Breath Analysis as a Technique in Clinical Chemistry

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Breath possesses unique advantages as a specimen for clinical chemical analyses, including the continuous equilibrium of gases and volatile substances between expired alveolar air and the pulmonary blood circulation. Substances amenable to analysis in breath include O2, CO2, CO, and other gases, volatile organic compounds, and many drugs with sufficiently high vapor pressures at physiological temperatures. Practical aspects of breath sampling and breath analysis are discussed, exemplified by breath-alcohol analysis. The requirements for obtaining breath samples in equilibrium with the pulmonary blood circulation are delineated, and experimental data are presented for the significant breath-sample characteristics bearing on design of breath collection and storage systems (end-expiratory temperature, breath volumes).

Additional Keyphrases: toxicology • alveolar and dead-space gas • analysis of volatiles

The adult lung is a most efficient boundary organ—as might be anticipated from the presence of $3 \times 10^8$ alveoli with a total surface area of about 70 m² in contact with capillaries, pulmonary blood flow consisting of the total cardiac output averaging about 6 liters/min, and a mean daily respiratory volume of $10^4$ liters (1). It follows that the breath should faithfully reflect the body burden and the concentration in the pulmonary capillary circulation of those substances that are capable of ready transfer across the alveolar–capillary membrane.

For many substances distribution equilibrium is, in fact, attained between the pulmonary circulation and the alveolar air. While recognition of this fact has led to widespread practical application of breath analysis to determination of the plasma $p_{CO_2}$ and estimation of the blood-alcohol concentration, only occasional use has been made of breath as a specimen for other analyses. With the recent development of a variety of rapid and sensitive chemical detectors, it is now possible to overcome the prior practical limitation of the low concentration in breath of many substances of clinical interest. Hence, breath analysis could become the preferred procedure for analysis for many normal body constituents, metabolic products, and exogenously introduced compounds.

This presentation seeks to delineate certain aspects of the current state-of-the-art of breath analysis for substances of clinical significance and to stimulate further advances in this field by indicating likely avenues for developments.

Breath Analysis—General Considerations

Breath, as a physiological specimen for routine use, offers several unique advantages: rapid, simple, non-traumatic and frequently repeatable sampling; real-time functional distribution equilibrium with the pulmonary circulation for many substances; potential for rapid analyses with greatly simplified primary separation steps, because the substances of interest are present in the gaseous or vapor state; and capability for immediate “field” analysis by portable, bedside devices for many substances, or for sample retention and subsequent laboratory analysis. There are also several inherent limitations: only substances capable of penetrating the capillary–alveolar membrane are present, often in low concentrations; cooperation is required from the patient for proper specimen collection (to an extent varying with the nature of the required breath specimen); precautions may be required to avoid sample contamination from extraneous sources such as eructation; and sampling and analytical results for some substances are potentially affected by respiratory disease or pulmonary insufficiency.

A wide range of substances of clinical significance has been identified as present in breath, and practical methods have been developed for analysis of many such substances in breath specimens. Representative current schemes for a variety of breath analyses (2–23) are listed in Table 1, and suggested applications that are considered practical at present are functionally grouped in Table 2.

Analysis of breath for alcohol, primarily to determine the absence or presence of alcoholic influence in traffic-law enforcement, is probably the most common application of breath analysis and has attained a high state of technical sophistication, especially with respect to the instrumental detection and quantitation of ethanol. Table 3 itemizes the major methods that have been used for alcohol analysis in commercially available instruments, to illustrate how various...
The methods are listed in chronological order of appearance during the past 40 years, with the six most recent having been utilized only within the past five years. In several automated breath-alcohol instruments (28–30), manual manipulations have been virtually eliminated and the results are obtained by mechanized, internally-controlled sequential operations. It has been possible recently to develop several highly reliable, sophisticated, yet moderately priced breath-alcohol devices. Table 4 lists various advanced features of current commercial devices for breath-alcohol analysis. Although not all of these features are found in any single current production instrument, they do illustrate the degree of success already attained in dedicated instrumentation for breath analysis in a cost range of $1000–$3000 (31). It deserves mention that these developments occurred for an application with demanding special requirements, including intended use by nontechnical personnel, in nonlaboratory environments, and under the probative value constraints of forensic sciences.

