Technische Universität München
Lehrstuhl für Ökologische Chemie und Umweltanalytik

Development of capillary electrophoretic methods for the determination of amines in metalworking fluid and atmospheric aerosols

Agnes Fekete

Vollständiger Abdruck der von der Fakultät
WissenschaftszentrumWeihenstephan für Ernährung, Landnutzung und Umwelt
der Technischen Universität München zur Erlangung des akademischen Grades eines
Doktors der Naturwissenschaften
genehmigten Dissertation

Vorsitzender: Univ.-Prof.Dr. rer. nat. habil. Wilfried Huber

Prüfer der Dissertation:
1. Univ.-Prof. Dr. rer. nat. Dr. h.c. (RO) Antonius Kettrup
2. Univ.-Prof. Dr. rer. nat. Dr. agr. habil. Dr. h.c. (Zorguldak Univ./Türkei) Harun Parlar
3. Prov-Doz Dr. agr. Dr. (INPL Vandeuvre-les-Nancy/Frankreich) Philippe Schmitt-Kopplin

Die Dissertation wurde am 13.01.2005
bei der Technischen Universität München eingereicht und durch die Fakultät
WissenschaftszentrumWeihenstephan für Ernährung, Landnutzung und Umwelt
am 12.04.2005 angenommen.

Acknowledgement

I would like to acknowledge the Deutschen Forschungsgemeinschaft and GSF Research Centre for Environmental and Health for the financial support that allowed me to undertake this Ph.D.

I would like to my "Doktorvater" Prof. Antounius Kettrup for his guidance and support.

Thanks to my project supervisors Dr. Majlinda Lahaniatis and Dr. Jutta Lintelmann for the help during my work.

My biggest thanks have to go to Dr. Philip Schmitt-Kopplin, Dr. Istvan Gebefügi and Dr. Jenö Fekete for their unconditional support.

Thanks to thank my colleagues and friends for the memories I will treasure, especially those in Attaching group.

I would like to thanks my family and my Friend for being there, supporting me and always having faith in me.

List of figures

List of tables

Abbreviations

2AB: 2-amino-1-butanol
μ_{eff}: effective mobility
$\mu_{eff,norm}$: normalised effective mobility
AA: acrylacid
AAS: atomic absorption spectroscopy
AC: automated colorimetry
AEE: 2-(2-aminoethoxy)-ethanol
AEPD: 2-amino-2-ethyl-1,3-propandiol
AIBN: isoazobisisobutylnitrile
AMP: 2-amino-2-methyl-1-propanol
ATOF-MS: aerosol time of flight mass spectrometry
BA_2: 1,4-diaminobutane
BA: butyl amine
BGE: background electrolyte
BMA: butyl methacrylate
BTA: 1,2,3-benzotriazle
CE: capillary electrophoresis
CEC: capillary electrochromatography
conduct: conduct: conductivity detectionS
CZE: capillary zone electrophoresis
d_{ae}: aerodynamic diameter
DAD: dioda-array detection
DEA: diethanolamine
DMBA: dimethyl-benzotriazole
DMOI: 4,4-dimethyloxazolidine
E_2A: diethyl amine
EA: ethyl amine
EC: elemental carbon

EChD=electrochemical detection
ECD: electrone capture detection
EDAO: 5-ethyl-3,7-dioxa-1-azabyciclo[3.3.0]octan
EDMA: ethylene dimethacrylate
EDTA: ethylenediaminetetraacetic acid
EEA: european environmental agency
EOF: electroosmotic flow
EPA: environmental pollution agency
f: relative sensitivity of internal standrard
FID: flame ionisation detector
FTIR: furrier transformation infrared spectroscopy
GC: gas chromatography
Grotan BK: N,N,N-tris(ß-hidroxyethyl)hexahydro-triazine
Grotan WS: N,N,N-tris(ß-hidroxypropil)hexahydro-triazine
Grotan OX: N,N-methylenbis(5-methyloxazolidine)
H^1-NMR: proton nuclear magnetic resonance
HULIS: humic like substances
IC: ion chromatography
ICP-AES: inductively coupled plasma with atomic emission spectroscopy
ICP-MS: inductively coupled plasma with mass spectroscopy
I.D.: inner diameter
I/O: ratio between indoor and outdoor aerosol particle concentration
LC: liquid chromatography
LD_{50}: lethal dosis
LIF: laser induced fluorescence
LLE: liquid-liquid extraction
LMW: low molecular weight
LOD: limit of detection
logP: octanol/water partition coefficient
LOQ: limit of quantification
M: molecular weight
M_2A: dimethylamine

MA: methyl amine,

MAC: maximally allowed concentration at working place

MBA: methyl-benzotriazole

MBT: 2-mercaptobenzothiazoles

MDEA: N-methyldiethanolamine

MEA: monoethanolamine

MEKC: micellar electrokinetic chromatography

MIPA: monoisopropanolamine

mol: molecule

MOR: morpholine

MS: mass spectroscopy

MT: migration time

MWF: metalworking fluid

n: repetition time

NDLEA: N-nitrosodiethanolamine

NMR: nuclear magnetic resonance

NPD: nitrogen-phosphor detection

OC: organic carbon

PA_2: 1,5-diaminopentane

PA: propylamine

PAH: polycyclic aromatic hydrocarbon

PAK: polycyclic aromatic keton

PAQ: polycyclic aromatic quinone

PIP: Piperazine

PIXE: proton induced X-ray emission analysis

PM: particulate matter

PTBBA: para-*tert*butyl-benzoic acis

Q: charge

R^2: regression coefficient

R_s: resolution

RSD: relative standard deviation

SEC: size exclusion chromatography

SFC: supercritical fluid chromatography

SP: spermidine

SPE: solid phase extraction

TC: total carbon

TEA: triethanolamine

UV: ultra violet

VOC: volatile organic carbon

VIS: visible

XRF: X-ray fluorescence spectrometry

1. Introduction and goals

Epidemiological studies showed that particles present in the atmospheric aerosol have hazardous effect even at low mass concentrations. Generally, diseases caused by inhalation or skin contact of ambient particles are associated with their individual components. However, not only the particulates have influence on the human health since it is closely connected to the vapour phase in which they are suspended due to gas-vapour motion, desorption and adsorption of individual compounds and heterogeneous reactions.

The chemical composition of the vapour phase is on the whole well characterized, but the content of particulate matter is more complex and strongly depends on several factors like its source, size and physical processes such as transformation and coagulation. The main components of the condensed phase are carbonaceous substances (carbonates, elemental and organic carbon), ammonia, nitrate, sulphate and metals. With decrease of the particle size the organic carbon faction became more dominant.

Lot of information have been reported about the individual organic substances present in the particulate phase containing carbon, hydrogen and oxygen but only a limited data was found about nitrogen containing organic substances. Moreover, the knowledge about their fate and transformation are limited because their chemical description is incomplete due to the absence of validated analytical methods.

That is why the aim of this work was to develop and improve analytical methods for the determination of organic substances containing nitrogen in atmospheric and inddustrial aerosol. In metal working industry the use of metalworking fluid (MWF) is indispensable to protect the tools. During the use of the fluid it is emerged into the air in a form of mist (aerosol) that causes several disorders like contact dermatitis, asthma or hypersensitivity pneumonitis among the workers. These diseases have been associated with individual compounds present in MWF aerosol thus the estimation of the exposure of these substances to the workers is negligible to protect the workers.

The selected solutes were derivatives of (i) benzotriazoles and benzothiazoles, (ii) hexahydrotriazines and oxazolidines, (iii) amino-alcohols and (iv) volatile and biogenic alkylamines because these hazardous chemicals are ingredients or by-products of MWF. It was also issued that the developed and validated method for determination of atmospheric air because aerosols formed inside and outside of the plant might be co-mingled by filtration processes.

The analytical procedure for the determination of saturated and unsaturated heterocyclic and aliphatic amines in atmospheric and MWF aerosol includes the sampling of the particulate matter and the targets present in the vapour phase, the sample preparation or extraction and analysis. The sample has to be investigated by separation techniques because of its complex nature.

Generally, gas chromatography (GC) or high performance liquid chromatography (HPLC) is applied for the determination of organic compounds in atmospheric aerosol. However, capillary electrophoretic methods have been recently validated for different individual components like carboxylic acids. The advantages of capillary electrophoresis (CE) beside low solvent consumption and high efficiency, is the ability to separate compounds that are traditionally difficult to be handled by HPLC or GC.

For the determination of highly polar and thermally instable compounds like the low molecular weight (LMW) carboxylic acids and bases with the chromatographic techniques, modifications (derivatisation) in the chemical structures are necessary that more difficult for routine analysis. These compounds can be easily separated with CE without any modification. Although only a few capillary electrophoretic methods has been developed and validated for the determination of organic amines in atmospheric and MWF aerosol because the selectivity and sensitivity of the chromatographic methods are sometimes better compared to CE. Thus, the second goal of the work among developing and validating analytical methods for the determination of organic compounds containing nitrogen in aerosol was to improve the performance of the capillary electrophoretic separations. To adopt capillary electrophoresis to analytic of small bases in aerosol (i) mobility scale transformation was aimed to use for improving the precision of identification and peak area (ii) the methods were planned to be MS compatible for orthogonal analysis and (iii) a in-line concentration with realisation of new material was purposed to increase the sensitivity of a method.

2. Atmospheric and metalworking fluid aerosol

Presence of aerosol in the life is negligible since this definition including wide range of phenomenon such as smoke, rainbow, mist, fog and spray. They have effect on the human health and our environment thus its characterisation investigation of its effect are among the central topics in current research works. It is impossible to describe all aspects that have been published because it is a very broad and complex field.

Since in this study it was aimed to develop capillary electrophoretic methods for the determination of different target compounds from aerosol, the already known information was collected in aspect of chemical composition and fate on the environment and human health. These data -that are summarized in this chapter- determine the targets had to be analysed and the matrices (known constituents) of the aerosol.

The other main point of the work was to improve CE to be a fitting analytical tool for aerosol analytic that require high selectivity and sensitivity of the method and the theory and properties of CE is described in chapter 3.3.

2.1 Atmospheric aerosol

Aerosol present in the atmosphere has been studied for a long time [1]. From the 18th century meteorologists and geographers examined the origin and properties of atmospheric aerosol because it was recognized that their presence facilitates the formation of rain and snow, and influences visibility (volcano eruption, red sunset etc.) and thermal radiation in the atmosphere [2].

On the other hand, since 1660 the so-called London smog, a special type of aerosol, has been known and described as “Hellish and dismall cloud of sea-coale”, but its impact on the human health was marginal until the 1950s. On 5th December 1952 a mixture of dense fog and sooty black coal smoke descended upon London and the four-day smog episode resulted in 4000 deaths, nearly three times the normal toll [3]. Not only this “smog-accident” happened at that decade; over Donora in the year of 1948 extraordinary high levels of sulphur oxide, sulphates and fluorides were built up in the air over three days causing poor visibility, and by the time the rain cleared the air, 20 people had died and thousands of others had been sickened [4]. Since these accidents, an enormous progress has been made in understanding the presence of particulates in the air.

Researches in the field of air pollution mainly focus on the improving combustion systems (since the predominance of emission of air pollutes results from combustion processes), broadening the knowledge of its health effect, study its physical properties and processes such as transport and transformation, developments in its sampling and its analytic for determination of the chemical composition.

2.1.1 Definition and classification of airborne aerosol

Aerosols are a mixture of small solid and/or liquid particles suspended in carrier gas (usually air) [5]. A great variety of terms such as dust, haze, fog, mist, drizzle, smoke and smog are in use to describe aerosol systems. The difference of these systems is the type and the stability of the particles thus this phase called also as particulate matter (PM) or condensed phase. However, the vapour phase and PM can not be totally separated since they are dynamically connected to each other how it was earlier mentioned.

Aerosols are classified in different ways focusing mainly on the physical-chemical properties of the condensed phase. The most frequently used categorisation is based on the distribution of the aerodynamic diameter (d_{ae}) of the particles. The aerodynamic diameter of a particle whatever its shape and density is defined as the diameter of sphere particles with the density of 1 g/cm^3 which sediments at the same rate as the particle in laminar flowing air. The size of atmospheric aerosol particles ranges from 0.002 μm till 100 μm, which cover the range of million (10^6) [5]. The division according to the size can be taking the consideration of (i) formation of PMs, (ii) deposition pattern in the lung and (iii) the regulation which consider the toxicity of the PM and its ingredients.

Classification according to the formation: From the formation aspect the distribution range is divided into: (i) the coarse mode cover particles with d_{ae} higher than 1 µm and (ii) fine mode when the d_{ae} of the particle is smaller than 1 µm as shown in figure 1 [6]. The fine particles can be subdivided into the nucleation mode (<0.1 µm) and the accumulation mode or Aitken particles (0.1-1 µm). Particles in the coarse mode are mainly formed by mechanical attraction processes. Particles in the nucleation mode have recently been emitted from processes involving condensation of hot vapours or freshly formed in the air by gas-to-particle conversion. These particles account for the major fraction of the aerosol number concentration, but because of their small size only for a small fraction of the mass. Particles in the accumulation mode originate from the growth of particles in the nucleation mode by coagulation or condensation of vapours on the existing particles.

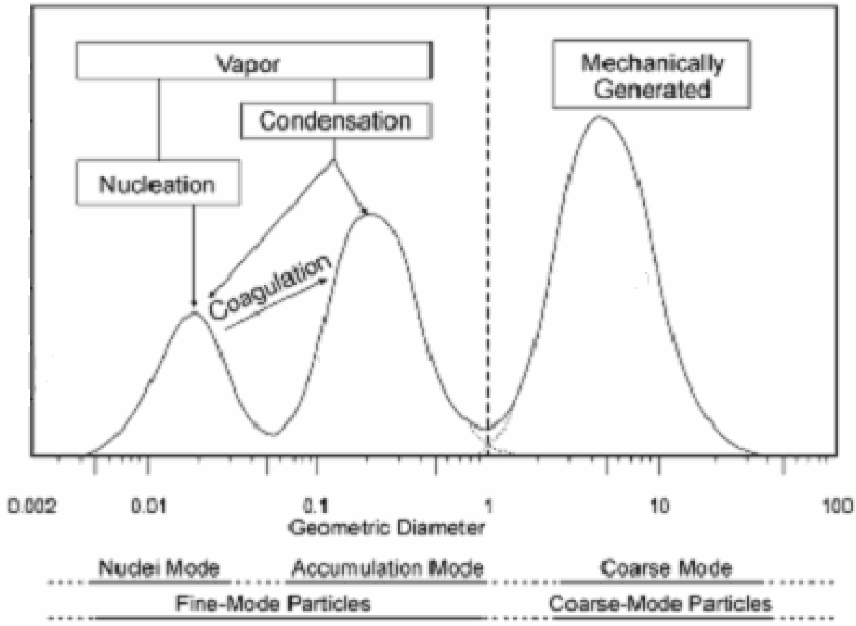


Figure 1: Classification of aerosol particles according to their size and forming process.

Classification according to the deposition in the respiratory tract: The PM fractions can be defined according to their deposition behaviour in the lung (figure 2) since the uptake into the body takes place mostly via respiratory organs [7].

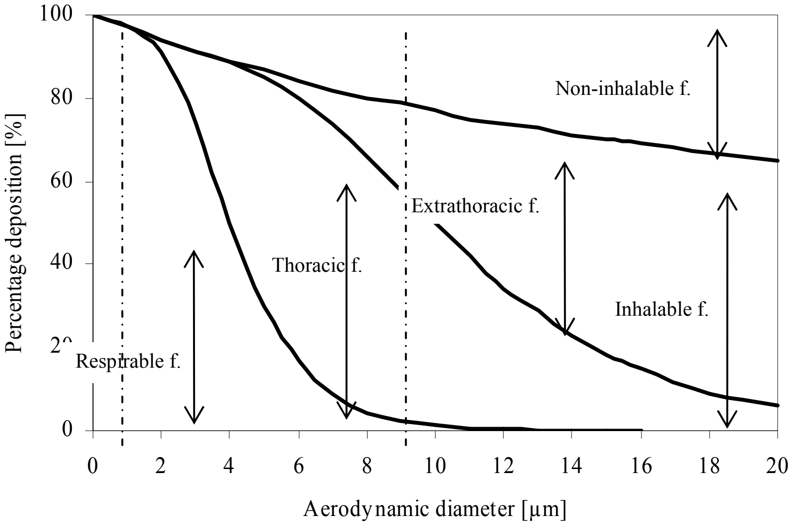


Figure 2: Deposition pattern of particulates in the respiratory tract in function of their size.

A part of the total particulates are inhaled and called inhalable fraction. The ratio of inhaled and non-inhaled fraction increases with decreasing of $d_{ae.}$ The inhalable fraction is divided into two, extrathoracic and thoracic fraction. Extrathoracic fraction includes the particles deposits in the region of nose, throat and larynx. The clearance of this fraction is complete within a few hours. Thoracic fraction comprises tracheobronchial and respirable fraction.

The tracheobronchial fraction is that aerosol fraction that deposits in the region of tracheobronchial tree and persists for up to several weeks.

When the particulates deposit in the alveoli and in the region of *bronchioli respiratorii* and they are defined as respirable fraction. The elimination of these particles from the lung takes place with half-times of up to years.

Classification according to the regulations: Environmental Protection Agency (EPA) and European Environment Agency (EEA) is now considering the fraction of PM10–2.5 as the indicator for coarse-fraction thoracic particles in conjunction with PM2.5 that address fine fraction thoracic particles (figure 2). This definition fits to the deposition pattern of aerosol particles and thus the measurements should reflect the deposition pattern of the particles in the respiratory tract too.

Classification according to the source of the particles: Aerosol particulates can be categorized into primary or secondary aerosols [8]. Secondary aerosol is particles formed within the air from the condensation of the gaseous substances presented in the air. Primary aerosol is those emitted directly in particulate form from their sources. They can be emitted as an attendant of human-activities or from processes in the nature. Thus primary aerosol is subdivided into anthropogenic and natural ones, however sometimes the distinction between these two classes is not possible (such as combustion of biomass or soul dust). Primary anthropogenic aerosols can be classified according to the place where they were formed and thus they are divided into outdoor and indoor aerosols. Outdoor aerosols are those forming in the atmosphere mostly during industrial processes (flay ash) and combustion processes, pulverisation of coal or rock crushing, solid waste disposal and transportation. The sources of atmospheric PMs are widespread. On global scale, natural sources play an important role since they are emitted from large areas such as the oceans or deserts. On a local scale, anthropogenic sources are likely to exceed the natural ones in case of cities and industrial areas such as combustion processes [9,10]; although some natural sources such as volcano eruption [11] or wildfires also play a role in the local concentration of particles.

Indoor aerosols are those forming and resting in separated places such as buildings, rooms, etc. and they can be further divided into domestic and occupational (industrial) ones. Domestic indoor aerosols refer particles generating during household processes such as cleaning or cooking. Industrial indoor aerosols such as metalworking fluid (MWF) aerosols are generated inside the plants during working process.

Geographic classification: Aerosol can be classified geographically into marine and continental aerosols depending on the place of the sampling. The further categorisation of continental aerosol is based on the elevation (mountain, valley etc), but they can be categorized on the distance of the station of large sources such as cities, power plants and major motorways.

Strong correlation was found between the class defined different ways and the chemical composition of the PM. For example, the organic carbon content of particulates was shown to be significantly different collected at natural background and at kerbside (next to the road). Significant difference in the content of condensed phase has been also found between the coarse and fine particles. The source of the particulates is more or less defines the main compounds of the emitted PM. That is why; the analysed atmospheric aerosol sample is always classified mostly based on the place of the sampling and size fraction of the PM.

2.1.2 Composition and stability of airborne aerosol

As it was mentioned before the condensed phase of the atmospheric aerosol has higher impact on the environment and human health as the vapour phase and thus its fully chemical characterisation is important.

The particles have a homogeneous chemical structure, but once the particles have been formed and emitted they are subjected to several transformation processes: physical and chemical modifications due to the coagulation of particles, condensation of vapours on the particles and surface reactions. These processes influence the size distribution and the chemical composition of the aerosol particles. The main constituents determined from PM are the total carbon content (TC), nitrate salts, sulphate salts, ammonia, mineral dust and metal ions [12-15].

Mineral dust includes definitively silica dioxide, aluminium oxide and calcium [16]. The generally presented metal ions are sodium, potassium, iron and heavy metals such as lead or chromium.

Carbonaceous components are divided into three groups: carbonates, elemental (EC) and organic (OC) carbon. EC is sometimes termed as “black carbon” or “soot” since it is the dark-coloured, low volatile carbon fraction that does not appreciably evolve without oxidants at temperatures below 700 °C [17]. It is usually produced by incomplete combustion of organic gases and particles. The chemical structure of EC is similar to the impure graphite and rarely occurs as a pure particle. OC is operationally defined as the difference between TC and EC [18] and appear to be the major component of PM [12]. The amount of the individual organic components depends on several parameters such as the size, source, sites (kerbside, urban, rural and background), residence time or the weather (temperature and humidity).

The importance of organic components present in PM is high since they are expected to effect cloud formation, aggregation of the particles, heterogenic reactions and affect the human health [19-21]. Thus the identification of organic contaminants adsorbed on the particulates at molecular levels is negligible [22,23]. Over 2800 compounds have been identified, but the full chemical description has not been achieved yet [24]. Rogge and his co-workers [25] investigated dichlormethane-methanol extract of the airborn aerosol and compared the identified organic solutes in function of PM constitution (figure 3).

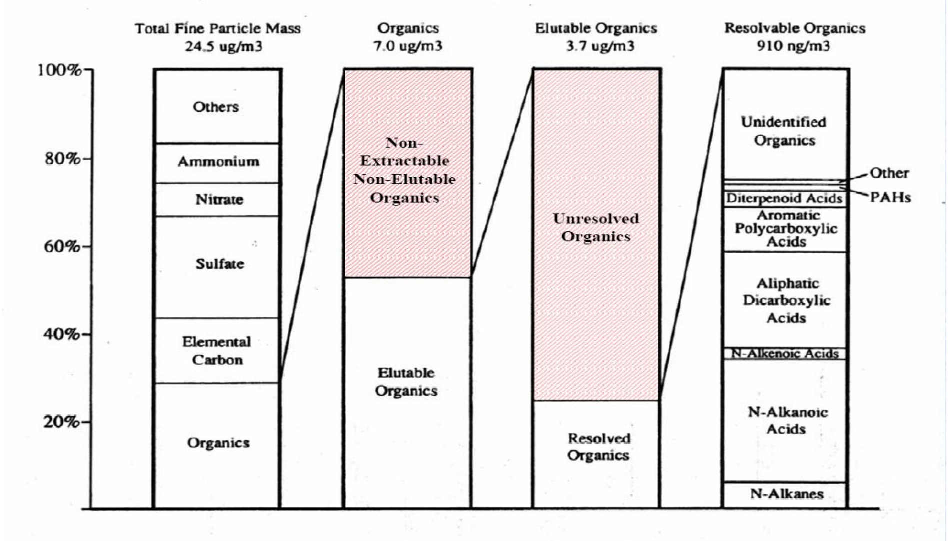


Figure 3: Chemical mass balance of fine-particle concentrations [25].

The identified components were n-alkanes, n-alkan-2-ones, n-alkanols, methyl n-alkanoates, n-alkyl nitriles, n-alkanals, n-alkanoic acids, levoglucosan, polyaromatic hydrocarbons (PAHs), but terpenoids, glyceryl esters and sterols were also found in minor amounts [26]. During the last years, the identification and quantification of these substances became more reliable and routinely. The non-extractable and unresolved fraction contains highly polar organic compounds.

Water soluble organics present in PM have strong effects in nucleation and cloud formation. They also influence the growth of fine particles in the respiratory tract [27]. The term “polar organics” is used to designate the oxygen-containing compounds such as alcohols, carboxylic acids, humic-like substances [28] and generally result from photo-oxidation of emitted or formed compounds [29]. Nitrogen containing organic compounds are also important components of PM [30,31].

The organic nitrogen content of the EC fraction was found between 10-14% depending on the particle size and source [32]. Nitrogen conctaining organic compounds divided into reduced and oxidised ones according to the electron-negativity of the nitrogen atom in the molecule. Organic solutes containing reduced nitrogen such as serine, glycine, alanine, valine, methylamine and ethylamine are found in gas and in particle phase of atmospheric aerosol too. In localized areas, the concentrations of methylamine and dimethylamine can range from 0.02-0.28 $\mu g/m^3$, and may exceed the ammonia concentration in the same air mass [33]. Major sources of primary reduced organic nitrogen include both natural such as soil humic substances, by-products of processes of vegetation and animal, and anthropogenic ones such as combustion processes, animal husbandry, food processing and waste treatment. No information has been found till now about the presence of polyamine derivatives in atmospheric aerosol in absence of developed analytical method. Once in the atmosphere, many of them react quickly with life time of 1-5 days [33]. They can be eliminated by hydroxyl radical abstraction forming hydroxyl-amines, amides, ketones or nitrosoamines [34].

Oxidised organic nitrogen can be subdivided into alkyl nitrates, nitriles, nitric acid esters, nitric acid diesters, hydroxyl nitric acid esters, peroxynitric acid esters and peroxycarboxylic nitric anhydrides. Aliphatic nitro compounds were detected at high concentration in both phases at urban regions however larger alkyl nitrates ($>C_5$) are likely to adsorb on the surface of particles. A few of these compounds are known to be formed during combustion of fuels and diesels, but the most are present as by-products. More than hundred aromatic nitro compounds are present in the atmosphere at detectable concentration. Some of these are in the gas phase, but the most have been found instead of constituents of aerosol particle. Their sources are mainly diesel exhaust and aluminium manufacture. Some are known as a result of photochemical reactions. Nitrophenols are formed by aqueous phase nitration reactions of phenol in cloud droplets [35] and nitro-PAHs are formed from PAHs. Many oxidised organic nitrogen have relatively long atmospheric lifetimes. No information was found about compounds containing nitrogen and oxygen or sulphur however they are probably formed during combustion processes.

The ambient residence time of atmospheric particles varies with their size. Larger particles are not readily transported across urban or broader areas, because they are generally too large to follow the air streams, and they tend to be easily removed by impaction. Coarse-mode particles can settle rapidly from the atmosphere with lifetimes from a few seconds to hours. Smaller-sized particles can have longer life-times and longer travel distances.

Atmospheric particles may eliminate by so called removal processes that are generally divided to dry and wet one. The dry removal mechanisms for scavenging aerosols from the atmosphere are impaction and diffusion sedimentation or gravitational settling. Wet scavenging processes are rainout and wash-out.

2.1.3 Fate and behaviour of atmospheric aerosol

Atmospheric PM have a big fate in different processes take place in the troposphere, stratosphere and have significant affects the human health. Additionally, these effects are strongly correlated to the individual components of the condensed phase. In this section its fate and behaviour will be shortly summarized.

2.3.1 Environmental effect of atmospheric aerosol

The natural and anthropogenic aerosols effect the environment locally and globally too. Local point of view they can change the visibility or acidify the environment and globally they may change the nutrient cycle or the climate.

Visibility impairment has been considered as the “best understood and most easily measured effect” of atmospheric aerosol [36]. Particles in the air influence the transfer of electromagnetic radiation in the visible range through the air by scattering and absorbing light. This manifests itself in changes in visibility (scattering) and coloration (absorbing). High concentration of particles degrades the visual appearance of distant objects to the observer.

Particulates in the atmosphere reflect the light and heat back into the space causing directly cooling effect [37,38]. On the other hand anthropogenic black carbon and hygroscopic materials present on the particulates provide a springboard for cloud formation [39]. Most clouds have warming effect by trapping light and heat in the atmosphere. The heated air makes the atmosphere more unstable, creating rising air, which forms clouds and bring rainfall to regions where the concentration of the particles is high. Despite extensive study, the effect of the condensed phase on the overall climate is still unclear.

Carbonaceous particles can cause blackening of the stone [40]. This is mainly due to the accumulation of carbonaceous particles on the gypsum layer (formed by the interaction of the stone with SO_2 and acid rain). The particles can also create ideal conditions, by acting as catalysts, for chemical reactions between the stone and other (gaseous) pollutants. They can act as gypsum nucleating agents. Carbonaceous particles, for example, having a large specific area and containing metals, play a role in the formation of crusts on the stone surface, as well as fly ash particles, containing several metals such as V, Fe, Cu, Cr and Mn, which catalyse the conversion of SO_2 into sulphate [38]. The process results in the conversion of calcite into the more soluble gypsum and is called sulphating. Some studies even show that gypsum formation on clean limestone is only possible after the deposition of dust, which then catalyses the oxidation of SO_2 in the presence of humidity.

Atmospheric aerosol inputs to the marine environment are an important source of nutrients like nitrogen and bio-available trace elements, such as Si, Mn, Fe, Co, Ni, Cu, and Zn, which play an important role in the primary production [38]. This effect is less pronounced in coastal areas or land-based water reservoirs due to a major contribution by run-off from the land. Apart from this vital function of these species, they can exhibit an adverse effect on the system. An excessive supply of nutrients to the sea may result in an increased biological activity, changes in species and phytoplankton succession, algae blooms, changes in the oxygen consumption in the water column, etc. The atmospheric supply of nitrogen occurs in the form of NH_3, but even more important in the form of particulate ammonium, nitrate salts and organic nitrogen. (For example the averaged annual deposition of nitrogen was estimated to 1.1 tonnes N/km^2 for the Danish waters[41].)

The deposited materials may not only play the role of nutrients, as mentioned above, but may be potentially toxic pollutants, like heavy metals and pesticides [42]. Large aerosol particles, despite their low number concentration, are important regarding pollution due to atmospheric deposition because of their large deposition rates and because they constitute a major fraction of the aerosol mass. Their effect is the highest in the proximity of the emission source. Pollution of more distant and remote regions by atmospheric deposition is more connected to smaller particles since these can be transported over longer distances. Even low fluxes of heavy metals are important since the deposited elements accumulate in the soil and can reach the level at which they become mobile in the environment [38]. This would have a variety of consequences on soil, forests, and water which would affect human health.

2.3.2 *Health effect of atmospheric aerosol*

Three types of studies have been used to estimate the health effects of atmospheric aerosol: toxicological, epidemiological, and human chamber studies [43]. Toxicological studies involve administering high doses of individual compound(s) to lab animals and measuring cause-effect relationships such as cancer rates or rates of other health endpoints. One obvious problem of these studies is figuring out how to take results from animals and transfer them to humans.

Because of the myriad uncertainties are associated with toxicological studies, human epidemiological evidence is generally preferred for adverse effect studies. Epidemiological studies are designed to show relationships between exposure to an agent and the onset of disease in human populations. Inadequate control of potential co-founding factors is a major criticism of these types of studies.

Human chamber studies involve placing healthy or susceptible human volunteers in controlled exposure settings to evaluate how atmospheric PM exposure influences physiological measures like lung function or heart rate variability. They provide important evidence in the mechanisms by which a pollutant can influence health but these studies cannot tell us anything about long-term exposures.

Health effects of airborne aerosol are considered both acute and chronic. Acute health effects include the diseases due to short-term exposure, and chronic effects are due to long-term exposure. For the case of premature mortality, “acute mortality” or “chronic mortality” simply refers to the exposure period.

Results of some epidemiological as well as animal toxicological studies and in-vitro experiments have supported the hypothesis that physical and chemical properties of the inhaled single particles are involved in toxic, genotoxic and carcinogenic health effect mechanism [44,45]. Particulate size, shape and electrical charge, particulate surface and solubility of adsorbed compounds are the most important factors that are correlated to the observed health effect. These parameters also substantially influence the particulate lung deposition and clearance rates [46]. Fine and ultra-fine particles penetrate the deep lung compartments, and have a very long clearance half time. By the large number per unit mass and their increased surface areas available for interactions with cells [47] and they are poorly taken up by lung macrophages and are capable of penetrating the pulmonary epithelium into the interstitium [48].

Historically, acute effect of high level PM exposure episodes on human mortality and morbidity is well documented such as London smog-episode at 1952. Several studies have demonstrated associations between daily mortality and short term (24 hour) concentrations of ambient PM. These studies suggest about 0.3%-1.6% increase in acute total mortality for a 10 $\mu g/m^3$ increase in PM10 [43,49]. As for acute morbidity effects, hospital admission studies showed that hospitalization due to chronic obstructive pulmonary diseases like pneumonia and bronchitis show a moderate but significant increase in relative risk at the range of 1.06-1.25 resulting from an increase of 50 $\mu g/m^3$ of PM10.

Links between PM concentrations and acute cardiovascular diseases such as cardiovascular death, myocardial infarctions and ventricular fibrillation have been also shown [50,51]. Long-term PM exposure could promote life-shortening chronic illnesses, but determination of life lost to PM exposure has not been established yet. Continuous exposure to lower pollution levels may result in chronic diseases like chronic bronchitis or even lung cancer.

Statistically and substantively significant report showed strong relationships between birth weight and maternal exposure to total suspended atmospheric particle [52].

Numerous controlled toxicological investigations of individual chemical species have clearly shown that specific constituents of ambient air PM (e.g. diesel exhaust particles, PAHs, oxy- and nitro-PAHs acidic aerosol, metal ions) are associated with adverse health effects [53].

Transition metal such as Fe, V, Ni, Ti etc. in interactions with cells may catalyze the production of hydroxyl free radicals via the Fenton reaction [54]. The free radical activity of particle surface could damage the cells by lipid peroxidation, protein oxidation, DNA strand breaks or antioxidant depletion [55].

PAH and halogenated aromatic hydrocarbons have been shown to pass through the placenta exposing the fetus to the contaminant body burden of the mother. Additionally they are supposed to be neurotoxic substances and they are also proven to be carcinogenic [53].

On the whole, the uptake of atmospheric aerosol particles into the body takes place mostly via the respiratory organs and cause variety of respiratory tract disorders. Additionally, not only the concentration and size distribution of the PM has effect on the human health but also its chemical composition as it was shown by several toxicological and epidemiological studies.

2.2 A type of industrial aerosol: metalworking fluid aerosol

Recently it has been suggested that indoor aerosol may pose more serious problem than outdoor aerosol since people generally spend more time indoor. People spend on average 87% of their time indoors, 7% in enclosed transit and 6% outdoors [56]. Thus the knowledge of the composition of inside aerosol at home or in working place is nesessary for estimating its risk on the human health.

Indoor aerosol contains particles formed inside the building or room during different activities but it also contains atmospheric aerosol transported from outdoor (figure 4).

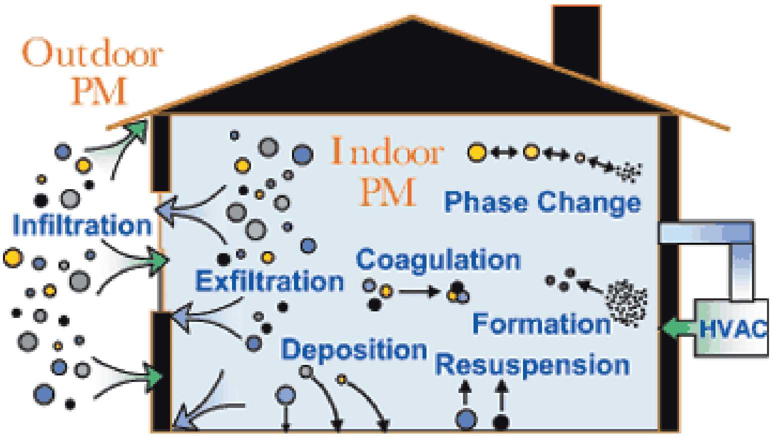


Figure 4: Transportation processes of PMs between indoor and outdoor [57].

The ratio between indoor and outdoor aerosol particle concentration (I/O) gives a method to analyze the origin and transport of indoor particles [58]. Tung and his co-workers [59] measured the I/O and found it between 0.69-0.86 when the ventilation was not operating and indoor sources were set to the minimum. It was assumed that the particles penetrated through the doors and window cracks. When the investigated place was relatively airtight and the human indoor activity was high this ratio increased to 41 [60] thus the indoor aerosol became dominant. The physical-chemical properties of aerosol formed inside closely depend on its source.

The indoor activities for generating particles are divided into household and occupational. Household aerosol might be generated during burning processes (smoking or open fireplace), cooking and cleaning activities such as hovering, sweeping or washing. Occupational indoor aerosols are mostly formed in the industry and its chemical composition strongly depends on the type of the producing process.

A special type of industrial indoor aerosol is the metalworking fluid (MWF) aerosol. Recently, it is intensively investigated since several disorders between exposed workers have been identified [61].On the other hand the monitoring of this aerosol is quite difficult since the possible hazard components which present in the aerosol are in a wide range due to the non-stable, complex property of MWF. Additionally, the particles are forming from oil-water emulsions thus wide range of tracers with property from the highly polar to the highly apolar one can present in the MWF aerosol.

2.2.1 Metalworking fluid aerosol

Metal working fluids (MWF) – often called coolants or cutting oils - are industrial lubricants used to reduce the friction between the cutting tool and the working spot and parallel carry away the generated heat, protect the surface characteristics, reduce wear, galling, surface adhesion or welding, and flush away the chips, fines and residues. Because of the versatile function of MWF, they are indispensable at manufacturing industries that perform different mechanic operations such as grinding, honing, forming, turning, boring, tapping, threading, gear shaping, reaming, milling, broaching, drilling and band sawing, otherwise the tools used in the process are quickly damaged and/or destroyed [62,63].

The worldwide estimated used amount is around 600 million gallons annually [64]. In Germany approximately 30 thousands tons of water-based MWF are produced annually [65].

During the use of the fluid, it is emerged into the working place by (i) the evaporation of fluid resulting from high temperature in the cutting zone, (ii) spin-of of the fluid from the work-piece due to rotational motion and (iii) the splash due to kinetic energy from fluid work-piece impact as a mist [66,67].

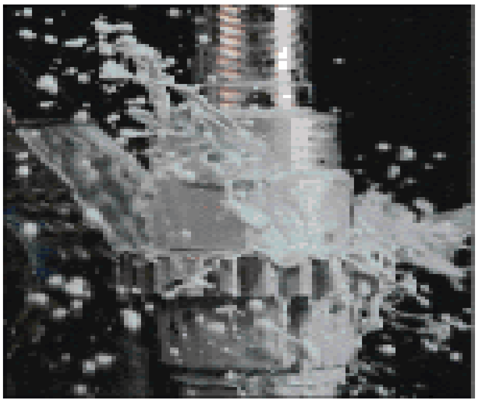


Figure 5: Generation of MWF aerosol during grinding.

The amount of generated mist mostly depends on the type, concentration and volatility of MWF, tramp oil level [68], velocity of the fluid, speed and wear of the tool [69,70]. Grinding and drilling operations produced higher exposures than turning and milling for water fluids [71,72]. The median aerodynamic diameter of the particles usually ranges from 3-6 μm in the grinding process [70] which is in fine-course mode and they deposit mainly in the tracheobranchial and alveolar regions when they are inhaled (thoracic fraction). The shape of the size distributions is unaffected by the velocity of MWF, speed of the working tool [70] but is affected by the type of the fluid and mechanical operation; e.g. grinding and turning produced the largest particles, whereas hobbing resulted in the smallest ones.

Occupational exposure to mineral oil MWF mist have to be less than 3 mg/m^3 (8h time-weighted average) and less than 1mg/m^3 for water-mix MWF mist [69,71]. These regulated exposure values do not necessarily represent the practice because the measured concentration of MWF particles is still relatively high in spite the fact that numerous improvements in MWF products, engineering controls, anti-misting technologies and industrial hygiene procedures have been developed [72-74].

The total particular mass was 1.23 mg/m^3 taking samples from 21 plants in the 1970s, 0.57 mg/m^3 in 15 plants in the1980s and 0.34-2.43 mg/m^3 from 5 plants in the 1990s [75]. From 322 personal samples taken in small machine shops the average measured MWF particulate concentration was between 0.06-1.29 mg/m^3 for the coarse fraction [76]. Another study showed that in 10 out of 79 small machine shops the MWF aerosol concentration was higher than the exposure limit of the thoracic fraction [77].

2.2.2 Chemical composition of MWF aerosol

The composition of MWF aerosol may include the MWF components of the fresh fluid and different contaminants due to abiotic and biotic processes taking place in the fluid. The comsumption of the droplets are continuously changing since various unpredictable processes take place such as photochemical reactions, microbial growth or absorption of gaseous solutes. To describe the composition of MWF aerosol, the knowledge of composition of the fluid and the possible processes which can differentiate the chemical and physical properties of in-use fluid is summarized since only a few publications have been found about the chemical characterisation of MWF aerosol.

The recently used cutting oils are grouped into four major classes according to their main components: (i) straight oils, (ii) soluble oils, (iii) semi-synthetic MWF and (iv) synthetic MWF.

Straight oil (or neat oil) MWFs are solvent-refined petroleum oils or other animal, marine, vegetable or synthetic oils used singly or combined with different additives. They are not designed to be diluted with water. Historically this is the oldest class of engineered MWF products. Recently, there have been attempts to use vegetable oils instead of mineral oil, which resulted in more environmental friendly lubricant due to their better biodegradability [78]. Straight oils provide excellent lubrication properties, good rust control and long sump life [79], but the disadvantage of the straight oil MWFs is the poor coolant ability.

Soluble oil (or emulsifiable oil) MWFs are combinations of 30-85% (V/V) severely refined lubricant-base oils and emulsifiers which may include other performance additives. The surface-active emulsifying agents provide the maintenance of the oil-water emulsion. The soluble oil concentrate is diluted with water at ratios of 1 part MWF to 5-40 parts in volume before use. Soluble oils provide good lubrication and better cooling as compared to straight oils. However, they may have poor corrosion control, may smoke and may have poor mix stability and/or short sump life [80].

Semi-synthetic MWFs contain 5-30% (V/V) of severely refined lubricant-base oils, higher proportion of emulsifiers and 30-50% (V/V) water. The transparent applied oil is made by dilution of 10 to 40 parts of water to 1 part concentrate in volume. Semi-synthetics offer good lubrication, good heat reduction, good rust control, and have longer sump life as compared to emulsifiable oil. Conversely, this class of coolants has a greater tendency to foam in soft water and can be unstable in hard water. They are the most complex MWFs because it can contain up to fifteen individual ingredients or mixtures.

Synthetic MWFs contain no petroleum oils, and can be water-soluble or water dispersible. The simplest synthetics are made with organic and inorganic salts dissolved in water. Others may be formulated with synthesized hydrocarbons, organic esters, polyglycols, phosphate esters and other synthetic lubricating fluids [81]. One part concentrate is diluted with 10 to 40 parts water in volume before the operation. Among the four classes of fluids, they offer the best rust protection and cooling capacity even at high speeds and feeds, they are the cleanest, have an excellent long sump life, are largely unaffected by hard water, eliminate smoking, reduce misting and provide detergent action and oxidative stability [82]. But usually they have the poorest lubricating ability, difficult to treat the waste of these fluids and they can foam in some applications.

Since MWFs have to provide versatile functions, all types of fluids are complex mixtures manufactured by hundreds of blenders. The physical-chemical properties and some individual ingredients are reported on the Material Safety Data Sheet of the product, but the exact chemical composition is concealed by the manufacturer because of the high competitive and proprietary nature of metalworking industry. The main ingredients according to their function that are generally used in all types of MWFs are shortly discussed in the following and summarized in appendix.

Base oils are commonly mineral oils. They are typically distillates of hydrotreated (hydrogenated) petroleum, and contain either, heavy and light fraction. Mineral oil contains hundreds or thousands of different hydrocarbons, which have naphthenic or paraffinic nature. The oil fraction is generally classified as “naphthenic” if its hydrocarbon components are not aromatic but contains at least one cyclic alkane, and “paraffinic” if all hydrocarbons in the fraction are derivatives of normal and/or branched alkanes. Soluble oil and semi-synthetic MWFs tend to contain naphthenic mineral oils, whereas straight oils are generally either paraffinic or naphthenic mineral ones. The “average” molecular weight for mineral oil used as MWF amounts to 455 g/mol for paraffinic and 310 g/mol for naphthenic oils, that corresponds to average carbon numbers of C_{30} and C_{23} respectively [83].

Most major additives of MWF are organic compounds, and vary widely in terms of hydrophobicity and polarity [84]. The primary types of additives are emulsifiers (and surfactants), extreme pressure agents, corrosion inhibitors, friction reduction agents, wetability agents, bactericides and alkaline reserve agents. Current trends in additives include elimination of chlorine-containing compounds, phenols, nitrites, amines, PCBs and heavy metals. Chlorine and sulphur containing additives were used extensively in the 1970s, but have been mostly eliminated except the high molecular-weight alkanes containing chlor used as extreme pressure additives [61] and petroleum sulphonates used as emulsifying agents [85].

Emulsifiers and surfactants are added to water-based MWFs (emulsifiable MWF and semi-synthetics) to disperse oil drops in the fluid due to the formation of spheres or “micelles”, and to reduce the surface tension of the fluid. Petroleum sulphonates have been widely used as emulsifiers in soluble oil MWF, but there has been ongoing concern that this compound will eventually be unavailable to MWF manufacturers. As a result, MWF suppliers are investigating alternative emulsifiers such as derivatives of ethanolamine or fatty acid amines [86-88].

Extreme pressure and anti-weld agents are used to guard against welding of the work piece with the metal tool in heavy duty machining. Extreme pressure agents (e.g. chlorinated paraffins) are chemically active compounds that react with metal surfaces at high temperature and pressure and create a protective layer. This layer has lower shear strength than the base metal and is preferentially sheared. The use of short chlorinated alkanes (from C_{10} to C_{13}) has been eliminated because of its suspected carcinogenicity, and long chain chloroalkanes remain in use (from C_{14} to C_{30}) [61]. However, health and environmental “friendly” compounds such as synthetic esters of carboxylic fatty acids, phosphoric and dithiophosphoric acid derivatives were tested and show the same efficiency as the conventionally used anti-weld agents [89,90].

Corrosion inhibitors form films on metal surfaces consisting of polar organic compounds or inorganic oxides to prevent the attack of acids and peroxides. These compounds are added to water-based cutting oils and are retained in the oil phase to inhibit their loss through evaporation. Sodium nitrite is one of the oldest and cheapest corrosion inhibitors. Aromatic polyamines, such as 1,2,3-benzotriazole, 5,6-dimetyl-benzotriazole, 5-metyl-benzotriazole, and 2-mercaptobenzothiazole are well known to inhibit the corrosion of copper alloys [91,92], but their antimicrobial activity had been also considered [93]. Para-*tert*-butyl-benzoic acid has also inhibiting action effect on corrosion of aluminium, iron and copper alloys.

Bactericides are intended to control the proliferation of bacteria, fungi, yeast and mould in the coolant to prolong the life of MWF, maintain its stability, reduce the odour and prevent the workers from microbial caused diseases [94]. US EPA lists more than 70 chemicals as a preservative and over 200 active products can be used as an antimicrobial agent in MWF. Because of the presence of a wide variety of micro organism species in the MWF and the effect of resistance on one preservative derivative [95], MWFs contain more than one biocide. The other important option is the dose of these antibacterial agents. If the amount of this agent is low, the micro organisms can overgrow, and when its amount is high, the averse risk of the active substance on the workers increase since these substances generally hazardous at high concentration.

Antimicrobial agents can be classified into two groups due to their activity: formaldehyde releasers [96] and non-formaldehyde releasers. The activity of these formaldehyde releasers occurs by formaldehyde production [95]. Non-formaldehyde-releasing antimicrobial agents used in coolants can be differentiated into two groups according to their chemical structure: (i) phenolic type such as 2-phenoxyethanol or p-chlor-*meta*-cresol and (ii) nitrated type such as Bronopol, Bioban or 2,2-dibronzo-3-nitropropionamide [97]. Generally, biocides from both groups are added into the MWF concentrate, since formaldehyde releasers are effective against bacteria, and the other one against fungi and moulds [98]. The effectiveness of these substances is limited, because they are more soluble in oil than in water and microbes usually grow in the water phase.

Alkaline reserve agents are used to keep the pH of water-based MWF between 8.5 and 9 to control bacterial growth (which is favoured at neutral pH), maintain the rust protection, and to retain emulsion stability and aid cleaning [99]. Earlier, derivatives of ethanolamine were used for this purpose, which have an emulsifier activity, too. The concentrate of the coolant typically contain 2-3% (V/V) monoethanolamine (MEA) and diethanolamine (DEA), and up to 25% (V/V) of triethanolamine (TEA). Recently other derivatives of aminoalcohols (or hydroxyalkilamines) have been attempted as alkaline reserve agents to eliminate the formation of nitrosodiethanolamine as it will be described later [72]. Recently, antimicrobial activity of hydroxyl-alkylamines has also been proven at alkaline pH [100].

Other ingredients are used as odorant, dye, anti-foaming agent, dispersant, oiliness agent or as plasticizer. The used additives in the MWF formulation has highlighted that there are a number of substances that give cause for concern, especially some surfactants and extreme pressure agents. Some reformulation may be required in order to prevent preparations being classified either as dangerous for the environment, harmful to aquatic organisms or may cause acute or chronic adverse effects in the human health.

2.2.3 Stability of MWF aerosol

The chemical composition of the diluted water-based MWF aerosol is continuously altered since several known and unknown processes such as contamination, biogen and abiogen reactions take place in the droplets. The main contamination sources that have influence on the quality of MWF are reviewed shortly in this chapter.

Abiotic contamination: during the use of cutting oils, the contamination of the fluid is inevitable and can accelerate breakdown of MWF. A common contamination source is the minor components of mineral oils such as the potential carcinogenic PAHs, and hetero-aromatic (with sulphur and/or nitrogen) organic compounds. Since 1950 these contaminants have now been virtually eliminated by petroleum refiners through hydrogenation and solvent extraction [72].

Another common source of contamination is the lubricants used for other machinery operations for example hydraulic fluids and greases. They are often called as “tramp oil”. These oils are often formulated products themselves, so by introducing different compounds like antioxidants, zinc dialkylthiophosphates and mercaptobenzothiazole into the MWF thus they can be present in the aerosol too.

Another general contamination facility is the presence of solid and dissolved metals. The concentration of dissolved metal is directly related to the quality and the surface of the working metal, operating temperature, type of the MWFs and the type of the machining operation. Soluble metals that may contaminate the MWF are aluminium, brass, lead, nickel, chromium; zinc, mercury, cobalt, copper and ductile irons [61]. In general, straight oils absorb fewer metals than water emulsions.

Biotic contamination: microbial growth as a substantial type of contamination has been known for a long time [101]. Once the container is opened and used, different microbes will enter from different sources (e.g. from tap water used for diluting the coolant concentrate; from machine operator's hand, sweat and saliva; from the atmosphere; from tramp oil; from the parts of the machine and from residues of the previous fluid) and they are growing in the system [102,103]. The degradation of MWF by microbial growth may result in changes of fluid viscosity, and the acid products of fermentation may lower the pH of the fluid, causing corrosion and leaks in the operation system and catalyse different reactions such as formation of formaldehyde or nitroso-amines.

Water-based MWFs are excellent nutritional sources for many kinds of bacteria and fungi. Water-mix sumps generally contained high bacterial level [104]. The predominant microbial species routinely recovered from MWF and its aerosols are virtually identical to those routinely recovered from natural water systems. As a group, they exhibit great nutritional diversity and complex interactions may occur among different member species or groups within the population. For example, the growth of one species may result in conditions that are more (or less) favourable to the subsequent establishment of other species. The elimination of one group of organisms may permit the overgrowth of another. All these factors contribute to the establishment of a unique microbial community and to the continuous changes in the population.

Although several data have been reported about the identification of micro organism species in the used MWF, the whole microbial population have not been defined yet [105-107]. Many researchers have suggested that aerobic bacteria are the primary colonisers of in-use fluids and thus its aerosol and *Pseudomonas* are the predominant population among the bacteria [108-111]. Once aerobic bacteria are growing, the second colonisers will be appearing such as sulphate reducing bacteria, anaerobic bacteria, yeasts and fungi. Most of the microorganism associated with MWF characterized either as nonpathogens (such as *Pseudomonas* species, *Pantoca agglomerans*, *Citrobactee freundii*, *Klebesiella pneumonie*, *Escherichia Coli*, *Proteus vulgari*) or as opportunistic pathogens such as some *Mycobacterium* species [112]. However, pathogenic organisms such as *Salmonella*, *Staphylococcus* and *Legionella* species have been isolated [113].

