EFFECTS OF RELATIVE HUMIDITY ON
INHALATION AEROSOLS

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submitted for the degree of Doctor of Philosophy
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September, 1981.

SUMMARY

Most drugs employed in inhalation therapy are water-soluble and when exposed to the high humidity of the respiratory tract will attempt to achieve thermodynamic equilibrium by exchange of water vapour and heat. Techniques for predicting the growth of a single water-soluble compound at 99.5% relative humidity from the physicochemical characteristics of its aqueous solution are described and results presented for several drugs currently used in inhalation therapy.

From this data, changes in particle size distributions at 99.5% relative humidity have been determined. Using a computer model of deposition in the human respiratory tract after mouth-breathing based on published data, the effect of this size change on the site and amount of deposition have been examined. Different deposition patterns are predicted for different salts of the same drug for the same original dry powder size distributions.

The techniques are not however applicable with multicomponent aerosols, for example those generated using a pressure pack. In this instance direct measurement of the size distribution under conditions of high humidity is desirable. The cascade impactor can be used to study the changes in distribution of drug amongst different aerodynamic size fractions of complex mixtures.

The design of a controlled temperature and humidity apparatus is described. The use of this apparatus has been validated using a single water-soluble model inhalation aerosol:- fluorescein disodium. The experimentally determined aerodynamic growth ratios have been compared with those predicted from the physicochemical characteristics of aqueous fluorescein disodium solutions.

The in vitro work predicts that changes in particle size due to hygroscopic growth will effect both site and amount of deposition. Chapter six is devoted to the development of a technique capable of providing information on the systemic availability of a compound administered in various inhalation aerosol formulations.

Good systemic availability $(Bf > 0.90)$ has been shown for intratracheal instillations of 8.2mg to 30.5mg of fluorescein$^=$ in aqueous solutions.

Keywords:

AEROSOL   HUMIDITY   ISO-OSMOTIC   IMPACTOR   BIOAVAILABILITY

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LIST OF CONTENTS

LIST OF FIGURES

Chapter 1 INTRODUCTION

The term aerosol has been used in many ways, creating some degree of confusion. Initially coined to describe colloids of solid or liquid particles in a gas, the term is now frequently used to describe the 'pressurised pack' (Ansel, 1976, Sciarra, 1975). In this thesis the term 'inhalation aerosol' shall be taken to mean a dispersion of solid or liquid in a gas intended for administration to the respiratory tract.

Inhalation aerosols have long been used in respiratory medicine for the management of respiratory disease. Commonly used aerosols include water or saline droplets to 'liquify' sputum, β_2 adrenergic agonists for bronchodilation and disodium cromoglycate and steroid aerosols for prophylactic treatment of asthma. To be effective aerosols of these drugs must deposit within the respiratory tract, whether or not their subsequent pharmacological response is brought about by local concentration of the drug in the respiratory tract (e.g. salbutamol probably exerts its anti-asthmatic effect locally in the respiratory tract (Walker et al, 1972) or by systemic concentrations after drug absorption from the respiratory tract (e.g. isoprenaline bronchodilator action may be due to circulating levels (Davies, 1975)). Deposition of aerosols within the respiratory tract is not a simple matter since the respiratory airways form an extremely efficient aerodynamic filter, which acts as an important pulmonary defence mechanism preventing naturally occuring airborne particulates from penetrating the lung (Proctor, 1964).

A number of factors influence the site and extent of deposition in the respiratory tract, these have been reviewed in detail by Morrow (1974). These include both physiological parameters, such as tidal volume, respiratory rate and residence time within the respiratory tract and particle characteristics such as particle size, shape and density.

The TASK group on Lung Dynamics (1966) recommended that data produced for aerosols of various sizes, shapes and densities be normalised by the use of the aerodynamic diameter (Da). This is defined as the 'diameter of a unit density sphere with the same settling velocity as the particle in question'. In this context unit density, do, means $1g\ cm^{-3}$. The aerodynamic diameter is given by

$$Da = Dv \sqrt{\frac{(dp.\ C(Dv))}{(S_f.\ do.\ C(Da))}} \quad = \quad Dg \sqrt{\frac{(dp.\ C(Dg))}{(do.\ C(Da))}}$$

Equation 1.1

where the first expression is for non-spherical particles of equivalent volume diameter, Dv, and the second for spherical particles of geometric diameter, Dg, with particle density, dp. The dynamic shape factor, S_f, is the ratio of the drag force on the non-spherical particle to the drag force on a sphere of equal volume. Stöber (1972) has reviewed the theoretical and experimental aspects of determining dynamic shape factors for spheroid, isometric and cluster aggregates

The general expression for the slip correction, C(D), to Stokes Law for small spherical particles of diameter, D, is (Fuchs, 1964).

-2-

$$C(D) = 1 + \frac{2L}{D} \left(x + ye^{-\cdot(ZD/2L)} \right) \qquad \text{Equation 1.2}$$

where L is the mean free path of air molecules and x, y and Z material dependent constants. For particles larger than 0.3μm diameter, $\left(x + ye^{-(ZD/2L)} \right)$ may be approximated to 1.26 (Raabe 1976). The accepted value for the mean free path of air molecules at 23° C and 760mm Hg is 0.0653μm. Substituting these values into Equation 1.2 and combining the result with Equation 1.1, the following equation is obtained for the aerodynamic diameter of spherical particles greater than 0.3μm diameter, where the particle density is in g cm^{-3} and the geometric particle diameter and aerodynamic diameter in μm.

$$Da = 0.0823 + \sqrt{(0.0068 + 0.1646 \, dpDg + dp \, Dg^2)}$$
$$\text{Equation 1.3}$$

From Equation 1.2 it is seen that the slip correction factor is a function of the mean free path of air molecules. Since the mean free path of gas molecules is inversely proportional to their concentration, from the ideal gas equation the mean free path of air molecules at temperature $T(^0 K)$ and pressure P (mm Hg) can be calculated as (Raabe, 1976)

$$L = 0.0653 \cdot \frac{760}{P} \cdot \frac{T}{296} \qquad \text{Equation 1.4}$$

The slip correction factor becomes most significant for small particles, if a spherical particle of 0.3μm geometrical diameter and of 1.5gcm^{-3} density is considered, the difference in the aerodynamic diameter of such a particle at 23° C and 37° C will be

less than 1%. The use of the aerodynamic diameter enables comparison to be made between deposition of different materials in the respiratory tract in most instances. However for fibrous particles the probability of interception or wall collision increases as the fibre length becomes a significant fraction of the airway diameter and thus for long thin fibres while the probability of deposition based on the particles aerodynamic diameter is low, the probability of interception may be high because of fibre length (Timbrell, 1972). The use of aerodynamic diameters is also only of limited use when deposition of submicron particles is considered. For these particles the principle mechanism for deposition is diffusion, a mechanism which is a function of the geometric diameter (cf the Stokes-Einstein Equation) of the particle rather than the aerodynamic diameter.

Air entering the respiratory tract will be conditioned until, in the pulmonary region (as defined by TASK, 1966), it is at 99.5% relative humidity, that is the saturated vapour pressure over isotonic body fluids, and at body temperature (Muir, 1972). Air temperature conditioning occurs mainly in the nasal passages and oral cavity (Walker 1961). Cole (1953) has shown that the mouth performs this function with an efficiency nearly equal to that of the nasal passages. As the inspired air is warmed, its water content will increase. By comparison of the water content and temperature of inspired air samples in the pharynx with the variation of water content of saturated air with temperature, Cole (1953) has shown that air in the pharynx is near saturation whatever temperature it has attained during its passage to the pharyngeal region. The recent calculations of Ferron (1981) have revealed that

as mass transport (in this instance of water vapour) is faster
than heat transport, supersaturation of the inhaled air might
occur in the trachea or first bronchus. Inhaled aerosol
particles will thus be subjected to high humidity conditions
almost immediately upon entry to the respiratory tract, even
before inspired air has attained complete temperature condition-
ing.

An aerosol particle not in thermodynamic equilibrium
with the surrounding atmosphere will attempt to attain   the
equilibrium state by condensation or evaporation of water
vapour and heat exchange. An aerosol particle containing
hygroscopic substances will, at equilibrium, form a droplet
whose humidity is equal to the humidity of the surrounding air.
As particle size is a determining factor in the site and
extent of deposition in the respiratory tract, condensation
growth of hygroscopic drug aerosol particles would be expected
to affect the site and dose of drug deposited in the respiratory
tract. Only 5 to 10% of the inhaled dose of disodium
cromoglycate is absorbed (Moss, 1970) it has been suggested
that this may be explained by the effects of condensation
growth (Gonda and Byron, 1978). The importance of condensation
growth on lung deposition clearly depends upon the rate of
growth (Ferron 1977). An indication that hygroscopic particles
grow sufficiently fast to affect lung deposition comes from
studies with aerosol of inorganic electrolytes (Walkenhorst
and Dautrebande, 1961; Wagner, 1975) and the recent work of
Martonen et al (1980) has shown that isoprenaline hydrochloride
particles grow at approximately the same rate as sodium chloride
in the 90 - 95% relative humidity range.

The TASK group (1966) suggest that the effect of condensation growth on hygroscopic aerosols can be considered by assuming that the solution formed behaves as an ideal solution. If we consider a dry particle of a single water-soluble compound with diameter, Do, mass, Mo and density do, undergoing condensation growth to form a droplet of diameter Dd, mass, Md and density dd, the ratio of the diameter of the equilibrium droplet to initial dry particle diameter will be

$$\frac{Dd}{Do} = \sqrt[3]{\frac{Md}{Mo} \cdot \frac{do}{d_d}} \qquad \text{Equation 1.5}$$

The mass of the droplet will equal the mass of the initial dry particle plus the condensed water, Mw

$$Md = Mo + Mw \qquad \text{Equation 1.6}$$

The mass of water can be described in terms of the number of moles of water, nw, and the molecular mass of water Ww

$$Mw = nw\,Ww \qquad \text{Equation 1.7}$$

and a similar expression will describe the mass of the dry particle in terms of the number of moles of solute, ns, and the molecular mass of the solute, Ws.

$$Mo = ns\,Ws \qquad \text{Equation 1.8}$$

Substituting Equations 1.6, 1.7 and 1.8 into Equation 1.5 and rearranging, the following expression for the growth ratio

is obtained

$$\frac{Dd}{Do} = \sqrt[3]{\frac{do}{d_d}\left(1 + \frac{ns}{nw} \cdot \frac{Ww}{Ws}\right)} \qquad \text{Equation 1.9}$$

At equilibrium the fractional humidity of the droplet will equal the fractional humidity of the surrounding air, H. If surface tension effects are negligible the humidity of the droplet will equal the water activity of the solution of concentration equal to that in the droplet. Assuming ideal solution behaviour the water activity, a, will equal the mole fraction of water

$$a = \frac{nw}{nw + ins} = H \qquad \text{Equation 1.10}$$

Rearranging Equation 1.10

$$\frac{nw}{ns} = \frac{iH}{1 - H} \qquad \text{Equation 1.11}$$

Substituting this expression into Equation 1.9, the following equation is obtained describing the growth ratio in terms of the molecular mass of water and solute, the density of the dry particle and droplet grown from it and the humidity of the surrounding atmosphere.

$$\frac{Dd}{Do} = \sqrt[3]{\frac{do}{d_d}\left(1 + \frac{Ww}{Ws}\left(\frac{iH}{(1-H)}\right)\right)} \qquad \text{Equation 1.12}$$

This expression assumes that the solute concentration is below

the solubility limit, that there are negligible surface tension
effects and that the solution formed behaves in an ideal manner.
A similar expression incorporating a term to allow for hydrated
solute molecules has been used by Ferron (1977). The accuracy
of the growth ratio obtained using this expression clearly
depends upon the solute forming an ideal solution. If physical
data is available it is obviously preferable to use this data
to determine the growth ratio. Neglecting surface tension
effects the equilibrium droplet will have the same concentration
as the bulk solution whose water activity is equal to the humidity
over isotonic body fluids at 37^0 C, that is the iso-osmotic
concentration , Me'. The concentration in the droplet in terms
of mass of solute and water (measured in grams) can be defined
by

$$Me' = \frac{Mo}{Ws} \cdot \frac{1000}{Mw} \qquad \text{Equation 1.13}$$

The mass of water in the equilibrium droplet will equal the
difference between the mass of the droplet and the dry particle

$$Mw = Md - Mo \qquad \text{Equation 1.14}$$

Combining Equations 1.13 and 1.14 and substituting the result
in Equation 1.5, the following expression is obtained to describe
the growth ratio in terms of iso-osmotic concentration, dry
particle density and droplet density.

$$\frac{Dd}{Do} = \sqrt[3]{\frac{d_o}{d_d} \left(1 + \frac{1000}{Ws\ Me'}\right)} \qquad \text{Equation 1.15}$$

The British Pharmaceutical Codex lists the depression of freezing point of 1% solutions of a number of substances commonly used in injections. The depression of freezing point of water due to solutes in blood serum has an average value of 0.52^0 C. Assuming Raoult's Law to be applicable, the relationship between depression of freezing point and concentration will be linear and the concentration of drug giving a freezing point depression of 0.52^0 C may be easily determined. Although water activity in electrolyte solutions is relatively insensitive to temperature (Robinson and Stokes 1959a) for systems such as disodium cromoglycate-water which undergo phase transitions (Cox et al, 1971), it is advisable to take measurements at body temperature and iso-osmotic concentrations found by freezing-point depression should be treated with some suspicion. A number of techniques are available for determining iso-osmotic concentrations at body temperature. Robinson and Stokes (1959b) have reviewed techniques for determining the vapour pressure of electrolyte solutions. However, for dilute solutions very accurate pressure measurements are required. Recently vapour pressure osmometry has been used to determine osmotic behaviour of a number of electrolytes found in atmospheric aerosol (Thudium, 1978). Using this technique water activities may be determined at body temperature.

Rigorously speaking, the concentration of solute in the droplet equilibrated to 99.5% relative humidity at 37^0 C will not be the same as the concentration of bulk solution giving a water activity of 0.995 at 37^0 C, as the humidity of the droplet, h will be greater than the water activity of the

solution because of surface tension effects and is given by
the Kelvin equation

$$h = a + \exp \left(\frac{4S\ Ww}{d_d \cdot RT \cdot Dd} \right) \qquad \text{Equation 1.16}$$

where S is the surface tension of the solution, d_d its density
R the universal gas constant and T the droplet temperature.
The exponential term becomes more significant for small diameters.
In order to investigate the likely overestimate of the growth
ratio by neglecting this term data relating the surface tension
of drug solutions to their concentration are required. If the
exponential term is designated by E^1 Equation 1.12 becomes
(Ferron, 1977)

$$\frac{Dd}{Do} = \sqrt[3]{\frac{do}{dd} \left(1 + \frac{Ww}{Ws} \left(\frac{i\ (H - E^1)}{1 - (H - E^1)} \right) \right)} \qquad \text{Equation 1.17}$$

if surface tension effects are considered. As the exponential
term includes the diameter of the droplet an explicit
expression for the growth ratio is not obtainable and the
growth ratio may be found in an iterative manner.

The growth ratios described (Equations 1.12, 1.15 and
1.17) are applicable to single water-soluble compounds of
initially dry particles. This type of aerosol would be
generated from powder insufflators such as the Spinhaler
(Fisons Ltd.). The Spinhaler is a tubular plastic device
which is loaded with a gelatine capsule containing the drug
mix (see Figure 1.1). Two metal pins pierce the capsule to
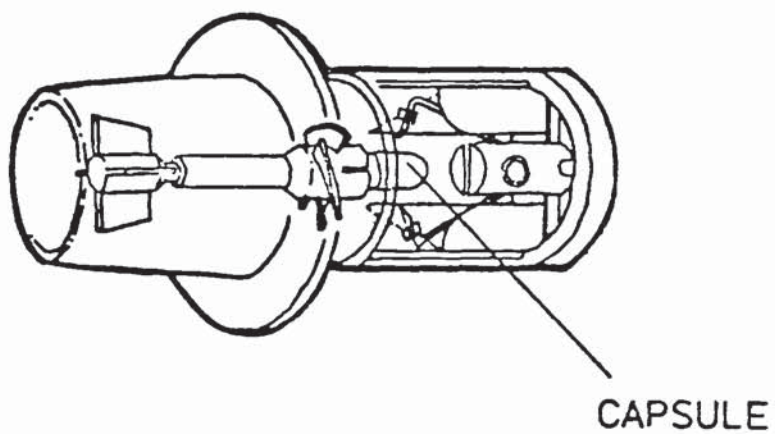
CAPSULE

provide an escape route for the capsule contents. When the user inhales, a rotor spins the capsule and powder is thrown out into the inhaled air stream. Lactose (30 - 60µm in diameter) is mixed with micronised drug to assist dispersion, some drug will adhere to the lactose particles but most of the drug will be dispersed as single particles or aggregates of drug alone (Bell et al, 1971).

Drugs may also be administered as aerosol droplets generated using airblast or ultrasonic nebulisers. If the simplest formula of water-soluble drug in water is considered, an equation to determine the growth or shrinkage of the aerosol droplet of initial diameter, Di can be derived. The ratio of the diameter of the equilibrium droplet, Dd, to the diameter of the initial droplet may be described in terms of the mass and density of the initial and equilibrium droplet

$$\frac{Dd}{Di} = \sqrt[3]{\frac{Mi}{Md} \cdot \frac{dd}{di}} \qquad \text{Equation 1.18}$$

where Mi and di are the mass and density of the initial droplet. If the mass of solute in the droplets is Mo, the initial molality, Mi' will be

$$Mi' = \frac{Mo}{Ws} \cdot \frac{1000}{(Mi-Mo)} \qquad \text{Equation 1.19}$$

Combining Equations 1.14, 1.18 and 1.19 the following expression for the ratio of the diameters of the initial and equilibrium droplet

$$\frac{De}{Di} = \sqrt[3]{\frac{\frac{di}{de}\left(1 - \frac{1000}{Ws\,Me}\right)}{\left(1 - \frac{1000}{Ws\,Mi}\right)}} \qquad \text{Equation 1.20}$$

Frequently however, nebuliser solutions are not of drug in water but may contain other solvents such as alcohols or glycols, and other formulating agents such as buffers, antioxidants preservatives and salts. If the nebuliser solution is formulated to be isotonic and if surface tension effects are assumed to be negligible, the droplets would be expected to neither grow or shrink. For small droplets where surface tension effect became significant the humidity above the isotonic droplet will be greater than 0.995 and some evaporation will occur.

The major domiciliary aerosol generator used in inhalation therapy is the pressure pack. The basic principles of pressurised products are extensively described by Sciarra (1975). Two types of pressurised inhalation aerosol packs are formulated. The active medicament may be dissolved in the liquified propellant usually with addition of cosolvents to assist drug solubility such as alcohols or glycols. Alternatively the active agent may be suspended as a micronised powder in the liquid propellant system. Surfactants such as lecithin, lanolin, oleyl alcohol or cholesterol derivatives are frequently added to prevent drug agglomerations. Lubricants such as isopropyl myristate, isopropyl palmitate allow the powder particle to flow through the valve minimising valve clogging. The moisture content in the pack can be minimised by inclusion of desiccants. Aerosols generated from pressurised packs will be polyphasic and it is not possible to determine the aerosol behaviour at high humidity

from physicochemical characteristics of the drug alone. In
these instances direct measurement of the change in size
distribution of the aerosol at high humidity is desirable.
Hiller et al have described the changes in particle size
distributions of aerosol of disodium cromoglycate (1980a),
Isoprenaline hydrochloride and sulphate (1980b) and isoethanine
sulphate and metaproterenol sulphate (1978) generated from
commercial products at high (95%) and low (21%) humidity.
The aerodynamic diameter distribution of the aerosols were
measured using the single particle aerodynamic relaxation
time analyser. This type of analysis will not however
distinguish between aerosol particles containing the compound
of interest, that is the drug, and aerosol particles void of
the medicament.

It is sometimes possible however, to infer which part
of the total distribution is due to drug aerosol particles.
For example, powder insufflators frequently incorporate an
inert excipient to improve the flow properties of the mixture
and facilitate aerosol generation. The average size of the
excipient particles is usually much larger than that of the
drug particles.

Impaction techniques allow the collection of aerosols
of various aerodynamic size fractions. Chemicals or
radioactive analysis of the deposits provides a means of
distinguishing the aerodynamic distribution of a particular
species of interest, in this case drug. Hiller et al (1978 .)
have suggested that the instability of water-containing
particles precludes the use of impaction techniques and
measurement in the suspended state is necessary in order to

assess accurately the effect of humidity on the size of
inhalation aerosols. Velocity changes in the jets (Hering
et al, 1978) will lead to variation in temperature in the gas
stream (Fuchs, 1978). The linear velocity in an impactor jet
can be calculated from the volume flow rate through the
impactor and jet diameter. The largest perturbation in
temperature will occur at the smallest diameter jets however,
the transit time through the smallest jets will be very small.
Calculations for the Delron DCI-6 cascade impactor reveal
that the transition time through the last two stages will be
0.35 and 0.15 milliseconds respectively. It is unlikely that
any substantial changes in particle size would occur on such a
short time scale. Hochrainer and Zebel (1981) have performed
similar calculations and examined this problem experimentally
and agree with this viewpoint. Indeed the cascade impactor
has been used as the sizing instrument in experiments examining
the effect of humidity on the size distribution of propylene
glycol (Davis and Bubb, 1978) and disodium cromoglycate
aerosols (Byron et al 1977).

In practise it is difficult to achieve the degree of
temperature control required to maintain the relative humidity
experienced in the respiratory tract. For example, in order
to obtain the growth ratio for a single water-soluble compound
with an accuracy of 5% at 99.5% relative humidity would
require temperature control within $\pm$ 0.015^0 C at 37^0 C. (Gonda
et al, 1981). Correspondingly studies on the effect of humidity
on particle size distributions of aerosol are performed at
sub-physiological humidity. Dry aerosols of a single water-
soluble compound exposed to increasing humidity will display

-15-

a distinct humidity at which particles will start to grow. If the droplet formed is then exposed to decreasing humidity, no abrupt reduction in particle size is observed, instead a continuous reduction in size takes place as a result of the droplet becoming super-saturated. This hysteresis phenomenon is well known for inorganic salt aerosols and has been observed by a number of investigators (Orr, 1954; Tang, 1977). Figure 1.2 illustrates the effect of humidity on the size of a hypothetical single water-soluble compound. At high humidity the growth ratio shows a steep dependence on humidity. This makes accurate prediction of the growth ratio at 99.5% humidity from direct measurement at lower humidities difficult.

Atmospheric aerosols, which consist of mixtures of inorganic water-soluble materials and insoluble materials, by comparison show a continuous growth character by gradual deliquescence of water-soluble material (Winkler, 1974) but still display steep dependence at high humidities. Studies with atmospheric aerosols have indicated that the particles show reduced water absorption when water-insoluble compounds are present (Winkler, 1974; Eisner et al, 1960; Snead and Zung, 1968). Similarly, it may be expected that pharmaceutical aerosols from pressure packs, which include water-soluble components would show reduced water absorption compared with the active component alone.

As particle size is a determining factor in the site and extent of deposition in the respiratory tract, condensation growth of hygroscopic drug aerosol particles would be expected to affect the dose deposited in the respiratory tract.

Figure 1.2 Growth ratio versus relative humidity of a hypothetical water-soluble aerosol.
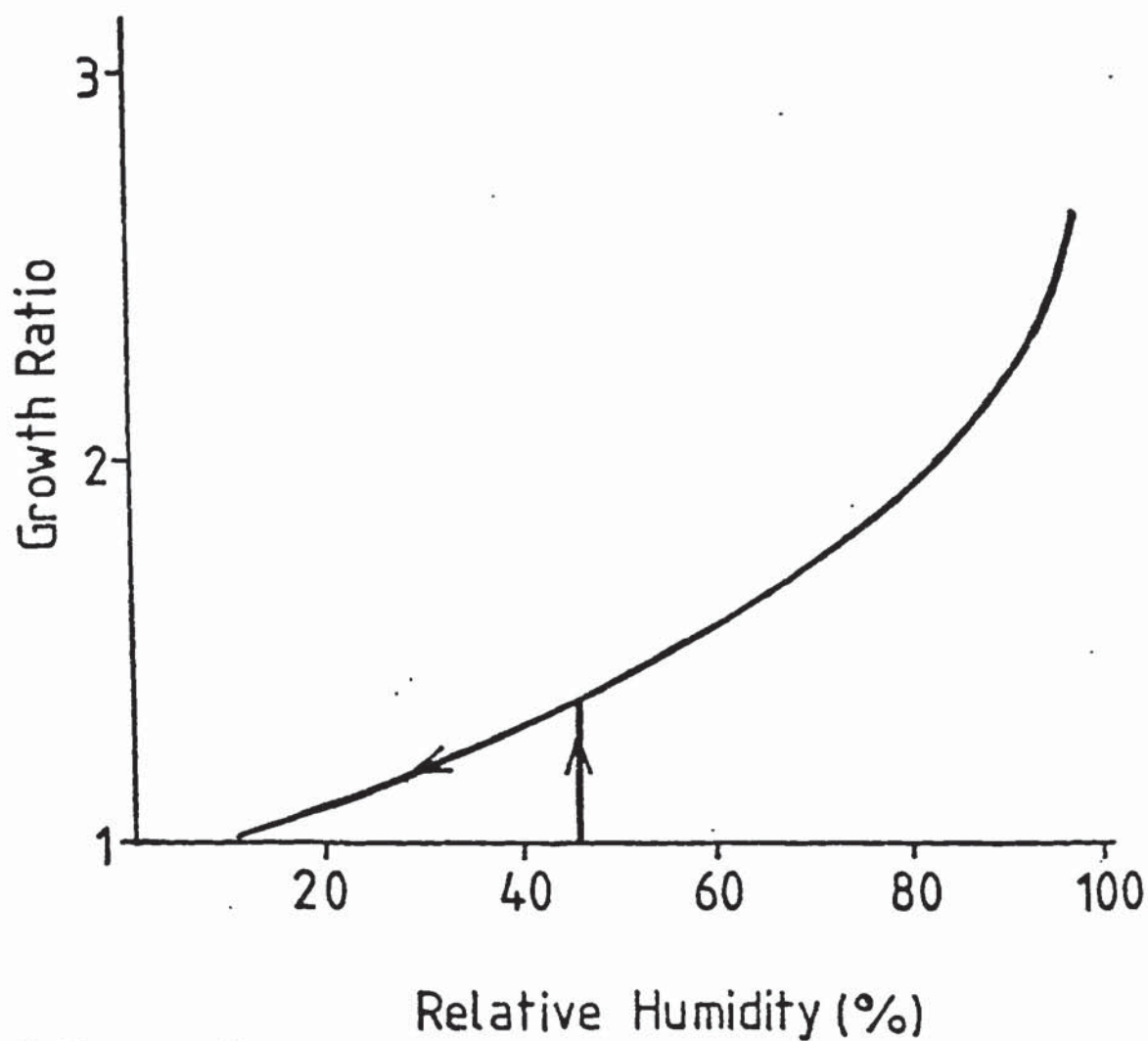
The magnitude of this effect may be examined using theoretical
models based on the deposition patterns of non-hygroscopic
aerosols.

Most pharmaceutical inhalation aerosols currently in use,
are intended for local activity within the respiratory tract.
However, the inhalation route would appear to offer a number
of advantages as an absorption site. The surface area is
approximately $30m^2$ (Hatch and Gross, 1964a), providing an
absorptive area comparable with that of the gastrointestinal
tract. Drugs administered by the inhalation route will not
be subject to large pH changes (compared with the gastro-
intestinal tract) nor such a large number of degradative
enzymes (Gonda and Byron, 1978). The inhalation route may be
useful in attaining rapid delivery of drugs designed to act
systemically. Indeed this route is already used to achieve
rapid onset of action of Ergotamine (Ergotamine Aerosol
Inhalation, British Pharmaceutical Codex, 1979). Chapter six
describes the development of a technique capable of providing
information on the systemic bioavailability of a compound
administered as an inhalation aerosol formulation to an
experimental animal.

Chapter 2   PREDICTION OF EQUILIBRIUM DIAMETERS OF INHALATION AEROSOLS
            IN THE RESPIRATORY TRACT

2.1   INTRODUCTION

In the introductory chapter expressions for the growth
ratios of aerosols of single water-soluble compounds in the
high humidity of the respiratory tract were described. The
purpose of this chapter is to determine the physicochemical
characteristics of several drugs currently used in inhalation
therapy and use this data to calculate the increase in
diameter of these drug aerosols in the respiratory tract.

The simplest expression for the growth ratio of dry
particles to droplets in equilibrium with a humidity of 99.5%
assumes that the solution formed behaves in an ideal manner
and that surface tension effects are negligible. (Equation
1.12). The main advantage of this expression is that only a
few compound specific parameters are required; the molecular
weight of the drug, the density of the dry powder˙ and the
density of the solution formed.

In order to obtain a more exact estimate of the growth
ratio knowledge of the water activity of aqueous solution of
drug at 37^0 C is required. Vapour pressure osmometry provides
a technique for the determination of water activity of solutions.
This technique has been used to determine the isotonic
equivalents of a number of drugs (Newburger, 1977).

