows a linear dependence of the volume on the EOF. The deviation of the drop volume is 10%. This technique is an easy method to determine the drop volume simultaneously when analysing the electropherogram.

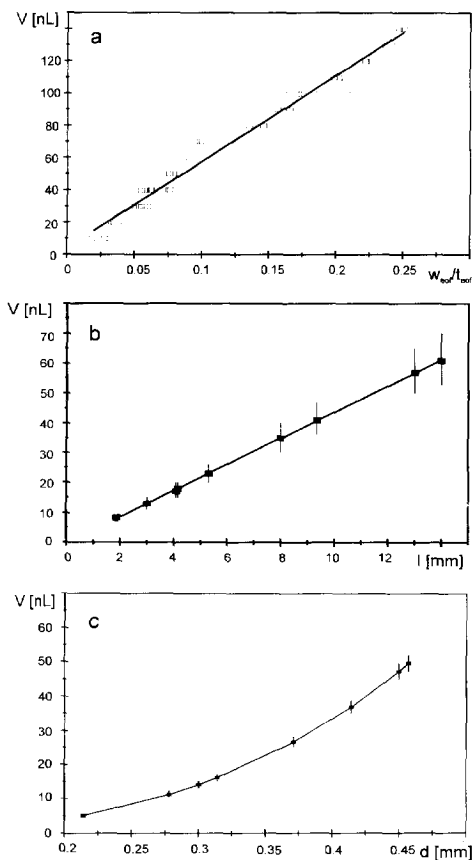


Fig. 2. Determination of the drop volume: (a) calculation of the drop volume directly from the electropherogram; (b) measurement of the length of the sample in a capillary; (c) calculation of the drop volume by measuring the diameter of a drop.

### 3.1.2. Determination of the volume of a drop by measuring the length of the liquid column in a capillary

The second way to determine the volume of a drop is by injecting a drop into a capillary with a known diameter and measuring the length of the liquid column (the calibration graph is shown in Fig. 2b). For this series of measurement a fused-silica capillary with an inner diameter of  $75 \mu\text{m} \pm 5 \mu\text{m}$  was used. This inaccuracy of about 7% results in a deviation of 13% of the drop volume. A further disadvantage of this method is that the drop and the electrolyte have same refractive indices. To determine the length of the liquid column it is necessary to inject first some nanoliter of a liquid with a different refractive index, for example paraffin oil.

Afterwards the drop can be introduced into the capillary. A sharp border between drop and paraffin oil is seen then. After measuring the length of the liquid column the drop has to be removed out of the capillary. For analysis the drop has to be injected a second time. This procedure is time-consuming and therefore not suitable for the analysis of individual raindrops.

### 3.1.3. Determination of the volume by measuring the diameter of a drop

Another method to determine the volume of a drop is by measuring the diameter of a drop with a scaled microscope. Because water drops remain their spherical shape in paraffin oil this technique is applicable to determine the volume of single raindrops. The scale in the eyepiece of the microscope was calibrated with a standard measure. Fig. 2c shows a calibration curve for drop volumes in the range of 0–70 nL. An error in reading of about 0.5 graduations of scale results in a deviation of 1–6% of the drop volume. This method is a simple means to determine the volume of individual raindrops because the diameter of a drop can be measured simultaneously when sample injection occurs.

For the analysis of individual raindrops the determination of the drop volume was carried out by measuring the diameter of the drop.

## 3.2. Calibration

Owing to varying sample volumes analysing single raindrops it is necessary to make calibration graphs for each drop size. To create comparable conditions synthetic raindrops were produced. Drops were formed of a standard solution by a sprayer system and directly introduced in a petri dish filled with ten times water-extracted paraffin oil. Injection and analysis was carried out as described in Section 2.4. The series of measurements resulted in calibration graphs for constant concentration and varying drop size. Fig. 3a exemplary shows a calibration curve for sodium with a concentration of  $41 \mu\text{M}$  ( $r^2 = 0.971$ ).

In order to get a calibration to each drop size it is necessary to combine multiple calibration graphs for different concentrations. A calibration curve of so-

