The advantages of standardizing exhaled breath-alcohol concentration to a reference respiratory gas—water vapor

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Abstract
Measuring the concentration of alcohol (ethanol) in exhaled breath (BrAC) provides a rapid and non-invasive way to determine the co-existing concentration in arterial blood (A-BAC). The results of breath-alcohol testing are used worldwide as evidence of excessive drinking, such as when traffic offenders are prosecuted. Two types of breath-alcohol analyzer are in common use; hand-held instruments used as preliminary screening tests of sobriety and more sophisticated evidential instruments, the results of which are accepted as evidence for prosecution of drunken drivers. Most evidential breath-alcohol analyzers are designed to capture the last portion of a prolonged exhalation, which is thought to reflect the alcohol concentration in substantially alveolar air. The basic premise of breath-alcohol analysis is that there is a physiological relationship between A-BAC and BrAC and close agreement between the two analytical methods. This article reviews the principles and practice of breath-alcohol analysis and introduces the concept of standardizing the results to a secondary physiological gas (water vapor), which therefore serves as an internal standard. The measured BrAC is thus adjusted to an alveolar air water content of 43.95 mg l⁻¹ at 37 °C. This has several advantages, and means that a sample of breath can be captured without the person having to blow directly into the instrument. Adjusting the breath-alcohol concentration to water vapor concentration also compensates for variations in temperature of the expired air. The contact-free method of sampling breath means that a mouthpiece is unnecessary and the test subject does not need to make a continuous end exhalation.

1. Introduction

Research dealing with the analysis of volatile organic compounds in human breath, endogenous and exogenous substances, continues to expand and this technique has found many applications in clinical and diagnostic medicine, such as for investigating various disease states [1–4]. However, the oldest and most well-established application of breath analysis is in law enforcement, such as when drunken drivers are prosecuted [5]. This development started in USA in the 1940s, because of constitutional issues related to obtaining samples of a person’s blood for analysis in criminal cases [6]. After alcohol was determined in a person’s breath, the result was converted into a presumed venous BAC (V-BAC) by assuming a constant blood/breath ratio (BBR) of alcohol of 2100:1 [7].

The first scientific article comparing V-BAC with BrAC was published in 1938 and the results were found to be highly correlated [6]. The test subject inflated a rubber bladder or balloon, hence capturing a sample of the mixed expired air for analysis. This breath samples was then passed through an oxidizing agent consisting of acidified potassium permanaganate. This reagent changed in color and indicated that the subject’s probable V-BAC was above a certain blood-alcohol threshold limit (0.15 g% or 1.5 g l⁻¹), which was the legal limit for driving at the time [8]. To obtain a more exact estimate of the V-BAC the volume of breath passing through the oxidizing agent was measured and after removal of water vapor its
CO₂ content was determined by weighing an absorbent material (Ascarite tube). The fraction of alveolar air in the mixed expired air sample was calculated assuming a CO₂ content of 5.5 vol%.

Whether or not alveolar air contained 5.5 vol% CO₂ depended on the person's pattern of breathing, the resistance to exhalation, any hyp- or hyper-ventilation and ventilation-perfusion mismatch (V/Q-mismatch) [9]. Later versions of this same analytical method sampled breath for analysis after a subject rebreathed the initial exhalation five times as a way to obtain an ethanol content representative of alveolar air [10]. The rebreathing of an initial exhalation should eventually provide a sample of breath comparable with respiratory gases [11]. However, this has since been shown to be an inaccurate assumption [12].

In 1954 the Breathalyzer was developed by Robert Borkenstein in USA and this instrument captured the last portion of a prolonged exhalation for determination of alcohol and estimation of blood-alcohol content [13]. After a moderate inhalation of room air, the subject made a continuous exhalation through a heated plastic tube and most of the breath was vented back into the atmosphere. When the subject stopped blowing into the instrument the last portion of the exhalation (52.5 ml) was trapped and the alcohol content determined by oxidation with chromic acid. The technique of sampling the end portion of a prolonged exhalation became the standard design feature with most subsequent instruments for breath-alcohol analysis.

The instruments currently used for breath alcohol analysis in law enforcement are classified as (a) hand-held preliminary breath-testers, which are used to screen drivers at the roadside, and (b) quantitative evidential breath-alcohol instruments, the results of which are used for prosecuting traffic offenders.

The analytical technology incorporated in most hand-held breath analyzers for determination of ethanol is electrochemical oxidation with a fuel cell detector. By contrast, most of the evidential breath-alcohol equipment's incorporate infrared spectrometry and multiple wavelength filters close to 3.4 μm (C–H bond stretching) or 9.5 μm (C–O bond stretching).

Infrared absorption technology relies on the fact that absorption of infrared light is directly proportional to the alcohol concentration according to the Lambert–Beer laws. The technique is fast, making it possible to measure the alcohol concentration during the course of a prolonged expiration [14]. However, since only alcohol content is being measured the end result depends on breath temperature and the person's breathing pattern prior to expiration.

The standard procedure when evidential breath alcohol tests are conducted is to observe the subject for 15 or 20 min during which time he or she is not allowed to have anything in the mouth. This deprivation time is important to ensure that the exhaled breath is not contaminated by any alcohol in the oral mucosa from a recent drink.

The test person is then instructed to make a prolonged expiration through a mouthpiece and spit-trap attached to the inlet tube of the breath-analyzer. This leads directly into the infrared chamber or cylinder for absorption of infra-red light. The subject makes a prolonged exhalation and it is the last portion of the breath that is captured for analysis. The test subject has to make a continuous exhalation at a certain minimum pressure and flow-rate for at least six seconds.

