A mathematical model for breath gas analysis of volatile organic compounds with special emphasis on acetone

Julian King · Karl Unterkofler · Gerald Teschl · Susanne Teschl · Helin Koc · Hartmann Hinterhuber · Anton Amann

Received: date / Accepted: date

J. King
Breath Research Institute of the Austrian Academy of Sciences
Dammstr. 22, A-6850 Dornbirn, Austria
E-mail: julian.king@oeaw.ac.at

K. Unterkofler
Vorarlberg University of Applied Sciences and
Breath Research Unit of the Austrian Academy of Sciences
Hochschulstr. 1, A-6850 Dornbirn, Austria
E-mail: karl.unterkofler@fhv.at

G. Teschl
University of Vienna, Faculty of Mathematics
Nordbergstr. 15, A-1090 Wien, Austria
E-mail: gerald.teschl@univie.ac.at

S. Teschl
University of Applied Sciences Technikum Wien
Hochstädtpl. 5, A-1200 Wien, Austria
E-mail: susanne.teschl@technikum-wien.at

H. Koc
Vorarlberg University of Applied Sciences
Hochschulstr. 1, A-6850 Dornbirn, Austria

H. Hinterhuber
Innsbruck Medical University, Department of Psychiatry
Anichstr. 35, A-6020 Innsbruck, Austria

A. Amann (corresponding author)
Innsbruck Medical University, Univ.-Clinic for Anesthesia and
Breath Research Institute of the Austrian Academy of Sciences
Anichstr. 35, A-6020 Innsbruck, Austria
E-mail: anton.amann@oeaw.ac.at, anton.amann@i-med.ac.at
Abstract  Recommended standardized procedures for determining exhaled lower respiratory nitric oxide and nasal nitric oxide (NO) have been developed by task forces of the European Respiratory Society and the American Thoracic Society. These recommendations have paved the way for the measurement of nitric oxide to become a diagnostic tool for specific clinical applications. It would be desirable to develop similar guidelines for the sampling of other trace gases in exhaled breath, especially volatile organic compounds (VOCs) which reflect ongoing metabolism.

The concentrations of water-soluble, blood-borne substances in exhaled breath are influenced by:

– breathing patterns affecting gas exchange in the conducting airways
– the concentrations in the tracheo-bronchial lining fluid
– the alveolar and systemic concentrations of the compound.

The classical Farhi equation takes only the alveolar concentrations into account. Real-time measurements of acetone in end-tidal breath under an ergometer challenge show characteristics which cannot be explained within the Farhi setting. Here we develop a compartment model that reliably captures these profiles and is capable of relating breath to the systemic concentrations of acetone. By comparison with experimental data it is inferred that the major part of variability in breath acetone concentrations (e.g., in response to moderate exercise or altered breathing patterns) can be attributed to airway gas exchange, with minimal changes of the underlying blood and tissue concentrations. Moreover, it is deduced that measured end-tidal breath concentrations of acetone determined during resting conditions and free breathing will be rather poor indicators for endogenous levels. Particularly, the current formulation includes the classical Farhi and the Scheid series inhomogeneity model as special limiting cases and thus is expected to have general relevance for other classes of volatile organic compounds as well.

Our model is a first step towards new guidelines for breath gas analysis of acetone (and other water-soluble compounds) similar to those for nitric oxide.

Keywords  breath gas analysis · volatile organic compounds · acetone · modeling

Mathematics Subject Classification (2000)  92C45 · 92C35 · 93C10 · 93B07
1 Introduction

Measurement of blood-borne volatile organic compounds (VOCs) occurring in exhaled breath as a result of normal metabolic activity or pathological disorders has emerged as a promising novel methodology for non-invasive medical diagnosis and therapeutic monitoring of disease, drug testing and tracking of physiological processes [2, 3, 61, 47]. Apart from the obvious improvement in patient compliance and tolerability, major advantages of exhaled breath analysis compared to conventional test procedures, e.g., based on blood or urine probes, include de facto unlimited availability as well as rapid on-the-spot evaluation or even real-time analysis. Additionally, it has been pointed out that the pulmonary circulation receives the entire cardiac output and therefore the breath concentrations of such compounds might provide a more faithful estimate of pooled systemic concentrations than single small-volume blood samples, which will always be affected by local hemodynamics and blood-tissue interactions [54]. Despite this huge potential, the use of exhaled breath analysis within a clinical setting is still rather limited. This is mainly due to the fact that drawing reproducible breath samples remains an intricate task that has not fully been standardized yet. Moreover, inherent error sources introduced by the complex mechanisms driving pulmonary gas exchange are still poorly understood. The lack of standardization among the different sampling protocols proposed in the literature has led to the development of various sophisticated sampling systems, which selectively extract end-tidal air by discarding anatomical dead space volume [34, 25, 13]). Even though such setups present some progress, they are far from being perfect. In particular, these sampling systems can usually not account for the variability stemming from varying physiological states.

In common practice it is tacitly assumed that end-tidal air will reflect the alveolar concentration \( C_A \) which in turn is proportional to the concentration of the VOC in mixed venous blood \( C_v \), with the associated factor depending on the substance-specific blood:gas partition coefficient \( \lambda_{b:air} \) (describing the diffusion equilibrium between capillaries and alveoli), alveolar ventilation \( V_A \) (governing the transport of the compound through the respiratory tree) and cardiac output \( Q_c \) (controlling the rate at which the VOC is delivered to the lungs):

\[
C_{\text{measured}} = C_A = \frac{C_v}{\frac{\lambda_{b:air}}{V_A} + \frac{V_A}{Q_c}}.
\]

(1)

This is the familiar equation introduced by Farhi [19], describing steady state inert gas elimination from the lung viewed as a single alveolar compartment with a fixed overall ventilation-perfusion ratio \( V_A/Q_c \) close to one. Since the pioneering work of Farhi, both equalities in the above relation have been challenged. Firstly, for low blood soluble inert gases, characterized by \( \lambda_{b:air} \leq 10 \), alveolar concentrations resulting from an actually constant \( C_v \) can easily be seen to vary drastically in response to fluctuations in blood or respiratory flow. While this sensitivity has been exploited in MIGET (Multiple Inert Gas Elimination Technique, cf. [79, 78]) to assess ventilation-perfusion inhomogeneity throughout the normal and diseased lung, it is
clearly problematic in standard breath sampling routines based on free breathing, as slightly changing measurement conditions (regarding, e.g., body posture, breathing patterns or stress) can have a large impact on the observed breath concentration \cite{17}. This constitutes a typical example for an inherent error source as stated above, potentially leading to a high degree of intra- and consequently inter-individual variability among the measurement results \cite{53}. It is hence important to investigate the influence of medical parameters like cardiac output, pulse, breathing rate and breathing volume on VOC concentrations in exhaled breath. In contrast, while highly soluble VOCs ($\lambda_{b:\text{air}} > 10$) tend to be less affected by changes in ventilation and perfusion, measurement artifacts associated with this class of compounds result from the fact that – due to their often hydrophilic properties – a substantial interaction between the exhalate and the mucosa layers lining the conducting airways can be anticipated \cite{6}. In other words, for these substances $C_{\text{measured}} \neq C_A$, with the exact quantitative relationship being unknown. Examples of endogenous compounds that are released into the gas phase not only through the blood-alveolar interface, but also through the bronchial lining fluid are, e.g., acetone and ethanol \cite{7,76}.

The emphasis of this paper lies on developing a mechanistic specification of end-tidal breath dynamics of highly soluble, blood-borne trace gases during different physiological situations (e.g., rest, exercise, sleep and exposure scenarios) using a prototypic compound. Our main focus will be on the ketone body acetone (2-propanone; CAS number 67-64-1; molar mass 58.08 g/mol), which is one of the most abundant VOCs found in human breath and has received wide attention in the literature. Being a natural metabolic intermediate of lipolysis \cite{31}, endogenous acetone has been considered as a biomarker for monitoring the ketotic state of diabetic and fasting individuals \cite{73,57,60,66}, estimating glucose levels \cite{20} or assessing fat loss \cite{38}. Nominal levels in breath and blood have been established in \cite{80,65}, and bioaccumulation has been studied in the framework of exposure studies and pharmacokinetic modeling \cite{84,37,49}. Despite this huge body of experimental evidence, the crucial link between breath and blood levels is still obscure. For instance, multiplying the proposed population mean of approximately 1 µg/l \cite{65} in end-tidal breath by the partition coefficient $\lambda_{b:\text{air}} = 340$ \cite{7} at body temperature appears to grossly underestimate observed (arterial) blood levels spreading around 1 mg/l \cite{80,84,53}. Furthermore, continuous profiles of breath acetone extracted during moderate workload ergometer challenges \cite{34} drastically depart from the behavior expected from Equation (1).

In contrast to previous studies which were mainly concerned with the dynamics of single breath tests \cite{76,57}, our model is simple in the sense that no detailed anatomical features of the respiratory tract are taken into account. Instead, by adopting the usual compartmental approach \cite{37,49}, our formulation proves to adequately explain a wide range of experimentally observed data while simultaneously maintaining a model structure amenable to sound mathematical analysis and further algorithmic treatment in the framework of systems theory. Although such models have been criticized for their underlying simplifications \cite{21} (e.g., regarding the cyclic nature of breathing), we believe that, if applied in proper way, they provide a practicable bridge
between formulations capturing short-term behavior as indicated above and phenomena that are characteristic for sampling scenarios extending over minutes to hours. As previously mentioned the latter typically comprise measurements of VOCs during distinct physiological states such as exercise and are expected to give additional information on the mid- to long-term kinetics of such compounds within various parts of the human body.

The physical model to be presented here is driven by the well founded theory covering soluble gas exchange in the single exhalation framework and aims at extending these ideas to a macroscopic level, thereby yielding a model capable of monitoring acetone-specific distribution processes over longer time periods via the respective breath concentrations.

2 Experimental basics

Breath acetone concentrations are assessed by means of a real-time setup designed for synchronized measurements of exhaled breath VOCs as well as a variety of respiratory and hemodynamic parameters. Our instrumentation has successfully been applied for gathering continuous data streams of these quantities during moderate workload ergometer challenges as well as in a sleep laboratory setting. Details are described elsewhere [34]. In brief, the breath-related part of the mentioned setup consists of a head mask spirometer system allowing for the standardized extraction of predefined exhalation segments which – via a heated and gas tight Teflon transfer line – are then directly drawn into a Proton-Transfer-Reaction mass spectrometer (PTR-MS, Ionicon Analytik GmbH, Innsbruck, Austria) for online analysis. This analytical technique has proven to be a sensitive method for quantification of volatile molecular species down to the ppb (parts per billion) range on the basis of “soft” chemical ionization within a drift chamber, i.e., by taking advantage of the proton transfer

\[ \text{H}_3\text{O}^+ + \mathbf{X} \rightarrow \mathbf{XH}^+ + \text{H}_2\text{O} \]

from primary hydronium precursor ions originating in an adjoint hollow cathode [41] [42]. Note that this reaction scheme is selective to VOCs with proton affinities higher than water (166.5 kcal/mol), thereby precluding the ionization of the bulk composition exhaled air, N\textsubscript{2}, O\textsubscript{2} and CO\textsubscript{2}. Count rates of the resulting product ions XH\textsuperscript{+} or fragments thereof appearing at specified mass-to-charge ratios \( m/z \) can subsequently be converted to absolute concentrations of the protonated compounds (see [64] for further details on the quantification of acetone as well as [34] for the underlying PTR-MS settings used). The carbon dioxide concentration \( C_{\text{CO}_2} \) of the gas sample is determined by a separate sensor (AirSense Model 400, Digital Control Systems, Portland, USA).

