# CHAPTER TWO

## GENERATION, SAMPLING AND PARTICLE SIZE MEASUREMENT

# CHAPTER 2 - GENERATION, SAMPLING AND PARTICLE SIZE MEASUREMENT

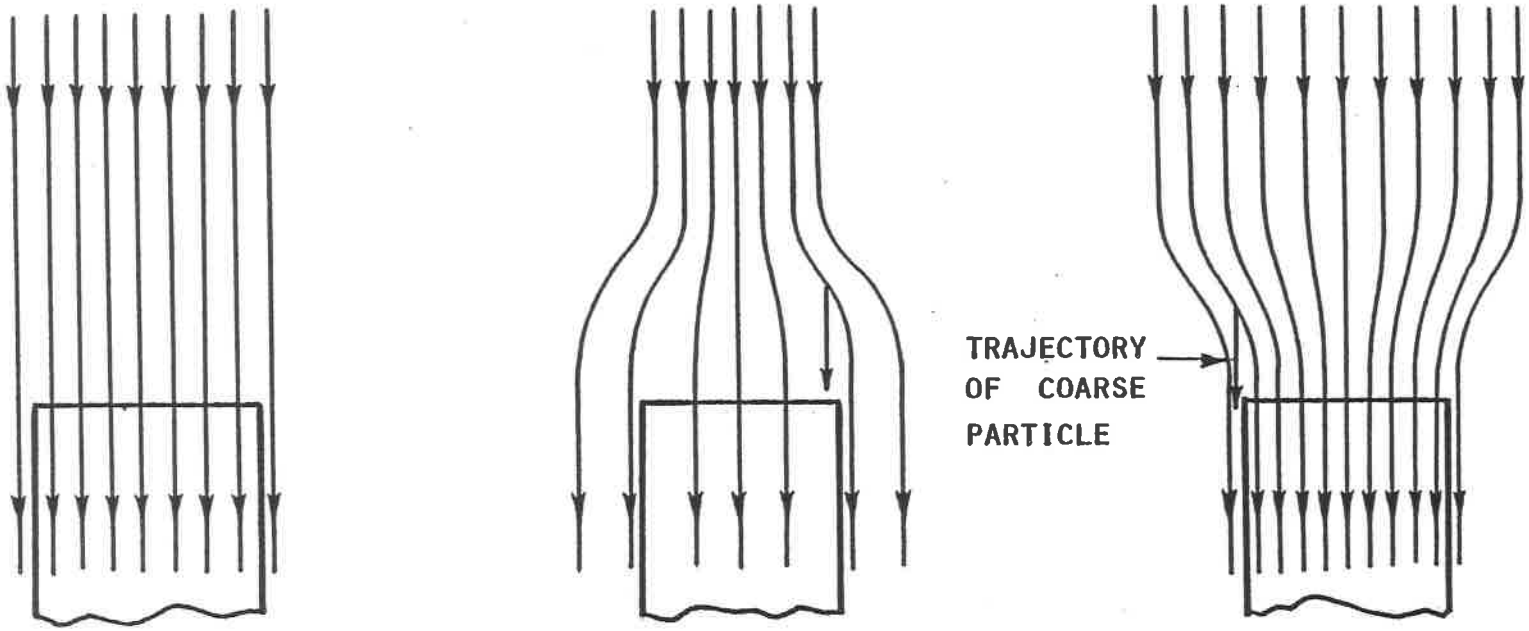
## 2.1 REVIEW

The properties of the aerosol govern the sampling methods and the choice of technique for size measurement. Sampling of monodisperse aerosols is relatively easy, provided that aggregation of the sample is avoided. The concentration of particles produced from the specialised generators is often low enough not to require dilution. A therapeutic aerosol is often highly concentrated; and, when generated by an MDI, is initially travelling at high velocities. The additional problems therefore include dilution and representative sampling by the measuring device. Polydisperse aerosols in an airstream require isokinetic sampling for representative size measurement. Fig. 2.0 illustrates this principle. The airflow rate within the sampling probe must match that of the airstream so that neither the largest or smallest particles are biased in the sample (Allen, 1975).

Various inlet designs have been reviewed which claim representative sampling from aerosol clouds in the respirable size range (Farthing, 1982; Wedding, 1982; Liu & Pui, 1981). Estimates have been made of sampling efficiencies for a particular inlet with variations in particle size, sampling and air velocities and sampling angles. (Tufto & Willeke, 1982; Vincent et al., 1982; Gibson & Ogden, 1977). The errors due to anisokinetic sampling have also been calculated on a theoretical basis (Selden, 1977; Addlesee, 1980).

One of the first attempts at solving the sampling problems of pressurised aerosols was published by Dixon (1966). It consists simply of a cylindrical chamber into which the aerosol is introduced prior to gravitational sedimentation of the particles onto microscope slides for size measurement.

Fig 2.0. ISOKINETIC SAMPLING




(A) ISOKINETIC SAMPLING; REPRESENTATIVE CONCENTRATION AND GRADING.

(B) SAMPLING VELOCITY TOO LOW; EXCESS OF COARSE PARTICLES.

(C) SAMPLING VELOCITY TOO HIGH; DEFICIENCY OF COARSE PARTICLES.

This method has been adapted for MDI's (Hallworth & Hamilton, 1976).

Several workers have developed complete systems for sampling and size measurement of aerosols (Davies, 1980a; Berres, 1979; Godfrey, 1974), but each has its disadvantages. For example, Berres (1979) used a chamber volume in excess of 5000l. for measurement of continuous spray aerosols of insecticides. A smaller volume would be required to provide an adequate particle concentration for measuring metered dose therapeutic aerosols. Davies (1980a) used a rectangular chamber which would probably have 'dead spaces' giving low concentration at the corners. Often aerosols must be stirred to provide efficient mixing with diluting air and allow homogenous sampling. It is advantageous to have a low background of particles in the chamber, particularly for optical counters, thus filtration and circulation of the air may be necessary (Vos & Thomson, 1974; Davies, 1980a).

The existing standard method for particle size measurement of pharmaceutical MDI's (BPC, 1973) consists of firing the inhaler at a microscope slide held 5cm from the actuator and perpendicular to the spray. This is a limited quality control test and cannot provide a complete size distribution because of high particle concentrations on the slide and loss of fine particles.

Sampling and size measurement of droplet aerosols such as those generated from nebulisers and solution type MDI's must take into account the environmental conditions. Control of temperature and humidity influences the degree and rate of evaporation of small volatile droplets. In ambient conditions, the residence time in the sampling system before size measurement will influence the droplet size.

The most common techniques for aerosol size measurement have

been developed for environmental and 'model' particles (Mercer, 1973; Bright, 1979), but many have been adapted for the size measurement of therapeutic aerosols (Bell, 1967). The methods may be divided into three main types; impaction, optical methods and microscopic/miscellaneous methods.

Cascade impaction methods were originated by May (1945) and measure the aerodynamic size distribution of aerosols by separation of particles in air due to their inertia. The instruments are so designed that the air velocity increases through stages of the instrument, thereby depositing smaller particles from the airstream at each stage. Many different designs have been published for the sampling of 'respirable' aerosols with flow rates varying from 50cm$^3$/min (Mercer et al., 1970) to 850 litres/min (Gussman et al., 1973). Theoretical studies have defined the ideal impactor design for optimum collection efficiency (Marple & Liu, 1975; Newton et al., 1977).

Low losses of aerosol by impaction on the walls rather than the collecting plates have been achieved in various designs (Mercer et al., 1970; May, 1975a). Recent modifications have included rotating plates to produce uniform deposits (Marple et al., 1981), combining impaction with photodetection for particle counting (Cooper & Spielman, 1974) and variation in size classification by opposing airflow jets (Pavlik & Willeke, 1978). Several authors have specifically designed impactors to match the particle size deposition curves of lung models proposed by the Task Group (Stevens & Churchill, 1973) and the ACGIH (Marple, 1978). (ACGIH - American Conference of Governmental Industrial Hygienists).

Although impactors have been used for size measurement of pressurised aerosols (Sciarra et al., 1969), few methods have been published using therapeutic aerosols, particularly from metered-dose inhalers (Bell et al., 1973; Nilsson et al., 1977; Hallworth & Andrews, 1976). Bell used a Multistage Liquid Impinger (May 1966a) and Nilsson used an Andersen sampler (Andersen, 1966) to size both suspension and solution MDI's. Hallworth & Andrews used three types of impaction devices for measurement of pharmaceutical MDI's employing a bent glass tubular throat as a simulated oro-pharynx.

Impactors have been used extensively for particle size measurement but the collection characteristics are not ideal. A particular size of particle is not all collected on a single stage. Many studies have evaluated the collection efficiencies in terms of shape of jet, upstream jet geometry and effect of gravity (Mercer, 1964; May, 1975b; Rao, 1975). Impactor performance may be expressed in terms of the square root of the inertial impaction parameter ($\psi$) (Mercer, 1963), and calibration curves established of $\psi$ vs stage collection efficiency (Cushing et al., 1979). One of the commonest errors in impaction methods is due to particle bounce or re-entrainment in the airstream after deposition, in either case causing particles to be deposited on a lower stage than expected (Esmen & Lee, 1980; Dzubay et al., 1976). Many authors have studied the effect of impaction surface on particle deposition and hence collection efficiency (Willeke & McFeters, 1975; Rao & Whitby, 1978; Barr et al., 1982).

Some of the problems with cascade impactors are overcome by using a liquid layer on each stage for particle collection. May (1966a) developed the Multistage Liquid Impinger for bacteriology, and this principle has been used in the development of a simpler, two-stage impinger for rapid

quality control of therapeutic aerosols (Hallworth et al., 1978).

An alternative impaction method uses centrifugal force for particle size measurement (Kotrappa & Light, 1972; Oeseburg & Roos, 1979; Martonen, 1982) which may prove a useful technique for sampling therapeutic aerosols.

Optical methods of size measurement for aerosol particles of a respirable size range include light-scattering, light diffraction and laser velocimetry. Lundgren et al., (1979) have published an excellent book on all forms of aerosol size measurement, with a useful section on light scattering counters. The first light-scattering instruments for size measurement of aerosol particles in the respirable size range used incandescent light sources (Rimberg & Keafer, 1970). More recent instruments use laser light which increases the resolution and the particle concentrations which may be measured (Roth et al., 1976). The advantages of light-scattering methods include the fact that single aerosol particles are measured in situ without the need for sampling and collection processes. This is particularly important for aerosols which may be influenced by sampling methods such as nebulised droplets. The disadvantages include the lack of particle differentiation with optical counters; each sampled particle is measured regardless of constituent materials. This is a problem for therapeutic aerosols which contain drug and excipient, which may be in separate particles.

The collection of scattered light in an optical counter may be in a near-forward or right-angle direction to the incident light. Generally, right angle scattering instruments have better full-range resolution than near forward scattering angle instruments, but are more affected by differences in refractive index. Liu et al. (1974) studied the

response characteristics of several commercially available optical particle counters using monodisperse aerosols. They found that the response of forward scattering counters (Royco 245 and Bausch & Lamb 40-1) was independent of refractive index for particles > 1μm. Several studies have evaluated response functions of light scattering instruments for particle size, shape and refractive index, and also for the specific geometrical factors and light source of the counter (Cooke & Kerker, 1975; Heyder & Gebhart, 1979; Ho & Bell, 1981). Particle shape (Schuerman et al., 1981; Jaggard et al., 1981) and the effects of humidity on measured size distributions have also been extensively studied (Farmer et al., 1981; Hanel, 1981, Covert et al., 1972). Petheram (1982) has shown that hygroscopic growth of aerosols can produce spurious results due to growth of particles into or out of, the measured size range. Many different light-scattering instruments have been developed (Wyatt & Phillips, 1972; Lewis & Lamothe, 1978; Suda & Handa, 1979). Modifications for particular purposes include the measurement of particle density (Sasaki et al., 1980) and the examination of mixed phase aerosols (Stow & Millener, 1980).

Knollenberg and coworkers have produced a range of laser light-scattering and light extinction instruments (Knollenberg, 1970; Schuster & Knollenberg, 1972; Knollenberg & Luechr, 1976; Pinnick & Auvermann, 1979). In common with many optical counters, they were originally developed for atmospheric aerosol measurement, and are now finding increasing use in laboratory research work concerned with deposition and toxicology of aerosols. Several workers have estimated the relationship between the measured optical aerosol sizes and the aerodynamic diameter of particles which is important for lung deposition studies (Harrison & Harrison, 1982; VanBuijtenen & Oeseburg, 1974; Marple & Rubow, 1976).

The paper by Davies et al. (1978) is among the few published studies which uses a forward light-scattering instrument (Royco 225) for particle size measurement of therapeutic aerosols generated from MDI's. This is probably due to the difficulties in sampling and in particle differentiation, as previously mentioned.

Hiller and coworkers have used a specialised optical counter, the single particle aerodynamic relaxation time analyzer (SPART) for size measurement of therapeutic aerosols (Mazumder & Kirsch, 1977; Mazumder et al., 1979). The instrument employs laser velocimetry and a microphone to measure phase lag of particles within an acoustic field. The aerodynamic size range measured is 0.1-10μm, at a maximum count rate of 200 particles per second. Therapeutic aerosols are sampled from a chamber to produce suitable concentrations, and subject to low and 'high' humidity for estimations of hygroscopic growth. Suspension and solution MDI's, powder aerosols and nebulised aerosols have been measured by this method. (Hiller et al., 1980c; Smith et al., 1980; Hiller et al., 1980d).

An additional method of measuring aerodynamic size by laser velocimetry has recently been published (Agarwal et al., 1981). This technique uses a two spot laser velocimeter which measures the velocity of particles as they leave an accelerator nozzle. The particle velocity is inversely proportional to aerodynamic diameter. Low concentrations are also required for this instrument; it has yet to be fully evaluated for therapeutic aerosol measurement.

Holography appears to be a promising method for particle size analysis of aerosols, and has the advantage of being able to study individual particles in detail (Thompson et al., 1967; Prikryl & Vest, 1982). The assessment of holograms has been facilitated by interfacing with an image

analysing computer (Bexon et al., 1976) and has been used to produce particle size and concentration data in a model of the human upper airways. (Gross & Peter, 1973).

Image analysing computers have been used to facilitate microscopic examination of drug particles in therapeutic aerosols (Hallworth & Hamilton, 1976) and of the input drug (Hallworth & Barnes, 1974). By utilising established formulae, measurement of liquid aerosol droplets may also be accomplished from their appearance on a microscope slide (Bexon & Ogden, 1974; Liu & Pui, 1981). Electron microscopy has been used for studying aerosol particle morphology (Bloch et al., 1982).

## 2.2 GENERATION OF AEROSOLS

### 2.2.1 Introduction

This section describes the methods used to generate aerosols which were employed in the in vivo studies. Polydisperse aerosols were produced from two types of nebuliser and from metered-dose inhalers. The nebuliser types were chosen for availability and simplicity of operation. All studies with radiolabelled nebulised solutions used a lead-shielded DeVilbiss nebuliser, and disposable nebulisers were used for non-radiolabelled aerosols (section 2.2.4). Air-driven nebulisers were used in preference to the ultrasonic type because they were quieter and provided sufficient output for in vivo dosing of rabbits and dogs.

Metered-dose inhalers (MDI) were used so that in vivo and in vitro correlations could be made of a type of aerosol which is predominant for inhalation therapy. The materials, equipment and expertise for manufacture of MDI's were available at Glaxo Group Research Ltd., and the formula-

tions studied contained micronised salbutamol base with similar particle size distributions to that in Ventolin Inhaler.

### 2.2.2 Metered-dose Inhalers

Fig. 1.4 shows a diagram of a metered-dose inhaler containing a suspension of drug particles. It consists of a metering valve crimped on to an aluminium can. Individual doses are measured volumetrically by a metering chamber within the valve. The contents of the can may be a suspension of micronised drug or a solution of drug in a mixture of fluorocarbon propellants, with a cosolvent such as ethanol if necessary. The latent heat of evaporation of the volatile propellants provides the energy for atomisation. The valve stem is fitted into an actuator incorporating a mouthpiece. The aerosol, consisting of propellant droplets containing drug, is delivered from the actuator mouthpiece at very high velocity, probably about $30ms^{-1}$ (Rance, 1974) and each metered-dose typically contains millions of drug particles (Hiller et al., 1980b). There is partial (15-20%) evaporation of propellant prior to exit from the atomising nozzle and further breakup of droplets beyond this point caused by the violent evaporation of the propellant (Wiener, 1958).

Ventolin Inhaler contains micronised salbutamol base in suspension in a mixture by weight of 28 parts of Propellant 11 (trichlorofluoromethane) and 72 parts of Propellant 12 (dichlorodifluoromethane). The MDI's used in this study were made to a similar formulation. All preparations were packed in aluminium cans (Presspart Ltd) and sealed with BK 300 valves (B/N25395, Bespak Ltd). They contained micronised salbutamol base (A/N 82145) with Priolene 6952 (commercial grade oleic acid, Unichema Ltd., A/N 16215) as a surfactant.

### 2.2.3 Alpine Zig-Zag Classifier

It was desirable to produce coarsely micronised salbutamol base for comparative *in vivo* studies to assess particle size differentiation. For this purpose, 2kg of unmicronised salbutamol base (B/N 1763) was micronised by a deliberately inefficient process so that a considerable proportion of the milled particles were larger than 5µm. This was achieved with a 4" microniser, the drug being fed in as rapidly as possible with the feed and grinding air pressures set at 20psi and 80psi respectively. The coarsely micronised material produced was then passed through an Alpine Zig-Zag classifier to remove the fine material in the mixture. The particle size distribution of the resultant micronised salbutamol had a larger mean size than standard micronised material, with a low percentage of fine particles.

The Alpine Zig-Zag classifier (Fig. 2.1) separates powder into two fractions by centrifugal elutriation, according to the aerodynamic particle sizes. The instrument is calibrated using limestone dusts.

$$d = d_i \sqrt{\frac{\rho_i}{\rho}} \qquad \text{Equation 2.1}$$

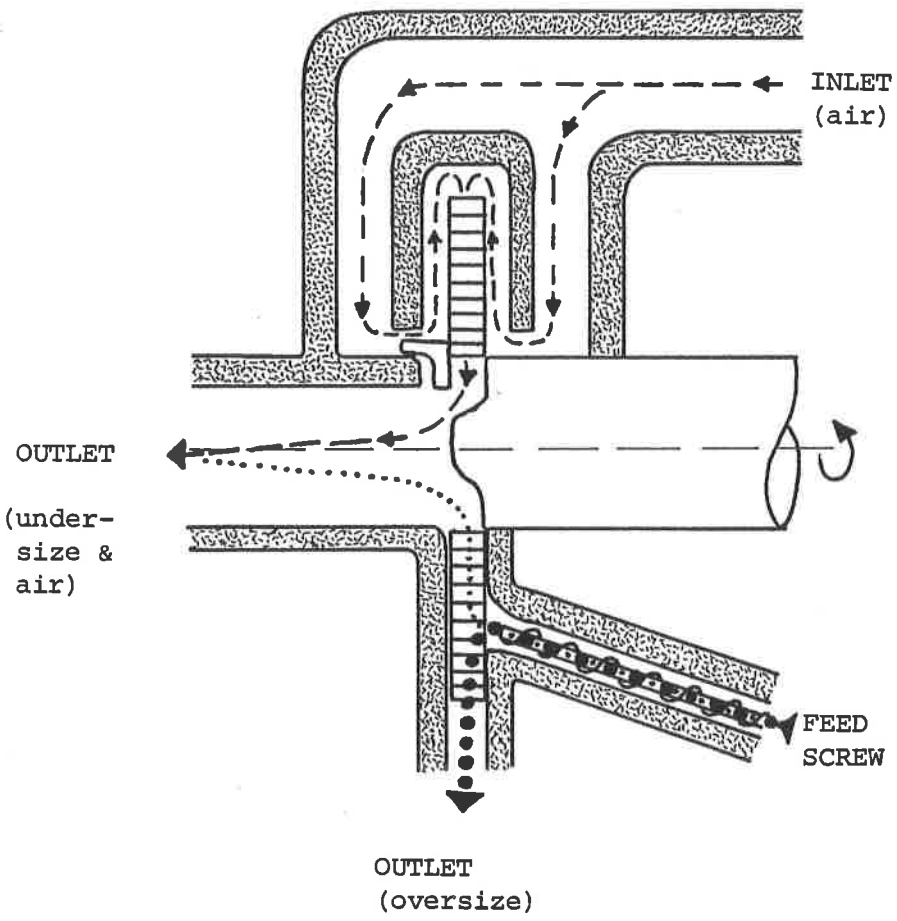
where $\rho_i$ = density of limestone ($2.7 gcm^{-3}$)

$\rho$ = density of sample (approx. $1.2 gcm^{-3}$)

$d_i$ = theoretical cut point of limestone (µm)

$d$ = required cut point (µm)

Fig 2.1 Section of Alpine Zig-Zag Classifier.