Although, evidential breath-alcohol instruments capture the last part of a prolonged expiration for analysis, which is considered equivalent to an alveolar breath sample, the measured BrAC tends to underestimate the alveolar alcohol concentration. For this reason, some investigators have proposed and tested a method that standardizes the alcohol concentration in breath to a reference gas of known concentration in alveolar air.

This paper reviews the physiological principles and practical application of a method that attempts to standardize breath alcohol concentration to the concentration in alveolar air of the physiological gases, water vapor, CO₂ and O₂.

2. Alcohol distribution in the body

For most practical purposes and under real-world situations alcohol is taken by mouth (oral ingestion), and gets absorbed into the portal venous blood from the stomach and upper part of the small intestine (duodenum and jejunum). Soon after drinking an alcoholic beverage, the alcohol concentration is initially highest in the stomach contents and the portal venous blood, which drains the gastrointestinal tract before passing through the liver. Hepatic enzymes start to metabolize alcohol immediately, but they are quickly saturated with substrate after the first couple of drinks, provided that portal V-BAC exceeds 0.015–0.020 g% (0.15–0.20 g l⁻¹).

After passage though the liver the hepatic vein blood drains into the inferior vena cava before entering the right side of the heart (right atrium), where it mixes with deoxygenated blood returning from peripheral tissues via the superior vena cava. This admixture of deoxygenated blood passes from the right atrium to the right ventricle and via pulmonary arteries into the pulmonary capillaries of the lungs.

Diffusion of gases and other volatile substances occur across the alveolar-capillary membrane depending on solubility of the substances in blood and their blood-air partition coefficients at body temperature (37 °C). The oxygenated blood, which has equilibrated with the alveolar air across the pulmonary alveolar-capillary membrane, returns to the left side of the heart via the pulmonary veins and is
pumped via the aorta throughout the entire vascular network of the body—the systemic circulation. The concentration of alcohol in this blood, the arterial blood (just equilibrated with air in the lung), governs the concentration reaching all body organs and tissues, including the brain, which ultimately determines alcohol-induced impairment of body functions and drunkenness.

When deoxygenated venous blood returns from peripheral tissues its alcohol concentration is depleted, owing to losses to organs and tissues, such as skeletal muscles, which act as a reservoir. Hence V-BAC is lower than A-BAC during the time alcohol is being absorbed from the stomach and distributed into the total body water compartment [15]. During the absorption phase of the BAC curves, a considerable amount of alcohol from the gastrointestinal tract replenishes the peripheral venous blood at the level of the portal vein before it again enters the liver and the heart. During the elimination phase, there is no alcohol being absorbed from the gastrointestinal tract and alcohol in peripheral venous blood decreases owing to losses through hepatic metabolism and dilution with blood returning from the hepatic arteries.

3. Alcohol concentration in arterial and venous blood

The above brief overview of ethanol’s distribution in the body makes it clear that the concentration in arterial blood is initially higher than in the venous blood returning from peripheral tissues and extremities. These differences become progressively smaller as more and more alcohol is absorbed and distributed throughout the total body water [16].

After drinking stops, alcohol continues to be absorbed for some time afterwards until a complete equilibration is reached between the concentration in the arterial and the venous blood circulation. At this time point the arterial and venous concentrations are the same for a brief instant [16]. During the entire post-absorptive phase, provided no additional alcohol is consumed, the V-BAC is slightly higher than the A-BAC. This follows because alcohol is continuously being cleared from the central blood compartment by metabolism in the liver, there is a feed-back from alcohol contained in the peripheral tissue water, especially the skeletal muscles.

Figure 1 compares the concentration-time profiles of ethanol in venous and arterial blood in one subject who drank 0.60 g alcohol/kg body weight on an empty stomach. The observed systematic differences, A-BAC > V-BAC during the first ∼60 min post-dosing and V-BAC > A-BAC at all later times, were confirmed in experiments with 12 other volunteers [17]. These temporal variations between arterial and venous blood were also confirmed in animal studies using rabbits [18].

4. Physiological principles

Breath-alcohol analysis furnishes an indirect way to estimate the concentration of alcohol in arterial blood and relies on the concept that highly hydrophilic (water soluble) substances in pulmonary capillary blood equilibrates almost instantly with alveolar air by diffusion across the alveolar-capillary membrane [19]. Since the alcohol concentration in the pulmonary capillary blood is the same as the concentration in the ejected systemic arterial blood, it is expected that the concentration of alcohol in the alveolar air reflects the concentration of alcohol in the arterial blood [16, 17, 20–25].

During a continuous exhalation, the concentration of water vapor and other highly water soluble substances, such as acetone and ethanol, decreases compared with the concentration of these substances in alveolar air. The concentration of alcohol in end-exhaled breath is therefore appreciably lower than the alcohol concentration in alveolar air and the arterial blood leaving the lungs [12, 19]. In part, this difference depends on a decrease in equilibrium temperature from ∼37 ℃ at the alveolar membrane to ∼34 ℃ as breath leaves the mouth. Another reason is the exchange of alcohol that takes place between respired air and the watery mucus surfaces covering the upper airways [26].

One theory mandates that there is a depletion of alcohol from the conducting airways during the inspiration of alcohol-free ambient air and this alcohol is later recovered to some extent from the subsequent alveolar air during exhalation. It does not appear possible to obtain a sample of end-exhaled breath with the same water vapor and/or alcohol concentration as in alveolar air, not even after rebreathing [12].