Fungi generally occur after pH decrease of the MWF and when organic matter is produced in the coolants, where saprophytic species can grow [114,115]. *Cephalosporium*, *Penicillium* and *Aspergillus* species have been commonly isolated from the in-use MWF. Yeasts and moulds are not the major microbial contaminant in cutting oils.

Abiotic byproducts: some ingredients of MWF are not stable. Base oil may degrade, when the operation temperature is excessive and PAHs may form. Recently low levels of benzo[a]pyrene (median 0.03µg/g) have been found in mineral oils [72]. Nitrated biocides have been shown to release nitrite that can act as a nitrosating agent in the formation of nitroso-amines. Formaldehyde releasers hydrolyze in water forming formaldehyde [96]. For example hydrolysis of Grotan BK which is the most known formaldehyde releaser used in MWFs is plotted in the following figure.

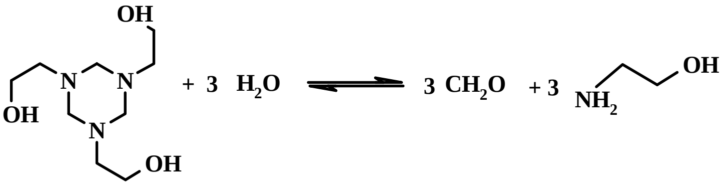


Figure 6: Reaction scheme of hydrolysis of Grotan BK.

The kinetic of the hydrolysis of Grotan BK was studied by proton nuclear magnetic resonance (H^1-NMR) spectroscopy at different conditions [116]. The determined half life time of this hexahydrotriazine derivative was 2.8 h at pH 11 and 0.1 s at pH 8, thus the reaction is not inhibited by alkaline condition as it is written in its material data sheet reported by the commercial distributor [117]. However, other parameters that have influence on the actual concentration of the released formaldehyde (such as electrolyte and bacterial content) have not been thoroughly studied. No data were found about the hydrolysis kinetics of the other derivatives of formaldehyde releasers used in MWF. Since it is known that formaldehyde is formed, the concentration of formaldehyde was closely determined in the working place area since high concentration of biocides in the fluid is necessary to have good effectiveness against bacteria [118]. The mean concentration of formaldehyde measured from 300 air samples was 0.5 mg/l [119] that is under its maximally allowed concentration (MAC) in the working place.

During grinding and machining operations small metal particles occur in the MWF. Thus vast area of nascent metal surface are generated which are chemically reactive. Many fluid components, especially corrosion inhibitors, are stripped from the mix by these sites and are replaced by largely unknown reaction products.

The oxidation of MWF oils and constituents can lead to the formation of acids, resins, varnishes and carbonaceous deposits [120]. Considering the human health effect, formation of N-nitroso compounds or nirosoamines, a class of notorious mutagenic, carcinogenic and teratogenic chemicals, was closely investigated. Nitrosoamines are generated by reaction of hydroxyl-alkylamines and nitrosating agents (mostly nitrite salt) [121]. The most investigated nitrosoamine measured in small machine shops was N-nitrosodiathanolamine (NDLEA). In simulated metalworking coolant, which contained both nitrite and DEA or TEA at pH 9, NDLEA formed at an initial rate of 11-6 mg/l per week, respectively. This rate was increased by the heating of fluids, by acidification and by addition of paraformaldehyde or formaldehyde releasers [122]. Thus EPA passed a rule prohibiting the use of nitrites in MWFs containing aminoalcohols. However, NDLEA have been found in aerosol produced from nitrite free coolants, since DEA and TEA were exposed to nitrite oxide in the air [123]. The determined concentration of NDLEA was between 200-30000 mg/l [124] in 1978, and 0.02-7.53 mg/l measured in 1997 [125]. Other nitrosoamines are also formed by oxidation of hydroxyl-alkyl-amines. Nitrosooxasolidines are formed in the fluid or in the aerosol containing MEA at high concentration. N-nitrosodiethanolamine and N-nitrosomorpholine were determined too, when the used fluid contained diglycolamine and the MWF was operated at a relatively high temperature (100 ^{o}C).

Biotic byproducts: some ingredients of MWF can be readily consumed by micro organism as primary substrates (e.g. fatty acids and monoethanolamine). Other solutes like mineral oil are consumed slower. Microbes might produce acids and gases. Hydrogen-sulphide and ammonia are produced at anaerobic condition and carbon dioxide at aerobic condition. Viable cells secrete organic compounds during physiological function, which are often proteins or polysaccharides (called exotoxins), and might stimulate the immune system. Endotoxins are heat-stable lipopolysacharide-protein complexes stayed in the cell envelopes of all gram-negative species, and released from cells generally as a result of the death of the cell or lysis [126]. Mycotoxins are toxic metabolites of fungi. If the cutting oils contain *Fusarium* or *Cephalosporium* species these toxins are produced at high concentration, which may cause dermatitis among the workers [127].

As a rule of thumb, it can be assumed that any material present at working places will wind up into the MWF aerosol, so some contaminants can be estimated when the working environment is charted. Since the composition of MWF aerosol is continuously changing, permanent monitoring is necessary for evaluating the hazard risk.

2.2.4 Health effects of MWF aerosol

Since 1976 more than 70 health hazard evaluations of industries with occupational exposures to MWF have been conducted [72] and it was accounted for 3% deaths among the workers exposed by MWF aerosol [128]. Skin disorders were the most frequently reported health problems, followed by mucous membrane irritation (complaints of eye, nose and throat irritation) and respiratory disorders since workers are exposed to MWF aerosol through skin contact and by inhalation.

Several skin disorders such as folliculitis, oil ance, keratoses, eczema, rashes, contact dermatitis and skin cancer have been reported [129,130]. The most commonly reported disorder from the above mentioned diseases is irritant contact dermatitis [131,132] which can be caused by individual components of MWF such as amino-alcohols [133-135], fatty acids [136] or glycolamines [137]. Many factors play a role in the development of contact dermatitis and other skin diseases among the workers such as the MWF class and additives used the microbial content of the work environment, humidity and temperature of the plan, type of the operation or inadequate cleansing of the skin after skin contact [138].

The risk that MWF aerosol pose for nonmalignant respiratory disease is high [72]. Occupational exposure to MWF aerosols may cause lipid pneumonia, hypersensitivity pneumonitis, asthma, acute airways irritation, chronic bronchitis and impaired lung function. Among these disorders hypersensitivity pneumonitis has recently been emerging as an important risk among workers exposed to MWF aerosol [139-141] and microbial 4contaminants are postulated to be most likely cause of this outbreaks [142-145].

Additionally, increased risk of work-related asthma has been also reported [72] and it mostly depends on the type of the MWF for the machining operation and the appearance of the known sensitizers in the aerosol. The known sensitizers are alkyl-amines, derivatives of ethanolamine, heavy metallic salts, endotoxins and formaldehyde.

One report showed consistent evidence that exposure to MWF aerosol at concentration at or above the controlled limit causes chronic respiratory symptoms for each class of MWF. Currently, no MWF aerosol component(s) have been identified causing adverse chronic effect.

Epidemiological studies have been shown that some MWF aerosols are associated with an increased risk of larynx, rectum [146], pancreas [147], skin [148], scrotum [149], breast [72], and bladder cancer [150]. However, these studies reflect most likely the cancer risk associated with exposure condition in the middle of the 70s.

Toxicological studies showed that the cancer risk among the MWF workers strongly depend on the MWF constituent such as PAHs or nitrosoamines. The carcinogenic effect of triethanolamine (TEA) and diethanolamine (DEA) have been also examined on mice and rats. After two years of dermal and inhalation studies, it was suggested that the ethanolamines are potential carcinogenic if delivered via inhalation route of exposure [61]. The carcinogenic effect of short-chain chlorinated paraffins was clearly proven [151]. MWF aerosols containing nitrosoamine had genotoxic effect [72].

Generally it can be concluded that these above-mentioned disorders are caused by different individual compounds are present in MWF aerosols (mostly additives of cutting oil) [152]. Thus efforts have been made to reduce the potentially hazardous components of MWF aerosols (mostly additives and impurities). However, in absence of validated analytical methods determined from MWF aerosol it has not been solved.

2.3 Analytical chemistry of atmospheric and MWF aerosol

The analytical chemistry of atmospheric and indoor aerosol is mainly based on the analysis of the condensed phase since this fraction is more complex and has bigger influence on the ecosystem and on human health. Once a sample of particles has been collected, its physical properties, chemical and biological composition can be determined. The sample may be analyzed as a whole called bulk analysis or each particle is investigated individually.

The most common tools for determination of physical properties of PMs are gravimetric and microscopic techniques. Gravimetric measurements determine the total mass of particles collected on filter by weighing the filter before and after sampling with a balance in a temperature- and humidity-controlled environment [72]. This technique is simple and cheap, but non-specific, thus negative and/or positive interferences due to the presence of other particulate and/or losses of organic particulate matter or water may occur. The estimated quantification limit of this method is generally 130 μg/filter, which fit to the sensitivity of a conventional balance [72]. Optical and electron microscopy are used for sizing, counting and identifying individual particles.

For visibility measurements the scattering and absorption coefficient are determined by nephelometers [153]. To have a complete physical characterisation of the aerosol system, its water content and its volatility have to be determined. Several methods have been developed for the determination of water content including thermodynamic model [154], microwave resonance measurements [155], using sensitive microbalance [156], β-gauge [157], conductivity detector [158] or tandem differential mobility analyzer. The determination of aerosol volatility till now can be only measured with indirect methods or with help of real time instruments [159].

In some applications the composition of individual particles has a great importance since not all inhaled particles have adverse effect on the health. The determination of solutes present on individual particles can be performed by electron, ion, laser or scanning Auger microbes.

Recently, so called real-time measurements have been come into the front. Prototype versions of mass spectrometers (MS) that determine the composition of individual particles in time have recently been developed; however till now these instruments do not provide correct quantification characteristics.

Also laboratory prototype instruments have been developed for determination of concentrations of total sulphur, nitrogen and carbon in function of time ranging from minutes to hours.

Bulk aerosol analytic for the determination of chemical composition of aerosol includes four steps: sampling, sample preparation, determination and data evaluation. Each step plays a role for having a reliable determination protocol.

The sampling of atmospheric and indoor aerosol generally include the collection of gas and/or particle phases separately. Several sampling processes result in non-adequate sample since negative or positive artefacts often occur. Sample preparation is also a “tender spot” since during the treatment a part of the analyte can be lost.

Chemical analysis comprises the determination of the particle components by using various analytical tools depending on target compounds. These methods should be selective and sensitive since the sample are complex mixtures with variable concentrations and compositions. Generally, separation techniques such as gas chromatography, liquid chromatography and capillary electrophoresis are widely used for the determination of organic and inorganic compounds.

Biological methods are used when dealing with viable particles or particle containing biologically active materials. Different staining techniques are used for differentiate various species under microscope, however recently more techniques have been developed for the so called bio-aerosol analysis.

2.3.1 Sampling of aerosol

The vapour and particulates can be collected parallel, however it is used for special cases since the instrumentation is difficult or not reliable. Denuder technique [160] is used when the interest of analyses focus on one substance class such as amines or semi-volatiles. Prototype instruments of steam-jet aerosol collector [161,162] have been published which collect both phases at the same time but separately with a help of water steam. Additionally, these techniques have less artefact comparing with the other sampling methods.

Atmospheric or indoor particles are often collected by drawing air through filter material using a pump. The material of the filters is usually polytetraflouroethylen, polyvinyl chloride, glass and quartz fibre. This is the simplest technique, but the possibility of negative and positive artefacts is the highest comparing with the other sampling techniques. The separation should be done under isokinetic conditions and by using size-selective inlets, which define the particle size fraction being sampled.

Cascade and virtual impaction is the method of choice for the fractionation of particles within the size range less than 10 μm. In virtual impactors an acceleration nozzle (jet) directs particles-laden air toward a collection pore, which is slightly larger than the jet. Particles larger than a certain size cross the streamlines due to their inertia and enter the collection probe, whereas smaller particles follow the deflected streamlines. Several special types of impactors focus on collecting fine and ultra-fine fractions, and high volume impactors have been also developed [163].

An alternative to the impactor is the cyclone in which particles are removed by centrifugal forces. The sampled aerosol moves in a helical path and particles with high inertia separate out by the centrifugal force while smaller particles continue with the air flow. In general, the cyclone does not yield such a sharp cut-off in size as the impactor, but due to its high loading capacity is a good choice when a large amount of particles has to be sampled.

Electrostatic collectors may be used as suitable alternatives for collecting mostly fine aerosol fractions. The air is drawn through the annular space, field charging occurs, and the aerosol is collected on the surface of the tubular electrodes [164]. The advantage of this sampling procedure is the lower probability of the generation of artefacts compared to filter sampling.

The convention of European Standard EN 481 [165] requires separate measurement of inhalable, thoracic and alveolar fractions for collection of indoor particulate matter. The sample should represent the aerosol inhaled by individual workers. Thus it should be small and light to be worn by the person. These thoracic samplers are used to estimate the risk of the emitted aerosol on the human health.

A personal cascade impactor has been used for measuring the size distribution of particulates [166]. Another developed thoracic sampler uses a cyclone-operated system at 1.6 l/min, which matches to the cut-point of the thoracic curve [167]. The third type of thoracic samplers used for occupational measurements collects the aerosol on open-pore foam [168]. They are sampling at flow rate of 7 L/min allowing the collection of short-term samples or more accurate full-shift samples, but they are still expensive.

Sampling artefacts or errors are formed by the retention and absorption of gases by the sample media and collected particles, volatilization of collected particles, gas-particles and particle-particle reactions within collected particles. Almost all sampling procedures influence the chemistry of PM. The measurement of semi-volatile materials, which can co-exist in the particulate and vapour phase, may be lost from the condensed phase causing negative artefacts. For example ammonium salts, nitrates, amines and PAHs. Adsorption of HNO_3, SO_2 or different gaseous organics (VOCs) onto the collected PM can increase its total amount and can change its composition causing positive artefacts. The adsorbed amount during the sampling period is affected by the material of the filter and the flow rate of the ambient air.

2.3.2 Sample preparation

Sample preparation protocol used for bulk analysis includes different steps depending on the determination technique and physical-chemical properties of the targets. Before identifying and quantifying individual substances from atmospheric or indoor aerosol, filters or sample holder medium containing the collected particulates have to be stored at low temperature in dark to inhibit the evaporation of volatile substances and to decrease the possibility of contamination and reactions. The sample is supposed to be treated at once after the sampling.

Generally, the target compounds are extracted with appropriate solvent by shaking, sonication, water-steam distillation, accelerated solvent extraction, supercritical fluid extraction, sub-critical water extraction, and microwave-assisted extraction or Soxhlet extraction.

After the extraction step, additional clean-up and/or pre-concentration may be added into the treatment process to increase the selectivity and/or sensitivity of the analytical procedure however the extraction step alone could be sufficient for a reliable method. Additional clean-up and pre-concentration can be liquid-liquid extraction, solid phase extraction (SPE) or evaporation of the sample solvent under vacuum or nitrogen steam.

2.3.3 Measurement of chemical composition of atmospheric aerosol

The identification and quantification of the composition of atmospheric PM is difficult and till now not fully resolved in spite the fact that several research focuses on it, although a systematic classification methods for characterisation have been improved. The characterisation process differentiates the determination according to the physical-chemical properties of the constituents such as the inorganic element analysis, EC and OC measurements, the determination of water soluble ions etc. [169]. The composition of the aerosol as mentioned before are effected by several factors such as the source of the emitted and formed particles that may be marine, urban, indoor, fly ash, biological and remote aerosol, the gas/particle motion, physical and chemical changes that are condensation, nucleation, evaporation, coagulation and/or heterogeneous reaction. In the following, developed method for determination of different chemicals will be shortly discussed.

Inorganic solutes: sodium, magnesium, aluminium, silicon, phosphorus, sulphur, chlorine, potassium, calcium, titanium, vanadium, chromium, manganese, iron, cobalt, nickel, copper, zinc, gallium, arsenic, selenium, bromine, rubidium, strontium, yttrium, zirconium, molybdenum, palladium, silver, cadmium, indium, tin, antimony, barium, lanthanum, gold, mercury, thallium, lead, and uranium are commonly analyzed from ambient air samples. These elements often indicate the sources of particles and several are considered to be toxic.

Elemental measurement methods include: (i) X-ray fluorescence (XRF) spectrometry [170]; (ii) proton induced X-ray emission (PIXE) analysis; (iii) instrumental neutron activation analysis; (iv) atomic absorption spectrophotometry (AAS); (v) inductively coupled plasma with atomic emission spectroscopy (ICP-AES); and (vi) ICP coupled with mass spectroscopy (ICP-MS).

These methods differ with respect to detection limits, sample preparation, and cost [171]. XRF and PIXE are the most commonly applied methods because the selective quantification of more than 40 detectable elements is possible, these techniques are non-destructive, and relatively inexpensive. AAS, ICP-AES, and ICP-MS are also appropriate for selective and sensitive ionic measurements after extraction of the sample.

Water-soluble ions: The most common water soluble solutes in ambient aerosol samples are sulphate, nitrate, ammonium and chloride. The determination processes of these targets base on ion chromatography (IC), automated colorimetry (AC) or CE.

In IC, the sample extract passes through an ion-exchange column which separates the ions in time usually connected with a conductivity detector. Prior to detection, the column effluent enters a suppressor column where the chemical composition of one element is altered, resulting in a matrix of low conductivity [172]. IC provides multi-species analysis for the anions.

AC applies different colorimetric analyzes for small sample volumes with automatic sample throughput and the most common measured ions are ammonium, chloride, nitrate, and sulphate.

CE recently has been more often applied for the determination of water-soluble inorganic and organic ions. CE methods for the determination of inorganic ions from aerosols have been recently reviewed [173]. The most procedures are based on indirect UV detection mode which is the most unselective one, and the typical detection limit is at concentration range of low mg/L. Methods have been improved and validated for the determination of selected cations using pre-derivatization and direct UV detection [174] that increase the sensitivity of the method

Carbonaceous substances: Three classes of carbon are commonly measured in ambient aerosol samples collected on quartz-fibre filters: (i) organic, volatilized or non-light absorbing carbon; (ii) elemental, black or light-absorbing carbon; and (iii) carbonate carbon.

Carbonate carbon (i.e., K2CO3, Na2 CO3, MgCO3, CaCO3) can be determined on a separate filter section by measurement of the carbon dioxide (CO2) evolved upon acidification [175].

Several analytical methods for the separation of OC and EC in ambient and source particulate samples have been evaluated [176,177]. These methods include: (i) solvent extraction of the organics followed by total carbon analysis; (ii) nitric acid digestion of the organics followed by total carbon analysis; (iii) absorption of radiation using an integrating plate to determine EC (variations of this method include infrared absorbance, Raman spectroscopy, and visible absorbance); (iv) thermal combustion including both temperature-programmed and step-wise pyrolysis followed by oxidation using either carbon dioxide or methane detection; and (v) a combination of thermal and optical methods. Chow and Watson [178] have been summarized different carbon analysis methods along with their measurement principles. Comparisons among the results of the majority of these methods show that they yield comparable quantities of total carbon in aerosol samples, but the distinctions between OC and EC are quite different.

Organic compounds are important components of PM, whether it is taken in urban, rural, or remote areas. Most of the particulate OC is believed to be found in the fine fraction. Analytical methods include mostly separation techniques; LC, GC, CE coupled with various detectors. Several studies [24,25,179,180] have been identified and quantified over 80 individual organic compounds in the fine and/or coarse particle fractions with use of GC-MS, including n-alkanes, n-alkanoic acid, one n-alkenoic acid, one n-alkanal, aliphatic dicarboxylic acids, aromatic polycarboxylic acids, polycyclic aromatic hydrocarbons (PAH), polycyclic aromatic ketones (PAK), polycyclic aromatic quinones (PAQ), diterpenoid acids, and some nitrogen-containing compounds.

Over 70% of the organic compounds in the fine aerosol at all sites (e.g. rural or natural background) have polar nature [181], however only few analytical methods have been developed for the determination of polar organics from atmospheric aerosol. Humic-like substances (HULIS) have been measured with different analytical tools. The structural studies measured with NMR, FTIR and UV/VIS exhibited that HULIS contained in air dust samples are small in their molecular size and rich in aliphatic and carbohydrate substructures compared to humic and fulvic acids from soil and aquatic system [182]. The same was concluded from CE measurements [183,184], but the molecular identity behind the peaks has been remained unknown yet.

Indirect UV is also used for determination of carboxylic acids such as formate, acetate, propionate, butanoate, oxalate, malonate, succinate, malate, glutarate, glycolate, pyruvate, suberate, glycoxylate and lactate analysed with GC, CE and IC [185-189]. The typical detection limits were tens of mg/l that shows a low sensitivity method. The advantages of CE over IC in the analysis of carboxylic acids are lower solvent consumption, better separation efficiency, lower analysis time. Beside a larger variety of low molecular weight (LMW) carboxylic acid can be determined with CE according to the different separation mechanism. However, in terms of detection limit, IC has an advantage over CE. Between CE and IC techniques, GC is the most frequently used technique for the determination of trace concentrations of emitted di-carboxylic acids measured from ambient air. Since the majority of the carboxylic acids exist in ionic polar forms which have relatively high boiling point and often poor thermal stability, they have to be converted into non-polar, volatile and thermally stable species such as esters before the GC separation. This method provides the best sensitivity, but the difficulty of automating the derivatization and extraction procedures, and the long analysis time are the disadvantages of GC methods comparing to CE.

Several publications were found for the determination of the total amount of dissolved organic nitrogen with use of Khjendal titration, UV photolysis, persulphate oxidation and high temperature chemical oxidation [190,191] but these methods cannot specify them at molecular level. To date, GC and HPLC methods are the most widely adopted techniques for the measurement low molecular weight primary and secondary amines (see table 1).

Table 1: Developed methods for the determination of LMW amines from ambient air.

Sampling	Sample preparation,	Method	LOD	Ref.
Onto filter	Derivatisation before analysis	CE-LIF	5 µg/l	192
-	-	MS	-	193
Adsorbent (silica)	Derivatisation before analysis	HPLC-ECD	3 µg/l	194
Trapping in H_2SO_4	Enrichment with membrane	GC-NPD	-	195
-	-	MS	-	196
-	Derivatisation before analysis	LC-MS/MS	0.2 mg/l	197
Adsorbent (C_{18})	Derivatisation before analysis	LIF	1 mg/m^3	198
Diffusion scrubber	Solid-liquid extraction	IC-conduct	0.5 µg/l	199
Solid phase microextraction		GC-FID	0.7 mg/m^3	200
Adsorbent (silica)	Solid-liquid extraction	GC-FID	20 mg/l	201

Abbreviations: EChD: electrochemical detection, NPD: nitrogen-phosphor detector, conduct: conductivity detector, LIF: laser induced fluorescence, -:not reported.

These methods have some inherent problems related to the difficulty in handling amines due to their high water solubility and volatility. For GC separation like in carboxylic acid analysis derivatisation is indispensable. For HPLC analysis the selection of the appropriate solid phase and the detection system makes the determination difficult, however several method has been developed with and without derivatization of analytes. Only few methods have been developed with use of CE from ambient air. Additionally these methods were developed for the analysis of vapour phase or of condensed separately. Recently, analytical procedures focusing on the identification and quantification of amino acids from atmospheric samples have been published [202-204]. Only some methods were developed for the determination of organic solutes containing oxidised nitrogen from aerosol samples. Most of them focus on determination of nitro-PAH determination [205-207]. A CE method was also developed for the determination of aromatic acids and nitrophenols [208]. This method can separate 17 solutes with low sensitivity to be useful for real sample analysis. Urea was also determined from rain samples and aqueous aerosol extracts by colorimetry [209,210].

On the whole, the analysis of inorganic ions and several apolar organic substances from atmospheric aerosol has been established. But the determination of highly polar organics present in the aerosol has not been resolved fully.

2.3.4 Measurements of chemical composition of MWF aerosol

The identification and qualification of individual substances in MWF aerosol represents a complex problem, since the exact composition of MWF concentrate is held back by the producers, and several unknown processes take place in MWF aerosol. The volatile and some semi-volatile compounds are usually found in vapour phase, but a part of these compounds are absorbed in the produced droplets. Soluble and semi-synthetic MWFs compose an oil-water emulsion, so the target organic components can be solved partly in both phases depending on their octanol/water partition coefficient (logP).

Only some reports deal with the analysis of MWF aerosols, and these focus on the characterization of MWF aerosols using Fourier transformation infrared spectroscopy (FTIR) or aerosol time of flight mass spectrometer (ATOFMS) [211], and do not focus on the determination of individual compounds absorbed in the condensed phase. In the following earlier developed analytical methods for determination of MWF ingredients is summarized.

Base oils: For the determination of the total amount of mineral oil present in coolants and MWF mist, fluorescence spectroscopy and FTIR methods have been developed [61,212]. A method, which scans the tetrachlormethane extraction at C-H valence stretching band with FTIR, is applicable to mineral oil aerosol, but not to the water soluble MWF aerosol. For eliminating the interferences of apolar compounds (since most of the organic group contribute to that vibration band) the method was improved by using a NaCl window, which enables a reliable estimation of the content of carbonyl compounds such as aldehydes, ketones and esters.

Several analytical methods have been developed for the identification of hydrocarbons present in mineral oil e.g. GC combined with flame ionisation detector (GC-FID) and MS [213-215], supercritical fluid chromatography (SFC) and HPLC [216]. Unfortunately, the determination of individual hydrocarbon species in mineral oil has not been technically feasible yet, because mineral oils contain lot of different hydrocarbons and a vast number of isomers having a mixture with virtually indistinguishable physical and chemical properties.

Emulsifiers and surfactants: different analytical techniques were improved for determination of petroleum sulphonates with use of ion-pair chromatography from wastewater of MWF [72] or ultraviolet (UV) spectrophotometry [217]. For the analysis of fatty acids, different methods were used such as SPE followed by HPLC separation [218], size exclusion chromatography (SEC) with refractive index detection or CEC with UV detection [219].

Extreme pressure agents and antiweld agents: An analytical procedure for screening chlorinated paraffins in technical fluids was developed and validated using GC with electron capture detection (ECD) after SPE. This method was adapted into MS detection, too [220].

Corrosion inhibitors: Several methods were developed for the determination of benzotriazoles such as 1,2,3-benzotriazole (BTA), methyl-benzotriazole (MBA) and dimethylbenzotriazole (DMBA) and 2-mercaptobenzothiazoles (MBT) including ultraviolet spectrophotometry [221], FTIR, aniodic chronopotentiometry [222], amperometric titration [223] and stripping voltammetry [224], that are not suitable for analysing the individual components from mixture. For the analysis of these substances in mixtures separation methods with use of HPLC and CE combined with UV detection were developed.

Table 2: Developed methods for the determination of corrosion inhibitors.

Sample	Sample preparation	Method	LOD [mg/l]	Ref.
MWF	Dilution	HPLC-UV	-	[225]
Coolant liquid	-	HPLC-UV	1	[226]
Coolant liquidF	-	HPLC-UV	-	[227]
MWF	Dilution	CE-UV	0.02–0.16	[228]
MWF	Dilution	CE-UV	-	[229]
MWF	Extraction	CE-DAD	1	[230,231]

Abbreviations: -: was not publicated, LOD: limit of detection, DAD: diodarray detection.

Biocides: Only a few methods have been developed for the identification and quantification of formaldehyde releasers. These methods are mostly based on the determination of the total amount of the released formaldehyde, so the differentiation between the individual compounds is not possible, and some interfering compounds such as acetaldehyde existed in the sample. However, a method for the determination of selected formaldehyde releasers from MWF was developed using CZE with indirect UV detection [231].

Micellar electrokinetic electrophoresis (MEKC) method combined with UV detection has been already developed for the determination of non-formaldehyde releasing preservatives such as phenol, 2-phenoxyethanol, p-chlor-*meta*-cresol, derivatives of isothiasolone, and 3-iod-2-propinyl-butyl-carbamate measured from MWF [232].

Alkaline reserve agents: Several methods were developed and improved for the determination of amino-alcohols using ion mobility spectrophotometer, GC, HPLC and CZE combined with different types of detection techniques with or without derivatization. Table 8 summarizes of the reported methods for the determination of amino-alcohols considering the matrices, sample preparation technique and some quantification characteristics.

Table 3: Developed methods for the determination of amino-alcohols.

Sample	**Sample preparation**	**Method**	**LOD [mg/l]**	**Ref.**
Aqueous solutions	Derivatisation	GC-FID	0.14	[233]
Air samples	Derivatisation	GC-FID	0.1	[234]
Aqueous solutions	Filtration	GC-FID	0.1	[235]
Aqueous slurries	Centrifugation, filtration	GC-NPD	0.05	[236]
Water	Filtration	IC-PAD	0.25	[237]
Air samples	Extraction, derivatisation	HPLC-LIF	1 µg	[238]
Vegetation	Extraction, centrifugation	LC—MS/MS	25 pg	[239]
MWF	Dilution	CE-indirect UV	0.25	[240]

Contaminants and by-products: Analysing the contaminants and by-products from MWF aerosol is more difficult than determining the additives, because of their low concentration and the scarcity of the knowledge of the structure. Up to the present few publications dealt with the determination of individual microbial antigens from MWF aerosol [241]. No more publication have been found about the determination of different contaminants and by-products present in MWF aerosols

Last but not least, several methods have been developed and validated for determination of ingredients and by-products from the MWF but only a few focus on the determination from MWF aerosol although it affects the human health.

3. Experimental methods

The developed analytical method includes (i) the aerosol sampling, (ii) the sample preparation and (iii) the CE measurement. The sampling was carried out using quartz filter to collect the condensed phase and using adsorbent for collecting highly polar and volatile compounds from the vapor phase. The collected materials were extracted by sonication in water to dissolve water soluble compounds present in the sample. The prepared samples were then directly injected into the CE instrument. For separation of the target components capillary zone electrophoresis (CZE) was chosen since the targets are Lewis acids or bases. The selected types for detection were diode array detection (DAD) and MS coupled on-line with CE. Direct UV was used for aromatic polyamines and indirect UV and MS for the unsaturated cyclic polyamines, amino-alcohols and alkyl amines. The concentrations of the targets were determined from the integrated peak areas using calculation techniques of calibration and internal standard. In this chapter the used instruments and chemicals and each analytical step will be closely described.

3.1 Sampling process and modelling of sampling

Lung and skin irritant hetero-aromatic amines (benzotriazoles and benzothiazoles) were suspected to be found in the condensed phase of MWF aerosol due to their low volatility. Amino-alcohols and alkyl-amines have high vapour tension thus they are supposed to be found in the vapour phase but might be also found in the condensed phase because of their high water solubility and low volatility of their salt.

The generally used flow rate was 1 l/min during the development and validation processes in line with the definition of inhalable dust fraction. During the method improvement the sampling durations were ranged from 20 minutes till 24 hours. Atmospheric temperature, atmospheric pressure and the moisture were recorded during the sampling.

Since benzotriazoles and benzothiazol are supposed to dissolve in fine droplets, the sampling efficiency on filter was determined. The filters were spiked with 100 μl standard solution of analytes (the volume capacity of the filter was maximum 200 μl). Then clean air was forced through the prepared filters by vacuum pumps for 2 hours, thus the sampled volume was 120L. This process was studied for determination of amino-alcohols and alkyl-amines too.

A model system for sampling the gaseous organic amines is based on active sampling was also studied. The standard solution of the selected compounds was set in exicator and the volatile amines were forced into the vapour phase by adding 1 mol/L NaOH. Then ambient air was continuously pumped through to purge and trap the alkyl-amines into the adsorbent.

3.2 Sample preparation

After the sampling, the quartz fibre filter was covered with 10 ml of a methanol/water mixture at a ratio of 30/70 (V/V%) in a suitable vessel. For further sample preparation, the vessel with the filter was treated for 15 minutes in ultrasonic bath. After the sample solution has cooled down, and the extraction solvent was evaporated under vacuum to a volume of 500 μl (pre-concentration factor of 20). The concentrated sample was then injected into the capillary. The same solution was used for the electrophoretic separation of saturated and unsaturated heterocyclic amines, hydroxyl-alkyl-amines and alkyl-amines present in the collected condensed phase.

The adsorbent for sampling of gaseous alkyl- and hydroxyl-alkyl-amines was a novel self-prepared poly(methacrylate-acrylate) co-polymer. For 10g adsorbent 1.6 g ethylene dimetacrylate (EDMA), 1.68 g butyl-methacrylate (BMA), 0.72 g acryl-acid, 0.6 g water, 3.84 g 1-propanol (PrOH), 1.56g and 1,4-butandiol ($BuOH_2$) and 40 mg isoazobisisobutylnitrile (AIBN) were measured into a glass holder. The solution was shaken till AIBN dissolved and then it was polymerised at 60 ^{o}C for 20 h. After polymerisation white, continuous bed was taken out from the oven. The monolith was put into a mortar and the solid was disintegrated. The particles were spheres with diameter between 100 μm and 1000 μm.

1 g solids were then filled into the extraction body and were conditioned with 1 ml methanol and then 1 ml water. After sampling, the cartridge containing the adsorbent was place onto a vacuum extractor and the adsorbed solutes were dissolved by 2 ml 0.3% HCl solution at flow velocity of 1ml/min. This elute was then directly injected into the CE instrument.

3.3 Capillary electrophoresis

Capillary electrophoresis was chosen as separation technique for identification and quantification of organic amines in atmospheric and MWF aerosol. CE has advantages of high separation efficiency, short analysis time, low sample and solvent consumption, low cost of the running and lower effect of matrices comparing with other separation techniques. In addition, CE can separate compounds that have been traditionally difficult to handle by HPLC for example highly polar and water soluble substances like the selected organic amines. However, there are still some barriers to overcome.

The major drawbacks in CE are the poor precision and low sensitivity in comparison to the chromatographic techniques. That could be also reason why CE is not as widespread applied technique such as GC or HPLC. Thus if these parameters are improved CE would have comparable performances as the chromatographic techniques. But to be able improve these characteristics, the theory of the separation and the instrumentation must be understood and thus they will be shortly described in this chapter.

Capillary electrophoretic separation occurs due to differences in mobility between the analytes under influence of an electrical field; some components move down the capillary faster than others. The basic instrumental set-up, as illustrated in figure 7, consists of a high voltage supply, fused silica capillary, buffer reservoirs, electrodes and an on-column detector.

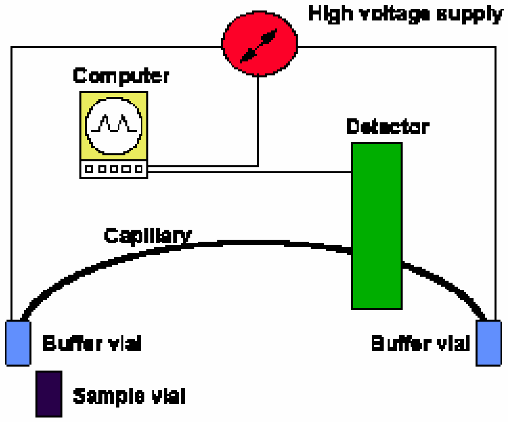


Figure 7: Scheme of a conventional CE instrument.

The sample is introduced into the capillary by hydrodynamic injection or by electro-kinetic injection. The hydrodynamic injection is performed by creating a pressure difference between the buffer and the sample reservoirs during dipping the appropriate end of the separation capillary into a sample solution.

Using electro-kinetic injection, voltage is applied (1-5 kV) for a time while the beginning of capillary is inserted in the sample reservoirs. This sampling mode is at some cases more sensitive than hydrodynamic injection. However the injected amount is matrix dependent and thus application of hydrodynamic injection is more popular.

High voltage (maximum 30 kV) is generated over the capillary for the separation of analytes and the charged components move through the capillary under the electric field. Positive components migrate to the negative electrode (cathode) and negative components migrate to the positive electrode (anode) at different velocity.

The mobility difference between the separated substances depends mostly on the analyte's charge and size.

$$\mu_{ep} = \frac{Q}{6\pi\eta r}, \qquad (1)$$

where μ_{ep} is the electrophoretic mobility of a substance, Q is the charge of the substance, η is the viscosity of separation medium and r is the hydrodynamic radius of the substance. The charge of ion strongly depends on pH of running buffer, and thus the migration velocity of the molecules also differs with the pH. The size is a combination of the sample component and the bounded water shield to the solute. This is the reason that small inorganic metal ions migrate with similar velocity as the volatile alkyl-amines.

Modifiers like as organic solvents, surfactants, cyclodextrins or polymers can be added into the separation buffer to enhance mobility differences between the target components. When cyclodextrin is added into the buffer the technique is called electro-kinetic chromatography and when surfactants are added it is micellar electro-kinetic chromatography. In these cases the separation depends also on the solubility of a solute since more apolar ones stays longer in the micelles produced by the surfactant. However, the separation can be modified by other factors. When the capillary is filled with a solid phase the technique is classified as capillary electrochromatography (CEC).

In most applications the capillary is by fused silica. This material contains on its surface silanol groups. These groups are slightly acidic thus they are negatively charged at alkaline condition. When high voltage is generated over the capillary, the positively charged buffer constituents migrate towards the negative electrode but during the migration they also interact with the negatively charged silanol groups producing a layer which bring the neutral and negatively charged compounds towards the cathod too.

This flow is called as electro-osmotic flow (EOF) which is proportional to the dielectric constant, zeta potential at the capillary-buffer interface and inversely proportional to the viscosity of the separation medium. Thus the mobility of the solutes depends not only the μ_{ep} also on the EOF as shown in equation 2 when the separation buffer does not contain any modifiers.

$$\mu_{app} = \mu_{ep} + \mu_{eo} , \qquad (2)$$

where μ_{app} is the apparent mobility of the compound and μ_{eo} is the mobility of EOF. Thus EOF play a big role in the separation. Generally, EOF can not be fully controlled small changes in the EOF cause big change in the migration velocity of the solute and poor precision.

However, EOF has also advantages. On one hand, negatively charged or acidic compounds can be also separated after the detection of positively charged or basic compounds without any modification of the instrumentation. On the other hand, the flow profile is flat because the positive charge density is high close to the capillary wall and there is no drop in the capillary. This causes no peak broadening like the parabolic flow profile in HPLC and GC, and that is one of the reasons why such a high resolution can be achieved in capillary electrophoretic separations. Under typical conditions, a CE separation invokes 50,000 to 500,000 theoretical plates, which is at least one or two magnitude higher than in HPLC and better than capillary GC.

The most commercial detection mode is based on spectrophotometry (UV/VIS, DAD or fluorescence). Spectrophotometric detectors used in CE might be an order of magnitude less sensitive compared to those available for HPLC because the former detectors use shorter path length. For enhancing the sensitivity, different stacking or sweeping techniques were developed. Additionally, the detection path length can be also elongated by the use of a bubble or Zet-detector cell. However these methods to improve the method sensitivity can not be used for all cases and the enhancing factor in some cases is not enough for trace analysis. For example bubble detector cell has enhancing factor of 3-5 and Z-cell has 10-30, and peak broadening effect often occurs. Moreover, these special detector cells cannot be used with all type of instrument. Thus other techniques should be developed for decreasing the detection limit of the capillary electrophoretic separations. Another detection system has been also coupled with CE such as MS, conductivity, electrochemical or radioactive detector.

Scheme of optimisation process: Since the selected analytes are Lewis acids or bases CZE or free solution CE was selected that is the simplest capillary electrophoretic technique. In CZE the separation is based on the apparent mobility of the solutes. At first, theoretical mobilities of selected analytes were calculated with a help of self-made program [255]. The program plotted the ratio of charge and molecule mass on the two third ($Q/M^{2/3}$) in the function of pH in accordance with equation 1. From that diagram the pH range was estimated in which the analytes are fully or partly charged.

Then the buffer system of the background electrolyte (BGE) was selected. The buffer capacity should be the maximum in the estimated separation range to have similar mobility of the targets for run to run. On the other hand, the mobility of the buffer electrolyte should be in the range of the analyte mobility to avoid the tailing of the peaks.

After selecting the buffer system, its optimal concentration had to be determined. The concentration of the buffer determines the EOF, current and peak symmetry. With increase of the concentration of running buffer the current increases since the resistance of the background electrolyte (BGE) decreases according to the Ohm equation.

After the concentration of BGE separation buffer was determined, the pH of running medium was optimized. After determining the optimal concentration and pH of the BGE the buffer system, organic modifiers like acetonitrile, methanol, ethanol etc. were added into the background electrolyte at different concentration and their effect on the separation quality was investigated.

Then capillary dimension like inner diameter (I.D.) and length were optimised. The first choice was always 57 cm total length (50 cm till the detector or effective length) and 75 µm I.D. Finally, the effects of applied voltage and temperature on the separation and current were investigated. The final separation protocol is summarized in chapter 4.

3.4 Problem of data interpretation and possible solutions

The data interpretation plays also an important role in the analytical process. In this step the target substances are identified and quantified. The identification in CE is generally based on migration time but other parameters correlated from the migration time are also used in practice. One type of transformed identification parameter is the effective mobility which was also calculated and the transformation process is described in the following chapter.

From the size of the peak, the injected concentration of the solutes can be determined. In separation techniques, peak height or peak area is used for determination of analyte concentration, and use of peak area is usually more reliable than peak height.

To assume the reliability of a whole method, the analytical characteristics of the targets were determined when (i) the analytes were solved as standard and (ii) when the target compounds were spiked in the matrix to study the matrix effect on the determination of the selected compounds.

3.4.1 Effective mobility scale transformation (identification)

One major disadvantage of CE compared with HPLC is its poor reproducibility of MT and peak area. The low precision of these parameters is often related to little changes in EOF due to uncontrollable alternations of the capillary surface, which lead to MT shifting. If the effect of EOF can be eliminated on the migration of the solutes the reliability of the identification and quantification would be dramatically improved.

Thus when the migration times are transformed into effective mobility ($\mu_{eff,}$ [$cm^2/V*s$]) the method is assumed to be more representative and precise [242]. The effective mobilities of target substances are determined with use of the following equation.

$$\mu_{eff} = \frac{L_d * L_t * (t_{eof} - t_m)}{V * t_{eof} * t_m}, \qquad (3)$$

where L_d is the effective length, L_t is the total capillary length, V is the applied voltage, t_{eof} is the time of the EOF and t_m is the MT of the solute.

Effective mobility value shows the charge of the compounds: if it is anion at the separation condition, its effective mobility is negative and positive when the analyte separated as cation. Moreover, this parameter is independent on the EOF, and capillary dimensions such as the applied voltage and the length of separation capillary. When every registered point of the electropherogram is transformed to effective mobility, effective mobility scale is arisen.

The effective mobility can be normalized ($\mu_{eff,norm}$) with the following equation, when a marker with known mobility is added to the injection solution:

$$\mu_{eff,norm} = \mu_{int} + \frac{L_d * L_t * (t_m - t_{int})}{V * t_{int} * t_m} \quad (4)$$

,

where μ_{int} is the mobility of internal standard and is the migration time of the internal standard. Normalized effective mobility scale (normalized μ-scale) is given, when the detected signal is plotted in the function of normalized effective mobility.

Mobility scale or normalized mobility scale can be used for quantification, since the transformation from time scale does not result in any loss of information due to the bit-to-bit correspondence [242]. On the other hand, better precision of peak area is expected when the electropherogram is transformed into mobility scale [242].

This transformation is not usual in other liquid phase separation technique but necessary in CE to overcome the variations of MTs and peak area. Considering the “CE-mode thinking” the measured electropherograms were transformed to effective mobility scale, and then the performance characteristics were determined and compared with the characteristics determined form the conventionally used time-scale. The transformation process was made by a program called GelTreat [243]. Thus it will be shown from different examples that this scale can be easily or routinely applied for capillary electrophoretic separations.

3.4.2 Determination of solute concentration (quantification)

The concentrations of the target compounds were calculated from electronically integrated peak area using calibration curve or internal standard technique. The integration was made using self-developed program called GelTreat [256] and the solvent used for calibration was identical as for the sample preparation. For calibration the integrated peak areas were plotted in a function of the theoretical concentration of standard solution.

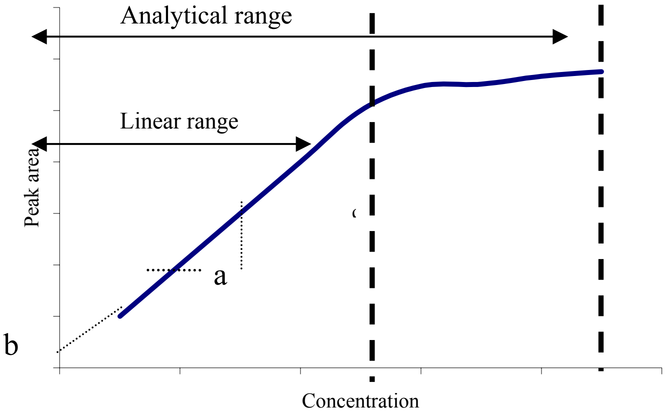


Figure 8: Peak area response in function of analyte concentration.

As shown in figure 8, peak area is proportional to analyte concentration at low range of the measured concentration. The linearity of peak area response is determined by regression coefficient with use of equation 3.

$$r^2 = \left(\frac{\Sigma(c_i - \bar{c}) * (A_i - \bar{A})}{\sqrt{\Sigma(c_i - \bar{c})^2 * (A_i - \bar{A})^2}}\right)^2, \qquad (5)$$

where c_i is the actual concentration of the target compounds, $\bar{c}$ is the averaged concentration, A_i is the peak area at actual concentration of target compounds and A is the averaged peak area. When the regression coefficient is higher than 0.99, linear dependency can be concluded and thus calibration can be used for determination of concentration of analytes from the integrated peak area with use of the following equation.

$$c = \frac{A - b}{a}, \qquad (6)$$

where A is the peak area, a is the slope of the linear slope, c is the concentration of the analyte and b is the y intercept of the trend line (see figure 9). The value of the slope and intercept of y-axis for each analytes should be determined by separating the analytes at minimum five different concentrations every measuring day.

Calibration can not be used for all cases since unpredictable parameters may affect the peak areas mostly when the substances measured from sample containing matrices. For example, small changes in the viscosity or ionic strength of the sample solution may influence the injected amount of analyte or injected volume of the sample solution. In these cases, so called internal standard method is used for eliminating the uncountable effects causing different peak areas integrated for the same concentration of target compounds.

This method is based on addition of known compounds called internal standard into the sample before the injection. The requirements against this compound are that (i) the real sample should not contain it, (ii) it should have same physical-chemical properties as the analytes and (iii) it should be separated from the target compounds. However, this technique can be only used when the peak area of analytes and internal standard is linearly dependent on their concentration since the process assumes constant value of the ratio of the calibration slopes of the analyte and internal standard (see equation 7).

$$f = \frac{a_i}{a_{st}} = \frac{A_i * c_{st}}{c_i * A_{st}} \rightarrow c_i = \frac{A_i * c_{st}}{f * A_{st}}, \qquad (7)$$

where f is the relative sensitivity, a_i is the slope of the target, a_{st} the slope of the internal standard, A_i is the peak area of the target, c_{st} [mg/l] is the concentration of the internal standard, c_i [mg/l] is the concentration of the target ad A_{st} is the peak area of the internal standard. For application of equation 7, the f has to be determined with determination of analytes with known concentration every measurement day. The use of internal standard might be as “indicator” of matrix on the separation of the analytes from real samples.

To determine the concentration of the analytes in the sampled air, the conditions of sampling and sample preparation have to take into account with using the following equation:

$$c_{air} = \frac{c_{sol} * V_{sol} * D_f}{V_{air}}, \qquad (8)$$

where c_{air} is the concentration of the solutes in the air, c_{sol} is the concentration of the solutes after the sample preparation, V_{sol} is the volume of the extraction solvent, D_f is the dilution or concentration rate before the analysis and V_{air} is the total volume of pumped air during the sampling.

3.4.3 Determination of analytical performance characteristics

The required analytical performance characteristics for validation were determined according to the standards DIN EN 482, EN 4500 and ISO/IEC Guide 25 to characterise the correctness and precision of the optimised method.

Resolution: This performance characteristic shows if the solutes are separated for reliable identification. The resolution (R_s) can be defined as a following:

$$R_S = 2 * \frac{t_{m,2} - t_{m,1}}{w_1 + w_2}, \qquad (9)$$

where $t_{m,I}$ is the identification parameter of compound i, and w_i is the peak width of compound i. If the R_s of the separated substances higher than 1.5, they can be differentiated from each other when they present in the sample.

Precision: The parameter describing the precision is the standard deviation (s^2) which was calculated with the equation 6.

$$s^2 = \sqrt{\frac{\Sigma(x_i - \overline{x})}{n-1}}, \qquad (10)$$

where x_i is the measured data, $\bar{x}$ is the average and n is the number of repetitions. For precision of identification and quantification the measurements were repeated three or five times at three different concentrations in the working range.

When the relative standard deviation (RSD) of identification parameter were determined between 1-2%, the method is concluded enough precise to use for real sample analysis. When the RSDs of peak areas that describe the repeatability of quantification were lower than 10% precision fits to the specific analytical requirement.

Linearity and working range: as it was described in the previous chapter, standard solutions at different concentrations were measured and the integrated peak areas were plotted in the function of the solution concentration. Trend line was drawn through the measured points and the correlation coefficient was determined to check the proportional response between peak area and analyte concentration.

The maximal allowed concentration (MAC) of targets determined the working range. MAC is defined as the maximum concentration of a chemical substance as gas, vapour or particulate matter in the workplace air [165]. When the measured concentration is lower than this value it is assumed not to be hazardous even when the person is exposed during a long period for 8 hours daily or 40 hours weekly. The linear range should cover the range of 0.1*MAC and 2*MAC.

Sensitivity: The limit of detection (LOD) and limit of quantification (LOQ) show the sensitivity of the method. They were calculated according to DIN 32645 [244] after calibration. LOD and LOQ are usually defined as the concentration at which the peak area three and ten times bigger, than the noise one (see equation11).

$$\mathrm{LOD} = \frac{3 * \mathrm{noise}}{\mathrm{a}} \qquad \mathrm{LOQ} = \frac{10 * \mathrm{noise}}{\mathrm{a}}, \qquad (11)$$

where noise in the averaged peak area of the noise in the baseline and a is the slope of the calibration curve. When LOQ was lower than the lowest point of the working range (0.1*MAC) the method was feasible for quantification.

Accuracy: The accuracy of the whole analytical procedure including the sampling, sample preparation and separation was characterized by the recovery and shows the correctness of quantification. The relative recovery (w) is defined as:

$$w = \frac{c_{theo} - c_{meas}}{c_{theo}} * 100\,\%, \tag{12}$$

where c_{theo} is the theoretical concentration, c_{meas} is the quantified concentration calculated from calibration curve.

When the recovery of the measured substance is 100%, the measured concentration is identical to the theoretical one. The recoveries were determined always for the overall procedure at three different concentrations of solutes. If the relative difference between the measured and theoretical amount was lower than 10% the method was correct for sample analysis.

Thus for having a reliable method that can be used for measuring unknown samples the performance characteristics which are selectivity, linearity, sensitivity, precision and accuracy were determined including the whole analytical process.

3.5 Study of matrix effect

Since the optimised and validated methods were aimed for determination of the target compounds from atmospheric and MWF aerosol, the effects of MWF constituents on the sampling, extraction efficiency and separation were studied.

At first, different MWF concentrates were diluted and injected to CE instruments. From the investigated coolants Polynor FMR produced by Rhenus and Wicopal-HS produced by Wilke were suitable, since no peaks were detectable at the investigated range and the diluted solution was a stable emulsion; white, non-transparent emulsion was formed from yellow transparent fluid after dilution and shaking.

For purpose of matrix effect investigation, three emulsions at different concentrations of MWF concentrate (0%, 1%, 3% of Polynor FMR and 0%, 5%, 10% for Wicopal in accordance with the practice) were prepared and then spiked with the target analytes. The final concentration of target was 10 mg/l in each emulsion. From the spiked emulsion 200 µl were injected to the quartz filter, and clean air was drawn through the filter at a flow rate of 1 l/min for 2 h. Then they were extracted in 5 ml water-methanol mixture with ultrasonic bath for 15 minutes. Then the solution was filtered and injected to CE instruments. The whole analytical process was repeated three times for each emulsion. Standard solution of analytes was always analysed between the prepared samples prepared to control the stability of the measurement.