Thus for a required cut point of:-

$$d = 4\mu m \qquad d_i = \frac{4}{\sqrt{2.7/1.2}} = 2.67\mu m$$

The speed of the rotor in the classifier, n, (rpm) and volumetric air flow rate, Q, ($m^3hr^{-1}$) through the classifier govern the theoretical cut point, according to the following equation:-

$$Q = 55\text{-}\frac{n}{1000} \qquad \text{Equation 2.2}$$

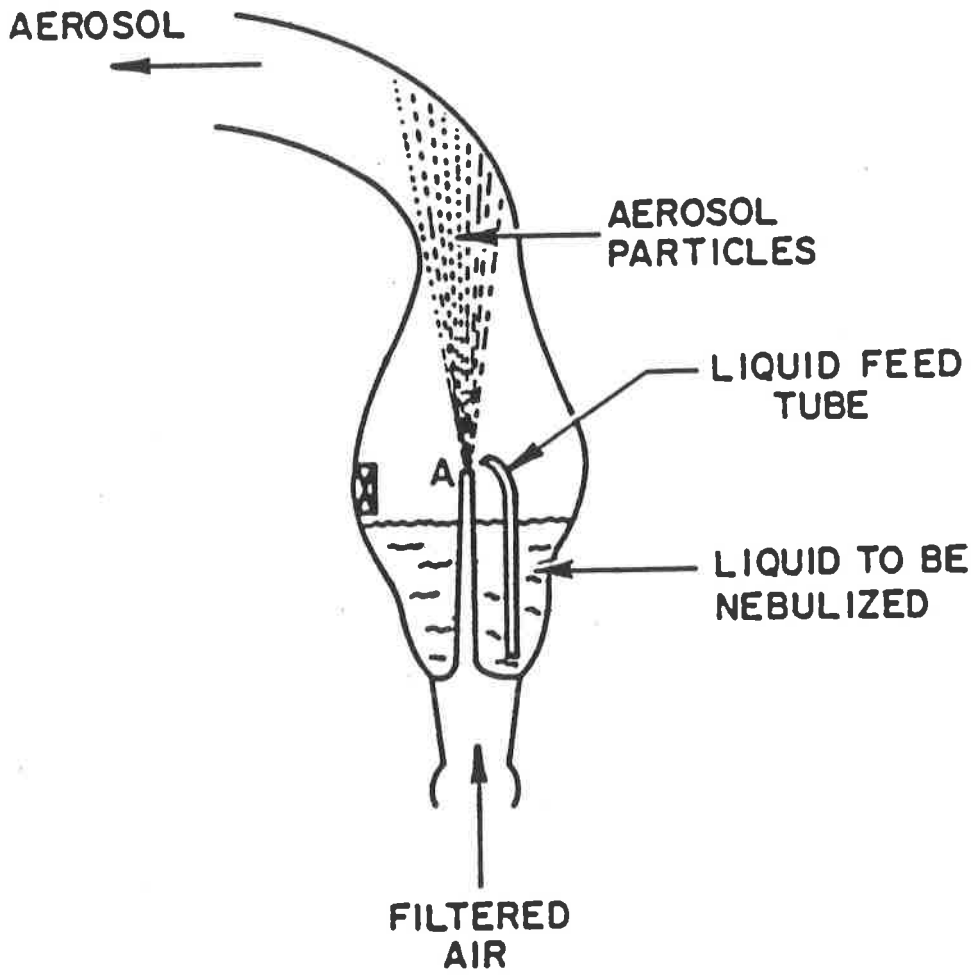
The required n for a theoretical cut point is obtained from a calibration curve. Thus for d = 4μm, n = 19500 rpm and Q = 35.5 $m^3hr^{-1}$.

The coarse and fine fractions from the classification were collected in tared amber glass bottles of about 1 litre capacity. The particle size distributions of the powder fractions were measured by microscopic and laser light-scattering methods (sections 2.3.2 and 2.3.3).

### 2.2.4 Nebulisers

Fig. 2.2 shows the principle of operation of a simple DeVilbiss air-jet nebuliser. A high velocity stream of filtered air is forced through a nozzle which forms a low pressure area at point A (see Fig. 2.2). This in turn draws liquid up through the feed tube, to be atomised at the jet.

Fig 2.2. DeVilbiss Nebuliser



Most of the droplets impact on the upper walls and flow back into the reservoir, whilst a small amount escapes to the outlet as a fairly fine polydisperse aerosol. The design of the nebuliser and the airflow rate influences the total output of aerosolised liquid and the droplet size distribution.

A DeVilbiss type nebuliser is incorporated in the shielded CIS design (Fig. 2.3) for generating aerosols from $\gamma$-radiolabelled solutions or suspensions. Secondary baffles are positioned above the atomising jet. The outlet of the nebuliser is joined by a ground-glass ball-joint to a 3-way valve to facilitate inspiration of supplementary air and expiration into an absolute filter. The whole instrument is encased in 5mm lead shielding. The nebuliser was operated at an airflow rate of 8 litres $min^{-1}$.

The output of this nebuliser was measured as 0.5ml $min^{-1}$ by loss of weight for 50% aqueous ethanol solution.

The Bennett twin-jet nebuliser is shown in Fig. 2.4. It operates at an airflow rate of 5L $min^{-1}$, on the same principle as the DeVilbiss type but has two liquid feed tubes and jets to increase the aerosol output.

2.2.5 The Spinning Disc Aerosol Generator
(Research Engineers Ltd., Shoreditch, London).

The spinning disc generator was used for manufacturing HSA microspheres (Section 3.2). Figs. 2.5 and 2.6 show the generator, manufactured by Research Engineers Ltd., London, and described by May (1949), which operates on the following principle:-

Liquid is fed at constant rate from a hollow needle to the centre of the rotor, which is driven by tangential air jets and typically revolves at about 1000 rps. A liquid film is

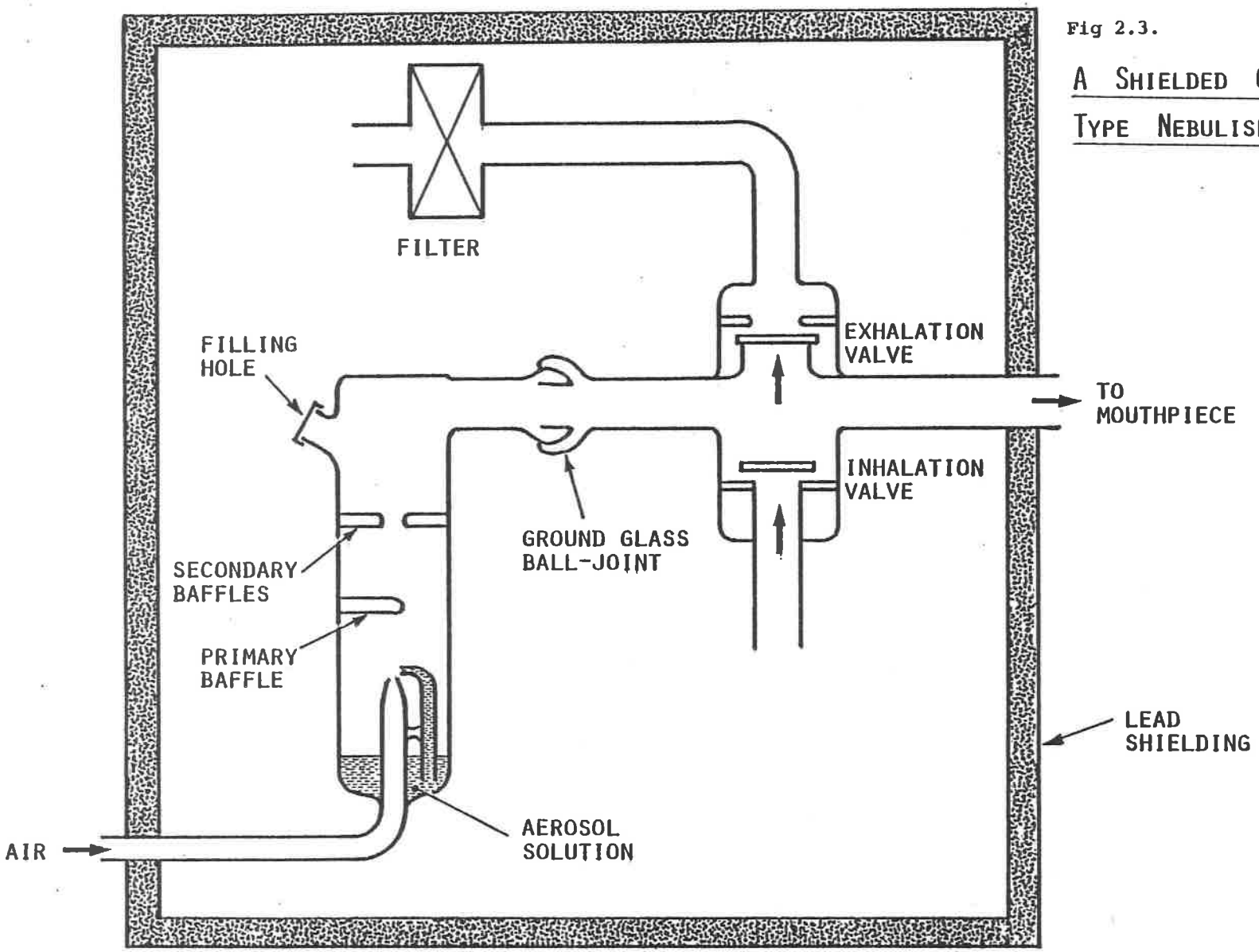



Fig 2.3.

A SHIELDED CIS DEVILBISS-TYPE NEBULISER.

Fig. 2.4. Bennett twin-jet nebuliser.

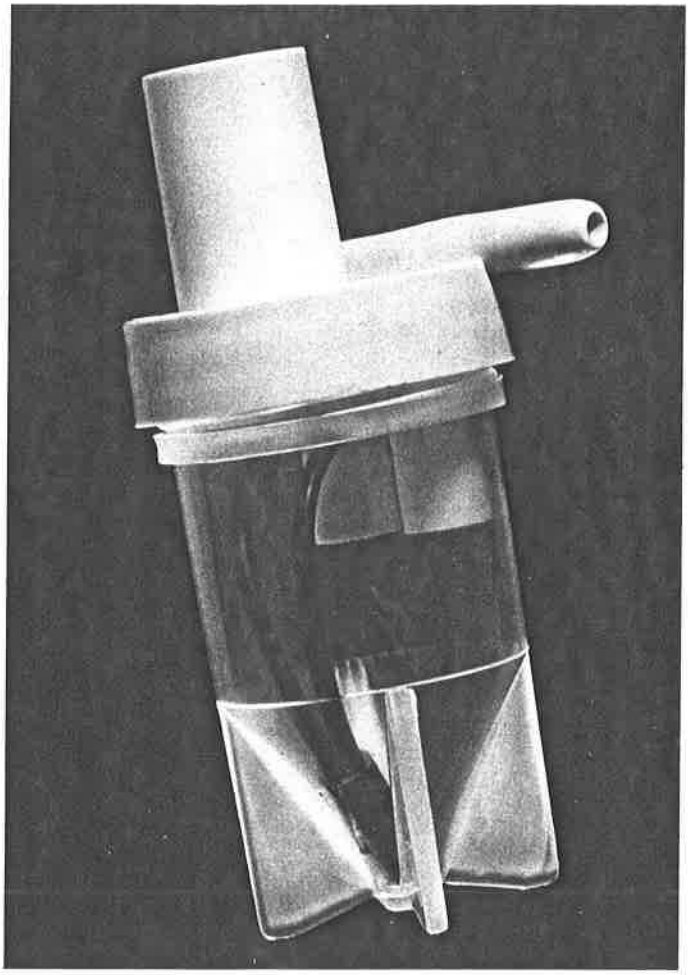


Fig. 2.5. Spinning disc aerosol generator.

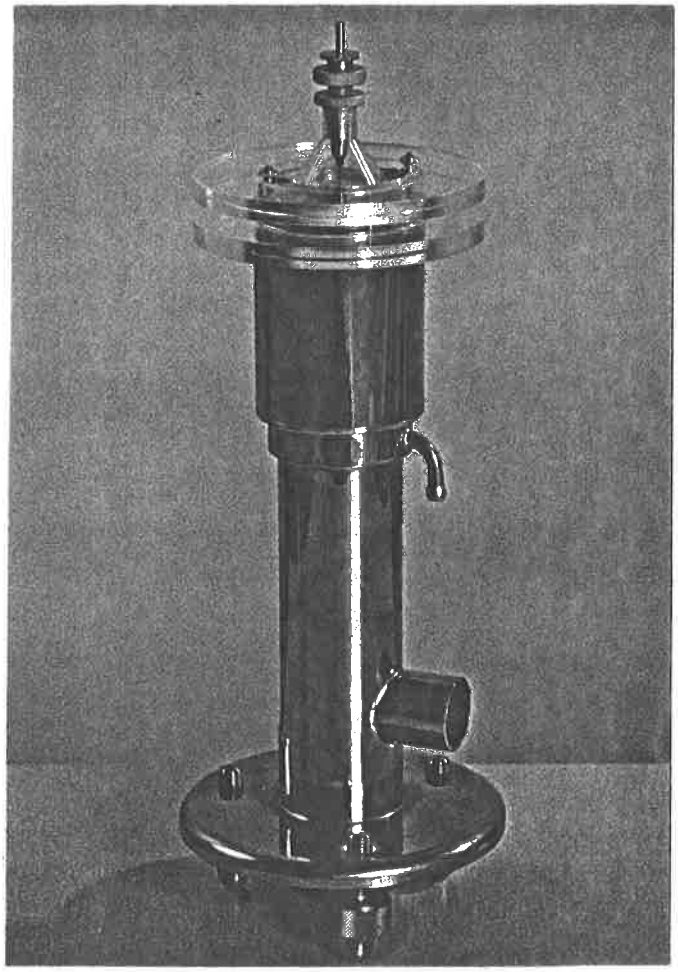
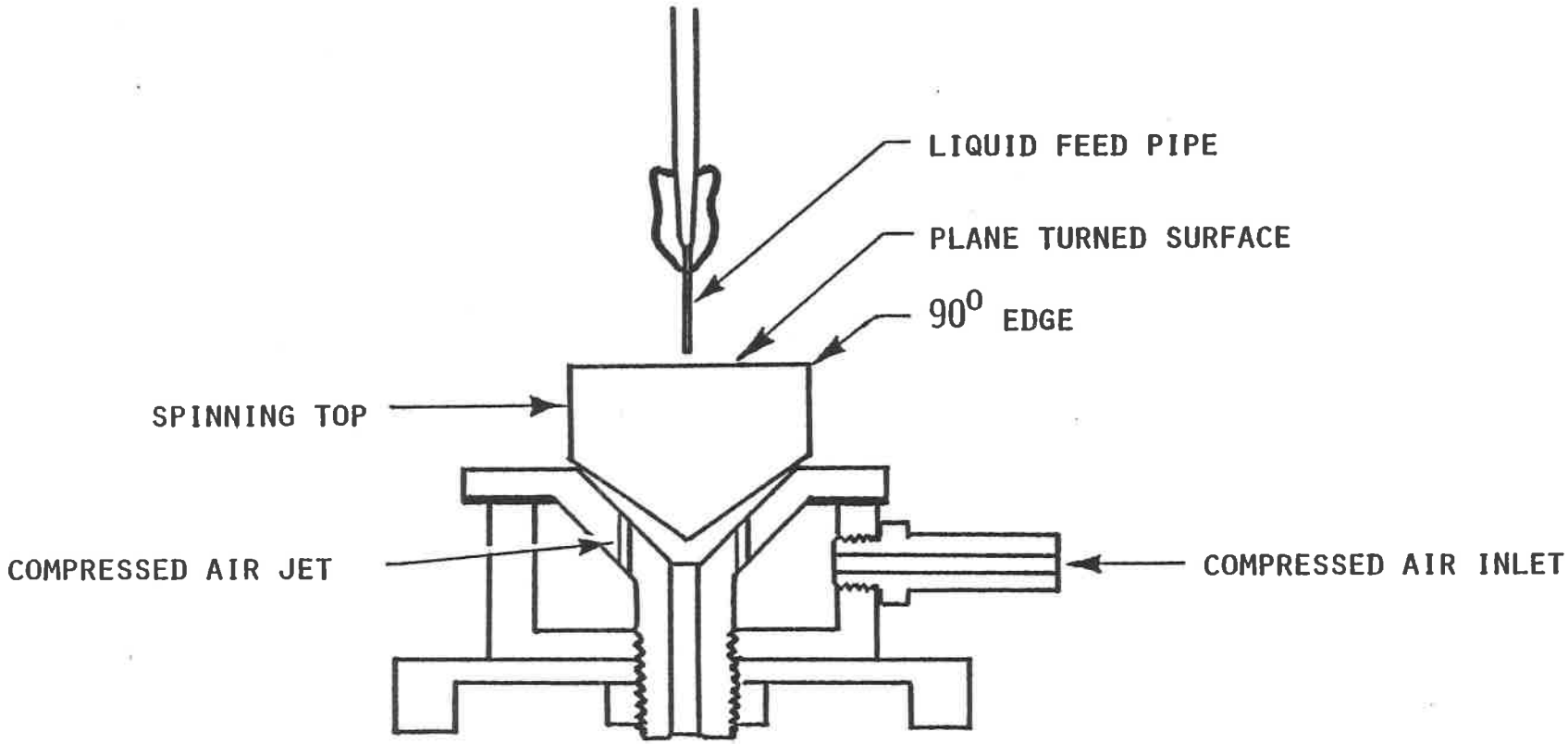


Fig 2.6. DIAGRAM OF SPINNING DISC APPARATUS



is formed which, at a suitable feed rate, ruptures and produces uniform liquid droplets at the edge of the rotor disc. A dry aerosol can be produced by feeding a colloidal suspension or dilute solution of the desired product to the rotor and allowing evaporation of solvent from the discharged droplets. The particle size of the aerosol may be predetermined by controlling the speed of rotation of the disc and the concentration of the solution or suspension feed.

The mean diameter of the primary droplets generated by a spinning disc (Walton & Prewett, 1949) is given by:-

$$d_w = \frac{K}{W}\frac{(T)^{\frac{1}{2}}}{D\rho} \qquad \text{Equation 2.3}$$

K = constant
W = angular velocity of disc (rad $s^{-1}$)
D = diameter of rotor (cm)
$\rho$ = density of solvent ($gcm^{-3}$)
T = liquid surface tension (dynes $cm^{-1}$)

The dried droplet size may be calculated from:

$$d_w^3 = \frac{d_d^3 \rho d}{C\rho_w} \qquad \text{Equation 2.4}$$

$d_w$ and $d_d$ - diameters of wet and dried droplets, respectively ($\mu m$).

$\rho_w$ and $\rho_d$ - density of solvent and substance to be aerosolised, respectively ($gcm^{-3}$).

C - concentration of feed solution or suspension (% in g/g).

An aerosol of the low-volatility ester, dibutyl phthalate, was produced from the spinning disc generator to test the sampling and particle size measurement systems used in these studies. The values of the parameters used in Equations 2.3 and 2.4 are shown in Table 2.1.

The theoretical primary droplet diameter was 24.7µm. Solutions of dibutyl phthalate in methanol were made up to concentrations of 0.2%, 2% and 5.3% by weight. The particle size distributions of the dried droplets from each solution were measured with the Andersen Sampler (section 2.3.1) and the PMS light-scattering instrument (section 2.3.2), following sampling from the aerosol generation chamber. The results are presented in section 2.3.4.

Table 2.1

| Equation 2.3 | Equation 2.4 |
|---|---|
| $K = 4.5$ (i) | $\rho_w = 0.791\ gcm^{-3}$ |
| $D = 2.618cm$ | $\rho_d = 1.043\ gcm^{-3}$ (iv) |
| $\rho = 0.791\ gcm^{-3}$ (ii) | $d_w = 24.7\mu m$ |
| $T = 22.65$ dynes $cm^{-1}$ | $C = 0.2$, 2 or 5.3 |
| $W = 6031$ rad $s^{-1}$ (iii) | |

Notes (i) average value for high speed air driven disc (May 1966a).
(ii) density of solvent, methanol
(iii) at 10 psi driving air pressure
(iv) density of dibutyl phthalate

## 2.3 SAMPLING AND PARTICLE SIZE MEASUREMENT

The problems of sampling aerosols to obtain a representative size distribution measurement are well known (Allen, 1975). Metered-dose inhalers produce particular sampling problems due to the high velocity, high concentration and inhomogeneity of the discharged aerosol spray. The droplets in the spray undergo rapid evaporation and the propellant must be allowed to evaporate if the drug powder particles are to be measured. Difficulties arise in avoiding loss of larger particles by sedimentation whilst taking a representative sample of the finer particles. This requires uniform mixing of the discharged spray with air, which may need to be filtered.

Nebulised aerosols are also difficult to sample representatively because of their unstable nature. A polydisperse aerosol of aqueous droplets is produced which changes in size distribution due to rapid evaporation. The evaporation rate is dependent on solute and solvent characteristics, the initial particle size distribution and the relative humidity (or solvent vapour pressure) of the dilution air.

### 2.3.1 The Andersen Sampler (Gelman Hawkesley Ltd., Northampton)

#### Introduction

Cascade impaction was selected as the prime particle sizing method because it not only measures aerodynamic size, the relevant size parameter for describing particle behaviour during inhalation, but specifically measures the particle size distribution of known components in an aerosol cloud. The Andersen Sampler (Andersen, 1966) was selected as a suitable cascade impactor for the following reasons:-

(i) It has adequate stages to achieve reliable size distributions. The use of small numbers of stages causes

greater errors due to interstage overlap (Mercer, 1964).

(ii) It has a suitable size range for inhalation aerosols (0.4-9μm) and operates at a realistic airflow of 28.3 l $min^{-1}$ for inhalation studies.

(iii) It has notably low wall losses (Rao, 1975).

(iv) This instrument is well-known in the field of aerosol research, allowing comparisons of results with published work.

(v) It is manufactured in a way which should give reproducible jet dimensions and is supplied pre-calibrated.

(vi) It has been shown to give satisfactory results with metered dose inhalers (Nilsson et al., 1977).

A schematic cross section of the Andersen Sampler is shown in Fig. 2.7. The particle size range collected at each stage depends on the orifice velocity, the distance between the orifices and the collection surface, and the collection characteristics of the preceding stage. The combination of a constant volumetric flow rate and successively smaller diameter orifices increases the air velocity in successive stages, resulting in the impaction of progressively smaller particles. Particles too small to be impacted on the last collection plate are collected in the backup filter which is an integral part of the sampler.

The Andersen Sampler has an optional preseparator, which removes particles greater than 10μm, before the aerosol enters the first stage. However, this was not thought to be as useful an inlet system as a glass throat, which mimics the human oropharynx (Hallworth and Andrews, 1976).