The efficacy of the exchanges of alcohol between the inhaled and exhaled air and the upper respiratory tract (URT) mucosa depends on pattern of breathing and how much alcohol the bronchial circulation has been able to supply to the mucosa, after its depletion by alcohol-free inspired ambient air, between inhalation and the following exhalation. This means that the pre-requisites for the Fahri equation, which applies to inert gases describing the conventional pulmonary gas elimination theory, are not applicable to water soluble substances like alcohol [12].

If the exchange of gases between the inhaled and exhaled air and the mucosa is taken into consideration together with the temperature dependency of the mucosa: air partition coefficient, the Fahri equation for inert gases can be modified for hydrophilic soluble substances like alcohol [12]. By using this modified equation the corresponding arterial blood/breath concentration ratio of ethanol can be calculated to be 2250. This was almost exactly the same as the value determined in-vivo in alcohol dosing studies.
Figure 1. Concentration-time profiles of ethanol in blood from a cubital vein (venous BAC, V-BAC) and a radial artery (arterial BAC, A-BAC) on the same arm. The one male subject drank 0.60 g kg\(^{-1}\) alcohol on an empty stomach and blood samples were taken via indwelling catheters for up to 6–7 h post-dosing (adapted and modified from Lindberg et al [17]).

Figure 2. Time course of the differences between BrAC \(\times 2251\) and the concentration of ethanol in arterial blood (A-BAC) and venous blood (V-BAC) in healthy subjects of both sexes (\(n = 15\)) after they drank 0.6 g kg\(^{-1}\) ethanol on an empty stomach. The mean \(\pm 95\%\) confidence interval at each time point are plotted (adapted and modified from Lindberg et al [17]).

(2251 \(\pm 46\)) when A-BAC was compared with BrAC [17]. By using this arterial blood/breath alcohol ratio, the arterial blood concentration can be estimated almost perfectly from measured BrAC [27].

Figure 2 shows that BrAC \(\times 2251\) agrees much better with A-BAC rather than with the co-existing V-BAC during the entire time that alcohol is measurable in the body. The first sample of breath taken very soon after end of drinking underestimated slightly the A-BAC (see explanation below), but thereafter, good agreement was found at all later times. By contrast, the relationship between BrAC and V-BAC was continuously changing as a function of time after the end of drinking. In short, the V-BAC/BrAC ratio was a moving target.

This plot shows that V-BAC is not causally related to BrAC and the differences change as a function of time after drinking. This systematic difference between BrAC and V-BAC is attributed to the fact that V-BAC reflects the alcohol concentration in the peripheral tissue water, mainly in the muscle compartments. The true physiological explanation is that V-BAC can never accurately reflect A-BAC nor BrAC [28, 29].

Since BrAC reflects the arterial BAC, the concept of using the arterial blood-breath ratio (A-BBR) to establish an A-BAC from BrAC is fundamentally correct. However, the statutory 'BAC' limits for driving refer to the concentration of alcohol determined in venous blood drawn from a cubital vein. This
concentration cannot be reliably predicted from the BrAC, because of the arterial-venous differences discussed above and illustrated in figures 1 and 2. There is a strong correlation between V-BAC and BrAC, although the magnitude of the differences depend on the phase of ethanol metabolism when tests are made.

During the absorption phase, BrAC × A-BBR is significantly higher than the V-BAC and in the post-absorptive phase the BrAC × ABBR is significantly lower than the V-BAC as shown in figure 2. The magnitude of over- and under-estimation depends on the value adopted for the BBR, which differs between countries and law enforcement agencies, since the BBR is incorrectly derived by comparing BrAC and the variable V-BAC values.

5. Breath expirograms

The concentration of a gas in the exhaled air of a single-breath can be determined and presented as a volumetric or a time-based expirogram.

5.1. Volumetric expirograms (CO₂, water vapor, and alcohol)

A volumetric expirogram is shown as a scatterplot of the exhaled concentration on the y-axis against the exhaled volume on the x-axis. This makes it possible to see the distribution of the gas concentration in relation to expired air volume.

5.1.1. The volumetric expirogram for CO₂

The volumetric single breath expirogram for carbon dioxide (CO₂), also known as the volumetric capnogram, has been extensively studied. Carbon dioxide is produced in the mitochondria during energy production. It reacts with water to form carbonic acid (H₂CO₃), which in the blood exists as hydrogen ions and bicarbonate ions. This process is reversed in the lungs, where CO₂ diffuses across the pulmonary capillary-alveolar membrane and is expelled in breath. Because of its low solubility, CO₂ interacts minimally with the water containing mucosa layer of the conductive airways. The exchange of CO₂ between blood and air occurs exclusively in the alveoli of the lungs [30].

During an exhalation, the first breath that leaves the mouth is free from CO₂ because it is essential air from the conducting airways (mouth, trachea, bronchi). There has been no exchanges of CO₂ between this first part of an exhalation and blood and is, therefore, also referred to as the dead space volume (VD of CO₂). The volume of the anatomical VD has been determined to be about 150 ml. The VD for CO₂ was determined to be 158 ± 37 ml in the study were water vapor and alcohol were simultaneously measured [31]. The VD was determined by a modification of the method described by Koulouris et al [32].

