Sampling Strategies for the Analysis of Reactive Low-Molecular Weight Compounds in Air

Hartmut Henneken

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SAMPLING STRATEGIES FOR THE ANALYSIS OF REACTIVE LOW-MOLECULAR WEIGHT COMPOUNDS IN AIR

DISSERTATION

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Prof. Dr. Uwe Karst and Dr. Martin Vogel

Für meine Eltern

Contents

Abbreviations

2-MP	1-(2-methoxyphenyl)piperazine
2-PP	1-(2-pyridyl)piperazine
A	area
ACGIH	American Conference of Governmental Industrial Hygienists
ACN	acetonitrile
ADS	2-([3-{2-[4-Amino-2-(methylsulfanyl)phenyl]-1-diazenyl}phenyl]-sulfonyl)-1-ethanol
ADSO	2-([3-{2-[4-amino-2-(methylsulfoxy)phenyl]-1-diazenyl}phenyl]sulfonyl)-1-ethanol
$ADSO_2$	ADS-based sulfone
AGC	automated gain control
AMMS	9-anthracenylmethyl methyl sulfide
APCI	atmospheric pressure chemical ionization
BOELV	binding occupational exposure limit value
c	concentration
CDL	curved desolvation line
CIS	coordination ionspray
CLND	chemiluminescence nitrogen detection
DBA	dibutyl amine
DEA	diethylamine
D_{BA}	diffusion coefficient
D_{BA}	diffusion coefficient of compound B in compound A
DMAP	4-(N,N-dimethylamino)pyridine

EC	electrochemistry
EIC	ethyl isocyanate
ESI	electrospray ionization
FCC	ferrocenecarboxylic acid chloride
Fc-PZ	ferrocenoyl piperazide
FLD	fluorescence detection
FSG	Fuller-Schettler-Giddings (equation)
FTICRMS	fourier transform ion cyclotron resonance-mass spectrometry
GC	gas chromatography
GF	glass fiber
HDI	hexamethylene diisocyanate
HP	hydrogen peroxide
HPLC	high performance liquid chromatography
HSE	Health and Safety Executive
ICA	isocyanic acid
IOELV	indicative occupational exposure limit value
IPDI	isophorone diisocyanate
iPIC	isopropyl isocyanate
K_h	Henry's law constant
L	length
L	liter
LC	liquid chromatography
LC-MS/MS	liquid chromatography tandem mass spectrometry
LOD	limit of detection
LOQ	limit of quantification

m/z	mass-to-charge ratio
MAC	maximaal aanvaarde concentratie (maximum allowable concentration)
MAK	maximale Arbeitsplatzkonzentration (maximum workplace concentration /8 h work-shift)
MAMA	9-(N-methylaminomethyl)anthracene
MAP	1-(9-anthracenylmethyl)piperazine
mAU	milli absorbance units
MDI	methylenebis(phenyl isocyanate)
MEL	maximum exposure limit
MFC	mass flow controller
MHP	methyl hydroperoxide
MIC	methyl isocyanate
MMNTP	4-methoxy-6-(4-methoxy-1-naphthyl)-1,3,5-triazine-2-(1-piperazine)
MNMA	N-methyl-1-naphthalenemethylamine
M_r	reduced mass
MRM	multiple reaction monitoring
MS	mass spectrometry
ms	millisecond
MTS	methyl-*p*-tolyl sulfide
MTSO	methyl-*p*-tolyl sulfoxide
NBDPZ	4-nitro-7-piperazinobenzo-2-oxa-1,3-diazole
NCO	isocyanate
NGV	nivågränsvärde (full working day limit value)

Abbreviations

NIOSH	National Institute for Occupational Safety and Health
nm	nanometer
NMA	1-naphthalenemethylamine
OEL	occupational exposure limit
OES	occupational exposure standards
OSHA	Occupational Safety and Health Administration
PAA	peroxyacetic acid
PAC	9-anthracenylmethyl 1-piperazinecarboxylate
PDMS/DVB	poly(dimethylsiloxane)/divinylbenzene
PhIC	phenyl isocyanate
ppb	parts per billion
ppm	parts per million
ppt	parts per trillion
psi	pound per square inch
PTFE	Teflon®
PUF	polyurethane foam
PUR	polyurethanes
PVDF	poly(vinylidene fluoride)
R	coefficient of linear regression
RD_{50}	10-minute exposure concentration producing a 50% respiratory rate decrease in mice or rats
RH	relative humidity
rpm	rounds per minute
RSD	relative standard deviation
RT	room temperature

s	second
SD	standard deviation
SDB	poly(styrene-divinyl benzene)
SIM	selected ion monitoring
SPME	solid phase microextraction
S_R	sampling rate
STEL	short term exposure limit
t	time
TBP	tributylphosphate
TDI	toluene diisocyanate
TGV	takgränsvärde (ceiling limit value
THF	tetrahydrofuran
TIC	total ion current
TLC	thin-layer chromatography
TLV	threshold limit value
TOF	time-of-flight
TPP	triphenyl phosphine
TPPO	triphenyl phosphine oxide
T_R	retention time
TRIG	total reactive isocyanate groups
TRYP	tryptamine; 3-(2-aminoethyl)indole
TWA	time weighted average
u	units
UV	ultraviolet
V	volt

VOC	volatile organic compound
v/v	volume-to-volume ratio
vis	visible
WEL	workplace exposure limit

Chapter 1

Introduction

1.1 Introduction and scope

Airborne hazardous substances often occur in various workplace atmospheres. In order to protect workers from health problems related to exposure to such compounds, recommended or mandatory occupational exposure limits (OELs) have been developed in many countries for airborne exposure to gases, vapours and particulates. For airborne exposures, there are three types of limits in common use:

- the time-weighted average (TWA) exposure limit - the maximum average concentration of a chemical in air for a normal 8-h working day and 40-hour week;
- the short-term exposure limit (STEL) - the maximum average concentration to which workers can be exposed for a short period (usually 15 minutes);
- the ceiling value - the concentration that should not be exceeded at any time.

In the European Union, the legal basis for the preparation of occupational exposure limits is contained in Directives 98/24/EC (on chemical agents) and 2004/37/EC (on carcinogens and mutagens). Indicative Occupational Exposure Limit Values (IOELVs) are adopted through Commission Directives

while Binding Occupational Exposure Limit Values (BOELVs) are adopted through Council and European Parliament Directives.

Furthermore, legally binding national TLVs are implemented, such as the Dutch MAC-values [1], the German MAK-values [2], the Swedish TGV- and NGV-values [3], or the British Workplace Exposure Limit (WEL) [4], which replaced the former Maximum Exposure Limits (MELs) and Occupational Exposure Standards (OESs) in April 2005.

In the United States, recommended exposure limits are developed and periodically revised by the National Institute for Occupational Safety and Health (NIOSH). These recommendations are then published and transmitted to the Occupational Safety and Health Administration (OSHA) for use in promulgating legal standards. Threshold limit values (TLVs) are also issued by the American Conference of Governmental Industrial Hygienists (ACGIH).

A limit value is useless if the compliance with regulations is not regularly or randomly controlled. Therefore, workplace atmospheres need to be routinely monitored and screened for hazardous conditions. Ideally, such a screening should be cheap, easy to perform, reliable and not disturbing the work process. In order to provide best protection for the worker, a fast analysis result should be available; ideally a direct-reading device should be used, allowing to take immediate measures in the case of hazardous conditions. However, often this is not possible even if state-of-the-art technology is used. Therefore, there is a continuous need for the development of new, improved

methods for the analysis of workplace atmospheres. Generally, the more toxic a substance is, the lower will be the applicable TLV, and hence the airborne concentration that needs to be reliably quantified. Thus, the challenges for the analytical procedures are often increasing with the toxicity of the analytes. The work presented in this thesis is mainly focused on isocyanates as group of analytes. These substances are well-known compounds in the field of occupational hygiene as world-wide largest known inducers of occupational asthma. Owing to their very high reactivity, isocyanates are also extremely toxic species [5]. Methyl isocyanate, e.g., was responsible for one of the largest tragedies in industrial history, as in 1984 an accidental release of large amounts into the surrounding air of a pesticide plant in Bhopal (India) happened, killing thousands of people and causing a series of follow-up health problems until today [6].

Isocyanates are already known for more than 150 years. However, the large scale industrial application started more than 80 years later, when the polymer industry discovered the ability of diisocyanates to react with polyols to yield polyurethanes (PUR) [5]. Soon, adverse health effects were reported from isocyanate exposure, and first methods for analysis were presented. Since then the OELs have been continuously lowered. This makes continuous improvement of existing methods and development of new analysis methods necessary, especially as so far no “best” method for all purposes and applications exists. In **chapter 2,** the development and recent advances in sampling and analysis of airborne isocyanates are reviewed to give more in-depth background information.

The goal of this thesis was to develop new and user-friendly sampling and analysis methods to be applied for the determination of airborne isocyanates and other workplace air contaminants. The focus was to be laid on passive sampling applications, but preferably solvent-free active methods should be developed and applied during the validation processes as independent reference methods as well. Owing to expertise and knowledge available in our group, as analytes and derivatizing agents of interest, isocyanates were selected to be analyzed by means of 4-nitro-7-piperazinobenzo-2-oxa-1,3-diazole (NBDPZ) derivatization [7], and peroxyacetic acid using the oxidation reaction of 2-([3-{2-[4-Amino-2-(methylsulfanyl)phenyl]-1-diazenyl}phenyl]-sulfonyl)-1-ethanol (ADS) to the respective sulfoxide ADSO [8]. In order to be able to carry out the experimental work, an appropriate test atmosphere generation system had to be constructed.

Chapter 3 describes the application of NBDPZ as derivatizing agent for airborne isocyanates. As model analyte, methyl isocyanate (MIC) was selected. Diffusive sampling was performed, using the commercially available UMEx 100 passive sampling device equipped with new NBDPZ impregnated glass fibre filters. As independent reference method, active sampling was performed, employing cartridges with 2-MP reagent-coated filters. The NBDPZ diffusive samplers were analyzed by means of LC-FLD as well as LC-MS/MS, while reference samples were only determined by tandem mass spectrometry.

The work described in **chapter 4** was based on the results discussed in chapter 3. The nature of the humidity problems that were encountered if NBDPZ coated glass fibre (GF) filters were used for diffusive sampling was elucidated and this drawback was solved by exchanging the filter material. A detailed comparative study of two filter materials (GF and poly(styrene divinyl benzene) (SDB) was carried out. Both materials were tested to be used as filter tapes for impregnation with NBDPZ as well as 2-MP, and the study was extended to cover methyl, ethyl (EIC) and phenyl isocyanate (PhIC) as analytes.

According to the results described in chapter 4, the SDB material was chosen as collector surface for further diffusive sampling experiments. A full validation of NBDPZ diffusive sampling methods for the determination of vapour-phase MIC, EIC and PhIC is presented in **chapter 5**. The target concentration range was placed between one tenth and 5 times the existing OELs of 5-10 ppb for the individual isocyanates. Dry and humid conditions were simulated in the laboratory and short-term sampling was performed as well as full work-shift length monitoring. The method was also tested to be applied for determination of isocyanic acid (ICA). As means of detection after LC separation, APCI-MS/MS (for NBDPZ samples) and ESI-MS/MS (for 2-MP samples) were selected, employing an ion-trap mass analyzer. For the analysis of PhIC-NBDPZ, a fluorescence detection method is presented as well.

In **chapter 6,** the synthesis and application of ferrocenoyl piperazide as a new derivatizing reagent for isocyanates is presented. A different approach for

sensitive detection of isocyanate derivatives is introduced by using liquid chromatographic separation, electrochemical oxidation/ionization and mass spectrometry (LC/EC/MS).

Chapter 7 shows the first implementation of a passive sampling technique for the determination of airborne peroxyacetic acid (PAA). The diffusive samplers were equipped with ADS-coated glass fibre filters. As the collection mechanism is not a true derivatization, but an oxidation reaction, thorough investigations are described with respect to the selectivity of the method towards hydrogen peroxide. Analysis was performed by means of reversed-phase HPLC and UV/vis spectroscopy, and real samples were collected during disinfection of a laboratory area.

General conclusions and future perspectives of using passive sampling devices for the determination and identification of reactive low-molecular compounds in air are found in **chapter 8**, which concludes this thesis.

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Chapter 2

Determination of Airborne Isocyanates[‡]

Isocyanates play a key role in the fields of industrial hygiene and workplace monitoring. Owing to their severe acute toxicity and sensitizing properties, analytical methods are required that provide high sampling efficiency and sensitivity in the low ppb down to the ppt range. The reactivity of isocyanates necessitates initial derivatization using a nucleophilic agent - mostly amines - for stabilization and enrichment, which is often followed by chromatographic separation and spectroscopic, electrochemical or mass spectrometric detection. Sampling strategies for airborne isocyanates comprise active, i.e. pumped, or passive, i.e. diffusive, methods, being strongly dependent on the respective application. While active methods mainly rely on impingers, reagent-coated filters or sampling tubes, passive samplers make use of reagent-coated filters, the surface of which is connected to the air sample by diffusion channels. As airborne isocyanates are prone to occur in different forms, i.e. as vapors, as aerosols or adsorbed on particulate matter, denuder sampling has been introduced, thus allowing the simultaneous collection of gaseous and aerosol isocyanates. In a first part, this review summarizes chemical methods and reagents, which have been introduced for the derivatization of airborne isocyanates. The advantages and drawbacks of the individual derivatization procedures as well as their combination with different

detection principles are evaluated. In a second part, most recent developments in the fields of air sampling for isocyanates, with special focus toward diffusive sampling, are reviewed and critically discussed.

‡ accepted for publication in H. Henneken, M. Vogel and U. Karst, *Anal. Bioanal. Chem.*, **2006**.

2.1 Introduction

Isocyanates are highly reactive compounds containing one or more isocyanate functionalities (–N=C=O) and are classified according to the number of free isocyanate moieties as mono-, di-, or polyisocyanates. Dependent on the character of the attached organic entity, they may be divided into aliphatic or aromatic isocyanates, the latter of which exhibits higher reactivity towards nucleophilic agents [1]. The most important isocyanates are shown in Fig. 2.1. Due to their high reactivity, both aliphatic and aromatic isocyanates are prone to undergo oligomerization. Dimerization is frequently observed, thus yielding cyclic or linear dimeric species [1]. Catalyzed by either acids or bases, also trimerization of alkyl and aryl isocyanates is obtained; e.g., methyl isocyanate forms a trimer that is present in two isomeric forms when trialkylphosphine is used as a catalyst [2]. Monomeric diisocyanates may yield homopolymers or, by reacting these monomers with co-polymers, e.g. polyols or polyamines, heteropolymers such as polyurethanes (PUR) or polyureas are synthesized. If polyols are pre-reacted with an excess of di- or polyisocyanates, a prepolymer is yielded, which is still reactive but less volatile.

Although isocyanates have already been known for more than 150 years [3], the main industrial application of isocyanates is based on the ability of diisocyanates to undergo polyaddition reactions with polyols to form polyurethanes (PUR), which was first described for toluene diisocyanate (TDI) by Bayer in 1937 [4,5]. After World War II, PUR have found widespread

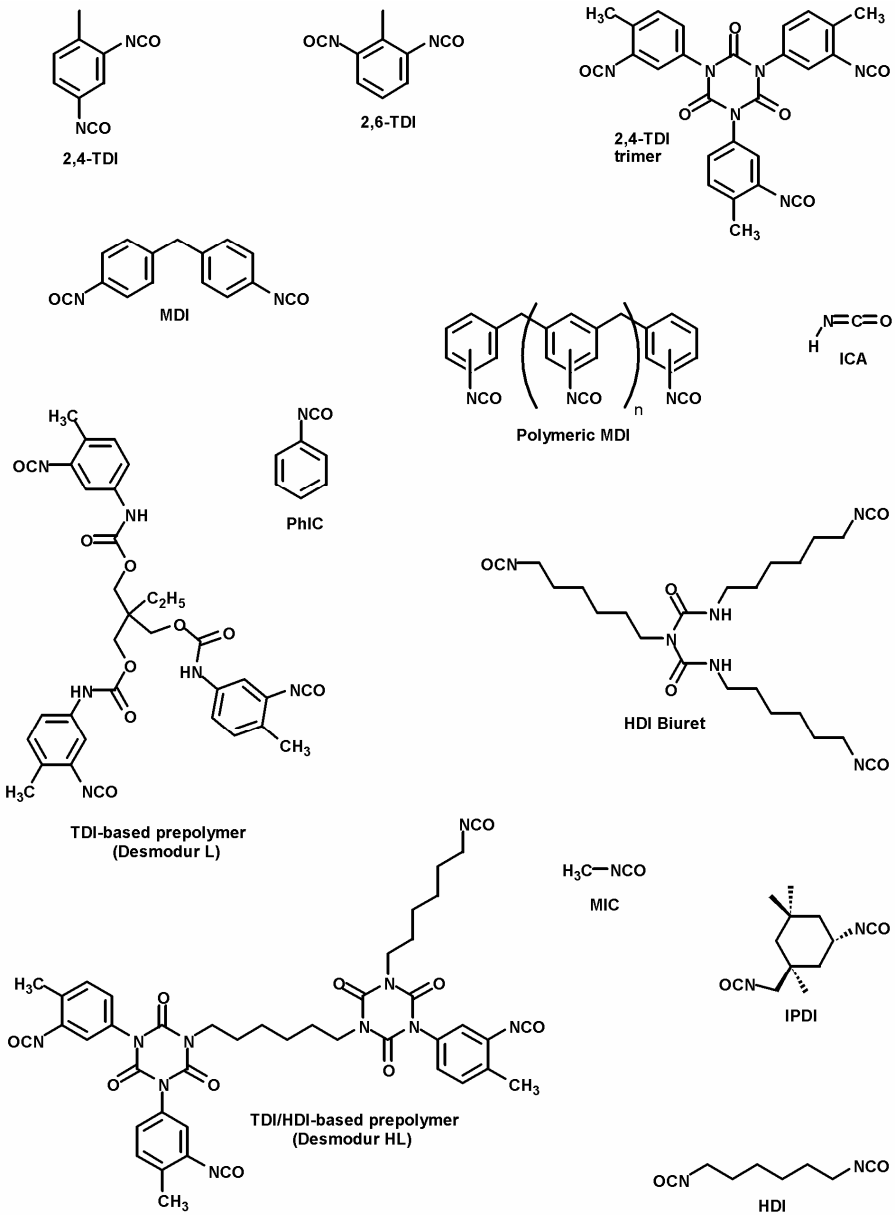

Fig. 2.1: A selection of commonly used mono- and diisocyanates, isocyanic acid, oligomeric and polymeric isocyanates as well as industrially relevant prepolymers.

application as rigid or elastic foams, plastics, elastomers, varnishes, coatings, paints, lacquers and adhesives. In addition, monoisocyanates are mainly used as intermediates during production of various pharmaceutical and agricultural products, e.g., for the production of sulfonylurea hypoglycemic agents or carbamate-based pesticides. Long-chain aliphatic monoisocyanates also find application for surface treatment of textile or paper materials. Generally, the large-scale industrial use of isocyanates is based on their high reactivity towards nucleophilic agents, e.g. alcohols or amines, often showing quantitative reaction yields without any side reactions [1]. Nevertheless, their reactivity is also associated with an acute toxicity. Soon after the industrial introduction of isocyanates, first adverse health effects were reported [6,7], followed by numerous publications describing various toxic effects related to isocyanate exposure, which were already extensively reviewed elsewhere [8-21]. Two general types of physiological effects have been observed: On the one hand, exposure to airborne isocyanates is associated with irritating effects to the skin, eyes, mucous membranes, and the respiratory system, starting from coughing and shortness of breath, up to pulmonary edema and even death at very high concentrations [22]. On the other hand, isocyanates also have sensitizing properties [23-25]. The so-called isocyanate asthma is the most commonly observed form of occupational asthma in industrialized countries [21]. A single exposure to a high-level isocyanate concentration may already be sufficient to develop an asthmatic condition. However, usually sensitization takes at least several weeks of exposure. Once a person is sensitized, even very low concentrations below existing occupational exposure limits (OEL) can trigger life-threatening asthma attacks. The

prevalence of isocyanate asthma in exposed workers ranges from 5 to 13% [21]. Legal authorities are nowadays well aware of the isocyanate hazards, with OELs being continuously lowered during the past decades. OELs for isocyanates have already been regulated to the lowest levels compared with any other hazardous organic compound, and worldwide applied OELs are in the range of 2-20 ppb [26-30]. The hazard assessment of isocyanates has not yet been completed, as new studies continuously require reevaluation of existing OEL. For example, in the Netherlands a further lowering down to values of 2 ppb is currently in progress [31]. Generally, two different approaches to settle maximum exposure levels are used. In some countries, e.g., Finland, Switzerland and the UK, the exposure limits are based on the number of total reactive isocyanate groups (TRIG), while in other countries, e. g., Germany, Sweden and the USA; exposure limits have been set for individual compounds. These approaches and existing limit values have recently been discussed in a critical review about exposure limits and metrics by Bello *et al.* in 2004 [32].

Despite the broad range of PUR applications and a large likelihood of contact with PUR products, there is relatively little risk of isocyanate exposure for persons outside the industries as the final reaction products are normally stable and non-toxic compounds at ambient conditions. Nevertheless, native isocyanates are also handled at non-production plants and, in some cases, they can remain on surfaces of newly made polyurethane products for an extended period., thus leading to a possible exposure risks. However, owing to their versatile properties regarding stability and biocompatibility, PUR-

based materials are even used for medical implants. As numerous studies have demonstrated, a large variety of isocyanate compounds are released when PUR is treated thermally or mechanically [33-38]. Therefore, occupational exposure to isocyanates is not only restricted to industries where isocyanates are produced. Most importantly, isocyanate emission occurs also at those workplaces where neither isocyanates nor polyurethanes are made [39,40], such as autobody repair shops [41], in the building industry, at flame-lamination plants, during spray-painting operations [42] or at other workplaces, where processes are performed such as welding, cutting, grinding or sanding of PUR-coated materials [43].

In order to monitor airborne isocyanate concentrations in workplace atmospheres, a large variety of analytical methods has been presented during the past decades, mostly based on chemisorption with different derivatizing agents in order to stabilize the reactive analytes and to improve detection properties. In fact, some attempts were made to detect isocyanates without derivatization [44,45], but owing to their high reactivity towards common nucleophiles [46], e.g. water, isocyanates are prone to undergo degradation during sampling, thus yielding only non-satisfactory limits of detection. Most importantly, polyols or polyamines that are used during polyurethane synthesis are a significant source of degradation during sampling. They present a part of a reacting aerosol and are prone to consume the isocyanate in the absence of a derivatizing agent, particularly during solventless sampling. In the past, a number of reviews with respect to the analysis of airborne isocyanates have been published [47-51]. However, most recent

developments in the field - especially with respect to air sampling - have not yet been summarized and are covered in detail within this review paper.

2.2 Derivatization and analysis of isocyanates

2.2.1 Colorimetric determination of isocyanates

First methods for the determination of isocyanates were mainly based on colorimetric techniques, which were, from today's point of view, mostly lacking selectivity and sensitivity [52-56]. A well-known procedure for the determination of isocyanates that had already been published in the 1950s is the Marcali method [56]. In this method, isocyanates are hydrolyzed using a mixture of acetic and hydrochloric acid. The yielded amines are diazotized, coupled to N-1-naphthyl ethylenediamine and detected photometrically as the respective azo dyes. The Marcali method was sufficiently sensitive for the determination of airborne TDI until the threshold limit value (TLV) was lowered from 650 to 130 $\mu g \cdot m^{-3}$ (from 100 to 20 ppb) in 1961 [57]. Owing to the derivatization involving the formation of an azo dye, this method is only applicable to aromatic isocyanates, and it is subject to interferences by aromatic amines. Furthermore, the Marcali method only allows determining a sum parameter of aromatic isocyanates because prior to detection no separation step is involved. A number of modifications have been presented in order to comply with the lower TLVs [58-61]. These and other colorimetric methods were extensively reviewed by Purnell and Walker [47]. As the Marcali method did not require expensive instrumentation, it had been further developed and modifications hereof were published still in the middle of the 1980s [62].

2.2.2 Fluorimetric determination of isocyanates

In 2000, Wang *et al.* published a method [63] for the fluorimetric detection of methyl isocyanate which is based on the Hantzsch reaction [64]. Methyl isocyanate is absorbed in acidified dimethylsulfoxide, and thus hydrolyzed to methylamine (Fig. 2.2). The latter is reacted with formaldehyde and acetyl-

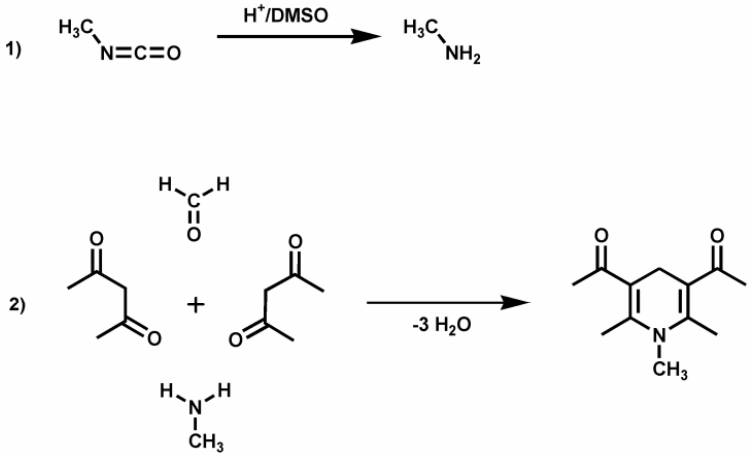

Fig. 2.2: Fluorimetric analysis of airborne methyl isocyanate (MIC). 1) In a first step, MIC is hydrolyzed in acidified solution to yield methyl amine. 2) The reaction of methyl amine, formaldehyde and two molecules of acetylacetone yields a highly fluorescent heterocyclic product (*N*-methyl-2,6-dimethyl-3,5-diacetyl-1,4-dihydropyridine).

acetone to form a heterocycle (*N*-methyl-2,6-dimethyl-3,5-diacetyl-1,4-dihydropyridine), which can be detected by means of fluorescence spectroscopy (λ_{exc} = 404 nm; λ_{em} = 474 nm). Instrumental limits of detection were reported to be 20 $\mu g \cdot L^{-1}$. However, the method proposed by Wang *et al.* has not yet been applied to real samples, and it is known from the literature

that the background signal typically limits the applicability of the method in practice [65]. Especially at workplaces where methyl isocyanate and amines are present, the Hantzsch approach is prone to yield false positives. Furthermore, as no chromatographic separation is involved, only a sum parameter can be determined in case of complex isocyanate mixtures.

2.2.3 Derivatization of isocyanates followed by chromatographic separation

Airborne isocyanates can be sampled and hydrolyzed in a phosphoric acid solution. The formed amines may be separated by means of either liquid [66] or gas chromatography [67]. However, the method does not allow differentiating between isocyanates and amines that may both be present in air samples. Therefore, modern analytical approaches for the determination of mono- and diisocyanates predominantly involve the derivatization with nucleophilic compounds such as alcohols or amines, which is followed by chromatographic separation and, in most cases, photometric [68-75], fluorimetric [76-78], electrochemical [75,79,80] or, more recently, also mass spectrometric detection [36,37,81-83]. Over the past 25 years, the use of amine-based reagents has proven to show best results with respect to the analysis of isocyanates [84,85]. In the following, the most commonly used derivatizing agents are described in their order of appearance in the literature. Chemical structures of the derivatizing agents are shown in Figure 2.3.

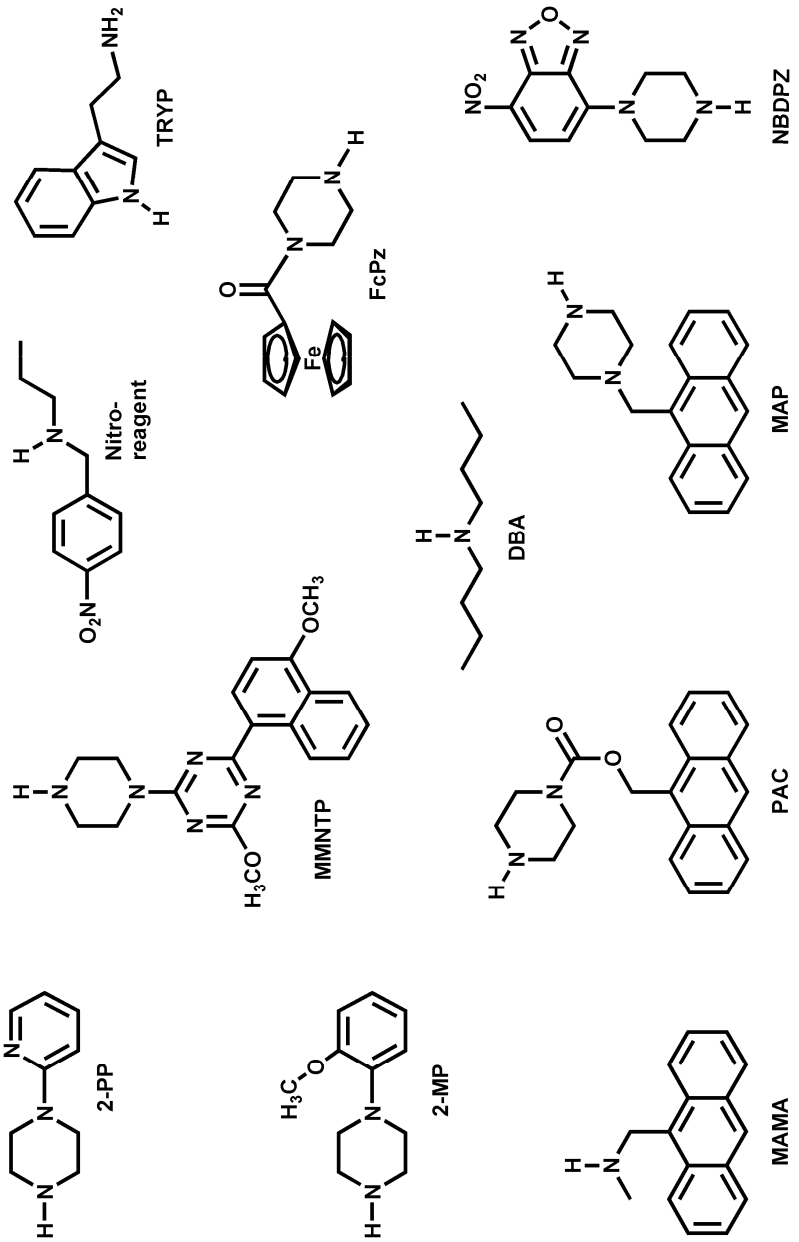

Fig. 2.3: Structures of selected amine reagents that have been introduced for the derivatization of isocyanates. Full names for the respective compounds are given in the text.

N-4-Nitrobenzyl-N-n-propylamine ("nitro reagent")

In 1971, Heuser *et al.* [86] presented a thin-layer chromatographic (TLC) method for the determination of free hexamethylene diisocyanate (HDI) monomer in polyurethane samples. The method is based on the reaction of the analytes with N-methyl-N-(4-aminobenzyl)amine, and it was the first method that made use of an amine for the derivatization of an isocyanate compound. Only three years later, Keller *et al.* presented the first method for the determination of airborne aliphatic and aromatic isocyanates by introducing N-4-nitrobenzyl-N-n-propylamine (nitro reagent) as a new reagent for TLC [68]. Subsequently, this method was also applied in combination with liquid chromatographic separation on reversed-phase columns [69]. Several methods employing the nitro reagent had been developed over a period of about 20 years [70-74,87]. Some methods [87,88] served as NIOSH standard methods for a while, but have meanwhile been replaced by other improved procedures [48]. Methods relying on the nitro reagent are lacking sensitivity and stability, as the reagent is relatively unstable and light sensitive [47]. The limits of detection that could be achieved with these methods were reported (for TDI) to range from 2.6 $\mu g \cdot m^{-3}$ (0.4 ppb) using the HPLC method up to 52 $\mu g \cdot m^{-3}$ (8 ppb) with the TLC method, each for a 10-L air sample.

Alcohols

Shortly after the introduction of the nitro reagent, alcohols were applied in order to convert the isocyanates into stable urethane compounds. In a first paper, methanol, ethanol and butanol were used by Kozlovski *et al.* for the analysis of aromatic isocyanates by means of TLC [89]. In 1980, Bagon and

Purnell reported an LC method, based on ethanol as derivatizing agent [90]. A detection limit of 2 $\mu g \cdot m^{-3}$ was reported for TDI and MDI, respectively. However, due to the lack of chromophors, photometric detection was mainly possible for aromatic analytes, and only few attempts were carried out making use of ethanol as derivatizing agents for LC. Dalene *et al.* described the derivatization of TDI in alkaline ethanol followed by LC separation and both UV/VIS and electrochemical detection with the latter showing a limit of detection of 0.1 $\mu g \cdot m^{-3}$ [91]. In a later paper, Skarping *et al.* presented a gas chromatographic (GC) method, using ethanol for derivatization and both nitrogen selective and mass spectrometric detection [92]. Using nitrogen-selective detection, a detection limit of 0.2 $\mu g \cdot m^{-3}$ has been reported [89].

1-(2-Pyridyl)piperazine (2-PP)

1-(2-Pyridyl)piperazine (2-PP) was introduced by Hardy and Walker as a derivatizing reagent for isocyanates [93], which offered better stability and higher molar absorptivities than the nitro reagent. In combination with thin-layer chromatography, a detection limit of about 2 $\mu g \cdot m^{-3}$ was achieved for HDI, TDI and MDI [94]. Furthermore, with LC methods the sensitivity could be further increased, and LODs were around 0.5 $\mu g \cdot m^{-3}$ [95,96]. For a total sampling volume of 1 m^3, even a detection limit of 9.0 $ng \cdot m^{-3}$ was reported [97]. Nowadays, especially in the United States, modifications of these 2-PP methods are still in use [98-100].

1-Naphthalene methylamine (NMA), N-methyl-1-naphthalene methylamine (MNMA), aniline, diethylamine

In the early 1980s, some new reagents were presented, which have only been rarely applied for real-sample analysis yet. 1-Naphthalene methylamine (NMA) was introduced as first reagent to be used in combination with LC-fluorescence detection, allowing a more sensitive detection compared to other methods known at that stage [101]. This method was reported to be 50 times more sensitive than the nitro reagent technique, and NMA was reported to be more reactive than the nitro reagent. However, unexplainable double peaks appeared in the chromatograms for air samples from spray tests using hexamethylene diisocyanate-biuret trimer. Furthermore, solubility problems were encountered for some NMA derivatives. Therefore, Kormos *et al.* proposed a further naphthalene-based fluorescent reagent, N-methyl-1-naphthalene methylamine (MNMA), which was found to be more versatile and showed a significantly improved chromatographic separation [102]. The detection limits were decreased by a factor of two for those aliphatic diisocyanates that had been covered by the NMA method. Detection limits for MNMA ranged from 1 $\mu g \cdot m^{-3}$ for HDI to 15 $\mu g \cdot m^{-3}$ for TDI. Nevertheless, MNMA suffered from instability and light sensitivity. In another study, aniline was suggested as derivatizing agent for isocyanates, but both detection limit and reagent stability were only poor [103]. Another paper described a method for the determination of airborne methylene bisphenyl diisocyanate (MDI) using diethylamine as derivatizing agent [104]. However, diethylamine is not suitable for air sampling operations due to its high vapor pressure, even

though the isocyanate derivatives of diethylamine could be easily baseline separated on reversed-phase columns [36].

9-(N-Methylaminomethyl)anthracene (MAMA)

In 1980, Sangö and Zimerson presented 9-(N-methylaminomethyl)anthracene (MAMA) as a novel reagent for the determination of isocyanates [76-78]. Owing to the anthracene backbone, sensitive fluorescence detection and, due to high molar absorptivities, sensitive photometric detection are possible. However, the reagent suffers from instability, and both reagent and derivatives are prone to decompose in the light. Nevertheless, MAMA has been among the most frequently applied derivatization reagents for isocyanates for about two decades. It has often been used for the determination of the total isocyanate group content in complex samples as the UV response of MAMA is nearly independent of isocyanate structure [105,106]. In contrast to UV detection, fluorescence responses of several isocyanate monomers derivatized with MAMA showed a strong dependence on the respective isocyanate structure [105].

1-(2-Methoxyphenyl)piperazine (2-MP)

Warwick *et al.* presented 1-(2-methoxyphenyl)piperazine (2-MP) [75] for the determination of isocyanates. For 2-MP, other abbreviations such as MOPIP, MOPP, MP and MPP are commonly used as well. Apart from photometric detection, the reagent and its derivatives can be detected electrochemically with good sensitivity showing LODs down to 0.2 $\mu g \cdot m^{-3}$. In contrast, UV/VIS detection yields LODs of about 5 $\mu g \cdot m^{-3}$. The 2-MP reagent has found

widespread application and many research groups have been using 2-MP-based methods during the past 25 years [107]. There are several standard methods, the most important one being the British MDHS 25/3, which is broadly accepted especially in European countries and probably the method most commonly used worldwide for the determination of isocyanates in air [108-110]. It has to be mentioned that the intent of MDHS 25/3 was for all isocyanate compounds to have a very similar response ratio of UV/VIS and electrochemical detection. However, it was found that the variability of the UV response of 2-MP derivatives made the detector response ratio unreliable for the identification of isocyanates in some cases [111]. As legislation in many countries nowadays requires the estimation of TRIG, and as for the majority of industrially used oligomeric isocyanates there are no standards available, most methods are not able to be used for quantification of total isocyanate groups. MDHS 25/3 quantifies these oligomers using an electrochemical detector, which oxidizes the methoxy group on the 2-MP reagent, thus giving a signal independent from the analyte backbone [75,112,113]. Therefore, quantification of oligomeric isocyanates may be based on the use of the corresponding monomer, e.g., the MDI monomer for oligomeric MDI. The application of 2-MP-based methods has been further increasing during the last years when new mass spectrometric detection methods became routinely available. Recently, some very sensitive tandem mass spectrometric methods were published that significantly decreased the detection limits when being compared to those of spectroscopic or electrochemical detection schemes [114-117]. The most sensitive method so far was described by Gagne *et al.* in 2005, who found quantification limits of 0.04 $\mu g \cdot m^{-3}$ (6 ppt) for HDI, 0.01 μgm^{-3}

(2 ppt) for 2,4-TDI and 0.03 $\mu g \cdot m^{-3}$ (5 ppt) for 2,6-TDI (for a 10-L air sample), using coordination ionspray (CIS) tandem mass spectrometric (MS/MS) detection based on the formation of lithium adducts [117]. Two years earlier, Vangronsveld and Mandel described an ion-trap MS/MS detection method that was about one order of magnitude less sensitive than this CIS method, but which, in terms of sensitivity, was still superior to any other photometric or electrochemical method [115]. However, with respect to TRIG determination, mass spectrometric quantification is troublesome, as only those compounds can be accurately determined of which standards are available. Even though identification of unknowns is simplified by the high selectivity of the mass analyzer, quantification can be difficult if no reference materials are available.

A different approach to increase the sensitivity was presented by Molander *et al.* in 2000 by using a miniaturized capillary LC system for the determination of 2-MP isocyanate derivatives [118,119]. Compared with the use of conventional columns, this technique offers enhanced mass sensitivity due to reduced dilution of the sample [120]. A similar work has also been published earlier by Brunmark *et al.*, who used MAMA as reagent [121]. The method published by Molander *et al.* [118] was reported to be more sensitive by a factor of three than the MAMA micro-LC method, and the LOD improvement compared with conventional LC was reported to account even for a factor of 30. In 1999, Rudzinski *et al.* compared the LC analysis of 2-MP derivatives of HDI and HDI-based oligomers with capillary zone electrophoresis (CZE) [122]. It was observed that detection limits for HDI obtained in CZE and LC were the same. LC was followed by photometric detection at 254 nm, while CZE

separation was detected at 185 nm. Despite the advantages of capillary electrophoretic separations such as low sample consumption, short analysis times and high resolution, typical drawbacks were reported such as limited stability of the capillary and problems with reproducibility due to pH sensitivity.