It is often necessary or desirable to collect breath specimens or selected breath components for subsequent analysis in a different location, or for retention for various purposes. Many procedures have been developed for this purpose, and typical systems for retention of whole-breath specimens or of breath components are listed in Table 5. These clearly fall into two principal categories: (a) Direct temporary storage of whole-breath (in collapsible polymeric or laminated containers, gas pipettes and syringes, vacuum canisters, or indium capsules), and (b) separation and retention by physical or chemical means (condensation, adsorption, or adsorption on suitable columns or in appropriate liquid reagents) of selected breath components of interest. For the latter category, it is necessary to measure or fix the breath volume from which the desired component is removed and stored. This can readily be accomplished with a field collection apparatus (41, 45) combined with suitable absorption or adsorption columns or by modifications of the indium encapsulation technique (26).
Table 4. Typical Features of Current Devices for Breath-Alcohol Analysis
- End-expiratory breath sampling
- Analysis by GC, IR, or solid-state sensing
- Direct digital concentration readout and result printout
- Rapid results (5-15 s) and short turn-around time (30-60 s)
- Direct or stored-specimen analysis capability
- Portable, self-contained, hand-held devices

Table 5. Typical Schemes for Collection and Storage of Breath Specimens or Breath Components

<table>
<thead>
<tr>
<th>Device or procedure</th>
<th>Application</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metal-Foil container</td>
<td>Alcohol</td>
<td>(32)</td>
</tr>
<tr>
<td>Rubberized &quot;Douglas&quot; bag</td>
<td>Respiratory gases</td>
<td>(33)</td>
</tr>
<tr>
<td>Polymeric film bag</td>
<td>Metabolic products</td>
<td>(34)</td>
</tr>
<tr>
<td></td>
<td>Alcohol</td>
<td>(35)</td>
</tr>
<tr>
<td></td>
<td>Halogenated hydrocarbons</td>
<td></td>
</tr>
<tr>
<td>Condensation of volatiles</td>
<td>Methanol</td>
<td>(37)</td>
</tr>
<tr>
<td></td>
<td>Volatile fatty acids</td>
<td>(7)</td>
</tr>
<tr>
<td>Glass gas pipette</td>
<td>Carbon monoxide</td>
<td>(38)</td>
</tr>
<tr>
<td></td>
<td>Alcohol</td>
<td>(39)</td>
</tr>
<tr>
<td></td>
<td>Trichloroethylene</td>
<td>(40)</td>
</tr>
<tr>
<td>Column adsorption on Activated charcoal</td>
<td>Volatile compounds</td>
<td>(80)</td>
</tr>
<tr>
<td>Ascarite</td>
<td>Carbon dioxide</td>
<td>(84)</td>
</tr>
<tr>
<td>Calcium chloride</td>
<td>Alcohol</td>
<td>(41)</td>
</tr>
<tr>
<td>Magnesium perchlorate</td>
<td>Alcohol</td>
<td>(42)</td>
</tr>
<tr>
<td>Silica gel</td>
<td>Alcohol</td>
<td>(43)</td>
</tr>
<tr>
<td>Indium encapsulation</td>
<td>Alcohol</td>
<td>(86)</td>
</tr>
<tr>
<td>Vacuum container</td>
<td>Alcohol</td>
<td>(44)</td>
</tr>
</tbody>
</table>

Breath Sampling

Proper breath sampling is fundamental to all applications of breath analysis, but this aspect of breath analysis has often been neglected as attention has been focused on the detection and quantitation of a component in the sample. Theoretically, several types of expired breath specimens are suitable for chemical analysis: expired alveolar air, end-tidal air, end-expiratory air, and re-breathed air. Mixed expired air is generally not suitable for breath-component analysis inasmuch as it contains variable proportions of alveolar air and of dead-space air, which does not reflect the body burden or pulmonary blood contents of respired compounds because a considerable part of the inspired air comes to rest in the conducting air passages and does not participate in (alveolar) gaseous exchange. For the same reason, CO₂ measurements cannot be used to determine the proportion of alveolar air in a mixed expired air sample (31).