After the VD is discarded, the CO₂ of breath comes exclusively from alveolar air. The position in the volumetric expirogram when CO₂ is measurable in expired air is called the transition zone. Since VD for CO₂ exceeds that of water vapor and alcohol, it indicates that they are coming from the conducting airway proximal to the alveoli. During exhalation, the CO₂ content of breath change by 9.13 ± 0.75% l⁻¹ of expired air, which is much greater than for water vapor and alcohol. Since CO₂ is an inert gas and has a very low blood/air partition coefficient of 3.0, the slope is mainly caused by ventilation to perfusion (V/Q) mismatch. End-expired concentration of CO₂ is normally 0.3 kPa lower than the partial pressure of CO₂ in the arterial blood and is caused by the normal ventilation/perfusion mismatch that exists in the lung (figures 3 and 4).

5.1.2. The volumetric expirogram for water vapor

Water vapor is present in ambient air depending on its humidity and temperature of the environment. The URT airway mucosa is rich in water. Inspired air takes up some water as it passes over the watery surface of the URT mucosa until it reaches 100% humidification at body temperature on reaching the alveolar region of the lungs.

Thermal changes in the airways may occur all the way down to the terminal bronchiole during extreme conditions of inhaled air. Regardless of the temperature and humidity of inhaled air the breath is warmed to body temperature (37 °C) and fully saturated with water vapor by the time the breath reaches the respiratory zones at the alveolar level [33, 34]. At normal body temperature of 37 °C the water content is 43.95 mg l⁻¹. During the following expiration a thermal exchange occurs between the warm exhaled intra-luminal air and the relatively cooler airway mucosa. The breath cools down to about 34–34.5 °C as it reaches the mouth and both water and alcohol to some extent condense onto the mucosa lining of the conducting airway [35]. However, the air is oversaturated in relation to its temperature (34 °C–34.5 °C), when it leaves the mouth because of the convective airflow in the URT (figure 5). Since water vapor always exists in the upper airways it starts to be exhaled almost immediately from the airways shown by its volumetric expirogram (figure 6).

Since there is water vapor in ambient air, depending on temperature and humidity, the concentration of water vapor in the volumetric expirogram starts from this concentration. The VD for water vapor has been determined to be 26 ± 10 ml [31], which indicates that it starts to enrich the expired air already in the most proximal part of the airways, where the transition zone is situated. It can also be seen that water vapor soon reaches a plateau concentration approximately 10% lower than its alveolar concentration of 43.95 mg l⁻¹ at 100% saturation and at 37 °C. The slope of expired water is minimal and has
Figure 3. Illustration of changes in the carbon dioxide (CO$_2$) partial pressures during mouth breathing. Ambient air is almost free of CO$_2$ before it equilibrates completely with the partial pressure of CO$_2$ in the pulmonary-capillary blood. Depending on ventilation/perfusion (V/Q) mismatch the expired end-tidal CO$_2$ is approximately 0.5 kPa lower than the partial pressure of CO$_2$ in arterial blood. The graph shows the dead space volume (VD) and transition zone for CO$_2$ (dashed red line) from analysis of volumetric expirogram (adapted and modified from Lindberg and Grubb [31]).

Figure 4. Shown is an authentic free volumetric single breath expirogram of CO$_2$. The dead space volume of CO$_2$ corresponds to the part of the tidal volume that comes from the mouth, trachea and bronchi. After approximately 158 ml of the tidal volume has been expired, the breath contains CO$_2$ representative of that in the alveolar air. The graph shows the maximal expired CO$_2$ concentration in end-tidal air and the partial pressure of CO$_2$ in arterial blood (adapted and modified from Lindberg and Grubb [31]).

been determined to be 1.07 ± 0.45% l$^{-1}$ expired air. The water vapor concentration in expired air never reaches the alveolar level, even when alveolar gas is expired. The mucosa in the conducting airway reabsorbs approximately 10% of the alveolar water vapor content during exhalation (figures 5 and 6).

5.1.3. The volumetric expirogram for alcohol
Ambient air is free from alcohol, although during inspiration it picks up some alcohol from the conducting airways before it reaches the alveolar space and equilibrates with the pulmonary blood. Depending on the concentration of alcohol in the water phase of blood (the active alcohol concentration), alcohol in arterial blood diffuses across the alveolar capillary membrane and equilibrates in less than 0.01 s with the alcohol in alveolar air. Since it is the alcohol concentration in the water fraction of blood that governs the driving force for diffusion, the blood/air partition ratio of alcohol ($K_{(\text{blood/air})}$) depends to some extent on hematocrit [19]. According to in-vitro studies, the mean (±SE) blood/air
Figure 5. Illustration of water vapor saturation in breath during mouth breathing. The water vapor content of inspired air is about 8.6 mg l$^{-1}$ assuming 50% saturated at ambient temperature (20 °C). The inspired air becomes fully saturated with water vapor in the alveoli at body temperature (37 °C). This corresponds to 43.95 mg l$^{-1}$ water. During expiration some of the water condenses on the airway mucosa as breath cools from 37 °C to 34 °C as it leaves the mouth. The graph shows the dead space volume (VD) and the transition zone (dashed red line) for water vapor (H$_2$O) from analysis of the volumetric expirogram (adapted and modified from Lindberg and Grubb [31]).

Figure 6. Shown is an authentic free volumetric single breath expirogram of water vapor. The dead space volume of water vapor corresponds to the part of the tidal volume that does not participate in water vapor exchange in the airway [32]. The graph shows the maximal expired and alveolar water vapor concentration at 37 °C. The dead space volume for CO$_2$ indicates when alveolar air reaches the mouth (adapted and modified from Lindberg and Grubb [31]).

