Fitting of single-exhalation profiles using a pulmonary gas exchange model—application to carbon monoxide

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Keywords: real-time breath gas analysis, carbon monoxide (CO), pulmonary gas exchange model, single-exhalation profile, laser absorption spectroscopy

Abstract
Real-time breath gas analysis coupled to gas exchange modeling is emerging as promising strategy to enhance the information gained from breath tests. It is shown for exhaled breath carbon monoxide (eCO), a potential biomarker for oxidative stress and respiratory diseases, that a weighted, nonlinear least-squares fit of simulated to measured expirograms can be used to extract physiological parameters, such as airway and alveolar concentrations and diffusing capacities. Experimental CO exhalation profiles are acquired with high time-resolution and precision using mid-infrared tunable diode laser absorption spectroscopy and online breath sampling. A trumpet model with axial diffusion is employed to generate eCO profiles based on measured exhalation flow rates and volumes. The concept is demonstrated on two healthy non-smokers exhaling at a flow rate of 250 ml s⁻¹ during normal breathing and at 120 ml s⁻¹ after 10 s of breath-holding. The obtained gas exchange parameters of the two subjects are in a similar range, but clearly distinguishable. Over a series of twenty consecutive expirograms, the intra-individual variation in the alveolar parameters is less than 6%. After a 2 h exposure to 10 ± 2 ppm CO, end-tidal and alveolar CO concentrations are significantly increased (by factors of 2.7 and 4.9 for the two subjects) and the airway CO concentration is slightly higher, while the alveolar diffusing capacity is unchanged compared to before exposure. Using model simulations, it is found that a three-fold increase in maximum airway CO flux and a reduction in alveolar diffusing capacity by 60% lead to clearly distinguishable changes in the exhalation profile shape. This suggests that extended breath CO analysis has clinical relevance in assessing airway inflammation and chronic obstructive pulmonary disease. Moreover, the novel methodology contributes to the standardization of real-time breath gas analysis.

1. Introduction
During the past decade, the advent of novel analytical techniques has intensified the interest in real-time detection of trace species in exhaled breath as alternative to offline analysis [1, 2]. In the context of this work, real-time breath gas analysis refers to controlled online breath sampling and subsequent quantitative biomarker detection with sufficient measurement time-resolution (usually sub-second) and precision to accurately resolve individual breath cycles. Compared to offline mixed- or end-tidal breath sampling, the advantages of the online approach include fast response, the possibility for continuous (inline) breath monitoring over longer time periods, and reduced risk for sample contamination due to pre-concentration and storage procedures. An additional benefit of breath-cycle-resolved detection is that single-exhalation profiles contain spatiotemporal information about the gas exchange in the respiratory tract. Coupled to suitable mathematical models of gas exchange [3], this enables biomarker source discrimination and non-invasive determination of physiological parameters, which can lead to improved data interpretation, a better understanding of the origin and biochemical pathways of biomarkers and, eventually, to novel breath tests.

The shape of an exhalation profile primarily depends on the locations of biomarker production and exchange in the respiratory tract (alveoli, airways, oral/nasal cavities), and the breath sampling
conditions (e.g. exhalation flow rate and volume, inhaled concentration, body position) [3]. In general, compounds with low water/blood solubility will exchange in the alveolar region, whereas highly water-soluble molecules will exchange in the airways. For example, both carbon monoxide (CO) and nitric oxide (NO) have a low water solubility, but exhaled CO originates mainly from the alveoli, leading to high end-tidal values [4], while exhaled NO to a large extent stems from ambient air and nasal/airway production, resulting in a characteristic maximum in the beginning of the exhalation [3, 5]. High initial concentrations can also be expected for ammonia (NH3), which mostly originates from the oral cavity, but the exceptionally high water solubility and the propensity to adsorption hamper quantitative detection and have so far prevented the reliable measurement of NH3 exhalation profiles [6].

Real-time breath analysis is routinely performed in clinical practice only for carbon dioxide (CO2) using miniature capnographs based on non-dispersive infrared (NDIR) spectroscopy. Compact optical set-ups can also be used for rapid measurement of the other major breath species, oxygen (O2) and water vapor (H2O) [7]. However, more sophisticated analytical techniques are needed for real-time analysis of the less abundant molecules. These methods include soft-ionization mass spectrometry (MS), such as selected-ion flow tube and proton transfer reaction MS [1], electrospray ionization MS [8], non-equilibrium dilution ion mobility spectrometry [3] and laser absorption spectroscopy (LAS) [2, 9].