3-(2-Aminoethyl)indole (tryptamine)

In 1987, Wu *et al.* introduced 3-(2-aminoethyl)indole (tryptamine, TRYP) as a derivatizing agent for isocyanates [123-130]. The reagent could be used with fluorescence and electrochemical detection, and the responses were fairly independent of isocyanate structure for both detector types, meaning that unknown isocyanate species could in principle be identified by their detector response ratios and quantified using a different derivative as standard substance. A NIOSH method had been developed that uses TRYP as derivatizing reagent [131]. Disadvantages are related to excitation in the low-wavelength range (λ_{exc}: 275 nm) where interferences from matrix constituents are prone to occur. Furthermore, primary amines are weaker nucleophiles than secondary amine, which leads to decreased reactivity toward isocyanates.

n-Dibutylamine (DBA)

Based on an ASTM method for the determination of the isocyanate content in polymer samples, which makes use of a derivatization with *n*-dibutylamine (DBA) followed by back titration [132] or a gas chromatographic determination of excess DBA [133], Skarping and co-workers investigated the derivatization of aromatic isocyanates with DBA [37,134]. Isocyanates were absorbed and

reacted in impingers filled with a solution of DBA dissolved in toluene. In contrast to chromophoric derivatizing agents, DBA can achieve a much higher derivatization rate which is mainly due to the fact that DBA can be used at much higher concentrations than these reagents. After evaporation of the solvent, the DBA derivatives were separated by means of reversed-phase LC and detected photometrically at 240 nm. As the reagent was lacking any chromophores, only aromatic isocyanates could be detected with sufficient sensitivity by this LC-UV/VIS method. LODs were reported to range from 0.5 - 0.8 $\mu g \cdot m^{-3}$ for a 15-L sample [134]. The DBA method could be expanded toward aliphatic isocyanates by introducing LC coupled to mass spectrometric (MS) detection [36,37,81-83]. For the hyphenation of chromatography and MS, either electrospray ionization (ESI) [36,81,82] or atmospheric pressure chemical ionization (APCI) [37] run in the positive-ion mode was applied. By monitoring the respective pseudo-molecular ions ($[M+H]^+$), instrumental detection limits between 0.1 $\mu g \cdot m^{-3}$ for HDI and 0.5 $\mu g \cdot m^{-3}$ for methyl isocyanate were reported [81]. Furthermore, the DBA method was applied in combination with LC and chemiluminescence nitrogen detection (CLND) [38,135]. However, detection limits for LC-CLND were higher than for LC-MS, and thus hardly suited to be used for air analysis [38]. Apart from the determination of aliphatic and aromatic isocyanates, DBA has also been used for the determination of airborne isocyanic acid (ICA) [136] and anhydrides as well [137]. Although the name implies that ICA is an acid, according to IUPAC, it has been classified as an isocyanate [138]. With respect to ICA, the analyte was also enriched in an impinger filled with a DBA solution in toluene, and subsequently, the ICA derivative was determined by means of either LC-MS

or LC-CLND. For LC-MS, instrumental limits of detection were in the nanomolar range while for CLND, the LOD was approximately four orders of magnitude higher [136]. Although DBA can be applied to selectively determine isocyanates in air samples also far below the occupational exposure limit (OEL) [139], DBA itself is highly volatile and sampling is mostly reported for the use of impingers filled with large amounts of organic solvents, thus making the method laborious and inconvenient for personal monitoring at workplaces. However, recently Marand *et al*. described a solvent-free sampler for airborne isocyanates, which consisted of a polypropylene tube whose inner wall was coated with a glass fiber filter coupled in series with another glass fiber filter [140]. Both filters were impregnated with DBA/acetic acid reagent solution. In comparison with an impinger-filter-based reference method, recoveries for various isocyanates formed during thermal decomposition of PUR ranged between 72% and 109%.

1-(9-Anthracenylmethyl)piperazine (MAP)

In 1996, Streicher *et al.* reported on a new derivatizing agent, which was developed in order to comply with the increasing demand for methods capable of determining TRIG [79,141-143]. As a derivatizing agent, 1-(9-anthracenylmethyl)piperazine (MAP) was developed. Similar to the MAMA reagent, an anthracene group is responsible for sensitive UV/VIS- and/or fluorescence detection, while the piperazine functionality is used for reaction with isocyanate species. The greater distance between the derivatizing moiety, the piperazine function, and the fluorescent moiety, the anthracene backbone, is intended to decrease the dependency of UV/VIS and

fluorescence response on the structure of the respective isocyanate [79]. Electrochemical as well as UV detection can be performed with very low compound-to-compound variabilities, while the fluorescence signal is not completely independent of structural aspects, and variations of more than 30% for different isocyanates were reported. MAP reaction rates - determined for the reaction with phenyl isocyanate - were compared with those of other reagents and found to be in the same range as for the 2-MP reagent, while TRYP and MAMA reacted by a factor of 3 and 4 slower. In an evaluation study covering the analysis of isocyanates in spray-painting operations, it was found that the results obtained using MAP are within a 95% interval with those obtained using 2-MP [144].

9-Anthracenylmethyl 1-piperazinecarboxylate (PAC)

In 2000, Roh *et al.* presented a different approach for the determination of total isocyanate content [145,146]. In a first step, isocyanates reacted with 9-anthracenylmethyl 1-piperazinecarboxylate (PAC) to give the corresponding urea derivatives. After removal of excess reagent, the isocyanate-PAC derivatives are treated with sodium thiomethoxide ($NaSCH_3$) in a second step (Fig. 2.4). Thus, for all isocyanates a defined reaction product, 9-anthracenylmethyl methyl sulfide (AMMS) is formed allowing the determination of TRIG. The PAC method circumvents the problem of different responses for different analytes as they had been mentioned for, e.g., MAMA or MAP. Nevertheless, the reactivity of PAC was reported to be lower than for most other reagents [145], and owing to the instability of sodium thiomethoxide solutions, derivatization and cleavage have to be carried out

under thoroughly selected conditions. Furthermore, the cleavage reaction to form the AMMS is not specific for isocyanate derivatives, meaning that reaction products of PAC and unknown substances could lead to false positives. Detection can be performed with UV/VIS or fluorescence detectors. However, data concerning the method's sensitivity have not yet been provided [145].

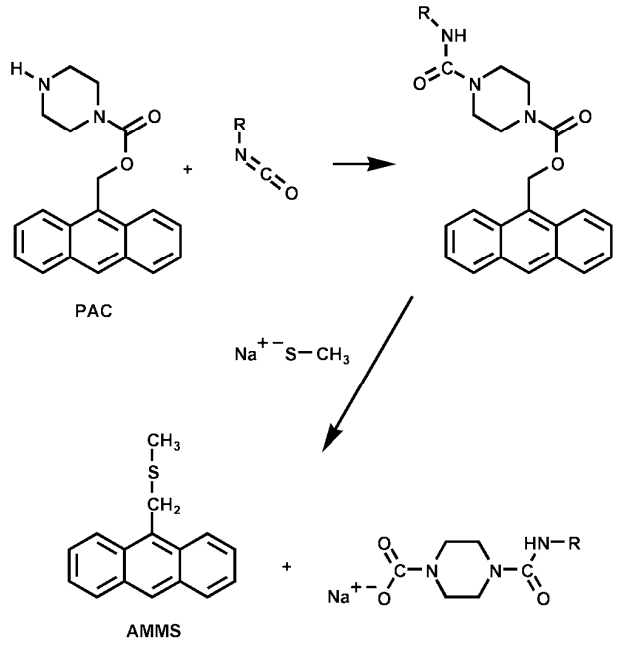

Fig. 2.4: The derivatization reaction of isocyanates with PAC yields a urea derivative that is subsequently cleaved by adding sodium thiomethoxide ($NaSCH_3$).

4-Nitro-7-piperazino-2,1,3-benzoxadiazole (NBDPZ)

In 2002, Vogel *et al.* reported 4-nitro-7-piperazino-2,1,3-benzoxadiazole (NBDPZ) as a new derivatizing agent allowing both sensitive UV/VIS and fluorescence detection [147]. Compared with other amine-based derivatizing

agents for isocyanate analysis, NBDPZ exhibits a strong red-shifted absorption maximum at 480 nm, while the molar absorptivity was reported to be about a factor of 4 better than for the 2-MP reagent. However, compared to MAMA's molar absorptivity at 254 nm, NBDPZ reached roughly a sixth of the MAMA value. The described fluorescence quantification limits were equivalent to about 20 ppt (~0.1 $\mu g \cdot m^{-3}$) – 150 ppt (~1.0 $\mu g \cdot m^{-3}$) of the respective isocyanate in air for a 10-L air sample depending upon the sampling and work-up procedure. Sensitive LC-tandem mass spectrometric analysis could also be carried out, thus lowering the LOQ about one order of magnitude compared with fluorescence detection [148]. The reagent showed only a limited stability in solution, while it was very stable when stored as a solid substance. In contrast, the derivatives were very stable both in solution and as solid substances.

4-Methoxy-6-(4-methoxy-1-naphthyl)-1,3,5-triazine-2-(1-piperazine) (MMNTP)

Recently, Werlich *et al.* presented 4-methoxy-6-(4-methoxy-1-naphthyl)-1,3,5-triazine-2-(1-piperazine) (MMNTP) as a derivatizing agent for isocyanates [149]. The reagent was systematically synthesized in a tailor-made approach [150] in order to obtain good chromatographic and spectroscopic properties of the reagent and its isocyanate derivatives. Subsequent to a three-step synthesis, MMNTP can be used with either UV/VIS or fluorescence detection. However, the sensitivity for fluorescence detection was not exceeding that of UV/VIS detection. Separation of MMNTP and its derivatives has been carried out on an octadecyl-modified silica column applying gradient elution at 10 °C with both organic and aqueous eluent constituents adjusted to pH 9.5.

Nevertheless, not all peaks could be resolved during chromatographic separation, as the 2,6-TDI and HDI derivatives could not be baseline separated.

Ferrocenoyl piperazide (FcPZ)

Very recently, Seiwert *et al*. introduced ferrocenoyl piperazide (FcPZ) as an alternative derivatizing agent for airborne isocyanates in combination with LC/electrochemistry/MS detection for sensitive and highly selective analysis [151]. FcPZ-isocyanate derivatives were separated by LC, ionized by post-column electrochemical oxidation and delivered into the mass spectrometer using an APCI interface, which was run in the "heated nebulizer" mode. The APCI source was operated at a very low potential of 0.1 kV (corona discharge off), which led to no further ionization of unwanted matrix components, and hence minimized the background signal. The instrumental limits of detection ranged from $6 \cdot 10^{-9}$ mol·L^{-1} for MDI to 20 10^{-9} mol·L^{-1} for methyl isocyanate. Baseline separation was obtained for eight monomeric isocyanate derivatives ranging from MIC to MDI [151].

Advantages and disadvantages of the different derivatization reactions and reagents are summarized in Table 2.1. Today, colorimetric methods play a negligible role, whereas amine reagent-based procedures in combination with LC separation, especially those using DBA, MAP, MAMA, 2-MP or NBDPZ, provide advantageous analytical performance.

Table 2.1: Summary of applied methodologies, advantages and disadvantages of different derivatization reagents and reactions

Method	Methodology	Advantages	Disadvantages	References
Marcali	UV/VIS	-Sum parameter determination for aromatic amines	-Formation of azo dyes -Limitation toward aromatic isocyanates -Lack of sensitivity	[47,56-62]
Hantzsch reaction	FLUO	-Fluorescence detection -Readily available analytical instrumentation required	-Interferences from amines -Background limitation -No LC separation	[63]
Nitro reagent	TLC, LC-UV/VIS	-Simultaneous determination of aliphatic and aromatic isocyanates	-Unstable reagent -Limited sensitivity -Moderate reactivity toward isocyanates	[47,48,68,70-74,87,88]
Alcohols	TLC, LC-UV/VIS, LC-ECD, GC-NSD, GC-MS	-Application of non-toxic reagents	-Mainly used for aromatic isocyanates -Low sensitivity -Use of volatile reagents	[89-92]
2-PP	TLC, LC-UV/VIS	-Higher stability than the nitro reagent	-Absorption in the low-wavelength range -Low molar absorptivity -Sensitivity toward oxidation	[93,94,98-100]
NMA, MNMA	LC-FLUO, LC-UV/VIS	-Fluorescence detection -Better sensitivity and reactivity than the nitro reagent	-Moderate chromatographic separation and solubility problems (NMA) -Instability and light sensitivity (MNMA)	[101,102]

Method	Methodology	Advantages	Disadvantages	References
Aniline	LC-UV/VIS	-Possibility for liquid chromatographic separation	-Poor detection limits -Oxidation of reagent during sampling	[103]
DEA	LC-UV/VIS	-Good separation on RP columns	-High volatility of the reagent -Limited to aromatic isocyanates	[36,104]
MAMA	LC-UV/VIS, LC-FLUO	-Fluorescence detection -Highly sensitive UV/VIS detection -TRIG determination by means of UV/VIS detection	-Light sensitivity of reagent and derivatives	[76-78,105, 106]
2-MP	LC-UV/VIS, LC-ECD, LC-MS/MS, CZE-UV/VIS	-Reagent stability and reactivity -Identification based on detector ratios (UV/VIS:ECD) -TRIG determination with ECD -Mass spectrometric and tandem mass spectrometric detection	-Restriction of TRIG determination to ECD -Absorption in the low-wavelength range -Low molar absorptivity -Limited reproducibility and sensitivity for CZE	[75,107-110, 112-119,122]
TRYP	LC-FLUO, LC-ECD	-Fluorescence detection -Structurally independent detector responses -Poor solubility in common solvents	-Fluorescence excitation in the low-wavelength range (275 nm) -Lower reactivity of the primary amine function compared to secondary amines	[123-130]
DBA	LC-UV/VIS, LC-MS, LC-MS/MS, LC-CLND	-High reactivity, in part due to high impinger concentrations -Good sensitivity in LC-MS and LC-MS/MS	-Limitation mainly to impinger sampling due to high volatility of the reagent -UV/VIS only possible for aromatic isocyanates	[36,37,81-83, 134-136,139, 140]

Method	Methodology	Advantages	Disadvantages	References
MAP	LC-UV/VIS, LC-FLUO, LC-ECD	-Rapid reaction with isocyanates -Fluorescence detection -ECD and UV/VIS responses nearly independent of isocyanate structures	-Limited solubility in commonly used LC eluents -Reagent not commercially available	[79,141,142-144]
PAC	LC-UV/VIS, LC-FLUO	-TRIG determination via simple measurement of one peak in a chromatogram	-Laborious derivatization and cleavage procedure -Low reactivity toward isocyanates -High potential for interferences from other electrophiles -Reagent not commercially available	[145,146]
NBDPZ	LC-UV/VIS, LC-FLUO, LC-MS/MS	-Strongly red-shifted UV/VIS absorption maxima -Fluorescence detection -Tandem mass spectrometric detection	-Limited reagent stability in solution	[147,148,176]
MMNTP	LC-UV/VIS, LC-FLUO	-Fluorescence detection	-Fluorescence LODs comparable to LODs in UV/VIS -Moderate chromatographic separation -Reagent not commercially available	[149]
FcPZ	LC-EC-MS	-Sensitivity	-No data available regarding stability and Reactivity -Reagent not commercially available	[151]

2.3 Sampling techniques for airborne isocyanates

Sampling, analysis and exposure assessment of airborne isocyanates are challenging tasks as they occur in a variety of chemical species, i.e. monomers, oligomers or polymers in workplace atmospheres. Moreover, they may be present as vapors, aerosols or adsorbed on particulate matter [51]. Workplace air - especially in production plants - is commonly a complex matrix comprising a broad range of other substances than isocyanates, e.g., alcohols, amines, oxidizers, dust particles and water. All of these are prone to interfere with sampling and analysis of isocyanates. Although this seems to be trivial it is important to note that a sampling method being suitable for one physical form or one specific application is not always suitable for another [152].

Briefly, air sampling methods can be classified into two major groups: On the one hand, there are active sampling methods, which are using sampling pumps to deliver the air sample to the collection device [153-155]. In these cases, the sample volume is based on flow rate and sampling time. On the other hand, there are passive sampling methods relying on diffusion, which do not require a pump [156-158].

2.3.1 Active sampling methods for airborne isocyanates

The continuous on-line monitoring of airborne isocyanates has not yet been established for routine analysis. Owing to quite low concentrations of, mostly complex, isocyanate mixtures in air, an enrichment step is required that is often incompatible with continuous monitoring and thus leading to low time

resolution. Nevertheless, already in the 1970s different continuous monitors were introduced most of which are reviewed in [159]. Monitor systems such as the MDA 7100, MDA Isologger or GMD instruments are said to be promising [50]. Nevertheless, reliable data and broad field test studies have not yet been published. In 1996, Dharmarajan published an evaluation study in which nine personal continuous paper-tape monitors for TDI were tested [160]. It turned out that most instruments failed at high relative humidity conditions, and both high and low TDI concentrations were underestimated.

Today, with respect to active, i.e. pumped, sampling, mainly the following sampling systems - in combination with or without one of the above-mentioned reagents - are used:

- impingers filled with an absorbing solution [80,91,107,129,130,142,161]
- sampling tubes filled with a reagent-coated solid [113,147]
- reagent-coated filter samplers [97,107,118,119,162,165]
- reagent-coated polyurethane foam (PUF) sponge samplers [166,167,168].

Among active sampling techniques, impingers filled with an absorbing solution have already been used for decades. They can be used either as pure absorbing devices, in this case derivatization of the enriched analytes is performed subsequent to air sampling, or, in case that derivatization of the reactive analytes is carried out directly in the sampling solution, as chemisorptive devices. Owing to the high reactivity of isocyanates, enrichment

and derivatization are mostly carried out simultaneously. Impinger sampling allows the use of large reagent excess, thus leading to advantageous derivatization kinetics and nearly quantitative recovery rates. However, the use of large amounts of organic solutions in combination with a bulky instrumental set-up renders impinger sampling a laborious and awkward method that is not very suitable for personal air monitoring at workplaces. The evaporation of solvent during sampling is a source of major concern. Therefore, most personal sampling methods rely on the application of solvent-free systems such as impregnated filters [97,107,118,119,162,164] or reagent-coated sorbent tubes [113,147]. However, in some cases, especially in fast-curing environments, the use of impingers may be necessary to guarantee complete derivatization. E.g., NIOSH Method 5525 sometimes uses an impinger filled with a non-volatile, non-toxic solvent that is removed prior to analysis by solid-phase extraction [143]. Although adsorptive enrichment of isocyanates, e.g. on Tenax-TA [44], was described as another sampling approach, it has turned out to be no alternative to chemisorptive enrichment. Reagent-coated sorbent tubes allow a longer contact time between analytes and derivatizing agents than filters do. However, sorbent tubes are limited by high back pressures at elevated flow rates. In contrast, filters allow higher sampling rates limited by shorter contact times. Different filter cassette geometries have been suggested, one of which is schematically shown in Fig. 2.5 [169]. Furthermore, filters can be easily coupled in series in order to separate aerosol-phase isocyanates from gas-phase isocyanates. In this case, the first filter is uncoated with the goal to remove aerosols while the second one is coated to derivatize the gaseous analytes [163,170].

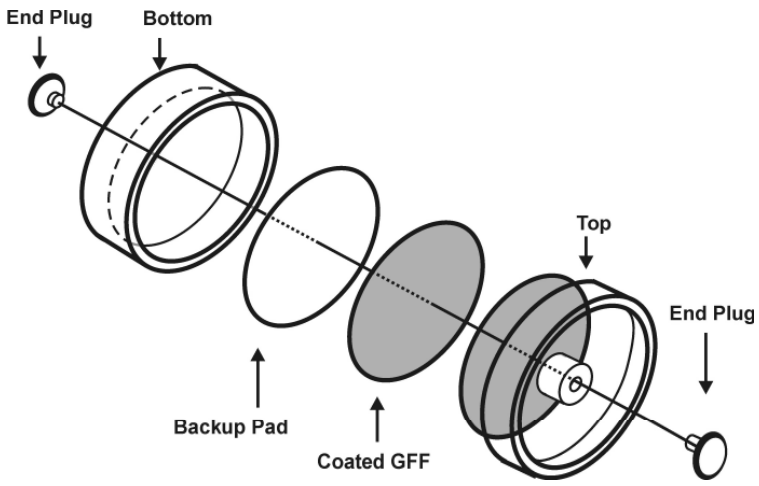

Fig. 2.5: Schematic set-up of a filter-housing system for air sampling (Iso-Chek® configuration [169]). A reagentless filter is placed in front, followed by a reagent-coated glass-fiber filter (GFF) that is placed in series with a backup pad.

Nevertheless, owing to both adsorption of vapors on the front filter and volatilization of aerosol-bound semi-volatile species towards the second filter, not in all cases a double filter set-up guarantees complete separation and determination of free and aerosol-bound isocyanates.

Several studies have been carried out to compare the sampling efficiencies of solvent-free samplers with those of impingers. The results have been reviewed and discussed by Streicher and co-workers [51,171]. In laboratory tests, solvent-free samplers often gave higher results than impingers, while in field comparisons this order was reversed, as the results obtained from impinger sampling indicated higher concentrations. Filters do sample both the gas and particulate phase, but the derivatization reaction is not always

efficient, which is mainly due a localized depletion of reagent and a poor mixing kinetics. The impinger methods used for isocyanates cannot completely collect particles smaller than 1.5 µm, while the gas phase is efficiently sampled. Whereas in the field many reactive species may compete for the derivatizing agent, in the laboratory usually water is the only species competing for the available isocyanate. Additionally, losses and overestimation can occur because of adhesion effects to walls and sampler material of filter cassettes. Depending on the kind of isocyanate exposure and expected particle size distribution, the appropriate sampling device or a combination of both should be considered.

To combine the advantageous reaction characteristics of impinger sampling with the easy-to-use filter sampling, polyurethane foam (PUF) coated sponge samplers [166,167] as well as natural sponge samplers [168] have been introduced. For the preparation of the PUF sampler, the coating reagent was dissolved in dimethylsulfoxide, and the sponge material was then coated with this solution. Thus, the isocyanates soaked through the sampler quickly react with an excess of derivatizing reagent in a solution environment [166]. Owing to its ease of use, especially when being compared to impinger sampling, the PUF sampler may be a promising alternative to established sampling techniques. However, only few studies on this topic have been yet published [166,167].

In brief, active sampling allows rapid and - owing to the possibility of large-volume sampling - sensitive determination of airborne isocyanates provided

that an appropriate reagent and an adequate sampler have been selected. Nevertheless, pumps have disadvantages of cost, size, possible break-down and frequent need for calibration [152].

2.3.2 Diffusive sampling methods for airborne isocyanates

In many workplace atmospheres, complex mixtures of aerosol-bound and vapor-phase isocyanates can be found. Sometimes, e.g. during autobody spray painting, aerosol-phase isocyanates represent even the primary inhalation hazard, while in other cases mainly vapor-phase isocyanates are of major concern. For monitoring of these vapor-phase isocyanates, diffusive or passive sampling devices offer an interesting alternative to pumped sampling. As stated above, active sampling procedures are not the most suitable for personal monitoring at workplaces - especially when it comes to sampling close to the breathing zone [148]. In these cases, diffusive samplers are easier to use, and no specially trained personnel are needed to carry out the measurement. Several techniques and sampling devices are known in order to obtain controlled diffusion [156]. While vapor-phase isocyanates diffuse sufficiently fast, aerosol-bound compounds are too slow to reach the collector surface of the passive sampling device. The sample volume is depending on sampling time and diffusion rate of the analyte molecule. According to Fick's first law of diffusion, the sampling rate (analyte uptake per time unit) is constant for one type of sampler, with small variations for different temperatures due to temperature dependency of the diffusion coefficients. The respective equations are summarized in Scheme 2.1.

$$J = \frac{dn}{dt} = -DA\frac{\partial c}{\partial x} \quad \textbf{(I)} \qquad J = \frac{m}{t} = DA\frac{C - C_0}{L} \quad \textbf{(III)}$$

$$-\frac{dc}{dx} = \frac{C - C_0}{L} \quad \textbf{(II)} \qquad \frac{m}{t} = DA\frac{C}{L} = SC \quad \textbf{(IV)}$$

dn: amount of analyte that passes area **A** in time **t**;
∂c/∂x: concentration gradient;
D: diffusion coefficient;
C_0: concentration at the collector surface; $C_0 = 0$, (due to immediate reaction on the collector surface);
S: sampling rate;
L: diffusion path length.

Scheme 2.1: Fick's first law of diffusion (I, II and III). Provided that the concentration of the analyte on the collector surface (C_0) equals zero, the sampling rate S is only dependent on the diffusion coefficient D and sampler geometry. The latter in expressed by area A and diffusion pathlength L.

Sampling rates must be empirically determined for each analyte, which often is very laborious. However, after laboratory and field validation, diffusive sampling devices are very powerful tools for personal exposure assessment, as they are very convenient and easy to use for non-educated personnel. Especially long-term sampling periods, e.g. full 8 h work-shifts can be easily carried out. Usually, used samplers can be sent by mail to a laboratory for analysis. Even though sampling rates could also be calculated from estimated diffusion coefficients [172], this method is erroneous and not recommended for unknown compounds. Generally, a negative bias can be expected if the

analytes are not exclusively present in vapor phase, because particles and aerosols are diffusing much slower and are thus not collected by means of diffusive samplers.

In the fields of isocyanate analysis, only a limited number of papers have yet been published describing the application of passive sampling devices. In 1989, Rando *et al.* described a passive sampler for the determination of toluene diisocyanate [173,174]. The set-up of the sampler is schematically shown in Fig. 2.6. It comprises a wind-screen on its top and an inner

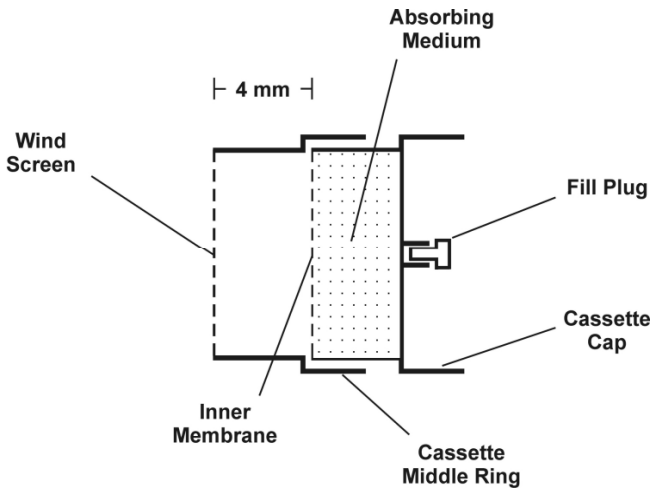

Fig. 2.6: Schematic set-up of a re-usable passive sampler. The wind screen is to avoid convective transport of air towards the surface of the absorbing medium.

membrane in order to guarantee that analyte transport towards the absorber surface is only based on diffusion. The absorbing medium is 0.5% sulfuric acid, which - upon contact with isocyanates - hydrolyzes them to give the

corresponding amines. Furthermore, the absorbing medium is spiked with sulfamic acid (HSO_3NH_2) to eliminate interferences from airborne nitrogen dioxide. Subsequent to sampling, hydrolyzed TDI is colorimetrically determined by means of a modified Marcali method [173]. The described passive sampler was reported to be suited for monitoring of occupational exposure doses greater than 10 ppb·h (~ 65 $\mu g \cdot m^{-3} \cdot h$) [174]. Although the sampler is re-usable and shows reliable sampling efficiency, sensitivity is limited by the Marcali method used for detection.

Recently, Levin and co-workers introduced a passive sampling system for airborne methyl isocyanate combining the sensitivity of the 2-MP method with a reliable sampling on reagent-coated glass fiber filters [175]. Impregnated filters (sample and control filter) are placed in a badge housing that is sealed by a screen and a sliding cover. To start sampling, the cover is removed, and for analysis, the control filter is used for the blank determination. Analysis was carried out by means of LC-MS/MS. Sampling rate was thus determined to be 15.6 $mL \cdot min^{-1}$, and LOD were 1 $\mu g \cdot m^{-3}$ (0.4 ppb) for a 15-min sampling and 3 $\mu g \cdot m^{-3}$ (1.3 ppb) for a 5-min sampling interval, respectively. The sampler was validated for relative air humidity between 20% and 80%, which turned out to have no influence on sampling rate.

Subsequently, Henneken *et al.* developed a similar passive sampler for MIC, which was based on the use of NBDPZ-coated glass fiber filters [148]. The respective sampler is schematically shown in Fig. 2.7. After elution of sample and control filter, quantification can be carried out either by LC-fluorescence

or LC-MS/MS detection. For fluorescence detection, the limit of detection was 1 $\mu g \cdot m^{-3}$ (0.4 ppb) for a 15 min sampling and 0.2 $\mu g \cdot m^{-3}$ (0.08 ppb) for an 8-h

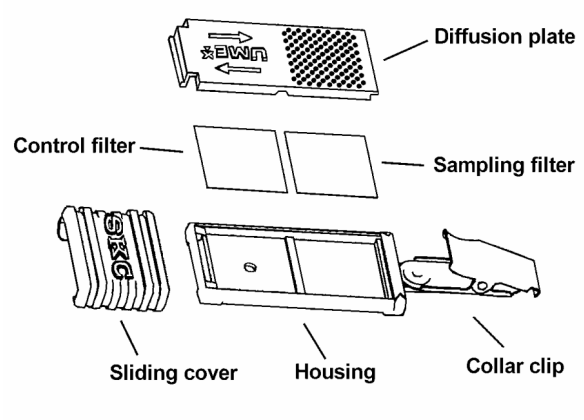

Fig. 2.7: A passive sampler based on the use of reagent-coated filters. Only the sampling filter is brought into contact with air, while the control filter is to determine the reagent background.

sampling period, respectively. For LC-MS/MS analysis, LOD were 0.4 $\mu g \cdot m^{-3}$ (0.17 ppb) for a 15-min sampling and 0.05 $\mu g \cdot m^{-3}$ (0.02 ppb) for an 8-h sampling interval, respectively. Although the NBDPZ sampler showed better sensitivity than the 2-MP filter sampler, sampling with a reproducible sampling rate was only applicable at low-humidity conditions around 20% RH [148], while at high humidity, sampling was failing. In a very recent paper, the same authors reported that the humidity problem could be overcome by changing the filter material from glass fiber to polystyrene divinylbenzene (SDB) [176]. Thus, sampling rates became independent of air humidity, and MIC sampling could also be carried out at high-humidity conditions. Furthermore, the same passive sampler based on NBDPZ-coated SDB filters was successfully

applied and validated for the determination of ethyl and phenyl isocyanate showing constant sampling rates at all humidity conditions [177].

Another sampling approach also relying on diffusion has been reported by Batlle *et al.* in 2001 [178]. They used solid-phase microextraction (SPME) with on-fiber derivatization for the determination of gaseous toluene diisocyanate. SPME utilizes a small, fused silica fiber with a polymeric coating and was already successfully applied to the determination of volatile organic compounds (VOCs), formaldehyde, and particulate matter in air [179]. For TDI analysis, dibutylamine was loaded onto a poly(dimethyl-siloxane)/divinylbenzene (PDMS/DVB) fiber coating. The loaded sampler was subsequently exposed to atmospheres of gaseous 2,4-TDI for 60 min. The TDI-DBA derivatives were determined by LC-MS/MS, and the linearity of the method ranged from 53 to 3,100 $\mu g \cdot m^{-3}$. The detection limit was approximately 2 $\mu g \cdot m^{-3}$ (0.25 ppb) for a 60-min sampling interval. In combination with the ease of use, SPME with on-fiber derivatization provides a wide linear range and sufficient limits of detection. Nevertheless, the reagent readily evaporates from the fiber coating, thus yielding only 25% of the initial DBA loading after three hours [178]. Furthermore, time consuming calibration is required for the analysis of unknown concentrations of TDI.

In a nutshell, passive sampling devices based on reagent-coated filters are easy-to-use and yield reproducible sampling rates in combination with low limits of detection even for short-term sampling. However, filter materials as well as derivatizing agent have to be thoroughly validated in advance. Special

attention has to be directed to the influence of relative humidity as has recently been reported [176]. SPME in combination with on-fiber derivatization is promising provided that a non-volatile derivatization reagent is used for fiber coating.

2.3.3 Denuder sampling of airborne isocyanates

Isocyanates are often simultaneously present as gas-phase analytes, dissolved in aerosols and adsorbed on particulate matter [51]. In many cases, it is important to know the amount of different physical states of airborne isocyanates in order to evaluate exposure control as well as the site of respiratory deposition or uptake [74]. The combined use of denuders and filters allows the simultaneous collection of mixtures of vapor and condensed-phase analytes. Denuders make use of the fact that gases and submicron particles significantly differ in their diffusivities. Thus, by using a column whose wall serves as an adsorbent, gases rapidly diffuse and collect on the column wall during their passage while - owing to their inertia - particles pass through and can finally be collected on a filter [74]. The schematic set-up of a denuder/filter system is shown in Fig. 2.8. In 1994, Rando and Poovey introduced a dichotomous sampler for the determination of vapor and aerosol MDI [74]. The sampler consisted of two annular denuders in series with an aerosol filter. The denuder walls were coated with the nitro reagent in order to chemisorb the gaseous analytes, while aerosol particles were collected on glass fiber filter that had also been impregnated with the nitro reagent. Subsequent to sampling, denuder and filter were analyzed separately by means of reversed-phase LC with UV/VIS detection at 254 nm. It could be

shown that at concentrations above 75 µg·m^{-3} MDI partitioned between vapor and aerosol, and the dichotomous sampler could efficiently separate the vapor fraction, collected on the denuders, and the aerosol phase, collected on the filter. Later, the same authors reported the development of a similar system for the determination of HDI-derived TRIG [180]. In this set-up, the denuder was coated with a mixture of MAMA and tributylphosphate (TBP); TBP was used in order to enhance the collection of HDI vapor.

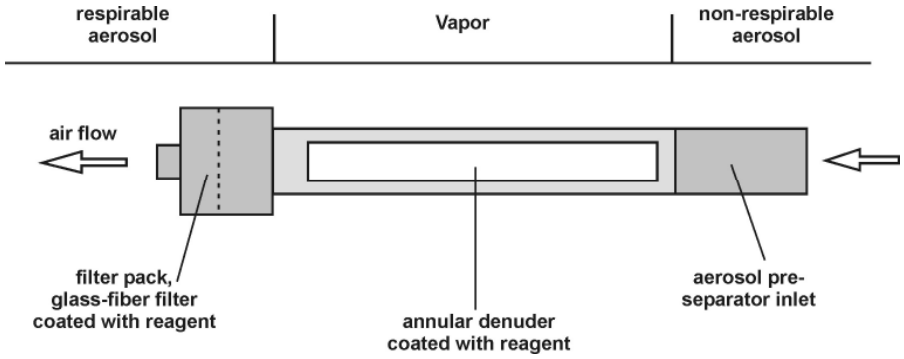

Fig. 2.8: Set-up of a dichotomous sampling device. An annular denuder sampling gaseous analytes is hyphenated to a filter, which is to collect aerosols and particulate matter.

Nordqvist *et al.* investigated the use of a DBA-coated cylindrical denuder for gaseous TDI [181] as well as for MDI aerosols [182]. While TDI sampling was carried out without placing a filter in series [181], MDI was sampled in a dichotomous set-up [182]. In both cases, the denuder stationary phase consisted of polydimethylsiloxane (SE-30), and quantitative analysis was carried out either by means of LC-MS/MS [181] or LC-MS [182]. The

denuder/filter system has also successfully been applied to the determination of TDI aerosols [183].

In summary, combined denuder/filter devices are a valuable tool for the simultaneous determination of aerosols and gas-phase isocyanates, and depending on the application, the best sampler geometry, annular or cylindrical, has to be selected.

2.4 Conclusions

Derivatizing strategies and most recent developments in the field of sampling of airborne isocyanates have been summarized and critically discussed. Over the last 30 years, a broad range of derivatizing agents has been introduced, and amine reagents have turned out to be best suited for rapid and quantitative derivatization. Among amine-based reagents, DBA, MAP, MAMA, 2-MP and NBDPZ have proven high sensitivity, sufficient stability and high reactivity toward isocyanates. However, none of these has yet been established to serve as a standard for analysis of airborne isocyanates. This is, on the one hand, mainly owing to the diversity of isocyanate air samples requiring different reagent performance and dedicated time-consuming method validation, and, on the other hand, owing to different legislative regulations and strategies in different countries. Today, the nitro reagent, alcohols or 2-PP are negligible when new method development is concerned, while for recently introduced reagents, such as MMNTP and FcPz, only few data are available yet. However, e.g., U.S. Occupational and Health

Administration (OSHA) continues to use 2-PP-based methods for the determination of isocyanates [98,99].

Active sampling applying impingers or sampling tubes is ideally suited for those air samples where high sensitivity is required, and where operations, e.g. in the automobile industries, have to be monitored. Although most sampling procedures still rely on active techniques, they are laborious and require bulky instrumentation as well as experienced personnel to carry out the analyses. Therefore, passive samplers have recently been introduced for low-molecular weight isocyanates and have turned out to be best suited for personal monitoring at workplaces where the individual isocyanate uptake of a worker has to be controlled. SPME is a promising alternative in the fields of diffusion-based methods; however, it has only been applied in combination with DBA yet. Further improvements in passive-sampler development for airborne isocyanates can be expected in the near future, and special focus has to be directed toward TDI, MDI and HDI. With respect to complex air samples containing gaseous as well as particulate or aerosol isocyanates, denuders were reported to provide good sampling efficiency in those cases, where simultaneous analysis is required. Annular and cylindrical denuders were described and successfully applied.

Although already made commercially available in the 1970s, automated systems for on-line sampling and analysis play only a minor role, and no fully validated system has been successfully introduced yet.

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Chapter 3

Diffusive Sampling of Methyl Isocyanate Using 4-Nitro-7-piperazino-benzo-2-oxa-1,3-diazole (NBDPZ) as Derivatizing Agent ‡

In this chapter, a diffusive sampling method for the determination of methyl isocyanate (MIC) in air is introduced. MIC is collected using a glass fiber filter impregnated with 4-Nitro-7-piperazino-benzo-2-oxa-1,3-diazole (NBDPZ). The formed urea derivative is desorbed from the filter with acetonitrile and analyzed by means of high-performance liquid chromatography (HPLC) using fluorescence detection (FLD) with λ_{ex} = 471 nm and λ_{em} = 540 nm. Additionally, a method was developed using tandem mass spectrometric (MS/MS) detection, which was performed as selected reaction monitoring (SRM) on the transition $[MIC\text{-}NBDPZ+H]^+$ (m/z 307) to $[NBDPZ+H]^+$ (m/z 250). The diffusive sampler was tested with MIC concentrations between 1 and 35 µg m^{-3}. The sampling periods varied from 15 minutes to 8 h, and the relative humidity (RH) was set from 20% up to 80%. The sampling rate for all 15 min experiments was determined to be 15.0 mL min^{-1} (using HPLC-FLD) with a relative standard deviation of 9.9% for 56 experiments. At 80% RH, only 15 min sampling gave acceptable results. Further experiments revealed that humidity did not affect the MIC derivative but the reagent on the filter prior

to and during sampling. The sampling rate for all experiments (including long term sampling) performed at 20% RH was found to be 15.0 mL min^{-1} with a relative standard deviation of 6.3% (N=42). The limit of quantification was 3 µg m^{-3} (LC/MS/MS: 1.3 µg m^{-3}) for 15 min sampling periods and 0.2 µg m^{-3} (LC/MS/MS: 0.15 µg m^{-3}) for 8 h sampling runs applying fluorescence detection.

‡ published in H. Henneken, R. Lindahl, A. Östin, M. Vogel, J.-O. Levin, U. Karst, *J. Environ. Monit,* **2003**, 5, 100 - 105.

3.1 Introduction

Monoisocyanates are widely used as intermediates during manufacture of various pharmaceutical and agricultural products. The widespread application of isocyanates is mainly based on their high reactivity towards nucleophilic agents, e.g. alcohols or amines, often showing quantitative reaction yields without any side reactions [1]. Methyl isocyanate (MIC) is primarily used in the production of carbamate-based pesticides. The toxicological effects of exposure to methyl isocyanate have been well explored after the Bhopal accident in 1984 and are associated with irritation of the respiratory system, the mucous membranes and the eyes [2-6]. Isocyanates in general have strong sensitizing properties and are supposed to be the most common inducers of occupational asthma [7]. Exposure can occur while handling the native compounds or heating and processing polyurethane (PUR) or other isocyanate-based products [8]. The low-molecular and highly volatile methyl isocyanate and isocyanic acid were found as degradation products resulting from PUR products that neither originally contained these substances nor have been used during manufacture [9]. In order to comply with the 5 ppb Threshold Limit Value (TLV) and to detect hazardous conditions it is important to monitor isocyanate concentrations in workplace atmospheres. Known procedures involve pumped sampling in combination with impingers, reagent-coated filters or impregnated sorbent tubes for collection of the analytes. Compounds containing amino groups, such as 1-(2-methoxyphenyl)piperazine (2MP), dibutylamine (DBA) and 1-(2-pyridyl)piperazine (2-PP) serve as derivatizing reagents [10-13]. Further known reagents are 9-(N-methylaminomethyl)anthracene (MAMA) or 1-(9-anthracenylmethyl)piperazine

(MAP) [14,15]. Most of these methods are well developed and sufficiently sensitive. However, they are very complicated, expensive and require a high degree of technical competence. Therefore, active sampling methods are less suitable for personal sampling to monitor occupational exposures. In order to perform sampling close to the breathing zone, it cannot be recommended to use impingers with possibly toxic solvents, which would represent an additional potential health risk to the performing person. In contrast, diffusive sampling offers several advantages when compared with pumped sampling. Diffusive samplers are easier to use than active sampling methods. No specially trained personnel is needed, and the sampling is much more comfortable for the performing person during work time. 8 h sampling periods can be done without inconvenience and no pumps (that have to be calibrated prior to sampling) are needed. However, regarding quantification, especially for short period sampling often going along with very low analyte concentrations, highly sensitive analytical methods are inevitable, such as LC/MS/MS. Recently, Zweigbergk *et. al.* presented a diffusive sampling method for the determination of methyl isocyanate in air based on the derivatization with 2-MP and subsequent analysis by means of LC/MS/MS [16]. Also recently, 4-Nitro-7-piperazino-benzo-2-oxa-1,3-diazole (NBDPZ) has been introduced as a derivatizing reagent for isocyanates [17,18]. On the basis of NBDPZ, methyl isocyanate can be determined by means of liquid chromatography with subsequent fluorescence, photometric or even mass spectrometric detection. The aim of this work was to develop a new user-friendly diffusive sampling method for the determination of airborne methyl isocyanate. The analysis should be performed using HPLC with fluorescence

detection (LC-FLD), but a method using the more sensitive tandem mass spectrometric detection should also be developed.

