IMPROVED BREATH ALCOHOL ANALYSIS WITH USE OF CARBON DIOXIDE AS THE TRACER GAS

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School of Innovation, Design and Engineering
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Akademisk avhandling

som för avläggande av teknologie doktorsexamen i elektronik vid Akademin för innovation, design och teknik kommer att offentligen försvaras fredagen den 10 september, 2010, 10.15 i Milos, Mälardalens högskola, Västerås.

Fakultetsopponent: Dr. Johannes Lagois, Dräger Safety AG & CO
Abstract

State-of-the-art breath analysers require a prolonged expiration into a mouthpiece to obtain the accuracy required for evidential testing and screening of the alcohol concentration. This requirement is unsuitable for breath analysers used as alcolock owing to their frequent use and the fact that the majority of users are sober drivers; as well as for breath testing in uncooperative persons.

This thesis presents a method by which breath alcohol analysis can be improved, using carbon dioxide (CO₂) as the tracer gas, offering quality control of the breath sample, enabling the mouthpiece to be eliminated, and bringing about a significant reduction in the time and effort required for a breath alcohol screening test. With simultaneous measurement of the ethanol and the CO₂ concentrations in the expired breath, the end-expiratory breath alcohol concentration (BrAC) can be estimated from an early measurement, without risk of underestimation.

Comparison of CO₂ and water as possible tracer gases has shown that the larger intra- and inter-individual variations in the (end-expiratory) concentration is a drawback for CO₂ whereas the advantages are a low risk of underestimation of the BrAC, and the limited influence from ambient conditions on the measured CO₂ concentration. The latter is considered to be of importance because the applications likely imply that the breath tests will be conducted in an uncontrolled environment, e.g., in a vehicle or ambulance. In emergency care, the measurement of the expired CO₂ concentration also provides the physicians with information about the patient’s respiratory function.

My hope and belief, is that with a more simple, reliable and, user-friendly test procedure, enabled with the simultaneous measurement of the CO₂ in the breath sample, the screening for breath alcohol will increase. An increased number of breath alcohol analysers installed as alcolocks and more breath alcohol tests conducted in emergency care, is likely to save lives and diminish the number and severity of injuries.
Sammanfattning

För bestämning av alkoholkoncentrationen i utandningsluften kräver dagens alkometrar att användaren gör en lång och kraftig utandning i ett minstycke. Vid användning av alkometrar som alkoläs och vid mätning på patienter är kravet på denna relativt ansträngande typ av utandning mindre lämplig av flera anledningar. Alkoläs används ofta och till största delen av nyktra förare, varav en icke försvarar andel upplever problem att genomföra ett utandningsprov. Dessutom minskar tillförlitligheten på mätningen vid provtagning på patienter med nedsatt förmåga att medverka.

Genom samtidig mätning av koldioxid i luftprovet kan användandet av alkometrar underlättas på flera sätt; nivå på uppmätt koldioxidkonsentration ger ett mått på kvalitén på utandningsprovet, beröringsfri mätning kan möjliggöras och kravet på utandningens tid och volym kan sänkas.

En jämförelse av koldioxid och vatten som alternativa referensgaser, har visat att till nackdel för koldioxid är den större variationen i endexpiratorisk (alveolär) konsentration mellan olika individer samt att nivån påverkas av fysisk ansträngning. Dock är fördelarna att koldioxidkonsentrationen i en utandning i mindre grad påverkas av omgivande faktorer och att risken för att en falskt låg alkoholkoncentration presenteras är lägre jämfört med om vatten används som referensgas. Dessutom ger den uppmätta koldioxidkonsentrationen den medicinska personalen en indikation om patientens respiratoriska tillstånd.

Min förhoppning och tro är att genom en enklare, mer användarvänlig och tillförlitlig mätproceduren, vilket kan ästadkommas med hjälp av koldioxid som referensgas, kommer acceptansen för och användandet av alkometrar att öka. Ett ökat antal installerade alkoläs och genomförda utandningsprov inom ambulans- och akutsjukvård, kan med stor sannolikhet leda till ett minskat antal skadade och döda i trafiken samt leda till färre allvarliga patientskador.
"Vissa dagar har det ingen betydelse alls om man kan stava till Tisdag."

- Kanin

"...eller förstår sig på alkohollunodningsanalys."

- Annika
Contents

1 Introduction

2 Breath alcohol analysis 21
   2.1 The pharmacokinetics of ethanol .................................. 23
   2.1.1 Absorption and distribution .................................. 23
   2.1.2 Elimination .................................................. 24
   2.2 The gas exchange ............................................... 25
   2.2.1 The exchange of ethanol .................................. 26
   2.2.2 Conditioning of inspired air ................................. 27
   2.2.3 The expirogram ............................................ 28
   2.2.4 Physiological factors that influence the gas exchange .... 31
   2.3 Summary breath alcohol analysis ................................ 34

3 Breath alcohol testing in practice 37
   3.1 The Blood:Breath Ratio ........................................ 37
   3.2 Traffic law enforcement ........................................ 38
   3.2.1 Alcolocks .................................................. 38
   3.3 Breath alcohol analysis in emergency care .................... 40
   3.4 Improved breath analysis with use of a tracer gas ........... 43

4 Sensor technologies for breath alcohol analysis 47
   4.1 Selectivity ..................................................... 47
   4.2 Sensor technologies ........................................... 48
   4.3 IR transmission spectroscopy .................................. 49

5 Research contribution 53
   5.1 Methods and materials .......................................... 53
   5.1.1 Investigation of the measurement method ................. 53
   5.1.2 Evaluation of the breath analyser prototypes .......... 55
   5.2 Results ....................................................... 58
### Contents

5.2.1 Investigation of the measurement method ............... 58  
5.2.2 Evaluation of the breath analyser prototypes ............. 69

6 Discussion 73

7 Future work 77  
7.1 New prototypes ............................................. 77  
7.2 Analysis of additional breath compounds ..................... 78

8 Conclusion 81

Bibliography 87
## Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \lambda_{ba} )</td>
<td>Blood:air partition coefficient</td>
</tr>
<tr>
<td>BAC</td>
<td>Blood alcohol concentration</td>
</tr>
<tr>
<td>BBR</td>
<td>Blood:breath ratio</td>
</tr>
<tr>
<td>BrAC</td>
<td>Breath alcohol concentration</td>
</tr>
<tr>
<td>CO(_2)</td>
<td>Carbon dioxide</td>
</tr>
<tr>
<td>COPD</td>
<td>Chronic obstructive pulmonary disease</td>
</tr>
<tr>
<td>EtOH</td>
<td>Ethanol</td>
</tr>
<tr>
<td>EtOH/CO(_2)</td>
<td>Ratio of the ethanol and carbon dioxide concentration</td>
</tr>
<tr>
<td>EtOH/H(_2)O</td>
<td>Ratio of the ethanol and water concentration</td>
</tr>
<tr>
<td>H(_2)O</td>
<td>Water</td>
</tr>
<tr>
<td>PCO(_2)</td>
<td>Partial pressure of carbon dioxide</td>
</tr>
</tbody>
</table>
Chapter 1

Introduction

Breath analysis provides an opportunity to non-invasively reflect the concentrations of many of the gases present in blood. The only restraint is that the substance of interest must have a sufficiently high vapour pressure at the prevailing temperature in the lungs and airways. Apart from analysis of expired respiratory gases, e.g. carbon dioxide in medical care, one of the more common breath analysis applications is quantification of the breath alcohol concentration (BrAC) for forensic purposes related to traffic law enforcement. The interest in breath alcohol analysis and need for on-the-spot determination of the BrAC grew with the increased number of vehicles on the roads and the knowledge that drink driving is closely associated with the occurrence of traffic accidents.

The drawbacks with existing state-of-the-art breath alcohol analysers are the need for regular calibration, and, from the user’s perspective, the requirement of a prolonged and often forced expiration into a single-use mouthpiece. The breath test procedure takes advantage from the fact that the BrAC increases with increased expired volume. For persons with a small vital capacity volume or with impaired pulmonary function, e.g. elderly women and persons diagnosed with chronic obstructive pulmonary disease (COPD), this requirement can lead to difficulties in achieving a valid breath test as the person becomes short of breath. Furthermore, in those cases where a person is unable to cooperate when testing, the steady increase of the BrAC during an expiration implies that the reliability of the measurement is lowered.
This thesis explains how the use of carbon dioxide as the tracer gas can improve the usability and reliability of breath alcohol analysers used in two applications: an alcohol ignition interlock, a so called alcocol, and in emergency care patients who cannot cooperate in breath testing.

Introduction to the use of carbon dioxide as the tracer gas
As with the BrAC, the concentration of CO₂ increases with the volume of air expired. With simultaneous measurement of CO₂ in the expired breath, the ratio between the measured CO₂ concentration and a reference value given by the end-expiratory (alveolar) CO₂ concentration can be calculated, and this ratio reflects the volume fraction expired in relation to the total lung volume or, in the case of contact-free measurement, the dilution of the sample of the breath.

With use of this ratio, an estimation of the end-expiratory BrAC can be made from the alcohol concentration measured in the breath sample. However, the measurement method relies on two assumptions, namely that the intra- and inter-individual variations in the end-expiratory (alveolar) CO₂ concentration are of acceptable size, and that the expiration profiles of the ethanol and CO₂ concentrations are correlated throughout an expiration.

A more user-friendly alcocol
The general attitude towards alcocol in Sweden has drastically changed in the last decade, and with that, their use has increased. Today alcocol are installed in taxis, buses and trucks for quality assurance of transport companies and interest in purchasing an alcocol for installation in private cars is increasing.

As a result of the increased interest and use of alcocol nationally, and internationally, the development of a new alcocol began in 2005. The aim was to develop a vehicle integrated alcocol that was more user friendly, with a reduced product-life-cycle cost, and increased calibration interval, in comparison to alcocol available on the market. The goal has been to improve different aspects of the usability by enabling contact-free measurements and more shallow expirations to be performed, features that are likely to appeal to frequent users, including both professional and non-professional drivers.

The project was lead by Autoliv Development AB and Hök Instrument AB, Imego and SenseAir AB were engaged as technical partners. The vehicles manufactures, Volvo Cars and AB Volvo, also took an
active part, especially concerning the usability aspects, Hök Instrument AB has mainly been involved in finding technical solutions, the design, assembly and evaluation of prototypes, the evaluation of usability and human-machine interface, and has been responsible for the clinical studies conducted for evaluation of the measurement method of using CO$_2$ as the tracer gas. Since January 2009, the project has entered its development phase, in which Hök Instrument AB is continuously involved as a partner to Autoliv Development AB.

**Improved breath alcohol analysis in emergency care**
In pre-hospital and emergency care, the paramedics, nurses and physicians often have to consider a multitude of diagnoses, possibly including alcohol intoxication. In these cases it is of benefit to assess the alcohol concentration in order to decide whether to include or exclude alcohol intoxication as a possible diagnosis. The rapid response and non-invasive nature of breath analysis is preferable to the invasive, time-consuming and more costly blood analysis. However, in uncooperative or unconscious patients, the reliability of BrAC measurement is decreased, and to my knowledge, there is no breath analyser available on the market today that can assess the quality of a breath sample.

With the knowledge gained concerning the tracer gas measurement method and the technical development within the alcolock project, a breath analyser for usage in emergency care is under development. This breath analyser uses the same technology as the alcolock, but with some technical changes with respect to the sampling of small and passively expired volumes. Upon the presentation of the measured CO$_2$ concentration, the user would be informed about the quality of the breath test and would thus be able to decide whether to conduct a re-test or not.
Aims of the thesis

The primary aim of this thesis was to investigate whether the measurement of expired CO₂ could be used for reliable estimation\textsuperscript{1} of the end-expiratory BrAC, under different respiratory conditions\textsuperscript{2} [Papers I and II].

A second and subsequent aim was to contribute to the development and the evaluation of prototypes of breath analysers designed for two areas of application: as alcolocks and for use in emergency care [Papers III and IV].

\textsuperscript{1}Reliable estimation: minimising the underestimation of the BrAC and offering sufficient accuracy for screening purposes.

\textsuperscript{2}Respiratory conditions can vary as a result of differences in anatomy and physiology, the expired volume, different breathing pattern, and because of variations in the environmental conditions.
List of publications

These papers are reprinted with kind permission from the publishers.

Paper I

Methodology investigation of expirograms for enabling contact free breath alcohol analysis
A. Jonsson, B. Hök, L. Andersson, and G. Hedenstierna

Paper II

Influences from breathing pattern on alcohol and tracer gas expirograms - implications for alcolock use
A. Kaisdotter Andersson, B. Hök, M. Ekström, and G. Hedenstierna
In press, Forensic Science International.

Paper III

Breath analyzer for alcolocks and screening devices
B. Hök, H. Pettersson, A. Kaisdotter Andersson, S. Haasl, and P. Akerlund

Paper IV

Improved breath alcohol analysis in patients with depressed consciousness
A. Kaisdotter Andersson, B. Hök, D. Rentsch, G. Rücker, and M. Ekström
In press, Medical & Biological Engineering & Computing.
Related work

Development of a breath alcohol analyzer for use on patients in emergency care
A. Jonsson, B. Håk, and M. Ekström
Proceedings of The World Congress of Medical Physics & Biomedical Engineering, München, 7-12 September, 2009.

