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The Use of Light Scattering Photometry in Aerosol Medicine

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ABSTRACT

Light scattering photometers in combination with monodisperse test aerosols permit continuous recording of the aerosol concentration close to the entrance of the respiratory tract during the whole course of a breathing cycle. Data of this kind together with simultaneous records of the respiratory volumes provide new possibilities in lung function tests. Different inhalation apparatuses based on light scattering have been developed in the last decades. They are classified according to their principle of operation. Requirements with respect to the aerosols are worked out and potential sources of error arising in inhalation photometry are analyzed on the base of experiments.

A novel 2-Mode-Photometer is described which can be operated in the photometric or analog mode at aerosol concentrations above 100 particles per cm^3 and in the counting mode below 10 particles per cm^3. Its applicability for various kinds of aerosol inhalation studies is demonstrated. With this device total deposition on human subjects can be measured at very low number concentrations. Under certain assumptions it can be also used as a sizing instrument for aerosol particles in the respiratory flow immediately at the entrance of the respiratory tract.

INTRODUCTION

Since the pioneering work of Altshuler et al. (1957) light scattering photometers in combination with monodisperse test aerosols are used in aerosol research to measure total deposition of aerosol particles in the human respiratory tract as well as to study gas mixing processes and deposition mechanisms inside the airways (Altshuler et al. 1959; Muir and Davies, 1967; Muir 1967; Altshuler 1969; Davies et al. 1972; Giacomelli-Maltoni et al. 1972; Heyder et al. 1973; Heyder et al. 1975; Gebhart et al. 1981; Heyder et al. 1987).

The photometer technique permits continuous recording of the aerosol concentration close to the mouth during the whole course of a breathing cycle. From such records and simultaneous measurements of respiratory volumes and flow rates the amount of aerosol in successive fractions of inspired and expired air

can be evaluated. Data of this kind are of interest since they can provide new possibilities for lung function tests.
In the present paper the possibilities and limitations of the photometer technique are analyzed on the base of experimental results. Various sources of error arising from photometric measurements during aerosol breathing are discussed. If properly used in association with well-defined aerosols this technique proves as a useful tool in lung investigations which allows a high degree of automation. Different photometric arrangements and applications presented may have a stimulating effect to introduce this technique not only in aerosol laboratories but also in the field of clinical research.

CLASSIFICATION OF THE TECHNIQUES

Different devices based on the principle of light scattering have been developed in the last decades to evaluate the number of particles in inspired and expired air of a subject. Photometric arrangements which measure the aerosol concentration in the main stream of inspired and expired air immediately at the entrance of the respiratory tract are in the following called on-line systems. They have the advantage that particle losses in connecting tubes and valves do not influence the results. The volume between mouthpiece and sensitive area of the photometer can be kept below 10 cm^3. Photometric measurements a certain distance away from the respiratory tract, on the other hand, have to take into account particle losses in tubes and valves which depend on particle size as well as on the geometry and flow pattern in the apparatus.

On-Line Closed Circuit System

This technique has been described by Muir and Davies (1967) and by Davies et al. (1972). Its principle of operation is shown in Figure 1.

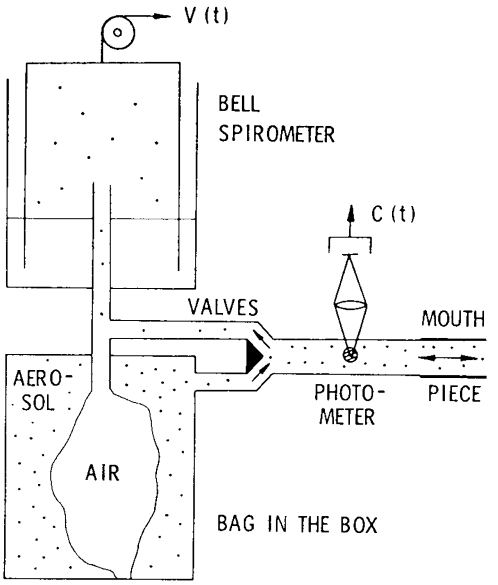


FIGURE 1. Schematic Diagram of the On-line Closed Circuit System.

A monodisperse aerosol is filled in a rigid chamber containing a thin rubber bag connected to a water-filled spirometer. The aerosol is inhaled from the chamber and exhaled through the spirometer into the rubber bag. Inspired and expired air are separated automatically by spring-loaded poppet valves at the end of the inhalation and exhalation path. Due to this closed circuit the total volume of air in the chamber remains constant and the spirometer indicates both the inspired and expired volumes, V(t). The aerosol concentration, c(t), is measured close to the entrance of the respiratory tract by a photometer and recorded alongside the spirometer signal delivered by a potentiometer circuit which includes the suspending wire of the bell. By turning a tap the subject can inhale filtered air instead of aerosol from a second chamber which is also part of the closed circuit system.
The amount of aerosol inhaled is obtained by multiplying the inhaled volume, V_i, by the constant concentration, co, of inhaled aerosol. The amount of aerosol exhaled is obtained by graphical summation of the aerosol contained in successive fractions of expired air. The ratio of the number of expired to inspired particles per breath is thus given by:

$$\frac{Ne}{Ni} = \frac{\sum_{1}^{m} \nu \, , \, c_\nu \cdot \Delta V_\nu}{c_o \, V_i} \qquad (1)$$

where m is the number of fractions in which the volume of expired air is devided and c_ν is the concentration in fraction ΔV_ν. The on-line closed circuit system indicates the volumes of a breathing maneuvre directly but the labour in analysing the records is considerable.

On -line Open Flow System-Analog Mode.

This system has been introduced in aerosol inhalation by Heyder et al. (1975; 1980). Its principle of operation is illustrated in Figure 2.

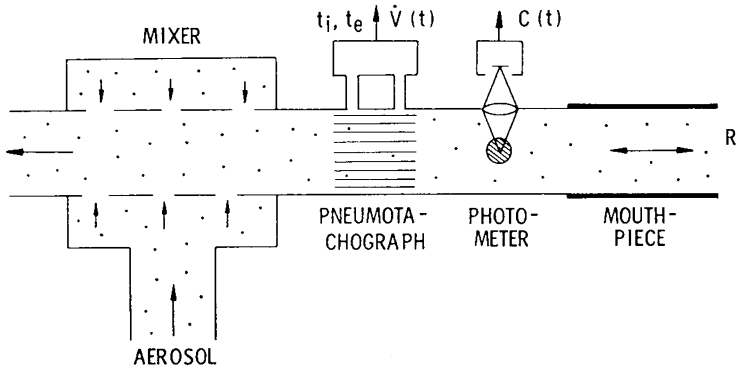


FIGURE 2. Schematic Diagram of the On-line Open Flow System Operated in the Analog Mode.

A continuous stream of a monodisperse aerosol passes through a mixing chamber to obtain a uniform particle concentration over the cross section of the inhalation-exhalation channel. The volumetric flow rate of the aerosol stream always exceeds the respiratory flow rate so that the exhaled aerosol is continuously removed from the mixer. Only about 30 cm^3 of aerosol corresponding to the volume between mouthpiece and mixer remain in the inhalation-exhalation-channel. From continuous recordings of flow rate, $\dot{V}(t)$, with a pneumotachograph and of particle concentration, c(t), with a photometer close to the mouth the ratio of the number of expired to inspired particles per breath, $\frac{Ne}{Ni}$, is calculated by:

$$\frac{Ne}{Ni} = \frac{\int_{(te)} \dot{V}(t)\ c(t)\ \delta t}{\int_{(ti)} \dot{V}(t)\ c(t)\ \delta t} \qquad (2)$$

where ti(te) is the inspiratory (expiratory) period.
For this purpose the electrical analog signals of the photometer and the pneumotachograph are fed to a computer for multiplication and integration. At the end of an inhalation experiment the inspired and expired volumes and the number of inspired and expired particles are printed out for each breath. The on-line analog system allows a high degree of automation but needs a careful calibration and adjustment of the photometer and the pneumotachograph.

On-line Open Flow System - Counting Mode.

An aerosol photometer which counts and classifies individual particles in the respiratory flow immediately at the entrance of the respiratory tract has been developed by Gebhart et al. (1980) and used by Heyder et al. (1980) for measurements of total deposition. Its principle of operation is demonstrated in Figure 3.