3.2 Exprimental part

Chemicals

Solvents used for HPLC analysis were methanol (HPLC gradient grade, J.T. Baker, Deventer, The Netherlands), acetonitrile (ultra gradient HPLC grade, J.T. Baker), water (purified using Milli-RQ systems, Millipore, Bedford, MA, USA), formic acid (98%, p.a., J.T. Baker) and ammonium acetate (98%, p.a., Merck, Darmstadt, Germany). For synthesis and filter coating, dichloromethane (p.a., J.T. Baker) and acetonitrile (HPLC grade S, Rathburn, Walkerburn, UK) were used. MIC-NBDPZ for calibration was prepared from methyl isocyanate (99%, Chem Service, West Chester, UK) and 4-Nitro-7-piperazino-benzo-2-oxa-1,3-diazole which was prepared from 4-chloro-7-nitro-benzo-2-oxa-1,3-diazole (98%, Fluka, Neu-Ulm, Germany) and piperazine (99%, Sigma-Aldrich, Steinheim, Germany) according to literature [17,18]. Toluene (J.T. Baker) used as solvent for pure MIC was dried with calcium hydride (Fluka, Buchs, Switzerland), distilled and stored over molecular sieves. 1-(2-methoxyphenyl)piperazine was obtained from Sigma (St. Louis, USA) and trideuterated 1-(2-methoxyphenyl)piperazine was delivered by Synthelec (Lund, Sweden). Acetic anhydride (99.5%, p.a.), propionic anhydride (98%), butyric anhydride (97%) and isopropyl isocyanate (99%) were obtained from Fluka (Neu-Ulm, Germany).

Synthesis

4-Nitro-7-piperazino-benzo-2-oxa-1,3-diazole (NBDPZ)

4-chloro-7-nitro-benzo-2-oxa-1,3-diazole (2.0 g, 10 mmol) in 120 mL dichloromethane was added dropwise to a stirred solution of 3.6 g (40 mmol) piperazine in 160 mL methanol. The NBDPZ precipitated as red crystals. It was filtered off and washed with cold water and methanol. The yield was 65%. The product was characterized by means of ^{1}H-NMR, IR, UV/Vis and fluorescence spectroscopy, mass spectrometry and elemental analysis [17].

Methyl isocyanate NBDPZ urea derivative

4-Nitro-7-piperazino-benzo-2-oxa-1,3-diazole (0.7 g, 3 mmol) was dissolved in 200 mL dichloromethane. 500 µL methyl isocyanate (9 mmol) were added rapidly with stirring. The MIC-NBDPZ precipitated as orange solid. Two mL methanol were added to remove excess MIC. The product was filtered off, washed with cold methanol and dried in a vacuum desiccator. The yield was 73%. The product was characterized by means of ^{1}H-NMR, IR, UV/Vis and fluorescence spectroscopy, mass spectrometry and elemental analysis [17].

Isopropyl isocyanate NBDPZ urea derivative (iPIC-NBDPZ)

NBDPZ (0.17 g, 0.7 mmol) was dissolved in 60 mL dichloromethane. 75 µL isopropyl isocyanate (0.8 mmol) were added rapidly with stirring. 40 mL n-hexane were added and the iPIC-NBDPZ precipitated as orange solid. Two mL methanol were added to remove excess isocyanate. The product was filtered off, washed with cold methanol and dried under reduced pressure. The yield was 49%.

Generation of standard atmospheres of methyl isocyanate

Methyl isocyanate spiked air was dynamically generated and controlled with respect to relative humidity (RH), temperature and concentration. A solution of pure MIC in toluene was injected into an evaporation chamber using a syringe pump (CMA/100, Carnegie Medicine, Stockholm, Sweden). The injection was performed through a nebulizer (J. E. Meinhard, Santa Ana, CA, USA). The airflow through the nebulizer was 0.5 L min^{-1}, and the aerosol from the nebulizer was mixed with air (4.5 L min^{-1}). After evaporation, the mixture was further diluted with humidified air and delivered to the exposure chamber with a total flow of 40 L min^{-1}. The airflows were controlled by a mass flow meter (Bronkhorst Hi-Tec, Ruurlo, The Netherlands) and the relative humidity was measured at the end of the exposure chamber (RH & T Indicator HMI 14, Vaisala, Helsinki, Finland). The concentration was always checked with a reference method described below, using six parallel ports which were mounted to the exposure chamber, thus allowing to take reference samples while diffusive sampling experiments were carried out. These experimentally determined values were always taken to calculate the uptake rate. The generation equipment with exposure chamber has been described previously [19,20].

Coated filters for diffusive sampling

NBDPZ (100 mg) was dissolved in 150 mL acetonitrile. Glass fiber filters (20 x 20 mm) were cut from round filters (type A/E, diameter 37 mm, SKC, Inc., PA, USA), put on a glass surface and impregnated two times with 100 µL of the reagent solution. The filters were subsequently allowed to dry for 20 minutes

under reduced pressure. One filter was placed under the sampling part of the sampler and another under the control part.

Coated filters for pumped sampling (reference method)

A pumped filter method described by Henriks-Eckerman et al. [21] was applied to verify the MIC concentrations of generated test atmospheres. Round glass fiber filters (GFB, diameter 25 mm, Whatman Ltd., Maidstone, UK) were placed on a glass surface and impregnated two times with 200 µL of a solution containing 500 mg 2-MP in 50 mL toluene (52 mmol L^{-1}). Afterwards, the filters were dried in a gentle stream of filtered air and finally stored in a refrigerator. Two filters were placed on top of each other in a Swinnex 25 filter cassette (Millipore, Milford, MA, USA). Six filter cassettes were connected to the ports on the exposure chamber. For a period of 15 min, samples were taken at a sampling rate of 0.2 to 0.3 L min^{-1}. For long-term experiments, this reference method was carried out twice, once in the beginning and once at the end of the test period, respectively.

Diffusive sampling

The diffusive sampler is schematically shown in Fig. 3.1. The housing, with dimensions of 86 x 28 x 9 mm, is made of polypropylene. Two impregnated filters are placed beneath a 2.9 mm thick screen. The part of the screen covering the sampling filter comprises 112 holes within a total area of 20 x 20 mm and with a diameter of 1.0 mm for each hole. A sliding cover was used to seal the holes when the sampler was not in use. The second filter (control filter) was used to quantify the methyl isocyanate blank. The sampler is

commercially available as UMEx 100 (with coated filters for sampling of formaldehyde) from SKC (Eighty Four, PA, USA).

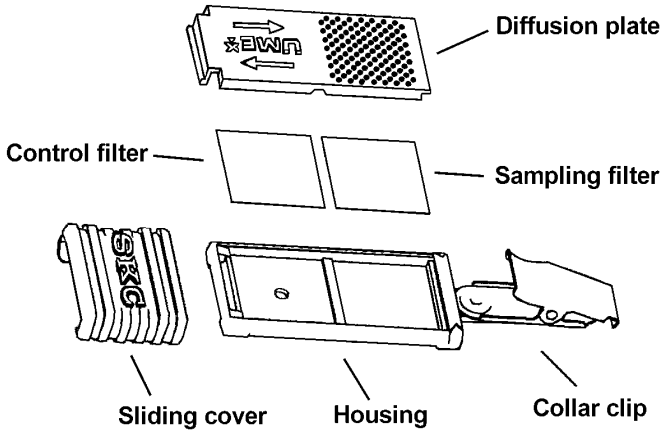

Fig. 3.1: Set-up of the UMEx diffusive sampler.

Diffusive sampling experiments were performed mainly according to EN 838[22] in the low concentration range of approximately 0.1 to 3 times the Swedish Time Weighted Averages (TWA) limit value, which is approximately 12 µg m^{-3} MIC (5 ppb). The Swedish Short-Term Exposure Limit (STEL) is set to 10 ppb MIC (23 µg m^{-3}) and equals the German TWA (MAK) value [23,24].

Laboratory validation

HPLC Instrumentation

The chromatographic system for LC/MS/MS analysis was delivered by Perkin Elmer (PE Series 200, Norwalk, CT, USA) and consisted of an autosampler and two micro pumps. The column outlet was coupled to a triple quadrupole mass spectrometer (API 2000, Applied Biosystems, Foster City, CA, USA)

with ESI (for the reference method) or APCI interface. Data were collected and analyzed using Analyst 1.1 Software (Applied Biosystems). The columns used were two GROM-SIL 80 ODS-7 pH columns with 4 µm particle size and 80 Å pore diameter (GROM, Herrenberg, Germany), 200 x 3 mm (for the reference method) and 60 x 4 mm for the MIC-NBDPZ method.

The chromatographic system used in combination with fluorescence detection was delivered by Waters (Milford, MA, USA) and consisted of two Millipore Model 510 pumps, a 717plus autosampler, a SAT/IN Communications Bus Module, a 474 Scanning Fluorescence Detector and an ERC 3415 degasser (Scantec, Gothenburg, Sweden). Data were collected using Millenium32 Chromatography Manager Version 3.05. The column used was an YMC-Pack Pro-C18 (YMC Co. Ltd., Kyoto, Japan), 150 x 4.6 mm, with 5 µm particle size and a pore diameter of 120 Å.

Filter Work-Up and HPLC Analysis

Reference Method

Exposed filters from one filter cassette were placed together in 4 mL HPLC vials containing 3 mL of 1-(2-methoxyphenyl)-piperazine (2-MP) in toluene (0.26 $mmolL^{-1}$). Unreacted 2-MP reagent was acetylated with acetic anhydride according to the procedure described in MDHS 25/3 [13] and trideuterated MIC-2-MP was added as internal standard. The samples were evaporated to dryness in a vacuum centrifuge (Speed-Vac 290, Savant, Farmingdale, NY, USA), redissolved in acetonitrile, centrifuged for 5 min at 5000 rpm (Uniequip UEC 13, Martinsried, Germany) and analyzed by means of LC/MS/MS. Later

experiments showed that the workup procedure could be simplified by leaving out the acetylating step, which did not affect the results. This was due to the fact that in this case only MIC was analyzed. If a more complex mixture of isocyanates had to be determined, this step could not be left out. By using 3 mL of a 2-MP solution (0.26 $mmolL^{-1}$) in acetonitrile instead of toluene for desorption, the LC/MS/MS analysis could be performed directly without evaporating and redissolving the samples.

The injection volume was 3 µL and the flow rate was set to 400 $\mu Lmin^{-1}$ applying a water/acetonitrile gradient (with 2 $mmolL^{-1}$ NH_4Ac in both eluents). After 5 min of isocratic elution at 60% acetonitrile, a linear gradient for 2 min to 95% acetonitrile was run subsequently.

The ESI capillary voltage was set to 5.5 kV and the added drying gas was heated to 320 °C. Selected Reaction Monitoring (SRM) was performed on the transition $[M+H]^+$ to $[2\text{-}MP+H]^+$, and data were collected with a dwell time of 200 ms. The analyte was quantified referring to the ratio between analyte and internal standard.

Diffusive samplers

Fluorescence detection

Sample and control filters of exposed diffusive samplers were transferred into separate HPLC vials and eluted with 3 mL acetonitrile. Subsequently, 150 µL aliquots were transferred into 200 µL vials and a standard solution of NBDPZ

derivative of isopropyl isocyanate (iPIC-NBDPZ) in acetonitrile was added as internal standard. These vials were retained for later MS/MS analysis.

The samples in the HPLC vials were injected directly into the HPLC system for analysis with fluorescence detection. The injection volume was 10 µL and the sample was eluted with 1.0 $mLmin^{-1}$ in a water/methanol gradient (with 0.25% (v/v) formic acid in both components). After 12 min of isocratic elution at 35% methanol, a linear gradient for 2 min to 100% methanol followed. The total time for the analysis was 30 min, including reequilibration. Conditions for fluorescence detection were λ_{ex} = 471 nm and λ_{em} = 540 nm.

Additionally, it was tested if the separation of NBDPZ from analyte derivative could be improved by addition of acid anhydrides to the sample. The anhydride was to react with excess NBDPZ from exposed diffusive samplers in order to elute after the MIC-NBDPZ. Therefore, an excess of appropriate anhydride was added to the vials containing the eluted filters in acetonitrile. The tested anhydrides were acetic, propionic and butyric anhydride. Propionic anhydride (PrAn) was found to be most suitable as the acetylated NBDPZ eluted just in front of the MIC-NBDPZ while the retention time of the butyric anhydride adduct was much longer than necessary. Thus, following the first analysis with LC-FLD, 1 µL of propionic anhydride was added to the sample solution to propionylate the NBDPZ reagent and the analysis was repeated.

Mass spectrometric detection

The solutions that were transferred to the 200 µL vials were taken for LC/MS/MS analysis. The injection volume was 10 µL and the sample was

eluted with 1.0 $mLmin^{-1}$ in a water/methanol gradient (with 0.5% (v/v) formic acid in both). After 5 min of isocratic elution at 20% methanol, a linear gradient for 2 min to 90% methanol followed. The total time for the analysis was 10 min, including reequilibration. The quadrupole was operated in the APCI(+) mode, the ionization voltage was set to 2.5 kV and the APCI temperature was set to 500 °C. Detection was performed as selected reaction monitoring (SRM) on the transition $[MIC\text{-}NBDPZ+H]^+$ (*m/z* 307) to $[NBDPZ+H]^+$ (*m/z* 250), and data were collected with a dwell time of 200 ms. The analyte was quantified referring to the ratio between analyte and internal standard (iPIC-NBDPZ). For the internal standard, SRM was performed on the transition $[iPIC\text{-}NBDPZ+H]^+$ (*m/z* 335) to $[NBDPZ+H]^+$ (*m/z* 250).

3.3 Results and discussion

The experiments performed are based on the derivatization reaction of NBDPZ with methyl isocyanate (Fig. 3.2). A reliable pumped sampling method

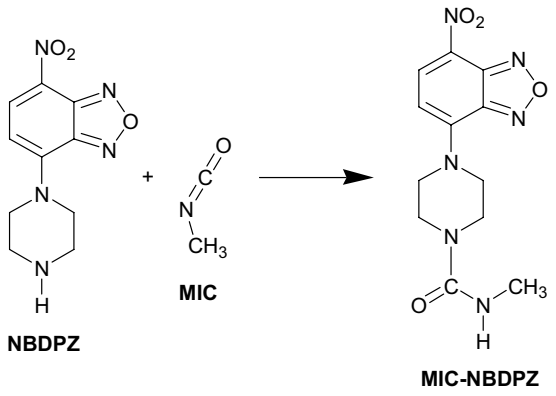

Fig. 3.2: Scheme of the derivatization reaction of NBDPZ with MIC.

with 2-MP coated filters was used as a reference method to validate the new NBDPZ diffusive sampler. The 2-MP reference method itself was validated using an independent DBA impinger method. This work is described in more detail in the paper of Zweigbergk *et. al.* [16].

Using NBDPZ as derivatizing reagent, fluorescence and mass spectrometric detection of isocyanate derivatives are possible as well as UV/Vis detection [18]. In Table 3.1, the instrumental limits of detection of different detection methods are listed. While UV/Vis detection does not show sufficient sensitivity for the given analytical problem, fluorescence and triple quadrupole MS detection are working well in the required concentration range.

Table 3.1: Instrumental limits of detection (LOD) of MIC-NBDPZ determined with different detection methods

Detection method	**LOD/ 10^{-9} mol·L^{-1}**	**LOD/ pg (analyte)**
Tandem-MS detection *m/z* (Q1) = 307 *m/z* (Q3) = 250	0.82	0.47
Fluorescence detection λ_{ex} = 471 nm; λ_{em} = 540 nm	6.5	3.7
UV/Vis detection λ = 480 nm	35	20

Since fluorescence detection can be performed by less experienced personnel and requires less expensive equipment than MS/MS spectrometry, attention has been primarily focused on that method although the developed

MS/MS method is very sensitive and highly selective. Nevertheless, most of the exposed and eluted diffusive samplers were analyzed applying both methods in order to obtain comparable results.

3.3.1 Fluorescence Detection

In order to achieve base-line separation of NBDPZ and its corresponding derivatives, it was necessary to add formic acid to the mobile phase to protonate the NBDPZ. Two problems had to be solved to obtain peaks that were easy to quantify:

As NBDPZ shows higher fluorescence intensity in acidic media than its urea derivatives do, as the reagent peak tails severely, and due to the fact that the eluted filter solution inherently contains a large excess of reagent, it is inevitable to choose an eluent mixture shifting the retention time of the MIC-peak well behind the reagent peak.

Additionally, during storage of the loaded diffusive samplers some interfering peaks appeared in the chromatogram. As these peaks had not been present in the chromatogram of the solution used for impregnation, the corresponding compounds were yielded after the impregnation process. As can be seen from Fig. 3.3, their intensity is increasing with storage time of the samplers (which were loaded immediately after impregnation). These interferences are acceptable provided that the extent shown in Fig. 3.3 is not exceeded. For that purpose, it is important to strictly follow the impregnation procedure described above, since first attempts with longer drying periods in a gentle

stream of filtered air led to interfering peaks of intensities that made quantification by means of fluorescence detection almost impossible.

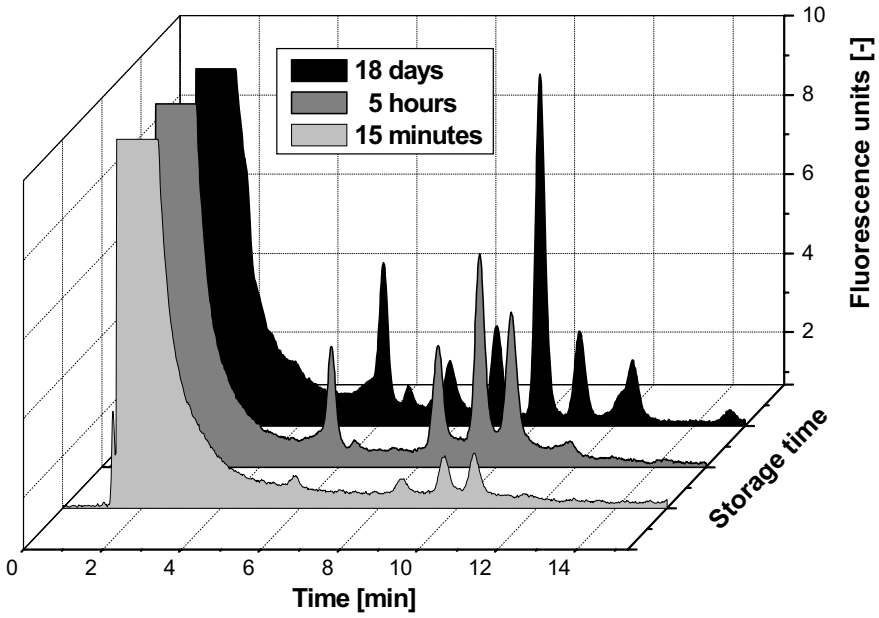

Fig. 3.3: Chromatograms of eluted NBDPZ coated filters from diffusive samplers stored for different time periods at room temperature.

Although several attempts were performed to identify the character of the interfering peaks by means of LC/MS/MS, the unknown compounds could not be structurally elucidated yet. As the addition of propionic anhydride had no influence on the retention times, some unknown components being present in air must have reacted with the piperazine functionality, blocking this position for propionylation or derivatization with isocyanates. Though only a small part of the reagent was affected in that way (the area of these disturbing peaks

accounted for only about 0.01-0.1% of the reagent peak area), this was well in the range of the analytes' peak areas.

Obviously, the influence of the facts stated above increased with a larger amount of reagent on the filter. Therefore, the NBDPZ amount applied for impregnation was thoroughly optimized: A fifty-fold excess based on the complete filter area and on an uptake rate of 15 mL min^{-1} during an 8 h experiment at 23 µg m^{-3} MIC was experimentally determined to be sufficient for quantitative collection of the analyte. This is equivalent to a range from LOD up to over 500 µg m^{-3} with 15 min sampling periods.
For the calculation of the minimum amount of reagent it must be considered that only one quarter of the filter surface is positioned beneath the holes and is thus accessible for the airborne analyte.

Based on the MIC concentration in the evaporation chamber given by the reference method and on the results obtained by analyzing the diffusive samplers, an uptake rate was determined for all experiments. The amount of MIC collected on the control part always accounts for about 10% of the sample part's result, which is owing to a leakage into the diffusive sampler that cannot be avoided, as the sampler is not completely tight. However, this is not crucial as the amount found on the control part is always to be subtracted from the amount found on the sample filter and the sampling rate is determined under these conditions. Fig. 3.4 shows chromatograms of a typical quantification of both filters from a diffusive sampler exposed to 19 µg m^{-3} MIC for 6 h.

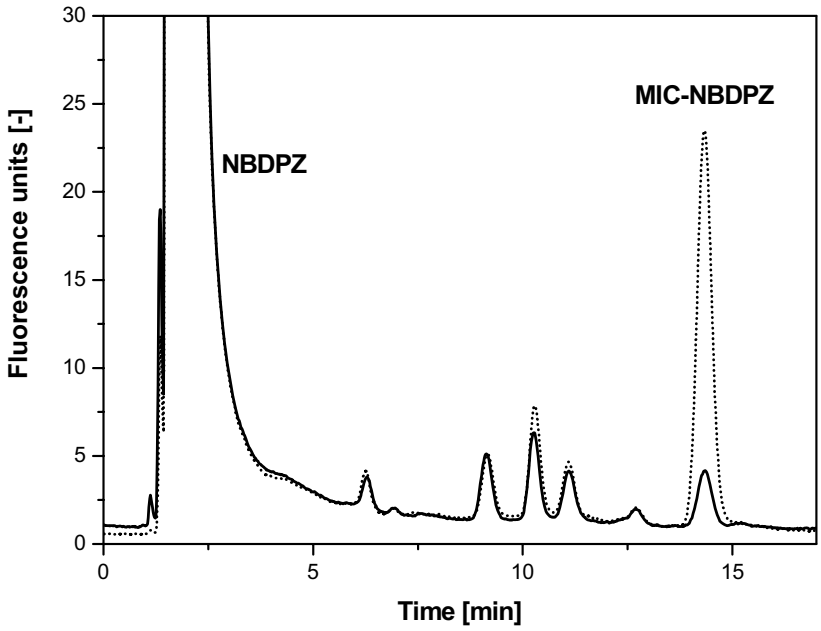

Fig. 3.4: Chromatograms of an eluted sample filter (dotted line) and an eluted control filter (full line) from a diffusive sampler exposed to 19 µg m^{-3} MIC (8 ppb) for 6 h.

The mean sampling rate for all 15 min experiments was determined to 15.0 mL min^{-1} with a relative standard deviation of 9.9% for 56 experiments. Only 15 min experiments yielded acceptable results at 80% RH. Under these conditions, 8 h experiments led to sampling rates ~ 1 mL min^{-1}. All results of the LC-FLD analysis are listed in Table 3.2.

The sampling rate for all experiments (including long term sampling) performed at 20% RH was found to be 15.0 mL min^{-1} with a relative standard deviation of 6.3% (N=42) (Table 3.2). The limit of quantification (LOQ) was 3 µg m^{-3} for 15 min sampling periods and 0.2 µg m^{-3} for 8 h sampling periods

Table 3.2: MIC concentrations of test atmospheres obtained by the pumped reference method and uptake rate of the NBDPZ diffusive samplers determined with fluorescence detection

			Pumped reference method		Diffusive sampler		
Measurement	**ST[a] /min**	**RH[b] (%)**	**c (MIC) /µg m^{-3}**	**RSD[c] (N = 6) (%)**	**SR[d] /mL min^{-1}**	**RSD (%)**	**N[e]**
1	15	20	25.8	7.3	15.8	6.1	9
2	15	80	27.8	2.5	14.8	8.9	6
3	15	20	34.7	2.3	13.7	2.8	6
4	15	20	17.8	2.0	14.4	1.8	6
5	15	20	4.4	8.6	14.9	3.9	6
6	15	80	15.1	6.2	13.6	15.8	6
7	15	80	3.3	4.5	15.1	13.8	5
8	16	80	32.4	1.9	15.5	6.9	6
9	15	80	16.7	2.8	14.1	10.3	6
10	356	20	18.9	3.3	15.0	2.9	9
11	480	20	1.4	7.0	8.4	2.1	6
12	480	20	17.5	2.1	15.9	3.8	6
13	480	80	1.2	6.9	1.0	13.9	6
14	480	80	16.8	3.4	1.1	15.6	6

[a]ST, sampling time. [b]RH, relative humidity. [c]RSD, relative standard deviation. [d]SR, sampling rate. [e]N, number of experiments.

using fluorescence detection. The LOQ was determined as ten times the mean standard deviation of six control filters exposed to MIC concentrations of 4.4 µg m^{-3} (for 15 min experiments) and 1.4 µg m^{-3} (for 8 h experiments).

It could be shown that the humidity did not affect the stability of the formed MIC derivative but the reagent itself, thus leading to decreased sample capacity of the filters. This is likely to be a physical problem, as the NBDPZ might be flushed from the filter surface into the filter. This theory is supported taking visual aspects into account: Impregnated filters (orange from NBDPZ, originally white) that were exposed to humidified air showed the pattern of the samplers' holes mapped in white on the filter surface. Table 3.3 shows the results of the experiment to examine the behavior of coated filters towards humidity:

Table 3.3: Diffusive samplers exposed to MIC-atmospheres without (A, D), before (B) and after (C) zero exposure to humidified air (80% RH) for 10 h

Series (N = 4)	MIC-NBDPZ (peak area units)
A	66600 ± 4200
B	60500 ± 3500
C	18000 ± 1200
D	63500 ± 2700

The samplers of series A were exposed to MIC only, of series B to MIC and subsequently to humidified air, of series C first to humidified air and then to MIC, and of series D again only to MIC. Each series comprised 4 samplers, MIC exposure was to 30 µg m^{-3} for 30 min at 20% RH, and exposure to

humidity was for 10 h at 80% RH. When the data sets were compared statistically, the Student t-test showed that the means of A, B and D were not significantly different at the 95% level.

The addition of propionic anhydride to the sample solution prior to analysis resolved the tailing problem, but the analysis time could not be reduced because the reaction products mentioned above were still present. The mean uptake rate was about the same as before, but the standard deviation was higher: The sampling rate was 14.8 mL min^{-1} with an RSD of 10.6% for all 15 min experiments (N=56) and 14.6 mL min^{-1} with an RSD of 8.9% for all experiments performed at 20% RH (N=42) (Table 3.4).

Table 3.4: Sampling rate of the NBDPZ diffusive samplers determined with fluorescence detection after addition of propionic anhydride [a]SR, sampling rate. [b]RSD, relative standard deviation

	Diffusive sampler	
Measurement	**SR /mL min^{-1}[a]**	**RSD (%)[b]**
1	15.5	6.4
2	15.9	5.9
3	14.5	4.3
4	14.7	6.2
5	12.6	11.0
6	14.0	11.0
7	15.2	5.2
8	15.4	8.0
9	13.7	5.8
10	15.3	3.9
11	8.8	3.1
12	14.9	4.5

[a]SR, sampling rate. [b]RSD, relative standard deviation.

It is not always reasonable to use this procedure, but in some cases it offers a possibility to improve the results: If the filter matrix is for any reason worse than illustrated in Fig. 3.3, the analyte peak might not be base-line separated from the previous peak (or could lay within the tailing) and could thus be difficult to quantify. To create such a situation, some filters were dried overnight in a gentle stream of filtered air after impregnation. These filters were spiked with MIC-NBDPZ and analyzed in the same way as exposed diffusive samplers, first without and subsequently with addition of propionic anhydride. The standard concentrations were chosen that number 3 and 4 equaled diffusive samplers exposed for 15 min to MIC concentrations at two times and exactly the TLV, respectively. As the results from Table 3.5 show, the recovery was much better after addition of PrAn, especially at low concentrations:

Table 3.5: Recovery of MIC-NBDPZ spiked samples (complex filter matrix) with and without addition of propionic anhydride (known concentration set to 100%)

Number	MIC-NBDPZ concentration /mol·L^{-1}	Recovery (direct analysis)	Recovery (after PrAn was added)
1	$8.9 \cdot 10^{-7}$	86.6 %	95.6 %
2	$8.9 \cdot 10^{-8}$	58.5 %	95.8 %
3	$3.6 \cdot 10^{-8}$	37.8 %	98.2 %
4	$1.8 \cdot 10^{-8}$	14.3 %	97.8 %
5	$7.1 \cdot 10^{-9}$	0 %	91.1 %

3.3.2 MS/MS Detection

If tandem mass spectrometry is used for detection, the selectivity is much higher. In this case, there are no tailing or coelution problems and the analysis time can be reduced to less than 10 minutes per run. The MS analysis was performed in the APCI(+)-mode as SRM on the transition $[MIC\text{-}NBDPZ+H]^+$ (*m/z* 307) to $[NBDPZ+H]^+$ (*m/z* 250) (Fig. 3.5).

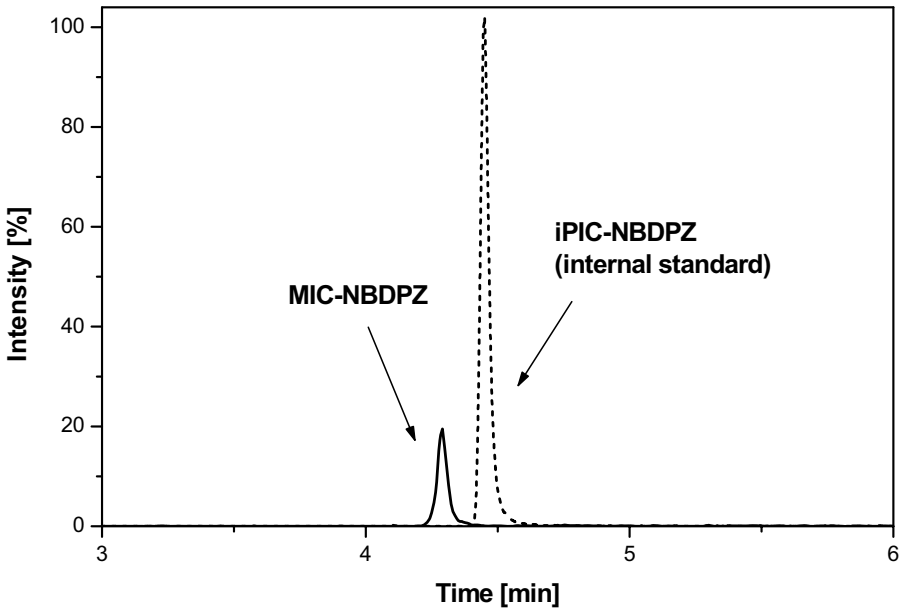

Fig. 3.5: LC-MS/MS analysis (APCI(+)) of an eluted sample filter from a diffusive sampler exposed to 10 ppb MIC (24 µg m^{-3}) for 15 min.

With MS/MS-detection, the sampling rate was determined to 11.8 mL min^{-1} with an RSD of 15.3 % for all MS experiments (N=56) (Table 3.6). This was significantly lower than the uptake rate obtained on the basis of fluorescence detection. That behavior could not be explained as always the same samples were analyzed with both methods. Since the deviation always showed the same tendency, the results could be evaluated without problems. The LOQ

was 1.3 µg m^{-3} for 15 min sampling periods and 0.15 µg m^{-3} for 8 h experiments.

Table 3.6: Sampling rate of the NBDPZ diffusive samplers determined by means of tandem mass spectrometry

	Diffusive sampler	
Measurement	**SR /mL min^{-1}[a]**	**RSD (%)[b]**
1	11.8	12.2
3	12.0	6.2
4	10.8	7.3
5	11.3	5.4
6	10.0	6.4
7	10.0	4.6
8	14.9	8.5
9	11.4	5.6
11	7.1	5.8
12	13.8	10.9

[a]SR, sampling rate. [b]RSD, relative standard deviation.

3.3.2 Shelf life

The shelf life of coated filters was investigated by storing impregnated filters for different time periods at room temperature prior to sampling. The recovery was 83% after 5 days, 60% after 11 days and only 17% after 50 days, with the results obtained from immediately prior to sampling impregnated filters set to 100%. Therefore, the storage time of impregnated filters prior to sampling must be minimized. No negative effect, e.g. loss of analyte was observed while storing the exposed samplers for five days at room temperature after sampling. This was expected, as it is known from the literature and from own experiments not shown in this chapter that the MIC-NBDPZ derivative is very

stable in solution and as solid compound over the complete period observed (more than 40 days) [18].

3.4 Conclusions

The experiments performed showed that the derivatization reaction of NBDPZ with methyl isocyanate can be used for collection of MIC in diffusive sampling devices with reagent-coated filters. As mentioned above, there is no passive sampling method known in the literature, which allows the determination of airborne MIC by means of HPLC/MS/MS and HPLC/FLD. A great advantage of the NBDPZ method is therefore the possibility to apply fluorescence detection for analysis in addition to the use of a more complicated and costly LC/MS/MS-system to obtain the required sensitivity. A problem is the strong dependence upon humidity, which inhibits long sampling periods if high relative humidity is present. The reduced shelf life limits the use of this diffusive sampler to applications that allow sampling within about one week from impregnation whereas the storage time from sampling to analysis is less problematic.

In summary, the developed method meets the requirements for a rapid determination if MIC concentrations in workplace atmospheres are in the range of the threshold values. Despite shelf-life problems, the NBDPZ diffusive sampler is well suitable for screening purposes.

3.5 References

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Chapter 4

Effects of Humidity and Filter Material on Diffusive Sampling of Isocyanates using Reagent-Coated Filters‡

Diffusive sampling of methyl isocyanate (MIC) on 4-nitro-7-piperazinobenzo-2-oxa-1,3-diazole (NBDPZ)-coated glass fibre (GF) filters is strongly affected by high relative humidity (RH) conditions. It is shown that the humidity interference is a physical phenomenon, based on displacement of reagent from the filter surface. In this chapter, this drawback has been overcome by changing the filter material to the less polar polystyrene divinyl benzene (SDB). A series of experiments was performed to compare the analyte uptake on the two filter materials for different sampling periods and analyte concentrations at both low and high RH conditions. Additionally, the materials were investigated as well for passive sampling of ethyl (EIC) and phenyl isocyanate (PhIC) with NBDPZ and 1-(2-methoxyphenyl) piperazine (2-MP) as alternative derivatizing agent. Using 2-MP, the mean GF/SDB response ratios were determined to be 1.02 for MIC (RSD: 6.1%) and 1.03 for EIC (RSD: 6.8%), whereas PhIC could only be determined on SDB filters. Using NBDPZ as reagent, the negative influence of high humidity disappeared when SDB filters were used instead of GF filters. Even at low RH conditions, sampling

with SDB material generally resulted in a higher analyte uptake than with GF filters. The GF/SDB response ratios were independent of sampling time or analyte concentration and were determined to be 0.70 (RSD: 4.7%) for MIC, 0.84 (RSD: 4.5%) for EIC and 0.95 (RSD 5.4%) for PhIC, meaning that the NBDPZ diffusive sampler based on SDB can be used at all humidity conditions without any restrictions.

[‡] published in H. Henneken, M. Vogel, U. Karst, *J. Environ. Monit,* **2006**, 8, 1014-1019.

4.1 Introduction

Occupational exposure to isocyanates may occur during spray-painting operations or at such workplaces where polyurethane (PUR) products are mechanically or thermally treated. Isocyanates are reactive and hazardous substances showing acute and chronic toxicity to the respiratory system [1]. They have been identified as one of the most common causes of occupational asthma in industrialized countries [2-4]. In the Netherlands, the legal threshold limit values (TLV) for all isocyanates are currently set to 5 ppb_v [5]. Many different methods for sampling and analysis of isocyanates have been presented in the past decades, mostly using amine reagents for derivatization of the highly reactive and unstable compounds. Among these, established reagents are secondary amines such as 1-(2-methoxyphenyl) piperazine (2-MP) [6] and 1-(2-pyridyl) piperazine (2-PP) [7]; while 4-nitro-7-piperazinobenzo-2-oxa-1,3-diazole (NBDPZ) [8] and ferrocenoyl piperazide (FcPZ) [9] have recently been introduced as derivatizing agents (Fig. 4.1). Most methods for the determination of airborne isocyanates are based on active collection of the analytes using either impingers filled with reagent solutions or reagent-coated filters for pumped sampling. Sampling is mostly followed by LC analysis with UV, electrochemical (EC), fluorescence or mass spectrometric detection or combinations hereof. In Europe, standard methods using 2-MP-coated filters or impingers with 2-MP solutions are commonly used [10-12], while in the US methods based on the 2-PP reagent are favored [13,14].

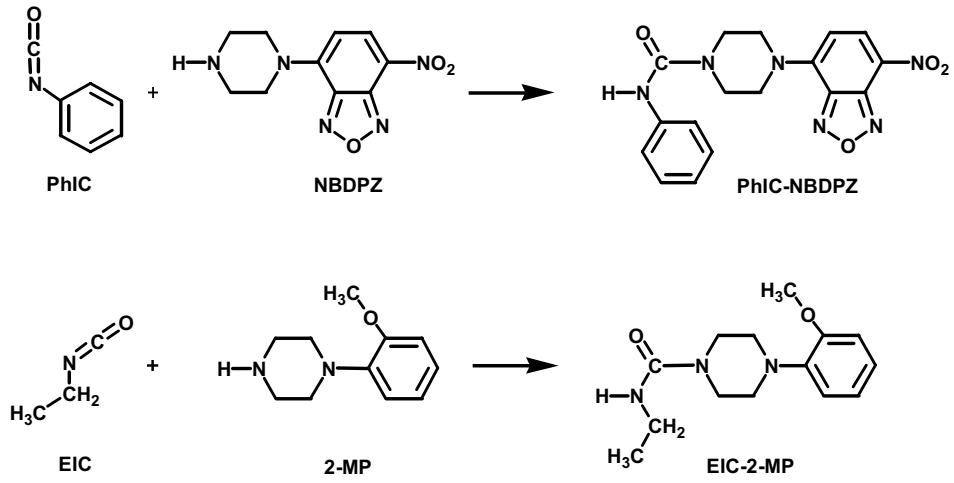

Fig. 4.1: Reaction schemes of phenyl isocyanate with NBDPZ and ethyl isocyanate with 2MP

As the analytes may occur both as vapor or aerosol, the accurate and sensitive determination of isocyanates and the assessment of isocyanate exposure are complex and difficult [15]. Often not only monomeric species are observed but complex mixtures that additionally contain oligomers and polymers. When PUR products are decomposed, complex mixtures of isocyanates, also including low molecular monomers such as methyl isocyanate (MIC), ethyl isocyanate (EIC) and phenyl isocyanate (PhIC) are found as degradation products in workplace environments [16-19]. Low molecular weight compounds predominantly occur in the vapour phase and are therefore well suited to passive sampling, which has been increasingly considered in recent years as a reliable standard procedure and good alternative to active sampling. Especially the ease of handling is a great advantage compared to active methods [20]. However, only little effort has been put into the development of diffusive sampling methods for the

determination of airborne isocyanates yet. In the late 1980s, Rando et al. described a passive dosimeter for toluene diisocyanate based on a modification of the colorimetric Marcali method [21,22]. Up to now, there are only two completely validated methods for methyl isocyanate (MIC) known from the literature [23,24]. Besides that, Batlle et al. described two methods for TDI and HDI based on diffusion-controlled sampling using SPME devices [25,26].