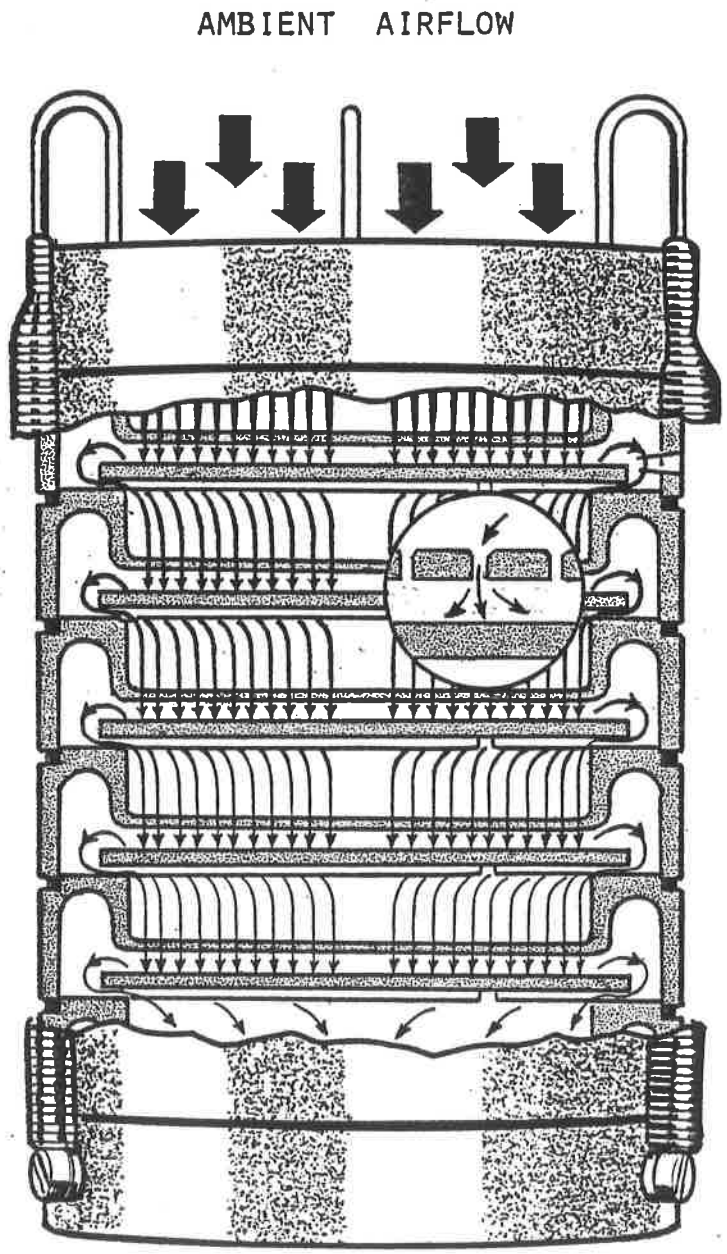



Fig 2.7.

SCHEMATIC CROSS SECTION OF THE ANDERSEN SAMPLER

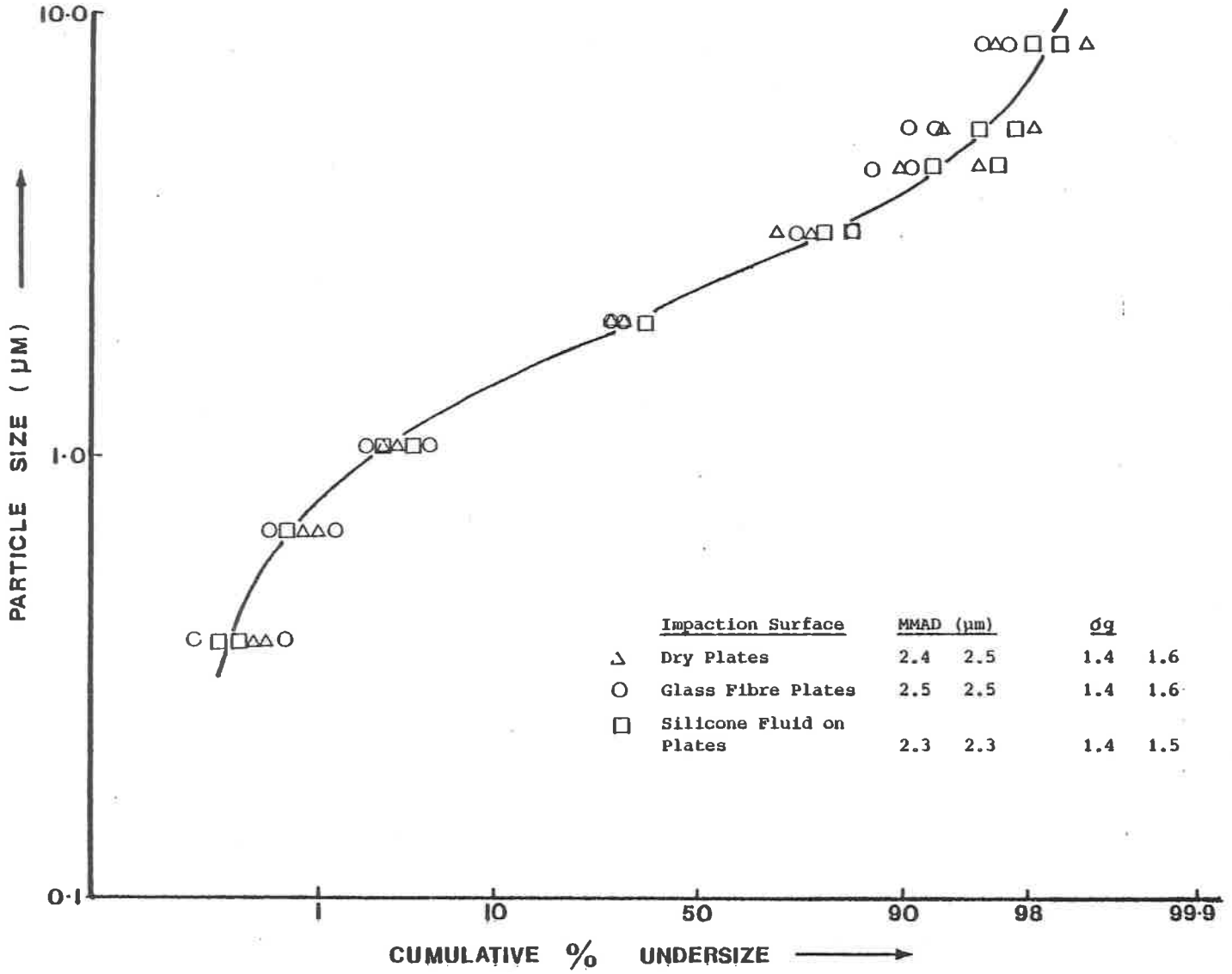
Experimental

In view of the known problem of particle bounce on impactor plates, the sampler was evaluated with three kinds of impaction surface, namely, dry stainless steel plates, oiled plates and glass fibre filter papers. The oily surface was produced by coating the stainless steel plates with a 50/50 mixture of 200 and 1000cS silicone fluids. The layer was approximately 2μm thick, except for stages 1 and 2 where it was approximately 5μm thick. The thickness was estimated from the plate areas and weight increase after coating. Each plate system was tested in duplicate by accumulating 40 successive shots of Ventolin Inhaler. This was the maximum number of shots which could be used without extraneous particles appearing between each jet deposit. The size distributions are illustrated graphically in Fig. 2.8 and are summarised below.

| Type of impaction surface | mean diameter $d_{gw}$ (μm) | geometric standard deviation $\sigma g$ |
|---|---|---|
| dry plates | 2.5, 2.4 | 1.6, 1.4 |
| oiled plates | 2.3, 2.3 | 1.5, 1.4 |
| GF/A filter paper | 2.5, 2.5 | 1.6, 1.4 |

The results show little difference for the three types of impaction surface except in the tails of the distributions, showing that the problems of particle bounce and re-entrainment described elsewhere (Rao, 1975) are not a problem for this type of aerosol. In view of the present results, much of the initial experimentation used dry, stainless steel plates for simplicity of technique.

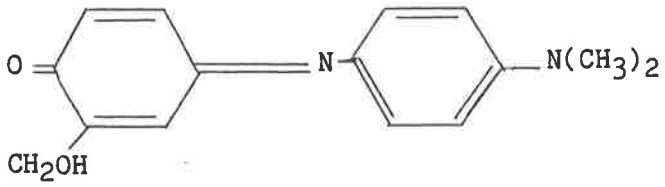
Fig 2.8 Ventolin Inhaler Particle Size Distribution by Andersen Sampler.



The general method for particle size analysis by Andersen Sampler was as follows. The sampler was assembled with clean, dry impaction plates, with a GF/A glass fibre filter paper in the filter holder and a clean, dry, glass throat coupled to the inlet with a rubber sleeve. With the pump drawing air through the sampler, 40 shots of the metered-dose inhaler were discharged into the throat, the can being shaken between each actuation and approximately four shots per minute being fired at regular intervals. The impactor was dissembled and each impaction plate washed with a suitable solvent. Methanol was used to rinse off the drug when assaying simultaneously for oleic acid. When the drug distribution alone was required, aqueous methanol (50%) was used. Details of the oleic acid assay are given in Section 3.3. Salbutamol was assayed colorimetrically by the following method:-

Each sample was made up to 85ml with distilled water and placed in a 250ml separating funnel. 4ml of each of three reagents were added in the following order: 5% w/v aqueous sodium bicarbonate solution, dimethyl p-phenylenediamine reagent and 8% w/v aqueous potassium ferricyanide solution. Under these conditions, salbutamol forms a p-coupled indoaniline blue dye (x) which is soluble in chloroform.

Structure X



The drug and reagent mixture was allowed to stand for 15 minutes in subdued light, the blue dye extracted with two 10ml volumes of chloroform, and made up to volume in a 20ml volumetric flask. The absorbance of this solution was measured spectrophotometrically at 605nm against chloroform. The content of salbutamol in each sample was calculated by reference to the colour produced from a standard solution of salbutamol containing approximately 100µg, accurately measured.

The salbutamol assay was done manually or by autoanalyser. In each case the same reagents were used and the absorbance measured at 605nm. If the autoanalyser was employed, aqueous or aqueous methanol solutions were essential, as pure methanol caused peak interference.

The sampling and assay methods were tested for reproducibility with Ventolin Inhaler. Twenty shots were fired into the Andersen Sampler on two separate occasions, each sample was assayed twice by autoanalyser. There were no measurable differences between the results, the size distribution having a mean diameter and geometric standard deviation of 2.2µm and 1.53, respectively.

The effect on the size distribution of aerosol from Ventolin Inhaler of sampling without a glass throat was demonstrated by using a 5 litre flask and the preseparator (Fig. 2.9). The flask was designed to minimise deposition of the aerosol by impaction, and thereby maximise the quantity of aerosol entering the Andersen Sampler. The experiment was repeated, and the samples assayed manually and by autoanalyser for direct comparison of the assay methods. The results are presented in Table 2.2 and shown graphically in Fig. 2.10.

Fig 2.9 Sampling Apparatus for the Andersen Sampler.

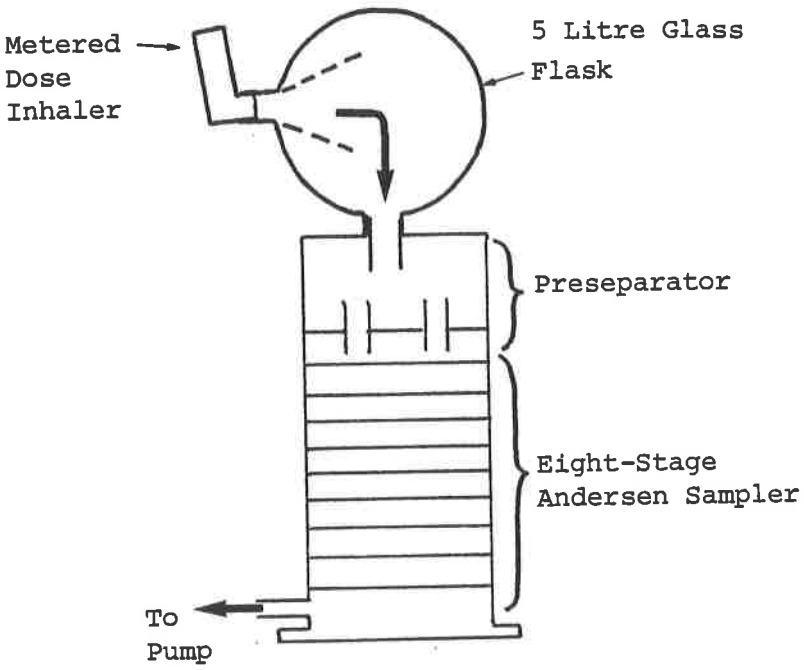


Table 2.2 Results comparing autoanalyser and manual assays of VENTOLIN aerosol, 20 shots

| Sample | Autoanalyser peak height (cm) | µg/ml salbutamol | total µg per stage | % in size range | % less flask & walls | Cumulative % undersize |
|---|---|---|---|---|---|---|
| Flask | 25.3 | 10.05 | 201.0 | 9.5 | | |
| Preseparator | 4.3 | 1.15 | 11.5 | 0.5 | 0.6 ) ) 1.9 | |
| 0 | 7.4 | 2.45 | 24.5 | 1.1 | 1.3 ) | 98.1 |
| 1 | 18.25 | 7.05 | 70.5 | 3.3 | 3.8 | 94.3 |
| 2 | 22.0 | 8.65 | 86.5 | 4.1 | 4.6 | 89.7 |
| 3 | 12.95 | 4.8 | 320.0 | 15.1 | 17.1 | 72.6 |
| 4 | 22.1 | 8.7 | 870.0 | 41.0 | 46.5 | 26.1 |
| 5 | 22.4 | 8.8 | 440.0 | 20.7 | 23.5 | 2.6 |
| 6 | 11.0 | 4.0 | 40.0 | 1.9 | 2.1 | 0.5 |
| 7 | 2.9 | 0.55 | 5.5 | 0.2 | 0.3 | 0.2 |
| 8 Filter | 2.5 | 0.4 | 4.0 | 0.2 | 0.2 | |
| Walls | 7.05 | 2.3 | 46.0 | 2.2 | | |
| TOTAL | | | 2119.5 | | | |

| Sample | Spectrometer reading (manual assay) | total µg per stage | % in size range | % less flask & walls | Cumulative % undersize |
|---|---|---|---|---|---|
| Standard | 0.286 | 99.8 | | | |
| Flask | 0.977 | 347.0 | 19.0 | | |
| Preseparator | 0.160 | 56.8 | 3.1 | 4.0 ) ) 6.2 | |
| 0 | 0.088 | 31.2 | 1.7 | 2.2 ) | 93.7 |
| 1 | 0.215 | 76.3 | 4.2 | 5.4 | 88.3 |
| 2 | 0.322 | 114.4 | 6.3 | 8.0 | 80.3 |
| 3 | 0.926 | 328.9 | 18.0 | 23.1 | 57.2 |
| 4 | 1.029 | 365.5 | 20.1 | 25.7 | 31.5 |
| 5 | 0.978 | 347.3 | 19.1 | 24.4 | 7.1 |
| 6 | 0.173 | 61.4 | 3.4 | 4.3 | 2.8 |
| 7 | 0.047 | 16.7 | 0.9 | 1.2 | 1.6 |
| 8 Filter | 0.064 | 22.7 | 1.2 | 1.6 | |
| Walls | 0.154 | 54.7 | 3.0 | | |
| TOTAL | | 1822.9 | | | |

Fig 2.10 Graph to Show Log-Probability Curves of Ventolin Aerosol (20 Shots) Assayed Manually and by Autoanalyser.

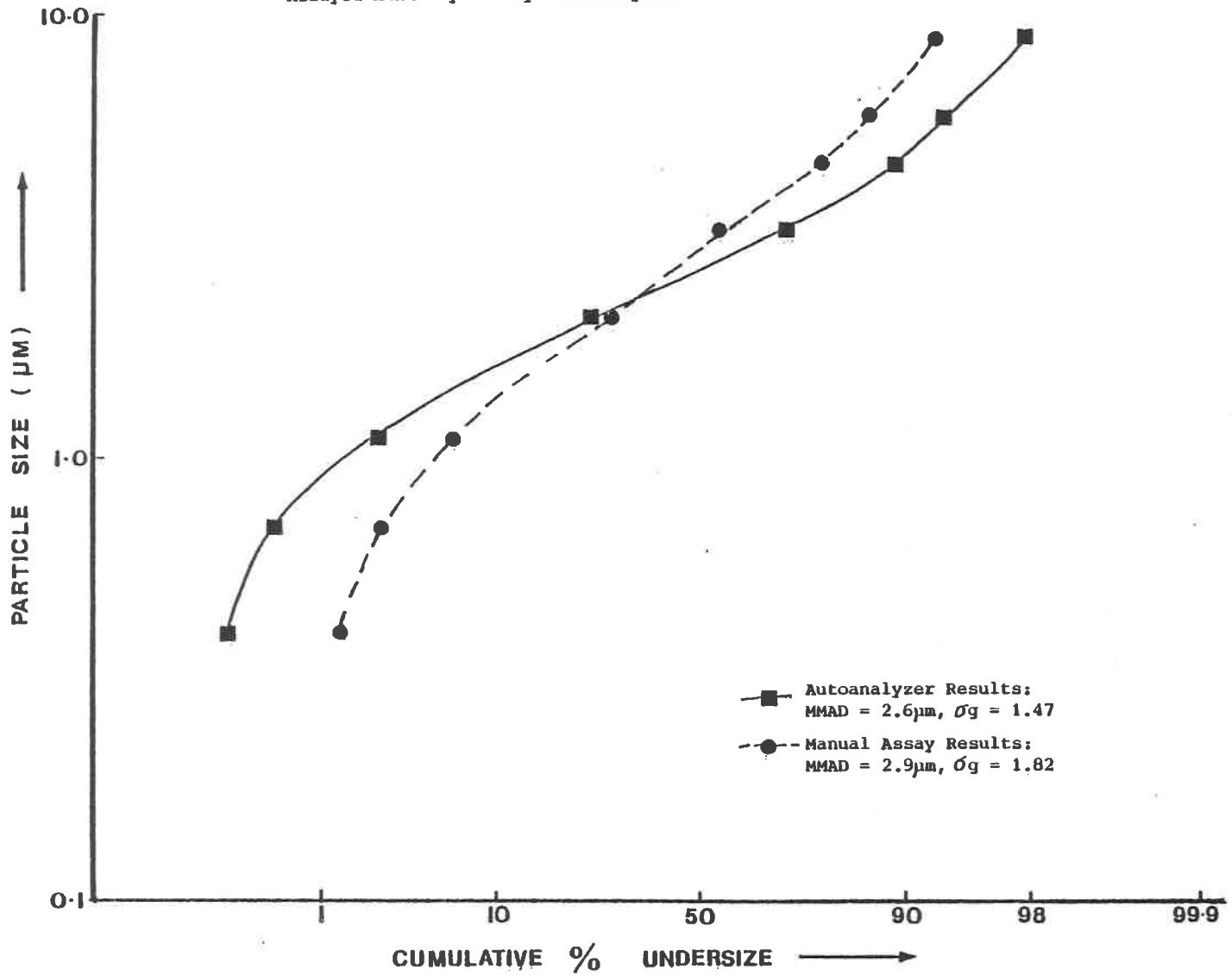


Table 2.3 summarises the results from experiments using the Andersen sampler with and without the preseparator fitted, and with different plate impaction surfaces. Comparing the mean diameter and standard deviation figures, they are all within the limits of experimental error, except for samples from experiments 8 and 9 in which salbutamol aerosols containing no oleic acid were used. The mean diameters of 1.7 and 1.75μm in these experiments are lower than the previous results using standard cans containing 10% oleic acid (average mean diameter 2.5μm).

Discussion

The Andersen Sampler proved to be a robust, reproducible method for measuring particle size distributions of metered-dose inhalers. The different methods of sampling into the instrument had no significant effect on the distribution obtained, therefore the glass throat was used throughout these studies.

The results obtained from aerosols containing no oleic acid show lower mean diameters than normal, and suggest that particle bounce may have occurred. The presence of oleic acid may prevent this problem, by increasing the stickiness of the drug particles, and perhaps explains the small effect of varying the impaction surface with Ventolin Inhaler. The results of Rao and Whitby (1978) demonstrating particle bounce were obtained with spherical, polystyrene particles which are elastic and more likely to bounce on impact. The Andersen Sampler was the only particle sizing method used routinely in these studies for assessing the distributions of gamma-radiolabelled aerosols since it was readily available, portable, relatively simple to use and provided aerodynamic sizes directly.