The alcohol and water contents of expired air depend on the completeness of equilibration with the surfaces of the conducting airways, but unlike CO$_2$ the concentrations are not much influenced by ventilation/perfusion inequality. Neither water vapor, alcohol, nor CO$_2$ reach the same concentrations as in alveolar air at the end of a prolonged exhalation or after rebreathing. This depends on the difference between body temperature and exhaled breath temperature and the high solubility of alcohol and water in the mucosa of the airways. The mucosa in the conducting airway reabsorbs approximately ~20% of the alcohol from alveolar air during exhalation and this amount seems to be fairly stable [27].

ratio for men was 1830 ± 7.8 compared with 1783 ± 8.1 for women at 37 °C (mean 1806 for both sexes) [36].

During expiration some of the alcohol in exhaled air gets reabsorbed onto the mucosa covering the conducting airways. The exhaled BrAC is always less than the concentration in the alveolar air (figure 7). In authentic volumetric expirogram, the anatomical VD of alcohol was calculated to be 62 ± 3 ml as illustrated in figure 8 [31]. The first part of an exhaled breath is an admixture of mouth air and ambient air and contains a low alcohol concentration. BrAC increases continuously during an exhalation at a rate of ~5.8 ± 1.4% l$^{-1}$ expired air [31].
Figure 7. Illustration of alcohol (ethanol) conditioning during mouth breathing. Ambient air is free from alcohol. The inspired air extracts some alcohol from the airways, before it becomes equilibrated with the partial pressure of alcohol in pulmonary blood reaching the alveolar air sacs at body temperature (37 °C). The partition coefficient of ethanol between blood and alveolar air is expected to be 1783–1830 at 37 °C and equilibration is almost instantaneous (<0.01 s) (0.76/0.00042 = 1809). During expiration, the concentration of alcohol in alveolar air decreases, owing to an interaction with the airway mucosa at a lower temperature. However, the airway mucosa regains some alcohol by diffusion from the bronchial circulation. Nevertheless, the concentration of alcohol in end-exhaled breath is approximately 20% lower than it was in the alveolar air. The expired alcohol concentration is also lower than would be expected based on differences in breath temperature (94% at 34.5 °C). The partition coefficient of ethanol between pulmonary arterial blood and expired breath is 2250 (0.76/0.0003377 = 2250). Also shown is the dead space volume (VD) and the transition zone (dashed red line) for alcohol (ethanol) determined from the volumetric expirogram (adapted and modified from Lindberg and Grubb [31]).

Figure 8. Shown is an authentic free volumetric single breath expirogram of alcohol. The dead space volume of alcohol corresponds to the part of the tidal volume that does not participate in alcohol exchange. The graph shows the maximal expired and calculated alveolar alcohol concentrations. The death space volume for CO₂ indicates when alveolar air reaches the mouth (adapted and modified from Lindberg and Grubb [31]).

5.2. Time-based expirogram
In time-based expirogram, which is a scatterplots of expired gas concentration on the y-axis against time on the x-axis the volume of exhalation is unknown and therefore the position of gas origin. If a strong flow-restriction is applied to the expiration it can take an undefined time until the dead-space volume is expired. This also implies that in time based expirograms the measured concentrations can vary depending on the air volume that has been allowed to be expired.

6. The bronchial arterial circulation
The position of the transition zone and the VD for alcohol is influenced by the bronchial arterial circulation. The alcohol in the mucosa of the
conducting airway comes partly from the bronchial circulation and partly from expired alveolar air rich in alcohol. When ambient air, free from alcohol is inspired, it absorbs alcohol that is held in the mucosa during its passage down to the alveoli. This inspired air can never reach alveolar levels of alcohol, due to the lower temperature in the URT and diffusion restriction across the wall in the conducting airway. During the following expiration the alcohol contained in alveolar air is reabsorbed by the mucosa that just has been alcohol depleted by the previous inspired air. At steady-state condition, between delivery of alcohol coming from the bronchial circulation, desorption, and absorption, approximately 20% of the alcohol in alveolar air is reabsorbed.

In the beginning before consumption of alcohol, the mucosa of the upper airways and oral cavity are alcohol-free. The alcohol concentration in the mucosa of the conducting airways increases slowly as the alcohol diffuses all the way from the bronchial circulation to the mucosa. Meanwhile, before the mucosa has been saturated with alcohol and steady-state condition has occurred, a higher amount of exhaled alcohol coming from the alveolar space is absorbed.

This can be shown by a shift in the transition zone of alcohol during the first 5 min after starting to drink alcohol [31]. This explains why the origin of the expired alcohol changes from near alveoli shortly after start of intake and moves up in the conducting airways as alcohol by the contribution of the bronchial circulation equilibrates within the airways. The position when alcohol starts to occur in the airway depends on the reabsorption capacity of the mucosa. This change in the reabsorption rate during the first 15–20 min of the uptake phase is also shown by a more pronounced deviation in the exhaled alcohol curve of the volumetric expirogram and a higher slope rate until an equilibration occurs between absorption, desorption and supply of alcohol by the bronchial circulation [31]. Water vapor, in contrast, is unlimitedly accessible to the expired air and has consequently a transition zone positioned high up in the airway and a very low VD. A-BAC is transiently higher compared with BrAC × 2251 during the first 15 min after alcohol is consumed [27], since more alcohol is reabsorbed during this time. The transition zone for alcohol stabilizes after approximately 15 min and this indicates that an equilibration between the air and the mucosa of the conducting airway has occurred. Most of this period of time after intake is, however, concealed by the presence of mouth alcohol, which is of minor practical importance 15 min after end of drinking.