The low occupational exposure limits for isocyanates require methods providing low limits of detection, especially as the sample volumes for diffusive sampling are inherently small; unless very long sampling periods are accomplished. However, analytical detection limits have been continuously lowered owing to the use of sensitive detection such as mass spectrometry [27-31]. In 2003, a diffusive sampling method for methyl isocyanate (MIC) based on MIC collection on 4-nitro-7-piperazinobenzo-2-oxa-1,3-diazole (NBDPZ)-impregnated glass fiber filters and fluorescence detection was published. The sampling rate was determined to be 15 $mL \cdot min^{-1}$. However, a limitation in atmospheres of high relative humidity was reported, which did not allow applying long-term sampling of MIC at such humid conditions [24]. The aim of the present work was to overcome this humidity limitation by using different filter materials that are coated with NBDPZ. Furthermore, it should be investigated whether passive sampling using NBDPZ filters can be applied to other isocyanates than MIC and whether this might also be affected by high humidity. Within this study, two types of filter materials have been tested. In order to compare the results obtained with an independent reference method,

experiments were also performed with diffusive samplers equipped with 2-MP-coated filters.

4.2 Experimental part

Chemicals

1-(2-Methoxyphenyl) piperazine (2-MP), ethyl and phenyl isocyanate (EIC, PhIC), formic acid and anhydrous toluene were purchased from Aldrich Chemie (Steinheim, Germany) in the highest quality available. Methyl isocyanate was obtained from Chem Service (West Chester, PA, USA). For the preparation of HPLC mobile phases, acetonitrile and water (both HPLC-S gradient grade) were obtained from Biosolve (Valkenswaard, The Netherlands). NBDPZ and its isocyanate derivatives were synthesized as described in [8]. Isocyanate derivatives of 2-MP were synthesized according to [10].

Diffusive sampler

The diffusive sampling device used in this study was a badge equipped with two reagent-impregnated filters of 20 x 20 mm each, one serving as sample part and the other as blank control. The sample filter was placed beneath a 2.9 mm thick diffusion plate containing 112 parallel diffusion channels (diameter 1 mm) through which the analyte reaches the collector surface. A solid plate covers the control filter and a sliding cover seals the diffusion plate when the sampler is not in use. The sampler is commercially available as UMEx 100 (with coated filters prepared for sampling of aldehydes and

amines) from SKC (Eighty Four, PA, USA). We replaced the original filter tapes with our own ones (see below).

Generation of test atmospheres

Isocyanate test atmospheres were generated by nebulizing standard solutions of isocyanates dissolved in anhydrous toluene into an air stream of known total flow, with all flows being adjusted by means of mass-flow controllers. The yielded aerosol was sprayed into an evaporation chamber and delivered to the test chamber, where the sampling experiments were performed. Dry air was delivered by a compressor model 2xOF302-40MD2 (Jun-Air, Nørresundby, Denmark) and could be humidified by leading parts of the air through gas-washing bottles filled with water. At the end of the exposure chamber, the relative humidity (RH) is measured with a handheld humidity meter. According to the values measured with the humidity meter, the flow of the humidified air stream was adjusted by means of mass flow controllers to generate the desired humidity. The whole system was made of Teflon®, glass and stainless steel to ensure maximum inertness. It was built according to similar equipment described in the literature [32,33].

Coated filters for diffusive sampling

NBDPZ (10 mg) was dissolved in 10 mL of acetonitrile. Glass fiber filters (type A/E, diameter 37 mm, SKC, Inc., PA, USA) or Empore SDB-XC extraction disks, diameter 90 mm (3M, St. Paul, MN, USA) were cut to 20 x 20 mm squares from round filters, put onto a glass surface and impregnated with 200 µL (in case of glass fiber) or 250 µL (in case of SDB material) of the reagent

solution. The filters were subsequently allowed to dry for 20 minutes under reduced pressure. One filter was placed under the sampling part of the sampler and another under the control part.

Regarding 2-MP impregnation, glass fiber or SDB filters were placed onto a glass surface and impregnated with 400 µL of a solution containing 500 mg of 2-MP in 50 mL of acetonitrile (52 mmol/L). Afterwards, the filters were dried under reduced pressure and finally stored in a refrigerator.

Diffusive sampling experiments

For direct comparisons of the performances of GF and SDB samplers, always at least 3 samplers of each type were simultaneously exposed to the same test atmosphere. As the relative difference between the uptake of the two filter materials was to be examined, no reference method was applied to verify the obtained concentration in air. From earlier experiences with the test equipment it was known that the generated isocyanate atmospheres were usually close to the expected concentrations (e.g., 92% of the calculated concentration for MIC, 98% of the calculated concentration for EIC) calculated based upon the concentration of the evaporated isocyanate standard solution. MIC, EIC and PhIC test atmospheres were generated in concentrations between 500 ppt_v and 30 ppb_v of the respective analytes, and diffusive sampling experiments were carried out at relative humidity (RH) conditions between 10 and 90%. Sampling periods were varied from 15 min up to 15 hrs. Two sets of experiments were performed using either NBDPZ or 2-MP as derivatizing reagent.

HPLC instrumentation

For HPLC analysis, a system comprising a binary gradient HPLC pump (HP1100 model GF1312A), an autosampler (HP1100 model G1313A) and a diode-array UV/Vis detector (HP1100 model G1315B; all from Agilent, Waldbronn, Germany) was connected to the mass spectrometric detector.

For separation of the 2-MP derivatives, a Prontosil® 120-5-C18-ace-EPS column with dimensions of 3 mm x 150 mm, particle size of 5 μm and pore size of 120 Å was selected (Bischoff Chromatography, Leonberg, Germany). NBDPZ derivatives were separated using a Prontosil® 120-5 Phenyl column with dimensions of 2 mm x 250 mm, particle size of 5 μm and pore size of120 Å (Bischoff Chromatography, Leonberg, Germany). For mass spectrometric detection, an Esquire 3000+ ion trap mass spectrometer (Bruker Daltonik, Bremen/Germany) equipped with an ESI interface (for analysis of 2-MP derivatives) and an APCI ion source (for NBDPZ derivatives) was used. All measurements were performed using the positive-ion MS detection mode.

Filter work-up

Sample and control filters of exposed diffusive samplers were transferred into separate vials and eluted with 3 mL of acetonitrile. Unlike SDB filter samples, glass fiber filter samples had to be centrifuged for 5 min at 5000 rpm prior to analysis in order to settle loose filter particles.

HPLC MS analysis

NBDPZ method

The injection volume was 5 µL, and the sample was eluted with 0.45 mL·min^{-1} in a water/acetonitrile gradient (with 0.05% (v/v) formic acid in the aqueous phase). Initially, a linear gradient from 30% to 40% ACN was applied within the first 5 min and subsequently to 100% ACN for another 5 min. After 1 min of isocratic elution with pure acetonitrile, a steep gradient for one minute back to 30% of ACN was applied. The total time for the analysis was 21 min, including re-equilibration. Tandem-mass spectra were recorded in the manual MS/MS mode, scanning from *m/z* = 100 to *m/z* = 700 employing the following APCI(+) MS parameters: Nebulizer pressure 50 psi, drying gas flow rate 5 L·min^{-1}, drying gas temperature 350°C, vaporizer temperature 450°C, corona current 4500 nA, V_{cap} 3115 V, capillary exit voltage 109.4 V, skimmer 40V, trap drive 35.0. Detection was performed as selected-reaction monitoring (SRM) on the transitions from the protonated isocyanate derivative [IC-NBDPZ+H]$^+$ (*m/z* = 307 for MIC; *m/z* = 321 for EIC and *m/z* = 369 for PhIC) to [NBDPZ+H]$^+$ (*m/z* = 250). Data were collected as neutral-loss scans of *m/z* = 57, *m/z* = 71 and *m/z* = 119, respectively with a maximum accumulation time of 200 ms. The analytes were quantified based on 6 point external calibrations run with each measurement. The resulting data were analyzed using DataAnalysis software version 3.1 (Bruker Daltonics).

2-MP method

The injection volume was 5 µL, and the sample was eluted with 0.3 mL·min^{-1} in a water/acetonitrile gradient (with 0.05% (v/v) formic acid in the aqueous

phase). A linear gradient from 30% to 90% ACN was applied within the first 2 min. After 3 min of isocratic elution with pure acetonitrile, the starting conditions were re-established within one minute. The total time for the analysis was 15 min, including re-equilibration. Tandem-mass spectra were recorded in the same way as described for the NBDPZ method, employing the following ESI-MS parameter settings: Nebulizer pressure 40 psi, drying gas flow rate 10 $L \cdot min^{-1}$, drying gas temperature 365°C, V_{cap} 5000 V, capillary exit voltage 109.8 V, skimmer 40V, trap drive 27.7.

4.3 Results and discussion

4.3.1 Humidity effects

NBDPZ as well as its isocyanate derivatives have proven to be stable compounds [8]. The secondary amine functionality within the piperazine group of NBDPZ is the only reactive moiety, and there is no reasonable route for a reaction with water. NBDPZ is even washed with water during the purification steps after synthesis [8]. Therefore, the negative influence of air humidity on passive sampling of MIC on NBDPZ-impregnated glass fiber filters is assumed to be a physical phenomenon. As von Zweigbergk *et al.* did not report a similar behavior in their work [23] when using the 2-MP reagent, an effect of humidity on the isocyanate test atmosphere concentration can be excluded. Also active reference sampling did not show any decomposition of the isocyanates even at high humidity conditions [23].

Based on these investigations, we concluded that the reason for the bad performance at high humidity consists in a continuous repelling of the

hydrophobic NBDPZ away from the polar glass fiber (GF) filter surface beneath the diffusion channels. This continuous repelling would also explain why short sampling periods were still yielding a more reasonable result for diffusive sampling of MIC, whereas longer ones are not possible to be applied at all [24].

On highly polar GF filters, air humidity might accumulate or condense on the filter surface. Hence, the use of a less polar filter material was considered to be a possibility to overcome the humidity drawback. In the following, polystyrene-divinyl benzene (SDB) filters were tested. When the coated and dried glass fiber and SDB filters were visually compared, one could observe a significant difference between both originally white filters: While the SDB filter showed a very intense yellow color as well as a perfectly smooth and homogeneous distribution over the whole surface, the GF filter looked more orange-red and textured.

After exposure to high humidity for 12 h, the GF sample filter exhibited the diffusion channel pattern slightly mapped in white on the filter surface, meaning that there was less reagent excess available for the analytes afterwards. For a diffusive sampler loaded with coated SDB filters, the surface afterwards looked as homogeneous as before.

The reagent's displacement from the GF filter surface is shown in Fig. 4.2, where a few microliters of water were applied to the centre of two impregnated GF and SDB filters. Obviously, NBDPZ is carried away from the GF material

by the water, while nothing happens on the SDB filter. SDB is so hydrophobic that the drop is not soaked up at all.

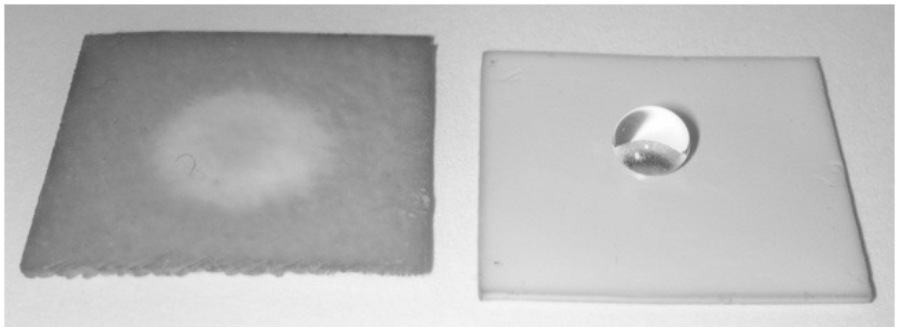

Fig. 4.2: A droplet of water, which was deposited onto a GF and a SDB filter, both impregnated with NBDPZ.

4.3.2 Diffusive sampling results

Initial diffusive sampling experiments of methyl isocyanate with NBDPZ-coated SDB filter tapes were promising, and the amount of MIC collected on this filter material was more than a factor of 10 higher than with the GF samplers (Fig. 4.3). In these experiments, relative humidity was set to 85% and sampling time was 480 min. Further experiments were then performed to confirm these preliminary findings for methyl isocyanate. In a second step, the use of NBDPZ-coated filters was extended towards passive sampling of ethyl and phenyl isocyanate. To compare the results obtained with passive sampling based on NBDPZ, additional experiments were subsequently accomplished using 2-MP impregnated filters (both SDB and GF material).

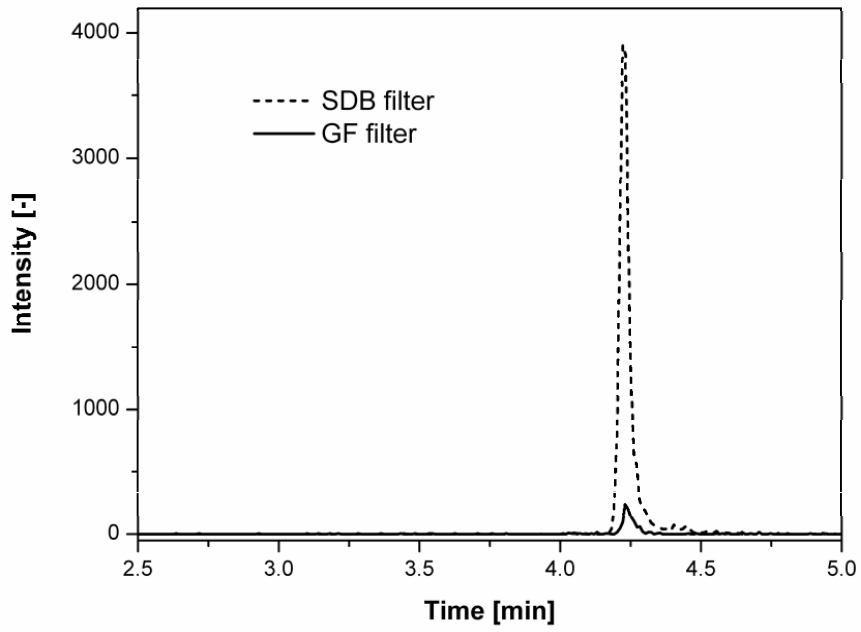

Fig. 4.3: Chromatograms of eluted SDB and GF sample filters from samplers simultaneously exposed to a MIC atmosphere of 5 ppb_v at 85% humidity. Sampling time was 8 h.

The results of the diffusive sampling experiments for methyl, ethyl and phenyl isocyanate obtained from badges loaded with NBDPZ-coated filter tapes are shown in Table 4.1, while results from 2-MP experiments are summarized in Table 4.2. The results are expressed as ratios of the amounts of isocyanate found on the GF filters and the SDB filters. Both filter materials were simultaneously exposed to the same test atmosphere. The absolute analysis results (expressed as sampling rate values) for both filter types are displayed in Table 4.3. These sampling rates are calculated based on the theoretical isocyanate concentration inside the test chamber, which may differ from the

real present concentration. Usually, the observed concentration is slightly lower, i.e. between 92% and 98% of the calculated value, which would then yield a higher sampling rate.

Table 4.1: Isocyanate response ratios between NBDPZ-coated GF and SDB filter types. Results are shown for both high-humidity (90%, shown above) and low-humidity (10%, shown below) conditions.

Time /min	RH (%)	*c*(MIC) (ppb)	MIC Ratio GF/SDB	*c*(EIC) (ppb)	EIC Ratio GF/SDB	*c*(PhIC) (ppb)	PhIC Ratio GF/SDB
15	90	15	0.53	—	—	20	1.04
90	90	30	0.37	30	0.58	30	0.96
120	90	20	0.42	20	0.61	20	1.12
240	90	5	0.42	5	0.58	5	0.96
300	90	1	0.22	1	0.38	1	1.24
360	90	2	0.26	2	0.52	2	0.95
480	90	5	0.05	—	—	5	0.79
800	90	—	—	2	0.48	2	0.95
		Mean	**0.32**		**0.53**		**1.00**
		SD	**0.16**		**0.09**		**0.13**
		RSD	**49.2%**		**16.2%**		**13.4%**
15	10	25	0.72	30	0.83	20	0.99
30	10	10	0.68	—	—	10	0.89
30	10	10	0.74	15	0.79	—	—
90	10	5	0.69	7	0.88	5	0.97
120	10	2	0.66	3	0.84	2	1.01
240	10	2	0.73	2	0.86	3	0.95
250	10	15	0.65	15	0.80	14	0.95
800	10	0.5	0.71	0.5	0.89	0.5	0.87
		Mean	**0.70**		**0.84**		**0.95**
		SD	**0.03**		**0.04**		**0.05**
		RSD	**4.7%**		**4.5%**		**5.4%**

Table 4.2: Isocyanate response ratios between 2-MP coated GF and SDB filters for MIC and EIC. As sampling of PhIC was not possible on GF filters, the GF/SDB values are excluded in this table.

Time/ min	RH (%)	*c*(MIC) (ppb)	MIC ratio GF/SDB	*c*(EIC) (ppb)	EIC ratio GF/SDB
20	10	10	1.01	10	1.13
30	10	10	1.14	10	1.00
30	10	20	1.01	20	1.09
45	90	30	0.94	30	1.07
45	10	15	0.99	15	1.03
60	90	20	1.01	20	1.00
60	10	5	1.13	5	1.14
200	90	1	1.03	1	1.05
240	90	5	0.99	5	0.94
240	10	2	0.99	2	0.98
280	10	8	1.01	8	1.06
300	90	1	0.94	1	1.01
		Mean	**1.02**		**1.03**
		SD	**0.06**		**0.07**
		RSD	**6.1%**		**6.8%**

In contrast to the absolute sampling rates determined for the respective filters materials, the sampling rate ratios (sampling rate on GF filters / sampling rate on SBD filters) are very precise values. For all experiments, these values are summarized in Table 4.4. The true isocyanate concentration does not play any role in this case as the two types of samplers were always simultaneously exposed to the same atmosphere.

Table 4.3: Calculated sampling rates

Reagent	**NBDPZ**						**2-MP**					
Analyte	**MIC**		**EIC**		**PhIC**		**MIC**		**EIC**		**PhIC**	
Filter type	**SDB**	**GF**	**SDB**	**GF**	**SDB**	**GF**	**SDB**	**GF**	**SDB**	**GF**	**SDB**	**GF**
RH conditions	all	low	all	low	all	all	all	all	all	all	all	all
Mean SR /mL min^{-1}	19.3	13.6	14.6	12.4	11.2	10.8	17.2	17.6	14.8	15.3	6.3	0.3
RSD (%)	9.9%	3.8%	7.1%	10.2%	14.0%	14.1%	15.7%	15.6%	14.9%	12.5%	32.1%	180.9%
N	66	29	58	27	66	52	36	36	36	36	36	36
Average RSD experiment^{-1} (%)	6.5%	4.9%	5.6%	4.7%	9.4%	8.5%	3.5%	5.8%	3.9%	6.4%	13.5%	58.3%

Table 4.4: Summarized GF/SDB response ratios for all analytes and reagents.

	RH conditions	Mean ratio GF/SDB	RSD (%)
MIC-2-MP	All	1.02	6.1
EIC-2-MP	All	1.03	6.8
NBDPZ-MIC	Low	0.70	4.7
NBDPZ-EIC	Low	0.84	4.5
NBDPZ-PhIC	Low	0.95	5.4
NBDPZ-MIC	High	0.32	49.2
NBDPZ-EIC	High	0.53	16.2
NBDPZ-PhIC	High	1.00	13.4

4.3.2.1 NBDPZ as reagent

Using NBDPZ-coated SDB filters, there was no significant difference of uptake rates for MIC, EIC and PhIC recognizable anymore between dry or humid conditions for both short and long-term sampling. Thus, the drawback at high humidity conditions, which had been observed on glass fiber filters, has been overcome by a change of filter material. As can be seen from Table 4.3, the calculated sampling rates were quite constant between the different experiments. Relative standard deviations were 9.9% for MIC experiments on SDB filters at all humidity conditions, 7.1% for EIC and 14.0% for PhIC, respectively. Regarding ethyl and phenyl isocyanate, no experimental data for passive sampling had been reported before. With this study, we therefore introduce the first passive sampling method for these analytes based on the use of NBDPZ.

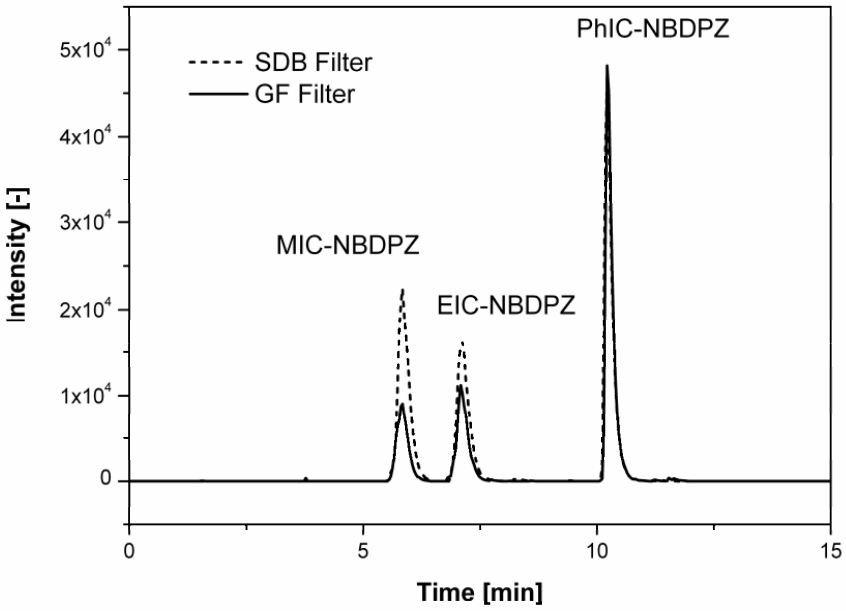

Fig. 4.4: Chromatogram of two eluted NBDPZ sample filters (GF and SDB) from samplers simultaneously exposed for 4 h to an atmosphere containing about 5 ppb_v MIC, 5 ppb_v EIC and 10 ppb_v PhIC at 90% RH.

Fig. 4.4 shows a separation of two eluted NBDPZ sample filters (GF and SDB) from a sampler, which had been exposed for 4 h to an atmosphere containing about 5 ppb_v MIC, 5 ppb_v EIC and 10 ppb_v PhIC at 90% RH. While for both methyl and ethyl isocyanate, the sampling efficiency on GF filters was affected by high humidity, phenyl isocyanate showed almost no difference between the two filter materials. A possible explanation for the different behavior of phenyl isocyanate can be based on the fact that aromatic isocyanates react faster with NBDPZ than aliphatic isocyanates do [34]. In case of PhIC, this means that the amount of accessible reagent on the filter surface may still have been

sufficient before back diffusion effects could have resulted in losses of analyte.

As can be seen from Tables 4.1, 4.3 and 4.4, even at dry conditions, there was a general difference in collection efficiency between the glass fiber and the polymer material. On GF filters, about 30% less methyl isocyanate was collected compared with SDB filters at dry conditions. For ethyl isocyanate it was 16% less and for phenyl isocyanate below 5% off the SDB value. However, these ratios were constant for all three analytes (RSD ~5%), meaning that the diffusive sampler could be used with either of the two filter materials at low humidity conditions.

The high standard deviations of the GF/SDB response ratios for the NBDPZ experiments at high RH conditions (Table 4.4) are based on the fact that the displacement of the reagent from the filter surface is a continuous process which starts at the same time as the sampling procedure. As these RSD values are calculated for the whole set of experiments (short and long sampling periods), they are rather a measure for the influence of humidity than for the precision of the individual experiments.

4.3.2.2 2-MP as reagent

Using 2-MP as coating reagent, the influence of humidity and filter material was negligible for methyl and ethyl isocyanate. The ideal value of 1 was within the standard deviation of the mean of the measured GF/SDB response ratios, which were determined to be 1.02 for MIC (RSD: 6.1%) and 1.03 for EIC

(RSD: 6.8%) (Table 4.2). If sampling rates were calculated for the 2-MP measurements, the value for MIC determined in this work was also correlating

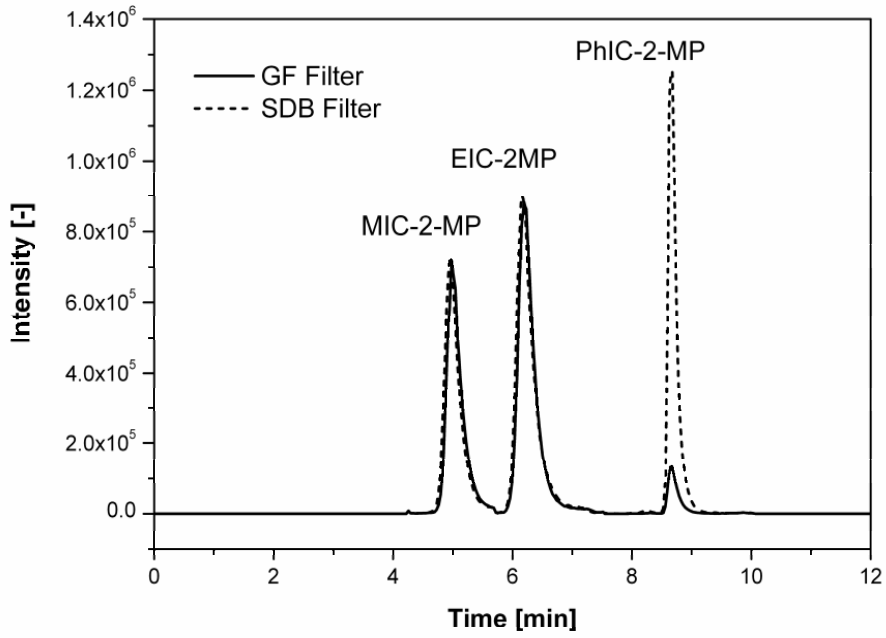

Fig. 4.5: Chromatogram of two eluted 2-MP sample filters (GF and SDB) from samplers simultaneously exposed for 4 h to the same atmosphere as described in Fig. 4.3.

well with literature data provided by von Zweigbergk *et al.* [23] who determined a sampling rate of 15.6 mL·min^{-1}, which was within 13% of the 17.6 mL·min^{-1} found in this study. The sampling rates for EIC and PhIC showed the same tendencies as seen for NBDPZ experiments, decreasing with increasing size of the analyte molecule (Table 4.3). However, for phenyl isocyanate diffusive sampling did not work at all on 2-MP-impregnated glass fiber filters, which is shown in Fig. 4.5. For all experiments on GF filters, the

collected amount of PhIC was much too low, thus leading to GF/SDB values << 1. This behavior was neither depending on humidity conditions, nor on PhIC concentrations nor on sampling time. Until now, there is no satisfying explanation for this. In contrast, diffusive sampling of phenyl isocyanate worked on SDB filters impregnated with 2-MP, even though an increase of the standard deviation within each experiment and also between different experiments (see Table 4.3) was observed.

4.4 Conclusions

It has been shown that the limitations at high relative humidity for diffusive sampling of methyl isocyanate on NBDPZ-impregnated glass fiber filters are related to a reagent displacement from the filter surface. By using less polar SDB filter tapes instead, the uptake rates become independent of humidity conditions. Furthermore, it was shown for the first time that NBDPZ diffusive sampling on SDB material is also suitable for the determination of airborne ethyl and phenyl isocyanate. When 2-MP is used as derivatizing agent, there is no humidity dependency, confirming the findings of von Zweigbergk et al. for MIC on GF filters [23]. No significant difference was found between the two filter materials regarding sampling of methyl and ethyl isocyanate. However, sampling of phenyl isocyanate is strongly dependent on the filter material used and can only be carried out on SDB filters.

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Chapter 5

Validation of a diffusive sampling method for airborne low-molecular isocyanates using NBDPZ-impregnated filters and LC-MS/MS‡

A diffusive sampling method for the determination of low-molecular isocyanates as their 4-nitro-7-piperazinobenzo-2-oxa-1,3-diazole (NBDPZ) derivatives using tandem mass spectrometry (MS/MS) after atmospheric pressure chemical ionisation (APCI) is presented. Isocyanic acid (ICA), methyl isocyanate (MIC), ethyl isocyanate (EIC) and phenyl isocyanate (PhIC) are collected on NBDPZ-impregnated polystyrene divinyl benzene (SDB) filter tapes. The method was validated for MIC, EIC and PhIC for concentrations between 0.5 ppb and 50 ppb at relative humidity (RH) conditions from 10% up to 90%. Validation was carried out by active sampling using 1-(2-methoxyphenyl) piperazine (2-MP) as derivatising agent. Sampling periods applied were between 15 min and more than 8 h. The sampling rates were determined to be 21.0 mL/min for MIC with a relative standard deviation (RSD) of 9.0% for 184 samplers, 15.6 mL/min for EIC (RSD 11.6%; N=154) and 11.5 mL/min for PhIC (RSD 8.4%; N=87). The limits of quantification were 1.4 ppb for MIC and 1.3 ppb for EIC and PhIC applying 15 min sampling

periods. Owing to high background signals, isocyanic acid could only be determined when it was present in concentrations in the high ppb range.

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5.1 Introduction

Isocyanates are important compounds in the field of occupational hygiene. Besides their high acute toxicity, isocyanates also show strong sensitizing properties, being considered as a major cause of occupational asthma in western countries. Sensitization usually takes at least several weeks of exposure, but then even very low concentrations below existing occupational exposure limits (OEL) could trigger life-threatening asthma attacks [1-7]. Today, isocyanates have been regulated to some of the lowest levels in workplace air for any organic compound, with OELs of 5–20 ppb [8-10]. In the Netherlands, the existing exposure limits for individual isocyanates will probably be lowered during 2006, while, e.g. in Switzerland the 5 ppb exposure limit for single compounds was extended to be applied for free isocyanate groups in 2005 [11,12]. Diisocyanates are predominantly used in the polymer industries during manufacture of polyurethane (PUR) products or as components for adhesives, paints and lacquers. Monoisocyanates are mainly used as intermediates for synthesis of pharmaceutical and agricultural products, such as pesticides and herbicides. It is known that under high-temperature conditions (above 200-300°C), isocyanates are released from usually stable compounds, such as PUR products. Conditions that are prone to cause the release of isocyanates are readily achieved during many work processes, e.g. welding, cutting and grinding. Isocyanates will also be set free during spray-painting and foam-blowing operations [13-17]. Additionally it has been shown that low-molecular isocyanates were detected in significant concentrations when other nitrogen containing materials are thermally decomposed [18,19].

In the past, a large variety of analytical methods for the determination of isocyanates has been reported, employing mainly derivatization procedures with secondary amines as reagents to stabilize the highly reactive analytes and to improve detection properties. The most common reagents include N-4-nitrobenzyl-N-n-propylamine ("nitro reagent") [20-22], 3-(2-aminoethyl)indole (tryptamine) [23-25], 1-(2-methoxyphenyl)piperazine (2-MP) [26-31], dibutylamine (DBA) [32-34], 1-(2-pyridyl)piperazine (2-PP) [35], 9-(N-methylaminomethyl)anthracene (MAMA) [36-38], 1-(9-anthracenylmethyl)-piperazine (MAP) [39-41] and 4-nitro-7-piperazinobenzo-2-oxa-1,3-diazole (NBDPZ) [42-44]. After liquid chromatographic separation, quantification is performed by means of UV/Vis absorbance, fluorescence, electrochemical (EC), mass spectrometric (MS) or tandem mass spectrometric (MS/MS) detection. The assessment of isocyanate exposure is challenging due to their varying occurrence in vapor form, as particles or aerosols, as well as monomers, oligomers and polymers with different number of free isocyanate groups. Therefore, method selection must be carefully matched to the individual situation. So far, no such thing as the best reagent for all purposes exists and new reagents are still currently being presented [45,46].

Regarding the determination of isocyanate concentrations in workplace atmospheres, up to now all routine and standard measurements are based on active (pumped) sampling methods. In order to be better suited for personal sampling, solvent free methods are gaining importance [47-49].

In recent years, passive sampling methods have proven great effectiveness, reliability and cost-efficiency in personal air monitoring applications for a large variety of airborne analytes [50]. For isocyanates, there are up to now only two fully validated passive methods for methyl isocyanate (MIC) known from the literature [43,51], as well as an early approach by Rando et al. [52] reporting a passive dosimeter for toluene diisocyanate (TDI) based on a modification of the colorimetric Marcali method [53]. Furthermore, Batlle et al. presented two methods for diffusion-controlled sampling of TDI and hexamethylene diisocyanate (HDI) using SPME devices [54,55]. The very low OEL for isocyanates cause strong requirements on the analytical methods, especially if diffusive sampling is used for collection. In this case, sample volumes are inherently small unless very long sampling periods are accomplished. Current research in the field of isocyanate analysis is more and more focusing on the development and application of highly sensitive mass spectrometric detection methods [28,32,56-58], which is compatible with applications that require high selectivity and sensitivity, such as diffusive sampling does. Recently, it has been shown that different filter materials as well as high and low relative humidity have a significant influence on diffusive sampling efficiency [59]. Furthermore, it could be shown that passive sampling for MIC, EIC and PhIC using NBDPZ-coated SDB filters is possible [59]. However, the method has not been validated yet and isocyanic acid as an important airborne pollutant has not been considered up to now.

The aim of this work was to develop a fully validated diffusive sampling method for the determination of airborne methyl (MIC), ethyl (EIC) and phenyl (PhIC) isocyanate, as well as isocyanic acid (ICA), using NBDPZ-coated

polystyrene divinyl benzene filter tapes as collection material. For validation, an active sampling method based on the use of 2-MP impregnated filters as independent reference method was performed.

5.2 Experimental part

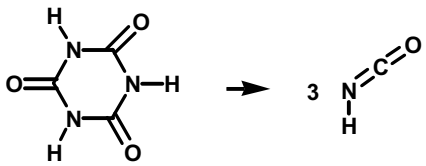
Chemicals

1-(2-Methoxyphenyl) piperazine (2-MP), cyanuric acid, ethyl and phenyl isocyanate (EIC, PhIC), formic acid, acetic anhydride and anhydrous toluene were purchased from Aldrich Chemie (Steinheim, Germany) in the highest quality available. Methyl isocyanate was obtained from Chem Service (West Chester, PA, USA) and caproic, valeric, isobutyric and acetic anhydride were delivered by Fluka (Buchs, Switzerland). Acetonitrile and water (both HPLC-S gradient grade) were obtained from Biosolve (Valkenswaard, The Netherlands). 4-Nitro-7-piperazinobenzo-2-oxa-1,3-diazole (NBDPZ) and its isocyanate derivatives were synthesized as described in reference [42]. 2-MP derivatives of isocyanates were synthesized according to reference [26].

Synthesis of isocyanic acid NBDPZ urea derivative

NBDPZ (50 mg) was dissolved in 200 mL of acetonitrile and placed in a gas-washing bottle that was connected to a round bottom flask containing cyanuric acid. A constant stream of nitrogen was led through the whole setup passing the cyanuric acid and bubbled slowly through the NBDPZ solution. Cyanuric acid was then thermally decomposed to yield ICA (Fig. 5.1) by carefully heating with a Bunsen burner for about 30 min. In order to collect retrimerized cyanuric acid and to prevent it from entering the NBDPZ solution, about 1 m of

winded and spiral shaped glass tubings were inserted before the gas-washing bottle. The latter was additionally secured with a glass wool plug. The decomposition of cyanuric acid was continued until no NBDPZ reagent was left unreacted in the gas-washing bottle, as monitored by LC with fluorescence detection. After completion, the reaction solution was evaporated to dryness, and the light orange solid ICA-NBDPZ urea derivative was recrystallized from acetonitrile for further purification. Identity and purity of the synthesized ICA-NBDPZ derivative was determined by LC, mass spectrometry and elemental analysis.



Cyanuric acid **ICA**

Fig. 5.1: Generation of isocyanic acid by thermal decomposition of cyanuric acid

Generation of standard atmospheres

Isocyanate test atmospheres were dynamically generated by continuous evaporation of defined amounts of analyte standard solutions into a constant stream of humidified air. For this purpose, a generation system was constructed based on the model of a similar setup described in the literature [60,61]. The nebulizer was a TR-50-C1 (J. E. Meinhard, Santa Ana, CA, USA), and the syringe pump (KD Scientific, Holliston, MA, USA) was operated at flow rates between 1 and 10 µL/min with syringe volumes from 0.5 up to 2.5 mL (SGE, Darmstadt, Germany). Air flows were 0.4 L/min through the nebuliser, additional 4.6 L/min added at the bottom end of the evaporation

chamber and further dilution with 35 L/min of humidified air. The Teflon®- and glass-made exposure chamber had dimensions of 7 x 5 x 100cm and comprised six sliding doors to introduce the diffusive sampling badges and seven ports for active reference sampling. Dry air was delivered by a compressor model 2xOF302-40MD2 (Jun-Air, Nørresundby, Denmark). All air flows were set and controlled with mass-flow controllers (EL-Flow® series F-201C and F-201AC, Bronkhorst Hi-Tec, Ruurlo, The Netherlands). The part led through the nebulizer was additionally adjusted using a rotameter valve (Cole Palmer, Vernon Hills, Illinois, USA). The flow of 35 L/min was split into four parallel channels, three of which were led through gas-washing bottles that were filled with water and placed in a tempered water bath (25 °C). These four flows were later re-united and could be adjusted individually by means of needle valves to allow rapid control of relative humidity conditions between 10 and 100% inside the test chamber. All tubing was made of Teflon® and all connections and valves were from stainless steel (Swagelok, Waddinxveen, The Netherlands) to ensure maximum inertness.

Preparation of isocyanate standards

Standard solutions for generation of isocyanate test atmospheres (except for ICA) were made by dissolving the respective isocyanate in anhydrous toluene. The concentrations of these stock solutions were determined by LC-UV/Vis, and the solutions were stored in the freezer. Diluted standards were made from stock solutions by further dilution with dry toluene to the required concentration range. As no stable solutions could be obtained in toluene for ICA, THF was used as solvent. In this case, the same procedure was applied

as for the synthesis of the ICA-NBDPZ derivative, except that pure THF without addition of NBDPZ was added to the gas washing bottle.

Diffusive sampler

The diffusive sampling batch used in this study is schematically described in reference [43]. The housing, with dimensions of 86 x 28 x 9 mm, was made of polypropylene. Two impregnated filters were placed beneath a 2.9 mm thick screen. The part of the screen covering the sampling filter comprised 112 diffusion channels (tubes) within a total area of 20 x 20 mm and with an orifice diameter of 1.0 mm for each hole, increasing slightly towards the collector surface. When the sampler was not in use, the diffusion channels were sealed by a sliding cover. The second filter (control filter) was used to quantify the isocyanate blank. The sampler is commercially available as UMEx 100 (with coated filters for sampling of formaldehyde and amines) from SKC (Eighty Four, PA, USA).

Coated filters for diffusive sampling

NBDPZ (10 mg) was dissolved in 10 mL of acetonitrile (4 mmol/L). Empore SDB-XC extraction disks, diameter 90 mm (3M, St. Paul, MN, USA) were cut into 20 x 20 mm squares, put onto a glass surface and impregnated with 250 µL of the reagent solution. The filters were subsequently allowed to dry for 20 minutes under reduced pressure. One filter was placed under the sampling part of the badge and another under the control part. For some experiments, glass fiber filters (type A/E, diameter 37 mm, SKC, Inc., PA, USA) were prepared the same way, except that 200 µL of the reagent solution were

applied to the filter. For passive sampling experiments with 2-MP as reagent, filters were prepared the same way using a solution of 100 mg 2-MP in 10 mL of acetonitrile for impregnation (52 mmol/L).

Coated filters for active reference sampling

Round glass fiber filters (GFB, diameter 25 mm, Whatman Ltd., Maidstone, UK) were placed onto a glass surface and impregnated with 400 µL of a solution containing 500 mg of 2-MP in 50 mL of acetonitrile (52 mmol/L). Afterwards, the filters were dried under reduced pressure and finally stored in a refrigerator. The same procedure was also applied to obtain NBDPZ-coated filters, using a reagent solution of 50 mg NBDPZ in 50 mL of acetonitrile instead (4 mmol/L).

Diffusive sampling experiments

The NBDPZ diffusive sampling experiments were performed mainly according to EN 838 [62]. The concentrations covered during this validation ranged from approximately 0.1 up to 10 times the threshold-limit value, which resembles isocyanate concentrations between 0.5 ppb and 50 ppb. For most experiments, 5 or 6 diffusive samplers were simultaneously exposed to test atmospheres of MIC, EIC and PhIC. Some experiments were done with isocyanate atmospheres containing only one compound; but typically all three analytes were evaporated from one standard mixture to allow simultaneous diffusive sampling experiments of MIC, EIC and PhIC. To control the homogeneity of the test atmospheres inside the exposure chamber and to determine the variation between the single sampler badges, one experiment

was carried out with 15 samplers in parallel. The sampling periods were mainly selected between 15 min and 8 hrs, and relative humidity was varied between 10 and 100%. Additional, 2-MP diffusive sampling experiments were performed on a smaller scale with a reduced quantity of parallel diffusive samplers of one kind (equipped with either SDB or GF filters). Sample and control filters of exposed diffusive samplers were transferred into separate vials and eluted with 2 mL (for SDB filters) or 3 mL (for GF filters) of acetonitrile. The glass fiber filter samples had to be centrifuged prior to analysis for 5 min at 5000 rpm (Labofuge A, Heraeus Sepatech, Osterode, Germany) in order to settle loose filter particles.