Mathematical models of pulmonary gas exchange in the respiratory tract were developed early on to enable determination of the fractional airway NO contribution [10, 11] and to improve the interpretation of CO2 [12] and ethanol [5] exhalation profiles. Physiological modeling was also used to better understand short-term changes in exhaled isoprene [13] and acetone [14] concentrations. In most of these model implementations, end-tidal biomarker levels were computed and compared to experiments. Only a few attempts have been made to compare fit simulations of entire expirograms to experimental real-time data [15–19]. In a recent work by Mountain et al least-squares fitting of O2, CO2 (and N2 tracer) profiles was employed to assess lung inhomogeneity [20].

The interest in exhaled breath CO (eCO) as biomarker for oxidative stress and respiratory diseases stems from the fact that the molecule is endogenously released as a byproduct of heme oxygenase (HO-1) activity [21]. While the main part of CO production is due to systemic heme degradation, with gas exchange in the alveoli, there are indications that HO-1 activity can also be induced locally in airway and lung tissue [22, 23]. Some studies reported elevated eCO levels due to local airway inflammation [24], infections [25] and chronic obstructive pulmonary disease (COPD) [26], but contradictory results were also obtained [27]. In general, exhaled CO also depends on recent environmental exposure, such air pollution and smoking [28], and on the molecular diffusion properties in the respiratory tract, which may deviate from normal in diseased cohorts. For example, in patients with severe COPD, the CO diffusing capacity is significantly reduced [29]. Conventional end-tidal eCO analysis with electrochemical sensors cannot resolve a potential small airway contribution or assess pulmonary diffusion, which hampers the interpretation of eCO concentrations outside the healthy population range.

To add value to eCO diagnostics, a compact LAS sensor for sensitive real-time detection of CO in exhaled breath and ambient air has recently been developed [4, 30]. Moreover, a trumpet model with axial diffusion (TMAD) has been adapted to, for the first time, simulate pulmonary gas exchange dynamics and single-exhalation profiles of CO during systemic elimination [31]. The exhalation profiles are calculated based on four parameters, namely the CO diffusing capacities and maximum fluxes in the airways and the alveolar region. In that study, simulated and measured exhalation profiles have been visually compared to roughly estimate the model parameters and predict the equilibrium CO concentrations in the two compartments.

In this work, a weighted, nonlinear least-squares fit of the model solution at the mouth grid point to the experimental CO exhalation profiles is used to extract the TMAD parameters. The purpose of applying a fit instead of a manual comparison, is to enable fast, reliable and precise extraction of the model parameters, including end-tidal CO, in a consistent way. Such strategy is little described in the context of breath gas analysis, but can greatly contribute to the standardization of (real-time) breath sampling and data evaluation. Precise and systematic profile analysis may also lead to improved gas exchange models and a better understanding of the biomarker physiology. Normal breathing and BH expirograms from two healthy non-smokers are analyzed to demonstrate the novel approach. The inter- and intra-individual variations in the model parameters, and the influence of acute exposure to elevated CO levels on the parameters are scrutinized. Furthermore, using simulations, it is shown that a small increase in airway CO flux and changes in the alveolar diffusing capacity, as may be anticipated in the diseased population, have distinct effects on the shape of exhalation profiles and can be resolved. The clinical relevance of extended CO breath analysis is discussed.

2. Materials and methods

2.1. Laser-based carbon monoxide sensor

Real-time detection of CO in breath and ambient air was achieved using a home-built mid-infrared tunable diode laser absorption spectroscopy (TDLAS) system
The spectrometer employed a distributed-feedback interband cascade laser (ICL, Nanoplus GmbH) in combination with a circular, low-volume multipass cell (MPC, IR Sweep, IRCell-4M) for absorption path length enhancement (4 m) and 2/f-wavelength modulation spectroscopy for noise-reduction. Breath samples were analyzed in the MPC at a pressure of 100 Torr and at close to room temperature (23 °C). The absorption spectra were scanned with a frequency of 140 Hz and averaged 10 times. This sensor provided selective, interference-free eCO quantification down to 9 ppb at a precision of 5 ppb and time-resolution of 0.1 s. The rapid gas exchange in the MPC (<0.1 s) required for real-time detection was guaranteed by the low MPC volume (38 ml) and the pumping speed (360 ml s⁻¹ at 100 Torr) of the vacuum pump (Leybold, Divac 1.4HV3C). True real-time capability of the system was previously confirmed by direct comparison of eCO₂ profiles measured by capnography and TDLAS [4]. The main components of the experimental setup are shown schematically in figure 1.

### 2.2. Online breath sampling system

Pulmonary gas exchange and exhaled biomarker concentrations strongly depend on the breath sampling conditions, including hyperventilation, body position and inhaled biomarker concentrations [32]. To minimize these effects and ensure repeatability, the sampling process was standardized using an advanced breath sampling system that controlled the breathing frequency and the inhalation and exhalation flow rates (IFR and EFR, respectively).

