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Proton-transfer-reaction mass spectrometry (PTR-MS) of carboxylic acids Determination of Henry's law constants and axillary odour investigations

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Abstract

Proton-transfer-reaction mass spectrometry (PTR-MS) was used as an analytical tool to measure gas-phase concentrations of short-chain fatty acids. Chemical ionisation of C_2–C_6 carboxylic acids by PTR-MS produced intense protonated molecular ions (with traces of hydrates) along with acylium ion fragments. Gas-phase concentrations were derived using the established method for calculating PTR-MS sensitivity factors. Henry's law constants of carboxylic acids for aqueous solutions at 40 °C were determined. Direct monitoring of volatile fatty acids, known to be associated with secretions from the human axilla, was performed via a specially designed transfer device situated in the axilla. Mass spectral data corresponded with the findings of a sensory assessor.

Keywords: PTR-MS; Chemical ionisation; Carboxylic acids; Henry's law constants; Axillary odour

1. Introduction

The early pioneering work of Shelley et al. [1] demonstrated that the typical strong axilla odour is released only from apocrine secretions and that bacteria resident in the axilla are required to generate the odourous compounds from their non-volatile precursors. Of the two dominant bacterial genera, Staphylococcus and Corynebacteria, present in the axilla the latter seems to be more associated with axillary odour formation [2–4]. The work of Zeng et al. [5,6] has implicated, from organoleptic and analytical evidence, that a mixture of C_6–C_{11} straight-chain, branched and unsaturated acids constitute the characteristic axillary odour. In pooled male axillary secretions the dominant analytical component was 3-methyl-(E)-2-hexenoic acid, which together with the balance of other short-chain fatty acids accounted for the characteristic axillary aroma. Further detailed studies isolated the fatty acid 3-hydroxy-3-methylhexanoic acid as well as the unsaturated acid from unhydrolysed axilla secretions [7]. Fatty acids, therefore, constitute a very important group of compounds contributing to developing axillary malodour.

Characterisation of short chain fatty acids is classically carried out by alkaline hydrolysis of axilla secretions followed by analysis using solid phase extraction and gas chromatography–mass spectrometry (GC–MS) [8]. Such methods provide only a point analysis of composition and are not readily amenable to monitoring the kinetics of the formation of this fatty acid family. The development of chemical ionisation techniques such as proton-transfer-reaction mass spectrometry (PTR-MS) has made the continuous monitoring of fast formation and release of volatile organic compounds (VOC) a manageable process. The technique is simple to operate and requires limited work-up procedure. Quantification of gas-phase organics is possible in the pptV–ppmV range, and aroma compounds can be monitored particularly in vitro

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but also in vivo in view of the general simplicity of the mass spectra usually associated with the soft ionisation procedure employed [9,10]. The related technique of atmospheric pressure chemical ionisation mass spectrometry (APCI-MS) has been applied to monitor the release of fragrance compounds from simulated dry and oily skin and real undisturbed human skin [11].

Herein we will characterise the ion chemistry involved in the PTR-MS chemical ionisation process of short-chain fatty acids and report on the application of PTR-MS to determine Henry's law constants of C_2–C_6 carboxylic acids. In addition, the technique will be utilised to monitor the evolution of fatty acids in human axillary odour.

2. Experimental

2.1. PTR-MS

A PTR-MS instrument (Ionicon Analytik GmbH, Innsbruck) operated at standard conditions (drift tube voltage: 600 V, drift tube pressure: 2.0 mbar, drift tube temperature: 60 °C) was used for the present study. A detailed description of the instrument is reported in literature [9,10]. PTR-MS is a chemical ionisation mass spectrometry technique based on proton transfer reactions from H_3O^+ ions to gaseous organic analytes. H_3O^+ primary ions are produced in a hollow cathode ion source and injected into a flow drift tube continuously flushed with analyte air. No time-consuming pre-concentration procedures that may alter the nature of the sample are required. On each collision between a H_3O^+ primary ion and an organic molecule, a proton H^+ is transferred thus charging the reagent molecule. Primary and product ions are mass analysed in a quadrupole mass spectrometer and detected by a secondary electron multiplier/pulse counting system. A heated (60 °C) Silcosteel^{TM} capillary inlet was used to minimise memory effects and to prevent condensation in the transfer line.