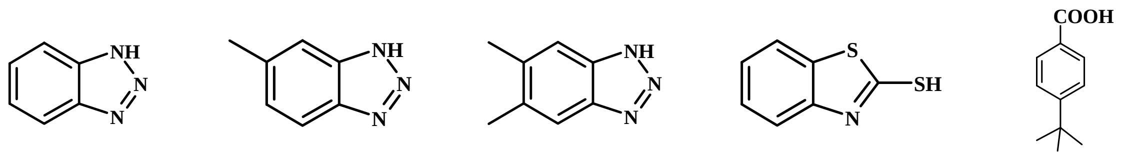
At first, the effect of matrices on the identification parameter was examined and compared, and then the quantification performance characteristics (recovery with RSDs) were determined and compared.

4. Results and discussion

Capillary electrophoretic methods were developed and validated for the determination of highly polar organics containing heteroatom –mainly nitrogen- from atmospheric and MWF aerosol. The target compounds of the methods were (i) saturated heterocyclic amines (ii) unsaturated heterocyclic amines and their by-products (derivatives of amino-alcohols) and (iii) LMW and biogenic alkyl-amines. These hazardous substances are MWF additives or by-products, thus they may be ingredients of MWF aerosol. Since these substance classes have different physical-chemical properties, their determination is discussed separately.

4.1 Determination of hetero-aromatic amines

The aim of the work was to develop and to validate analytical processes for the determination of hazardous heteroaromatic amines from MWF aerosols. The selected compounds for the development of capillary electrophoretic method were 1,2,3-benzotriazole (BTA), 5-methyl-1H-benzotriazole (MBA) and 5,6-dimethylbenzotriazole (DMBA), 2- mercapto-benzothiazole (MBT) and p-*tert*-butyl-benzoic acid (PTBBA).



BTA MBA DMBA MBT PTBBA

Figure 9: Chemical structure of benzotriazoles and benzothiazole.

They are commonly used as copper inhibitors in coolants, as light stabilizers in photographic films and as bactericide agents in MWF. Since they are synthetic organic compounds they cannot be found in the nature. However, similar structures such as benzoisothiazole, methyl-indole or methyl-thiobenzothiazole have been identified from the humic substance fractions of atmospheric air particles [245]. The adverse health effects of the derivatives of benzotriazoles, MBT and PTBBA have been evaluated based on the available data on humans as well as on experimental animals. BTA and MBT have been shown to induce tumours [246,247] and irritate the eyes and the skin. DMBA, MBA and PTBBA were evaluated as skin sensitizer substances [152]. The MAC at working areas was established to 2.08 mg/m^3 for PTBBA, to 4 mg/m^3 for MBT; and no MAC values have been established for DMBA, MBA and BTA [152].

BTA, MBA and DMBA contain three nitrogen atoms side by side in cyclic system, thus they are anions at high pH in spite the fact that amines are generally Lewis bases. MBT can be grouped into amides that are weak acids. PTBBA is a derivative of organic acid. Thus these solutes are anions at alkaline condition and can be separated with free solution capillary electrophoresis. They are polar compounds although their solubility in water is limited since their octanol/water coefficient is between 2 and 3 (see Appendix), so they are double or triple time more soluble in apolar solvent than in water.

An earlier developed capillary electrophoretic method [243,244] for the determination of BTA, MBA and DMBA in MWF aerosol was at first studied. The process permits the quantification of the target compounds when present as particles in a concentration range of 0.2 to 4 mg/m^3. For sampling the MWF aerosol, the occupational air was pumped through a quartz fibre filter with suitable sampling pump and PM was collected on the filter. For stabilization, the loaded quartz fibre filters were covered with double distillate water directly after sampling and for complete extraction before analysis the filters covered with water were treated in ultrasonic bath. Analytical determination was carried out by CE coupled with DAD. Standard solutions of MBA, BTA and DMBA were used for calibration. Applying the published method, the following electropherogram was detected.

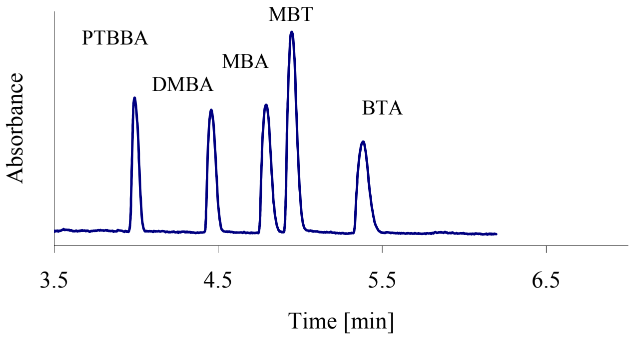


Figure 10: Electropherogram of selected benzotriazoles and benzothiazole at analyte concentration of 10 mg/l.

The critical substances in the separation were MBA and MBT, thus the resolution of these two compounds was examined. The R_s were between 1.6 with RSD of 14% calculated from five repetitions. The high RSD of resolution shows that the separation of the targets is imprecise. On the other hand the R_s values were near to the acceptable limit thus the identification and the quantification of MBA and MBT from real sample is not suggested.

Precision of identification: In order to check the reliability of the identification, the precision of MT of PTBBA, DMBA, MBA, MBT and BTA were evaluated for within-day measurements. The run-to-run RSDs of MTs ranged between 1.3-4.0%, and were independent on the concentration of the solutes. Generally, the lowest RSD was determined for the first peak (PTBBA) and the highest for the last one (BTA). The day-to-day precision were plotted in table 4, where the determined median, measured minimum and maximum of MTs were shown.

Table 4: Day-to-day precision of MT independently on the analyte concentration (n=25).

Box and whisker plot	*Substance*	**MT [min]**	**RSD [%]**
	**PTBBA**	4.9	5.1
	DMBA	5.6	6.2
	MBA	6.2	7.0
	MBT	6.4	7.2
	BTA	7.1	8.3

The determined RSD values were high that represent low day-to-day precision. The same trend on the RSDs in the function of peak sequence was observed as for within-day precision: the lowest RSD was determined for PTBBA and the highest for BTA. The differences of MTs determined from capillary to capillary were between 6-9% for each substance that was higher than those described in the literature (4-6% for fused-silica tubes with 50 to 250 µm I.D. [87]).

Because of the quite high RSDs of day-to-day precision, the influence of the buffer prepared on different days was investigated. The determined difference on the MTs of the targets was 10% meaning that the MTs were strongly influenced by the preparation of the BGE.

Quantification performance characteristics: for determining the quantification characteristics the method was performed as published. The working range that fit to the MAC of the targets was set between 0.208 mg/m^3 and 4.16 mg/m^3 in the air that is equal to 2.5 mg/l and 50 mg/l in the extraction solution. The quantification performance characteristics were determined at concentration of 0.208 mg/m^3, 2.08 mg/m^3 and 4.16 mg/m^3 and they were summarized when the characteristics were independent on the analyte concentration. The measurements were always five times repeated. The following table summarize the values of averaged quantification performance characteristics.

Table 5: Quantification performance characteristics at range between 2.5-50 mg/l (n=5).

	linearity	**precision**	**sensitivity**		**accuracy**
	r^2	RSD [%]	LOD [mg/l]	LOQ [mg/l]	w [%]
PTBBA	0.995	6.30	0.10	0.35	(72)
DMBA	0.998	6.36	0.17	0.56	89
MBA	0.993	5.21	0.12	0.41	89
MBT	0.995	8.84	0.06	0.19	(75)
BTA	0.999	5.01	0.13	0.43	89

The peak areas were linearly dependent on the solute concentration in the working range, thus calibration can be used for quantification when the analytes are solved in high purity water. The RSDs of the peak areas were concentration independent and were under 10% however the precision of quantification was not excellent. The detection limits were in the upper µg/L range, and the LOQs were lower than the lowest point of the required working range (2.5 mg/l). However increased sensitivity would allow for determination at trace level. The accuracies of determination of benzotriazole derivatives were concentration independent but the recoveries were near to the required limit. For the other two substances (PTBBA and MBT) systematic concentration dependence on the recovery was observed. The recovery of PTBBA decreased with the increase of its concentration, and the opposite effect was found for MBT. Additionally, the accuracy of MBT and PTBBA was poor and did not fit to the specified analytical requirement.

Real samples were taken in a metal working plant, but none of these corrosion inhibitors was detectable in spite the fact that the fluid contained BTA at a concentration of 0.1% (w/w) (1000 mg/L). It might be possible that (i) the sampling time might be too short and/or (ii) the matrices influenced the extraction efficiency or determination process. Both explanation are reliable, since the ventilation system of the plant was properly thus only a small part of the produced MWF droplets stays permanently in the occupational air. On the other hand, the analyte could retarding in the filter since they are 2-3 times more soluble in apolar solvents than is water and no matrix effect was investigated.

Several drawbacks were observed during the measurement of standard solution of PTBBA, DMBA, MBA, MBT and BTA such as (i) poor resolution of MBA and MBT, (ii) poor run to run, day to day and capillary to capillary precision of MTs, (iii) poor and dependent accuracy of PTBBA and MBT and (iv) no matrix effect was investigated.

Moreover a special capillary so called extended light capillary was used to decrease the detection limit. This capillary can not be used for every commercial CE instrument thus the method can not be applied in every laboratory without any modification. Since these disadvantages were identified, the purpose of the research work was to improve the analytical method to get a more reliable method, which can be used for routine determination of these hetero-aromatic corrosion inhibitors from MWF aerosol.

4.1.1 Study of the sampling process

When samples were taken in a plant where MWF is used for cutting, no any target compounds were identified in spite the fact that the used coolant contained 1000 mg/l of BTA. It was assumed that the sampling time (2h) was too short. Thus the sampling time was increased from 2h to 24h, and the reliability of the sampling procedure with longer duration time was studied. The aim of this study was to determine the sample loss during long period sampling. Clean air was forced through the immersed filters containing different amounts of analytes (25 µg, 250 µg and 500 µg for each target compounds) at different sampling durations (2h, 12h, 24h). Then these filters were extracted and injected into the CE. The recoveries of PTBBA, DMBA, MBA, MBT and BTA with RSDs were determined from three repetitions.

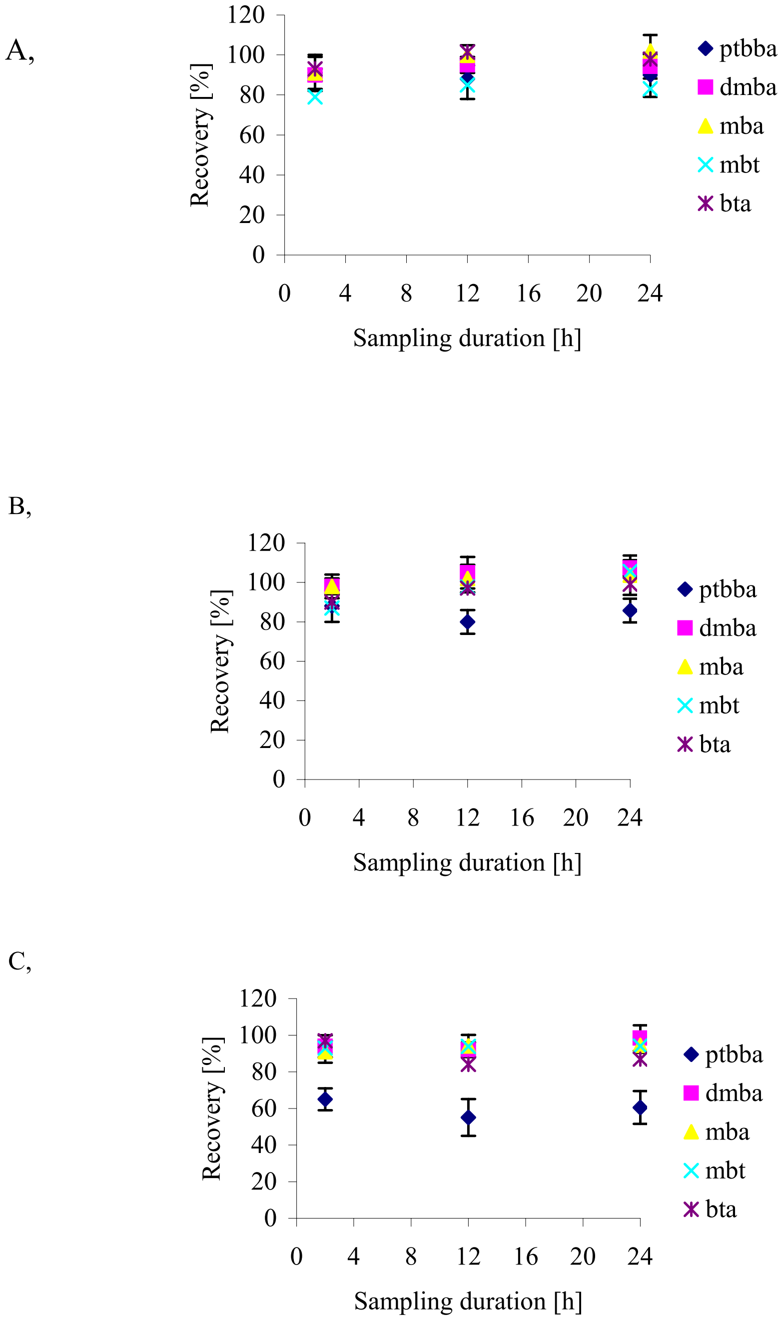


Figure 11: Recoveries in funtion of sampling duration when the filter was immersed with A, 25 µg; B, 250 µg and C, 500 µg of each target compounds repeated three times.

As shown in figure 11, no systematic alteration was observed in the recovery values measuring different amounts of PTBBA, DMBA, MBA, MBT and BTA when the sampling time increased from 2 h to 24 h. However, the recovery of PTBBA systematically decreased with increasing of its concentration. This phenomenon was also observed during the investigation of the tested method. The determined RSDs of target compounds were between 4-7% for each concentration. Consequently, it is recommended to increase the sampling time, and from the practical point of view, the selected maximum duration time is 24 h.

4.1.2 Improvement on the sample preparation

In the developed method, the immersed filters were extracted with 10 ml high purity water to dissolve the water-soluble components. Unfortunately, the recovery of PTBBA systematically decreased with increase of its concentration (see figure 11). This can be cause by the low water solubility of PTBBA. The octanol-water partition coefficient (logP) of PTBBA is 3.85 meaning that it is more than thousand times better soluble in apolar organic solvent than in water. Thus it was supposed that the solubility of PTBBA reached its equilibration when its concentration was increased, hence a portion of the total amount stayed on the surface of the quartz filter after the extraction process.

Thus the recovery of PTBBA can be increased with changing the polarity of the extraction solvent. In practice, the extraction solvent should accomplish several analytical requirements, such as (i) the final extraction solvent of the sample should be compatible with the separation buffer, (ii) the solutes must be soluble in the extraction solvent and (ii) the solvent must be pure. All above mentioned requirements can be fulfilled if a methanol-water mixture is used for the extraction of the filters. To find the optimal extraction solvent, the methanol content of extraction solvent was set from 10% (V/V) to 50% (V/V). Higher percentage of methanol was not reasonable, since the solvent of the sample plug and separation buffer dramatically differ from each other. The determined recoveries with the corresponding RSDs in function of extraction solvent are plotted in figure 12.

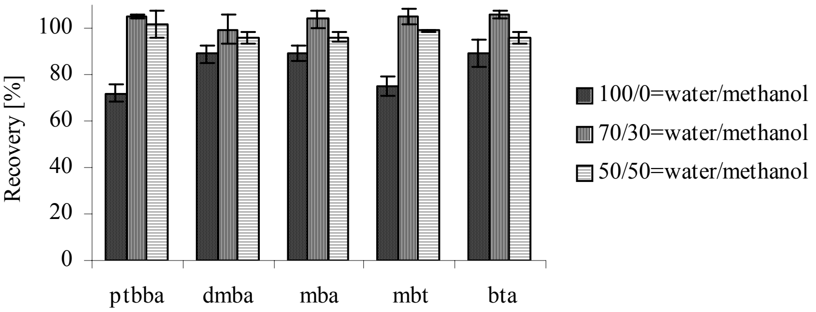


Figure 12: Extraction efficiency in function of methanol content in water at analyte concentration of 10 mg/L (n=5).

The recoveries increased when the extraction solvent contained methanol comparing to the results determined from water extraction. No difference between the recoveries was observed when the methanol content was increased from 30% to 50%. The final extraction solvent had a methanol content of 30% (V/V) since it is more compatible to the BGE. The RSDs were between 1-6% for each target compound.

Addition of pre-concentration step: An additional step in the sample preparation was developed to decrease the determination limit of BTA, DMBA, MBA, MBT and PTBBA, because the measurements of real samples showed that the earlier validated method is not enough sensitive. Thus a pre-concentration step was developed into the method which based on the evaporation of solvent under vacuum from 10 ml to 0.5 ml (concentration factor: 20). This pre-concentration step is theoretically suitable, because the volatility of water and methanol is higher than that of the target substances. Presumably, the total amount of methanol evaporates since methanol-water mixture does not compose azeotrop mixture. However, this step can be used only with restrictions. It is unnecessary if the concentration of the analytes is higher then 2.5 mg/l and it has to be omitted if the concentration of PTBBA is higher than its water solubility (28 mg/l).

Standard solutions of PTBBA, DMBA, MBA, MBT and BTA were prepared at concentration of 1.5 mg/l, 0.7 mg/l and 0.2 mg/l; 10 ml from these solutions were pre-concentrated to 0.5 ml under vacuum in water bath at 40^{o}C. The average duration time of evaporation was 40 min. The recoveries with their RSDs were obtained from five replications at three different concentrations of target substances. The calibration curves were plotted with standard solutions solved in distillate water, since it was assumed that methanol evaporates.

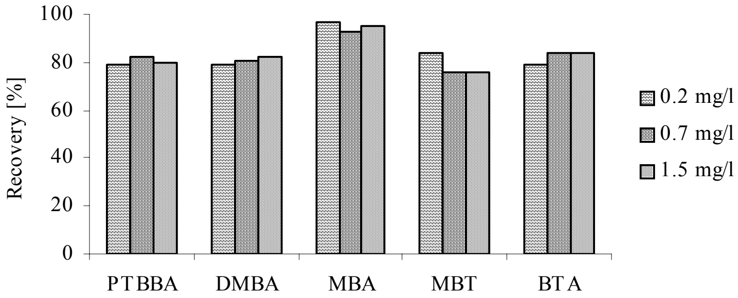


Figure 13: Recoveries of benzotriazoles and benzothiazole at different stadard concentrations applying pre-concentration during sample preparation.

The additional pre-concentration step leads to lower recoveries for PTBBA, MBT, BTA and DMBA comparing the average recoveries. However, pre-concentration is necessary for analysing real samples because the targets are minor components of MWF aerosol.

6.3 Optimisation of separation and determination of performance characteristics

The investigation of the migration behaviour of the analyte is generally important for the optimisation of the CE separation. The mobility of a certain analyte can be influenced by different factors, such as the pH of the buffer, the content of organic modifiers in BGE etc.

At first, the ratio of charge and molecular weight on the two third of the solutes in function of pH were determined by using a self-made program (see figure 14).

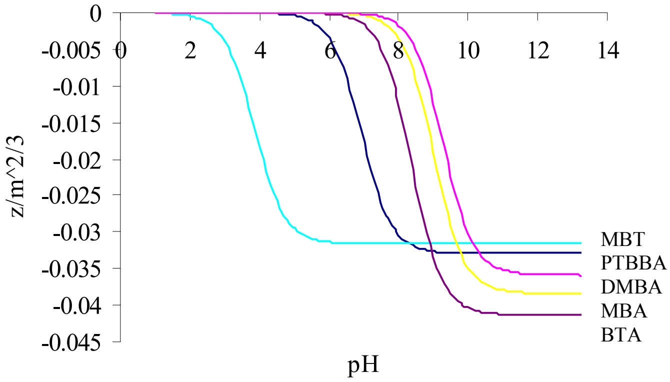


Figure 14: Theoretical migration behaviour of benzotriazoles and benzothiazole.

According to the theoretical calculation the pH of BGE should be more than 11 for having a robust separation method for the selected compounds. This value is quite high for practical point of view since only two buffer systems (phosphate or CAPS) has buffer capacity at this range. CAPS was selected since the current was lower as for phosphate buffer [244].

The theoretical prediction was then compared with the measured migrations by varying the pH of BGE in a range of 10-11.5. Typical electropherograms at different running medium are shown in the following figure.

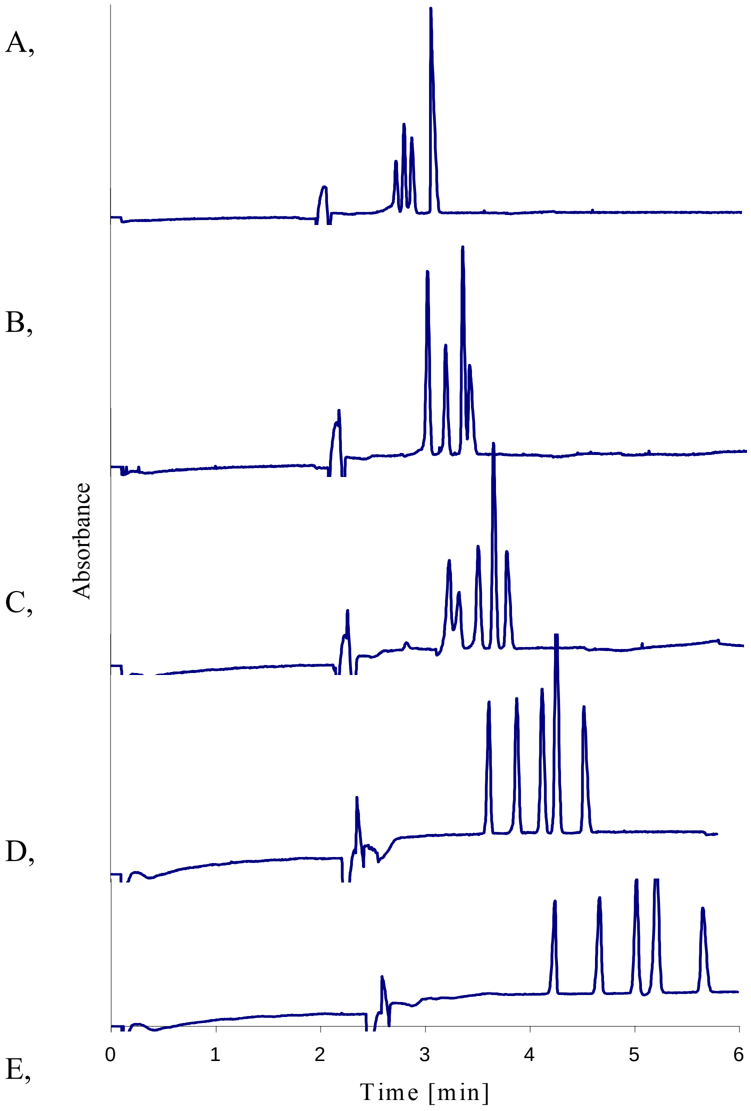


Figure 15: Electropherogram of PTBBA, DMBA, MBA, MBT and BTA separated with CAPS buffer at A, pH 10.0; B, 10.2; C, 10.5, D, 11.0 and E, 11.5.

The experimentally determined migration behaviour in the function of pH was identical than the predicted one. PTBBA, MBA, MBT, DMBA and BTA were baseline separated when pH of BGE was higher than 11.0. At pH values between 10 and 11, the peaks were close together and only four from the five analytes were separated. Comparing the peak sequence with the theoretical prediction (figure 14) difference in the case of MBT was observed

For further examination the pH of the buffer was set to 11.4, since at this condition the target compounds are baseline separated and the buffer capacity is higher than at pH 11.75 resulting better precision of the method. Moreover, the separation time decreased.

The presence of organic solvents (such as methanol or acetonitrile) in BGE could also influence the separation, so both solvents were added separately to the BGE, and the resolution of critical substances (MBA and MBT) was investigated in the presence of these organic modifiers at pH 11.4 (see figure 16).

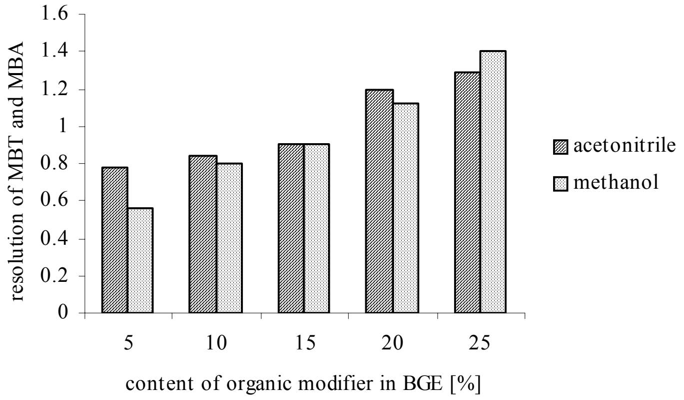


Figure 16: Resolution of MBA and MBT in presence of organic modifier (acetonitrile and methanol) in BGE at percentage of 5%-25%.

With increasing concentration of organic modifier in BGE the separation time increased. While the separation time at 5% of methanol or acetonitrile in the buffer was 6 minutes, it was 15 minutes at 25%. The increase of separation time causes peak widening, and hence poorer resolution and higher detection limit. Thus the content of organic modifier in BGE was not increased above 25 V/V%. The resolution of critical substances increased from 0.8 to 1.4 with increase of concentration of methanol or acetonitrile and no difference was observed in the resolution when methanol or acetonitrile was added into the BGE. Considering the separation time and resolution of MBA and MBT 15% acetonitrile was chosen for further analysis.

Finally the separation voltage and cartridge temperature was changed, and their effect on the separation of the selected solutes was investigated. The separation time decreased when the voltage or the cartridge temperature increased, but no effect on the resolution was observed.

Conventional capillary was used for separation purpose instead of extended light capillary to can use the developed method in any CE instrument. The I.D. was 50 µm thus the current was decreased instead of 75 µm I.D. However, with decrease of I.D. the sensitivity also decrease since less volume is injected and the path length is shorter. The effective length of capillary was shortened from 50 cm to 40 cm to speed up the separation.

After optimization of a capillary electrophoretic method for the determination of PTBBA, DMBA, MBA, MBT and BTA the analytical performance characteristics were determined and the following electropherogram was typical when standard solutions at concentration of 10 mg/l were injected into the CE instrument.

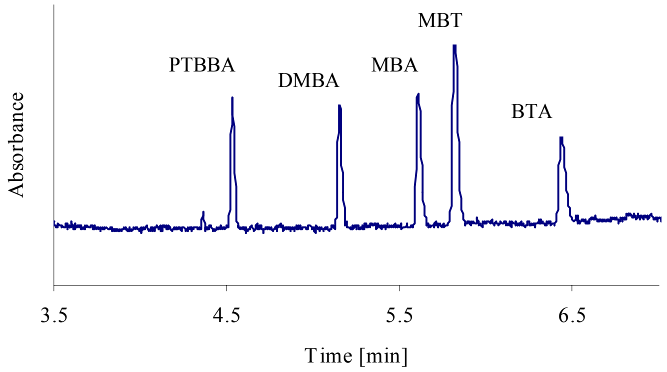


Figure 17: Typical electropherogram of benzotriazoles and benzothiazole at final condition.

The target substances were separated in 7 minutes. The observed peaks were narrower comparing the peak measured with extended light capillary and the critical substances were also fully separated.

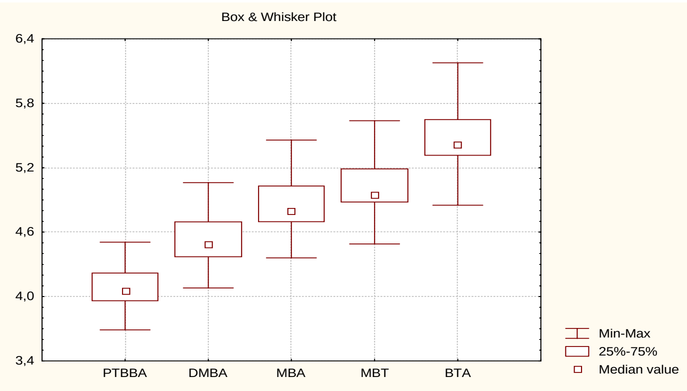
Resolution: In spite the fact that the critical substances were more than baseline separated, the resolution factor for this pair was determined. The determined R_s of critical substances using conventional capillary were more than 2. The RSD of the resolution from five repetitions were under 2%.

In case of the extended light capillary, R_s were near to the required limit and its RSD were more than 10 %. Thus, better resolution of MBT and MBA was achieved using conventional capillary.

Precision of identification parameter: The run-to-run RSD values were between 1% and 3% for each selected compounds. They were independent on their concentrations but were dependent on sequence of the peaks. The lowest RSDs were determined for PTBBA (first peak) and the highest for BTA (last peak).

The day-to-day precision was also investigated thus the RSDs of the MTs of the target compounds were determined from one measuring day to the other one.

Table 6: Migration times with RSDs of analytes independently on their concentrations (n=25).

Box and whisker plot		**MT [min]**	**RSD [%]**
	**PTBBA**	4.59	4.86
	DMBA	5.09	5.51
	MBA	5.52	6.01
	MBT	5.73	6.26
	BTA	6.29	6.97

The determined day-to-day RSDs varied between 5-7% and were lower comparing the results with extended light capillary (see table 4). The RSDs for examination of capillary-to-capillary precision were determined between 5-7% that meets with the electrophoretic practice, and were lower than measured with extended light capillary.

Linearity and working range: PTBBA has the lowest MAC values among the target compounds, which is 2,08 mg/m^3, so the minimum concentration has to be measured is 0,208 mg/m^3 (0.1*MAC) and the maximum is 4,16 mg/m^3 (2*MAC). The determined analytical range and the linearity is shown in figure 18.

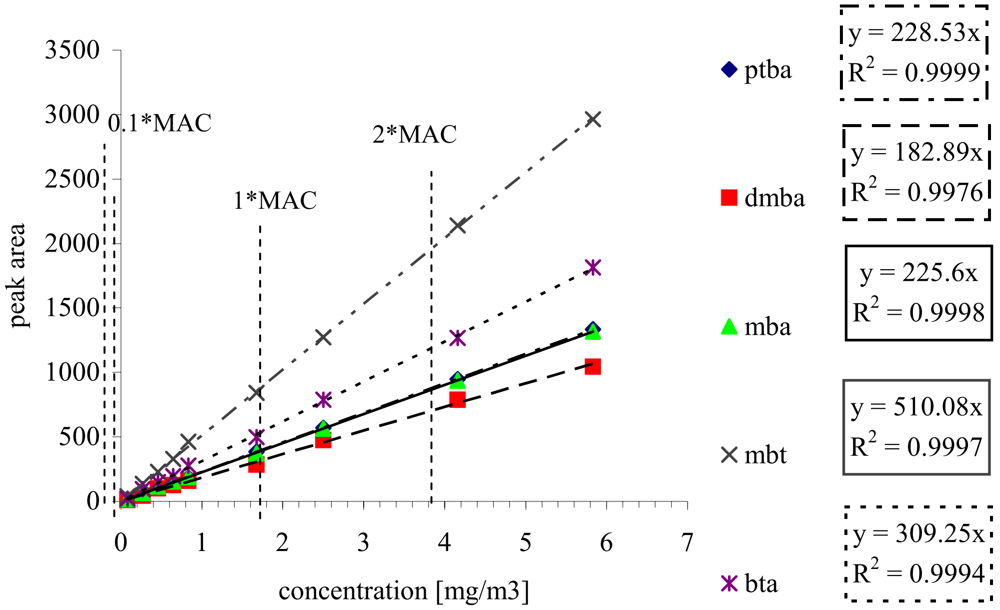


Figure 18: Linearity and working range of a method for the determination of target compounds.

Precision of quantification: The RSDs of the whole analytical method was determined from five repetitions at three different concentrations of the analytes.

Table 7: RSDs of peak area determined for the overall procedure (n=5)

Substances	**RSDs [%] of analytes**		
	0.21 mg/m^3	**2.08 mg/m^3**	**4.17 mg/m^3**
PTBBA	10.02	7.39	9.83
DMBA	6.36	7.40	10.3
MBA	8.83	10.40	9.90
MBT	9.31	9.75	10.84
BTA	8.27	10.16	11.04

The determined RSDs of injection were between 2-7%, and it was independent on the concentration of PTBBA, DMBA, MBA, MBT and BTA. The RSDs of the whole method, as shown in table 7, were quite high for each target compound (~ 10%).

Sensitivity: The LOD and LOQ for solution and 120 l pumped air were determined as described in the experimental part (see section 3.4.3).

Table 8: LOD and LOQ of a method for the determination of benzotriazoles and benzothiazole.

Substances	**LOD**		**LOQ**	
	µg/m^3	**µg/L**	**µg/m^3**	**µg/L**
PTBBA	2.15	25.5	7.5	90
DMBA	2.85	33.5	8.5	102
MBA	2.15	25.5	7.0	84
MBT	0.95	11.5	3.0	36
BTA	1.55	18.5	5.0	60

The LODs were between 0.23-0.62 mg/l in solution. The highest sensitivity was determined for MBT and the lowest for PTBBA.

The determined LOQ were lower than the lowest point of the working range, thus this method is also suitable for quantification. As it was expected, the LOQ using conventional capillary was 3-5 times higher than as for extended light capillary.

Accuracy: The recoveries of the whole process for the determination of the selected targets were determined at three analyte concentrations and the measurements were repeated five times.

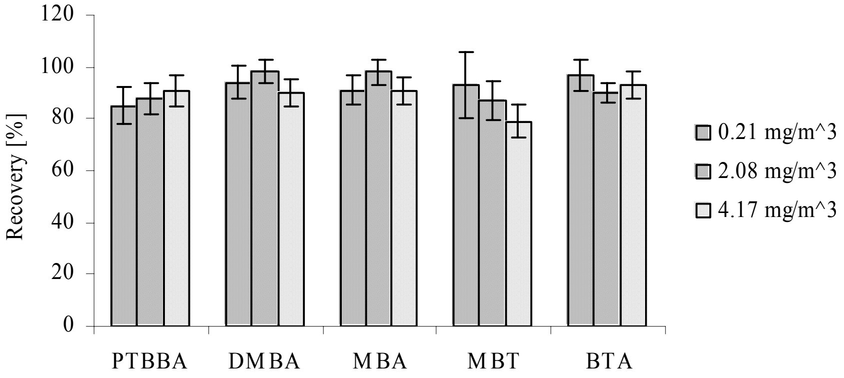


Figure 19: Recovery of a method for the determination of benzotriazoles and benzothiazole at three analyte concentration (n=5).

The recoveries of benzotriazole derivatives were more than 93%, and for PTBBA and MBT were lower. The recovery values were independent on the concentration of PTBBA, BTA, MBA and DMBA, but for MBT the recovery decreased with decrease of its concentration.

4.1.4 Improvement on data interpretation

The measured electropherograms, were transformed to effective mobility scale (see section 3.4.1). After transformation of the time scales, the peaks were integrated too and thus the performance characteristics were determined and compared to each other. For mobility scale transformation vanillin was added into the sample as a marker since it is not used in the metal industry, migrates between PTBBA and DMBA and has absorbanvnce on both detection wavelength (236 nm for PTBBA and MBT and 277 nm for BTA, MBA and DMBA).

The peak sequence of the analytes did not change after transformation of electropherogram into effective mobility scale. A typical effective mobility scale of PTBBA, DMBA, MBA, MBT and BTA is shown in figure 20.

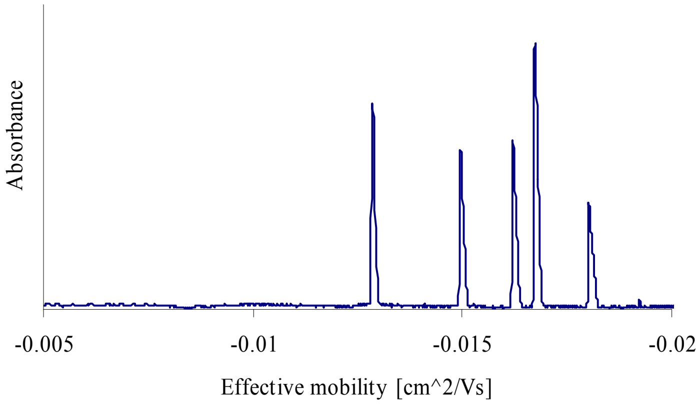
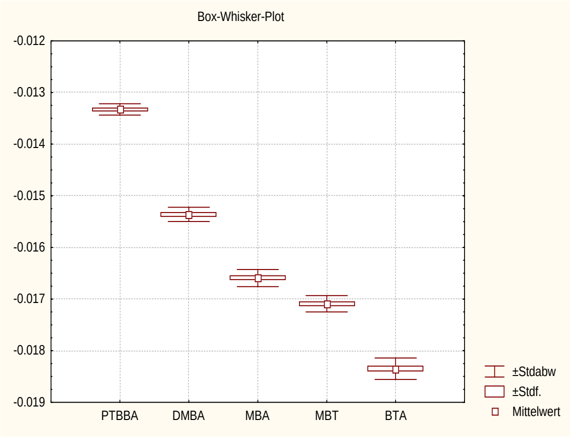


Figure 20: Typical effective mobility scale of benzotriazoles and benzothiazoles at concentration of 10 mg/l for each analytes.

Precision of identification: As it was shown in table 6, the difference between the measured maximum and minimum migration times measured at different days was quite big (more than one minute). The measured MTs of analytes were transformed into effective mobility and the determined values with RSDs are summarized in the following table.

Table 9: Effective mobilities of benzotriazoles and benzothiazole with RSDs independently on their concentration repeated 25 times.

Box and whisker plot		μ_{eff} [cm^2/Vs]	RSD [%]
	**PTBBA**	-0.031	0.86
	DMBA	-0.0151	0.77
	MBA	-0.0164	0.82
	MBT	-0.0169	0.86
	BTA	-0.0182	0.94

The day-to-day RSDs of identification parameter decreased from 4%-7% to 0.8%-1.4% when the time scale was transformed into effective mobility scale.

Moreover, the capillary-to-capillary RSDs of MTs also decreased after transformation of MTs from 5-7% to 1-2%. Thus transformed effective mobilities for the identification of the selected amines are more applicable for routine analysis too.

Linearity and working range:. The linearity of calibration curve at the working range was determined after transformation of electropherogram into effective mobility scale (see figure 21). The measured curves fit to the linear range for each target analytes when the peaks were integrated in mobility scale.

The slope of BTA and PTBBA changed when comparing to the slope values from the electropherogram. The slope of BTA decreased and for PTBBA increased after mobility scale transformation. In CE the peak area depends on the time of the detection since the speed of the mobile phase is not constant. After transformation of electropherogram to effective mobility scale this effect is on the whole eliminated and thus the slope of the substance migrate slowest decrease with the decrease of the peak area. Thus the slope of the calibration curve depends only on the spectrometric absorption coefficient when mobility scale is used for calibration.

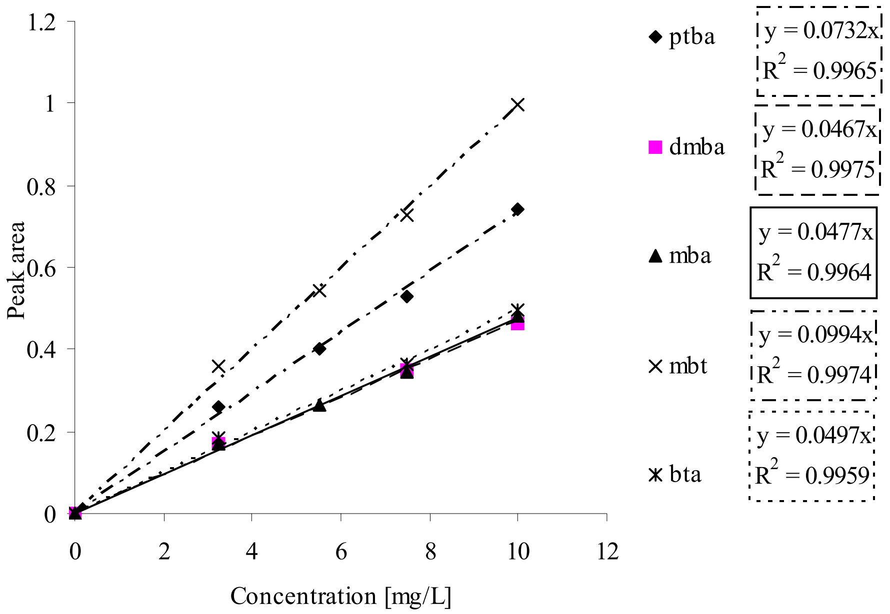


Figure 21: Calibration curves of benzotriazoles and benzothiazole using effective mobility scale.

Precision of quantification: The repeatability of peak area using transformed μ-scale were the same as for the time scale (electropherogram). The RSDs for the whole analytical process were between 6-9% and were independent on theanalyte concentration.

Sensitivity: The LOD and LOQ were also identified when effective mobility scale was used for their calculation. The LODs were 0.010 mg/l for PTBBA, 0.016 mg/l for DMBA, 0.015 mg/l for MBA, 0.007 mg/l for MBT and 0.013 mg/l for BTA. Small increase of detection limit of BTA was observed. The possible reason of this phenomenon is the same as the decrease of the slope.

Accuracy: The recoveries of the whole method using μ-scale were also determined at three different concentrations of the target compounds.

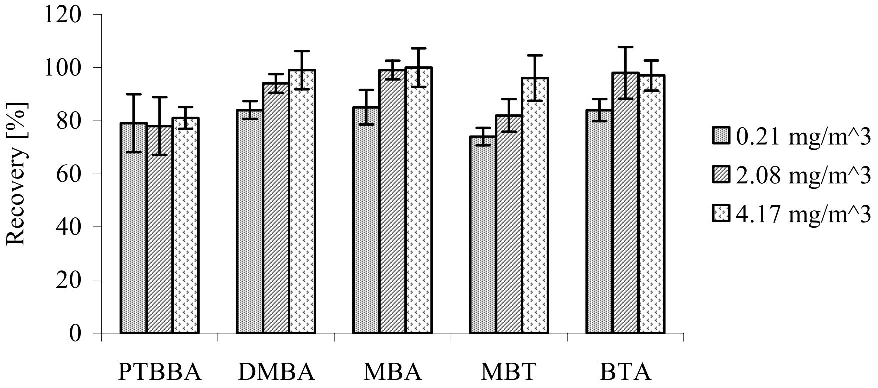


Figure 22: Recovery of a method using effective mobility scale.

As it is summarized in figure 18 and 22, no difference was observed in the recoveries determined from time scale and effective mobility scale. Thus both scales can be used for the quantification of the selected saturated heterocyclic amines.

On the whole, the identification is more reliable after transformation of the electropherogram into the effective mobility scale. Moreover, the dimensions of the calibration curves were dependent only on the spectrometric properties of the targets and independent on the migration properties.

Each performance characteristics were in accordance with the analytical requirement thus mobility scale transformation can be used to quantify PTBBA, DMBA, MBA, MBT and BTA from atmospheric and MWF aerosol.

4.1.5 Study of matrix effect

The investigation of the matrix effect on the optimised method is indispensable, because the composition of MWF aerosol is very complex and the matrices are unidentified, thus a lot of unpredictable parameter -such as paraffin oil content or presence of complexing agents and solids- can affect the analysis of the selected amine. On the other hand, the method does not contain any clean-up and emulsion may have an additional influence on the correctness of the determination of BTA, MBA, DMBA, MBT and PTBBA.

If the sample compounds interact with the capillary wall, the EOF may change (mostly when the separation pH is high) and thus the MTs of the analytes also change. In CE this effect is also called as matrix effect. In other separation techniques this effect is at least one order less. MWF concentrate was added to the standard solution of corrosion inhibitors at different ratios for investigation of this special effect.

<u>*Migration time change with concentration of MWF and difficulties in identification*</u>: The samples prepared from emulsions, that contained different amount of MWF concentrate, were injected step by step. The following figure shows the electropherograms and mobility scales, when the samples contained different concentrations of the MWF (1% and 3% (V/V)).

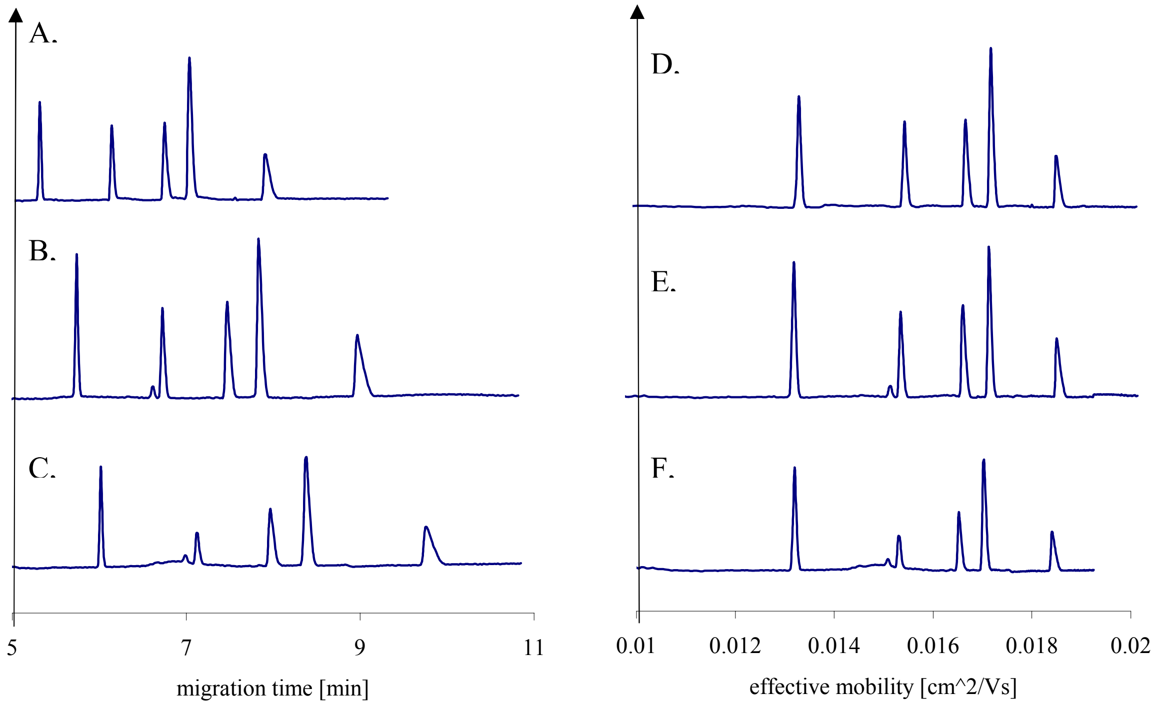


Figure 23: Electropherogram of the benzotriazoles and benzothiazole when the sample contain A, no MWF B, 1% MWF concentrate and C, 3% MWF concentrate and transformed effective mobility scale when the sample contain D, no MWF E, 1% MWF concentrate and F, 3% MWF concentrate.

As it shown in figure 23/A,B and C, a shifting of MTs was observed with the increase of MWF concentration. This effect might be caused by ”blocked” silanol groups due to the interaction between the silanol groups on the surface of the capillary and MWF ingredients because the EOF decreased with the increase of the MWF content. To see, whether the capillary surface changed temporally or permanently, standard solution without MWF was always injected between the measurements of spiked samples. No migration shift was observed at case of the separations of standard solutions. It might be concluded that the change of the MT is caused by temporary “blocked” silanol groups and the surface condition of the capillary between the runs is reconstructed.

Anyhow, the identification of target compounds in real sample based on migration time at least is questionable. It must be stated that in real sample the presence of matrices interacting with the surface of the fused silica tube cannot be avoided even the most sophisticated clean-up procedure is done before the separation.

When the electropherograms were transformed to μ-scale, no observable changes in the mobility of analytes with the function of content of MWF concentrate were determined (see figure 34/D,E and F).

As mentioned before, CE is a high effective separation technique, although its main drawback the the high RSDs of migration time and peak area. Therefore the migration time shift should be closely investigate since it is an inherent character of CE when real sample is injected. To investigate the adequate or stochastic character of MT shifting, the experiment was repeated three times and the MTs with RSDs were plotted in figure 24.

Surprisingly, the effect of emulsifiable MWF content on the migration behaviour of BTA, DMBA, MBA, MBT and PTBBA was different when the experiment was repeated at different measuring days. While in the first time it was positive, in the second time negative shifting on MTs of the analytes was observed in function of MWF concentration (the differences of MTs were out of the RSDs), and in the last repetition no effect on the MT was observed. Thus the matrix effect of MWF on the MTs is assumed to be stochastic. At least, the variation of migration time is uncontrollable. In HPLC experiments it was found that the molecule form of the silanol groups on the surface of stationary phase depend on the previous conditions of the mobile phase. In analogy, the same silanol groups exist on the surface of the fused silica capillaries and the precondition procedure is assumed not to be enough sufficient to get the same conditions from one measuring day to the other.

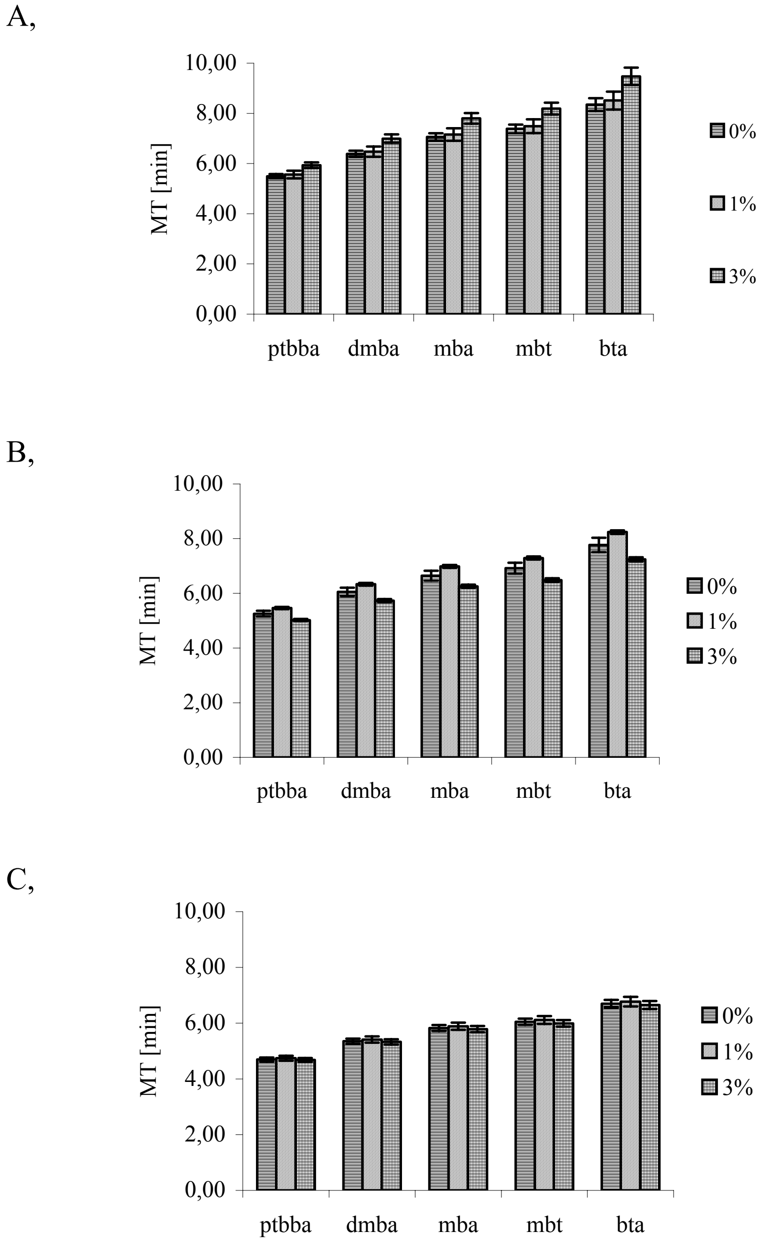


Figure 24: MTs of analytes in function of MWF content repeated 3 different days (A, B and C) and repeated three times per measuring day. Abbreviations: 0%= standard solution without MWF, 1% and 3%= 1% and 3% MWF concentrate in the standard solution of analytes.

The effective mobilities of the analytes with RSDs were determined in function of MWF concentration. In the following figure the mobilities of first two experiment day were summarized since in the third measuring day no difference was observed.