The online breath sampler (figure 1) comprised a flow meter (Phillips Respironics, FloTrak Elite Module), a mainstream capnograph (Phillips Respironics, Capnostat 5) and a Teflon buffer tube of length 15 cm and volume 30 ml. A mouthpiece made of Teflon and a disposable anti-bacterial filter (GVS, Eco Maxi Electrostatic Filter, 4222/701) were mounted at the inlet. The flow meter was a fixed orifice differential pressure type, which potentially can evaluate more than 60 respiratory parameters on a breath by breath basis including the flow rate (range -5000 to +5000 ml s⁻¹, resolution 0.2 ml s⁻¹), volume (range -1000 to +4000 ml, resolution 1 ml) and airway pressure (range -150 to +150 cmH₂O, resolution 0.05 cmH₂O). The capnograph was a NDIR single beam optical device with a CO₂ measurement range of 0%–19.7% and a resolution of 0.1%.

A two-way non-rebreathing valve (Rudolph Inc.) was connected to the outlet of the buffer tube to separate the inspiration and expiration routes, as subjects performed both inhalation and exhalation through the breath sampler. In order to restrict IFR and EFR close to desired values, suitable orifices of different diameters were installed at the inlet and outlet ports of the two-way valve. A LabVIEW computer interface with audiovisual indicators helped the subjects to maintain the intended IFR/EFR and breathing frequency (6 or 3 breaths/min for normal breathing and breath-holding, respectively) according to the protocol specified in section 2.6. A portion of the inhaled and exhaled breath was continuously extracted from the buffer tube and led to the MPC of the TDLAS sensor at a flow rate of 50 ml s⁻¹ (set by the vacuum pump speed).

Figure 2 shows typical respiratory data sets recorded over a single breath-cycle during normal breathing for the two healthy, non-smoking subjects. For a breathing frequency of 6 breaths/min and an IFR/EFR of around 250 ml s⁻¹ (average indicated by a dashed line), the inhaled/exhaled volumes were close to 1250 ml. A clear inter-individual difference in airway pressure and end-tidal CO₂ concentration can be observed. In all experiments, the respiratory data and ambient air CO (Cₐmb) were continuously recorded during the breath cycles and later used as input parameters to the mathematical model of pulmonary CO gas exchange dynamics.

### 2.3. Pulmonary gas exchange model

The TMAD is based on a one-dimensional, trumpet-shaped representation of the respiratory tract following Weibel’s symmetrically bifurcating lung structure [33, 34] rescaled for a total airspace volume of 3700 ml...
and using the latest anatomical data [31]. Figure 3 shows a schematic drawing of the TMAD with the main model parameters indicated.

The governing equation, which accounts for the axial gas transport and radial sources and sinks as a function of time and axial distance \( z \) along the trumpet, can generally be written as

\[
\begin{align*}
\left\{ A_{c,aw}(z) + \left[ \frac{N_{aw}(z)}{N_{t}} \right] A_{c,A} \right\} c_{awCO} &= T(V, D_{CO,air}, z) \\
&+ S(J'_{awCO}, D'_{awCO}, J'_{ACO}, D'_{ACO}, z),
\end{align*}
\]

(1)

where \( A_{c,aw} \) is the airway cross-sectional area (cm\(^2\)), which follows a power-law relation (\( \sim 1/z^2 \)) towards the lower airway generations (towards the mouth), \( A_{c,A} \) is the total cross-sectional area of the alveolar compartment (cm\(^2\)), the function \( T \) describes the convective bulk flow and axial diffusion, and the function \( S \) represents the flux to and diffusion from the airway and alveolar regions per unit axial distance. The function \( T \) is given by

\[
T(V, D_{CO,air}) = -V \frac{dC_{CO}}{dz} + D_{CO,air} \frac{d}{dz} \left[ A_{c,aw}(z) \frac{dC_{CO}}{dz} \right],
\]

(2)

where \( V \) is the volumetric (inhalation and exhalation) flow rate (ml s\(^{-1}\)), \( D_{CO,air} \) the molecular diffusivity of CO in air (cm\(^2\) s\(^{-1}\)). The function \( S \) has the form

\[
S(J'_{awCO}, D'_{awCO}, J'_{ACO}, D'_{ACO}) = (J'_{awCO} - D'_{awCO} C_{CO}) \left[ 1 - \frac{N_{aw}(z)}{N_{max}} \right] + (J'_{ACO} - D'_{ACO} C_{CO}) \frac{N_{aw}(z)}{N_{t}}.
\]

(3)

where \( J'_{awCO} \) and \( J'_{ACO} \) are the maximum fluxes per unit axial distance (pl s\(^{-1}\) cm\(^{-1}\)) and \( D'_{awCO} \) and
The mass balance equation, equation (1), is solved numerically using the method of lines, and provides the distribution of CO in the respiratory tract during inhalation, exhalation and breath-holding with spatial and temporal resolutions of 1 mm and 0.01 s, respectively. Single-exhalation profiles are extracted from the first simulated grid point (z = 0) representing the mouth. Details on the model parameters, the boundary conditions and the numerical solution can be found in [31].