In highly simplified terms, an expirate may be considered to consist of physiological dead-space gas and alveolar gas. In subjects without respiratory disease or pulmonary dysfunction, the composition of the expired air is, therefore, approximately constant after the dead-space gas has been expelled, reflecting the alveolar-air phase of expiration (the so-called "alveolar plateau"). The practical problem is how to know when this has occurred during any single expiration or how to arrange matters so that breath-specimen collection occurs only after all physiological dead-space gas has been discarded. It is impossible consistently to obtain expired alveolar air by collecting the breath remaining after discard of any fixed volume of dead-space air. However, four different schemes can provide practical means for determining when the alveolar plateau has been reached during a continuous expiration, or for securing breath specimens that are essentially alveolar or equivalent to alveolar air in composition:

- The expiration can be monitored with a rapidly responding analysis device sensitive to a suitable breath component (e.g., carbon dioxide or oxygen) until breath composition becomes essentially constant, or the rate of composition change approaches zero.
- A phenomenon that is known to have a time course that parallels changes in breath composition (e.g., breath temperature) can similarly be monitored with rapidly responding instrumentation.
- A small or moderate breath volume (e.g., 250 ml or less) can be collected at or near the end of a prolonged, uninterrupted full expiration, by means of a device that traps an end-expiratory specimen.
- A small or moderate breath volume can be exhaled into a suitable collapsible container, and inspired and expired several times until this "re-breathed air" has attained a composition identical or equivalent to alveolar air, with respect to the component of interest.

In the practical application of such breath sampling schemes, consideration must be given to the presence of water vapor in breath specimens, and to breath temperature, volume, and pressure factors. Experimental data bearing on design and use of such breath sampling systems follow.

Materials and Methods

Studies were conducted on healthy volunteer human subjects (40 δ, age 21–50 years; 15 ơ, age 23–46 years) by methods previously described (46) for measurement of breath volumes and temperature.

Composition of expired air in each expiration of healthy subjects was measured by continuous mass-spectrometric analysis, in the single-breath test mode, with a Model 1100 Medical Gas Analyzer (Perkin-Elmer Corp., Medical Instruments, Pomona, Calif. 91767). The CO₂ and O₂ content of the expired air at constant breath flow rates were recorded, in percent by volume, against time.

Carbon dioxide content of expired breath was de-
terminated in physiological samples (at body temperature, without removal of water vapor) and under ambient conditions\(^1\) by nondispersive infrared absorbance measurement with a Model KK146 Godart Capnograph (Instrumentation Associates, Inc., New York, N. Y. 10023). The capnograph response for each complete expiration was recorded on a Model 194 10-inch potentiometric strip-chart recorder (Honeywell, Inc., Ft. Washington, Pa. 19034). After a period of resting-state normal respiration, the nonfasting, standing subjects performed a maximum exhalation following a normal inspiration. The breath CO\(_2\) was monitored, using a by-pass mouthpiece, with the flow-through cell of the capnograph (0.44-ml sample-cell volume, 2 liters/min flow-rate, 80 ms full-scale response).

**Results**

Key characteristics of the principal method we used in these studies, including their precision in nonbiological reference systems, have been previously reported by us (46).

Table 6 summarizes the breath temperature and breath volume data. All temperatures shown were recorded at the end of an expiratory vital-capacity maneuver\(^2\) and are, therefore, end-expiratory temperatures. The breath volume data consist of the maximum expiratory volume measured by an expiratory forced vital capacity maneuver\(^2\) in standing subjects, and of the maximum expiratory volume in the same subjects after a normal inhalation. Volumes shown are for breath at physiological temperature, essentially saturated with water vapor, and at ambient barometric pressure.

A typical single-breath mass-spectrometric expirogram for CO\(_2\) and O\(_2\) is shown in Figure 1.

Figure 2 illustrates a typical simultaneous in vivo recording of expiratory temperature and expiratory CO\(_2\) during a single continuous full expiration after a normal inhalation. The same simultaneous measurements for an in vitro system are shown in Figure 3, illustrating the thermistor response to a nearly instantaneous transfer from a 23.3 °C to a 34.5 °C medium and capnograph response to nearly instantaneous change in gas flow from CO\(_2\)-free air to medical gas of nominal 5.3% (by vol) CO\(_2\) content flowing at 2 liters/min.

**Discussion**

The several methods used in this study have good precision, as previously reported (46), and the variability found in the human subjects studies is, thus, not attributable to the methods of measurement.