Reference method

A pumped filter method described by Henriks-Eckerman et al. [63] was modified and applied to determine the isocyanate concentrations of generated test atmospheres. Two 2-MP-impregnated filters were placed on top of each other in a Swinnex 25 filter cassette (Millipore, Milford, MA, USA). Three or four filter cassettes were connected in parallel to the active sampling ports of the exposure chamber with a second filter cassette connected in series as backup. For a period of 10-30 min, samples were taken at pump flow rates between 0.3 to 0.5 L/min, using an air sampling pump (Thomas Diaphragm, H/D Pump, Environmental Monitoring Systems, Charlston, SC, USA). Parallel sampling was achieved by splitting the tubing into several parallel pathways, each provided with glass-capillary tubes (inner diameters between 0.45 and 0.6 mm) in order to maintain a constant pressure drop during sampling. The pump flow was adjusted with a needle valve, placed between pump and

"splitting tree". The flow through each cartridge was determined prior and subsequent to the sampling using a DryCal DC-Lite flow calibrator (Bios, Butler, NJ, USA). For short-time exposure experiments, the active reference method was executed simultaneously with diffusive sampling. For longer experiments, it was carried out several times during the generation of the test atmosphere to verify its stability.

Exposed filters from one filter cassette were placed together in 4 mL LC vials and eluted with 4 mL of acetonitrile. The filter particles were allowed to settle down using a centrifuge for 5 min at 5000 rpm and analyzed by means of LC-MS/MS.

The sampling procedure employing cartridges equipped with NBDPZ coated filters remained the same, except that in addition to LC-MS/MS detection, UV/Vis and fluorescence detection were applicable as well.

HPLC instrumentation and analysis

NBDPZ spectroscopic methods

The chromatographic system for LC-UV/Vis and LC-fluorescence analysis of NBDPZ urea derivatives was delivered by Shimadzu (Duisburg, Germany) and consisted of the following parts: two LC-10AS pumps, GT-104 degasser unit, SIL-10A autosampler, sample loop with variable injection volume of up to 50 µL, SUS mixing chamber (0.5 mL), CTO-10ACvp column oven, SPD-M10Avp diode-array detector, RF-10AXL fluorescence detector, CBM-10A controller unit and Class LC-10 software version 1.63.

For determination of the phenyl isocyanate NBDPZ urea derivative from diffusive sampling experiments, the following method was used: The column was a ProntoSIL® 120-3-C18 ace-EPS; particle size 3 µm; pore size 120 Å; column dimensions 150 mm x 4.6 mm (Bischoff Chromatography, Leonberg, Germany). The injection volume was 10 µL, and the column was run with a flow rate of 1 mL/min in a water/acetonitrile gradient (with 0.05% (v/v) formic acid in the aqueous phase). Starting conditions were 20% acetonitrile, followed by a linear gradient to 70% acetonitrile at 15 min and 100% acetonitrile at 16 min. After one minute, the starting conditions were re-established within another minute. Total analysis time was 25 min including re-equilibration. Conditions for fluorescence detection were: λ_{ex} = 471 nm and λ_{em} = 540 nm. Absorption was measured at 480 nm.

For simultaneous analysis of all isocyanate NBDPZ urea derivatives from active sampling experiments, the following method was used: The column was a ProntoSIL® 120-5-Phenyl; particle size 5 µm; pore size 120 Å; column dimensions 250 mm x 3 mm (Bischoff Chromatography). The injection volume was 10 µL, and the column was run with a flow rate of 0.8 mL/min applying a water/acetonitrile gradient (with 0.05% (v/v) formic acid in the aqueous phase). Starting conditions were 20% ACN. 5 min of isocratic elution were followed by a linear gradient to 80% ACN at 20 min and back to the starting conditions within another minute. Total analysis time was 25 min including reconditioning of the column.

MS/MS methods

For LC-MS/MS analysis, a system comprising a binary gradient HPLC pump (HP1100 model GF1312A), an autosampler (HP1100 model G1313A) and a diode-array UV detector (HP1100 model G1315B; all Agilent, Waldbronn, Germany) was connected to the mass spectrometric detector.

For separation of both the 2-MP and NBDPZ derivatives, a ProntoSIL® 120-5 phenyl column with dimensions of 2 mm x 250 mm, particle size of 5 μm and pore size of 120 Å was selected (Bischoff Chromatography).

For mass spectrometric detection, an Esquire 3000+ ion trap mass spectrometer (Bruker Daltonics, Bremen, Germany) was used, equipped with an ESI interface (for analysis of 2-MP derivatives) or an APCI source (for NBDPZ derivatives). All MS measurements were performed in the positive ion mode. The analytes were quantified by 6 point external calibrations run with each series of measurements. The resulting data were analyzed using DataAnalysis software version 3.1 (Bruker Daltonics).

MS method for NBDPZ

The injection volume was 5 µL, and the sample was eluted with 0.45 mL/min of a water/acetonitrile gradient (with 0.05% (v/v) formic acid in the aqueous phase). Starting conditions were 30% acetonitrile, followed by a linear gradient to 40% acetonitrile within 5 min and subsequently to 100% acetonitrile within another 5 min. After one minute, a steep gradient for one minute back to the starting conditions was applied. The total time for the

analysis was 21 min, including re-equilibration. Tandem mass spectra were recorded in the manual MS/MS mode, scanning from *m/z* 100 to *m/z* 700 employing the APCI-MS parameter settings as shown in Table 5.1. Detection was performed as selected-reaction monitoring (SRM) on the transitions from the protonated pseudomolecular ion of the isocyanate derivative $[(IC\text{-}NBDPZ)+H]^+$ (*m/z* 293 for ICA; 307 for MIC; 321 for EIC and 369 for PhIC) to $[NBDPZ+H]^+$ (*m/z* 250), and data were collected as constant neutral loss scans of (*m/z* 43, 57, 71 and 119, respectively) with a maximum accumulation time of 200 ms. Time segments were programmed based on UV/Vis retention

Table 5.1: Summarized List of APCI-MS parameters for Esquire 3000+.

Parameter	Settings
Nebulizer gas (N_2) pressure	50 psi
Dry gas (N_2) flow	5 L/min
Dry gas (N_2) temperature	350 °C
Vaporizer temperature	450 °C
Corona current	4500 nA
Capillary high voltage	3115 V
Capillary exit voltage	109.4 V
Skimmer voltage	40.0 V
Octopole 1 voltage	12.0 V
Octopole 2 voltage	1.7 V
Octopole amplitude	150.0 Vpp
Lens 1 voltage	-5.0 V
Lens 2 voltage	-60.0 V
Trap drive level	35.0

times, in which different precursor ions were selected: From 0 to 4 min, the eluent was diverted into waste; from 4 to 5.4 min, *m/z* 293.1 (NBDPZ-ICA) was selected; from 5.4 to 6.7 min, *m/z* 307.1 (NBDPZ-MIC); from 6.7 to 8.2

min, *m/z* 321.1 (NBDPZ-EIC); from 8.2 to 9.6 min, *m/z* 335.1 (NBDPZ-iPIC); from 9.6 to 12 min, *m/z* 369.1 (NBDPZ-PhIC); and from 12 to 20 min the eluent was again diverted into waste. The NBDPZ-iPIC signal was used as internal standard in some experiments.

MS method for 2-MP

The injection volume was 5 µL, and the sample was eluted with 0.3 mL/min applying a water/acetonitrile gradient (with 0.05% (v/v) formic acid in the aqueous phase). Starting conditions were 25% acetonitrile, followed by a linear gradient after one minute to 85% acetonitrile within 3 min. After two minutes, the starting conditions were re-established within one minute. The total time for the analysis was 15 min, including reequilibration. Tandem mass spectra were recorded in the same way as described for the NBDPZ method, employing the ESI-MS parameter settings as shown in Table 5.2. The

Table 5.2: Summarized List of ESI-MS parameters for Esquire 3000+.

Parameter	Settings
Nebulizer gas (N_2) pressure	40 psi
Dry gas (N_2) flow	10 L/min
Dry gas (N_2) temperature	365 °C
Capillary high voltage	5000 V
Capillary exit voltage	109.8 V
Skimmer voltage	40.0 V
Octopole 1 voltage	12.0 V
Octopole 2 voltage	1.7 V
Octopole amplitude	142.5 Vpp
Lens 1 voltage	-5.0 V
Lens 2 voltage	-60.0 V
Trap drive level	27.7

selected precursor segments were in this case: From 0 to 4.1 min into waste; from 4.1 to 5.6 min, *m/z* 250.0 (2-MP-MIC); from 5.6 to 7.6 min, *m/z* 264.0 (2-MP-EIC); from 7.6 to 10.1 min, *m/z* 312.0 (2-MP-PhIC); and from 10.1 to 15 min again into waste.

Nano-electrospray-Fourier transform ion cyclotron resonance-mass spectrometry

All nano-ESI-FTICRMS experiments were carried out using a LTQ FT Fourier transform ion cyclotron resonance hybrid mass spectrometer (Thermo Electron, Bremen, Germany), equipped with a 7.0 Tesla actively shielded superconducting magnet and nano-ESI source. The instrument was operated in the positive ionization mode. Ion transmission into the linear trap and signal intensity was automatically optimised for maximum ion signal of the EIC-NBDPZ. The parameters were: source voltage 0.8-1.0 kV, capillary voltage 26 V, capillary temperature 200 °C, and tube-lens voltage 100 V. Full scan FTICR mass spectra in the mass range *m/z* 100-500 were acquired with an automated gain control (AGC) of $2 \cdot 10^5$. The resolving power of the FTICR mass analyser was set to 200,000 (FWHM at *m/z* = 400). Accurate mass measurements were carried out by a zoom scan in a narrow mass window (± 5 Da at AGC = $5 \cdot 10^4$ and a resolving power of 50,000). The instrument was calibrated externally using 0.01% solution of 85 % phosphoric acid in water/methanol (1/1; v/v).

5.3 Results and discussion

5.3.1 NBDPZ method

Earlier it has been shown [43] that MIC can be analyzed using diffusive sampling and fluorescence detection. In the present study, we have developed a method for PhIC. Acetic anhydride was found to be suitable for reagent excess deactivation, thus diminishing the interfering tailing of the NBDPZ peak. Fig. 5.2 shows the analysis of eluted sample and control filters from a diffusive sampler exposed to 30 ppb PhIC for 45 min. In that

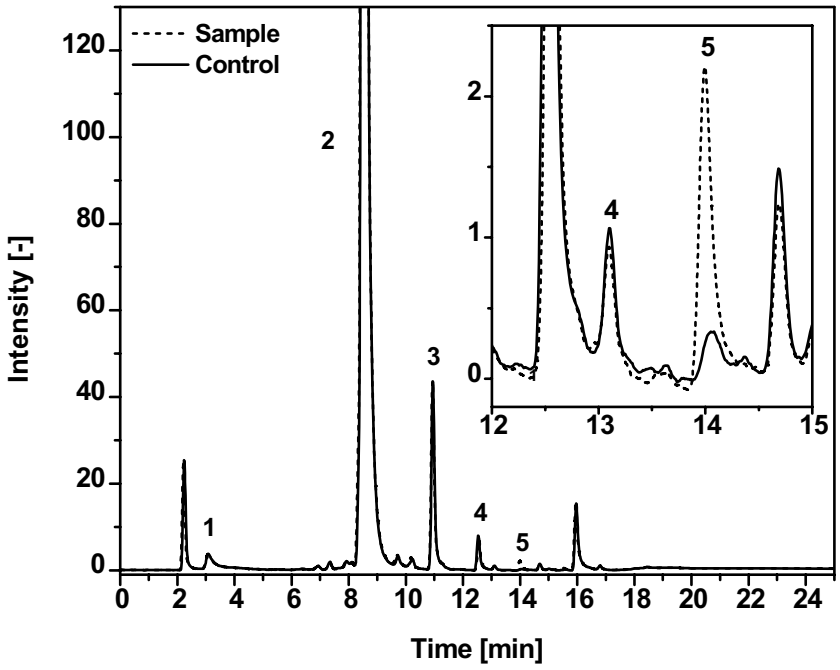


Fig. 5.2: Fluorescence analysis of a diffusive sampler exposed for 45 min to 30 ppb of PhIC; Peak assignment: Isocyanic acid background signal (1), acetylated NBDPZ excess (2), propionylated NBDPZ (3), unknown reaction product from anhydride impurity (4) and PhIC-NBDPZ derivative (5).

concentration range, fluorescence detection can well be applied, but owing to tailing problems and matrix interferences, both related to the reagent excess, the use of mass spectrometry for detection is much more favorable.

In order to minimize the interferences from reagent excess, an examination was carried out, whether the analysis could be improved by the addition of different acid anhydrides. As can be seen from the chromatograms in Fig. 5.3,

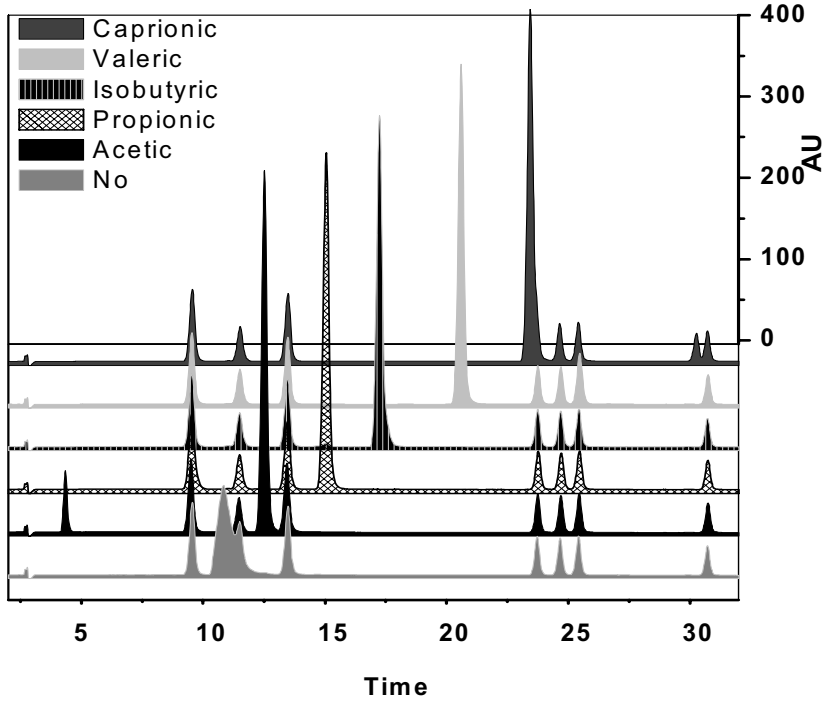


Fig. 5.3: HPLC separation of different isocyanate derivatives (ICA, MIC, EIC, 2,6-TDI, HDI, 2,4-TDI and MDI) and a small 2-MP reagent excess reacted with various anhydrides; phenyl column; 4mM ammonium formate/ ACN

the retention time of the deactivated reagent compound can be shifted into regions where it was not interfering with the analysis anymore, which enables

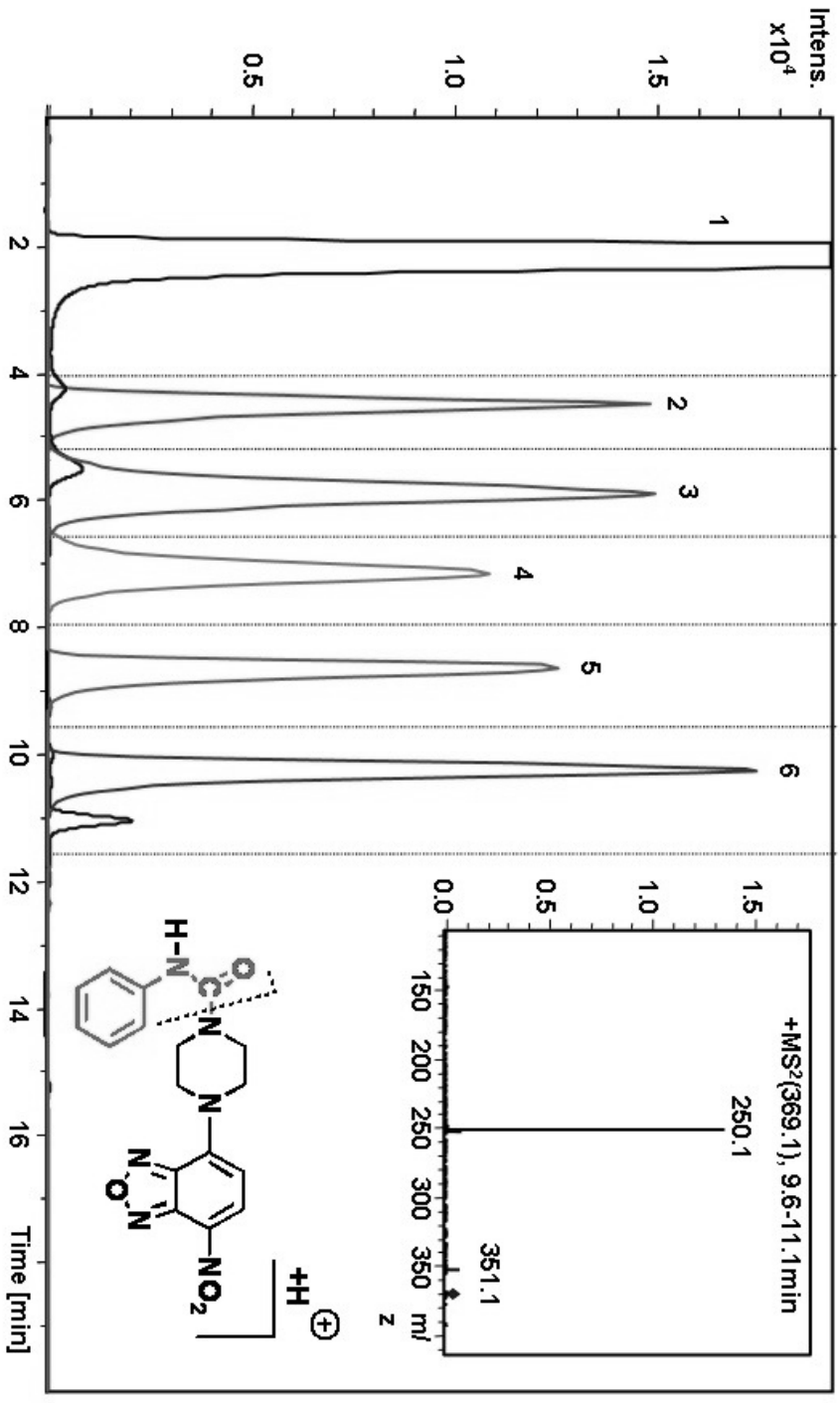
us to chose between a variety of anhydrides depending on the expected retention time for each individual analyte. The example in Fig. 5.3 shows UV chromatograms of the 2-MP reagent deactivated with different acid anhydrides. However, almost all tested anhydrides also contained traces of other anhydrides, which will then still lead to coelution problems if the sample concentration is very low, as it is often the case for diffusive sampling applications. Due to the low detection wavelength of 254 nm, the respective free acids appeared also in the chromatograms and were possible interferences, which is not the case if NBDPZ is detected at 480 nm.

The LC method applying optical detection of NBDPZ derivatives, which was developed for the determination of phenyl isocyanate had an analytical limit of detection (LOD) of $1.8 \cdot 10^{-8}$ mol/L for fluorescence and $5.9 \cdot 10^{-8}$ mol/L for UV/Vis detection, determined as a signal intensity of three times the noise. The limit of quantification (LOQ), determined as a signal intensity of ten times the noise, was $6.0 \cdot 10^{-8}$ (for FLD) and $2.0 \cdot 10^{-7}$ mol/L (for UV/Vis), respectively. The calibration function had a correlation coefficient of linear regression (R) of 0.99986 and a linear range of more than 3 decades above the LOQ. The fluorescence LOQ is equivalent to a concentration of about 10 ppb PhIC in air collected by 60 min diffusive sampling at an estimated uptake rate of 10 mL/min.

When tandem mass spectrometry was applied, the tailing of the reagent excess that caused a severe drawback in fluorescence detection became negligible. Only the large reagent excess must not be allowed to enter the

mass spectrometer in order to avoid an overload and a contamination of the system. It was not necessary to add any anhydrides for reagent excess deactivation, as the addition of formic acid to the LC eluent sufficiently shifted the retention time of the reagent well in front of its derivatives. The first chromatographic window (0 to 4 min) was directed to the waste using a six-port switching valve, thus allowing only the analyte derivatives to enter the mass spectrometer. Baseline separation of the different analytes is favorable, as for every analyte, a different precursor ion is selected for fragmentation to obtain the best sensitivity (Fig. 5.4).

To investigate the influence of ion suppression or other matrix effects on the analysis result, isocyanate standard solutions with and without the addition of a reagent-impregnated blank filter have been analyzed. No difference between the two data sets was observed, which means that the diffusive sampler analysis results are not influenced by any background signal (data not shown). The limits of quantification (LOQ) and limits of detection (LOD) were determined by assessment of the signal-to-noise ratios of 3:1 for the LOD and 10:1 for the LOQ (Table 5.3). The calibration functions always had correlation coefficients of linear regression (R) of 0.99975 and better. The linear range comprised more than three decades starting at the LOQ. The NBDPZ quantification limits corresponded to concentrations of about 1.5 ppb PhIC in air collected by 15 min diffusive sampling at an estimated sampling rate of 10 mL/min; of about 1.3 ppb EIC (15 min at 16 mL/min); and of about 1.5 ppb MIC (15 min at 20 mL/min).


Intens.
x10⁴
1.5
1.0
0.5
1
2
3
4
5
6
2
4
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8
10
12
14
16
Time [min]
+MS²(369.1), 9.6-11.1min
250.1
351.1
0.0
0.5
1.0
1.5
150
200
250
300
350
m/z
H-N
O
N
N
N
O
N
NO₂
+H

Fig. 5.4: Chromatograms of a typical separation of a mixture showing the NBDPZ reagent excess (1; UV trace at 480 nm) and mass traces of ICA (2), MIC (3), EIC (4), iPIC (5) and PhIC (6). The MS^2 fragmentation pattern and mass spectrum for the PhIC derivative is shown as example. Vertical lines illustrate segment borders for precursor ion selection.

5.3.2 2-MP method

The use of 2-MP in combination with tandem mass spectrometry as described in this chapter is a modification of a method presented by Vangronsveld et al. [57] However, in their work, quantitative method performance data was only provided for the analysis of the most common diisocyanates, while ICA, MIC and PhIC were only treated qualitatively or semiquantitatively. In the present chapter, MS parameters have been optimised for the analysis of the MIC-2-MP derivative, and LODs for MIC-2-MP, EIC-2-MP and PhIC-2-MP have been determined in the same way as described for the NBDPZ method (data also shown in Table 5.3). Correlation coefficients of linear regression (R) were 0.999 and better, and a linear range of more than three decades starting at the LOQ was obtained. The sensitivity was in the same range as described by Vangronsveld et al. [57] for 2-MP diisocyanate derivatives.

Table 5.3: MS/MS limits of detection and quantification.

	NBDPZ		2-MP	
Derivative	**LOD/ mol L^{-1}**	**LOQ/ mol L^{-1}**	**LOD/ mol L^{-1}**	**LOQ/ mol L^{-1}**
ICA	$6.0 \cdot 10^{-9}$	$2.0 \cdot 10^{-8}$	---	---
MIC	$2.8 \cdot 10^{-9}$	$9.3 \cdot 10^{-9}$	$8.7 \cdot 10^{-10}$	$2.9 \cdot 10^{-9}$
EIC	$1.9 \cdot 10^{-9}$	$6.3 \cdot 10^{-9}$	$1.5 \cdot 10^{-9}$	$5.1 \cdot 10^{-9}$
PhIC	$1.3 \cdot 10^{-9}$	$4.3 \cdot 10^{-9}$	$1.4 \cdot 10^{-9}$	$4.6 \cdot 10^{-9}$

5.3.3 Active sampling results

2-MP reference method

The active 2-MP method was very robust, as the analysis results from 3-4 simultaneously taken samples from different positions in the test chamber showed average standard deviations of 2.5% for MIC, 2.3% for EIC and 3.2% for PhIC. Initial experiments with up to six parallel samples showed no significant improvement compared to experiments with 3-4 samples, meaning that the reduced number was sufficient to obtain reliable results. These results also demonstrate that the test-atmosphere composition was homogeneous inside the whole exposure chamber. The overall recovery was 92% of the calculated concentration for MIC, 98% for EIC and 82% for PhIC. The test atmospheres were stable over a period of several hours, as air sampling at different points in time gave identical results within the above mentioned standard deviations. The concentrations determined by the 2-MP reference method were generally taken as basis for the calculation of the respective diffusive sampling rates.

Active NBDPZ method

Active sampling using NBDPZ-impregnated filters was applied the same way as with 2-MP except that the amount of reagent was about a factor of 10 smaller. This was due to the fact that NBDPZ coating solutions were lower in concentration because the solubility of NBDPZ in acetonitrile is limited. Quantitative collection was obtained only for PhIC, and no breakthroughs into the backup filter were seen. The mean deviation between the samplers at different sampling positions was 2.7%. For aliphatic isocyanates, significant breakthroughs had been detected even in a second backup filter. The amount of MIC collected on the backup filters was between 26 and 50% of the sample filter value, and on the second backup between 15 and 48%. For EIC the results showed breakthroughs of 11-39% for the first backup and 4-33% for the second. This means that the active NBDPZ method is only suitable for the determination of airborne phenyl isocyanate.

5.3.4 Diffusive sampler validation

Methyl isocyanate

In order to determine the diffusive sampling rate for methyl isocyanate, 36 series of experiments were conducted using NBDPZ-impregnated SDB filters. MIC concentrations were varied between 0.4 and 31 ppm, which were determined with the active 2-MP filter method. For all 36 experiments, the average diffusive sampling rate was determined to be 21.2 mL/min with a relative standard deviation (RSD) of 5.9% (Table 5.4). If all single samplers from all experiments were considered, the mean sampling rate was 21.0 mL/min with a standard deviation of 9.0% (N=184). The RSD of the individual

samplers within one experiment averaged 6.2%. One experiment was carried out using 15 parallel samplers, in which the standard deviation was determined as 7.3%, demonstrating the good reliability and reproducibility of the passive method.

Ethyl isocyanate

As the respective diffusion rates are directly depending on the size of the analyte molecules, it is expected that the sampling rate for ethyl isocyanate is smaller than the one for MIC. For experimental determination of the sampling rate, 30 experiments were carried out in this case, covering a concentration range between 0.5 and 41.1 ppm of EIC in the exposure chamber. The overall passive sampling rate for EIC was found to be 15.6 mL/min with an RSD of 9.9% for the mean values of each experiment, and 15.6 mL/min ± 11.6% for all 154 individual samplers. The standard deviation within each experiment was 6.2% in average, while the one experiment of 15 simultaneously exposed samplers gave an RSD of 3.4% (Table 5.5).

Phenyl isocyanate

The sampling rate of PhIC was expected to be the lowest of all isocyanates that are included in this study. 37 diffusive sampling experiments were carried out to determine that sampling rate (concentration range: 0.4 to 47.7 ppm; Table 5.6). The sampling rate calculated on the basis of the 2-MP reference method was 12.8 mL/min. However, the relative standard deviation between the individual experiments was up to 41.5% for all 187 samples, which would not be acceptable anymore for unrestricted application. As the 2-MP reference

measurements (based on active sampling) additionally revealed PhIC test-atmosphere concentrations that varied from 50% up to 120% of the calculated value, we concluded that not the diffusive sampling was the major problem, but more likely the 2-MP reference method.

This assumption was corroborated by the results of those 16 experiments, during which active sampling was additionally performed using the NBDPZ method for phenyl isocyanate. Based on this method, the sampling rate was determined to 11.5 mL/min, while the standard deviation was 8.4% (Table 5.7). This would be well acceptable for diffusive sampling applications. As the results of the active NBDPZ method came much closer to the calculated value than the 2-MP method, this method was supposed to be better suited as a reference. This failure of the active 2-MP reference method for PhIC has not been reported yet and needs a more in-depth elucidation in the future.

Six additional experiments with a total number of 29 diffusive samplers were carried out using LC-fluorescence for analysis. In these experiments, the PhIC concentration was varied between 2 and 30 ppb and sampling periods ranged from 45 min up to 8 hrs. The sampling rates obtained by this procedure gave an average result of 10.7 mL/min (RSD=7.4%; N=29), which was in the same range as the results obtained by MS/MS detection if the error margins are considered.

Table 5.4- first part: NBDPZ diffusive sampling results of MIC on SDB filters.

Experiment	*c*(MIC)/ ppb	ST[a] / min	RH[b] (%)	SR[c] / mL min^{-1}	RSD[d] (%)	N[e]	Reference yield (%)	RSD (%)
1	0.4	815	10	21.21	5.9	6	95.3	2.4
2	0.7	300	90	19.62	7.7	6	96.9	2.1
3	1.2	200	90	21.12	6.2	5	81.7	1.0
4	1.7	370	90	21.67	5.6	2	80.2	2.3
5	1.8	240	10	21.69	7.8	4	81.9	3.2
6	1.9	920	90	20.51	3.0	2	90.9	3.0
7	2.0	80	90	20.35	7.2	5	92.3	1.6
8	2.2	30	10	21.30	7.9	5	100.5	1.7
9	4.1	40	10	21.90	4.8	5	95.1	1.9
10	4.2	60	10	21.71	7.5	4	77.0	1.7
11	4.5	93	90	19.51	4.3	5	82.5	1.5
12	5.3	240	90	21.76	8.1	4	81.5	5.3
13	5.6	90	10	19.79	5.3	5	91.3	5.6
14	5.7	30	10	21.53	6.7	6	93.6	2.3
15	6.2	30	10	21.42	5.5	5	95.9	1.9
16	7.4	60	10	22.95	10.7	7	94.0	5.9
17	8.5	280	10	19.27	3.6	5	88.5	1.1
18	8.8	20	10	22.45	4.4	4	80.1	2.2
19	9.6	30	90	21.86	6.3	5	88.7	1.8

[a]ST, sampling time. [b]RH, relative humidity. [c]SR, sampling rate. [d]RSD, relative standard deviation. [e]N, number of parallel samplers.

Table 5.4- second part: NBDPZ diffusive sampling results of MIC on SDB filters.

Experiment	*c*(MIC)/ ppb	ST[a] / min	RH[b] (%)	SR[c] / mL min^{-1}	RSD[d] (%)	N[e]	Reference yield (%)	RSD (%)
20	10.0	30	10	20.77	13.4	4	99.9	3.3
21	10.6	30	10	20.16	7.9	5	97.7	3.0
22	11.0	30	10	20.33	5.9	4	113.1	0.8
23	11.9	253	10	21.92	4.5	6	98.4	2.2
24	13.3	45	10	20.62	4.3	4	82.1	3.5
25	15.4	15	10	21.95	8.4	5	94.7	1.7
26	17.5	180	10	18.76	7.3	15	90.6	2.1
27	18.6	120	90	21.28	5.3	6	85.1	1.6
28	20.0	15	90	20.43	6.7	4	109.9	2.5
29	20.1	30	10	20.20	2.6	4	103.5	1.2
30	20.9	60	90	23.17	3.6	6	95.9	0.9
31	26.1	20	10	23.36	7.6	3	85.9	3.9
32	26.4	35	10	21.93	2.3	4	86.8	5.0
33	29.9	60	10	19.46	2.5	3	100.0	3.2
34	30.0	90	90	24.05	4.0	6	91.4	2.5
35	30.1	47	90	21.95	11.5	6	86.5	3.1
36	31.0	120	10	19.30	5.4	9	95.7	0.9
			mean:	**21.15**			**91.8**	**2.5**
			SD:	**1.25**				
			RSD:	**5.89%**				

[a]ST, sampling time. [b]RH, relative humidity. [c]SR, sampling rate. [d]RSD, relative standard deviation. [e]N, number of parallel samplers.

Table 5.5- first part: NBDPZ diffusive sampling results of EIC on SDB filters.

Experiment	c(EIC)/ ppb	ST[a] / min	RH[b] (%)	SR[c] / mL min^{-1}	RSD[d] (%)	N[e]	Reference yield (%)	RSD (%)
1	0.5	815	10	16.33	4.41	6	92.7	2.59
2	1	300	90	14.89	11.25	6	103.8	4.09
3	1.6	200	90	14.27	8.64	5	88.0	0.70
4	2.4	370	90	15.29	7.97	3	89.0	5.61
5	2.5	80	90	18.22	3.31	5	103.0	1.38
6	2.6	240	10	15.10	7.53	4	97.0	5.48
7	2.7	920	90	13.61	0.60	2	99.0	2.32
8	3.1	30	10	14.49	11.88	5	115.7	0.56
9	5.6	90	90	17.22	6.49	5	92.0	2.03
10	5.8	40	10	14.51	10.40	5	108.1	2.79
11	6	60	10	16.20	3.57	4	88.9	1.84
12	7.4	240	90	15.60	6.89	4	91.3	5.16
13	7.9	280	10	12.65	6.99	5	98.9	1.54
14	8.7	30	10	13.82	10.28	5	108.3	2.44
15	10.1	30	10	14.04	5.18	4	123.0	1.29
16	12	60	10	14.11	11.12	7	81.8	0.79
17	12.1	20	10	18.22	3.91	4	89.4	1.90

[a]ST, sampling time. [b]RH, relative humidity. [c]SR, sampling rate. [d]RSD, relative standard deviation. [e]N, number of parallel samplers.

Table 5.5 second part: NBDPZ diffusive sampling results of EIC on SDB filters.

Experiment	c(EIC)/ ppb	ST[a] / min	RH[b] (%)	SR[c] / mL min^{-1}	RSD[d] (%)	N[e]	Reference yield (%)	RSD (%)
18	12.2	30	90	18.71	4.06	5	100.0	1.02
19	14.2	30	10	14.76	2.02	5	105.8	0.88
20	15.5	250	10	15.17	5.12	6	105.2	3.68
21	17.2	30	10	13.91	6.65	4	105.4	1.15
22	18.1	45	10	16.79	2.43	4	89.8	1.98
23	20.8	15	10	15.64	4.71	5	103.3	1.56
24	23.1	180	10	15.95	3.38	15	96.3	1.30
25	23.5	120	90	16.32	5.90	6	86.8	1.82
26	26.2	60	90	15.90	7.68	6	96.6	0.78
27	37.1	90	90	18.74	4.39	6	86.0	3.38
28	37.4	45	90	15.60	8.29	6	93.8	2.29
29	40.6	20	10	16.80	3.75	3	96.3	4.08
30	41.1	35	10	15.70	6.50	4	97.7	3.87
			mean:	**15.62**			**97.8**	**2.3**
			SD:	**1.55**				
			RSD:	**9.92%**				

[a]ST, sampling time. [b]RH, relative humidity. [c]SR, sampling rate. [d]RSD, relative standard deviation. [e]N, number of parallel samplers.

Table 5.6- first part: NBDPZ diffusive sampling results of PhIC on SDB filters; concentrations from 2-MP reference method.

Experiment	c(PhIC)/ ppb	ST[a] / min	RH[b] (%)	SR[c] / mL min^{-1}	RSD[d] (%)	N[e]	Reference yield (%)	RSD (%)
1	0.4	815	10	22.08	7.2	6	57.5	2.2
2	1.0	300	90	11.41	16.9	5	87.7	5.0
3	1.3	200	90	6.90	14.2	5	56.3	2.2
4	2.2	80	90	21.28	6.0	4	71.4	0.5
5	2.8	240	10	15.37	4.5	4	79.8	6.0
6	2.9	920	90	11.66	6.7	3	86.5	4.7
7	3.1	370	90	13.52	11.4	3	81.4	4.9
8	3.9	30	10	5.72	27.6	5	110.9	3.9
9	5.0	480	90	—	2.4	5	—	—
10	5.5	60	10	16.37	4.8	4	62.1	9.8
11	5.8	90	90	16.50	3.5	5	76.8	1.1
12	6.9	90	10	17.84	2.3	5	55.6	5.7
13	7.4	30	10	19.01	3.5	5	59.8	10.3
14	7.4	280	10	10.14	7.0	5	77.8	4.2
15	8.2	40	10	5.47	17.7	5	116.1	0.9
16	9.0	30	10	3.58	12.5	4	90.8	0.9
17	9.6	240	90	11.38	5.2	4	90.5	7.0
18	10.0	30	10	—	6.7	6	—	—
19	11.8	20	10	17.46	4.2	4	65.0	4.8
20	12.0	30	10	6.21	24.2	5	113.3	4.4

[a]ST, sampling time. [b]RH, relative humidity. [c]SR, sampling rate. [d]RSD, relative standard deviation. [e]N, number of parallel samplers.

Table 5.6second part: NBDPZ diffusive sampling results of PhIC on SDB filters; concentrations from 2-MP reference method.

Experiment	c(PhIC)/ ppb	ST[a] / min	RH[b] (%)	SR[c] / mL min^{-1}	RSD[d] (%)	N[e]	Reference yield (%)	RSD (%)
21	13.5	30	90	16.20	9.4	4	88.8	1.2
22	14.3	250	10	20.24	5.1	6	73.7	0.9
23	17.7	60	10	13.92	7.0	6	70.6	1.9
24	18.0	45	10	15.56	4.5	4	67.9	1.5
25	20.0	15	90	—	3.5	4	—	—
26	20.7	30	10	5.27	18.1	5	117.3	2.5
27	22.2	120	90	14.37	16.4	6	62.2	2.1
28	23.0	30	10	3.46	15.2	4	116.3	1.1
29	23.0	180	10	15.77	8.6	15	90.0	2.5
30	24.6	60	90	13.85	24.1	6	68.9	1.4
31	29.6	90	90	20.36	9.2	6	52.1	1.3
32	29.8	45	90	16.43	32.4	6	52.4	2.6
33	30.0	45	10	10.00	6.9	5	—	—
34	31.5	15	10	5.57	29.0	5	118.9	2.7
35	46.8	20	10	10.53	0.6	3	95.6	2.6
36	46.8	35	10	9.58	5.5	4	95.6	2.6
37	47.7	120	10	12.68	10.3	6	95.2	1.3
			mean:	**12.81**			**82.0**	**3.2**
			SD:	**5.32**				
			RSD:	**41.53%**				

[a]ST, sampling time. [b]RH, relative humidity. [c]SR, sampling rate. [d]RSD, relative standard deviation. [e]N, number of parallel samplers.

Table 5.7: NBDPZ diffusive sampling results of PhIC on SDB filters; concentrations from NBDPZ reference method.

Experiment	SR[a]/ mL min^{-1}	RSD[b] (%)	N[c]	Reference yield (%)	RSD (%)
1	12.20	7.2	6	104.0	1.5
4	13.40	6.0	4	113.4	5.3
5	12.76	4.5	4	96.3	2.6
9	10.38	2.4	5	100.0	—
10	10.74	4.8	4	95.0	4.0
11	12.42	3.5	5	102.1	14.7
12	10.72	2.3	5	92.7	3.0
13	11.84	3.5	5	95.8	4.2
18	12.00	6.7	6	100.0	—
19	12.03	4.2	4	94.7	3.4
21	12.18	9.4	4	118.1	3.8
22	11.17	5.1	6	133.4	1.2
23	11.38	7.0	6	86.4	2.9
24	10.44	4.5	4	95.1	3.9
25	11.19	3.5	4	136.2	2.5
29	9.85	8.6	15	131.3	3.6
mean:	**11.54**	**5.2**		**105.9**	**4.0**
SD:	**0.97**				
RSD:	**8.40%**				

[a]SR, sampling rate. [b]RSD, relative standard deviation. [c]N, number of samplers.

Isocyanic acid

Due to background interferences, the quantification of isocyanic acid (ICA) derivatives is problematic. For the 2-MP method, a large amount of ICA derivative was already found in the reagent solution that had been used for filter impregnation. Although there was almost no ICA background seen in the reagent solution, there was a similar problem observed for the NBDPZ method, too. After impregnation of both SDB and GF filters with NBDPZ, a background peak appeared that coeluted with the ICA-NBDPZ peak and

revealed the same fragmentation pattern in the MS/MS mode. The background signal was equivalent to a detector response from concentrations greater than 10 ppb for ICA collected by 120 min diffusive sampling at an estimated uptake rate of 25 mL/min. This makes reliable quantification in that range very difficult.

To verify the nature of these interfering compounds, nano-ESI-FTICRMS experiments were carried out for the determination of exact masses of these compounds. The experiments revealed that the interferences were indeed the respective ICA-2-MP and ICA-NBDPZ derivatives, as no compounds with different elemental composition could be detected. This was quite unexpected and demands for a closer examination in the future.