2.4. Nonlinear least-squares fitting implementation

The solution of the TMAD at the mouth grid point is fitted to the experimental eCO profiles using a weighted, nonlinear least-squares algorithm implemented in MATLAB. There are four open parameters representing the sinks and sources in the airway and the alveolar region, namely $J_{awCO}$, $D_{awCO}$, $I_{ACO}$ and $D_{ACO}$, which denote the total CO concentration, $D'_{awCO}$, $J_{ACO}$ and $D_{ACO}$ in units of pl s$^{-1}$ and pl s$^{-1}$ ppb$^{-1}$, respectively. These four parameters affect the exhala-
tion profile shape in different ways (no mutual dependencies) and are uniquely determined in the fitting process. Starting values for the open TMAD parameters have previously been estimated [31]. As mentioned above, other input data to the model were the actual IFRs and EFRs, inhaled/exhaled volumes and the inhaled CO concentration.

Due to the steep eCO increase in exhalation phase II, which represents the transition between the conducting airways and the alveolar region, this phase is particularly sensitive to discrepancies between the anatomical data assumed in the TMAD and the actual lung structure of the subject providing the sample. Therefore, a good fit cannot be expected in this region and more weight was put on the data points in phases I and III, which are the dominant regions for evaluation of the gas exchange in conducting airways and alveoli, respectively. The first 4% of the expirogram data points (corresponding to phase I) were weighted 20 time more and the last 74% (corresponding to phase III) 60 times more than the rest of the profile (phase II). Since the data acquisition rate and exhaled volume were kept constant, the relative amount of data points in each exhalation phase was the same regardless of exhalation flow rate and time. The TMAD fitting parameters, $J_{awCO}$, $D_{awCO}$, $I_{ACO}$ and $D_{ACO}$, were free to vary in the range of 100–500 pl s$^{-1}$, 1.0–1.6 pl s$^{-1}$ ppb$^{-1}$, $5 \times 10^{2}$–$2 \times 10^{8}$ pl s$^{-1}$, and $300$–$5 \times 10^{3}$ pl s$^{-1}$ ppb$^{-1}$, respectively.

For healthy, non-smoking subjects, the airway CO contribution is usually very small and the airway TMAD parameters ($J_{awCO}$ and $D_{awCO}$) cannot be accurately determined from normal breathing profiles. During a BH maneuver, however, there is more time for airway tissue CO to diffuse into the gas stream, which increases the sensitivity to the airway parameters. Thus, in this work, $J_{awCO}$ and $D_{awCO}$ were first determined from an expirogram recorded after 10 s BH, and then fixed in the fits to the normal breathing exhalation profiles. The alveolar and airway (tissue) CO concentrations predicted for equilibrium conditions, $C_{ACO}$ and $C_{awCO}$, were obtained from the ratios $J_{ACO}/D_{ACO}$ and $J_{awCO}/D_{awCO}$, respectively. The computational time for fitting a typical normal breathing eCO profile recorded at an EFR of 250 ml s$^{-1}$ and an exhalation volume of 1250 ml was around 45 s on a standard office PC. For a 10 s BH profile, the time was 4–5 min As expected, the computational time increases with decreasing IFR and EFR, and increasing BH time.

2.5. Controlled human exposure to carbon monoxide

The effect of CO exposure on the eCO profiles and TMAD parameters was investigated in a human exposure study including intermittent exercise. The subjects stayed in a controlled environment exposure chamber (18 m$^3$) [36] for 2 h, breathing a mixture of 10 ± 2 ppm CO in air derived from a 300 ppm CO gas standard (AGA Gas AB) by dilution with air. The expected increase in blood carboxyhemoglobin (COHb) concentration due to the CO exposure was calculated using the differential Coburn–Forster–Kane (CFK) equation [37] and healthy non-smoker blood and lung properties. An increase in COHb level of around 60% compared to normal (up to 1.3% saturation from an initial value of 0.8%) was predicted for the 2 h exposure assuming an alveolar ventilation rate of 301 min$^{-1}$. The COHb level can also be calculated from the measured alveolar CO concentration ($C_{ACO}$) using an empirical relationship given by [38]

$$COHb = 0.63 + 0.16 C_{ACO}$$

where $C_{ACO}$ is in ppm, and COHb is obtained in units of % saturation.