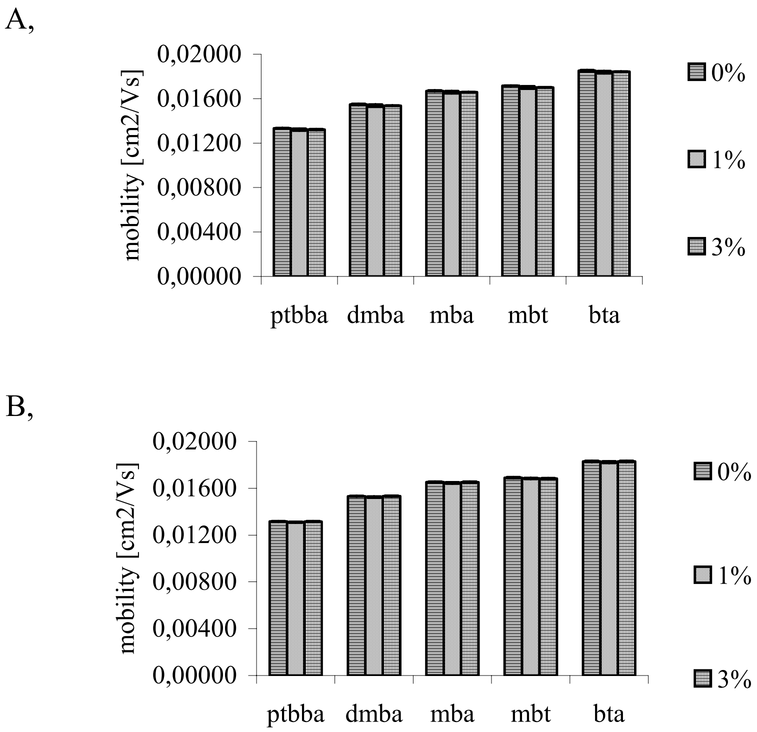


Figure 25: Effective mobilities of analytes in function of MWF content measured at two different days (A and B) and repeated three times per measuring day. Abbreviations: 0%= standard solution without MWF, 1% and 3%= 1% and 3% MWF concentrate in the standard solution of analytes.

When the electropherograms were transformed to μ-scale, the effective mobility values of PTBBA, DMBA, MBA, MBT and BTA were independent on the amount of the MWF concentrate. When the effective mobilities were normalized, no differences among the determined values in function of MWF content were observed.

The RSDs of μ_{eff} and $\mu_{eff,norm}$ of PTBBA, DMBA, MBA, MBT and BTA were also determined from three replications.

Table 10: RSDs of MT and μ_{eff} of the selected substances in function of MWF content. Abbreviations: 0%= standard solution without MWF, 1% and 3%= 1% and 3% MWF concentrate in the standard solution of analytes.

		0% MWF	**1% MWF**	**3% MWF**
PTBBA	MT	6.53	6.95	10.13
	μ_{eff}	1.48	1.65	1.39
DMBA	MT	7.30	7.75	11.83
	μ_{eff}	1.66	1.76	1.55
MBA	MT.	7.92	8.43	13.03
	μ_{eff}	1.58	1.52	1.52
MBT	MT	8.13	8.74	13.67
	μ_{eff}	1.53	1.59	1.42
BTA	MT	8.98	9.75	15.60
	μ_{eff}	1.55	1.57	1.44

The RSDs of MTs were between 6-15%, and increased with the increase of MWF content and with the migration time order. The RSD of effective mobilities varied between 1.4-1.7%. The repeatability of the effective mobilities was independent on the MWF content and migration sequence of the selected substances.

Consequently, the only way to get qualitative reliable results for real sample analysis is the mobility scale transformation.

Quantification: Effect on the analyte peak area of matrices was also investigated. For this purpose recoveries with RSDs were determined in spiked solutions. The standard solutions contained 1% and 3% of MWF concentarte. Since effective mobility scale is negligible for identification and effective mobility scale can be used for quantification (see chapter 4.1.4), this was used for determination of analyte concentration from spiked sample. The determined mean recoveries with RSDs in function of MWF content are summarized in figure 26.

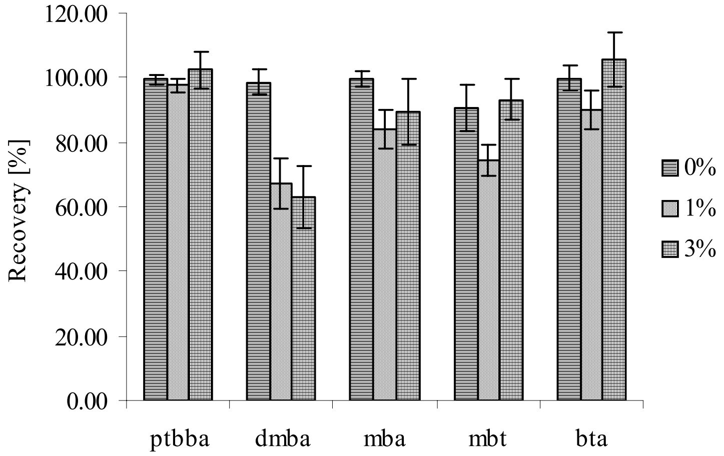


Figure 26: Recoveries with RSDs (n=3) of a method in function of MWF content determined from effective mobility scale. Abbreviations: 0%= standard solution without MWF, 1% and 3%= 1% and 3% MWF concentrate in the standard solution of analytes.

The recovery of PTBBA was between 96-101% for each MWF content in the emulsion, thus matrices had no effect on the accuracy of PTBBA. The determined RSDs for PTBBA were 2-7%, which is lower than the acceptable limit.

The biggest influence of the matrices on the accuracy was determined for DMBA, since the recoveries varied between 59-78% for all content of MWF with RSDs 5-10%. The average recovery of MBA varied between 76-84% with variations of 4-7%. The same trend was determined for MBT as for MBA. The accuracy of BTA was also not influenced by the presence of MWF, since the recoveries were determined between 92-97% for each MWF concentration with RSDs of 4-7%.

Thus the accuracy of PTTBA, MBA, MBT and BTA was not influenced by the matrices of the MWF. DMBA can be quantified with lower reliability from MWF aerosol since the recoveries of DMBA were depended on the concentration of MWF content in the spiked samples.

Theoretical interpolation: Considering the measurements, it was assumed that the accuracy of PTBBA, DMBA, MBA, MBT and BTA determined from MWF emulsion depending on their solubility properties. Thus mean recoveries of these solutes measured from the emulsions were plotted in function of their octanol-water partition coefficient (logP).

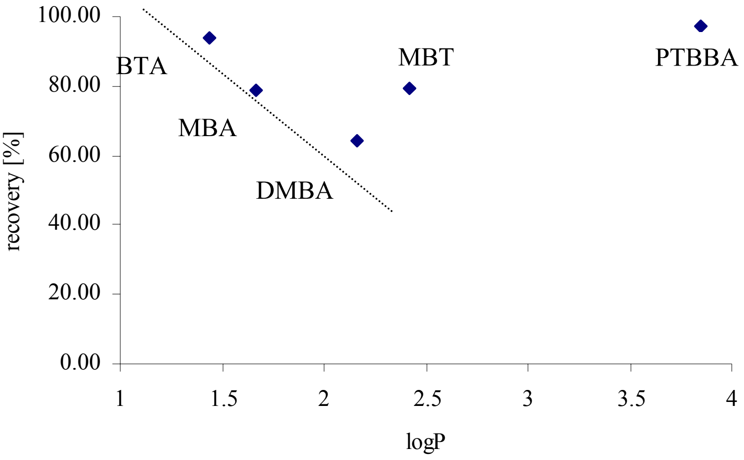


Figure 27: Recoveries of benzotriazoles and benzothiazole in function of their logP.

As shown in figure 27, the accuracy of benzotriazole derivatives from MWF emulsion depending linearly on their water solubility (logP). The cases of MBT and PTBBA are different, since the recoveries were independent on their logP value.

The optimised method were also characterised when the injected solution contained matrices. The identification performance characteristic was excellent when the electropherograms were transformed into mobility scale.

The quantification parameters met with the analytical requirement at case of PTBBA, BTA, MBA and MBA but the extraction efficiency of DMBA were dependent on the concentration of matrices present in the MWF.

4.1.6 Final protocol

Table 11: Final protocol for the determination of selected saturated heterocyclic amines in MWF aerosols.

Sampling:	With air pump at flow rate of 1 L/min maximum 24h onto a quartz filter.
Sample preparation:	Extract the filter with 10 ml 30:70= MeOH: water mixture with ultrasonic bath then concentrate to 0.5 ml under vacuum.
Capillary:	Fused silica, I.D. 50 μm, effective length 40 cm, total length 47 cm.
Detection wave length:	236 nm for PTBBA and MBT; 277 nm for BTA, MBA and DMBA detector cell size: 100*800 μm.
Temperature:	Cartridge set to 30 ^{o}C.
BGE:	25 mmol/l 3-cyclohexylamino-1-propanesulfonic acid (CAPS), 15% (V/V) acetonitrile, pH= 11.4.
Capillary preconditioning:	2 min pressure with 0.1 mol/L NaOH, then 3 min with BGE.
Injection:	Hydrodynamic injection of sample 50 mbar for 10 s (V_{inj}= 14 nl).
Voltage:	23,5 kV.
Replenishment:	Both separation vials and injection vial after each injection.
Capillary preconditioning: **(new capillary)**	Pressure flush with 0.1 mol/L NaOH for 30 min when it is used at first time
Storage capillary: **(for next measuring day)**	Pressure flush with 0.1 mol/L NaOH for 15 min before and after the measuring day
Storage capillary: **(more then one week)**	Pressure flush with 0.1 mol/L NaOH for 15 min followed by high purity water for 15 min, then with air for 5 min.
Marker	Vanillin
Scale	Using the effective mobility scale transformed from electropherogram
Determination of concentration:	With five-point calibration curve in the working range between 2.5 mg/l and 50 mg/l determined every measuring day

4.1.7 Conclusion

A published method for the determination of PTBBA, DMBA, MBA, MBT and BTA from MWF aerosols was studied, and several drawbacks were recognised that made the method not reliable for the analysis of real sample. Thus the goal of the project was to improve the method the selected hetero-aromatic amines in MWF aerosols for having a reliable determination process.

The reliability of the long-term sampling was investigated since the suggested two hour duration time was not enough for real measurements. From a practical point of view 24 h was chosen as maximum one. The recoveries of analytes were in an acceptable range (~90%) for three concentrations of PTBBA, DMBA, MBA, MBT and BTA when the sampling time was increased. The RSDs of peak areas were between 4-7%. Thus longer sampling time as 2 h can be recommended for the determination of hetero-aromatic corrosion inhibitors from MWF aerosols.

Since the recovery of PTBBA was not independent on its concentration (decreasing with increasing of its concentration), the extraction solvent was changed to improve the accuracy of the sample preparation. When the polarity of the extraction solvent was changed by addition of methanol, the recoveries of this solute was independent on its concentration.

A pre-concentration step was also developed for decreasing the determination limit of these analytes, which is based on the evaporation of extraction solvent under vacuum. The determined recoveries at three concentration levels at range of upper ng/l of the selected compounds were ~80%.

The migration behaviour of PTBBA, DMBA, MBA, MBT and BTA was also investigated. After the theoretical prediction, migration behaviour of these substances in function of pH and organic modifier present in BGE, separation voltage and temperature was investigated. The optimised pH of BGE was 11.4. At this pH the separation puffer has still buffer capacity and the targets are fully charged. Acetonitrile and methanol influenced the migrations of PTBBA, DMBA, MBA, MBT and BTA and considering the separation time 15% acetonitrile was added into the final separation electrolyte.

The method using conventional capillary and modified separation buffer was then validated. The selectivity of critical substances (MBA and MBT) was better when conventional capillary was used comparing with the extended light one.

The run-to-run RSDs of MTs were between 1-3% for each analyte and were independent on their concentration. However, dependency of RSD values on the migration time order of the peaks was observed. The day-to-day and capillary-to-capillary RSDs were 5-7%, which was lower than using extended light capillary.

The working range was set between 0.208 mg/m^3 and 4.16 mg/m^3 that cover the range of 0.1*MAC and 2* MAC of PTBBA. The calibration curve in the working range showed an excellent linearity because the regression coefficients were more than 0.999.

The RSDs of peak area for the whole analytical method were between 6-10% for each analytes at three different concentrations.

The LOD and LOQ using conventional capillary was approximately 3 times higher as determined for extended light capillary, but the specified LOQ of analytes were at least ten times lower than the lowest point of the working range.

The recoveries of PTBBA, DMBA, MBA, MBT and BTA for the whole analytical method at different concentrations were more than 90%, which met with the analytical requirement and were the same as measured with use of extended light capillary.

It can be recommended to apply conventional capillary for analysing these hetero-aromatic amines, because the analytical performances of this method is in acceptable range and it can be applied to any CE instrument.

During the validation of the original method, quite poor precision of identification was determined, which decrease the reliability of peak assign. This poor reproducibility are often related to small changes of EOF from one measurement to the other. Thus the electropherograms were transformed into mobility scale, which is independent on the EOF. The qualification and quantification parameters of the method were also determined using effective mobility scale.

The within-day precision of qualification was at least 10 hold improved when the time scale is transformed to effective mobility scale. This was also observed for day-to-day and capillary-to-capillary precision of identification.

The calibration curves fitted to the linear model, when effective mobility scale is used for quantification. The RSD values for the whole analytical process using mobility scale were independent on the substance concentration and were between 6%-9%. The recoveries of the selected solutes were the same, when they were determined from electropherogram.

Finally, matrix effect on the analysis was investigated, since MWF is a complex mixture and no clean-up was added into the method.

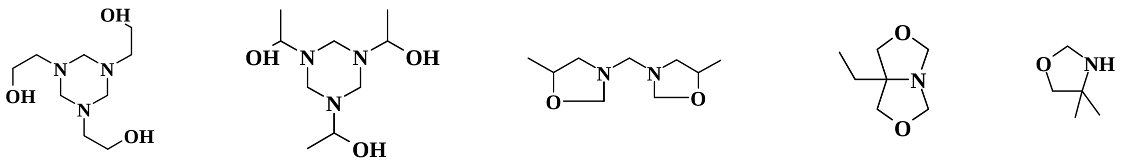
Random changes in the day-to-day repeatability of MTs in function of MWF concentration in the emulsion were observed. But no adequate changes were observed in function of MWF content when effective mobility scale was used for peak identification. The RSDs were also decreased from 6-16% to 1-1.5% when effective mobility scales were transformed from electropherograms.

Effect of MWF on the accuracy of DMBA was determined, thus this solute can only semi-quantified for MWF aerosols. The quantification of the other selected substances was not affected by MWF content.

To sum up the improved method is more suitable for analysing PTBBA, MBA, MBT, DMBA and BTA from MWF aerosols. However, it is suggested to improve the sample preparation to quantify DMBA with higher reliability. For that purpose addition of more apolar solvent into the extraction solvents is suggested since DMBA three times more soluble in apolar solvent than in water. To decrease the duration of sample pre-treatment SPE is suggested as pre-concentration step. When anion-exchange is used for SPE, matrices with more apolar and cationic properties would be eliminated thus the effect of “silanol blocking” during the capillary electrophoretic separation would be also minimised. Additionally, more target components like 1-hydroxy-2-pyridinthion or 2(3H)-benzothiazolthion can be added into the method that has the same separation properties as the derivatives of benzotriazoles and benzothiazole.

4.2 Determination of heterocyclic amines and their by-products

In the metalworking industry, microbes living in the MWFs provoke a huge problem since they deteriorate the fluid effectiveness and more diseases among the workers were connected directly to the microbial contamination of the fluid. Thus producers always add anti-microbial agents into the MWF to prevent the microbial growth. The used formaldehyde releasers are 5-ethyl-3,7-dioxa-1-azabyciclo[3.3.0]octan (EDAO), 4,4-dimethyloxazolidine (DMOI), N,N,N-tris(ß-hidroxyethyl)-hexahydro-triazine (Grotan BK), N,N,N-tris(ß-hidroxyl-propil)-hexahydro-triazine (Grotan WS) and N,N-methylenbis(5-methyloxazolidine) (Grotan OX).



Grotan BK Grotan WS Grotan OX EDAO DMOI

Figure 28: Chemical structures of hexahydrotriazines and oxasolidines used as bactericide.

Their determination from MWF aerosol is important since they act by releasing formaldehyde. Formaldehyde is carcinogenic to rats by inhalation [61], it is an airway irritant and recognized as a possible cause of occupational asthma [248]. However, formaldehyde releasers themselves pose risk on the human health because they strongly evoked sensory and pulmonary irritation in mice. Moreover, it was shown that Grotan BK may cause allergic contact dermatitis [72]. No MAK values have been established for these target compounds in spite the fact that they are recognised as skin and lung irritant substances [152].

4.2.1 Test separation of formaldehyde releasers with a published method

A capillary electrophoretic method was developed by Trappfor their determiantion [231]. They are weak Lewis bases; therefore they are theoretically protonised at low pH. But they are not stable at acidic conditions. Thus the pH of separation buffer was chosen at 7, because it was assumed that these compounds are protonised at this condition. Since these compounds have no chromophor groups, indirect UV detection was applied by addition of an absorbing co-ion, often called *probe,* into the BGE. The absorption, mobility and concentration of the probe are crucial because they influence the method performances such as peak shapes, efficiency and sensitivity [249]. Imidazole was chosen as a *probe* since it has good buffer capacity at pH 7, that ensure better repeatability of MT and peak area and has similar mobility as the selected unsaturated heterocyclic amines.

At first, the separation was carried out with the published separation parameters described in table 12 and the following electropherogram (figure 29) was observed.

Table 12: Published separation condition of hexahydrotriazines and oxasolidines.

Capillary	Uncoated silica-tubes, effective length 40 cm, 75 µm i.d.
Voltage	20 kV
Detection	214 nm
Temperature	30 °C
BGE	40 mmol/L imidazole, 20 mmol/L α-hydroxy-isobutyric acid, pH=7
Injection	Hydrodynamic; water for 1s and sample for 1.5 s at 50 mbar

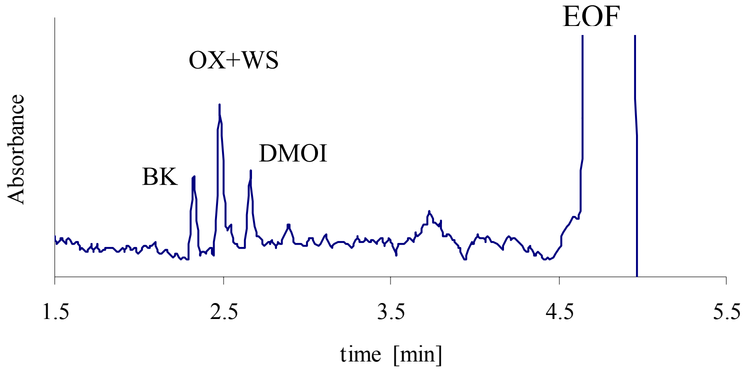


Figure 29: Electropherogram of hexahydrotriazines and oxasolidines.

The selected formaldehyde releasers were separated within 3 minutes, but from the five peaks only three of them were detected. On the other hand, the sensitivity of the method was poor. Thus improvement of the method for the separation of EDAO, DMOI and the Grotan products was goaled to achieve better separation selectivity and sensitivity.

4.2.2 Improvement of the separation of heterocyclic amines

Since the published method had several drawbacks, the improvement of the capillary electrophoretic separation was aimed. At first the anion of the buffer was changed from α-hydroxyl-isobutyric ion to chloride since it was not necessary to use weak acid for setting the pH of the BGE. According to the measurements there no any changes were observed, thus the buffer system was changed to imidazole/imidazole chloride. The buffer capacity of the new system was better, and the handling of hydrochlorid acid is more comfortable because of the cheapness and α-hydroxy-isobutyric acid has unpleasant smell.

The effect of buffer pH on the migration behaviour of the target compounds was then investigated. At first, the theoretical migration behaviour in function of pH was calculated

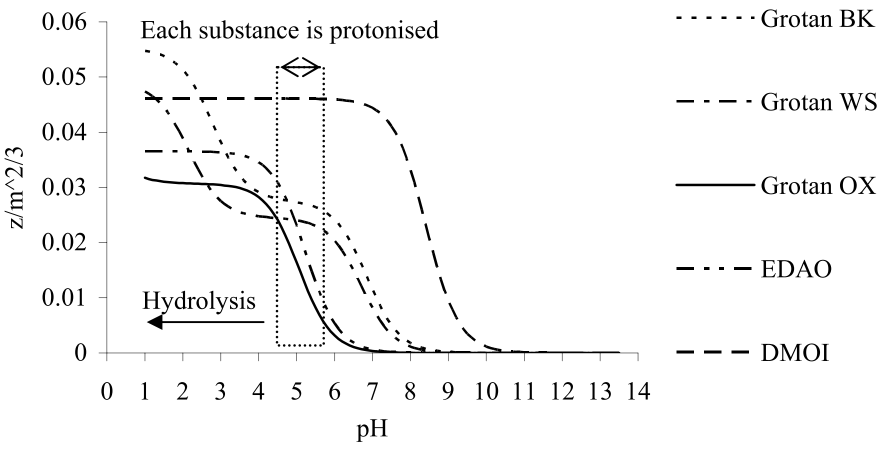


Figure 30: Theoretical mobility of hexahydrortiazines and oxasolidines.

Baseline separation can be achieved if the pH is lower than 7, but then the separation is sensitive to little changes of buffer pH, and the EDAO and Grotan OX migrate theoretically close to the EOF. Unfortunately, only DMOI is fully charged at pH range of 6-7 according to the calculation.

The theoretical prediction was controlled in practice by varying the pH of BGE in a range from 6 to 8 (see figure 31). For investigation a standard solution of EDA, DMOI, Grotan BK, WS and OX (50 mg/L every component) was prepared and separated at different pH.

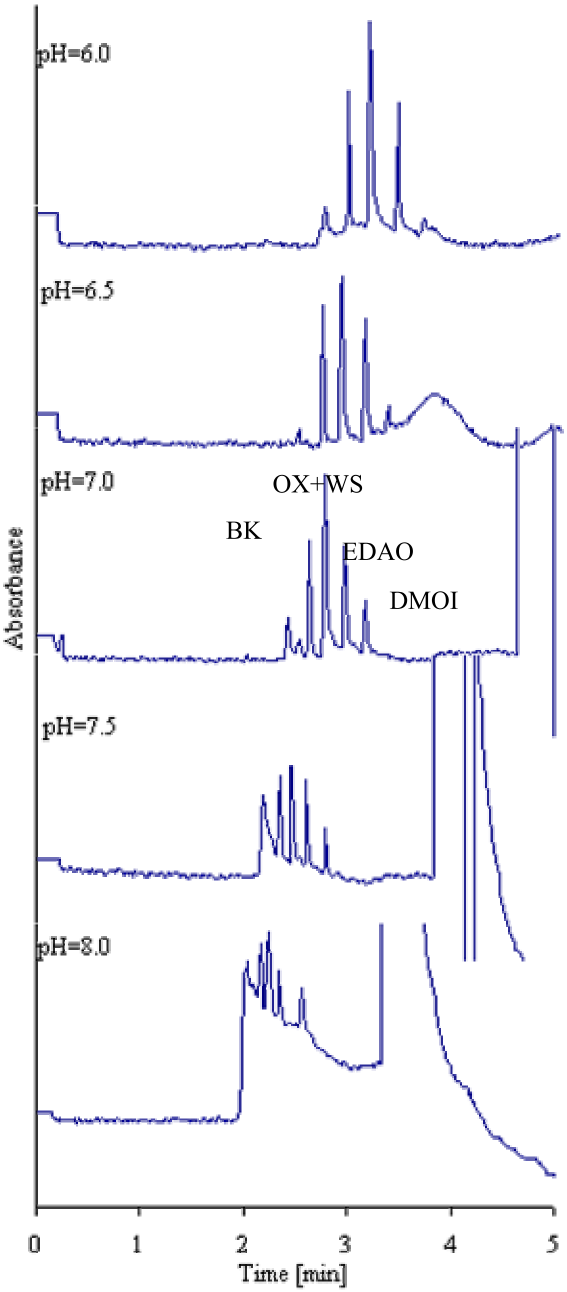


Figure 31: Migration behaviour of hexahydrotriazines and oxasolidines in function of pH.

When the pH of the BGE was increased from 6 to 8 the separation time was decreased from 3.5 minutes to 2.5 minutes and the EOF decreased from 6.4 min to 4.1 min too. When the buffer pH was above 7 a system peak appeared in front of the peaks of target components which is one of the disadvantage of the indirect UV detection technique [250]. The size of the system peak dramatically increased with the increase of pH. Grotan OX and WS co-migrate at each buffer pH used for separation hence more changes in the separation condition were aimed to separate them.

The experimentally investigated migration behaviour of the selected heterocyclic amines was different than it was predicted with the theoretical model. From the theoretical model DMOI was the fastest and according to the measurements it was the slowest from the selected substances. Moreover, the apparent mobilities were near to each other when they were separated.

Organic modifier was added into the BGE to increase the resolution of the analytes and to separate Grotan OX and WS from each other. For this purpose, different water soluble organic solvents such as acetonitrile (ACN), tetrahydrofurane (THF), methanol (MeOH) and propanol (PrOH) at different concentrations (V/V%) were added to the electrolyte, and the resolution of the first two peaks was determined to characterize the separation.

Table 13: Resolution of Grotan BK and Grotan OX in function of organic modofier in BGE.

	ACN	**THF**	**MeOH**	**PrOH**
0%	1.27	1.27	1.27	1.27
10%	2.00	2.14	1.67	2.31
20%	1.64	2.5	2.33	4.00
30%	1.40	-	6.00	-
40%	0.77	-	3.09	-
50%	-	-	2.00	-

The R_s values decreased with the increase of ACN content. The presence of THF had improvement on the separation quality, but the handling of THF is unpleasant. The best resolution was reached when MeOH was added to the separation buffer as an organic modifier to the BGE. The resolution values in function of methanol concentration go through a maximum at a concentration of 30 V/V%. This phenomenon caused by the boardening effect as described in section 4.13. Propanol had also a significant influence on the separation qualification, but its water solubility is limited and current problem often occurred during the separation. Unfortunately, no further changes in the resolution of Grotan OX and WS have been observed when any type of organic substance was added to the separation buffer.

Other parameters were also investigated such as (i) the capillary length: 40 cm and 50 cm to detector, (ii) the cartridge temperature: 25 °C and 30 °C, (iii) and the applied voltage: 20 kV, 25 kV and 30 kV. The separation time increased with the length of the capillary. With increasing separation voltage the baseline became unstable, thus 20 kV was chosen for separation. No big difference was observed, when the cartridge temperature was changed.

The final operation conditions for the capillary electrophoretic determination of EDAO, DMOI, Grotan BK, OX and WS are defined in table 14:

Table 14: Final condition for determination of hexahydrotriazines and oxasolidines.

Capillary	Uncoated silica-tubes, 40 cm to the detector, 75 μm I.D.,
Detection	214 nm, 100*800 μm detector cell
Temperature	30 °C
BGE	30 mmol/L imidazole/imidazole chlorid, 30 V/V% methanol, pH=7
Capillary preconditioning	0.1M NaOH (3min)+BGE (4min)
Injection	Hydrodynamic injection of sample at 50 mbar for 10 s
Voltage	20 kV

4.2.3 Difficulties occurring during separation of hexahydrotriazines and oxasolidines

Grotan OX and WS co-migrated even if the separation parameters, that may influence the separation, were changed. The mass, charge and molecule radius of these substances is not identical, thus theoretically they should have been separated. Thus the chemical-physical properties of these two compounds were compared to find the reason of this phenomenon.

Since indirect UV detection is the less selective detection technique [92], it may be possible that the observed peaks did not belong to the formaldehyde releasers but to their hydrolysis products since their hydrolysis by-product is the same (monoisopropanolamine (MIPA)). Moreover, the other heterocyclic amines may also hydrolyse and monoethanolamine (MEA) forms during the hydrolysis of Grotan BK, 2-amino-2-methyl-1-propanol (AMP) from DMOI and 2-amino-2-ethyl-1,3-propandiol (AEPD) from EDAO.

To check this assumption, standard solution of these hydroxyl-alkyl-amines at the same concentration were injected into the capillary and separated with the separation condition developed for hexahydrotriazines and oxasolidines.

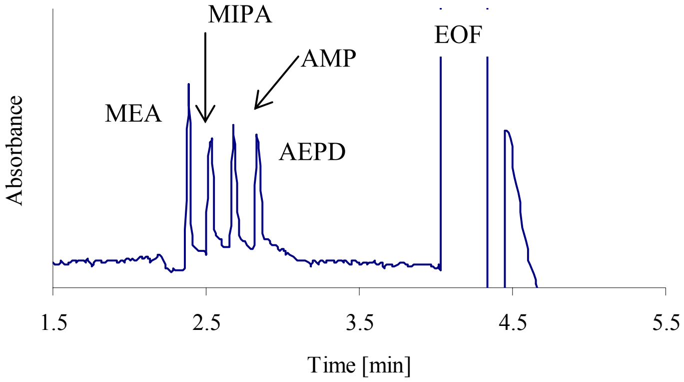


Figure 32: Electropherogram of the by-products (derivatives of amino-alcohols).

The hexahydrotriazines and oxasolidines and their hydrolysis products migrated together (see figure 31 and 32). The peak height of the formaldehyde releasers and the corresponding amino-alcohols were not the same; it was always lower for Grotan BK, EDAO and DMOI. Because it was not clear which substances were detected during the separation, the concentrations of these substances could not be determined.

The ratio of charge/mass$^{2/3}$ of MEA, MIPA, AMP and AEPD was also calculated as a function of pH to see if the thoretical model fits to the measured mobilities illustrated in figure 32.

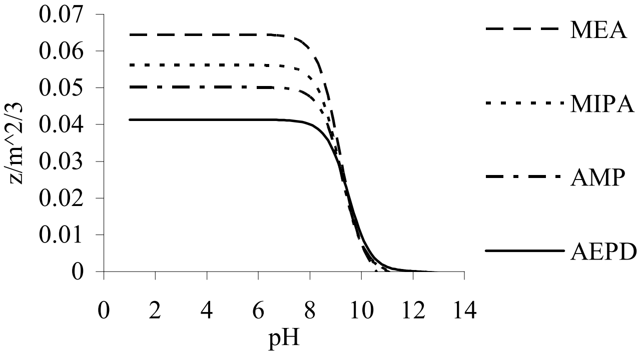


Figure 33: Theoretical mobility of hydroxyl-alkyl-amines.

The theoretical model corresponds to the measured separation when solution of MEA, MIPA, AEPD and AMP was injected into the CE instrument. Thus it can be assumed that MEA, MIPA, AMP and AEPD were detected when standard solution of DMOI, EDAO and Grotans were injected and separated.

Since the stock solutions of formaldehyde releasers were made every week, it was supposed that they hydrolyse fully during the storage, and thus the by-products were analysed. Thus integrated peak areas were determined in function of time after solving the formaldehyde releasers in high purity water. The solutions of substances at were injected one by one immediately and then every 15 minutes through 1.5 hour period. No adequate differences were observed in the peak areas for each target compounds.

Then EDAO, DMOI, Grotan BK, WS and OX were solved in methanol and acetonitrile at the same concentration to try to eliminate the hydrolysis reaction, but the observed migration times were the same as detected for water solutions. It seems that MEA, MIPA, AEPD and AMP were measured when solution of hexahydrotriazines and oxasolidines were injected.

4.2.4 Purity investigation of heterocyclic amines

It was assumed that the hydrolysis products were detected when water solution of hexahydrotriazines and oxasolidines were injected. The question is whether they are impurities of the industrial materials or if they are formed during the analytical process. However, the peaks are generally wide and asymmetric if the substances are formed during the separation, and in this case the peaks are sharp and symmetric. MS and H^1-NMR were chosen as analytical tools to investigate what was really separated.

Before kinetic examination of hydrolysis of DMOI, EDAO, Grotan BK, WS and OX, the impurity of the bought materials was investigated. The presence of hydroxyl-alkyl-amines as an impurity is possible, since the products may contain water in trace, which initiate the formation of the appropriate hydroxyl-alkyl-amines.

Water solutions of formaldehyde releasers at concentration of 50 mg/L were injected one by one into the MS immediately after the preparation of standard solutions. The flow injection system was adapted from CE-MS and capillary was used to introduce the sample fluid into the MS. Sheath liquid was also used to serve as the electric connection for the ionization source and to complement the required flow rate.

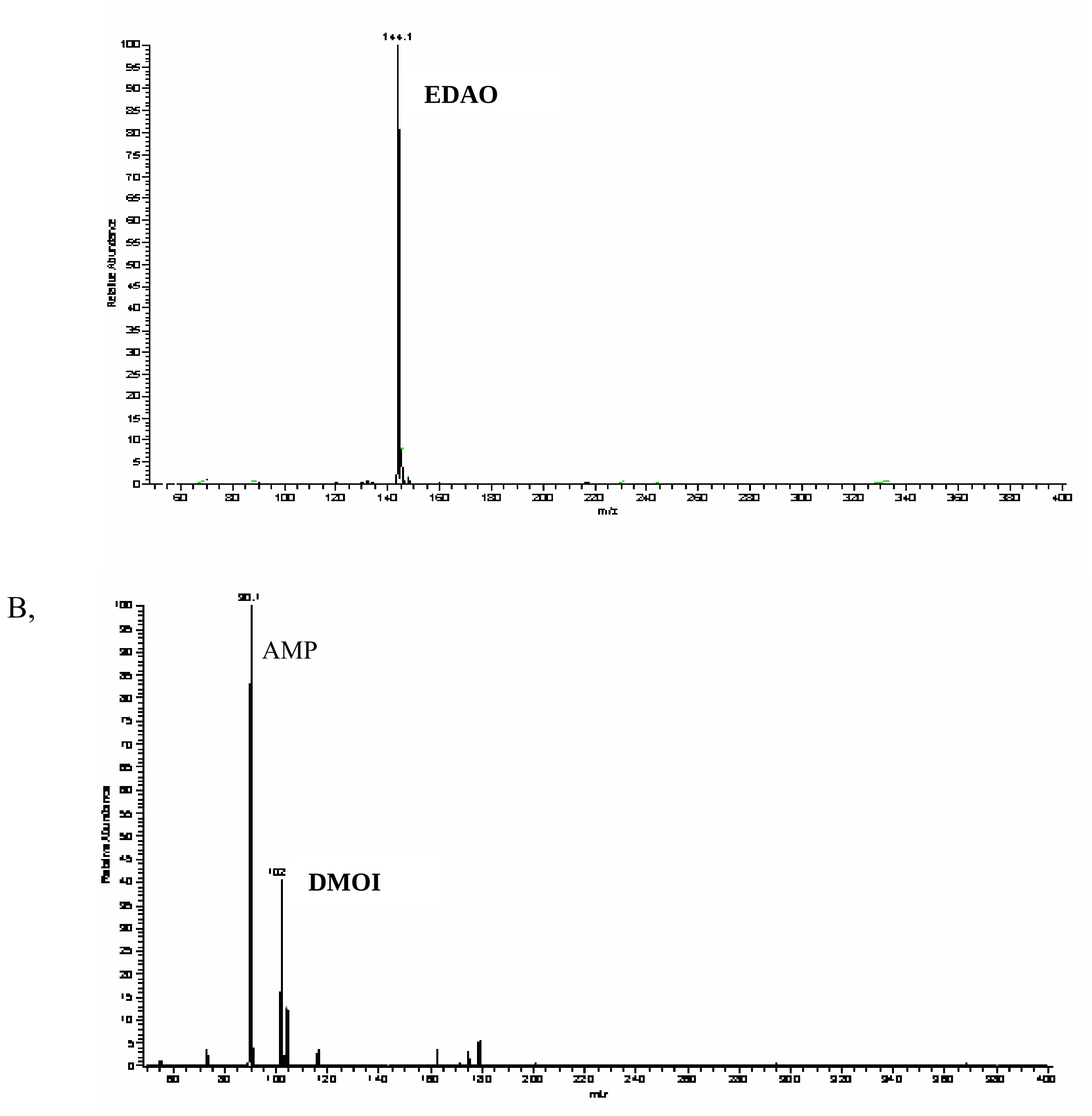


Figure 34: Mass spectra of water solution of A, EDAO and B, DMOI.

EDAO is assumed to be pure because only its molecule peak was detected and the molecule fragment of its hydrolysis products was not observed. In case of DMOI, the relative ambudance of its by-products was double as the DMOI peak.

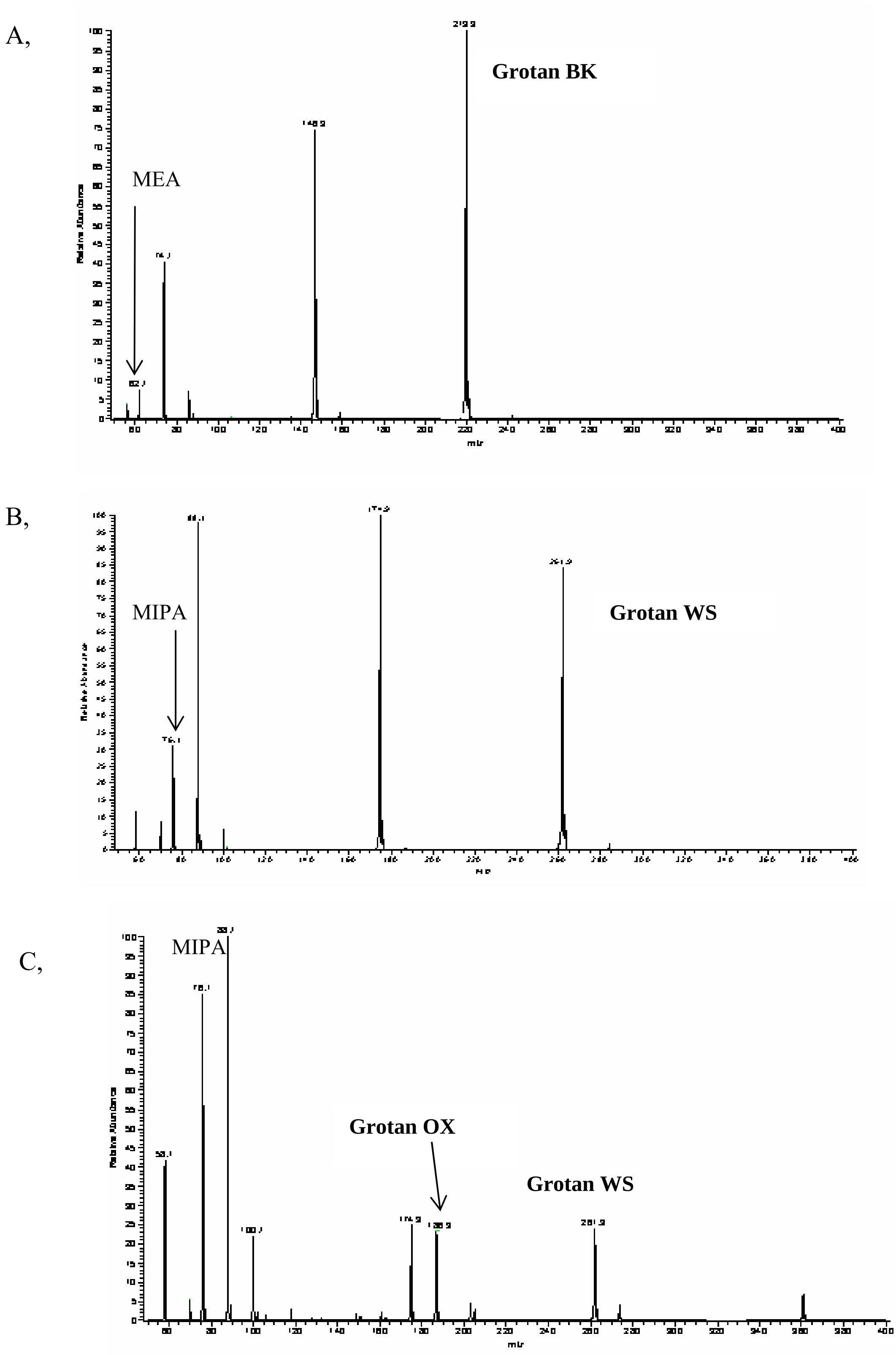


Figure 35: Mass spectra of water solution of A, Grotan BK; B, Grotan WS and C, Grotan OX.

According to the mass spectrw of Grotan-BK, the reference material probably contain mostly the preservative itself, since the main peak belongs to the ionised molecule, and the peaks at m/z of 147 and 74 may be the fragments ($[CH_3\text{-}NH\text{-}C_2H_4\text{-}OH]_{n=1,2}$) of the target compounds. However, a small peak at m/z of 62 -the molecule peak of MEA- was also detectable. The same trend was observed for Grotan WS as for Grotan BK, however the peak of MIPA was higher. The MS spectrum of Grotan OX is more complicated; it seems that the bought material is mixture of Grotan WS and Grotan OX and the relative ambundance of MIPA is relatively high (85%).

Unfortunately, it cannot be concluded that the bought materials contains amino-alcohols originally, since the hydrolysis of these biocides can be catalysed by the applied condition such as high temperature or acidity of the sheath liquid. On the other hand, no fingerprints of these formaldehyde releasers were found in the MS library, thus it cannot be told exactly if the measured spectra are spectra of a mixture or not.

For further impurity examination of EDAO, DMOI and Grotans H^1-NMR was selected. At first, the chemicals were solved in acetone in which supposedly no hydrolisation takes place and the analytes are soluble in acetone. Only the spectra of Grotan OX was not clear, which is in accordance with MS measurement. For the other substances it can be assumed that the bought materials of these formaldehyde releasers were pure which do not correspond with the results measured with MS.

4.2.5 Investigation the hydrolysis of cyclic amines

Since purity study with MS and NMR gave the same results only for EDAO, its hydrolysis was at first investigated. The reference material of EDAO was solved in water, and was immediately measured with NMR. Then the spectrum of this solution was measured every 15 minutes after the preparation of the solution in three hour period, and finally the same solution was measured two weeks after the solution. During this period no changes in the NMR spectra were observed, thus EDAO did not hydrolyse in high purity water. Contrarily, EDAO hydrolyses when its water solution was injected into the CE instrument and detected with indirect UV detection. The difference between the two techniques was that the injected plug sample was mixed with BGE during CZE separation, and BGE contain electrolytes at high concentration.

Thus it was supposed that the reaction type of the hydrolysis is S_N2 nucleophile substitution reaction, where proton or hydroxide i at higher concentration than found in high purity water is negligible for the initiation of hydrolysis. The supposed reaction is self-catalysed, since proton is also forming. The reaction scheme for EDAO is plotted in figure 36 and this scheme is also supposed for DMOI and for the Grotan products.

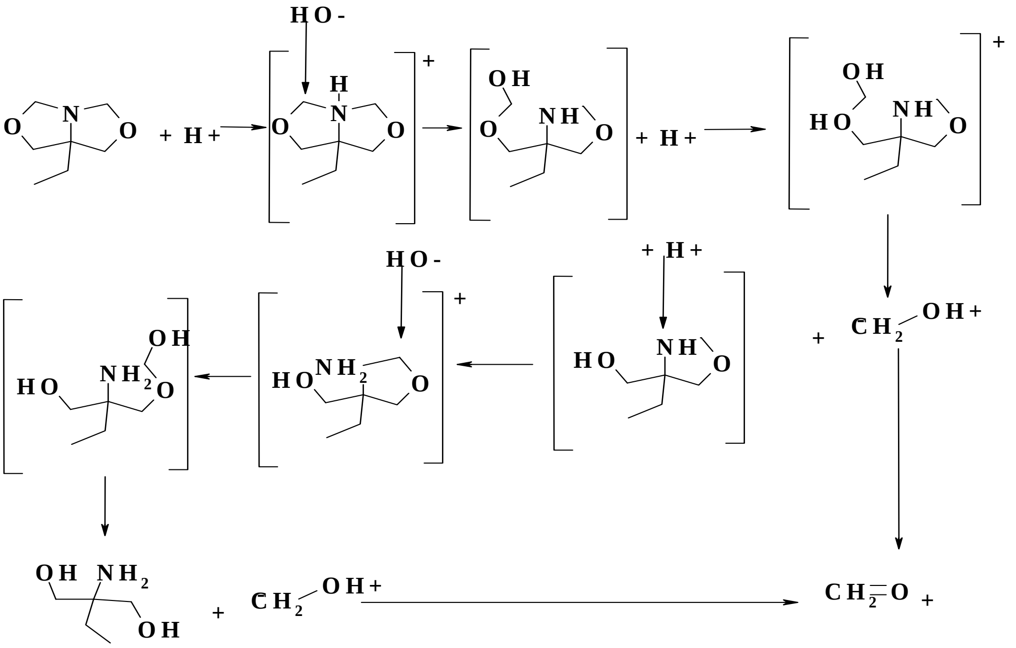


Figure 36: Supposed reaction scheme of hydrolysis of EDAO.

To confirm this theory, EDAO was solved in water and the pH of the solution was set to 3 and injected immediately into NMR instrument. Then the spectra were compared with spectra measured in water solution. The difference between the spectra measured at pH 7 and 3 was conspicuous, but the reaction was too fast for further investigation. The result of this experiment corresponded with the reported reaction kinetics of hydrolysis of Grotan BK [129].

For further study EDAO and Grotan WS were selected (however the other substances were also investigated). They seemed to be more stable according to the flow injection MS study. EDAO and Grotan WS were solved in NaOH solution (pH=13) one by one, and were measured with proton- NMR. The alakaline medium of the solvent was important to slow down the hydrolysis.

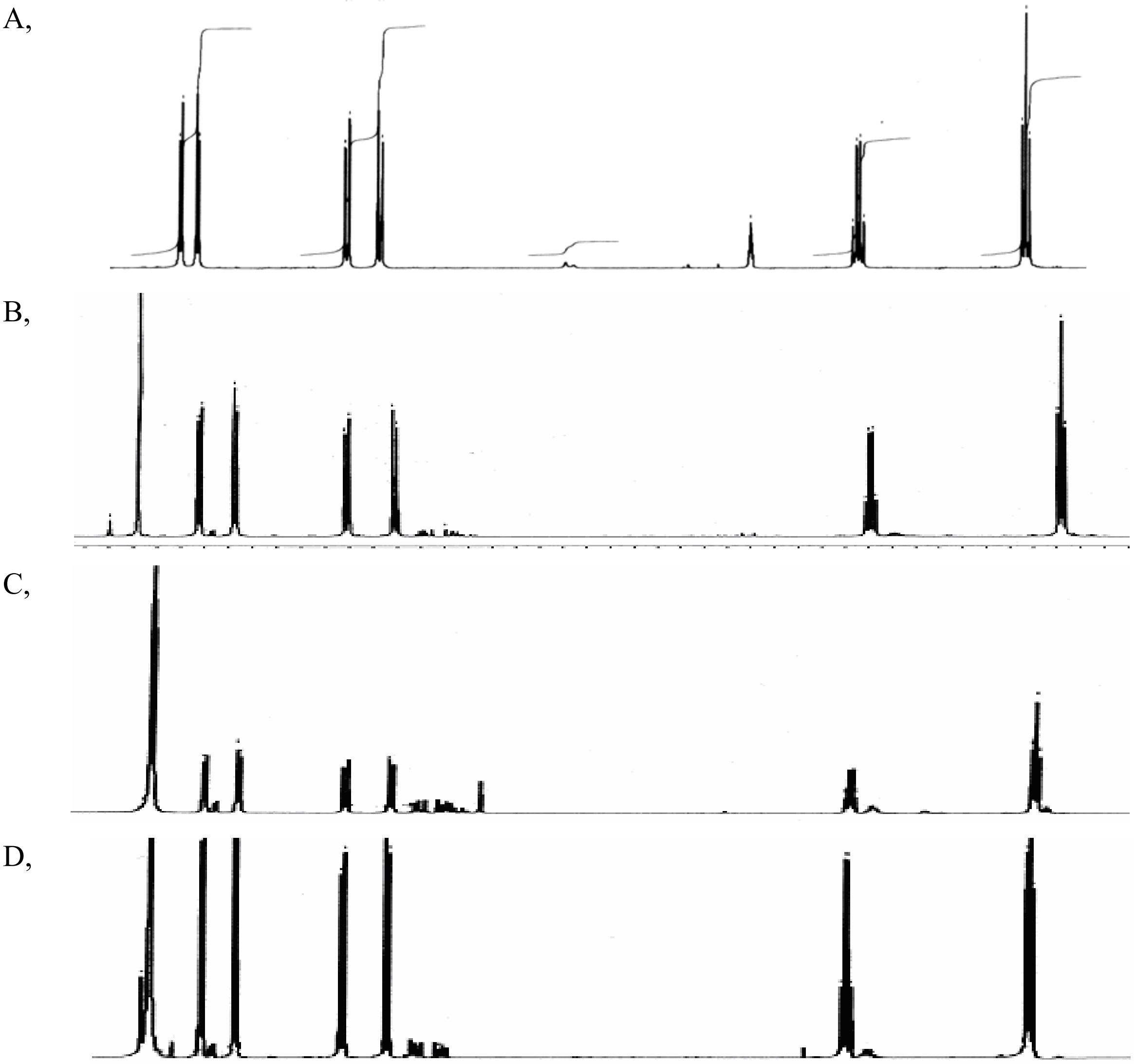


Figure 37: NMR spectrum of EDAO A, solved in acetone measured 30 min after the solution; B, solved in 0.1 mol/l NaOH measured 30 min after the solution; C, solved in 0.1mol/l NaOH measured 1.5 year after the solution and D, solved in NH_4CO_3 measured 30 min after the solution.

In the NMR spectra of EDAO small peaks at 4.4 ppm, 3.3-3.5 ppm and at 1.4-1.5 ppm were detected, which were always in front of the EDAO peaks. As it shown in figure 37, the measured solution of EDAO seemed to be a mixture of main and tracer components. The small peaks can belong to the end-product of the hydrolysis reaction according to the NMR spectrum of AEPD. After one and a half year the NMR spectra of the same solution was measured and the same spectrum was observed. The small peaks were higher and new peak at 3.2 ppm was additionally observed. The tracer peaks were observed when EDAO was solved in 20 mmol/l NH_4CO_3 solutionand measured 30 min after the solution. Thus the type and concentration of electrolyte have influence in the hydrolysation.

The measured spectrums of Grotan WS solved in different solvents are shown in the following figure.

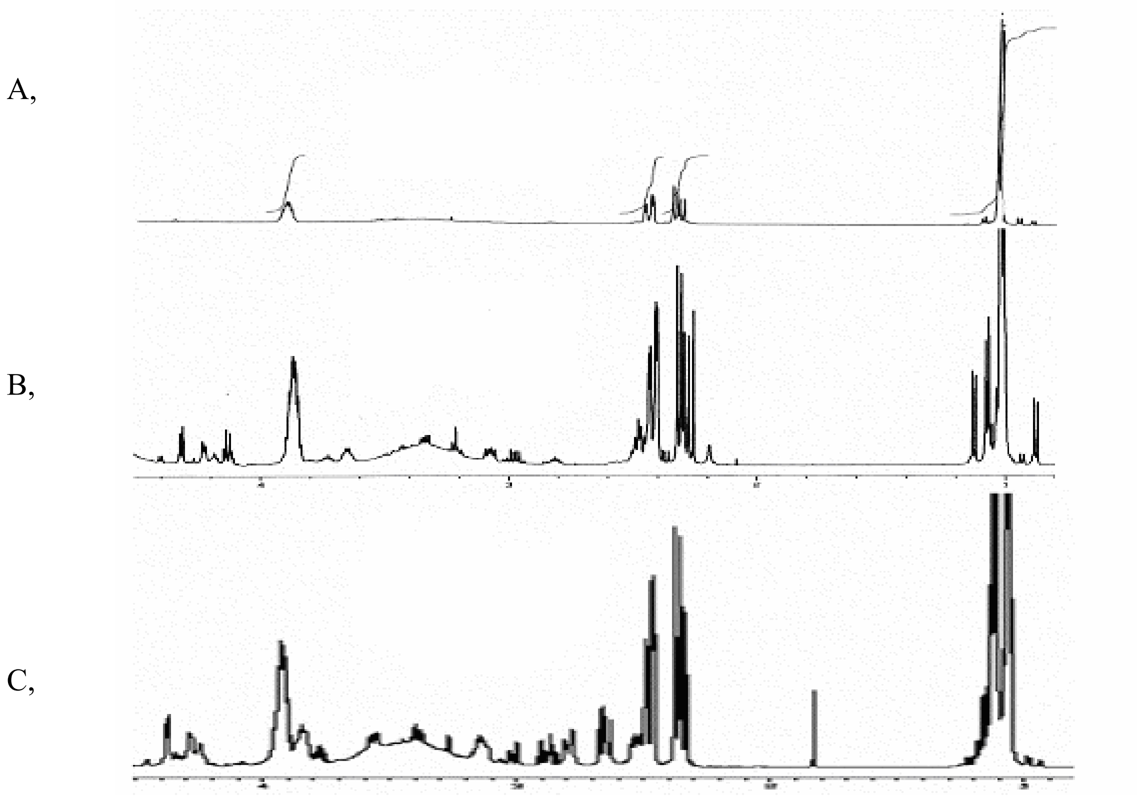


Figure 38: NMR spectrum of Grotan WS A, solved in 0.1mol/l NaOH measured 30 min after the solution; B, solved in 0.1mol/l NaOH measured 1.5 year after the solution and C, solved in NH_4CO_3 measured 30 min after the solution.