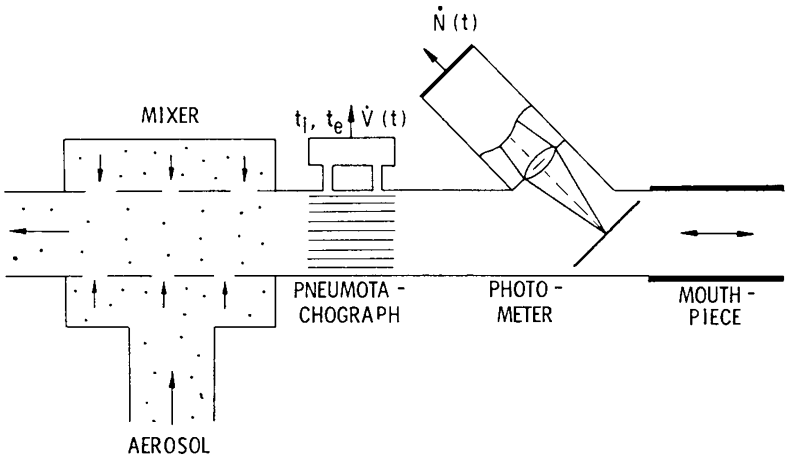


FIGURE 3. Schematic Diagram of the On-line Open Flow System Operated in the Counting Mode

The system is supplied with aerosol (monodisperse or polydisperse) via a mixing chamber like in Figure 2. The sensitive area of the photometer is formed by a sheet of Laser-light, which covers almost the whole cross section of the inhalation-exhalation-channel and is inclined at a certain angle to the direction of the aerosol flow.

A particle traversing the light sheet produces a flash of scattered light which is received by a photomultiplier and converted into an electrical pulse. The pulse is analyzed according to its height and stored in a multichannel device whereby the signals during inspiration and expiration are collected separately. The time signals controlling the period of inspiration, ti, and expiration, te, and the volumetric flow rate, $\dot{V}(t)$, are obtained from a pneumotachograph. A computer counts the number of signals during inspiration and expiration and calculates the ratio of the number of expired to inspired particles per breath according to:

$$\frac{Ne}{Ni} = \frac{\int_{(te)} \dot{N}(t)\ \delta t}{\int_{(ti)} \dot{N}(t)\ \delta t} \qquad (3)$$

where $\dot{N}(t)$ is the particle stream (count rate) monitored by the photometer. The inspired and expired volumes, Vi resp. Ve, are achieved by integrating the volumetric flow rate $\dot{V}(t)$. At the end of a breathing experiment the quantities, Vi, Ve, Ni, Ne, $\frac{Ne}{Ni}$, $1 - \frac{Ne}{Ni}$ are printed out for each breath together with their means $\pm$ SD.
Since the sensitive area of the photometer does not cover the whole cross section of the inhalation-exhalation-channel Ni and Ne are not the total numbers of particles inhaled and exhaled but a representative fraction which is equal for inhalation and exhalation so that the ratio as expressed by equ. 3 is measured correctly. The on-line counting system works straight forward and can be combined directly with modern computer technique. For correct measurements the flow velocity through the sensitive area, A, has to be representative for the whole cross section of the aerosol channel. This is achieved by a sheet-like sensing volume which covers a large part of the cross section of the channel. To avoid counting losses due to coincidences the particle number concentration has to be kept below a certain limit.

Stationary Open Flow System

A system of this kind has been described by Giacomelli-Maltoni et al. (1972) and applied by Heyder et al (1978) for intercomparison measurements of total deposition. Its principle of operation is explained in Figure 4. A continuous stream of a monodisperse aerosol passes a mixing chamber to obtain a uniform particle concentration over the cross section of the aerosol channel. Before a breathing experiment the mouthpiece is closed and aerosol is sucked through a photometer by a pump via a spirometer. The flow rate through the photometer is constant and corresponds to the mean flow rate of aerosol in the following experiment. The photometer then indicates the particle number concentration, xc_o, where c_o is the mean particle number concentration available for inhalation and x is the mean probability that a particle escapes deposition while it is transported from the mouthpiece to the photometer. During breathing inspired and expired aerosols are separated by a system of valves close to the mouthpiece. The expired aerosol is also sucked through the photometer with the same flow rate as used for the measurement of xc_o. A pneumotachograph indicates the inspiratory volumetric flow rate which after integration yields the tidal volume. The bell spirometer serves as a mixing chamber for the expired aerosol so that after a number of breaths a stationary state is achieved and the photometer reads a constant aerosol concentration, xc, where c is the mean expired aerosol concentration. The ratio of the number of expired to inspired particles during steady

state breathing is then given by:

$$\frac{Ne}{Ni} = \frac{xc}{xc_o} = \frac{c}{c_o} \qquad (4)$$

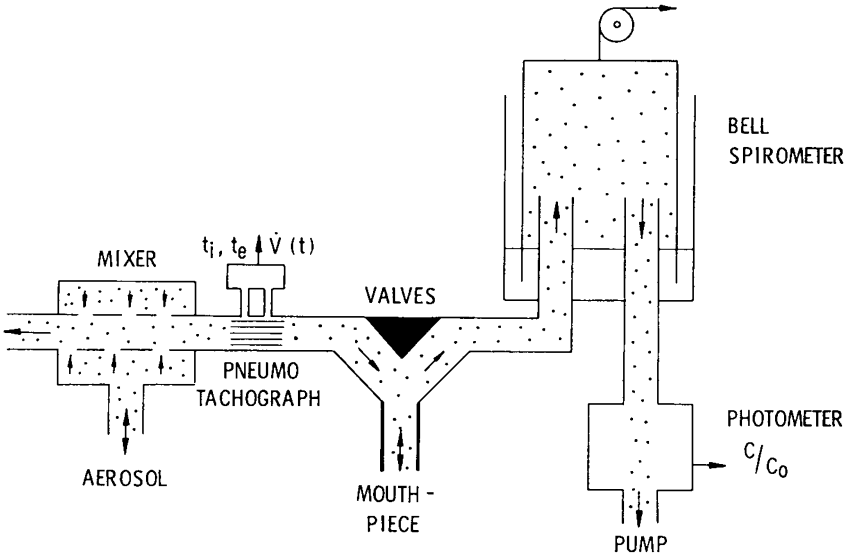


FIGURE 4. Schematic Diagram of the Stationary Open Flow System.

The stationary open flow system is useful for total deposition measurements during steady state conditions but not appropriate for single breath experiments. If the photometer in Fig. 4 is replaced by a classifying instrument like an optical particle counter (OPC) or an aerodynamic particle sizer (APS) deposition measurements can be also carried out with polydisperse aerosols. Particle losses in the apparatus while recording c have to be equal to those while recording c_o. To adjust for equal losses it proved necessary not only to establish the same mean flow rate but to simulate the whole oscillating flow pattern of the subject while measuring c_o (Heyder et al. 1978).

Bag-Collection System

An arrangement which collects the expired aerosol in a bag has been proposed by Palmes and Wang (1971) for measuring the aerosol recovery after single-breath inhalations. Its principle of operation is shown in Figure 5. A three-way valve system is employed to join a mouthpiece by choice to a bell spirometer or the one of two rigid boxes, each containing a rubber bag. The boxes are connected to the spirometer so that changes in the volumes of the bags can be recorded. Before an experiment bag B_1 is filled with a monodisperse aerosol through the mouthpiece. Then the subject takes the required breath of aerosol from bag B_1 and exhales into bag B_2. For the evaluation of the number of inhaled and exhaled particles a sampling and counting unit consisting of a photometer and a pump is used. The pump maintains a constant flow rate, $\dot{V}_{ph}$, through the photometer. While emptying the bags the photocurrent is integrated and the resulting voltage is monitored on a voltmeter. To determine the number of exhaled particles, Ne, all particles from bag B_2 have to be sucked through the photometer. Therefore,

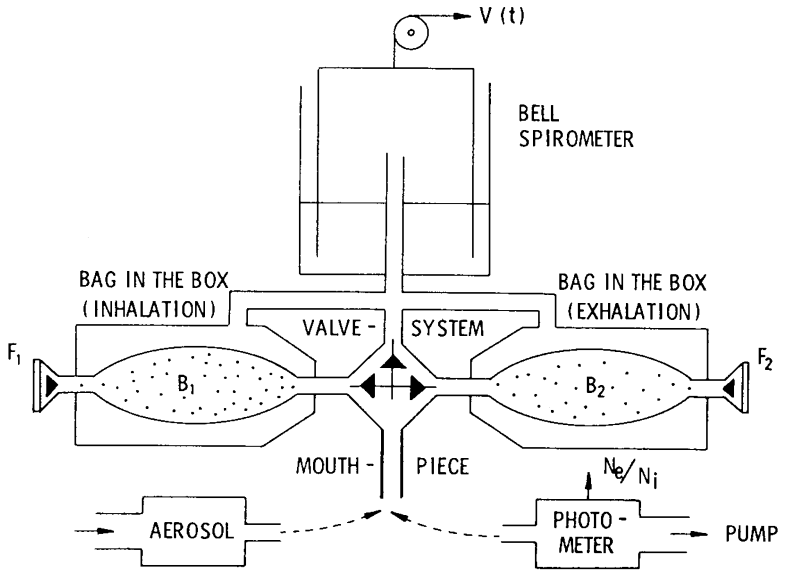


FIGURE 5. Schematic Diagram of the Bag-Collection System.