2.6. Human subjects and study protocol

Two healthy, male non-smokers (subjects 1 and 2, aged 42 and 37 years, respectively) participated in the pilot-study. No diary restrictions were imposed on the subjects, but they were asked not to exercise for 3 h prior to the test. All eCO measurements were conducted between 1 pm and 3 pm on weekdays in late October in Umeå, Sweden. Two different breathing maneuvers were considered. The first maneuver, here referred to as ‘normal breathing’, comprised 5 s inhalation of ambient air at 250 ml s$^{-1}$ IFR, followed by 5 s exhalation at 250 ml s$^{-1}$ EFR. This resulted in inhalation/exhalation volumes of about 1250 ml, depending on how well the
subjects followed the audiovisual indicators. The second maneuver consisted of 10 s inhalation at 120 ml s$^{-1}$ IFR, followed by 10 s breath-holding and 10 s of exhalation at 120 ml s$^{-1}$ EFR. These two breathing maneuvers were also performed 7 min after the end of the 2 h exposure to 10 ppm CO. During the exposure in the chamber, the subjects alternated between 15 min of moderate cycling and 15 min of rest. The sequence of breath cycles was recorded by performing twenty successive normal breathing maneuvers. All breath samples were given through the mouth, while subjects were sitting upright. The study protocol was approved by the Regional Ethical Review Board at Umeå University (2017/306-31 and 2018-35-23 M).

3. Results

3.1. Least-squares fitting of single-exhalation profiles

Figure 4 presents measured, normal breathing CO exhalation profiles (red markers, every 2nd data point shown) from subjects 1 and 2, together with weighted TMAD curve fits (blue lines) and fit residuals (lower panels). The corresponding breath sampling data for these expirograms is shown in figure 2. Time zero indicates the start of the exhalation. The prevailing ambient air CO concentration was continuously sampled during inhalation. In figure 4(a), the three exhalation phases are indicated by Roman numerals. C$_{amb}$—ambient air CO.

Figure 4. Weighted, nonlinear least-squares TMAD fits (solid lines) to measured normal breathing eCO profiles (markers) from healthy non-smoker subjects 1 and 2. The average EFRs are indicated. The lower panels show the residuals of the fits. For clarity, only every 2nd experimental data point is shown in the region of the fit. In panel (a), the three exhalation phases are indicated by Roman numerals. C$_{amb}$—ambient air CO.

Figure 5. Weighted, nonlinear least-squares TMAD fits (solid lines) to measured 10 s breath-holding eCO profiles (markers) from healthy non-smoker subjects 1 and 2. The average EFRs are indicated. The lower panels show the residuals of the fits. For clarity, only every 2nd experimental data point is shown in the region of the fit.

Figure 5. Weighted, nonlinear least-squares TMAD fits (solid lines) to measured 10 s breath-holding eCO profiles (markers) from healthy non-smoker subjects 1 and 2. The average EFRs are indicated. The lower panels show the residuals of the fits. For clarity, only every 2nd experimental data point is shown in the region of the fit.
3.2. Intra-individual variations in the TMAD parameters

The repeatability of the breath sampling procedure and robustness of the TMAD fitting routine were studied by looking at the intra-individual variations of the model parameters under normal breathing conditions.

As presented in figure 6, sequences of twenty consecutive breath-cycles were measured for both subjects (left panels) and analyzed using TMAD fits. The right panels in figure 6 show the TMAD fits (blue lines) to five of the measured eCO profiles (red markers). The TMAD parameter mean values and coefficients of variation (CV) extracted from fits to all twenty profiles are given in table 2. The mean IFR and EFR were 242 ml s\(^{-1}\) and 243 ml s\(^{-1}\) for subject 1, and 263 ml s\(^{-1}\) and 258 ml s\(^{-1}\) for subject 2, respectively. The mean ambient air CO concentrations were 115 ppb and 149 ppb, respectively. The intra-individual CV of \(J_{ACO}\) and \(D_{ACO}\) were less than 6% for subject 1 and less than 5% for subject 2. Inter-individually, the mean value of \(J_{ACO}\) for subject 1 is almost twice that of subject 2, whereas there is less than 9% difference in the mean \(D_{ACO}\).

3.3. Exposure to carbon monoxide

Exposure to exogenous CO gives rise to an increase in blood COHb, which, in turn, results in elevated eCO concentrations during elimination [28, 37]. We hypothesize that, for healthy non-smokers, exposure will mainly affect the maximum CO flux, but not the lung diffusion properties. Figure 7 shows measured single-exhalation eCO profiles (markers) for the two subjects before and 7 min after the 2 h exposure to 10 ± 2 ppm CO, together with least-squares fits (lines) to the experimental data. In each case, the airway TMAD parameters \(J_{awCO}\) and \(D_{awCO}\) have first been determined from fits to the corresponding BH curves (not shown). The extracted parameters are presented in table 3.