In addition to the breath concentration profiles of acetone, it will be of importance for us to have at hand a continuous estimate of the corresponding sample water vapor content \( C_{\text{water}} \). As has been put forward in the literature, the water dimer (\( \text{H}_3\text{O}^+ \) \( \text{H}_2\text{O} \)) forming in the drift chamber as a result of clustering can be used for this purpose [82] [4]. More specifically, the pseudo concentration signal at \( m/z = 37 \) calculated according to Equation (1) in [64] using a standard reaction rate constant of \( 2.0 \times 10^{-9} \text{ cm}^3/\text{s} \)
yields a quantity roughly proportional to sample humidity. Slight variations due to fluctuations of the (unknown) amount of water clusters forming in the ion source are assumed to be negligible. Absolute quantification can be achieved by comparison with standards containing predefined humidity levels. Such standards with $C_{\text{CO}_2}$ and $C_{\text{water}}$ varying over the experimental physiological range of 2 - 8 % and 2 - 6 %, respectively, were prepared using a commercial gas mixing unit (Gaslab, Breitfuss Messtechnik GmbH, Harpstedt, Germany), resulting in a mean calibration factor of $2.1 \times 10^{-4}$ and $R^2 \geq 0.98$ for all regressions. It has been argued in [32] that the aforementioned pseudo concentration can drastically be affected by the carbon dioxide concentration, which, however, could not be confirmed with our PTR-MS settings. Although the computed water content is slightly overestimated with increasing $C_{\text{CO}_2}$, the sensitivity was found to stay within 10 % of the mean value given above. Nevertheless, we recognize that this approximate method for determining water vapor levels can only serve as a first surrogate for more exact hygrometer measurements. Despite the fact that molecular oxygen is not protonated, the breath oxygen concentration $C_{\text{O}_2}$ – relative to an assumed steady state value of about 100 mmHg in end-tidal (alveolar) air during rest [44] – within one single experiment can still be assessed by monitoring the parasitic precursor ion $O_2^+$ at $m/z = 32$ (adjusted to be below 2 % of $H_3O^+$), resulting from a small amount of sample gas entering the ion source with subsequent ionization of $O_2$ under electron impact [54]. For normalization purposes, the respective count rates are again converted to pseudo concentrations.

Table 1 summarizes the measured quantities relevant for this paper. In general, breath concentrations will always refer to end-tidal levels, except where explicitly noted. Moreover, an underlying sampling interval of 5 s is assumed for each variable.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Symbol</th>
<th>Nominal value (units)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cardiac output</td>
<td>$\dot{Q}_c$</td>
<td>6 (l/min) [48]</td>
</tr>
<tr>
<td>Alveolar ventilation</td>
<td>$\dot{V}_A$</td>
<td>5.2 (l/min) [83]</td>
</tr>
<tr>
<td>Tidal volume</td>
<td>$V_T$</td>
<td>0.5 (l) [83]</td>
</tr>
<tr>
<td>Acetone concentration</td>
<td>$C_{\text{measured}}$</td>
<td>1 ($\mu$g/l) [65]</td>
</tr>
<tr>
<td>CO₂ content</td>
<td>$C_{\text{CO}_2}$</td>
<td>5.6 (%) [44]</td>
</tr>
<tr>
<td>Water content</td>
<td>$C_{\text{water}}$</td>
<td>4.7 (%) [23]</td>
</tr>
<tr>
<td>O₂ content</td>
<td>$C_{\text{O}_2}$</td>
<td>13.7 (%) [44]</td>
</tr>
</tbody>
</table>

3 Acetone modeling

3.1 Preliminaries and assumptions

Classical pulmonary inert gas elimination theory [19] postulates that uptake and removal of VOCs take place exclusively in the alveolar region. While this is a reasonable assumption for low soluble substances, it has been shown by several authors that exhalation kinetics of VOCs with high affinity for blood and water such as acetone...
are heavily influenced by relatively quick absorption and release mechanisms occurring in the conductive airways, see, e.g., Ref. [5]. More specifically, due to their pronounced hydrophilic characteristics such compounds tend to interact with the water-like mucus membrane lining this part of the respiratory tree, thereby leading to pre- and post-alveolar gas exchange often referred to as wash-in wash-out behavior. The present model aims at taking into consideration two major aspects in this framework.

3.1.1 Bronchial exchange

It is now an accepted fact, that the bronchial tree plays an important role in overall pulmonary gas exchange of highly (water) soluble trace gases, affecting both endogenous clearance as well as exogenous uptake. For instance, Anderson et al. [7] inferred that while fresh air is being inhaled, it becomes enriched with acetone stored in the airway surface walls of the peripheral bronchial tract, thus leading to a decrease of the acetone pressure/tension gradient between gas phase and capillary blood in the alveolar space. This causes an effective reduction of the driving force for gas exchange in the alveoli. In contrast, during exhalation the aforementioned diffusion process is reversed, with a certain amount of acetone being stripped from the airstream and redeposited onto the previously depleted mucus layer, thereby decreasing overall acetone elimination as compared to purely alveolar extraction. Similarly, exposition studies suggest a pre-alveolar absorption of exogenous acetone during inhalation and a postalveolar revaporization during expiration, resulting in a diminished uptake of highly soluble VOCs than what would be expected if exchange occurred completely in the alveoli [84, 37, 74].

In accordance with previous modeling approaches, we consider a bronchial compartment separated into a gas phase and a mucus membrane, which is assumed to inherit the physical properties of water [37, 39] and acts as a reservoir for acetone. Part of the acetone dissolved in this layer is transferred to the bronchial circulation, whereby the major fraction of the associated venous drainage is postulated to join the pulmonary veins via the postcapillary anastomoses [44], cf. Fig. 2. A study by Morris et al. [51] on airway perfusion during moderate exercise in humans indicates that the fraction \( q_{\text{bro}} \in [0, 1] \) of cardiac output \( \dot{Q}_c \) contributing to this part of bronchial perfusion will slightly decrease with increasing pulmonary blood flow. According to Figure 3 in that contribution and assuming that \( \dot{Q}_{c}^{\text{rest}} = 6 \text{l/min} \) we can derive the heuristic linear model

\[
q_{\text{bro}}(\dot{Q}_c) := \max \{0, q_{\text{bro}}^{\text{rest}}(1 - 0.06(\dot{Q}_c - \dot{Q}_{c}^{\text{rest}}))\}. 
\]  

(2)

The constant \( q_{\text{bro}}^{\text{rest}} \) will be estimated in Section 4. As a rough upper bound we propose the initial guess \( q_{\text{bro}}^{\text{rest}} = 0.01 \) [44]. We stress the fact that the bronchial compartment just introduced has to be interpreted as an abstract control volume lumping together the decisive sites of airway gas exchange in one roughly homogenous functional unit. These locations can be expected to vary widely with the solubility of the VOC under scrutiny as well as with physiological boundary conditions [5].
3.1.2 Temperature dependence

The solubility of acetone in the mucus surface of the airways is strongly affected by temperature, which in turn can be assessed by taking into account absolute sample humidity as follows. Passing through the conditioning regions of the upper airways, inhaled air will be warmed to a mean body core temperature of approximately 37 °C and fully saturated with water vapor, thus leading to an absolute humidity of bronchial and alveolar air of about 6.2 % at ambient pressure. During exhalation, depending on the axial temperature gradient between the lower respiratory tract and the airway opening, a certain amount of water vapor will condense out and reduce the water content \( C_{\text{water}} \) in the exhalate according to the saturation water vapor pressure \( P_{\text{water}} \) (in mbar) determined by local airway temperature \( T \) (in °C) \([46,24]\). The relationship between these two quantities can be approximated by the well-known Magnus formula \([68]\)

\[
P_{\text{water}}(T) = 6.112 \exp \left( \frac{-17.62T}{243.12 + T} \right),
\]

valid for a temperature range \(-45 °C \leq T \leq 60 °C\). For normal physiological values of \( T \), the resulting pressure is sufficiently small to treat water vapor as an ideal gas \([59]\) and hence by applying Dalton’s law we conclude that absolute humidity \( C_{\text{water}} \) (in %) of the exhalate varies according to

\[
C_{\text{water}}(T) = 100 \frac{P_{\text{water}}(T)}{P_{\text{ambient}}},
\]

where \( P_{\text{ambient}} \) is the ambient pressure in mbar. Inverting the above formula, the minimum airway temperature \( T_{\text{min}} = T_{\text{min}}(C_{\text{water}}) \) during exhalation becomes a function of measured water content in exhaled breath. From this, a mean airway and mucus temperature characterizing the homogenous bronchial compartment of the previous section will be defined as

\[
\bar{T}(C_{\text{water}}) := \frac{T_{\text{min}}(C_{\text{water}}) + 37}{2},
\]

corresponding to a hypothesized linear increase of temperature along the airways. Note that this assumption is somehow arbitrary in the sense that the characteristic temperature of the airways should be matched to the primary (time- and solubility-dependent) location of airway gas exchange as mentioned above. Equation (5) thus should only be seen as a simple ad hoc compromise incorporating this variability.

The decrease of acetone solubility in the mucosa – expressed as the water:air partition coefficient \( \lambda_{\text{muc:air}} \) – with increasing temperature can be described in the ambient temperature range by a van’t Hoff-type equation \([70]\)

\[
\log_{10} \lambda_{\text{muc:air}}(T) = -A + \frac{B}{T + 273.15},
\]

where \( A = 3.742 \) and \( B = 1965 \) Kelvin are proportional to the entropy and enthalpy of volatilization, respectively. Hence, in a hypothetical situation where the absolute sample humidity at the mouth is 4.7 % (corresponding to a temperature of \( T \approx 32 °C \)
and ambient pressure at sea level, cf. \[46,23\]), local solubility of acetone in the mucus layer increases from \(\lambda_{\text{muc:air}}(37 \, ^\circ\text{C}) = 392\) in the lower respiratory tract (cf. \[46\]) to \(\lambda_{\text{muc:air}}(32 \, ^\circ\text{C}) = 498\) at the mouth, thereby predicting a drastic reduction of airstream acetone concentrations along the airways. The above formulations allow to assess this reduction by taking into account sample water vapor as a meta parameter. This meta parameter reflects various influential factors on the mucus solubility \(\lambda_{\text{muc:air}}\) which would otherwise be intricate to handle due to a lack of information, such as local airway perfusion, breathing patterns, mucosal hydration and thermoregulatory events which in turn will affect axial temperature profiles. In particular, \(\lambda_{\text{muc:air}}\) for the entire bronchial compartment will be estimated via the mean airway temperature \(\bar{T}\) as

\[
\lambda_{\text{muc:air}}(C_{\text{water}}) = \lambda_{\text{muc:air}}(\bar{T}(C_{\text{water}})).
\]

The strong coupling between sample humidity and exhaled breath concentrations predicted by the two relationships (3) and (6) is expected to be a common factor for all highly water soluble VOCs. In the framework of breath alcohol measurements Lindberg et al. \[40\] indeed showed a positive correlation between these two quantities along the course of exhalation, which can also be observed in the case of acetone, cf. Fig. 1.

Variations of the acetone blood:air partition coefficient \(\lambda_{\text{b:air}} = 340\) \[7,18\] – dominating alveolar gas exchange – in response to changes in mixed venous blood temperature, e.g., due to exercise, are ignored as such changes are necessarily small \[16\]. Hence, \(\lambda_{\text{b:air}}\) will always refer to \(37 \, ^\circ\text{C}\). Similarly, the partition coefficient between mucosa and blood is treated as constant defined by

\[
\lambda_{\text{muc:b}} = \lambda_{\text{muc:air}}(37 \, ^\circ\text{C})/\lambda_{\text{b:air}},
\]

resulting in a value of 1.15. Note, that if the airway temperature is below \(37 \, ^\circ\text{C}\) we always have that

\[
\lambda_{\text{muc:air}}/\lambda_{\text{muc:b}} \geq \lambda_{\text{b:air}},
\]

as \(\lambda_{\text{muc:air}}\) is monotonically decreasing with increasing temperature, see Equation (6).