when the bag is almost deflated filtered air must be drawn in by an additional valve to flush aerosol particles trapped in the collapsing bag. The reading of the voltmeter at the end of flushing is then proportinal to:

$$Ne = \dot{V}_{ph} \cdot \int_{(B_2)} c(t)\ \delta t \qquad (5)$$

To determine the number of inhaled particles, Ni, the aerosol concentration, c_o, in bag B_1 has to be multiplied by the inspired volume, V_i. The concentration, c_o, is obtained by dividing the reading on the voltmeter by the volume change, V_s, when aerosol from bag B_1 is sampled. The ratio of the number of expired to inspired particles per breath is then given by:

$$\frac{Ne}{Ni} = \frac{\dot{V}_{ph} \cdot \int_{(B_2)} c(t)\ \delta t}{\frac{V_i}{V_s} \cdot \dot{V}_{ph} \int_{(B_1)} c(t)\ \delta t} = \frac{\int_{(B_2)} c(t)\ \delta t}{\frac{V_i}{V_s} \cdot \int_{(B_1)} c(t)\ \delta t} \qquad (6)$$

Particle losses in the bags during the time of breathing and sampling have to be taken into account. About equal volumes in both bags, at the end of the breath-

ing experiment simplify this correction since particle losses also depend on the volume of the bags.

REQUIREMENTS AND SOURCES OF ERROR

Errors in the measurement of the ratio of expired to inspired particle number, $\frac{Ne}{Ni}$, by means of photometry can arise from the light scattering properties of small particles, from an incorrect aerosol supply,from changes in temperature and humidity of the aerosol, from the limited range of linearity of the photometer and from instrumental dead spaces.

Light Scattering Properties of Aerosol Particles

Let an aerosol with particle number concentration, c, and diameter probability density function f(d) pass through a photometer with sensitive volume, V_m, and illumination, I_o. The flux of light, P, scattered by the assembly of particles into the receiver aperture, $\Delta\Omega$, is then given by:

$$P = I_o \cdot V_m \, c \int_0^\infty f(d) \cdot S(d,m,\lambda,\Delta\Omega)\, \delta d \qquad (7)$$

where S $(d,m,\lambda,\Delta\Omega)$ is the partial scattering cross section of a spherical particle with diameter, d, and refractive index, m, at the wavelength, λ. The sensitive volume, V_m, the receiver aperture, $\Delta\Omega$, and the wavelength, λ, are fixed by the photometer design. If the reading of the photometer now varies one can generally not distinguish whether the number concentration, c, the function, f(d), or the optical constants, m, of the particle material have changed. Since only the change in the number concentration, c, is to be measured the size distribution function f(d) and the refractive index, m, of the particles have to be the same during inspiration and expiration. Therefore monodisperse aerosols consisting of non-hygroscopic particles have to be used in combination with photometry. To be sure that the function, f(d) is not altered by the filter characteristic of the respiratory tract the geometrical standard deviation of these aerosols should be below 1.15. Exceptions are the on-line counting system and the stationary open flow system in combination with a counting and sizing instrument for the particles. In this case all particles above a certain detection limit are counted regardless of their optical properties and size distribution, so that the ratio $\frac{Ne}{Ni}$, is measured correctly even for polydisperse aerosols.

Aerosol Supply

Before an aerosol enters the photometer it has to be thoroughly mixed so that the aerosol concentration passing through the sensitive area of the potometer is representative for the whole cross section of the aerosol channel. This condition has to be fulfilled for all types of photometrical arrangements. In most experiments it is also necessary that the aerosol concentration offered for inhalation is constant. Thus the term 'total deposition' refers to the mean probability of an inspired particle being deposited in the whole respiratory tract by collection on airway surfaces (Heyder et al. 1986). This convention requires a constant number concentration c_o of the inspired aerosol during each inspiration and throughout the entire experimental run. Therefore the inhalation apparatus has to be supplied with a homogeneous stream of aerosol or-if stored in chambers - a uniform distribution of the aerosol concentration has to be established inside the vessel.

Growth of Hygroscopic Particles

Aerosol particles consisting of purely hygroscopic substances grow in size within the respiratory tract so tremendously (Ferron, 1977) that not only their optical properties but also their deposition probabilities are obviously changed. Nevertheless hardly detectable errors in the measurements can arise from the up-take of water within the respiratory tract as will be shown in the following examples.

1. Monodisperse inhalation aerosols are usually generated by controlled condensation of vapours of oily, non-hygroscopic liquids upon foreign nuclei (Stahlhofen et al. 1975). Using a polydisperse nuclei source the smaller nuclei may remain uncoated after condensation (Kelvin-Effect). During inspiration these uncoated nuclei do not influence the photometer reading, since for particles much smaller than the wavelength of light the scattering cross section of a particle decreases with d^6. During expiration, however, these nuclei contribute to the photometer signal if they are hygroscopic and have grown up to droplets. Figure 6 shows the modulation of a photometer signal during breathing of NaCl-nuclei with a CDM of 0.015 µm and a concentration of $8 \cdot 10^4$ cm^{-3}.

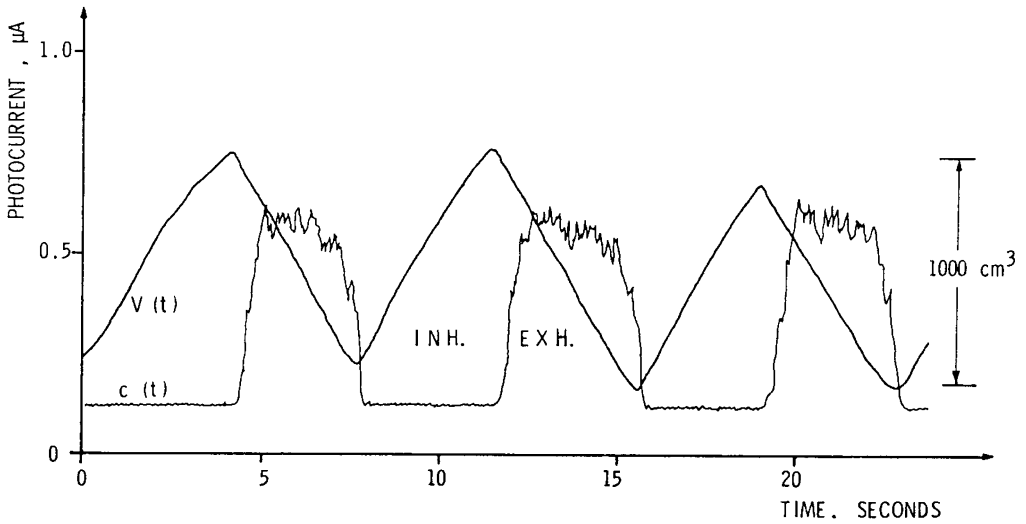


FIGURE 6. Effect of Growth of NaCl-Nuclei in the Respiratory Tract upon the Photometer Reading.

During inspiration the photometer signal is identical with the base line for filtered air whereas in the exhalation phase a photocurrent of about 0,5 µA is measured. To avoid errors in the evaluation of particle deposition the contribution of uncoated nuclei to the photometer response has to be negligible against the signal of the aerosol particles in use. No attention has to be payed to this problem if non-hygroscopic nuclei (Fe, AgCl) are used.

2. Latex aerosols are generated by atomizing latex suspensions which in the undiluted state contain about 10 per cent polystyrene and 2 per cent hygroscopic stabilizer (Fuchs, 1973). During atomization droplets containing particles as well as empty droplets are formed. After drying either particles covered by a shell of stabilizer or residuals consisting of stabilizer remain. If the stabilizer is not eliminated by extreme dilution of the suspension with distilled water the remaining residuals behave in a breathing experiment like uncoated, hygroscopic nuclei whereas the solid particles take up a thin sheet of water inside the respiratory tract (see Figure 17).

3. If solid particles are inhaled which have only a small hygroscopic component as for instance iron-oxide particles containing a small rest of chloride, the particles are only covered with a thin film of water inside the respiratory tract. This amount of water is to small to change the aerodynamic behaviour of the particles but large enough to change their optical properties. As consequence the same deposition probability is found for iron-oxide particles and for oil droplets (di-2-ethylhexyl sebacate) of the same aerodynamic size if the measurements are carried out with the on-line counting system (Heyder et al. 1980). A comparison of the size distribution spectra of the inspired and expired iron-oxide particles, however, shows (Figure 18) that the center peaks are displaced by about 15 channels indicating a small change in the scattering cross section of the particles. Therefore an anlog signal of the photometer would have been modified by the optical properties of the particles, too.