Compared to the control values, \(J_{ACO}\) was substantially increased after exposure (by factors of 2.7 and 4.9 for subjects 1 and 2, respectively), which suggests elevated COHb levels. Indeed, the COHb values calculated from \(C_{ACO}\) using equation (4) were increased accordingly and similar to those predicted with the CFK model (section 2.5) considering ventilation rates of 26 l min\(^{-1}\) and 36 l min\(^{-1}\) for subjects 1 and 2, respectively. For both study participants, a slightly elevated equilibrium airway (tissue) CO concentration was found after exposure. The alveolar diffusing capacities, on the other hand, did not change significantly for subject 1 (6% increase), and not at all for subject 2.

3.4. Simulation of respiratory disease conditions

The potential of the novel methodology to assess health conditions connected to respiratory diseases was investigated by simulating eCO profiles that could be expected from subjects with airway inflammation (increased \(J_{awCO}\)) and an obstructive lung disease (decreased \(D_{ACO}\)). We hypothesize that a curve fit to highly precise and time-resolved TDLAS data, as presented in this study, can be used to extract parameters that reflect changes in the exhalation profile shape associated with the diseases.

Figure 8(a) presents eCO profiles (10 s BH, 240 ml s\(^{-1}\) IFR/EFR) for a healthy subject (blue solid lines) and for a subject with three-fold increased maximum airway flux (red dashed line). Figure 8(b) shows a comparison between CO expirograms (normal breathing, 240 ml s\(^{-1}\) IFR/EFR) for a healthy subject (blue solid line) and for a subject with an alveolar CO diffusing capacity reduced to 40% of normal with (red dashed line) and without (green dashed-dotted line) simultaneously increased COHb level (controlled by means of \(J_{ACO}\)). COHb is believed to be elevated in COPD [39], but this may not always be the case. The initial TMAD parameters representing a healthy subject were taken from the average data of subject 1 given in table 2. Ambient air CO concentrations and mean inhaled/exhaled volumes were assumed to be 100 ppb and 1200 ml, respectively. For all cases, the TMAD parameters used for the simulated CO expirograms are provided in table 4.

4. Discussion

The TMAD is a rather complex 1D model of pulmonary gas exchange with a reasonably realistic anatomical structure and inclusion of axial diffusion. It is shown for CO, two healthy non-smokers and different breathing patterns that the numerical solution of such model for the gas concentration at the mouth can be fitted to experimental real-time breath data with excellent results (figures 4–7). Compared to a mere comparison as reported in [31], curve fitting enables rapid and more precise determination of the unique TMAD parameters. In combination with a highly precise analytical method, such as LAS, this provides the possibility to resolve small inter- and intra-individual variations in eCO due to health or sampling conditions. The TMAD can be adapted to suit different biomarkers by considering their physical and physiological properties and corresponding initial estimates of the model parameters.

In principle, all four TMAD parameters can be extracted from a fit to a single-exhalation profile measured during systemic CO elimination at close-to-normal ventilation and fixed EFR. For healthy subjects, however, the airway contribution is small and the sensitivity to the airway TMAD parameters \(J_{awCO}\) and \(D_{awCO}\) accordingly low for normal breathing profiles. By analyzing BH profiles, on the other hand, the airway parameters can be obtained with reasonable precision and used to determine the alveolar TMAD.
Table 1. Gas exchange parameters extracted from the TMAD fits to the experimental data in figures 4 and 5, and corresponding respiratory data. ETCO—end-tidal CO concentration, V—average of inhaled/exhaled volume, ETCO₂—end-tidal CO₂ concentration.

| Figures | $I_{\text{awCO}}$ | $D_{\text{awCO}}$ | $I_{\text{ACO}}$ | $D_{\text{ACO}}$ | ETCO | $C_{\text{ACO}}$ | $C_{\text{awCO}}$ | $V_I$ | $V_E$ | $V$ | ETCO₂ | $C_{\text{amb}}$ |
|---------|-------------------|------------------|------------------|------------------|-------|-----------------|-----------------|-------|-------|------|--------|-------------|
| 4(a)    | 192               | 1.6              | $2.21 \times 10^7$ | 10 461           | 1969  | 2113            | 120             | 242   | 246   | 1201 | 6.4    | 120        |
| 4(b)    | 291               | 1.6              | $1.23 \times 10^7$ | 10 867           | 1050  | 1136            | 182             | 255   | 257   | 1289 | 5.8    | 154        |
| 5(a)    | 192               | 1.6              | $1.35 \times 10^7$ | 6361            | 2062  | 2118            | 120             | 115   | 119   | 1121 | 7.7    | 90         |
| 5(b)    | 291               | 1.6              | $8.37 \times 10^6$ | 6434            | 1248  | 1301            | 182             | 130   | 128   | 1177 | 6.1    | 127        |