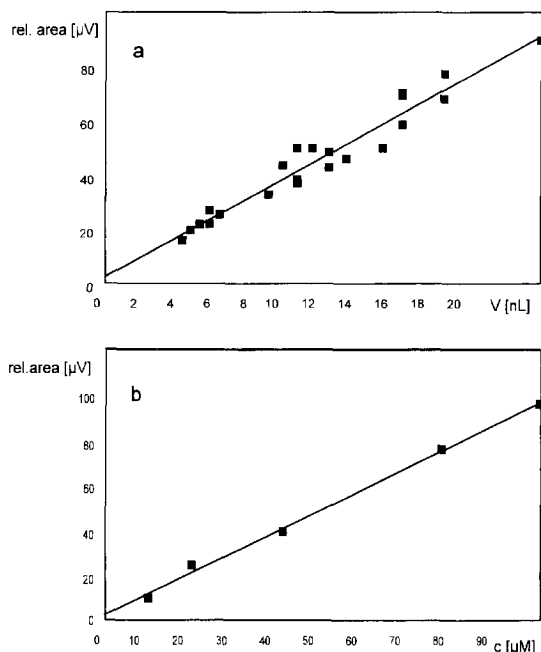


Fig. 3. Examples of calibration graphs: (a) calibration graph for sodium with a concentration of  $41 \mu\text{M}$ ,  $r^2=0.971$ ; (b) calibration graph for sodium for a constant volume of 10 nL,  $r^2=0.996$ .

dium for a volume of 10 nL is presented in Fig. 3b ( $r^2=0.996$ ).

### 3.3. Analysis of individual raindrops

Sampling of raindrops was performed using the "Guttalgor method" (see Experimental, Section 2.3). Samples were taken in Darmstadt, Germany. The frozen droplets of the sieve with the mesh width 0.2 mm were directly introduced in paraffin oil. Afterwards the cations  $\text{NH}_4^+$ ,  $\text{K}^+$ ,  $\text{Na}^+$ ,  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$  and  $\text{Zn}^{2+}$  were analysed using CZE (see Section 2.4). The drop volumes ranged from 4 to 14 nL. Fig. 4 shows for example an electropherogram of an individual raindrop. The volume of the drop was  $8 \pm 0.5$  nL.

In order to obtain concentration distributions of trace elements in individual raindrops (see Section 1) several raindrops have to be analysed. One concentration distribution for potassium is demonstrated in Fig. 5. 28 raindrops were analysed, each of a volume of  $7 \pm 1$  nL. Fig. 5 shows a bimodal distribution showing one maximum at  $0-3 \mu\text{M}$  and a

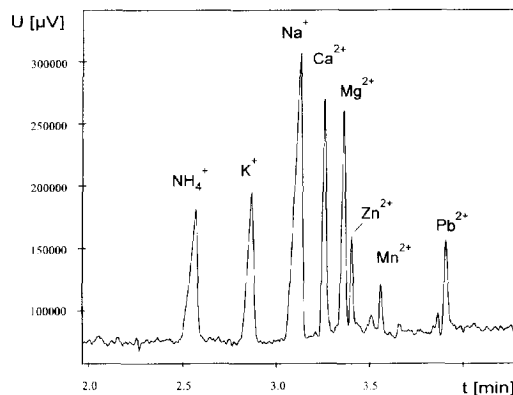


Fig. 4. Electropherogram of an individual raindrop sample:  $8 \pm 0.5$  nL ( $\text{NH}_4^+$ :  $87 \mu\text{M}$ ;  $\text{K}^+$ :  $143 \mu\text{M}$ ;  $\text{Na}^+$ :  $275 \mu\text{M}$ ;  $\text{Ca}^{2+}$ :  $60 \mu\text{M}$ ;  $\text{Mg}^{2+}$ :  $65 \mu\text{M}$ ;  $\text{Zn}^{2+}$ :  $22 \mu\text{M}$ ); electrolyte: 4 mM 4-N-methylaminophenol, 4 mM 18-crown-6, pH 6.5; capillary: 74 cm (60 cm to detector)  $\times$  50  $\mu\text{m}$  I.D.; voltage: 25 kV; detection: indirect UV detection (220 nm).

second maximum at  $9-12 \mu\text{M}$ . The second maximum at  $9-12 \mu\text{M}$  is due to evaporation, aerosol scavenging and gas scavenging (see Section 1) whereas the first maximum at  $0-3 \mu\text{M}$  is caused by coalescence and break up of large raindrops ( $\geq 0.5 \mu\text{L}$ ) which have low concentrations of trace elements [5–12]. A proof for these conclusions can be given by analysing size fractions (see Section 2.3) of the same rain event. The analysis of the size fraction collected on the sieve with mesh width 1 mm (volume  $\geq 0.5 \mu\text{L}$ ) results in a concentration of  $3 \mu\text{M}$  for potassium. This value agrees with the first maximum at  $0-3 \mu\text{M}$  and is a proof of the break up of large raindrops.

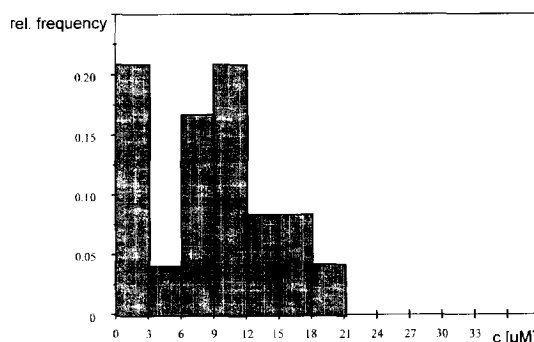


Fig. 5. Concentration distribution for potassium: 28 raindrops of  $7 \pm 1$  nL.