Figure 2.1 is a schematic diagram of the vapour pressure
osmometer. It consists of a heat insulated chamber containing
a pair of thermistors which are connected to a wheatstone bridge.
Using filter paper wicks and a reservoir of distilled water
vapour concentration near saturation is maintained within the

chamber. Syringes containing solution and solvent are kept at approximately chamber temperature within a heated head. Droplets of solution and solvent (in this instance water) may be applied to the thermistors by lowering the syringe needles into the chamber. The wheatstone bridge is initially zeroed after applying solvent droplets approximately equal in diameter to both the test and reference thermistors. Figure 2.2a illustrates saturation vapour pressure, p, as a function of temperature, T, for pure water and for a solution. When droplets of solution and solvent are applied to the thermistors they attempt to achieve equilibrium with the vapour pressure of the chamber, p^1, by evaporation and condensation of water vapour. The water vapour concentration within the chamber will be near to saturation vapour pressure and so little change will occur to the solvent droplet. The vapour pressure of the solution droplet will be lower than the chamber and so water vapour will dondense onto the solution droplet raising its temperature to $T + \Delta T$, when the vapour pressure of the solution equals that of the chamber. The temperature difference established, ΔT, is measured by means of the thermistors. It has been shown theoretically that the temperature difference is proportional to the negative of the natural logarithm of water activity (-ln a) (Thudium, 1978). The droplets applied to the thermistors are large enough for the condensing water vapour to have negligible effect on the concentration of the solution droplet (Whatley, 1966). Waste droplets of solution are washed into the chamber reservoir in the apparatus used in this study (KG Dr. Ing Herbert Knauer, West Germany) and so during a course of readings the vapour pressure of the chamber will be lowered. Figure 2.2b

may be used to illustrate the establishment of the temperature difference between the solution and solvent droplets when the vapour pressure of the chamber is less than the saturation vapour pressure. In this instance the solvent droplet will attempt to attain equilibrium by evaporation of water vapour, correspondingly the solvent droplet will cool until at the temperature of $T - \triangle T_1$ its vapour pressure equals that of the chamber . The vapour pressure of the solvent droplet, in this example, is still lower than that of the chamber. Condensation of water vapour onto the solvent droplet will occur until at a temperature of $T + \triangle T_2$ its vapour pressure equals that of the chamber. The fall in temperature of the solvent droplet plus the rise in temperature will equal the same temperature difference, $\triangle T$, described in Figure 2.2a. This may be confirmed experimentally by determining the established temperature difference for the same solution at the beginning and at the end of a set of experimental readings.

Solutions of surface active materials might be expected to give false results using the vapour pressure osmometer because of possible effects the surface film may have affecting heat and mass transfer in an unequal manner. However, Fineman and McBain (1948) have shown that addition of a small amount of potassium laurate, sufficient to lower surface tension appreciably without appreciably lowering vapour pressure, to 0.1Molal potassium chloride solutions did not affect the temperature difference between the solution and solvent droplets. Indeed, vapour pressure osmometry has been used to determine the critical micelle concentration of aqueous solutions of detergents (Huff et al, 1951).

In order to determine the magnitude of the Kelvin effect on the growth ratio it is necessary to determine the surface tension of aqueous solutions of the compounds used in this study. The surface tension was determined using the Wilhelmy plate technique (Adamson, 1967). The basis of this technique is that a thin plate will support a meniscus whose weight, measured statically or by detachment is given by

$$m_t = m_p + S.e \qquad \text{Equation 2.1}$$

where m_t and m_p are the total mass and the mass of the plate respectively; S the surface tension of the solution and e, the perimeter of the plate.

2.2   EXPERIMENTAL

2.2.1   Vapour Pressure Osmometry

2.2.1.1   Calibration of vapour pressure osmometer

The instrumental response of the vapour pressure osmometer used in this study (K G Dr. Ing Herbert Knauer, West Germany) at various sensitivity settings was calibrated against electrolytes and non-electrolyte solutions of known water activity. Data were directly available for solutions at 25^0 C (Robinson and Stoke,1959a) either tabulated as water activity or in terms of osmotic coefficient. Water activity, a, may be calculated from osmotic coefficient, O, by

$$- \ln a = 0.018 \; i \; M \; O \qquad \text{Equation 2.2}$$

where i is the number of ions formed by the compound and M the solution concentration in moles per kg of water (Robinson and Stoke, 1959 d).

Osomotic coefficients for sodium chloride solutions of greater than 1 molal at 37^0 C were obtained from the data of Gibbard et al (1974). Other values were obtained by extrapolation between available data at 25^0 C and the nearest temperature to 37^0 C available in the literature. The values used in the calibration are tabulated in Appendix 1.

Solutions of known water activity were prepared from analytical grade sodium, potassium and calcium chlorides and sucrose with deionised water.

2.2.1.2 Preparation of test solutions

Disodium cromoglycate (supplied by Fisons, Pharmaceutical Division, Loughborough) was dried in a vacuum oven at 170° C for 18 hours, then allowed to cool over phosphorous pentoxide (Attiga et al, 1979). Solutions of known molality were prepared from this dried powder and deionised water.

Isoprenaline sulphate and hydrochloride were used as supplied by the manufacturer (Riker Laboratories, Loughborough). Solutions of isoprenaline salts decompose on exposure to air as indicated by the appearance of a pink coloured solution. Solutions of isoprenaline sulphate and isoprenaline hydrochloride of known molality were prepared with deionised water therefore just prior to measurement.

Only limited quantities of salbutamol sulphate (Glaxo Group Research, Ware) were available. Solutions of known molarity were prepared by dilution of a 0.15 molar stock solution in deionised water.

2.2.1.3 Measurement of water activity

The apparatus was allowed to equilibrate for at least one hour before any measurements were made. The instrument was initially zeroed by placing solvent onto both reference and test thermistors. Once a constant instrumental reading was obtained, the wheatstone bridge was balanced to give a zero reading. Measurements were then made by placing droplets of solution onto the test thermistor and solvent onto the reference thermistor of similar size (approximately 4mm in diameter). The size of the droplets could be observed through

a window into the test chamber. The most dilute solutions
were measured first. At least three measurements were made
of instrumental response with each solution. The solution
droplet was then washed from the test thermistor with several
drops of the next most concentrated solution before the
determination of instrumental response to that solution.

2.2.2   Density Measurements

2.2.2.1 Density of dry powders

The density of the dry powders used in this study were
measured by air comparison pycnometry (Beckman, Model 930).
Approximately thirty grams of each powder was accurately
weighed into the sample chamber and the volume of air displaced
by the powder determined. Only 2 grams of salbutamol sulphate
was available for density determination and this is reflected
in the accuracy of the measurements. The density of each powder
was determined three times.

2.2.2.2 Density of Solutions

The density of aqueous solutions of the compounds used
in this study at 37° C were determined using a specific gravity
bottle. The volume of the bottle at 37° C was determined by
filling the bottle with deionised water and leaving it in a
water bath at 37° C for 1 hour to equilibrate. Excess solution
was removed, the bottle dried externally and allowed to cool
before weighing. The volume of the bottle could then be
calculated from the weight of water held in the bottle and the
tabulated density of water at 37° C (Robinson and Stokes, 1959).

The specific gravity bottle could then be used to determine
the density of aqueous solutions of drug at 37^0 C in a similar
manner. Each determination was made three times.

2.2.3   <u>Surface Tension Measurements</u>

The surface tension of aqueous solutions of the compounds
used in this study were determined using the Wilhelmy plate
technique (Adamson, 1967). The plate used in these studies
was 10mm high, 5mm wide and 0.025mm thick and made of 99.95%
pure platinum (Goodfellow metals, Cambridge). The plate was
roughened slightly to enhance wetting. Before each measurement
the platinum plate was cleaned by heating to red heat and
used immediately it had cooled (Bewig, 1965). The cleaned
plate was hung from the beam of a microbalance (CI Electronics
Ltd., Mark 2B). This arrangement was counter balanced in air
by a weighing pan and weights hung from the other balance arm.

Twenty five millilitres of the test liquid, which had
been previously warmed to 37^0 C was placed in a jacketted beaker
maintained at 37^0 C using a water circulator-heater (Chiller-
Thermo Circulator, Churchill Ltd.). The beaker was placed on
a movable platform which was raised until the plate was just
below the surface of the liquid. The platform was then lowered
slowly until the plate detached from the liquid surface. The
increase in weight on the balance arm was read from an indicating
meter and chart recorder. All measurements were made at
least three times. The meter readings obtained were calibrated
using solutions of known surface tension shown in Table 2.1 .

Test solutions were prepared with deionised water from dried disodium cromoglycate (see section 2.2.1.2) and isoprenaline sulphate, isoprenaline hydrochloride and salbutamol sulphate as supplied by the manufacturers.

2.3 EXPERIMENTAL RESULTS

2.3.1 Vapour Pressure Osmometry

2.3.1.1 Calibration of vapour pressure osmometer

The temperature difference between the solution and solvent droplets in the vapour pressure osmometer has been shown to be proportional to the negative of the natural logarithm of water activity (Thudium, 1978). The instrumental response (percent full scale deflection, % FSD) is directly proportional to the temperature difference. Figure 2.3 shows the instrumental response at an instrumental sensitivity setting of 16, versus $-\ln a$ for sodium, potassium and calcium chloride and sucrose at 25° C. The results could be satisfactorily described by a rectilinear plot of the form

$$\% \text{ FSD} = 3329 \, (-\ln a) - 0.8 \qquad \text{Equation 2.3}$$

with a correlation coefficient of greater than 0.999 $(n = 57)$. The theoretical relationship therefore appears to apply to 1:1 and 1:2 electrolytes and non-electrolyte solutions.

Similar rectilinear plots with correlation coefficients of greater than 0.998 ($n > 15$), were obtained at 37° C for several instrument sensitivity settings. The slope and intercepts of these calibration curves are given in Appendix 2. The calibration curves were used to determine the water activities of test solutions.

2.3.1.2 Water activity of Test solutions

The water activity (a) versus concentration (M) for aqueous solutions of isoprenaline hydrochloride, isoprenaline sulphate and salbutamol sulphate at 37^0 C and disodium cromoglycate at 37^0 C and 25^0 C are shown in Figure 2.4. The solid lines indicate the linear approximations used in the computation of growth ratios taking into account the Kelvin effect (section 2.4).

The water activity versus concentration plots for isoprenaline hydrochloride, isoprenaline sulphate and salbutamol sulphate could be satisfactorily described by rectilinear plots. Included on Figure 2.4 are the lines for ideal solution behaviour assuming dissociation into 2 ions, e.g. isoprenaline hydrochloride or 3 ions, c.f. disodium cromoglycate and the sulphates of isoprenaline and salbutamol. It is readily seen that all aqueous solutions of the drugs examined show apparent deviation from ideality.

The relationship between water activity and concentration for disodium cromoglycate solutions was complex. For the concentration range examined water activity appears to plateau at concentrations exceeding 0.22 Molal at 37^0 C and 0.13 Molal at 25^0 C. These transition points correspond to a phase change between solution and mixed solution and mesophase (Cox. et al, 1971)

2.3.2 Density Results

2.3.2.1 Density of dry powders

The mean and standard deviation of three determinations

of dry powder density determined by air comparison pycnometry
are given in Table 2.2. The density of salbutamol sulphate
could only be determined to $\pm$ 0.5gcm^{-3} as insufficient sample
was available for more accurate determination.

2.3.2.2 Density of aqueous drug solutions

Figure 2.5 illustrates the density of aqueous solutions
of disodium cromoglycate, isoprenaline hydrochloride, isoprenaline
sulphate and salbutamol sulphate. The mean results of three
determinations and the least mean square regression line are
illustrated. Each determination was within 0.0002gcm^{-3} of
the mean value. For the concentration range considered, the
density of the aqueous solutions could be satisfactorily
described by rectilinear plots of density versus concentration.

2.3.3 Surface Tension Measurement
2.3.3.1 Calibration of Instrument

Figure 2.6 shows instrumental response (Y) versus surface
tension (S). The mean of three determinations is illustrated,
all individual readings were within $\pm$ 1 instrumental response
unit. Instrumental response versus surface tension could be
satisfactorily described by a rectilinear plot. The least
mean square regression line may be described by

$$Y = 1138 \ S + 0.15 \qquad \text{Equation 2.4}$$

where S is the surface tension in Nm^{-1}.

The correlation coefficient of this line was 0.999 (n = 24).
This calibration line was used to determine the surface tension
of test solutions.

2.3.3.2 Surface tension of aqueous drug solutions

Figure 2.7 illustrates the surface tension of drug solutions.
The reproducibility in replicate determinations of surface
tension by measurement of the force on the immersed plate was
about 0.0001 Nm^{-1}. The solid lines in Figure 2.7 show the
linear approximations used to compute the growth ratios taking
into account the Kelvin effect.

It is interesting to note that the surface tension of
aqueous disodium cromoglycate solutions decrease rapidly with
increasing concentration above 0.13 Molal at 25^0 C and 0.21
Molal at 37^0 C, corresponding to the transition points from
solution to mixed solution and mesophase (Cox, 1971). These
values compare favourably with the transition points for this
phase change obtained from water activity data of 0.13 Molal
at 25^0 C and 0.22 Molal at 37^0 C (see Figure 2.4).

2.4 CALCULATION OF GROWTH RATIOS

Examination of the plots of water activity versus concentration (Figure 2.4) reveals that all the compounds studied here show appreciable deviation from ideality. The experimentally determined isosmotic concentrations show greater than 10% deviation from those predicted on the basis of 'ideal' solution behaviour and full ionization. The growth ratio of an initially dry spherical particle to a droplet in equilibrium with a relative humidity of 99.5% at 37° C may be calculated assuming 'ideal' solution behaviour and full ionization using Equation 1.12 and from experimental isosmotic concentrations using Equation 1.15. Both these equations neglect surface tension and so the results refer to the limit of large spherical particles. The calculated ratios are given in Table 2.3 and reveal that the deviation from ideality observed for these compounds is sufficient to affect the growth ratio markedly.

From a deposition viewpoint it is the increase in aerodynamic diameter which is important. It is useful therefore to express the growth ratio in terms of aerodynamic diameter. As the growth ratios determined from experimental isosmotic concentrations refer to the limit of large spherical particles, it is reasonable to neglect the Cunningham slip factor in the determination of aerodynamic diameters. The aerodynamic growth ratio, $(^{Dd}/Do)a$, may be calculated from

$$\left(\frac{Dd}{Do}\right)_a = \sqrt{\frac{dd}{do}} \cdot \left(\frac{Dd}{Do}\right) \qquad \text{Equation 2.5}$$

where dd and do are the densities of the equilibrium droplet
and initial dry particle respectively and Dd/Do is the ratio
for geometric diameters calculated from Equation 1.15. As
the increase in geometric diameter due to condensation of
water is accompanied by a decrease in particle density, the
aerodynamic growth ratio will be less than the growth ratio
in terms of geometric diameter. The calculated aerodynamic
growth ratios are given in Table 2.3.

When surface tension effects are considered no single
growth ratio will apply to all particle sizes. Growth
ratios considering the Kelvin effect were therefore calculated
for individual particle diameters using an iterative computer
programme. For each drug the experimental results for
surface tension, water activity and density were expressed as
functions of concentration using the linear approximations
shown in Figures 2.4, 2.5 and 2.7. The programme starts with
the isosmotic concentration of each drug at 37° C and using the
Kelvin equation calculates the humidity above the droplet.
For small droplets the fractional humidity in this instance
will exceed 0.995. The drug concentration is then increased
in an incremental manner and the droplet humidity recalculated.
When the droplet humidity is less than 0.995, the concentration
is reduced to the previous value and the size of the incremental
step reduced. In this manner the concentration at which a
droplet of known diameter has a fractional humidity equal to

0.9950000 ± 0.0000001 is determined. The growth ratio can
then be calculated using Equation 1.15 for the droplet size
under consideration and the procedure repeated for the next
particle size.

The magnitude of the Kelvin effect relative to aerodynamic
diameter is illustrated in Figure 2.8 where (Da) i refers
to the aerodynamic diameter calculated from the isosmotic
concentration and (Da) e, the aerodynamic diameter calculated
including the Kelvin effect. It is apparent that this effect
is only significant for very small particles (initially less
than 1μm) which are unlikely to occur in appreciable quantities
in most currently employed inhalation aerosols (Byron et al,
1978; Davis, 1978; Hallworth and Andrews, 1976; Mercer et al,
1968; Ruffin et al, 1978).

2.5   DISCUSSION

The water activity of disodium cromoglycate, isoprenaline
hydrochloride, isoprenaline sulphate and salbutamol sulphate
in aqueous solution has been determined by vapour pressure
osmometry. In all instances there was a significant deviation
from 'ideal' solution behaviour. This was most marked for
disodium cromoglycate which undergoes a phase transition from
solution to mixed solution and mesophase. This phase change
is indicated by a plateau in water activity. The concentration
at which this phase change occurs has been shown to be
temperature dependent (Cox et al, 1971). Furthermore, at
25^0 C the water activity at the plateau is higher than the
value for isotonic saline, whereas the converse is true at
37^0 C. In the light of this observation it is advisable that
determinations of iso-osmotic equivalents should be made at
physiological temperatures.

The most exact expression for condensation growth ratios
takes into account the increased vapour pressure above curved
surfaces of small droplets, the Kelvin effect. In this instance
explicit expressions for the growth ratio of all particle
sizes is not possible, the growth ratio for each particle
size may be found in an iterative manner.

Determination of growth ratios from the experimentally
determined iso-osmotic concentrations, neglecting the Kelvin
effect will lead to overestimates. For the compounds studied
here this overestimation is less than 5% for particles initially
greater than 1μm in diameter. Submicron particles are unlikely

to occur in appreciable quantities in most currently employed

inhalation aerosols. Reasonably accurate estimates of the growth

ratios can therefore be obtained from the iso-osmotic concentrations

at 37° C for pharmaceutical aerosols.

2.6   TABLES

Table Captions

Table 2.1   Reference solutions used to calibrate surface tension

apparatus (Handbook of Chemistry and Physics, 1978)

Table 2.2   Density of dry powders determined by air comparison

pycnometry

Table 2.3   Growth ratios calculated assuming ideal solution

behaviour and full ionization or based on experimental

iso-osmotic concentration at 37° C. The Kelvin effect

is neglected and the results refer to the limit of

large spherical particles.

Table 2.1   Reference solutions used to calibrate surface tension
             apparatus (Handbook of Chemistry and Physics, 1978).

Solution	Temperature ^{0}C	Surface Tension (S) mNm^{-1}
Acetone	20	23.9
Benzene	20	28.9
20% w/w acetone in water	25	41
10% w/w acetone in water	25	48.9
5% w/w acetone in water	25	55.5
Water	40	69.6
Water	30	71.2
Water	20	72.75

Table 2.2   Density of Dry powder determined by air comparison
            pycnometry

Compound	Density (gcm^{-3})
Disodium cromoglycate	1.623 ± 0.001
Isoprenaline hydrochloride	1.325 ± 0.003
Isoprenaline sulphate	1.362 ± 0.001
Salbutamol sulphate	1.50 ± 0.5 *
* insufficient sample for more accurate determination	

Table 2.3 Growth ratios calculated assuming ideal solution behaviour and full ionized (Equation 1.12) or based on experimental iso-osmotic concentration at 37° C (Equation 1.15). The Kelvin effect is neglected and the results refer to the limit of larger spherical particles.

Drug	Geometric diameter growth ratios		Aerodynamic diameter growth ratios	
	Assuming ideal solution behaviour	Calculated from iso-osmotic concentration	Assuming ideal solution behaviour	Calculated from iso-osmotic concentration
Disodium cromoglycate	3.26	2.67	2.60	2.12
Isoprenaline hydrochloride	3.40	3.28	2.97	2.85
Isoprenaline sulphate dihydrate	3.02	2.76	2.59	2.37
Salbutamol Sulphate	3.1 *	2.96	2.5 *	2.41

* Sample too small for greater accuracy

2.7   <u>FIGURES</u>

<u>Figure Captions</u>

Figure 2.1   Schematic diagram of the vapour pressure osmometer

Figure 2.2   Saturation vapour pressure, p, versus temperature, T,
             for solvent and solution

Figure 2.3   Instrumental response of the Knauer vapour pressure
             osmometer (sensitivity setting 16) versus the
             negative of the natural logarithm of water activity
             at 25^0 C for sodium chloride (O), potassium chloride
             (●), calcium chloride (Δ) and sucrose (x).

Figure 2.4   Water activity versus molality for aqueous solutions
             of isoprenaline hydrochloride at 37^0 C (◇), isoprenaline
             sulphate at 37^0 C (◆), salbutamol sulphate at 37^0 C
             (Δ) and disodium cromoglycate at 37^0 C and 25^0 C (●).
             The solid lines indicate the linear approximations
             used to compute equilibrium diameters with the inclusion
             of the Kelvin effect. Also shown are the lines for
             ideal solution behaviour assuming dissociation into
             2 ions (••••) – cf isoprenaline hydrochloride or
             3 ions (⋯⋯) – cf disodium cromoglycate and sulphates
             of isoprenaline and salbutamol.

Figure 2.5   Density of aqueous solutions of disodium cromoglycate
             (O), salbutamol sulphate (x), isoprenaline hydrochloride
             (●) and isoprenaline sulphate (Δ) at 37^0 C. The solid
             lines illustrate the linear approximations used to
             determine droplet equilibrium diameters with the
             inclusion of the Kelvin effect. The mean of three
             determination is illustrated.

Figure 2.6   Calibration of surface tension apparatus, instrumental
             response versus surface tension. The mean of three
             determinations is illustrated, individual readings
             were within $\pm$ 1 instrumental response limit of the
             mean.

Figure 2.7   Surface tension against molality at 37° C for aqueous
             solutions of disodium cromoglycate (●), isoprenaline
             hydrochloride (▲), isoprenaline sulphate (△) and
             salbutamol sulphate (○). The solid lines indicate
             linear approximations used to compute the equilibrium
             diameters with inclusion of the Kelvin effect.

Figure 2.8   Percentage error caused by neglecting the Kelvin effect
             in calculation of equilibrium droplet size: $(Da)_e$
             and $(Da)_i$ - aerodynamic diameters calculated with and
             without the Kelvin correction respectively. Results
             for isoprenaline sulphate (____), isoprenaline
             hydrochloride(--.--.), salbutamol sulphate (---)and
             disodium cromoglycate (-.-), all at 37° C

Figure 2.1 Schematic diagram of the Vapour Pressure Osmometer

Wheatstone bridge

Syringe

Blotting-paper

Thermistors

Heat insulation

Water
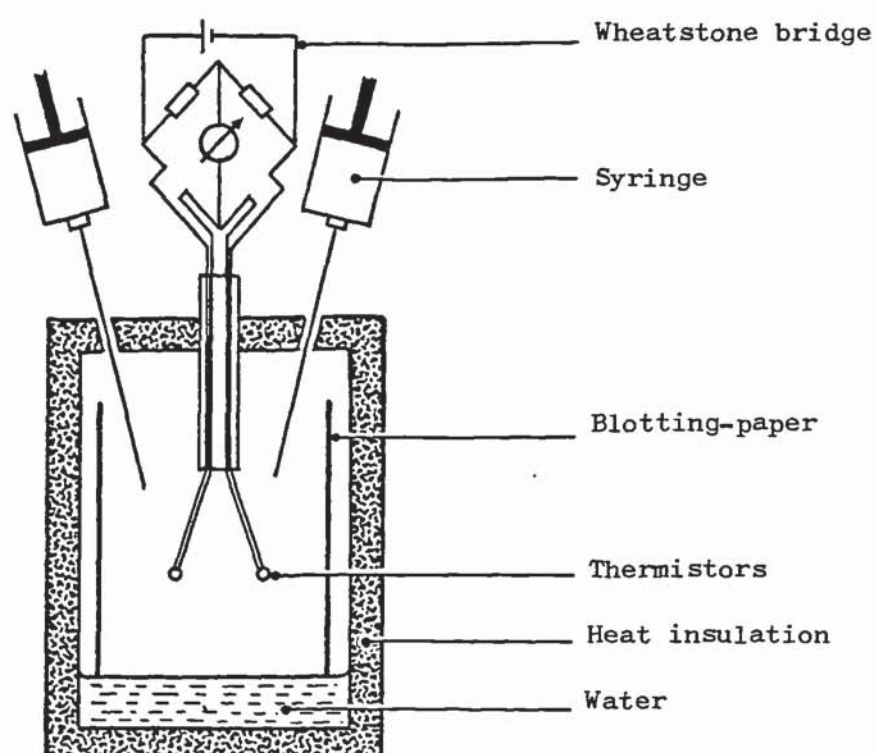
Figure 2.2: Saturation vapour pressure p, versus temperature, T, for solvent and solution.

a)

b)

$$\Delta T_1 + \Delta T_2 = \Delta T$$
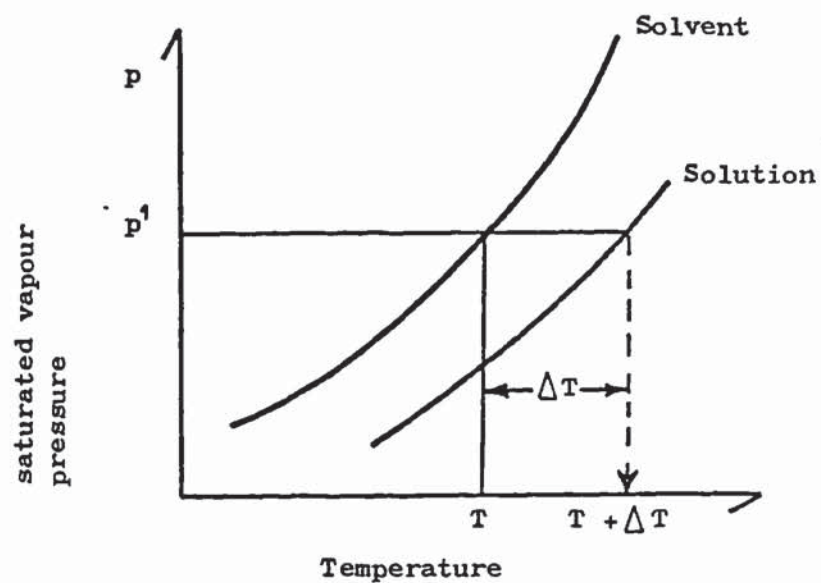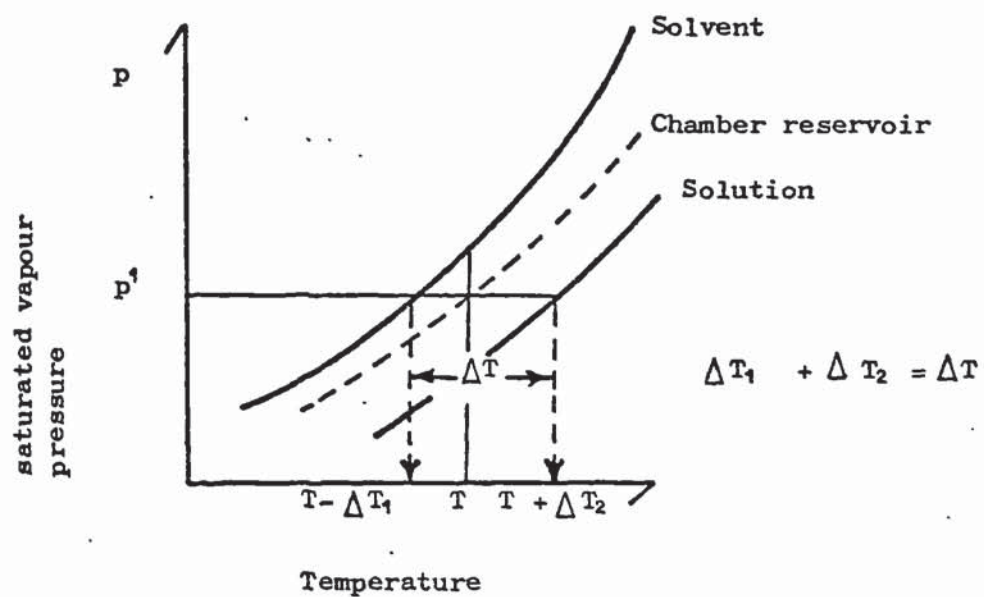
Figure 2.3 Instrumental response of the Knauer vapour pressure osmometer (sensitivity setting 16) versus the negative of the natural logarithm of water activity at 25^0 C for sodium chloride (O), potassium chloride (●), calcium chloride (Δ) and sucrose. (x).

Figure 2.4 Water activity versus molality for aqueous solutions of
isoprenaline hydrochloride at 37° C ($\Diamond$), isoprenaline sulphate
at 37° C ($\diamondsuit$), salbutamol sulphate at 37° C ($\triangle$) and disodium
cromoglycate at 37° C ($\circ$) and 25° C ($\bullet$). The solid lines
indicate the linear approximations used to compute equilibrium
diameters with the inclusion of the Kelvin effect. Also shown
are the lines for ideal solution behaviour assuming dissociation
into 2 ions ($\bullet\,\bullet\,\bullet\,\bullet$) - cf isoprenaline hydrochloride, or 3 ions
($\cdots\cdots$) - cf disodium cromoglycate and sulphates of isoprenaline
and salbutamol.
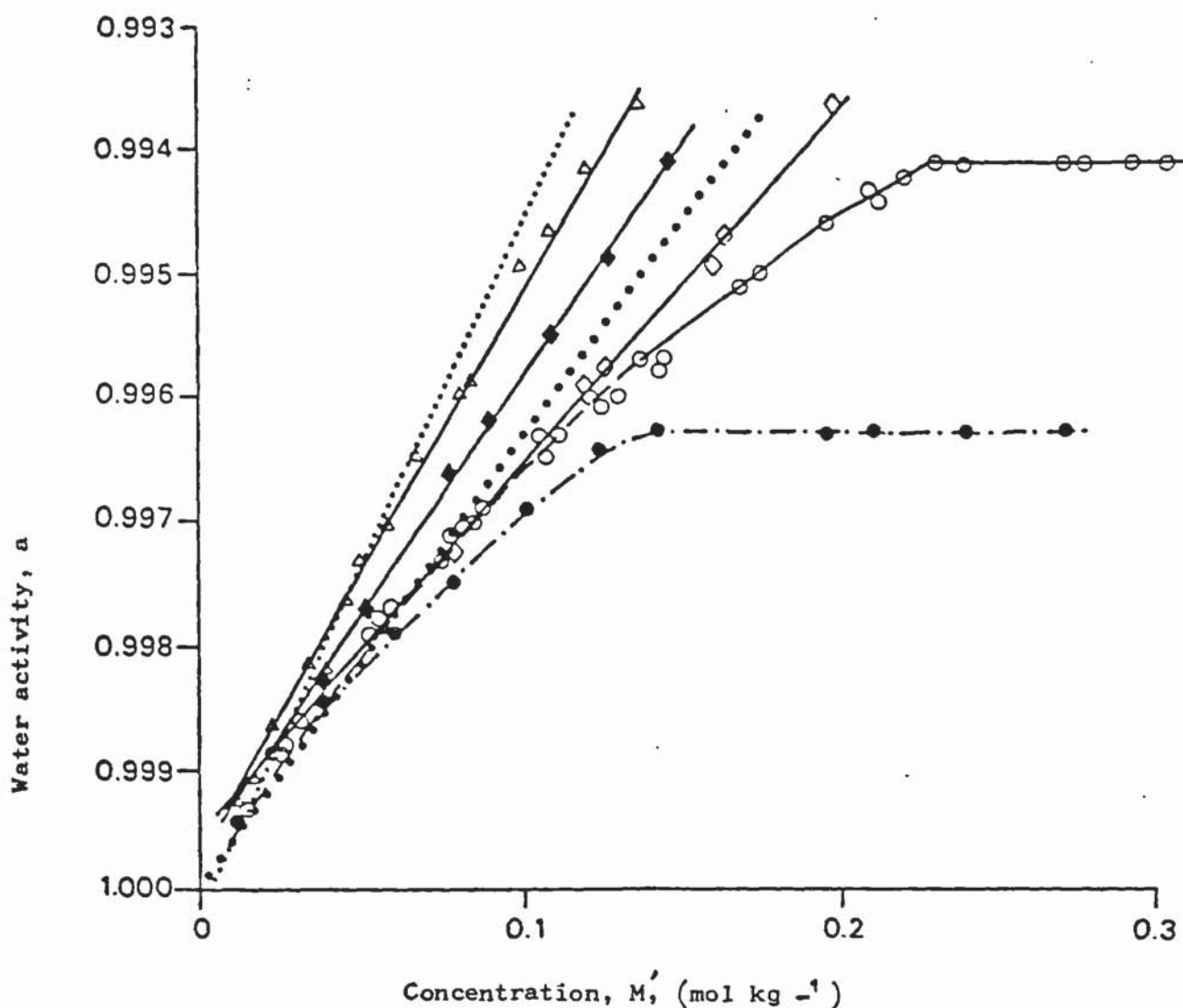
Figure 2.5 : Density of aqueous solutions of Disodium cromoglycate (O),
salbutamol sulphate (x) isoprenaline hydrochloride (●) and
isoprenaline sulphate (Δ) at 37°C. The solid lines illustrate
the linear approximations used to determine droplet equilibrium
diameters with the inclusion of the Kelvin efeect. The mean
of three determinations is illustrated.