Breath alcohol sensor for emergency care
J. Steggo, A. Kaisdotter Andersson, and B. Håk

Physiological and usability aspects of breath alcohol estimation
A. Kaisdotter Andersson, B. Håk, and H. Pettersson

Additional papers not included in this thesis

Evaluation of antidecubitus mattresses
A. Jonsson, M. Lindén, M. Lindgren, L-Å. Malmqvist, and Y. Bäcklund

Skin temperature effects on the skin blood flow at areas prone to pressure sore development
A. Jonsson, M. Lindgren, and M. Lindén

New sensor design made to discriminate between tissue blood flow at different tissue depths at the sacral area
A. Jonsson
Technical report, Department of Computer Science & Electronics, Mälardalen University, Västerås.

A technique based on laser Doppler flowmetry and photoplethysmography for simultaneously monitoring blood flow at different tissue depths
My contribution

Papers I and II
I have taken an active part in the design of the studies, collected and analysed all data, and have written the manuscripts in collaboration with the other authors.

Paper III
I have contributed to the research result concerning the measurement method of using CO₂ as the tracer gas, and have taken part in the writing of the manuscript.

Paper IV
I initiated and designed the study, collected and analysed the data, and have written the major part of the manuscript.
Chapter 2

Breath alcohol analysis

The alcohol concentration in breath is correlated to the concentration in blood. This is a fact that has been known since 1874 when Austie presented the first result of quantitative analysis of expired ethanol. In 1927 Bogen suggested breath analysis as a test for alcohol intoxication [1], followed by results from Liljestrand and Linde [2], who presented the first ratios between ethanol concentration in the blood and the breath, from in vivo and in vitro studies performed at different temperatures.

With knowledge about the relationship between the BAC and the BrAC, often termed the Blood:Breath Ratio (BBR), the BAC can be estimated from the measured BrAC. However, the value obtained for the BBR depends on many factors and the conversion includes many fallacies. The factors that influence the measured BrAC and thus the BBR will be further discussed in this thesis. As far as the alcohol is concerned, the mechanisms included in the body’s absorption, distribution and elimination of alcohol are explained in the field of pharmacokinetics, and the field of pharmacodynamics explains what alcohol does to the body and mind [3, p.48]. The pharmacokinetics of alcohol will be briefly described, whereas the pharmacodynamics of alcohol will not be considered further with in this thesis. The effects corresponding to a specific alcohol concentration will vary between different occasions of alcohol consumption and from one individual to another, see Table 2.1. The effect depends on the individual’s habit of consuming alcohol, their physical condition and general state of health, as well as their intake of food and water.
Table 2.1: Some of the effects that might occur at the different stages of acute alcoholic influence and intoxication. The summary is made with respect to effects' relevance for driving and in medical assessment. The summary is based on [4, 3].

<table>
<thead>
<tr>
<th>BAC</th>
<th>General effects and medical symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.3-1 %&lt;sub&gt;e&lt;/sub&gt;</td>
<td>Euphoria</td>
</tr>
<tr>
<td></td>
<td>Relaxation</td>
</tr>
<tr>
<td></td>
<td>Some sensory-motor impairment</td>
</tr>
<tr>
<td></td>
<td>Loss of efficiency in critical performance test</td>
</tr>
<tr>
<td>1-2 %&lt;sub&gt;e&lt;/sub&gt;</td>
<td>Excitement</td>
</tr>
<tr>
<td></td>
<td>Slurred speech</td>
</tr>
<tr>
<td></td>
<td>Increase reaction time</td>
</tr>
<tr>
<td></td>
<td>Decreased sensory response</td>
</tr>
<tr>
<td></td>
<td>Increased sensory-motor incoordination</td>
</tr>
<tr>
<td></td>
<td>Impaired balance</td>
</tr>
<tr>
<td></td>
<td>Drowsiness</td>
</tr>
<tr>
<td>2-3 %&lt;sub&gt;e&lt;/sub&gt;</td>
<td>Stupor</td>
</tr>
<tr>
<td></td>
<td>Markedly muscular incoordination, inability to stand and walk</td>
</tr>
<tr>
<td></td>
<td>Markedly decreased response to stimuli</td>
</tr>
<tr>
<td></td>
<td>Increase pain threshold</td>
</tr>
<tr>
<td></td>
<td>Nausea and vomiting</td>
</tr>
<tr>
<td></td>
<td>Depressed consciousness, sleep or stupor</td>
</tr>
<tr>
<td>&gt;4 %&lt;sub&gt;e&lt;/sub&gt;</td>
<td>Coma</td>
</tr>
<tr>
<td></td>
<td>Impairment of circulation and respiration</td>
</tr>
<tr>
<td></td>
<td>Complete unconsciousness or coma</td>
</tr>
<tr>
<td></td>
<td>Possible death</td>
</tr>
</tbody>
</table>
2.1 The pharmacokinetics of ethanol

2.1.1 Absorption and distribution

Ethanol is a small molecule that mixes completely with water, thereby making diffusion possible throughout the body. The majority, approximately 80%, of the ethanol is absorbed by diffusion in the intestine and the rest is absorbed in the stomach [3, 5]. The amount of ethanol absorbed is regulated by the concentration gradient (the chemical concentration potential) over the gastric lumen. After absorption from the stomach or the intestine, the ethanol molecules enter the blood circulatory system, via the portal vein and the liver, which distributes the alcohol throughout the body.

When the absorption and distribution of ethanol is complete, the concentration in the blood and breath attains its maximum, $C_{\text{max}}$. The maximal concentration reached in blood after drinking alcoholic beverage depends not only on the amount of alcohol consumed, but also on the length of time over which it was consumed, and the body’s rate of absorption, distribution and elimination of ethanol on that occasion [3]. The time taken to reach $C_{\text{max}}$ for a given distributed dose of ethanol is dependent on the rate of absorption of ethanol from the intestine, which is dependent on numerous factors and is subject to large inter- and intra-individual differences. One factor considered to influence the rate of absorption is the gastric emptying, the time until the alcoholic beverage leaves the stomach and enters the duodenum. The rate of gastric emptying is dependent of factors such as whether the stomach is empty or full, whether the beverage had a high or low concentration of alcohol and carbohydrates, smoking and also the presence of various medical conditions. The distribution system for alcohol is such that the absorption process on the arterial blood side will be completed before the venous side. This means that $C_{\text{max}}$ is achieved earlier in arterial than venous blood, a difference referred to as the arterial-venous (A-V) concentration difference [6, 7, 3, 8]. The size of the A-V difference depends on the way alcohol is administered (i.e. whether it is by oral ingestion or venous infusion) as well as the rate of administration [6]. When all the alcohol has been absorbed and the distribution throughout the body is complete, the arterial and the venous concentration will be the same [6]. This is referred to as the A-V crossover point. The time at which the A-V crossover takes place depends on the blood flow to tissues
and organs and is approximately 90 minutes; thereafter the concentration in venous blood is higher than in the arterial blood [9, 3, 8, 6]. With physical activity the blood flow to muscles increases owing to vasodilation in activated muscles [16], which implies that the concentration in the arterial blood, and thus also the breath, will decrease, whereas the alcohol concentration in the venous blood increases [3]. As yet unpublished results from experimental work conducted as a part of this thesis has shown a decrease in the BrAC after physical activity on an exercise bike, see Section 5.2.1.

2.1.2 Elimination

The elimination of ethanol occurs through oxidative metabolism in organs and tissue, and might already start in the stomach (first-pass metabolism), nevertheless, it occurs primarily in the liver [1, 5, 3]. The primary active enzymes involved in the oxidative metabolism of ethanol are alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH). The final metabolites are water and carbon dioxide, with acetaldehyde and acetic acids as intermediate metabolites. In addition, a small fraction of the ethanol is excreted via breath, sweat and urine.

Widmark’s extensive work on the pharmacokinetics of ethanol showed among other things an average ethanol elimination rate for a moderate drinker of 0.15 mg g⁻¹ hr⁻¹ (β-slope) from the blood [11]. These results are in agreement with those obtained from later research [12, 13, 14]. Corresponding mean values for the elimination rate are found in breath analysis (approx. 0.08 mg l⁻¹ hr⁻¹) [9, 15], cf. Figure 2.1. The elimination rate experiences inter-and intra-individual variation, one explanation for the inter-individual differences is the individuals’ drinking habits [16]. Indications have been found that the elimination rate might increase with an increase in the dose of ethanol administered [8, 16, 14], and that the elimination rate is slightly, but significantly higher in women than in men [9, 15, 14]. The higher rate in women can be explained by the fact that the liver has a larger mass in comparison to the body weight compared to the ratio for men [3]. Despite the fact that only a small fraction of the alcohol will be eliminated through the breath [3, 5, 1], the BrAC correlates well with both the arterial and the venous BAC [2, 9, 7, 8, 6]. However, in accordance with the pharmacokinetics of ethanol and the physiological principles of alcohol gas exchange, the concentration-time profile of the BrAC is more closely correlated to the concentration-time
Figure 2.1: The BrAC concentration curve as a function of time for one individual after ingestion of alcohol. The β-slope is 0.09 mg l⁻¹ h⁻¹.

profile of arterial and capillary blood than that of venous blood, especially up to the point of A-V crossover [9, 7, 8, 6].

2.2 The gas exchange

The anatomy of the respiratory organ and the mechanism of gas exchange across cell membranes enable not only the exchange of respiratory gases, e.g., oxygen and carbon dioxide, but also other endogenously and exogenously breath compounds. After distribution of alcohol throughout the body, the blood in the pulmonary arteries and capillaries will contain molecules of alcohol. This constitutes a driving force of movement of alcohol molecules into the alveoli, where the concentration, the partial pressure, of alcohol is zero. The passive transport of a solute or water across a membrane is determined by the driving force, the prevailing electrochemical potential difference. The electrochemical potential difference is a combination of the concentration gradient of the solute (the chemical potential), and in the case of charged solutes, also the difference
in electrical potential that exists across the cell membrane [10].

The ability for a solute, \( X \), to cross the cell membrane is expressed through the permeability coefficient \( (P_x) \), which is determined by its solubility in the membrane lipid (the lipid water partition coefficient) and its ability to move within the membrane (the diffusion coefficient). The diffusion coefficient is determined by the molecular weight, the solubility of the gas in water, the diffusion area, and the thickness of the membrane. An increase in the thickness of the mucous membrane from pulmonary disease or inflammations, e.g., asthma, will negatively affect the gas diffusion, and also therefore the ability to achieve equilibrium between the mucous membrane/blood and breath [17, 18, 19].

The net flux of the solute \( X \), \( J_x \), in the case of simple concentration driven diffusion can be expressed by Fick’s law:

\[
J_x = P_x (c_{x_o} - |c_{x_i}|)
\]

(2.1)

where \( c_{x_o} \) and \( c_{x_i} \) are the concentrations of \( X \) outside and inside the cell, respectively. Fick’s law implies that when the concentration gradient for a solute, \( X \), is zero (i.e., \(|c_{x_o}| - |c_{x_i}| = 0\)), the net flux will also be zero.

According to Henry’s law (Henry, 1803), the partial pressure of a solute is proportional to its concentration in the liquid phase. The proportionality constant, termed the partition coefficient (\( \lambda \)), defines the distribution of a substance between two media at equilibrium; in breath analysis these media are blood and air (and the corresponding partition coefficient is written \( \lambda_{b:a} \)). The \( \lambda \) is also referred to as the solubility coefficient since it represents the ratio of the solubility of a substance in two media. By definition, \( \lambda \) is dependent on temperature (\( T \)) and pressure (\( P \)); \( \lambda = \lambda(T, P) \).

### 2.2.1 The exchange of ethanol

For the first twenty to thirty years of breath alcohol analysis, the exchange of ethanol was considered to only take place in the lung alveoli. This assumption was based on the belief that the gas exchange for alcohol was similar to that of the respiratory gases and the latter was known to occur entirely in the alveoli. In the alveoli, the respiratory gases diffuse over the alveolar-capillary membrane into the inspired air as long as a concentration gradient exists and the low partition coefficient implies that the concentration is not altered during expiration. With this assumption the end-expiratory concentration reflects the concentration in
the alveoli and, thereby, the concentration in the capillary and arterial blood.