#### 4. Conclusion

We have demonstrated a new method to analyse individual raindrops by CZE and to determine simultaneously the volume of the analysed raindrops. This system allows the storing and handling of raindrops in the nanoliter range without any contamination. Analysis occurred using a new developed microinjection system. Further different methods to determine the volume of individual raindrops have been shown.

This newly developed technique allows the determination of concentration distributions for individual raindrops in smaller ranges of radii than previous methods [5,6,9,11]. Therefore future works using this new developed method have to give statistical assurance for the interpretation of rain events.

#### References

- [1] H.R. Pruppacher and J.D. Klett, in *Microphysics of Clouds and Precipitation*, Reidel, Dordrecht, 1978.
- [2] A. Flossmann, T. Cvitas, D. Möller and G. Mauersberger, in: *EUROTRAC: Clouds: Models and Mechanisms*, 1995.
- [3] J.S. Turner, *Quart. J. Roy. Meteor. Soc.*, 81 (1955) 418–429.
- [4] D. Wötzel and H.W. Georgii, *J. Geophys. Res.*, 75 (1970) 1727–1731.
- [5] K. Bächmann, I. Haag, U. Sprenger, K.-H. Steeg, K. Steigerwald, B. Bastian and A. Röder, *Fresenius J. Anal. Chem.*, 340 (1991) 548–552.
- [6] K. Bächmann, A. Röder and I. Haag, *Atmos. Environ.*, 26A (1992) 1795–1797.
- [7] K. Bächmann, I. Haag, T. Prokop, A. Röder and P. Wagner, *J. Aerosol. Sci.*, 24 (1993) S421–S422.
- [8] K. Bächmann, I. Haag and A. Röder, *Atmos. Environ.*, 27A (1993) 1951–1958.
- [9] K. Bächmann, I. Haag, T. Prokop, A. Röder and P. Wagner, *J. Chromatogr.*, 643 (1993) 181–188.
- [10] K. Bächmann, P. Ebert, I. Haag, T. Prokop, A. Röder and K. Steigerwald, in *Proceedings of EUROTRAC Symposium '94*, 1994, p. 1101–1104.
- [11] K. Bächmann, P. Ebert, I. Haag, T. Prokop and K. Steigerwald, in *Final report: Tropospheric Oxidation Mechanisms*, Leipzig, September 1994, p. 403–408.
- [12] K. Bächmann, I. Haag and K. Steigerwald, *Atmos. Environ.*, 29A (1995) 175–177.
- [13] R.A. Wallingford and A.G. Ewing, *Anal. Chem.*, 59 (1987) 1762–1766.
- [14] R.A. Wallingford and A.G. Ewing, *Anal. Chem.*, 60 (1988) 1972–1975.
- [15] R.T. Kennedy, M.O. Oates, B.R. Cooper, B. Nickerson and J.W. Jorgenson, *Science*, 246 (1989) 57–63.
- [16] T.M. Olefirowicz and A.G. Ewing, *Anal. Chem.*, 62 (1990) 1872–1876.
- [17] T.M. Olefirowicz and A.G. Ewing, *Chimica*, 45 (1991) 106–108.
- [18] B.L. Hogan and E.S. Yeung, *Anal. Chem.*, 64 (1992) 2841–2845.
- [19] T.T. Lee and E.S. Yeung, *Anal. Chem.*, 64 (1992) 3045–3051.
- [20] S.D. Gilman and A.G. Ewing, *Anal. Chem.*, 67 (1995) 58–64.
- [21] H.T. Chang and E.S. Yeung, *Anal. Chem.*, 67 (1995) 1079–1083.
- [22] F.E. Regnier, D.H. Patterson and B.J. Harmon, *Trends Anal. Chem.*, 14 (1995) 177–181.
- [23] J.A. Janowski, S. Tracht and J.V. Sweedler, *Trends Anal. Chem.*, 14 (1995) 170–176.
- [24] E. Litborn, A. Emmer, J. Roeraade, presented at the 7th International Symposium on High Performance Capillary Electrophoresis, Würzburg, 29 January–2 February, 1995.
- [25] E. Litborn, A. Emmer and J. Roeraade, presented at the 8th Symposium on High Performance Capillary Electrophoresis, Orlando, FL, 21–25 January, 1996.
- [26] I. Haumann and K. Bächmann, personal communication.
- [27] M. Gratzl and C. Yi, *Anal. Chem.*, 65 (1993) 2085–2088.