### 3.1.3 Bronchio-alveolar interactions

In a series of modeling studies \[76,5\], the location of gas exchange (from gas phase to liquid phase and vice versa) has been demonstrated to shift between bronchial and alveolar regions depending on the solubility of the compound under investigation. While during tidal breathing exchange for substances with blood:air partition coefficient \(\lambda_{\text{b:air}} \leq 10\) takes place almost exclusively in the alveoli, it appears to be strictly limited to the bronchial tract in the case of \(\lambda_{\text{b:air}} \geq 100\). Transport for VOCs lying within these two extremes distributes between both spaces. Likewise, for fixed \(\lambda_{\text{b:air}}\), location of gas exchange is expected to vary with breathing patterns. As has been concluded by Anderson et al. \[7\], airway contribution to overall pulmonary exchange of endogenous acetone is about 96% during tidal breathing, but only 73% after inspiration to total lung capacity. The rationale for this reduction is that while more proximal parts of the mucosa lining are being depleted earlier in the course of inhalation by loosing acetone to the inhale, saturation of the airstream with acetone
Fig. 1 Correlation between breath acetone concentrations and breath water content during free exhalation at an approximately constant rate of 150 ml/s. Water-acetone pairs in the third panel correspond to the two linear Phase 3 segments as described in [7]. The high sampling frequency is achieved by limiting the PTR-MS measurement cycle to only three mass-to-charge ratios (with corresponding dwell times given in brackets): \( m/z = 21 \) (5 ms), \( m/z = 37 \) (2 ms) and \( m/z = 59 \) (10 ms).

is continuously shifted towards the alveolar region. Furthermore, it can be argued that the magnitude of this shift increases with volumetric flow during inhalation as equilibration of fresh air with the mucus layer in regions with high flow rates might not be completed. From a reversed viewpoint this would be consistent with the observation made in [7] that end-exhaled acetone partial pressures increase with exhaled flow rate.

Such smooth transitions in the location of gas exchange can be incorporated into the model by including a diffusion process describing the interaction between bronchial and alveolar compartment (cf. Fig. 2), which is similar to the strategy found in the theory of stratified or series inhomogeneities developed by Scheid et al. [62]. According to the approach presented there, mentioned inhomogeneities arise...
from the fact that while gas flow in the upper parts of the respiratory tree is primarily dominated by convection, axial diffusion becomes the decisive factor in the terminal airspaces. This can also be thought of as incomplete mixing of tidal volume with the functional residual capacity, thus leading to an effective increase of alveolar deadspace. It will be shown that reinterpreting stratified inhomogeneity and the stratified conductance parameter \( D \) (having units of volume divided by time and taking values in \([0, \infty)\)) in the framework of soluble gas exchange allows a proper description of bronchio-alveolar interactions. The alveolar region itself is represented by one single homogenous alveolar unit, thereby neglecting ventilation-perfusion inequality throughout the lung. In the case of VOCs with high \( \lambda_{b:\text{air}} \) this constitutes an acceptable simplification, since the classical Farhi equation predicts a minimal influence of local ventilation-perfusion ratios on the corresponding alveolar concentrations. Uptake and elimination of VOCs to and from the bronchio-alveolar tract during inhalation and exhalation is governed by the alveolar ventilation \( \dot{V}_A \).

### 3.1.4 Body compartments

The systemic part of the model has been adapted from previous models \([36, 49]\) and consists of two functional units: a liver compartment, where acetone is endogenously produced and metabolized, as well as a tissue compartment representing an effective storage volume. The latter basically lumps together tissue groups with similar blood:tissue partition coefficient \( \lambda_{b:\text{tis}} \approx 1.38 \), such as richly perfused tissue, muscles and skin \([7, 49]\). Due to the low fractional perfusion of adipose tissue and its low affinity for acetone, an extra fat compartment was not considered. The fractional blood flow \( q_{\text{liv}} \in [0, 1) \) to the liver is assumed to be related to total cardiac output by

\[
q_{\text{liv}}(\dot{Q}_c) := 0.034 + 1.145 \exp(-1.387 \dot{Q}_c / \dot{Q}_{c\text{rest}}),
\]

obtained by exponential fitting of the data given in \([49]\). The rate \( \dot{M} \) of acetone metabolism is assumed to be proportional to liver concentration, i.e.,

\[
\dot{M} = k_{\text{lin}} \text{bw}^{0.75} C_{\text{liv}} \lambda_{b:\text{liv}} =: k_{\text{met}} C_{\text{liv}} \lambda_{b:\text{liv}},
\]

where \( \text{bw} \) denotes the body weight in kg and the kinetic rate constant \( k_{\text{lin}} \) is obtained by linearization of the Michaelis-Menten kinetics proposed in \([36]\), i.e., we expect a value of

\[
k_{\text{lin}} := v_{\text{max}} / k_m \approx 0.0037 l / kg^{0.75} / \text{min}.
\]

Taking into account nominal mixed venous blood concentrations of approximately 1 mg/l for healthy volunteers as well as an apparent Michaelis-Menten constant \( k_m = 84 \text{ mg/l} \) this will result in a good approximation for most situations encountered in practice (except for extreme cases, e.g., severe diabetic ketoacidosis or starvation ketosis where plasma concentrations up to 500 mg/l have been reported \([57, 60]\) and hence metabolism can be expected to reach saturation). Other ways of acetone clearance such as excretion via the renal system are neglected \([84]\). Endogenous synthesis of acetone in the liver is assumed to occur at some rate \( k_{\text{pr}} > 0 \) depending on current lipolysis \([31]\). In particular, fat catabolism is considered a long-term mechanism compared to the other dynamics of the system, so that \( k_{\text{pr}} \) can in fact be assumed constant during the course of experiments presented here (less than 2 hours).
3.2 Model equations and a priori analysis

3.2.1 Derivation

In order to capture the gas exchange and tissue distribution mechanisms presented above, the model consists of four different compartments. A sketch of the model structure is given in Fig. 2 and will be detailed in following.

Model equations are derived by taking into account standard conservation of mass laws for the individual compartments, see Appendix A.1.2. Local diffusion equilibria are assumed to hold at the air-tissue, tissue-blood and air-blood interfaces, the ratio of the corresponding concentrations being described by the appropriate partition coefficients, e.g., $\lambda_{b:\text{air}}$. Unlike for low blood soluble compounds, the amount of highly soluble gas dissolved in local blood volume of perfused compartments cannot generally be neglected, as it might significantly increase the corresponding capacities. This is particularly true for the airspace compartments. Since reliable data for some local blood volumes could not be found, in order not to overload the model...
with too many hypothetical parameters, we will use the effective compartment volumes \( \tilde{V}_{\text{bro}} := V_{\text{bro}} + V_{\text{muc}} \lambda_{\text{muc:air}} \), \( \tilde{V}_{\Lambda} := V_{\Lambda} + V_{\text{c:air}} \lambda_{\text{b:air}} \), \( \tilde{V}_{\text{liv}} := V_{\text{liv}} + V_{\text{liv,b}} \lambda_{\text{b:liv}} \) as well as \( \tilde{V}_{\text{tis}} := V_{\text{tis}} \) and neglect blood volumes for the mucosal and tissue compartment, see also Appendix A.1.2. According to Fig. 2 as well as by taking into account the discussion of the previous subsections, for the bronchial compartment we find that

\[
\frac{dC_{\text{bro}}}{dt} \tilde{V}_{\text{bro}} = \dot{V}_{\Lambda} (C_{I} - C_{\text{bro}}) + D (C_{A} - C_{\text{bro}}) + q_{\text{bro}} \dot{Q}_{c} (C_{a} - \lambda_{\text{muc:air}} C_{\text{bro}}),
\]

(13)

with \( C_{I} \) denoting the inhaled (ambient) gas concentration, while the mass balance equations for the alveolar, liver and tissue compartment read

\[
\frac{dC_{\Lambda}}{dt} \tilde{V}_{\Lambda} = D (C_{\text{bro}} - C_{\Lambda}) + (1 - q_{\text{bro}}) \dot{Q}_{c} (C_{\bar{v}} - \lambda_{\text{b:air}} C_{\Lambda}),
\]

(14)

and

\[
\frac{dC_{\text{liv}}}{dt} \tilde{V}_{\text{liv}} = k_{pr} - k_{me} \lambda_{\text{b:liv}} C_{\text{liv}} + q_{\text{liv}} (1 - q_{\text{bro}}) \dot{Q}_{c} (C_{a} - \lambda_{\text{b:liv}} C_{\text{liv}}),
\]

(15)

and

\[
\frac{dC_{\text{tis}}}{dt} \tilde{V}_{\text{tis}} = (1 - q_{\text{liv}}) (1 - q_{\text{bro}}) \dot{Q}_{c} (C_{a} - \lambda_{\text{b:liv}} C_{\text{tis}}),
\]

(16)

respectively. Here,

\[
C_{\bar{v}} := q_{\text{liv}} \lambda_{\text{b:liv}} C_{\text{liv}} + (1 - q_{\text{liv}}) \lambda_{\text{b:liv}} C_{\text{tis}}
\]

(17)

and

\[
C_{a} := (1 - q_{\text{bro}}) \lambda_{\text{b:air}} C_{\Lambda} + q_{\text{bro}} \lambda_{\text{muc:air}} C_{\text{bro}} / \lambda_{\text{muc:b}}
\]

(18)

are the associated concentrations in mixed venous and arterial blood, respectively. Moreover, we state that measured (end-tidal) breath concentrations equal bronchial levels, i.e.,

\[
C_{\text{measured}} = C_{\text{bro}}.
\]

The decoupled case \( D = q_{\text{bro}} = 0 \) will be excluded further on in this paper as it lacks physiological relevance.

### 3.2.2 Steady state relationships and interpretation of the stratified conductance parameter \( D \)

Assume that the system is in steady state and we know the associated end-tidal breath concentration \( C_{\text{measured}} = C_{\text{bro}} \). Furthermore, we define the bronchial and alveolar ventilation-perfusion ratio to be

\[
r_{\text{bro}} := \frac{\dot{V}_{\Lambda}}{q_{\text{bro}} \dot{Q}_{c}} \quad \text{and} \quad r_{\Lambda} := \frac{\dot{V}_{\Lambda}}{(1 - q_{\text{bro}}) \dot{Q}_{c}},
\]

respectively. If \( D = 0 \) we deduce that

\[
C_{\text{measured}} = C_{\text{bro}} = r_{\text{bro}} C_{I} + \frac{(1 - q_{\text{bro}}) \lambda_{\text{b:air}} C_{\Lambda}}{(1 - q_{\text{bro}}) \lambda_{\text{muc:air}} + r_{\text{bro}}} = r_{\text{bro}} C_{I} + \frac{(1 - q_{\text{bro}}) C_{a}}{(1 - q_{\text{bro}}) \lambda_{\text{muc:air}} + r_{\text{bro}}} = r_{\text{bro}} C_{I} + C_{a},
\]

(20)
corresponding to purely bronchial gas exchange. On the other hand, for $D \to \infty$ it can be shown by simple algebra that

$$C_{\text{measured}} = C_{\text{bro}} = \frac{C_A}{q_{\text{bro}} \lambda_{\text{muc:air}} + (1 - q_{\text{bro}}) \lambda_{\text{b:air}} + r_A} = \frac{C_A}{q_{\text{bro}} \lambda_{\text{muc:air}} + (1 - q_{\text{bro}}) \lambda_{\text{b:air}} + r_A}.$$

In the following, let $C_1 = 0$. Note that then in both cases the physiological boundary condition $C_\infty \geq C_A$ is respected. Substituting $q_{\text{bro}} = 0$ into (21) yields the well-known Farhi equations describing purely alveolar gas exchange.