Temperature and Moisture Content of the Aerosol

A change im temperature and moisture content changes the volume of an aerosol which results in a corresponding variation of its concentration. Applying the equations for ideal gases the relative change of the aerosol volume, $\Delta V/V$, can be estimated according to (Muir and Davies, 1967):

$$\frac{\Delta V}{V} = \frac{(760 - \eta 47)}{(760 - 47)} \frac{310}{(273 + \vartheta)} - 1 \qquad (8)$$

Equation (8) assumes that the expired aerosol entering the photometer has a temperature of 37°C and is saturated with water vapour at pressure p_S (37°C) = 47 mm Hg. Figure 7 shows the relative change of the aerosol volume, $\Delta V/V$, i.e. the diluting effect, if during inspiration the aerosol has a temperature ϑ (°C) and a water vapour pressure $\eta \cdot p_S(37°C)$. The diagram demonstrates that the dilu-

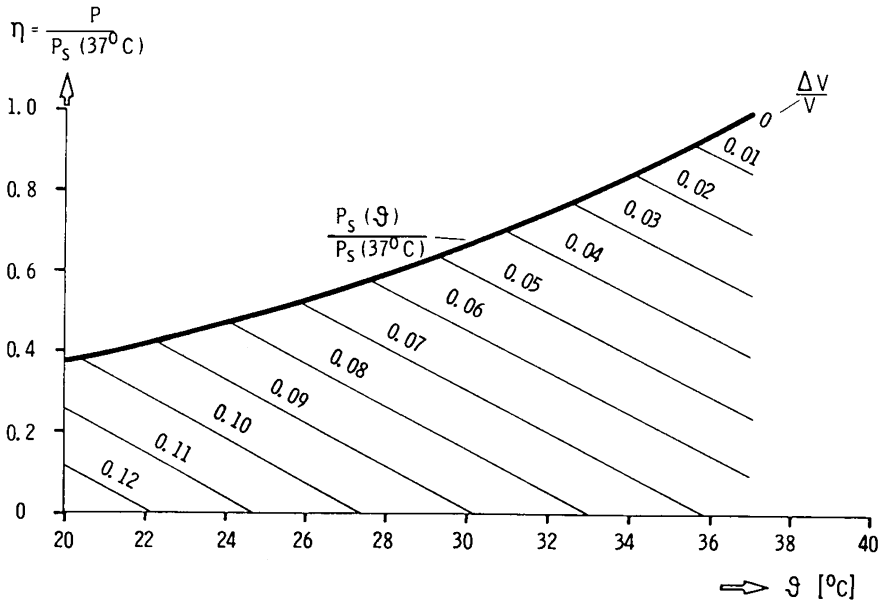


FIGURE 7. Effect of Temperature and Moisture Content on Aerosol Volume

ting effect can only be neglected if the aerosol offered for inhalation is warmed up to body temperature and humidified above about 80% rel. humidity. In

an experiment where the aerosol is inspired at room temperature (23°C) and expired at body temperature the photometer indicates a degrease of the concentration of about 10% without having lost any particle in the respiratory tract. Beyound that, volume changes of the air with temperature and moisture content also influence the reading of the bell spirometer.

Condensation of Water Vapour during Exhalation

When breathing dry air at room temperature the upper part of the respiratory tract remains below body temperature (Walker et al. 1961). As aerosol from the alveolar region passes through the upper airways supersaturation is achieved and water may condense not only on the walls of the respiratory tract but also on aerosol particles. To avoid supersaturation within the upper airways the whole respiratory tract has to be maintained at body temperature by warming the inhaled aerosol to 37°C and saturating it with water vapour. Supersaturation inside the photometer can have two effects: a change of the size and the optical properties of the aerosol particles due to condensation of water on the particles and an increase of the stray-light background due to condensation of water on optical elements. Therefore in all on-line systems the mouthpiece and the photometer have to be warmed up slightly above body temperature. In the stationary open flow system the whole aerosol path between mixer and photometer has to be brought to body temperature in order to prevent condensation of water on the expired particles. Otherwise particle losses in the apparatus while measuring the expired concentration, c, may differ from those while measuring the inspired concentration, c_o.

Limited Range of Linearity

A photometer in the analog mode determines the number concentration of an aerosol indirectly. The measurements are correct as long as N particles in the sensitive volume, V_m, yield an N-fold signal of a single particle. This range of linearity is limited at high concentrations by multiple scattering and at low concentrations by the stray-light background of the optical chambers (see Figure 8). Stray-light in the photometer originates from optical elements like lenses and glass windows and from Rayleigh-scattering of the air molecules. A measure of the stray-light background is the photometer response in the presence of filtered air. As can be seen from Figure 8 a certain concentration ration, $c_1/c_2 = P_1-P_s/P_2-P_s$, is only measured correctly by the photometer if the contribution of the stray-light, P_s, is either negligible or substracted from the responses. Otherwise a wrong ratio c_1/c_2 is obtained. In all cases where the analog signal is fed to an electronic device for multiplication or integration the photometer has to be adjustied to zero-response for filtered air by means of a potentiometer. No compensation of the stray-light signal is necessary as long as the photometer is operated in the counting mode. A main source for stray-light is the entrance window of the illuminating light. Since particles (dust and inhalation aerosols) precipitate on the glass of this window the stray-light background is not a constant quantity and has to be checked from time to time.

Instrumental Dead Space

The concentration, c_o, provided for inhalation, is not available at the mouth immediately at the onset of inspiration since the space between mouthpiece and separating system (valves or mixer) is filled either with expired aerosol or filtered air. This instrumental dead space (volumes between 16 and about 40 cm^3) influences the evaluation of particledeposition in two different ways. In the bag-collection and the on-line closed circuit system, the quantity of aerosol inspired is obtained by multiplying the concentration, c_o, during inspiration by

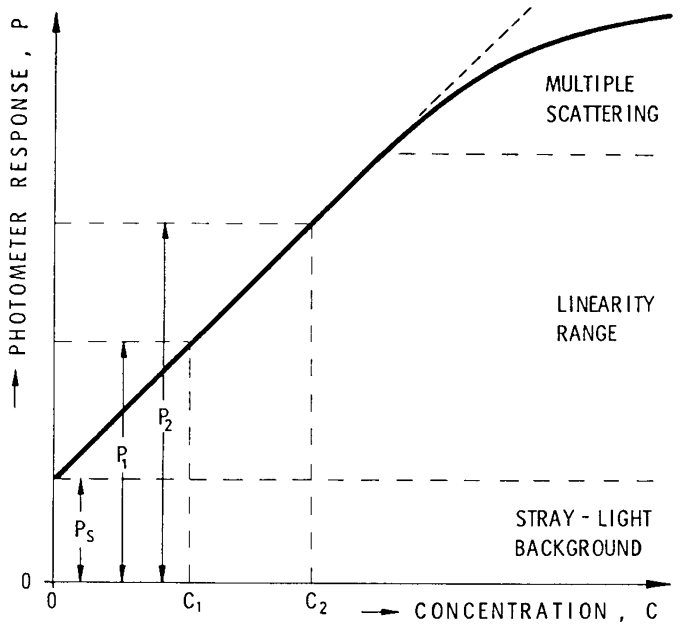


FIGURE 8. Linearity Range of a Photometer

the inspired volume, V_i, recorded on the bell spirometer. In order to get the actual amount of aerosol inhaled the inspired volume has to be corrected by the volume of the dead space. In the on-line analog and the on-line counting system the amount of inspired aerosol is measured correctly even in the presence of an instrumental dead space. But there is another source of inaccuracy which has to do with the experimental conditions for the determination of total deposition during steady breathing. Such measurements require a constant particle concentration of the inspired aerosol during the whole period of inspiration and throughout the entire breathing experiment (Heyder et al. 1980). Since an instrumental dead space reduces the aerosol concentration at the beginning of an inspiration different concentration profiles in inspired air resulting in different total deposition data are obtained.

CHARACTERISTICS OF A 2-MODE-PHOTOMETER

In the last decade the photometer already presented in Figure 3 has been reconstructed as a 2-Mode-Photometer for aerosol inhalation studies (Gebhart et al. 1980; 1983). It can be operated in the analog mode above 100 particles per cm^3 and in the counting mode below 10 particles per cm^3. With this device total deposition on human subjects can be measured at very low number concentrations. Under certain assumptions it can be also used as a particle size spectrometer which classifies aerosol particles in the main stream of inspired and expired air immediately at the entrance of the respiratory tract.

Opto-mechanical Arrangement

The parallel beam of an argon ion Laser (2W) traverses a system of diaphragms and cylindrical lenses to form within the sensitive volume a sheet of light of 80 µm thickness and 15 mm height (Figure 9). The sheet of light is inclined at an angle of 60° to the direction of the aerosol flow and covers almost the whole cross section of the aerosol channel (Ø = 16 mm).