The first researchers to obtain results indicating a lower expired BrAC than the expected alveolar BrAC were Liljestrand and Linde [2] who observed that the alveolar air was cooled during expiration and that re-equilibration must therefore occur during expiration. The reason for this lower end-expiratory BrAC was further investigated by Wright and colleagues [20], who revealed that the lower BrAC could not, solely, be explained by re-equilibration with respect to the lower breath temperature, but that it was also a result of gas exchange over the mucous membrane in the airways in addition to the alveolar gas exchange. During inspiration and expiration, equilibrium between the air and the mucous membrane will occur in accordance with the prevailing partition coefficient [21]. The location of gas exchange is determined by the $\lambda_{b:a}$; gases with $\lambda_{b:a} < 10$ are considered to be entirely exchanged in the alveoli, whereas $\lambda_{b:a} > 100$ implies that the exchanges occur entirely in the airways [22, 23]. Values of $\lambda_{b:a}$ in the range of 1700 to 2200 have been found for temperatures of 34 °C and 37 °C and for different haematocrit values [24]. In comparison, approximate values of the $\lambda_{b:a}$ for oxygen and CO$_2$ are 0.7 and 3, respectively [18]. A temperature coefficient of approximately 6.5% per 1°C change in temperature at equilibrium has been found for solutions of ethanol in water, plasma and whole blood [24, 25]. Corresponding values from in vivo studies are 5.7% [26] and 7% [27] per 1°C change in breath temperature.

In addition to the breath temperature, the $\lambda_{b:a}$ is dependent on the water content of the blood and thus the level of haematocrit [24]. For ethanol this implies that a higher haematocrit (implying a lower water content in the blood) might give rise to a slightly increased BrAC, than for a subject with exactly the same BAC but a lower haematocrit level. The level of haematocrit differs between individuals and is generally lower in women.

2.2.2 Conditioning of inspired air

The airways are responsible for the conditioning of the inspired air with respect to heating and humidifying [19, 28, 29]. When air with a temperature lower than the tissue temperature (approximately 37 °C) is inspired, the temperature of the mucous membrane of the airways is cooled both through direct loss of heat energy and evaporation [19, 28].
With decreased temperature and water content in the inspired air, a larger part of the airways has to be involved in order to condition the inspired air [28]. During expiration, the breath which was saturated with water in the alveoli (at 37 °C) is cooled as it passes through the respiratory tract. This results in condensation on the mucous membranes on the surrounding airway walls, a mechanism that helps to decrease the loss of water from the body which would otherwise have occurred [28]. This alteration of the inspired breath greatly affects the temperature and humidity of the mucous membrane and, thereby, also influences the exchange of ethanol which occurs there.

2.2.3 The expirogram

The change in the expired gas concentrations throughout an expiration is illustrated with an expirogram, as displayed in Figure 2.2. There are three distinct phases in an expirogram which will be referred to as Phases I, II and III: in Phase I, the concentration of the solute is zero or very low. This is followed by Phase II, where the measured concentration increases rapidly. The third and final phase is characterized by a slow but steady increase of the concentration of the solute in the measured gas until the expiration ceases. The characteristics of the phases are related to the physiology of the airways and lungs and the prerequisites for gas exchange for whichever gas is being monitored. This implies that there are differences between the expirograms of water, and gases with high (e.g., ethanol) and low (e.g., the respiratory gases) partition coefficients.

In the expirogram, Phase I corresponds to the period during which the air originating from the dead space of the apparatus and the anatomical dead space for the specific gas is measured. The size of the anatomical dead space will depend on where the gas exchange takes place and thus on the gas’s solubility in water [22]. As the main exchange occurs higher up in the airways with increased $\lambda_{Va}$, the length of phase I will decrease with increased $\lambda_{Va}$ [22, 30]. For ethanol, the gas exchange is considered to start after the oropharynx [31], which gives a considerably smaller anatomical dead space and thus make phase I shorter for ethanol than for the respiratory gases [30, 21], as evident in Figure 2.2. Water is exchanged in the entire respiratory organ, and is more or less abundant, which implies that water is already present in the expired air at the beginning of expiration.
Figure 2.2: An expirogram illustrates the increase in the concentrations of ethanol, CO\textsubscript{2} and water, in this case during a vital capacity breath test, plotted with respect to the expiration time (a) and the expired volume (b).
Phase II of the expirogram reflects the increasing concentration of the gas in the expired breath as time passes; the gas free air from the anatomical dead space becomes mixed with air which has participated in the gas exchange. The difference in the time to the onset of the third and final phase\(^1\) for different gases and also the influence from differences in the breathing pattern have been studied in Papers I and II, see Section 5.2.1.

Phase III shows a steady positive slope for the gas concentration that continues until expiration stops and the air flow decreases [31, 19, 32]. However, the reasons for the positive slope in phase III are different for the respiratory gases and for ethanol. For respiratory gases, phase III is referred to as the alveolar phase, and the positive slope is related to the increased fraction of alveolar air expired [33, 19]. For respiratory gases the end-expiratory concentration is considered to reflect the alveolar concentration [19]. The slope of the expirogram for CO\(_2\) may also reflect an uneven distribution in the ventilation, reflecting the fact that some regions in the lung are better ventilated than others. Thus, obstructive pulmonary diseases lead to an increase in the slope in phase III [19], as evident in Figure 5.3.

For ethanol, the explanation for the characteristics of phase III is more complex and is related to the continuous exchange of ethanol over the mucous membrane during expiration. According to Fick’s law, a net flow exists to attain equilibrium in both the alveoli and the airways. This gives a net flow of ethanol molecules towards the inspired ethanol-free air already during inspiration, which implies that when the inspired air reaches the alveoli, the concentration gradient between the blood and the breath has decreased. This results in a smaller contribution from the alveoli gas exchange than from the airway gas exchange.

The fact that the partition coefficient is temperature dependent implies that the equilibrium in the airways will be influenced by the prevailing temperature and water content of the mucous membrane, which is not only influenced by the body temperature, but also changed during inspiration and expiration [28, 29, 20, 34, 2, 21]. During expiration, the mucous membrane will not only re-absorb heat and water from the now warm and humid air [28], but also ethanol as it attains equilibrium with the now cooler mucous membrane [2, 20, 30]. The extent of the re-absorption is determined by the size of the air-to-mucous concentration

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\(^1\)Defined as the point where the value of the time derivative is half of its maximum value.
gradient [22], which is determined from the partition coefficient [22],
inspiration depth [35, 17], the temperature and humidity of inspired
air and the mucus membrane [34, 20], the replenishment of ethanol
from the bronchial blood flow [20, 36], and the length of the inspiratory
pause\(^2\) [17]. The positive slope evident in phase III is, therefore, related
to the fact that the ethanol deficit of the mucus membranes will
decrease with increased expired volume. The results of simulations made
indicate that as a result of the airway exchange, the BrAC might be ap-
proximately 15-20\% lower than the alcohol concentration in the alveoli,
even after complete expiration to residual volume [18, 35].

Regardless of the reason, the expirograms of both CO\(_2\) and ethanol
show a steady positive slope during phase III, which means that the
measured concentrations of the gases will increase with an increase in the
expired volume. The similarities and differences between experimentally
obtained expirograms for ethanol, water and CO\(_2\) have been further
investigated in Papers I and II, see Section 5.2.1.

### 2.2.4 Physiological factors that influence the gas exchange

Since ethanol is mainly exchanged in the airways, the expired BrAC
strongly depends on parameters that are related to the actual breathing
pattern, which might affect the condition in the airways. The most
determining factors of the BrAC are the prevailing temperature, both at
the alveolar-capillary membrane interface and the air-mucus membrane
interface, and the expired volume [2, 20, 21, 31, 35, 34, 32]. In addition,
the measured BrAC will be affected by the length of the inspiratory
pause [24, 32], the depth of the inspiration\(^3\) [35], and the respiratory
rate [37, 17, 32, 38]. Analyses of ethanol and endogenous breath acetone
has given rise to the suggestion that the exhalation flow might influence
the measured concentrations [39, 17]. The effect of the breathing pattern
on the expired BrAC makes manipulation of the breath alcohol reading
possible in those cases where the user performs a non-supervised breath
test. Hyperventilation decreases the BrAC, whereas a re-breathing pro-
cedure\(^4\) and breath-holding increase the BrAC in comparison to a vital

\(^2\)The pause between inspiration and expiration

\(^3\)The depth is directly related to the inspired volume of air.

\(^4\)A breath sampling method which implies breathing several times into a collecting bag.
capacity breath test [38, 37, 17, 32], see also results in section 5.2.1. Mulder and Neuteboom [37] concluded that the length of the provocative manoeuvre does not seem to have a great influence on the measured BrAC, but that the influence seems to increase with increased alcohol concentrations. No such indications have been found in our studies where the test subject had BrACs of around the legal limit for drunk driving in Sweden, 0.2 mg g⁻¹, see Figure 2.3 [As yet unpublished material].

![Figure 2.3: The percentage change in BrAC in comparison to the BrAC measured after a the vital capacity breath test. [Unpublished results from data collected in Paper II.]](image)

**Temperature**

The temperature of the breath and mucous membrane, which determines the λbr, can be drastically influenced by the breathing pattern and the characteristics of the inspired air [28, 34, 17, 35, 18, 22, 30, 20]. The temperature in the alveoli corresponds to the core body temperature of approximately 37 °C, but during expiration, the breath temperature will decrease. The mean temperature of the breath is approximately 34.5 °C [40, 21, 41, 42]. No large changes in the temperature were found with variation in the expired volume, a range of 33.3-34.4 °C have
been found for expirations of 500 to 4500 ml [21]. After inspiration of cold/dry and warm/moistened air mean temperatures of 33.2 °C and 36.2 °C, respectively, have been found [34]. Inspiration of dry air (cold and warm) is found to give lower BrAC, due to heat vapourisation lost from the mucous membrane [32, 34]; see also Section 5.2.1 for results from experimental breath testing performed after visits in a chilly outdoor environment.

**Expired volume**
Ethanol's interaction with the mucous membrane in the airways implies that the BrAC will increase with an increase in the expired volume [31, 21, 34, 17, 20, 35]. This means that the correlation between the BrAC and the BAC is stronger for larger expired volumes and procedures of re-breathing [21, 20, 35, 17, 32]. This is the reason why a prolonged expiration, ensured by the requirement of attaining a certain volume, is requested in breath analysers for evidential tests and screening. A subject with small lung volume will have to exhale a greater fraction of the vital capacity to fulfill the requirement for the expired volume than others would have to, and thus their measured BrAC will be higher than that of a subject with similar BAC, but a larger vital capacity. This is the reason why the determination of the BrAC at a certain volume has been attained has been questioned with respect to its validity and fairness [22, 18, 35].

**Inspiration depth**
The depth of the inspiration, the inspired volume, will determine how much ethanol is absorbed from the mucous membrane during inspiration, and thus the size of the ethanol deficit of the mucous membrane [35]. This implies that during expiration after a large inspiration, the mucous membrane will have to reabsorb more ethanol from the expired air, and thus the increase in ethanol concentration with expired volume will be slower. From simulations Hlastala and Andersen [35] have found that a decrease in inspired volume is found to increase the BrAC. The influence of the depth of inspiration has also been briefly tested and discussed by Wright et al. [26] and Jones [17]. Our results indicate that breath tests with only a little or no inspiration performed prior to exhalation (e.g. the tidal volume and the low maximum expiration breath tests) gave a higher BrAC after two seconds compared to the vital capacity expiration performed in the conventional manner after a complete inspiration, see Table 5.3.
Inspiratory pause
An increased length of the inspiratory pause will not increase the amount of diffused gas over the alveolar/capillary interface and the mucous membrane of the airways since it is a process completed much faster. However, it has been suggested that with increased inspiratory pause means that a longer time is available for the mucous membrane to recover in temperature and for the bronchial blood circulation to replenish the mucous membrane with ethanol [17]. This decreases the air-to-mucous ethanol gradient and the need for the mucous membrane to re-absorb ethanol from the expired breath [32, 17], and thus gives an earlier increase in the expired BrAC, see Figure 2.4 (c). Thus is utilised in the re-breathing procedure, which has been suggested as a preferable breath-testing procedure [24, 32, 41, 20].

Respiratory rate
A high respiratory rate leads to an increased loss of heat and water from the airways and, thereby, cooling of the mucous membrane [28]. A decreased temperature increases the mucous membrane’s solubility to ethanol, and thus also the diffusion of alcohol to the inspired air. This creates a large ethanol deficit in the mucous that will have to be restored during expiration [17]. Hyperventilation is found to lower the BrAC [17, 37, 32]; Section 5.2.1 presents results found in conjunction with the work conducted for this thesis regarding hyperventilation and physical activity.

2.3 Summary breath alcohol analysis
This chapter has explained the exchange of gases within the respiratory organ and has highlighted the fact that the gas exchange of ethanol occurs under unstable conditions as far as the temperature and humidity in the respiratory tract are concerned. It has become obvious that the exchange of ethanol - and thus the BrAC to be measured - is influenced by many factors related to both the physiology and the performance of the breath test. Furthermore the CO₂ in the expired breath is affected by the volume of the air expired and provocative breathing manoeuvres, such as hyperventilation and breath-holding [19, 43], whereas the abundance of water in expired breath implies that its concentration is only affected to a minor extent, as illustrated in Figure 2.4 and also Table 5.3.
Figure 2.4: The characteristics of the expirograms for three different types of breath test performed by the same test subject: a vital capacity breath test (a), a breath test after hyperventilation (b), and a breath test after 30 seconds of breath-holding (c). For ease of comparison the scales are identical.
Chapter 3

Breath alcohol testing in practice

3.1 The Blood:Breath Ratio

In the early years of breath alcohol analysis, the relationship between the BAC and the BrAC was believed to be constant, valid for all individuals and all types of breath tests performed. In their pioneering work [2], became the first to present a comparison between the measured BAC and the measured BrAC, and they found a ratio of 2000:1. These results were followed by further studies made by Harger and colleagues [41, 27], which resulted in a suggested ratio of 2100:1 [41].