Figure 2.6 Calibration of surface tension apparatus, instrumental
response versus surface tension. The mean of three deter-
minations is illustrated, individual readings were with $\pm$
1 instrumental response unit of the mean.
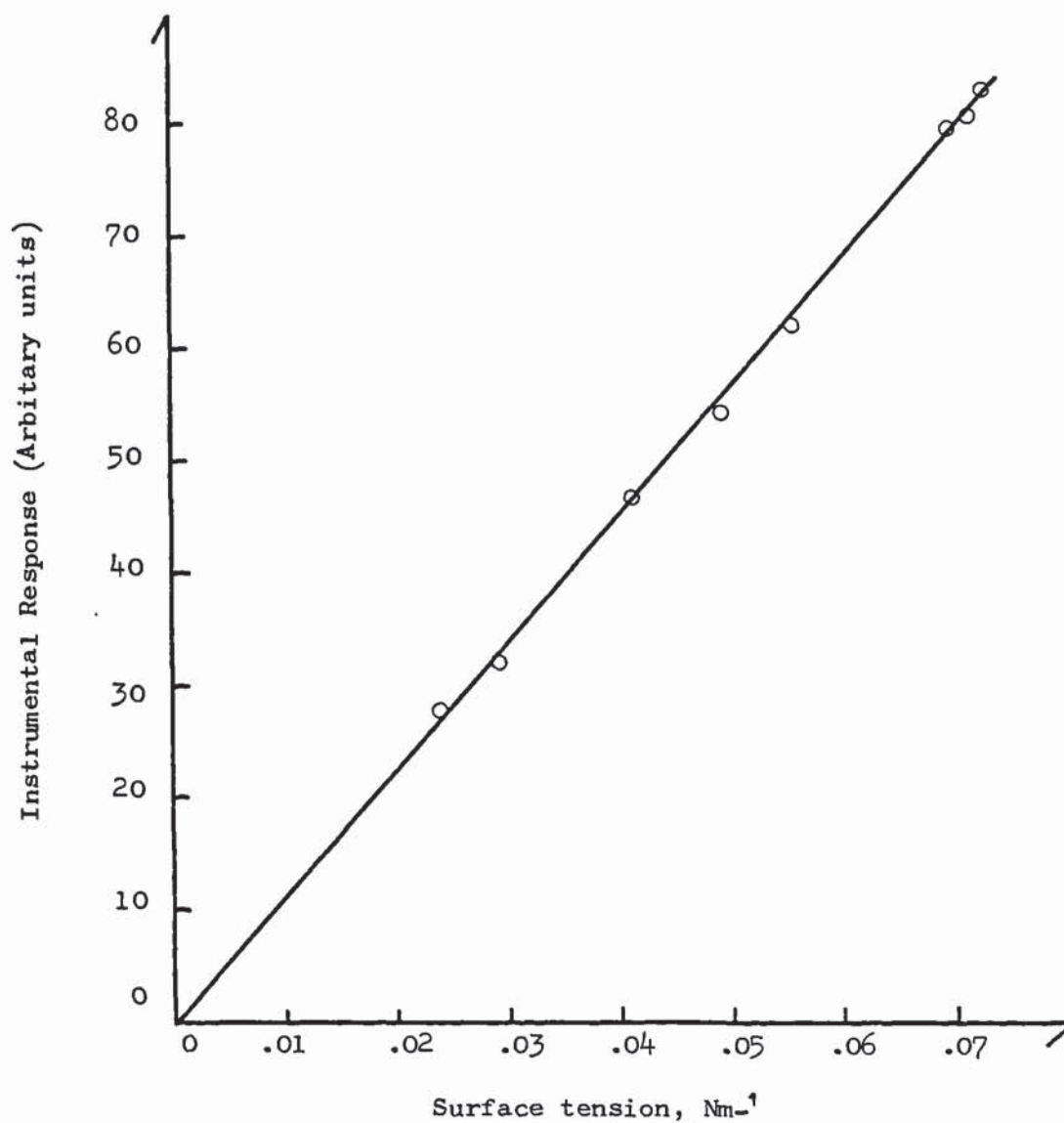
Figure 2.7   Surface tension against tension molality at 37° C for aqueous
             solutions of disodium cromoglycate (●), isoprenaline hydro-
             chloride (▲), isoprenaline sulphate (Δ) and salbutamol sulphate
             (○). The solid lines indicate the linear approximations used
             to compute the equilibrium diameters with inclusion of the
             Kelvin effect.
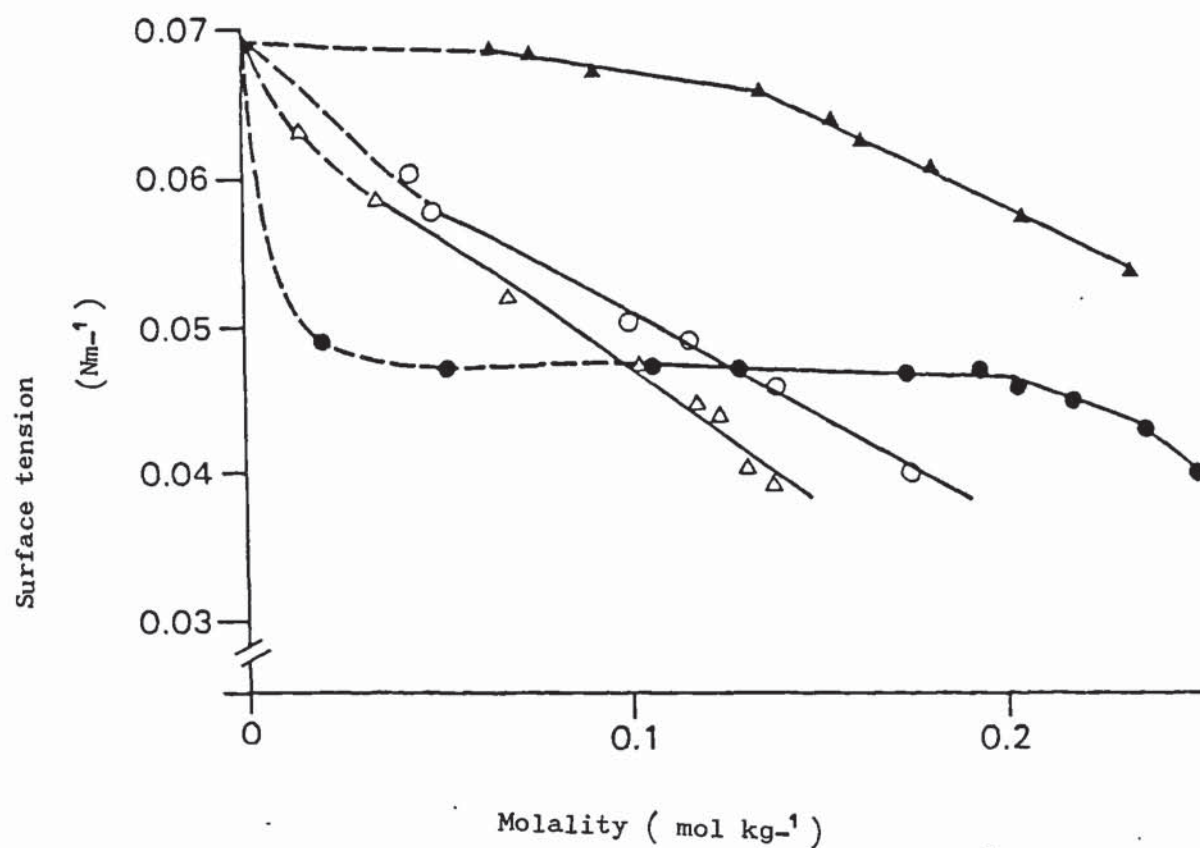
Figure 2.8 Percentage error caused by neglecting the Kelvin effect in calculation of equilibrium droplet size: $(D_a)_e$ & $(D_a)_i$ - aerodynamic diameters calculated with and without the Kelvin correction, respectively. Results for isoprenaline sulphate (———), isoprenaline hydrochloride —--.---.), salbutamol sulphate (---) and disodium cromoglycate (-.-), all at 37° C
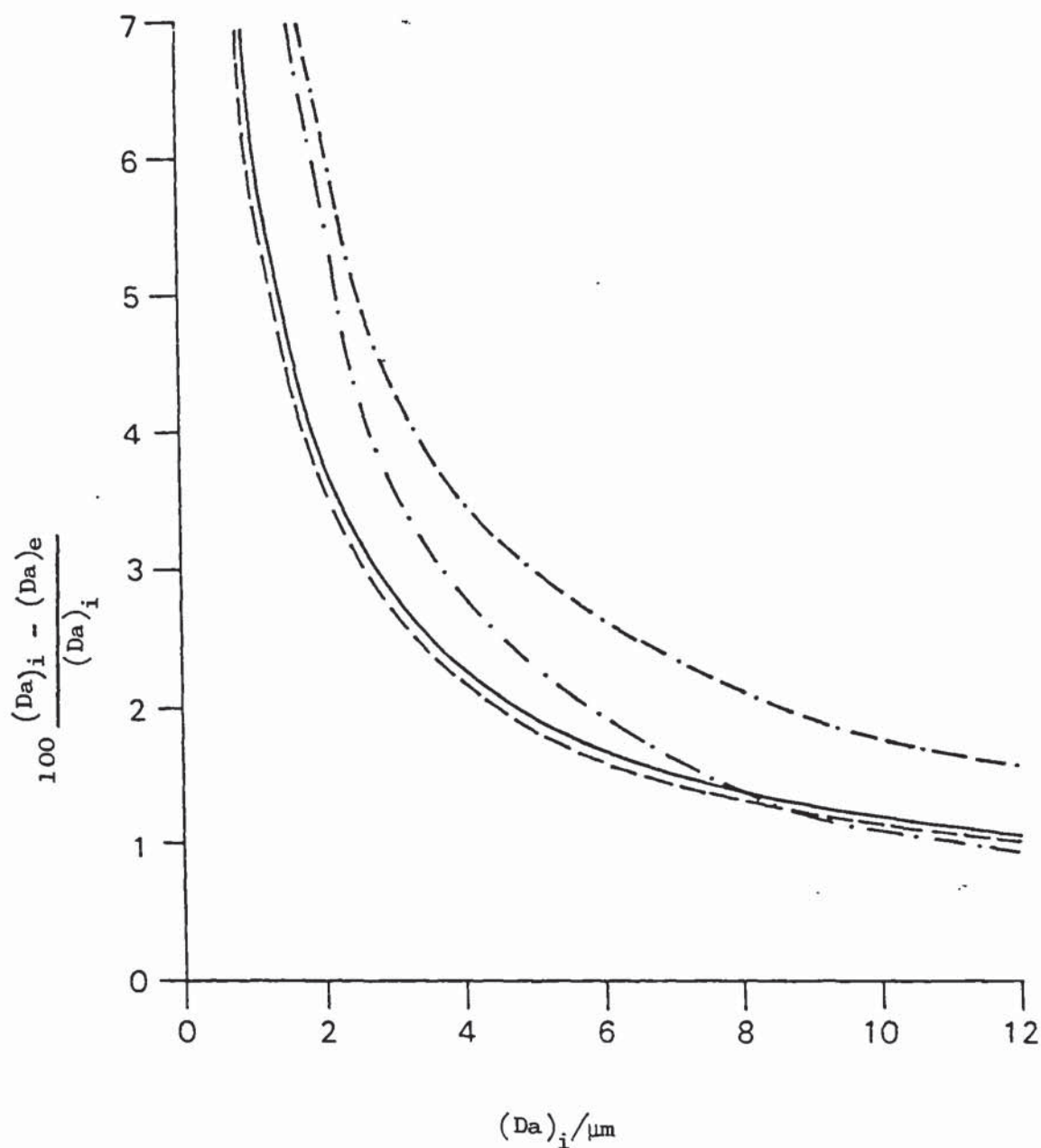
Chapter 3   PREDICTED DEPOSITION OF INHALATION AEROSOLS IN THE RESPIRATORY TRACT

3.1   INTRODUCTION

It has been shown that the drugs examined are likely to more than double in aerodynamic diameter when exposed to the high humidity of the respiratory tract. As the site and extent of deposition is a function of aerodynamic diameter this increase in diameter is likely to affect the available dose of drug administered. Large particles may deposit higher in the respiratory tree and be subject to ciliary clearance with subsequent swallowing. However smaller particles which would initially be expected to be exhaled may be deposited in the respiratory tract because of the increase in aerodynamic diameter. It is useful therefore to quantify the changes in deposition that may arise from condensation growth.

Deposition in the respiratory tract is known to be determined by both physiological parameters and aerosol particle characteristics. The experimental determination of aerosol deposition is by no means easy, indeed large discrepancies in reported results have been attributed to uncontrolled experimental variables and poor experimental technique (Davies, 1974). Lippmann (1977) has reviewed the available experimental data and found that elimination of data collected using doubtful techniques reduced scatter, but in trials involving more than one subject there was considerable individual variation between subjects which may be attributed to differences in the morphology of the lungs.

Experimental determinations have revealed differences in tracheobronchial deposition between healthy smokers and non-smokers (Lippmann, 1977; Stahlhofen et al, 1980) and deposition patterns in patients with respiratory diseases would be expected to differ from deposition in the healthy, normal population.

Predictive models of deposition have mainly been developed to estimate the impact of environmental pollutant aerosols. The TASK model (1966) and its successor (ICRP, 1979) have been developed to determine lung dosimetry after exposure to radioactive aerosols. As the usual mode of breathing is through the nose these models use data for nose deposition in healthy volunteers as their basis (Pattle, 1961). In inhalation therapy the drug aerosol is normally inhaled through the mouth, frequently a breath-holding manoeuvre is also employed. A more realistic model for determining trends in inhalation therapy deposition should take this into account. Davies (1980) has developed an empirical model for total deposition during mouth breathing for individuals, in which the model parameters are obtained from a large number of experiments on each individual. For particles below 3μm diameter, this model would indicate the dose deposited in the tracheobronchial and pulmonary regions of the respiratory tract (as defined by TASK, 1966). Above this diameter when head deposition becomes significant total deposition no longer reveals the tracheobronchial and pulmonary dose. This type of approach could however be useful when an individual patients respiratory characteristics can be found (for example with the hospital patient) to determine the dose of therapeutic aerosol likely to

deposit in the tracheobronchial and pulmonary regions.

Models applicable to the whole population or discrete populations (for example certain age groups or disease states) could be useful in designing aerosol formulations to optimise tracheobronchial and or pulmonary deposition.

The model used in this study (developed by Dr. I Gonda) is based on empirical descriptions of regional deposition in healthy volunteers breathing through the mouth. Currently this model does not include the breath-holding manoeuvre. The absolute numerical values obtained using this model are therefore unlikely to give true values for the deposition of a drug administered by the inhalation route. The model doses however provide a means of testing the difference which very rapid hygroscopic growth could make to the regional deposition of an inhaled aerosol.

3.2 SIZE DISTRIBUTIONS OF INHALED AEROSOLS

The cumulative weight distribution with regard to equivalent volume diameter for micronised disodium cromoglycate (DSCG) was obtained using a Coulter Counter (Model ZB, Coulter Electronics Ltd) with a 50μm orifice. The electrolyte system used was ammonium thiocyanate in isopropyl alcohol presaturated with DSCG and filtered through a 0.1μm pore cellulose filter (MF-Millipore, Millipore (UK) Ltd). As the number of counts per channel did not change with time, it was assumed that the electrolyte system had no effect on the original size distribution of the powder. The results obtained are shown in Figure 3.1.

Assuming the particles to be spherical, the equivalent volume diameters were converted to aerodynamic diameters by means of Equation 1.3 and the dry powder density determined by air comparison pycnometry (see section 2.3.3.1.). The size distribution of the droplets that would be formed in equilibrium with the humidity of the respiratory tract was determined using the physicochemical data described in Chapter 2 and the iterative programme described in that chapter (i.e. they include the Kelvin effect). The cumulative weight distribution versus aerodynamic diameter is shown in Figure 3.2 and may be adequately described by a lognormal distribution with a mass median aerodynamic (MMAD) of 5.45μm and geometric standard deviation (σ_g) of 3.11. The equilibrium droplet distribution could also be described by a lognormal distribution with MMAD of 11.26μm and σ_g of 3.18.

The cumulative weight distribution versus equivalent volume diameter of micronised isoprenaline sulphate determined using a Coulter Counter was supplied by Riker Laboratories. Isoprenaline hydrochloride was said to have a similar size distribution. The aerodynamic diameter size distributions for the dry particles and equilibrium droplets were calculated including the Kelvin effect using the physicochemical data described in Chapter 2. The aerodynamic dry particle size distributions for the two isoprenaline salts will differ as the two salts have different densities (see section 2.3.3.1). The distributions may be approximated by two normal distributions: for example, the cumulative weight distribution versus aerodynamic diameter of dry isoprenaline sulphate powder may be described by a normal distribution with a mass median aerodynamic diameter of 3.77μm and a standard deviation of 1.34μm for particles below 6.2μm and a normal distribution with MMAD of - 56.32μm and standard deviation of 33.48μm for particles greater than 6.2μm. These approximated distributions are shown as the solid lines in Figure 3.4 and 3.5.

3.3 DEPOSITION MODEL FOR MOUTH INHALATION

A model of deposition for mouth inhalation developed by Dr. Gonda based on published empirical data and the TASK model was used in this study.

Experimental data for aerosol deposition in the head during mouth breathing (Lippmann, 1977) when plotted against Da^2 If, where Da is the aerodynamic diameter and If the mean inspiratory flow rate, may be approximated by two intercepting rectilinear lines (Mercer, 1975). The mean inspiratory flow rate for any particular tidal volume, Iv and respiratory rate, r, may be calculated from

$$If = 2 \times Tv \times r \qquad \text{Equation 3.1}$$

So, for example, at 15 respirations per minute and a tidal volume of 0.75 litres, the mean inspiratory flow rate will be

$$If = 2 \times 0.75 \times 15 \qquad 1 \text{ min}^{-1}$$
$$= 22.5 \qquad 1 \text{ min}^{-1} \qquad \text{Equation 3.2}$$

The intercept for the two rectilinear lines correspond to an aerodynamic diameter of $4.99\mu m$ for a tidal volume of 0.75 litres and 15 respirations per minute, resulting in a discontinuity in the curve for head deposition at that value (see Figure 3.6).

Lippmann (1977) has also shown that tracheobronchial deposition, when expressed as the fraction depositing of that entering the trachea, versus log Da^2 If may also be described by a rectilinear plot. The experimental data in Lippmann's study did not extend below approximately 10% deposition, corresponding to an aerodynamic diameter of $3\mu m$ at a tidal volume of 0.75 litres and 15 respirations per minute. The model therefore draws upon the TASK model (TASK group, 1966) for tracheobronchial deposition of particles below $3\mu m$. The data from the TASK model was corrected for nose inhalation by

$$TB = \frac{TBn}{(1 - Hn)} \qquad \text{Equation 3.3}$$

where TB is the fractional tracheobronchial deposition of the amount of aerosol entering the trachea and TBn and Hn the depositing fractions in the tracheobronchial and head regions after nose inhalation respectively. The tracheobronchial deposition for mouth inhalation, TBm was calculated from

$$TBm = TB \, (\, 1 - Hm) \qquad \text{Equation 3.4}$$

where Hm is the deposition in the head region after mouth inhalation. The values for TBm were fitted using NONLIN (Metzler, 1969) to an empirical equation of the form

$$TBm = \frac{1 + a^1 \, Da + b^1 \, Da^2 + c^1 \, Da^3}{d^1 + e^1 \, Da + f^1 \, Da^2 + g^1 \, Da^3} \qquad \text{Equation 3.5}$$

where a^1 , b^1 , c^1 , d^1 , e^1 , f^1 and g^1 are empirical constants

The deposition in the tracheobronchial region for particles less than $0.01\mu m$ aerodynamic diameter is set at the deposition value at $0.01\mu m$ in this model.

The values for pulmonary deposition used in this model have similarly been determined from the TASK model. Mercer (1975) has determined that the TASK model will tend to over-estimate pulmonary deposition at the expense of tracheobronchial deposition. When total deposition, that is head deposition plus tracheobronchial and pulmonary deposition, exceeded 1, the deposition in the pulmonary region, Pm, was described by

$$Pm = 1 - (TBm + Hm) \qquad \text{Equation 3.6}$$

The model at 1.45 litres and 2.15 litres tidal volumes and 15 respirations per minute are illustrated in Figures 3.7 and 3.8 respectively.

3.4   CALCULATED DEPOSITION OF INHALED AEROSOLS

The fraction of the dose, F, deposited in the head, tracheobronchial and pulmonary regions of the respiratory tract have been calculated from the probability of deposition, $P(Da)$, of a particle with aerodynamic diameter, Da, and the mass size distribution function, $f(Da)$, integrated with respect to aerodynamic diameter.

$$F = \int_0^\infty P(Da) \cdot f(Da) \cdot d\,Da \qquad \text{Equation 3.7}$$

The integral was evaluated numerically.

In calculating the effects of condensation growth on the regional deposition of an inhalation aerosol, it has been assumed that equilibrium is instantaneously attained on the entry of the aerosol into the respiratory tract.

The calculated deposition values are summarised in Table 3.1.

<u>Table 3.1</u>: Calculated deposition fractions in the head tracheobronchial
and pulmonary regions of dry powders and equilibrium droplets
of DSCG, Isoprenaline hydrochloride and Isoprenaline Sulphate

Disodium cromoglycate

	Dry Powder			Equilibrium Droplets		
	750ml	1450ml	2150ml	750ml	1450ml	2150ml
Head	0.35	0.45	0.51	0.57	0.67	0.73
Tracheobronchial	0.20	0.16	0.16	0.18	0.13	0.12
Pulmonary	0.27	0.25	0.21	0.17	0.14	0.11
Total	0.82	0.86	0.88	0.92	0.94	0.96

Isoprenaline hydrochloride

	Dry Powder			Equilibrium Droplets		
	750ml	1450ml	2150ml	750ml	1450ml	2150ml
Head	0.09	0.18	0.27	0.55	0.74	0.83
Tracheobronchial	0.23	0.26	0.27	0.31	0.18	0.12
Pulmonary	0.49	0.44	0.37	0.10	0.07	0.04
Total	0.81	0.88	0.91	0.96	0.99	0.99

Isoprenaline Sulphate

	Dry Powder			Equilibrium Droplets		
	750ml	1450ml	2150ml	750ml	1450ml	2150ml
Head	0.09	0.19	0.28	0.45	0.65	0.76
Tracheobronchial	0.23	0.26	0.30	0.35	0.23	0.17
Pulmonary	0.48	0.43	0.36	0.16	0.10	0.06
Total	0.80	0.88	0.94	0.96	0.98	0.99

3.5 DISCUSSION

These calculations are an attempt to quantify the effect of condensation growth on regional deposition. It has been assumed that the size distribution from the commercial aerosol generators (for DSCG the spinhaler [R] and for the isoprenaline salts pressure pack generators) will be similar to those of the micronised dry powders used in the preparation of these dosage forms. This is probably an over optimistic view, Bell et al (1971) has shown that the output from the spinhaler will consist of drug particles adhering to the lactose particles included in Intal capsules to improve flow from the capsule, some aggregates of drug particles as well as primary drug particles.

It has also been assumed that equilibrium is attained instantaneously; however calculations for the deposition of soluble inorganic aerosols by Ferron (1977) indicate that considerations of growth rates may alter the calculated deposition pattern. In the absence of data on the rate of condensation growth of pharmaceutical aerosols, models for real time growth would be based on simple electrolyte systems, and thus result in further uncertainty on the absolute deposition values obtained. Because of these assumptions and the simplicity of the model employed the trends of deposition are of more significance than the absolute values obtained. The calculated deposition fractions illustrate that while total deposition increases with increasing tidal volume, this is mainly because of increased head deposition with correspondingly

less dose available for tracheobronchial and pulmonary regions. As would be expected, for small differences in the particle size distributions, as for the dry powders of the isoprenaline salts, the calculated deposition fractions are very similar. When hygroscopic growth is considered the potential differences between the two isoprenaline salts become more apparent. The calculations reveal that given the same initial particle size distribution of the generated aerosol, isoprenaline sulphate would be expected to deliver more drug to the tracheobronchial and pulmonary regions, than the hydrochloride salt.

The results also predict that less aerosol would be exhaled if the aerosols form equilibrium droplets compared with the aerosol remaining as a dry powder in the respiratory tract. The deposition model used here does not include the breath-holding manoeuvre which is commonly employed in inhalation therapy. With this manoeuvre small particles which are exhaled during normal breathing, can deposit in the lower part of the respiratory tract given sufficient time (Byron et al, 1977). Consequently the predicted difference in exhaled aerosol due to condensation growth is unlikely to be observed in reality.

It should also be noted that the deposited dose may not in fact reflect the systemically available dose. In the tracheobronchial region the deposited dose will be subject to competing ciliary clearance and absorption. In the pulmonary region the deposited dose will be subject to competing absorption and lymphatic clearance.

3.6   FIGURES

Figure Captions

Figure 3.1   Cumulative mass size distribution of Disodium
              Cromoglycate (DSCG). Percent weight undersize
              versus equivalent volume diameter of dry powder.

Figure 3.2   Cumulative mass size distribution of DSCG. Percent
              weight undersize versus aerodynamic diameter of
              the dry powder (o) and droplets in equilibrium
              with 99.5% relative humidity (●).

Figure 3.3   Cumulative mass size distribution of Isoprenaline
              Sulphate (courtesy of Riker Laboratories). Percent
              weight undersize versus equivalent volume diameter
              of dry powder.

Figure 3.4   Cumulative weight versus aerodynamic diameter of
              Isoprenaline Sulphate of the dry powder (o) and
              droplets in equilibrium with 99.5% relative humidity
              (●).

Figure 3.5   Cumulative weight versus aerodynamic diameter of
              Isoprenaline Hydrochloride of the dry powder (o)
              and droplets in equilibrium with 99.5% relative
              humidity (●).

Figure 3.6   Model for deoposition: Fraction deposited versus
              aerodynamic diameter, 750ml tidal volume, 15
              respirations per minute for mouth breathing Total
              deposition (_____), head deposition _._),
              tracheobronchial deposition (---) and pulmonary
              deposition (.....).

Figure 3.7   Model for deposition: Fraction deposition versus
              aerodynamic diameter, 1450ml tidal volume, 15
              respirations per minute for mouth breathing. Total
              deposition (_____), head deposition _._), tracheo-
              bronchial deposition (---) and pulmonary deposition
              (.....).

Figure 3.8   Model for deposition: Fraction deposited versus
              aerodynamic diameter; 2150ml Tidal volume, 15
              respirations per minute, mouth breathing. Total
              deposition (___), head deposition (_._), tracheo-
              bronchial deposition (---) and pulmonary deposition
              (.....).