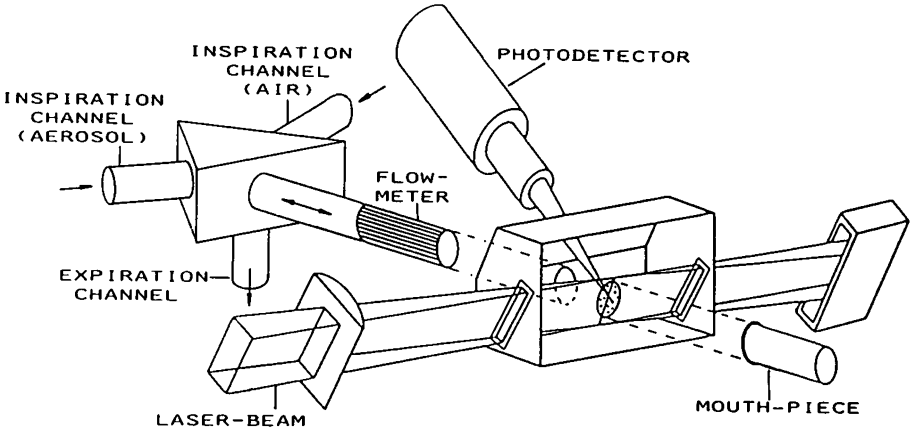


FIGURE 9. Opto-mechanical Arrangement of the 2-Mode-Photometer

The sheet-like laser beam enters the metallic housing of the photometer through a glass window, passes through a slit-channel of 8 cm length, crosses the aerosol channel and vanishes via a second slit-channel in a light trap. The light flashes scattered from single particles crossing the light sheet are received by a photomultiplier and converted into electrical pulses. Details of the receiver unit are shown in Figure 10. It consists of a microscope objective, a field stop, a field

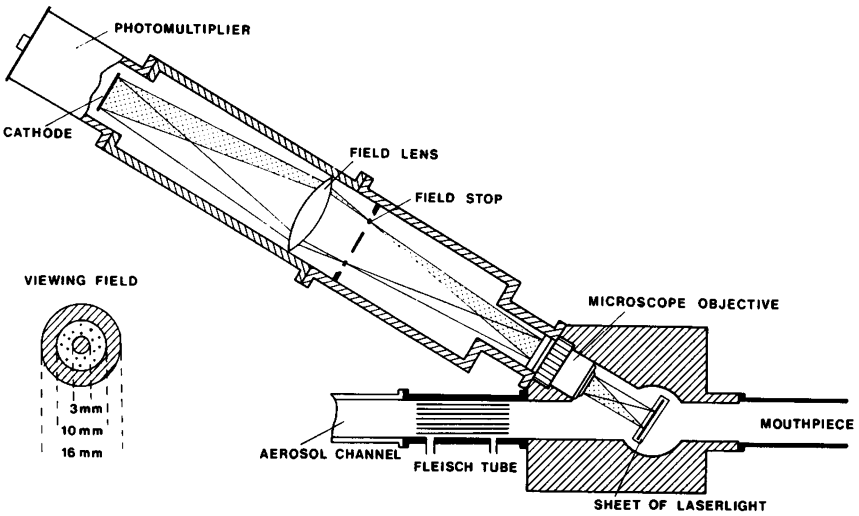


FIGURE 10. Receiver Unit of the 2-Mode-Photometer

lens and a photomultiplier tube. The light scattered by the particles is collected by the microscope objective (f = 25 mm; aperture angle: 12°) under a mean scattering angle of 90°, whereby the sensitive volume is imaged into the plane of the field stop. Since the sheet of light coincides with the object plane only those particles are illuminated which are imaged sharply. The field stop confines the sensitive area of the photometer and keeps away stray-light from the photomultiplier. The size of the field stop allows about half of the number of particles traversing the total cross section of the channel to be seen by the photomultiplier. The field lens images the aperture of the microscope objective onto the cathode of the photomultiplier so that all light flashes originating from

single particles strike the same area of the cathode regardless of their position inside the sensitive volume.

Electronic Equipment

A schematic diagram of the electronic circuits is shown in Figure 11. The time signals for the onset of inspiration and expiration are supplied from the differential pressure transducer of the pneumotachograph. A discriminator circuit separates the time intervals of inspiration, t_i, from those of expiration, t_e, and feeds the signals for external triggering to the integrators, the counter and the pulse height analyser.The analog signal of the pneumotachograph represents the respiratory volumetric flow rate, $\dot{V}(t)$,which can be recorded continuously and which after integration yields the inspired and expired volumes (V_i, V_e). For triggering and controlling purposes the analog signals of the volume are cut in small equidistant intervals of $\Delta V = 0{,}6\ cm^3$ whereby each volume change ΔV during a breathing cycle generates a pulse.
In the analog mode the instantaneous aerosol concentration c(t) is represented by an analog voltage which is fed to an analog-digital-converter (ADC). For each ΔV-pulse the ADC delivers a corresponding binary-number of the concentration c_ν which is stored together with the ΔV-pulses in a computer separately for inspiration. The computer calculates the ratio of expired to inspired particle number according to:

$$\frac{Ne}{Ni} = \frac{\sum \nu, c_{e\nu} \Delta V_\nu}{\sum \nu, c_{i\nu} \Delta V_\nu} \qquad (9)$$

and records the curve c(V) in steps ΔV on a graphic monitor. In addition, the concentration c(t) can be drawn directly with an u.v.-light recorder.

In the counting mode all electrical pulses of the photomultiplier above a certain pulse height are counted.During normal breathing pulse durations between 50 and about 500 µs are achieved. These pulses are fed to the ADC of a pulse-height-analyser, classified according to their height and stored separately during inspiration and expiration. The output of the ADC is controlled by a digital 2-level-comparator. All pulses being selected by the comparator are counted in electronic counters to obtain Ne/Ni.
By means of a volume-controlled system of solenoid and poppet valves the respiration channel can be either supplied with filtered air or aerosol (see Figure 9). For certain applications instead of total-breath inhalations a pulse or bolus of aerosol can be automatically injected into the inspiratory flow at a preset volume (V_{i1}, V_{i2}).

Range of Linearity and Sensitivity

The simple proportionality of the scattered light flux to the number of particles inside the sensitive volume holds only if independent scattering by separate particles is considered and multiple scattering can be neglected. Estimations show that a mutual particle distance of a few diameters is a sufficient condition for independent scattering (van de Hulst, 1957). The influence of multiple scattering on the response of this photometer has been calculated under the following assumption: (I) The particles are spheres with refractive index m = 1,45 and scatter independently. (II) The intensity of the illuminating beam passing through the aerosol channel is reduced by extinction, whereby the extinction is partially compensated by forward scattering. (III) On its way from the particle to the receiver aperture the scattered light is attenuated by other particles inside the aerosol channel.

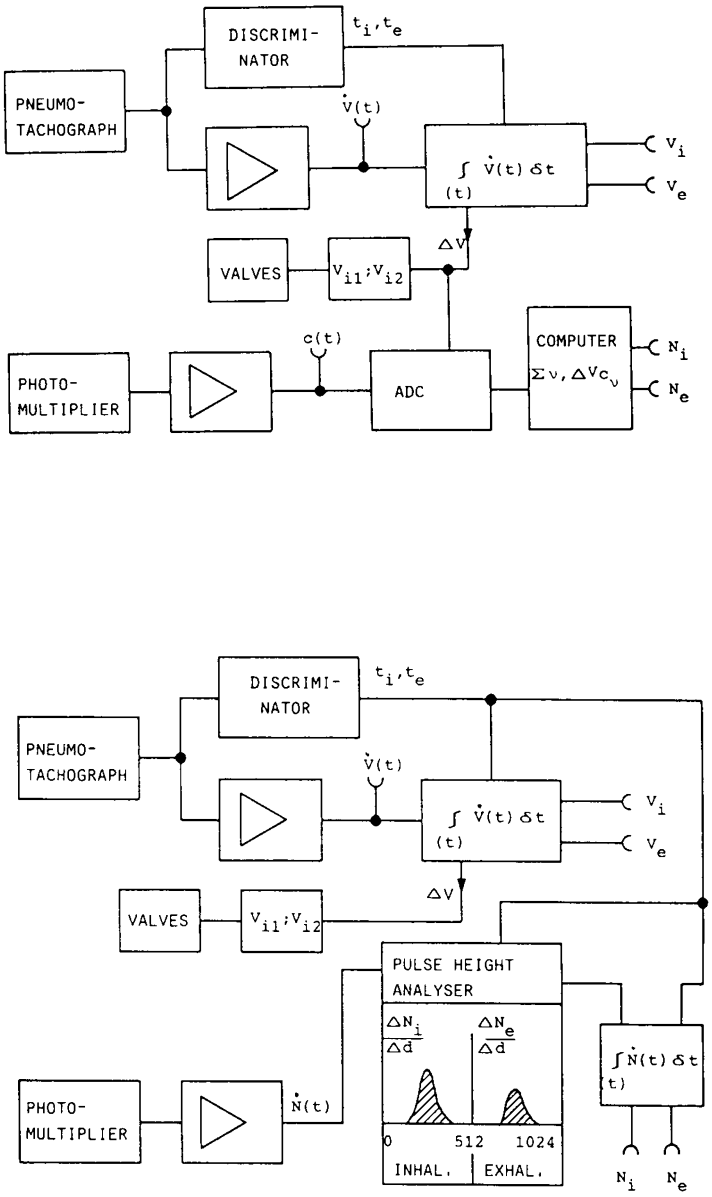


FIGURE 11: Block diagram of the Electronic Circuit of the 2-Mode-Photometer operated in the Analog Mode (above) and in the Counting Mode (below).