2.2. In vitro study

Acetic, propanoic, butyric, isobutyric, valeric, isovaleric and caproic acid were obtained from Sigma-Aldrich at >99% purity. Aqueous solutions were prepared at concentrations of 10, 50, 100 and 150 ppm. A 10 ml solution aliquot was placed in a temperature stabilised (T = 40 °C) 40 ml glass vial (Supelco) capped with a Teflon septum. The headspace was flushed with 16 ml/min of air and fed to the PTR-MS for analysis. Reported product ion abundances are mean values of data obtained at different mixing ratios.

2.3. In vivo study

A two-port glass bell axilla-sampling device (Fig. 1) was used to sample the headspace of human axillae. Air was supplied via the first port; analyte air was withdrawn at 16 ml/min via the second port. A grid of heating wire was mounted on the outer surface of the glass bell. The bell and wiring were covered with a thermally conductive epoxy material to achieve a homogeneous temperature distribution and provide sufficient electrical insulation. During measurements the sampling device was heated to 40 °C to prevent condensation. Before each measurement the bell was cleaned with purified water and heated to 100 °C to evaporate any organic residues. For each axilla 10 mass spectra (20–150 amu, 0.2 s dwell time/mass) were recorded. Background signals from the instrument and the supply air were subtracted.

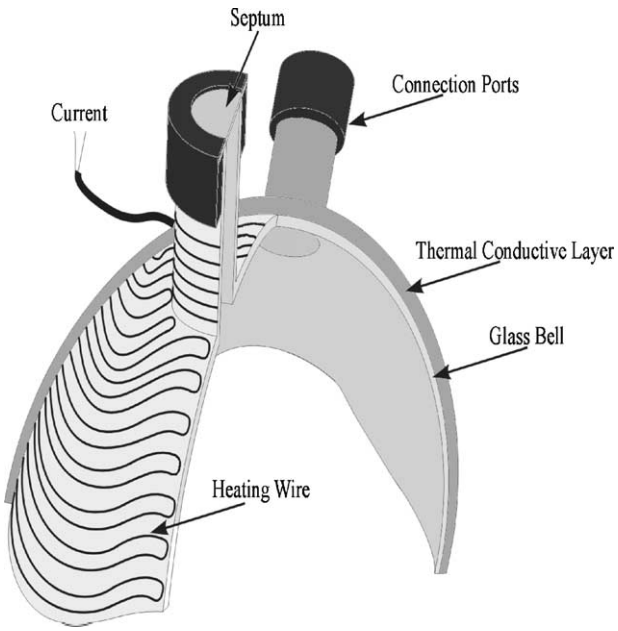

Fig. 1. Axilla headspace sampling device.

In addition to PTR-MS analysis, a sensory assessor made a subjective analysis of the axilla odour.

Measurements were carried out on five test persons. The measurement protocol required the application of a specific deodorant for a period of 2 weeks prior to the first measurement, and subsequently application of a specific antiperspirant for 1 week prior to the second measurement. Alcohols present in deodorants and antiperspirants reduce the bacterial number and thus also axilla odour, but only antiperspirant inhibits their re-growing by reducing axillary humidity. Dietary restrictions and prohibition on showering, smoking and alcohol consumption were imposed for the sampling and pre-sampling days.

3. Results

3.1. Ion chemistry

Chemical ionisation of short-chain carboxylic acids (RCOOH; R=CH_3, C_2H_5, n-C_3H_7, i-C_3H_7, n-C_4H_9, i-C_4H_9, n-C_5H_{11}) by PTR-MS produced protonated carboxylic

acid ions ($RCOOHH^+$), traces of the respective hydrates ($RCOOHH^+ \cdot H_2O$) and acylium ions (RCO^+) resulting from H_2O loss upon protonation. Relative abundances (>0.5%) of product ion signals are listed in Table 1. ^{13}C signals have not been included in the table. The proton affinity of H_2O is 165.0 kcal mol^{-1}, while proton affinities of carboxylic acids are in the 190–193 kcal mol^{-1} range [12]. This results in an exothermic proton-transfer reaction

$$H_3O^+ + RCOOH \rightarrow RCOOHH^+ + H_2O \quad (1)$$

In PTR-MS, the dissociation to form acylium ions

$$H_3O^+ + RCOOH \rightarrow RCO^+ + H_2O + H_2O \quad (2)$$

is more favourable than under thermal conditions, where dehydration is amounting to only 10% [13]. In the flow drift tube of the PTR-MS instrument, the mean relative collision energy between reagents and the mean relative collision energy between ions and buffer gas are suprathermal resulting in enhanced fragmentation. $RCOOHH^+ \cdot H_2O$ ions may be formed in subsequent association reactions

$$RCOOHH^+ + H_2O \xrightarrow{M} RCOOHH^+ \cdot H_2O \quad (3)$$

where M is a third body (N_2, O_2) to remove the excess energy from the formed complex.