The spectra of Grotan WS was at first not clear since the peaks that belong to the ring were not clear enough. This can be caused by the too frequent vibration of the ring and additionally they may be covered by the vibration of the hydroxyl group. According to the two dimensional NMR measurements, it was supposed that the NMR spectrum of Grotan WS was measured and not that of MIPA. Vibrations of other compound besides the peaks of Grotan WS were also observed when the solute was solved in NH_4CO3 solution as shown in figure 38/C.

Similar spectrum was observed for Grotan BK as for Grotan WS. This is in accordance with the structural similarity. The spectrum of Grotan OX is very complex, and in correspondence with its MS spectrum not only one substance was found in the bought material. The mixture contains at minimum three compounds according to the NMR investigation; typical peaks at 3.9 ppm, 2.3 and 2.45 ppm were observed that may be peaks of Grotan WS. In case of DMOI different spectra was measured than it was expected. In the spectra of DMOI the typical vibration picture of ring were not observed, thus it might be that at once after the solution the ring opens or the ring frequency is too high for observing it.

For further study CE-MS was chosen as analytical tool for kinetic study. Water solution of the selected heterocyclic amines were injected and separated with CE-MS and the initial and by-products were detected as can be seen in figure 39.

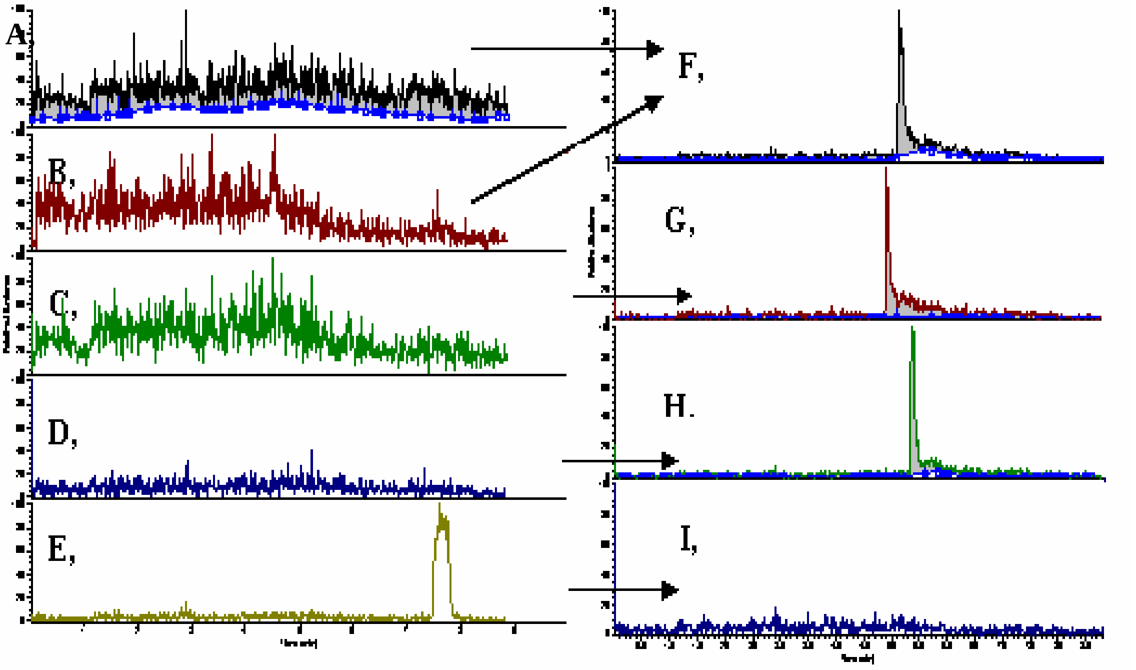


Figure 39: CE-MS electropherogram of initial and end-products 25 minutes at molecule m/z peaks of A, Grotan WS (262); B, Grotan OX (187); C, Grotan BK (220); D, DMOI (102); E, EDAO (144); F, MIPA (76); G, MEA (62); H, AMP (90) and I, AEPD (106).

When water solutions of DMOI and Grotan products were injected and separated with CE-MS, they were not detected 25 minutes after the solution and only their hydrolysis products were able to be detected. Moreover, the peaks of hydroxyl-alkyl-amines were not bright what is usual for substances form during the separation process; only small tailing of the peaks was observed.

EDAO seemed to be quite stable, however 35 minutes after the preparation the peak of AEPD was also detected. EDAO migrates with EOF because change in the current was observed. Thus the formaldehyde releasers may not be protonised at separation pH of 7 that is possible since they are secondary or tertiary amines. This means that they can not be separated with capillary zone electrophoresis.

The peak areas of the hydrolysis products were determined and plotted in function of time.

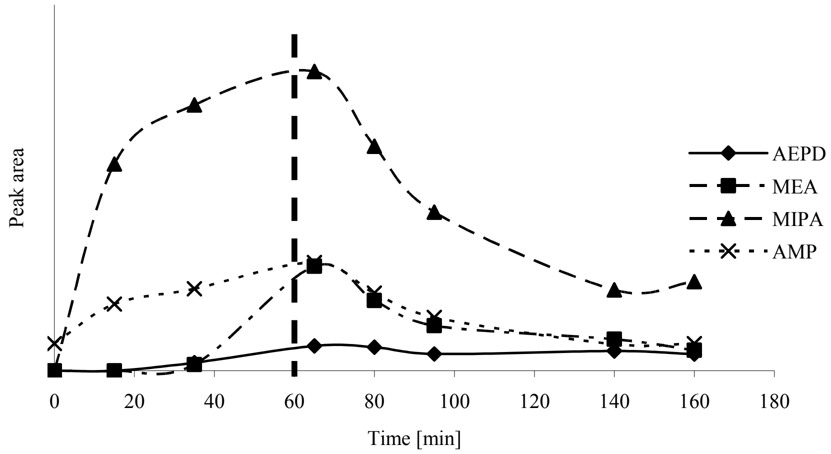


Figure 40: Peak areas of hydrolysis products in function of time.

In the first hour the peak areas were increasing with the time, but then systematic decreasing was detected. When the experiment was repeated the same tendency was measured. Till now, no satisfactory explanation of this tendency has been taken. The biggest problem is that the source of the hydroxyl-alkyl-amine formation can not be separated exactly since they can form also during the contact with seath liquid.

However, according to NMR measurement the water solutions of these formaldehyde releasers are stable and the initial materials does not contain the amino-alcohol (except DMOI). If it is supposed that they not hydrolyse in high purity water, they can only form during the separation. But why systematic tendency in the peak areas in function of reaction time can be concluded? If it is supposed that notwithstanding the hydrolisation takes place in water medium, the decrease of the peak area after one hour should be taken in consideration. It may be possible that the end-products were consumed or evaporated since their vapour pressure are high.

On the whole, the EDAO, DMOI and Grotan products can not be directly separated and detected with capillary electrophoretic method, but since their hydrolysis are adequate at acidic condition they can be measured indirectly when the sample does not contain originally the appropriate amino-alcohols.

According to the H^1-NMR and MS measurements it can be concluded that even at alkaline condition these formaldehyde releasers hydrolyse, and during the reaction amino-alcohols are forming. The reaction speed mostly depends on the pH of the medium and the concentration of electrolyte.

The theory was also supported with *ab-initio* calculations (B3LYP 3-21g* and B3LYP 6-311+g**), and the reaction energy of the hydrolysis of the formaldehyde releasers in gas phases were between 2 and 6 kcal/mol.

4.2.6 Optimization of a method for the determination of amino-alcohols

Since it was assumed that MEA, MIPA, AEPD and AMP were measured with CE combined with indirect UV detection when the standard solution of EDAO, DMOI, Grotan BK, WS and OX was injected, it was aimed to optimise a CZE-indirect UV method for the determination of these alkanolamines. The target substances were extended with diethanolamine (DEA), triethanolamine (TEA), morpholine (MOR), 2-(2-aminoethoxy)-ethanol (AEE), 2-amino-1-butanol (2AB), N-methyldiethanolamine (MDEA) because they may be ingredients of MWF. Piperazine (PIP) was selected as internal standard, since it is not supposed to be found in MWF aerosol and have similar migration property as the targets. MAC values have been established only for MEA at concentration of 5.1 mg/m^3 and the other substances are in the list of hazardous substances [152].

The goal was to optimise the separation parameters for the determination of hydroxyl-alkyl-amines that is MS compatible. It was proven that the indirect detection mode is not selective enough for the determination of these solutes, because the quantification limit is too high and interference effects may occur such as the presence of alkali metals and alkaline earth [93]. Using MS as a detector the LOD would be decreased, and the most interference effects would be also eliminated. To have an MS compatible method the following parameters should be considered: (i) the BGE should be volatile and (ii) the applied voltage should be low, since the current has to be as low as possible.

The initial operation conditions for the separation of the selected hydroxyl-alkyl-amines were identical to those used for the formaldehyde releasers. The *probe* of BGE was not changed, since the peaks measured with imidazole and imidazole-chloride buffer were sharp and symmetric. The BGE when MS detection is used does not contain *probe* (that have chromophor group), but for indirect UV detection mode it is indispensable. Thus the probe should not interact with the target compounds to have an MS compatible buffer, which can be also applied for indirect UV detection. The migration behaviour of MEA, MIPA, AEPD and AMP was identical from theoretical prediction and experimental separation (figure 32 and 33), so it was assumed that no interaction exist between *probe* and analytes.

At first the concentration of the probe was investigated, because it has big influence on the detection limit and the noise of the baseline [263]. The concentration of imidazole was increased from 10 mmol/L to 100 mmol/L in the separation buffer. The noise of the baseline and the peak height increased with the increase of buffer concentration. For further study the buffer concentration of 20 mmol/L was the optimum.

To have MS compatible background electrolyte, the buffer anion was changed from chloride to acetate. According to the measurements no differences was observable in the electropherograms using the two anions (chloride and acetate) in the BGE.

After the buffer system was set, the migration behaviour in function of pH of BGE was investigated, because at neutral pH only 6 peaks were separated from the 11 solutes. The pH of BGE was decreased from 7 to 3, and analytes at concentration of 10 mg/L were separated using these buffers. It was not possible to decrease the pH of BGE below 3 because of the buffer capacity is negligible. The separation time and also the resolution of the selected amino-alcohols increased with the decrease of pH of BGE. The peak numbers were 8 at pH bellow 4, and under pH 3.5 the resolution of AEE, 2AB, DEA, MDEA and AMP were increased. Since the best resolution was reached when the pH of the BGE was 3.5, this buffer system was chosen for the following study.

To achieve separation of the five critical substances (AEE, 2AB, DEA, MDEA and AMP), organic solvents were added to the BGE. According to the study of the effect of organic modifiers on the separation of the heterocyclic amines methanol, ethanol and 2-propanol were added to the separation buffer at ratios from 0 V/V% to 40 V/V%. The best separation was achieved, when the organic modifier was ethanol at volume of 25% since four peaks from the 5 critical substances were baseline separated.

The separation voltage and temperature were also investigated. The separation voltage was increased from 20 kV to 30kV, and the cartridge temperature was decreased from 30 °C to 25°C. Only a small effect of these parameters on the separation was found and acceptable baseline (flat and less noise) were got when the measurements were carried out at 20 kV and 30 °C.

Thus for final separation 20 mmol/l imidazole/imidazole-acetate buffer was chosen at pH of 3.5 solved in ethanol/water mixture at ration of 25%/75% (V/V). The typical measured current was 19 µA, which is low enough for applying the method for MS detection. A typical baseline corrected electropherogram is shown in figure 41 when standard solution of analytes was injected into the CE and detected with indirect UV.

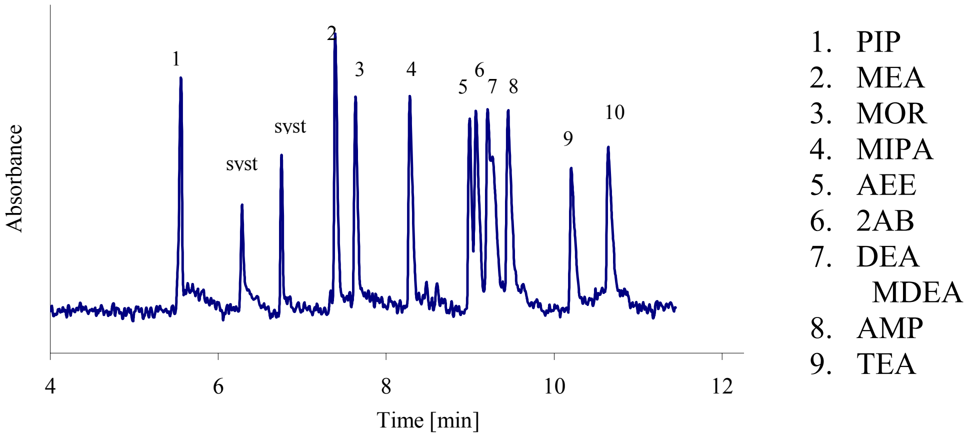


Figure 41: Baseline corrected electropherogram of hydroxyl-alkyl-amines determined with CE-indirect UV. Abbreviation: syst=system peak.

The analytes were separated within 11 minutes. Two system peaks at 6.28 min at 6.74 min was observed when water solutions of hydroxyl-alkyl-amines were injected. PIP, MOR, MEA, MIPA TEA and AEPD were fully separated and AEE, 2AB and AMP were baseline separated. Unfortunately, MDEA and DEA were separated only partly, thus the quantification parameters were not determined for these solutes.

4.2.7 Performance characteristics of CE using indirect UV detection

The separation parameters were evaluated from electropherogram and normalised effective mobility scale. The selected internal standard was PIP that was added into the sample solution before the injection.

Resolution: R_s were calculated for neighbour peaks step by step and the results are summarized in figure 42.

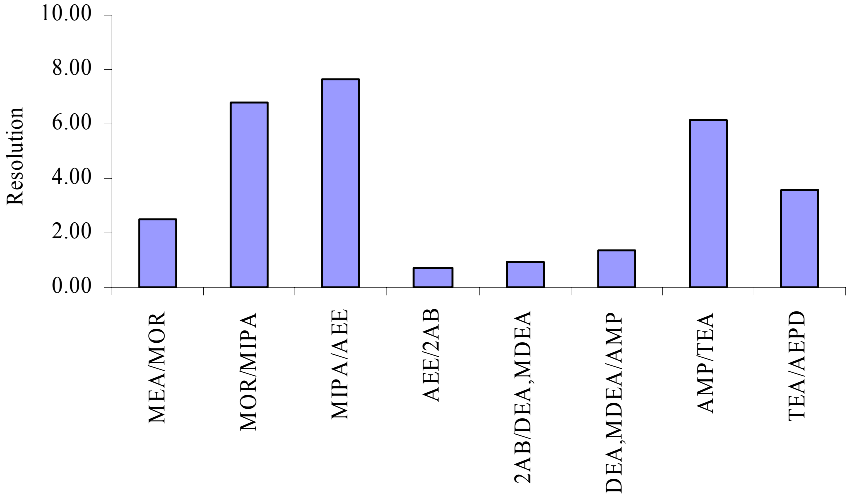


Figure 42: R_s of hydroxyl-alkyl-amines using indirect UV detection.

The Rs values were the highest for MIPA/AEE and the lowest for AEE/2AB. The Rs values for AEE/2AB, for 2AB/DEA+MDEA and for DEA+MDEA/AMP were close to one, thus the identification of these substances should be taken carefully because of the interference with the matrices might be happen. The capillary was not overload in the investigated concentration range because the resolution was independent on the solute concentrations.

Precision of identification: The within-day repeatability of the identification parameters was calculated from five measurements at three analyte concentrations and summarized in table 15.

Table 15: MT and µeff,norm with RSDs from 5 repetitions.

	Migration time		**Effective mobility**	
	MT [min]	**RSD [%]**	**$\mu_{eff,norm}$ [cm2/Vmin]**	**RSD [%]**
PIP	5.57	0.94	1.53	-
MEA	7.35	0.56	1.02	0.33
MOR	7.60	0.48	0.97	0.38
MIPA	8.23	0.69	0.85	0.43
AEE	8.92	1.09	0.74	0.55
2AB	9.02	0.63	0.73	0.58
DEA, MDEA	9.19	0.36	0.71	0.53
AMP	9.43	0.38	0.68	0.57
TEA	10.18	0.50	0.59	0.75
AEPD	10.62	0.39	0.54	0.82

The RSDs of MTs of the selected hydroxyl-alkyl-amines were under 1% showing an excellent precision of this method. The RSD values were determined between 0.03-0.82% for normalised effective mobility.

Linearity and working range: The working range was established by MAC values for selected hydroxyl-alkyl-amines. The lowest one was established for MEA which is 5.1 mg/m3 that is equal to 61 mg/L for a sampled volume of 120L. Thus the linear range should cover the concentration from 6.1 mg/L to 122 mg/L. The calibration curves using electropherogram and effective mobility are shown in table 16.

Table 16: Calibration parameters applying electropherogram and mobility scale.

	Migration time			**Effective mobility**		
	Equation	**R^2**	**f**	**Equation**	**R^2**	**f**
PIP	A=0.50*c+0.041	0.998	-	A= 1.88*c-2.48	0.996	-
MEA	A=0.67*c+0.025	0.999	1.42	A=1.46*c-0.82	0.997	0.78
MOR	A=0.48*c+0.29	0.999	4.05	A=0.94*c+0.91	0.998	0.55
MIPA	A=0.62*c+0.35	0.999	1.34	A=1.04*c+0.56	0.999	0.58
AEE	A=1.05*c-0.91	0.992	2.41	A=1.38*c+2.02	0.999	0.83
2AB	A=0.853*c+0.57	0.994	2.13	A=1.214*c+1.07	0.998	0.52
AMP	A=0.53*c+0.32	0.999	1.18	A=0.65*c-0.47	0.999	0.33
TEA	A=0.45*c+1.56	0.999	1.14	A=0.75*c-0.16	0.986	0.35

With both scales for each analytes the regression coefficients were higher than 0.998. The determined relative sensitivities for internal standard were between 0.6-1.3 for each selected amino-alcohols and the values were independent on the analyte concentration (no capillary overload).

Precision of quantification: The RSDs of the peak areas calculated from five measurements were under 7% for both scales. No significant differences were observed in the precision of the quantification between the time- and mobility-scale.

LOD and LOQ: The quantification limits for the amino-alcohols were determined between 0.6 -1.5 mg/L using electropherogram and normalised effective mobility scale. The concentration value of quantification limit was 5 mg/l in solution and 0.46 mg/m3 when 120 l air is pumped through the filter. The LOQ was lower than the lowest point of the working range.

Accuracy: The recovery values are used for evaluation of accuracy. The calculations of recovery were determined using time scale and normalised effective mobility scale. Two data interpretation technique was used for determination of analyte concentation from peak area. They are calibration and internal standard.Internal standard is more useful in some cases mostly when real sample is analysed. However the linearities should be also determined to be able to apply internal standard.

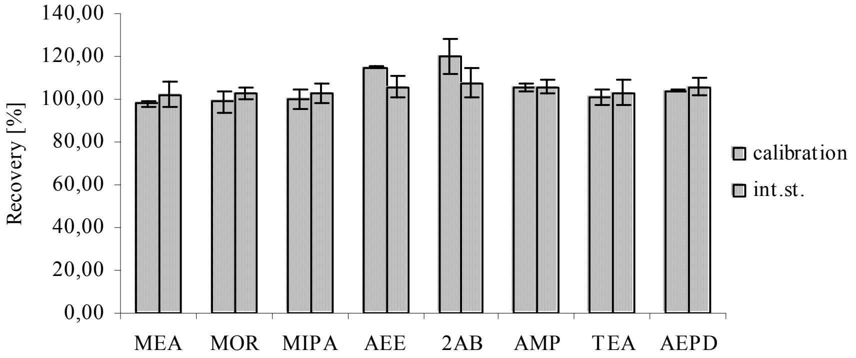


Figure 43: Recovery with RSDs of a method determined from normalised effective mobility scale (n=5). Abbreviations: int.st.: internal standard.

The recoveries were independent on the calculation methods and on the analyte concentration. The mean recovery values were determined between 93 % and 111% which correspond to the specified analytical method.

On the whole, all performance characteristic met with the specified requirement except for DEA and MDEA which were not separated. These solutes must be determined as a sum of two solutes. For separation and calculations of quantification effective mobility scale is suggested and both techniques, calibration and internal standard, are suitable for determining the concentration of the selected hydroxyl-alkyl-amines.

4.2.8 Performance characteristics of CE using MS detection

The method optimised for indirect UV detection was used for CE-MS measurements. The only difference was that the cation of the BGE was ammonium ($NH4^+$) instead of imidazole.

After tuning the MS condition the capillary temperature was set to 150 °C, the capillary voltage to 3.5 kV and the distance between the capillary outlet and MS inlet was 48.05 mm. The typical current of MS was detected at 11 µA; and the spray was stable during the measurements.

Applying flow injection mode the molecule peaks of the selected hydroxyl-alkyl-amines was determined. MS spectra when mixture solution of amino-alcohols is was introduced is shown in figure 44.

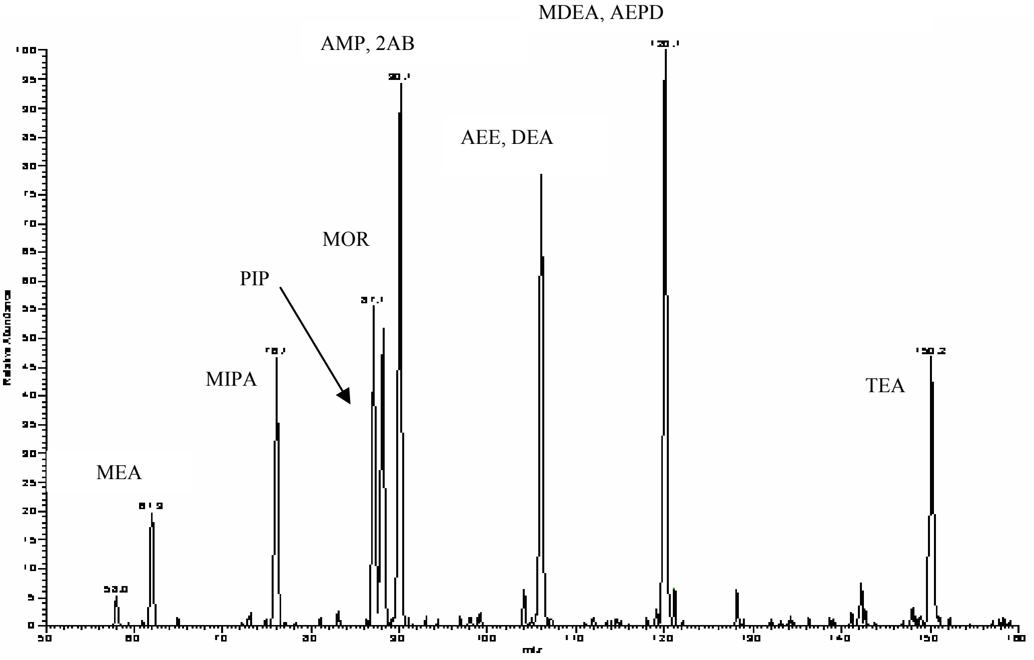


Figure 44: MS spectra of water solution of selected amino-alcohols.

The detected peaks belong to the molecule peak of the amino-alcohols. Unfortunately, AMP and 2AB, AEE and DEA, MDEA and AEPD have the same molecular weight, but it was supposed to be separated from each other. During the separation SCAN mode was used, and m/z peak at 62±0.5 for MEA, 76±0.5 for MIPA, 87±0.5 for PIP, 88±0.5 for MOR, 90±0.5 for AMP and 2AB, 106±0.5 for AEE and DEA, 120±0.5, 150±0.5 was selected fo ther identification and the integration of the detected peaks.

To see whether the CE condition optimised for indirect UV detection mode fits to CE-MS the concentration and pH of the buffer system (10, 15 and 20 mM NH4OAc/AcOH at pH of 5.0, 4.3 and 3.3), the type of organic modifier like methanol, ethanol at volume ratio of 10%, 20% and 30% and the separation voltage (15 kV, 20 kV, 25 kV) were tested. According to the measurements, it can be concluded that the final condition optimised for indirect UV is equal to the optimal condition with MS detection mode. A typical electropherogram is shown in figure 45 when standard solution of selected hydroxyl-alkyl-amines was measured with CE-MS.

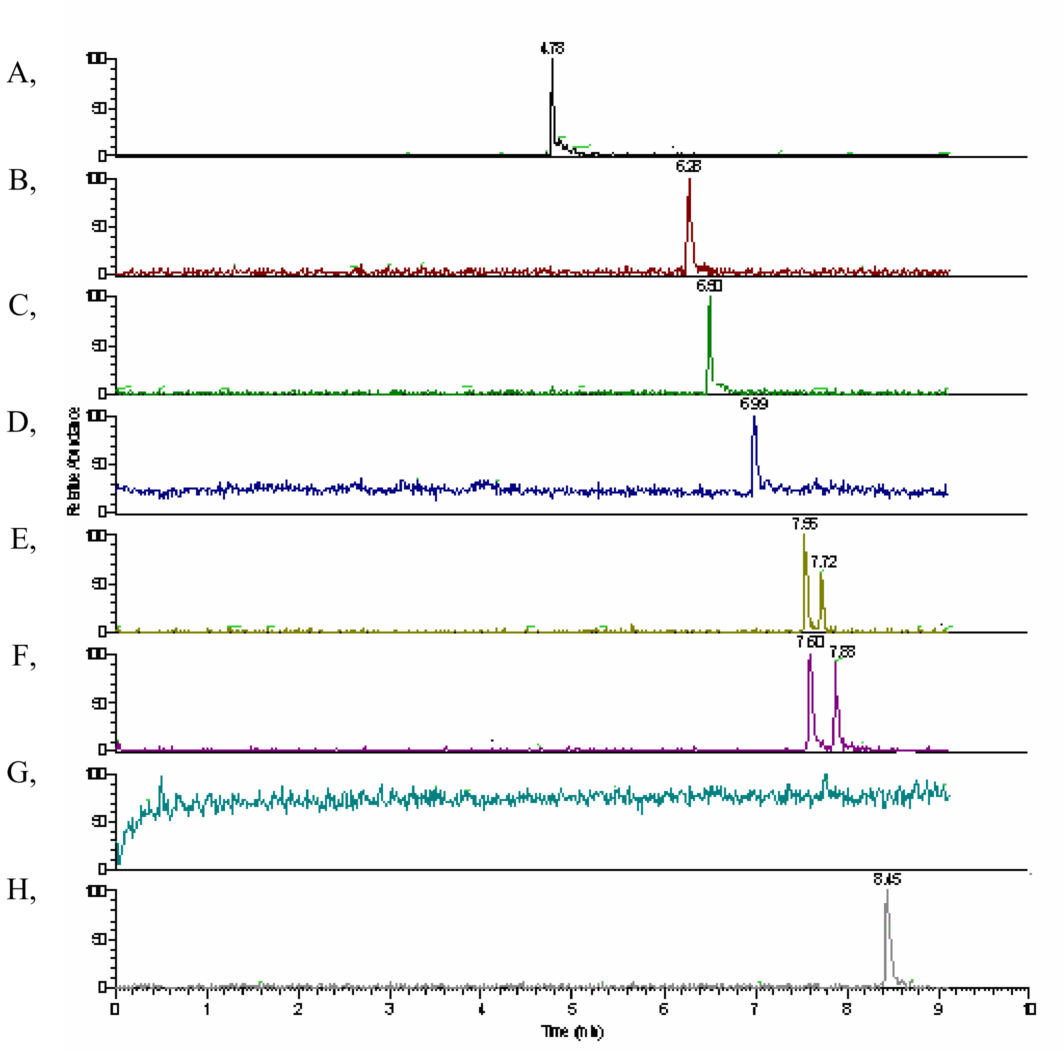


Figure 45: Elelectropherograms of A, PIP at m/z 87; B, MEA at m/z 62; C, MOR at m/z 88; D, MIPA at m/z 76; E, AEE and DEA at m/z 106; F, 2AB and AMP at m/z 90; G, MDEA and AEPD at m/z 120 and H, TEA at m/z 150.

During the separation m/z peaks at 61, 78, 103, 120 and 142 were continuously detected. Unfortunately the molecule peak of MEA, MIPA, MDEA and AEPD interfere thus the MS detection is less sensitive for MEA and MIPA and MDEA and AEPD could not be detected at all. Small tailing of the hydroxyl-alkyl-amine peaks were observed.

The performance characteristic of a CE-MS method for the determination of amino-alcohols was also determined as described in chapter 3.4.3.

Resolution: R_s was examined for AEE/DEA and 2AB/AMP since these analytes had the identical detection fragment. The resolution factor were independent from their concentration and were 2.1±3% and 2.2±5% which meets with the specified analytical requirement. Thus the performance characteristics of quantification for these substances were also determined.

Precision of identification: The precision of migration time was carefully investigated because the CE connected with MS had no coolant system. When the coolant effectiveness is poor the Joule heating may cause higher changes in EOF and thus in MT. The calculated RSDs of MT measured from five repetitions are summarized in figure 46.

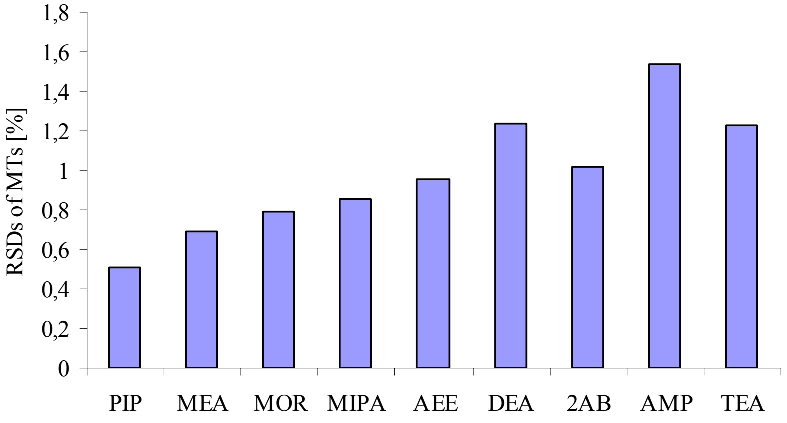


Figure 46: Precision of identification of CE-MS method for the determination of amino-alcohols.

The within-day RSDs were between 0.6-1.5% and were independent on the concentration of analytes. This quite good repeatability may be consequence of low separation current and the suppressed EOF (because of the low separation pH).

Small increase in the RSD values was determined with increase of MT. On the whole, the precision of MTs fulfil the specified analytical requirement.

Linearity and sensitivity: the determined linear curve with their regression coefficients were summarized in table 17 for each selected derivative of amino-alcohols.

Table 17: Linearity, LOD and LOQ of hydroxyl-alkyl-amines determined with CE-MS.

	Equation of calibration curve	**R^2**	**f**	**LOD [mg/L]**	**LOQ [mg/L]**
PIP	A=1.28*c-2.00	0.995	-	0.17	0.57
MEA	A=0.64*c -0.37	0.999	0.61	1.18	3.94
MOR	A=1.34*c -0.16	0.991	1.23	0.41	1.36
MIPA	A=1.08*c -0.34	0.998	1.06	3.53	8.91
AEE	A=6.70*c -0.13	0.998	0.67	0.33	1.11
2AB	A=0.30*c +0.23	0.993	0.34	0.73	2.44
DEA	A=1.00*c -0.05	0.999	1.01	0.15	0.55
AMP	A=0.76*c -0.27	0.997	0.76	0.20	0.67
TEA	A=1.44*c -0.31	0.990	1.30	0.22	0.75

The regression coefficients of the selected analytes in the working range were higher than 0.990, so the detection response linear with analyte concentration.

The determined detection limits were between 0.15-0.73 mg/L except for MIPA and MEA as it was expected. Using MS for detection 5-10 times lower concentration was able to be detected than with indirect UV detection.

Accuracy and precision: The recoveries were determined with use of two data interpretation technique and summarized in the following figure.

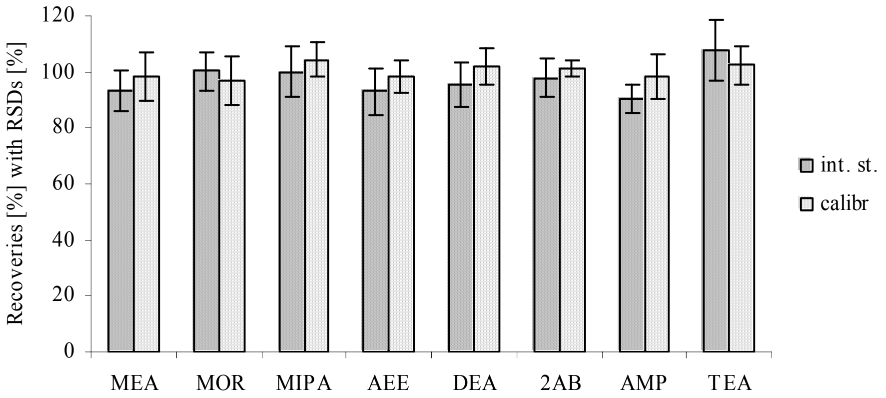


Figure 47: Recoveries with RSDs of CE-MS method for determination of hydroxy-alkyl-amines (n=5).

The recoveries were concentration independent and the averaged values were found between 97% and 104% for each analytes when calibration technique was applied and from 93% till 108% when internal standard method was used for calculation of the solute concentration.

The RSDs of the peak area for the whole method were between 3-6% that shows an excellect precision of quantification.

CE-MS method adapted from CE-indirect UV can be used for the identification and the quantification of the selected hydroxyl-alkyl-amines with high selectivity, higher sensitivity, good precision and accuracy (recovery).

4.2.9 Study of matrix effect

The extraction efficiency were determined in function of MWF concentration as written in section 3.5, however no effect of the emulsion system was expected due to the high water solubility of the targets. The MTs in function of MWF present on the filter is shown in figure 48.

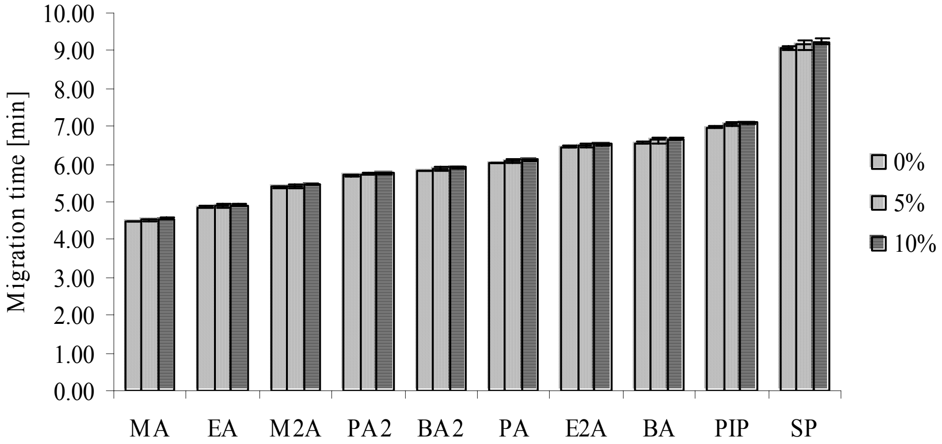


Figure 48: Migration times of hydroxyl-alkyl-amines in function of MWF concentration (n=5). Abbreviations: 0%= standard solution without MWF, 1% and 3%= 1% and 3% MWF concentrate in the standard solution of analytes.

No MT shifting was observed in the function of concentration of MWF emulsion. The repeatability of the identification parameters was independent on MWF concentration and the RSDs were between 0.40-1.31% for each compounds (see figure 48). Thus no effect of matrices on the identification of hydroxyl-alkyl-amines was determined.

The effect of MWF on the precision of quantification and accuracy was also determined the same way as written in section 3.5. Recoveries with RSDs calculated from three repetitions were determined in function of MWF concentration in the standard solution of selected hydroxyl-alkyl-amines. In the case of study of matrix effect, calibration was used for determination of analyte concentration from peak area. The averaged recoveries for the whole analytical process are summarized in the following figure.

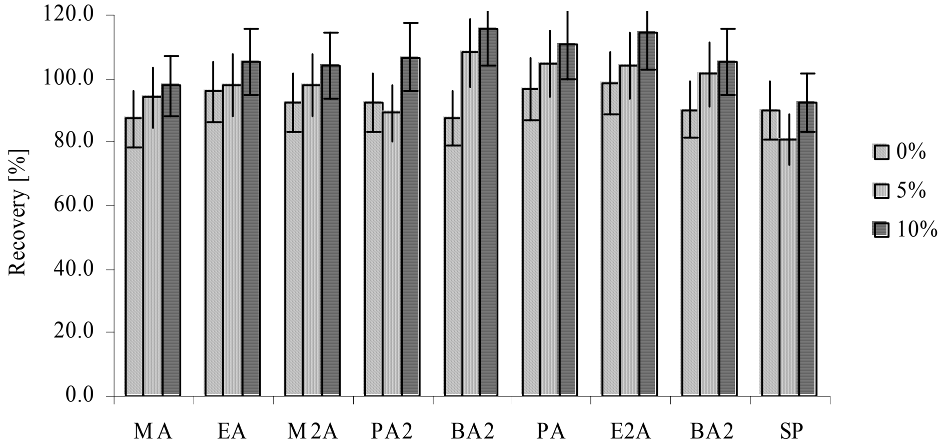


Figure 49: Recoveries with RSDs of a method in function of MWF content (n=5). Abbreviations: 0%= standard solution without MWF, 1% and 3%= 1% and 3% MWF concentrate in the standard solution of analytes.

The recoveries and their RSDs in spiked MWF emulsion sample were independent on the concentration of matrices. The mean recoveries of PIP; MEA, MOR, MIPA, AEE, 2AB, AMP, TEA and AEPD using calibration varied between 95-110%. The RSDs of peak area from three repetitions were under 7%.

4.2.10 Final protocol

Table 18: Final protocol for the determination of amino-alcohols in atmospheric and MWF aerosol.

Sampling:	With air pump at flow rate of 1 L/min, time duration of 2h onto a quartz filter.
Sample preparation:	Extract the filter with 10 ml 30:70= MeOH: water mixture with ultrasonic bath.
Capillary:	Fused silica, I.D. 75 µm, effective length 50 cm, total length 57 cm.
Detection wave length:	Indirect detection, 214 nm, Detector cell size: 100*800 µm.
Temperature:	Cartridge set to 30 °C.
BGE for aminoalcohols with indirect UV detection:	20mM imidazole/ imidazole acetate, 25 V/V% ethanol, pH=3.5
BGE for aminoalcohols with MS detection	20 mM ammoniumacetate/acetic acid, 25 V/V% ethanol, pH=3.5
Capillary preconditioning:	2 min pressure with 0.1 mol/L NaOH, then 3 min with BGE.
Injection:	Hydrodynamic injection of sample 50 mbar for 10 s (V_{inj}= 59 nl).
Voltage :	20 kV.
Replenishment:	Both separation vials and injection vial after each injection.
Capillary preconditioning: (new capillary)	Pressure flush with 0.1 mol/L NaOH for 30 min when it is used at first time
Storing of capillary: (for next measuring day)	Pressure flush with 0.1 mol/L NaOH for 15 min before and after the measuring day
Storing of capillary: (more then one week)	Pressure flush with 0.1 mol/L NaOH for 15 min followed by high purity water for 15 min, then with air for 5 min.
Marker	Piperazine
Scale	Using the transformed effective mobility scale from time scale
Determination of concentration:	With five-point calibration curve in the working range between 6.1 mg/l and 122 mg/l determined every measuring day Or internal standard by addition of 10 mg/l PIP into the sample before injection

4.2.11 Conclusion

The aim of this study was to develop and validate CZE method combined with indirect UV detection for the determination of “amino-alcohol type” formaldehyde releasers (DMOI, EDAO, Grotan BK, WS and OX) in MWF aerosol. After several CE parameters were investigated for optimisation purpose, only four solutes were separated. Grotan WS and OX co-migrated, in spite the fact that their molecule size and ion-charge that have influence on the capillary electrophoretic separation are not identical. Since indirect UV detection technique is the least selective, it was possible that the determined peaks did not belong to the formaldehyde releasers but to their hydrolysis products. For this purpose, the by-products, which are derivatives of hydroxyl-alkyl-amines were injected and separated applying the same conditions as for formaldehyde releasers. The measured migration times of the formaldehyde releasers and the coherent by-products were the same. The kinetics of hydrolysis of these substances was then suggested for further examination to know what was really separated when standard solution of formaldehyde releasers were injected into the CE.

Before kinetic study of the hydrolysis of the selected biocides, purity of the reference materials of DMOI, EDAO, Grotan BK, WS and OX was investigated to see if they contain amino-alcohols or not. At first, MS was chosen for this purpose. When the water solutions of these substances were measured one by one, the molecule peaks of the by-products were observed at case of DMOI, Grotan BK, WS and OX. Because the source of amino-alcohols was unknown (from the materials or formed during the analysis), another analytical tool was chosen for further examination. It was supposed that NMR spectra can give more reliable information of the original solutes compared to MS because of the experimental conditions would not cause hydrolysis of the investigated materials. According to their NMR spectra the standard solutions of formaldehyde releasers were amino-alcohol free when EDAO, DMOI and Grotans were solved in acetone.

After the purity examination of the reference materials the reaction kinetic of formaldehyde releasing was studied. No big changes in the NMR spectra in time were observed when EDAO was solved in high purity water. When the pH of the solvent was decreased, the measured spectra was totally different comparing the spectra measured at purity water medium. Since the iinvestigation of the reaction was not possible at low pH, the formaldehyde releasers were solved in sodium hydroxide solution (pH=13). In this case hydrolysis was also observed for each substance, but the reaction slowed down dramatically: two weeks after solving the standard materials in sodium hydroxide solution only a small change in the peak area of the tracer was observed. The reaction sped up when EDAO and Grotan WS was solved in buffer thus the hydrolisation depend not only the pH of the medium but the concentration of electrolytes.

Since MEA, MIPA, AMP and AEPD were measured when the formaldehyde releasers were injected into the separation capillary, it was planned to optimise a method for the determination of these hydroxyl-alkyl-amines from MWF aerosol. The number of analytes was extended from 4 to 11 because more amino-alcohol derivates may be found in MWF used as additives. The main aim was to improve a method that is MS compatible since indirect UV detection mode is the least selective and therefore the most subject to interferences. For this purpose the composition of the BGE was changed, and with the final separation parameters were MS compatible.

The analytical performance characteristics were determined including the identification and quantification. DEA and MDEA were not separated, thus these substances can be only identified from real samples using CE-indirect UV method. AEE, 2AB, MDEA+DEA and AMP were only baseline separated, and their mean resolution factors were near to the required limit. Both identification parameters, the migration time and normalized effective mobility, were precise when the analytes were solved in test mixtures and in spiked samples.

The linearity were determined at range between 6.1 mg/L and until 122 mg/L since this range cover the 0.1*MAC-1*MAC of MEA for sampled volume of 120L. The precision of quantification was determined between 1-6% when time- and mobility-scale was used for integration, and the RSDs of peak area were independent on analyte concentration. The detection limits of PIP; MEA, MOR, MDEA, AEE, 2AB, DMBA, AMP, TEA and AEPD were 1.5 mg/L for both scales applying indirect UV detection. The recoveries of the CE-indirect UV method for determination of selected hydroxyl-alkyl-amines were near to 100% thus the method is enough reliable for quantification.

The method developed for indirect-UV detection for determination of selected amino-alcohols were theoretically MS compatible, since the BGE was volatile and the measured current was enough low. Thus the method was also adapted for MS detection. The only difference was the type of cation in BGE; the electrolyte was changed from imidazole, which was at the same time the *probe,* to ammonium.

According to the measurements, no difference between the peak sequence was observed when MS and indirect-UV detection was connected to the CE instrument. Unfortunately MDEA and AEPD were not identified and quantified since with their mze molecule peak at 120 interfere with BGE or sheath liquid. Additionally, decreased sensitivity was observed for MIPA and MEA due to the inference effect.

Applying CE-MS the resolution between AEE/DEA and 2AB/AMP was enough for having a reliable identification for these substances in real samples. The precision of migration time was high since the within-day RSDs were under 1.5%. The quantification performance characteristics were also determined and the values for linearity, precision of peak area and accuracy fulfil the specified requirements. According to the measurements, both, calibration and internal standard are useful for interpretation technique for the determination of MEA, MOR, MIPA, AEE, DEA, 2AB, AMP and TEA. The detection limit were determined between 0.17 and 1.18 mg/L expect for MIPA that had higher one (3.5 mg/L) and were 5-10 times lower than determined with indirect-UV detection.

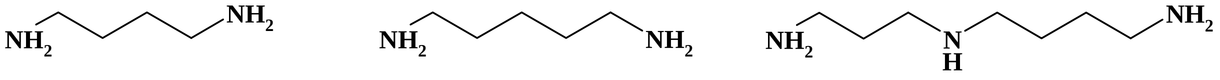
Finally, the effect of matrices (MWF emulsion) on the extraction and separation efficiency was investigated. According to the measurements, no any significant difference was observed in the identification parameter and in the recoveries as a function of MWF concentration.

This means that the method can be applied for determination of the selected hydroxyl-alkyl-amines from MWF aerosol. With this method formaldehyde releasers can not be determined directly and only their hydrolysis products can be quantified and determined. Unfortunately, the hydrolysis products might be found in MWF aerosol originally, thus it can not be told correctly whether MEA, MIPA, AEPD and DMOI were found originally in MWF aerosol or produced during the sample preparation. For that purpose selective sample preparation or HPLC separation of formaldehyde releasers is suggested since they are quite stable in pue solvent. On the other hand, vapour phase should be also sampled because hydroxyl-alkyl-amine derivatives are volatile thus they might be found in the vapour phase of the aerosol.

4.3. Determination of volatile and biogenic alkyl amines

Capillary electrophroretic method the determination of alkyl-amines including sampling and sample preparation in atmospheric and indoor aerosol was developed. These substances are still in the interest because of their odoriferous properties and in they influence the nitrogen cycling, nutrient transfer, and atmospheric acidity in the ecosystem. On the other hand they have acute and chronic effect on the human health. Recent works show that chemical compounds of low molecular weight alkyl-amines, cause occupational asthma [251] and block the voltage-dependent calcium channel in human embryonic kidney cells [252]. Moreover, the structure toxicity relationship for several aliphatic amines has been also reported [253,254].

The selected alkyl amines were methyl-amine (MA), ethyl-amine (EA), propyl-amine (PA), butyl-amine (BA), dimethyl-amine (M_2A) diethyl-amine (E_2A) as low molecular weight (LMW) primer and secondary amines and cadaverine (PA_2), putrescin (BA_2) and spermidine (SP) as biogenic amines (see figure 50). The MAC values has been established for MA at 13 mg/m^3, for EA at 9.4 mg/m^3, for BA at 15 mg/m^3, for M_2A at 3.7 mg/m^3 and for E_2A at 15 mg/m^3 [152].



BA_2 PA_2 SP

Figure 50: Chemical structure of selected biogenic alkyl-amines.

The vapour pressures of these substances are high, thus they are found in dynamic equilibrium between vapour and condensed phase in the atmosphere. Thus the sampling have to contain the collection of both phases to determine the total amount of these analytes presented in aerosol system.

The sample preparation was adapted from the earlier developed one described in section 6.2 because the aim of the work was to can determine as much targets as it possible form the polar extract

4.3.1 Study of the sampling of alkyl amines

The sampling of alkyl amines derivatives from atmospheric or indoor aerosols should be divided into two. They may adsorb on the surface of solid particles and/or absorbed in droplets present in the air and they also may precipitate as a low volatile salts. Quartz fibre filter with suitable pump was used for sampling of the condensed alkyl-amines from ambient air. However, evaporation of alkyl-amines during sampling may cause negative artefact in the determined amount in the particulate phase.

On the other hand they may be found as vapour due to their high tension and the emitted LMW amines are mostly gaseous. Thus the already used collector containing the quartz filter for the collection of the particles was supplemented with a holder containing the adsorbent for collecting the amines form the vapour phase. The adsorbent may collect also the evaporated part from the condensed phase thus the total amount of alkyl amines presented in the atmospheric and MWF aerosol can be determined.

4.3.1.1 Preparation of novel adsorbent

Selection of proper adsorbent for primary and secondary LMW amines is difficult because of their high polarity. If the adsorbent is highly polar silica or aluminium oxide (Al_2O_3), the elution may be not quantitative and when apolar adsorbent is used such as C18 the adsorption might be too weak to have reliable sampling. The other possibility is to use ion-exchange material, although its effectiveness is also questionable because the great majority of the gaseous amines are in neutral form. Thus surface with functional group that can be form H-bridge with the amine group seemed to be the best solution because of the quite strong bounding and full desorption is possible by protonising them. Other requirements beside the high adsorption and desorption efficiency are the high porosity, high stability and low cost.

Instead of testing commercially available materials, it was aimed to prepare a polymer with high selectivity to the organic amines. Modified polymethacrylate was chosen as solid phase because the initial materials used for preparation are cheap and commercially available; no special instrument is needed for the preparation; easy to modify its surface properties and the polymer itself very stable against extreme conditions such as strong alkaline or acidic aqueous solutions or organic solvents.

This continuous bed usually used as stationer phases in CEC. The solid monoliths have been prepared from butyl-methacrylate and ethylene-dimethacrylate and studied extensively for the separation of basic pharmaceuticals [255], polycyclic aromatic hydrocarbons [256], benzene derivatives [257] and model compounds of humic substances [258]. The preparation of the polymerisation process is well known [259].

The polymerisation solution contains three parts: (i) monomers (generally 40 %(m/m) of the total volume), (ii) initiator (generally 1%(m/m) of the monomer volume) and (iii) porogens (generally 60%(m/m) of the total volume) in which the monomers are soluble but the polymer not. Usually, the monomers contain 40% ethylene dimetacrylate (EDMA) as cross linking agent, 60% butyl-methacrylate (BMA) as the adsorbent and wetability agent which is usually 2-acrylamido-2-methylpropanesulfonic acid (AMPS). The porogen comprising generally 10% water, 64% 1-propanol (PrOH) and 26% 1,4-butandiol ($BuOH_2$) however other porogen solution have been also used. The initiator used for thermal initiated polymerisation is isazobisisobutylnitrile (AIBN). For the purpose of active sampling of alkyl-amines this solid phase was not suitable, since the targets did not adsorb on the solid phase. Thus some alterations in the monomer composition were suggested for having a suitable adsorbent for the sampling of amines. Acrylic acid (AA) was added into the monomer solution in place of butyl-methacrylate at different ratios since the carboxylic acid group form H-bridge with amine group. Scheme of possible structure of the monolith is shown in figure 51.