Table 2.3 Table to show comparison of results from experiments using VENTOLIN inhaler and the Andersen sampler.

| Experiment No. | Number of Shots | Assay - manual or autoanalyser | Salbutamol aerosol dgw (μm) | $\sigma g$ | Notes |
|---|---|---|---|---|---|
| 1 | 20 | M | 2.3 | 1.67 | |
| 2 | 20 | M | 2.8 | 1.82 | with preseparator |
| 3 | 20 | AA | 2.65 | 1.5 | with preseparator |
| 4 | 40 | AA | 2.5 | 1.56 | |
| 5 | 40 | AA | 2.3 | 1.53 | oiled plates |
| 6 | 40 | M | 2.45 | 1.58 | GF/A filter paper |
| 7 | 30 | M | 2.35 | 1.68 | oiled plates |
| 8 | 30 | M | 1.75 | 1.78 | without oleic acid |
| 9 | 30 | M | 1.7 | 1.77 | oiled plates, without oleic acid |

## 2.3.2 Laser light-scattering Instruments

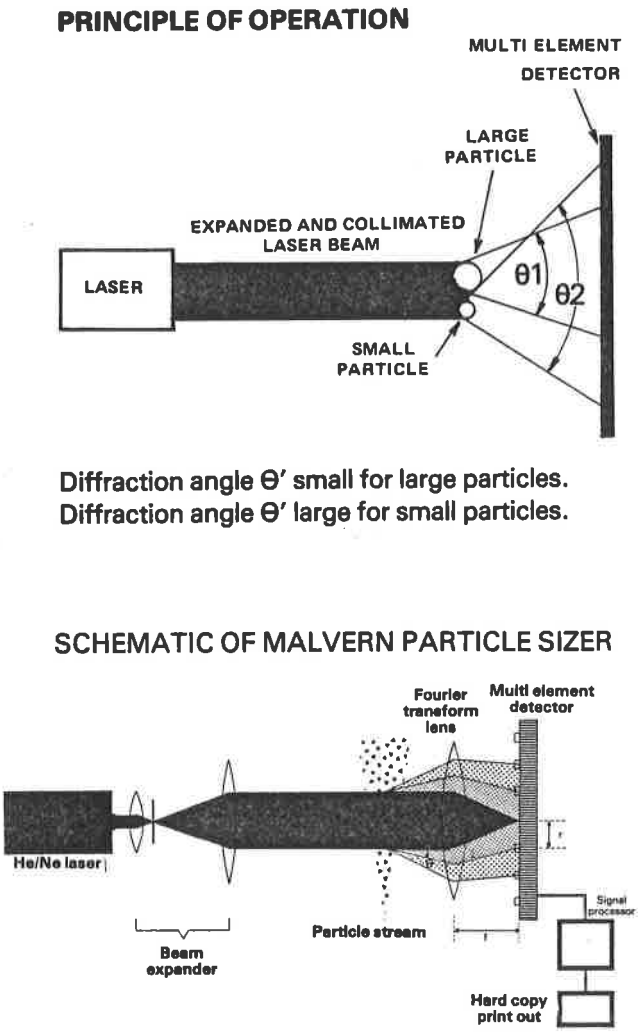
### Introduction

Light-scattering was explored as an auxiliary method of particle size analysis because of its great potential advantage of rapidity. It does, however, measure on the basis of projected area diameters and care must be taken in attempting to compare the results with aerodynamic sizes. Laser light-scattering methods are relatively new, and have rarely been described for assessing inhalation aerosols (Hiller et al., 1978a; Davies et al., 1980). Instruments employing laser light sources were considered to be better than white light instruments since size resolution is greater due to the monochromatic illumination and uniform energy density of a laser (Lundgren et al., 1979). Four laser sizing instruments were considered, three of which are commercially available instruments and one, the 'SPART' system, which has been used extensively for metered-dose aerosols (Hiller et al., 1978a; Hiller et al., 1980a).

### Selection of the sizing instrument

1. The Malvern ST1800S (Malvern Instruments Ltd., Spring Lane, Great Malvern, Worcs.) measures the diffraction pattern produced by the passage of a laser beam through an aerosol cloud. It is based on the principle that the diffraction angle produced by a particle is inversely proportional to the size of the particle. A Fourier transform lens focuses coaxially all the light rays with the same diffraction angle at a distance from the axis which is proportional to the diameter of the particle (Fig. 2.11). The light energy distribution is measured by detectors at various radial distances from the axis and is used to calculate the aerosol particle size distribution.

Fig 2.11. The Malvern ST1800S Particle Sizer.




The main disadvantages of this instrument are:

(a) it does not measure individual particles, but rather the total diffraction in each size class, and there are complex corrections applied in apportioning the diffracted light correctly to each size class.

(b) the size range of 1.2-100μm (using a single lens) does not permit the lower size fractions of inhalation aerosols to be measured.

(c) The results are calculated by assuming that the data fit the Rosin-Rammler distribution. This equation has the disadvantage of using a double-log function which may artificially reduce the scatter of the results, and is not necessarily the best equation to use for a variety of aerosols (Allen, 1975).

(d) Although aerosol sampling is rapid, the data handling processes take several minutes for each calculated distribution which is inconvenient for extensive experiments.

(e) Particles below the minimum size range of the instrument can cause severe interference with the measured size distribution due to Rayleigh light scattering (Butters and Wheatley, 1982). Initial results with this instrument show that approximately 50% of the particle distribution by number for Ventolin Inhaler is below 1.2μm.

2. The Royco Model 226 (Gelman Hawkesley Ltd., Northampton) provides a number distribution of an aerosol cloud by measuring the amount of light scattered at 90° by individual particles, which is proportional to their projected area diameters.

The size range of 0.1-6.1μm is divided into 15 size channels, and the results are printed out in a 6-digit display of counts in each size channel (Fig. 2.12). Unfortunately the upper size limit of the range is too low to provide a complete weight distribution from metered dose inhalers. The sampling tube is badly designed for sampling from a large chamber, being located in the side of the instrument and immovable. Also, measurements based on 90° scattered light are sensitive to the refractive index of the aerosol particles (Lundgren et al. 1979).

Fig. 2.13 shows the log-probability plots of particle size distribution of Ventolin Inhaler, which gave mean diameters by number and weight of 0.28μm and 3.8μm respectively.

3. The SPART analyser (Hiller et al., 1978a) measures aerodynamic diameters of particles in the size range 0.2-10μm at a maximum count rate of 200 particles per second. The aerodynamic diameter, $d_a$, is related to the aerodynamic relaxation time, $T_p$, by the equation:

$$T_p = d_a^2 C_{ca} / 18 \eta \qquad \text{Equation 2.5}$$

where $C_{ca}$ - slip correction factor

$\eta$ - dynamic viscosity of air.

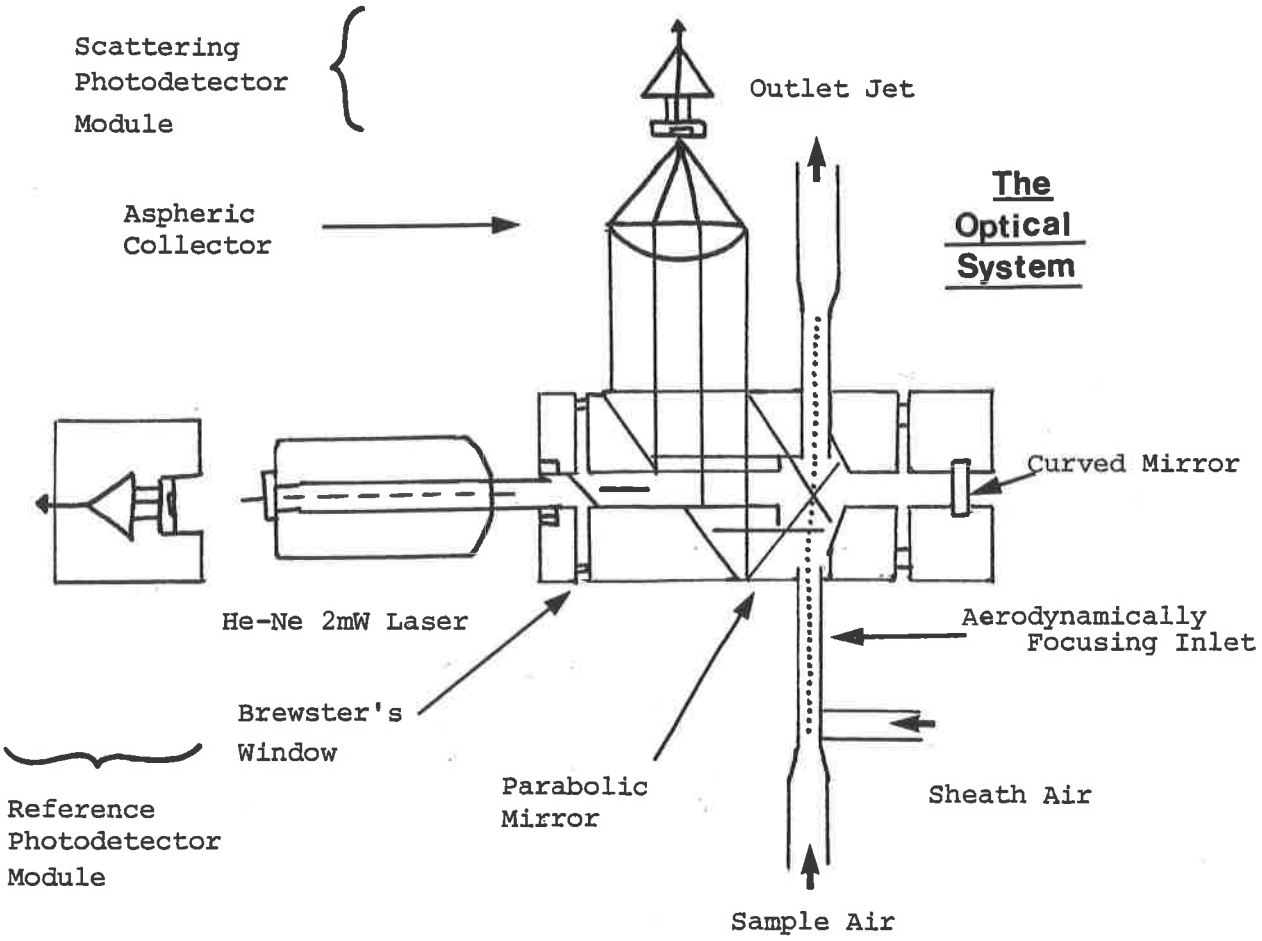
and the relaxation time, $T_p$ is determined by:

$$\theta = \tan^{-1} ( \omega T_p)$$

where $\theta$ - relative phase lag

$\omega$ - frequency of acoustic excitation

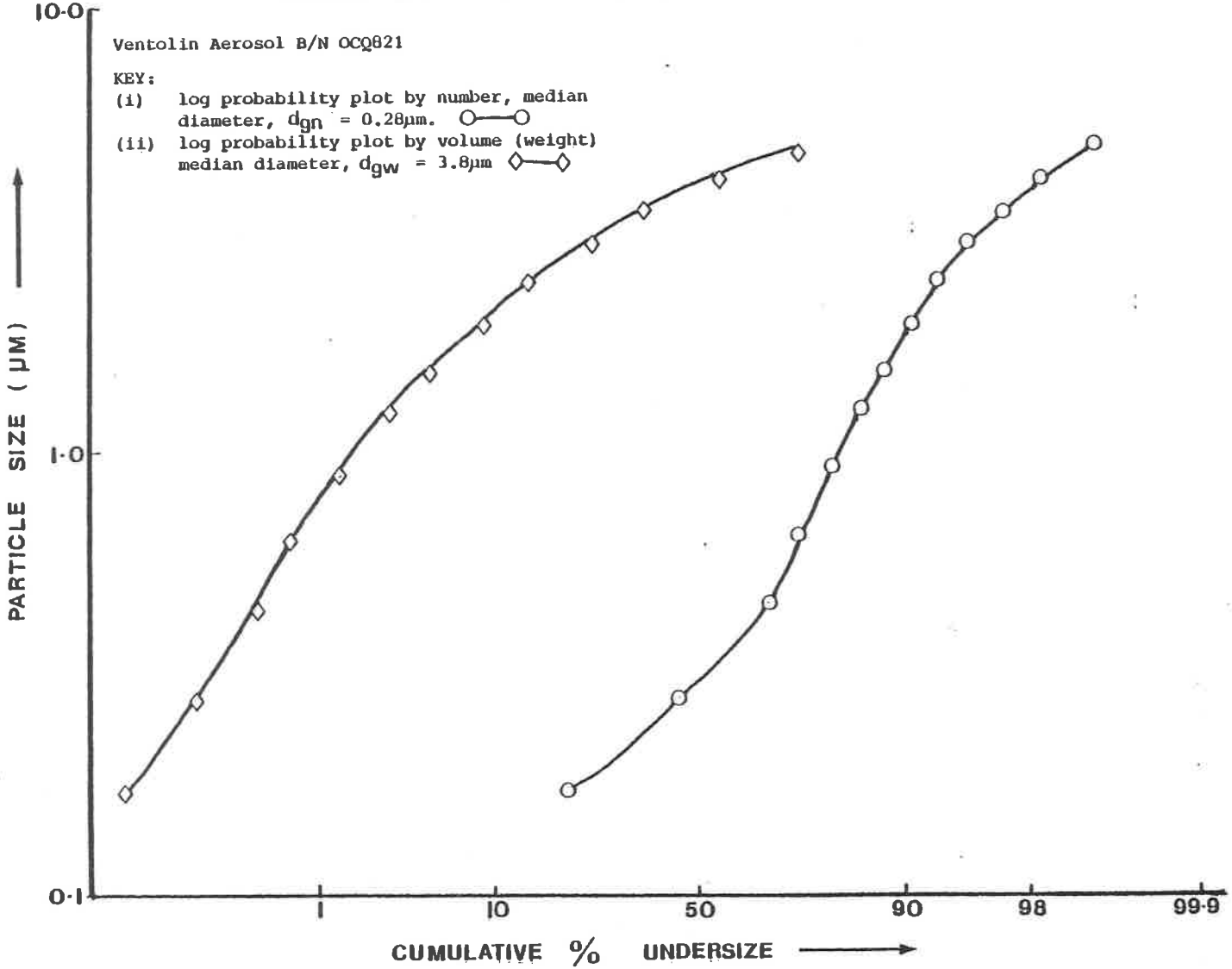
Fig 2.12 Details of the Royco 226 Particle Sizer.




Size Range = 0.1 to 6.1 Micrometres.

| Channel | Size (Micrometres) |
|---|---|
| 1 | 0.10 to 0.17 |
| 2 | 0.17 to 0.27 |
| 3 | 0.27 to 0.42 |
| 4 | 0.42 to 0.62 |
| 5 | 0.62 to 0.87 |
| 6 | 0.87 to 1.17 |
| 7 | 1.17 to 1.52 |
| 8 | 1.52 to 1.92 |
| 9 | 1.92 to 2.37 |
| 10 | 2.37 to 2.87 |
| 11 | 2.87 to 3.42 |
| 12 | 3.42 to 4.02 |
| 13 | 4.02 to 4.67 |
| 14 | 4.67 to 5.37 |
| 15 | 5.37 to 6.12 |
| 16 | Over 6.12um |

Fig 2.13 Graph to Show Log-Probability Plots by Volume (weight) and Number of Ventolin Aerosol. 10 Shots. (Measured by Royco Model 226).



The relaxation time of individual particles and droplets in real time is determined by measuring the phase lag of suspended particles in air with respect to acoustic excitation. A laser Doppler velocimeter measures the Doppler shift of light scattered by an oscillating particle passing through the sensing volume and a microphone measures the acoustic velocity field of air in a standing wave. The difference between these two values is the relative phase lag $\Theta$.

Unfortunately, this instrument is not commercially available, but was considered for use in the present study. It has been used by the manufacturers to produce mass distribution results from metered-dose inhalers which compare well with cascade impactor results. The low aerosol concentrations required, necessitating typical sampling times of about 10 minutes to provide sufficient counts for statistical accuracy, present possible problems for achieving representative sampling.

The SPART analyser has been used to measure the effect of humidity on the aerosol size distributions from MDI's in an attempt to estimate the hygroscopic growth of bronchodilator drug aerosols (Hiller et al., 1980).

4. PMS-probe models CSASP-100 and ASASP-X (Particle Measuring Systems Inc., Boulder, Colorado, U.S.A.). Both of these models, designed to work together, were used by the manufacturers of the instruments to assess the particle size distribution of Ventolin Inhaler. The ASASP-X has essentially the same sampling system and optics as the Royco 226, although a high energy density in the laser cavity of the former enables a low size range of 0.08-3.0µm to be achieved. However it was not necessary to use the ASASP-X in conjunction with the CSASP-100 since the lower limit of the latter instrument (0.3µm) was adequate for the envisaged use. The CSASP-100 (subsequently referred to as 'PMS') measures in a

size range of 0.3-20μm which is divided into 60 size channels divided equally into four overlapping sub-ranges (Table 2.4). The photomultiplier collects laser light scattered by each particle at narrow forward angles (Fig. 2.14). This gives less sensitivity to the refractive index and morphology of the aerosol particles than wider angle or 90° light scatter. The scattered light is a function of the particle projected area. The sampling system is in the form of a miniature wind tunnel which accelerates the particles to a flow rate of $6.22ms^{-1}$ at the point of intersection with the laser beam, and high particle concentrations can be handled (Knollenberg 1976).

Figure 2.15 shows an approximate number size distribution for Ventolin Inhaler, incorporating the results from both probes, and determined by adding together cumulative counts in arbitrary size ranges. Correct computation over all four sub-ranges of a probe is difficult because of the overlapping sub-ranges and the different size channel widths. The mean size by number from this distribution is approximately 0.26μm, which agrees well with the result from the Royco 226 of 0.28μm.

The PMS instrument (probe CSASP-100, data processing unit PDS-300) was selected for the present study, since both the Malvern and Royco instruments did not cover the lower and upper limits respectively of the required size range. The optical design of the PMS minimised errors due to particle refractive index and shape, and the built-in sampling system was versatile enough to handle different types of aerosol. The PMS was purchased with a microcomputer to analyse the particle counts and to calculate several parameters from each aerosol sample.

Table 2.4 Details of the PMS Instrument

| | |
|---|---|
| Size Ranges: | Four ranges covering 0.3-20μm<br>Range A 0.30-0.75μm<br>Range B 0.50-2.75μm<br>Range C 1.00-12.25μm<br>Range D 2.00-20.0μm |
| Number of Size Channels: | 15 (x 4) |
| Minimum Detectable Size: | 0.3μm |
| Size Resolution: | 0.03μm typical (Range A) |
| Sampling capability: | Sample area: 0.018mm$^2$<br>Volume sampling rate: 0.13cm$^3$s$^{-1}$ |
| Laser: | He-Ne high order multi-mode |

Fig 2.14 OPTICAL SYSTEM DIAGRAM PMS Instrument

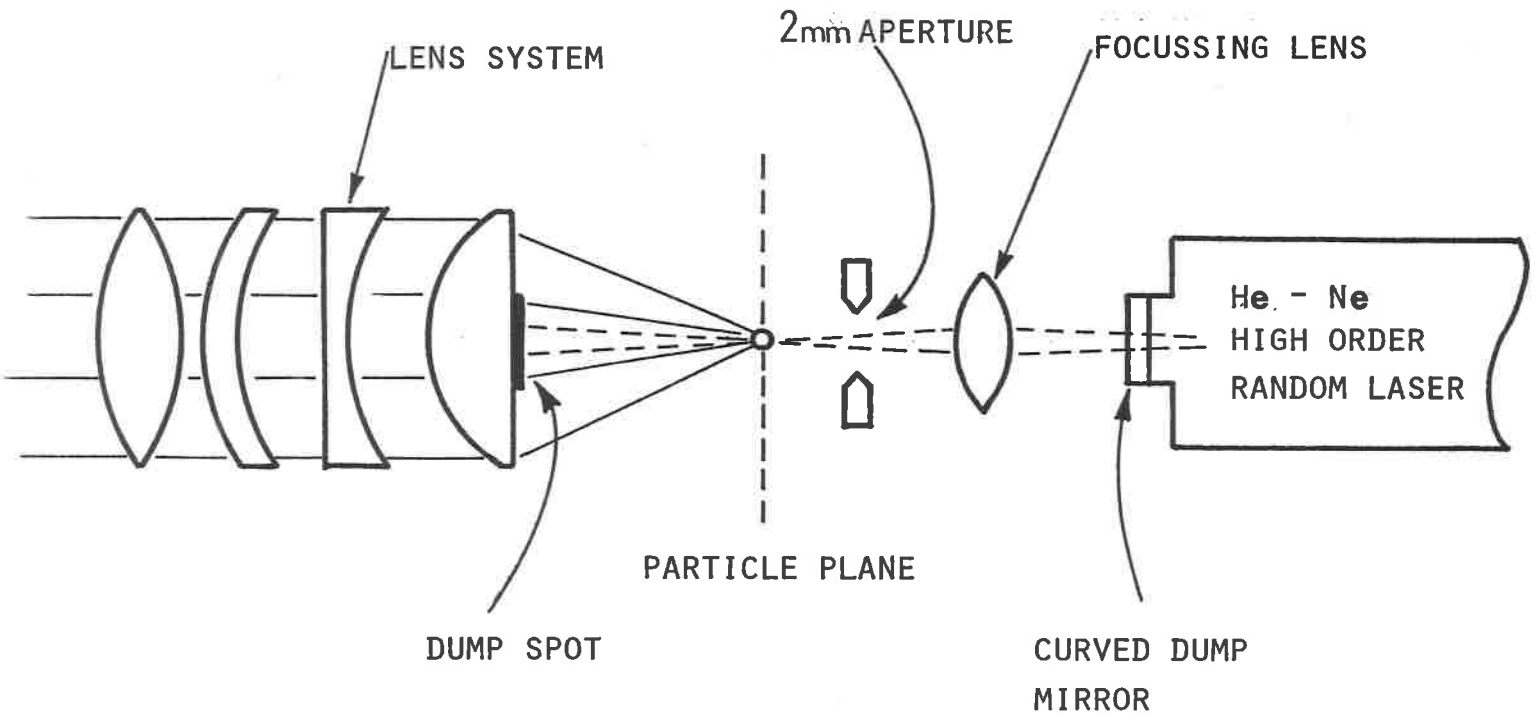
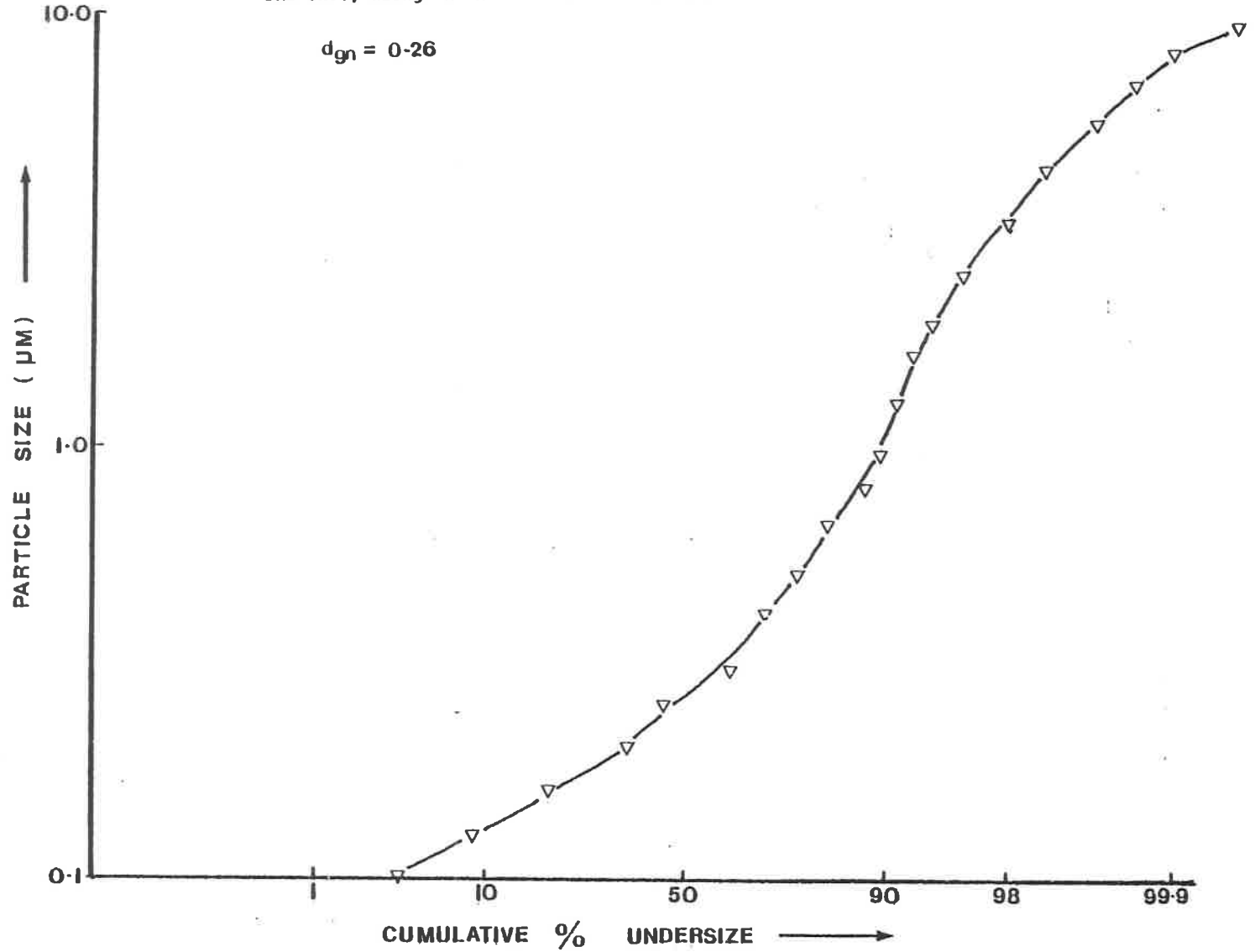


Fig 2.15 Graph to Show the Log-Probability Plot of Ventolin Aerosol as Measured by PMS Inc., using the ASASP and CSASP Probes.