Figure 3.1 Cumulative mass size distribution of disodium cromoglycate.
% weight undersize versus equivalent volume diameter of dry
powder.
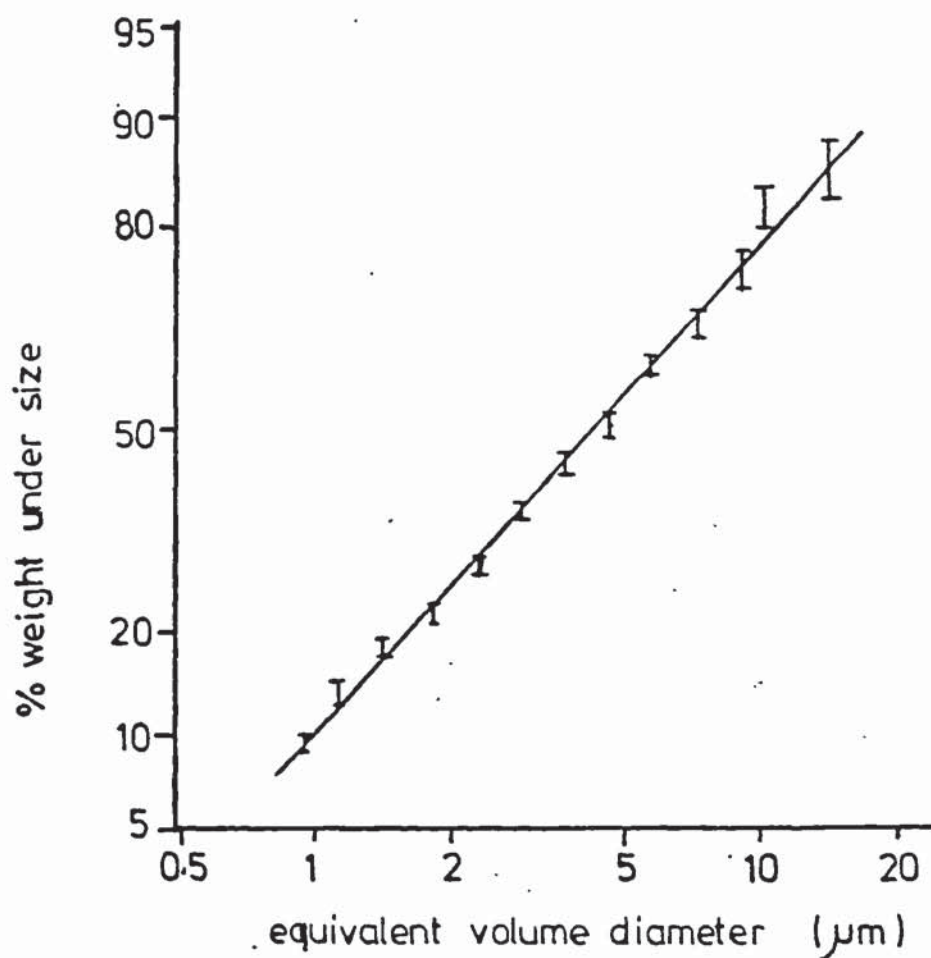
Figure 3.2 Cumulative mass size distribution of disodium cromoglycate
% weight undersize versus aerodynamic diameter of the dry
powder (o) and droplets in equilibrium with 99.5% relative
humidity (•).
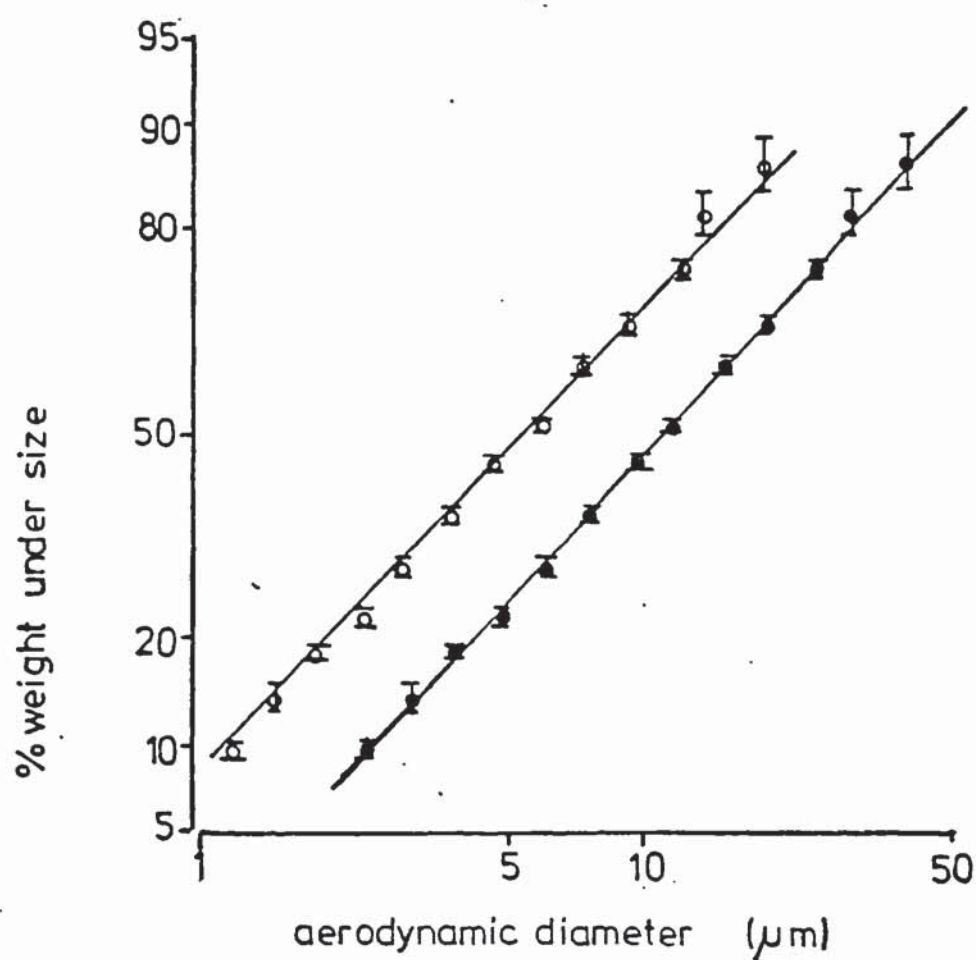
Figure 3.3 Cumulative mass size distribution of Isoprenaline sulphate (courtesy of Riker Laboratories) % weight undersize versus equivalent volume diameter.
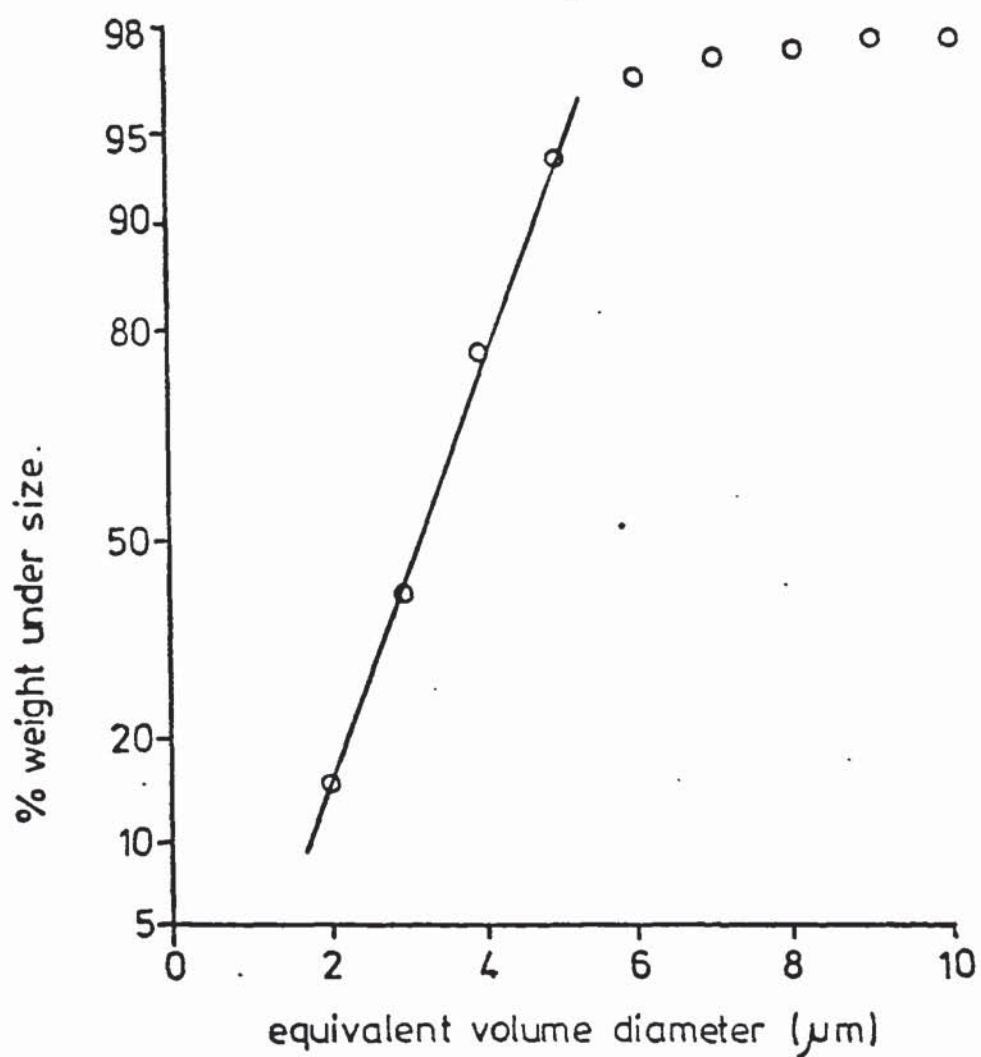
Figure 3.4 Cumulative weight versus aerodynamic diameter of Isoprenaline sulphate of the dry powder (o) and droplets in equilibrium with 99.5% relative humidity (•)

Figure 3.5 Cumulative weight versus aerodynamic diameter of Isoprenaline hydrochloride of the dry powder (o) and droplets in equilibrium with 99.5% relative humidity (•).

A: POWDER
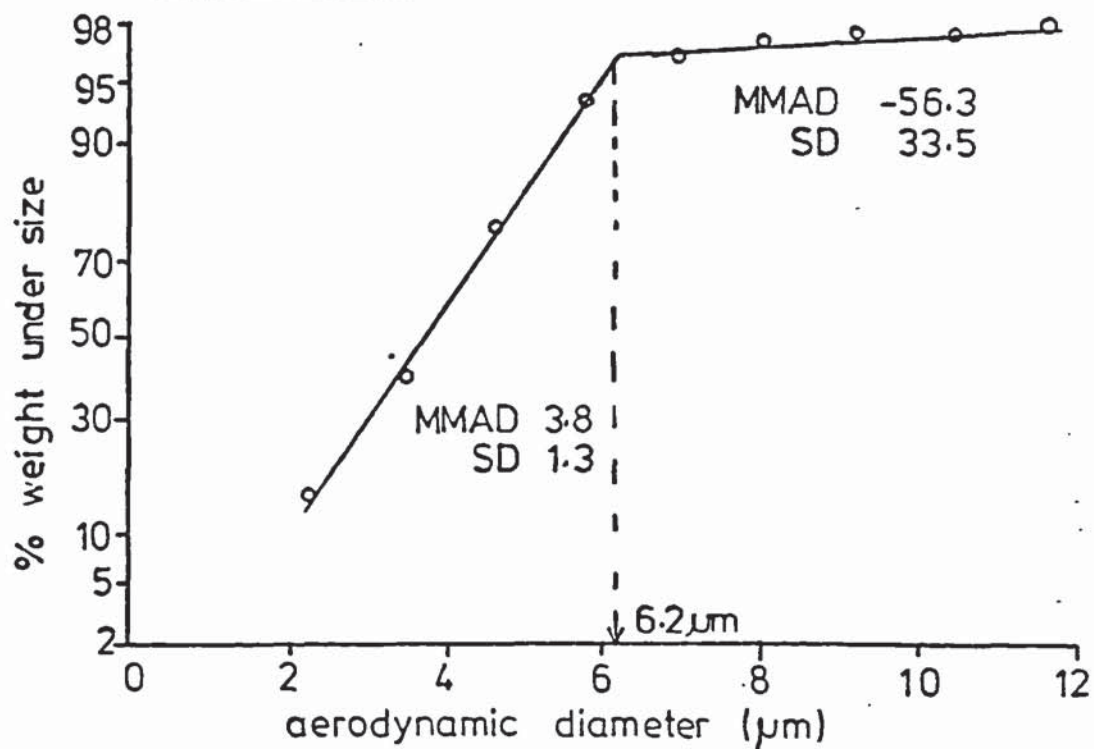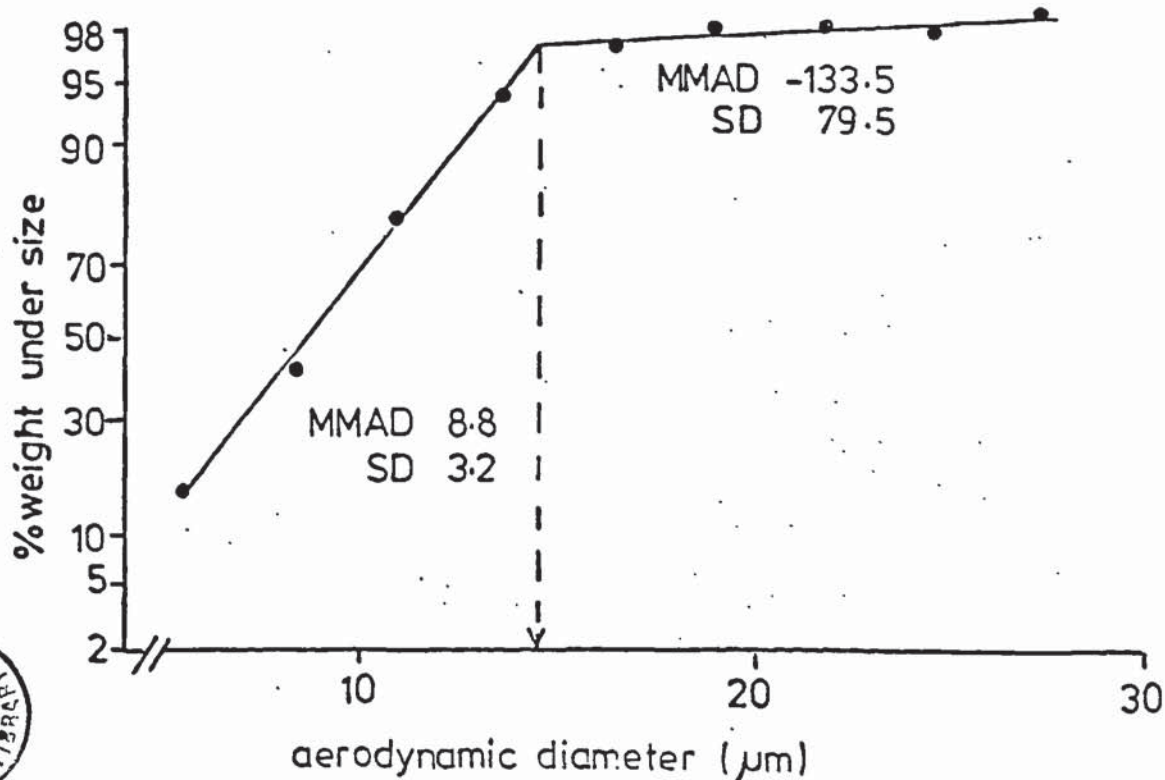
MMAD −55·5
SD 33·0

MMAD 3·7
SD 1·3

6·2 μm

aerodynamic diameter (μm)

% weight under size

B: EQUILIBRIUM DROPLETS

MMAD −157·6
SD 93·7

MMAD 10·4
SD 3·7

17·2 μm

aerodynamic diameter (μm)

% weight under size
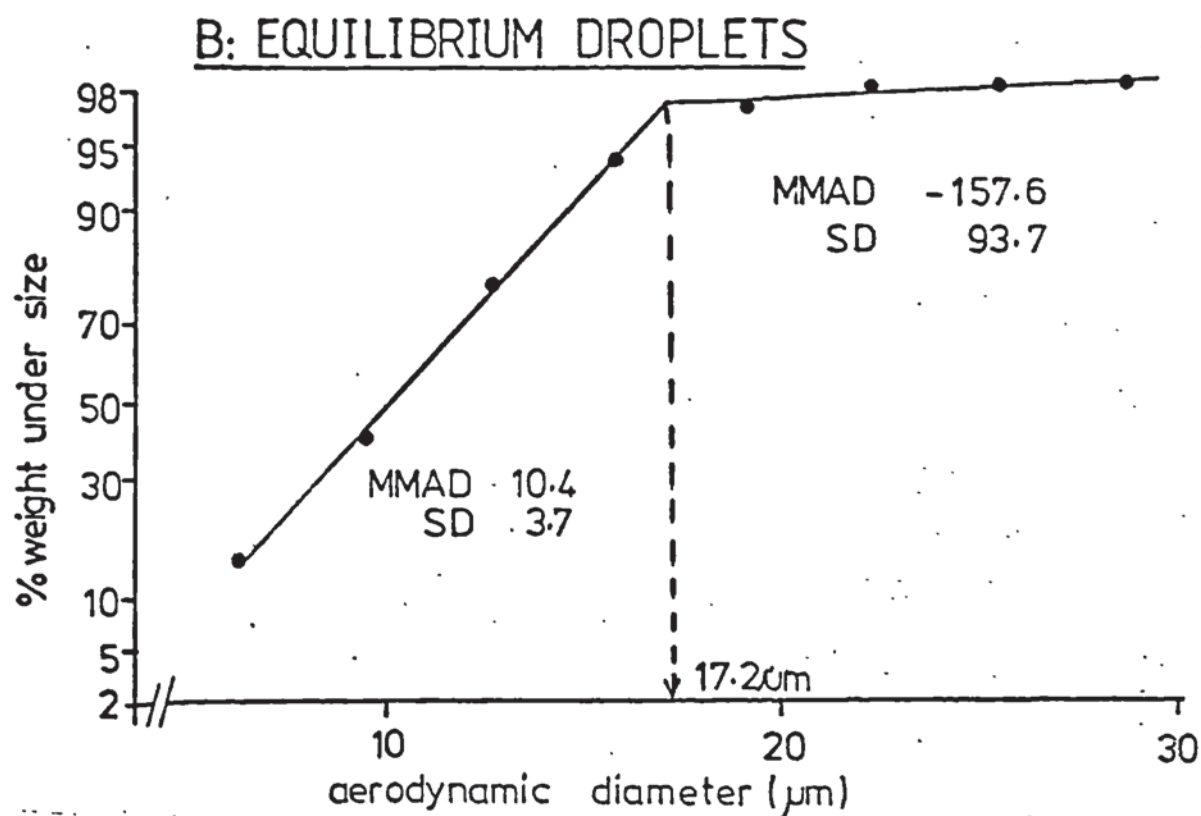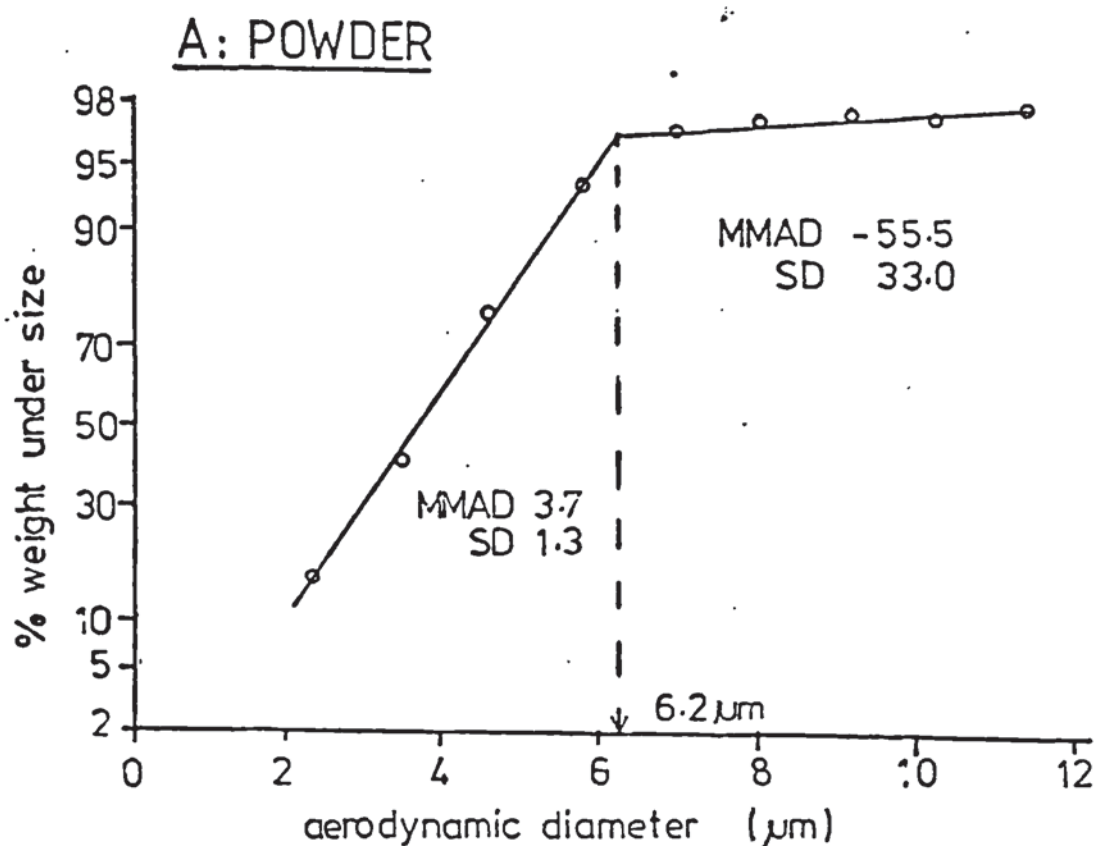
Figure 3.6 Model for deposition: Fraction deposited versus aerodynamic diameter, 750ml Tidal volume, 15 respiration min^{-1} for mouth breathing, Total deposition (_____), head deposition (_·_) tracheobronchial deposition (---) and pulmonary deposition (....).
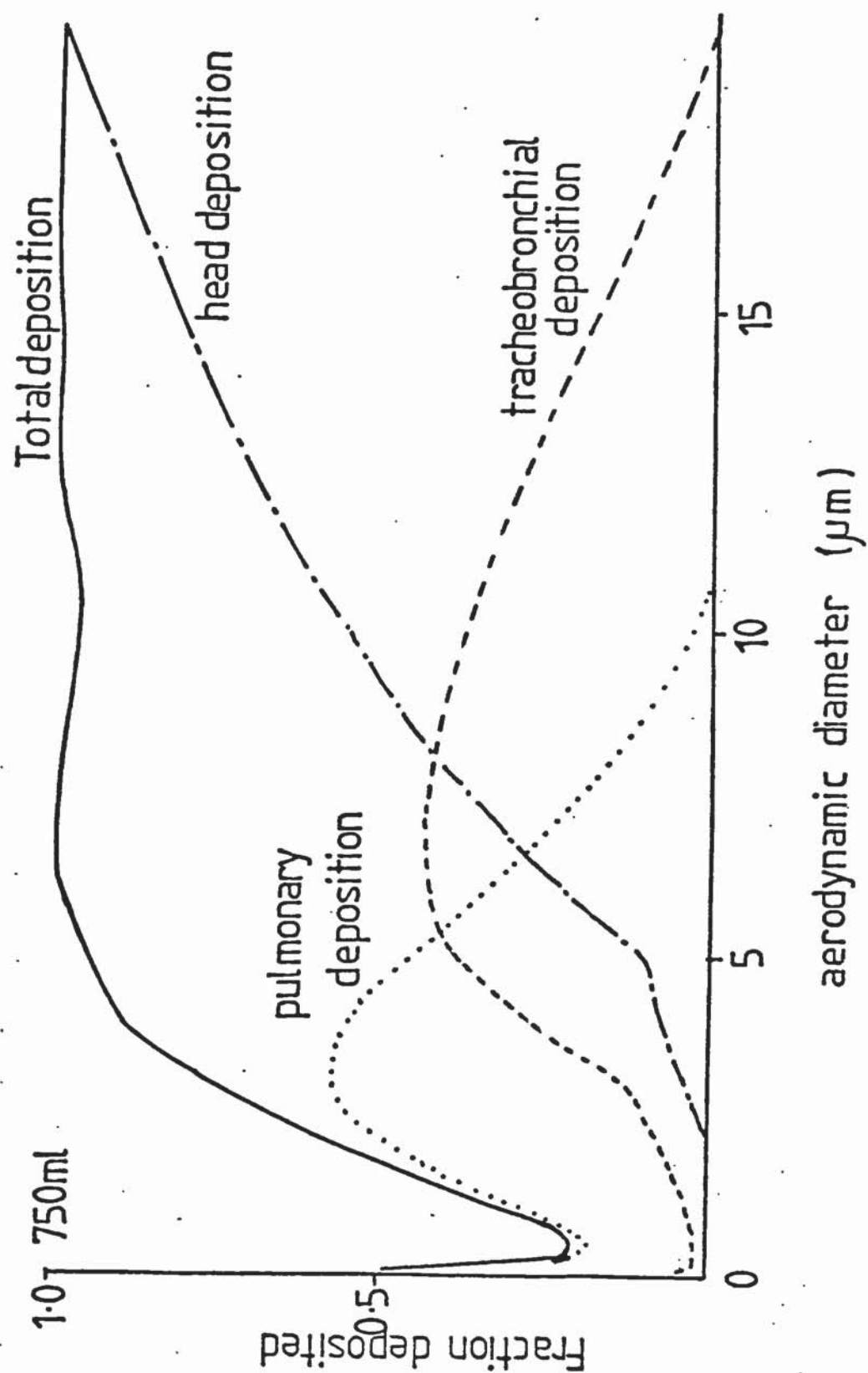
Figure 3.7 Model for deposition: Fraction deposited after mouth breathing, 1450ml tidal volume, 15 respirations per minute, mouth breathing. Total deposition (___) head deposition (_._), tracheobronchial deposition (----) and pulmonary deposition (....).

Figure 3.8 Model for deposition: Fraction deposited versus aerodynamic diameter; 2150ml Tidal volume, 15 respirations per minute, mouth breathing. Total deposition (____), head deposition (_._), tracheobronchial deposition (---) and pulmonary deposition (....).

Chapter 4   AEROSOL SIZING BY CASCADE IMPACTORS
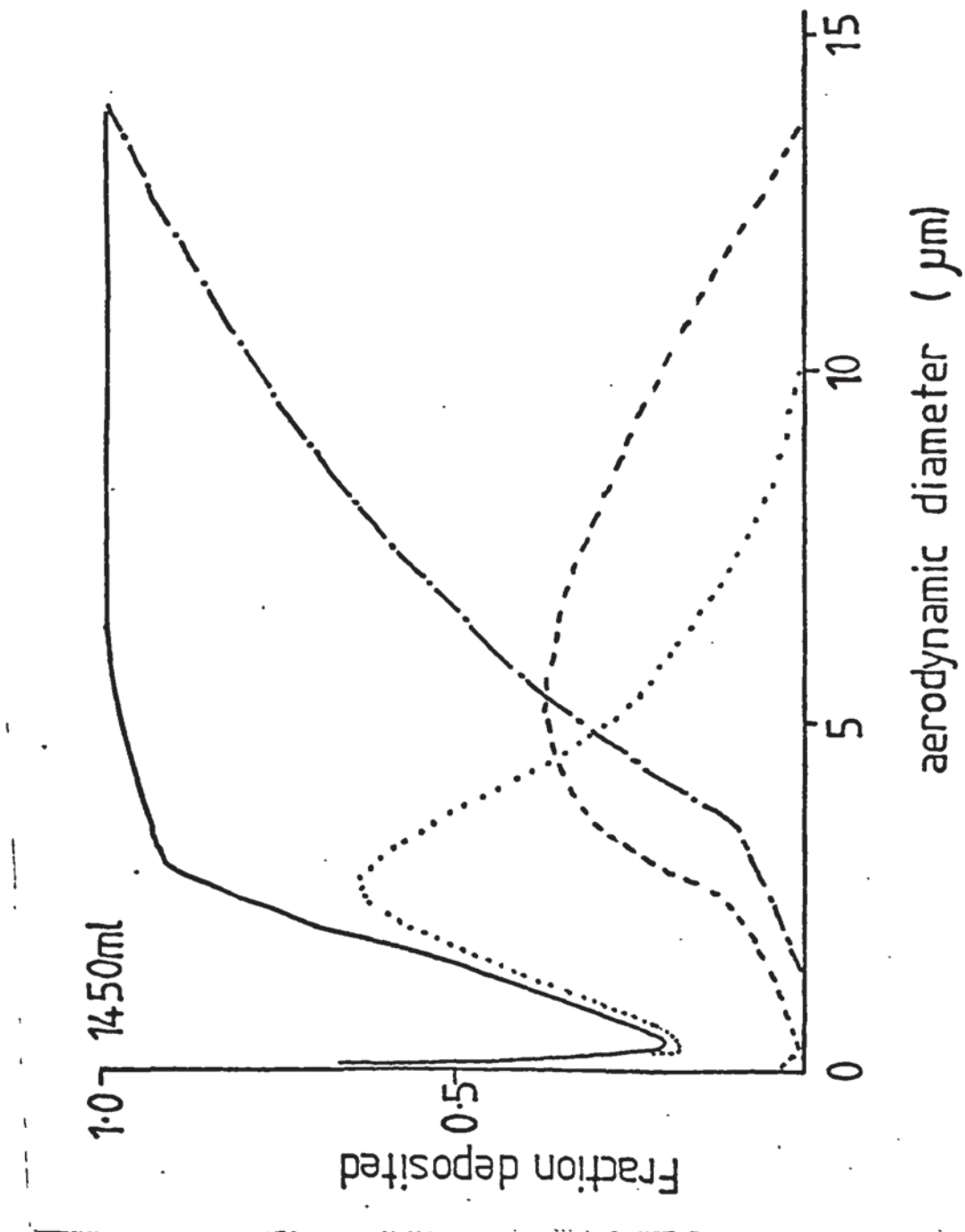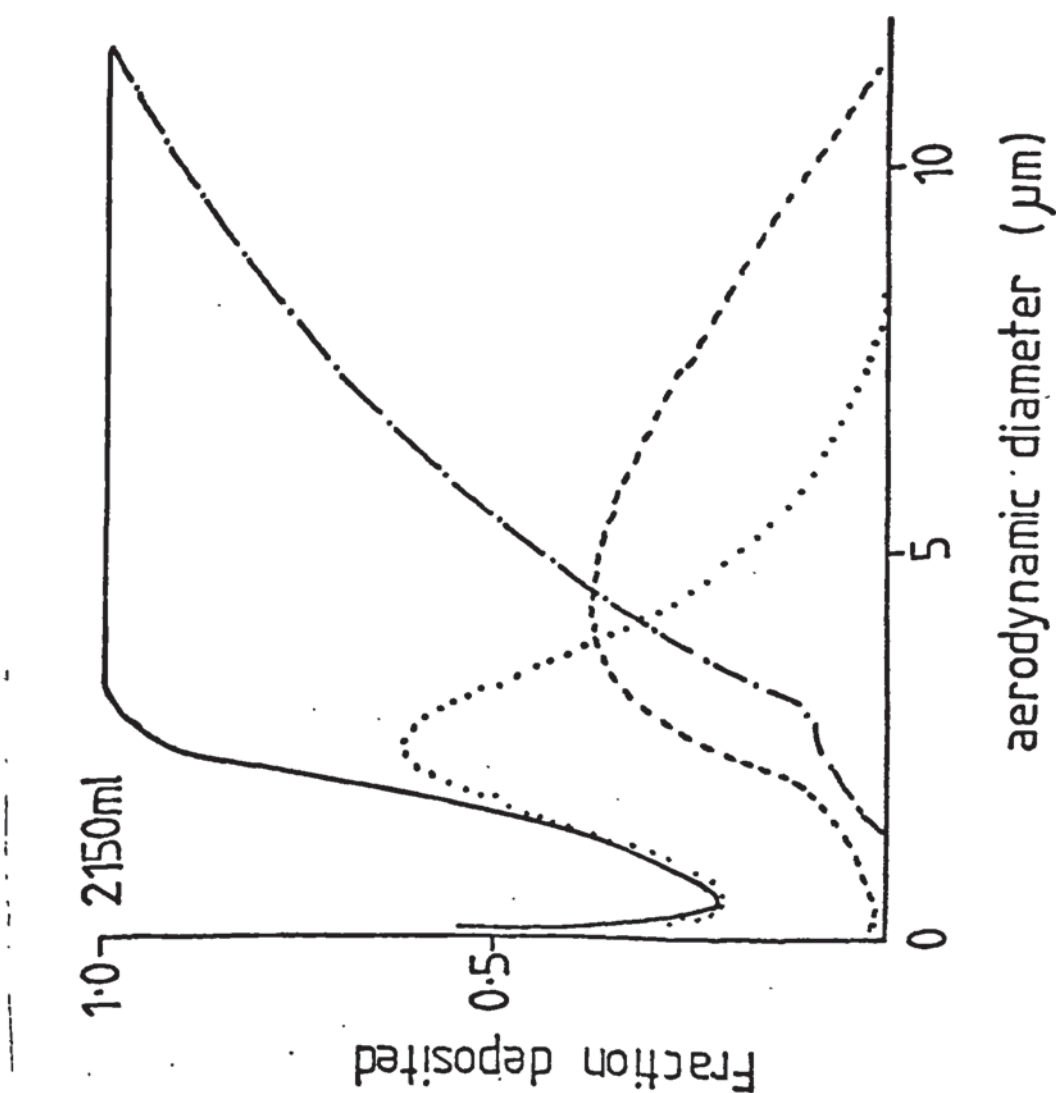
4.1   INTRODUCTION

The major domicilliary aerosol generator used in inhalation
therapy is the pressure pack. Aerosols generated from the
pressure pack will be polyphasic and so it is not possible to
predict the aerosol behaviour at high humidity on the basis
of the physicochemical measurements described in chapter 2 for
pure medicament alone. In these instances direct measurement
of changes in the aerosol size distribution at high humidity
are desirable. From a therapeutic viewpoint it is the behaviour
of aerosol particles which contain the drug which are of interest.
For this reason the use of a sizing device capable of dis-
tinguishing between drug containing aerosol particles and those
void of medicament is desirable. Impaction techniques allow the
collection of aerosols in various aerodynamic size fractions.
Chemical or radioactive analysis of the deposits provides a
means of distinguishing the aerodynamic size distribution of
the drug.