Owing to these findings, only one diffusive sampling experiment was performed for ICA, exposing the samplers to a very high concentration of about 650 ppb for 1 h in order to obtain a sample peak that was very large in comparison to the background signal. In that experiment, a sampling rate of 25.7 mL/min was determined for isocyanic acid, and the standard deviation between the 6 samplers was 2.9%. This is to be considered as a preliminary result, since no reference method was applied. The ICA standard concentration was determined using the NBDPZ fluorescence method directly before the atmosphere was generated. The experiment showed that diffusive sampling also works for ICA, with a larger sampling rate than methyl isocyanate. However, prior to a full validation for ICA sampling, the reason for the interference has to be elucidated and minimized.

5.4 Conclusions

NBDPZ-coated polystyrene divinyl benzene (SDB) filters can be applied for the diffusive sampling of vapor-phase MIC, EIC and PhIC. A validation of passive sampling has been carried for all three analytes. The determined sampling rates are independent of analyte concentration and relative humidity conditions; they rates are decreasing with increasing size of the analyte molecule, which fully complies with expectations from diffusion theory. Regarding the MS/MS method presented in this study, the LOQ was 1.5 ppb for the individual isocyanates when 15 min sampling periods were accomplished. For 8-h measurements, this corresponds to concentrations of below 50 ppt, which is 1% of the existing occupational exposure limit (OEL). Generally, the use of mass spectrometry is strongly recommended because of higher sensitivity and better selectivity. The fluorescence method for phenyl isocyanate showed a significantly lower LOQ and was not suitable for unrestricted application with passive sampling at concentrations below the OEL because of interferences resulting from the large reagent excess.

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Chapter 6

Ferrocenoyl Piperazide as Derivatizing Agent for the Analysis of Isocyanates and Related Compounds Using Liquid Chromatography/Electrochemistry/Mass Spectrometry (LC/EC/MS)‡

Ferrocenoyl piperazide is introduced as new pre-column derivatizing agent for the analysis of various isocyanates in air samples using reversed-phase liquid chromatographic separation, electrochemical oxidation/ionization and mass spectrometry. The non-polar derivatives can be separated well using a phenyl-modified stationary phase and a formic acid/ammonium formate buffer of pH 3, which yields excellent separations especially for one problematic group of isocyanates consisting of 2,4- and 2,6-toluylenediisocyanate (2,4- and 2,6-TDI) and hexamethylenediisocyanate (HDI). Electrochemical oxidation at low potentials (0.5 V vs. Pd/H_2) leads to formation of charged products, which are nebulized in a commercial atmospheric pressure chemical ionization (APCI) source, with the corona discharge operated only at low voltage. Limits of detection between 6 and 20 nmol/L are obtained for the isocyanate derivatives, and calibration is linear over at least two decades of concentration. The method is applied for the analysis of air after thermal

degradation of a polyurethane foam, and it is demonstrated that it is suitable as well for the analysis of carboxylic acid chlorides and of isothiocyanates.

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6.1 Introduction

Isocyanates (R-N=C=O) nowadays find widespread application in the manufacture of pharmaceuticals, pesticides and polyurethanes (PUR). Isocyanate-based polymers have found many applications, e.g. as paints, adhesives, insulations, sealants, textile fibers, lacquers and finishes. The industrial use of isocyanates is mainly based on their high reactivity towards nucleophilic agents, e.g. alcohols or amines, often showing quantitative reaction yields without any side reactions [1]. However, this reactivity causes a high toxicity. When inhaled, isocyanates can bind to human tissues, proteins and DNA, forming toxic adducts and metabolites which may cause adverse health effects especially to the respiratory system, including acute pulmonary edema, chronic obstructive pulmonary diseases and bronchial asthma [2,3].

Occupational exposure can occur at many workplaces while handling the native compounds, during spraying isocyanate-based paints or when heating and processing polyurethane (PUR) or related products [4]. It is known that not only those diisocyanates are released that were used during manufacture, but complex mixtures containing also lower isocyanates as degradation products [5].

In order to comply with the 5 ppb Threshold Limit Value (TLV), identification and sensitive quantification of mono- and diisocyanates in workplace air is very important. As the analytes are very reactive, direct spectroscopic methods are not applicable to the analysis of individual isocyanates and derivatization methods must be used. Early methods were based on

colorimetric techniques with restricted selectivity and sensitivity [6], while modern analytical approaches focus on derivatization with nucleophilic reagents, followed by liquid chromatographic (LC) separation and photometric or fluorimetric detection. In the past decades, compounds containing amine functionalities, such as 1-(2-methoxyphenyl)piperazine (2-MP) [7-12], 9-(N-methylaminomethyl)anthracene (MAMA) [13,14], 1-(9-anthracenylmethyl) piperazine (MAP) [15,16], 1-(2-pyridyl)piperazine (2-PP) [17] and 4-nitro-7-piperazinobenzo-2-oxa-1,3-diazole (NBDPZ) [18] were introduced as derivatizing reagents. In recent years, the use of mass spectrometry as detection technique became more and more important because of its high selectivity and low limits of detection. A sensitive MS method makes use of dibutylamine (DBA) as derivatizing reagent [4,5,12,19]. While DBA is only suited for MS detection due to the lack of chromophoric or fluorophoric groups, other reagents that were previously used with photometric detection are now applied with increased sensitivity in tandem MS detection [20,21].

Ferrocene-based derivatizing agents have been used in combination with chromatographic separations and selective detectors since long. Gas chromatography (GC) of ferroceneboronates of diols and related compounds with electron impact mass spectrometric detection was described by Brooks and Cole [22]. Rolfes and Andersson derivatized phenols with ferrocenecarboxylic acid chloride, separated the derivatives by GC and used the highly selective atomic emission detector for quantification [23,24]. The majority of publications on ferrocene-based derivatizing agents, however, describes the use of LC with electrochemical detection [25-33]. Shimada et al.

[25] introduced N-substituted ferrocene-containing maleimides for the derivatization of thiols with subsequent LC and dual-electrode coulometric detection. Fatty acids were determined by the same group based on derivatization with a bromoacetylferrocene, LC and electrochemical detection [26]. Similar approaches were introduced for the determination of brassosteroids using ferroceneboronic acid by Gamoh et al. [27], and for amino acids, peptides and proteins by Eckert and Koller [28,29], Cox et al. [30] and Shimada et al. [31,32]. Lo et al. recently presented ferrocenehexanethiol for the pre-column derivatization of microcystins [33].

Van Berkel et al. introduced the use of the electrospray interface itself as electrochemical reactor after derivatization of various groups of analytes with ferrocenes [34-37]. Electrochemical oxidation of the derivatives leads to formation of the respective ferrocinium ions, which are detected in the mass spectrometer with excellent limits of detection, as demonstrated for the analysis of alcohols in food extracts [34] and plant oils [35]. The fragmentation pathways of the derivatives were elucidated in further work of the same group [36]. Ferroceneboronic acid was used by Van Berkel et al. to analyze alkenes after their oxidation to diols [37], and by Williams et al. to determine several neutral mono- and disaccharides [38] and various estrogens [39]. While all of these methods do not involve a separation, Karst et al. used the combination of pre-column derivatization with ferrocenoyl azide, LC separation, electrochemical conversion and MS detection for the determination of alcohols and phenols [40]. The method could be combined with rapid separations on

very short LC columns [41] and was applied for the analysis of alcohols and phenols in mineral oils [42].

Based on this work, it was considered to develop a dedicated ferrocene-based method for the analysis of isocyanates with reversed-phase liquid chromatography, electrochemical conversion and mass spectrometry. The respective data are presented within this chapter.

6.2 Experimental part

Chemicals

Ferrocenecarboxylic acid, 4-(N,N-dimethylamino)pyridine (DMAP), piperazine, acetyl chloride, most isocyanates, propyl isothiocyanate, formic acid and ammonium formate were purchased from Aldrich Chemie (Steinheim, Germany) in the highest quality available. Methyl isocyanate was obtained from Chem Service (West Chester, PA, USA). For the preparation of the mobile phases for HPLC, acetonitrile and water (both HPLC-S gradient grade) were obtained from Biosolve (Valkenswaard, The Netherlands).

Instrumentation

All HPLC-MS-experiments were performed on a LCMS QP8000 single quadrupole mass spectrometer equipped with an atmospheric pressure chemical ionization (APCI) source connected to a SCL-10Avp controller unit, DGU-14A degasser, two LC-10 ADvp pumps, SUS mixing chamber (0.5 ml), SIL-10A autosampler and a SPD-10AV UV/vis detector. The equipment used for on-line electrochemical oxidation was obtained from ESA (Chelmsford,

MA, U.S.A.) and comprised a Coulochem II electrochemical detector and a model 5021 conditioning cell. For the protection of the working electrode, a PEEK in-line filter (ESA) was mounted between column and electrode.

LC conditions

A binary gradient of acetonitrile and a formic acid/ammonium formate buffer (20 mM, pH = 3) was used for the liquid chromatographic separation. The column employed for the MS measurements was a NC-03 Prontosil 120-5-PHENYL (Bischoff chromatography, Leonberg, Germany) with 5 µm particle size, 120 Å pore size, 250 mm length and 3.0 mm i.d.. Injection volume was 10µL. As flow rate, 0.6 ml/min was selected. The following gradient profile was selected for all measurements:

t (min)	0.03	15	23	25	34	36	37	39
c(CH_3CN) (%)	20	50	55	60	90	90	20	stop

MS conditions

The APCI interface was used as heated nebulizer. Therefore, an APCI probe voltage of only 0.1kV was applied. The nebulizer flow rate was set to 2.5 ml/min, the APCI temperature was set to the highest value possible (500 °C) and the curved desolvation line (CDL) temperature to 300°C. The CDL voltage was set to -5 V, the deflector voltages to +35 V and the detector voltage to 1,7 kV.

Conditions for Cyclic Voltammograms

FcPZ or the derivatized isocyanates were dissolved in a mixture of 10 ml ammonium formate buffer (0.1 M) and 10 ml acetonitrile to form a 0.32 to 0.37 mM solution. After 5 min of stirring under a nitrogen atmosphere, the stirrer was turned of and cyclic voltammograms were recorded in a potential range from -1000 to 1000 mV with a scan rate of 50 mV/s.

Synthesis of the derivatizing agent

In order to derivatize isocyanates, ferrocenoyl piperazide (m = 298 Da) was synthesized. Ferrocenecarboxylic acid chloride was prepared according to literature [23,24,40]: At room temperature, a solution of 1.43 ml (16.6 mmol) of oxalyl chloride in 30 ml of toluene was added to a stirred suspension of 3.0 g (13.0 mmol) of ferrocene carboxylic acid and catalytic amounts (3.5 mg) of DMAP in 35 ml of toluene. The reaction mixture was stirred for 1 h at room temperature. The colour changed from orange to dark red. The solution was heated to 80°C to complete the reaction. After evaporation of the solvent, the residue was extracted several times using warm pentane (T = 30 °C). Ferrocenecarboxylic acid chloride precipitated as dark red, cubic crystals and was used without further purification. A solution of ferrocenecarboxylic acid chloride (500 mg, 2 mmol) in 60 ml dry CH_2Cl_2 was added dropwise to a solution of piperazine (680 mg, 8 mmol) in 20 ml dry CH_2Cl_2 over a period of half an hour under cooling in an ice bath. Afterwards, it was stirred for 1 h at room temperature, before a part of the solvent was evaporated and the solution was filtered in order to remove the excess of piperazine. Subsequently, the solution was evaporated to dryness. As a side product,

bis(ferrocenoyl)piperazide may be obtained, which is only poorly soluble in ether. Therefore, the crude product was suspended in ether and the solution was filtered and evaporated. The obtained yellow to orange substance was a relatively clean derivatization reagent with only small amounts of side product. Further cleaning of the substance by preparative column liquid chromatography in portions of 500 mg on a stationary phase of silica gel 100 from Fluka (Buchs, Switzerland) with only methanol as eluent yielded 720 mg (25 %) of pure derivatization reagent.

Synthesis of the derivatives

The derivatives of monoisocyanates and isothiocyanates were prepared as follows: An excess of the respective monoisocyanate or isothiocyanate (0.4 mmol) was dissolved in 15 mL of anhydrous toluene and the derivatizing reagent (60 mg, 0.2 mmol) in anhydrous toluene (30 ml) was added. The mixture was stirred at room temperature for 30 min. Afterwards, 2 ml methanol were added to destroy the remaining isocyanates. The solvent was removed by evaporation. By this method, the derivatives of methyl isocyanate, ethyl isocyanate, phenyl isocyanate and Fc-PZ-propyl isothiocyanate were synthesized.

For the synthesis of Acetyl-FcPZ, 125 mg (0.42 mmol) Fc-PZ in 30 mL toluene and 23 µL (32 mg, 0.42 mmol) acetyl chloride were reacted. A catalytic amount of 3.5 mg DMAP were added. The mixture was stirred for 4 h to complete the reaction. The further procedure was performed as in case of the monoisocyanates.

For the derivatization of diisocyanates, equimolar amounts of ferrocenoylpiperazide in respect to the isocyanate functionalities were dissolved in anhydrous toluene, mixed with the dissolved isocyanate and stirred at room temperature for 30 min. The solvent was removed by evaporation and orange solids were obtained. According to this procedure, the derivatives of 2,4-TDI; 2,6-TDI; HDI; MDI and IPDI were synthesized.

Characterization of the synthesized substances

Ferrocenoyl piperazide: ^{1}H-NMR (δ/ppm, 60 MHz, $CDCl_3$): 1.73 (s, 1H, NH); 2.85 (t, 4H); 3.71 (t, 4H); 4.24 (s, 5H); 4.26 (dd, 2H); 4.54 (dd, 2H); IR (cm^{-1}, KBr): 3306 (m), 3074 (w), 3001 (w), 2974 (w), 2859 (w),1617 (s), 1540 (s), 1470 (m), 1410 (m), 1285 (s), 1263 (w), 1237 (w), 1173 (w), 1006 (m), 824 (m); ESI-MS (*m/z*): 299 $[M+H]^+$

Fc-PZ-MIC: ^{1}H-NMR (δ/ppm, 60 MHz, $CDCl_3$): 2.84 (d, 2H); 3.49 (m, 4H); 3.67 (m, 4H); 4.23 (s, 5H); 4.31 (m, 2H); 4.56 (m, 2H); IR (cm^{-1}, KBr): 3366 (m), 3083 (w), 2905 (w), 1608 (s), 1549 (s), 1470 (s), 1414 (s), 1262 (s), 1172 (w), 1147 (w), 1105 (w), 1001 (m), 816 (m); ESI-MS (*m/z*): 356 $[M+H]^+$

Fc-PZ-EIC: ^{1}H-NMR (δ/ppm, 60 MHz, $CDCl_3$): 1.15 (t, 3H); 3.48 (m, 6H); 3.67 (m, 4H); 4.32 (s, 5H); 4.41 (dd, 2H); 4.56 (dd, 2H); IR (cm^{-1}, KBr): 3359 (m), 3090 (w), 2975 (w), 2859 (w); 1616 (s), 1539 (s), 1470 (m), 1410 (m), 1265 (s), 1173 (w), 1141 (s), 1105 (w), 1006 (m), 824 (m); ESI-MS (*m/z*): 370 $[M+H]^+$

Fc-PZ-PIC: ^{1}H-NMR (δ/ppm, 60 MHz, DMSO): 3.54 (m, 4H); 3.66 (m, 4H); 4.27 (s, 5H); 4.40 (m, 2H); 4.60 (m, 2H); 7.26 –7.44 (m, 5H); IR (cm^{-1}, KBr): 3316 (m), 3069 (w), 2921 (w), 2856 (w), 1637 (s), 1594 (s), 1537 (s), 1470 (w), 1444 (s) 1404 (w), 1244 (s), 1171 (w), 1147 (w), 1105 (w), 993 (m), 821 (m), 754 (s); ESI-MS (*m/z*): 418 $[M+H]^+$

Fc-PZ-MDI: ^{1}H-NMR (δ/ppm, 60 MHz, $CDCl_3$): 3.52 (m, 8H); 3.65 (m, 8H); 3.84 (m, 2H); 4.23 (s, 10H); 4.34 (m, 4H); 4.55 (m, 4H); 6.88 – 7.21 (m, 8H); IR (cm^{-1}, KBr): 3433 (m), 3097 (w), 2915 (w), 2853 (w); 1645 (s), 1595 (s), 1513 (s), 1471 (s), 1413 (s), 1242 (s), 1172 (w), 1105 (w), 993 (m); 991 (w); 820 (m); 728 (m); ESI-MS (*m/z*): 847 $[M+H]^+$

Fc-PZ-IPDI: ^{1}H-NMR (δ/ppm, 60 MHz, $CDCl_3$): 0.96 –1.10 (m, 15H); 1.7 (m, 1H); 3.46 (m, 8H); 3.71 (m, 8H); 4.24 (s, 10H); 4.33 (m, 4H); 4.57 (m, 4H); IR (cm^{-1}, KBr): 3355 (m), 3086 (w); 2900 (m); 1622 (s); 1538 (s); 1471 (s); 1410 (m); 1251 (s); 1171 (w); 1105 (w); 1003 (m); 821 (w); ESI-MS (*m/z*): 819 $[M+H]^+$

Fc-PZ-HDI: ^{1}H-NMR (δ/ppm, 60 MHz, $CDCl_3$): 1.01 – 1.41 (m, 8H); 3.34 (m, 4H); 3.47 (m, 8H); 3.68 (m, 8H); 4.25 (s, 10H); 4.34 (m, 4H); 4.55 (m, 4H); IR (cm^{-1}, KBr): 3333 (m), 3085 (w), 2922 (w), 2856 (w), 1617 (s), 1549 (s), 1465 (s), 1409 (s), 1254 (s), 1170 (m), 1105 (s), 823 (m); 757 (m); 731 (m); ESI-MS (*m/z*): 765 $[M+H]^+$

Fc-PZ-2,4 TDI: ^{1}H-NMR (δ/ppm, 60 MHz, DMSO): 2.02 (s, 3H); 3.58 (m, 8H); 3.68 (m, 8H); 4.26 (s, 10H); 4.39 (m, 4H); 4.60 (m, 4H); 7.05 (m, 3H); IR (cm^{-1}, KBr): 3254 (w), 3084 (w), 2909 (w), 2855 (w), 1622 (s), 1507 (s); 1471 (s), 1411 (s), 1249 (s), 1170 (m), 1105 (w), 1003 (m); 822 (w); 781 (w); 757 (w); ESI-MS (*m/z*): 771 $[M+H]^+$

Fc-PZ-2,6 TDI: ^{1}H-NMR (δ/ppm, 60 MHz, DMSO): 2.36 (s, 3H); 3.53 (m, 8H); 3.70 (m, 8H); 4.26 (s, 10H); 4.39 (m, 4H); 4.57 (m, 4H); 7.10 (m, 3H); IR (cm^{-1}, KBr): 3324 (w), 3095 (w), 2909 (w), 2857 (w), 1669 (s), 1646 (s), 1598 (s), 1516 (s), 1472 (s), 1416 (s), 1254 (s), 1174 (w), 1105 (w), 1003 (m); 819 (m); 760 (m); ESI-MS (*m/z*): 771 $[M+H]^+$

Fc-PZ-propyl isothiocyanate: ^{1}H-NMR (δ/ppm, 60 MHz, $CDCl_3$): 0.98 (t, 3H); 1.75 (m, 2H); 3.54 –3.91 (m, 10H); 4.26 (s, 5H); 4.36 (m, 2H); 4.58 (m, 2H); IR (cm^{-1}, KBr): 3313 (m), 3084 (w), 2955 (w), 2922 (w); 2862 (w), 1600 (s), 1542 (s), 1467 (s), 1411 (m), 1389 (m); 1348 (m); 1222 (w), 1105 (w), 1076 (w), 1008 (m), 809 (w); 761 (w); ESI-MS (*m/z*): 400 $[M+H]^+$

Acetyl-Fc-PZ: ^{1}H-NMR (δ/ppm, 60 MHz, $CDCl_3$): 2.14 (s, 3H); 3.69 (m, 8H); 4.25 (s, 5H); 4.35 (dd,2H); 4.54 (dd, 2H); IR (cm^{-1}, KBr): 3445 (m), 3073 (w), 2919 (w); 1626 (s), 1603 (s); 1451 (s), 1434 (s), 1360 (m), 1282 (m); 1282 (m); 1249 (s), 1171 (m), 1105 (w), 1002 (m), 862 (w); 816 (w); ESI-MS (*m/z*): 341 $[M+H]^+$

Air sampling was performed using a two-channel air sampler pump (model 1067) from Supelco (Bellefonte, PA, USA). Flow rates were determined using a DryCal DC-lite flow calibrator from Bios (Butler, NJ, USA).

The generation of an isocyanate-containing atmosphere was performed in dry glassware to avoid hydrolysis of the reactive compounds. A 500 mL round bottom flask was partly filled with a commercially available MDI based PUR foam (E-Coll from E/D/E GmbH, Wuppertal, Germany). After letting the foam harden and dry for 30 min, the flask was connected to an impinger containing 24.3 mg (0.08 mmol) Fc-PZ in 50 mL of acetonitrile. For 20 min, the flask was heated with a Bunsen-type burner to decompose the PUR material. This procedure is simulating a process where heat is applied to PUR foams, such as welding of insulated water pipes. During this time period, air samples of 200 mL/min were continuously taken from the flask and bubbled through the impinger.

6.3 Results and discussion

6.3.1 Synthesis

Ferrocenoyl piperazide (Fc-PZ) was synthesized as a new derivatizing agent for the analysis of isocyanates following a two-step route, which is presented in Fig. 6.1. The first step consisted of the reaction of the commercially available ferrocenecarboxylic acid to the respective ferrocenecarboxylic acid chloride (FCC) by using oxalyl chloride and catalytic amounts of N,N-dimethylaminopyridine. This procedure was introduced by Rolfes and Andersson [23,24], but for this work, a slightly modified version by Karst et al.

[40] was applied. FCC was then reacted with an excess of piperazine under formation of Fc-PZ. Despite the excess of piperazine, small amounts of bis(ferrocenoyl)piperazide are formed as side product. Therefore, purification of the reagent was carried out by preparative column liquid chromatography. The reagent was characterized by means of ^{1}H-NMR and IR spectroscopy as well as electrospray mass spectrometry (ESI-MS). The respective data are listed in the Experimental Section.

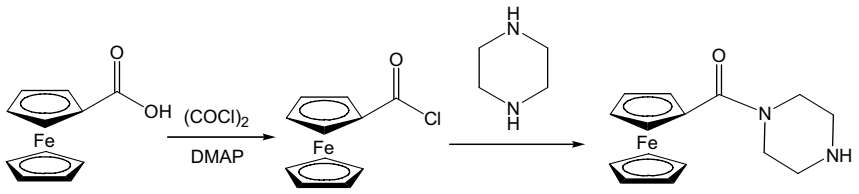


Fig. 6.1: Synthesis of Fc-PZ.

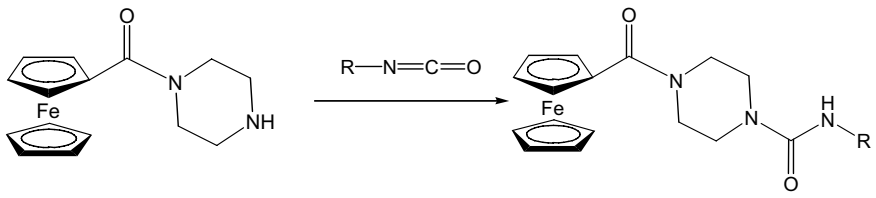


Fig. 6.2: Synthesis of the isocyanate derivatives of Fc-PZ.

For calibration purposes, a series of isocyanate derivatives was synthesized according to Fig. 6.2. The reaction between equimolar amounts of Fc-PZ and isocyanates rapidly yielded the desired products, which were characterized as in case of the reagent. The derivatives of methyl isocyanate (MIC), ethyl isocyanate (EIC), phenyl isocyanate (PhIC), methylenebis(4,4´-phenylisocyanate) (MDI), isophorone diisocyanate (IPDI), hexamethylene

diisocyanate (HDI) and the 2,4- and 2,6-isomers of toluylene diisocyanate (2,4-TDI and 2,6-TDI) were obtained this way.

6.3.2 Chromatographic separation

The chromatographic separation of the derivatives was optimized in the following. For the planned electrochemical conversion of the derivatives after separation, it is important to use a conductive mobile phase, which contains volatile buffers. From earlier work on the analysis of ferrocene-derivatized alcohols and phenols, it was known that a binary gradient consisting of acetonitrile and an aqueous formic acid/ammonium formate buffer with pH 3 fulfils these requirements [40-42]. Initial tests confirmed that this mobile phase is suitable as well for the separation of the derivatized isocyanates. Various stationary phases were tested, and it became evident that the separation of the derivatives of the aliphatic isocyanates can easily be performed on different reversed-phase columns. However, the separation of the two TDI derivatives and the HDI derivative was difficult, as was reported earlier for the separation of NBDPZ-derivatives of these isocyanates [18]. As in this case, a phenyl-modified stationary phase proved to be better suited for the baseline separation of all three derivatives than C_{18}-modified columns. The chromatogram of a standard mixture consisting of the derivatives of MIC, EIC, PhIC, IPDI, 2,4-TDI, HDI, 2,6-TDI and MDI using UV/vis and electrochemistry/MS detection is provided in Fig. 6.3. The optimization of the electrochemistry/MS detection is described in detail below. When comparing the relative signal intensities in UV/vis and MS detection, it becomes evident that in most cases, a good correlation is obtained. However, for the

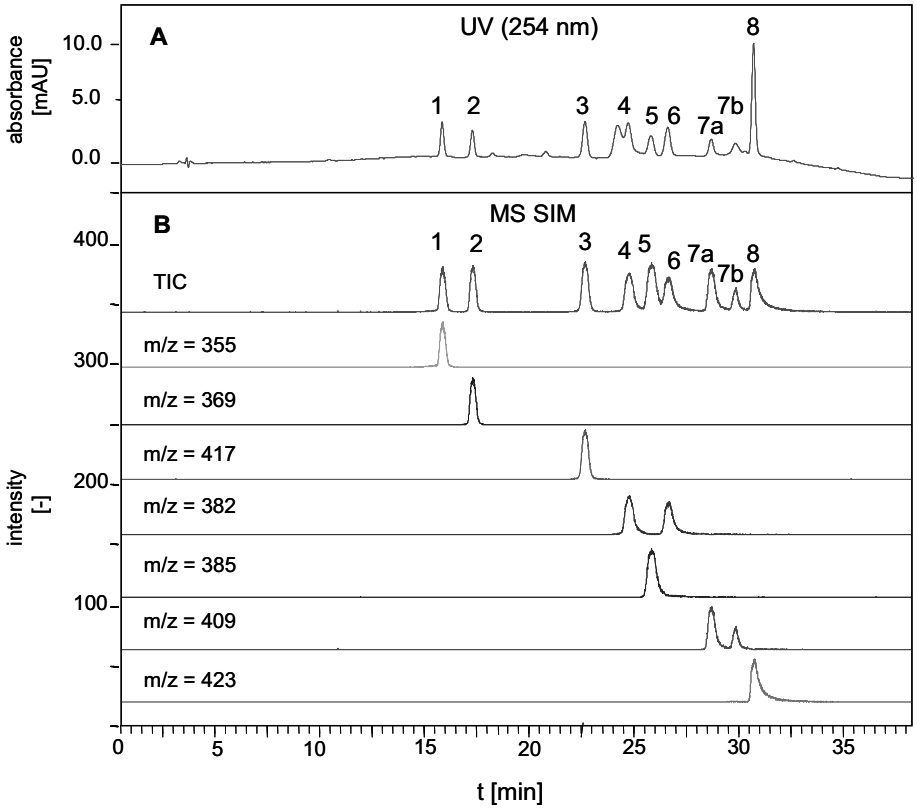
derivatives of the aromatic isocyanates (2,4-TDI, 2,6-TDI and MDI), the relative intensity of the UV absorbance signal is larger due to the strong absorbance of the aromatic rings at the detection wavelength. The chromatogram proves that all of the investigated derivatives can be well separated under the selected conditions. Between the two TDI isomers, the derivative of HDI elutes. For IPDI, two structural isomers are observed at slightly different retention times. With this separation, the analysis of real samples should be possible. This was investigated in detail as described later in this chapter.

6.3.3 LC/EC/MS

When analyzing the derivatives with LC/ESI-MS or LC/APCI-MS, but without electrochemical pretreatment in the positive ion mode, the $[M + H]^+$ pseudomolecular ions were observed in all cases. Signal intensities were poor due to the limited polarity of the analytes. For electrochemistry/MS measurements, a porous glassy carbon cell with very large surface area was used with the goal to obtain a high conversion rate of the ferrocene

Fig. 6.3: Separation of a mixture of Fc-PZ isocyanate derivatives, concentrations between $1.4*10^{-5}$ and $2.0*10^{-5}$ M for monoisocyanate derivatives and between $8.0*10^{6}$ and $9.2*10^{-6}$ M for the derivatives of diisocyanates. Peak assignment: Fc-Pz-MIC (1), Fc-Pz-EIC (2), Fc-Pz-PIC (3), Fc-Pz-2, 6 TDI (4), Fc-Pz-HDI (5), Fc-Pz-2, 4 TDI (6), Fc-Pz-IPDI (7 a,b), Fc-Pz-MDI (8). (A): UV chromatogram recorded at 254 nm; (B): MS chromatogram, selected ion monitoring (SIM) mode, potential of the electrochemical flow cell 0.5 V vs. Pd/H_2.

derivatives. This electrochemical cell uses Pd as counter electrode, and a Pd/H_2 reference system. All potentials provided in the following were determined against this reference electrode, with the mobile phase used for the LC separations (see above). The commercial APCI interface was used without any technical modifications, but with the corona voltage switched to only 0.1 kV, in the "heated nebulizer" or "Thermospray" mode. This allows improving the selectivity, as no further unselective ionization process increases the background signal.



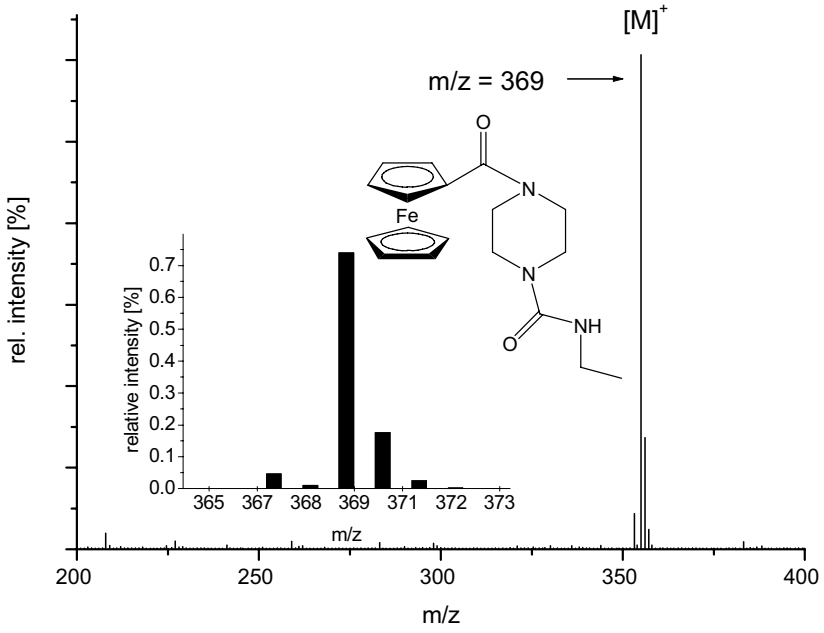


Fig. 6.4: Mass spectrum of Fc-PZ-EIC obtained with electrochemical oxidation. Inserted are the chemical structure of the analyte as well as the calculated isotopic pattern.

The mass spectra for selected derivatives using LC/electrochemistry/MS under these conditions were recorded in the following. Fig. 6.4 shows the mass spectrum of the EIC derivative, the structure of which is inserted. Due to the electrochemical conversion, the ferrocinium ion is formed, which leads to the $[M]^+$ signal as base peak. Compared with the approach without electrochemical conversion, signal intensities are much higher, typically by a factor of 50. Under these conditions, the $[M + H]^+$ ion does not contribute significantly to the signal. The inserted mass spectrum shows the calculated abundance of the masses under consideration of the isotopic pattern of the elements. This correlates well with the measured isotopic pattern.

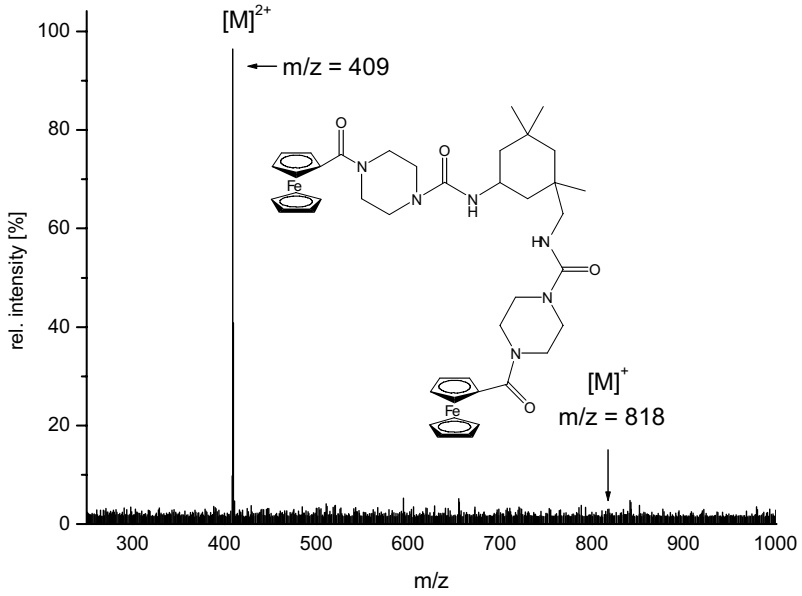


Fig. 6.5: Mass spectrum of Fc-PZ-IPDI obtained with electrochemical oxidation. Inserted is the chemical structure of the analyte.

For all derivatives of the diisocyanates, $[M]^{2+}$ ions are predominant, while $[M]^{+}$ ions are not detected at all. The mass spectrum of the IPDI derivative is presented in Fig. 6.5, in which its chemical structure is inserted. The dual charge of the derivative is proven by the isotopic pattern of the peak (not shown). Obviously, both ferrocene groups are oxidized during this experiment, as no peak is observed for the singly charged oxidation product at *m/z* = 818. This is marked by an arrow in Fig. 6.5.

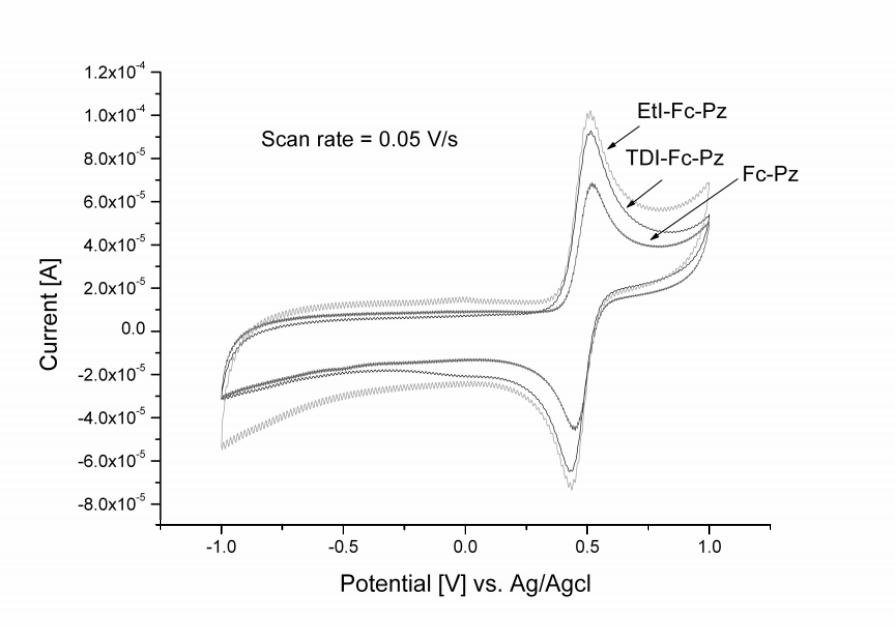


Fig. 6.6: Cyclic voltammogram of Fc-PZ (0.35 mM), Fc-PZ-EIC (0.37 mM), and Fc-PZ-2,6 TDI (0.32 mM) in an 1:1 mixture of acetonitrile and ammonium formate buffer.

To study the electrochemical behavior of the ferrocenoyl piperazides, cyclic voltammetry is performed in a mixture of acetonitrile and ammonium formate buffer. The redox system shows a reversible one-electron oxidation of the ferrocene derivative to the ferrocinium cation (Fig. 6.6). The half-wave potential for the derivatization reagent is found to be 480 mV vs. Ag/ AgCl. As expected, the influence of the individual isocyanate derivatives was neglectable and the potentials were found to be in the same range.

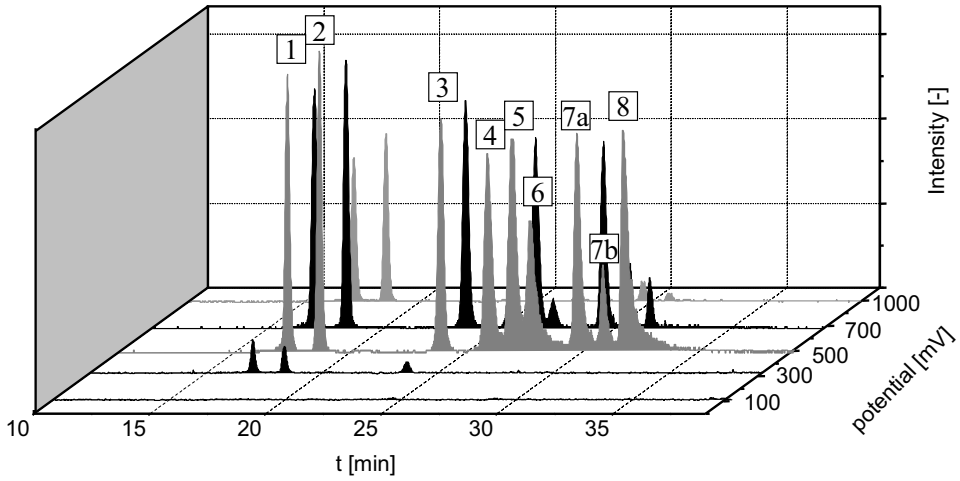


Fig. 6.7: Dependency of the signal intensity on the applied electrochemical potential varied between 0.1 to 1.0 V vs. Pd/H_2, selected ion monitoring (SIM) mode, peak assignment: Fc-Pz-MIC (1), Fc-Pz-EIC (2), Fc-Pz-PIC (3), Fc-Pz-2,6-TDI (4), Fc-Pz-HDI (5), Fc-Pz-2,4-TDI (6), Fc-Pz-IPDI (7 a,b), Fc-Pz-MDI (8).

The dependency of the obtained signal on the applied potential is presented in Fig. 6.7. Small signals are already obtained for the monoisocyanates at a potential of 0.3 V vs. Pd/H_2, while the diisocyanates are not detected at all under these conditions. The highest signals for all compounds were observed at 0.5 V, and this potential was therefore selected for all further measurements. This is consistent with cyclic voltammetric measurements. In the range up to 0.7 V, only a minor decrease of the signal is observed for all derivatives. With further increase of the potential, however, the derivatives of aromatic isocyanates rapidly lose signal intensity, while the derivatives of the

aliphatic isocyanates (MIC, EIC and, to a lesser extent HDI and IPDI), still can be detected at 1.0 V. This indicates that the aromatic ring is involved in further oxidation processes of the derivatives.

The analytical figures of merit were determined for these derivatives. The respective data are listed in Table 6.1. Limits of detection range from 6-20 nmol/L, limits of quantification from 20-50 nmol/L. The linear ranges extend over two decades for all derivatives.

Table 6.1: Analytical figures of merit for the investigated isocyanates using the Fc-PZ method.