Sampling Methods

Sampling methods for the PMS used small dilution chambers for the collection of an MDI spray and delivery of a sample to the inlet of the instrument. A 5 litre spherical glass flask was used, with inlet and outlet tubes of 2.5cm inside diameter positioned perpendicular to each other (Fig. 2.16). A flask of this volume was chosen to minimise aerosol losses by impactional deposition of the discharged spray on the walls.

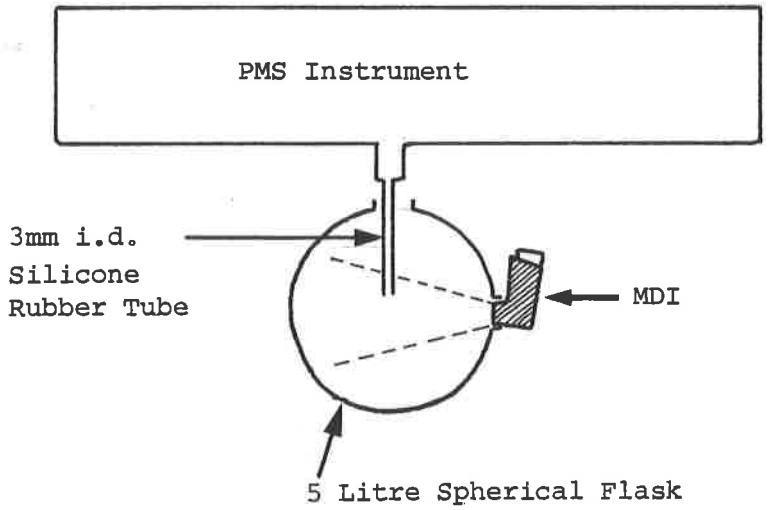



Fig. 2.16 Sampling of MDI via 5 litre flask.

The aerosol was sampled into the instrument either via the 2.5cm outlet or via a straight 3mm i.d. silicone rubber tube positioned so that the inlet end was in the centre of the flask. Alternatively, a cylindrical drum of about 10 litres volume was used with 2.5cm diameter inlet and outlet parts at the ends. A 15cm length of flexible plastic tube of 2.5cm inside diameter delivered aerosol to the laser beam.

These small sampling chambers have the advantage over the large aerosol generation chamber (section 2.3.4) of providing an adequate aerosol concentration from single shots of MDI's to give statistical accuracy in particle numbers with short sampling times. A short sampling interval of 10s for each subrange on the PMS minimised possible aerosol disproportionation errors due to sedimentation losses of the larger particles. Low background counts were achieved by flushing the chambers with filtered air between samples to give less than 50 particles in 1 minute of sampling in the lowest size channel.

Experimental

The four sub-ranges of the PMS are fitted together by the microcomputer by eliminating the overlapping size channels giving the lowest signal-to-noise ratio. Alternatively, any single range can be computed. A disadvantage of this instrument is that although all four ranges were sampled automatically, they were counted sequentially and not simultaneously, which increased the period of sampling, when the four ranges were measured together. This problem was alleviated with MDI's by firing a separate shot into the sampling flask before measuring each sub-range.

The microcomputer (PMS-PDS300) and printer (Axiom Model EX810 printer/plotter) supplied with the instrument provide on a particle number and particle weight basis, frequency histograms, cumulative size distributions, calculated mean diameters, concentration of particles by number and mass and the total number of particles measured (Fig 2.17). Single print-outs of the raw data or any other programmed output may be obtained separately.

Measurement of metered-dose inhalers

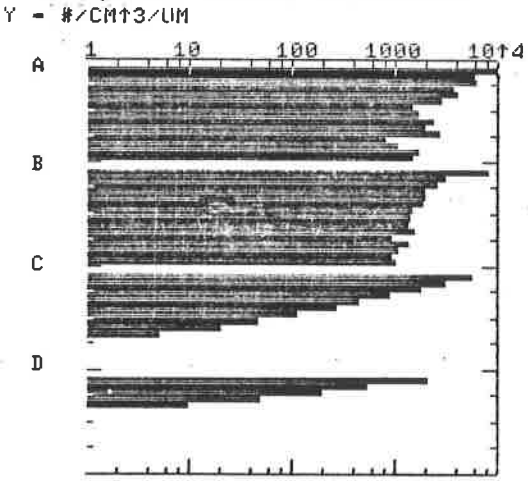
Most measurements were taken using the four sub-ranges

Fig 2.17 Typical Print-Out from PMS Instrument.
(Note: As the size channels of the sub-ranges overlap, the overall shape of the histograms may be misleading).

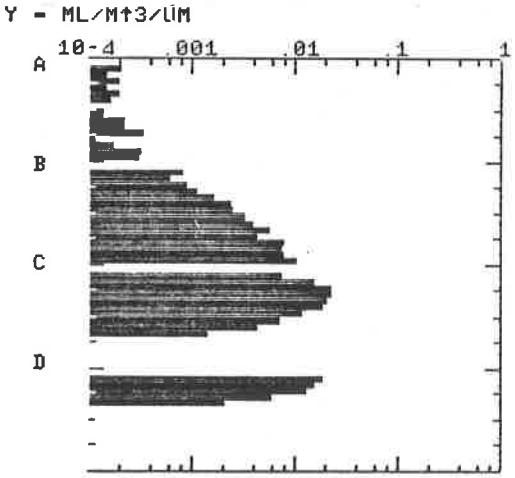
RAW DATA

```
TIME OF DAY                              15:37:39
SAMPLE/RANGE LENGTH                00:00:02.00
A ACTIVITY(%)                           0   0   0   0
R,B RAW POPULATION
A 0  0000000  0000077  0000044  0000046
A 4  0000028  0000031  0000021  0000011
A 8  0000013  0000018  0000015  0000020
A12  0000006  0000008  0000013  0000011
B 0  0000000  0000315  0000118  0000098
B 4  0000077  0000076  0000074  0000058
B 8  0000056  0000052  0000060  0000036
B12  0000053  0000041  0000036  0000039
C 0  0000000  0001080  0000603  0000347
C 4  0000177  0000089  0000052  0000022
C 8  0000009  0000004  0000001  0000000
C12  0000000  0000000  0000000  0000000
D 0  0000000  0000641  0000171  0000062
D 4  0000015  0000003  0000000  0000000
D 8  0000000  0000000  0000000  0000000
D12  0000000  0000000  0000000  0000000
```

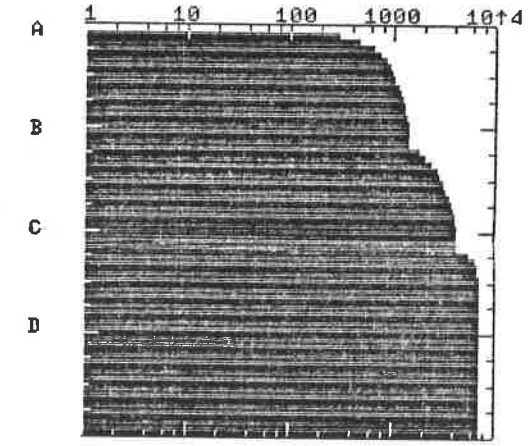
FREQUENCY NUMBER HISTOGRAM



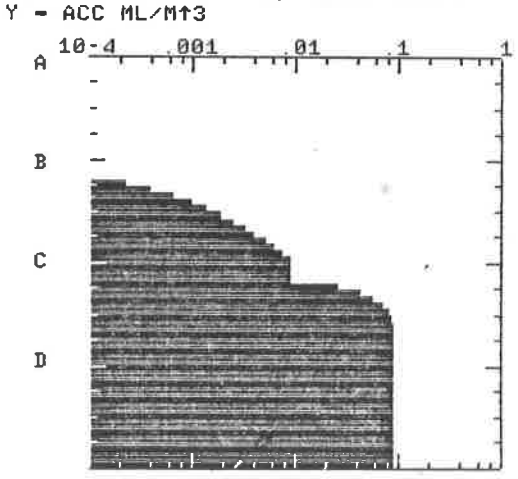

FREQUENCY VOLUME (mass) HISTOGRAM




CUMULATIVE NUMBER HISTOGRAM




CUMULATIVE VOLUME (mass) HISTOGRAM




SUMMARY OF COMPUTED MEAN DIAMETERS, CONCENTRATION

```
TIME OF DAY                              15:37:39
SAMPLE/RANGE LENGTH                00:00:02.00
A ACTIVITY(%)                           0   0   0   0
RAW POPULATION                        .4827E 04
CONCENTRATION(#/CM↑3)                 .6998E 04
GEO MEAN DIA BY CONC(UM)                 1.641
MASS(GM/M↑3)                          .9018E-01
GEO MEAN DIA BY MASS(UM)                 4.022
```

(A-D) sequentially, with a 10s time interval, sampling from a 5 l spherical flask through a 3mm inlet tube as previously described (Fig. 2.18). One shot of the MDI was fired into the chamber at the beginning of each sub-range, and the background count was reduced by flushing the flask with filtered air before firing the inhaler.

The PMS results for Ventolin Inhaler are summarised in Table 2.5. Results from batches 1 and R-01/03 are very consistent both within and between batches. These batches were sampled via the narrow inlet and 5 litre flask, and the mean diameter by weight is similar to that found in batch 9HR554, sampled in an identical manner. However sampling batch 9HR554 via a wide inlet to the PMS instrument reduces the mean diameter results, although the number of samples used is too small to confirm this.

Problem of non-drug particles

A major disadvantage of all light-scattering spectrometers for use with inhalation products, particularly MDI's, is that all particles in the sample are counted irrespective of their nature. For example, surfactant particles and those representing contaminants from the formulation and packaging components are included. Drug particle distributions can be obtained by subtracting placebo particle distributions from separate metered-dose inhalers prepared without the drug. However, this approach is only valid on a statistical basis for a number of cans, because the non-drug particle distribution was found to vary considerably from can to can.

A study of the size distribution of non-drug particles in Ventolin Inhaler involved manufacture of placebo and propellant-only cans using components that had undergone additional cleaning processes. Thus the aluminium cans were scrubbed inside using a wire brush and propellant 11, and the valves were rinsed in propellant 11. Results from several batches of MDI's with different non-drug particle counts are shown in Table 2.6. Mass frequency histograms of these batches are shown in Fig. 2.19.

Fig 2.18. PMS laser light-scattering instrument.

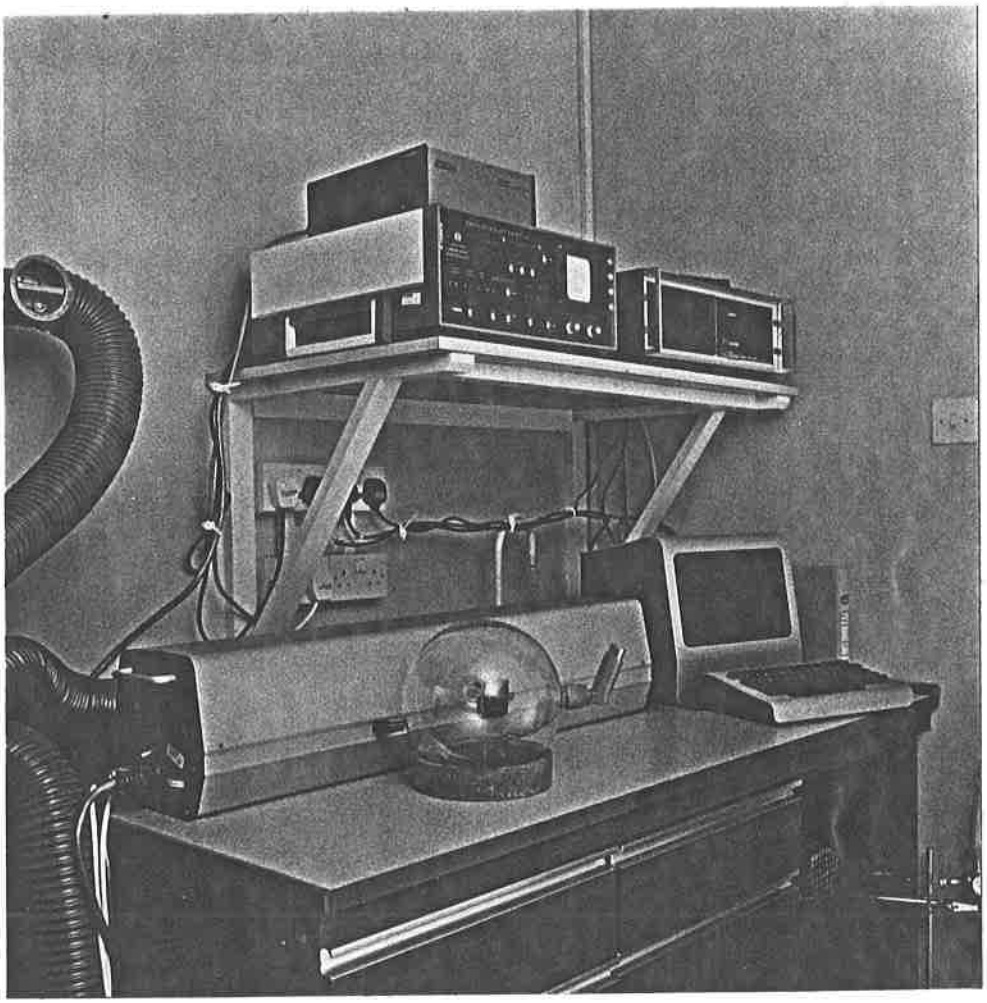


Table 2.5 Summary of PMS results for the mean particle size of Ventolin Inhaler

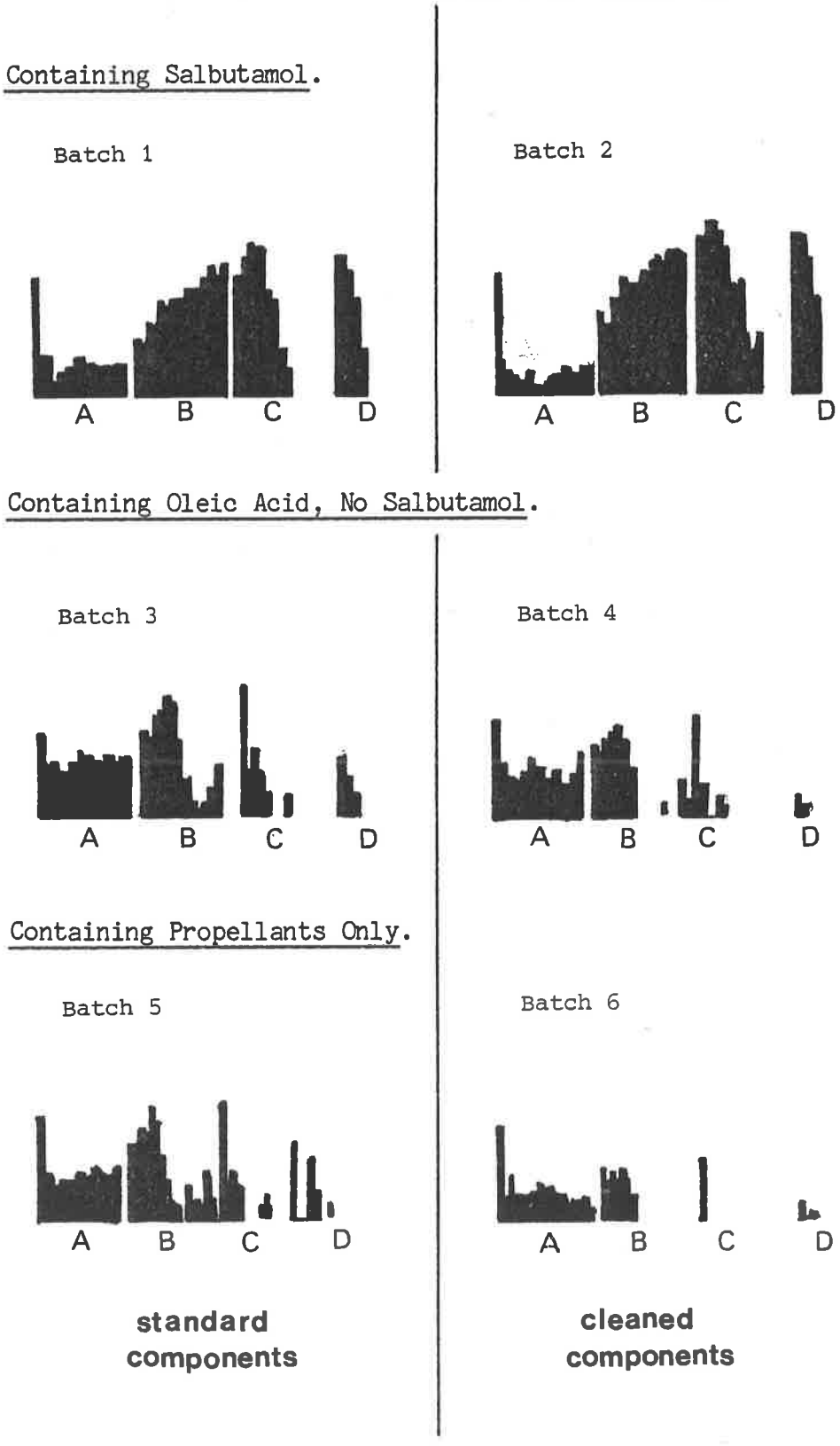
| Sample | | Mean diameter by number $d_{gn}$ (µm) | | | Mean diameter by weight $d_{gw}$ (µm) | | |
|---|---|---|---|---|---|---|---|
| | | Geometric Mean | S.D. | Range | Geometric Mean | S.D. | Range |
| Batch 1 Table 2.6 | (n=12) | 0.65 | 1.19 | 0.51-0.89 | 3.29 | 1.06 | 2.90-3.64 |
| R-01/03 | (n=12) | 0.69 | 1.12 | 0.59-0.84 | 3.34 | 1.08 | 2.84-3.74 |
| B/N 9HR554 narrow inlet 5l. flask | (n=3) | 1.14 | - | 1.12-1.17 | 3.25 | - | 3.17-3.37 |
| B/N 9HR554 wide inlet 5l. flask | (n=3) | 0.49 | - | 0.45-0.55 | 2.43 | - | 2.32-2.50 |
| Total samples | (n=30) | 0.71 | 0.18 | 0.45-1.17 | 3.23 | 0.33 | 2.32-3.74 |

Table 2.6 Summarised PMS results from batches of MDI's containing different amounts of non-drug particles (mean and S.D. of mean diameters).

| Ref. | Containing salbutamol | Containing oleic acid | Standard or cleaned components | number of samples (a) | mass concentration (g $cm^{-3}$) (b)x$10^{-3}$ | $d_{gn}$ (µm) | $d_{gw}$ (µm) |
|---|---|---|---|---|---|---|---|
| 1 | Yes | Yes | Standard | 12 | 12.5 | 0.66 (0.12) | 3.30 (0.19) |
| 2 | Yes | Yes | Cleaned | 12 | 14.6 | 0.73 (0.17) | 3.31 (0.34) |
| 3 | No | Yes | Standard | 11 | 4.2 | 0.60 (0.08) | 2.26 (0.47) |
| 4 | No | Yes | Cleaned | 10 | 1.3 | 0.43 (0.04) | 1.63 (0.23) |
| 5 | No | No | Standard | 12 | 4.1 | 0.59 (0.04) | 2.27 (0.42) |
| 6 | No | No | Cleaned | 11 | 0.7 | 0.39 (0.03) | 1.14 (0.28) |

(a) for each batch, 3-4 samples from each of 3 cans
(b) a density of 1 g $cm^{-3}$ is assumed for the particles.