The results in Figure 12 show that the onset of multiple scattering depends on the concentration as well as on the diameter of the particles. Down to concentrations of 10^2 cm^{-3} the photometer can be operated well in the analog mode. In the range below 10 cm^{-3} particles are counted individually. The smallest detectable particle size in the counting mode mainly depends on the electronic noise and is about 0.3 µm (see Figure 15).

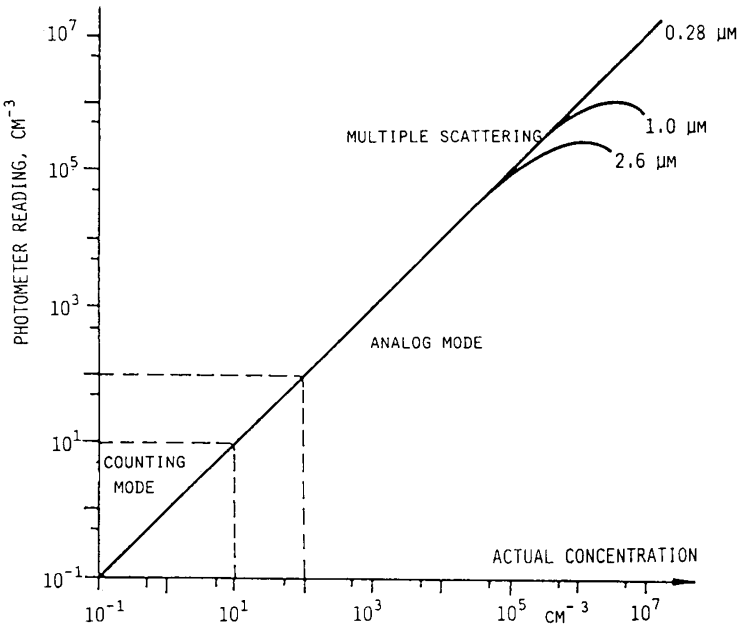


FIGURE 12. Onset of Multiple Scattering and Concentration Ranges of the 2-Mode-Photometer

The sensitivity resp. lower detection limit in the analog mode is given by the amount of stray-light reaching the photomultiplier. With the entrance window of the primary beam a certain distance away from the sensitive volume and a field stop inside the receiver unit the stray-light can be reduced to a level which is determined by the Rayleigh-scattering of the gas molecules in the sensitive volume. The photometer is adjusted well when the base line for filtered air changes with the composition of the carrier gas. In this case the variation of the photometer signal due to the Co_2-content in expired air is the limiting factor for analog measurement of aerosol concentration. In Figure 13 the response of the photometer to a pulse of 100% CO_2 is compared with the signal produced by di-2-ethylhexyl-sebacate particles with a diameter of 0.08 µm and a concentration of

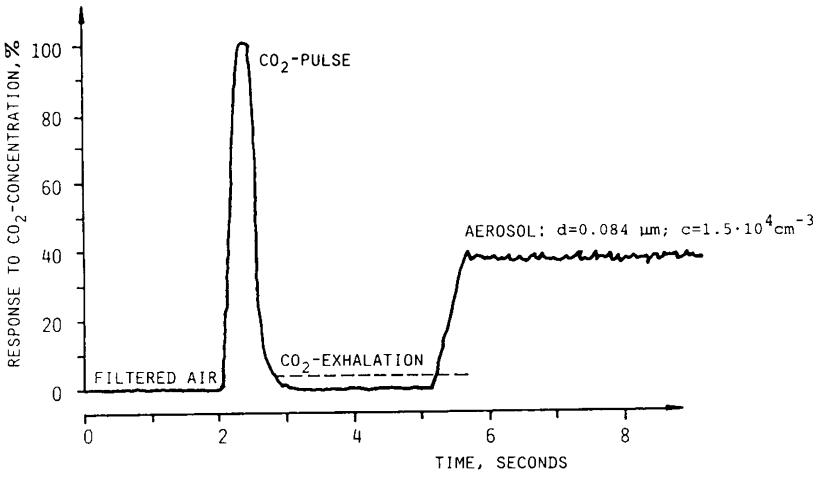


FIGURE 13. Lower Detection Limit in the Analog Mode of the 2-Mode-Photometer due to CO_2-Content in Expired Air.

1.5 10^4 cm^{-3}. The response to the aerosol is about 10 times higher than the level corresponding to about 4% CO_2 in expired air (dashed line). This gives experimental evidence that down to particle diameters of 0.08 µm aerosol concentrations can be measured in the analog mode provided their number concentration exceeds some 10^4 cm^{-3}.

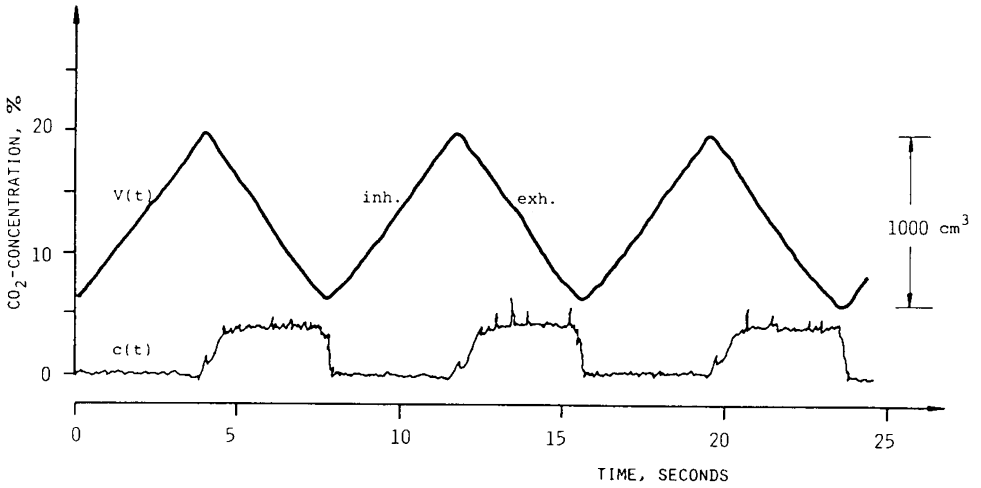


FIGURE 14. Concentration Profile of CO_2 in Expired Air Recorded with the 2-Mode-Photometer.

In Figure 14 an amplified signal of the photometer during breathing of filtered air is plotted alongside with the inspired and expired volumes. The diagram indicates the capability of the instrument to measure the concentration of CO_2 and other carrier gases by means of Rayleigh-scattering.

Rayleigh-Scattering of Gases

For dielectric particles much smaller than the wavelength of light dipole theory can be applied so that the flux of scattered light per unit solid angle, q, is given by (van de Hulst, 1957):

$$q = I_o \frac{8\pi^4}{\lambda^4} \alpha^2 (1 + \cos^2\Theta) \qquad (10)$$

I_o is the intensity and λ the wavelength of the illuminating light, Θ the scattering angle and α the polarizability of the particle. For randomly oriented gas molecules the mean polarizability, α, of a single molecule is connected with the macroscopic refractive index, M, of the gas according to (Born and Wolf, 1965):

$$\alpha = \frac{(M^2 - 1)}{4\pi C_m} \qquad (11)$$

where C_m is the number concentration of the molecules.
Thus the ratio of the scattered light fluxes for two different gases is given by:

$$\frac{q_1}{q_2} = \frac{(M_1^2 - 1)^2}{(M_2^2 - 1)^2} \qquad (12)$$

Macroscopic refractive indices of some gases at λ = 0.546 µm and the corresponding fluxes of scattered light relative to air are listed in Table 1. The theoretical predictions in Table 1 have been confirmed experimentally by sucking

TABLE 1 . Rayleigh-Scattering of Different Gases in Relation to Air

	air	He	Ne	H_2	H_2O	Ar	Kr	CO_2	Xe
$(M - 1) \cdot 10^6$	292	34.89	67.25	139.6	252.7	282.3	428.7	450.5	705.5
Φ_i / Φ_{air}	1	0.014	0.053	0.228	0.75	0.934	2.15	2.38	5.84

different filtered gases through the photometer (see also Figures 13 and 14). Fortunately the cross-sensitivity of the photometer to water vapour is negligibly small. Replacing all air molecules by water vapour at normal pressure (760 mm Hg) results in a decrease of the photometer response of 25%. Air saturated with moisture at body temperature (p_s = 47 mm Hg), however. reduces the signal only by about 1.5% compared to dry air. Since in all experiments the inspired air has at least a rel. humidity of 80% variations of the photometer signal due to different moisture contents are below 0.5%.