As carboxylic acid samples were prepared in aqueous solutions, the H_3O^+ primary ion signal was partially converted into $H_3O^+ \cdot H_2O$ ions through association reactions. The $H_3O^+ \cdot H_2O/H_3O^+$ ratio was $\leq$0.25 at 40 °C H_2O saturation. Ligand switching reactions from $H_3O^+ \cdot H_2O$ result in the formation of $RCOOHH^+ \cdot H_2O$ ions

$$H_3O^+ \cdot H_2O + RCOOH \rightarrow RCOOHH^+ \cdot H_2O + H_2O \quad (4)$$

Due to suprathermal conditions $RCOOHH^+$ ions may be formed in dissociative ligand switching reactions

$$H_3O^+ \cdot H_2O + RCOOH \rightarrow RCOOHH^+ + H_2O + H_2O \quad (5)$$

or collision-induced dissociation of $RCOOHH^+ \cdot H_2O$ ions. RCO^+ ions are not produced from $H_3O^+ \cdot H_2O$ at quasi-thermal energies (Wisthaler, unpublished results). This pathway can, however, not be excluded at suprathermal energies.

The reported product abundances (Table 1) were obtained at 40 °C H_2O saturation; product abundances may change slightly with different humidity conditions.

In mixture analysis, interferences from a number of compounds (e.g., hydroxy-alkanals, hydroxy-ketones, ethers) may impede the quantitative detection of protonated carboxylic acid ions. PTR-MS should thus be regarded as an on-line monitoring tool; compound identification by a complimentary technique such as GC–MS is essential.

3.2. Quantification

PTR-MS instruments are usually calibrated using test gas atmospheres that contain known concentrations of organic compounds. In previous studies, dynamically diluted VOC mixtures from standard gas cylinders have been used ([10], and references therein). This static calibration method (dilution of a pure compound to a defined gas volume) cannot, however, be used for carboxylic acids as these compounds are rapidly lost from the gas-phase. Dynamic procedures such as the permeation method [14] or the diffusion method [15] have been deployed to generate standard gas mixtures of carboxylic acids. These calibration techniques, however, suffer from long equilibration times and pronounced memory effects, and very constant temperature, pressure and flow rate must be maintained in all parts of the calibration unit and delivery tubing to ensure quantitative carboxylic acid calibrations. In this study, it was not attempted to generate test gas atmospheres with known concentrations of carboxylic acids. Quantification was based on calculated response factors as described in Lindinger et al. [9] and de Gouw et al. [16]. In brief, pseudo-first order kinetics applies for the proton-transfer reaction (Eq. (1)) occuring in the flow drift tube and VOC concentrations are quantitatively related to the detected ion signals via the equation

$$VOC = \frac{1}{kt} \frac{VOCH^+}{H_3O^+} \quad (6)$$

where k is the reaction rate coefficient of the proton-transfer reaction, t is the ion residence time in the drift tube, and H_3O^+ and $VOCH^+$ are primary and product ion (including fragments) signal count rates. Ion count rates are corrected for mass discrimination in the extraction and detection system. Mass discrimination factors are obtained by adding a large amount of a compound and by measuring the decrease

Table 1
PTR-MS product ion signals of short-chain fatty acids

Compound (molecular weight)	*m/z* (relative abundances)	*m/z* (relative abundances)	*m/z* (relative abundances)
Acetic acid (60)	43 (92,2)	61 (100)	79 (3,7)
Propionic acid (74)	57 (47,1)	75 (100)	93 (1,3)
Butyric acid (88)	71 (31,7)	89 (100)	107 (1,8)
Isobutyric acid (88)	71 (5,0)	89 (100)	107 (0,9)
Valeric acid (102)	85 (33,7)	103 (100)	121 (1,2)
Isovaleric acid (102)	85 (29,1)	103 (100)	121 (1,5)
Caproic acid (116)	99 (59,9)	117 (100)	135 (2,0)