However, with the knowledge we have today about the differences between arterial, venous and breath alcohol concentration over time, the re-equilibrium occurring during expiration and the continuously on-going gas exchange in the upper airways, as well as the knowledge that it is impossibility to standardize a breath sample, it is easy to understand that there is nothing like a constant ratio between the concentrations in the two media. To emphasize the fact that the ratio of blood and breath alcohol concentration depends on many factors, the term Blood:Breath Ratio (BBR) was introduced in breath alcohol analysis instead of using the partition coefficient. Multiple studies have been undertaken to investigate the BBR and a strong debate about the appropriateness of using a BBR value of 2100:1 has been going on over the years.
3.2 Traffic law enforcement

With an increased use of alcohol and numbers of vehicles, the need for simple on-the-spot analysis of alcohol grew, and became feasible through the advances in breath alcohol analysis and sensor technology [3]. In the late 1930s the first breath alcohol analysers for roadside screening in traffic were developed [44, 45]. These first devices used reagents which changed their colour upon reaction with alcohol, and the presence of alcohol in the breath could be detected. In parallel with the first available breath analyser for traffic law enforcement, Norway (1936) and Sweden (1941) became the first countries to state statutory limits of BAC in the traffic safety legislation [44].

In 1972, experts within the field of breath alcohol analysis met and came to the conclusion that a BBR of 2100:1 was to be recommended for use in law enforcement and for calibration of the breath analysers [44], and in 1979, the U.S. government made an amendment to the Uniform Traffic Law and Ordinance, section 11-902-1a, that read "Alcohol concentration shall mean either grams of alcohol per 100 ml of blood or grams of alcohol per 210 litres of breath". This amendment was made to stop the need for conversion from BrAC to BAC. In Sweden statutory BrAC limits for driving under the influence of alcohol were imposed in 1989 and, thenceforth from then used in parallel with the BAC limits. However, the statutory limits stated for BrAC are derived from the limits stated for BAC, which implies that a specific BBR of 2100:1 value has been accepted. Today the statutory limit for drunk driving in Sweden is 0.2 mg g\(^{-1}\) in blood or 0.1 mg l\(^{-1}\) in breath.

3.2.1 Alcolocks

In addition to the use of breath alcohol analysers by the police for screening and evidential testing of potentially drunk drivers, breath alcohol analysers are used as ignition interlocks, i.e., referred to as alcolocks. In Sweden, the general attitude toward alcolocks has become more positive since the beginning of the 21st century, partly because of widespread awareness of the prevalence and consequences of drunk driving. At the end of 2009 the total number of alcolocks sold in Sweden was almost 60,000. The use of alcolocks in commercial transportation is considered so important that many communities and counties in Sweden do not purchase transportation services from companies that do not have alcolocks.
installed in their vehicles. The installment of alcoclocks has quite simply
turned out to be part of the quality assurance of transport companies.
Compared to many other countries, Sweden has come exceptionally far
where the use of alcoclocks for increased traffic safety is concerned, but
France, Slovenia and the United States are examples of other countries
that are also taking action and assigning resources at a national level
to increase the usage of alcoclock. In addition to the use of alcoclocks
in commercial and private vehicles, drivers convicted of drunk driving
are offered the possibility of applying to the Swedish national alcoclock
programme for conditional driving licence withdrawal. The programme
started as a trial in certain parts of Sweden in 1999 and, after evaluation
of this trial it was decided to roll out the programme nationwide in 2003.
Similar alcoclock programs for convicted drivers exist in other countries.

Unlike the breath tests performed for screening or evidential test-
ing, alcoclocks are intended for use by non-professional users without any
external supervision. This requires that the breath analyser is easy to
handle, understand and use, but it also gives the user a possibility to
attempt to influence the BrAC measurement deliberately. Manipulation
can occur with respect to the installation of the device, by using an arti-
ficial breath sample or an active charcoal filter which absorbs ethanol, or
by the performance of a provocative breath manoeuvre. A vast majority
of drivers [46] are sober and, thereby also a majority of the alcoclock users
are likely to be sober, and, therefore, to avoid the alcoclock becoming a
nuisance it is of importance that the handling of the unit and the breath
test procedure are quick and simple for these drivers. There are two
main issues that limit the user-friendliness of state-of-the-art alcoclocks:
the mouthpiece and the criteria of the breath test performance, which
originates from the requirement that a sufficiently accurate measure-
ment is obtained for forensic purposes. The mouthpiece comprises both
a hygienic and a handling aspect, the handling aspect is even more pro-
nounced in commercial vehicles where there are multiple drivers than in
privately owned cars. As far as the breath test procedure is concerned,
a frequent user or a user with impaired respiratory function is likely to
find it difficult to make a sufficiently strong expiration for long enough.
In Sweden, approximately 20% of the middle-aged women experience
difficulties in achieving an approved breath test with the screening in-
struments used by the police. A more user-friendly alcoclock than the
alcoclocks available on the market today may further increase the accep-
tance and use of alcoclocks.
3.3 Breath alcohol analysis in emergency care

More than one patient in five in need of emergency care is under the influence of alcohol, and the number increases significantly for nighttime admittance and for trauma patients [47, 48, 49, 33, 50, 51, 52, 53]. The medical assessment of a patient with a depressed grade of consciousness and an inability to communicate is difficult, but it becomes more complex if alcohol is involved since alcohol-induced inebriation can diminish the appearance and severity of pathological symptoms owing to the suppression of the patient’s sensory affirmations [53, 47].

However, what is even more problematic is the fact that the condition of many of the patients encountered in pre-hospital and emergency care could easily be falsely believed to be the result of consumption of alcohol. The high prevalence of patients under the influence of alcohol and the fact that some clinical conditions introduce behavior that resembles the behavior of an alcohol intoxicated person, makes this risk considerable. Some of the more common pathological conditions that can be mistaken for alcohol inebriation are listed in Table 3.1. In addition, cannot be uncommon that patients, for example, with a diabetes induced hypoglycemia/hyperglycemia, or having a heart attack, are admitted to the hospital after a minor intake of alcohol.

Determining whether alcohol is or is not involved in a patient’s clinical condition from clinical signs alone has been found to be inaccurate and unreliable [49, 54, 52, 55], therefore in a situation where the paramedics and the physician considers a multitude of diagnoses, early quantification of the patient’s blood or breath alcohol concentration is considered to be important for the prevention of a delayed diagnosis or misdiagnosis [49, 47, 56, 57, 48, 4, 58, 55, 59, 53, 60, 61, 52]. With a negative alcohol analysis or confirmation of a low alcohol concentration, the uncertainties concerning the involvement of alcohol can be eliminated, and a continued medical assessment secured [61, 48]. However, a positive test does not imply that the medical assessment can be stopped until other possible medical conditions have been ruled out [49, 57, 47, 61, 4, 48, 62].

Many of the emergency patients are unwilling or unable to cooperate for a breath alcohol test [49, 50, 57], and in these patients, the accuracy and reliability of a test conducted with a conventional breath analyser is negatively affected [63, 56, 48, 54, 64]. The unknown volume of the collected nasal or oral breath sample and a deficiency of knowledge about
whether or not the sample was diluted, contributes to the uncertainty of the relationship between the measured values and the real end-expiratory BrAC [56]. However, there is no concentration that consistently defines intoxication for all individuals, which means that each measured BrAC has to be assessed in the context of the patient’s appearance, alcohol-influenced anamnesis, and general health of state. This means that, for medical use, the requirement is lower for an accurate measurement than for forensic purposes [57, 65, 33, 48, 61].

Research studies have found that patients who are intoxicated by alcohol are more likely to be intubated, undergo additional diagnostic procedures and be monitored than patients who are not considered to be intoxicated [52]. Early and rapid quantification of the blood or breath alcohol concentrations, in pre-hospital care and at the emergency departments, is likely to enable better triage, and thus more efficient use of resources in the form of medical competence, time, equipment and beds [4]. These are effects that favour both the patient and the hospital’s economy.

With this insight into the problems encountered in emergency care, it becomes obvious that point-of-care alcohol analysis in patients would be of great benefit and that breath analysis is preferable over costly and time-consuming blood analysis. But for increased use in emergency medicine, more reliable breath alcohol analysis for all patients must be ensured regardless of their ability and willingness to co-operation and their expiration volume.
Table 3.1: Some of the more common medical conditions that might introduce behaviour that resembles the behaviour of an alcohol intoxicated person. The summary is based on [59, 4, 62].

<table>
<thead>
<tr>
<th>Main medical condition</th>
<th>More specified medical condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intoxication</td>
<td>Other alcohols than ethanol</td>
</tr>
<tr>
<td>Methanol</td>
<td></td>
</tr>
<tr>
<td>Drugs</td>
<td></td>
</tr>
<tr>
<td>Carbon monoxide</td>
<td></td>
</tr>
<tr>
<td>Metabolic causes</td>
<td>Hypo-/hyperglycemia</td>
</tr>
<tr>
<td></td>
<td>Alcoholic and diabetic ketoacidosis</td>
</tr>
<tr>
<td>Infectious diseases</td>
<td>Sepsis</td>
</tr>
<tr>
<td>Meningitis</td>
<td></td>
</tr>
<tr>
<td>Encephalitis</td>
<td></td>
</tr>
<tr>
<td>Neurological causes</td>
<td>Seizure disorders e.g. epilepsy</td>
</tr>
<tr>
<td></td>
<td>Cerebrovascular accidents</td>
</tr>
<tr>
<td>Psychological illness</td>
<td></td>
</tr>
<tr>
<td>Respiratory causes</td>
<td>Hypoxia</td>
</tr>
<tr>
<td></td>
<td>Respiratory depression</td>
</tr>
<tr>
<td></td>
<td>Carbon dioxide narcosis</td>
</tr>
<tr>
<td>Trauma</td>
<td>Concussion</td>
</tr>
<tr>
<td></td>
<td>More severe head injury</td>
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<tr>
<td>Other</td>
<td>Hypotension</td>
</tr>
<tr>
<td></td>
<td>Hypo-/hyperthermia</td>
</tr>
<tr>
<td></td>
<td>Dehydration</td>
</tr>
<tr>
<td></td>
<td>Hyper/hypothyroidism</td>
</tr>
</tbody>
</table>
3.4 Improved breath analysis with use of a tracer gas

The reliability and accuracy of the breath alcohol measurement will be negatively affected if it is not possible to sample the end-expiratory breath [66, 20]; which is the case in persons who cannot be instructed or are unable to perform a complete expiration [67, 68], in persons with impaired pulmonary functions or patients with depressed levels of consciousness [69, 64, 63]. In addition, the breath sample can be diluted, which is likely to occur in sampling from non-cooperative persons when a tight seal is not achieved against the nostril or mouth [56, 70, 68].

Since there is no way to standardise a breath test procedure performed by humans or to use the same measurement criteria in all individuals, methods to decrease the influence of physiological prerequisites, various breathing techniques, and the mixing of alveolar and dead space air have been presented over the years. The re-breathing procedure is found to negate the influence of provocative breath manoeuvres performed prior to the breath test and the temperature of the inspired air, and has, therefore, been suggested to improve breath analysis, not only in cooperative persons but also in sleeping and unconscious patients [26, 32, 26, 63]. In order to attain equilibrium, the sampling time has to be prolonged to collect multiple expirations, during which time the user breathes through a mouthpiece or a mask. A re-breathing procedure does not improve the usability and is not appropriate for use on patients, since the placement of a mask over a patient’s face might cause the patient stress, irritation or anxiety and interfere in the medical treatment.

In both the fields of alcolock usage and breath analysis for medical purposes, a lowering of the requirement for the expired volume while maintaining the reliability of the measurement would improve the user-friendliness. Simultaneous measurement of a tracer gas in expired breath, has been found to improve breath analysis, both with respect to differences in physiological prerequisites and shallow expirations. CO₂ has been used as a tracer gas in the analysis of breath acetone [70] and breath hydrogen [68, 71, 67, 66], and in occupational exposure studies [72, 73]. In breath alcohol analysis for traffic law enforcement, CO₂ [45, 41, 74], O₂ [75], and water [7] have been used as tracer gases.
CO₂ as the tracer gas can be used in two different manners; as a quality indicator of the breath sample or, for estimation of the end-expiratory BrAC.

**CO₂ as a quality indicator of the breath sample**
The concentration of CO₂ increases with the expired volume, which enables the measured value to be used as an indicator of whether the expiration was sufficiently long for a reliable determination of the concentration of the breath compound of interest to be made, or if the breath test was contaminated with dead-space air or ambient air. Franzblau et al. [73] and Vreman et al. [71] have used CO₂ as a tracer gas in this manner.