* Directly measured; all other parameters are derived from the model fits.
values for normal breathing. As a consequence of the weighted fitting strategy, a good overall fit is achieved, but a small discrepancy in exhalation phase II remains (figures 4–7). Possible reasons for this discrepancy are differences between assumed and actual morphologic data (number and distribution of alveoli, airway and

Table 2. Mean values and coefficients of variation (CV (%)) = Standard deviation/Mean × 100 of the TMAD and respiratory parameters derived from the 20 breath-cycles shown in figure 6 for subjects 1 (S1) and 2 (S2). ETCO—an-end-tidal CO concentration, V—in-average of inhaled/exhaled volume.

|       | Subject 1 S1 | Subject 2 S2 |
|-------|--------------|--------------|
|       | J_{awCO} pl s^{-1} | D_{awCO} pl s^{-1} ppb^{-1} | J_{ACO} pl s^{-1} | D_{ACO} pl s^{-1} ppb^{-1} | ETCO ppb | C_{ACO} ppb | V_I ml | V_E ml | V ml | ETCO2 % | C_{amb} ppb |
| CV    |——|——|5.1|5.8|2.1|2.2|2|2|3.4|1.5|5|
|       | 192|1.6|2.04 \times 10^{7}|9652|1943|2120|242|243|1196|6.5|115|
|       | 291|1.6|1.20 \times 10^{7}|10515|1063|1139|263|258|1301|5.8|149|

* Directly measured; all other parameters are derived from the model fits.
lungs, cross-sectional areas, lung symmetry, dead space), and that not all gas mixing mechanisms, as well as ventilation heterogeneity, are accounted for in the one-dimensional model. Moreover, in reality, lung volume and breath gas flow rate are not constant during inhalation and exhalation. A potential, slight instrumental delay during the rapid CO increase in phase II may also contribute to the deviation \[4\].

The observed eCO and TMAD parameters (tables 1–3) are in good agreement with the established end-tidal CO range for healthy non-smokers (1–3 ppm) \[21\], and the recently reported theoretical estimates and experimental TMAD parameter values. In general, due to the diffusion limited CO gas exchange, end-tidal CO is always lower than the predicted alveolar equilibrium CO concentrations, and \(J_{ACO}\) and \(D_{ACO}\) are significantly lower after BH than for normal breathing. The absolute values obtained for the alveolar CO diffusing capacity are larger than those obtained in the clinical standard \(D_{LCO}\) test, mainly due to the significantly different experimental approach (average over exhalation during systemic elimination versus inhalation of high

### Table 3. Physiological parameters of subjects 1 and 2 determined with extended eCO analysis before and 7 min after a 2 h exposure to 10 ± 2 ppm CO in air.

|                      | Subject 1 |                  |                  |                  |                  |                  |                  |
|----------------------|-----------|------------------|------------------|------------------|------------------|------------------|------------------|
|                      | Before    | After            |                  |                  |                  |                  |                  |
|                      | exposure  | exposure         |                  |                  |                  |                  |                  |
| \(I_{awCO}\) pl s\(^{-1}\) | 265       | 350              | 2.08 \times 10\(^7\) | 10 383           | 10 383           | 5.71 \times 10\(^7\) | 2115             |
| \(D_{awCO}\) pl s\(^{-1}\) ppb\(^{-1}\) | 1.6       | 1.6              |                  |                  |                  |                  |                  |
| \(J_{ACO}\) pl s\(^{-1}\) | 9837      | 5106             |                  |                  |                  |                  |                  |
| \(D_{ACO}\) pl s\(^{-1}\) ppb\(^{-1}\) | 1965      | 5495             |                  |                  |                  |                  |                  |
| ETCO \(C_{ACO}\) ppb | 1123      | 219              |                  |                  |                  |                  |                  |
| \(C_{awCO}\) ppb    | 166       | 219              |                  |                  |                  |                  |                  |
| \(COH_b\) %        | 5.8       | 5.6              |                  |                  |                  |                  |                  |
| \(pl s\(^{-1}\) ppb\(^{-1}\) ppb ppb ppb ppb % \(\times \) % | 1976      | 5133             |                  |                  |                  |                  |                  |

### Table 4. TMAD parameters (based on table 2, subject 1) used to generate the synthetic eCO profiles shown in figure 8.