Coincidence Losses in the Counting Mode

The simultaneous presence of several aerosol particles inside the sensitive volume, V_m, leads to count losses so that the actual particle concentration, c, is indicated too low. Using Poisson statistics the apparent decrease in concentration, $-\Delta c$, can be estimated by the expression:

$$- \Delta c = c \, (1 - e^{-V_m c}) \simeq V_m c^2 \qquad (13)$$

Considering the ratio of expired to inspired concentration, c/c_o, the error due to coincidence losses is partially compensated and one gets:

$$\Delta \left(\frac{c}{c_o} \right) = \frac{c}{c_o} \cdot \left\{ \frac{V_m c_o \left(1 - \frac{c}{c_o} \right)}{1 - V_m c_o} \right\} \qquad (14)$$

For $V_m c_o$ = 0.06 and c/c_o = 0.5 the absolute error in the concentration ratio is $\Delta (c/c_o)$ = 0.016. The sensitive volume has a thickness of $8 \cdot 10^{-3}$ cm and a sensitive area of about 0.8 cm^2 so that the volume is $V_m = 6.4 \cdot 10^{-3}$. Substituting this volume in the equation: $V_m c_o$ < 0,06 yields as restriction for the aerosol concentration in the counting mode: c_o < 10 cm^{-3}.

Resolution Power

The resolution power of the photometer in the counting mode describes its capability to distinguish between adjacent size fractions. It mainly depends on the homogeneity of the illumination. To obtain a homogeneous illumination of the particles only the central part of the Gaussian-shaped energy profile of the laser beam is used to form the sheet of light. In optical particle counters where the sensitive volume is cut out of the aerosol stream by means of a field stop (without using an aerosol nozzle) particles touching the edge of the viewing field reduce the resolution power. If the particles are illuminated by a

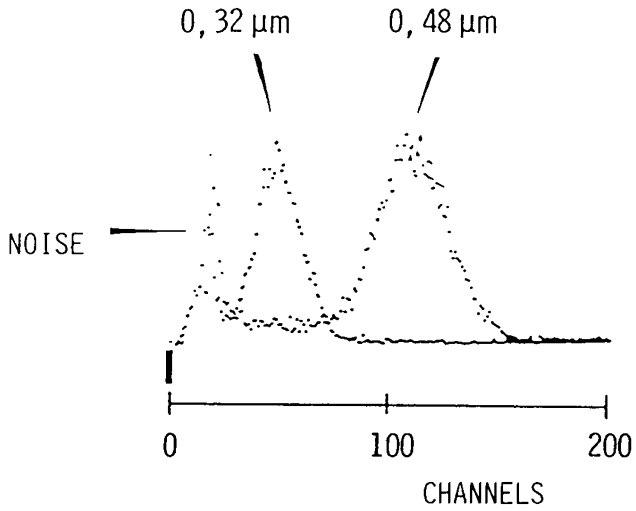


FIGURE 15. Resolution Power and Lower Detection Limit of the 2-Mode-Photometer in the Counting Mode.

parallel pencil of light most of them are imaged unsharply into the plane of the field stop. They are then partially shadowed by the edge of the stop and give rise to broad distributions of the recorded spectra even for monodisperse aerosols. With a sheet of light for the illumination, however, only sharp particles are seen in the plane of the field stop and the edge effect influences less than 2% of the particles. To evaluate the resolution power of the photometer spectra of monodisperse polystyrene latex spheres have been recorded in the multichannel analyser. The example in Figure 15 demonstrates that particles with diameters of 0.318 µm and 0.488 µm are separated clearly. Thus the Photometer can be used not only to count but also to classify particles in inspired and expired air. Below channel 20 the noise counts for filtered air appear.

Recording of Aerosol Concentration Profiles

Continuous records of the aerosol concentration c(t) resp. c(V) in the analog mode have useful applications in single-breath and pulse inhalation studies. Since aerosol particles differ from tracer gases by more than five orders of magnitude in their intrinsic motion, particle concentration profiles in expired air provide new possibilities for lung function tests. To throw some light on the mechanisms of particle transport from inhaled to lung air during breathing Muir (1976) proposed investigations of particle concentration profiles in exhaled air after periods of breath holding. Such information has been also extracted from single-breath experiments by Gebhart et al. (1981). The dispersion of boluses of half-micron aerosols in expired air as function of lung depth is a significant index for mechanical mixing of air volumes during a respiratory cycle (Heyder et al. 1987; Ramm et al, 1987). In normal lungs the volume of inspired air preserves its identity in a remarkable manner: the volume inspired first is expired last and vice versa. Airways obstruction, however, results in a highly dispersed and asymmetric pulse of aerosol in expired air (Gebhart et al. 1981a; Stahlhofen et al. 1986).

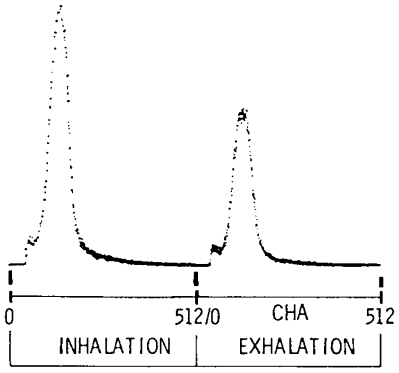
Measurement of Total Deposition

A typical experimental run which illustrates the determination of total deposition in the counting mode is given in Figure 16. The figure contains a specification of the breathing manoeuvre, the particle size distributions measured in inspired and expired air and a print-out of computer calculated data. The subject

AEROSOL PARTICLES : POLYSTYRENE SPHERES , d = 2.02 µm

BREATHING PATTERN : V_t = 1000 cm^3, f = 15 min^{-1}, τ = 2 s, Q = 500 $cm^3 s^{-1}$

TOTAL DEPOSITION : DE = 0.405



N_i	V_i	N_e	V_i-V_e	N_e/N_i	I-N_e/N_i
3672	874	2164	0	0.589	0.411
3591	869	2146	8	0.597	0.403
3562	870	2158	13	0.605	0.395
3664	880	2154	-16	0.588	0.409

FIGURE 16. Measurement of Total deposition in the Counting Mode

inhaled polystyrene spheres of 2,02 µm diameter under standardized breathing conditions. The stabilizer in the latex suspension was extremely diluted so that the same size spectra were obtained during inhalation and exhalation. The printed data represent four successive breaths under steady state conditions after the completion of the wash-in. For each breath the number of counts during inspiration and expiration, N_i and N_e, the inspired volume, V_i, the difference between inspired and expired volume, $V_i - V_e$, the ratio, N_e/N_i, and the deposition per breath, $1 - N_e/N_i$, are listed. Since about half of the total number of particles passing through the aerosol channel are counted the subject inhaled about 7000 particles per breath resp. per liter which corresponds to a concentration of only 7 cm^{-3}. Human deposition data evaluated with the analog method at relatively high concentrations and with the counting method after extreme dilution of the aerosol have been found to be in good agreement (Heyder et al. 1980). The counting method has the advantage that the lung burden originating from particulate matter can be kept extremely low.