**Estimation of the end-expiratory BrAC with use of CO₂**
The measurement method for the estimation of the end-expiratory concentration is based on calculation of the ratio between the measured tracer gas concentration in the breath sample and its corresponding end-expiratory (alveolar) concentration. The ratio reflects the fraction of expired volume in relation to a complete expiration to the residual volume, or in the case of unintentional dilution of the sample or contact-free measurement, it reflects the dilution factor. The ratio is then used for calculation of the estimated end-expiratory concentration according to equation 3.1, where CO₂ is used as the tracer gas for the estimation of the end-expiratory BrAC.

\[
BrAC_{End-exp} = BrAC_{Meas} \cdot \frac{CO₂_{End-exp}}{CO₂_{Meas}}
\] (3.1)

In non-forensic breath analysis applications, the method of normalising with the use of CO₂ has been found to eliminate the need for a rebreathing procedure or for end-expiratory breath sampling [66], reducing the influence of differences in breathing patterns [70, 72] and from dilution of the breath sample [70, 68], and allowing more shallow expirations to be performed and that with maintain reliability [72]. The reduced influence from different breathing patterns and dilution contributes to an increase reproducibility [68, 67, 66], factors that are found to improve breath analysis in uncooperative persons, for example children [67, 68].

In breath alcohol analysis, the validity of normalising using CO₂ as the tracer gas has been questioned over the years since the method relies on the assumptions that expirograms for ethanol and the CO₂ are simi-
lar, and more importantly, small intra- and inter-individual variations in the alveolar PCO$_2$. The intra-individual variations over time are small, and at rest the individual variations in the end-tidal CO$_2$ concentration for healthy individuals are considered to be modest [76, 77]. However, the expired CO$_2$ concentration is influenced by the breathing pattern and is increased by physical activity [19], see also section 5.2.1. This implies that inter-individual variations in the alveolar/end-expiratory CO$_2$ exist [66, 45, 40]. In 1967, the American National Safety Council recommended that the use of CO$_2$ for the estimation of the alveolar alcohol concentration for traffic law enforcement ceased [45], because for forensic purposes, the variation in the end-expiratory CO$_2$ concentration was considered to be too large to make normalisation with respect to CO$_2$ eligible [40].

Earlier evaluations of the increase in the concentration of ethanol [40] and acetone [76] over the time of an expiration, are found to correlate with the increase in CO$_2$ concentration. However, for validity of the tracer gas method the concentration ratio between the compound of interest and CO$_2$ has to be relatively stable during an expiration, regardless of the breathing pattern. Niu et al. [66] and Kien et al. [68] have observed altered ratios between breath hydrogen and CO$_2$ after engaging in intentionally provocative procedures involving hyperventilation or breath-holding.

Dubowski [42] found that the concentration of CO$_2$ and the breath temperature reach the maximal (termed alveolar) plateau almost at the same time. This led to the suggestion that the end-expiratory breath temperature would be a better predictor of when alveolar air is exhaled because of its smaller intra- and inter-individual variations in comparison to CO$_2$. In line with this, water has been suggested as an appropriate tracer gas for improvement of the correlation between BrAC and arterial BAC, and to enable contact-free measurements to be made [7]. The method is based on the assumption that, at the end of a vital capacity breath test, the expired breath is saturated with water, which at 37°C implies an approximate concentration of 44 mg L$^{-1}$.

The development and the stability of the ratios of ethanol to CO$_2$ and, ethanol to water, have been investigated within this thesis, see Figures 5.5 and 5.6, as well as the variations in the end-expiratory concentrations of CO$_2$ and water between subjects, see Table 5.2.
Chapter 4

Sensor technologies for breath alcohol analysis

Three different types of sensor are used in non-laboratory based breath alcohol analysers: semiconductors, electrochemical (fuel-cells), and infrared (IR) spectroscopy sensors. For traffic law enforcement, only instruments based on fuel-cells and IR spectroscopy, or a combination of the two technologies, are used. The reliability of the BrAC measurement is dependent on three important issues concerning the sensor used for breath alcohol measurement: the sensor’s specificity for ethanol, the accuracy of the measurement, and possible degradation of the sensor element over time.

4.1 Selectivity

A couple of thousand compounds have been detected in breath, many of them only in very small concentrations [78]. In general, a breath sample contains approximately 200 different compounds, both endogenously and exogenously produced [79, 78, 80]. If present, and in sufficiently high concentrations, some of these compounds might interfere with the measurement of ethanol.

The high selectivity of ethanol is of importance in breath alcohol analysis for forensic and medical purposes. However, the number of evidential tests in which a high concentration of an interfering substance
have been detected is low, and are primarily related to elevated concentrations of acetone [81, 82]. Acetone is found in relatively high concentrations in breath [33, 79], and the concentration is elevated in fasting, undernourished people and those on low-carbohydrate diets, with diabetes, and in alcoholics [33, 83, 84, 85]. A high concentration of acetone in the breath also often occurs after intake of technical alcohol containing fluids such as cocker fuels or windscreen washer fluid, owing to their content of isopropanol (2-propanol) and methyl ethyl ketone [33, 86]. Acetone, together with isopropanol and methyl ketone, is considered to be the most critical substance for interference in breath alcohol analysis [33, 81]. The requirement of selectivity for alcolocks is specified in the European industrial standard, EN 50136-2 [87], in the form of limits on the concentration of twelve substances under which the alcolock is not allowed to block the ignition.

4.2 Sensor technologies

Within a fuel-cell, the expired alcohol is oxidised into acetic acid, which results in free electrons that create an electrical current proportional to the concentration of alcohol. Breath analysers based on electrochemical oxidation provide relatively good selectivity for ethanol since ethanol only responds to other alcohols and not to the additional compounds found in breath.

Semiconducting sensors often employ a Taguchi gas sensor [3]. The sensor element within a Taguchi cell is an N-type semiconductor. When the sensor element is exposed to certain combustible compounds in the breath, e.g. ethanol, these gases are absorbed onto the bead and the electrical conductivity increases in proportion to the concentration of the absorbed gases. This implies that Taguchi cells respond to all combustible endogenous and exogenous breath compounds; e.g. acetone and cigarette smoke. As a result of their low sensitivity and the risk of interference, semiconducting breath analysers should be used restrictively.

Any intentional and unintentional chemical reactions, e.g. with breath alcohol and sulphur compounds in ambient air, that occurs within fuel-cells and semiconductors leads to degradation of the active surfaces. The degradation of the surface increases the recovery time of the sensor and decreases the sensitivity of the sensor, which affect the long-time
4.3 IR transmission spectroscopy

Infrared transmission spectroscopy is based on the substances' absorption of IR light at different wavelengths. The absorption of light originates from specific vibrations and rotations of the molecules [88]. The characteristic structure of a molecule gives it a specific absorbance signature, which enables substances existing in the same gas mixture or solution to be differentiated.

The ratio of the intensity of the transmitted light (I), and the incident light (I₀), at a given frequency is called the transmittance (T), of the sample:

\[ T = \frac{I}{I_0} \]  \hspace{1cm} (4.1)

The Beer-Lambert law states that the transmitted intensity is directly proportional to the molar concentration of the substance (J), and the length of the optical path (l):

\[ I = I_0 \cdot 10^{-\varepsilon[J]l} \]  \hspace{1cm} (4.2)

The proportionality coefficient, the molar absorption coefficient (ε), depends on the frequency of the incident light. The Beer-Lambert law can also be expressed as the absorbance (A), of the sample, at a given wavenumber (v):

\[ A(v) = -\log[I(v)/I_0(v)] = -\log T(v) = \varepsilon(v)cd \] \hspace{1cm} (4.3)
When using a wavenumber interval for detection the total absorption is calculated as the integrated absorption coefficient:

\[ A(v) = \int \varepsilon(v) c dv \quad (4.4) \]

To achieve a suitable sensitivity of the concentration measurement, the choices of wavelength and optical path length are critical. The optical path has to be related to the absorption at the given wavelength, and the concentration level to be measured. Figures 4.1 and 4.2 illustrate the wavelength spectra for ethanol and CO₂. The strong absorbance of ethanol around 3.3 to 3.5 µm and the sufficiently high and appropriate wavelength of 4.2 µm for CO₂, make these commonly used wavelengths for quantification of these two gas concentrations. The use of these wavelength makes an optical path of the order of decimetres and millimetres sufficient for qualitative measurement of ethanol and CO₂, respectively.

The strong absorption of ethanol around 3.3 to 3.5 µm has been assigned to the C-H stretch vibrations of the molecule, whereas the absorption of CO₂ is related to the antisymmetric stretches between the C and two O atoms [88]. C-H bindings are found in most endogenous and exogenous substances in expired air which implies that they all of these substances are strong absorbers in the same wavelength region as ethanol [88]. With the use of the unique absorption characteristics of each substance, selectivity is enabled through comparison of the absorption at a minimum of two different wavelengths. Ethanol also strongly absorbs in the wavelength interval around 9.5 µm, which corresponds to the stretch and bending vibrations of the -OH bond [44]. In this wavelength range the risk of interference from other endogenous and exogenous substances is considerably lower than in the range around 3-4 µm [3, 89], cf. Figure 7.2.
Figure 4.1: The wavelength spectra for ethanol in the interval 3 to 4 µm, at a concentration of 0.1 mg L⁻¹, with an absorption path of 200 mm. The simulations are made using absorption spectra from the Pacific Northwest National Laboratory, PNNL.

Figure 4.2: The wavelength spectra for carbon dioxide in the interval 4.1 to 4.45 µm, at a concentration of 76.3 mg L⁻¹ (4.2 kPa), with an absorption path of 5 mm. The simulations are made with use of absorptions spectra from the Pacific Northwest National Laboratory, PNNL.
Chapter 5

Research contribution

5.1 Methods and materials

5.1.1 Investigation of the measurement method

The methodology studies [Papers I and II] address the two physiological assumptions on which the tracer gas method relies, namely, small intra- and inter-individual variations in the end-expiratory tracer gas concentration, and similarities in the time-dependent development of the ethanol and the tracer gas concentration during an expiration. An additional assumption is made regarding the use of a tracer gas: that ethanol and the tracer gas are mixed with ambient air to a similar extent. Investigation of this assumption has not been addressed either in Paper I or Paper II where undiluted air was analysed, whereas, the breath samples analysed in Paper III (and to some extent also in Paper IV) were mixed with ambient air, and thus the validity of this assumption has been indirectly evaluated in these two studies.

The characteristics of the expirograms and the influence of different types of breath test have been studied to investigate the validity of using CO₂ and water as tracer gases [Papers I and II]. The comparisons were made with expirograms recorded from breath tests performed in an evidential breath alcohol analyser equipped with a mouthpiece¹, to exclude the influence of mixing the expired breath with ambient air. The normal set-up of optical filter in the evidential breath alcohol analyser

¹Evidenzer, Nancpuls, Sweden
uses five different filters to assure high selectivity for ethanol [90].
Additional detection of \( \text{CO}_2 \) and water demanded the filter set-up to be
modified by the supplier. In the first study [Paper I], the instrument
used two optical band-pass filters with center wavelengths at \( 3.41 \, \mu \text{m} \)
and \( 3.80 \, \mu \text{m} \) for the determination of the ethanol concentration [90]. In
order to avoid interference from expired methane in the quantification
of the ethanol concentration, the filter set-up for ethanol was changed
to \( 3.47 \, \mu \text{m} \) in combination with \( 3.80 \, \mu \text{m} \) prior to the second methodo-
logy study [Paper II]. In both studies, filters with center wavelengths at
\( 2.57 \, \mu \text{m} \), and \( 2.77 \, \mu \text{m} \) enabled determination of the water and the \( \text{CO}_2 \)
concentration. During a breath test, the breath analyser continuously
recorded the concentration of gases with a sampling rate of 10 Hz.

In the first methodology study [Paper I] the intra- and inter-individual
variations in the \( \text{CO}_2 \) and the \( \text{H}_2\text{O} \) concentrations obtained at the end
of a vital capacity expiration and the characteristics of the expirograms
of ethanol, \( \text{CO}_2 \) and water, after shallow expirations (tidal volume) and
vital capacity expirations (as explained in Table 5.1), were investigated
for healthy subjects (\( n=21 \)) and for patients with impaired respiratory
function (\( n=9 \)). The respiratory function of the subjects was tested with
spirometry\(^2\). In the follow-up study [Paper II], the influence of provoc-
ative breathing manoeuvres was investigated (see Table 5.1) in healthy
subjects (\( n=30 \)).

In both studies the subjects constituted a group with a large variation
in age and bodily constitution. To achieve a BAC of the order of the
legal limit for drink driving in Sweden (0.02\%), the subjects consumed
0.3 gram ethanol per kilo body mass in the form of wine or spirit (either
straight or mixed with juice or soft drinks). The breath test procedure
started thirty minutes after ingestion, and no specific consideration was
given to whether or not the subject had entered the post-absorption
phase. However, the relatively short duration of the procedure ought
to not to have had any considerable influence on the measured BrACs. The
clinical studies were approved by the Regional Ethical Review Board in
Uppsala, Sweden, and written informed consent was obtained from the
test subjects.