Fig 2.19 Mass Frequency Histograms of MDI's with Different Non-Drug Particle Counts.
(for batch details see Table 2.5; A,B,C,D refer to sub-ranges, see Table 2.4



The proportion of the mass of particles measured which is contributed by non-drug particles is clearly significant. Comparison of results from Batches 3 and 5 indicates that the presence of oleic acid has little effect on the non-drug particle size distributions if standard components are used. It is also apparent from the results on Batch 6, containing no drug or oleic acid, that the mean number diameters of active cans do not give a true impression of the number distribution of drug. However for practical purposes, Batches 1 and 2 show that the type of components used in an active formulation have no effect on the measured mean mass diameter.

The inclusion of non-drug particles in the size distribution, and the difficulties in computation from four overlapping sub-ranges having different channel widths, meant that the raw data obtained for MDI's could not be fitted to any recognised statistical particle size distribution model. This presented difficulties in comparing one set of data with another by any distribution parameters other than mean size.

The data acquisition system of the PMS is currently being modified using an interface link to a PRIME mainframe computer. The raw data from the PMS are fitted to a bimodal log-normal model size distribution (representing the distributions of drug and oleic acid) to provide sizing parameters for the population without biasing the sample by eliminating size channels. The program also uses data from placebo MDI's to estimate the true size distribution of the drug particles.

When the additional programming was used to assess the contribution of non-drug particles to the distribution, the corresponding diameters by number and weight increases significantly in value, to 1.8, 2.1μm and 3.8, 4.1μm respectively.

MDI's manufactured with salbutamol fractionated by particle size in a zig-zag classifer. Size measurements of aerosols prepared in MDI's with classified fractions of salbutamol (section 2.2.3) gave the results (including non-drug particles) as shown below. The results show that only a small degree of size separation was achieved. The 'coarse' fraction was subsequently used to manufacture MDI's which were compared in vivo by aerosol deposition studies with those made with standard micronised salbutamol.

| Classified fraction | $d_{gn}$ (μm) | $d_{gw}$ (μm) |
|---|---|---|
| 'coarse' | 3.5, 3.7, 4.2 | 7.6, 8.7, 8.4 |
| 'fine' | 3.3, 3.2 | 7.0, 5.5 |

These results are compared with a microscope method of measurement in Table 2.7.

### 2.3.3 Microscopic Methods

#### Sampling Methods

The only existing compendial pharmaceutical methods for the particle size measurement of metered dose inhalers (B.P.C and U.S.P.) depend on firing one shot of the aerosol perpendicularly against a microscope slide held at 5cm from the mouthpiece. The slide is then rinsed with carbon tetrachloride and examined microscopically for impacted particles over 20μm diameter. This is a limited quality control test and is incapable of being adapted to provide a size distribution due to the uneven particle distribution on the slide and the loss of finer particles. The BPC method was occasionally used in this study as a rough check on the quality of the metered dose inhalers being studied.

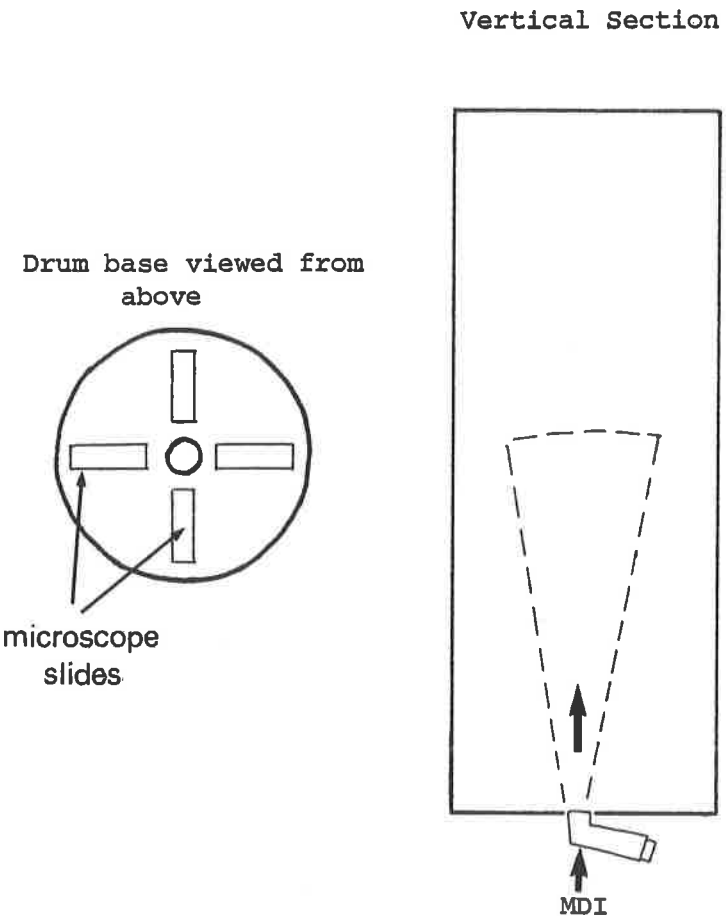
In general, the Dixon drum microscopy method was used which is slow but provides reasonably consistent results. This method has been adapted from the original technique for continous spray aerosols (Dixon, 1966) to allow measurement of metered-dose inhalers (Hallworth & Hamilton, 1976). The aerosol is discharged into a cylindrical chamber and allowed to settle over a period of several hours onto clean microscope slides (Fig. 2.20). About 10-30 shots of the MDI are required in the drum to give adequate particles for counting. The size analysis is facilitated by the use of a Quantimet 720 automatic image analyser.

The aerosol mean particle size obtained by this sedimentation method using either manual microscopy or the Quantimet is often approximately double the aerodynamic size found by cascade impactor methods. The difference is probably mainly due to anisodiametric particles having a preferred orientation on the slide, giving projected area diameters greater than those implied by the equivalent spherical volume. A disproportionately large fraction of the fine particles collect on the walls of the settling drum but these do not alter the derived volume distribution appreciably.

Quantimet system

The Quantimet 720 image analyser was used to scan the microscope slides and detect particles by difference in contrast from the background. A monitor screen enabled those particles which were being detected to be seen by eye. The particle images were measured in a single horizontal dimension in picture points and allocated to preset size intervals. The images were scanned automatically in each field of view and the data stored for subsequent computer analysis. The method is slow, as it takes about one hour to scan a microscope slide and obtain an estimate of size distribution, in addition to the prolonged time for aerosol settling in the sampling drum. A typical print-out of the

Fig 2.20 Dixon Drum Method.



computed size distributions for a Ventolin Inhaler is shown in Fig. 2.21. The computer programme calculates the mean diameters by assuming a log-normal distribution. This may lead to considerable errors with some samples which do not follow such a distribution. The lower size limit is restricted to about 1.1µm because of focussing/detection problems below this size, so that the smaller particles are not counted. However, this does not cause much error in size distributions on a mass basis. The particles must provide good image contrast which requires correct focussing of the microscope. When aggregates of particles are seen visually on the monitor screen they tend to be detected as a single large particle. If the particles assume a random orientation on the slides, the results should indicate the approximate projected area diameter distribution in spite of the unilateral scanning. Manual microscopy based on equivalent circle areas gives similar results and so tends to confirm this approximation. Typical mean diameters measured for Ventolin Inhaler are 2.9µm and 7.4µm by number and weight respectively.

Size measurement of the classified 'coarse' and 'fine' fractions of salbutamol by the Quantimet system are compared in Table 2.7. The results from the Quantimet for these aerosols compare very well with those from the PMS instrument.

Table 2.7 Mean diameters of classified salbutamol in MDI's, measured by the PMS and Quantimet Instruments

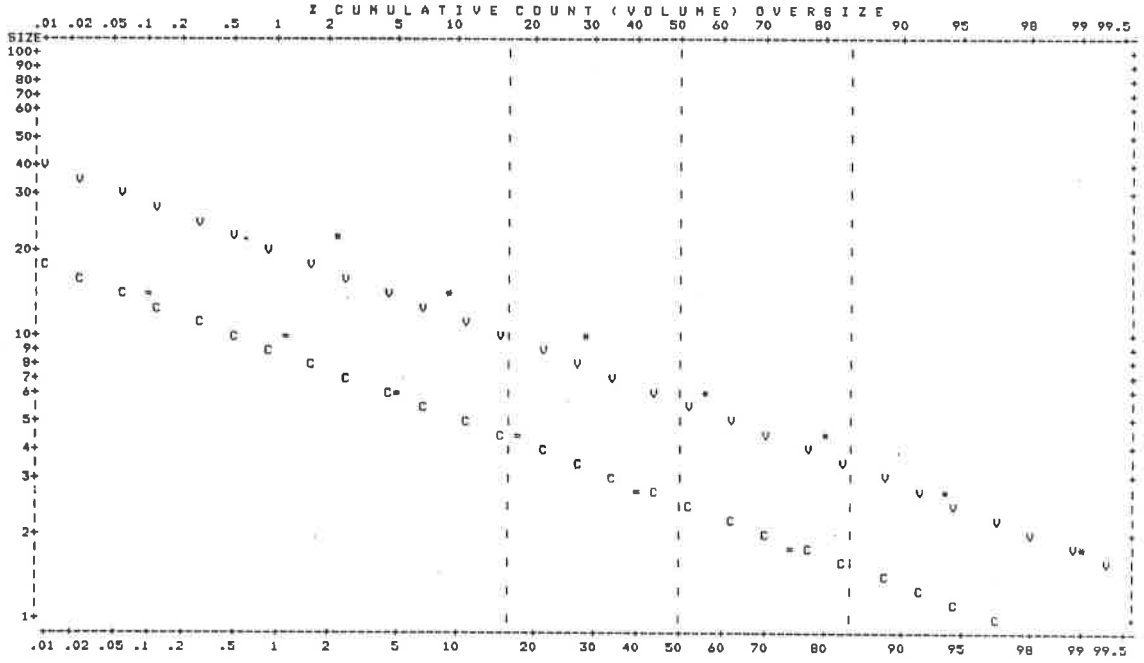
| Classified | Sizing method | $d_{gn}$ (µm) | $d_{gw}$ (µm) |
|---|---|---|---|
| 'Coarse' | PMS | 3.5, 3.7, 4.2 | 7.6, 8.7, 8.4 |
| | Quantimet | 3.7 | 8.7 |
| 'fine' | PMS | 3.3, 3.2, | 7.0, 5.5, |
| | Quantimet | 3.3 | 7.0 |

PAGE 2045

| LOWER SIZE BOUNDARY (UM) | Σ COUNT / UNIT AREA | SE | Σ CUMULATIVE COUNT | Σ VOLUME / UNIT AREA | SE | Σ CUMULATIVE VOLUME | NO. OF FIELDS FOR VOLUME SE = 2Σ |
|---|---|---|---|---|---|---|---|
| 1.1 | 26.3 | 0.5 | 100.0 | 0.9 | 0.0 | 100.0 | 0 |
| 1.8 | 32.8 | 0.5 | 73.7 | 5.1 | 0.1 | 99.1 | 1 |
| 2.8 | 23.2 | 0.4 | 40.9 | 13.5 | 0.3 | 94.0 | 8 |
| 4.3 | 12.6 | 0.3 | 17.7 | 24.6 | 0.5 | 80.5 | 29 |
| 6.4 | 4.1 | 0.2 | 5.1 | 26.9 | 0.8 | 55.9 | 62 |
| 9.6 | 0.9 | 0.1 | 1.0 | 19.8 | 1.0 | 29.0 | 96 |
| 14.2 | 0.1 | 0.1 | 0.1 | 6.7 | 1.1 | 9.2 | 110 |
| 21.3 | 0.0 | 0.1 | 0.0 | 2.3 | 1.1 | 2.3 | 126 |

DLN = 3.01 SE = 0.01
DVN = 4.17 SE = 0.02
DVS = 5.83 SE = 0.06
DGN = 2.60 SE = 0.01
DGV = 6.90 SE = 0.11

NV = 0.263E 11 / CC
SV = 1.03 / MICRON
TOTAL = 50184




ASSUMING THE PARTICLE-SIZE DISTRIBUTION IS LOG-NORMAL:

GEOMETRIC S.D. = 1.68

& THE HATCH-CHOATE EQUATIONS GIVE:

| | PERCENTILE 15.9Σ | 50Σ | 84.1Σ |
|---|---|---|---|
| COUNT | 4.37 | 2.60 | 1.55 |
| VOLUME | 9.77 | 5.82 | 3.47 |

Run on 14/04/82 at 12:27:12

PAGE 2046

Fig 2.21. Quantimet print-out.

## 2.3.4 Aerosol Generation Chamber

### Introduction

The aerosol generation chamber (AGC) was primarily developed to allow simultaneous size measurement of aerosols by the PMS and the Andersen Sampler. It was designed for generation of aerosol from the spinning disc generator or for collection of discharged aerosols from metered dose inhalers. Exploratory studies on nebulised aerosols were also an envisaged use of the AGC.

The chamber was designed to be large enough to minimise impaction on the walls of the discharged spray from an MDI. The dimensions of the AGC were also governed by the requirements of the spinning disc generator. The volume and diameter of the chamber was designed to allow evaporation of the large primary droplets without wall impaction when the droplets are flung off the edge of the disc. The liquid feed to the disc and adjustments to the needle governed the right-angled design of the air inlet and the position of the door and windows in the chamber.

The AGC is shown in Figs. 2.22 and 2.23. The chamber consists of a stainless steel parallel-sided cylinder welded to two conical end pieces and has a volume of 119 litres. An inlet for aerosol samples from MDI's or nebulisers is situated approx. 10cm from the top of the cylinder and may be sealed when not in use. Fittings for supporting the spinning disc generator, supplying pressurised air and removing exhaust air are placed in the cylinder walls; all fittings may be removed so that the empty chamber is available when the generator is not used.

The sampling outlets for particle size measurement from the chamber were positioned in the bottom cone and orientated

Fig 2.22. AEROSOL GENERATION CHAMBER

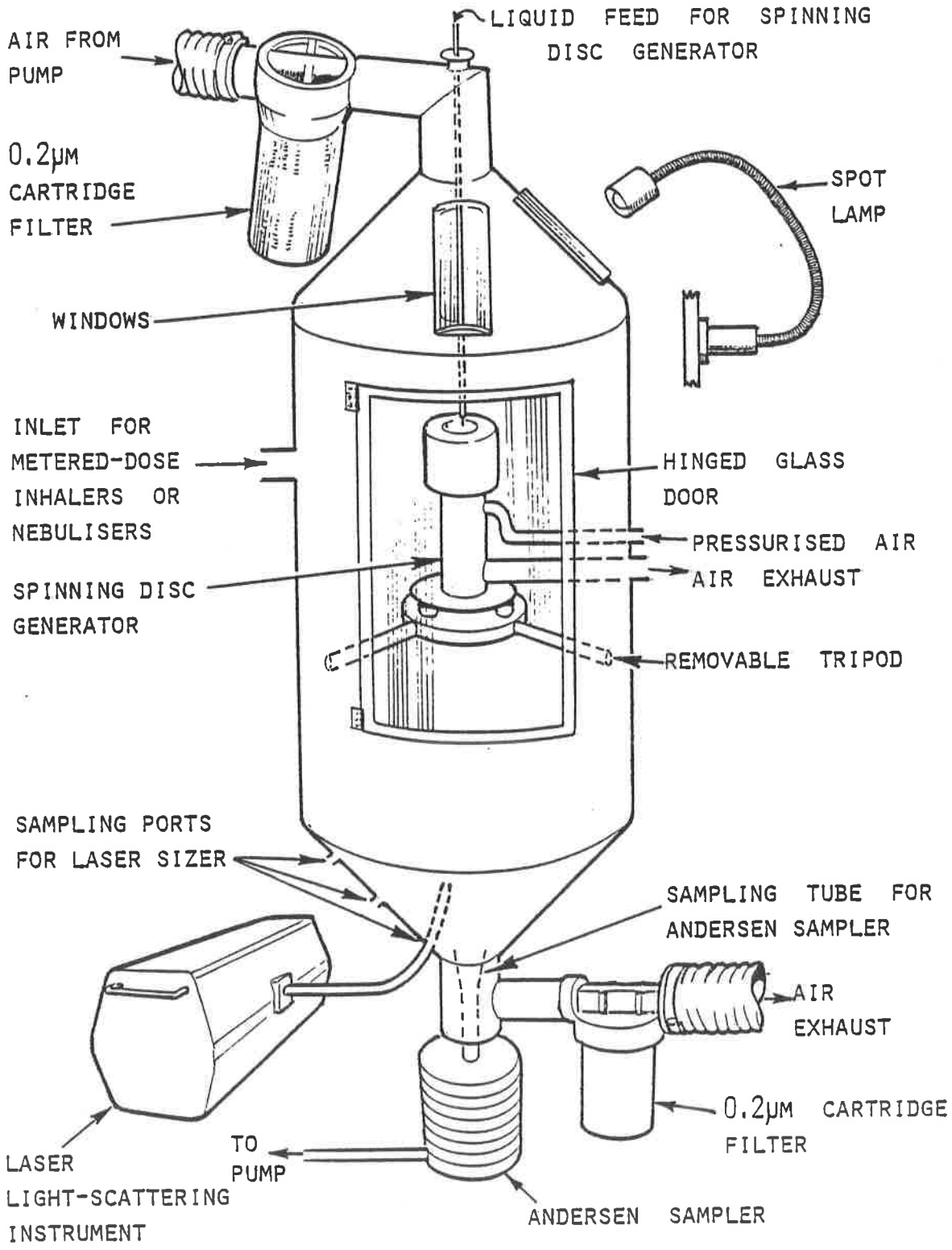
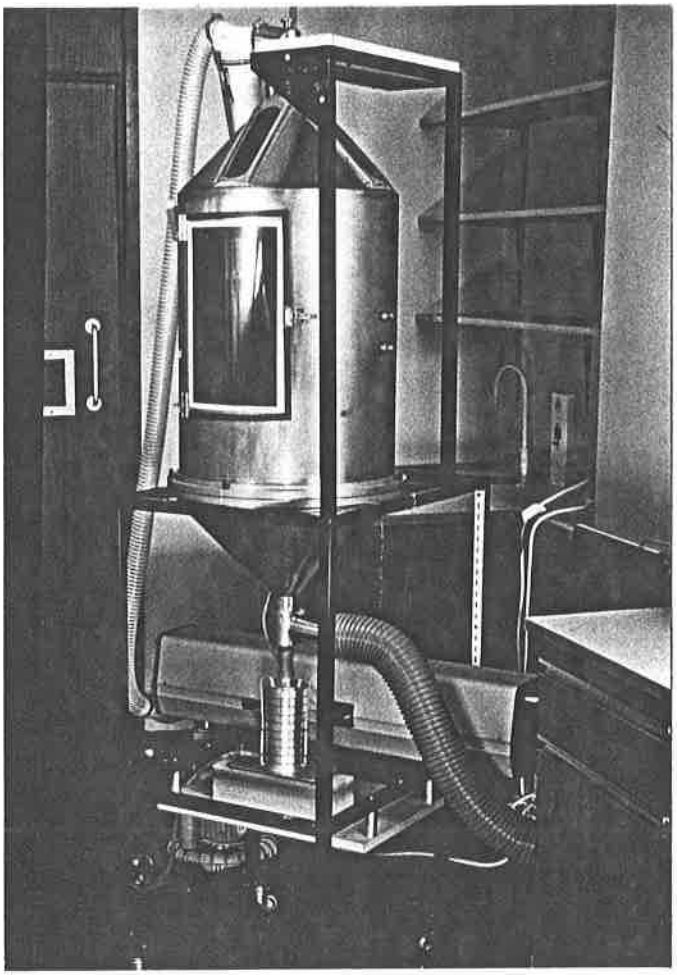


Fig 2.23. Aerosol Generation Chamber.



vertically to minimise sedimentation errors. A pump and 0.2μm cartridge filter were used to provide filtered dilution air if required; the air was introduced at the top of the chamber and withdrawn via an identical filter from the bottom cone.

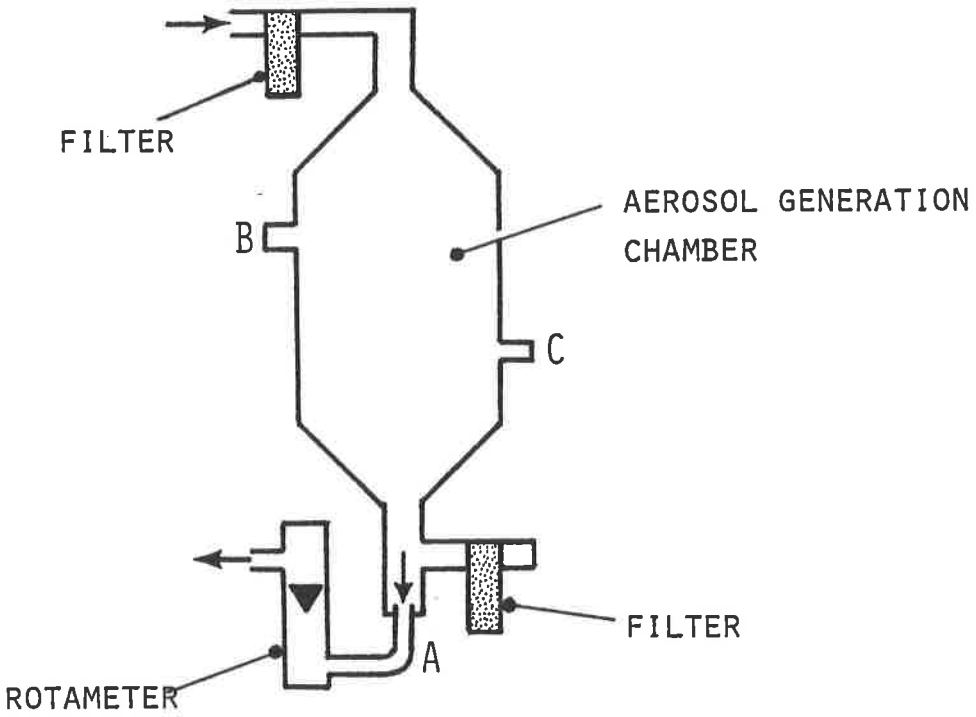
Environmental measurements in the chamber

Airflow The flow rate in the chamber was measured with rotameters fitted into the chamber outlets as shown in Fig. 2.24. The results are consistent within the limits of the rotameter sensitivity and errors such as a slight leakage from the chamber, which was difficult to eradicate.