Thus, $D$ defines the location of gas exchange in accordance with Section 3.1.3, while $q_{\text{bro}}$ determines to what extent the bronchial compartment acts as an inert tube. In this sense, Equations (13)–(16) define a generalized description of gas exchange including several known models as special cases. This hierarchy is summarized in Fig. 3.

For perspective – as has been rationalized in the case of acetone in Section 3.1.3 and is likely to be a common characteristic for all highly water soluble VOCs – the stratified conductance parameter $D$ will be close to zero during rest and is expected to increase with tidal volume and/or flow rate [77,5]. With hindsight, as will be justified in Section 4.2, we propose to model this dependency as

$$D := k_{\text{diff},1} \max\{0, V_T - V_{T\text{rest}}\} + k_{\text{diff},2} \max\{0, V_A - V_{A\text{rest}}\}, \quad k_{\text{diff},i} \geq 0. \tag{22}$$

We stress the fact that if $D$ is close to zero, calculating blood levels from $C_{\text{measured}}$ by using Equation (20) or taking advantage of this expression for normalization and correction purposes is an ill-posed problem due to the possibly large influence of the term $r_{\text{bro}}$ as well as the uncertainty regarding $q_{\text{bro}}$ and $\lambda_{\text{muc:air}}$. Particularly, from the aforementioned facts this means that measured breath concentrations of acetone (and generally highly water soluble substances) determined during resting conditions and free breathing can be rather misleading indicators for endogenous levels, even if
sampling occurs under well defined standard conditions, for instance – as is common practice – using \( \text{CO}_2 \)- and/or flow-controlled extraction from the end-tidal exhalation segment [34,13]. This has first been recognized in the context of breath alcohol measurements revealing experimentally obtained blood-breath concentration ratios of ethanol during tidal breathing that are unexpectedly high compared with in vitro partition coefficients [30]. An elegant approach proposed to circumvent this problem is isothermal rebreathing [30,55], which aims at creating conditions where (cf. Equation (20))

\[
C_{\text{measured}} = C_1 = C_A = C_{\text{bro}} = \frac{r_{\text{bro}} C_{\text{bro}} + (1 - q_{\text{bro}}) C_\bar{v}}{(1-q_{\text{bro}}) \lambda_{b:air} + r_{\text{bro}}} = \frac{C_\bar{v}}{\lambda_{b:air}} = \frac{C_a}{\lambda_{b:air}} \quad (23)
\]

from which the mixed venous blood concentration can easily be determined by multiplying the measured rebreathing concentration with the anticipated blood:air partition coefficient at body temperature. Further discussion of this technique is postponed to another paper (in preparation). Another idea to arrive at a more reliable estimation of endogenous acetone levels obviously might be to mimic steady states corresponding to a situation where \( D \) is large, so that Equation (21) can be assumed to hold approximately. The natural experimental framework here are workload challenges as described in Section 4.2.

However, a general word of caution is in order at this point, certainly limiting the practical value of aforementioned steady state calculations. Firstly, experimental data in breath gas analysis usually corresponds to a transient state of the system, despite the fact that the breath concentration itself might appear to be reasonably constant, compare, e.g., with Fig. 4. Moreover, one equilibrium alone does usually not provide enough information to uniquely determine all endogenous variables of interest. Thus, estimation has to be based on dynamic behavior rather than steady states, which will be the topic of Section 4.

3.2.3 A priori analysis

Equations (13)–(16) can be written as a time-varying linear inhomogeneous ODE system

\[
\dot{c} = A(u, \theta) c + b(u, \theta) =: g(u, c, \theta) \quad (24)
\]

in the state variable \( c := (C_{\text{bro}}, C_A, C_{\text{liv}}, C_{\text{tis}})^T \), which is dependent on a time-independent parameter vector \( \theta \) as well as on a vector \( u := (V_A, Q_c, V_T, \lambda_{\text{muc:air}}(C_{\text{water}}), C_1) \) lumping together all measured variables. The associated measurement equation reads

\[
y = C_{\text{measured}} = (1, 0, 0, 0) c =: h(c). \quad (25)
\]

In the present paragraph we are going to discuss some qualitative properties of the system presented. Firstly, as the system is linear, for any given initial condition \( c(0) \) there is a unique global solution. Moreover, \( A \) is a Metzler matrix, meaning that all off-diagonal entries are non-negative and \( b \geq 0 \). Consequently \( c_i \geq 0 \) for every component and it follows that the trajectories remain non-negative. Thus, (24) constitutes a positive system with the state \( c \) evolving within the positive orthant \( \mathbb{R}_{>0}^n := \{ c \in \mathbb{R}^n, c_i > 0, i = 1, \ldots, n \} \), where \( n = 4 \).
Proposition 1 All solutions of (24) starting in $\mathbb{R}_{>0}^n$ remain bounded.

Proof This can be shown by considering the total mass $m := \sum \tilde{V}_i c_i \geq 0$ and noting that
\[ m = k_{pr} - k_{met} \lambda_{b:liv} C_{liv} + V_A (C_1 - C_{bro}). \] (26)
Taking into account positivity of the solutions and the involved parameters this shows that $C_{liv}$ is bounded from above for bounded $C_I$, since the assumption that $C_{liv}$ is unbounded yields a contradiction. Analogously, $C_{bro}$ and $C_A$ can be shown to be bounded by considering $\tilde{m} := \tilde{V}_{bro} C_{bro} + \tilde{V}_A C_A + \tilde{V}_{tis} C_{tis}$ and similarly for $C_{tis}$.

Furthermore, it can be proven that under physiological steady state conditions, i.e., for constant $u$, the above system has a globally asymptotically stable equilibrium $c_0 := -A^{-1}b$. To this end it suffices to show that the time-invariant matrix $A$ is Hurwitz, i.e., all real parts of the associated eigenvalues are negative.

Proposition 2 Suppose $u$ is time-independent. Then the real parts of all eigenvalues of $A(u, \theta)$ are non-positive. They are strictly negative if $\det(A(u, \theta)) \neq 0$.

Moreover, $\det(A(u, \theta)) = 0$ if and only if either $\dot{Q}_c$ vanishes or two of the quantities $\dot{V}_A, q_{liv}, k_{met}$ vanish.

Proof For this it is sufficient to confirm that $A$ is diagonally dominant, i.e., there exists a vector $z > 0$ such that the row vector $z^T A$ is non-negative. This is a simple consequence of the fact that in such a case $A$ can be shown to be similar to a matrix $\tilde{A}$ which has the claimed property. Indeed, if we define the diagonal matrix $U := \text{diag}(z_1, \ldots, z_n)$ and set $\tilde{A} := UAU^{-1}$, it holds that
\[ \tilde{a}_{jj} + \sum_{i \neq j} |\tilde{a}_{ij}| = a_{jj} + \sum_{i \neq j} \frac{a_{ij} z_i}{z_j} = \frac{1}{z_j} (z^T A) j \leq 0 \] (27)
for all $j$ since $A$ is Metzler. Moreover, note that $\tilde{a}_{jj} < 0$ for all $j$. Hence, the first claim will follow from Gershgorin’s circle theorem. The required vector $z$ follows from
\[ (\tilde{V}_{bro}, \tilde{V}_A, \tilde{V}_{liv}, \tilde{V}_{tis}) A = (-\dot{V}_A, 0, -k_{met} \lambda_{b:liv}, 0). \]
Moreover, the only case when the real part of an eigenvalue can vanish is when it lies on the boundary of a circle touching the imaginary axis, that is, when an eigenvalue is zero. This proves the first part.

To show the second part one verifies that
\[ \det(A) = \dot{Q}_c (\vartheta_1 \dot{V}_A + \vartheta_2 k_{met} q_{liv} \dot{Q}_c + \vartheta_3 \dot{V}_A k_{met}) \] (28)
with $\vartheta_j > 0$.

This completes the minimal set of properties which necessarily should to be satisfied in any valid model of concentration dynamics. In particular, global asymptotical stability for the autonomous system ensures that – starting from arbitrary initial values – the compartmental concentrations will approach a unique equilibrium state once the physiological inputs $u$ affecting the system are fixed. Obviously, this is of
paramount importance when aiming at the description of processes exhibiting pronounced steady states (which in the context of breath gas analysis corresponds, e.g., to the situation encountered during rest or constant workload [34]) and a prerequisite for orchestrating reproducible experiments. Hence, in the context of VOC modeling, any approach not incorporating this property will lack a fundamental characteristic of the observed data.

Remark 1 We stress the fact that if the rate of metabolism is described by saturation rather than linear kinetics, i.e., if the term \( k_{\text{met}} C_{\text{liv}} \lambda_{b:liv} \) in Equation (15) is replaced by

\[
M = \frac{v_{\text{max}} C_{\text{liv}} \lambda_{b:liv}}{k_m + C_{\text{liv}} \lambda_{b:liv}},
\]

with \( v_{\text{max}}, k_m > 0 \), the system becomes essentially nonlinear. However, all conclusions drawn so far remain generally valid. Firstly, by applying the Mean Value Theorem note that the Michaelis-Menten term above is Lipschitz and so again a global Lipschitz property holds for the right-hand side of the resulting ODE system. Positivity and boundedness of the solutions for arbitrary inputs \( u \) can be established in analogy with the arguments in the last paragraph. Effectively, in order to show boundedness from above, we first note that Equation (26) now reads

\[
\dot{m} = k_{pr} - \frac{v_{\text{max}} C_{\text{liv}} \lambda_{b:liv}}{k_m + C_{\text{liv}} \lambda_{b:liv}} + V_A (C_I - C_{\text{bro}})
\]

and as a result \( C_{\text{bro}} \) is unconditionally bounded if \( V_A > 0 \). From Equation (13) it then follows that \( C_A \) must be bounded and subsequently \( C_v \) (and hence \( C_{\text{liv}} \) as well as \( C_{\text{tis}} \)) are bounded using Equation (14). In the case \( V_A = 0 \), the inequality

\[
k_{pr} \leq v_{\text{max}}
\]

is necessary and sufficient for boundedness. Necessity is easily deduced from the fact that \( k_{pr} > v_{\text{max}} \) implies \( m > 0 \) and consequently \( m \) (i.e., at least one component \( c_i \)) is unbounded. Conversely, Equation (31) ensures that \( C_{\text{liv}} \) is bounded and hence the same arguments as in the linear case apply.