The concentrations after two seconds of expiration and at the end
of each recorded breath test were extracted from the expirograms for
further analysis. The different types of breath test performed were

\(^2\)MasterScope, Jaeger, Spirofarma Cardiopulmonary, Sweden
Table 5.1: Description of the five types of breath tests performed by the test subjects in the second methodology study [Paper II]. FRC = functional residual capacity, RV = residual volume, TFC = total lung capacity.

<table>
<thead>
<tr>
<th>Breath test</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tidal volume</td>
<td>Normal insp. (0.5 l), slow exp. (0.5-1.0 l)</td>
</tr>
<tr>
<td>Slow max. exp.</td>
<td>No insp., slow exp. from FRC to RV</td>
</tr>
<tr>
<td>Vital capacity</td>
<td>Insp. to TLC, and forced exp. to RV</td>
</tr>
<tr>
<td>Hypervent.</td>
<td>5 insp. to TLC, 4 exp. to FRC, 5th exp. to RV</td>
</tr>
<tr>
<td>Breath-holding</td>
<td>Insp. to TLC, 30s. of breath hold, slow exp. to RV</td>
</tr>
</tbody>
</table>

compared to determined the differences in the expired volume, duration, the two-second and end gas concentrations, breath temperature [only in Paper II], and the time to the onset of the final phase of the expirogram\(^3\) for each of the three gases.

For evaluation of the tracer gas method, the end-expiratory BrAC was estimated from the two-second and end gas concentrations of ethanol and CO\(_2\) and, ethanol and water, respectively. The estimated end-expiratory BrAC was compared to the measured end-expiratory BrAC after the vital capacity expiration. For the end-expiratory (alveolar) tracer gas concentration values, the mean value of the end concentrations of the vital capacity breath tests performed by the subjects were used, cf. Table 5.2.

The vital capacity breath test is the recommended breath test procedure for the evidential breath analyser and therefore the values obtained with this breath test were used as reference, which enabled paired t-test analyses to be conducted in both studies. For the comparison of values between healthy individuals and the patients, analyses of variance (ANOVA) were performed [Paper I].

### 5.1.2 Evaluation of the breath analyser prototypes

Evaluation of the performance of two types of breath analysers prototypes which use the measured CO\(_2\) concentration for estimation of the end-expiratory BrAC have been done: one prototype enabling contact-free measurement in the application of an alcolock [Paper III], and

\(^3\) As defined in Section 2.2.3
prototypes for use in medical care [Paper IV]. Evaluation of the performance through bench tests was in focus in Paper III, whereas in Paper IV the performance of the prototypes was evaluated from breath tests performed by patients.

Paper III, and in part also Paper IV, present the design of the breath analyser with respect to the IR transmission spectroscopy technology, the optical measurement cavity including a IR source and thermopiles, and the measurement procedure and algorithm. One of the main features with these prototypes is the optical cavity which enables a long optical path length to be achieved within a cavity of small dimensions and thereby introducing the possibility of a hand-held IR spectroscopy breath analyser. Optical bandpass filters with center wavelengths of 3.45 µm and 4.26 µm have been used for quantification of the ethanol and CO₂ concentration, respectively. Figure 4.1 and 4.2 show the absorption spectra of the wavelength intervals of use, with absorption paths of 200 mm for ethanol and 5 mm for CO₂. The use of a single wavelength for detection of ethanol in these prototypes did not secure complete selectivity against other endogenous and exogenous breath compounds.

The technical differences between the two types of prototypes are mostly related to the breath sampling and the measurement algorithm. In the prototype to be used as an alcolock, contact free measurement was enabled, as demonstrated in Figure 7.1, whereas in the prototype intended for medical use, a mask was used for sampling of the expired breath from the mouth and nose in cooperative and uncooperative patients. To prevent contamination of the devices and in-between successive test performed by the test subjects enrolled, a single-use bacterial filter was attached between the device and the mask. Since minimal dead space volume and minimal hinder for inspiration were required, and the measurement method allows minor dilution of the breath sample, a mask of small size was used despite the fact that it did not fit tightly to the nose and mouth.

To investigate whether enhanced transport of the breath sample into the measurement cell would improve the quality of the breath alcohol analysis in uncooperative patients, two prototypes of the medical breath analyser were evaluated: one in which the expired breath was passively transferred into the measurement cell and one which was equipped with

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4 TCO2, size 2, Intersurgical Ltd., U.K.
5 IsoGard® Filter Small Gibeck, Teleflex Medical, Malaysia
a pump\textsuperscript{6} for active transport of the breath sample.

The performance of the breath analyser was evaluated in conjunction with the assessment of the measurement method in both areas of application. In the alcclock prototype, one alcohol-imbibed test subject performed a series of contact-free breath tests [Paper III], and the prototypes for medical usage were evaluated in a pre-clinical test, in which the patients cooperated when conducting the breath tests according to their ability and grade of consciousness [Paper IV].

A total of eighty-seven breath tests (of which fifty-nine were conducted with the active sampling prototype) were collected from thirty-seven patients who sought medical care at an emergency care clinic. This pre-clinical study was approved by the ethical committee at the University Hospital in Rostock, Germany.

In both types of prototype, the criteria for an approved breath test was set with respect to a threshold level of PCO\textsubscript{2}, and in the medical prototype, this criteria was used in conjunction with the requirement that the onset of the final phase had occurred\textsuperscript{7}.

The evaluation of the performance of the alcclock prototype took the form of a comparison between the end-expiratory BrAC estimated from the ethanol and CO\textsubscript{2} concentrations measured in freely expired and diluted air, and the end-expiratory BrAC measured in undiluted air with a reference breath analyser\textsuperscript{8} [Paper III]. For the evaluation of the performance of the prototypes for medical use [Paper IV], the most suitable reference was considered to be the BAC since it is the most commonly used analysis method in medicine and enables accurate measurement regardless of the subjects cooperation. The BrAC measured at the end of the expiration and the estimated end-expiratory BrAC were compared to the venous BAC. The BAC was determined with the alcohol dehydrogenase method. In both studies a reference value for CO\textsubscript{2} concentration of 4.8 kPa was used and the designs of both studies enabled analyses of paired samples to be performed.

\textsuperscript{6}Diaphragm pump 2002G, Rietschle Thomas Fuchheim GmbH, Germany

\textsuperscript{7}As defined in Section 2.2.3

\textsuperscript{8}Evidenzer, Nanopuls AB, Sweden
Table 5.2: The mean and standard deviation of the partial pressure of \( \text{CO}_2 \) (\( \text{PCO}_2 \)) and the water concentration (\( \text{H}_2\text{O} \)), measured at the end of the vital capacity breath test. The mean concentrations are those measured with the reference instrument (the Evidenzer, Nanopuls AB, Sweden) during the last second of expiration. The paper in which the results are presented is written within square brackets.

<table>
<thead>
<tr>
<th></th>
<th>Mean ( \text{PCO}_2 ) (kPa)</th>
<th>Standard dev. (kPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients [I]</td>
<td>3.9</td>
<td>0.7</td>
</tr>
<tr>
<td>Healthy ind. [I]</td>
<td>4.4</td>
<td>0.5</td>
</tr>
<tr>
<td>Healthy ind. [II]</td>
<td>4.3</td>
<td>0.6</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Mean ( \text{H}_2\text{O} ) (mg l(^{-1}))</th>
<th>Standard dev. (mg l(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients [I]</td>
<td>39</td>
<td>1.2</td>
</tr>
<tr>
<td>Healthy ind. [I]</td>
<td>40</td>
<td>1.0</td>
</tr>
<tr>
<td>Healthy ind. [II]</td>
<td>40</td>
<td>1.1</td>
</tr>
</tbody>
</table>

5.2 Results

5.2.1 Investigation of the measurement method

The variation in the measured gas concentrations

The standard deviation of the end-expiratory \( \text{PCO}_2 \) after a vital capacity breath test was significantly larger (approximately ±11-17%) than the standard deviation of the water concentration (approximately ±3%), as displayed in Table 5.2. The end-expiratory \( \text{PCO}_2 \) (\( p<0.01 \)) and water concentrations (\( p<0.01 \)) were found to be significantly lower in patients with impaired respiratory function, than in healthy individuals [Papers I and II].

The changes in gas concentrations arising from the provocative breath manoeuvres, were compared to the measured end concentration after the vital capacity breath tests for ethanol, water, and \( \text{CO}_2 \), and these relative changes in concentrations are summarised in Table 5.3. The results show that a user can deliberately decrease the measured BrAC, but with simultaneous changes being brought about in the concentration of the two tracer gases.

To further investigate if the concentration of the two tracer gases can be influenced by factors other than only the breathing pattern, additional
5.2 Results

Table 5.3: The relative changes in gas concentrations measured at two seconds and at the end of expiration for the different types of breath tests performed, compared to the measured end concentration at the vital capacity breath test procedure. (NS p>0.05; *p<0.05; ** p<0.01; *** p<0.001)

<table>
<thead>
<tr>
<th>Breath test</th>
<th>Ethanol conc. (mg l⁻¹)</th>
<th>Last sec.</th>
<th>FCO₂ (kPa)</th>
<th>Last sec.</th>
<th>H₂O conc. (mg l⁻¹)</th>
<th>Last sec.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vital cap.</td>
<td>0.11±0.05 84±6%</td>
<td>0.14 ± 0.06 100%</td>
<td>3.1±4.4 74±1%</td>
<td>4.3±6.6 100%</td>
<td>38.6±1.3 95±2%</td>
<td>48.1±1.1 100%</td>
</tr>
<tr>
<td>Tidal vol.</td>
<td>8±1% 6±1% 100%</td>
<td>8±10% 3±10% 100%</td>
<td>9±14% 4±10% 100%</td>
<td>9±14% 7±10% 100%</td>
<td>95±3% 65±3% 100%</td>
<td>95±3% 65±3% 100%</td>
</tr>
<tr>
<td>Slow max.exp.</td>
<td>9±1% 9±6% NS</td>
<td>9±15% 9±15% NS</td>
<td>113±15% 113±15% NS</td>
<td>97±15% 97±15% NS</td>
<td>100±15% 100±15% NS</td>
<td>100±15% 100±15% NS</td>
</tr>
<tr>
<td>Hypervent.</td>
<td>8±8% 4±8% NS</td>
<td>4±12% 4±12% NS</td>
<td>9±12% 9±12% NS</td>
<td>9±12% 9±12% NS</td>
<td>95±15% 95±15% NS</td>
<td>95±15% 95±15% NS</td>
</tr>
<tr>
<td>Breath hold.</td>
<td>10±8% 10±8% NS</td>
<td>12±1% 12±1% NS</td>
<td>12±1% 12±1% NS</td>
<td>12±1% 12±1% NS</td>
<td>10±8% 10±8% NS</td>
<td>11±8% 11±8% NS</td>
</tr>
</tbody>
</table>

Experiments involving physical exercise, outdoor tests and smoking were conducted within the work carried out for this thesis. The test subjects consumed a similar amount of alcohol to the test subjects in the two methodology studies. The results of these experimental tests are as yet unpublished.

To study the influence of physical activity on the expired gases, six test subjects performed one minute and ten minutes of cycling on an exercise bike, after which they performed the breath tests. As assumed, physical activity strongly increased the expired PCO₂, whereas the water concentration decreased (mean 16%), see Figure 5.1; note the x-axis. Both gas concentrations returned to normal values shortly after the physical activity ceased. To study any possible effects of the physical activity on the BrAC, four of the six test subjects involved consumed alcohol and then cycling for one minute on the exercise bike. This short period of physical activity was found to decrease the expired BrAC by almost 30%, as shown in Figure 5.1. An increase in the expired PCO₂ as a result of physical activity is as anticipated [19], and the latter is in agreement with results from Schmutte et al. [38], which showed that physical exercise and hyperventilation lower the BrAC.

The results from a minor experimental set-up performed with four
healthy individuals indicated that the water and ethanol gas concentrations are influenced by the properties of the inspired air. The subjects performed vital capacity breath tests and extensive hyperventilation procedures both indoors and outdoors, at an outside temperature of about +1 °C. The results show that, with extensive hyperventilation at low temperatures outside, the ethanol and water concentrations in the expired breath were approximately 16% and 6% lower than the already decreased values obtained from hyperventilation indoors, see Figure 5.2. The lower water concentration at the end of the expiration, and the increased positive slope of phase III found in the expirogram, is likely related to the loss of water from the upper respiratory tract during hyperventilation, which becomes more pronounced in cold and dry air. After a couple of minutes of breathing air at room temperature, the water concentrations had returned to normal values, whereas the BrAC remained at a lower level. The decreases in PCO₂ induced as a result of hyperventilation were also considerable. An hypothesis is that the smaller decrease in PCO₂ after hyperventilation outdoors may reflect a reduced delivery of CO₂ to the alveoli, possibly an effect of vasoconstriction in the pulmonary bed.