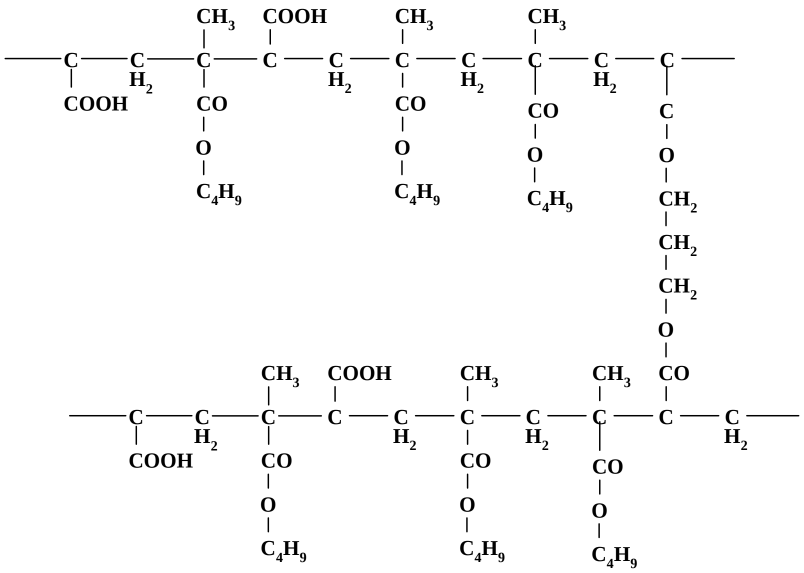


Figure 51: Possible structure of poly(acrylate-methacrylate) co-polymer.

For characterizing the self prepared polymer, the affectivity of adsorption and desorption was tested with 0.5 ml standard solution of alkyl amines at 10 mg/L (thus the total target amount is 9*5=40 μg). The elution volume was at first identical to the adsorption one. The amount of the solid phase was always 400 mg packed in polyethylene cylinder body. The preparation parameters of the monolith are summarized in the following table:

Table 19: Composition of monomer solutions.

Name	**DMBA**	**BTA**	**AA**	**H_2O**	**PrOH**	**$BuOH_2$**
MonE(100)	40%	0%	60%	10%	64%	26%
MonF(50)	40%	30%	30%	10%	64%	26%
MonJ(40)	40%	24%	36%	10%	64%	26%
MonI(30)	40%	42%	18%	10%	64%	26%
MonG(25)	40%	45%	15%	10%	64%	26%
MonA(0)	40%	60%	0%	10%	64%	26%

At first, sorption properties of Mon**A**(0), Mon**F**(50) and Mon**E**(100) was characterised. Mixture of the selected alkyl-amines did not adsorb on Mon**A**(0) at all thus further examination had not been done. When Mon**F**(100) and Mon**E**(50) was used for extraction, all targets were trapped since no amines were detected in the wash-out. The adsorbed compounds then were eluted with different solvents systematically such as methanol, water, methanol-water mixture at different ratios, 0.01 mol/l NaOH solution (pH 12) and 0.01 mol/L hydrochloric acid (HCl) solution (pH 2). No amine peaks were observed at case of Mon**E**(100) in the elute for each elution solvent. When Mon**F**(50) was used for SPE the same results was observed when pure solvents and NaOH solution was used for desorption purpose. Only at case of elution with HCl solvent was the amine peaks detectable but the recoveries of the targets were less than 30%. Thus the adsorption property of the solid phase was changed because all extreme cases can be used for elution had been tried.

Since it was assumed that the interaction between the solid phase and the targets were due to the carboxylic acid group the amount of acrylic acid in the monomer solution was decreased to 15% (Mon**G**(25)). When the same standard solution of amines was added into the solid phase as for Mon**F**(50), the wash-out contained alkyl-amines above the quantification level. The wash-out contained 10%-20% of the injected amount of the alkyl-amine mixture.

Therefore the concentration of AA in the monomer solution was increased to 18% (Mon**I**(30)) and 36% (Mon**J**(40)). The peaks of organic amines in the wash-out were not detectable, thus it was supposed that the when the monomer contained more than 18%(m/m) AA the adsorption capacity is enough high for real application.

4.3.1.2 Use of novel adsorbent for sampling of alkyl-amines

For characterizing the desorption properties of Mon**I**(30) of the analytes the same elution solvents was tested as described above. The amines were detected in elutes when acidic medium (pH 2) was used for eluting. This may happen due to the protonisation of the analytes that increase water solubility of the analytes. However, the elution volume had to be increased from 0.5 ml to 1 ml, factor two, to elute the total adsorbed amount of alkyl-amines.

To sum up the results, the best monolith composition was achieved when the ratio between BTA and AA were 70/30 from the adsorption and the elution point of view and the selected elution solvent was 2 ml of 0.01 mol/l HCl solution when 1g solid phase was used. The extraction efficiency was investigated according to the determination of recoveries of standard solution of MA, EA, M2A, BA2, PA2, PA, E2A, BA and SP (see figure 52).

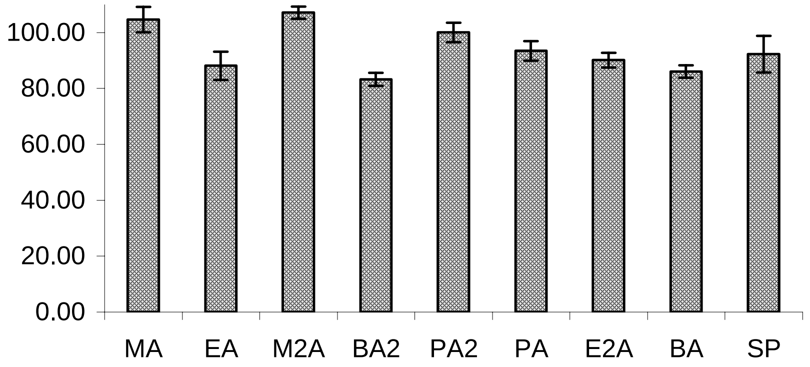


Figure 52: Extraction efficiency of alkyl-amines from monolith MonI (n=5).

Since the analytes are in vapour phase during their collection, the adsorbent was tested for the gaseous amines with use of a model system. The system based on evaporation of target compounds by adding NaOH and clean air was purged through the solution containing the mixture of target compounds (see section 5.1).

For characterizing the model system that aimed to purge and trap the selected organic amines the following parameters were examined: (i) the air volume that volatilize the total amount of amines (ii) loss of the analytes from the adsorbent with the air flow (iii) determination of the breakthrough concentration that determine the maximum trapped amount of the selected analytes. 10 μg for each analytes (1 ml of 10 mg/l solution) were volatilized and the recoveries of MA, EA, PA, BA and E_2A were determined in function of air volume that was purged through the solution contain the targets. The used standard solutions were always measured after the sampling to determine the evaporated amount (full or not).

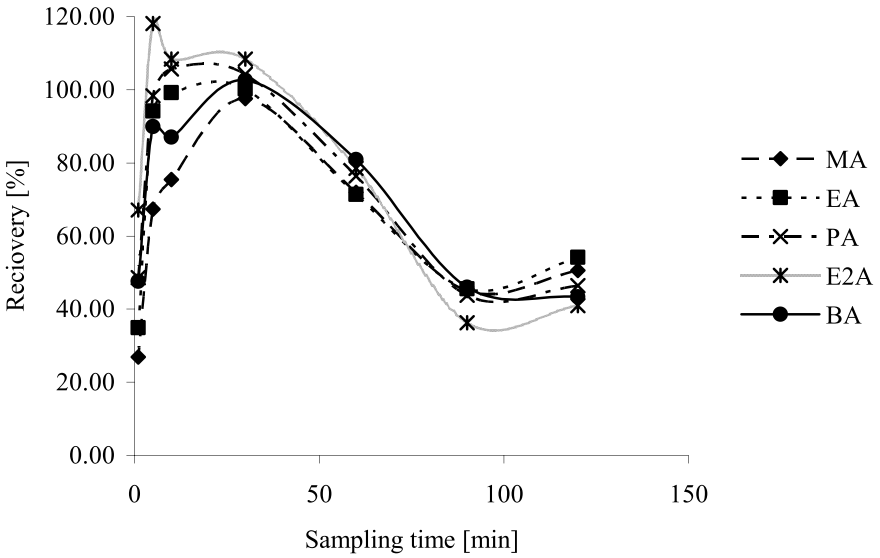


Figure 53: Determination of the maximum sampling volume.

After purging 5 l air through the solution the total amount of the targets were volatilized and trapped in the sampling tube containing the adsorbent. Between 5 and 60 l the loss of the analytes were in the acceptable range, however after volume of 40 l significant decrease in the recovery were observed. The experiment was repeated using different concentrations of LMW amines and the same phenomenon was observed. Thus for further experiments the sampled volume were set to 40 l for 1 g polymer.

The determination of breakthrough concentration was also investigated with the same experimental condition (see figure 54).

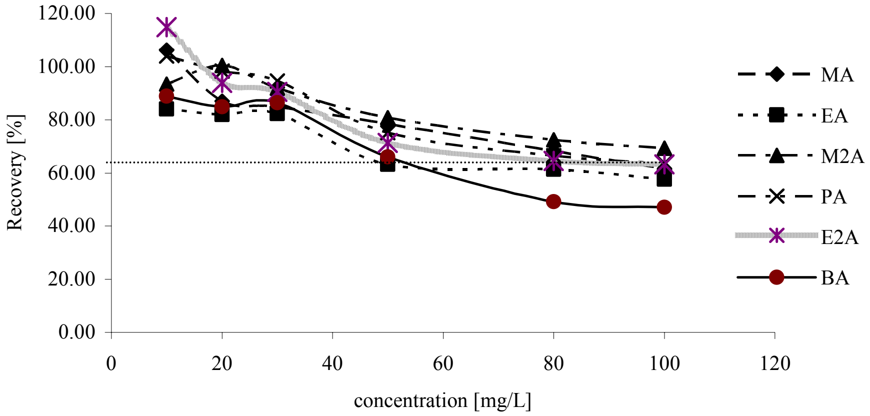


Figure 54: Determination of the breakthrough concentration of LMW amines.

The breakthrough concentration was determined between 40 µg and 75 µg when 1 g sorbent used for sampling of 40 l ambient air at flow rate of 1 l/min. This is equal to air concentration of 2.00 mg/m^3 and 3.75 mg/m^3 when each analytes are present in the sample at same concentration. The RSDs in the measurable concentration range were between 2% and 8% that met with the specified analytical requirement.

The capacity of the adsorbent may increase with decreasing of the backpressure after the cartridge because the volatile substances are evaporated with the air flow with the increase of the backpressure. One possible mode to decrease the backpressure is to increase the porosity of the adsorbent. When the porosity is higher the surface/volume ratio also increases thus increasing of the adsorption capacity of solid phase are supposed. This can be solved in two possible ways: (i) to decrease the diameter of the solid particles or (ii) preparation of the monolith in the cartridge. In the latter case a continuous bed is forming which backpressure was also high. On the whole, this novel adsorbent can be used as alternative to the standard procedures [215].

4.2.2 Development of the sample preparation

The sample preparation process includes the extraction step and off-line pre-concentration step was also proposed (see section 6.2). The extraction of the selected amines from filter on which the particles are collected is the same as for the extraction of hetero-aromatic amines. The filter was covered with 10 ml methanol water mixture at volume ratio of 30:70 and then the solution was sonicated for 15 min. The solution then was acidified to decrease the negative artefact by protonising the amines into their non-volatile salt. The extraction efficiency was examined by determination of recoveries with RSDs. The recoveries were independent on the concentration thus they were summarized and plotted in figure 55.

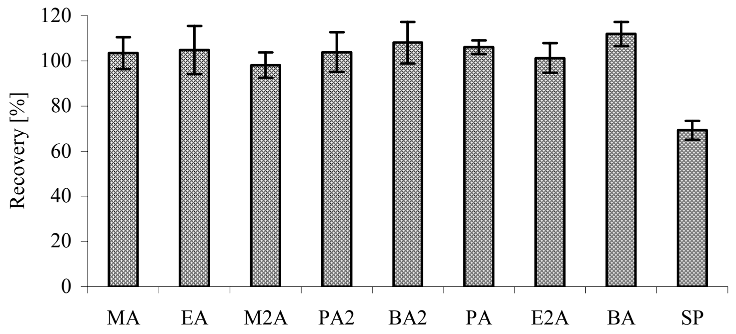


Figure 55: Extraction efficiency of LMW and biogenic amines from filter (n=5).

The measured recovery values were between 98-112% for MA, EA, PA, BA, M_2A, E_2A with RSDs of 3-10% calculated from five repetitions. The accuracy of SP from filter was poor, thus this solute can be determined with low reliability (semi-quantification).

The pre-treatment process of the amines collected in the vapor phase includes the elution of target compounds with proper solvent. The characteristics of the elution process have already been shown in figure 52. The elution characteristic was also examined when the concentration of the loaded sample onto 1 g solid phase was increased.

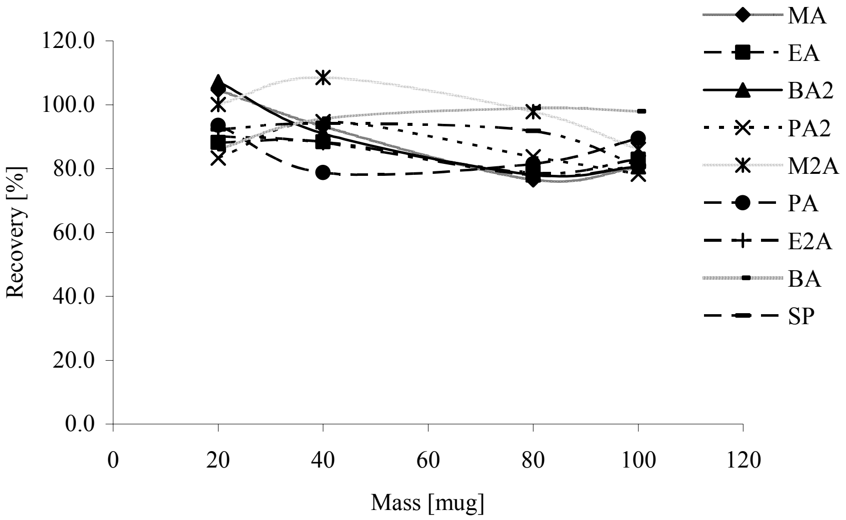


Figure 56: Determination of the mass capacity of LMW and biogenic amines on 1 g MonI.

The recoveries were higher than 80% when mass range of 20-100 μg for mixture of each selected amines were loaded onto 1 g solid phase when 40 l air was drown through the sampling system.

Preliminary examination was done for off-line pre-concentration by evaporation of solvent of the extraction solution from 10 ml for filter sample and 2 ml for adsorbent sample to 0.5 ml. The mean recoveries with RSDs of standard solution of the selected amines were determined. According to the measured results (see figure 57), the loss was 40% with standard deviations of 4-17% for each analytes, thus this step was not added into the method.

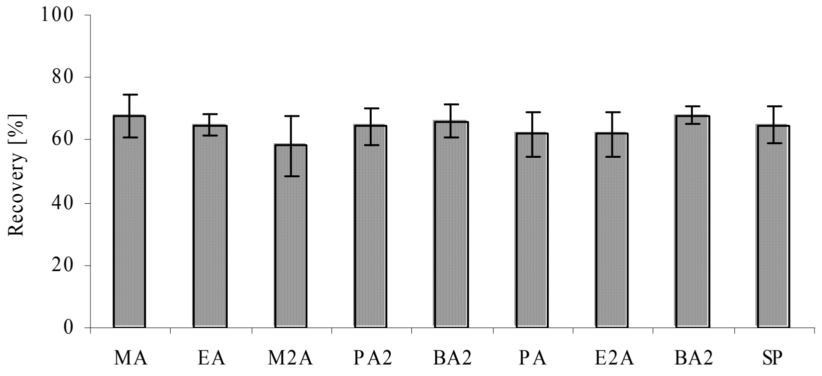


Figure 57: Accuracy and precision of the pre-concentration step.

On the whole, the sample preparation step includes only the extraction of the target compounds from filter sample collected the particulate matter and from the adsorbent on which the gaseous amines are adsorbed. Its advantage is that the negative artifact is minimized because the pretreatment process includes only one step. Since no clean-up and pre-concentration step was added into the process the possibility of interference is higher.

4.3.3 Development of the capillary electrophoretic separation

The chemical and physical properties of the alkyl amines are similar to hydroxyl-alkyl-amines, thus the method developed for determination of amino-alcohols (described in chapter 4.2.6) was at first used for the separation of aliphatic amines. The background electrolyte contained imidazole which was the *probe* for indirect UV detection added into acetate buffer at pH 3.3.

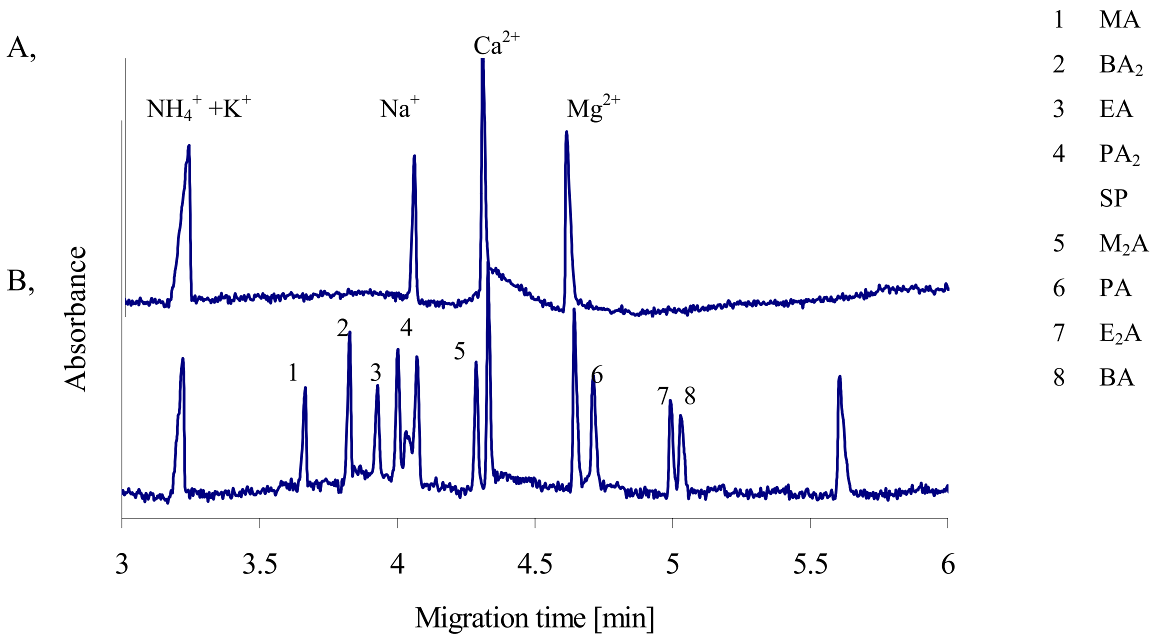


Figure 58: Electropherogram of the A, inorganic metals and NH_4^+ and B, mixture of inorganic metals and alkyl-amines.

The LMW and biogenic amines were detected in 5.5 minutes. MA, M_2A, PA BA_2, EA, E_2A and BA were baseline separated and PA_2 and SP were not separated when standard solution of the selected analytes were injected into the CE. Additionally, more drawbacks were observed. On one hand they may interfere the peaks of the amino-alcohols however the selected ones migrated from 5.5 min to 10.6 min.

On the other hand it was supposed that the system peak at 4.1 min belong to sodium ion (Na^+) because inorganic ions can be separated with use of indirect UV detection. This was tested and water solution of inorganic cations such as Na^+, kalium (K^+), ammonium (NH_4^+), calcium (Ca^{2+}) and magnesium (Mg^{2+}) was separated with the same condition as for alkyl amines.

After identification of the peaks, it was observed that the inorganic metal ions and the selected amines were migrated together (see figure 58). NH_4^+ and K^+ ion migrated before the analytes, but the other inorganic ions migrated in the range of the selected aliphatic amines. Na^+ peak was detected between EA and PA_2 peaks, the Ca^{2+} peak was too near to the peak of M_2A and Mg^{2+} migrated close to PA. Thus it was aimed to change the separation condition to eliminate the interference of Na^+, Ca^{2+}, Mg^{2+} and improve the selectivity of PA_2 and SP.

One possible way to improve the separation was the addition of modifier into the background electrolyte. Since organic solvent in the buffer had only a small effect on the separation of hydroxyl-alkyl-amines, complexation agent was selected. In some cases crown ethers are used as modifier.

Other possible complexation agent is ethylene-diamine-tetraacetic acid (EDTA) that binds to the metal ions. The equilibrium or formation constants for most metals, especially the transition metals, are very large, hence the reactions are shifted to the complex, but no complex is forming with single charged ions such as Na^+, K^+ and $NH4^+$. EDTA at concentrations from 1 mmol/l to 15 mmol/l was added into the background electrolyte to improve the selectivity of PA_2 and SP and to eliminate the influence of multiple charged inorganic ions. In the following figure the mobilities of the selected amines were plotted in the function of concentration of EDTA in the running buffer.

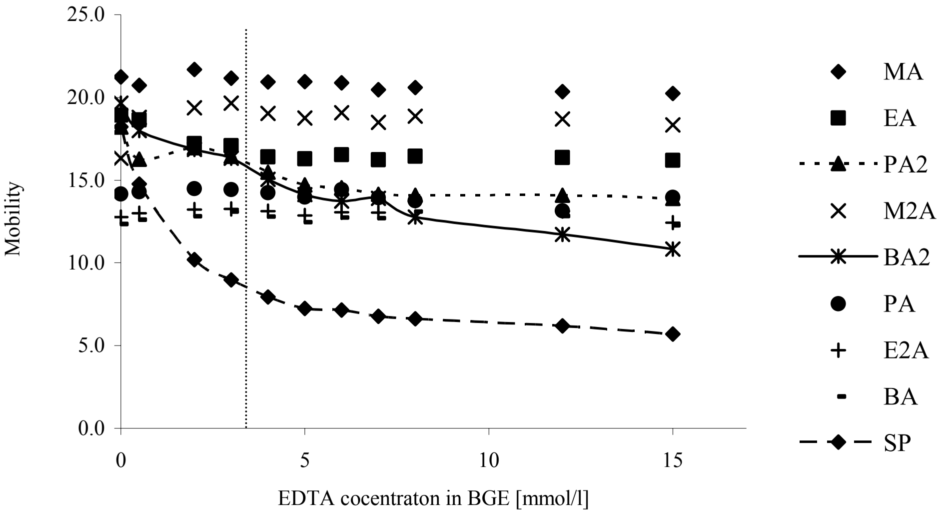


Figure 59: Effect of EDTA in the running buffer on the mobility of alkyl amines.

Presence of EDTA in the running buffer had effect on the migration of PA2, BA2 and SP and the selected alkyl amines were separated from each other. The selected biogenic amines containing minimum two primary amine groups and thus they have complex ability to EDTA. The LMW alkyl-amines have one chargeable group thus no complexation between the targets and EDTA was not observed.

The final concentration of EDTA in the running buffer was set to 4 mmol/l since the selectivity did not change in a small compass when the EDTA concentration was higher. On the other hand side, peak broadening was observed with increase of EDTA concentration in the separation electrolyte.

When solution of metal ions were injected into the capillary and separated with running buffer containing EDTA, only Na^+, K^+ and $NH4^+$ were detected due to the formation of stable complex between EDTA and multiple charged metal ions. The peak of K^+ and $NH4^+$ are not interfering the separation of the selected alkyl-amines because they migrate before the selected alkyl-amines. The only drawback was that Na^+ still migrated similarly than the selected organic amines; however the mobility difference between M_2A, Na^+ and EA increased.

The mobility behavior of hydroxyl-alkyl-amines was also investigated when the running buffer contained EDTA and the following Vaseline corrected electropherogram was observed.

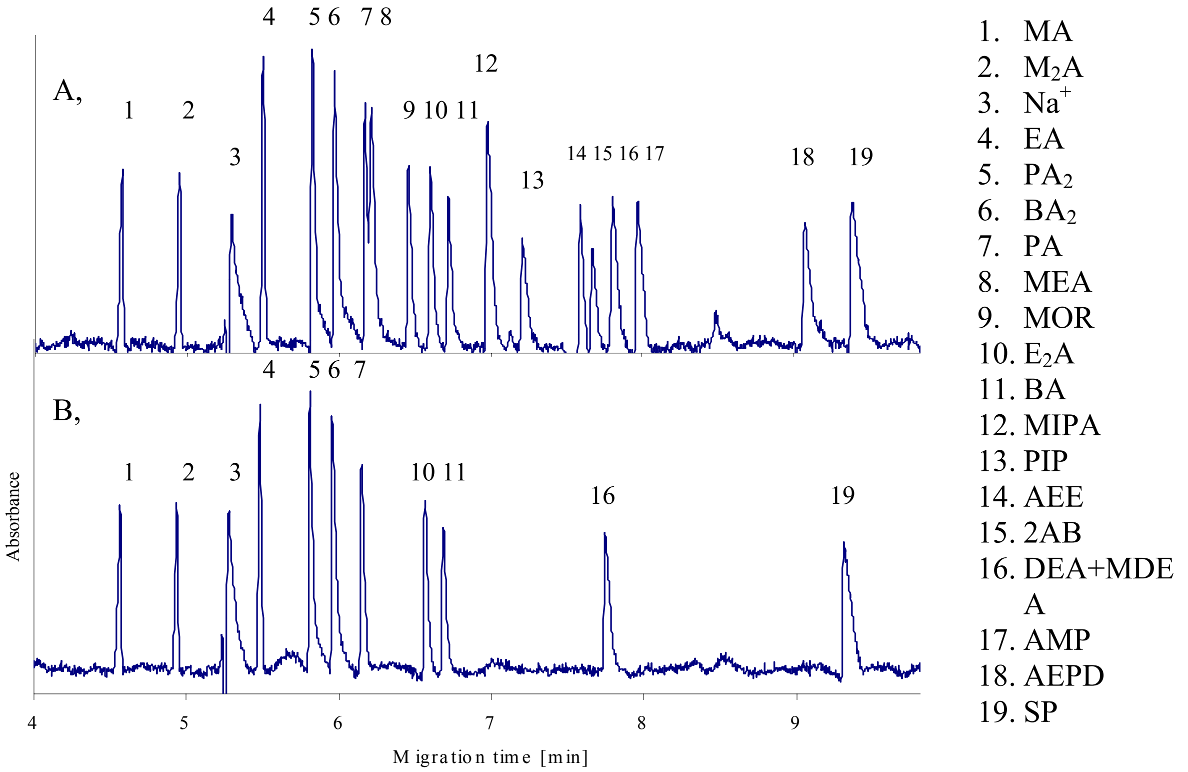


Figure 60: Electropherogram of A, mixture of selected alkyl-amines and hydroxyl-alkyl-amines and B, alkyl-amines separated with BGE containing EDTA.

When standard solution of alkyl-amines and hydroxyl-alkyl-amines was separated the migration of PA and MEA was similar and thus they were not baseline separated. The other targets were baseline separated and small tailing was observed for peaks migrated slower.

8.4 Determination of the performance characteristics

The performance characteristics of a method for determination of MA, M_2A, EA, PA_2, BA_2, PA, E_2A, BA and SP with use of CE coupled with indirect UV detection were determined. The internal standard was the same as for hydroxyl-alkyl-amine analysis (PIP). The validation procedure described in chapter 3 was done for the whole analytical procedure. As in the previous methods, normalized effective mobility scale was also transformed from the electropherogram and the validation parameters were calculated with calibration and internal standard too.

Resolution: The R_s was determined for each analytes, however only one the critical substance pair (E_2A and BA) was observed from the electropherogram.

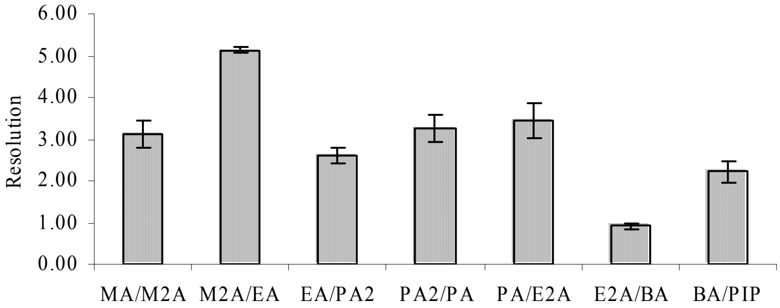


Figure 61: Resolution of LMW and biogenic alkyl-amines (n=5).

The resolution factors were more than 1.5 except for E_2A and BA thus the peak identification of these solutes should be closely investigated.

Precision of identification: The precision investigation was done neglecting the analyte concentration because no overloading effect was observed. Effective mobility was also calculated from the migration time and the marker for normalisation was PIP.

Table 20: MT and $\mu_{eff,norm}$ with RSDs of alkyl-amines (n=5).

	Migration time		**Effective mobility**	
	MT [min]	**RSD [%]**	**μeff,norm [cm2/Vmin]**	**RSD [%]**
MA	4.26	0.84	0.0208	0.72
M2A	4.59	0.90	0.0189	0.64
EA	5.05	1.12	0.0167	0.54
PA2	5.29	1.29	0.0156	0.37
PA	5.61	1.26	0.0144	0.26
E2A	5.95	1.44	0.0133	0.28
BA	6.04	1.37	0.0130	0.36
SP	8.01	1.99	0.0083	0.70

The RSDs of MTs of selected alkyl-amines were 1-2% that shows an excellent precision of the method. However increase in the RSD values with the peak order was observed. This phenomenon was not observed when normalised effective mobility was used for identification and the RSDs values decreased less than 1%.

The day-to-day reproducibility was worse since the RSDs were 8% for each analytes when migration time was used for identification. Therefore, it is better to rely on normalised effective mobility because day-to-day RSDs were 1-2%.

Working range and linearity: The working range was defined by the MAC value of the targets. The lowest MAC of the selected alkyl-amines was established for M2A at 3.7 mg/m3 which equal to 29.65 mg/L for filter and 74 mg/L for adsorbent when the sampled air volume is 40 L. Thus the working range was set between 2.96 mg/L to 59.3 mg/L.

For determination of the linearity and calibration different concentrations of standard solutions were injected. The equations of the calibration curves and relative sensitivity of internal standard using electropherogram and $\mu_{eff,norm}$ are shown in the table 21.

Table 21: Equation of calibration curves with their regression coefficients and relative sensitivity of internal standard using electropherogram and effective mobility scale.

	Migration time			**Effective mobility**		
	Equation	**R2**	**f**	**Equation**	**R2**	**f**
MA	A=7.79*c+7.49	0.999	0.77	A=6.21*c+0.65	0.0998	1.7
M2A	A=6.39*c+2.96	0.994	0.54	A=4.10*c+0.50	0.998	1.04
EA	A=12.39*c+4.39	0.995	1.01	A=6.70*c-6.54	0.996	1.59
PA2	A=17.15*c-6.65	0.997	0.92	A=4.33*c+1.76	0.997	1.33
PA	A=8.22*c+2.23	0.996	0.68	A=3.56*c-4.96	0.999	0.86
E2A	A=10.75*c+3.25	0.999	0.91	A=3.81*c+3.10	0.999	1.02
BA	A=12.91*c-5.82	0.994	0.77	A=2.72*c+8.53	0.995	0.85
SP	A=16.35*c+5.82	0.995	1.45	A=3.12*c+6.70	0.999	0.90

Precision of quantification: The repeatability of peak area of normalised effective mobility scale was the same as for the time scale (electropherogram). The RSDs for the whole analytical process were 1%-10% and were independent on the concentration of analytes. The RSDs of peak area are summarized in table 22.

Sensitivity: The LOD and LOQ for the amines were determined between 1.9 and 3.1 mg/L determined from electropherogram and normalised effective mobility scale which is lower than the lowest point of the working range. They were higher as determined for hydroxyl-alkyl-amines since the addition of the organic modifier caused peak broadening thus increase in the detection limit. The limits were quite high, but still fit to the working range.

Accuracy: The accuracy was determined for the whole analytical process that includes the collection of the vapour and condensed phase. When the model system was used that is based on the evaporation of the amines, no analyte was determined from the filter extract thus the recoveries were determined in elute. The recovery values were calculated at different concentrations for both scales. Since the recoveries were independent on the analyte concentration they were summarized in table 22.

Table 22: Recoveries and RSDs of CE-indirect UV method for the determination of alkyl-amines calculated with calibration and internal standard using electropherogram and effective mobility scale (n=5).

	Migration time		**Effective mobility**	
	W [%]± RSD[%] from calibration	**W [%] ± RSD [%] from int.st.**	**W [%]± RSD[%] from calibration**	**W [%]± RSD[%] from int.st.**
MA	97±5	100±4	106±1	102±5
M2A	103±8	106±8	95±3	100±7
EA	100±1	105±1	98±5	105±9
PA2	93±10	95±13	104±8	104±7
PA	104±4	104±5	98±3	100±2
E2A	109±6	107±6	105±5	107±8
BA	103±5	106±5	109±9	102±3
SP	93±11	90±9	91±5	91±7

The recoveries were between 93-109% for both scales and for both types of data interpretation. Thus both, calibration and internal standard can be used for quantification of the alkyl amines from real sample.

4.3.5 Performance characteristics of CE using MS detection

The CE-MS method described for determination of hydroxyl-alkyl-amines was tested for separation of the aliphatic amines since MS is more selective than indirect UV detection. Moreover, interference of inorganic ions are eliminated. However, MA, M2A and EA are not able to be detected since their molecular weights are lower than 50 g/mol. The separation condition was identical as used for hydroxyl-alkyl-amines described in chapter 4.2.8. A typical electropherogram of the separated and detected alkyl amines is shown in the following figure.

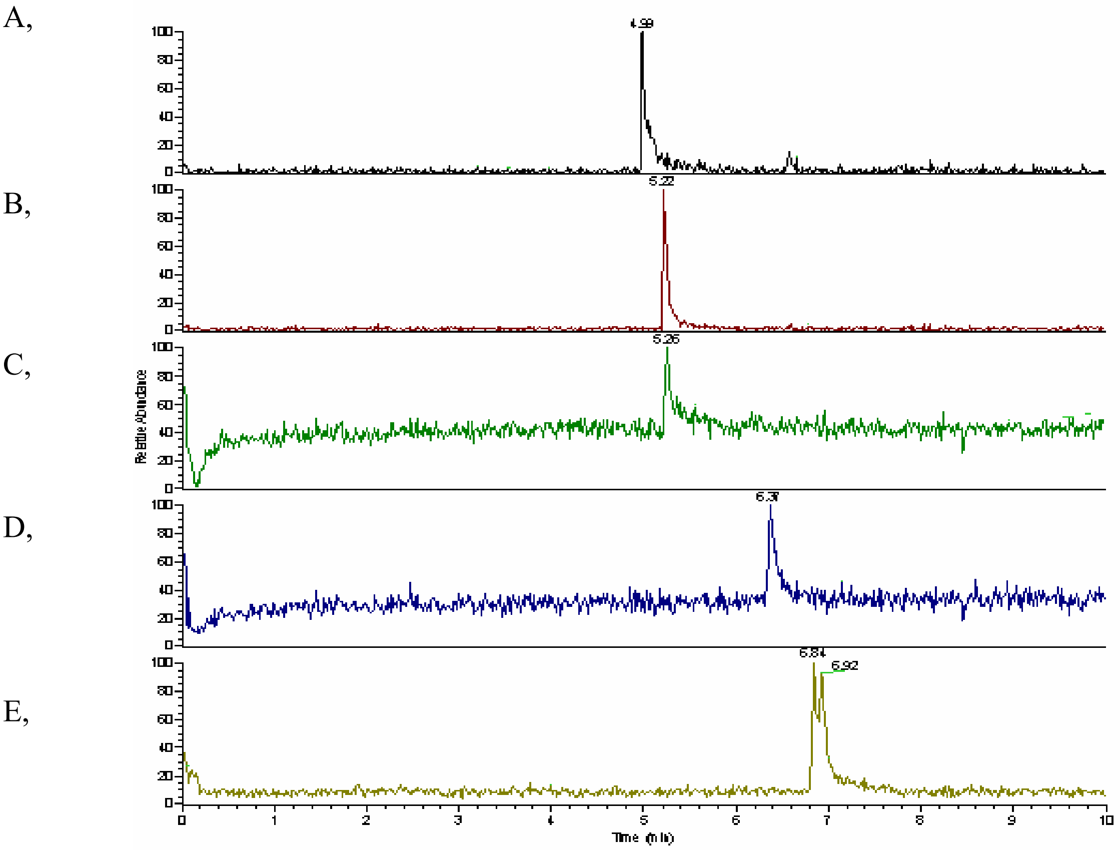


Figure 62: Elelectropherograms of A, BA_2 at m/z 89; B, SP at m/z 146; C, PA_2 at m/z 103; D, PA at m/z 60 and E, E_2A and BA at m/z 74.

The target substances were separated within 7 minutes. The same interfering m/z peaks were observed as for hydroxyl-alkyl-amines thus poorer sensitivity was observed. The other drawback is that E_2A and BA were not baseline separated with BGE without EDTA.

Resolution: R_s was determined only for E_2A and BA since their detection m/z peak was identical. The determined value was under the expectable limit (0.82) thus the differentiation between the two substances and their quantification is uncertain.

Precision of identification: Stable migration times (within day RSDs less than 0.8%) were calculated with use of this method. Because of the high repeatability of the identification parameter, the possibility of miss-identification is low.

Linearity and sensitivity: Linearity of the calibration curve and LOD and LOQ were also calculated measured with CE coupled with MS detection.

Table 23: Equation of calibration curves with regression coefficient and sensitivity of a method for the determination of alkyl-amines.

	Equation*106 with regression coefficient		**f**	**LOD [mg/L]**
BA2	A=4.54*c-3.80	0.995	0.39	1.32
SP	A=9.48*c-9.93	0.996	0.77	0.85
PA2	A=5.88*c+1.04	0.999	0.55	7.93
PA	A=6.86*c-3.65	0.996	0.54	5.79

The standard calibration graphs of peak areas were linear with correlation coefficient of 0.995 in the working range for all the amines. The relative sensitivity of internal standard for the target amines were constant thus no mass overloading effect was observed. RSDs of the internal standard constant were between 6-11% calculated from five repetitions.

The detection limit of a CE-MS method for the detectable amine compounds was the same as for indirect UV detection. Thus the sensitivity was not improved when the detection system was changed to MS.

Accuracy and precision: The accuracy and precision of the CE-MS method for the determination of selected and detected alkyl-amines were described by determining the recoveries at different concentration. The recovery values with RSDs are summarized in the following table applying both calculation modes (calibration and internal standard).

Table 24: Recoveries and RSDs of CE-MS method for the determination of alkyl-amines (n=5).

	Recoveries [%] with RSDs [%]	
	Calibration	**Internal standard**
BA2	100±2	99±2
SP	100±5	99±7
PA2	98±6	95±6
PA	99±4	95±7

The recovery values showed an excellent accuracy for both calculation methods. Thus both can be applied for quantification of real sample containing SP, BA_2, PA_2 and PA. The RSD values of the peak areas were between 6% and 9%.

For summarization, only six selected LMW and biogenic amines can be identified and four can be quantified from the eight ones with CE coupled MS. The performance characteristics of a method for the four detectable analyte met with the specified requirement.

4.3.6 Investigation of matrix effect

The migration behaviour and recoveries of the analytes were examined as a function of MWF concentration in standard solution with use of CE-indirect UV method was used since each target compounds can be detected.

Matrix effect was studied as described in chapter 3.5 and MTs behaviour as a function of MWF concentration in the spiked sample is shown in figure 63.

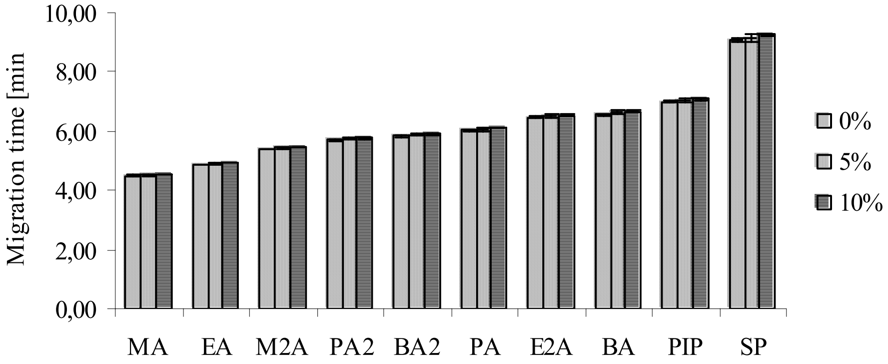


Figure 63: MTs of analytes with RSDs in function of MWF content (n=5). Abbreviations: 0%= standard solution without MWF, 5% and 10%= 5% and 10% MWF concentrate in the standard solution of analytes.

No MT shifting was observed in the function of concentration of MWF emulsion. The repeatability of the identification parameter was MWF concentration independent and the RSDs were between 0.4- 1.31% for each compounds. Thus no effect of matrices on the identification of the selected alkyl-amines was determined.

The effect of matrices on the quantification were also determined and summarized. Since the calibration curves showed linear correlation with the peak areas in the function of analyte concentration calibration was used for quantification.

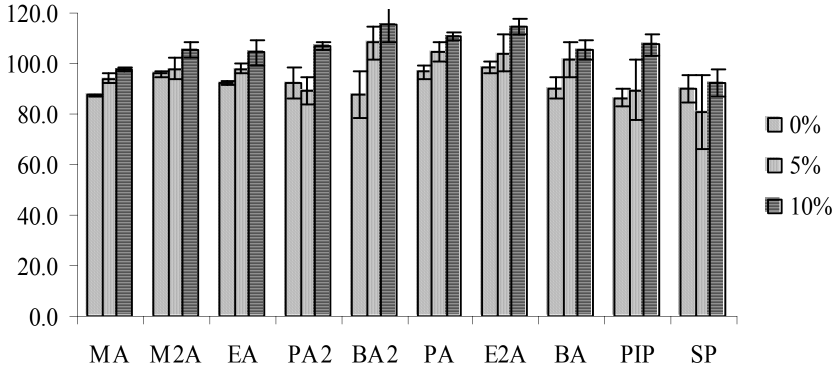


Figure 64: Effect of matrices on the recoveries and RSDs of a method (n=5). Abbreviations: 0%= standard solution without MWF, 5% and 10%= 5% and 10% MWF concentrate in the standard solution of analytes.

The recoveries and their RSDs in spiked MWF emulsion sample were independent on the concentration of matrices and analytes. The average recoveries of selected biogenic and LMW amines using calibration were between 90-111% which meets with the specified analytical requirements. The RSDs of the peak area of the whole process were under 10% when the measurements were repeated five times.

On the whole, no silanol blocking effect was observed in function of the MWF matrices at low separation pH since no MT shifting was observed when the concentration of MWF in the standard solution of alkyl-amines was increased. Moreover, MWF constituents did not affect the recoveries thus the method is suitable for determination of volatile and biogenic amines determined from MWF aerosol.

4.3.7 Analysis of real sample

To test of a method developed for determination of selected alkyl-amines, samples were taken outside and the whole analytical process was carried out as described in the final protocol.

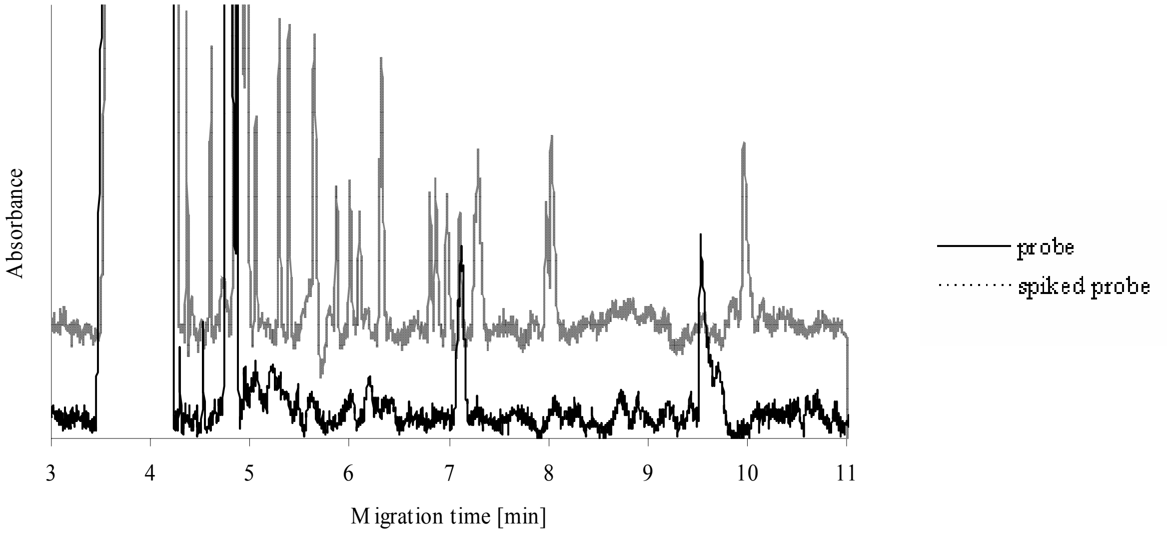


Figure 65: Electropherogram of atmospheric air sample and spiked sample.

Shifting in migration times were observed when the electropherograms measured with and without spiking thus the electropherograms were transformed into effective mobility scale.

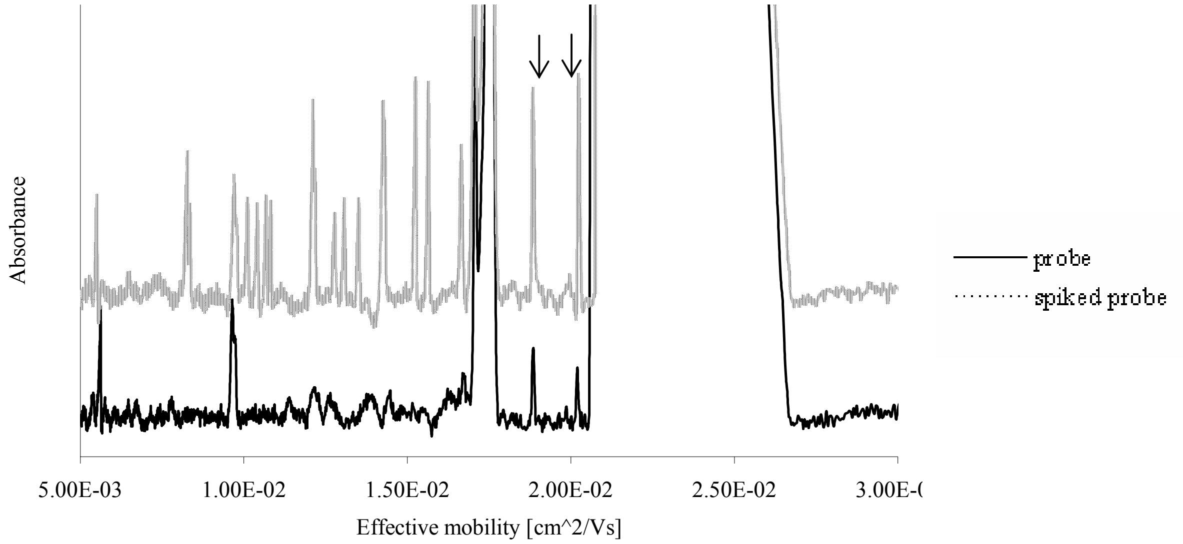


Figure 66: Effective mobility scale of atmospheric sample air sample and spiked sample.

The shifting of the peaks was eliminated when the electropherogram is transferred into effective mobility scale. With mobility scale the identification of the peaks from real probes is more reliable as can be seen from the figure 65 and 66.

In the filter extract no peak was observed. The air probe contained MA and M_2A since the normalised mobilities of the peaks were the same as in the spiked sample. The peak areas were under the quantification limit. In the electropherogram one huge and three smaller peaks were observed. The big one supposedly is peak of NH_4^+, the other one may be the peak of Na^+ and the other two one is still unidentified.

4.3.8 Development of a novel at-line pre-concentration step

The performance of the method developed for determination of biogenic and LMW alkyl amines had a drawback of its relatively poor sensitivity. In spite the fact that the linear range fitted to the MAC range it did not fit to the perception level in the nose of the amines which range from 1mg/m^3 for NH_3 to 20 µg/m^3 for PA [261]. Thus it was aimed to add a pre-concentration step into the method to increase the method sensitivity. At-line solid phase extraction was selected for pre-concentration and this is at the same time function as clean-up.

The developed system is divided into three parts: (1) concentrator, (2) separator and (3) detection part.

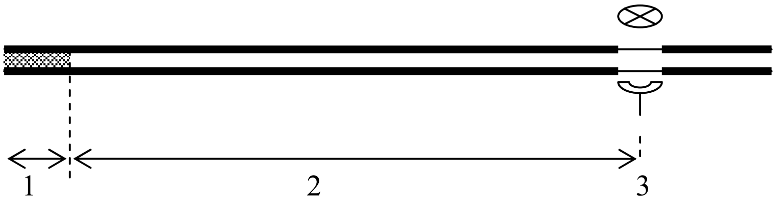


Figure 67: Scheme of the at-line pre-concentration system. Abbreviations: 1, concentrator; 2, separation and 3, detection parts.

The system is similar as used for capillary affinity chromatography, but it differs in the concentrator part. On one hand the adsorbent is planned to polymerised in-situ into the capillary and not pressed between the ends of capillaries. Thus the use of frits which are walls between the opened tube and tube containing solid phases are eliminated causing better repeatability and robustness.

The material of the concentrator is identical as used for sampling of vapour targets (MonI(30)) that is selective for amines. The other main difference is the injection mode. In affinity chromatography when the length of the solid phase is more than 1 mm, electro-kinetic injection has to be applied since the back pressure of the solid phase is too high to can introduce the sample solvent with pressure. The drawback of electro-kinetic injection mode is that matrix dependent injection volume thus for complex mixture analysis is not reliable. Thus it was aimed to have a solid phase which backpressure is low for hydrodynamic injection. When monI(30) was polymerised in the capillary with length of 2-15 mm, the backpressure of the solid phase was too high and thus no solvent could be drawn through with pressure at 20 psi (~0.14 bar). Thus it was aimed to increase the total porosity of a material to decrease the monolith backpressure.

At first, the ratio of monomer and porogen was changed. The volume of porogen in the monomer solution was increased from 60% to 70% and 80%, but no change in the porosity was reached. The monoliths are built up in one chain so they can fill up the capillary in which they are polymerised since monoliths have their own tertiary structure that shrank during the polymerisation. Since they are polymerised in open tube, a proportion of porogen volume was extruded and thus the increase of the porogen did not increased the porosity of the monolith.

So another way was aimed to decrease the backpressure of the material. The idea was to add an insoluble material in the monomer solution which defines the shape, size and the amount of the "routes" in the monolith.

Silica-gel was chosen for that purpose since they are spheres with defined diameter and stable at polymerisation condition. Additionally, silica-gel soluble in NaOH solution till monI(30) not, thus after the polymerisation the silica-gel can be removed by rinsing the capillary with NaOH forming an highly porous material. Silica-gel with 3 μm diameter and the monomer solution were mixed at different ratios (0.1, 0.5, 1 and 2) but after the polymerisation silica-gels were not able to be dissolved. This could be caused by the uncontrollable inhomogeneous of the two phases during the polymerisation process.

Since it seemed that the removing of the solid phase is not possible, instead of adding solid material an insoluble liquid was added into the monomer solution which produce a stable emulsion and thus homogeneous reaction solution. The liquid was water solution containing emulsifying agent at high concentration. This polymerisation process called as oil-in-water or water-in-oil polymerisation producing a high internal phase emulsion monolith.

Thus among the micro-pores formed from the porogen, and mega- and giga-pores are forming due to the presence of micelles. After the polymerisation process, the water with emulsifying agents was washed out. During the polymerisation process the solution in the capillary was shaken with use of ultrasonic bath to avoid the phase separation. When the length of the monolith in the capillary was 1.0 cm, solvent could force through with use of high pressure. In the electron-microscopic photos the macro- and mega-pores can be clearly seen.