In order to establish the existence of a globally asymptotically stable equilibrium point for fixed \( u \) we adopt a result on monotone systems taken from Leenheer et al. [39], see also [29]. For monotone systems in general with applications to biological systems we refer to [67,8]. Firstly, note that the steady state relation associated with Equation (15) now becomes

\[
0 = C_{\text{liv}}^2 + a_1 C_{\text{liv}} + a_0
\]

with \( a_0 < 0 \). Thus, there exists a unique positive steady state solution for the liver concentration \( C_{\text{liv}} \) and consequently the same can be confirmed to hold true for the other components of \( c \) as well. As a consequence, it follows that the model has a unique equilibrium point in the non-negative orthant \( M := \mathbb{R}_+^n \). Moreover, note that

\[
\frac{\partial \nu_i}{\partial c_j} \geq 0 \text{ for } i \neq j, \ i, j \in \{1, \ldots, n\}.
\]
i.e., the system is cooperative. This term stems from the fact that a given component is positively affected by the remaining ones. For linear systems, an equivalent characterization is that the corresponding system matrix $A$ is Metzler. A well-known result on this type of systems asserts that the associated semiflow $\Phi : \mathbb{R}_{\geq 0} \times \mathbb{R}^n \to \mathbb{R}^n$, $(t, c_0) \mapsto \Phi_t(c_0) := c(t)$ is monotone with respect to the natural (componentwise) partial order on $\mathbb{R}^n$ given by

$$c \preceq z \text{ if and only if } c_i \leq z_i, \; i \in \{1, \ldots, n\}.$$  

That is, $\Phi$ preserves the order of the initial conditions (see [67], Prop. 3.1.1), i.e., for $c_0, \tilde{c}_0 \in \mathbb{R}^n$ the condition $c_0 \preceq \tilde{c}_0$ implies that $\Phi_t(c_0) \leq \Phi_t(\tilde{c}_0)$ for $t \in [0, \infty)$. As a third requirement, since all trajectories are bounded and the system evolves within the closed state space $M \subset \mathbb{R}^n$ it follows that for every $c \in M$ the corresponding semi-orbit $O(c) := \{\Phi_t(c), \; t \geq 0\}$ has compact closure. In summary, we are now in the situation to apply Theorem 5 of [39] (by choosing $X = M$), which asserts that the aforementioned properties are sufficient for the unique equilibrium point in $M$ to be globally attractive.

One of the main purposes of the proposed model is to provide a basis for estimating (unknown) compartment concentrations $c$ as well as certain acetone-specific parameters $\theta_i \in \{k_{pr}, k_{met}, v_{\text{max}}, k_{m}, k_{\text{diff},1}, k_{\text{diff},2}, q_{\text{rest}}\}$ from the knowledge of measured breath concentrations $y$. Often over-looked, a necessary requirement in this framework is a priori (or structural) identifiability/observability of the model, which basically checks that in an ideal context of an error-free (i.e., “correct”) model and noise-free measurements there exist functions $u$ (or, in other words, conductible experiments) such that the associated output $y$ (the accessible data) carries enough information to – at least locally – enable an unambiguous determination of all unknown states and parameters. Particularly, this avoids an over-parameterization of the model. As the time evolution of the system (13)–(16) for a given $u$ is fixed once the initial conditions $c_0$ at the start of the experiment are known, the analysis of a priori identifiability/observability hence amounts to studying (local) injectivity of $y$ with respect to $c_0$ and the parameters $\theta_i$ under scrutiny. Evidently, if such a property does not hold then any attempt to reliably estimate these quantities is doomed to failure from the start, as two entirely different parameter combinations can yield exactly the same data. In the present context, generic a priori identifiability/observability for $c_0$ and any selection of parameters $\theta_j$ as above was confirmed by interpreting the latter as additional states with time derivative zero and subjecting the augmented (possibly nonlinear, but control-affine) system to the Hermann-Krener rank criterion [26] (see Appendix [A.2]), which can be seen as a nonlinear analog of the Kalman observability criterion. Intuitively, generic a priori identifiability/observability guarantees that “almost every” experiment represented by some choice of $u$ allows to distinguish the “true” values of $c_0$ and $\theta_i$ from any other possible choice lying close to these values by giving rise to a unique process response. It is evident that generic local observability is indeed the best we can hope for in our context. For instance, looking at the metabolizing liver compartment of the model (13)–(16), if we set $\theta_1 := k_{pr}$ and $\theta_2 := k_{\text{net}}$ and interpret them as additional states having time derivative zero, the augmented model obviously will not be locally observable if $c_0$ is an equilibrium state.
associated with some constant input \( u \). This is simply due to the fact that \( k_{pr} \) and \( k_{met} \) cannot be determined simultaneously from the single steady state equation related to Equation (15) and constitutes a typical example where the matrix \( J \) in (49) becomes rank-deficient for a specific choice of the initial conditions.

### 4 Model validation and estimation

#### 4.1 Simulation of exposure data and model calibration

Validation and refinement of the decisive but uncertain nominal values for the kinetic rate constants of metabolism and production, the fractional bronchial blood flow as well as for the equilibrium tissue levels \( c_0 \) at rest is carried out by fitting the corresponding model response to the Series 1 acetone exposure scenario studied by Wigaeus et al. [84,49]. This in vivo benchmark data set consists of pooled observed acetone concentration profiles in exhaled breath, arterial and venous blood of eight healthy male volunteers during and after exposure to \( C_I = 1.3 \text{ mg/l} \) acetone over a period of two hours. We assume that the exposure starts after ten minutes of quiet tidal breathing, i.e., the inhaled concentration is given by the scaled indicator function

\[
C_I(t) = 1.3 \chi_{[10,130]}(t)
\]

and set alveolar ventilation and cardiac output to constant resting values \( \dot{V}_{A,rest} = 6 \text{ l/min} \) and \( \dot{Q}_{c,rest} = 5.8 \text{ l/min} \), respectively [49,36]. Tissue volumes and partition coefficients are as in Table 3 for a male of height 180 cm and weight 70 kg. Furthermore, acetone metabolism is assumed to follow a Michaelis-Menten kinetics with a fixed apparent Michaelis constant \( k_m = 84 \text{ mg/l} \) (see Section 3.1.4).

Our aim is to determine the parameter vector \( \theta = (v_{max}, k_{pr}, D_{rest}, q_{bro}) \) as well as the nominal endogenous steady state levels \( c_0 \) by solving the ordinary least squares problem

\[
\arg\min_{c_0, \theta} \sum_{j=1}^{n} (C_{a,j} - C_a(t_j))^2, \quad \text{s.t.} \quad \begin{cases} g(u_0, c_0, \theta) = 0 \quad \text{(steady state)} \\ c_0, \theta \geq 0 \quad \text{(positivity)} \\ q_{bro} \leq 0.05 \quad \text{(normalization)} \\ C_a(0) = 1 \text{ mg/l} \quad \text{(endogenous arterial level)} \end{cases}
\]

Here, \( g \) is the right-hand side of the ODE system (13)–(16), \( C_{a,j} \) is the measured arterial blood concentration at time instant \( t_j \), whereas the predicted arterial concentration \( C_a \) is defined as in Equation (18). The proposed endogenous level of \( C_a(0) = 1 \text{ mg/l} \) is in accordance with other values given in the literature [31,84]. The above minimization problem was solved by implementing a multiple shooting routine [15] in Matlab. This iterative method can be seen as a generalization of the standard Gauss-Newton (i.e., single shooting) algorithm, designed to avoid divergence issues of the latter due to large residuals. For further details as well as convergence and stability properties we refer to [14,58]. The necessary derivatives of the trajectories with respect to \( (c_0, \theta) \) were computed by simultaneously solving the associated variational equations [22]. Starting values were chosen to be uniformly distributed in the interval \([0, 1] \) and convergence was assumed to be achieved when the maximum componentwise relative change between two successive parameter refinements was less than...
0.01 %. Fig. 4 shows that the calibrated model is capable of convincingly reproducing the pooled data given in [84].

![Graph showing modeled vs. measured concentrations](image)

**Fig. 4** Model fitted to the Series 1 exposure data published by Wigaeus et al.. First panel: observed versus predicted arterial concentrations. Second panel: observed versus predicted breath concentrations $C_{measured} = C_{bre}$.  

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Description</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>$v_{\text{max}}$</td>
<td>0.31 (mg/min/kg^{0.75})</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$k_{\text{pr}}$</td>
<td>$\leq 2$ (mg/min)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$D_{\text{rest}}$</td>
<td>0 (l/min)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$q_{\text{rest}}$</td>
<td>1 (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$C_{\text{bre}}, C_{\text{Ar}}, C_{\text{IIC}, C_{\text{Ar}}}$</td>
<td>(mg/l)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Moreover, literature or anticipated values of $c_0$ and $\theta$ compare favorably to those obtained by the aforementioned minimization, cf. Table 2. In particular, the fitted value of the gas exchange location parameter $D_{\text{rest}} = 0$ agrees well with what is expected from the theoretical discussion in Section 3.2.2. The rate at which endogenous acetone forms in healthy adults as a result of normal fat catabolism is not known. However, from the published study on acetone metabolism in lean and obese humans during starvation ketosis by Reichard et al. [60] a rather conservative upper bound for $k_{\text{pr}}$ can be derived to lie in the range of 2 mg/min. A diminished fractional bronchial perfusion $q_{\text{bre}}$ might reflect the fact that the assumption of diffusion equilibrium be-
tween the mucosa lining and the deeper vascularized sections of the airway wall is rather stringent, so that actually less acetone is transported away from the peripheral bronchial tract via the bloodstream. On the other hand, $q_{\text{bro}}^{\text{rest}} = 0.01$ refers to the entire bronchial circulation rather than only the part contributing to anatomic right-to-left shunt as discussed in Section 3.1.1. The fitted steady state value for the measured breath concentration $C_{\text{measured}}(0) = C_{\text{bro}}(0) = 0.0016$ mg/l during normal breathing at rest is only slightly higher than the observed levels spreading around 500 ppb (corresponding to about 0.0011 mg/l) [65,34]. The population spread of the fitted parameters within the study cohort could be assessed, e.g., by a Bayesian [50] or mixed effects approach [35], which, however would be beyond the scope of this paper. Here, the major aim rather is to demonstrate the flexibility of the model in covering a wide spectrum of different experimental scenarios.

Fig. 4 illustrates the necessity of taking into account the conducting airways as an additional compartment for exchange of highly soluble substances. In particular, the observed data profiles sharply contrast the classical Farhi inert tube description predicting arterial blood concentrations $C_A$ to be directly proportional to (alveolar) breath concentrations $C_A = C_{\text{measured}}$. On the other hand, from the viewpoint of pre-alveolar uptake as discussed in Section 3.1.1, accumulation of exogenous acetone in the systemic circulation is expected to be delayed due to the small contribution of bronchial blood flow to overall perfusion.

In the following, the fitted value of $k_{\text{lin}} = \nu_{\text{max}}/k_m = 0.0074$ l/kg$^{0.75}$/min is interpreted as an intrinsic property of acetone metabolism in the liver, while inter-individual variation is introduced by multiplication with the 0.75 power of body weight. Hence, $k_{\text{met}}$ as in Equation (11) is viewed as a constant known parameter. Similarly, for identifiability reasons $q_{\text{bro}}^{\text{rest}}$ will be fixed at its fitted value 0.0043, cf. Section 4.2.

4.2 Ergometer datasets and comparison with other models

A primary motivation for this work was to develop a model capable of explaining observed acetone profiles in exhaled breath resulting from endogenous levels (i.e., while breathing of an atmosphere with negligible acetone content) during moderate workload ergometer challenges, cf. [34]. As has been discussed there, end-tidal acetone levels closely resemble the profile of alveolar ventilation respectively inhalation volume, showing abrupt increases respectively drops in the range of 10 – 40 % at the onsets and stops, respectively, of the individual workload periods, see Fig. 5(a), first panel. Similarly, a series of auxiliary experiments carried out by means of the same instrumental setup showed that increasing tidal volume during rest results in increased breath acetone concentrations while increasing respiratory frequency has a less pronounced impact, cf. Fig. 5(b). Both effects appear to support the hypothesis of Section 3.1.3 that acetone exchange is strongly influenced by volume and speed of inhalation and hence suggest that any model not incorporating this mechanism will fail to reproduce these results. In particular, from the viewpoint of the classical Farhi description, the profile in Fig. 5 is rather counter-intuitive. For instance, during hyperventilation, acetone supply from the bloodstream tends to stay constant or rise
only slightly, while a drastically increased ventilation enhances dilution of the alveolar air and therefore is expected to decrease the corresponding breath concentration. Indeed, it can be shown that the Farhi model, which corresponds to a special case of our model by setting $q_{beo} = 0$ and $D \to \infty$ is not capable of recovering the measured profiles.