7. Flow-restriction

If the breath-alcohol analyzer is fitted with a mouthpiece that restricts flow of breath (back-pressure), the exhalation slope of the alcohol concentration becomes steeper and was found to be 9.2 ± 2.8% l⁻¹ expired air [37] compared with an unrestricted expiration when it was found to be 5.8 ± 1.4% l⁻¹ expired air [31]. The slope of BrAC and the difference between BrAC and A-BAC are related to the level of flow-restriction and the increase in back-pressure it causes (see figure 9). The BrAC is lower when a flow-resistive mouthpiece is used. The slope increased continuously and significantly more (0.017 (SD = 0.006) mg l⁻¹) compared with an exhalation with a non-restrictive mouthpiece (0.008 (SD = 0.004) mg l⁻¹) (p < 0.0001). Already after 0.5 s the mean BrAC had almost reach peak level during a free exhalation compared with an exhalation against flow-restriction, where the BrAC still increases after 4 s. Figure 9 shows peak BrAC at end-expiration was higher without flow-restriction, despite a non-significant higher mean expiratory volume.

Most evidential breath-alcohol tests require making a prolonged forced exhalation into the instrument via a disposable mouthpiece against a specified resistance (back-pressure) for a time necessary to discard about 1.5–2 l exhaled air. This is to ensure obtaining a sample of deep lung or alveolar air for analysis. Expiration of this volume still underestimate the alveolar concentration of alcohol as explained above and figures 7 and 8. In practice the manufacturers of alcohol breath analyzers have introduced a technique to use a flow-restricted back-pressure during the sample of the exhaled air and thereby try to entail a prolonged exhalation in order to get a sample of the last portion of the exhalation. However, this may augment the breathing pattern dependency, both by pressurizing the air in the upper airways and increasing the passage and interaction times between alcohol in breath and the mucosa of the airways (see figure 9) [38]. In addition, the flow restriction may impede the expiration and reduce the expired volume significantly, especially in subjects with significant obstructive or restrictive lung disease. The use of disposable mouthpieces is also associated with high costs for expenditure items.

8. Temperature of expired air

Some jurisdictions use temperature corrected alcohol concentrations to compensate for the decrease in temperature from 37 °C to approximately 34.5 °C within the URT airways during the expiration. This may partly compensate for the reabsorption of water vapor, which is instantaneously and unlimited accessible in the airways and therefore highly temperature dependent. However, some of the reabsorption of water vapor occurs deep in the airways, where the gases move by diffusion. In the URT airways the airflow becomes convective, which inhibits a temperature dependent reabsorption of the water molecules to the airway wall, even if the saturation level has been
Figure 9. A flow-restrictive mouthpiece attenuates the breath alcohol concentration profile as shown in the single breath volumetric expirograms. Subjects blew into warmed mouthpieces, one without restriction and two with flow restriction (0.3 l s\(^{-1}\) respective 0.35 l s\(^{-1}\)) and the shortest 2 s (1.5 l s\(^{-1}\)) without flow restriction (adapted and modified from a poster abstract presented at a conference in Seattle 2007 and attached as a supplemental file).

exceeded (see figure 5). Since the bronchial circulation is limited to only 1% of the cardiac output and there is a restriction to diffusion, due to the distance, there will be a deficit of alcohol in the mucosa of the conducting airways. This causes a higher grade of resorption of alcohol compared with water vapor at similar temperatures (see figure 7). Temperature correction does not fully compensate for the alcohol that is reabsorbed by the airways mucosa.

9. Standardization of breath alcohol to another respiratory gas

The principle by standardizing BrAC to another exhaled reference gas, dates back to the 1930s as described with results produced using the Drunkometer instrument. By using a gas which has a known concentration in the alveolar space (reference gas), we can determine its concentration in the expired air together with expired alcohol. By assuming that both dilute and vary in the same manner during its passage through the airways, we can determine the dilution level of the reference gas and transfer this dilution to BrAC. We can, thus, indirectly determine the concentration of alcohol that exists in the alveolar compartment. Standardization enables subjects to blow into the analyzer without the need of a flow-resistive mouthpiece and facilitates concurrently the ability to completely exhale, not only the tidal volume, but most of the vital capacity of the lung. This is especially advantageous for people with respiratory diseases. Since the dilution is detected by the analyzer close to the mouth, the disposable mouthpiece is not needed with less expenditure item costs [39]. Different expired reference gases, such as CO\(_2\), water vapor and O\(_2\) have been tested and used.

9.1. Standardization to CO\(_2\)

The CO\(_2\) concentration in exhaled breath varies widely between and within subjects depending on pattern and depth of breathing, amount of lung ventilation etc [40]. The normal laboratory limits are specified to be in the range 4–6 kPa. Since CO\(_2\) is much less soluble in water that alcohol, it is less influenced by airway temperature changes and is not taken up by the water-rich mucosa in the walls of the conducting airway to the same extent as alcohol.

Figure 10 shows how CO\(_2\) and water vapor vary in expired air in seven subjects after they made a free expiration.

The elimination of an inert gas from the alveoli depends only on solubility and V/Q ratio of the lung units from which it originated as predicted by the Farhi equation [30].

The slope of exhaled CO\(_2\) is steeper compared with water and alcohol [31]. However the increase in the concentration of CO\(_2\) depends on V/Q mismatch and not in decrease in temperature and resorption of the substance to the mucosa, which is the reason for the increase in the slope seen for water vapor and alcohol. The use of CO\(_2\) as a respiratory gas to standardize the alcohol exchange is not recommended because it neither compensates for changes in temperature nor the interaction within the water rich lining of the airways. The partition coefficient of three between blood and air for CO\(_2\) is very different from that of alcohol of 1806 [41].