Airflow pattern The pattern of airflow within the chamber was studied qualitatively by observing the pattern of smoke from a smoke pencil. Fig 2.25 is a diagrammatic representation of the airflow. The airflow at the inlet to the chamber was not uniform and demonstrates the typical pattern exhibited by flow of gas round a bend (Allen, 1975). The right angle bend was essential in the design to allow fitting of the liquid inlet for the spinning disc generator, which had to be directly over the centre of the disc. A 10cm extension on the inlet tube caused the airflow to be less turbulent although it was still not uniform across the tube. However, using the minimum output of air from the pump, the air in the chamber was 'gently turbulent' and appeared to mix well. The smoke cloud appeared uniform within 20 seconds. After input of aerosol at the top of the chamber, initial PMS counts occurred within 10-20s, indicating that the aerosol sample had travelled the length of the chamber in that time.

Pressure measurements were taken in the chamber, as significant deviations from atmospheric pressure would induce errors in particle size measurements since the Andersen

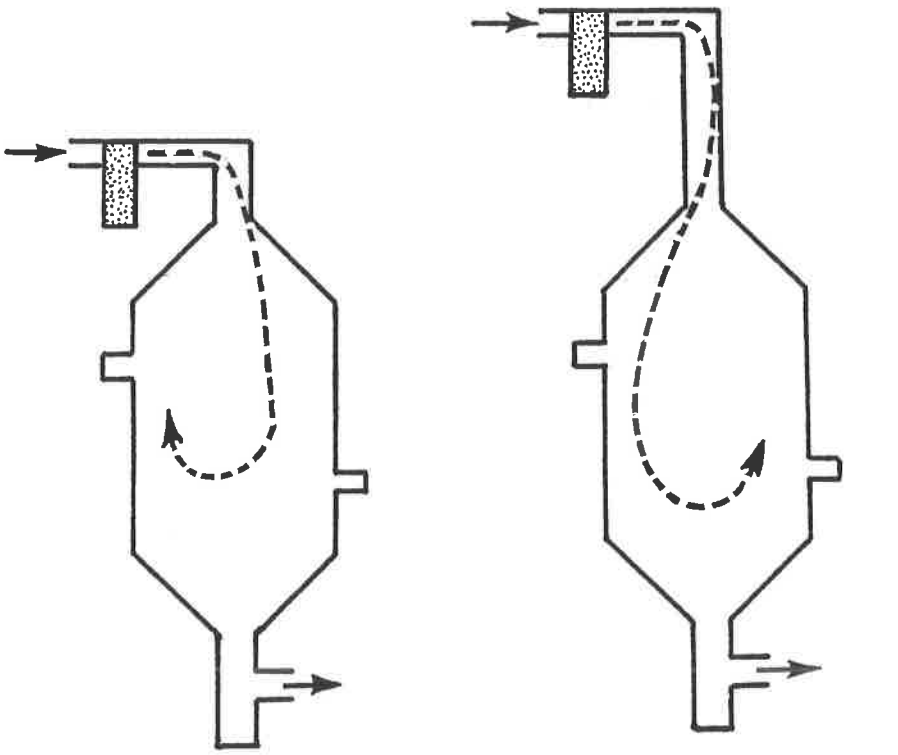
Fig 2.24. FLOW RATE MEASUREMENTS




| | ROTAMETER MEASUREMENTS AT ALTERNATIVE OUTLETS (LITRES/MINUTE) | | |
|---|---|---|---|
| | A | B | C |
| MAXIMUM AIRFLOW | 24 | 30 | 26 |
| MINIMUM AIRFLOW | 1.5 | 2.2 | 1.5 |

Fig 2.25.

AIRFLOW PATTERNS IN THE AEROSOL GENERATION CHAMBER



STANDARD SYSTEM

WITH 10CM INLET EXTENSION

(MAXIMUM AIRFLOW CONDITIONS)

PATTERN OF AIRFLOW

Sampler is calibrated at atmospheric pressure. Fig. 2.26 shows the measurements of pressure within the chamber at minimum and maximum airflow from the pump. A water manometer was used to measure the mean chamber pressure, as shown. At minimum airflow with the Andersen Sampler fitted and sampling at 30 l $min^{-1}$ the deviation from atmospheric pressure is extremely small (approx. 0.02%). Minimum airflow in the chamber was used for all size distribution measurements of aerosols.

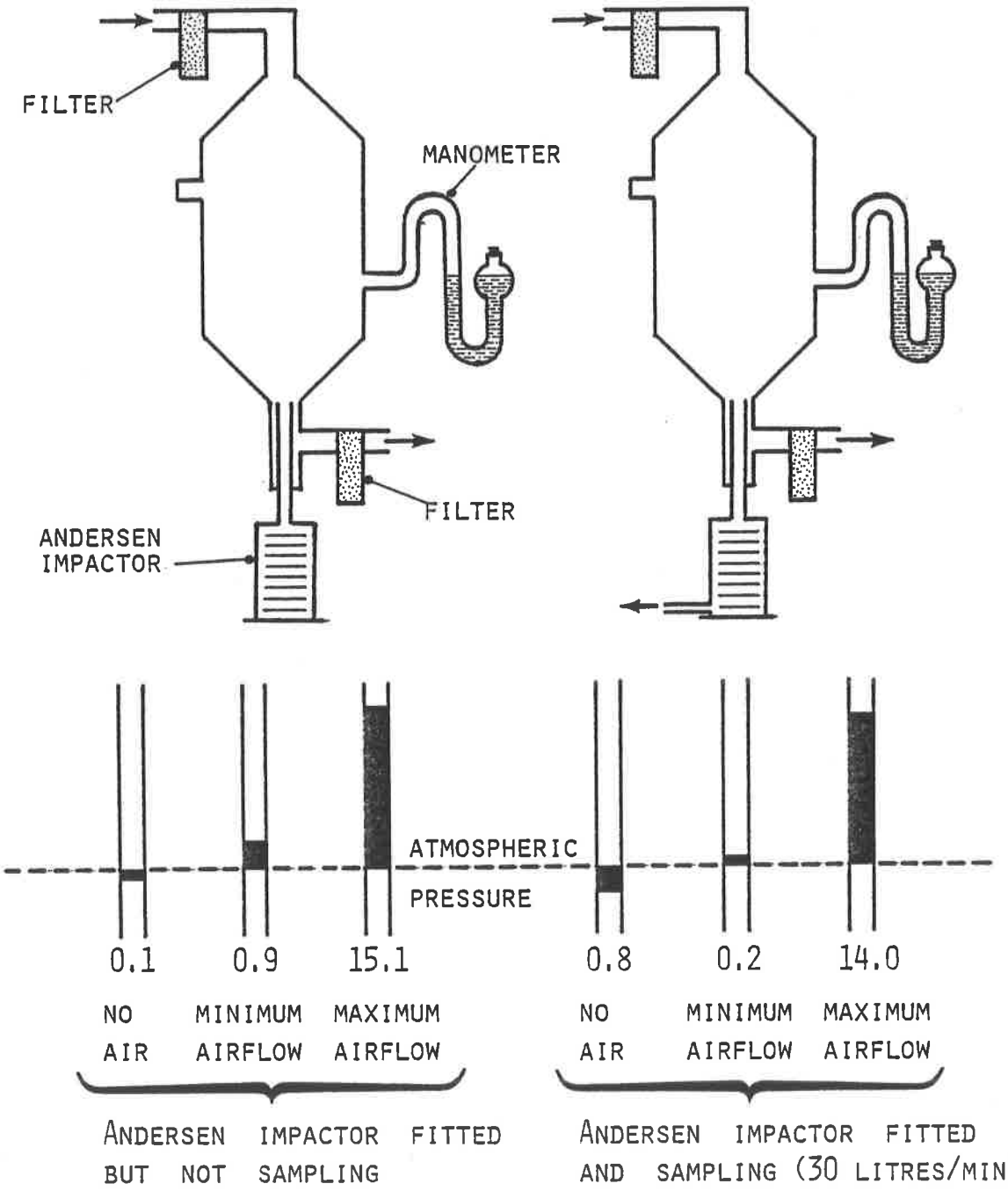
Temperature and humidity Since the aerosol generation chamber was not designed to provide controlled temperature and humidity, it was necessary to monitor these under different conditions of use.

The range of humidities measured (35-60%rH) would govern the degree and rate of evaporation of volatile droplets, thereby influencing their apparent particle size. This is particularly true of very small droplets which have a high vapour pressure (Tang, 1977). The temperature and humidity in the AGC were measured using a 1-100°C thermometer suspended in the centre of the chamber and a humidity sensor placed in the lower outlet of the chamber (Rotronic Hygroskop DMS-100 probe). The Hygroskop sensor consists of a small, defined quantity of a hygroscopic salt held between two platinum electrodes on a quartz plate. The impedance of the system changes with humidity and can be measured using a high frequency signal. A temperature compensator is also built in. The sensitivity of the instrument is $\pm$ 2%rH.

Fig. 2.27 shows the variation in temperature and humidity which were found in the chamber with the input of filtered air at maximum and minimum airflow (ie. 24 and 1.5 l $min^{-1}$ respectively, measured at the lowest outlet). Clearly, the conditions in the chamber varied dramatically with different airflow rates from the pump. The air was warmed gradually

Fig 2.26.

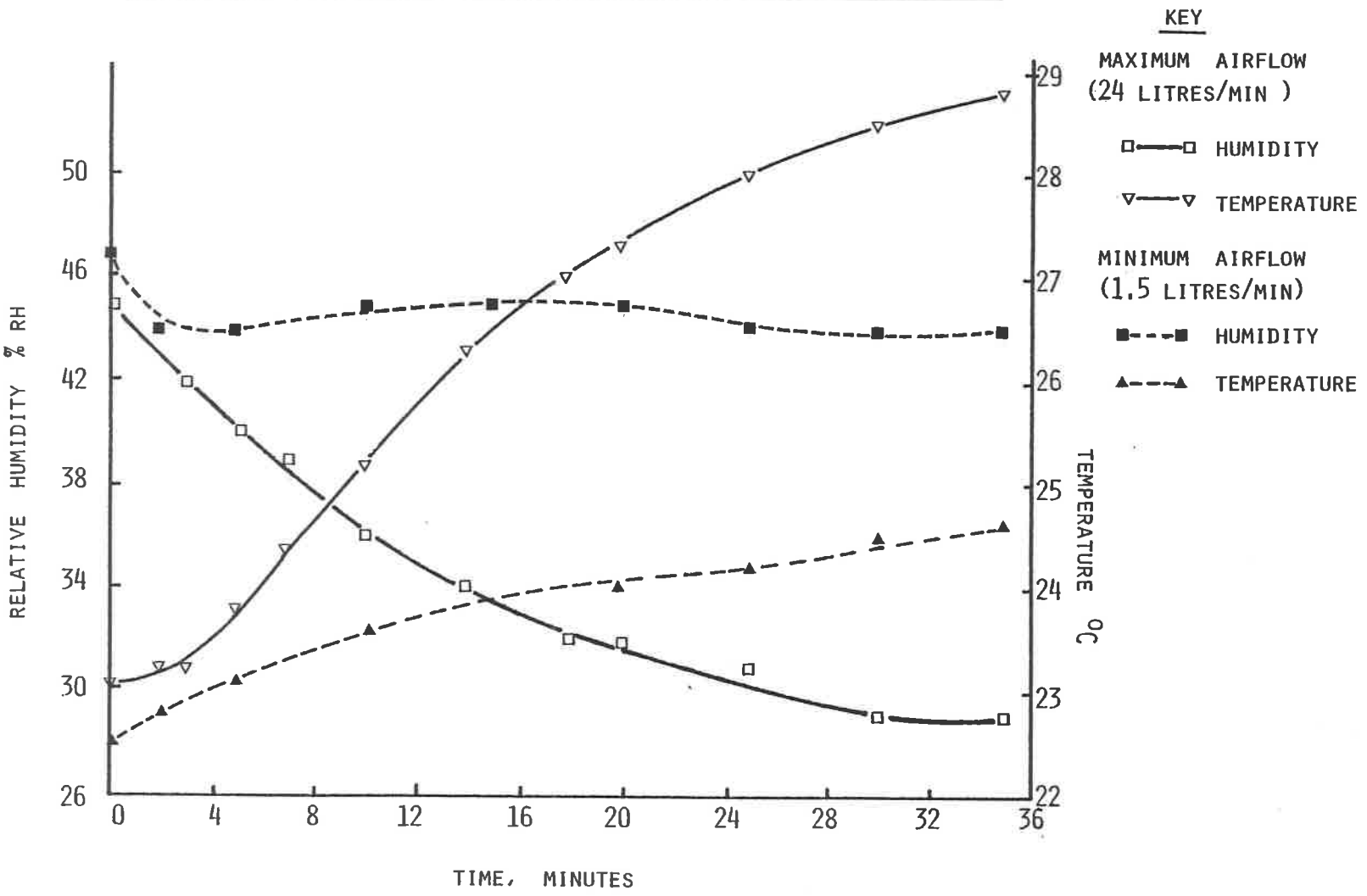
PRESSURE MEASUREMENTS IN THE AEROSOL GENERATION CHAMBER.




NOTE : FIGURES REFER TO PRESSURE IN $cmH_2O$

Fig 2.27.

VARIATIONS IN TEMPERATURE AND HUMIDITY AT TWO AIRFLOW RATES



as the pump continued operating; with minimum airflow the temperature increased by approximately 1.5°C, and at maximum airflow the increase was 5.5°C. The corresponding decreases in humidity were 3% rH and 16% rH, respectively.

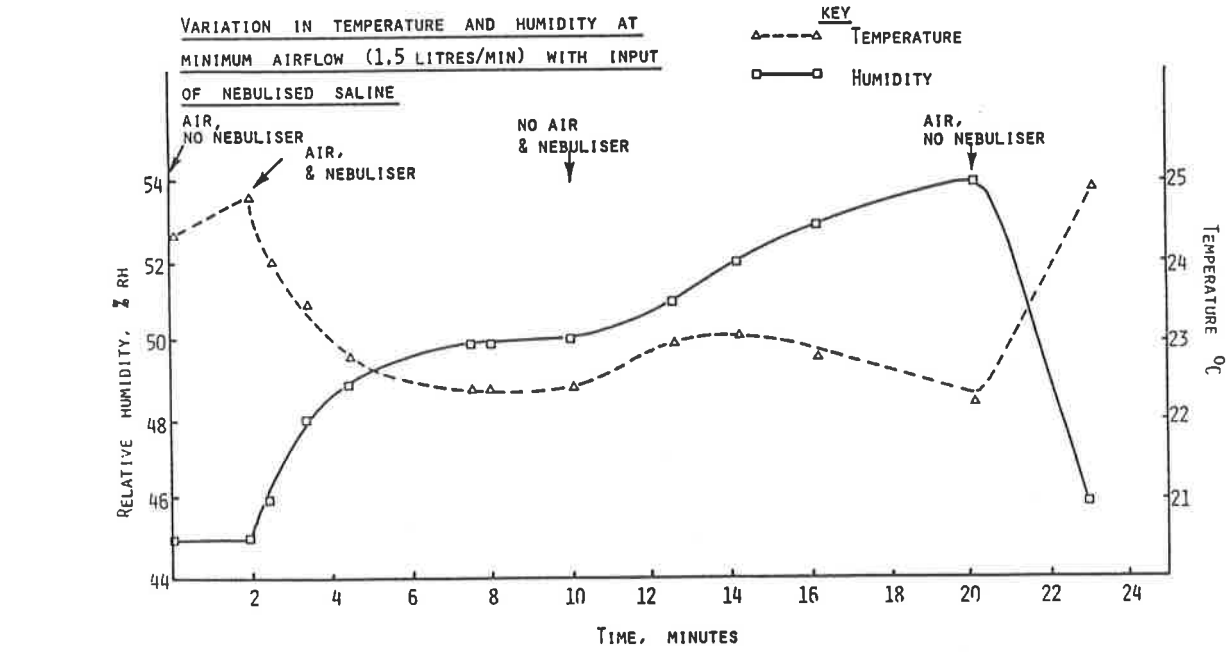
Fig. 2.28 show the changes in temperature and humidity at minimum and maximum airflow with the additional effect of input nebulised saline. Nebulised saline was used to demonstrate the large changes in humidity with input of an aerosol of aqueous droplets and to estimate the effects of these changes on the particle size of the aerosols subsequently measured. At maximum airflow, the humidity fell and the temperature rose sharply even though nebulised saline was introduced into the chamber. The 'masked' effect of the evaporation of nebulised saline was reversed when the pump was switched off and the humidity rose by 10%rH in 3 minutes. At minimum airflow, the humidity rose and the temperature fell with input of nebulised saline into the chamber, and a plateau in results was approached after 8-10 minutes. This plateau indicates that the latent heat required for evaporation of the droplets was exactly met by the heat contained in the warm input air from the pump. Furthermore, if aqueous nebulised aerosols are sampled from the chamber, it is likely that the particle size results would be unreliable until the equilibrium time is reached, which would be indicated by a plateau in the temperature and humidity measurements in the chamber.

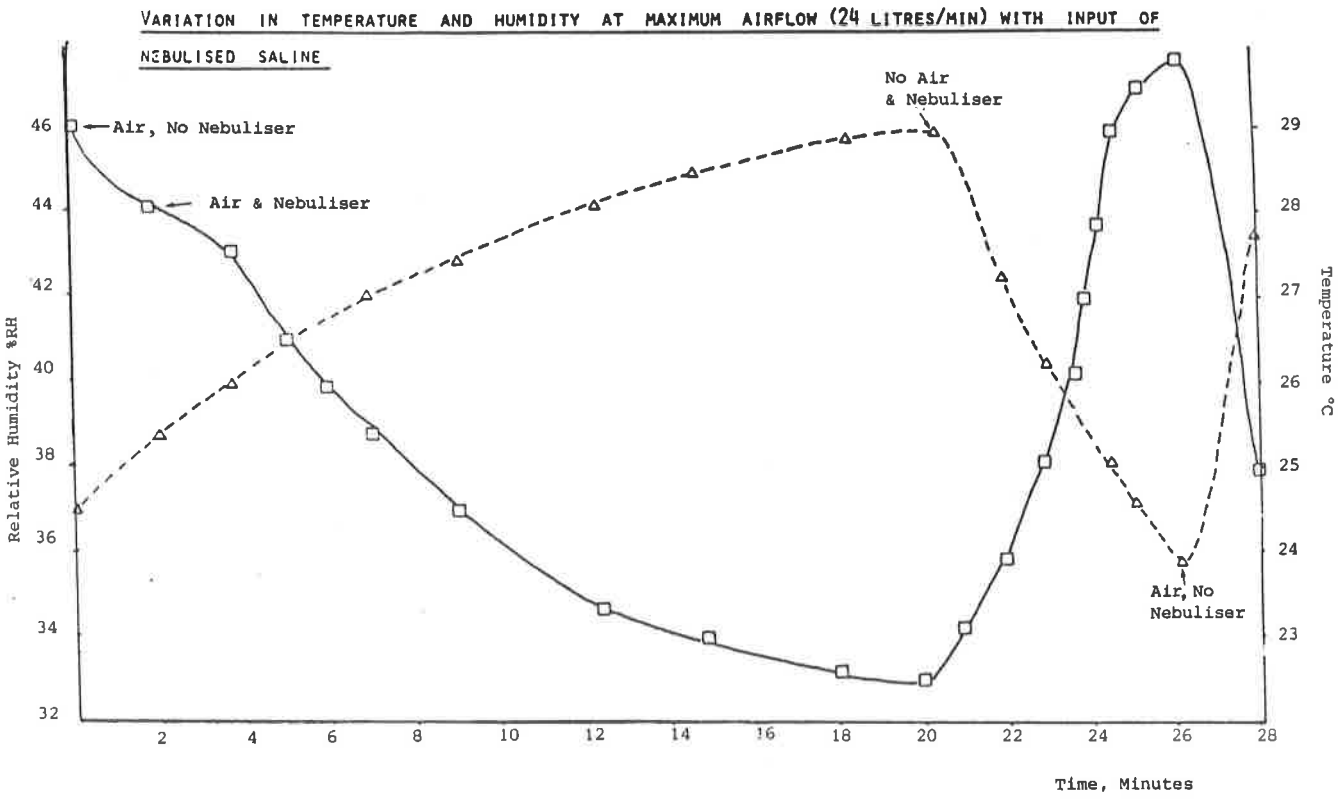
Particle Size Measurement

(a) Nebulised 0.9% sodium chloride solution.

Nebulised saline was introduced into the AGC from a Bennet twin-jet nebuliser which was operated at 5 l $min^{-1}$ airflow. The chamber airflow was at the minimum level, the manometer reading was 26 mm $H_2O$ (2mm Hg). The aerosol was sampled

Fig 2.28. Variation in temperature and humidity with input of nebulised saline at minimum and maximum airflows in the AGC.



at mid-height in the bottom cone, via the 3mm probe into the PMS laser sizer. The results are presented graphically in Fig. 2.29.