The cascade impactor was the first instrument specifically
developed for the separation of an heterogeneous aerosol cloud
into several fractions each containing only particles between
certain size limits (May, 1945). A schematic diagram of the
instrument is shown in Figure 4.1a. It consists of a series
of jets, each followed by an impaction slide, and is based on
the principle that particles in a moving airstream will impact
on a slide placed in the path of the airstream if their

momentum is sufficient to overcome the drag exerted by the airstream as it moves around the slide. Each jet is smaller than the preceding one, so the velocity of the airstream and that of the aerosol particles is increased as the aerosol cloud advances through the impactor. Consequently smaller particles eventually acquire enough momentum to impact on a slide. The final jet stage is followed by a filter to capture 'all' particles escaping impaction on the slides (Laskin, 1949).

When gravitational and electrical effects are small compared to inertial effects, the impaction process can be characterised by a single impaction parameter, Ψ , as derived by Ranz and Wong (1952).

$$\Psi = \frac{dp.\ V.\ Dp^2}{18.g.Dj} \qquad \text{Equation 4.1}$$

where Dj is the diameter of the impaction jet, Dp. the diameter of the aerosol particle; V, the velocity of the jet; dp, the density of the aerosol particle and g the gas viscosity.

The relationship between the impaction parameter, Ψ and the impaction efficiency, E, will depend on the ratio of the jet to slide distance J, to jet diameter (Mercer and Stafford, 1969) and Reynolds number, Re (Marple, 1979).

$$E = \Psi \left(\frac{J}{Dj}, \quad Re \right) \qquad \text{Equation 4.2}$$

The Battelle cascade impactors used in these studies (DCI5 and

DCI6, Delron Research Company, Powell, Ohio,) have been designed

so that the ratio of jet to slide distance to jet diameter is

constant (Mitchell and Pilcher 1959). These impactors, in

common with many others, have been calibrated by determining

impaction efficiency versus impaction parameter for one stage, .

then using Equation 4.1 to obtain calibration curves for all other

stages (Mitchell and Pilcher, 1959). However, the Reynolds

number of each jet is not constant throughout the impactor (see

Table 4.1). Recent studies with other cascade impactors have

revealed the inadequacies of this calibration technique (Cushing,

1979; Rao and Whitby, 1978b; Gonda et al. 1981). It was there -

-fore felt necessary to calibrate the cascade impactor used in

this study at the operating flow rates.

It has been shown that rebound can be a serious problem if

the collection surfaces are not coated with a soft layer to

cushion the impact of the particle (Mercer and Stafford, 1969).

The performance of several types of coating have been assessed

in various models of cascade impactors (Rao and Whitby, 1978a;

Rao and Whitby, 1978b; Cushing, 1979; Groom and Gonda, 1980).

Other sources of operating error may arise from disaggregation

of loose particle aggregates which would be aerodynamically

equivalent to a large single particle. If the sampling duration

is too shòrt (less than 10 seconds) systematic errors will

occur mainly because of the effect of build up and decay of

flow rates on impaction efficiencies (Blyth and Picknett, 1966).

Particles not in direct contact with the collection surface

may be re-entrained by the passing airstream (Corn and Stein,

1965). Wall losses or interstage losses, are caused by particles

depositing on surfaces other than the collection surface. Mitchell and Pilcher (1957) have shown that this can be reduced to less than 4% by proper design of cascade impactors. These non-ideal collection characteristics of cascade impactors are illustrated schematically in Figure 4.1b.

4.2   EXPERIMENTAL PROCEDURES

4.2.1   Generation of monodisperse aerosols

Aerosols used for calibrating the cascade impactors were
generated using a vibrating orifice monodisperse aerosol
generator (Model 3050, Thermosystems Inc., St Paul Minnesota)
with a Harvard syringe pump (compact infusion pump, Model 975,
Harvard Apparatus Millis, Mass) and frequency generator (B
and K Precision solid state Model E - 310B Sine/Square wave
generator, Dynascan Corp, West Belle Alaine, Chicago, Illinois).
This generator is based on the instability and break up of a
cylindrical liquid jet (Rayleigh, 1878 and 1879). By applying
a mechanical disturbance at constant frequency with sufficient
amplitude, the jet will break up into equal sized droplets.
To form a monodisperse aerosol these uniform droplets must be
dispersed and diluted before coagulation occurs. Since one
droplet is produced per cycle of disturbance, the droplet
volume is given by the ratio of the liquid flow rate Q and the
disturbance frequency f, so that the droplet diameter, Dd, is
given by

$$Dd = \left(\frac{6 \ Q}{\pi \ f} \right)^{1/3} \qquad \text{Equation 4.3}$$

If a solution containing a non-volatile solute is sprayed through
the orifice and the solvent allowed to evaporate, non-volatile
particle of the solute are obtained. The particle diameter,
Dp is given by

$$Dp = C^{1/3} . Dd \qquad \text{Equation } 4.4$$

where C is the volumetric concentration of the solute in the solution.

Any non-volatile impurities present in the solvent will cause an error in the calculated aerosol particle diameter, which may be corrected by

$$Dp' = \left(1 + \frac{N}{C}\right)^{1/3} Dp \qquad \text{Equation } 4.5$$

where Dp' is the corrected particle diameter and N the volumetric concentration of the non-volatile impurity in the solvent (Berglund and Lui, 1973).

The aerosol particles used for calibration of the cascade impactors were solid fluorescein disodium particles and dioctyl phthalate, DOP, droplets containing less than 1% fluorescein disodium by weight, as a fluorescent tracer for quantitative analysis of deposited particles. The fluorescein disodium aerosols were prepared by dissolving the solute in analytical grade methanol. DOP aerosols were prepared by dissolving DOP in analytical grade isopropyl alcohol and adding fluorescein disodium to give a final ratio of fluorescein disodium to DOP of 1:1000 by weight. Following evaporation of solvent the desired fluroescein disodium tagged DOP aerosols were obtained. The size and solid nature of the fluorescein disodium aerosols were examined by light microscopy (Olympus model BHB, Olympus

Optical Company Ltd, Tokyo) and scanning electron microscopy
(Cambridge 150a, Pye Unicam). For light microscopy samples
were collected on coated round glass slides by impaction.
Samples for scanning electron microscopy were collected on
adhesive coated aluminium stubs. The stubs were coated with
gold by the sputter coating technique. The sample stub was
placed under a gold plate electrode at a distance of about
5cm. After evacuation of the chamber, argon gas was introduced
around the sample to act as an ion carrier. Ionisation of
the gold was produced by applying a high voltage across the
gold plate electrode and sample stub causing gold ions to be
deposited over the sample.

4.2.2   Quantitative determination of Fluorescein

4.2.2.1 Effect of pH on the absorbance of Fluorescein

         A series of standard concentration (7.96 μm) fluorescein
solutions were prepared with pH 2.8 to 12.0 Sorensen's glycine
buffer (Sorensen, 1909). The absorbance of each solution was
determined at 492nm, using 1cm path length, matched spectro-
photometric cells in a Pye Unicam SP 500 Series 2 ultraviolet
and visible spectrophotometer.

4.2.2.2 Determination of emission wavelength maximum

         The percentage transmission of a 5.5ng per ml solution
of fluorescein in pH 12.0 glycine buffer was measured at

emission wavelengths between 486nm and 525nm, with an
excitation wavelength of 484nm in an Amino Bowman Spectro-
fluorimeter (Type 4-8202, American Instrument Company Inc)
using 10mm spectrophotofluorimeter cells (transmission matched)
with lids (Type 6010, code colour green, Helma (England) Ltd).

4.2.2.3 <u>Determination of excitation wavelength maximum</u>

The percentage transmission of a 5.5ng per ml solution
of fluorescein in pH 12.0 glycine buffer was measured at an
emission wavelength of 516nm, while changing the excitation
wavelength between 460nm and 520nm.

4.2.2.4 <u>Quantitative determination of Fluorescein</u>

Calibration curves of relative fluorescent intensity
(with reference to a standard of known concentration) versus
concentration were constructed for concentrations of anhydrous
fluorescein$^=$ ranging from 1 to 55ng per ml in pH 12.0 Sorensen's
glycine buffer. Excitation and emission wavelengths of 486nm
and 516nm respectively were employed. The standard reference
solution contained a known concentration of fluorescein$^=$ of
approximately 6ng per ml. Relative instensity (RI), with
reference to the standard solution was calculated according
to the following equation

$$RI = \frac{Z}{Z_s} \qquad \text{Equation 4.6}$$

where Zs is the % transmission of the standard solution and Z_1 the % transmission of the test solution.

4.2.2.5 <u>Fluorescein recovery from the collection surfaces</u>

Clean glass slides (Quartz Grinding Company) porous stainless steel slides (Delron Research Company Powell, Ohio) and glass fibre filters (Type AF, Gelman) were spiked with 50μl of an aqueous fluorescein solution containing 100ng of anhydrous fluorescein⁼ per ml. After drying, fluorescein was recovered by ultrasonic extraction with $5cm^3$ of pH 12.0 glycine buffer for 10 minutes . The extract from the glass fibre filter was filtered through a 0.22μm pore size millipore filter to remove any suspended glass fibre fragments.

With the petroleum jelly (Silkolene 783/L, Dalton and Co) and silicone fluid (Dow-Corning 200/60 000) coated slides a double extraction technique was employed (Hering, 1978). The fluorescein disodium and coating material were ultrasonically extracted with $5cm^3$ of benzene (analytical grade) for 10 minutes. Five cm^3 of pH 12.0 glycine buffer was added and the test tube shaken to mix the two solvents. The fluorescein dissolved in the buffer layer which could be removed for fluorimetric analysis (see section 4.2.2.4) once the two immiscible solvents had separated. In this manner potential fluorescent quenching by the collection substrate could be prevented.

4.2.3   Preparation of collection surfaces

Clean glass discs were prepared by washing round glass slides (Quartz Grinding Company Ltd) in chromic acid/sulphuric acid, followed by several rinses with double distilled water. The slides were then polished with a lens cloth which had been previously soaked in alcohol. The clean glass discs were then allowed to dry.

Porous stainless steel discs (Delron Research Co) were cleaned in an ultrasonic bath with several changes of distilled water, then placed in an drying oven.

Silicone fluid (Dow Corning 200/60000) and white petroleum jelly (Silkolene 783/L, Dalton & Co) coated discs were prepared by spreading 400µl of 10% w/v solutions of silicone fluid and petroleum jelly in benzene (analytical grade) onto clean glass slides. Evaporation of the benzene leaves a thin smooth coat of silicone fluid or petroleum jelly on the glass slide.

4.2.4   Determination of collection efficiencies

Figure 4.2 is a schematic diagram of the experimental set up used to determine collection efficiencies. The monodisperse aerosol generator was set up to generate particles in the range 0.3µm to 26µm. For each size the generator was vented for five minutes, after shifting the syringe pump gear to the final position to ensure steady liquid flow.

Flow rates through the DCI5 and DCI6 cascade impactors are controlled by critical orifices. In the DCI6 impactor this is beyond the filter stage and will give a flow rate of 12.5 litres min^{-1} if a vacuum of greater than 17 inches of mercury is applied. In the DCI5 cascade impactor the fifth stage acts as the critical orifice when a vacuum of greater than 17 inches of mercury is applied, giving a flow rate of 1.1 litres min^{-1}.

Collection efficiencies were determined by comparison of the mass of fluorescein on the collection surface to the total mass on the collection surface and glass fibre filter.

4.3   RESULTS

4.3.1   Monodisperse aerosol generation

Figure 4.3 is a scanning electromicrograph of a fluorescein disodium particle generated using the vibrating orifice generator. Solutions containing more than 1% w/v fluorescein disodium in methanol were found to block the 20μm orifice, thus setting an upper limit of 9μm to the aerosol particles of fluorescein disodium which could be prepared. Large particles could be generated using dioctyl phthalate. Table 4.2 gives the operating conditions for the Berglund-Lui vibrating orifice monodisperse aerosol generator and the range of droplet sizes that can be prepared using this instrument.

4.3.2   Quantitative determination of Fluorescein

4.3.3.1   The effect of pH on the absorbance of Fluorescein

Figure 4.4 shows the effect of pH on the absorbance (at 492nm) of a 7.96μm solution of fluorescein. The apparent spectrophotometric pKa (bearing in mind that fluorescein is dibasic) is 5.7. Consideration of the Hendersen-Hasselba.ch equation reveals that at pH 7.7 and above fluorescein is more than 99% ionised. Solutions for quantitative determination of fluorescein were subsequently prepared in pH 12.0 glycine buffer.

4.3.2.2 Determination of Emission Maximum

The percentage transmission of the 5.5ng per ml solution
of fluorescein$^=$ in pH 12.0 glycine buffer at a fixed excitation
wavelength varied with emission wavelength. The results of a
single determination are shown in Figure 4.5 and clearly show
an emission wavelength maximum at 516nm.

4.3.2.3 Determination of Excitation Maximum .

At a fixed emission wavelength the percentage transmission
of a 5.5ng per ml fluorescein$^=$ solution determined using an
Aminco Bowman spectrofluorimeter varied with excitation wavelength.
Figure 4.6 shows the percentage transmission versus excitation
wavelength; an excitation wavelength maximum occurs at 486nm.

4.3.2.4 Quantitative determination of fluorescein

Calibration curves of relative intensity versus concentration
of fluorescein disodium, such as that shown in Figure 4.7, were
rectilinear for concentrations up to 63ng per ml. The solid line
in Figure 6.2 is the best fit by linear regression to the data for
RI versus concentration of fluorescein disodium in buffer.
Repetitions of the experiment enabled $\geqslant$ 5% errors to be ascribed
to concentrations $\leqslant$ 2.0ng per ml of fluorescein disodium. This
limit was determined by the method of accuracy of coefficients
of the line of regression (Topping, 1962) using data from an
experiment performed in quintuplicate. More than 5% errors are
incurred in concentrations determined for relative intensities

≤ 0.3 (Equation 4.6).

4.3.2.5 Fluorescein recovery from collection surfaces

It was found that extracts from petroleum jelly coated
slides, in the absence of fluorescein disodium registered a
reading on the fluorimeter versus a buffer blank. It was necessary
to establish a calibration curve for fluorescein disodium in the
presence of water soluble extracts from 40mg of petroleum jelly.
Calibration curves of relative intensity versus concentration of
fluorescein disodium in the presence of the water soluble
extracts from 40mg of petroleum jelly could not be distinguished
from calibration curves obtained in buffer alone provided the
fluorimeter was first zeroed with an appropriate reference
solution and that the standard solution included these water
soluble extracts.

Table 4.3 shows the recovery of fluorescein disodium from
the collection surfaces. Except for the slight modification
required to assay fluorescein disodium recovered from petroleum
jelly coated slides, satisfactory recovery and the absence of
any quenching phenomena has been shown.

4.3.3 Collection Efficiency of Different Collection Surfaces

Figure 4.8 shows the collection efficiency of the first
stage of the DCI5 impactor with various types of collection
surfaces of solid fluorescein disodium particles of various
aerodynamic diameters. The solid line indicates the manufacturers

-85-

calibration curve (Mitchell and Pilcher, 1958). The mean and standard deviation of three determinations are indicated.

On clean glass slides the collection efficiency did not exceed 70% and there is some evidence of a decrease in collection efficiency for particles of higher aerodynamic diameters.

At low aerodynamic diameter the collection efficiency onto porous stainless steel slides is greater than for any of the other surfaces examined. However for this type of slide the maximum collection efficiency observed was only 60%. This type of broad collection efficiency curve has been reported by researchers using glass fibre discs (Hering, 1978). One possible explanation of the higher collection efficiency at low aero-dynamic diameters is the penetration of the air stream into the porous matrix of the plate and subsequent retention of aerosol particles within the porous matrix (Rao and Whitby, 1978b).

The maximum collection efficiency observed on white petroleum jelly coated plates was 80% and approached 100% for silicone fluid coated slides. Consequently, the slides treated with the latter material were used in subsequent experiments.

4.3.4   Calibration of DCI 6 impactor

Figure 4.9 to 4.14 show the collection efficiencies versus aerodynamic diameter for stages 1 to 6 of the DCI-6 cascade impactor using silicone fluid coated glass slides. The mean and standard deviation of three determinations is shown .

The collection efficiencies could be empirically described by curves of the form

$$E = A^1 \tanh\left(\frac{Da - B^1}{2C^1}\right) + D^1 \qquad \text{Equation 4.7}$$

The best estimates for the empirical constants A^1, B^1 C^1 and D^1 were determined by non-linear least mean square fitting (Metzler, 1969). The best fits to the experimental data is indicated as the solid lines in Figures 4.9 to 4.14. Best fits to the upper and lower limits (mean $\pm$ standard deviation) are indicated on the figures by the dashed lines.

4.4   DATA INVERSION

The traditional method of interpreting cascade impactor data is to assume that the collection efficiency of each stage can be approximated to a step function passing through the 50% collection efficiency (D_{50}) of the stage. Examination of the observed collection efficiencies (Figures 4.9 to 4.14) reveals that the efficiency curves deviate significally from a step function and even with the best collection surfaces some rebound or re-entrainment occurs. Consequently traditional data treatment may lead to biased estimates of size distribution. The problems with this bias and limitations of previous methods of correction, were discussed by Cooper and Spielman (1976), Cushing et al (1978) and Gonda and Groom (1981).

If we consider an aerosol of mass size distribution $f(x)$ with respect to aerodynamic size, x, entering a cascade impactor, neglecting wall losses the mass of aerosol, M_1, collected on the first stage will be

$$M_1 = \int_0^\infty f(x) \cdot E_1(x) \, dx \qquad \text{Equation 4.8}$$

where $E_1(x)$ is the collection efficiency of the first stage with respect to aerodynamic diameter, x. The mass size distribution of the aerosol passing onto the second stage will be

$$f(x) - f(x) \cdot E_1(x)$$
$$= f(x) \left[1 - E_1(x) \right] \qquad \text{Equation 4.9}$$

To simplify notation the variable x will be neglected for the efficiency term. If E_2' is the collection efficiency of the second stage with respect to x, the amount of aerosol collected on the second stage will be

$$M_2 = \int_0^\infty f(x).E_2 \left[1 - E_1\right] dx \quad \text{Equation 4.10}$$

At the n th stage the mass collected will be

$$Mn = \int_0^\infty f(x) En \left[1 - E_{(n-1)}\right] \ldots \ldots \left[1 - E_1\right] dx \quad \text{Equation 4.11}$$

Empirical descriptions of the collection efficiency curves for each stage of the DCI-6 cascade impactor were obtained as previously described. The final filter stage was assumed to have a collection efficiency of 100%. These equations can be used to calculate the theoretical percentages of aerosol deposited on the six stages and the final filter for any assumed particle size distribution (Fuchs, 1978 ; Gonda and Groom, 1981).

The calculation of the theoretical amounts landing on each stage is aided by the following interactive computer programme: The user decides upon a particular type of distribution function which may be characterised by two parameters, for example the log-normal distribution. A range of parameter values is selected of 10 mass median diameters and 10 geometric standard deviations for a log-normal distribution. The sum of the squares of the differences between the theoretical and experimental deposition results using average efficiencies for each stage are computed. The programme gives a two dimensional error map for the range of

parameters from which a narrower range of parameters may be selected, potentially ambiguously assigned distributions arising from multiple local minima are thus avoided (Huang et al, 1970; Gonda and Groom 1981). The programme is then rerun with a narrower range of parameters. For the best fit the residue values are shown to ascertain the random nature of errors. A systematic trend in errors indicates that a different type of size distribution should be attempted.

4.5   <u>EXPANSION HUMIDITY IN THE CASCADE IMPACTOR</u>

Gas flowing through the cascade impactor will gain velocity
at jet inlets and lose velocity at the exit. As the gas loses
velocity it will correspondingly experience a temperature change.
It is necessary to determine whether the increase in humidity
arising from the temperature change is likely to affect the size
distribution of the aerosol passing through the impactor.

Linear velocities in the impactor may be calculated from
the volume flow rate, F' (which for the DCI-6 cascade impactor
is 12.5 litres per minute) and the cross-sectional area. Thus
for a jet of diameter Dj, the linear velocity, V will be

$$V = \frac{4 \; F'}{\pi \; Dj^2} \qquad \text{Equation 4.12}$$

The linear velocity at each of the stages of the DCI-6 impactor
is given in Table 4.1. The maximum diameter of the DCI-6
impactor is 7.6×10^{-2} m. The linear velocity at the maximum
cross-section will therefore be 4.57×10^{-2} m sec^{-1}. Assuming
that the change in linear velocity is totally absorbed by a
temperature change of the gas (adiabatic isoentropic process)
the temperature change may be calculated from (Hering et al,
1978)

$$Cp' \cdot dT = - V \, dV.$$

$$Cp' \, (T_2 - T_1) = - \frac{(V_2^2 - V_1^2)}{2} \qquad \text{Equation 4.13}$$

where C_p' is the specific heat, T_1 and V_1 the temperature and linear velocity at the maximum cross-sectional area respectively; and T_2 and V_2, the temperature and linear velocity at the jet.

The specific heat may be approximated from (Coulson and Richardson, 1977)

$$C_p' = 1.00 + 1.9 \ H \qquad kJ \ kg^{-1} \ K^{-1} \qquad \text{Equation } 4.14$$

where H is the fractional relative humidity. At high humidity therefore

$$C_p' \approx 2.9 \ K \ J \ kg^{-1} \ K^{-1} \qquad \text{Equation } 4.15$$

At all stages $V_2 \gg V_1$ and so V_1 may be neglected from Equation 4.13. The temperature change at each stage may therefore be calculated from

$$\Delta T = \frac{V_2{}^2}{2 \ x \ C_p'} \qquad\qquad \text{Equation } 4.16$$

It is apparent therefore that the temperature change at each jet will be less for humid air than for dry air. The temperature change at each jet for humid air, ΔT_H and for dry air, ΔT_D, is given in Table 4.4.

It can be seen that the largest perturbation occurs at the final stage.

In order to assess the effect these temperature changes are likely to have upon the aerosol cloud it is necessary to determine the 'transit time', that is the time the aerosol is

likely to experience an increased humidity arising from the temperature drop. The DCI-6 cascade impactor is designed so that the jet-to-slide distance at all stages is $^3/_8$ Dj. A minimum 'transit time' t_{min}, may therefore be regarded as the time to traverse this distance. The maximum 'transit time' t_{max} may be calculated from the maximum length of the jet which is approximately 2×10^{-2}m for all stages. The maximum and minimum transit times are given in Table 4.4.

Significant changes in the aerosol size is unlikely to occur in a time scale of less than 1 msec. From Table 4.4 it is obvious that when t_{max} is greater than 1msec the maximum temperature change ΔT_D is less than 0.05K.

These calculations therefore indicate that the cascade impactor is a suitable instrument for investigating the effect of humidity on the particle size distribution of hygroscopic aerosols. Hochrainer and Zebel (1981) have reached the same conclusion after examining this problem both theoretically and experimentally.

4.6   CONCLUSION

These experiments have confirmed the observations of other researchers (Rao and Whitby 1977a, 1977b; Cushing et al 1979) that a suitable coating on the collection surface is necessary in order to obtain collection efficiency curves that approach 100% collection efficiency. Silicone fluid (200/60000) was shown to be the best coating of those examined here. However, even with this type of coating there was some evidence that the collection efficiency declined after reaching a maximum value approaching 100%, presumably through rebound and re-entrainment of the larger particles. In the light of these observations it would be wise to avoid overburdening the stages and only collect amounts of aerosols on any one stage in the order of the loading limits suggested by Mercer (1966). These loading limits represent the amount of aerosol of average size of the range likely to reach the stage which could be collected as a single layer in the area of the jet.

The D_{50} values obtained experimentally were found to vary from the manufacturer's calibration and are shown for each stage in Table 4.4 with the manufacturer's values for comparison. The DCI-6 cascade impactor, in common with many others, has been calibrated by determining impaction efficiency versus impaction parameter for one stage, then using Equation 4.1 to obtain calibration curves for all other stages (Mitchell and Pilcher, 1959). However, when the collection efficiencies obtained experimentally are plotted against the square root of the impaction parameter, $\sqrt{\Psi}$, (Figure 4.15) a shift in the relation-

ship is seen from stage to stage. The value of $\sqrt{\psi}$ corresponding to the D_{50} value used in the manufacturers calibration was 0.29 (Mitchell and Pilcher, 1959). While this is consistent with the experimental values obtained here for stages 4, 5 and 6, it does not adequately describe the results obtained for the first three stages. These observations indicate the necessity of calibration at the operating flow rates. Adiabatic expansion of air in the jets of the impactor will lead to temperature changes and corresponding changes in relative humidity. Theoretical consideration of this potential problem reveals that particle size changes are unlikely to occur because of the short time spent by particles in the jets. The cascade impactor is therefore a suitable instrument for size analysis of hygroscopic aerosol particles.

4.7   TABLES

TABLE LEGENDS

Table 4.1 Characteristics of Battelle Cascade Impactors, DCI-5 and DCI-6 cascade impactor; jet diameter, Dj; Reynolds number, Re; and linear velocity, V_1 for each stage (Hering et al, 1978)

Table 4.2 Operating conditions for the Berglund Lui, model 3.050, vibrating orifice monodisperse aerosol generator.

Table 4.3 Manufacturer's calibration and experimentally obtained D_{50} values for each stage of the DCI-6 cascade impactor.

Table 4.4 Adiabatic perturbations in the DCI-6 cascade impactor Transit times: t_{min}, the time taken to traverse the jet to slide distance; t_{max}, the time taken to traverse the jet length. Temperature changes: ΔT_H, the temperature change for high humidity air; ΔT_D, the temperature change for dry air.

Table 4.1 Characteristics of Battelle Cascade Impactors, DCI5 and DCI6.

DCI-5	flow rate 1.05Lmin⁻¹		
Stage Number	Dj (cm)	Re	V (m/s)
1	0.249	560	3.5
2	0.140	990	11
3	0.099	1400	22
4	0.064	2170	54
5	0.036	3500	164

DCI-6	flow rate 12.45Lmin⁻¹		
Stage Number	Dj (cm)	Re	V (m/s)
1	1.364	1160	1.4
2	0.859	1850	3.6
3	0.541	2930	9
4	0.341	4650	23
5	0.216	7340	57
6	0.141	11250	133

Table 4.2 Operating conditions for the model 3050 vibrating orifice monodisperse aerosol generator.

Orifice diameter	Syringe capacity	Gear position	Liquid feed rate ($cm^3 min^{-1}$)	Frequency range (KHz)	Droplet diameter range (μm)
20	50	18	0.139	40-80	38-48
10	20	18	0.071	130-260	21-26
5	10	18	0.0406	300-600	12-16

Table 4.3 Manufacturers' calibration and experimentally obtained D_5 values for the stages of the DCI-6 cascade impactor.

Stage Number	D_{50} value (μm)	
	Manufacturers value	Value obtained by least mean square analysis of experimental data
1	16	11.2 ± 0.7
2	8.0	5.5 ± 0.5
3	4.0	3.3 ± 0.3
4	2.0	1.95 ± 0.1
5	1.0	0.94 ± 0.05
6	0.5	0.51 ± 0.03

Table 4.4 Adiabatic perturbations in the DCI-6 cascade impactor. Transit times: t_{min}, the time taken to traverse the jet to slide distance; t_{max}, the time taken to travel the jet length and temperature changes, ΔT_H, the temperature change for high humidity air, and ΔT_D, the temperature change for dry air, at each of the stages.

Stage Number	$10^3 t_{min}$ (sec)	$10^3 t_{max}$ (sec)	ΔT_H (K)	ΔT_D (K)
1	3.60	14.1	0.0003	0.001
2	0.90	5.6	0.002	0.006
3	0.22	2.2	0.014	0.041
4	0.056	0.88	0.089	0.26
5	0.014	0.35	0.55	1.60
6	0.004	0.15	3.04	8.83

4.8   <u>FIGURES</u>

<u>FIGURE LEGENDS</u>

<u>Figure 4.1</u> Schematic diagram of aerosol collection by cascade impactor a) Ideal collection efficiencies and b) non-ideal collection efficiencies.