By using the dilution of CO\(_2\) from a predefine level the end-expired alcohol concentration may be calculated as BrAC × (≈5 kPa/measured CO\(_2\)), however, this technique was extensively investigated very early by several researchers, but the CO\(_2\) levels in expired air showed unacceptable inter-individual
Figure 10. The variations of water vapor and CO$_2$ at different expired volumes. Shown as mean ± 1.96 × SD (adapted and modified from a poster abstract presented at a conference in Oslo 2010 and attached as a supplemental file).

Differences for use in law enforcement. It only uses the fact that all lungs have a variable degree of V/Q mismatch and a gradient between the arterial partial pressure of CO$_2$ and end-tidal CO$_2$.

Dubowski [40] discussed the problem with normalizing the expired alcohol to the CO$_2$ content of breath already in 1967. Among other things he pointed to the large inter-subject variations depending on pattern of breathing, especially hyperpnoea or apnea. This opinion was endorsed by the US National Safety Council, who recommended that the practice should be discontinued [9, 42, 43]. Nevertheless, this concept still reappears as a way to determine alcohol in breath without the use of mouthpieces, such as in connection with a user-friendly vehicle integrated system [44].

The between subjects coefficient of variation (CV% (SD/mean)) for water vapor was 2.0%–2.2% and for CO$_2$ 20.6%–21.9% at 1.0–2.5 l. Linear regression models for water vapor and CO$_2$ show a significant within subject variability for CO$_2$ due to a positive slope of the expired single breath curve (figure 10) ($p = 0.0001$).

9.2. Standardization to oxygen (O$_2$)

The concentration of oxygen in inspired air remains practically constant at 20%–21% at sea level and can be compared with the expired concentration of oxygen which is ~15%, as a result of uptake and consumption in the body. The partition coefficient between blood and air for oxygen ($K_{blood/air}$) is 0.1 and gas exchange occurs almost exclusively in the alveoli where there are no interaction with the mucosa of the upper airways. The efficient of the uptake of oxygen into the blood is dependent on the level of oxygen consumption, perfusion and ventilation mismatch, and is independent of temperature.

A breath alcohol tester measures the oxygen concentration in breath to determine the amount of dilution during free exhalation without a mouthpiece. Alcohol in the breath was measured with an metal oxide semiconductor and oxygen with ‘a limiting current type zirconia solid electrolyte oxygen sensor’. The precision and accuracy was lower than with current fuel cell detector and further investigation considered necessary [45]. Use of this technology compensates for dilution with ambient air and thereby furnishes a non-invasive method of sampling of breath for analysis of alcohol without the need for attaching a mouthpiece.

9.3. Standardization to water vapor

The advantage of using water vapor as a reference gas is that breath is saturated to 100% in the deep lung alveolar air and at a constant concentration of 43.95 mg l$^{-1}$ at 37 °C (normal body temperature). The water-vapor in expired air is maintained at a stable concentration with low variations (figure 10) [31]. Alcohol has similar physicochemical characteristics to water vapor, such as a high partition coefficient and temperature dependency [46, 47]. The disadvantage is that the water vapor concentration in ambient air has to be measured in order to determine the dilution fraction for correcting the alcohol content accordingly. It is not possible to standardize alcohol by merely dividing the alveolar air concentration of water vapor with end-expired water vapor. There is, in addition, a difference in the exchange of water vapor and alcohol in the airways, since water vapor is almost instantaneously accessible in the airway tissue contrary to alcohol, which is restricted and dependent on the bronchial circulation.

The saturated gas concentrations at equilibrium for alcohol and water vapor are both highly
dependent on the temperature in the airways. The temperature is indirectly affected by the breathing pattern [46]. The alveolar air is at body temperature [34, 48], but during the process of exhalation the temperature decreases when it meets the inhaled ambient air, which usually is cooler [47]. This cooling of the breath is important to consider for both water vapor and alcohol concentrations, because the solubility coefficient is 5.34%–5.45% per 1°C change in the temperature between 34°C and 37°C for water vapor and 6.20%–6.40% per 1°C between 34°C and 37°C for alcohol [15, 19]. However, since alveolar alcohol and water vapor are influenced by temperature changes in the same way, it is possible to standardize the alcohol content to the concentration of water vapor at 37°C, which corresponds to the alveolar water vapor content of 43.95 mg l⁻¹, thereby, decreasing the temperature dependency to 0.9%/1°C. This makes it unnecessary to measure body temperature and correct the measurement for differences in temperature [17].

The alcohol concentration in the alveolar space is mixed with a water vapor concentration of 43.95 mg l⁻¹ at a body temperature of 37°C. Alcohol and water vapor are thus influenced in the same way by temperature, air-flow and pressure in the airway during an exhalation. However, the availability of alcohol is limited compared with water in the conducting airways, since the bronchial circulation only comprises 1% of the whole cardiac output and has diffusion limitation due to a longer diffusion distance compared with the unrestricted diffusion across the pulmonary capillary-alveolar membrane.

This contrasts with water vapor, which is freely available from the airway surfaces all along the airway and URT [31]. This lack of alcohol in the mucosa of the conducting airways compared with water, leads to a higher resorption fraction of alcohol compared with water vapor at similar temperatures. If the gas exchange between the mucosa in the conducting airways is taken into consideration together with the temperature dependence of the mucosa/air partition coefficient the Farhi equation for inert gases is not valid, but has to be modified [12]. With use of this modified equation the arterial alcohol BBR can be calculated as 2250 [12] and is almost exactly in agreement with experimental findings of 2251 [17]. As alcohol equilibrates with a blood/air ratio at 37°C of approximately 1806 in the alveoli and the alcohol BBR is found to be 2251, it is assumed that approximately 20% of the alcohol is lost to the mucosa during the expiration process. Accordingly, by standardizing the BrAC to the water vapor concentration of 43.95 mg l⁻¹ and use a BBR of 2250 the alveolar concentration of alcohol can be determined and it precisely agrees with the arterial alcohol concentration [27].