The geometric mean size, particularly by mass, becomes more consistent after 10 minutes, as demonstrated by the level portion of the graph (Fig. 2.29). These results show that the saturated vapour pressure of saline in the chamber is approached after this time period and thus the rate of shrinkage of the droplets is decreased.

(b) Nebulised dibutyl phthalate

Table 2.8 shows results obtained from the PMS instrument when sampling nebulised dibutyl phthalate via the 3mm inlet probe. The Bennet twin-jet nebuliser was used with the probe positioned in the bottom cone of the AGC in three different locations, as shown. The geometric mean sizes show consistency in each group, and overall with mean size by number. The geometric mean size by weight shows a slight increase in the lower sampling position. This is probably due to variations in airflow pattern within the chamber.

Fig. 2.30 shows a log-probability plot of particle size of nebulised dibutyl phthalate vs. cumulative % undersize by weight collected on each stage of the Andersen impactor. The size distribution of the aerosol was measured by gravimetric or spectrometric assay of the deposits on each stage. The gravimetric assay involved measuring the weight gain on filter papers placed on each stage of the impactor. The spectrometric assay involved washing the impactor plates with methanol and measuring the absorbance of the solutions at 275nm. The geometric mean diameter by weight of these results compares very well with the PMS results, measured simultaneously, as shown in Table 2.8.

Fig 2.29.

VARIATION IN GEOMETRIC MEAN SIZE OF NEBULISED SALINE WITH TIME, MEASURED IN THE AEROSOL GENERATION CHAMBER

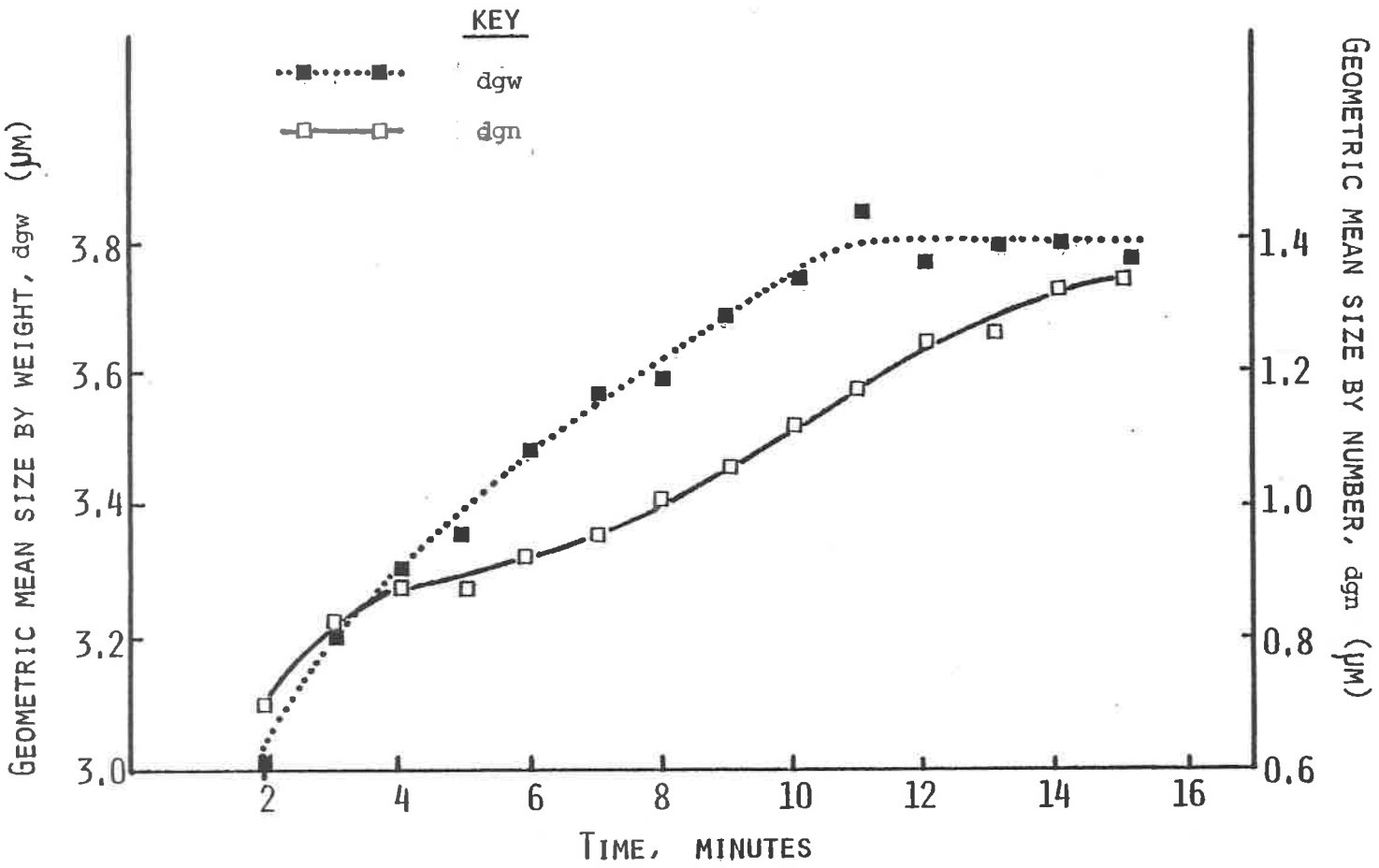
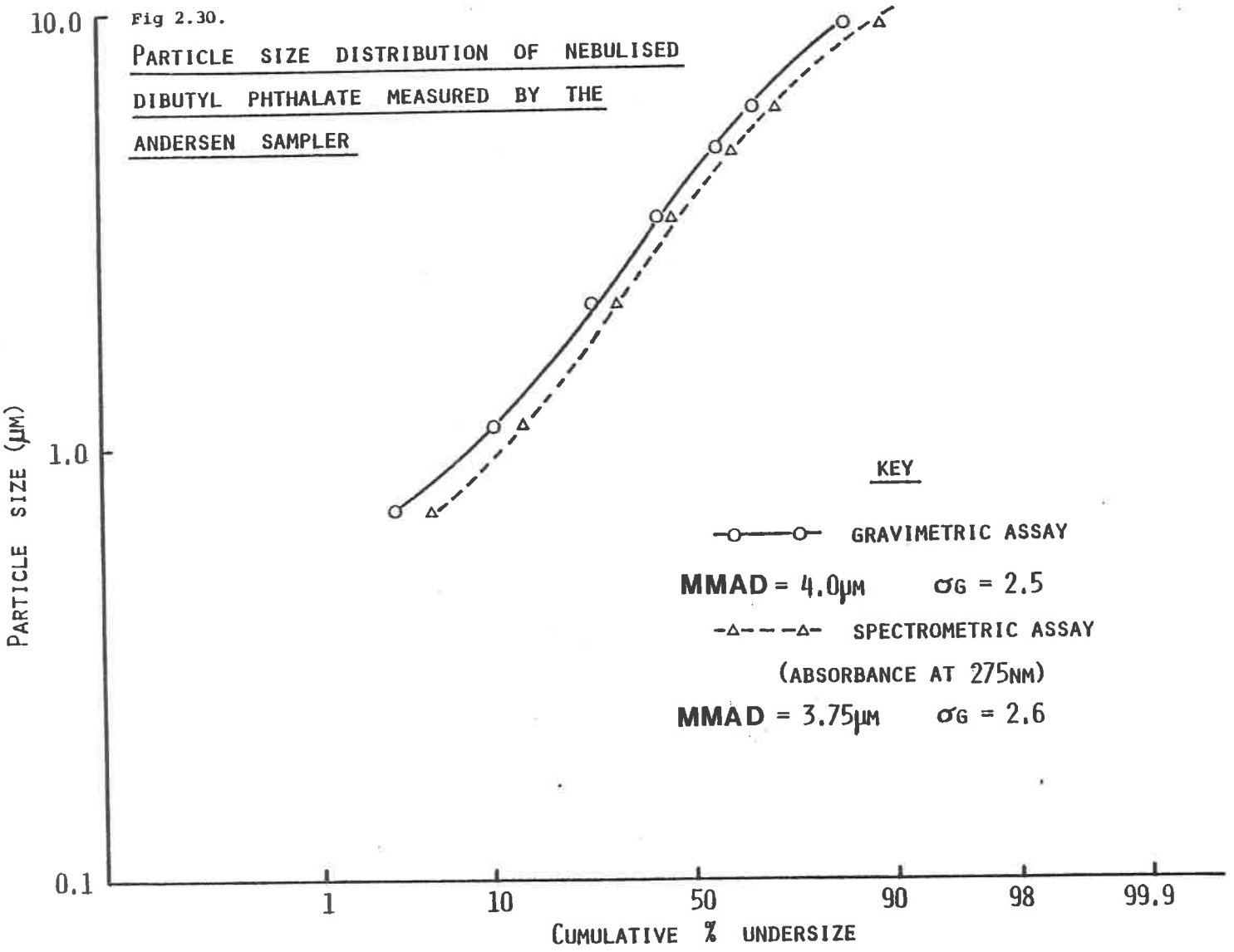


Table 2.8 PMS laser sizer results of nebulised dibutyl phthalate

| Sampling Method (see Fig. 2.22) | | Geometric mean sizes (μm) (geometric mean and S.D.) | |
|---|---|---|---|
| | | $d_{gn}$ | $d_{gw}$ |
| Lowest outlet in AGC | (n = 5) | 0.54 (1.20) | 4.62 (1.04) |
| Middle outlet in AGC | (n = 5) | 0.43 (1.03) | 3.98 (1.10) |
| Highest outlet in AGC | (n = 5) | 0.42 (1.13) | 3.67 (1.05) |
| Overall mean results for the whole chamber | (n =15) | 0.46 (1.18) | 4.07 (1.12) |

Fig 2.30.

PARTICLE SIZE DISTRIBUTION OF NEBULISED DIBUTYL PHTHALATE MEASURED BY THE ANDERSEN SAMPLER



(c) Dibutyl phthalate droplets from the spinning disc generator.

Section 2.2.5 describes the spinning disc generator and the equations used to calculate the dried droplet sizes generated from three concentrations of dibutyl phthalate. The results from particle size measurement of the aerosols by the Andersen Sampler and the PMS are shown in Table 2.9. The measured mean sizes compare fairly well with the calculated droplet diameters, particularly at the lowest concentration. The geometric standard deviation of the mass distribution was estimated from the Andersen results as 1.4-1.5. Clearly, this shows that improvements to the spinning disc system are required to increase the monodispersity of the generated aerosol. Undoubtedly this has contributed to the variations in results. The larger mean sizes measured by the Andersen Sampler may therefore be due to sampling larger particles than the calculated mean size because of the range of the distribution. Conversely, the PMS results are less than the measured sizes by Andersen Sampler, since the laser instrument measures distributions by number and samples particles at the lower end of the calculated size range, which are then taken into account in the computed mean diameter by mass. In addition, the larger particles in the aerosols from higher concentrations may not be efficiently sampled by the Andersen device because of its upper size limit of 9µm.

(d) Ventolin Nebuliser Solution

The nebuliser solution was filtered and nebulised using a Bennett twin-jet nebuliser into the chamber with minimum airflow from the pump and the exhaust fiter fitted. The aerosol was sampled into the PMS laser sizer via a 3mm probe fitted in the 'mid'-position in the bottom cone. The

Table 2.9 Summary of the mean particle sizes of spinning disc generated droplets of dibutyl phthalate measured by the PMS instrument and the Andersen Sampler.

| Concentration of dibutyl phthalate solution (% by weight) | PMS Instrument | | Anderson Sampler | Calculated droplet diameter (μm) |
|---|---|---|---|---|
| | $d_{gn}$ (μm) | $d_{gw}$* (μm) | $d_{gw}$ (μm) | |
| 0.2% | 0.35<br>0.34 | 2.18<br>2.34 | 4.7 | 2.87 |
| 2.0% | 0.36<br>0.37 | 7.14<br>8.37 | 8.6 | 6.19 |
| 5.3% | 0.42<br>0.40 | 9.40<br>9.39 | >9.0 | 8.56 |

* a density of 1 $gcm^{-3}$ is used in the programmed calculations in the PMS instrument. (actual density for the droplets = 1.043 $gcm^{-3}$, giving a conversion factor of 0.986 or 1.5% error).

temperature and pressure in the chamber were 23.5°C and 33mm$H_2O$. Measurements were taken every minute for the period 3-10 mins after nebulisation commenced. The geometric mean sizes by number and weight respectively were 0.38µm (S.D. 1.09) and 0.59µm (S.D. 1.13), for eight measurements. Assuming the measured sizes represent dry particles, the initial droplet size may be calculated from Equation 2.4. This gives a mean size by weight of 5.7µm which compares well with the PMS measurement with sampling from a 5 L flask, as shown in Table 2.10. The results from the AGC are very consistent and probably represent almost dry particles rather than solution droplets. Unfortunately this is not a realistic measurement for inhalation products since the aerosol would be inhaled directly with less time for the droplets to dry compared with a large chamber.

Ventolin Nebuliser solution sampled from the aerosol generation chamber showed similar results irrespective of the diameter of the inlet to the PMS (and thus the flow rate of sample) as shown in Table 2.10. However when the solution is sampled via a 5 litre flask the mean diameter by weight increases to 5.6µm, indicating that this is a measure of the initial droplet distribution from the nebuliser before evaporation commenced. This conclusion is confirmed by agreement with the calculated initial droplet diameter by weight (Equation 2.4) of 5.7µm, assuming the measurements from the AGC samples represent dry particles.

The chamber size, flow rate into the PMS (inlet size), changes in relative humidity and the initial size distribution will all affect the results. Clearly, in the absence of very carefully controlled and standardised conditions, only crude comparative measurements are possible and for these it is important to use a constant sampling system and method.

Table 2.10 Particle size measurement of Ventolin Nebuliser solution via various sampling methods.

| Method of Sampling | number of samples | Geometric mean size (µm) (and S.D.) | |
|---|---|---|---|
| | | $d_{gw}$ | $d_{gn}$ |
| From the AGC using 3mm probe | 8 | 0.59 (1.13) | 0.38 (1.09) |
| From the AGC using 20mm inlet | 7 | 0.87 (1.09) | 0.41 (1.08) |
| From a 5L flask using 20mm inlet (no airflow) | 5 | 5.62 (1.19) | 0.35 (1.05) |

(e) Ventolin Inhaler

After cleaning the AGC using filtered air, the inhaler was fired into the chamber at the rate of 2 shots in each 15 seconds with periodic shaking of the can, a 15 second sampling time interval on each subrange in the PMS and the airflow through the AGC at a minimum. 8 shots were fired per minute for 5 minutes and sampling continued for a further 5 minutes with a print-out every minute. The results are shown in Table 2.11. Sampling into the PMS on 3 separate occasions was as follows:

(i) Sampling was via the wide tubing (20mm) into the Andersen outlet to the wide inlet of laser probe. (at atmospheric pressure).

(ii) Sampling was via a 3mm probe in the middle outlet to the wide inlet of the laser probe (pressure as measured by the water manometer approx. 3.3cm $H_2O$).

(iii) Sampling as in (ii) but only after 5 mins aerosol generation into still air, then the pump was switched on and sampling started.

Table 2.11 PMS laser sizer results for Ventolin Inhaler - the effect of the sampling method

| Sampling method | number of samples | Geometric mean size (µm) (and S.D.) | |
|---|---|---|---|
| | | $d_{gn}$ | $d_{gw}$ |
| (i) | 10 | 0.49 (1.12) | 3.76 (1.05) |
| (ii) | 9 | 0.47 (1.15) | 3.63 (1.12) |
| (iii) | 6 | 0.36 (1.04) | 2.58 (1.22) |

The results from Ventolin Inhaler show the effect of non-uniform sampling. As the chamber pressure increases with use of a smaller tube the mean size by weight decreases indicating that some large particles are lost instead of being sampled. A proportion of the particle losses may be due to wall losses on the narrower tubing, in spite of efforts to minimise the length of, and the amount of curvature in, the tubing. The result from iii) has a higher standard deviation in the mean size; this perhaps indicates inefficient mixing of the aerosol, when the MDI is fired into still air. The mean sizes by weight are comparable with results produced by the Andersen Sampler, which typically have a range of 2.5 - 3.0μm. However, this is fortuitous as the size parameter measured by the Andersen Sampler (aerodynamic size) differs from that by light-scattering (projected area diameter).

## 2.4 DISCUSSION

Two major methods of particle size measurement have been established for estimates of mean diameters of inhalation aerosols, particularly metered-dose inhalers. The Andersen Sampler was used successfully for measuring in detail the aerodynamic particle size distributions of all the metered-dose aerosols used in the present study. This impaction method was the only particle sizing technique used for assessing the activity and mass size distributions of radiolabelled MDI's used in the in vivo experiments in the present study.

A cascade impactor was used in a similar application by Alison (1982) who radiolabelled a nebulised anaesthetic aerosol and studied the particle size distribution by measuring the activity present on each stage of the impactor. The present study is the only work known to the author which

uses the Andersen Sampler to study two size distributions simultaneously. The drug mass and $\gamma$-activity size distributions were measured for all aerosols used in the in vivo studies (Section 4.3.1); and the drug and oleic acid mass size distributions were studied in the validation work for radiolabelling the excipient (Section 3.3).

The PMS instrument has been used for rapid assessment of various sampling methods for MDI's and estimates of the contribution of non-drug particles to the number and mass distributions from MDI's obtained by a light-scattering method. Both the impaction and light-scattering methods have been used for measuring the mean particle sizes of the aerosol discharged from Ventolin Inhaler.

The summarised results of mean diameters measured by different methods are shown in Table 2.12 and include results from a microscopic method of measurement. Comparison of the measured values must necessarily consider the different particle parameters measured. The PMS and Quantimet systems both estimate the size distribution in terms of an assumed log normal distribution and are measured as projected area diameters. The mass distributions are then derived by the Hatch-Choate equation. However one measures airborne particles, and the other measures particles settled on a microscope slide. This may explain the difference in results, as 'preferred orientation' of particles on the slide should give larger mean sizes. The mean diameter by weight obtained from the Quantimet instrument is similar to the values of 6.6μm and 7.4μm measured by Hallworth and Hamilton (1976).

The Andersen Sampler selectively measures mass distributions of drug particles, whereas the light-scattering methods also count other particles unless appropriate corrections for these are applied. As the Andersen results are measured as aerodynamic sizes, it is expected, if one ignores possible

Table 2.12 Summary of mean particle diameters of Ventolin Inhaler measured by different methods.

| Sizing Method | Samples | Geometric mean diameters (μm) | |
|---|---|---|---|
| | | dgn | dgw |
| Andersen Sampler | n = 9 various sampling methods | geometric | Range 2.3-2.9 mean 2.53 S.D. 1.09 |
| PMS Instrument | n = 13 various sampling methods | geometric | Range 0.3-1.5 mean 0.59 S.D. 1.61 / Range 2.3-4.5 Mean 3.32 S.D. 1.19 |
| PMS with programming | (ie. subtracted non-drug particles individual results) | 1.8, 2.1 | 3.8, 4.1 |
| Quantimet | | 3.0 | 7.4 |

errors due to non-drug particles, that larger mean sizes would be shown by the projected area determination of the PMS instrument. Such differences between aerodynamic and optical diameters were demonstrated by Van Buijtenen & Oeseburg (1974) and Harrison & Harrison (1982). They concluded that the light-scattering diameter could be larger by a factor of 1.2-2.4, due to the shape and optical inhomogeneities of the particles. The good agreement of results between the two sizing instruments in the present study suggests that with random orientation in the aerosol the particles are detected by the laser as fairly round in shape. An alternative possibility is that there is significant loss of large particles in sampling to the PMS.

The mean diameters for Ventolin Inhaler produced by the PMS laser light-scattering instrument used in the present study are comparable with published results from other workers using similar inhalation aerosols and optical spectrometers. Davies et al. (1978) and Hiller et al. (1980a) both found mean diameters by mass between 2 and 3.5μm. It should be noted that the two optical instruments used by these authors measure projected area diameter and aerodynamic diameter respectively, hence particle density and shape will have a different influence for the two instruments. The density and shape factors also apply to comparisons of results between the PMS instrument and the Andersen Sampler in the present study. However, for density the conversion factor $(1/\text{density of drug})^{1/3}$ is small because the density of micronised bronchodilator drugs is close to unity. For salbutamol, with a density of 1.17 gcm-3 (measured by autopycnometry using helium at 5psi), the conversion factor is 0.949, giving only a 5% difference between the aerodynamic size measured by the Andersen Sampler and the projected area diameter measured by the PMS instrument.

The results from size measurements of samples from the

aerosol generation chamber show the limitations of this sampling system, for example the random airflow patterns generated within the chamber, the need to monitor temperature and pressure changes as well as careful selection of the type of sampling probe and its position within the chamber. However, providing careful consideration of the many factors involved is undertaken, realistic particle size measurements of inhalation products can be achieved in a few minutes, which compares favourably with existing methods.

Probably the most valuable results from the PMS instrument experiments are those which reveal the large number of non-drug particles below 1µm diameter discharged from MDI's. This has not been reported previously but was also apparent in studies by Dr. Hiller using the SPART aerosol analyser (private communication). Methods have been developed for reducing this 'foreign particle' count substantially by cleaning the aerosol packaging components. The total particulate mass of such particles is small above about 1µm diameter. This means that the background particles do not significantly affect the apparent mean diameter by weight of salbutamol. However this non-selective measurement of all particles in a sample is a disadvantage of the PMS instrument when attempting to measure particles of a known drug in aerosols containing excipients or more than one drug.

Metered-dose inhalers with low numbers of non-drug particles are at present required for the model-fitting program developed for the PMS instrument. The standard PMS program combines the four sub-ranges by eliminating size channels to calculate approximate results. The model-fitting program uses data from all size channels, thereby producing a more accurate result. The advantage of a very rapid measurement of particle size makes the PMS instrument potentially valuable for this type of research.