<u>Figure 4.2</u> Schematic diagram of the apparatus for determination of collection efficiencies.

<u>Figure 4.3</u> Scanning electromicrograph of a fluorescein disodium particle generated using the vibrating orifice generator.

<u>Figure 4.4</u> Effect of pH on absorbance of 7.6μM solution of fluorescein disodium at 492nm.

<u>Figure 4.5</u> Percentage transmission of 5.5ng per ml solution of fluorescein versus emission wavelength (excitation wavelength, 486nm).

<u>Figure 4.6</u> Percentage transmission of 5.5ng per ml solution of fluorescein$^=$ versus excitation wavelength (emission wavelength 516nm.

<u>Figure 4.7</u> Relative intensity (Equation 4.6) versus concentration of fluorescein$^=$ in buffer (pH 12).

<u>Figure 4.8</u> Collection efficiency versus aerodynamic diameter for stage 1 of DCI5 cascade impactor

<u>Figure 4.9</u> Percentage collection efficiency (100 xE) versus aerodynamic diameter (Da) for stage 1 of DCI6 cascade impactor manufacturer's calibration (-.-), mean efficiency (_) and maximum and minimum contours (_ _ _) obtained by least mean squares fitting of the experimental data to Equation 4.7. The mean and standard deviation (n=3) of the experimental values are indicated

Figure 4.10 Percentage collection efficiency (100 x E) versus aerodynamic diameter (Da) for Stage 2 of DCI6 cascade impactor: Manufacturer's calibration (-.-), mean efficiency (_) and maximum and minimum contours (---) obtained by least mean squares fitting of experimental data to Equation 4.7. The mean and standard deviation (n=3) of the experimental values are indicated

Figure 4.11 Percentage collection efficiency (100 x E) versus aerodynamic diameter (Da) for Stage 3 of DCI6 cascade impactor: Manufacturer's calibration (-.-), mean efficiency (_) and maximum and minimum contours (---) obtained by least mean squares fitting of the experimental data to Equation 4.7. The mean and standard deviation (n=3) of the experimental values are indicated

Figure 4.12 Percentage collection efficiency (100 x E) versus aerodynamic diameter (Da) for Stage 4 of DCI6 cascade impactor: Manufacturer's calibration (-.-), mean efficiency (_) and maximum and minimum contours (---) obtained by least mean square fitting of the experimental data to Equation 4.7. The mean and standard deviation (n=3) of the experimental values are indicated

Figure 4.13 Percentage collection efficiency (100 x E) versus aerodynamic diameter (Da) for Stage 5 of DCI6 cascade impactor: Manufacturer's calibration (-.-), mean efficiency (_) and maximum and minimum contours (---) obtained by least mean square fitting of the experimental data. The mean and standard deviation (n=3) of the experimental values are indicated

Figure 4.14 Percentage collection efficiency (100x E) versus aerodynamic diameter (Da) for Stage 6 of DCI6 cascade impactor: Manufacturer's calibration (-.-), mean efficiency (_) and maximum and minimum contours (---) obtained by least mean square fitting of the experimental data to Equation 4.7. The mean and standard deviation (n=3) of the experimental values are indicated

Figure 4.15 Mean experimental collection efficiency versus square root of impaction parameter (Ranz and Wong, 1952) for the DCI-6 cascade impactor.

Figure 4.1 Schematic diagram of aerosol collection by cascade impactor a) Ideal collection efficiencies and b) non-ideal collection efficiencies.

Figure 4.2 Schematic diagram of the apparatus for determination of collection efficiencies.

vent

Stage of cascade impactor

collection slide

filter

To vacuum pump

Kr⁸⁵ Aerosol neutralizer

Vibrating orifice mono disperse aerosol generator

clean, dry air supply

Syringe pump
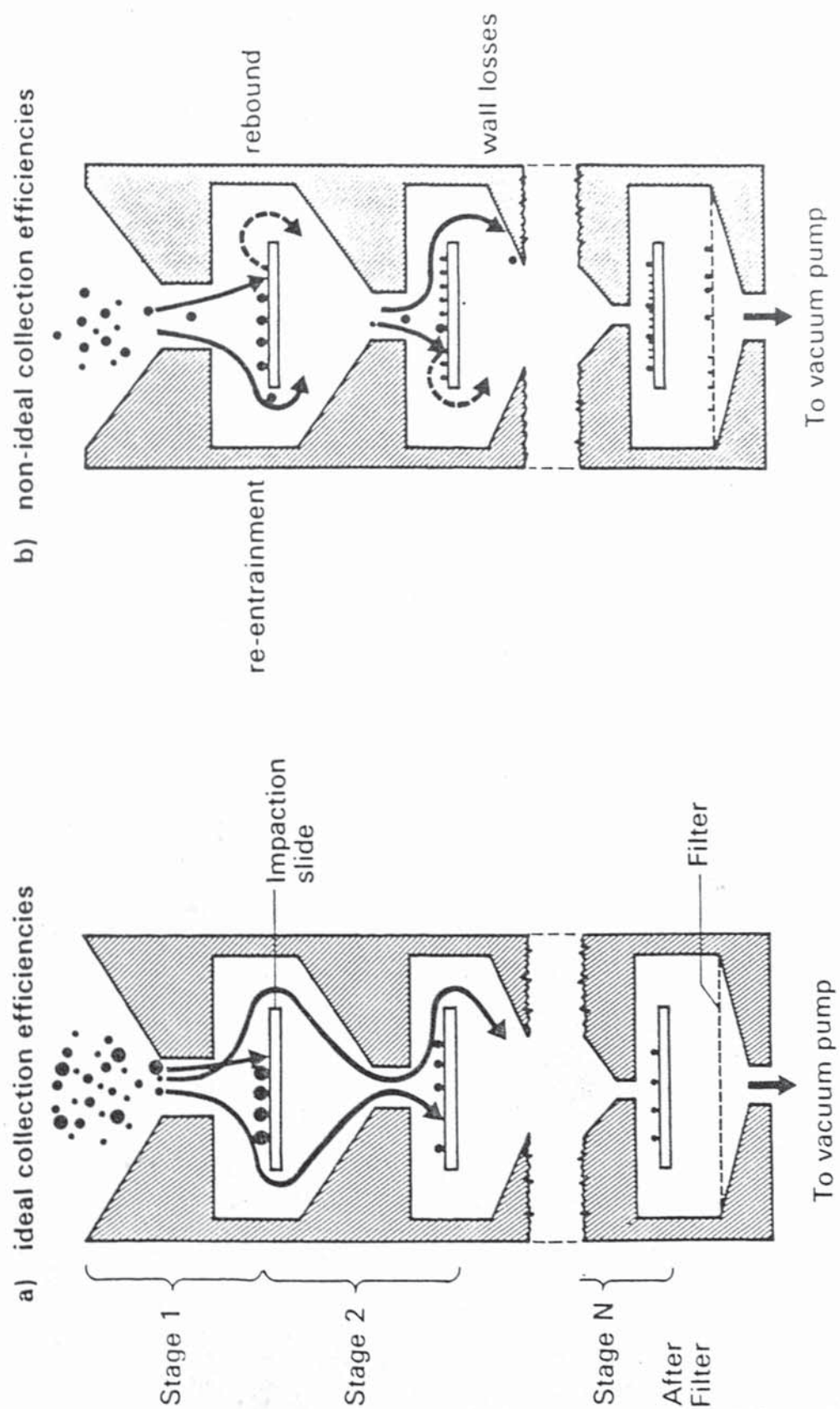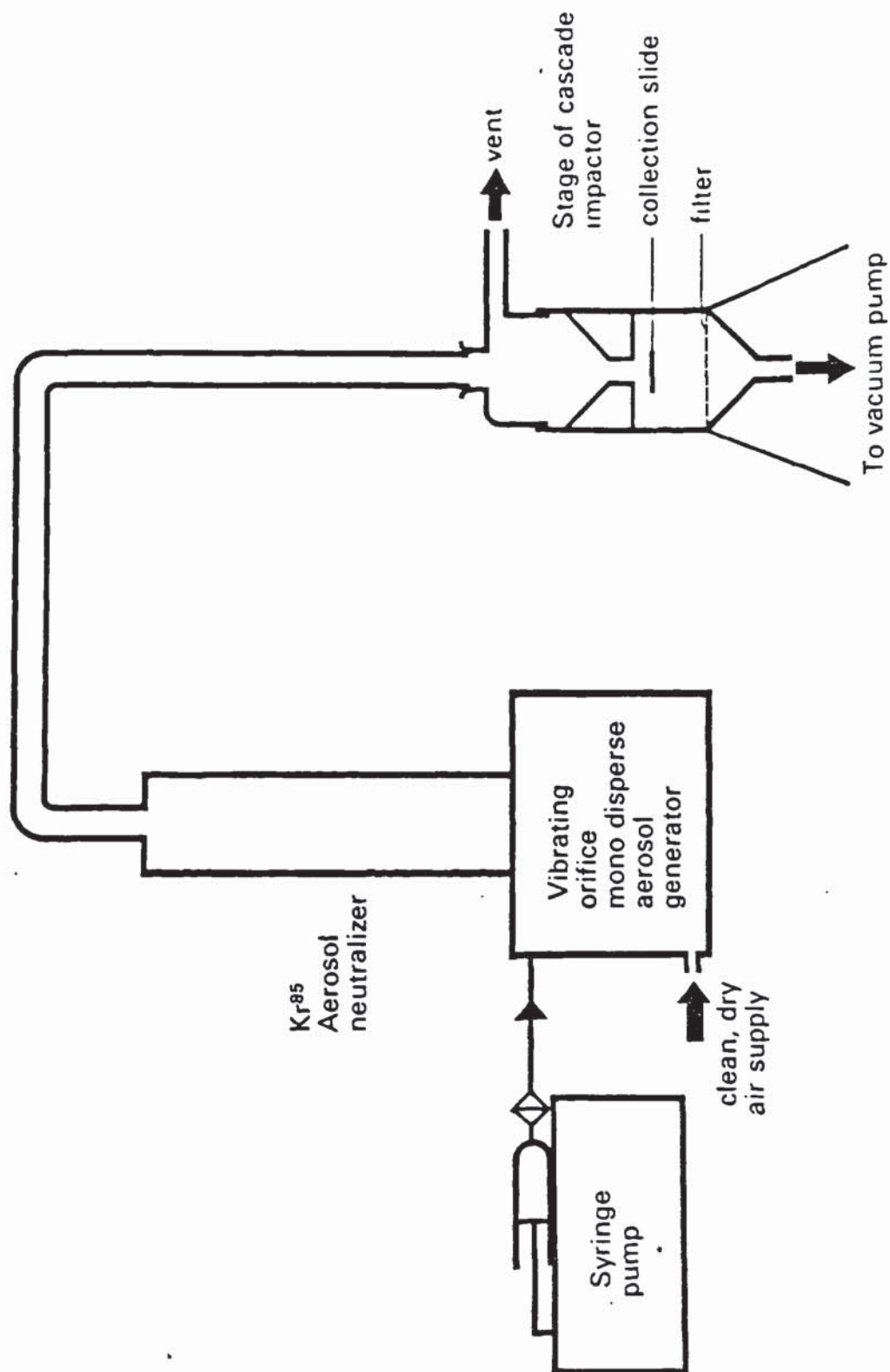
Figure 4.3 Scanning electromicrograph of fluorescein disodium particle generated using the vibrating orifice generator.
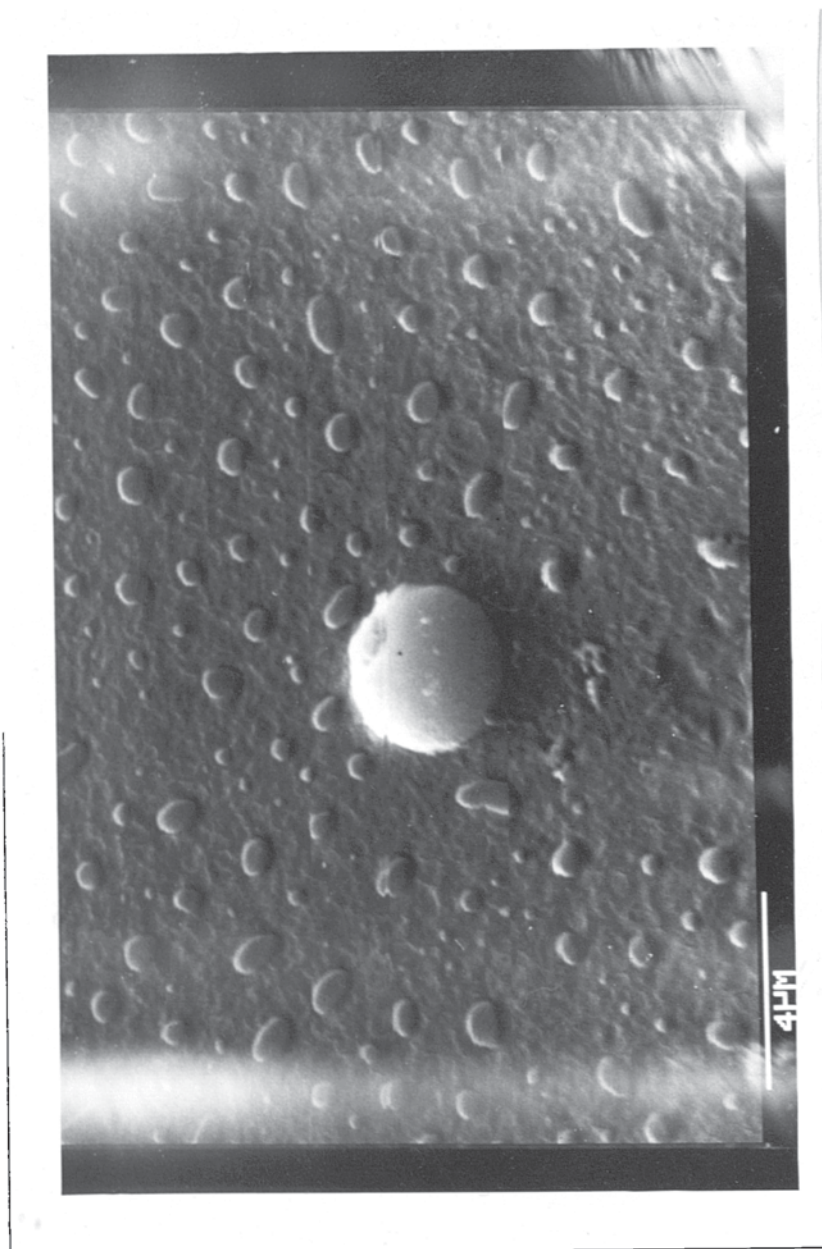
Figure 4.4 Effect of pH on absorbance of Fluorescein disodium at 492nm.
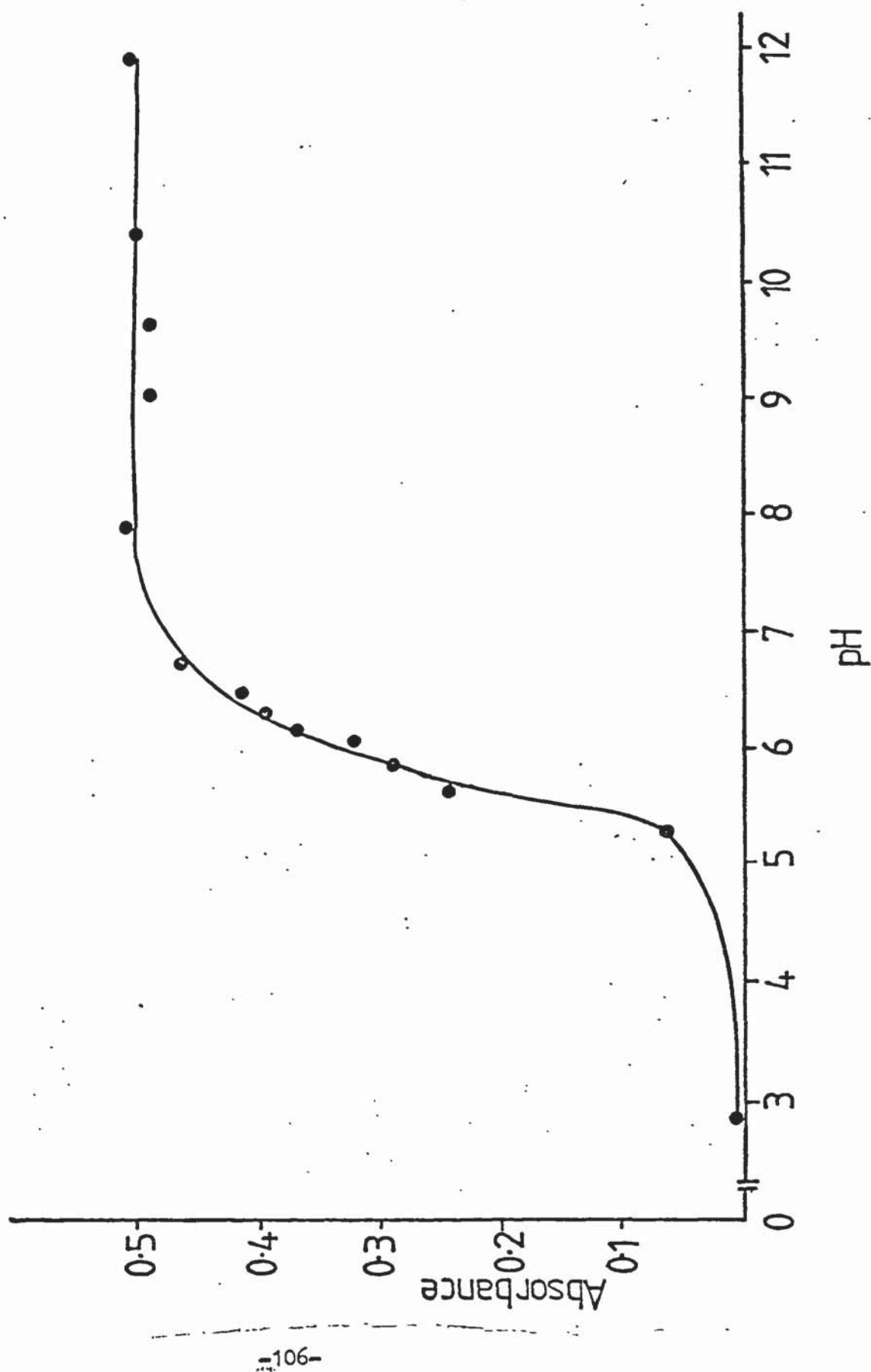
Figure 4.5 % Transmission of 5.5ng per ml solution of fluorescein= versus emission wavelength (excitation wavelength, 486nm).
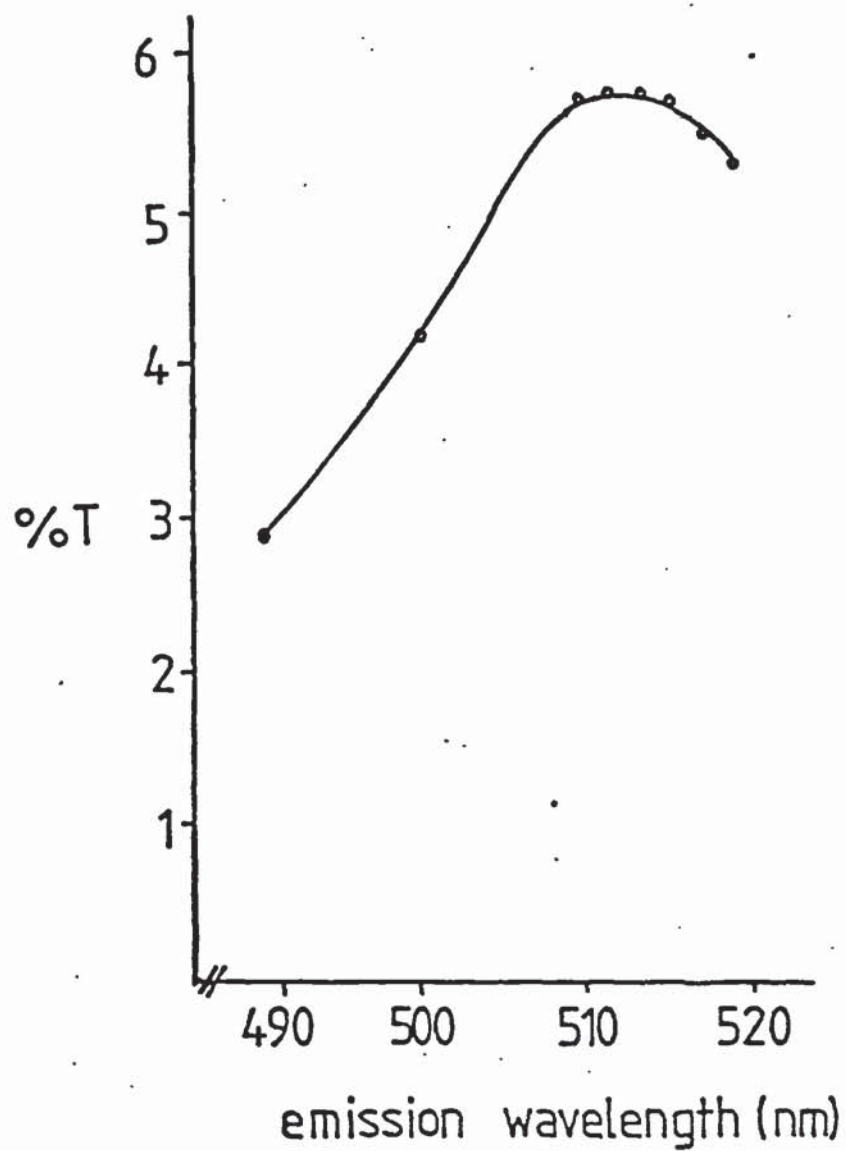
Figure 4.6 % Transmission versus excitation wavelength of 5.5ng per ml solution of fluorescein⁼ (emission wavelength 516nm).
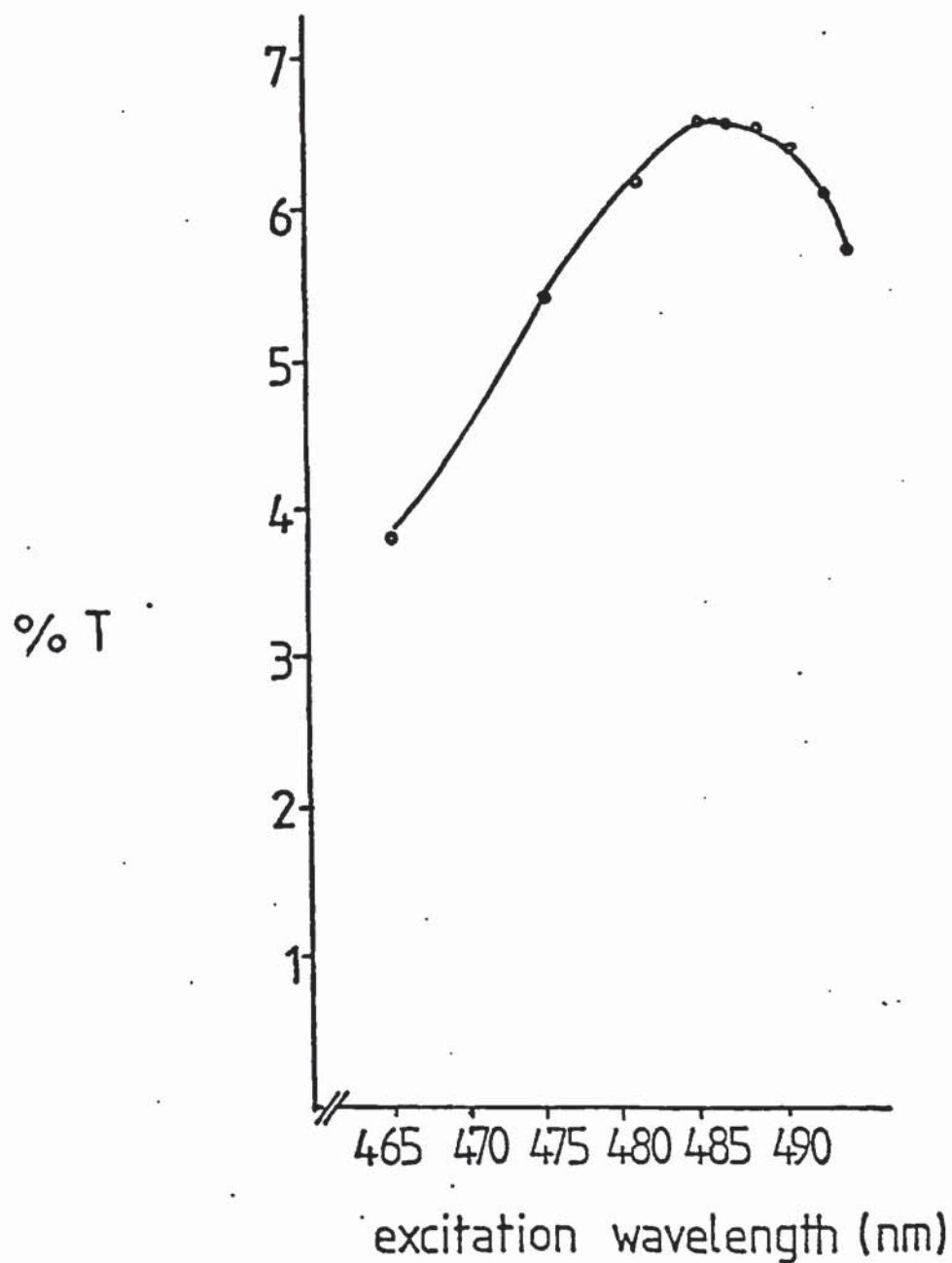
Figure 4.7 Relative Intensity (Equation 4.6) versus concentration of fluorescein= in buffer (pH 12). The solid line is the 'best fit' by linear regression. Each relative intensity was determined in quintriplicate.

Figure 4.8 Collection efficiency versus aerodynamic diameter for stage 1 of DCI5 cascade impactor.

The curve is the manufacturer' calibration

□ silicone fluid coated glass slide

✕ porous stainless steel slide

◀ white petroleum jelly coated glass slide

● clean glass slide

mean and standard deviation (n = 3)

Figure 4.9 Percentage collection efficiency (100 x E) versus aerodynamic diameter (Da) for stage 1 of DCI6 cascade impactor manufacturer's calibration (-.-), mean efficiency (_) and maximum and minimum contours (---) obtained by least mean squares fitting of the experimental data to Equation 4.7. The mean and standard deviation (n=3) of the experimental values are indicated

Figure 4.10 Percentage collection efficiency (100 x E) versus aerodynamic
diameter (Da) for stage 2 of DCI6 cascade impactor: Manufacturer's
calibration (-..-), mean efficiency (_) and maximum and minimum contours
(---) obtained by least mean squares fitting of experimental data to
Equation 4.7. The mean and standard deviation (n=3) of the experimental
values are indicated

Figure 4.11 Percentage collection efficiency (100 x E) versus aerodynamic diameter (Da) for Stage 3 of DCI6 cascade impactor: Manufacturer's calibration (-.-), mean efficiency (_) and maximum and minimum contours (---) obtained by least mean squares fitting of the experimental data to Equation 4.7. The mean and standard deviation (n=3) of the experimental values are indicated

Figure 4.12 Percentage collection efficiency (100x E) versus aerodynamic diameter (Da) for Stage 4 of DCI6 cascade impactor: Manufacturer's calibration (-.-), mean efficiency (_) and maximum and minimum contours (---) obtained by least mean square fitting of the experimental data to Equation 4.7. The mean and standard deviation (n=3) of the experimental values are indicated

Figure 4.13 Percentage collection efficiency (100 x E) versus aerodynamic diameter (Da) for Stage 5 of DCI6 cascade impactor: Manufacturer's calibration (-.-), mean efficiency (_) and maximum and minimum contours (---) obtained by least mean square fitting of the experimental data. The mean and standard deviation (n=3) of the experimental values are indicated

Figure 4.14 Percentage collection efficiency (100 x E) versus aerodynamic diameter (Da) for Stage 6 of DCI6 cascade impactor: Manufacturer's calibration (-.-), mean efficiency (_) and maximum and minimum contours (---) obtained by least mean square fitting of the experimental data to Equation 4.7. The mean and standard deviation (n=3) of the experimental values are indicated

Figure 4.15 Mean experimental collection efficiency versus square root of impaction parameter (Ranz and Wong, 1952) for the DCI-6 cascade impactor.

Chapter 5   THE EFFECT OF RELATIVE HUMIDITY ON THE PARTICLE SIZE OF
            INHALATION AEROSOLS

5.1   INTRODUCTION

        In Chapter 2 a technique for predicting the behaviour of

single water-soluble compounds in the high humidity of the

respiratory tract has been described. This technique is however

only applicable to aerosols generated as dry powders (for example

from powder insufflators) or as nebuliser solutions of drug and

water alone. However, nebuliser solutions may contain other

solvents such as alcohols or glycols and other formulating

agents such as buffer salts, antioxidants and preservatives.

For example Davis has examined water-propylene glycol (Davis, 1978a)

and water-propylene glycol-ethanol (Davis et al, 1978b) systems

as potential vehicles for nebuliser solutions of a test steroidal

compound of limited water solubility. The most widely used

aerosol generator in inhalation therapy is the 'pressurised pack'.

The drug may be dissolved in the liquified propellant, usually

with the addition of cosolvents or may be suspended as micronised

powder in the liquid propellant system. Surfactants are

frequently added to prevent drug agglomeration. Lubricants may

be included to improve powder flow through the valve. Desiccants

may be added to minimise moisture content. For complex nebuliser

solution systems and aerosols generated from pressurised packs

it is not possible to determine the behaviour of the aerosol

under high humidity conditions from the physicochemical

characteristics of the drug alone. In these instances direct

-118-

measurement of the change in the size distribution of the aerosol at high humidity is desirable.

In practise it is difficult to achieve the degree of temperature control required to maintain the relative humidity experienced within the respiratory tract. For example, in order to obtain the growth ratio of a single water-soluble compound with an accuracy of 5% at 99.5% relative humidity would require temperature control within $\pm$ 0.015° C at 37° C (Gonda et al, 1981). Correspondingly, studies on the effect of relative humidity on the particle size of aerosols are performed at sub-physiological humidities.