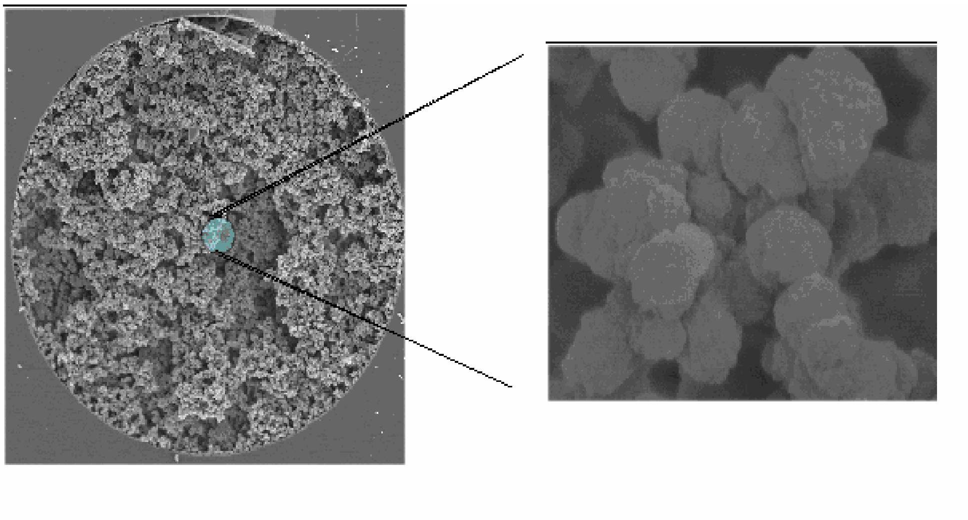


Figure 68: Electromicroscopical picture of the concentrator.

To check the applicability of the capillary MA, M_2A, EA and BA_2 was injected at different volume at 0.5 psi. The following electropherogram was detected when standard solution of these targets at concentration of 0.1 mg/l was injected for 90s that is 9 times higher than as used conventionally

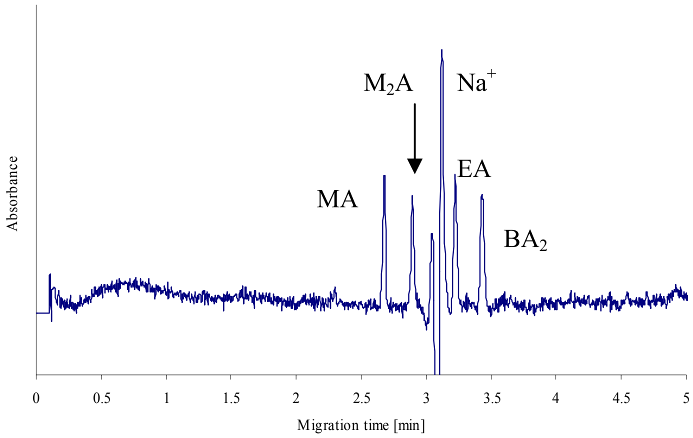


Figure 69: Baseline corrected electropherogram of 0.1 mg/l target solution by applying at-line pre-concentration and injection duration of 90 s.

The repeatability was tested to know whether the same volume is injected when the injection duration is the same. The injection times were 30s and 90 s and the RSDs of the peak areas were between 3-8% calculated from five repetitions which shows good precision at upper µg/l range.

The injection volume was increased from 30s to 540s. With the increase of the injection volume the current decreased since the sample solvent filled up the capillary. According to the Ohm equation the resistance und thus the current change with the increase of the water plug after the injection. With the decrease of the current the migration time increased as described in chapter 3.3, thus the peak areas were corrected.

When the injection volume was more than 420s, peaks were detected but the current was too low and thus peaks became broadened. Thus it should be solved that before the separation the separation part filled up only with the electrolyte. This might be possible with a reserve rinsing of a BGE till the end of the concentrator part after injection.

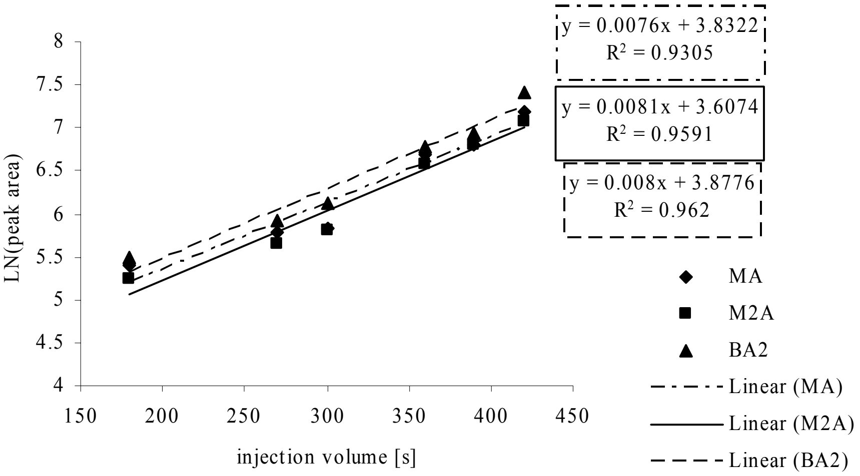


Figure 70: Corrected peak areas in the function of injection volume.

More development in the system should be done such as the optimisation of the constitution of the monomer solution and polymerisation process and then the system performances like porosity, adsorption capacity, linear velocity etc should be determined.

This novel at-line pre-concentration can be applied with use of conventional CE instruments and sample at high volume can be introduced. Moreover, with the composition of the monomer solution the monolith adsorbent properties can be differed.

4.3.9 Final protocol

Table 25: Final protocol for the determination of LMW and biogenic alkylamines.

Sampling:	With air pump at flow rate of 1 L/min with time duration of 40 min onto a quartz filter and 1g poly(acrylate-methacrylate) adsorbent.
Sample preparation:	Extract the filter with 10 ml 30:70= MeOH: water mixture with ultrasonic bath. Elute from the adsorbent with 2 ml 0.3% HCl solution
Capillary:	Fused silica, I.D. 75 μm, effective length 50 cm, total length 57 cm.
Detection wave length:	Indirect detection, 214 nm, Detector cell size: 100*800 μm.
Temperature:	Cartridge set to 30 ^{o}C.
BGE for aminoalcohols with indirect UV detection:	20mM imidazole/ imidazole acetate, 4 mmol/l EDTA, 25 V/V% ethanol, pH=3.5
BGE for aminoalcohols with MS detection	20 mM ammoniumacetate/acetic acid, 25 V/V% ethanol, pH=3.5
Capillary preconditioning:	2 min pressure with 0.1 mol/L NaOH, then 3 min with BGE.
Injection:	Hydrodynamic injection of sample 50 mbar for 10 s (V_{inj}= 59 nl).
Voltage :	20 kV.
Replenishment:	Both separation vials and injection vial after each injection.
Capillary preconditioning: (new capillary)	Pressure flush with 0.1 mol/L NaOH for 30 min when it is used at first time
Storing of capillary: (for next measuring day)	Pressure flush with 0.1 mol/L NaOH for 15 min before and after the measuring day
Storing of capillary: (more then one week)	Pressure flush with 0.1 mol/L NaOH for 15 min followed by high purity water for 15 min, then with air for 5 min.
Marker	Piperazine
Scale	Using the transformed effective mobility scale from time scale
Determination of concentration:	With five-point calibration curve in the working range between 3 mg/l and 60 mg/l determined every measuring day Or internal standard by addition of 10 mg/l PIP into the sample before injection

8.9 Conclusion

The developed and validated method can be used for the determination of MA, M_2A, EA, E_2A, PA and BA as selected LMW amines and B_2A, P_2A and SP as biogenic amines from air at concentration range from 0.37 mg/m^3 to 7.4 mg/m^3. The vapour phase and the particulate matter of the aerosol were investigated because these amines may present in both phases. The collection of the PM is based on filter sampling and the amines in vapour phase are adsorbed into a self-prepared novel adsorbent.

The sample preparation contains only the extraction having a fast procedure and the possibility of loosing the target during the treatment is minimal.

The determination process based on capillary electrophoretic separation coupled with indirect UV and MS detection of the substances without derivatisation. For identification the MTs and $\mu_{eff,norm}$ were used and for quantification of the pollutants calibration and internal standard method were applied.

Quartz filter was used for sampling the course and fine particle fraction with a help of suitable pump at flow rate of 1 l/min the same as used for determination of benzotriazoles, benzothiazoles and amino-alcohols. To collect the gaseous LMW alkyl-amines poly(methacryalte-acrylate) co-polymer was developed that is specific for amines. The composition of the polymer influenced the adsorption of alkyl-amines and it was found that butyl-methacryalte and acrylic acid at ratio of 70/30 (m/m%) in the polymerisation solution gave the best concentration efficiency.

This adsorbent has the advantages of the cheapness and easy preparation and can be used at higher flow rate for longer time as the standard procedure. Moreover a mixture of organic amines was examined for sampling purpose till in the standard method the target is only M_2A. The breakthrough amount of the mixture of the selected amines was determined at 60 µg for each analytes at sampled volume of 40 l.

The method of extraction of the targets from the filters was identical as for beznotriazoles, benzothiazoles and amino-alcohols. The extraction from the adsorbent was eluted with water at low pH. The extraction efficiency was excellent with both cases since the recoveries were between 95% and 101% for filter samples and 90%-103% for the gaseous amines except for spermidine (73%). The sample pre-treatment does not include clean-up and/or pre-concentration step.

The determination process of the alkyl-amines was the same as for amino-alcohols since they have similar chemical-physical properties. However, he separation condition was altered because of the interference of inorganic metal ions and poor resolution of the targets. A modifier, EDTA, was added into the separation buffer which has complex ability with the multiple and positively charged molecules. With adding EDTA, Ca^{2+} and Mg^{2+} were not detectable and NH_4^+, K^+ and Na^+ were detected in the migration range of akyl-amines. However, the migration differences of the inorganic ions and alkyl-amines increased thus the interference effect of inorganic ions was eliminated by adding EDTA in the separation buffer. Additionally, the separation of the biogenic amines became sufficient since they migrated slower when EDTA was added into the BGE.

The identification and quantification characteristics of the modified method met with the analytical requirements. The RSDs of MTs and effective mobilities were under 1% for each analytes and thus both parameters were used for identification. The peak area-concentration curve showed excellent linearity in the determined working range which was from 0.1*MAC and 2*MAC of M_2A. The precision of the peak area was bellow 10% when the peak areas were determined from electropherogram and effective mobility scale. The LOD of a method was quite high so pre-concentration step must be added into the process. The method was accurate since the recoveries at different concentration were between 93-107% when the peak area was determined from time and mobility scale applying calibration and internal standard for quantification.

When MS was used for detection only 6 compounds were detectable from the nine targets and 4 was quantifiable. The sensitivity of the method was the same as indirect UV detection mode since interference of sheath liquid was observed. Thus derivatisation of the alkyl amines is suggested for selective and sensitive detection with MS. The performance characteristic fitted to the analytical requirements for the four quantifiable substances (SP BA_2, PA_2 and PA).

No effect of MWF on the EOF was found in the identification and quantification characteristics when the optimised method was used for spiked samples. Real sample was also taken and MA and M_2A were identified when the electropherogram was transferred into effective mobility scale.

Since the detection limits of the method with both connected detector type (indirect UV and MS) were high, at-line pre-concentration and clean-up step was developed. The capillary used in CE distinct a concentrator, a separation and a detection part. The system can be classified as a hyphenation of CZE and capillary electro-chromatography (CEC).

The concentrator based on solid phase extraction and the material was the same monolith as used for sampling of the gaseous amines. It has advantage of the easy filling and the polymerisation takes place in the capillary. Thus frit is not necessary and the length of the solid phase depend only the preparation step.

Problems of the concentrator unit were observed when conventional polymerisation process was used. The backpressure of the solid phase was too high to apply hydrodynamic injection and the repeatability of electro-kinetic injection was not acceptable when spiked samples were injected. This drawback was eliminated with a newly polymerised monolith with a special type of polymerisation process. Therefore the composition of the monomer and as a direct consequence the monolith differed significantly compared to the monolith used for CEC.

The monolith contained giga-, mega- and micro-pores and thus the total porosity increased causing extreme high permeability and low backpressure. Thus hydrodynamic injection with high volume duration was possible to apply because of the new material. It was found that at least fifty times higher volume can be injected to the capillary. This phenomenon was direct evidence for high interaction of solutes and the newly synthesised monolith.

Moreover, the adsorbed amines can be derivatised on the solid phase onto a fluorescence active compounds having more sensitive method for determination of primary and secondary alkyl amines. This step is also a clean-up because the apolar substances present in the matrix do not adsorb onto the surface of the adsorbent.

This system is proposed to use for others target with different chemical properties as alkyl amines since the groups interacts with the targets can be changed by changing the monomers in the polymerisation solution. The optimisation and the characterisation of the system have to be further investigated. In the future it is planned to use the developed at-line concentration system for other target compound combined with different detection modes.

5. Summary

Public health is affected by several factors. One of these is the inhaled aerosol because several disorders have been correlated to its individual components; however its chemistry is very broad and complex field. Thus it is impossible to study all chemical aspects in one research work. In my study, organic amines were selected as target compounds determined from atmospheric and a special type of occupational aerosol produced from metalworking fluid (MWF).

In chemical point of view, the class of organic amines is very broad containing hundreds of natural and synthetic components. Therefore careful selection of analytes was carried out. The main criterions of the selection were the correlation between the composition of the used MWF and its aerosol and their hazardous property. In this respect not only the original composition of the fluid but the decomposition processes of this complex sample had to be also studied. Three substance classes were selected for developing and validating the determination process from MWF and atmospheric aerosols. These were: (i) benzotriazoles and benzothiazoles as saturated heterocyclic amines, (ii) hexahydrotriazines, oxasolidines as unsaturated heterocyclic amines and their hydrolysis products that are derivatives of amino-alcohols and at least (iii) volatile and biogenic alkyl-amines. They are used as additives in MWFs or by-products of abiotic and biogenic processes present in MWF aerosol.

The determination of these pollutants is quite difficult because of their high polarities and chemical reactivity. These two properties highly influence the analytical process including the sampling, sample preparation and the choice of the separation process. That can be a reason that only a few publications have been found about the determination of these organic amines from atmospheric and MWF aerosols. To fulfill the aim of the work, selective methods were improved and validated according to the generally accepted criteria. If the specified validation parameters met with the analytical requirements, the improved method could be also used for investigation of the solutes from aerosol.

The other goal of this dissertation is to improve capillary electrophoretic (CE) separations to be a reasonable choice for the determination of highly polar organic amines. The first aspect that decreases the reliability of a technique is the unfeasible use of conventional evaluation based on time detection called migration time of the target substances in the presence of unfavorable matrices that drift the electro-osmotic flow (EOF). The second aspect was to improve the sensitivity of the capillary electrophoretic methods for the determination of organic amines present in MWF and atmospheric aerosols at trace level. To introduce CE into high sensitive techniques a novel version of at-line concentration was developed allowing high volume injection having better sensitivity of the method.

At first, analytical process was improved and validated for the determination of aromatic polyamines namely 1,2,3-benzotriazole (BTA), 5-methyl-1H-benzotriazole (MBA) and 5,6-dimethylbenzotriazole (DMBA) and 2-mercaptobenzothiazole (MBT). They are used as corrosion inhibitors in MWFs. They irritate the skin and eyes of the workers and may induce tumors. Because of their low volatility and high stability they appear in the condensed phase of MWF aerosol thus the particulate matter is collected onto quartz filter. Then the targets were extracted in water-methanol mixture by sonication of the filter containing the sample. To increase the sensitivity of the method the extraction solvent was evaporated under vacuum and the final solution was injected into the CE instrument.

The substances were fully separated with alkaline buffer containing organic modifier within 6 minutes. The electropherograms were transformed into an earlier developed scale so called effective mobility scale. With use of this scale the precision of identification was approximately 10 hold increased. The RSDs were between 4.9-7.0% for migration time and 0.86-0.94% when effective mobility was used for identification.

Effective mobility scale can be used for quantification by integrating the peaks after transformation. Comparing the quantification characteristics like linearity, repeatability of peak area, detection limit and accuracy no differences were observed after mobility scale transformation. Because all specific analytical characteristics of the whole analytical procedure met with the analytical requirements when standard solutions of saturated amines were injected into the CE, the reliability of a method was studied in the presence of matrices.

When the injected samples were complex mixture drift in the EOF was observed in the electropherogram thus the peak identification was not possible. This could be caused by “silanol blocking” of matrices or not satisfactory condition of the capillary before separation. When the electropherograms were transformed into the effective mobility scale the identification of the peaks became reliable since the effective mobilities of targets are independent on the EOF and capillary dimension. Additionally, no alternations were observed in the recoveries calculated from spiked samples. Thus the method applying effective mobility became more reliable and thus the method can be adapted for routine analysis.

The second selected analytes were non-saturated heterocyclic polyamines namely 5-ethyl-3,7-dioxa-1-azabyciclo[3.3.0]octan (EDAO), 4,4-dimethyloxazolidine, N,N,N-tris(ß-hidroxyethyl)hexahydro-triazine (Grotan BK), N,N,N-tris(ß-hidroxypropil)hexahydro-triazine (Grotan WS) and N,N-methylen-bis(5-methyloxazolidine) (Grotan OX). These substances are also ingredient of MWFs used as bactericides due to their ability of releasing formaldehyde. Their determination in MWF aerosol is negligible since their hazardous property was proven.

These substances are chemically reactive since they hydrolyze at acidic conditions thus they were separated at neutral pH. It turned out that even at neutral condition the hydrolysis products that are derivatives of hydroxyl-alkyl-amines were measured. Thus the reaction kinetics of hydrolisation was studied. According to the results measured with NMR and MS it was concluded that even at alkaline pH the hydrolisation -at which these substances theoretically are stabile- speed up with increase of electrolyte concentration present in the solvent. That is why the by-products were separated when solutions of these bactericides were injected into the CE (separation buffer contains high concentration of electrolytes).

Thus the by-products of these bactericides that are monoethanolamine, monoisopropanolamine, 2-amino-2-methyl-1-propanol, and 2-amino-2-ethyl-1,3-propandiol (AEPD) can be determined with CE. The number of the targets were increased with diethanolamine, triethanolamine, morpholine, 2-(2-aminoethoxy)-ethanol, 2-amino-1-butanol, N-methyldiethanolamine (MDEA) since they are used as alkaline reverse, emulsifier and bactericide agents in MWF and thus they may be found in its aerosol. The sampling and sample preparation process was the same as for the hetero-aromatic amines thus from the same extraction solution each, saturated and unsaturated heterocyclic polyamines and hydroxyl-alkylamines can be determined.

The selected hydroxyl-alkylamines have no chromophor groups thus they were detected with indirect UV and MS detection. At first, indirect UV detection was used for optimize a MS compatible method. Each parameter such as separation current and peak sequence was the same when the method developed for indirect UV detection was adapted to MS detection having a more selective and sensitive determination process. The only difference was that two targets (AEPD and MDEA) were not detectable because interference of the sheath liquid at the detected m/z fragment occurred. The within day RSDs of migration times were between 0.6-1.5% and no big improvement was observed when electropherograms were transformed into effective mobility scale. The working range fit to the linear range. The determined recovereries of the whole analytical method were 93%-108% with RSDs of 3% -9%.

Since each characteristic fulfilled the requirements when standard solutions were measured, effect of matrices on the separation was investigated. No migration time and EOF shifting was observed as a function of MWF content because the ionization of silanol groups on the surface of the capillary wall at the separation pH was suppressed and thus the influence of EOF on the migration minimized.

The third part of the work was to develop a sensitive method for determination of alkyl-amines that are methyl-amine, ethyl-amine, propyl-amine, butyl-amine, dimethyl-amine, diethyl-amine as low molecular weight primer and secondary amines and 1,5-diaminopentane, 1,4-diaminobutane and spermidine as biogenic amines.

The sampling had to be extended with collection of gaseous alkyl-amines from atmospheric and MWF aerosol. For this purpose selective sampling with use of newly developed monolith was improved. The total breakthrough concentration of the used 1g poly(methacrylate-acrylate) co-polymer was 3 mg/m^3 for each target compounds when 40 L air was drawn through at flow rate of 1 L/min. The mixture of neutral alkyl-amines were fully desorbed with water at low pH (acidified) and elutes were directly injected into the CE. The collection of the condensed phase of the aerosol and the extraction process was the same as applied for saturated polyamines.

The capillary electrophoretic method was the same as used for hydroxyl-alkyl-amines and the same sensitivity was determined for MS than with indirect UV detection since the molecule fragments of the targets were too low for sensitive detection (interference of sheath liquid was determined). However, modification in the composition of the running buffer was necessary when CE was coupled with indirect UV detection. A complex agent, EDTA, was added into the buffer to eliminate the interference of inorganic ions because they migrated with the alkyl-amines and to increase the resolution of the selected alkyl-amines.

After re-optimisation of the capillary electrophoretic method, it was validated for volatile and biogenic alkyl-amines. The repeatability of a method showed good precision of migration time and effective mobility. The RSDs of migration times of the selected amines were 1-2% and 0.3-0.7% when effective mobility was used for identification. The determination range fitted to the linear range, and the relative sensitivities for internal standard were independent on the analyte concentration. The precision of the whole analytical process was between 1-11% when electropherogram and 1-9% when effective mobility was used for integration of the peaks. The whole method was also accurate since the recovery values were near to 100%. The developed and validated method permits the determination of these nine analytes from MWF and atmospheric aerosol.

The LOD of the method was between 1.9 mg/l and 3.1 mg/l that show low sensitivity. Thus pre-concentration step was planned to add to the method. Since loss of the solutes was determined during evaporation of the extraction solvent, at-line concentration based on solid phase extraction was developed. A plug of poly(methacrylate-acrylate) co-polymer was polymerised in the beginning of the capillary and the organic amines were adsorbed on the solid phase. The high porosity of the monolith allowed high permeability (low backpressure) and thus hydrodynamic injection can be used. With use of the novel system at least 50 times more sensitive method was achieved allowing the trace level determination of the volatile and biogenic amines.

Real samples were also taken and in spite the fact that no migration time shifting was observed when spiked samples were measured shifting in the migration time was observed. When the electropherograms were transformed into effective mobility scale the identification of alkyl-amines was more reliable. Thus the transformation process is also important when alkyl-amines and hydroxyl-alkyl-amines are determined from aerosols.

To summarize the results, methods were improved and validated for determination of saturated and unsaturated heterocyclic amines, amino-alcohols and alkyl-amines. The process includes the sampling of the vapor and the condensed phase, sample preparation and capillary electrophoretic separation. The same solvent containing the extracted target compounds after sampling can be used for determination of each organic amine since only the separation condition differs from each other.

Novel steps were developed and studied to increase the precision and sensitivity of capillary electrophoretic separations. It was shown that effective mobility scale can be used for quantification thus this scale is more feasible. Secondly, the sensitivity of capillary electrophoretic method for determination of organic amines was improved by introducing concentrator in the beginning of the capillary at plug length of 1 cm. As far as we know, it was the first time that so long solid phase plug was applied with combination of hydrodynamic injection due to a special polymerization process causing highly porous continuous bed.

In the future, it is supposed to improve the developed capillary electrophoretic methods to increase their sensitivity and more compounds are suggested to add into the method because more thousand natural and synthetic amines are known.

6. Reference

[1] Smith K.R., Jantunen M.: “Why particles?” *Chemosphere* **49** 867 (2002*)*

[2] Preining O., Davis E.J. “History of Aerosol Science” Proceedings of the Symposium on the History of Aerosol Science, Wien, ISBN 3-7001-2915-7 (2001)

[3] Stone, R., “Counting the cost of London’s Killer Smog,” *Science*, **298** 2106 (2002).

[4] Snyder, L. P., *The Death-Dealing Smog Over Donora, Pennsylvania: Industrial Air Pollution, Public Health, and Federal Policy, 1915–1963*,Ph.D. dissertation, University of Pennsylvania (1994).

[5] IUPAC Compendium of Chemical Technology 2nd Edition (1997)

[6] Singh H.B. “Composition, Chemistry and Climate of the Atmosphere” Van Nostrand Reinhold, New York (1995).

[7] European Committee for Standardization: EN 481 “Workplace atmospheres-Size fraction definitions for measurement of airborne particles” Brussel (1993).

[8] d’Almeida G.A., Koepke P. and Shettle E.P. “Atmospheric Aerosols, Global Climatology and Radiative Characteristics.” Deepak Publishing, Virginia (1991).

[9] Vouitsis E., Ntziachristos L., Samaras Z.: “Particulate mass measurements for low emitting diesel powered vehicles: what is next?” *Progress in enenergy and Combustion Science* **29** 635 (2003)

[10] Morawska L, Zhang J.F.: “Combustion sources of particles 1. Health relevance and source signatures.” *Chemosphere* **49** 1045 (2002).

[11] Thordarson T., Self S.: “Atmospheric and environmental effects of the 1783-1784 Laki eruption: A review and reassessment.” *Journal of Geophysical Research-Atmospheres* **108** D1 (2003).

[12] Putaud J. P., Raes F,. Van Dingenen R., Bruggemann E., Facchini M. C., Decesari S., Fuzzy S., Gehrig R., Huglin C., Laj P., Lorbeer G., Maenhaut W., Mihalopoulos N., Mulller K., Querol X., Rodriguez S., Schneider J., Spindler, ten Brink H., Torseth, K. Wiedensohler, A.: „A europian aerosol phenomenology-2: chemical characteristics of particulate matter at kerbside, urban, rural and background sites in Europe” *Atmospheric Environment* **38** 2579 (2004).

[13] Tsai Y. I,, Cheng M.T.: “Characterisation of chemical species in atmospheric aerosols

in metropolitan basin." *Chemosphere* **54** 1171 (2004).

[14] Sheesley, R. J. Schauer, J. J. Chowdhury, Z. Cass, G. R. Simoneit, B. R. T.: "Characterisation of organic aerosols emitted from the combustion of biomass indigeous to South asia." *Journal of Geophysical research-Atmospheres* **108** D9 (2003).

[15] Heintzenberg J., Covert D.C., Van Dingenen R.: "Size distribution and chemical composition of marine aerosols: a compilation and review" *Tellus series B-Chemical and Physical Meteorology* **54** 1104 (2000).

[16] Mouli P.C., Mohan S.V., Reddy S.J.: "A study on major inorganic ion composition of atmospheric aerosols at Tirupati" *Journal of Hazardous Materials* **96** 217 (2003).

[17] Chow J.C., Watson J.G., Antony L.-W., Chen W., Arnott P., Moosmüller H.: "Equivalence of Elemental Carbon by Thermal/Optical Reflectance and Transmittance with Different Temperature Protocols" *Environmental Science and Technology* **38** 4414 (2004).

[18] Pöschl U.:"Aerosol particleanalysis: challenges and progress" *Analytical and Bioanalytical Chemistry* **375** 30 (2003).

[19] Peterson R.E., Tyler B.J.: "analysis of organic and inorganic species on the surface of atmospheric aerosol using time-of-flight secondary ion mass spectrometry (TOF-SIMS)." *Atmospheric environment* **36** 6041 (2002).

[20] Jacobson M.C., Hansson H.C., Noone K.J., Charlson R.J.: "Organic atmospheric aerosols: Review and state of the science" *Reviews of Geophysics* **38** 267 (2000).

[21] Yu S.C.: "Role of organic acids (formic, acetic, pyruvic and oxalic) in the formation of cloud condensation nuclei (CCN): a review" *Atmospheric Research* **53** 185 (2000).

[22] Falkovich A.H., Schkolnik G., Ganor E., Rudich Y.: „Adsorption of organic compounds pertinent to urban environments onto mineral dust particles" *Journal of Geophysical research-Atmospheres* **109** D2 (2004).

[23] Ellison G.B., Tuck A.F., Vaida V.: "Size and composition distribution of atmospheric particles in southern California" *Environmental Science and Technology* **33** 3506 (1999).

[24] Mazurek M.A.: "Molecular identification of organic compounds in atmospheric complex mixtures and relationship to atmospheric chemistry and sources" *Environmental Health Perspectives* **110** 995 (2002).

[25] Rogge W.F., Mazurek M.A., Hildemann L.M., Cass G.R., Simoneit B.R.T.: ”Quantification of organic aerosols at molecular level; identification, abudance and seasonal variations” *Atmospheric Environment* **27A** 1309 (1993).

[26] Bin Abas M.R., Rahman N.A., Omar N.Y.M.J., Maah M.J., Abu Samah A., Oros D.R., Otto A., Simoneit B.R.T.: “ Organic composition of aerosol particulate matter during a haze episode in Kuala Lumpur, Malaysia” *Atmospheric Environment* **38** 4223 (2004).

[27] Broday D.M., Georgopoulos P.G.: “Growth and deposition of hygroscopic particulate matter in the human lungs” *Aerosol Science and Technology* **34** 144 (2001).Ka

[28] Aumont B., Madronich S., Bey I., Tyndall G. S.: Contribution of secondary VOC to the composition of aqeous atmospheric particles: A modelling approach.” *Journal of Atmospheric Chemistry* **35** 59 (2000).

[29] Kalberer M., Paulsen D., Sax M., Steinbacher M., Dommen J., Prevot A. S. H., Fisseha R., Weingartner E., Frankevich V., Zenobi R., Baltensperger U.: „Identification of polymers as major components of atmospheric organic aerosols.“ *Science* **303** 1659 (2004).

[30] Zhang Q., Anastasio C., Jimemez-Cruz M.: „Water soluble organic nitrogen in atmspheric fine particles (PM.2.5) from northern California.“ *Journal of Geophysical research-Atmospheres* **107** D11 (2002).

[31] Nolte C.G., Schauer J.J., Cass G.R. Simoneit B.R.T.: Chemical composition of aerosol during particle formation events in boreal forest” *Tellus Series B-Chemical and Physical Meteorology* **53** 380 (2001).

[32] Chiari M., Del Carmine P., Lucarelli F., Mareazzan G., Nava S., Paperetti L., Prati P., Valli G., Zuechiatti A.: “Atmospheric aerosol characterisation by Ion Beam Analysis techniques: recent improvements at the Van de Graf laboratory in Florence.” *Nuclear Instruments and Methods in Physics Research Section B* **219-20** 166 (2004).

[33] Schade G.W., Crutzen P.J.: “Emission of aliphatic amines from animal husbandry and their reactions; potential source of N_2O and HCN.” *Journal of Atmospheric Chemistry* **22** 319 (1995).

[34] Atkinson R.K.R., Darnall A.C., Lloyd A.C., Winer A.M:, Pitts J.N.,: “Kinetics and mechanism of the reaction of hydroxyl radical with organic compounds in the gas phase.” *Advanced Photochemistry* **11** 375 (1979).

[35] Luttke J., Scheer V., Levsen K., Wunsch, G., Cape, J.N., Hargreaves, K.J., Storeton West, R.L., Acker, K., Wieprecht, W., Jones, B.. “Occurrence and formation of nitrated phenols in and out of cloud.” *Atmospheric Environment* **31** 2637 (1997).

[36] Watson J.G.: “Visibility: Science and regulation” *Journal of the Air and Waste management Association* **52** 628 (2002).

[37] Grambsch A.: “Climate change and air quality” report of EPA´s Global Change Research Program (1997).

[38] Hoornaert S., van Grieken R.: „Atmospheric aerosol particles: A review on sources, sinks and effects“ Atmospheric deposition and ist impact on ecosystems proceedings of symposium held in Tel-Aviv on 2000, Edited by van Grieken R., Shevah Y., Antwerpen, Belgium (2002)

[39] Penner J.E., Dong X., Chen Y.: “Observational evidence of a change in radiative forcing due to the indirect aerosol effect” **427** 231 (2004).

[40] Hicks B.B. *Wet and Dry Surface Deposition of Air Pollutants and their Modelling. Conservation of Historic Stone Buildings and Monuments*. National Academic Press, Washington DC (1982).

[41] Hertel O, Skjoth C. A., Ellermann T., Skov H., Frohn L.M. “ Atmospheric nitrogen deposition in Denmark.” in “Atmospheric deposition and its impact on ecosystem2, edited by van Grieken R. and Shevah Y., Antwerpen, Belgium (2002).

[42] Van Jaarsveld J.A., Van Aalst R.M.. Onderdelinden D. “*Deposition of heavy metals from the atmosphere into the North Sea*.” National Institute of Public Health and Environmental Hygiene, Report no. 842015002, Bilthoven, the Netherlands (1986)

[43] Review of the national ambient air quality standards for particulate matter: policy assessment of scientific and technical information, United States Environmental Protection Agency, California, USA, (2001).

[44] Spurny K.R.: “Chemical mixtures in atmospheric aerosols and their correlation to lung diseases and lung cancer occurrence in the general population.” *Toxicology Letters* **88** 271 (1996).

[45] Dockery D.W.: ”Epidemiologic evidence of cardiovascular effets of particulate air pollution” *Environmental health Perspectives* **109** 483 (2001).

[46] Oberdörster G., Ferin J., Lehnert B.E. " Correlation between particle size, *in vivo* particle persistence and lung injury." *Environmental Health Perspectives* **102** 173 (1995).

[47] Ferin J., Oberdörster G., Penney D.P., Sonderholm S.C. *et.al.*: "Increased pulmonary toxicity of ultrafine particles" *Journal of Aerosol Science* **21** 381 (1990).

[48] Ferin J., Oberdörster G., Sonderholm S.C., gelein R.: „Pulmonary tissue access of ultrafine particles" *Journal of Aerosole Medicine* **4** 57 (1991).

[49] Goodman P.G., Dockery D.W. Clancy L.: "Cause-specific mortality and the extended effects of particulate pollution and temperature exposure" *Environmental Health Perpectives* **112** 179 (2004).

[50] Samet J.M., Zeger S.L., Dominici F., Curriero F., Coursac I., Dockery D.W. Schwartz J., Zanobetti A.: „The national morbidity, mortality and air pollution study II: Morbidity and mortality from air pollution in the United States." *Research reports of the Health Effect Institute* **2** 5 (2000).

[51] Peters A., Liu E., Verrier R.L., Schwartz J., Gold D.R., Mittelman M, Baliff J., Oh J.A., Allen G., Monahan K., Dockery D.W.: „Air pollution and incidence of cardiac arrythmia." *Epidemiology* **11** 7 (2000).

[52] Wang X., Ding H., Ryan L., Xu X.: „Association between Air Pollution and Low Birth Weight: A Community-based Study" *Environmental Health Perspectives* **105** 514 (1997).

[53] Wormley D.D., Ramesh A., Hood D.B.: "Environmental contaminant-mixture effects on CNS development, plasticity, and behaviour." *Toxicology and Applied Pharmacology* **197** 49 (2004).

[54] Li X.Y., Gilmour P.S., Donaldson K., MacNee W.: "Free radical activity and pro-inflammatory effects of particulate air pollution *in vivo* and *in vitro*." *Thorax* **51** 1216 (1996).

[55] Donaldson K., Beswick P.H., Gilmour P.S.: "Free radical activity associated with the surface of particles" *Toxicology Letters* **88** 293 (1996).

[56] Jenkins P.L., Phillips T.J., Mulberg J.M., Hui S.P.: "Activity patterns of Californians: use of and proximity to indoor pollutant sources." *Atmospheric Environment* **26A** 2141 (1992).

[57] EETD Newletter “Understanding the indoor concentrations of outdoor aerosols in residences.” *http://eetd.lbl.gow/newsletter/nl14/ResidenceAersols.html* (2003).

[58] Koponen I.K., Asmi A., Keronen P., Puhto K., Kulmala M.: „Indoor air measurement campaign in Helsinki, Finland 1999-the effect of outdoor air pollution on indoor air” *Atmospheric environment* **35** 1465 (2001).

[59] Tung T.C.W., Chao C.Y.H., Burnett J.: “A methodology to investigate the particulate penetration coefficient through building shell.” *Atmospheric Environment* **33** 881 (1999).

[60] Tu K., Knutson E.O.: “Indoor outdoor aerosol measurements for two residential buildings in New Jersey.” *Aerosol Science and Technology* **9** 71 (1988).

[61] National Institute for Occupational Safety and Health (NIOSH), Criteria for a Recommended Standard: Occupational Exposure to Metalworking Fluids, U.S. Department of Health and Human Services, Cinncinnati, Ohio (2002).

[62] Stanford M., Lister P.M.: “The future role of metalworking fluids in metal cutting operations.” *Industrial Lubrication and Tribiology* **54** 11 (2002).

[63] De Chiffre L., Belluco W.: “ Investigations of cutting fluid performance using different machining operations” *Lubrication engineering* **58** 22 (2002).

[64] Glenn T.F., van Antwerpen F.: “Opportunities and market trends in metalworking fluids.” *Lubrication Enineering* **54** 31 (1998).

[65] Hübner J.: „Aufbau moderner Kühlshmierstoffe.“ *Tribiologie und Schmierungstechnique* **41** 97 (1994).

[66] Bell D.D., Chou J., Nowag L., Liang S.Y.: “Modeling of the environmental effect of cutting fluid” *Tribology Transactions* **42** 168 (1999).

[67] Thornburg J., Leith D.: “Mist generation during metal machining” *journal of Tribiology –Transactions of the Asme* **122** 544 (2000).

[68] Turchin H., Byers J.P.: “Effect of oil contamination on metalworking fluid mist.” *Lubrication Engineering* **56** 21 (2000).

[69] Simpson A. T., Stear M., Groves J. A., Piney M., Bradley S. D., Stagg S., Crook B.: “Occupational exposure to metalworking fluid mist and sump fluid contaminants” *Annals of Occupational Hygiene* **47** 17 (2003).

[70] Thornburg J., Leith D.: “Mist generation during metal machining.” *Journal of Tribology-Transactions of the Asme* **122** 544 (2000).

[71] Piacitelly G.M., Sieber W.K., O'Brien D.M., Hughes R.T., Glaser R.A., Catalano J.D.: “Metalworking fluid exposures in small machine shops: an overview.” *Aihaj* **62** 356 (2001).

[72] National Institute for Occupational Safety and Health (NIOSH), Criteria for a Recommended Standard: OccupationalExposure to Metalworking Fluids, U.S. Department of Health and Human Services, Cinncinnati, Ohio (1998).

[73] Yacher J.M., Heitbrink W.A., Burroughs G.E.: “Mist control at a machining center, part 2: Mist control following installation of air cleaners” *American Industrial Hygiene Association Journal* **61** 282 (2000).

[74] Ball A.: “A survey of metalworking fluid mist in manufacturing mist.” *Lubrication Engineering* **53** 18 (1997).

[73] Ross A.S., Teschke K., Brauer M., Kennedy S.M.: “Determinants of exposure to metalworking fluid aerosol in small machine shops” *Annals of Occupational Hygiene* **48** 383 (2004).

[74] Dasch J.M., Ang C.C., Mood M., Knowles D.: “Variables affecting mist generation from metal removal fluids (c)” *Lubrication Engineering* **58** 10 (2002)

[75] Yacher J.M., Heitbrink W.A., Burroughs G.E.: “Mist control at a machining center, part 2: Mist control following installation of air cleaners” *American Industrial Hygiene Association Journal* **61** 282 (2000).

[76] Ross A.S., Teschke K., Brauer M., Kennedy S.M.: “Determinants of exposure to metalworking fluid aerosol in small machine shops” *Annals of Occupational Hygiene* **48** 383 (2004).

[77] Piacitelly G.M., Sieber W.K., O'Brien D.M., Hughes R.T., Glaser R.A., Catalano J.D.: “Metalworking fluid exposures in small machine shops: an overview.” *Aihaj* **62** 356 (2001).

[78] Ollinger C.: “Self-emulsifying, bio-based lubricant…Naturally better.” *Tribology and Lubrication Technology* **60** 40 (2004).

[79] Weindel H.F.: “Elements of selecting and using metalworking fluids. In: Improving production with coolants and lubricants.” Society of Manufacturing Engineers, Dearborn (1982).

[80] Fruzier D.: “Cutting fluid application for today’s materials. In: Improving production with coolants and lubricants.” Society of Manufacturing Enginerrs, Dearborn (1982).

[81] CRC handbook of lubrication, theory and practice tribiology. Booser ER, ed. Vols. 1-2 Boca Raton, FL. CRC Press, Inc., (1985).

[82] Passman F.J.: "Controlling microbial contamination in metalworking fluids." *Conference on metal Working Fluids* Cinncinati Ohio (1992**).**

[83] Anderson J.E., Kim B.R., Mueller S.H., Lofton T.V.: Composition and analysis of Mineral Oils and Other Organic Compounds in Metalworking and Hydraulic Fluids. *Critical Reviews in Environmental. Science and Technology* **33** 73 (2003).

[84] Jones A.M.: "Improved chemical additives in MW fluids" *Industrial lubrication and Tribology* **55** 15 (2003).

[85] Herdan J.M.: "Lubricating Oil Additives and the Environment-an Overview" *Lubrication Science* **9** 161 (1997).

[86] Bluestein C., Bluestein B.R.: "Petroleum Sulfonates in Anionic Surfactants, Part II", ED Lienfeld W.M., Marcel Dekkel Inc., 315 (1976).

[87] Miller P.R., Patel H.: "Using complex polymeric esters as multifunctional replacements for chlorine and other additives in metalworking." *Lubrication Engineering* **53** 31 (1997).

[88] Quitmeyer J.A.: "Amine carboxylates: Additives in metalworking fluids" *Lubrication Engineering* **52** 835 (1996).

[89] Eastwood J.: "EC dangerous Preparations Directive – applications to metalworking fluids and ingredients." *Industrial Lubrication and Tribology* **54** 296 (2002).

[90] Yamanaka Y., Hayama M., Oi T., Imai J., Satoh M.: "Development of new grinding fluid for CBN grinding wheels - Part III: Study of concentration of metalworking additives on grinding performance." *Lubrication Engineering* **54** 24 (1998).

[91] Schmitt T.M., Muzher E.S.: "Determination of 2-mercaptobenzothiazole, tolyltriazole and benzotriazole in coolant formulations by liquid chromatography." *Talanta* **28** 777 (1981).

[92] Bastidas M., Pinilla P., Canon E., Polo J.L., Miguel S.: "Copper corrosion inhibition by triphenylmethane derivatives in sulphuric acid media." C*orrosion Science*. **45** 427 (2003).

[93] Pillard D.A., Cornell, J.S., Dufrense D.L., Hernandez M.T.: "Toxicity of benzotriazole and benzotriazole derivatives to three aquatic species." *Water Research* **35** 557 (2001).

[94] Rossmoore H.W.: “Effect of microbial growth products on biocide activity in metalworking fluids.” *International Biodeterioration* **27** 145 (1991).

[95] Sondossi M., Rossmoore H.W., Williams R.: “Relative formaldehyde resistance among bacterial survivors of biocide-treated metalworking fluid (Reprinted)” *International Biodeterioration and Biodegradation* **48** 286 (2001).

[96] Brutto P.E., Pohlman J.L., Ryan A.M., Smith R.: “Formaldehyde control in metalworking fluids preserved with triazine biocide” *Lubrication Engineering* **52** 8 (1996).

[97] Passman F.J., Summerfield J., Sweeney J.: “Field evaluation of a newly registered metalworking fluid biocide (c)” *Lubrication Engineering* **56** 26 (2000).

[98] Thamm H.: “Formaldehyde and formaldehyde releasers in metalworking fluids: the current state of art.” *Allergologie* **20** 232 (1997).

[99] Bienkowski K.: “Coolants and Lubricants: Staying Pure” *Manufacturing Engineering* **110** 55 (1993).

[100] Sandin M., Mattsby-Baltzer I., Edebo L.: “Control of microbial growth in water-based metal-working fluids.” *International Biodeteration* **27** 61 (1991).

[101] Benett E.O., Wheleer H.O.: “Survival of bacteria in cutting oil.” *Applied Micribiology* **2** 368 (1954).

[102] Kachan V.I.: “Preventing microbial deterioration of cutting fluids.” *Soviet Engineering Research* **7** 68 (1987).

[103] Morton L.H.G., Gillatt J.W., Warrilow E.O., Greenahalg M. A.: “A potential method for the recognition of metalworking fluid spoilage organism.” *International Biodeterioration Bulletin* **19** 39 (1983).

[104] Virji M.A., Woskie S.R., Sama S.R., Kriebel D., Eberiel D.: “Identifying the determinants of viable microorganisms in the air and bulk metalworking fluids” *Aihaj* **61** 788 (2000).

[105] van der Gast C.J., Whiteley A.S., Lilley A.K., Knowles C.J., Thompson I. P.: “Bacterial community structure and function in a metal-working fluid.” *Environmental Microbiology* **5** 453 (2003).

[106] Skerlos S.J., Skerlos L.A., Aguilar C.A., Zhao F.: “Expeditious identification and quantification of mycobacteria species in metalworking fluids using peptide nucleic acid probes” *Journal of Manufacturing Systems* **22** 136 (2003).

[107] Adekambi T., Colson P., Drancourt M.: “rpoB-based identification of nonpigmented and late-pigmenting rapidly growing mycobacteria” *Journal of Clinical Microbiology* **41** 5699 (2003).

[108] Sandin M., Mattsby-Baltzer I., Edebo L.: “Control of microbial growth in water-based metal-working fluids.” *International Biodeteration* **27** 61 **(**1991).

[109] Baecker A.A.W., Roux K.L., Holy A.V.: “Microbiological contaminants of metal-working fluids in service.” *South African Journal of Science,* **85** 293 (1981).

[110] Baker C.A., Claus G.W., Taylor, P.A.: “Predominant bacteria in an activated sludge reactor for the degradation of cutting fluids.” *Applied and Environmental Microbiology* **46** 1214 (1983)**.**

[111] Mattsby-Baltzer I., Sandin M., Ahlstrom B., Allenmark S., Edebo M., Falsen E., Pedersen K., Rodin N., Thompson R.A., Edebo, L.: “Microbial growth and accumulation in industrial metal-working fluids.” *Applied and Environmental Microbiology* **55** 2681 (1989).

[112] Wilson R.W., Steingrube V.A., Bottger E.C., Springer B., Brown-Elliott B.A., Vincent V., Jost K.C., Zhang Y.S., Garcia M.J., Chiu S.H., Onyi G.O., Rossmoore H., Nash D.R., Wallace R.J.: “Mycobacterium immunogenum sp nov., a novel species related to Mycobacterium abscessus and associated with clinical disease, pseudo-outbreaks and contaminated metalworking fluids: an international cooperative study on mycobacterial taxonomy” *International Journal of Systematic and Evolutionary Microbiology* **51** 1751 (2001).

[113] Herwaldt L.A., Gorman G.W., McGrath T, Toma S., Brake B., Hightower A.W.: “A new Legionella species, Legionella feeleii species Nove, casues Potiac fever in an automobile plant.” *Annals of International Medicine* **100** 333 (1984).

[114] Hill E.C.: “Biodegradation of hydrocarbon based products in industrial use.” *Developments in Biodegradation of Hydrocarbons, Vol. 1*, Applied Science Publishers, Watkinson R.J., Barking (1978).

[115] Devon J.: “Fungicides and biostable metalworking fluids” *Chimica Oggi-Chemistry Today* **16** 38 (1998).

[116] Bakke J.M., Buhaug J., Riha J.: “Hydrolysis of 1,3,5-tris(2-hydroxyethyl)hexahydro-s-triazine and Its Reaction with H_2S.” *Industrial and Engineering Chemistry Research* **40** 6051 (2001).

[117] Safety Material Data sheet of Grotan BK, Scüelke & Mayr

[118] Linnainmaa M., Kiviranta H., Laitinen J., Laitinen S.: "Control of workers' exposure to airborne endotoxins and formaldehyde during the use of metalworking fluids" *Aiha Journal* **64** 496 (2003).

[119] Cohen H.: "A study off formaldehyde exposures from metalworking fluid operations using hexahydro-1,3,5-tris(2-hydroxyethyl)-s-triazine." Symphosium proceedings: the industrial metalworking environment; assessment and control, Dearborn M.I., Detroit, (1995).

[120] Kray L.R., Kane P.T.: "studies on coolant degradation and development of a laboratory test method for proceeding soluble oil emulsion oxidation stability" *Lubrication engineering* **54** 15 (1998).

[121] Keefer L.K:, Roller P.P.: "N-nitrosation by nitrite ion in neutral and basic medium" *Science* **181** 1245 (1973).

[122] Loeppky R., Hansen T. J., Keefer L.K.: "Reducing nitrosoamine contamination in cutting fluids" *Food and Chemical Toxicology* **21** 607 (1983).

[123] Ducos P., Gaudin R.: "N-Nitrosodiethanolamine urinary excretion in workers exposed to aqueous metalworking fluids." *International Archives of Occupational and Environmental Health* **76** 591 (2003).

[124] Williams D.T., Benoit F., Kuzika M.: "The determination of N-nitrosodiethanolamine in cutting fluids." *Bulletin of Environmental Contamination Toxiology* **20** 206 (1978)

[125] Fadlallah S., Cooper S.F., Perrault G., Truchon G., Lesage J.: "Presence of N-nitrosodiethanolamine contamination in Canadian metal-working fluids." *The Science of the Total Environment* **196** 197 (1997).

[126] Galanos C., Freudenberg M.A., Lüderitz O., Rietschel E.T., Westhpal O.: "Chemical, physicochemical and biological properties of bacterial lipopolysacharides." Biomedical applications of the horseshoe crab, Alan R. Liss Inc., New York, (1979).

[127] Bhavanishankar T.N., Rhames N.P., Shantha T.: "Deramal toxicity of *Furasium* toxins in combinations." *Arch. Toxicology* **61** 241 (1988).

[128] O'Brien D.M., Piacitelli G.M., Sieber W.K., Hughes R.T., Catalano J.D.: "An evaluation of short-term exposures to metalworking fluids in small machine shops" *Aihaj* **62** 342 (2001).

[129] Taibjee S.M., Foulds I.S.: “Microorganism-induced skin disease in wokers exposed to metalworking fluids.” *Occupational Medicine-Oxford* **53** 483 (2003).

[130] Semple S., Cherrie J., Dick F.: “Microorganism-induced skin disease in workers exposed to metalworking fluids” *Occupational Medicine-Oxford* **53** 484 (2003).

[131] Piebenga W.P., van der Walle H.B.: “Allergic contact dermatitis from 1-[2-(2,4-dichlorophenyl)-2-(2-propenyloxy) ethyl]-1H-imidazole in a water-based metalworking fluid” *Contact Dermatitis* **48** 285 (2003).

[132] Gruvberger B., Isaksson M., Frick M., Ponten A.N.N., Bruze M.: “Occupational dermatoses in a metalworking plant” *Contact Dermatitis* **48** 80 (2003).

[133] Geier J., Lessmann H., Frosch P.J., Pirker C., Koch P., Aschoff R., Richter G., Becker D., Eckert C., Uter W., Schnuch A., Fuchs T.: “Patch testing with components of water-based metalworking fluids” *Contact Dermatitis* **49** 85 (2003).

[134] Bhushan M., Craven N.M., Beck M.H.: “Contact allergy to 2-aminoethanol (monoethanolamine) in a soluble oil” *Contact Dermatitis* **39** 321 (1998).

[135] Blum A., Lischka G.: “Allergic contact dermatitis from mono-, di- and triethanolamine” *Contact Dermatitis* **36 166** (1997).

[136] Jensen C.D., Andersen K.E.: “Allergic contact dermatitis from a condensate of boric acid, monoethanolamine and fatty acids in a metalworking fluid” *Contact Dermatitis* **49** 45 (2003).

[137] Geier J., Lessmann H., Graefe A., Fuchs T.: “Contact allergy to diglycolamine in a water-based metalworking fluid” *Contact Dermatitis* **46** 121 (2002).

[138] Awosika-Olumo A.I., Trangle K.L., Fallon L.F.: “Microorganism-induced skin disease in workers exposed to metalworking fluids” *Occupational Medicine-Oxford* **53** 484 (2003).

[139] Hodgson M.J., Bracker A., Yang C., Storey E., Jarvis B.J., Milton D., Lummus Z., Bernstein D., Cole S.: “Hypersensitivity pneumonitis in a metal-working environment” *American Journal of Industrial Medicine* **39** 616 (2001).

[140] Freeman A., Lockey J., Hawley P., Biddinger P., Trout D.: “Hypersensitivity pneumonitis in a machinist” *American Journal of Industrial Medicine* **34** 387 (1998).

[141] Kreiss K., CoxGanser J.: “Metalworking fluid-associated hypersensitivity pneumonitis: A workshop summary” *American Journal of Industrial Medicine* **32** 423 (1997).

[142] Gordon T.: “Metalworking fluid - The toxicity of a complex mixture” *Journal of Toxicology and Environmental Health-Part a* **67** 209 (2004).