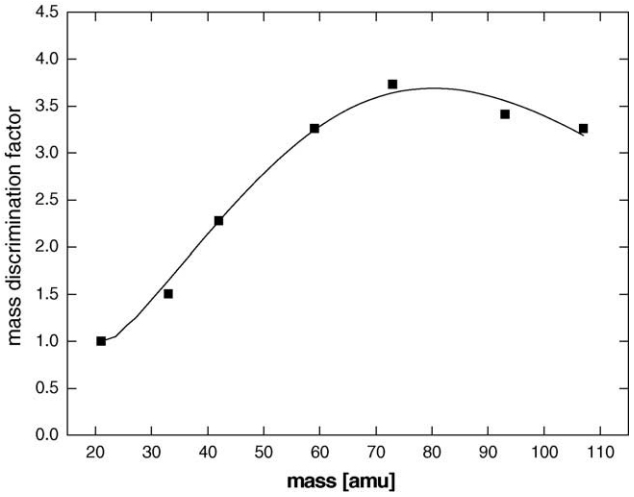

Fig. 2. Mass discrimination curve of the PTR-MS instrument.

of the H_3O^+ signal and the corresponding increase of the product ion signal. The measured mass discrimination curve is shown in Fig. 2. The curve shape is explained by low extraction efficiency for low molecular weight ions. The measured ion residence time was $100 \pm 5\,\mu s$. The reaction rate coefficient can be calculated using the parameterised trajectory formulae given by Su and Chesnavich [17], provided that dipole moment and molecular polarisability of the neutral reagents are known. A reaction rate coefficient of $2.0 \pm 0.1 \times 10^{-9}\,cm^3\,s^{-1}$ was calculated for acetic, propionic and butyric acid for suprathermal energies [18]. A reaction rate of $2.0 \times 10^{-9}\,cm^3\,s^{-1}$ was generally used for all investigated carboxylic acids, implying a higher uncertainty for isobutyric, valeric, isovaleric and caproic acid. For quantification, the influence of $H_3O^+ \cdot H_2O$ was neglected. This also implies a somewhat higher uncertainty. The estimated accuracy is ±15% for acetic, propionic and butyric acids and ±30% for isobutyric, valeric, isovaleric and caproic acids. Linearity of response in the ppmV regime was tested for all compounds. Results, exemplified for valeric and caproic acid, are shown in Fig. 3.

3.3. Applications

3.3.1. Determination of Henry's law constants

The Henry's law constant (HLC) is most commonly defined as

$$\mathrm{HLC} = \frac{c_a}{p_g}\,(\mathrm{M/atm}) \tag{7}$$

where M is mol/dm^3 in the aqueous phase, c_a is the concentration of a species in the aqueous phase, and p_g is the partial pressure of the same species in the gas phase. A common experimental approach for measuring HLCs relies on static headspace sampling to determine p_g at a known concentration c_a. The in vitro experiments performed in this study determined p_g of carboxylic acids in the headspace of aqueous solutions at known concentrations c_a. Although not optimised for accurate measurements of HLCs, these experiments allowed determination of HLCs of carboxylic acids for aqueous solutions at 40 °C. HLCs may be overestimated as they are deduced from a dynamic process rather than from a system at equilibrium. Measured HLCs are listed together with literature values for 25 °C [19] in Table 2. For acetic, valeric and caproic acids HLC temperature dependence is known and 40 °C values have been derived. Values agree within −30%/+55%.

3.3.2. Detection of carboxylic acids in the headspace of human axillae

A glass bell axilla-sampling device coupled to the PTR-MS instrument was successfully deployed to measure organic compounds in the headspace of human axillae. No