Additional indications that the water concentration in expired breath might be influenced by external conditions were found in nine test subjects who performed a vital capacity breath test prior to and after smoking outdoor. Smoking outside at a temperature in the range of +5-10 °C gave a significant (p<0.05) decrease in the water concentration in comparison to the concentration in the breath test performed prior to smoking outside [As yet unpublished results]. The mean relative decrease was similar to the decrease found in the outdoor tests described above (approximately -3%). It cannot be excluded that the decrease in water concentration was a combination of the effects from inspiration of cold air and smoking, and the limited number of observations calls for caution with the interpretation without further experiments being performed. No significant changes in the expired PCO₂ was found from smoking outdoors.

The fact that the CO₂ is entirely exchanged in the alveoli implies that, in healthy subjects, the PCO₂ is, aside physical activity, mainly influenced by the breathing pattern, whereas the airway gas exchange of water and ethanol, the means that changes in temperature and in the relative humidity of the surrounding environment influence the expired concentrations.
Figure 5.1: The relative mean changes in CO₂ and H₂O concentrations during physical activity on an exercise bike. The vertical bars represent the standard deviation. Six test subjects were enrolled for the 'sober' test and four for the post-drinking tests. [As yet unpublished results.]

**Characteristics of the expirograms**

The length of phases I and II⁹, and therefore the time until the onset of phase III, is different for ethanol, CO₂, and water [Paper I and II]. The gases occur in expired breath in the following order: water, ethanol and CO₂, which corresponds to their respective location of the gas exchange. The order in which the gases appear in expired breath does not seem to be affected by provocative breathing manoeuvres. The time to the onset of the final phase for water and ethanol is more or less stable, regardless of breathing manoeuvres, whereas the time to the onset of this phase for CO₂ is altered [Papers I and II]. The onset of the CO₂ expirogram occurred significantly later for a tidal volume breath test than for the vital capacity breath test (p<0.001) and significantly earlier, and thus closer in time to the onset for the water and ethanol expirograms, after hyperventilation (p<0.01) and breath-holding (p<0.001), see Table 5.4 [Paper II]. The influence of repetitive breathing or hyperventilation, on the time to onset of the expiratory curve is related to the mixing of dead-

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⁹As defined in section 2.2.3
Figure 5.2: The relative mean changes found in ethanol, CO₂, and H₂O concentrations after breath test performed indoor and outdoor, as compared to the concentration measured at the end of a indoor vital capacity breath test. Four test subjects were enrolled for these tests. The vertical bars represent the standard deviation. Because of the on-going elimination of ethanol, the concentrations changes measured in the breath test performed after the second indoor vital capacity (VC) breath test have been related to the concentrations measured at the second VC breath test. [As yet unpublished results.]
Table 5.4: The time to onset of the final phase for the three gas expi- programs, with respect to the different types of breath test performed. (NS p>0.05; * p<0.05; ** p<0.01; *** p<0.001)

<table>
<thead>
<tr>
<th>Breath test</th>
<th>$H_2O$</th>
<th>Ethanol</th>
<th>$CO_2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vital capacity</td>
<td>0.76±0.08</td>
<td>0.80±0.08</td>
<td>1.07±0.25</td>
</tr>
<tr>
<td>Tidal volume</td>
<td>0.82±0.07</td>
<td>0.84±0.18</td>
<td>1.25±0.21</td>
</tr>
<tr>
<td>Slow maximum expiration</td>
<td>0.79±0.07</td>
<td>0.81±0.08</td>
<td>1.18±0.29</td>
</tr>
<tr>
<td>Hyperventilation</td>
<td>0.75±0.06</td>
<td>0.78±0.07</td>
<td>0.98±0.16</td>
</tr>
<tr>
<td>Breath-holding</td>
<td>0.73±0.07</td>
<td>0.75±0.06</td>
<td>0.85±0.11</td>
</tr>
</tbody>
</table>

space air with air which has participated in the gas exchange. Breath- holding implies an increase in the time for the mucous membrane to recover and mixing of air, which leads to the presence of expired gases, especially $CO_2$, higher up in the respiratory tract.

For all three gases, and after all the types of provocative manoeuvres tested, the onset of the final phase occurred within two seconds of the start of the expiration; this indicates that, from then on the increase in concentration is more steady. The steady positive slope of the final phase implies that after two seconds of a vital capacity breath test the concentrations obtained for ethanol, $CO_2$ and water amount to approximately 85±7%, 78±8%, and 95±2% of the end-expiratory concentrations [Paper I]. This verifies that a measurement after a short and shallow expiration of two seconds, will result in a false low BrAC.

The differences in the relative two-second concentration found between the three gases, imply that there must be differences in the slope of the final phase of the expirogram for the three gases. These differences in steepness are related to the different mechanisms underlying the gas exchange, and can be influenced by different types of breathing patterns [As yet unpublished results, from analyses of the data included in Papers I and II]. To enable comparison of the slope of the final phase for the different gases, the slopes were normalised with respect to the time elapsed from two seconds to the end of each expirogram. Analyses of
Figure 5.3: The difference in the normalised slopes for phase III between patients with respiratory impairment and healthy test subjects, for the expirograms of CO₂, ethanol and H₂O (* p<0.05; ** p<0.01). (Result from analysis on the data included in Paper I).

The expirograms showed that the normalised slope obtained for ethanol and CO₂ were approximately three and five times steeper than the slope obtained for the water expirogram, respectively, see Figure 5.3. The slope of the CO₂ expirograms recorded from patients with respiratory impairments were significantly steeper than that of the healthy subjects’ expirograms (ANOVA: p<0.05), whereas the normalised slopes of the ethanol expirograms were steeper in the healthy subjects than in the patients (p<0.01) (analyses performed on the data included in Paper I). These differences are probably related to the impaired ventilation in the patients, which does not influence the airway exchange of either ethanol or water to the same extent.

Compared to ethanol and water, the CO₂ expirogram had the steepest slope for all types of breath tests, except after breath-holding, as is evident in Figure 5.4. With no preceding inspiration or after a period of breath-holding, the concentrations further up in the in the airways become higher, which is noticeable in the expirograms through the significantly lower steepness of the final phase for all three gases; compare with the slow maximum expiration and breath-holding breath tests as evident in Figure 5.4.
Figure 5.4: The differences in the normalised slope for phase III for the expirograms of CO\textsubscript{2}, ethanol and H\textsubscript{2}O for different types of breath tests (*** \(p<0.001\)). (Result from analysis on the data included in Paper II). 

The similarities between the expirograms for ethanol and the respective tracer gas during an expiration can be illustrated by the correlation between the measured concentrations. Despite the continuous increase in concentration during the final phase, the onset of this phase implies that the value of the ratio between the two pairs of gases; EtOH/CO\textsubscript{2} and EtOH/H\textsubscript{2}O, become more stable. The similarities and differences in the characteristics of the expirograms result in a linear correlation between the ethanol and CO\textsubscript{2} concentration after the onset of the final phase, whereas a non-linear relationship prevails between the ethanol and water concentration, see Figure 5.5 (a). The fact that the slope of the ethanol expirogram is less steep than that of the CO\textsubscript{2} expirogram, but steeper than the slope for the H\textsubscript{2}O expirogram, means that the value of the EtOH/CO\textsubscript{2} ratio increases over time, whereas the EtOH/H\textsubscript{2}O ratio decreases over time, as illustrated in Figure 5.5 (b). A ratio that slightly increases with the expiration time, as the EtOH/CO\textsubscript{2} ratio does, means that if the user performs a short/shallow breath test, there is little risk that the end-expiratory BrAC estimated will be falsely low. The stabilisation of the two ratios over time after a hyperventilation and a breath-holding procedure are illustrated in Figure 5.6 parts (b) and (c), respectively.
Figure 5.5: The similarities and differences in the development of the gas concentration during an expiration, illustrated through (a) the relationship between the ethanol and the respective tracer gas concentration, and (b) the development of the ratio of ethanol and the respective tracer gas concentration; EtOH/CO₂ and EtOH/H₂O.
Figure 5.6: The altering in the ratio of ethanol and CO₂ (EtOH/CO₂) and ethanol and H₂O (EtOH/H₂O) for three breath tests performed by the same test subject: the vital capacity breath test (a), a breath test after hyperventilation (b), and a breath test after 30 seconds of breath-holding (c). The stabilisation of the ratio is related to the onset of the final phase of the gas expirograms.
Figure 5.7: The relative change in the estimated end-expiratory BrAC with the use of CO₂ and H₂O as the tracer gases in comparison to the two-second reference BrAC, for the different breathing manoeuvres (NS p>0.05; * p<0.05; ** p<0.01; *** p<0.001). [Paper II]

Summary

The fact that it is possible to deliberately decrease the BrAC by small expiration volumes and by hyperventilation has been confirmed with the results presented in Table 5.3 and in Figure 5.7. The intra- and inter-individual variations in the end-expiratory PCO₂ and in the water concentration have been presented in Table 5.2, and the influence on the end-expiratory PCO₂ and the water concentration after adopting different breathing and from environmental factors have been presented.

With estimation of the end-expiratory BrAC, from the two-second concentration of ethanol and CO₂, the risk of a false low BrAC, which might imply a false unblocking in an alcohol, after a shallow expiration after a full inspiration (short vital capacity breath test) and hyperventilation is eliminated, whereas an overestimation of the BrAC is introduced after a period of breath-holding, as illustrated in Figure 5.7. The false high estimated BrAC after breath-holding is attributable to the fact that the PCO₂ experiences a larger relative increase than the BrAC. The use of water as the tracer gas only slightly corrects for the underestimation of the end-expiratory BrAC [Paper II].
5.2.2 Evaluation of the breath analyser prototypes

Figure 5.8 shows the magnitude of the signals from the ethanol and CO₂ channels from two sequential breath tests performed by one intoxicated subject [Paper III]. The magnitude of the output signals was influenced by the dilution of the breath sample, expressed as the mixing ratio seen in the insets of Figure 5.8. The degree of mixing of the breath sample with ambient air is proportional to the axial distance to the inlet of the prototype, whereas the degree of mixing increased significantly with small lateral misalignments [Paper III]. The ethanol signal is approximately linear with the concentration, whereas the signal for CO₂ decreases sub-linearly with increased concentration. There were minor differences in the sensitivity and the absorbance of the ethanol and CO₂ signals for the three prototypes, as can be seen in Table 5.5. However, the signal level for ethanol of the passive sampling medical prototype was decreased during the pre-clinical tests.

For the evaluation of the two different types of prototypes the estimated end-expiratory BrAC was compared with the alcohol concentration determined with the relevant reference analysis method; breath and blood alcohol analysis. Strong correlations were found between the estimated BrAC and the reference BrAC for the alkoclock prototype (r=0.91), see Figure 5.9 (a). The result for the pre-clinical test showed that the BrAC estimated with the active sampling prototype was strongly correlated with the BAC (r=0.89), see Figure 5.9 (b).

With use of a BBR of 2100:1, the equation of the regression line of the plot in Figure 5.9 (a) indicates that on average the estimated end-expiratory BrAC was approximately 22% higher than the BrAC measured in undiluted breath sample with the reference breath analyser, whereas the estimated end-expiratory BrAC in the less cooperative patients (b) on average underestimated the BAC with approximately 16%. These are indications from a limited number of breath test performed with these prototypes, larger studies with improved prototypes need to be conducted before an eventual systematic error in the estimation can be appointed and thus also possibly adjusted for.

However, the standard deviations found for the two prototypes, 0.05 mg/l and 0.3 mg/g respectively, are of acceptable size with respect the the early generation of prototype and for their respective application area. Note that the level of the standard deviations for the two prototypes is not comparable, because two different reference analysis
Figure 5.8: The signals from the ethanol and CO₂ channels during two sequential breath tests performed by a test subject. The difference in the degree of mixing with ambient air and the corresponding difference in magnitude of the signals are related to the distance from the breath analyser inlet, and the characteristics of the expiration [Paper III].

methods and because of the difference in type of expirations performed. The larger standard deviation found in the patient group might also indicate that the uncertainty in the estimation of the end-expiratory BrAC increases, since the inter-individual variation in PCO₂ is probably larger among patients.
Table 5.5: Calibration data of the alcolock prototype and the passive and active sampling medical (med.) breath analyser prototypes used in the pre-clinical tests. The data presented for the medical prototypes are the results of the calibration performed after the pre-clinical tests. The sensitivity and absorbance of the ethanol and the CO₂ detectors have been determined at 1 mg l⁻¹ and 0.9 kPa, respectively.