|                      | \(J_{awCO}\) pl s\(^{-1}\) | \(D_{awCO}\) pl s\(^{-1}\) ppb\(^{-1}\) | \(J_{ACO}\) pl s\(^{-1}\) | \(D_{ACO}\) pl s\(^{-1}\) ppb\(^{-1}\) | ETCO \(C_{ACO}\) ppb | \(C_{awCO}\) ppb | \(COH_b\) % |
|----------------------|-----------------------------|----------------------------------------|-----------------------------|----------------------------------------|----------------------|----------------|----------------|
| Increased maximum airway CO flux (three-fold) | 600                        | 1.6                                    | 2.00 \times 10\(^7\) | 10 000                                  | 1976                 | 2000           | 375            | 0.95          |
| Case 1: Decreased alveolar diffusing capacity (40% of normal); constant alveolar \(J\)—increased \(COH_b\) | 200                        | 1.6                                    | 2.00 \times 10\(^7\) | 4000                                   | 3315                 | 5000           | 125            | 1.43          |
| Case 2: Decreased alveolar diffusing capacity and alveolar \(J\) (both 40% of normal)—constant \(COH_b\) | 200                        | 1.6                                    | 8.00 \times 10\(^6\) | 4000                                   | 1327                 | 2000           | 125            | 0.95          |

\* Obtained from the empirical formula in equation (4).
CO concentrations are close to airways is negligible, and the equilibrium airway CO, the contribution from and gas exchange with the healthy subjects, and given the low water-solubility of estimate the diffusing capacity and because morphological models tend to over-
nimate from a daily variation in physiological parameters
ations in TMAD parameters between the different
jects. In contrast, the difference in alveolar diffusing capacity is not equally distinct. In general, the slight
jects was observed
onding difference in ventilation rate between the sub-
more rapidly, when subjects breath faster. A correspond-
tation rate during exposure, i.e. COHb increases
fact that the rise in COHb depends on the alveolar ven-
tilation rates of 26 l min$^{-1}$ and 36 l min$^{-1}$ for subjects 1 and 2, respectively, the CFK model predicts COHb values comparable to those calculated from alveolar CO using equation (4). Importantly, it is shown here for the first time that also the maximum airway CO flux is increased (if only slightly) after exposure. Furthermore, the results indicate that the alveolar diffusing capacity is not affected by the exposure.

A reduction in alveolar diffusing capacity on the order of what might be expected in COPD patients seems to clearly affect the shape of a normal breathing expirgram, in particular the slope of exhalation phase III (alveolar slope), but also the absolute eCO level (figure 8(b)). Any additional change in blood COHb leads to further alterations of the profile shape. A minor increase in maximum airway flux only affects the eCO profile (exhalation phase I) if a breath-hold-
ing maneuver is conducted (figure 8(a)). Clinically relevant changes in airway and alveolar parameters can be resolved using the fitting methodology. The COHb levels calculated from alveolar CO for Case 1, again using equation (4), conform with the COHb values previously determined in COPD patients [39].

Exhaled breath CO levels outside the normal range may originate from variations in endogenous produc-
tion (systemic or locally induced HO-1 activity), recent exposure to exogenous CO sources, or can be caused by changes in pulmonary conditions. The extended breath CO analysis approach proposed here constitutes a first step towards being able to locate CO sources in parts of the respiratory system other than the alveoli, and to distinguish whether eCO reflects blood-borne CO (COHb, including exogenous sources) or lung diffusion properties. Accurate determination of eCO parameters is of importance in applications such as non-invasive assessment of COHb and red blood cell lifespan [41], oxidative stress monitoring, and early diagnosis of respiratory dis-
ases. Advances in non-invasive physiological mon-
toring can help to elucidate the role of CO as cellular signaling molecule and therapeutic agent, and lead to a better understanding of the CO physiology. However, prior to applying the methodology in medical research and clinical applications, the healthy population baseline of the TMAD parameters needs to be established in larger cohort studies.

5. Conclusions

A novel approach to evaluate real-time breath data was introduced that involves least-squares fitting of com-
plete expirograms using a trumpet-shaped lung model with axial diffusion to simulate the dynamics of pulmonary gas exchange. It was demonstrated for carbon monoxide that, in addition to end-tidal CO, maximum CO fluxes, diffusing capacities and expected equilibrium concentrations in airways and alveolar region can be extracted from single-
exhalation profiles measured at normoventilation during systemic CO elimination. LAS and well-
controlled online breath-sampling were employed for precise and accurate real-time detection of CO in breath and ambient air. In a pilot-study with two healthy non-smokers, fractional CO contributions from airways and alveoli were distinguished for the first time. The expirogram shape and model parameters showed good repeatability with low intra- and inter-individual variation. Acute exposure to elevated CO levels only affected the maximum CO fluxes, but
not the diffusing capacities, in the respiratory tract. Simulations indicated that extended breath CO analysis has the potential to assess health conditions related to inflammatory and obstructive lung diseases.

Acknowledgments

The authors gratefully acknowledge financial support from the Swedish Research Council (2013-6031) and the Kempe Foundations (SMK-1446).

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