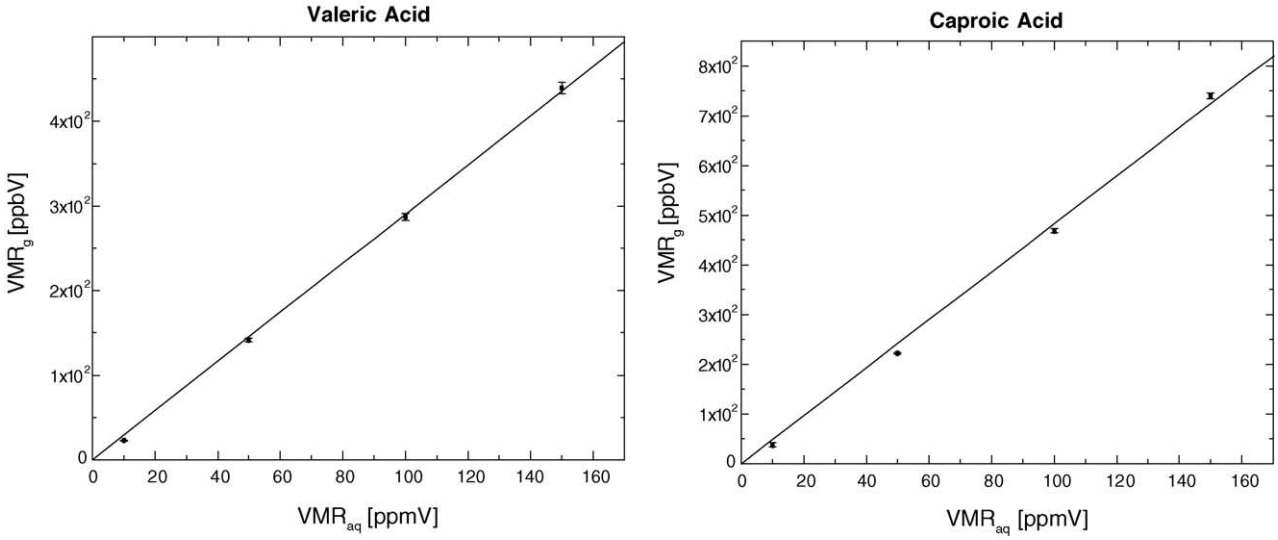

Fig. 3. Linearity curve for carboxylic acid measurements. VMR_g volume mixing ration in the gas phase and VMR_{aq} volume mixing ratio in the aqueous phase.

Table 2
HLCs of short-chain fatty acids

Compound	HLC (experimental, 40 °C) [M/atm]	HLC(literature, 25 °C) [M/atm]	HLC(literature, 40 °C) [M/atm]
Acetic acid	1.44E + 03	5.50E + 03	2.00E + 03
Propionic acid	1.49E + 03	5.70E + 03	–
Butyric acid	9.86E + 02	4.70E + 03	–
Isobutyric acid	9.69E + 02	1.10E + 03	–
Valeric acid	1.22E + 03	2.20E + 03	8.00E + 02
Isovaleric acid	1.14E + 03	1.20E + 03	–
Caproic acid	7.62E + 02	1.40E + 03	4.90E + 02

Literature values are taken from [19].

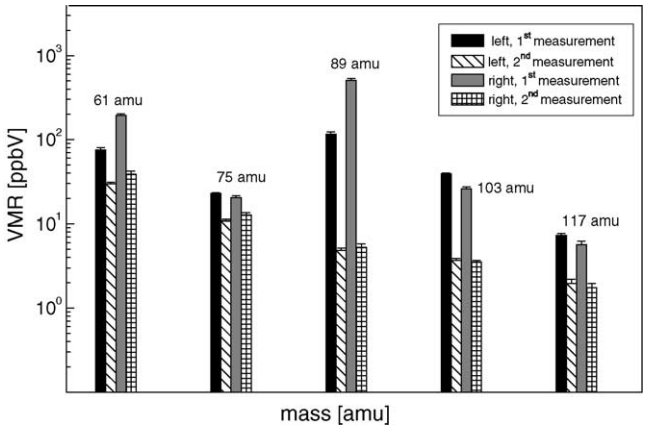

Fig. 4. Axilla headspace volume mixing ratios (VMRs) of short-chain fatty acids. Signals corresponding to acetic acid (61 amu), propionic acid (75 amu), butyric acid isomers (89 amu), valeric acid isomers (103 amu) and caproic acid (117 amu) are shown.

quantifiable effect by the treatment with an antiperspirant was observed for four of the five test persons. However, only one test person adhered to the instructions and restrictions imposed by measurement protocol. Based on this finding only observations made on this single subject are presented. A large proportion of the ion masses showed a statistically significant decrease after one week of antiperspirant treatment. Fig. 4 shows the signals corresponding to acetic acid (61 amu), propionic acid (75 amu), butyric acid isomers (89 amu), valeric acid isomers (103 amu) and caproic acid (117 amu). As pointed out previously, the presence of interfering species cannot be excluded and given volume mixing ratios should be regarded as upper limits. A significant decrease is observed for these acids over the timescale of the experiment. The results obtained for both axillae were similar. During the initial measurements some of the masses of the right axilla showed an increase relative to the left position. The data generated from the single volunteer generally corresponded with the findings of the sensory assessor both in terms of the overall aroma impact and with the observation of a stronger odor from the right axilla.