![Graph](image_url)

**Fig. 5** Typical profile of end-exhaled acetone concentrations in response to the following physiological regime: rest (0-3 min), ergometer challenge at 75 W (3-12 min), rest (12-20 min), hyperventilation with increased tidal volume (20-22 min), rest (22-25 min), high-frequency hyperventilation (25-28 min), rest (28-30 min).
The same holds true for the physiological compartment model introduced by Mörk et al. [49], which has been designed for the purpose of explaining acetone exhalation kinetics during rest and exercise and has been validated for exposure scenarios at hand of the Wigaeus et al. datasets presented in the last section. The model of Mörk et al. is implemented by replacing the mass balance Equations (13)–(14) of the respiratory tract with Equations (4)–(7) of that paper. The body compartments remain unchanged. Additional parameter values are adopted as given in [49]. Initial compartment concentrations as well as \( k_{pr} \) for the models of Mörk et al. and Farhi et al. model are calculated by solving the associated steady state equations at \( t_0 = 0 \) given \( C_I \equiv 0 \) and \( C_{bro}(0) = 1.3 \mu g/l \). Similarly, the response for the present model is computed by solving the ordinary least squares problem

\[
\text{argmin}_{c_0, k_{pr}, \alpha} \sum_{j=0}^{n} (y_j - C_{bro}(t_j))^2, \quad \text{s.t.} \quad \begin{cases} g(u_0, c_0, k_{pr}, \alpha) = 0 \quad \text{(steady state)} \\ c_0, k_{pr}, \alpha \geq 0 \quad \text{(positivity)} \\ C_{bro}(0) = 1.3 \mu g/l \quad \text{(initial level)} \end{cases}
\]

on the time interval \([t_0, t_n] = [0, t_{\text{max}}]\). Here, \( y_j = C_{\text{measured}, j} \) is the observed acetone concentration at time instant \( t_j \) and \( \alpha = (\alpha_1, \ldots, \alpha_{m-1}) \) is a coefficient vector for the piecewise linear spline function

\[
\hat{D}(t) := \sum_{i=1}^{m-1} \alpha_i S_i(t), \quad S_i(t) := \begin{cases} \frac{t - s_{i-1}}{s_i - s_{i-1}} & t \in [s_{i-1}, s_i] \\ \frac{s_{i+1} - t}{s_{i+1} - s_i} & t \in [s_i, s_{i+1}] \\ 0 & \text{otherwise} \end{cases}
\]

used for approximating the time-varying stratified conductance parameter \( D \in [0, \infty) \) on \( m \) subintervals covering the time span \([t_0 = s_0, t_{\text{max}} = s_m]\). The nodes \( s_i \) are chosen to result in an equidistant partition of about 0.5 min, cf. Fig. 5 third panel. For simulation purposes the measured physiological functions are converted to input function handles \( u \) by applying a local smoothing procedure to the associated data and interpolating the resulting profiles with splines. The aforementioned optimization problem was solved as described in Section 4.1. Fig. 5 summarizes the results of these calculations. The visually good fit can formally be assessed by residual analysis. Plots of the resulting residuals versus time and versus model predictions clearly exhibit random patterns, suggesting that the assumptions of i.i.d., homoscedastic additive measurement errors underlying ordinary least squares methodology might be reasonable [11]. Furthermore, no statistically significant autocorrelation among the residuals or cross-correlation between the residuals and the measured inputs could be detected, indicating that the model has picked up the decisive dynamics underlying the data [43].

While all three models will describe the steady state at rest, only Equations (13)–(16) tolerably capture the entire observable dynamical behavior. The second panel in Fig. 5 clearly reveals the physiological mechanism underlying the step-shaped dynamics of breath acetone concentrations in response to constant load exercise and hyperventilation: during rest and tidal breathing, measured (bronchial) levels markedly differ from the alveolar ones due to the effective diffusion barrier between the two spaces represented by a value of \( D_{\text{rest}} \approx 0 \). As soon as tidal volume and/or respiratory frequency are increased, this barrier – as reflected in the fitted profile of \( \hat{D} \)
will vanish to some extent according to the processes discussed in Section 3.2.2, causing \( C_{\text{bro}} = C_{\text{measured}} \) to approach a value closer to \( C_A \). In contrast, \( C_A \) itself as well as the breath acetone profiles simulated by means of the other two models remain relatively constant. This is consistent with the behavior expected from the Farhi formulation, predicting minimal sensitivity of alveolar concentrations with respect to fluctuations of the ventilation-perfusion ratio for highly soluble trace gases. In other words, the major part of short-term variability observable in breath acetone concentrations during free breathing can be attributed to airway gas exchange, with minimal changes of the underlying blood and tissue concentrations. In particular, note that with the present model the observed acetone dynamics can again be captured by applying a constant endogenous production rate of approximately 0.17 mg/min. The above-mentioned reasoning appears to agree with previous observations in the literature, where the excretion (defined as the ratio between steady state partial pressures in mixed expired air and mixed venous blood) of acetone has been demonstrated to increase during moderate exercise [63].

The high degree of interplay between \( D \) and \( V_A \) as well as \( V_T \) discernible in the third panel of Fig. 5 suggests to characterize the time-dependency of \( D \) via these two respiratory variables, e.g., as proposed in Equation (22). By employing non-negative least square methods we find that \( k_{\text{diff,1}} = 17.67 \) and \( k_{\text{diff,2}} = 0.81 \) with the associated model again giving a good agreement with the observed data. This parameterization might be used to reduce the originally infinite dimensional estimation problem for \( D \) to two additional parameters. While in Section 3.2.3 it has been confirmed that the model is structurally locally observable with respect to the initial conditions and \( \theta \subseteq \{ k_{pr}, k_{met}, k_{\text{diff,1}}, k_{\text{diff,2}}, q_{\text{bro}}^{\text{rest}} \} \), it remains to be investigated if the data corresponding to exercise or hyperventilation tests as presented above is rich enough to admit a joint numerical estimation of all these variables. This amounts to studying the a posteriori identifiability (also termed practical identifiability or estimability) of the model given the specific experimental situation. For an overview of related concepts we refer to [28]. Intuitively, it should be clear from the approximately constant profile of \( C_{lv} \) that \( k_{pr} \) and \( k_{met} \) can hardly be assessed simultaneously, as both values are coupled via a single steady state equation associated with Equation (15). Using similar reasoning, smaller values of \( q_{\text{bro}}^{\text{rest}} \) (leading to larger steady state concentrations \( C_A(0) \)) can be compensated for by smaller constants \( k_{\text{diff},i} \) to yield an almost identical model output. A more formal investigation of this situation can be carried out by calculating the correlation coefficients between the sensitivities of the model output with respect to these parameters. Values of these indices associated with \( (k_{met}, k_{pr}) \) as well as \( (k_{\text{diff,1}}, q_{\text{bro}}^{\text{rest}}) \) and \( (k_{\text{diff,2}}, q_{\text{bro}}^{\text{rest}}) \) are close to 1 or -1, thereby revealing a substantial degree of collinearity between the corresponding sensitivities and providing strong indications that a proper estimation of any of these pairs on the basis of moderate exercise challenges cannot be anticipated, cf. Appendix A.2. This observation is clearly problematic, as particularly \( q_{\text{bro}}^{\text{rest}} \) and the two diffusion constants \( k_{\text{diff},i} \) have to be regarded as highly uncertain parameters which are not accessible by direct measurement. In a subsequent paper, we will review isothermal rebreathing as a powerful experimental technique for mitigating this type of ill-conditionedness.
4.2.1 Discussion and critical remarks

The main intention of this section is to critically review and clarify some of the assumptions underlying the model derivation as well as to indicate some potential improvements of the present formulation.

**Diffusion equilibrium in the bronchial tract.** While it is commonly agreed upon that the transport of inert gases between the alveolar space and end-capillary blood is perfusion-limited [78], a similar premise in the case of airway gas exchange remains less certain. Experimental and theoretical evidence appears to favor the view that although a diffusion equilibrium might be attained at the air-mucus interface [76, 37], the bronchial epithelial tissue can constitute an effective diffusion barrier between the mucus lining and the bronchial circulation. The magnitude of this diffusional resistance is probably substance-specific, with an inverse relation to molecular weight [72]. In this sense, the fractional bronchial blood flow $q_{bro}$ has to be interpreted as effective perfusion of those mucosal tissue layers for which an instantaneous equilibrium with air can be achieved [37]. Specifically, $q_{bro}$ might differ for distinct compounds. While in the special case of acetone animal models seem to support the assumption of a complete equilibration between airstream and bronchial circulation [72], in order to extend the validity of the model over a wider range of highly water soluble VOCs it might hence be necessary to include a diffusion limitation between these two compartments. The statistical significance of this generalization might then be assessed by employing residual based comparison techniques for nested models as described in [11, 10]. However, at the current stage of research and given the limited data on the behavior of breath trace gases having similar physico-chemical characteristics like acetone, we prefer to maintain a parameterization as parsimonious as possible.

**Continuous ventilation and temperature dependence.** Due to the tidal nature of breathing, bronchial as well as alveolar gas concentrations will vary throughout the breathing cycle, following a roughly periodical pattern during normal breathing. These variations can be captured by considering two separate mass balance systems, describing the dynamics of the associated concentrations for each inhalation and exhalation phase, respectively [45, 37, 5]. While such microscopic formulations are of paramount importance for resolving events within one individual respiratory cycle, when looking at the mid- to long-term behavior of breath VOCs we are rather interested in the global dynamics of the averaged compartmental concentrations. This approach leads to models of continuous ventilation with a unidirectional gas stream and has the enormous applicatory advantage of reducing the model structure to one single mass balance system. Similarly, variable temperature distributions in the bronchial compartment are represented by a single mean temperature $\bar{T}$ according to Equation (5), thereby lumping together our ignorance regarding the exact temperature profile along the airways. Different functional relationships might be investigated here.

In summary, this paper introduces a novel compartmental description of pulmonary gas exchange and systemic distribution of blood-borne, highly blood and
water soluble VOCs, which faithfully captures experimentally determined end-tidal acetone profiles for normal healthy subjects during free breathing in distinct physiological states. Additionally, the model has been validated in the framework of an external exposure scenario published in the literature and illuminates the discrepancies between observed and theoretically predicted blood-breath ratios of acetone during resting conditions, i.e., in steady state. In this sense, the present model provides a good compartmental perspective of acetone exhalation kinetics and is expected to contribute to a better understanding of distribution, transport, biotransformation and excretion processes of acetone in different functional units of the organism as well as their impact on the observed breath concentration. The emphasis of this work has been laid on deriving a sound mathematical formulation flexible enough to cover a wide spectrum of possible VOC behavior, while simultaneously maintaining physiological plausibility as well as a clear-cut physiological interpretation of the involved parameters. Care has been taken to keep the parameterization as parsimonious as possible, thereby constructing a well-founded “minimal” model respecting fundamental physiological boundary conditions, such as boundedness of the involved dynamic components and the existence of a globally asymptotically stable equilibrium state. While a complete sensitivity and practical identifiability analysis was beyond the scope of this paper and has to be matched to the particular experimental framework in which the model will be used, general concepts from structural and practical identifiability have been exploited in order to provide some indications regarding the information content of the observable breath level with respect to the endogenous situation.