Growth Studies of Hygroscopic Particles

Separate recordings of the particle size distributions in inspired and expired air include the possibility to indicate any change of the scattering cross section of the particles during breathing. Since the scattering cross section depends on the size and composition (optical properties) of the particles changes of these parameters can be observed immediately. Figure 17 shows the particle size distributions of polystyrene spheres of 1.01 µm diameter in the inspired and expired air of a subject during steady breathing. The particles were produced by atomization of a highly diluted latex suspension. From the overlap of both spectra it can be seen that their center peaks (CP) agree within four channels. On the other hand the increased count rate in the lower channels of the expired particle spectrum indicates that some of the residuals originating from empty droplets have grown up above threshold within the respiratory tract. Monodisperse iron oxide particles are usually produced by atomization of an aqueous suspension of colloidal iron oxide with a spinning top generator (Stahlhofen et al. 1979). For the preparation of the suspension iron chloride dissolved in water is converted into insoluble, colloidal iron oxide by hydrolysis. To remove the dissolvable chloride completely the aqueous iron oxide colloid has to be thoroughly dialyzed. The effect of the dialysis on the behaviour of the iron oxide particles in the res-

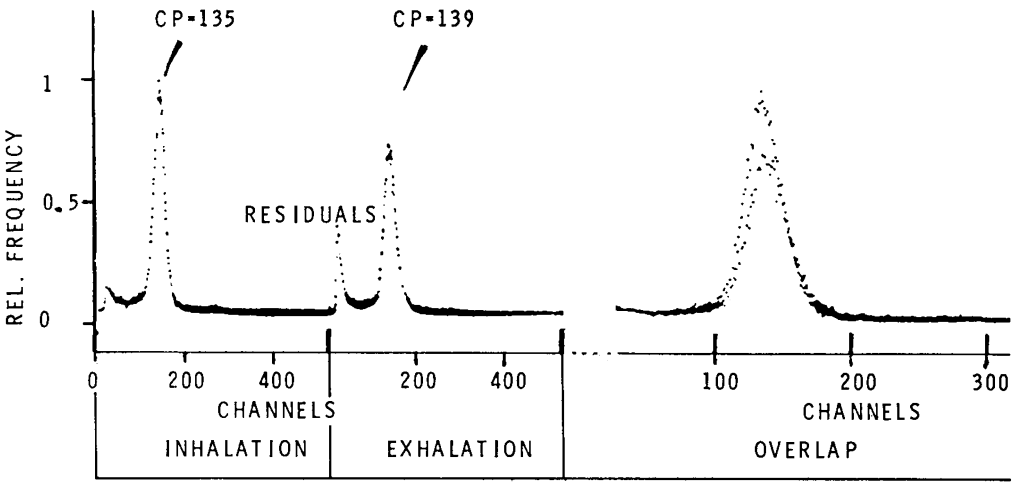


FIGURE 17. Hygroscopicity Test of Polystyrene Spheres Produced from a Highly Diluted Latex Suspension; CP: Center Peak.

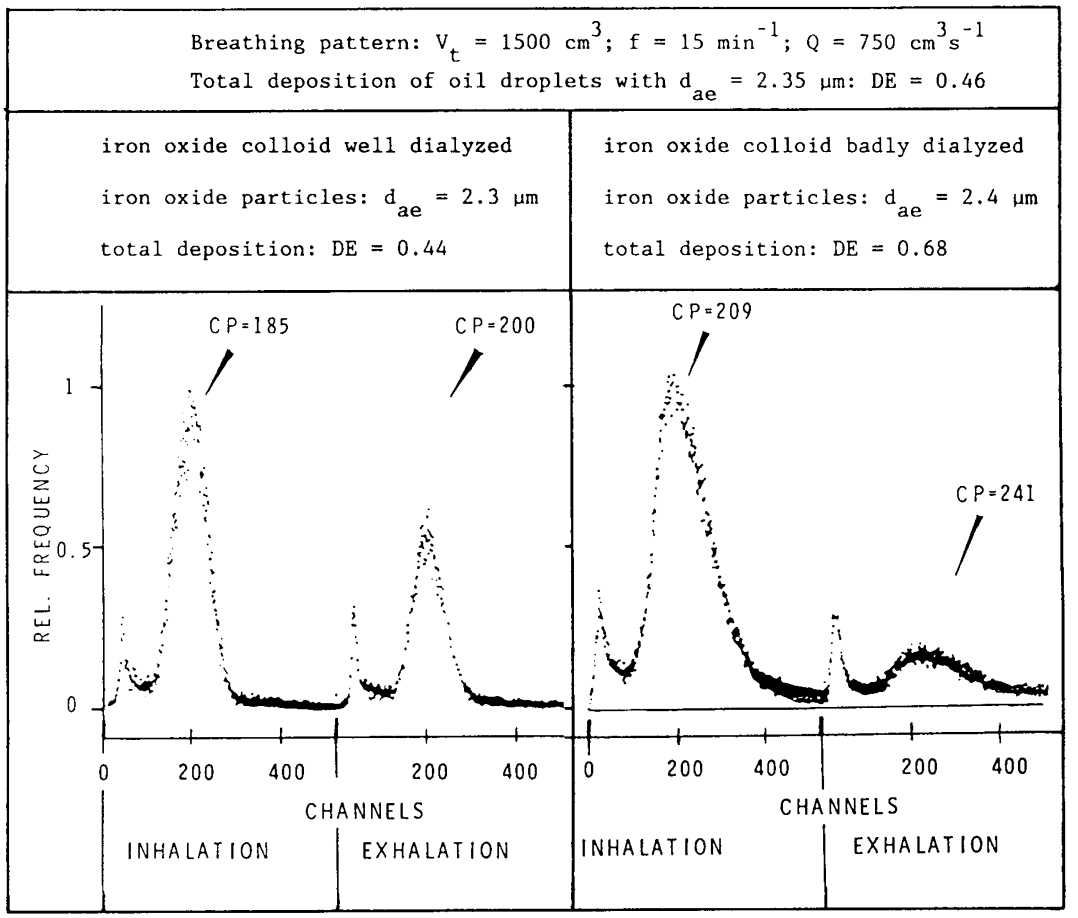


FIGURE 18. Hygroscopicity Test of Iron Oxide Particles Produced from a Well and a Badly Dialyzed Colloidal Solution.
CP: Center Peak of the Size Distribution.

piratory tract is demonstrated in Figure 18. With a well dialyzed iron oxide colloid for the production of the particles the measured total deposition of those particles (DE = 0.44)) is in good agreement with corresponding values obtained for oil droplets of the same aerodynamic size. Nevertheless the center peaks of both spectra are shifted by about 15 channels. This indicates that even after a careful dialysis the iron oxide particles (ρ = 3,2 g cm^{-3}) take up a thin film of water which does not change the aerodynamic diameter of the particles but affects their light scattering properties. The iron oxide particles produced from a badly dialyzed colloid on the other hand grow up within the respiratory tract to such an extend that not only their scattering cross section but also their aerodynamic behaviour are entirely changed. Consequently the measured total deposition of DE = 0.68 is much higher than the corresponding value for non-hygroscopic particles.

So far the photometer has proved as a sensitive monitor for hygroscopic properties of aerosol particles. For a quantitative analysis boluses of monodisperse NaCl-particles (150 cm^3) have been injected into the inspiratory flow of different subjects, transported into a defined lung depth and expired again with the same flow rate as during inhalation (Anselm et al. 1986). To obtain the diameter of the expired aqueous NaCl-droplets from their scattering signals theoretical response functions of the photometer have been calculated for various refractive indices ($1.33 < m < 1.38$) and matched with experimental calibration curves of test standards. Figure 19 compares growth factors measured for 0.7 um-NaCl-particles with theoretical predictions when exposing these particles at time zero to different relative humidities (Ferron, 1977).

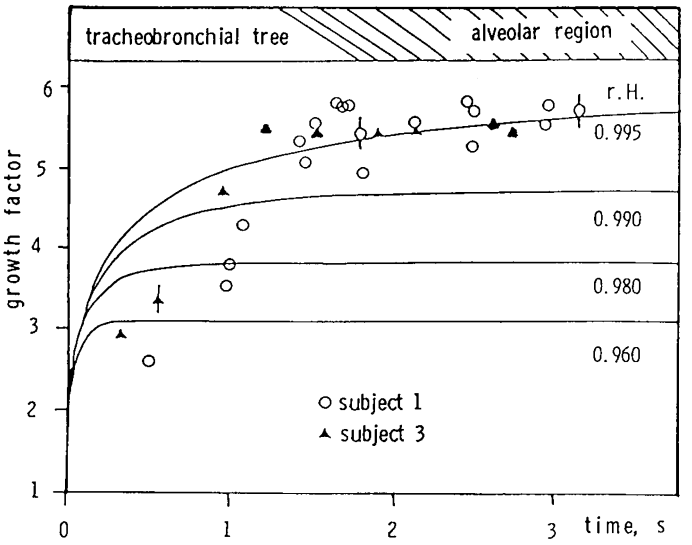


FIGURE 19. Growth Factor of Expired Aqueous NaCl-droplets as Function of Mean Residence Time in the Lungs after Inhalation of 0.7 um-NaCl-Particles in Comparison to Theoretical Predictions for Different Relative Humidities r.H. after Ferron (1977).

It has been found that 0.7 um-NaCl-particles are expired from the alveolar region as aqueous NaCl-droplets with a mean diameter of 3.85 um which corresponds to a growth factor of 5.5.

This work is dedicated in honour of Professor Dr. Wolfgang Pohlit on the occasion of his 60th Birthday.

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