4. Summary and conclusion

Chemical ionisation of C_2–C_6 carboxylic acids by PTR-MS produced intense protonated molecular ions. Minor chemical ionisation pathways were dehydration to give acylium ions, and hydration to form protonated carboxylic acid hydrates. Quantification of carboxylic acids was based on calculated sensitivity factors and not on dynamic calibration methods, which are technically difficult to realise. Henry's law constants for aqueous solutions at 40 °C were measured and compared reasonably well with known literature values for acetic, propionic and butyric acids. A glass bell axilla-sampling device was successfully coupled to the PTR-MS instrument to measure axillary odour. PTR-MS data generated from a test person who had strictly adhered to the instructions imposed by the measurement protocol generally corresponded with the findings of a sensory assessor. Inverse trends between antiperspirant treatment and signals for selected fatty acids were observed. The obtained results indicate that PTR-MS can be used to monitor the evolution of axillary malodour.

Opportunities exist for further exploitation of the PTR-MS technology as an extension to the work described here. Future studies may investigate the dynamics of formation and release of axillary malodourants and how this process is modified by external physical and psychological stimuli such as stress, anti-microbials, other actives and inhibitors. In addition, the dynamics of the deposition and substantivity of added fragrance(s) in malodour masking or a combination of fragrance masking with the above mentioned treatments may be studied.

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References

[1] W.B. Shelley, H.J. Hurley, A.C. Nichols, Arch. Dermatol. Syphilol. 68 (1953) 430.
[2] J.J. Leyden, K.J. McGinley, E. Hoelzie, J.N. Labows, A.M. Kligman, J. Invest. Dermatol. 77 (1981) 413.
[3] N. Shehadeh, A.M. Kligman, J. Invest. Dermatol. 41 (1963) 1.
[4] P.J. Jackman, W.C. Noble, Clin. Exp. Dermatol. 8 (1983) 259.

[5] X.N. Zeng, J.J. Leyden, H.J. Lawley, K. Sawano, I. Nohara, G. Preti, J. Chem. Ecol. 17 (1991) 1469.
[6] X.N. Zeng, J.J. Leyden, J.G. Brand, A.I. Spielman, K. McGinley, G. Preti, J. Chem. Ecol. 18 (1992) 1039.
[7] C. Zeng, A.I. Spielman, B.R. Vowels, J.J. Leyden, K. Biemann, G. Preti, Proc. Natl. Acad. Sci. U.S.A. 93 (1996) 6626.
[8] A. Natsch, H. Gfeller, P. Gygax, J. Schmid, G. Acuna, J. Biol. Chem. 278 (2003) 5718.
[9] W. Lindinger, A. Hansel, A. Jordan, Int. J. Mass Spectrom. Ion Proc. 173 (1998) 191.
[10] C.N. Hewitt, S. Hayward, A. Tani, J. Environ. Monit. 5 (2003) 1.
[11] P.J. Dunphy, in: A. Hansel, T.D. Märk (Eds.), Proceeding of the 1st International Conference on Proton Transfer Reaction Mass Spectrometry and its Applications, Igls, Innsbruck, Austria, 18–23 January 2003, p. 58.
[12] E.P. Hunter, S.G. Lias, J. Phys. Chem. Ref. Data 27 (1998) 413.
[13] P. Spanel, D. Smith, Int. J. Mass Spectrom. Ion Proc. 172 (1998) 137.
[14] J. Viidanoja, T. Reiner, A. Kiendler, F. Grimm, F. Arnold, Int. J. Mass Spectrom 194 (2000) 53.
[15] S. Paulson, G. Orzechowska, H. Ngoyen, Eos. Trans. AGU 83(47) Fall Meet. (Suppl.) (2002) (abstract A61B-0072).
[16] J. de Gouw, C. Warneke, T. Karl, G. Eerdekens, C. van der Veen, R. Fall, Int. J. Mass Spectrom. 223 (2003) 365.
[17] T. Su, W.J. Chesnavich, J. Chem. Phys. 76 (1982) 5183.
[18] R.D. Nelson Jr., D.R. Lide Jr., A.A. Mariott, CRC Handbook of Chemistry and Physics, CRC Press, Boca Raton, 1991.
[19] I. Khan, P. Brimblecombe, S.L. Clegg, J. Atmos. Chem. 22 (1995) 285.