Analyte	LOD/ (nM)	LOQ/ (nM)	RSD/ (%) (N = 3; c = 0,2 µM)
Fc-Pz-MIC	20	50	2.2
Fc-Pz-EIC	16	50	4.0
Fc-Pz-PIC	13	40	1.1
Fc-Pz-2,6 TDI	7	20	5.9
Fc-Pz-HDI	12	40	2.1
Fc-Pz-2,4 TDI	10	30	4.5
Fc-Pz-IPDI	13	40	4.3
Fc-Pz-MDI	6	20	7.1

6.3.4 Air sampling application

To investigate the suitability of the reagent for the analysis of real samples, the thermal degradation of MDI-based polyurethane foam was studied. It is known from literature that, besides the isocyanate which was used for synthesizing the polymer, lower isocyanates may be observed during this procedure [43]. This simulates a welding process at a metal pipe, which is insulated with polyurethane material. Sampling was performed with an

impinger, which was filled with 50 mL of a 1.6 mM solution of Fc-PZ in acetonitrile. The air samples were pumped through the impinger at a flow rate of 300 mL/min. Initial experiments with known amounts of nebulized isocyanates proved that the recovery was quantitative under these conditions. In Fig. 6.8, the results of the mass spectrometric analysis of the sampling impinger are presented. As a very large amount of MDI was released, the sample was diluted for the determination of MDI by a factor of 1000 prior to injection into the LC/MS system. It should be noted that some peak tailing is obtained for MDI in this experiment. It is assumed that this is due to absorption or precipitation of the derivative on the cell material and subsequent slow release by oxidation at these high concentrations. This effect was only observed for very high concentrations of those derivatives with the lowest polarity. PhIC was found in very high concentrations as well. As was expected from literature data [43], isocyanic acid (ICA), MIC, EIC and propyl isocyanate are all detected as degradation side-products at low concentrations in the undiluted impinger solution. However, the selected sampling arrangement does not allow a quantitative evaluation of these data. The asterisks indicate unidentified compounds, which occurred in the UV trace, but which could not be detected by mass spectrometry under the conditions used for this work. Therefore, it can be excluded that these peaks are isocyanate derivatives.

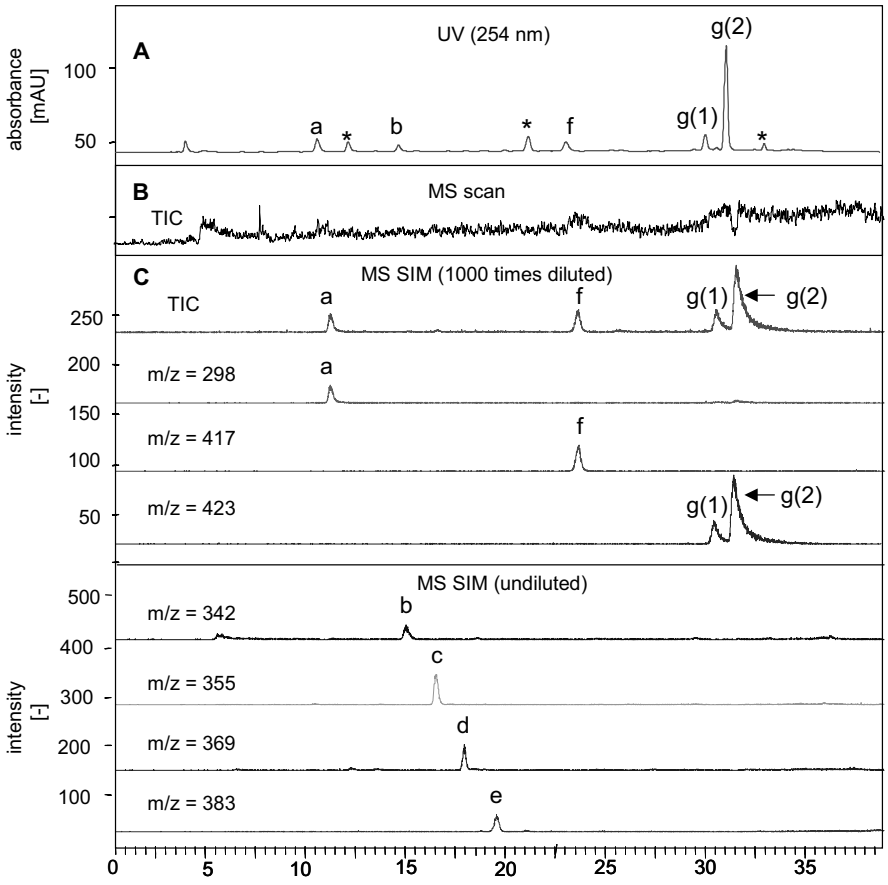


Fig. 6.8: Chromatogram of a thermally treated PUR sample. Peak assignment: Fc-Pz (a), Fc-Pz-ICA (b), Fc-Pz-MIC (c), Fc-Pz-EIC (d), Fc-Pz-PrIC (e), Fc-Pz-PIC (e), Fc-Pz-MDI (g(1), g(2)), (A): UV chromatogram recorded at 254 nm; (B) MS chromatogram scan mode m/z = 200 - 2000 (C): MS chromatogram, selected ion monitoring (SIM) mode, electrochemical flow cell 0.5 V vs. Pd/H_2. The asterisks indicate unidentified compounds, which occured in the UV trace, but which could not be detected by mass spectrometry.

6.3.5 Derivatization of isothiocyanates and acid chlorides

It is obvious that a piperazine function is, in principle, also capable of reacting with isothiocyanates or acid chlorides. To investigate the possibility to apply Fc-PZ for this purpose as well, the respective derivatives of propyl isothiocyanate and acetyl chloride were synthesized and characterized (see above). The mass spectrum of the propyl isothiocyanate derivative of Fc-PZ after electrochemical oxidation is presented in Fig. 6.9. Inserted are the calculated isotopic pattern and the chemical structure of the derivative. Again, the $[M]^+$ peak is predominant, but the $[M]^+$ of the reagent itself is observed as fragment at *m/z* = 298.

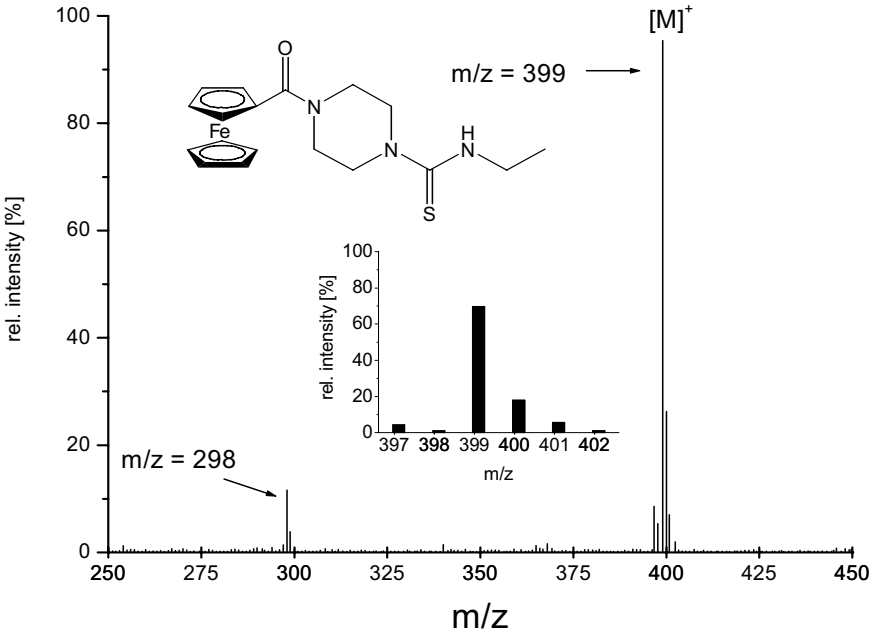


Fig. 6.9: Mass spectrum of Fc-PZ-propyl isothiocyanate obtained with electrochemical oxidation. Inserted are the chemical structure of the analyte and the calculated isotopic pattern.

The mass spectrum of the Fc-PZ derivative of acetyl chloride after electrochemical oxidation is presented in Fig. 6.10, with the inserted chemical structure and calculated isotopic pattern. As in the previous cases, the $[M]^+$ peak dominates. As in case of the isocyanate derivatives, but in contrast to the isothiocyanate derivative, no fragmentation is observed. It is

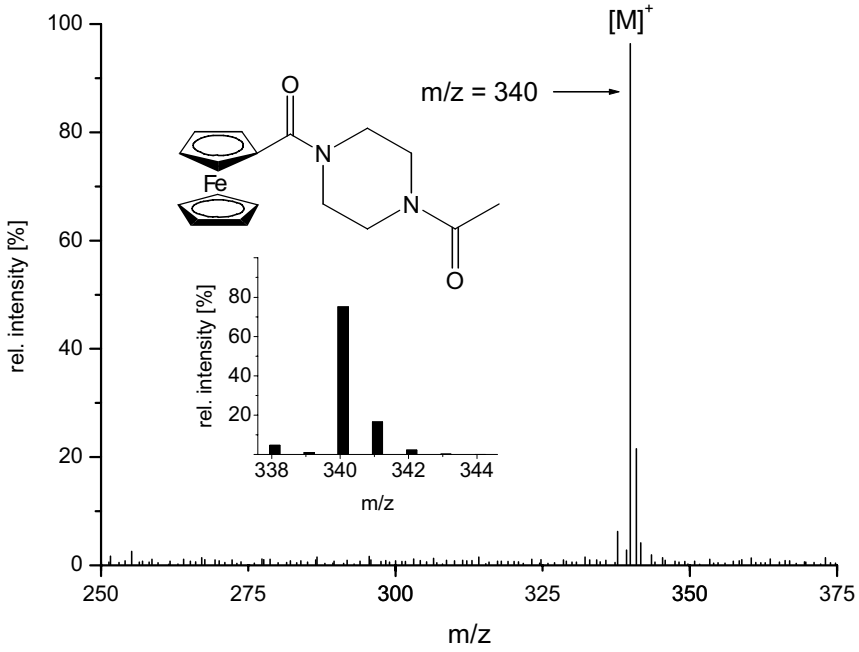


Fig. 6.10: Mass spectrum of acetyl Fc-PZ obtained with electrochemical oxidation. Inserted is the chemical structure of the analyte as well as the calculated isotopic pattern.

therefore evident that the reagent is in principle suitable to be used for the analysis of this group of compounds as well. These findings are also important to evaluate possible interferences in the analysis of isocyanates in real samples using related reagents, which were mostly carried out with LC and

UV/vis detection in previous times. In this case, misinterpretations of unknown peaks in the chromatograms as isocyanates are possible. The use of LC/MS is therefore strongly recommended to increase selectivity.

6.4 Conclusions

Fc-PZ has been introduced as promising new reagent for the analysis of isocyanates and, possibly, isothiocynates and carboxylic acid chlorides by LC/electrochemistry/MS. Considering the used LC/MS instrument, limits of detection are very good. Future work should be directed to the development of more suitable air sampling devices, e.g., reagent-coated test tubes and/or passive sampling devices. Furthermore, the use of tandem mass spectrometry should allow to significantly improve the limits of detection and the selectivity of the method. Fragmentation experiments of the doubly charged diisocyanate derivatives, possibly under formation of singly charged fragments with higher *m/z* ratio than the parent compound, could lead to a very high selectivity. Applications to the analysis of isocyanates in liquid samples also appear to be possible, as it could be expected that polyurethane prepolymers and related compounds with a larger number of isocyanate functionalities will react with several Fc-PZ molecules and will then be charged multiply after electrochemical oxidation. This would allow to analyze (pre)polymers of comparably high masses, if high-resolution mass spectrometry, e.g., with a TOF instrument, would be available.

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Chapter 7

Passive Sampling of Airborne Peroxyacetic Acid[‡]

The first passive sampling device for the determination of airborne peroxyacetic acid (PAA) is presented. 2-([3-{2-[4-Amino-2-(methylsulfanyl) phenyl]-1-diazenyl}phenyl]-sulfonyl)-1-ethanol (ADS) is used to impregnate glass fiber filters, and the reagent is oxidized by PAA to the corresponding sulfoxide ADSO. After elution of the filters, ADS and ADSO are separated by reversed-phase HPLC and detected by UV/vis absorbance. Limit of detection is 30 ppb, limit of quantification is 90 ppb (for 30 min sampling) and the linear range comprises two orders of magnitude. Thorough investigations were carried out with respect to the selectivity of the method towards hydrogen peroxide, and air samples were analyzed successfully after disinfection of a laboratory area.

[‡] published in H. Henneken, L. Assink, J. De Wit, M. Vogel and U. Karst, *Anal. Chem.,* **2006**, 78, 6547-6555.

7.1 Introduction

Due to its oxidative properties, peroxyacetic acid (PAA) has been established in recent years as an important disinfectant, sterilant and sanitizer in the food and beverages industries and for medical applications. Furthermore, it is frequently applied as bleaching agent and/or disinfectant in textile, pulp and paper industries [1]. It has found increasing attention as a disinfectant for wastewater effluents as well. The proven effectiveness against a wide range of microorganisms and decomposition into environmentally beneficial and biodegradable compounds, such as water, oxygen and acetic acid, are important properties. One of its major advantages compared with other sanitizers is the applicability under cold conditions, such as 5°C, without experiencing any cold temperatures failure [2].

Peroxyacetic acid is highly irritating to the skin, nose, throat and lungs. Higher exposure may cause pulmonary edema, while eye contact can cause severe irritation and burns leading to permanent damage. High or repeated exposure may also cause liver and kidney damage. In many industrial and medical applications, a release of PAA cannot be fully avoided. Therefore, the airborne concentration must be monitored on a regular base to prevent workers from being exposed to hazardous levels of PAA. In 1998 the European Parliament adopted a directive on the placing of biocidal products on the market, in which peroxyacetic acid is included [3]. So far, however, no official occupational exposure limits, such as threshold limit values (TLV) and time-weighted average (TWA) for PAA have been established. Therefore, many chemical and pharmaceutical companies use 1 ppm as an internal threshold value for

the PAA workplace concentration, thus following the existing TLV for hydrogen peroxide (HP) as related compound. Recently, Gagnaire et al. published results based on bioassays and RD50 data, demonstrating that the irritant potency of PAA by far exceeds the one of HP, proposing a lower short term exposure limit (STEL) and TWA of 0.5 and 0.2 ppm, respectively [4].

Under equilibrium conditions, PAA consists of a mixture of peroxyacetic acid, hydrogen peroxide, acetic acid and water, and these substances will often coexist in workplace environments. Therefore, suitable methods must be capable for the determination of PAA in the presence of varying concentrations of H_2O_2.

Most methods published for the analysis of PAA are focusing on liquid-phase analysis, based on titrations [5,6], photometry [7-9], potentiometric analysis [10,11], gas chromatography [12-14], liquid chromatography without [15,16]and with derivatization (oxidation) [17-22], and capillary electrophoresis [23,24]. In recent years, only a few methods for gas-phase analysis of peroxyacetic acid were presented [25-27]. Among the air-sampling methods, only active sampling is proposed for analyte collection. So far, no diffusive sampling methods for PAA or even for HP are known in the literature, although they are known to be excellent tools for workplace monitoring of other reactive analytes [28,29] due to their easy handling and attractive analytical figures of merit.

In the 1970s, the first diffusive sampling devices were introduced for air analysis of gaseous components by Palmes et. al. [30,31]. The so-called “Palmes´ Tube” was a precursor of most of the devices used today. Generally, the analytes reach the collector surface by diffusion down their (for a tube: linear) concentration gradient. Based on Fick’s law of diffusion [32], a certain diffusive sampler will collect one specific analyte always with the same constant sampling rate S_R = DA/L at equal temperature and pressure (D: diffusion coefficient; A: cross sectional area; L: length of diffusion path). The diffusion coefficient is temperature and pressure dependent, but effects on the sampling rate are usually negligible if sampling is performed at ambient conditions.

A particular plus of diffusive sampling devices is their high acceptance by the workers, because no loud and inconvenient sampling pumps have to be transported during the work shift, but rather a very small and lightweight device, which does not limit the action of the workers. The major reason for the lack of such methods is the fact that the analytes are very instable and that no classical derivatizing agents are known, which could react with the analytes under formation of a stable and detectable product. This is combined with the very low sampling rates for passive sampling devices, which lead to a significantly decreased limit of detection for the analytes especially for short-term monitoring.

Therefore, the goal of this work was to develop the first passive sampling method for the analysis of PAA at workplaces, which should be characterized

by excellent selectivity and low limits of detection below the suggested TLVs. The development and application of such a method are presented within this chapter.

4.2 Experimental part

Chemicals

All chemicals, unless specified otherwise below, were obtained from Aldrich (Steinheim, Germany) in the highest quality available. A stabilized catalase solution (ASC Super G) was obtained from Mitsubishi Gas Chemical Company, Inc. (Tokyo, Japan). Hydrochloric acid was delivered by Acros Organics (Geel, Belgium), acetic acid analytical grade and potassium permanganate Titrisol (0.02 M), as well as sodium thiosulfate Titrisol (0.1 M), sodium nitrite and ethanol p.a. were obtained from Merck (Darmstadt, Germany). Acetonitrile used for HPLC analysis was from Biosolve (Valkenswaard, The Netherlands). Wofasteril® disinfectant solution was obtained from Kesla Pharma (Wolfen, Germany). The syntheses of 2-([3-{2-[4-amino-2-(methylsulfanyl)phenyl]-1-diazenyl}phenyl]-sulfonyl)-1-ethanol (ADS) and of 2-([3-{2-[4-amino-2-(methylsulfoxy)phenyl]-1-diazenyl}phenyl]sulfonyl)-1-ethanol (ADSO) were carried out as described in the literature [21].

Safety considerations

PAA and hydrogen peroxide are strong oxidizers, and their concentrated solutions should neither be mixed with reducing agents nor with organic substances including solvents. Samples containing very high peroxide concentrations should therefore be diluted prior to the derivatization reaction.

Liquid samples

Five different industrial disinfectant solutions containing PAA and H_2O_2 as well as the PAA and HP solutions delivered by Aldrich, were analyzed for their PAA content. Two different derivatization methods based on oxidation of ADS [21] and methyl-p-tolyl sulfide (MTS) [19] and were applied to all samples. For that purpose, the samples were diluted 1:1000 with 0.01 M acetic acid and subsequently derivatized as described in the literature. The analytical parameters (HPLC) are described below. The analysis was repeated three times per sample solution.

HPLC instrumentation and analysis

The chromatographic system for LC-UV/VIS analysis was delivered by Shimadzu (Duisburg, Germany) and consisted of the following components: two LC-10AS pumps, GT-104 degasser unit, SIL-10A autosampler, sample loop with variable injection volume of up to 50 µL, SUS mixing chamber (0.5 mL), CTO-10ACvp column oven, SPD-M10Avp diode array detector, CBM-10A controller unit and Class LC-10 software version 1.63.

For liquid chromatographic analysis, the following columns were used: Column 1: Discovery® RP 18 (Supelco, Deisenhofen, Germany); particle size 5 µm; pore size 120 Å; column dimensions 150 mm x 4.6 mm. Column 2: ProntoSIL 120-5-C8 (Bischoff Chromatography, Leonberg, Germany); particle size 5 µm; pore size 120 Å; column dimensions 53 mm x 3 mm. Column 3:

Discovery® RP18 guard column (Supelco); particle size 5 µm; pore size 120 Å; column dimensions 10 mm x 4.6 mm.

Table 7.1: Profiles of binary gradients[a]

Gradient :							
time (min)	0	0.4	0.7	1.8	2.5	(stop)	
C_A (%)	33	45	100	33	33		
Gradient B							
time (min)	0	5	6	10	11	15	(stop)
C_A (%)	45	45	100	100	45	45	
Gradient C							
time (min)	0	6	7	8	13	(stop)	
C_A (%)	20	60	85	20	20		
Gradient D							
time (min)	0	4	5	6	(stop)		
C_A (%)	20	80	20	20			

[a] Conditions: flow, 1 mL/min; T, ambient; (A) acetonitrile and (B) water.

For separation, binary gradients with the profiles shown in Table 7.1 were chosen. For the liquid chromatographic analysis of aqueous samples containing both PAA and HP, modifications of the MTS/Triphenyl phosphine (TPP)-method for simultaneous quantification of both analytes19 were applied, using column 1 and gradient B (injection volume 10 µL), as well as column 3 with gradient A (injection volume 5 µL) for the development of a fast separation method. UV detection was performed at 225 nm in both cases.

For the quantification of only PAA (from liquid and air samples) using the ADS reagent, the method described by Effkemann et al.21 was modified using column 1 and gradient C (injection volume 10 µL), as well as column 2 with gradient D (injection volume 5 µL). The detection of the sulfide and the sulfoxide was performed at their absorption maxima at 427 nm (for the sulfide) and 410 nm (for the sulfoxide). All samples were injected in triplicate and an external 6-point standard calibration was run with each series of samples.

Titration

A two-step titration method [5,6] was used to determine the concentrations of PAA stock solutions: 1 mL of sample solution (diluted with water, if necessary) was added to 100 mL water and 4 mL concentrated H_2SO_4. Hydrogen peroxide was first titrated with 0.02 M $KMnO_4$ solution until the color of the solution turned pale rose. Then, an excess of solid KI was rapidly added and the solution was again titrated with 0.01 M Na_2SO_3 until only a pale brown color remained. After addition of one drop of 1% (w/v) starch solution in water, the titration was continued until complete decolorization.

Generation of test atmospheres

PAA and H_2O_2 test atmospheres were dynamically generated by continuous evaporation of defined amounts of analyte standard solutions into a constant stream of humidified air. For this purpose, the following system (see Fig. 7.1) was built according to a similar setup for other analytes described in literature [33,34]: PAA and H_2O_2 solutions are continuously injected through a nebulizer (TR-50-C1, J. E. Meinhard, Santa Ana, CA, USA) into a glass made

evaporation chamber, using a syringe pump (KD Scientific, Holliston, MA, USA) at flow rates between 1 and 10 µL/min with syringes from 0.5 up to 2.5 mL (SGE, Darmstadt, Germany). The analyte solution is nebulized at the

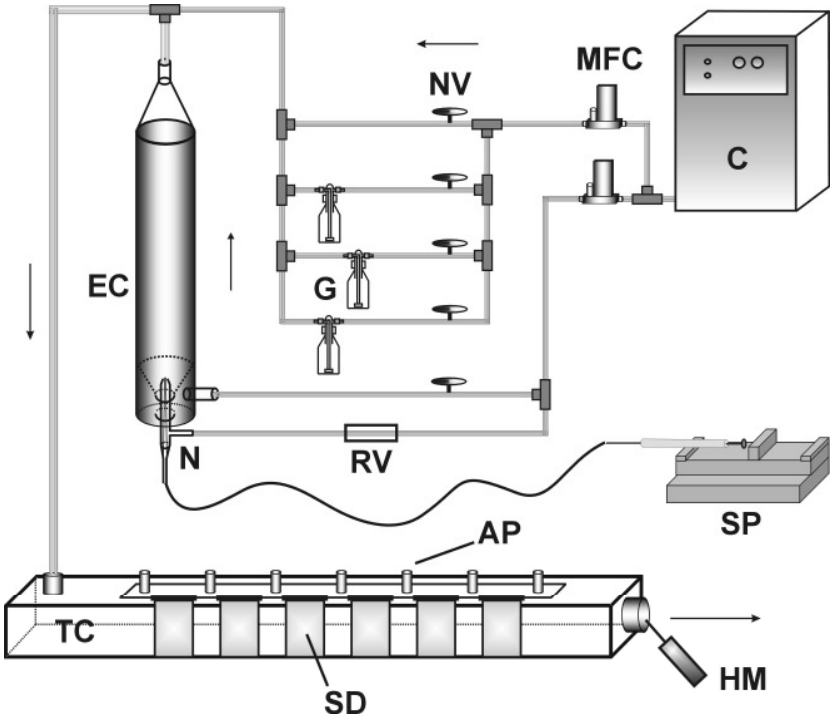

Fig. 7.1: Assembly of the test atmosphere generation system: C, compressor; MFC, mass-flow controllers; NV, needle valves; G, gas washing bottles filled with water; RV, rotameter valve; SP, syringe pump; N, nebulizer; EC, evaporation chamber; TC, test chamber; AP, active sampling ports; SD, sliding doors for passive samplers; HM, humidity meter.

nozzle tip applying an air stream of 400 mL/min through the nebulizer. The produced aerosol is evaporated and carried through the evaporation chamber with 4.6 L/min of air added at the bottom end of the chamber. This air/analyte

mixture is then further diluted with 35 L/min of humidified air and delivered into a Teflon® and glass made exposure chamber with dimensions of 70 x 50 x 1000 mm, which comprises 6 sliding doors to introduce the passive sampler badges and 7 ports for active reference sampling. At the end of the exposure chamber, the relative humidity (RH) is measured with a handheld humidity meter. Dry air is delivered by a compressor model 2xOF302-40MD2 (Jun-Air, Nørresundby, Denmark). All air flows are set and controlled with mass-flow controllers (EL-Flow® series F-201C and F-201AC, Bronkhorst Hi-Tec, Ruurlo, The Netherlands), while the part flowing through the nebulizer is additionally adjusted using a rotameter valve (Cole Palmer, Vernon Hills, Illinois, USA). The 35 L/min flow is split into four parallel channels, three of which are led through gas washing bottles that are filled with water and placed in a heated water bath (25 °C). These four flows are later reunited and can be adjusted individually by means of needle valves to vary the humidity conditions between 10 and 90% RH inside the test chamber. All tubing is made of Teflon® and all connections are of stainless steel (Swagelok, Waddinxveen, The Netherlands) to ensure chemical inertness.

Diffusive sampler setup

The passive sampling device used in this study is schematically shown in reference [35] (and Fig. 3.1). The polypropylene housing has dimensions of 86 x 28 x 9 mm. Two ADS reagent impregnated glass fiber filters are placed beneath a 2.9 mm thick screen. The part of the screen covering the sample filter comprises 112 holes within a total area of 20 x 20 mm and with an entry diameter of 1.0 mm for each hole. The diameter of these diffusion channels is

increasing slightly towards the collector surface, making a larger surface area accessible for the analytes. A sliding cover was used to seal the diffusion channels when the sampler was not in use. The second filter (control filter) was used to quantify the background signal. The sampler is commercially available as UMEx 100 (with coated filters prepared for sampling of aldehydes or amines) from SKC (Eighty Four, PA, USA).

Preparation of coated filters for diffusive sampling

Round glass fiber filters (type A/E, diameter 37 mm, from SKC) were cut into 20 x 20 mm square pieces and placed onto a clean glass plate. Subsequently, each piece was impregnated with 200 µL of a solution of 22 mg ADS in 25 mL of acetonitrile (2.5 mM). The plate was then transferred into a desiccator, and the filters were dried for 20 min under reduced pressure. Two filters each were placed into one diffusive sampling badge, one as sample filter and the other as control part and the sliding cover is closed until the sampler is used.

In this work, the following other filter types were also tested and prepared the same way as described above: GF/C glass fiber filters, diameter 70 mm (Whatman, Brentford, UK); Empore SDB-XC extraction disks, diameter 90 mm (3M, St. Paul, MN, USA); hydrophobic PTFE membranes type 11807 (Sartorius, Göttingen, Germany); Durapore hydrophobic PVDF membranes, type HVHP (Millipore, Milford, MA, USA).

Passive sampling experiments

In order to comply with internationally recognized validation procedures for diffusive samplers, 6 samplers were exposed to the respective test atmosphere in parallel. The common validation process usually covers a range from 1/10 up to 3 times of the existing threshold limit value, which in the case of PAA would be from 100 ppb to 3 ppm. Due to the limited stability of diluted standard solutions (see Results and Discussion), no stable test atmospheres could be generated below 500 ppb. Therefore, experiments were carried out with test atmospheres between 0.5 and 8 ppm PAA at relative humidity conditions between 15 and 85%, mainly applying sampling periods between 15 and 30 min. To investigate the cross reactivity towards hydrogen peroxide, pure H_2O_2 atmospheres were generated as well.

Active reference method

To verify the PAA concentration in the exposure chamber, an active impinger method described by Effkemann et al. [25] was chosen to serve as independent reference. Two impingers were filled with acidified aqueous solutions of ADS and connected in series to the exposure chamber. Air samples were pumped through these solutions at flow rates between 200 and 300 mL/min for 15 min. Two Model 1067 Dual Channel Ambient Air Sampler pumps from Supelco (Bellefonte, PA, USA) were used, allowing for four parallel samples. Active reference samples were taken directly before or after the diffusive sampling experiments and the pump flow through the impingers was calibrated prior to and after the sampling using a DryCal DC-Lite flow calibrator (Bios, Butler, NJ, USA).

Active filter method

An active filter method was also tested for suitability to serve as reference. In these tests, two ADS impregnated filters (Round GF/B glass fiber filters, diameter 25 mm, from Whatman) were placed on top of each other in a Swinnex 25 filter cassette from Millipore. Two cassettes were connected in series to check for a possible breakthrough. PAA air samples were drawn through these cartridges at a rate of about 400 mL/min for 15 minutes.

Sample work-up for analysis

The control and sample filters from exposed diffusive samplers were transferred into separate 4 mL vials and covered with 3 mL acetonitrile each. After 30 min elution time, the vials were centrifuged for 10 minutes at 5000 rpm to settle loose material from the filter and the supernatant was analyzed by means of HPLC-UV/Vis. Active reference samples taken with filter cartridges were treated the same way, except that 4 mL acetonitrile were used for elution. The impinger samples did not require any pretreatment and were ready for HPLC analysis after transferring aliquots into appropriate vials.

7.3 Results and discussion

7.3.1 Selection of the reagents

According to experience of our and other groups [35-38] on the analysis of other reactive organics in air samples, the use of reagent-impregnated glass fiber filters for collection of PAA with the UMEx diffusive sampling badge was considered first. For HPLC analysis, two reagents are known from literature, MTS [17] and ADS [21], both of which contain a sulfide group that is selectively oxidized by PAA to yield the respective sulfoxide. Triphenyl

phosphine (TPP) can be added to the reaction mixture after completed reaction of PAA to either remove the HP, or even for its determination by quantifying the formed triphenyl phosphine oxide (TPPO) (Fig. 7.2) [19].

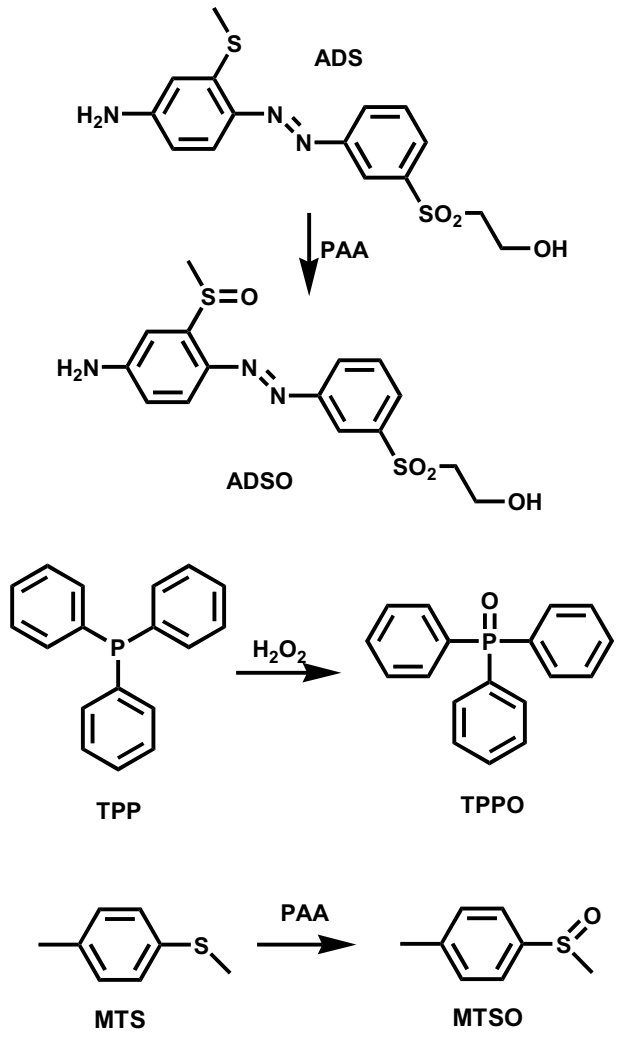

Fig. 7.2: Chemical structures and reaction schemes of ADS, ADSO; TPP, TPPO; MTS, MTSO.

Because of more favorable spectroscopic properties and a lower vapor pressure, ADS appears to be better suited to be used on filters in a diffusive sampling device. Moreover, the ADS method offers a slightly lower limit of detection, which is beneficial due to the inherently very small sample volumes that come along with passive sampling methods.

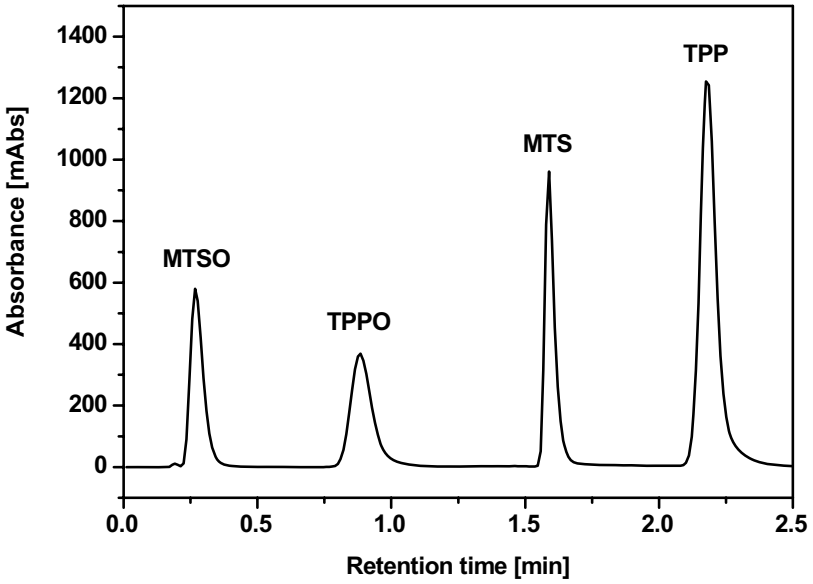

Fig. 7.3: Fast LC separation of a derivatized PAA and HP solution: Peaks: MTSO (1), TPPO (2), MTS (3), TPP (4).

7.3.2 HPLC analysis and method validation

The ADS method was tested in comparison with a procedure based on MTS oxidation and with the titration method for the analysis of a series of liquid samples. Goal was to investigate the reliability and applicability for this specific application. The MTS-TPP method for simultaneous PAA and H_2O_2 determination was carried out on a C18 column with dimensions of 150 mm x

4.6 mm. As indicated by a chromatographic resolution far better than required, the used column appeared to be by far overdimensioned and the separation system was scaled down to achieve shorter retention times. Pinkernell et al. [19] used a C8 column with dimensions of 70 mm x 3 mm for this separation problem, allowing for an analysis time of 5 min per chromatographic run. We could perform the analysis in half of this time by using a 10 mm x 4.6 mm guard column with excellent separation of all four components (Fig. 7.3). For the ADS/ADSO separation, it was not possible to scale the column down to the same extent, because baseline separation could not be achieved on the 10mm-column. The column used during first tests and method evaluation contains a C18 material and has dimensions of 150 mm x 4.6, resulting in a total analysis time of 13 minutes including reequilibration. The column used later for most series of experiments contains a C8 material and has dimensions of 53 mm x 3.0 mm, thus allowing for a higher throughput due to reduced analysis times of 6 minutes per run.

The results of all 3 methods correlated very well with each other (Table 7.2, Fig. 7.4). The main difference between the two HPLC methods was the higher standard deviation of the results obtained when using the MTS method. The improved limit of detection of the ADS method known from literature could be confirmed from external calibration data (not shown), allowing quantification down to concentrations of $1.7 \cdot 10^{-7}$ mol/L, with a linear concentration range of more than 3 orders of magnitude. These results showed that the ADS method is well suited for accurate and robust quantification of PAA samples using the equipment available in our laboratory.

Table 7.2: PAA content of six different disinfectant solutions determined with both HPLC methods (based on ADS and MTS oxidation): RSD, relative standard deviation (N=3).

	MTS method c(PAA) (mass-%)	RSD (%)	ADS method c(PAA) (mass-%)	RSD (%)	Label (mass-%)	Density (g/mL)
Sample 1	15.3	5.2	15.2	1.3	16.0	1.12936
Sample 2	8.2	8.3	8.4	1.9	8.5	1.10512
Sample 3	4.2	16.2	4.5	3.7	5.0	1.08109
Sample 4	4.5	13.1	4.9	3.5	5.0	1.11421
Sample 5	15.8	4.6	15.8	1.2	16.0	1.13210
Sample 6	43.0	2.4	42.4	1.2	32.0	1.14703

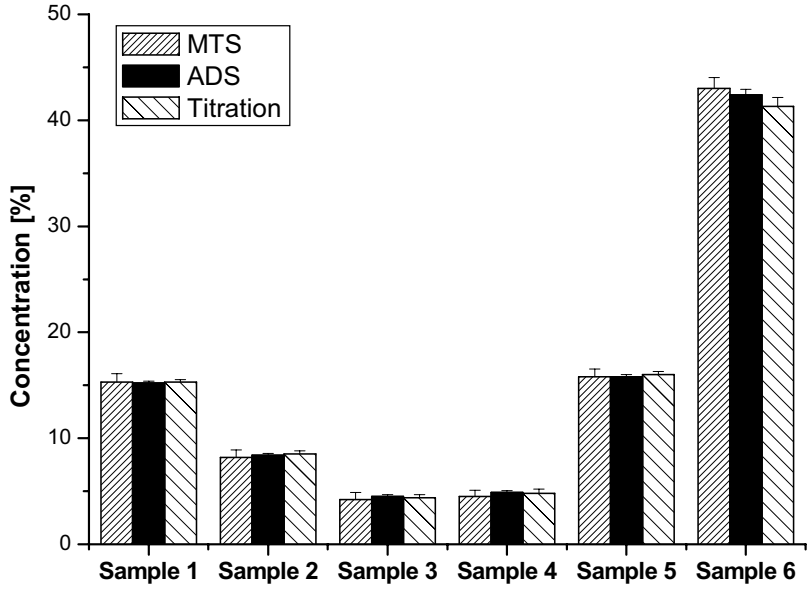

Fig. 7.4: Comparison of the PAA analysis results of 6 different industrial PAA solutions determined applying the ADS and MTS HPLC methods, as well as the titration method.

7.3.3 Active reference

To verify the PAA concentration inside the exposure chamber during the diffusive sampling experiments, an independent reference method had to be applied. First tests showed that an active method with air samples drawn through cartridges loaded with ADS impregnated glass fiber filters is not suitable for this purpose. The collection efficiency was poor, as the amount of ADSO found on the backup filters was equal to the one on the sample filters (Fig. 7.5). Presumably, the contact time between analyte and derivatizing agent was too short for a quantitative reaction yield. Therefore, the active impinger method was chosen to serve as independent reference. Applying this method, no significant breakthrough of PAA into the backup impinger was

detected (Fig. 7.6) and the recovery was in the range of 95% of the expected concentration. This also proved that the generation system works well for PAA as the analyte is effectively evaporated and delivered into the exposure chamber.

Owing to the complex and time-consuming setup of four parallel impingers mounted to the chamber, it was not possible to perform the reference and diffusive sampling experiments simultaneously.

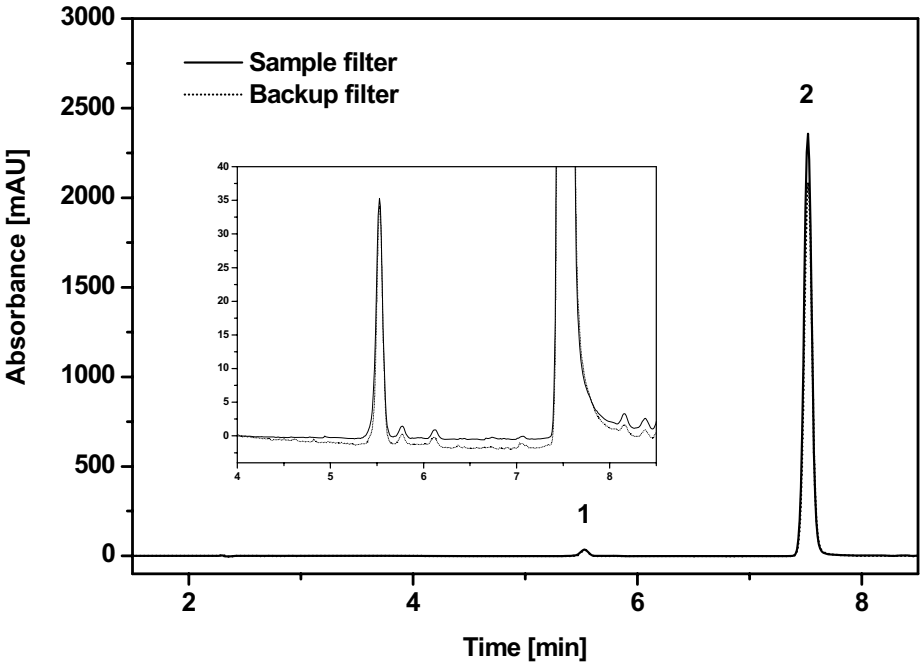

Fig. 7.5: HPLC analysis of an active PAA air sample drawn through ADS impregnated filters. The chromatograms shown are from the eluted filters taken out of the sample and backup cartridges: Peaks: ADSO (1), ADS (2).