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<tr>
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<tbody>
<tr>
<td><strong>Sensitivity</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ethanol (µV per (mg l⁻¹))</td>
<td>9.3</td>
<td>3.5</td>
<td>7.1</td>
</tr>
<tr>
<td>CO₂ (µV per kPa)</td>
<td>54</td>
<td>49</td>
<td>59</td>
</tr>
<tr>
<td><strong>Absorbance</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ethanol (% per (mg l⁻¹))</td>
<td>2.0</td>
<td>2.2</td>
<td>2.3</td>
</tr>
<tr>
<td>CO₂ (% per kPa)</td>
<td>10.6</td>
<td>15</td>
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The strong correlation and the standard deviations found represents sufficient evidence of the performance of these first generation prototypes for their development to be pursued for their respective areas of application. Additional conclusions drawn from the evaluations of the prototypes are that the resolution of the ethanol signal and the signal-to-noise ratio for both the ethanol and the CO₂ signals need to be improved, and for use on non-cooperative patients, a decreased dead space volume is likely to improve the performance and correlation with the BAC.
Figure 5.9: The relationship between the BrAC estimated using CO₂ as the tracer gas and the alcohol concentration measured with the reference method. Figure (a) shows a strong correlation ($r=0.91$) between the BrAC estimated from contact-free measurements and the end-expiratory BrAC measured with a reference breath analyser (n=45) (the Evidenzer, Nanopuls AB, Sweden). The tests were performed by one intoxicated subject [Paper III]. Figure (b) shows a strong correlation ($r=0.89$) between the BrAC estimated from the measurements with the active sampling medical prototype and the BAC. The analysis is based on 59 breath tests from 37 patients who performed the expirations according to their ability [Paper IV].
Chapter 6

Discussion

The reason for using breath analysers as alcolocks or in medical care, it is simply to save lives.
For many years the large number of fatal injuries occurring in road accidents related to drunk driving has been the subject of debate.
In Sweden, the total number of fatal injuries from traffic accidents continues to decrease; in 2009, the number was 355 [91], of which more than 25% were in alcohol related accidents [92, 46]. However, it has come to our knowledge that, for approximately 2000 of the patients admitted to Swedish emergency departments each year, fast and reliable alcohol analysis could make a difference of decisive importance, with respect to irreversible injuries or fatality [an estimation presented by MD Urban Säfvenberg, Uppsala University Hospital, 2010-04-29]. This means that recommended and frequent use of breath alcohol analysis in emergency medicine for securing of continued medical assessment, could be as important for lifesaving in hospitals, as it is for traffic safety, not to mention the improved outcome for many patients not at risk of dying.

In an alcolock, a breath test must be conducted routinely before driving, and for a driver with a BrAC over the legal limit for drink driving, the alcolock will automatically block the ignition. For the wide acceptance and use of alcolocks, the handling and breath test procedure ought to constitute a minimal effort for the sober driver, which in Sweden constitute 99.8% of the total traffic flow [46]. The results of this thesis have shown that with use of CO₂ as a tracer gas, contact-free screening measurement can be enabled, and bringing about a significant reduction
in the duration and volume of the expiration required for a breath test. For a completely sober driver, no alcohol will be detected in the expired breath, and for a driver who has consumed alcohol, estimation of the end-expiratory BrAC will minimise the risk of a falsely low BrAC, after shallow expiration and procedures involving hyperventilation. For the driver who has in fact consumed alcohol and from a first breath test is considered to lie within an interval around the legal limit, I am of the opinion, that request of a second, more demanding breath test for increased measurement reliability cannot compose too large an obstacle. In addition, simultaneous measurement of CO₂ in the breath sample makes manipulation through the use of artificial breath gas solutions more difficult.

The inter-individual variations in the end-expiratory PCO₂ do introduce a multiplicative error in the estimation of the end-expiratory BrAC. The larger inter-individual variation (the standard deviation) in PCO₂ and the water concentration found in patients with pulmonary diseases, in comparison to healthy subjects [Paper I], is probably a result of differences in severity of the respiratory impairment. The significantly lower mean PCO₂ found for the patient group, than for the healthy test subjects is not representative for patients with obstructive pulmonary diseases in general. Persons with fibrosis are likely to have lower levels of PCO₂ in the expired breath, whereas in severe cases of COPD, the PCO₂ in expired breath will be increased [93].

The fact that a larger inter-individual variations in the expired PCO₂ in patients can be expected implies that the method of normalising might be less appropriate for the medical applications. However, for medical use the requirement of accuracy is subordinated since the large individual variation in the tolerance of alcohol means that the measured BrAC has to be considered in the context of the specific patient, see Figure 2.1.

In itself, the presentation of the expired PCO₂ considerably improves the reliability of breath alcohol analysis in non-cooperative patients and provides the medical staff with support in the decision whether a re-test is needed or not. The presentation of the measured PCO₂ value also gives a supplementary value as an indication of the patient’s respiratory function. Measurement of the end-tidal PCO₂ in check-ups after intubation and for monitoring in emergency, intensive and post-operative care is common and of importance [94, 77].
With respect to the small intra-individual difference in the end-expiratory concentration, water has a clear advantages over CO₂ as the tracer gas. In a controlled indoor environment, the expired breath is more or less saturated with water from the beginning of expiration, regardless of the manner of breathing. Such abundance of water in expired breath implies that the concentration cannot be influenced to the same extent from the breathing manoeuvres, as the ethanol concentration, and therefore the possibility to compensate for provocative breath tests, is limited, as can be understood from Figure 5.7.

In an uncontrolled environment, e.g. in a private vehicle or an ambulance, or in the case of contact-free measurement, the influence from the ambient air on the expired gas concentrations cannot be controlled or prevented. In this respect CO₂ has an advantage over water as tracer gas. The CO₂ concentration outdoors (approximately 400 ppm) and in well ventilated rooms and vehicle compartments with good indoor air quality (<850 ppm) [95] is low, which implies that the contribution from ambient air can only constitute less than 2% of the end-expiratory PCO₂.

The relative humidity in ambient air, on the other hand, will change with the weather condition and the geographical location, and the variations can be large. The experimental results from the tests outside in chilly air showed that the water content of the expired air was influenced by the properties of the inspired air, whereas no systematic influences in the PCO₂ could be found. This is likely a result of the differences in location of the gas exchange within the respiratory organ for the two gases. In addition to the minor influence by the manner of breathing, the water concentration measured in the expired breath will be influenced by the properties of the ambient air, and might also be altered if condensation occurs in a cold mouthpiece or, in the case of contact-free measurement, at the inlet of the device.

To summarize, CO₂ is considered to be an appropriate tracer gas for indication of the quality of the breath sample and/or for estimation of the end-expiratory BrAC in breath alcohol analysis for the applications of an alcolock and for use in medical care.
Chapter 7

Future work

7.1 New prototypes

At present new prototypes of the alcolock and the medical prototype are being assembled. The new hand-held alcolock prototype, seen in Figure 7.1, contains a new optical measurement cell with an increased optical path length for increased sensitivity of ethanol. The wavelength interval used for the quantification of the ethanol concentration has been changed to around 9.5 µm to improve selectivity [89]. Figure 7.2 shows the low level of absorption from acetone, in comparison to the absorption of ethanol at a wavelength of 9.5 µm. Evaluation of the performance of the alcolock including bench testing is ongoing, and tests involving test subjects are planned for the near future.

From the result of the pre-clinical tests, it as concluded that the medical prototype was to enhance the breath sampling with use of a fan. Apart from the change of wavelength to 9.5 µm, the technical changes made to the new medical prototype include a significant reduction of the total dead-space volume. This prototype will be used together with an intended receptor of small volume, which is attached to the inlet of the hand-held device with a bacterial filter in-between, see Figure 7.3. In the interest of minimising the dead-space, a new optical measurement cell of smaller dimensions has been developed. With the decreased dead-space volume, the end-expiratory breath is more likely to enter the measurement cell, and the smaller dead-space has been proven to reduce the response time, and thereby enabling improved real-time acquisition
of the respiratory signal. Together with our clinical partners, both in pre-hospital care and at emergency departments, it is planned to commence trials with this new prototype during the autumn of 2010. Besides evaluation, of the performance of the prototypes, we address questions relating to the usability aspects of the device and the benefit of breath alcohol analysis in emergency care in the trials.

7.2 Analysis of additional breath compounds

Technology based on IR spectroscopy offers high selectivity to ethanol, which is of importance in breath alcohol analysis for traffic law enforcement, but this technology also offers the possibility to detect other breath compounds of interest, whether they are endogenously produced or inhaled from the surroundings (exogenous compounds). Intake of other alcohol containing fluids for inebriation purposes, e.g. cooker fuels, windshield washer-fluids, and antifreeze fluids is not unusual among alcohol dependent persons and patients seeking emergency care, so detection of these substances might be useful in the medical assessment [33], but intoxication with these substances is likely to be less common among drivers. Use of drugs, on the other hand, is an increasing problem both in medical care and in traffic, since it strongly impairs a person’s ability to drive safely. Recently, a Swedish research group presented a method for detection of amphetamines in exhaled breath [96]. Detection of amphetamines is especially beneficial with respect to traffic safety, since amphetamines are the most commonly used class of drugs among drivers [97].

The use of breath analysis in medicine is predicted to increase [98, 99, 100], thanks to technical development and the non-invasiveness of the method. During recent years, the research for biomarkers indicating pathological conditions has increased and some interesting biomarkers have been identified [98, 99, 100]. The concentration of isoprene is used to reflect the excretion of cholesterol [101]. Nitrogen oxide is a marker for pulmonary inflammations e.g. asthma and [102], and increases in the concentration of ammonia are related to hepatic disease and uremia [33, 100]. Rapid and simple detection of increased levels of carbon monoxide (CO) in expired breath has been suggested for medical triage at the emergency department [103], and could possibly also be used at the scene of fires. Carbon monoxide poisoning should be considered in patients
with CO concentration above 6 ppm in expired breath (4.9 μg L⁻¹)[103].
The elevated CO concentration in smokers [103], can also be used for
evaluation of attempts to stop smoking.

We believe that in pre-hospital and emergency care, a hand-held and
user-friendly device that can detect the presence of some of the more
useful breath compounds and, perhaps also quantify the concentrations,
is likely to be of great benefit in medical assessment and to support
decision in the triage of patients.
Figure 7.2: The spectra shows the possibility to avoid interference from expired acetone with use of a wavelength of 9.5 µm. Simulations made with use of absorption spectra from the Pacific Northwest National, PNNL.

Figure 7.3: The latest prototype of the hand-held breath alcohol analyser for medical use. (Design: Newlight Designers and Maaxdesign, Photo: Per Åkerlund)
Chapter 8

Conclusion

From the results of this thesis I would like to make the following conclusions:

- By the use of CO$_2$ as the tracer gas the risk of underestimating the BrAC is minimized in the cases of shallow expiration and corresponding provocative breathing pattern, both in the view of the intra- and inter-individual variations [Papers I and II].

- Hand-held prototypes which enable contact-free measuring and more reliable measurement of the BrAC in the case of shallow and passive expiration have been developed and evaluated. Their performance has been found to be adequate for the continuing development aiming at applications in alcolocks and emergency care [Papers III and IV].
Acknowledgement

This thesis work has made it possible for me to combine my interests in medicine and engineering science. For me, technical biomedical applications make more sense as they often make a difference. The best thing possible would be if the results of this thesis were to make a difference, if only for one person. Then all the work, time and effort I have put into this thesis would be really worth something.

Once I said "I will never continue to do research", I guess I should have considered the saying "Never say never". But "never" would have turned out to be true and I would have missed the opportunity to possibly make a difference, if it wasn't for some people:

My co-supervisor, Dr. Bertil Hök, during these three years you have shared your experience and inspired me. The discussions we have had and the support you have given me have been invaluable. You have believed in me, allowed me to take my own initiative and to be the "softer" engineer - Thank you! I look forward to see where these projects will take us.

My co-supervisor Prof. Göran Hedenstierna, you have contributed to this thesis with your extensive knowledge in the field of respiratory physiology, and your "questioning" questions and your help in writing have been very valuable. I am grateful and pleased to have been one of your many PhD students.

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Without the support from the project leader of the alcolock project, Håkan Pettersson at Autoliv Development AB, I would not have been given the possibility to look deeper into breath analysis. Thank you for the interest you have shown in my work!

My colleagues at Hök Instrument AB, without you there would not have been any prototypes for me to test, no photos or absorption spectra to include in this thesis, and the spontaneous unsober experimental tests would have required longer time for recruitment. I would also like to thank the company board for the support given and constructive discussions.

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I would also like to acknowledge our clinical partners at the emergency departments at The University Hospital in Uppsala and Södersjukhuset in Stockholm, and Hagersten's ambulance station run by Folk Ambulans AB. Thank you for the interest you have shown in our project and for very interesting, and for me fruitful, discussions. I look forward to our continued collaborations!
However, this research could not have been done without the persons who have participated in the studies. Thank you for your cooperation!

On a more personal level I would like to express my great gratitude to my dear family and friends that just by existing give me so much joy!

My "big" big brother Urban, you are nowadays also engaged in my professional life. Your experiences as a paramedic as well as your interest and participation in our project are very valuable to me. Thanks for the love you constantly show me.

My "little" big brother Pelle, I am thankful for the warmth and care you and your family show me. But you owe my one guinea pig trial!

My dear Mum and Dad, there are no words that can express my gratitude over the love, care and encouragement you constantly give me. I can just hope that I manage to return some of it!

Dear Kim, I am so grateful for the love and strength you give me every second of every day. You make me a better person and from now on, I promise to be a more calm and present wife!

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Bibliography