[143] Falkinham J.O.: “Mycobacterial aerosols and respiratory disease.” *Emerging Infectious Diseases* **9 763 (2003).**

[144] Weiss L., Pue C., Lewis R., Rossmoore H., Fink J., Harney J., Trout D.: “Respiratory illness in workers exposed to metalworking fluid contaminated with nontuberculous mycobacteria” *Jama-Journal of the American Medical Association **287*** 3073 (2002).

[145] Falkinham J.O.: “Mycobacterial aerosols and respiratory disease” *Emerging Infectious Diseases* **9** 763 2003.

[146] Sullivan P.A., Eisen E.A., Woskie S.R., Kriebel D., Wegman D.H., Hallock M.F., Hammond S.K., Tolbert P.E., Smith T.J., Monson R.R.: “Mortality studies of metalworking fluid exposure in the automobile industry: VI. A case-control study of esophageal cancer” *American Journal of Industrial Medicine* **34** 36 (1998).

[147] Bardin J.A., Eisen E.A., Tolbert P.E., Hallock M.F., Hammond S.K., Woskie S.R., Smith T.J., Monson R.R.: “Mortality studies of machining fluid exposure in the automobile industry .5. A case-control study of pancreatic cancer” *American Journal of Industrial Medicine* **32** 240 (1997).

[148] McKee R.H., OConnor D.J.: “Dermal carcinogenicity studies of metalworking fluids” *Lubrication Engineering* **52** 97 (1996).

[149] Agalliu I., Eisen E.A., Kriebel D., Quinn M.M., Wegman D.H.: “Exposure to metalworking fluids and prostate cancer incidence in the automobile industry.” *American Journal of Epidemiology* **157** s79 (2003).

[150] Calvert G.M., Ward E., Schnorr T.M., Fine L.J.: “Cancer risks among workers exposed to metalworking fluids: A systematic review” *American Journal of Industrial Medicine* **33** 282 (1998).

[151] Canadian Priority Substances List Assesment Report (1993).

[152] Deutsche Forschungsgemeinschaft, List of MAK and BAT values 2001, Comission for the Investigation of Health Hazards of Chemical Compounds in the Work Area, Report No. 37, (2001).

[153] McMurry P-H.: “A review of atmospheric aerosol measurement” *Atmospheric Environment* **34** 1959 (2000)

[154] Pilinis C., Seinfeld J.H., Grosjean, D.: “Water content of atmospheric aerosols.”

Atmospheric Environment **23** 1601 (1989).

[155] Ho W., Hidy G.M., Govan R.M.: "Microwave measurements of the liquid water content of atmospheric aerosols." *Journal of Applied Meteorology* **13** 871 (1974).

[156] Hitzenberger R., Berner A., Dusek U., Alabashi R.: "Humidity-dependent growth of size-segregated aerosol samples." *Aerosol Science and Technology* **27** 116 (1997).

[157] Speer R.E., Barnes H.M., Brown R.: "An instrument for measuring the liquid water content of aerosols." *Aerosol Science and Technology* **27** 50 (1997).

[158] Lee C.T., Hsu W.C.: "A novel method to measure aerosol water mass." *Journal of Aerosol Science* **29** 827 (1998).

[159] Brock C.A., Hamill P., Wilson J.C., Honsson H.H., Chan K.R.,." Particle formation in the upper tropical troposphere: A source of nuclei for the stratospheric aerosol." *Science* **270** 1650 (1995).

[160] Kitto A-M.N., Colbeck I.: "Filtartion and Denuder technique." In Analytical Chemistry of Aerosols" K.R. Spurny, Boca Raton USA (2000).

[161] Khylstov A., Wyers G.P., Slanina J.: "The steam-jet collector." *Atmsopheric Environment* **29** 2229 (1995).

[162] Orsini D.A., Ma Y., Sullivan A., Sierau B., Baumann K., Weber R.J.: "Refinements to the particle-into-liquid sampler (PILS) for ground and airborne measurements of water soluble aerosol composition" *Atmospheric Environment* **37** 1243 (2003).

[163] Koch W., Dunkhorst W., Lödding H.: „Respicon^{TM}-3: a new personal measuring system for size segregated dust measurements at worplaces" *Gefahrstoffe-Reinhalt Luft* **57** 177 (1997).

[164] Spurny K.R.: "New concepts for sampling, measurement, and analysis of atmospheric aerosol" In Analytical Chemistry of Aerosols" K.R. Spurny, Boca Raton USA (2000).

[165] Liu S., Dasgupta P.K.: "Automated system for chemical analysis of airborne particles based on corona-free electrostatic collection." *Analytical Chemsitry* **68** 3638 (1996).

[166] CEN EN 481: Festlegung von Konventionen von Partikelngrößen zur Messung von schwebstoffen am Arbeitsplatz. Beuth Verlag, berlin (1993).

[167] Leith D, Leith F.A., Boundy M.G.: "Measuring the concentration of mineral oil mists." In: Symposium proceedings: the industrial metalworking environment, assessment and control. Dearborn, (1995).

[168] Kenny L.C., Gussmann R.A.: “Characterisation and modelling of a family of cyclone aerosol preseparators.” *Journal of Aerosol. Science* **28** 677 (1997).

[169] Görner P., Fabries J.F.,Wrobel R.: “Thoracic fraction measurements of cotton dust.” *Journal of Aerosol Science* **25** 487 (1994).

[170] Wilson W.E., Chow J.C., Claiborn C., Fusheng W., Engelbrecht J., Watson J.G.: “Monitoring of particulate matter outdoors” *Chemosphere* **49** 1009 (2002)

[171] Watson J.G., Chow J.C., Frazier C.A.: “X-ray fluorescence analysis of ambient air samples.” In:, Elemental Analysis of Airborne Particles. Landsberger, S., Creatchman, M., Newark, (1999).

[172] Chow, J.C.: “Critical review: Measurement methods to determine compliance with ambient air quality standards for suspended particles.” *Journal of Air and Waste Management Association* **45** 320 (1995).

[173] Chow, J.C., Watson, J.G.: “Ion chromatography”. In:, “Elemental Analysis of Airborne Particles”, Landsberger, S., Creatchman, M. (Eds.) Newark (1999).

[174] Timerbaev A.R., Dabek-Zlotorzynska E., van den Hoop M.A.G.T.: “Inorganic environmental analysis by capillary electrophoresis.” *The Analyst* **124** 811 (1999).

[175] Dabek-Zlotorzynska E., Aranda-Rodrigez R., Buykx S.E.J.: „Development and validation of capillary eletrophoresis fort he determination of selected metal ions in airborne particulate matter after sequencial extraction.” *Analytical and Bioanalytical Chemistry* **372** 467 (2002).

[176] Johnson D.L., McIntyre B.L., Fortmann R.C., Stevens R.K., Hanna R.B.,. A chemical element comparison of individual particle analysis and bulk analysis methods. Scanning Electron Microscopy **1** 469 (1981).

[177] Cadle S.H., Groblicki P.J., Stroup D.P.: “An automated carbon analyzer for particulate samples.” *Analytical Chemistry* **52** 2201 (1980).

[178] Stevens R.K., McClenny W.A., Dzubay T.G., Mason M.A., Courtney W.J.: “Analytical methods to measure the carbonaceous content of aerosols.” In: “Particulate carbon: atmospheric life cycle.”, Wolff G.T., Klimisch R.L., New York, (1982).

[179] Chow J.C., Zielinska B., Watson J.G., Fujita E.M., Richards H.W., Neff W.D., Dietrich D., Hering S.V.: "Northern front range air quality study. Ambient measurements" vol. A. Cooperative Institute for Research in the Atmosphere, Colorado State University, Fort Collins, CO. 1998.

[180] Azevedo D.D., dos Santos C.Y.M., Neto F.R.D.: "Identification and seasonal variation of atmospheric organic pollutants in Campos dos Goytacazes, Brazil" *Atmospheric environment* **36** 2383 (2002)

[181] Simoneit B.R.T.: "A review of biomarker compounds as source indicators and tracers for air pollution." *Environental Science and Pollution respectives* **6** 159 (1999).

[182] Zappoli S., Andracchio A., Fuzzi S., Facchini M.C., Gelencser A., Kiss G., Krivacsy Z., Molnar A., Meszaros E., Hansson H.-C., Rosman K., Zebühr: "Inorganic, organic and macromolecular components of fine aerosol in different areas of Europe in relation to their water solubility." *Atmospheric environment* **33** 2733 (1999).

[183] Havers N., Burba P., Lambert J., Klockow D.: "Spectroscopic characterisation of humic-like substances in airborne particulate matter." *Journal of Atmospheric Chemistry* **29** 45 (1998).

[184] Havers N., Burba P., Klockow D., Klockow-Beck A.: "Characterisation of humic-like substances in airborne particulate matter by capillary electrophoresis." *Chromatographia* **47** 619 (1998).

[185] Krivacsy Z., Kiss Gy., Varga B., Galambos I., Sarvari Zs., Gelencser A., Molnar A., Fuzzi S., Facchini M.C., Zappoli S., Andracchio A., Alsberg T., Hansson H.C., Persson L.: "Study of humic-like substances in fog and interstitial aerosol by size-exclusion chromatography and capillary electrophoresis." *Atmospheric Environment***34** 4273 (2000).

[186] Dabek-Zlotorzynska E., McGrath M.: "Determination of low-molecular-weight carboxylic acids in the ambient air and vehicle emissions: a review." *Fresenius Journal of Analytical Chemistry* **367** 507 (2000).

[187] Krivacsy Z., Molnar A., Tarjanyi E., Gelencser A., Kiss Gy., Hlavay J.: "Investigation of inorganic ions and organic acids in atmospheric aerosol by capillary electrophoresis." *Journal of Chromatography* **781** 223 (1997).

[188] Tam W.F.C., Tanner P.A., Law P.T.R., Bächmann K., Pötzsch S.: "Uso of capillary electrophoresis in the analysis of aerosol and bulk/dry deposition collected on daily basis." *Analytica Chimica Acta* **427** 259 (2001).

[189] Dabek-Zlotorzynska E., Piechowski M., McGrath M., Lai E.P.C.: "Determination of low-molecular-mass carboxylic acids in atmospheric aerosol and vehicle emission samples by capillary electrophoresis." *Journal of Chromatography A.* **910** 331 (2001).

[190] Souza S.R., Vasconcellos P.C., Carvalho L.R.F.: "Low molecular weight carboxylic acids in an urban atmosphere: Winter measurements in Sao Paulo City, Brazil." *Atmospheric Environment* **33** 2563 (1999).

[191] Li Q.F., Yu J.Z.: "Determination of total aerosol nitrogen by thermal evolution." *Aerosol Science and Technology* **38** 382 (2004).

[192] S. E. Cornell and T. D. Jickells "Water-soluble organic nitrogen in atmospheric aerosol: a comparison of UV and persulfate oxidation methods" *Atmospheric Environment* 33 833 (1999).

[193] Dabek-Zlotorzynska E., Maruszak W.: "Determination of dimethylamine and other low-molecular-mass amines using capillary electrophoresis with laser-induced fluorescence detection" *Journal of Chromatography B* **714** 77 (1998).

[194] Makela J.M., Yli-Koivisto S., Hiltunen V., Seidl W., Swietlicki E., Teinila K., Sillanpaa M., Koponen I. K., Paatero J., Rosman, K.: Hameri, K.: "Chemical composition of aerosol during particle formation events in boreal forest" *Tellus Series B-Chemical and Physical Meteorology* **53** 380 (2001).

[195] Santagati N.A., Bousquet E., Spadaro A., Ronsisvalle G.: "Analysis of aliphatic amines in air samples by HPLC with electrochemical detection." *Journal of Pharmaceutical and Biomedical Analysis* **29** 1105 (2002).

[196] Grönberg L., Pövkist P., Jönsson J.A.: "Measurement of aliphatic amines in ambient air and rainwater." *Chemsophere* **24** 1533 (1992).

[197] Murphy D.M., Thomson D.S.: "Chemical composition of single aerosol particles at Idao Hill: Positive ion measurements." *Journal of Geophysical research* **102** 6341 (1997).

[198] Claeson A.-S., Östin A., Sunesson A.-L.: "Development of LC-MS/MS method for the analysis of volatile primary and secondary amines as NIT (naphthylisocyanate) derivatives." *Analytical and Bioanalytical Chemistry* **378** 932 (2004).

[199] Martinez Y.M., Falco P.C., Hernandez R.H., Andres J.V.: „Evaluation of C_{18} adsorbent cartridges for sampling and derivatization of primary amines.” *Analytica Chimica Acta* **502** 235 (2004).

[200] Chang I.-H., Lee C.-G., Lee D.S.: „Development of an autometed method for simultaneous determination of low molecular wieght amines and ammonia in air by diffusion scrubber coupled to ion chromatography.” *Analytical Chemistry* **75** 6141 (2003).

[201] Namiesnik J., Jastrzebska A., Zygmunt B.: „Determination of volatile amines in air by solid-phase microextraction coupled with flame ionisation detection.“ *Journal of Chromatography A* **1016** 1 (2003).

[202] NIOSH Manual of Analytical Methods, fouth edition, method nr. 2010 (1994).

[203] Mace K.A., Artaxo P., Duce R.A.: “Water-soluble organic nitrogen in Amazon Basin aerosols during the dry (biomass burning) and wet seasons.” *Journal of Geophysical Research-Atmospheres* **108** D16 (2003).

[204] Mace K.A., Duce R.A., Tindale N.W.: “Organic nitrogen in rain and aerosol at Cape Grim, Tasmania, Australia” *Journal of Geophysical Research-Atmospheres* **108** D11 (2003).

[205] Mace K.A., Kubilay N., Duce R.A.: “Organic nitrogen in rain and aerosol in the eastern Mediterranean atmosphere: An association with atmospheric dust” *Journal of Geophysical Research-Atmospheres* **108** D10 (2003).

[206] Jinhui X., Lee F.S.C.: „Quantification of nitrated polynuclear aromatic hydrocarbons in atmospheric particulate matter.” *Analytica Chimica Acta* **416** 111 (2000).

[207] Jinhui X., Lee F.S.C.: „Analysis of nitarted polynuclear aromatic hydrocarbons.“ *Chemosphere* **42** 245 (2001).

[208] Bamford H.A., Bezabeh D.Z., Schantz M.M., Wise S.A., Baker J.E.: “Determination and comparison of nitrated-polycyclic hydrocarbons measured in air and diesel particulate reference materials.” *Chemosphere* **50** 575 (2003).

[209] Rudolph J., Stupak J.: “Determination of aromatic acids and nitrophenols in atmospheric aerosols by capillary electrophoresis.” *Journal of Chromatographic Science* **40** 207 (2002).

[210] Mace K.A., Duce R.A.: “Determination of urea in atmospheric aerosols and natural waters - A cation exchange method” *International Journal of Environmental Analytical Chemistry* **82** 341 (2002).

[211] Cornell S.E., Jickells T.D., Thornton C.A.: “Urea in rainwater and atmospheric aerosol.” *Atmospheric Environment* **32** 1903 (1998).

[212] Trapp I., Famulok T., Risse U., Kettrup A.: “FTIR-screening of carbonyl compounds in metalworking fluid aerosols.” *Fresenius Journal of Analytical Chemistry* **362** 409 (1998).

[213] Wang Z., Fingas M.: “Developments in the analysis of petroleum hydrocarbons in oils, petroleum products and oil-spill-related environmental samples by gas chromatography.” *Journal of Chromatography A* **774** 51 (1997).

[214] Schoenmakers P.J., Oomen J.L.M.M., Blomberg J., Genuit W., van Velzen G.: “Comparison of comprehensive two-dimensional gas chromatography and gas chromatography -mass spectrometry for the characterization of complex hydrocarbon mixtures” *Journal of Chromatogy .A*, **892** 29 (2000**)**.

[215] Beens J., Brinkman U.A.T.: “The role of gas chromatography in compositional analyses in the petroleum industry.” *TrAC, Trends in Analytical Chemistry* **19** 260 (2000).

[216] Rudzinski W.E., Aminabhavi T.M.: “Review on extraction and identification of crude oiland related products using supercritical fluid technology” *Energy Fuels* **14** 464 (2000).

[217] Sun S., Boyd S.A.: “Sorption of Nonionic Organic Compounds in Soil-Water Systems Containing Petroleum Sulfonate-Oil Surfactants.”, *Environonmental Science Technology* **27** 1340 (1993).

[218] Almen R., Mantelli G., McTeer P., Nakayama S.: “Application of high-performance liquid chromatography to the study of the effect of microorganisms in emulsifiable oils”, *Lubrication Engineering* **38** 99 (1982).

[219] de Oliveira M.A.L., Micke G.A., Bruns R.E., Tavares M.F.M.: “Factorial design of electrolyte systems for the separation of fatty acids by capillary electrophoresis” *Journal of Chromatogphy A* **924** 533 (2001).

[220] Randegger-Vollrath A.: “Determination of chloronated paraffins in cutting fluids and lubricants” *Fresenius Journal of Analytical Chemistry* **360** 62 (1997).

[221] Mansfield F., Schmitt T., *Corrosion* **49** 322A (1973).

[222] Korpics C.J., *Material Performance* **13** 36 (1974).

[223] Jones H.M., Woodcoock J.T., *Can. Met. Quart.,* **12** 497 (1973).

[224] Fogg A.G., Ismail R., Ahmad R., Banica F.G.: "Cathiodic stripping voltammetric determination of 2-mercaptobenzothiazole using the catalyc cobalt peak." *Talanta* **44** 491 (1997).

[225] Iob A., Al-Yousef F., Tawabini B.S., Mohammed A.I., Abbas N.M.: "Simultaneous determination of benzotriazole copper inhibitor and microbiocidal isothiazolinenones by heigh-perfromance chromatography." *Journal of Chromatography A.* **661** 245 (1994).

[226] Hawn G.G., Diehl P.A., Talley C.P.: "High Performance Liquid Chromatographic Determination of Aromatic Triazole Corrosion Inhibitors in Aqeous Solutions." *Journal of Chromatography Science* **19** 567 1981).

[227] Schmitt T.M., Muzher E.S.: "Determination of 2-mercaptobenzothiazole, tolyltriazole and benzotriazole in coolant formulations by liquid chromatography**."** *Talanta*, **28** 777 (1981).

[228] Schubert B.A., Hohaus E., Dengel H.S., Riepe W., Maurer W.: „Bestimmung von Korrosioninhibitoren und Bioziden in wassermischbaren Kühlschmierstoffen mit Hilfe der Kapillarelectrophorese." *Gefahrstoffe - Reinhaltung der Luft* **57** 511 (1997).

[229] Chen C.C., Lin C.E.: "Analysis of copper corrosion inhibitors ba capillary zone electrophoresis." *Ananlytica Chimica Acta* **321** 215 (1996).

[230] Breuer D., Fischer K., Hansen K., A.Fekete, Lahaniatis M., Schmitt-Kopplin Ph.: Benzotriazole (1,2,3-Benzotriazole, 5-Methyl-1H-benzotriazole, 5,6-Dimethylbenzotriazole), Analytische Methoden Band 1, Deutchen Forschungsgemeinschaft, Senatskommosion zur Prüfun gesundheitsschädlicher Arbeitsstoffe-Arbeitsgruppe „Analytische Che mie" 13. Lieferung, Ausgabe (2003).

[231] Dissertation von Trapp I.: Kühlschmierstoff-Analytic, Screening von Aerosolen und Dämpfen sowie Quantifizierung toxikologish relevanter Additive in Arbietsluft und Gebrauchslösung, München, Technical Universität *(2001).*

[232] Schubert B.A., Hohaus E., Dengel H.S., Riepe W., Maurer W.: „Quantitive Bestimmung von Bioziden und Lösungsvermittlern in wassermischbaren Kühlschmierstoffen mittels Micellarer Electrokinetischer Chromatographie.“ *Gefahrstoffe - Reinhaltung der Luft* **62** 155 (2002).

[233] Choy E.T., Meisen A.: “Gas chromatographic detection of diethanolamine and its degradation products” *Journal of Chromatography* **187** 145 (1980).

[234] Langvardt P.W., Melcher R.G.: “Determination of ethanol- and isopropanolamines in air at parts-per-billion levels” *Analytical Chemistry* **52** 669 (1980).

[235] Dawodu O.F., Meisen A.: “Gas chromatographic analysis of alkanolamine solutions using capillary and packed columns” *Journal of Chromatography* **629** 297 (1993).

[236] Greene E.A., Coy D.L:, Fedorak P.M.: ”Laboratory Evaluations of Factors Affecting Biodegradation of Sulfolane and Diisopropanolamine” *Bioremedation* **3** 299 (1999).

[237] “Determination of Diethanolamine and Triethanolamine in Surface Finishing.” In: Waste water and Scrubber Solutions, *Application Update AU126R, Dionex, Sunnyvalle,* (1991).

[238] Serbin L., Birkholz D.: “A sensitive analytical procedure for the determination of primary and secondary alkanolamines” *American Industrial Hygiene Association Journal* **56** 66 (1995).

[239] Headley J.V., Peru K.M., Dickson L.C.: “Ion-exchange electrospray ionization liquid chromatography mass spectrometry and tandem mass spectrometry of alkanolamines in wetland vegetation exposed to sour-gas contaminated groundwater” *Rapid Communication of Mass Spectrometry* **13** 730 (1999).

[240] Schubert B.A., Hohaus E., Dengel H.S., Riepe W., Maurer W.: „Quantifizierung vom Alkanolaminen in wassermischbaren Kühlschmierstoffen mit Hilfe der Kapillarelectrophorese.“ *Gefahrstoffe - Reinhaltung der Luft* **56** 393 (1996).

[241] Lewis D.M., Janotka E., Whiitmer M.P., Bledsoe T.A.: “Detection of microbial antigens in metal working fluids.” *International Biodeterioration & Biodegradation*, **47** 89 (2001).

[242] Schmitt-Kopplin Ph., Garmash A.V, Kudryavtsev A.V., Menzinger F., Perminova I.V., Hertkorn N., Freitag D., Petrosyan V.S., Kettrup A.: **“**Quantitative and qualitative precision improvements by effective mobility-scale data transformation in capillary electrophoresis” *Electrophoresis* **22** 78, (2001).

[243] Kudryavtsev A.V., Perminova I.V., Petrosyan V.S.: “Size-exclusion chromatographic descriptors of humic substances” *Analytica Chimica Acta* **407** 193 (2000).

[244] Deutches Institut für Normung e.V. (DIN), DIN 32645-Chemische Analytic-Nachweis-, Erfassungs- und Bestimmungsgrenze. Beuth Veral, Berlin, (1994).

[245] Subbalakshmi Y., Patti A.F., Lee G.S.H., Hooper M.A. “Structural characterisation of macromolecular organic material in air particulate matter using Py-GC-MS and solid state ^{13}C-NMR” *Journal of Environmental Monitoring* **2** 561 (2000).

[246] Gold L.S., Slone T.H., Stern B.R., Bernstein R.: “Comparison of target organs of carcinogenity for mutagenis and non-mutagenic chemicals.” *Mutation Res.* **296** 75 (1993)**.**

[247] Stouten H., Rutten A.A.J.J.L., van de Gevel I.A., De Vrijer F.: “The Nordic expert Group for Criteria Documentation of Health Risks from Chemicals and the Dutc Expert Comitee on Occupational Standards. 126. 1,2,3-Benzotriazole”, *Arbete och Hälsa,* **24** 1 (2000).

[248] Chan-Yeung M., Malo J.L.: “Occupational asthma.” *New England Journal of Medicine* **333** 107 (1995).

[249] Macka M., Johns C., Doble Ph., Haddad P.R.: “Indirect Photometric Detection in CE using Buffered Electrolytes – Part I, Principles.” *LCGC,* **19** 38 (2001).

[250] Matchett W.H., Brumley W.C.: “Preconcentration of aliphatic amines from water determined by capillary electrophoresis with indirect UV detection.” *Journal of Liquid Chromatography and Related Techniques* **20** 79 (1997).

[251] R.M. Agius: “Why are some low-molecular-weight agents asthmagenic?” *Occupational Medicine* **15** 369 (2000).

[252] A.M. Beedle, G.W. Zamponi: “Block of voltage-dependent calcium channels by aliphatic monoamines.” *Biophysical Journal* **79** 260 (2000).

[253] G.D. Sinks, T.A. Carver, T.W. Schultz: “Structure-toxicity relationships for aminoalkanols: A comparison with alkanols and alkanamines” *Sar and Qsar in Environmental Research* **9** 217 (1998).

[254] H. Greim, D. Bury, H.J. Klimisch, M. Oeben-Negele, K. Ziegler-Skylakakis: “Toxicity of aliphatic amines: Structure-activity relationship.” *Chemosphere* **36** 271 (1998).

[255] D. Hindocha, N.W. Smith: “The analysis of basic pharmaceutical compounds by capillary electrochromatography using continuous bed stationary phase.” *Chromatographia* **55** 203 (2002).

[256] J.L. Liao, N. Chen, C. Ericson, S. Hjertén: “Preparation of continuous beds derivatized with one-step alkyl and sulfonate groups for capillary electrochromatography” *Analytical Chemistry* **68** 3468 (1996).

[257] E.C. Peters, M. Petro, F. Svec, J.M.J. Fréchet: “Molded rigid polymer monoliths as separation media for capillary electrochromatography. 2. effect of chromatographic conditions on the separation“ *Analytical Chemistry* **70** 2296 (1998).

[258] Ping G., Schmitt-Kopplin P., Hertkorn N., Zhang W.B., Zhang Y.K., Kettrup A.: “Separation of selected humic degradation compounds by capillary electrochromatography with monolithic and packed columns” *Electrophoresis* **24** 958 (2003).

[259] Svec F., Tennikova T.B., Deyl Z.: “Monolithic materials: preparation, properties and applications.” Netherland (2003).

[260] M. Devos, F. Patte, J. Rouault, P. Laffort and L. J. Van Gemert: “Standardized Human Olfactory Threshold”, IRL Press, New York, (1990).

7. Appendix

7.1 Chemical composition of MWF aerosol

Name	**Substances**	**Function**	**Straight oil [% (V/V)]**	**Soluble oil [% (V/V)]**	**Emulsifiable [% (V/V)]**	**Synthetic [% (V/V)]**
Water		Coolant, diluent	-	5-40*	10-40*	10-40*.
Base oil		Carry lubrication	60-100	30-85	5-30	-
Emulsifier	•Petroleum sulfonates •Fatty acid soaps •Ethanolamines •Nonionic ethoxylates •Synthetic sulphonates •Fatty acid amides	Emulsify	-	5-20	5-10	5-10
Extreme pressure additives	•Sulfurized hydrocarbons •Sulfurized fatty acid esters •Chlorinated paraffins •Chlorinated waxes •Chlorinated esters •Phosphate esters •Zinc dithiophosphate •Lead naphthenate	Act as a reaction lubricant films to prevent welding	0-40	0-20	0-10	0-10
Alkaline reserve	•Alkanolamines •Alkali hydroxides	Act as a buffer control	-	2-5	2-5	2-5
Surfactant wetting agent	•Alkoxylated alcohols •Alkoxylated nonylphenols	Reduce the surface tension	0-10	5-20	10-20	10-20
Chelating agents	•Sodium EDTA •Phosphonates •Gluconates	Tie up ions in solution	-	0-1	0-1	0-1

Name	**Substances**	**Function**	**Straight oil [% (V/V)]**	**Soluble oil [% (V/V)]**	**Emulsifiable [% (V/V)]**	**Synthetic [% (V/V)]**
Coupling agents	•Hexylene glycol •Short chain esters •Fatty alcohols	Stabilize	-	1-3	1-3	1-3
Viscosity index improvers	•Polyethers •Polyvinyl alcohols	Maintain the viscosity	+	-	-	-
Detergent	•Metal sulphonate •Metal phenate	Prevent the deposition	+	+	+	+
Corrosion inhibitors	•Amine carboxylate •Amine dicarboxylates •Boramides •Arylsulfonamido acids •Sodium molybdate •Sodium metasilicates •Succinic acids •Benzotriazoles •Benzothiazoles •Thiadiazoles	Prevent rust film barrier	0-10	3-10	10-20	10-20
Plasticizer	Polymeric esthers Polymeriy ethers	Reduce the tackiness	-	+	+	+
Biocides	•Triazines •Oxazolidines •Nitromorpholine •Sodium omadine •Bromonitriles •Substituated phenols •Halogen carbamates •Isothiazolones	Control bacterial and fungal contaminants	-	0-2	0-2	0-2
Antimist agent	•Polybutenes •Polyacrylates •Polyethylene oxide •Shear stable polymers	Reduce the misting	+	+	-	-

Name	**Substances**	**Function**	**Straight oil [% (V/V)]**	**Soluble oil [% (V/V)]**	**Emulsifiable [% (V/V)]**	**Synthetic [% (V/V)]**
Metal passivator	•Metal dithiophoshates •Aromatic amines •Metal phenates	Prevent the staining	+	-	-	-
Oiliness agent	•Fatty acid esters •High M.W. esters •Glycol esters •EO/PO copolymers •Natural riglycerides	Increase the film strength	+	-	-	-
Dispersants	•Alkenyl succinimides •Succinate esters •Alkyl-acrylic polymers	Prevent the fine agglomeration and the deposit formation	+	-	-	-
Anti-foaming agents	•Silicones •Waxes •Cacium nitrate •Calcium acetate	Prevent the foaming	0-500	0-500	0-500	0-500
Dyes	•Azo types •Flourescein	Colour the coolant	-	0-500	0-500	0-500
Odorant	•Citrus products •Aldehydes	Mask the odour	+	+	+	+

Abbrevuations: -: not present in this MWF class; +: usually present in this MWF class; *: dilution factor, part/ 1 part concentrate

7.2 Properties of the target compounds

7.2.1 Saturated heterocyclic amines

Name:	1H-benzotriazole	5-methyl-benzotriazole	5,6-dimethyl-benzotriazole	2- mercapto-benzothiazole	p-tert-butyl-benzoic acid
Abbreviation:	BTA	MBA	DMBA	MBT	PTBBA
CAS-number:	95-14-7	136-85-6	4184-79-6	149-30-4	98-73-7
Mol. formula:	C6H5N3	C7H7N3	C8H9N3	C7H5NS2	C11H14O2
Mol. weight:	119.1 g/mol	133.1 g/mol	147.1 g/mol	167.2 g/mol	178.2 g/mol
Melting point:	98.5 oC	80 oC	154 oC	181 oC	164 oC
Boiling point:	204 oC	160 oC	-	-	-
logP:	1.44	1.67	2.16	2.42	3.85
Vapor pressure:	5.3 kPa at 20 oC	3.99 kPa at 20 oC	-	0.0619 Pa at 25 oC	0.0477 Pa at 25 oC
pKa:	8.37	11.63*	11.93*	6.93	4.4
Henry's law constant:	-	-	-	3.6e-8 m3/mol at 25oC	2.8e-7 m3/mol at 25oC
Physical form:	White crystal	Yellow crystal	Dark yellow powder	Yellow needle	White crystal
MAC:	-	-	-	4 mg/m^3	2.08 mg/m^3
LD$_{50}$:	0.5-0.9 g/kg to rat,	>1 mg/kg to guinea pig; 1.6 g/kg to rat	-	0.1 g/kg to rat, 1.1 g/kg to mice	1.9 g/m^3/4h to rat by inhalation, 0.47 g/kg to rat orally
Health risk:	Contact dermatitis.	Irritating the lung, skin and eye.	Have not been fully investigated.	Skin and eye sensitizer.	Irritate the skin and eye.

Abbreviations: mol: molecule, -: not found, *: calculated, LD$_{50}$: lethal doses

7.2.2 Unsaturated heterocyclic amines

Name:	5-ethyl-3,7-dioxa-1-azabyciclo [3.3.0] octan	4,4-dimethyl-oxazolidine	N,N,N-tris(ß-hidroxyethyl)-hexahydro-triazine	N,N,N-tris(ß-hidroxy-propil)-hexahydro-triazine	N,N-methylenbis (5-methyl-oxazolidine)
Abbreviation:	EDAO	DMOI	Grotan BK	Grotan WS	Grotan OX
CAS-number:	7747-35-5	51200-87-4	4719-04-4	25254-50-6	66204-44-2
Mol. formula:	C7H13NO2	C5H11NO	C9H21N3O3	C12H27N3O3	C9H18N2O2
Mol. weight:	143.1 g/mol	101 g/mol	219.2 g/mol	261.3 g/mol	186 g/mol
Boiling point:	75 °C	-	-	-	116 ^{o}C
logP:	0.4*	-0.01*	-0.61*	0.46*	-0.11
Vapour pressure:	-	-	25 hPa at 20 oC	25 hPa at 20 oC	< 1 hPa at 20 oC
pKa:	4,7*	8.78*	5.85*	8.29*	6.06*
Henry's law constant:	-	-	-	-	-
Physical form:	Fluid, transparent, odourless	Fluid, transparent, odourless	Yellow fluid, amine smell	Yellow fluid, amine smell	Yellow fluid, amine smell
MAC:	-	-	-	-	-
LD$_{50}$:	-	1.4 g/kg to rabbit following skin contact; 0.95 to rat orally.	0.93 g/kg to rat following oral feeding; 2 g/kg to rat following skin contact.	-	0.9 g/kg to rat;
Health risk:	Immuno-toxicant, Skin sensitizer	-	Skin sensitizer,	-	Skin and eye sensitizer.

Abbreviations: mol: molecule, LD50: lethal doses, -: not found, *: calculated

7.2.3 Aminoalcohols

Name:	Mono-isopropanol-amine	Mono-ethanolamine	2-amino-2-methyl-1-propanol	2-amino-2-ethyl-1,3-propandiol	N-methyl-diethanol-amine
Abbreviation:	MIPA	MEA	AMP	AEPD	MDEA
CAS-number:	78-96-6	141-43-5	124-68-5	115-70-8	105-59-9
Mol. formula:	C3H9NO	C2H7NO	C4H11NO	C5H13NO2	C5H13NO2
Mol. weight:	75.11 g/mol	61.08 g/mol	89.14 g/mol	119.1 g/mol	119.1 g/mol
Boiling point:	160 ^oC	171 ^oC	165 ^oC	152 ^oC	247 ^oC
logP:	-0.96	-1.31	-0.74	-0.6	-1.5
Vapour pressure:	0.47 Hg mm at 25 ^oC	0.404 Hg mm at 25 ^oC	1 Hg mm at 25 ^oC	-	0.0002 Hg mm at 25 ^oC
pKa:	9.94	9.5	10.2	8.8	8.52
Henry's law constant:	$4.88e^{-10}$ m^3/mol at 25 ^oC	$3.25e^{-8}$ m^3/mol at 25 ^oC	$6.48e^{-10}$ m^3/mol at 25 ^oC	-	3.14e-11 m3/mol at 25 ^oC
Physical form:	Colourless liquid, ammonia odour	Colourless liquid, ammonia odour	Colourless crystalline mass	Colourless viscous liquid	Colourless viscous liquid
MAC:	-	5.1 mg/m^3	-	-	-
LD_{50}:	1.71 g/kg to rat following oral feeding; 1.64 g/kg to rabbit through skin	1.72 g/kg to rat following oral feeding; 1.00 g/kg to rabbit after skin contact.	2.9 g/kg to rat following oral feeding; > 2 g/kg to rabbit after skin contact.	-	4.78 g/kg to rat following oral feeding.
Health risk:	Skin and eye sensitizer; causes burns	Skin and eye sensitizer	Skin and eye sensitizer, causes burns, irritate lung	-	Irritating eyes, skin and lung

Abbreviations: mol: molecule, LD50: lethal doses, -: not found.

Name:	Diethanol-amine	Triethanol-amine	Morpholine	2-(2-amino-ethoxy)-ethanol	2-amino-1-butanol
Abbreviation:	DEA	TEA	MOR	AEE	2AB
CAS-number:	111-42-2	102-71-6	110-91-8	929-06-6	96-20-8
Mol. formula:	C4H11NO2	C6H15NO3	C4H9NO	C4H11NO2	C4H11NO
Mol. weight:	105.14 g/mol	149.19 g/mol	87.12 g/mol	105.1 g/mol	89.14 g/mol
Boiling point:	268 ^{o}C	335 ^{o}C	128 ^{o}C	223 ^{o}C	178 ^{o}C
logP:	-1.43	-1.00	-0.86	-2.24*	-0.70
Vapour pressure:	2.8e-4 Hgmm at 25 oC	3.59e-6 Hgmm at 25 oC	10.1 Hg mm at 25 oC	-	-
pKa:	8.96	7.76	8.49	9.17*	9.52
Henry's law constant:	3.87e-11 m^3/mol at 25 ^{o}C	7.05e-13 m^3/mol at 25 ^{o}C	1.16e-6 m^3/mol at 25 ^{o}C	-	-
Physical form:	Colorless liquid.	Light yellow, viscous liquid	Colourless liquid	Colourless liquid.	Colourless liquid
MAC:	-	-	-	-	-
LD_{50}:	620 μL/kg to rat orally; 7,640 μL/kg to rabbit through skin	8 g/kg to rat after oral treatment	1.45 g/kg to rat orally, 8 g/kg/8h to rat by inhalation; 1.22 g/kg to rabbit through skin.	3 mg/kg to rat orally, 1.19 μl/kg to rabbit through skin contact	-
Health risk:	Skin and respiratory tract irritant, burn the eyes.	Skin and eye sensitizer.	Tumorogen and mutagen, causes burns, affect the kidney and liver.	Immuno-toxicant, neuro-toxicant, skin and eye sensitizer	Causes burnes in eyes and skin.

Abbreviations: mol: molecule, LD50: lethal doses, -: not found, *: calculated

7.2.4 LMW and biogenic alkylamines

Name:	Methyl-amine	Ethyl-amine	Propyl-amine	Butyl-amine	Dimethyl-amine
Abbreviation:	MA	EA	PA	BA	M2A
CAS-number:	74-89-5	75-04-7	107-10-8	109-73-9	124-40-3
Mol. formula:	CH5N	C2H7N	C3H9N	C4H11N	C2H7N
Mol. weight:	31.06 g/mol	45.09 g/mol	59.11 g/mol	73.1 g/mol	45.09 g/mol
Boiling point:	-6.3 °C	16.5 °C	47.2 °C	77 °C	6.8 °C
logP:	-0.57	-0.13	0.48	0.97	-0.38
Vapour pressure:	2650 Hgmm at 25 °C	1050 Hgmm at 25 °C	310 Hgmm at 25 °C	93 Hgmm at 25 °C	1520 Hgmm at 25 °C
pKa:	10.6	10.9	10.7	10.8	10.7
Henry's law constant:	1.1e^{-5} m^3/mol at 25 °C	1.2e^{-5} m^3/mol at 25 °C	1.5e^{-5} m^3/mol at 25 °C	1.7e^{-5} m^3/mol	1.7e^{-5} m^3/mol at 25 °C
Physical form:	Gas with ammonia-like odour	light yellow liquid	Colourless Hygroscopic liquid with pungent odour	colourless to yellow liquid	colourless gas with strong ammonia-like smell
MAC:	13 mg/m^3	9.4 mg/m^3	-	15 mg/m^3	3.5 mg/m^3
LD$_{50}$:	100 mg/kg to rat orally, 5 g/kg/h to rat by inhalation	0.4 g/kg to rat orally, 0.39 g/kg to rabbit through skin	0.57 g/kg to rat orally, 0.56 g/kg to rabbit through skin	0.366 g/kg to rat orally, 0.8 g/kg to mice by inhalation	0.69 g/kg to rat orally, 0.24 g/ kg to guinea pig orally,
Health risk:	Irritate the respiratory tract, skin and eyes.	Corrosive: may cause burns to the eye, destructive of mucous membranes	Harmful if swallowed or inhaled, and in contact with the skin; causes burns	Harmful to skin, eye und lung, causes severe burns.	Harmful by ingestion, skin contact or inhalation, may cause permanent eye damage

Abbreviations: mol: molecule, LD50: lethal doses, -: not found.

Name:	Diethyl-amine	Cadaverine	Putrescin	Spermidine
Abbreviation:	E2A	PA2	BA2	SP
CAS-number:	109-89-7	462-94-2	110-60-1	124-20-9
Mol. formula:	$C_4H_{11}N$	$C_5H_{14}N_2$	$C_4H_{12}N_2$	$C_7H_{19}N_3$
Mol. weight	73.14 g/mol	102.18 g/mol	88.15 g/mol	145.25 g/mol
Boiling point:	55 ^oC	179 ^oC	158 ^oC	145 ^oC
logP:	0.58	-0.15	-0.70	-0.66
Vapor pressure:	237 m^3/mol at 25 ^oC	-	4.12 m^3/mol at 25 ^oC	-
pKa:	11.1	10.45*	10.8	10.5*
Henry's law constant:	2.5e-5 Hgmm at 25 ^oC	-	1.8e-9 Hgmm at 25 ^oC	-
Physical form:	Colourless liquid with ammonia-like odour	Colourless to light yellow liquid with a very unpleasant smell	Colourless liquid	Low melting-point solid
MAC:	15 mg/m^3	-	-	-
LD50:	0.54 g/kg to rat orally, 4 g/kg/4h to rat by inhalation	Toxicology not fully investigated.	0.46 g/kg to rat orally, 1.58 to rabbit through skin	Toxicology not fully investigated.
Health risk:	Corrosive - causes burns; skin, eye and respiratory tract irritant	Causes burns, destructive of mucous membranes	Readily passes through the skin, exposure may affect cell growth rate, may cause reproductive defects	Corrosive - causes burns, harmful to skin, eyes and respiratory tract

Abbreviations: mol: molecule, LD50: lethal doses, -: not found, *: calculated.

7.3 Instruments and chemicals

For sampling: -air pump: Desaga, Model GS-312 and Desaga, Model GS-212;

-quartz filter: Schleicher & Schuell, ∅ 37mm, Ref. Nr. 10373240.

For sample preparation: -ultrasonic bath: Bandelin Sondrex RK 100;

-rotadesk

-filtration: Schleicher & Schuell, ∅ 13mm, d_p= 0.45μm

-extractor

For solution preparation: -balance: Sartorius, Model BA210S and Model ME215S.
-magnetic stirrer: Roth, R1000;
-pH-meter: Sartorius, Professional Meter PP-20;
-distillation equipment: Milli-Q Plus, Millipore S.A. 67120;
-pipettes: Eppendorf 10-100 μl and Eppendorf 100-1000 μl;
-syringes: Hamilton 50 μl and 250 μl pipettes;
-beakers, glass pipettes, glass vial holders, graduated flasks, water-jet vacuum pump for filtration glass device.

Capillary electrophoresis: -capillary electrophoresis: Beckman P/ACE 5510 and P/ACE 2100 coupled with on-line UV and DAD detection

-capillaries: -Agilent, CE ext. light path cap., 50 μm inner diameter, 40cm 2/PK, Product Nr. G1600-60232B.

-Polymicro, conventional silica tube, 50 μm and 75 μm inner dimeter, Product Nr.: TSP050375 and TSP075375

For purity examination: -mass spectrometer, Finnigan LCQ Duo

-NMR, Bruker Br 500/52

Chemicals:

1H-benzotriazole	Merck, w>99%, Order. Nr. 8.23315.0100.
5-metyl- benzotriazole	Aldrich, w=98%, Order. Nr. 19630-4.
5,6-dimetyl- benzotriazole	Fluka, w>99%, Order. Nr. 39617.

2-mercaptobenzothiazole	Fluka, w~99%, Order. Nr. 63720.
p-tert-butyl-benzoic acid	Merck, w>99%, Nr. 8,20238,0100.
Grotan BK, OX and WS	Schuelke & Mayr.
EDAO	Aldrich, 97%, Order No.: 41,779-3.
DMOI	Aldrich, 75 wt% in water, Order No.: 43,615-1.
Monoethanolamine	Merck, Order No.: K15270343
Diethanolamine	Merck, 98%, Order No.: ZA779305
Triethanolamine	Merck, 98%, Order No.: K15993379
Piperazine	Sigma, Order No.: 113H0243
Morpholine	Sigma, Order No.: 43H03831
2-amino-1-butanol	Acros, 98%, Order No.: 66610
2-amino-2-ethyl-1,3-propandiol	Sigma, Order No.: 20H07641
2-amino-2-methyl-1-propanol	Sigma, >95%, Order No.: 63H09421
N-methyldiethanolamine	Fluka
1-amino-2-propanol	Aldrich, >95%, Order No.: 32293-032
2-(2-amonoethoxy)-ethanol	Becker, Order No.: A009880101
Propylamine	Fluka, >99.5%, Order No.: 82098
Butylamine	Fluka, >99,5%, Order No.: 19482
Spermidine	Aldrich, 99%, Order No.:S382-8
1,5-Diaminopentane	Aldrich, 95%, Order No.: D2,260-6
1,4-Diaminobutane	Aldrich, 99%, Order No.: D1,320-8
Diethylamine	Fluka, >99,7%, Order No.:31729
Dimethylamine hydrochloride	Aldrich, 99%, Order No.: 12,636-5
Ethylamine hydrochloride	Fluka, >99%, Order No.: 02960
Methylamine hydrochloride	Aldrich, 99%, Order No.: 24,101-6
Imidazole	Aldrich, 99+%, Order No: 43,615-1.
α-hydroxy isobutyric acid	Merck, >98%, Order No.: 8.41764.0025
3-cyclohexylamino-1-propanesulfonic acid (CAPS)	Sigma, Order Nr. C6070.
Ethylen glycol dimethacrylate	Fluka, >97%, Order No: 03808
Butyl methacrylate	Fluka, >99%, Order No.: 64110
Acrylicacid anhydrous	Fluka, >99%, Order No.: 01730

3-(trimethoxylsilyl)propyl methacrylate	Fluka, Selectrophere, Order No: 64208
α,α`-Azoisobutyronitrile	Fluka, >98%, Order No.: 11630
1,4-Butandiol	Fluka, 99%, Order No: 18960
1-Propanol	Merck, for analysis, Order No.: 1.762397
Hydrochloric acid	Merck, 32%, Order No.: Z380619
Acetic acid	Merck, 100%, Order No.: 1.02432
Ethanol	Merck, for analysis, Order No.: 100983.1000
Propanol	Merck: Order No.:1762397
Methanol	Riedel de Häen, HPLC gradient, Order Nr. 34485.
Acetonitrile	Riedel de Häen, HPLC gradient, Order Nr. 34851.
Tetrahydrofurane	Merck, Order No.:548001
Acetone	Riedel de Häen, HPLC gradient, Order Nr. 34480.
High purity water	Fresh made with Milli-Q Plus, Millipore S.A. 67120

EUROPEAN CURRICULUM VITAE

PERSÖNLICHE DATEN

Name	ÀGNES FEKETE
Adresse	AM SCHAEFERANGER 9/04, D-85764, OBERSCHLEISSHEIM, DEUTSCHLAND
Telefon	+49 (179) 7768186 (privat); +49(89) 3187 4618 (geschäftlich)
Fax	
E-Mail	agnes.fekete@gsf.de; a-fekete@freemail.hu
Nationalität	Ungarin
Geburtsdatum	03.02.1978

UNIVERSITÄRE FORTBILDUNG

• Zeitraum	seit Oktober 2001
• Name und Typ der Fortbildungseinrichtung	• Wissenschaftszentrum in Weihenstephan für Ernährung, Landnutzung und Umwelt; Fakultät der Technischen Universität in München, Deutschland • Institut für Ökologische Chemie im GSF-Forschungszentrum für Gesundheit und Umwelt in München, Deutschland
• Forschungsgebiet	Titel der Doktorarbeit: *“Entwicklung von kapillarischen elektrophoresischen Methoden für die Bestimmung von Aminen in Kühlschmierstoffen und atmospherischen Aerosolen.“*
• Angestrebter Titel	PhD. rer. nat.
• Forschungsaktivitäten	• Analytische Chemie • Kapillarelektrophoresisch • Kapillarelektrophorese mit Maßspektrometrie • Probenvorbereitung • Entwicklung von Methoden zur Analyse von gesättigten und ungesättigten heterozyklischen und aliphatischen Aminen in Kühlschmierstoffen und atmosphärischen Aerosolen • Monolithische Polymer Vorbereitung • Kapillare Elektrokromatographie

• Zeitraum	1994-2001
• Name und Typ der Fortbildungseinrichtung	• Fakultät für Chemieingenieurwesen in der Technischen Universität in Budapest, Ungarn • Fakultät für Bioingenieurwesen im Institut für Umweltschutz in der Technischen Universität in Budapest, Ungarn
• Forschungsgebiet	Titel der Diplomarbeit: “ *Phosphorus Stabilized Carbenes: Theoretical Predictions”*
• Abschluss	Bioingenieurin
• Forschungsaktivitäten	Computationale Chemie, *ab-initio* Kalkulation, Carbenes, Isodesmic Reaktionen

WEITERE FORSCHUNGSAKTIVITÄTEN

• Zeitraum	1999-2001
• Name und Adresse des Arbeitgebers	Institut für Anorganische Chemie in der Technischen Universität in Budapest, Ungarn
• Tätigkeitsbereich	Forschungsassistentin
• Hauptforschungsgebiet und Forschungstätigkeit	• *ab – initio* Kalkulation • Reaktionskinetische Untersuchungen mit Hilfe von Erkenntnissen aus der theoretischen

Chemie

• Zeitraum	1997-1998
• Name und Adresse des Arbeitgebers	Institut für Analytische Chemie in der Technischen Universität in Budapest Ungarn Kromatographische Forschungsgruppe
• Tätigkeitsbereich	Forschungsassistent
• Hauptforschungsgebiet und Forschungstätigkeit	Entwicklung von Forschungsmethoden zur Analyse von Rattengift Substanzen

VERÖFFENTLICHUNGEN

Fekete A., Kettrup A. (2004) Vortrag über die Entwicklung von Bestimmungsverfahren zur Untersuchung von Korrosionsinhibitoren durch Kühlschmierstoffe am Arbeitsplatz. Frühlingsjahrsakademie Nr. 16, München

Fekete A., Schmitt-Kopplin Ph., Lahaniatis M., Kettrup A. (2004) Application of the mobility scale transrormation in routine analysis. 17th International Symphosium on Microscal Separation and Capillary Electrophoresis, Salzburg

Breuer D., Fischer K., Hansen K., Fekete, A., Lahaniatis M., Schmitt-Kopplin Ph. (2003) Benzotriazole (1,2,3-Benzotriazole, 5-Methyl-1H-benzotriazole, 5,6-Dimethylbenzotriazole). In: Analytische Methoden Bd. .1, Deutsche Forschungsgemeinschaft; Senatskommission zur Prüfung gesundheitsschädlicher Arbeitsstoffe; Arbeitsgruppe „Analytische Chemie”

Fekete A., Nyulaszi L. (2002) Phosphorus stabilized carbenes: theoretical predictions. Journal Organometallic Chemistry, S. 278-284

Fekete A. (2001) Vortrag über: Stability examination of phosphorus stabilized carbenes (awarded). XXV. National Student Conference, Section of Theoretical Chemistry, Gödöllö

WEITERE PERSÖNLICHE KOMPETENZEN

MUTTERSPRACHE	Ungarisch
FREMDSPRACHENKENNTNISSE	
	DEUTSCH
• Leseverständnis	Gut
• Schreibfähigkeit	Grundkenntnisse
• mündliche Kommunikationskompetenz	Grundkenntnisse
	ENGLISCH
• Leseverständnis	Gut
• Schreibfähigkeit	Gut
• mündliche Kommunikationskompetenz	Gut
SOZIALKOMPETENZ	Assistentin im Institut für Anorganische Chemie in der Technischen Universität in Budapest, Ungarn Unterricht von klassischer qualitativen Analyse und chemische berechnung

TECHNISCHE KOMPETENZEN	Fundierte Kenntnisse in Word, Gaussian 98, Statistica, Pallas, ADC, Gel Treat und HTML
FÜHRERSCHEIN	Kategorie B (seit 1995)
ZUSÄTZLICHE INFORMATIONEN	Spezielles wissenschaftliches Interessengebiet Instrumentale analytische Chemie Methodenentwicklung und Validation Kapillarelectrophoresisch Monolithische Polymere im kapillarische Elektrochromatographie, Kirale Separation
	Referenzen
	Prof. Dr. rer. nat. Dr .h. c. Kettrup, A. Institutsleiter für Ökologische Chemie und Umweltanalytik in der Technischen Universität in München
	Prof. Dr. Nyulaszi, L. Institutsleiter für Anorganische Chemie in der Technischen Universität in Budapest

Neuherberg, 09.01.2005

…………………………………….
Àgnes Fekete