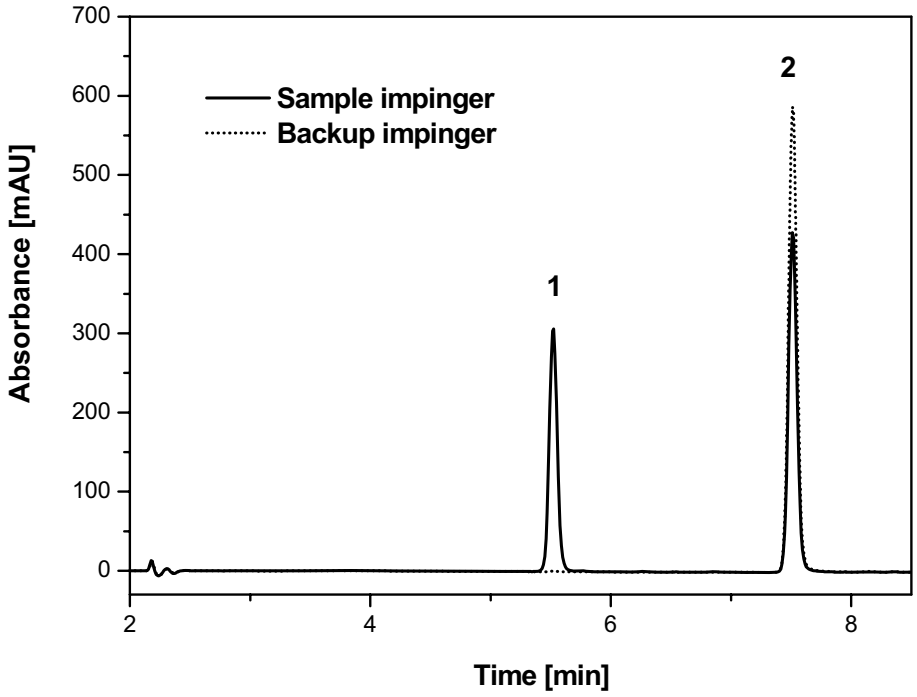

Fig. 7.6: LC separation of sample and backup solutions obtained from active impinger sampling of a PAA test atmosphere: Peaks: ADSO (1), ADS (2).

7.3.4 Stability of standard solutions for test atmosphere generation

A challenge for the generation of PAA atmospheres is the instability of PAA standard solutions. If the original highly concentrated PAA solution is diluted with water or acetic acid, decomposition may take place at least partly due to the dilution of stabilizers in the sample. This is observed by the formation of gas bubbles in the syringe (Fig. 7.1), sometimes amounting to up to half of the syringe volume within 1 h. The most critical issue in this procedure is not the loss in PAA concentration itself, but the large volume effect of the yielded gas in the syringe, which leads to an uncontrollable injection through the nebulizer.

Generally, the standards should always be freshly diluted from stable stock solutions.

Regardless of all measures taken, it was not possible to generate sufficiently stable PAA test atmospheres below a concentration of 500 ppb in air. However, this is not surprising, as to the best knowledge of the authors there are no methods described in the literature, in which stable test atmospheres at such low concentrations can be achieved. Hecht et al. recently e.g. described a generation system for controlled PAA atmospheres where the lowest reported PAA concentration was 1.9 ppm [39].

In order to perform reproducible diffusive sampling experiments with exposure times of 30 min, it is crucial to generate test atmospheres that are stable for at least 90 min, due to the facts that the reference method cannot be applied simultaneously and that the system should be allowed at least 30 min for equilibration before the experiments are started. On the one hand, the syringe speed (analyte standard flow through the nebulizer) should be set as high as possible in order to minimize the effects of gas bubbles yielded inside the syringe. On the other hand, the concentration of the standard solution should be as high as possible (ideally non-diluted) for maximum stability. Both cases are tending towards high concentrations in the exposure chamber, which is not desired. To achieve lower concentrations, a compromise must be found. Very often, even the same procedure yielded different stable atmospheres at different days, as obviously catalytic effects of impurities in the syringe or the solvents used for dilution did accelerate the degradation process. However,

this effect was easily observed by watching the formation of gas bubbles in the syringe. In such cases the experiment was aborted and restarted with new standards and syringes.

7.3.5 Diffusive sampling

The PAA passive sampling rate (S_R) needs to be determined experimentally during the validation process. It can be calculated from the amount of ADSO found on the filters, and from exposure times and known concentrations of the test atmospheres. During field application, the unknown concentration can then be determined from the ADSO analysis result, exposure time and the sampling rate now known from validation.

As stated earlier, a temperature and pressure dependency of the sampling rate is related to the diffusion coefficient, but the effect on the sampling rate is usually negligible if sampling is performed at ambient conditions. For example, the sampling rate for PAA is expected to increase or decrease for about 6% if the temperature during sampling is changed from 20 to 30 or 10 °C, respectively (determined from the calculated diffusion coefficient according to the Fuller-Schettler-Giddings equation described further below).

Initial diffusive sampling experiments

First diffusive sampling tests proved the general applicability of ADS impregnated glass fiber filters for diffusive sampling of PAA. Fig. 7.7 shows a chromatogram of eluted control and sample filters from a diffusive sampler exposed for 2 h to a test atmosphere of 5 ppm (16 mg m^{-3}) PAA. The

chromatogram of the control filter showed an ADSO peak (T_R= 5.5 min) that represented approximately 10% of the sample filters peak area. This is not only a background signal, but also caused by a literature-known [35,38] leakage into the diffusive sampler, which cannot be avoided, as the sampler is not completely tight at the two outside corners. However, this is not crucial, as the control filter value is subtracted from the sample filter value and the sampling rate is always calculated under these conditions. The evaluation of this first test resulted in a preliminary sampling rate of 9.1 mL/min.

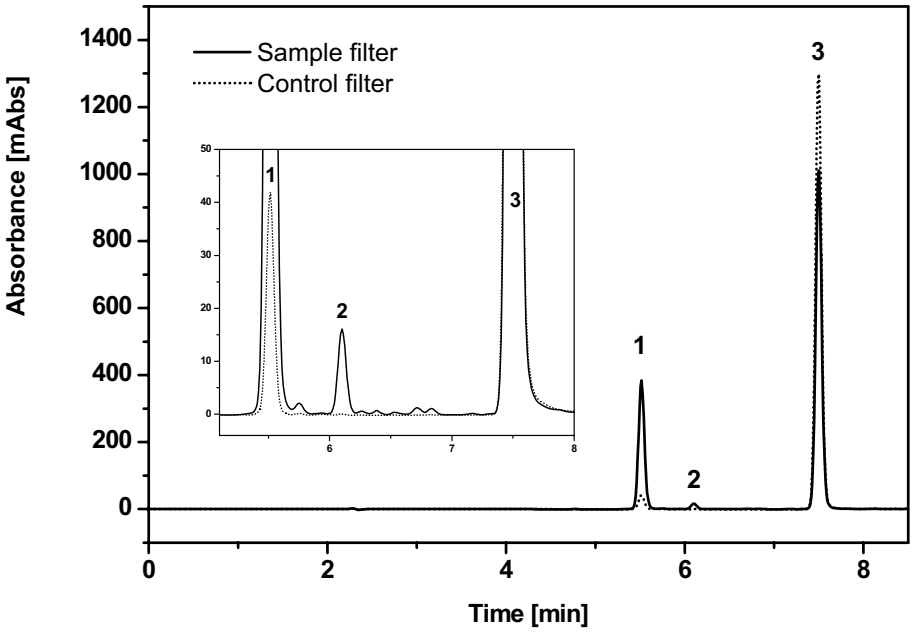

Fig. 7.7: HPLC analysis of a passive PAA air sample. The chromatograms show the separation of eluted control and sample filters from a diffusive sampler exposed to 5 ppm PAA for 2 h. Peaks: ADSO (1), $ADSO_2$ (2), ADS (3).

The ADS sulfide reagent always contains, due to oxidation during the synthesis, a small contamination of about 0.2% of the sulfoxide. Moreover, it was found that the background signal increased during the impregnation procedure, possibly due to catalytic effects of the larger surface. Therefore, it was important that the control and sample filters for one badge were always prepared simultaneously and treated in the same way before use.

The sample filter chromatograms sometimes also revealed a third distinct peak (T_R= 6.2 min; Fig. 7.7) that was related to the sulfone ($ADSO_2$) as a further oxidation product from ADSO. This was confirmed by means of mass spectrometry, as mass traces could be assigned to each peak that differed by a mass to charge ratio (*m/z*) of 16, thus representing one additional oxygen atom each (ADSO ($[M+H]^+$): m/z = 368; $ADSO_2$ ($[M+H]^+$): *m/z* = 384; ADS ($[M+H]^+$): *m/z* = 352).

Comparison with diffusion theory

In theory, the sampling rate could also be calculated using the Fick law of diffusion (see above) and the Fuller, Schettler and Giddings (FSG) correlation (eq. 7.1) for estimation of binary diffusion coefficients [40]. However, in practice these theoretical values may differ from the experimentally determined sampling rates.

$$D_{BA} = \frac{0.001 \cdot T^{1.75} \cdot \sqrt{M_r}}{p(V_A^{1/3} + V_B^{1/3})^2} \qquad (7.1)$$

The FSG-method is based on the regression formula where D_{BA} is the diffusion coefficient of compound B in compound A in cm^2/s, T is the temperature in K, p is the pressure in atm, M_r a function of the molecular weights M_A and M_B of compounds A and B, V_A and V_B are the molar volumes of air (A) and the gas (B) in question. M_r is equal to $(M_A+M_B)/M_AM_B$. V_B can be estimated from volume increments associated with each element in the compound. These increments give the volume (cm^3) per mole of atom present.

If the preliminary result of S_R(PAA) = 9.1 mL/min was compared to a theoretically calculated value for PAA using the FSG correlation, it was found to be far less than the calculated value of 19.4 mL/min. Based on own and other groups´ experiences with other analytes and the same diffusive sampling device [35,38], the experimentally determined sampling rate was expected to be only slightly lower than the calculated one, e.g. 92% for formaldehyde [34], or 73% for methyl isocyanate [38].

Examination of sampler saturation

The relatively high control filter value raised suspicion that the selected reagent excess was too small. Another experiment was then performed to examine the effect of the reagent excess; this time at a PAA concentration of 3 ppm. Three series of samplers were exposed to this test atmosphere for 1, 2 and 3 h respectively in order to find out if there is a saturation effect.

The results are shown in Table 7.3: The determined sampling rates were continuously decreasing with longer sampling periods, while the $ADSO_2$ peak increased in that order. During the 1-h experiment, no sulfone was formed. This clearly indicated that, as long as the ADS excess was sufficient, only ADSO was formed. If the ratios between the ADSO peak areas of the control filters and their corresponding sample filters were considered, it could be seen that the control filter fraction increased from 7% to 17%. If the measured control values for all three experiments were set to be 7% and the respective sample amounts were extrapolated on that basis, the resulting sampling rates corresponded well with each other and were all in the range of 14 mL/min for this set of experiments, which is in the expected range. These are further indications that the amount of reagent on the filter was not sufficient for longer sampling periods than 60 minutes, especially in combination with high PAA concentrations.

Table 7.3: Examination of the effect of the sampling time (t) on the determined sampling rate (SR), on the amount of $ADSO_2$ yielded and on the percentage of ADSO found on the control filter (CF) compared to the sample filter (SF) (control filter percentage, CFP). c(PAA) was 3 ppm, and N=4 for each experiment.

t (min)	SF peak area $ADSO_2$	SR (mL/min)	RSD (%)	CF peak Area ADSO	CFP (%)	SR (for CFP = 7%)
60	x	14.2	1.1	16000	7.0	14
120	7300	8.3	12.5	26000	9.9	13
180	13400	5.2	18.3	45000	17.0	14

However, if the reagent excess would be increased, the background signal would interfere stronger with the determination of lower concentrated atmospheres. Therefore, the sampler was mainly tested with respect to short-term sampling (15-30 min), in order to cover the required concentration range around the target concentration of 1 ppm. As discussed earlier, this has the advantage that the generation of stable atmospheres is the easier the shorter the required period is.

Tests of different filter materials

Prior to a more extensive validation, it was tested if the choice of another filter material had an influence on the sampling rate. For this purpose, filters were cut from polystyrene-divinyl benzene (SDB), polyvinylidene fluoride (PVDF) and Teflon® disks. Also comparable GF filters from a different manufacturer were examined. The Teflon® membranes could not be impregnated at all, as the reagent solution refused to wet the material. The SDB filters were impregnated the same way as the glass fiber filters, but the HPLC analysis revealed an almost complete conversion of the ADS into ADSO, even without exposure to any peroxides. This was probably due to traces of peroxides that were left as impurities on the SDB material from its production process. For the other filter types, however, the diffusive sampling experiments showed no difference compared with the original glass fiber filters.

7.3.6 Determination of PAA sampling rate

The results for all PAA passive sampling experiments performed at concentrations between 0.5 and 8 ppm are summarized in Table 7.4. The

sampling rate was determined to be 15.6 mL/min with a relative standard deviation of 4.7% (based on the mean results of the different experiments). If all analysis results of each single diffusive sampler were averaged, the mean sampling rate was found to be 15.7 mL/min with a slightly higher standard deviation of 9.2% (N = 88). This standard deviation incorporates all instrumental and experimental errors that were accumulated during sampling and analysis, including the preparation and cutting of filters and the generation of test atmospheres. The relative humidity conditions were varied between 15 and 85% and did not show a significant influence on the diffusive sampler's performance. If a recovery was calculated based on the determined mean sampling rate, most results were within 10% of the expected value.

7.3.7 Concentration range and storage stability

As stated earlier, the sampler could not be tested with test atmospheres below 500 ppb, meaning that test atmospheres in the range of the detection limit could not be experimentally reached in the laboratory. Limiting factors are the LOD and the background signal from the reagent excess. In this case, the background signal is always present above the analytical LOD, thus being the effective limiting factor. To estimate the sampler's experimental LOD, the ADSO peak areas of all HPLC injections from all control filters of the 500 ppb experiment were taken (N=15) and their standard deviation was determined. The mean uptake rate of 15.7 mL/min and three times this standard deviation as hypothetical peak area value were used to calculate back to a PAA concentration in air, which would represent the LOD. The same calculation was accomplished with 10 times the standard deviation to determine the LOQ.

Table 7.4: PAA sampling rates (SR) of the diffusive sampler, determined at different PAA concentrations, for different sampling periods (t), at different relative humidity conditions (RH)[a]

c(PAA) (ppm)	t (min)	RH (%)	N	recovery (%)	SR (mL/min)	SD (mL/min)	RSD (%)
0.5	30	15	5	102.2	15.95	0.52	3.3
0.7	45	15	6	98.4	15.36	0.60	3.9
1.0	30	15	6	103.3	16.12	1.56	9.7
1.0	30	85	6	99.3	15.50	0.95	6.1
2.0	30	15	6	94.4	14.74	0.71	4.8
2.0	30	15	6	120.2	18.77	0.70	3.7
2.8	60	15	4	90.8	14.18	0.16	1.1
5.0	30	15	5	96.1	15.00	1.40	9.3
5.0	15	15	6	107.8	16.82	0.92	5.5
5.0	15	15	8	110.8	17.30	1.10	6.4
5.3	15	85	4	96.8	15.11	0.10	0.7
5.4	30	15	5	92.2	14.39	0.78	5.4
6.4	30	15	5	94.1	14.69	0.36	2.5
6.4	30	85	4	95.5	14.91	0.23	1.5
6.4	15	15	6	101.9	15.90	1.10	6.9
8.0	15	15	6	96.6	15.08	0.59	3.9
Average of mean values:					**15.61**	**0.74**	**4.7**
Average of all individual samplers (N=88):					**15.73**	**1.45**	**9.2**

[a]SD, standard deviation; RSD, relative standard deviation; recovery based on mean sampling rate and expected concentration.

According to these results, the detection limit of the ADS diffusive sampler is 30 ppb, while the quantification limit was determined to be 90 ppb (based on 30 min sampling). Therefore, this diffusive sampling device is capable to fully cover the required concentration range down to 0.1 ppm. However, it would be advantageous to reduce the reagent excess if such low concentrations are expected, thus minimizing the negative effect of the background signal. The analytical method would allow an LOQ of 25 ppb under these conditions, or even lower, if the filter elution volume was reduced from 3 to, e.g. 2 mL.

ADS and its oxidation product ADSO are known to be stable compounds. A bulk amount of ADS was used for more than half a year without significantly increasing ADSO content. For the investigations described in this chapter, filters were impregnated and stored at most one week prior to sampling, while analysis was usually performed within 24 h after sampling, in some cases after a few days. Filters and exposed samplers were always stored in the refrigerator (in the dark) in sealed vessels. Every diffusive sampler contains a sealed control filter for blank subtraction; thus, the contribution of any slowly proceeding oxidation that is not caused by the sampling procedure will be eliminated during the evaluation process.

7.3.8 Cross reactivity towards H_2O_2

First semi-quantitative experiments revealed that there was a significant cross reactivity towards hydrogen peroxide. This stood in contradiction to our experience with liquid-phase reactions, where a 10000-fold excess of H_2O_2 over PAA was needed to give the same response. However, it was not totally

unexpected, as Effkemann et al. reported a decrease in selectivity on coated sorbent cartridges: In that case, a 100-fold excess of HP gave the same signal as from PAA, which was already by a factor of 100 lower compared to reactions in aqueous solutions [25]. Interferences resulting from other oxidants, e.g. ozone or methyl hydroperoxide (MHP), which are mainly associated with atmospheric chemistry, are not expected in the case of PAA sampling. Usually, ozone concentrations are in the low-ppb range, while MHP is normally found in the sub-ppb range. Thus, even if these compounds were present at relevant indoor workplaces, their contribution to ADS oxidation would be neglectable.

A full series of experiments was then performed to evaluate the extent of the HP cross reactivity. During these experiments, 6 identical samplers were exposed to pure H_2O_2 atmospheres between 1 and 11 ppm. To verify the HP concentration inside the test chamber, ‘online gas titrations’ were performed in some cases: Gas washing bottles filled with dilute acidic permanganate solutions were connected to the exposure chamber and continuous air samples were drawn through at known flow rates until the pale pink color disappeared. If this point was difficult to visualize, it could be verified by titrating this solution back to the first pale pink color with a permanganate solution.

The average sampling rate determined for H_2O_2 was found to be 2.45 mL/min with a standard deviation of 29% (see Table 7.5). This relatively high standard deviation could be due to the fact that the absolute amount of ADSO formed is

very small. Also, the requirement of immediate reaction (c_0=0) at the collector surface might not be fulfilled in this case, which means that the diffusion theory might also be overlaid by kinetic effects and back diffusion might occur. The experimental series at 1.1, 5.7 and 10.3 ppm were performed directly after each other, setting up the test atmosphere just by increasing the syringe flow. In that series, the formation of gas bubbles inside the syringe was negligible. The excellent correlation within that series indicated that the mean value of all experiments is a good description of the real cross reactivity.

Table 7.5: HP sampling rates (SR), determined for different sampling periods (t) and at different HP concentrations

c(HP) (ppm)	**t (min)**	**SR (mL/min) (N=6)**
1.0	240	2.73 ± 0.32
1.1	15	2.26 ± 0.38
2.5	60	3.47 ± 0.66
3.4	15	2.94 ± 0.45
5.7	15	2.26 ± 0.18
6.8	15	3.46 ± 0.42
6.8	15	1.27 ± 0.44
10.2	15	1.97 ± 0.20
10.3	15	2.25 ± 0.22
11.4	15	1.92 ± 0.39
	average	2.45 ± 0.70

If the FSG correlation is used to calculate an uptake rate for HP, a value of 36.2 mL/min is found. This is approximately by a factor of 15 higher than the experimentally determined sampling rate, which proves that there still is a significant difference in selectivity for ADS oxidation by PAA and H_2O_2, but that this difference is too small to allow for an unrestricted application in an

environment of fully unknown composition. However, it must be stated that because of the Henry's law constants (K_h), the cross reactivity towards HP might be insignificant if gaseous PAA shall be determined from evaporation out of diluted aqueous solutions that contain both PAA and HP: O'Sullivan et al. [41] measured K_h(PAA) = 837 M/atm and K_h(HP) = $8.33 \cdot 10^4$ M/atm, which means, according to Henry's law ($C_{perox} = K_h \cdot p_{perox}$) that at equal concentrations PAA has a partial pressure (p) that is 100 times higher than the one of HP.

A large number of experiments were performed attempting to minimize this cross reactivity. One approach was impregnating the filters with manganese dioxide in addition to the ADS reagent in order to use its ability of catalytical HP decomposition without affecting the PAA. For this purpose, permanganate and sodium hydroxide solutions were applied to the filters. By doing so, the yielded MnO_2 was strongly attached to the glass fiber material of the filters. Several steps of this procedure were varied, such as the concentrations of the permanganate or the NaOH solutions. Different washing steps with water, acetic acid, acetonitrile or acetone (and combinations thereof) were used in order to control the pH value on the filter. As a second approach, different attempts were made to impregnate the filters with catalase, which is also known for selective HP decomposition. First test in pure HP atmospheres were promising, as no oxidation of ADS was observed on both, catalase and MnO_2 modified filters (Table 7.6), which was exactly the goal of this experiment.

Table 7.6: ADSO peak areas from HPLC analyses of ADS diffusive samplers simultaneously exposed to the same pure HP atmosphere[a]

filter impregnated with	ADSO peak area
ADS only	62000
catalase + ADS	700
manganese dioxide + ADS	300

[a] The filters were additionally impregnated with manganese dioxide or catalase in order to decompose the HP.

Table 7.7: ADSO peak areas from HPLC analyses of ADS diffusive samplers simultaneously exposed to the same PAA atmosphere[a]

filter impregnated with	ADSO peak area (N = 3)
ADS	180000 ± 10%
catalase + ADS	75000 ± 12%
manganese dioxide + ADS	60000 ± 60%

[a] The filters were additionally impregnated with manganese dioxide or catalase in order to decompose the HP fraction present in the test atmosphere.

However, when such modified samplers were exposed to PAA atmospheres a strong effect on the PAA sampling performance was observed as well. Although some PAA exposure experiments looked promising, the reproducibility was not sufficient and the deviations within single experiments were extremely high (sometimes amounting up to 300%). In some cases, the

oxidation of ADS by means of PAA was completely inhibited on MnO_2 or catalase treated filters, while other tests indeed revealed a significant reaction with PAA (Table 7.7), but with a reaction yield that was lower than expected compared with the original method on pure ADS impregnated filters. Because of these uncertainties, it seems preferable to keep the original procedure without filter modification.

7.3.9 Field application

The ADS diffusive sampler was tested in a field application. For this purpose, a commercially available PAA-based disinfectant solution was used for area (floor and shelf) disinfection in a well-ventilated room of ~20 m^2. Approximately one-third of the floor was treated. The disinfectant was diluted and used strictly according to the instructions that were provided by the manufacturer. The concentrated disinfectant solution contained 40.3 % PAA (Label 40 ± 2%) and 11.5% H_2O_2. A 0.25% dilution was used and the residence time was 30 min.

Three sets of three diffusive samplers each were placed at three different positions in the room. The samplers were positioned close to the floor and on top of the shelf. The diffusive samplers were analyzed and gave the following results, provided as average concentration and its standard deviation: The samplers on the floor indicated a PAA concentration of 2.30 ± 0.34 ppm and 1.69 ± 0.14 ppm, while the result obtained from the shelf series was 1.36 ± 0.18 ppm. Concentration data obtained by passive sampling experiments were compared with a pumped reference method, in which the PAA

concentration was determined to ~1 ppm. However, it is important to state that the sampling positions of active and diffusive samplers had to be different in order not to remove the analyte from the air by the reference. These results cannot be compared directly, as the sampling positions were different and in contrast to the validation experiments, the PAA concentration in the room was certainly not homogeneous due to ventilation. As expected, the concentration close to the floor is higher than on the shelf, which was positioned at the best-ventilated position in the room. The standard deviations within one set of samplers were relatively small, indicating the good reproducibility of the measurements.

7.4 Conclusions

A passive sampling method has been developed for the determination of gas phase peroxyacetic acid. It is based on diffusion-controlled collection of PAA on ADS-impregnated glass fiber filters. Even though similar procedures are known for a wide variety of analytes, this was the first time that a passive method was proposed for the sampling and analysis of airborne peroxides. The mean uptake rate for peroxyacetic acid was determined to 15.7 mL/min ± 9.2% (N=89) and no significant deviation was observed at relative humidity conditions between 15 and 85%. However, a cross reactivity towards hydrogen peroxide was observed and found to be 2.45 mL/min, which means a certain limitation in terms of applicability, as the approximate concentration of airborne hydrogen peroxide must be estimated. This assessment is simplified by the fact that PAA has a strong penetrative and characteristic odor, which can be easily recognized above concentrations of 1 ppm.

However, if a signal was falsely interpreted as PAA-caused (instead of HP), this would from the workplace safety point of view reveal an even more serious issue, as that would mean an exposure to much higher hydrogen peroxide concentrations than expected. As diffusive sampling methods are, because of their ease of handling generally best suited for screening purposes, a positive result should be in any case reaffirmed by other independent methods before measures are taken. The general applicability of the new method has been demonstrated by performing a determination of PAA during a room disinfection process with a commonly used and commercially available disinfectant solution.

7.5 References

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Chapter 8

Concluding Remarks and Future Perspectives

A variety of active and passive sampling methods for the sensitive determination of airborne isocyanates and peroxyacetic acid has been presented in this thesis. A novel derivatization reagent has been introduced for the analysis of airborne isocyanates, allowing sensitive determination with reduced matrix-interferences due to the application of a technique hyphenating liquid chromatography, electrochemical on-line oxidation and mass spectrometry.

The general applicability of tube-type diffusive sampling devices equipped with reagent-coated filter tapes for the analysis of airborne isocyanates and peroxyacetic acid was successfully demonstrated in this thesis. The challenges associated with diffusive sampling, however, were of a different kind for both groups of analytes. While isocyanates are among the substances being regulated to the lowest occupational exposure limits of all reactive organic compounds, the target concentration range for the determination of peroxyacetic acid was almost three orders of magnitude higher. Therefore, the sensitivity of the analytical methods was the crucial factor for the analysis of passive isocyanate samples, which often yielded sample solutions that were in the range of the analytical LOQ due to the inherently very small sample volumes. As in the case of peroxyacetic acid no true derivatization procedure

is known, a chemisorption method based on PAA-induced oxidation of ADS to ADSO has been used. Thus, a non-specific reaction product was yielded, which made it impossible to distinguish between different sources of oxidation. The specifity of the oxidation reaction and stability of the reagent towards other oxidants (especially hydrogen peroxide) was therefore particularly considered.

In order to be able to perform reproducible and reliable air sampling experiments, stable test atmospheres were required. For this purpose, a dynamic generation system has been constructed using analyte solutions that were nebulized into continuous air streams.

After elucidation and resolution of the initially experienced humidity problem, the full validation of the NBDPZ diffusive sampling methods for MIC, EIC and PhIC showed that the determined sampling rates were constant for different humidity conditions and concentration ranges. Tandem mass spectrometric (MS/MS) detection was found to be the method of choice, even though fluorescence detection works well for more concentrated samples. However, due to the fact that even the higher concentrated passive samples still contained only small amounts of analytes, the superior selectivity and sensitivity of MS/MS detection makes its use inevitable for unrestricted application of the isocyanate passive sampler.

It can be expected that future generations of mass spectrometers are even providing better sensitivity. Especially state-of-the-art triple-quadrupole

tandem MS instruments are supposed to be superior to the used ion trap instrument. In particular, a faster screening for unknown isocyanate derivatives would be possible, as the ion trap always had to be loaded with one target mass within a certain elution time window in order not to lose sensitivity.

However, in the case of diffusive sampling, even if unknown substances could be identified and quantified, the specific sampling rate must be known to re-calculate the correct concentration in air.. As the concentration of all isocyanates in air (TRIG) should be increasingly determined instead of individual concentrations, one single method will certainly be only a part of a complex exposure measurement and assessment. Diffusive sampling is naturally only suited for vapor-phase analytes, as particulates or aerosols are having much lower diffusion rates. As the abundance and probability of airborne isocyanates in aerosol form increases with molecular size, diffusive sampling will be best applicable for the lower molecular species, such as the analytes covered by the work described in this thesis.

Future work could be directed towards comparison and application studies of passive and active sampling systems at real workplaces. Possibly, certain patterns of isocyanate fragment distribution can be associated with typical work processes, so that in the future some low molecular isocyanates could be used as markers for the TRIG concentration, e.g. PhIC for decomposition products from MDI-based materials. Ratios between the concentrations of several low-molecular isocyanates could be used as well. However this

requires a huge amount of reliable data from many different workplace monitoring applications.

The new Fc-PZ reagent presented in this thesis shall be part of more in-depth laboratory and field measurements. As all piperazine reagents that were presented could in principle also be used for the analysis of airborne anhydrides, this might be an interesting field for further research. First promising tests of analysis methods were performed during the work of this thesis. However, in this study anhydrides were exclusively used to remove reagent excess and were not considered as analytes.

With respect to the determination of airborne peroxides, diffusive sampling applications are certainly very attractive. Therefore future work should be directed towards the elimination of the cross interference resulting from hydrogen peroxide.

Summary

Summary

Within this thesis, new sampling and analysis strategies for the determination of airborne workplace contaminants have been developed. Special focus has been directed towards the development of air sampling methods that involve diffusive sampling.

In an introductory overview, the current state-of-the-art of sampling and analysis of airborne isocyanates is reviewed. The most important derivatization reagents are introduced, and their application for air analysis with special emphasis on sampling techniques and detection methods is presented.

In a first study, it could be shown that the derivatization reaction with NBDPZ can be used for diffusive sampling of methyl isocyanate (MIC), if reagent-impregnated glass fibre filters were used as collector surface. The method developed was the first one allowing the determination of airborne MIC by means of HPLC-MS/MS and HPLC-FLD. The developed tandem-mass spectrometric method allowed for very sensitive detection down to concentrations of $8 \cdot 10^{-10}$ mol/L, and best performance was obtained at low relative humidity conditions. If long term sampling was carried out at high-humidity conditions, decreased sampling rates were observed.

During further work, it could be shown that the humidity interference was a physical problem, caused by displacement of the hydrophobic NBDPZ reagent away from the glass fibre filter's surface. A comparison of two different filter materials turned out that the use of less polar SDB filter tapes resulted in higher and reproducible sampling rate values. Furthermore, this study was successfully extended to cover ethyl and phenyl isocyanate as well, being the first time that diffusive sampling was applied for these analytes. In addition, diffusive sampling experiments were performed with 2-MP as derivatizing agent, showing no humidity dependency for both filter types, but difficulties for the determination of phenyl isocyanate.

A validation of the new passive sampler for MIC, EIC and PhIC was executed using NBDPZ-coated SDB filters. The individual sampling rates have been determined for different conditions and concentrations. The measured sampling rates were independent of analyte concentration and relative humidity conditions and were decreasing with increasing size of the analyte molecule. It could further be shown that vapor-phase isocyanic acid could also be determined by diffusive sampling, but owing to background problems, ICA could only be quantified when it was present in concentrations in the high ppb range. For the determinations, ion-trap MS/MS LC methods were used, employing electrospray ionization (for 2-MP) and atmospheric pressure chemical ionization (for NBDPZ). The 2-MP method was used as reference after active sampling using reagent-coated filters. A similar active method was

also developed for NBDPZ, however it was only suitable to be used for PhIC sampling.

Ferrocenoyl piperazide was presented as a new pre-column derivatizing agent for the analysis of isocyanates using reversed-phase liquid chromatographic separation, electrochemical oxidation/ionization and mass spectrometry. As only the ferrocene-based derivatives were ionized in the electrochemical cell, MS detection could be performed without interferences from matrix components and background signals, and therefore, very sensitive detection could be achieved considering the used single quadrupole instrument.

The first passive sampling method has been developed for the determination of gas phase peroxyacetic acid (PAA), based on chemisorption of PAA on ADS-impregnated glass fiber filters. The reagent was oxidized by PAA to the corresponding sulfoxide ADSO, and the samples were analyzed by means of LC-UV/vis. The determined sampling rate was constant in the range around 1 ppm, with no interference from humidity being discovered, and the detection limit was ~30 ppb. Thorough investigations were carried out with respect to the selectivity of the method towards hydrogen peroxide observing a minor cross reactivity, which is not crucial for the desired application, but means a certain limitation, as the approximate concentration of airborne hydrogen peroxide should be estimated. The applicability of the new method has been demonstrated by successfully analyzing air samples taken during disinfection of a laboratory area.

Samenvatting

Samenvatting

Dit proefschrift beschrijft de ontwikkeling van nieuwe bemonsterings- en analysestrategieën voor de bepaling van schadelijke stoffen in de lucht op de werkplek. De nadruk ligt voornamelijk op de ontwikkeling van diffusieve luchtbemonsteringsmethodes.

De huidige en nieuwste technieken voor bemonstering en analyse van isocyanaten in de lucht worden getoond in het inleidende overzicht. Daarnaast worden de belangrijkste derivatiseringsreagentia geïntroduceerd en hun toepassingen met betrekking tot luchtanalyse worden besproken. In het bijzonder worden er bemonsteringstechnieken en detectiemethodes behandeld.

Eerste studies toonden aan dat de derivatiseringsreactie met NBDPZ gebruikt kan worden voor diffusieve bemonstering van methylisocyanaat (MIC) wanneer de collectoroppervlakte uit glasvezelfilters, geïmpregneerd met het reagens, bestaat. Dit leidde tot de ontwikkeling van de allereerste methode voor de bepaling van MIC in de lucht met behulp van HPLC-MS/MS en HPLC-FLD. Met de tandem-MS-methode is de detectie zeer gevoelig (met detectielimieten rond $8 \cdot 10^{-10}$ mol/L) en de beste resultaten worden verkregen bij lage relatieve luchtvochtigheid. Daarnaast werd een dalende snelheid van

monstername waargenomen bij het uitvoeren van lange termijn bemonstering in aanwezigheid van hoge luchtvochtigheid.

Tijdens het onderzoek werd aangetoond dat de bovengenoemde vochtigheidsinterferentie een fysiek probleem is, veroorzaakt door de afstoting tussen het hydrofobe NBDPZ-reagens en de glasvezelfilteroppervlakte. De vergelijking tussen twee verschillende filtermaterialen liet zien dat het gebruik van minder polaire SDB-filters leidde tot hogere en reproduceerbare snelheden van monstername. Daarnaast werd diffusieve bemonstering voor het eerst met succes toegepast om ethyl- en phenylisocyanaat te detecteren. Bovendien werden er diffusieve bemonsteringsexperimenten uitgevoerd met het 2-MP-reagens, waarin aangetoond werd dat de prestaties van beide filtermaterialen onafhankelijk zijn van de vochtigheid. Echter, de bepaling van phenylisocyanaat verliep moeizaam.

De nieuwe passieve sampler voor MIC, EIC en PhIC werd getest met behulp van SDB-filters, geïmpregneerd met NBDPZ. Om dit doel te bereiken werden de individuele snelheden van monstername bepaald voor verschillende concentraties en onder verschillende omstandigheden. De resultaten toonden aan dat de gemeten bemonsteringsdebieten onafhankelijk zijn van de analietconcentratie en de relatieve vochtigheid en er werd een afname van de snelheid van monstername bij stijgende grootte van het analietmolecuul geconstateerd. Daarnaast wezen de verkregen resultaten erop dat het isocyaanzuur (ICA) in de gasfase ook door diffusieve bemonstering kan worden bepaald. Echter, als gevolg van de achtergrondproblemen, kon ICA

alleen worden gekwantificeerd wanneer het in concentraties in het hoge ppb-gebied aanwezig was. De bepaling werd uitgevoerd met behulp van ion-trap MS/MS LC-methodes, gebruikmakend van electrospray ionization (voor 2-MP) en atmospheric pressure chemical ionization (voor NBDPZ) als ionisatiebronnen. Nadat de actieve bemonstering met reagentgeïmpregneerde filters werd uitgevoerd, werd de 2-MP-methode als referentie gebruikt. Ook werd er een soortgelijke actieve methode ontwikkeld voor NBDPZ, maar deze is alleen geschikt voor PhIC bemonstering.

Ferrocenoylpiperazide werd voorgesteld als een nieuw pre-column reagens om isocyanaten te analyseren met behulp van reversed-phase vloeistofchromatografie, elektrochemische oxidatie/ionizatie en massaspectrometrie. Omdat alleen de ferrocene gebaseerde derivaten werden geïoniseerd (in de elektrochemische cel), kon de MS-detectie zonder storingen van zowel de matrixcomponenten als de achtergrondsignalen worden uitgevoerd. Dit leidde tot een zeer gevoelige detectie, zelfs met het gebruikte single-quadrupool instrument.

De eerste passieve bemonsteringsmethode werd ontwikkeld voor de bepaling van peroxyazijnzuur (PAA) in de gasfase, gebaseerd op PAA chemisorptie op ADS-geïmpregneerde glasvezelfilters. Het reagens werd door PAA geoxideerd om sulfoxide ADSO te vormen. Vervolgens werden de monsters geanalyseerd door middel van LC-UV/vis. De bepaalde snelheid van monstername was constant in het concentratiegebied van 1 ppm, er werden geen vochtigheidsinterferenties geconstateerd en de detectielimiet was ~30

ppb. Een zorgvuldig onderzoek werd uitgevoerd om te bekijken wat de selectiviteit van de methode t.o.v. waterstofperoxide was en een geringe kruisreactiviteit werd waargenomen. Hoewel deze kruisreactiviteit niet essentieel is voor de gewenste toepassing, kan deze toch worden gezien als een beperking omdat er een schatting van de concentratie waterstofperoxide in de lucht noodzakelijk is. De toepasbaarheid van deze nieuwe methode werd aangetoond door het analyseren van luchtmonsters, die tijdens het desinfecteren van een laboratorium werden genomen.

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Curriculum Vitae

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Education

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1983 – 1992	Priv. St. Josef Gymnasium der Kapuziner in Bocholt, Germany
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1993 – 1994	College year at Lassen College, Susanville, CA, USA
1994 – 2002	Studies of chemistry at the Westfälische Wilhelms-Universität Münster, Germany
10/1999	Intermediate exam
01/2002	Graduation with the thesis entitled "*Entwicklung eines Passivsammlers für die Bestimmung von Methylisocyanat in Luft mit 4-Nitro-7-piperazino-benz-2-oxa-1,3-diazol als Derivatisierungsreagenz*"

02/2002	Begin of the dissertation in analytical chemistry at the Universiteit Twente in Enschede, Netherlands, with Prof. Dr. Uwe Karst
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List of Publications

Original Papers

1. H. Henneken, R. Lindahl, A. Östin, M. Vogel, J.-O. Levin and U. Karst, *Diffusive Sampling of Methyl Isocyanate Using 4-Nitro-7-piperazinobenzo-2-oxa-1,3-diazole (NBDPZ) as Derivatizing Agent*, J. Environ. Monit. **2003**, 5 (1), 100-105.

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2. *Entwicklung einer Passivsammelmethode zur Bestimmung von Methylisocyanat in Luft*, InCom **2003**, Düsseldorf, Germany.

3. *Humidity Effects on Passive Air Sampling of Methyl Isocyanate Using 4-Nitro-7-piperazinobenzo-2-oxa-1,3-diazole (NBDPZ) as Derivatizing Agent*, Artefacta **2003**, Munich, Germany.

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5. *Diffusive Sampling of Isocyanates*, Pittcon **2005**, Orlando, USA.

6. *Diffusive Sampling of Low Molecular Isocyanates*, Airmon **2005**, Loen, Norway.

7. *Determination of Airborne Isocyanates by HPLC with Fluorescence and MS Detection,* HPLC **2005**, Stockholm, Sweden.

8. *Active and Diffusive Air Sampling of Low Molecular Isocyanates,* NWO Congres Studiegroep Analytische Scheikunde **2005**, Lunteren, The Netherlands.

9. *Active and Passive Sampling and Analysis of Airborne Isocyanates and Peroxides,* Analytical Seminar at the University Münster **2006**, Münster, Germany.

Poster Presentations:

1. *Validation of 4-Nitro-7-piperazinobenzo-2-oxa-1,3-diazole as a New Reagent for Diffusive Sampling of Methyl Isocyanate,* Airmon **2002**, Lillehammer, Norway.

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3. *Sensitive Derivatization Methods for the Determination of Airborne Isocyanates,* ISC **2002**, Leipzig, Germany.

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6. *Passive Sampling of Low Molecular Monoisocyanates*, NWO Congres Studiegroep Analytische Scheikunde **2003**, Lunteren, The Netherlands.

7. *Development of a Passive Sampling Method for Determination of Peroxyacetic Acid in Workplace Atmospheres*, Analytica **2004**, Munich, Germany.

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