The behaviour of aerosols with varying humidity is pertinent to both the behaviour of aerosols in the respiratory tract and in cloud formation. A number of controlled humidity experiments are described in the literature. In his studies with atmospheric aerosols, Winkler (1974) controlled the humidity by lining a tube mounted prior to a cascade impactor with a filter paper wetted with a saturated salt solution. The equilibrium humidity above a saturated salt solution is constant (Kaye and Laby, 1973), however for the incoming aerosol to be exposed to a constant humidity, it is necessary for the flow rate to be sufficiently slow for diffusion of water vapour from the filter paper into the body of the aerosol cloud to occur. The filter paper reservoir should also contain sufficient water to accomodate this diffusion without drying the filter paper.

A number of commercial humidity cabinets are available, these are basically of two designs. In one the required humidity is

produced by a tray of saturated salt solution. Again sufficient
water must be available to humidify the cabinet without exhausting
the reservoir. Fans may be incorporated to induce good mixing
within the cabinet. This type of cabinet however, usually takes
some time to re-equilibrate if perturbed. In the second type
of cabinet the humidity is monitored by wet and dry bulb thermometers.
When the humidity falls below the desired level, water is sprayed
over the cabinet heaters to supply additional water vapour.
Because of the lag time before the wet-bulb thermometer re-
equilibrates, the humidity within this type of cabinet will tend
to fluctuate around the required humidity. The periodicity of
these fluctuations will tend to be longer than the time required
for the aerosol droplets to equilibrate.

In their studies on the behaviour of inorganic salt aerosols
Tang et al (1977a, 1977b, 1977c) used a controlled humidity
system whereby air saturated with water is mixed with dry air to
produce the required humidity. In their continuous flow system
only the humidity of the air which will be mixed with the aerosol
is controlled rather than that of the whole cabinet as in
commercial designs.

Dry aerosol particles of a single water-soluble compound
exposed to increasing humidity, will at a distinct humidity, the
deliquescence point, undergo an abrupt increase in size as a
saturated droplet is formed. If the droplet formed is then
exposed to a reduced humidity, a continuous reduction in size
takes place as a result of the droplet forming a supersaturated
solution. This hysteresis phenomenon is well documented for

inorganic salt aerosols (Orr, 1954; Tang, 1977b). It is important therefore to consider the direction of the humidity change below the deliquescence point of a single water-soluble compound. At high humidity the aerosol will undergo large change in size for small changes in humidity. This makes prediction of growth ratios at 99.5% relative humidity difficult from direct measurement of size changes at lower humidities.

Atmospheric aerosols, which generally consist of mixtures of inorganic water-soluble materials and insoluble materials by comparison, a continuous growth character by gradual deliquescence of water-soluble material (Winkler 1974). These aerosols still however display large size changes for small humidity changes at high humidity.

This chapter describes the development of a controlled temperature and humidity apparatus and its use in characterising the behaviour of a model inhalation aerosol, fluorescein disodium with varying humidity. For comparison the predicted growth ratio of fluorescein disodium with varying relative humidity could be calculated from physicochemical data using the techniques described in Chapter Two.

5.2   CONTROLLED TEMPERATURE AND HUMIDITY APPARATUS

5.2.1   Design considerations

The relative humidity over saturated salt solutions is
constant and relatively insensitive to temperature (Robinson
and Stokes, 1959a). The passage of air through saturated salt
solutions at the same temperature therefore provides a relatively
simple method of generating an airstream of known moisture
content. The limits to the temperature control in order to
reduce errors in the growth ratio Dd/Do to less than 5% were
calculated as follows:

Consider the passage of air through a bubble humidifier
containing saturated potassium sulphate solution at $(37.00 \pm
0.05)^0$ C into an equilibrium chamber maintained at $(37.00 \pm
0.05)^0$ C. The maximum lower deviation in relative humidity
from 96% (the equilibrium humidity over saturated potassium
sulphate) solutions will correspond to the humidifier maintained
at the lower limit of the temperature range, that is 36.95^0 C
and the equilibrium chamber at the upper limit of the temperature
range, 37.05^0 C. The saturated water vapour at 36.95^0 C is
46.96 Torr (Handbook of Chemistry and Physics, 1978),
correspondingly the water vapour pressure of air after its
passage through the bubble humidifier will be 45.96 Torr. At
37.05^0 C the saturated water vapour pressure is 47.20 Torr.
The relative humidity in the equilibrium chamber will therefore
be $((45.96/47.20) \times 100)$ % = 95.5%. The upper limit to relative
humidity will correspond to the opposite temperature limits
resulting in a humidity of 96.5%.

Assuming ideal solution behaviour and negligible surface tension effects the growth ratio Dd/Do may be calculated from Equation 1.12. For a solute of molecular weight of 400 Daltons forming 3 ions in solution and with the density of the dry particle equal to that of the equilibrium droplet, the growth ratio, Dd/Do at 96% relative humidity will be 1.619, at 95.5% relative humidity the growth ratio will be 1.569 and at 96.5% relative humidity the growth ratio will be 1.677. Thus with temperature control of $\pm$ 0.05° C at 37.00° C the growth ratio can theoretically be determined with less than $\pm$ 3% error at 96% relative humidity.

The humidity bottles were placed in a water bath whose temperature was controlled by a proportional temperature controller (YSI Model 72, Cole Palmer Instruments) using a 1 Kilowatt immersion heater and a pyrex tubular glass thermistor probe (YSI Model 404, Cole Palmer Instruments). The equilibrium chamber was heated by pumping water from a reservoir, through a water jacket. The temperature of the reservoir was maintained using a second proportional temperature controller (YSI Model 72, Cole Palmer Instruments) with a stainless steel thermistor probe (YSI 410, Cole Palmer Instruments). The required temperature is controlled on these instruments by setting dials. The temperature difference required to give 100% power to the heater is controlled by a bandwidth setting. This must be turned to take into account the lag time of the system being controlled. If the bandwidth is too narrow the controller will switch fully on when the detected temperature is below the required temperature then switch fully off when the detected temperature is equal

to the required temperature. However, because of the lag time
of the system the water temperature would not have finished
rising. In this instance regular large cyclic fluctuations
in temperature may occur. Figure 5.1 shows the temperature
fluctuations observed in the growth chamber reservoir when a
bandwidth of 0.5^0 C was set on the temperature controller. By
trial and error the bandwidth giving minimal temperature
fluctuations was found to be 1.5^0 C. The temperature control
dials of the proportional temperature controllers were marked
in degrees centigrade and the markings were within 0.5^0 C of
the controlled temperature. For the controller used for the
humidifier water bath the dial setting corresponding to 37^0 C
was found to be 37.27^0 C and for the controller used for the
growth chamber reservoir it was found to be 37.31^0 C.

The water bath, reservoir and growth chamber were placed
in a box whose walls were insulated with $^1/_4$ '' thick expanded
polystyrene sheeting. Using a proportional controller built
by Mr. D. Briggs of the Pharmacy Department of the University
of Aston in Birmingham (see Appendix 3) connected to a 375
watt fan heater (Calor, model 92-01) and a bead thermistor,
the temperature of the outer box was maintained at $(36.5 \pm$
$0.1)^0$ C. Circulation within the box was assisted by the use
of a second fan (Pifco, Spinair) in the upper part of the box.

The humidifier water bath and growth chamber reservoir
were insulated using expanded polystyrene sheeting. All
connecting air pipes and water pipes were insulated to minimise
heat loss. Using the above experimental conditions the water
bath and reservoir temperature could be controlled at $(37.00 \pm$

0.05$)^0$ C. Initially air was bubbled directly from the inlet

line through the bubble humidifiers but measurement of the air

temperature at the outlet revealed that using this procedure

the air temperature was still some 0.5^0 C below that of the water

bath. This problem was overcome by passing the air through

a glass coil situated in the humidifier water bath before its

passage through the bubble humidifiers. This procedure brought

the air temperature to within 0.1^0 C of the water bath temperature

before its passage through the bubble humidifiers. The measured

air temperature at the outlet was found to be $(37.00 \pm 0.05)^0$ C

using the improved procedure.

5.2.2   Final Design

A schematic diagram of the controlled temperature and

humidity apparatus is shown in Figure 5.2. The equilibrium

chamber with an internal volume of approximately 3 litres was

constructed from glass. At a flow rate of 12.5 litres per minute

this provided a residence time of 15 seconds. The aerosol

inlet into the equilibrium chamber was arranged tangentially

to encourage mixing of the aerosol and humidified air. Both

the equilibrium chamber and aerosol inlet were surrounded by

water jackets and insulated with polystyrene sheeting. Water

from the temperature controlled reservoir was passed through the

water jackets of the equilibrium chamber and aerosol inlet using

a water pump (Charles Austin, model CP25P).

Filtered compressed air was preheated by passing it through

a glass coil in a water bath maintained at $(37.00 \pm 0.05)^0$ C.

The preheated air was then passed through bubble humidifiers containing saturated salt solutions. The humidified air was passed through a glass sinter filter to remove any particulate matter or droplets before its passage into the equilibrium chamber.

5.3   EXPERIMENTAL

The behaviour of aerosol of single-water soluble compounds in the high humidity of the respiratory tract can be predicted from the physicochemical properties of solutions of the compound using the methods described in Chapter 2. In order to validate the use of the controlled temperature and humidity apparatus for more complex aerosols the behaviour of a single water-soluble model inhalation aerosol was investigated and compared to calculations based on its measured physicochemical properties.

5.3.1   Prediction of equilibrium growth of fluorescein disodium aerosols from bulk solution data

The water activity of aqueous fluorescein disodium solutions of concentrations approaching that of the saturated solution at 37^0 C, were determined using the vapour pressure osmometer as described in section 2.2.1.

Density measurements at 37^0 C were also made on these solutions using the techniques described in Chapter 2.

The density of dry fluorescein disodium powder was determined by air comparison pycnometry.

The relative humidity over a saturated fluorescein disodium solution at 37^0 C was determined using a dew-point hygrometer (E, G and G, model 911) at the output from bubble humidifiers containing the saturated solution.

5.3.2   Nebuliser characteristics

A compressed air nebuliser (Bird, Mark 7, BOC Medical
Supplies) was used to generate the experimental aerosols in this
study. The mass flow rate of air through compressed air
nebulisers is a function of the air pressure before the orifice
(Mercer, 1968). A schematic diagram of the experimental set-
up used to determine flow-rate versus gauge pressure of the
inlet line is shown in Figure 5.3. The gauge pressure of the
inlet line was controlled by a needle valve (G A Planton Ltd)
and measured using a mercury manometer. The flow rate was
measured using a flow meter (G A P meter, G A Planton). Control
of the operating pressure is important as there is some evidence
that the size distribution of the aerosol produced by compressed
air nebulisers is a function of operating pressure (Postendorfer,
1977). The total output from the nebuliser was determined by
measuring the weight loss of the nebuliser filled with a 0.032
molal aqueous fluorescein disodium solution. The total output
of the nebuliser will comprise of aerosol droplets escaping
from the nebuliser and solvent vapour evaporating from the bulk
solution which includes, previously impacted droplets that spread
as a thin layer of solution over the inner surface of the
nebuliser as they drain back into the reservoir. A loss of
water vapour would produce an increase in concentration of the
solution in the nebuliser. If an increase in concentration
occurs it is possible to determine aerosol output and solvent
vapour output by mass balance (Davis, 1978a). Correspondingly
the concentration of the remaining solution was determined after

each experiment.

In order to determine the original droplet size distribution from the nebuliser, a 0.191 Molal aqueous fluorescein disodium solution was nebulised at an inlet gauge pressure of 300mm of mercury. The aerosol was mixed with dry, filtered air to facilitate solvent evaporation and the size of the residual fluorescein disodium particles determined using a DCI-6 cascade impactor. The amount of fluorescein landing on each silicone fluid coated slide was determined by fluorimetry of the aqueous extract (see section 4.2.2.5). The cascade impactor is calibrated in terms of aerodynamic diameter, Da. Assuming that the residual dry particles are spherical, this is related to the geometric diameter of the residual dry particle, Dp by (TASK 1966)

$$C(Da).do.Da^2 = C(Dp).dp.Dp^2 \qquad \text{Equation 5.1}$$

where $C(D)$ is the Cunningham slip factor for a particle with diameter, D, and dp the density of the dry particle. The mass of the dry particle in terms of particle diameter and density, assuming the particle to be spherical will be

$$mp = \frac{4}{3} \pi \left(\frac{Dp}{2}\right)^3 . dp \qquad \text{Equation 5.2}$$

The concentration of fluorescein disodium in the nebuliser solution was equivalent to 1 gram of fluorescein disodium for each 14.9 grams of solution. The mass of the droplet, md, from

which the residual dry particle originated will therefore be

$$md = \frac{4}{3} \pi \left(\frac{Dp}{2}\right)^3 dp \cdot 14.9 \text{ (grams)} \qquad \text{Equation 5.3}$$

and may also be described in terms of the droplet diameter Dd, and density, dd, by

$$mf = \frac{4}{3} \pi \left(\frac{Dd}{2}\right)^3 dd \qquad \text{Equation 5.4}$$

Equating Equations 5.3 and 5.4, the ratio of the diameters of the original droplet and residual dry particle may be described by

$$\frac{Dd}{Dp} = \sqrt[3]{\frac{dp}{dd} \cdot 14.9} \qquad \text{Equation 5.5}$$

The size distribution of the original droplet output may therefore be calculated from the size distribution of the residual dry particles.

5.3.3   Fluorescein disodium aerosol size distributions with varying Relative Humidity

The controlled temperature and humidity apparatus described in section 5.2 was set to a temperature of $(37.00 \pm 0.05)^{\circ}$C. The temperatures in the growth chamber and water baths were monitored using precision thermistors (YSI 44031, Sasco). The thermistor used to measure the water bath temperature was

embedded in a protective araldite coat. When the chamber was at the required temperature filtered air was passed at 12.5 litres per minute through the bubble-humidifiers containing the required saturated salt solution. The humidity within the growth chamber was monitored using a dew-point hygrometer (E, G and G, model 911). When the humidity had established itself at the required level the pump to the cascade impactor was switched on to fill the impactor with humidified air. Before passing the aerosol into the chamber, the pump drawing air into the dew-point hygrometer was switched off to prevent any aerosol particles passing into the sensing chamber, which could result in erroneous readings.

There is some evidence that the size distribution of the aerosol produced by compressed air nebulisers is a function of operating pressure (Postendorfer, 1977). The nebuliser output was vented initially to prevent sampling of atypically sized aerosols arising from pressure surges. A nebuliser solution containing 0.032 molal fluorescein disodium was employed. The flow rate through the bubble humidifiers was reduced to 8 litres per minute and sample of the nebuliser output passed into the equilibrium chamber for ten seconds. The flow rate to the bubble humidifiers was then returned to 12.5 litres per minute. After two minutes collection to ensure capture of the aerosol, the pump to the cascade impactor was switched off. The quantity of fluorescein disodium landing on each slide was determined by fluorimetry of the aqueous extract (see section 4.2.2.5). After suitable dilution, the fluorescence of the residual nebuliser

solution was measured. For each humidity level the experiment
was repeated three times. The saturated salt solutions used
and the relative humidity over them at equilibrium are given in
Table 5.1.

5.3.3.1 Humidity levels during aerosol sizing experiments

The introduction of the aerosol in its carrier gas into
the equilibrium chamber will necessarily perturb the established
humidity system. The aerosol is only introduced into the
equilibrium chamber in a short burst (10 seconds) to avoid
overloading of the cascade impactor. This is insufficient time
for the dew-point hygrometer to detect and register the humidity.

In order to determine the exposure humidity the following
series of experiments were performed. The nebuliser output
at 4.5 litres per minute was mixed in the equilibrium chamber
with humidified air from the bubble-humidifiers at a flow rate of
8 litres per minute, for 15 minutes. The resulting humidity
was measured using a dew-point hygrometer (E, G and G, model 911).
In order to prevent aerosol particles entering the sensing
chamber the end of the inlet tubing to the dew-point hygrometer
was plugged with glass fibre wool. The experiment was repeated
with each of the saturated solutions used in section 5.3.3.

5.4   RESULTS AND CALCULATIONS

5.4.1   Predicted Growth Ratios for fluorescein disodium aerosols with varying relative humidity.

It has been shown in section 2.4 that the magnitude of the Kelvin effect relative to aerodynamic diameter is only significant for very small particles. In the calculation of growth ratios of fluorescein disodium aerosols with varying relative humidity the Kelvin effect has been neglected. The growth ratio at the required relative humidity may be calculated from

$$\frac{Dd}{Do} = \sqrt[3]{\frac{do}{dd} \left(1 + \frac{1000}{Ws\ M} \right)} \qquad \text{Equation 5.6}$$

where M is the concentration of fluorescein disodium (in moles per kg of water) which has a water activity equal to the fractional humidity under consideration. The derivation of Equation 5.6 is analogous to Equation 1.15. As the data from the cascade impactor sizing studies are in terms of aerodynamic diameter, it is useful to express the growth ratio in terms of aerodynamic diameter. By neglecting the Kelvin effect, the growth ratios obtained refer to the limit of large spherical particles, it is reasonable therefore to neglect the Cunningham slip factor in the determination of aerodynamic diameters. The aerodynamic growth ratio, $(^{Dd}/Do)a$, may be calculated from

$$\left(\frac{Dd}{Do} \right)_a = \sqrt{\frac{dd}{do}} \cdot \left(\frac{Dd}{Do} \right) \qquad \text{Equation 5.7}$$

Figure 5.4 illustrates the water activity of aqueous
fluorescein disodium solutions as a function of concentration.
The mean water activity of at least three determinations is
given in the figure. The individual determinations were within
0.003 of the mean value on all points.

The humidity over saturated fluorescein disodium solutions
at $(37.00 \pm 0.05)^0$ C was found to be $(53.7 \pm 0.2)\%$. If a particle
of dry fluorescein disodium were exposed to an increasing
humidity, a sudden increase in diameter would therefore be
expected at $(53.7 \pm 0.2)\%$ relative humidity as the saturated
solution was formed.

The density of aqueous fluorescein disodium solutions at
37^0 C is shown in Figure 5.5. Each determination was made in
triplicate, individual determinations were within 0.0005g cm^{-3}
of the mean value on all points. The density of dry fluorescein
powder was found to be (1.490 ± 0.001)g cm $^{-3}$ by air comparison
pycnometry.

From these experimental data it was possible to calculate
the growth ratios for fluorescein disodium aerosols with varying
humidity using Equations 5.6 and 5.7. The calculated aero-
dynamic growth ratios versus increasing humidity are illustrated
in Figure 5.6.

5.4.2   Nebuliser Characteristics

Figure 5.7 illustrates the air flow rate versus inlet
gauge pressure and Figure 5.8 the total nebuliser output

versus inlet gauge pressure for the nebuliser (Bird, Mark 7, BOC Medical Supplies) used in these studies. At an inlet gauge pressure of 300 mm of mercury, the value used in the subsequent aerosol sizing experiments, the air flow rate was 4.5 litres per minute. Measurement of the concentration of the residual nebuliser solution after fifteen minutes continuous operation showed no detectable deviation from the initial concentration, indicating that the total output in this instance could be taken as aerosol output. However, it was observed that the nebuliser cooled during operation indicating that some evaporation was taking place, this was insufficient to be detected by the assay technique employed here.

The size distribution of the residual dry aerosol from a 0.191 Molal fluorescein disodium solution nebulised at 300mm of mercury gauge pressure was found by analysis of the fractional deposition pattern on the stages of the DCI-6 cascade impactor using the programme described in section 4.4. The size distribution could be satisfactorily described by a log-normal distribution with a mass median aerodynamic diameter of (3.02 ± 0.02) μm with a geometric standard deviation of (1.38 ± 0.06). Correspondingly, the size distribution of the dry particles in terms of geometric diameter would be log-normal with a mass median diameter of (2.48 ± 0.02),m. The geometric mass median diameter of the original droplets calculated from Equation 5.5 was (6.96 ± 0.05)μm with a geometric standard deviation of (1.38 ± 0.06).

5.4.3   Fluorescein disodium aerosol size distributions with varying relative humidity

In these experiments the concentration of fluorescein disodium used in the nebuliser was 0.0325 Molal. The ratio of the residual dry particle to original droplet size will therefore be

$$\frac{Dd}{Dp} = \sqrt[3]{\frac{1.49}{1} \quad 82.877} = 4.98 \quad \text{Equation 5.8}$$

The original droplet particle size distribution for an aqueous 0.0325 molal nebuliser solution from the Bird (Mark 7) nebuliser operating at a gauge pressure of 300mm of mercury was assumed to be log-normally distributed with a mass median geometric diameter of $(6.96 \pm 0.05)\mu m$ with a geometric standard deviation of (1.38 ± 0.06) based on the results from a 0.191 molal nebuliser solution. The residual dry particle from a 0.0325 molal nebuliser solution will have a calculated mass median geometric diameter of $(1.40 \pm 0.01)\mu m$ with a corresponding mass median aerodynamic diameter of $(1.71 \pm 0.01)\mu m$.

The particle size distributions of the fluorescein disodium aerosols exposed to varying relative humidity were also found to be satisfactorily described by log-normal distributions by analysis of the fractional deposition on the stages of the DCI-6 cascade impactor using the programme described in section 4.4. The determined mass median aerodynamic diameters and geometric standard deviations are given in Table 5.2. Included in Table 5.2 are the aerodynamic growth

ratios with reference to the average mass median aerodynamic diameter of the residual dry particle of 1.71μm.

The average measured resultant humidity level and the range from the mixing experiment (see section 5.3.3.1) are given in Table 5.3. These values give an indication of the exposure humidity within the main body of the aerosol cloud during the sizing experiments. The concentration of water from the nebuliser at an operating pressure of 300mm of mercury and a nebuliser solution of 0.0321 molal fluorescein disodium is 0.0286g per litre. This exceeds the concentration of water from the bubble humidifiers when they contain sodium hydroxide (0.00308g per litre) and potassium carbonate (0.01890g per litre) so the resultant humidity is higher than the equilibrium humidity over the saturated solution in the bubble humidifier. For the remaining saturated salt solution used, the concentration of water from the nebuliser humidifier will be less than the concentration of water in the airstream from the bubble humidifiers. The resultant humidity of the mixed airstream is therefore less than the equilibrium humidity over the saturated solutions in this instance. However, the aerosol cloud is preceded by and followed by air at the equilibrium humidity over the saturated salt solution in the bubble humidifier. The humidity at the periphery of the aerosol cloud will therefore tend to these values.

Figure 5.9 show the mean and standard deviation of the experimentally determined aerodynamic growth ratios for fluorescein disodium aerosols with varying humidity. The

growth ratios are plotted against the average humidity levels
obtaining in the subsequent mixing experiments (see Table 5.3).
The horizontal bars illustrate the range of humidity experienced
by the aerosol cloud. For comparison the calculated aerodynamic
growth ratios for fluorescein disodium aerosols based upon
physicochemical characteristics of aqueous solutions is included
as the solid line in Figure 5.9. Above the deliquescence
point, $(53.7 \pm 0.2)\%$ relative humidity, there is good correlation
between the calculated and experimentally determined aerodynamic
growth ratios. The experimental aerosols were generated from
a 0.0325 molal solution which had a water activity of
approximately 0.998. The aerosols will therefore be decreasing
in size at each of the experimental humidities. The results
at humidities below the deliquescence point indicate that there
is a hysteresis as a supersaturated solution is formed. This
phenomenon has been previously described for inorganic electrolyte
aerosols (Orr, 1954; Tang, 1977).

5.5   DISCUSSION

The design of a controlled temperature and humidity
cabinet has been described. The major problem in the use of the
apparatus arises from the addition of carrier gas bringing
aerosol into the equilibrium chamber perturbing the previous
established humidity. In the experiments described here the
problem was further complicated by the need to limit the flow
from the nebuliser to a 10 second burst to avoid overloading of
the cascade impactor. The experimental aerosol cloud was
therefore preceded and followed by air at the humidity above the
saturated salt solutions used in the bubble humidifiers. The
results have therefore been interpreted taking into account
the extremes of humidity the aerosol cloud would have
experienced. The results indicate good correlation between
the predicted and observed growth ratios above the deliquescence
point. Below the deliquescence point the results indicate a
hysteresis with reducing relative humidity as a supersaturated
solution is formed. This phenomenon has been previously
described with inorganic aerosols (Orr, 1954; Tang, 1977).

A more constant humidity could be achieved by the use
of a continuous mixing system. The problem of overloading
the cascade impactor slides could be overcome by either reducing
the output from the nebuliser or by introducing a by-pass
device between the equilibrium chamber and the cascade impactor.

Reduction in nebuliser output could be achieved by
decreasing the inlet gauge pressure. The inlet gauge pressure
used in the experiment described in this chapter was 300mm of

mercury. This corresponded to a total output from the nebuliser of 0.029 grams per litre per minute, allowing only a ten second input into the equilibrium chamber if overloading of the cascade impactor was avoided. From examination of Figure 5.8 if the inlet gauge pressure were reduced to 50mm of mercury, the total output would only be reduced to 0.01 grams per litre per minute, allowing a maximum input time of only thirty seconds.

An alternative method of reducing the effective nebuliser output would be to allow only part of the total output to pass into the equilibrium chamber. This could be achieved by the use of a small bore sampling tube at the nebuliser outlet with the remainder of the aerosol passing to waste. The sample of aerosol passing into the equilibrium chamber need not necessarily be representative of the nebuliser output providing that the size fraction taken was constant between experiments. Problems could however occur in data interpretation, if the sampling device for example excluded particles above a certain size then it may be difficult to transform the fraction collected on each stage of the cascade impactor into a clearly defined size distribution as the original log-normal distribution, for example, would be truncated. Similarly, if a by-pass device was used between the equilibrium chamber and cascade impactor, data interpretation would be easier if the sample collected by the cascade impactor were representative of the whole size distribution.

The reduction in effective nebuliser output would have the advantage of enabling higher humidity levels within the equilibrium chamber to be achieved as the carrier gas input would also be reduced.

5.6   TABLES

5.6.1   Table Captions

Table 5.1 Humidifier solutions and the relative humidity above their saturated solutions at 37° C.

Table 5.2 Particle size distributions of fluorescein disodium aerosols exposed to varying humidity.

Table 5.3 Measured resultant humidity levels from mixing experiment (section 5.4.3.1)

Table 5.1 Humidifier solutions and the Relative Humidity above their saturated solutions at 37° C

Salt	Humidity over saturated solution (Robinson and Stokes, 1959)
NaOH	7.03
K_2CO_3	42.76
K Br	80.71
K NO_3	92.48
$K_2Cr_2O_7$	98.0

Table 5.2 Particle size distributions of fluorescein disodium aerosols exposed to varying humidity

Humidity		Mass median aerodynamic diameter	Geometric standard deviation	Aerodynamic growth ratio
	Range			
15.5	7.03–15.9	1.85	1.80	1.08
		2.05	1.42	1.20
		1.975	1.52	1.15
49.7	42.76–50.0	1.95	1.58	1.14
		1.98	1.50	1.15
		1.96	1.48	1.16
72.3	71.8–80.71	2.22	1.37	1.30
		2.13	1.62	1.25
		1.95	1.76	1.14
81.7	81.0–92.48	2.08	1.62	1.22
		2.26	1.42	1.32
		2.18	1.62	1.27
92.3	91.3–98.0	2.58	1.34	1.51
		2.40	1.32	1.40
		2.17	1.37	1.27

Table 5.3 Measured resultant humidity levels from mixing experiment
           (section 5.4.3.1)

Bubble-humidifier saturated solution	Measured humidity (% relative humidity)	
	Average	Range
NaOH	15.5	15.2-15.9
$K_2 CO_3$	49.7	49.5-50.0
K Br	72.3	71.8-72.8
KNO_3	81.7	81.1-82.4
$K_2 Cr_2 O_7$	92.3	91.4-93.3

5.7   FIGURES

5.7.1   Figure Captions

Figure 5.1 Temperature fluctuations in the equilibrium chamber reservoir using a bandwidth of 0.5^0 C on the proportional temperature controller.

Figure 5.2 Schematic diagram of the controlled temperature and humidity apparatus.

Figure 5.3 Determination of flow rate through the Bird nebuliser versus inlet gauge pressure: schematic diagram of apparatus.

Figure 5.4 Water activity of aqueous fluorescein disodium solution versus concentration.

Figure 5.5 Density of aqueous fluorescein disodium solutions at 37^0 C.

Figure 5.6 Calculated aerodynamic growth ratio versus relative humidity for fluorescein disodium.

Figure 5.7 Bird nebuliser operating characteristics: air flow rate versus inlet gauge pressure. Single determinations are shown.

Figure 5.8 Bird nebuliser operating characteristics: total output versus inlet gauge pressure.

Figure 5.9 Aerodynamic growth ratio versus relative humidity of fluorescein disodium aerosols.

Figure 5.1 Temperature fluctuations in the equilibrium chamber reservoir using a bandwidth of 0.5°C on the proportional temperature controller.

Figure 5.2 Schematic diagram of the controlled temperature and humidity apparatus.