The basic breath-sample characteristics shown in Table 6 are of considerable practical significance for breath sampling and breath-storage procedures. Information regarding the temperature of expired breath is required to determine the minimum constant temperature at which breath collection, storage, and rebreathing devices must be kept in order to prevent condensation of water vapor and consequent loss of condensable or water-soluble breath components, and to apply appropriate Charles’ law corrections to the breath volumes for calibration and analysis. This information is also of significance in applica-

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1. The ambient barometric pressure over the duration of these measurements varied from 979 \(\times\) 10\(^5\) to 987 \(\times\) 10\(^5\) Pa (734.7 to 740.7 Torr) (corrected for temperature and gravity) and room temperatures varied between 24.0 and 24.2 °C. The instruments were frequently recalibrated, at ambient conditions, at the time breath samples were analyzed.

2. Vital capacity (VC) is the volume of air that can be expelled during a maximal expiration after a maximal inspiration; Forced vital capacity (FVC) is the VC maneuver performed with expiration as forceful and rapid as possible. In normal subjects, VC equals FVC.
Table 6. Human Breath Characteristics: End-Expiratory Temperatures,
Measured at the Mouth, and Expiratory Volumes

<table>
<thead>
<tr>
<th>Subjects</th>
<th>n</th>
<th>End-expiratory temperature, °C</th>
<th>Forced vital capacity, ml</th>
<th>Maximum exhalation after normal inhalation, ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Men</td>
<td>40</td>
<td>32.41-35.57</td>
<td>2245-6550</td>
<td>1180-4550</td>
</tr>
<tr>
<td>Women</td>
<td>15</td>
<td>33.53-35.69</td>
<td>1825-3200</td>
<td>1480-3000</td>
</tr>
<tr>
<td>Total</td>
<td>55</td>
<td>32.41-35.69</td>
<td>1825-6550</td>
<td>1180-4550</td>
</tr>
</tbody>
</table>

Fig. 3. Typical simultaneous recording of temperature and CO₂ for an in vitro system, illustrating the rapid response to essentially instantaneous changes in the measured variables.

attained, presumably indicative of the alveolar plateau. Any portion of the remaining breath will have essentially the same composition and can be used for breath analysis. From the data in Table 6, a mean breath volume of 2690 ml must be discarded before sampling to obtain a substantially expired alveolar air sample in forced vital capacity sampling; and a mean breath volume of 1818 ml must be discarded for the same result in sampling a maximum exhalation after normal inspiration. These data demonstrate that an earlier accepted authoritative statement that “an expiration of 400 cc will completely wash out the respiratory dead space of a normal resting adult, which is estimated as 125 to 200 cc. Therefore, in such persons all the air expired after the first 400 cc is alveolar air” (47) is quite incorrect and misleading. The data in Table 6 show clearly that no single fixed volume discard can assure collection of expired alveolar breath, and even end-expiratory breath collection schemes thus require either knowledge of the subject’s actual expiratory volume at the time of sampling or arrangements to retain the utmost terminal portion of an uninterrupted expiration.

We had observed that the rise in breath temperature, measured with rapidly-responding instruments, approximately paralleled the rise in CO₂ content (or alcohol content after alcohol intake) of the breath during continuous full expiration. As illustrated in Figure 2, which is typical of the findings, both end-expiratory temperature and end-expiratory CO₂ attain their respective alveolar plateaus at or near the same time. Figure 3 illustrates performance of the same temperature and CO₂ sensors in tracking abrupt changes in an in vitro system. The rapid rise to the respective final values indicates that the slower responses shown in Figure 2 are the result of biological phenomena rather than of instrumental artefacts. The slightly slower initial increase in the breath temperature, compared with the time-course of CO₂, is probably largely attributable to the differences in response time of the respective sensors. The capnograph has a response time of 80 ms full-scale (in the 0–10% by vol range), while the time constant of the thermolinear probe, designed for air temperature measurements, is given as 600 ms.