Acknowledgements The research leading to these results has received funding from the European Community’s Seventh Framework Programme (FP7/2007-13) under grant agreement No. 217967. Julian King is a recipient of a DOC fellowship at the Breath Research Institute of the Austrian Academy of Sciences. Anton Amann greatly appreciates the generous support of the Member of the Tyrolean regional government Dr. Erwin Koler and the Director of the University Clinic of Innsbruck (TILAK) Mag. Stefan Deflorian. Gerald Teschl acknowledges support from the Austrian Science Fund (FWF) under Grant No. Y330.

A Appendix

A.1 Physical preliminaries

The following paragraphs briefly summarize the fundamental physical principles underlying the present model derivation. Further discussion largely follows standard textbooks on thermodynamics and biological mass transport; see for instance [75].

A.1.1 Diffusion and Henry’s Law

Fick’s first law of diffusion states that the net flux \( J \) quantifying diffusional transport of a gas species \( X \) between two spatially homogenous control volumes (i.e., compartments in which \( X \) can be postulated to behave uniformly) \( A \) and \( A' \) is proportional to the associated difference in partial pressure \( P_X \), i.e.,

\[
J = k_{\text{diff}}(P_X|A - P_X|A'),
\]

where the non-negative diffusion constant \( k_{\text{diff}} \) has dimensions of amount (in mol) divided by pressure and time. If the gas under scrutiny is in dissolved state rather than in gas phase, \( P_X \) is referred to as gas tension,
defined as the partial pressure that would be exerted by the gas above the liquid if both were in equilibrium (e.g., oxygen tension in blood). In the following we will omit the subscript \(X\). Equation (34) can also be expressed via a difference in concentrations. We will distinguish the following three cases:

(i). Firstly, if \(A\) as well as \(A'\) represent a gaseous medium and \(X\) can be treated as an ideal gas (which will be a general premise in the following) then, by the ideal gas law, \(P\) can be written as, e.g.,

\[
P_A = C_A RT,
\]

where \(C_A\) is the concentration in \(A\) and \(R\) and \(T\) denote the gas constant and absolute temperature, respectively. Hence,

\[
J = k_{\text{diff}} (C_A RT - C_A' RT) = k_{\text{diff}} (C_A - C_A'),
\]

where \(k_{\text{diff}}\) is defined by the above equation.

(ii). Similarly, if both \(A\) and \(A'\) are liquids, we can make use of Henry’s law stating that the amount of a gas \(X\) that can dissolve in a liquid at a particular temperature is directly proportional to the respective tension, viz.,

\[
C = H P.
\]

Here the solute- and solvent-specific Henry constant \(H = H(T)\) is inversely related to temperature. Substituting the last expression into Equation (34) yields

\[
J = k_{\text{diff}} (C_A H_A RT - C_A' H_A' RT) = k_{\text{diff}} (C_A - C_A' H_A' RT).
\]

(iii). Finally, let us assume that \(A'\) represents a liquid, while \(A\) is a gas volume. The prototypic example for this situation in our context is the blood-gas interface between respiratory microvasculature and the alveoli. In this case by combining (35) and (37) we arrive at

\[
J = k_{\text{diff}} RT (C_A - C_A' H_A' RT) = k_{\text{diff}} (C_A - C_A' H_A' RT).
\]

In case of a diffusion equilibrium, i.e., \(J = 0\), we deduce from Equation (35) that

\[
\frac{C_A'}{C_A} = \frac{H_A'}{H_A} = \lambda_{A' A},
\]

where the positive dimensionless quantity \(\lambda_{A' A}\) is the so-called partition coefficient between \(A'\) and \(A\) (e.g., \(\lambda_{\text{blood:fat}}\)). Accordingly, the reciprocal value is denoted by \(\lambda_{A A'}\). Analogously, from (39) the ratio between the liquid phase concentration and gas phase concentration of \(X\) in equilibrium is given by

\[
\frac{C_A'}{C_A} = \frac{H_A'}{H_A} RT.
\]

The partition coefficient \(H_A' RT\) is again denoted by \(\lambda_{A' A}\) (e.g., \(\lambda_{\text{blood:air}}\)) and is a measure of the solubility of the gas \(X\) in the solvent \(A'\), thereby allowing a classification of \(X\) as low or highly soluble with respect to \(A'\).

A.1.2 Compartmental mass transport

As above, consider two homogenous compartments \(A\) and \(A'\) with volumes \(V_A\) and \(V_A'\), respectively. Gas dynamics within these two compartments are governed by conservation of mass, stating that the rate at which the compartmental amount (e.g., \(V_A C_A\)) of \(X\) changes per time unit equals the rate of transfer into the compartment less the rate of removal from the compartment. For instance, the process of diffusion between \(A\) and \(A'\) as discussed before can be seen as a special case of this scheme with \(J\) (expressed in terms of concentrations) combining both diffusional inflow and outflow. Hence, if \(A\) and \(A'\) interact by diffusion and both are affected by additional input rates \(I\) and effluent output rates \(O\) (which might also comprise possible sources or sinks, respectively), then, assuming that the respective compartment volumes remain constant over time, the mass balances read

\[
V_A \frac{dC_A}{dt} = -J + I_A - O_A
\]
and
\[
V_A \frac{dC_A}{dt} = J + \dot{I}_A - \dot{O}_A, \tag{43}
\]
respectively. If the diffusion constant is sufficiently large to make \( J \) the clearly dominant term in the above equations, i.e., if diffusion is fast compared to the other dynamics influencing the system, \( C_A \) as well as \( \dot{C}_A \) will instantaneously approach their steady state values and a permanent diffusion equilibrium can be assumed to hold between \( A \) and \( A' \). In this case, by replacing \( C_A \) in (43) with \( C_A \dot{\lambda}_{A,A'} \) according to Equation (40), we see that the ODE system above simplifies to the one-dimensional differential equation
\[
(V_A \dot{\lambda}_{A,A'} + \dot{V}_A) \frac{dC_A}{dt} = \dot{I}_A - \dot{O}_A + \dot{I}_A - \dot{O}_A, \tag{44}
\]
The factor \((V_A \dot{\lambda}_{A,A'} + \dot{V}_A)\) can be seen as “effective” volume \( \dot{V}_A \) of the combined compartment. In the framework of physiological modeling this practice is routinely followed when setting up mass balance equations describing the kinetics of an inert gas \( X \) within a functional entity of the human body composed of two or more subcompartments. For perspective, consider the alveolar tract where the branching capillary network of the respiratory microvasculature promotes a fast equilibration between end-capillary blood \( A' \) and gas phase \( A \) even under moderate exercise conditions [78]. In other words, the transport of \( X \) within the alveolar tract is perfusion-limited rather than diffusion-limited. A common error here is to generally neglect the contribution of \( V_A \dot{\lambda}_{A,A'} \) to the effective alveolar volume as \( V_A \approx 0.15 \) l is argued to be much smaller than \( V_A \approx 3.1 \) l. Note, however, that this is only acceptable for low soluble inert gases with blood:air partition coefficient less than 1. Similarly, for most tissue groups, owing to their high degree of vascularization the intracellular space \( A \) can be assumed to rapidly equilibrate with the extracellular space \( A' \) (including the vascular blood and the interstitial space). In this case, a venous equilibrium is said to hold, i.e., the concentration \( C_A \) of \( X \) in blood leaving the tissue group is related to the actual tissue concentration \( C_A \) via Equation (40).

Note that the assumption of well-mixing is central to compartmentalization, and can often only be justified heuristically, either by examining factors that contribute to rapid distribution (e.g., convection) or by considering small volumes. If heterogeneity within a compartment is expected to be substantial or the main focus is on the spatial distribution of \( X \), general mass transport equations leading to PDEs have to be employed.

### A.2 A primer on local observability

For the convenience of the reader, the following section serves as a self-contained overview of the concept of (local) observability for analytic, single-output state-space models which are affine with respect to a given set \( \mathbf{u} = (u_1, \ldots, u_p) \) of bounded input functions \( u_j : [0, T] \to \mathbb{R}, \ j = 1, \ldots, p \), i.e., systems of the form
\[
\begin{align*}
\mathbf{x} &= \mathbf{g}(\mathbf{x}) + \sum_{j=1}^{p} \mathbf{g}_j(\mathbf{x}) u_j, \quad \mathbf{x}(0) = \mathbf{x}_0, \tag{45} \\
y &= h(\mathbf{x}).
\end{align*}
\]
Here it is assumed that \( \mathbf{x}(t) \) evolves within an open set \( M \subseteq \mathbb{R}^n \), \( h : M \to \mathbb{R} \) is real analytic, and the \( \mathbf{g}_j : M \to \mathbb{R}^n \) are analytic vector fields. We specialize to the case where the inputs are constant and hence can be interpreted as additional known parameters of the system
\[
\begin{align*}
\mathbf{x} &= \mathbf{g}(\mathbf{x}), \quad \mathbf{x}(0) = \mathbf{x}_0, \\
y &= h(\mathbf{x}). \tag{Σ}
\end{align*}
\]
Furthermore, it is required that a unique solution exists, e.g., by assuming that \( \mathbf{g} \) is globally Lipschitz on \( M \). Since \( \mathbf{g} \) is assumed to be analytic, the solution will also be analytic with respect to both \( t \) and \( \mathbf{x}_0 \). The output \( y = y(t, \mathbf{x}_0) \) is viewed as a function of the initial condition \( \mathbf{x}_0 \). It will be analytic as well. Equations (Σ) are a valid description for many biological processes under constant measurement conditions and represent a sufficient framework for the type of models and experiments considered here. Further exposition follows the treatment in [26,52,69] and [9].
Intuitively, observability (or a priori identifiability of the initial condition seen as unknown parameter of the model) characterizes the possibility to unambiguously determine $x_0$ (and hence the entire dynamics of $x$) from the exact knowledge of $y$, i.e., in the ideal context of error-free measurements. In particular, local observability states that $x_0$ can be instantaneously distinguished from any of its neighbors by using the system response. This is made explicit in the following definition.

**Definition 1** Let $V \subseteq M$. Two initial conditions $x_0, \tilde{x}_0 \in V$ are $V$-indistinguishable ($x_0 \sim_V \tilde{x}_0$) if for every $T > 0$ such that the corresponding trajectories remain in $V$ it holds that

$$y(t, x_0) = y(t, \tilde{x}_0)$$

for all $t \in [0, T]$. The initial conditions $x_0$ and $\tilde{x}_0$ are called indistinguishable if they are $V$-indistinguishable for any such $V$. A system is called locally observable at $x_0 \in M$, if there exists a neighbourhood $V \subseteq M$ of $x_0$ such that all points in $V$ are $V$-indistinguishable.