Inspired air contains a water vapor concentration of approximately 5–15 mg l⁻¹, depending on ambient temperature and humidity. Water vapor has a very high partition coefficient and interacts freely with the water-rich lining of the mucosa in the airways. Since the alcohol concentration in ambient air is zero and the water concentration in ambient air is 5–15 mg l⁻¹ a straight division between the end-tidal BrAC and end-tidal water vapor concentration is not possible [17]. The dilution fraction of water is the difference between end-expired minus ambient divided with end-expired water vapor concentration.

By simultaneously measuring the water vapor and alcohol in ambient and expired breath air their momentary concentrations can be shown during the expiration course. It can be seen that the expired concentration deviates during the first part of the expiration, which is caused by the difference in VD for alcohol and water vapor.

As deep-lung air is expired the concentration merges at an end-expired point, representing the alveolar plateau. From this point a linear relationship can be constructed to ambient concentrations and the dilution factor can be determined for both water vapor and alcohol at any volumes [39]. In the breath-analyzer this is done by a wash-out, which dilutes alcohol and water vapor similarly [39]. The wash-out restores the measured concentrations to ambient conditions, and improves the determination of the linear regression line. As long as water vapor concentration in expired air has not reached 43.95 mg l⁻¹, it can be expected that the expired air has been diluted or lost some of its water vapor and temperature during the expiration process. The BrAC corresponding to the alveolar air concentration is then determined by extrapolating the expired BrAC to a water vapor concentration existing in the alveoli of 43.95 mg l⁻¹ at 37°C (figure 11).

The limited amount of alcohol in the airways results in a higher degree of reabsorption of alcohol compared with water vapor. The concomitant BrAC at 43.95 mg l⁻¹ water vapor does not fully standardize for all the absorption of alcohol in the airways. BrAC, therefore, does not represent a true alveolar alcohol concentration, but underestimates the alveolar alcohol concentration by approximately 20% [31]. However, the finding, that BrAC predicts almost exactly the arterial blood alcohol concentration, indicates that BrAC still reflects a stable fraction of the alveolar alcohol concentration [27]. A direct accurate measurement of alveolar alcohol concentration is, therefore, not essential for a valid measurement to reflect the alcohol concentration in arterial blood.

The results of standardized breath-alcohol analysis are highly correlated with the arterial blood-alcohol content determined by gas chromatographic methods. The analysis of alcohol in breath was equally precise as the determination of A-BAC [27]. This was verified when tests were done using free exhalations.
without use of a mouthpiece and when a mouthpiece was attached to the breath analyzer [39].

Even when the test subject provided contact free exhalations at varying distances between the mouth and the breath analyzer, it was possible to predict almost precisely the A-BAC [39]. The method of standardizing BrAC to the alveolar air water vapor concentration at 37 °C is the only technique thoroughly tested against the arterial blood alcohol concentration (A-BAC) in human drinking experiments. BrAC is almost always compared with V-BAC, although the latter reflects the alcohol concentration in the extremities being drained, such as the skeletal muscles (antecubital vein) and not the alcohol concentration reaching the central nervous system. In addition, breath analyzers are usually calibrated under conditions that do not simulate actual expired human breath [27, 49]. Variability in the venous blood to breath alcohol concentration ratio is much greater than the arterial blood to breath alcohol concentration ratio. The use of water vapor as a reference gas also improves the possibilities to directly detect mouth alcohol during breath alcohol analysis [50].

10. Concluding remarks

The principle of standardizing exhaled BrAC to alveolar-air water vapor concentration has been described previously [17, 19]. The technology compensates for dilution with ambient air and enables, thereby, a non-invasive method of contact-free breath sampling and without the need of a mouthpiece. Because only a passive exhalation is necessary, this makes it a lot easier for people to comply with the breath sampling procedure, because there is no resistance to exhalation and blow time is minimal.

Our method of standardization to water vapor compensates for variations in the temperature of breath, but difference in physiological and physico-chemical properties of water and ethanol, means that it does not fully compensate for the alcohol exchanges taking place within the airways. After standardization of BrAC to water vapor at 37 °C, the precision of analysis was not different from determination of A-BAC by headspace gas chromatography [27]. There was excellent agreement between A-BAC and BrAC but not so for V-BAC and BrAC. Since the technology requires simultaneous measurements of water vapor, carbon dioxide and alcohol, it guarantees that alveolar air is being sampled for analysis and not breath contaminated with mouth alcohol [50].

The A-BAC should be the most relevant toxicologically test of alcohol impairment and drunkenness, because it reflects brain exposure to alcohol. However, the sampling of arterial blood is invasive and not very practical for routine forensic purposes, such as evidence for prosecution of drunken drivers. The method of breath analysis described in this article serves as a proxy for determination of the A-BAC. The concentration of alcohol in venous blood (V-BAC), on the other hand, represents the concentration of alcohol contained in the tissue water compartment. The analysis of breath is a non-invasive procedure and close agreement between BrAC standardized to the alveolar water vapor and A-BAC makes this an ideal way to monitor brain exposure to alcohol.
Data availability statement
No new data were created or analyzed in this study.

Statement of authorship
The enclosed manuscript has been written and approved by both authors.

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