3 Time constant, the standard measure of temperature-probe response time, is the time required for a probe to indicate 63% of a newly impressed temperature change.
It thus appears feasible to use breath-temperature measurement by means of rapidly responding device to indicate when the alveolar plateau has been reached and the sample for breath analysis should be taken. The regular and predictable nature of the breath-temperature–time curve would allow, by means of relatively simple electronic circuitry, the rate of temperature change or a function thereof to trigger valving for automated collection of end-expiratory, substantially alveolar breath samples without knowledge of the available breath volume and independent of technician judgment or skill or of complete subject cooperation. A coincident end-expiratory breath temperature indication could be used, if desired, to signal the possibility of undetected abnormalities in deep-body temperature that might impair or vitiate the validity of an instrument calibration or result conversion dependent on biological factors.

Once proper sampling is achieved, attention can again be directed to advances in recognition, identification, and quantitation of breath components. Systematic observations on exhalation of drugs were first recorded by Cushny (48) in 1910, but the field has been largely neglected since. The respiratory tract is a major entry route into the body for gases and vapors, and it seems reasonable to consider that this transport is reversible for many more substances than so far shown. High orders of sensitivity and, often, specificity are required for analysis in breath of those substances only present in trace quantities. Stewart et al. [e.g., Stewart and Erley (17)] have shown that infrared analysis of breath with cells of long pathlength can be used effectively to detect halogenated hydrocarbons, alcohols, ethers, ketones, and gases, and this technique can undoubtedly be refined and extended with modern instrumentation. Gas chromatography is an inherently useful procedure for analysis of many organic compounds and could well become universally usable for analysis in breath of compounds in ng/liter concentrations by use of enrichment or concentrating techniques based on physical adsorption, molecular sieving, or easily reversible chemical reactions. Zlatkis et al. (49) have recently reported use of a new porous polymer adsorbent, Tenax GC, which efficiently adsorbs and desorbs a large number of volatile organic compounds. This adsorbent has been used for air and breath sampling with an adsorbent trap that is subsequently inserted directly into a gas-chromatograph injector inlet for desorption and analysis. Such a system could facilitate breath analysis for volatiles regardless of their original concentration, by repetitive sampling of fixed volumes of end-expiratory breath to the extent required. Demonstration of reproducible patterns in particular metabolic aberrations or disease states could then permit useful application of breath analysis by gas chromatography even in the absence of identification of all components found.

Mass-spectrometric analysis holds great promise for breath analysis. Combined gas chromatography-mass spectrometry has been shown capable of simple and rapid qualitative and quantitative analysis of ethanol and other low-molecular-weight compounds (50), with quantitation accuracy of better than ±5%, ample for many biomedical applications. In a preliminary feasibility study, Green (51) demonstrated that a mass spectrometer coupled to multi-stage Llewellyn–Arnold separators as the enrichment device could be used to detect diethyl ether, dimethyl sulfoxide, ethanol, and methylparafynol in breath after introduction into the body of small quantities. It was also possible to identify and quantitate many other common drugs including cannabinoids, chlorpromazine, methamphetamine, opiates) in air at µg/liter or ng/liter concentrations. It has now been repeatedly shown that drugs and their metabolites and other physiologically important compounds can be identified and quantitated in nanogram quantities in microliter samples of such specimens as plasma, urine, breast milk, and amniotic fluid (52, 53). Such quantities of many compounds are likely to be present in breath after therapeutic intake or from metabolic sources. Breath therefore becomes a practical specimen for a large number of substances of clinical interest, probably including most compounds with melting points to 75 °C or boiling points to 250 °C, as well as sublimating compounds. Such drugs as the amphetamines, chloral hydrate, ethchlorvynol, glyceryl trinitrate, and methadone should be readily detectable in breath, and recent developments in simplified mass spectrometry and mass fragmentography for drug analysis in biological samples (54, 55) should significantly advance these studies.

It also seems probable that solid-state devices such as the Taguchi gas-sensor, now used successfully for breath-alcohol screening tests (56), will soon be made more specific, to complement their present rapidity of response and high sensitivity, and thus become useful for many additional breath analysis applications. Because of their rapid response, both mass spectrometry and these sensors, as well as infrared absorbance measurement for certain compounds, lend themselves well to real-time dynamic breath measurements. Peak-holding techniques can then be used to sense attainment of the alveolar plateau by following the breath concentration of the measured compound and to indicate its alveolar plateau concentration.

Breath analysis is a clinical chemical technique of great merit and exceptional potential.

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References