In other words, local observability characterizes the fact that $x_0 \rightarrow y(., x_0)$ is injective for arbitrarily small $T$. For the sake of illustration, consider the system

$$\begin{pmatrix} \dot{x}_1 \\ \dot{x}_2 \\ \dot{x}_3 \end{pmatrix} = \begin{pmatrix} -(x_2 + x_3)x_1 \\ 0 \\ 0 \end{pmatrix}, \quad y = x_1,$$

then

$$y = x_1, 0 \exp(-(x_2, 0 + x_3, 0)t).$$

Evidently, this toy model is not locally observable as the slightly perturbed initial conditions $\hat{x}_{2,0} := x_{2,0} + \varepsilon$ and $\hat{x}_{3,0} := x_{3,0} - \varepsilon$ result in exactly the same response $y$ for any $\varepsilon > 0$. If we interpret $x_1$, $x_2$, and $x_3$ as constant parameters, the above situation corresponds to a typical case of overparameterization, i.e., adding $x_3$ does not provide a more detailed description of the observable output. On the other hand, from the reversed viewpoint of estimation, this means that we will never be able to simultaneously estimate both $x_{2,0}$ and $x_{3,0}$ from the available process data but can only assess their sum. An equivalent characterization of this fact can be given in the context of sensitivities, i.e., the partial derivatives of the output with respect to the parameters. Indeed, we immediately see that

$$\frac{\partial y}{\partial x_{2,0}} - \frac{\partial y}{\partial x_{3,0}},$$

i.e., the sensitivities are linearly dependent, which is well-known to preclude a joint estimation of both parameters. Intuitively, the reason for this is that from the Taylor expansion of first order

$$y(x_{2,0}, x_{3,0}) \approx y(x_{2,0}, x_{3,0}) + \frac{\partial y}{\partial x_{2,0}} \varepsilon - \frac{\partial y}{\partial x_{3,0}} \varepsilon = y(x_{2,0}, x_{3,0}),$$

which again confirms that any small change in $x_{2,0}$ can be compensated for by an appropriate change in $x_{3,0}$ to yield almost the same output. A rigorous statement of this fact in the general unconstrained case is given in [12]. In contrast, as will be illustrated henceforth, local observability guarantees linear independence of the derivatives of $y$ with respect to $x_0$, which also is a necessary requirement for the broad class of numerical parameter estimation schemes based on the minimization of a given cost functional (e.g., in ordinary least squares methodology) using first-order information.

In more complex models, where an analytical expression for $y$ can usually not be derived, we have to resort to differential-geometric methods for investigating local observability. These tools will only be developed to the extent necessary for stating the final observability criterion, but are usually defined in a much more general framework than the one presented here.

For this purpose, first note that using the chain rule, the time derivative of the output equals the Lie derivative $L_gh$ of the output function $h$ with respect to the vector field $g$, i.e.,

$$\dot{y} = (\forall h \cdot g := L_gh$$
which again is analytic. Here, \( \nabla = (\frac{\partial}{\partial x_1}, \ldots, \frac{\partial}{\partial x_n}) \) denotes the gradient and \( \cdot \) denotes the scalar product in \( \mathbb{R}^n \). Similarly, by setting \( L_g^{(k)} h = h \) and iteratively defining \( L_g^{(k)} h = L_g^{(k-1)} h \) for \( k \geq 1 \) we immediately see that

\[
y^{(k)}(x_0) := \frac{\partial^k}{\partial t^k} y(t, x_0) \big|_{t=0} = L_g^{(k)} h(x_0).
\]

By our analyticity assumption on the system (\( \Sigma \)), the system response \( y \) is analytic and hence \( y \) can be represented by a convergent power (Lie) series around \( t = 0 \):

\[
y(t, x_0) = \sum_{k=0}^{\infty} \frac{t^k}{k!} y^{(k)}(x_0) = \sum_{k=0}^{\infty} \frac{t^k}{k!} L_g^{(k)} h(x_0).
\]  (47)

As a result, \( x_0 \) will only be \( V \)-distinguishable from \( x_0 \) for any \( V \) if we can find at least one \( k \geq 0 \) with the property that

\[
L_g^{(k)} h(x_0) \neq L_g^{(k)} h(x_0).
\]

This motivates the following two definitions. The linear space over \( \mathbb{R} \) of all iterated Lie derivatives

\[
\mathcal{L}(x_0) := \text{span} \{ L_g^{(k)} h(x_0), k \geq 0 \},
\]

is called the observation space of the model. Moreover, let \( \nabla \mathcal{L} := \{ \nabla H, H \in \mathcal{L} \} \). The famous Hermann-Krener criterion [26] now states that the model is locally observable at \( x_0 \) if

\[
\text{dim} \nabla \mathcal{L}(x_0) = n.
\]  (48)

Note that the sufficient condition [45] is equivalent to finding \( n \) indices \( k_j, j = 1, \ldots, n \) such that the gradients \( \nabla L_g^{(k_j)} h(x_0) \) are linearly independent. In fact, it turns out [7] that only the first \( n \) iterated Lie derivatives have to be computed in order to decide if such indices exist.

Summing up yields a simple rank criterion for local observability at a fixed point \( x_0 \), namely

\[
\text{rank} J(x) := \text{rank} \left( \nabla L_g^{(0)} h(x_0)^T, \ldots, \nabla L_g^{(n-1)} h(x_0)^T \right) = n.
\]  (49)

Note that for a linear system

\[
\begin{align*}
x &= Ax, \\
y &= Cx
\end{align*}
\]

the above condition simplifies to

\[
\text{rank} \left( C^T, A^T C^T, \ldots, A^{(n-1)} C^T \right) = n,
\]

which is the well-known criterion introduced by Kalman (see, e.g., [69]). Obviously, if the linear model is locally observable at one particular initial condition, local observability holds for every other initial condition as well.

To conclude this section let us assume that \( g \) and \( h \) belong to the field of rational functions in \( x = (x_1, \ldots, x_n) \) over \( \mathbb{R} \), i.e., \( g, h \in \mathbb{R}(x_1, \ldots, x_n) \) with no real poles in \( M \). Such rational models constitute an acceptable simplification for most biological processes describing mass balance systems and including standard kinetic mechanisms (e.g., Michaelis-Menten terms). Consequently, from the structure of the model [45] the entries of the matrix \( J \) defined in [49] belong to \( \mathbb{R}(x, u) \) and hence the rank of \( J(x, u) \) can be defined as the maximum size of a submatrix whose determinant is a non-zero rational function. This so-called generic rank can easily be determined by symbolic calculation software. Hence, if the generic rank of \( J \) is \( n \), the rank over \( \mathbb{R} \) of \( J(x, u) \) evaluated at one specific point \( (x, u) \in \mathbb{R}^{n+p} \) will also be \( n \) except for certain singular choices that are algebraically dependent over \( \mathbb{R} \). As these special points constitute a set of measure zero, we can conclude that if the generic rank of \( J(x, u) \) equals \( n \), almost every experiment corresponding to a choice of constant input variables \( u \) will suffice to immediately distinguish a particular unknown initial condition \( x_0 \) from its neighbors by giving rise to a distinct system output \( y \). Obviously, this is a necessary prerequisite in any attempt to identify \( x_0 \) from measured data. This powerful result is strengthened further by applying the Universal Input Theorem for analytic systems due to Sussmann [71].
ensuring that if a pair of unknown initial conditions can be distinguished by some (e.g., constant) input, it can also be distinguished by almost every input belonging to \( \mathcal{C}^\infty([0,T]), T > 0 \) (see also [81] for a rigorous statement as well as a self-contained proof). Note, that in the context of physiological models this is the appropriate class of functions for capturing a wide range of applicable physiological conditions.

Note that the line of argumentation remains valid for analytic \( g \) and \( h \). However, the generic rank in this case usually has to be estimated, e.g., by computing the rank of \( J \) evaluated at some randomly assigned points \((x, u) \in \mathbb{R}^{n+p}\). While from the above an affirmative outcome of the rank test guarantees that every initial condition within a neighborhood of the “true” value \( x_0 \) will yield a distinct output \( y \) when conducting a generic experiment with smooth inputs, the effective reconstruction of \( x_0 \) from partially observed and error-corrupted data remains a challenging problem. For instance, despite a positive result of the rank criterion, nothing can be said about the degree of linear independence between the derivatives of the output with respect to the initial conditions. In fact, the associated sensitivity matrix can still be rank-deficient in a numerical sense, indicating a high correlation among some of the components to be recovered. This is the context of practical identifiability or estimability by means of numerical estimation schemes, which will particularly depend on the specific experimental situation.
### A.3 Nomenclature

**Table 3** Basic model parameters and nominal values during rest; LBV denotes the lean body volume in liters calculated according to $\text{LBV} = -16.24 + 0.22 \text{bh} + 0.42 \text{bw}$, with body height (bh) and weight (bw) given in cm and kg, respectively [49].

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Symbol</th>
<th>Nominal value (units)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Compartment concentrations</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>bronchioles</td>
<td>$C_{\text{bro}}$</td>
<td>1 ($\mu$g/l) [65]</td>
</tr>
<tr>
<td>alveoli</td>
<td>$C_A$</td>
<td></td>
</tr>
<tr>
<td>arterial</td>
<td>$C_a$</td>
<td>1 (mg/l) [84,31]</td>
</tr>
<tr>
<td>mixed-venous</td>
<td>$C_{\text{lv}}$</td>
<td></td>
</tr>
<tr>
<td>liver</td>
<td>$C_{\text{tis}}$</td>
<td></td>
</tr>
<tr>
<td>inhaled (ambient)</td>
<td>$C_I$</td>
<td></td>
</tr>
<tr>
<td><strong>Compartment volumes</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>bronchioles</td>
<td>$V_{\text{bro}}$</td>
<td>0.1 (l) [49]</td>
</tr>
<tr>
<td>mucosa</td>
<td>$V_{\text{muc}}$</td>
<td>0.005 (l) [49]</td>
</tr>
<tr>
<td>alveoli</td>
<td>$V_A$</td>
<td>4.1 (l) [49]</td>
</tr>
<tr>
<td>end-capillary</td>
<td>$V_{C'}$</td>
<td>0.15 (l) [27]</td>
</tr>
<tr>
<td>liver</td>
<td>$V_{\text{liv}}$</td>
<td>0.0285 LBV (l) [49]</td>
</tr>
<tr>
<td>blood liver</td>
<td>$V_{\text{liv},b}$</td>
<td>1.1 (l) [50]</td>
</tr>
<tr>
<td>tissue</td>
<td>$V_{\text{tis}}$</td>
<td>0.7036 LBV (l) [49]</td>
</tr>
<tr>
<td><strong>Fractional blood flows</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>fractional flow bronchioles</td>
<td>$q_{\text{bro}}$</td>
<td>0.01 [44]</td>
</tr>
<tr>
<td>fractional flow liver</td>
<td>$q_{\text{liv}}$</td>
<td>0.32 [49]</td>
</tr>
<tr>
<td><strong>Partition coefficients at body temperature</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>blood:air</td>
<td>$\lambda_{\text{b:air}}$</td>
<td>340 [118]</td>
</tr>
<tr>
<td>mucosa:air</td>
<td>$\lambda_{\text{muc:air}}$</td>
<td>392 [70,36]</td>
</tr>
<tr>
<td>blood:liver</td>
<td>$\lambda_{\text{b:liv}}$</td>
<td>1.73 [50]</td>
</tr>
<tr>
<td>blood:tissue</td>
<td>$\lambda_{b:\text{tis}}$</td>
<td>1.38 [7]</td>
</tr>
<tr>
<td><strong>Metabolic and diffusion constants</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>linear metabolic rate</td>
<td>$k_{\text{met}}$</td>
<td>0.0074 (l/kg$^{0.77}$/min) [fitted]</td>
</tr>
<tr>
<td>saturation metabolic rate</td>
<td>$V_{\text{max}}$</td>
<td>0.31 (mg/min/kg$^{0.75}$) [36]</td>
</tr>
<tr>
<td>apparent Michaelis constant</td>
<td>$k_m$</td>
<td>84 (mg/l) [50]</td>
</tr>
<tr>
<td>endogenous production</td>
<td>$k_{\text{pr}}$</td>
<td>0.19 (mg/l) [fitted]</td>
</tr>
<tr>
<td>stratified conductance</td>
<td>$D$</td>
<td>0 (l/min) [fitted]</td>
</tr>
</tbody>
</table>

### References