Figure 5.3 Determination of flow rate through the Bird nebuliser versus inlet gauge pressure: Schematic diagram of apparatus.

flowmeter

needle valve

compressed
air inlet

Bird
nebulizer

mercury
manometer
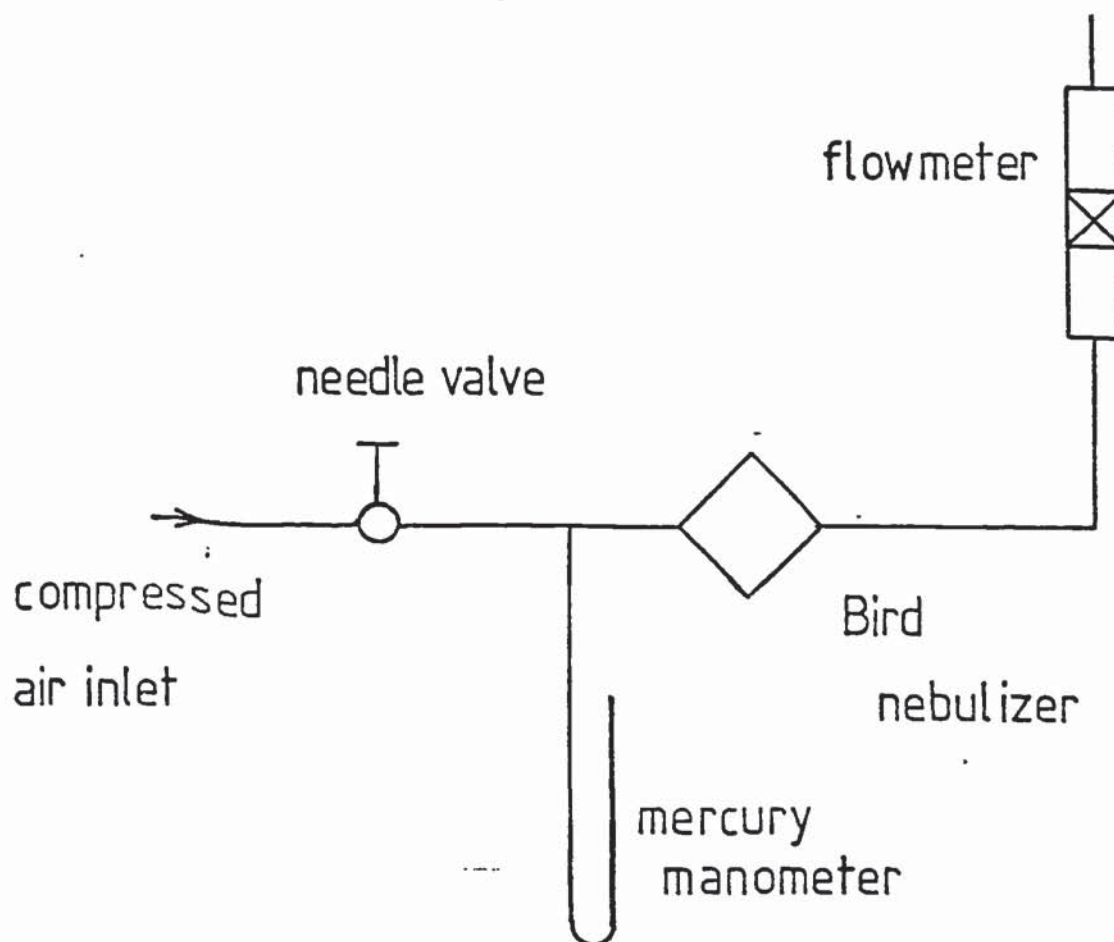
Figure 5.4 Water activity of aqueous fluorescein disodium solution versus concentration. The mean of at least three determinations is illustrated. Individual determinations were within 0.003 of the mean value on all points.
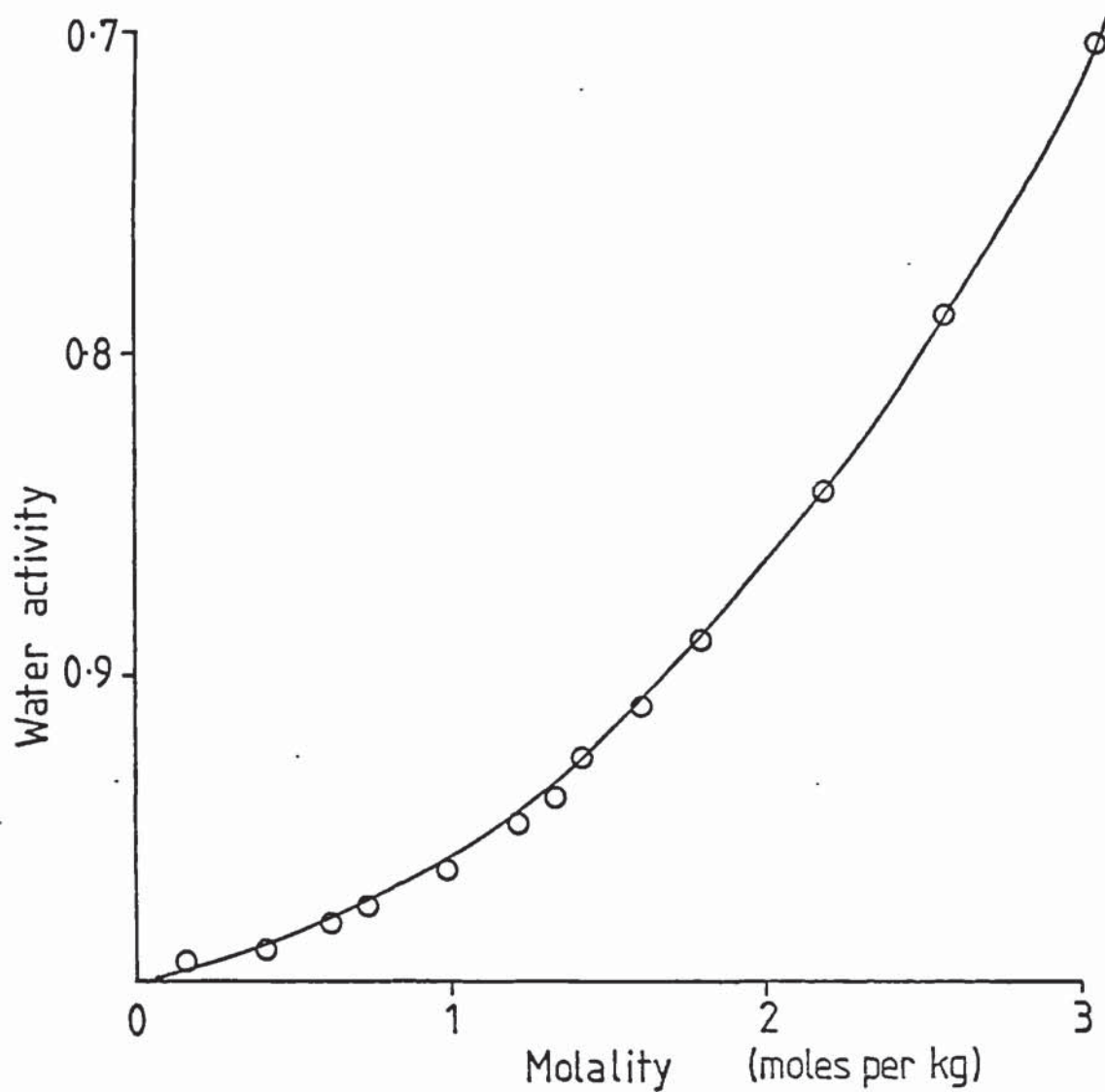
Figure 5.5 Density of aqueous fluorescein disodium solutions at 37° C
The mean value of three determinations is illustrated. Individual
determinations were within 0.0005g cm-^{3} on all points.
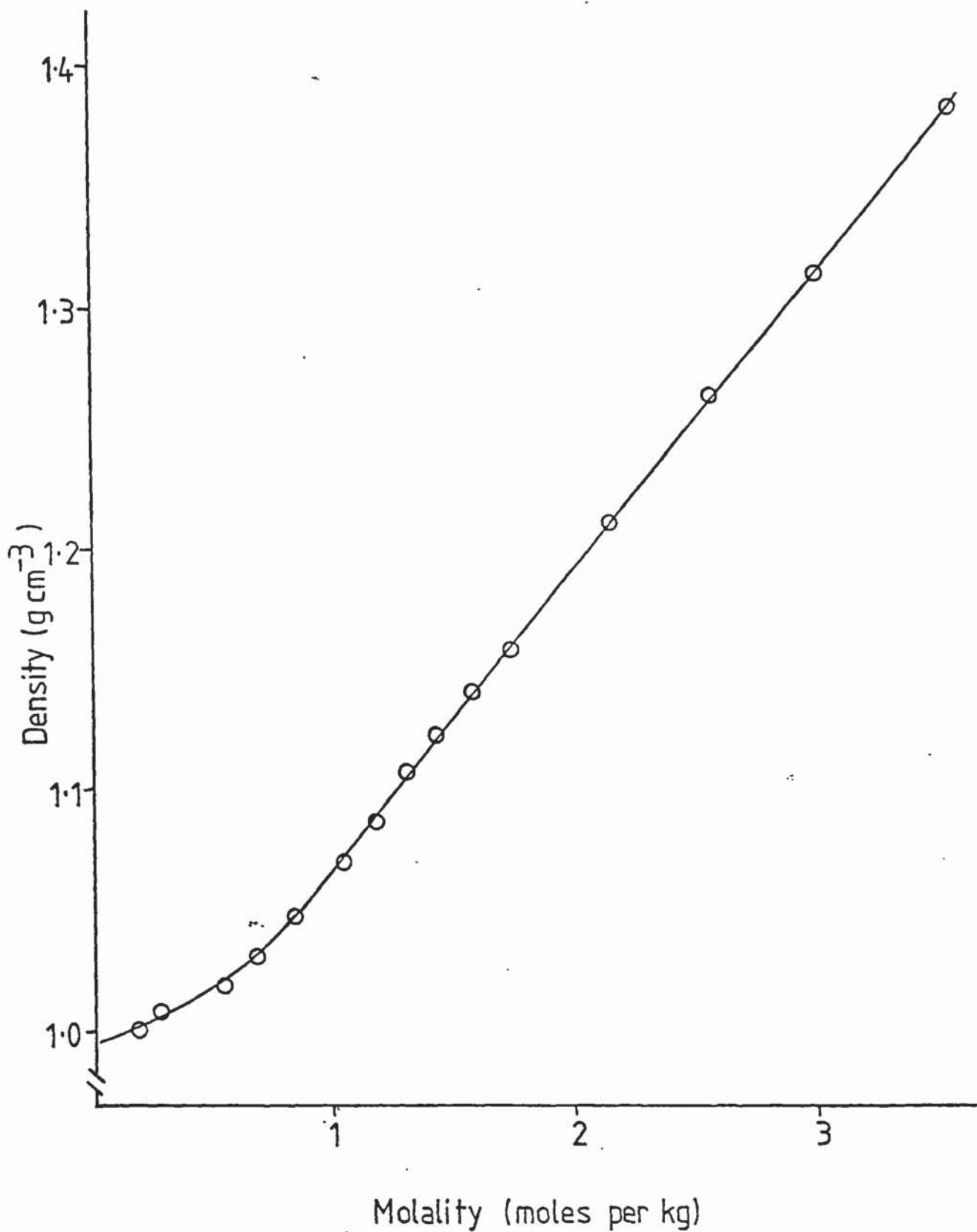
Figure 5.6 Calculated aerodynamic growth ratio versus relative humidity for fluorescein disodium.
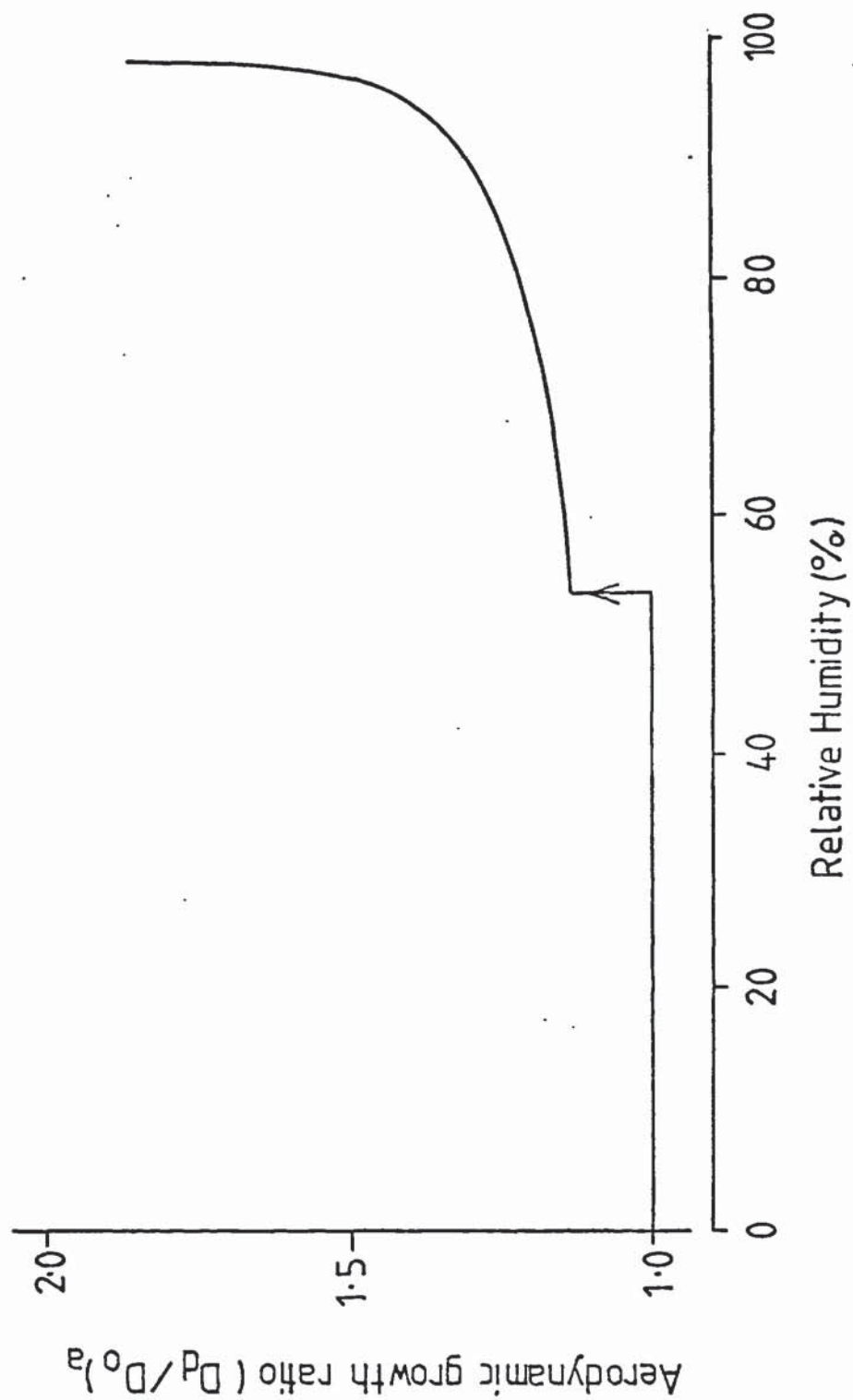
Figure 5.7 Bird nebuliser operating characteristics: air flow rate versus inlet gauge pressure. Single determinations are shown.
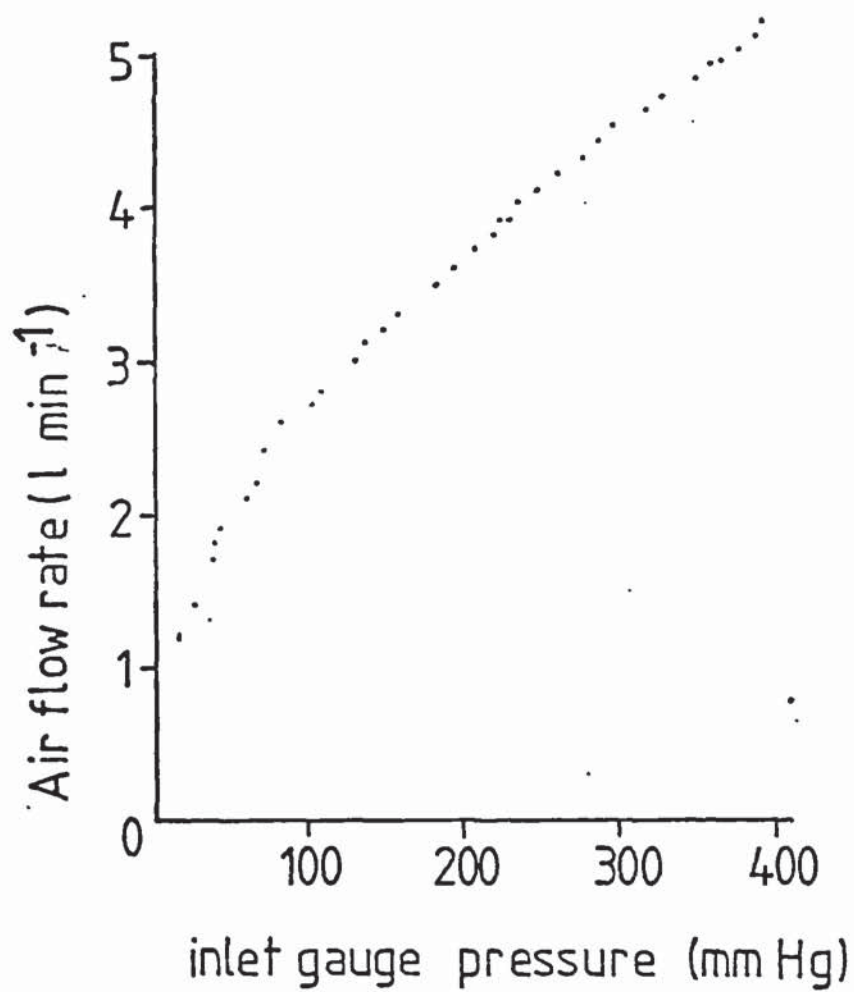
Figure 5.8 Bird nebuliser operating characteristics: total output versus inlet gauge pressure
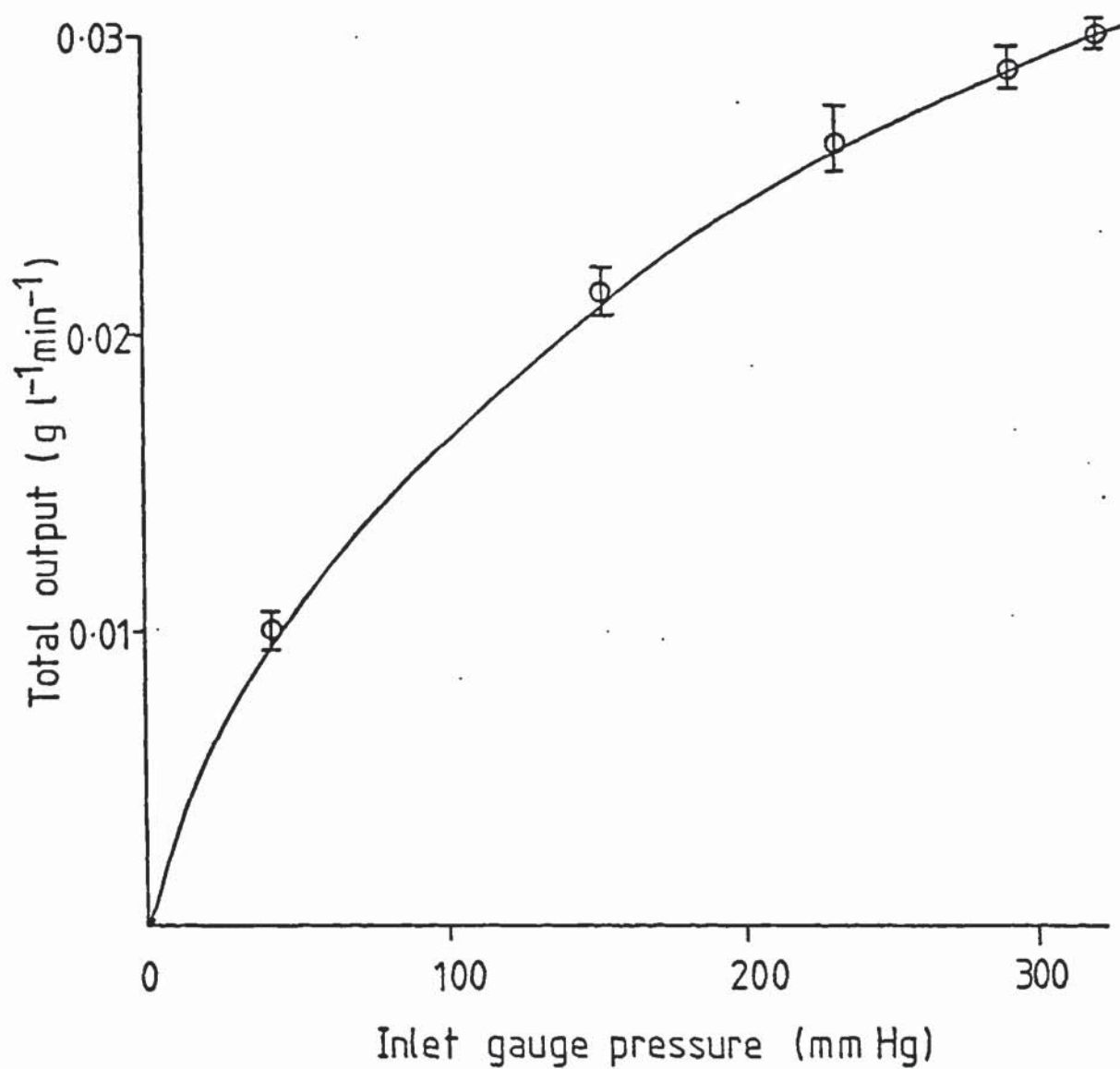
Figure 5.9 Aerodynamic growth ratio versus relative humidity for
fluorescein disodium aerosols. The mean and standard deviation of three
determinations plotted against average humidity is illustrated. The
horizontal bars show the range of humidity at the extremes of the
aerosol cloud. The solid line illustrates the calculated aerodynamic
growth ratio based on the physicochemical characteristics of aqueous
fluorescein disodium solutions.
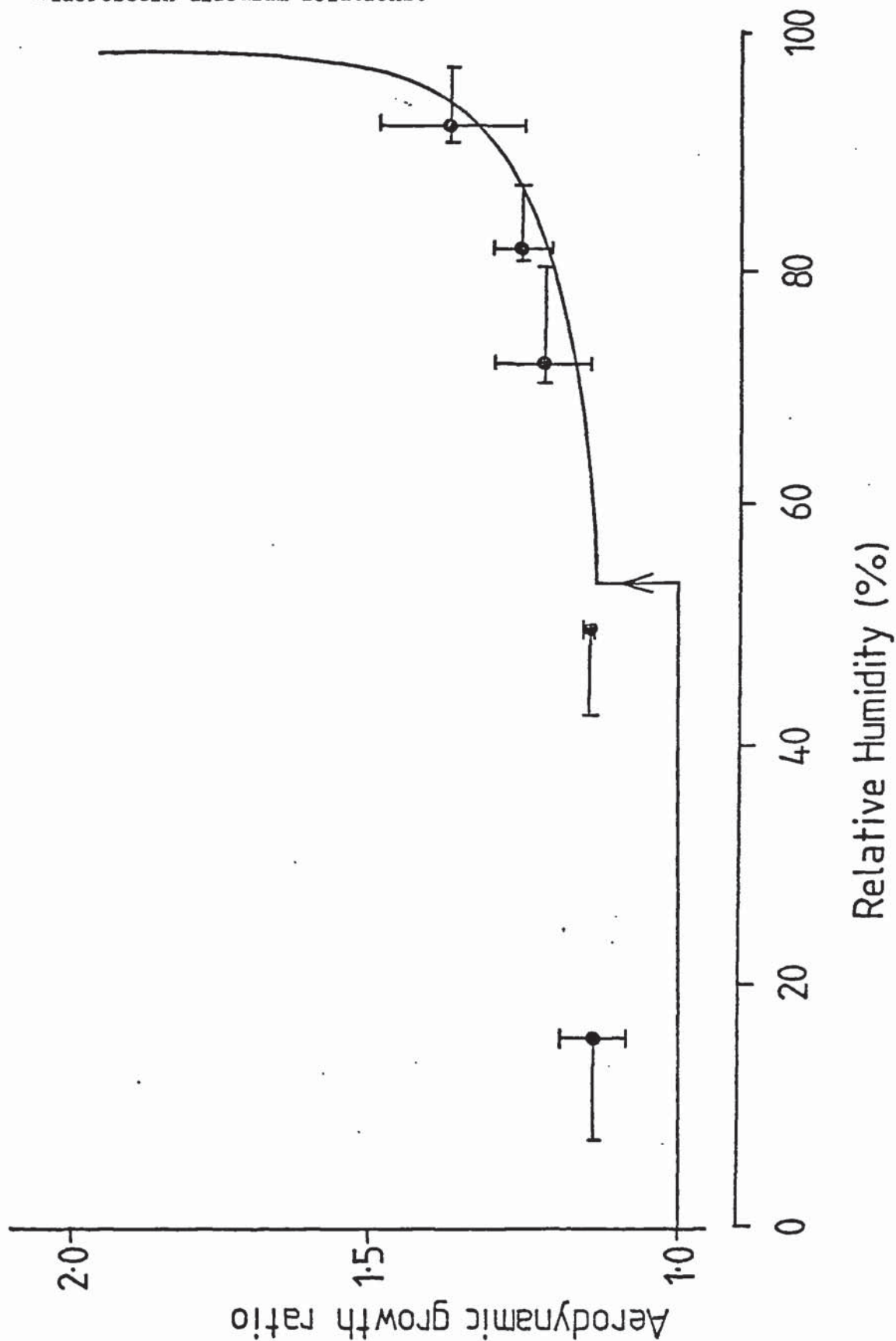
Chapter 6   Development of an _in vivo_ model for optimising systemic

drug delivery by inhalation aerosol administration.

6.1   INTRODUCTION

The lungs offer many advantages as an absorption site for
the systemic delivery of drugs. The surface area of the lungs
is approximately $30m^2$ (Hatch and Gross, 1964a), providing an
absorptive area comparable with that of the gastrointestinal
tract. Drugs administered by the inhalation route will not
be subject to large pH changes (compared with the gastro-
intestinal tract) nor such a large number of degradative
enzymes (Gonda and Byron, 1978). Sodium cromoglycate has been
shown to be practically unabsorbed from the gastrointestinal
tract but a competitive, saturable, carrier-type transport
mechanism has been implicated in its absorption from the
respiratory tract (Gardiner and Schanker, 1974). Moreover,
large molecules, such as proteins, have been shown to pass
through the alveolar wall intact (Schultz et al, 1964; Wigley
et al, 1971). Despite these observations, most of the pharma-
ceutical inhalation aerosols currently in use are intended for
local activity within the respiratory tract, a fact which
probably reflects the difficulties associated with deposition
of a known dose in the pulmonary and tracheobronchial regions
(as defined by TASK, 1966). Moreover, aerosols designed for
bronchodilator (local) activity may well require different
particle size distributions for maximum deposition than those
designed for maximum alveolar deposition with subsequent
systemic absorption.

The inhalation route may be useful in attaining rapid delivery of drugs designed to act systemically. Indeed this route is already used to achieve rapid systemic onset of action of Ergotamine (Ergotamine Aerosol Inhalation, British Pharmaceutical Codex, 1979).

The purpose of the work to be described in this chapter was to develop a technique capable of providing information on the systemic availability of a compound administered in various inhalation aerosol formulations to an experimental animal. Such a technique should enable the effects of respiratory (e.g. tidal volume, inspiratory rate, expiratory time) and formulation variables (such as the particle size distribution, particulate shape) and the effects of hygroscopic growth to be investigated.

The fate of an inhalation aerosol depends on respiratory parameters and particulate characteristics. An aerosol particle may be deposited on the inspiratory or expiratory cycle or during a period of breath-holding (Gorman and Hall, 1973). Expiration of small particles is also a possibility. In the tracheobronchial region deposited drug will be subject to parallel competing mucociliary clearance (and subsequent swallowing) and absorption. The quantities cleared and absorbed will depend upon the relative rates of ciliary transport and absorption. Similarly drug particles deposited in the pulmonary region will be subject to systemic absorption and lymphatic clearance. For highly water soluble compounds however, it is anticipated that lymphatic clearance should be negligible (Hatch and Gross, 1964b).

Most of the work in the literature documents deposition of non-pharmaceutical aerosols in the lungs of humans and experimental animals respiring normally. Little work has been performed to delineate the deposition of pharmaceutical aerosol systems. Some work has been performed with the intention of investigating the effects of formulation (Sterling and Batten, 1969), methods of aerosol generation (Shenfield et al, 1974; Stauder and Hidinger, 1980) and modes of inhalation (Newhouse and Ruffin, 1978) upon pharmacological effects within the respiratory tract of drugs administered in inhalation aerosols. In the absence of precise dose-response relationships, however, it is impossible to learn a great deal about the available dose of such drugs in the respiratory tract.

Established pharmacokinetic techniques enable determination of systemic bioavailability and drug input functions (for example, Nyberg, 1977) given plasma concentration, C_p, versus time, t, profiles for compounds administered by any route. The only prerequisite is that the intrinsic pharmacokinetics (which reflect the behaviour of the compound once in the circulation) of the compound must be known' in order to determine the amount absorbed versus time profile (Notari, 1980a). Bioavailability, which reflects both the amount absorbed and the speed of absorption, would be expected to change as a function of changes in drug deposition in the respiratory tract if a compound were administered by this route.

This chapter is devoted to the description and collection of a 'data base' which will enable the development of an *in vivo* model capable of estimating the bioavailability and input function of a marker compound administered in inhalation aerosols under standardised conditions to Beagle dogs. Subsequent to method development and evaluation, monodisperse aerosols may be administered via an endotracheal tube (eliminating the possibility of mouth deposition) to the respiratory tract. The bioavailability of the marker compound included in the aerosol formulation will be compared to the dose retained by the animal. This can be determined by measurement of the amount of inhaled aerosol and that exhaled and retained in the apparatus. It is hoped furthermore, that the speed of absorption will give some indication of the site of deposition (Schanker, 1978).

A marker compound was selected which had low toxicity, no pharmacological effect within the respiratory tract, insignificant metabolism and a chemical structure enabling the development of a highly sensitive assay procedure in biological fluids. Disodium fluorescein (I) fulfils all of these criteria. It has been used clinically in humans to determine circulation time (Lange and Boyd, 1942; Winsor et al, 1947; Neller and Schmidt, 1945). It has been reported to be excreted intact and as the glucuronide in urine and in bile in rats (Hanson, 1952; Webb et al, 1962). Fluorescence has been reported in the gall bladder of dogs after inhalation of Fluorescein (Calderwood et al, 1974).

Figure 6.1 illustrates the chemical structure of Fluorescein disodium (I) and Fluorescein= (II).

In order to be able to determine drug input functions (amount absorbed versus time profiles) after aerosol administration, it is first necessary to establish the pharmacokinetics of the compound in the experimental animal to be used. Moreover, the compound must be shown to be absorbed via the lungs. The primary purpose of this chapter is to describe the results of an intravenous dose ranging study in a Beagle dog in order to delineate I's intrinsic pharmacokinetics. With a view to administering I in inhalation aerosols, intratracheal instillation of I in aqueous solution was used to show its absorption from the lungs. These experiments are described and discussed in the light of possible use of inhalation aerosols of I as a means of investigating the effects of formulation and respiratory variables upon the systemic availability of I in Beagle dogs.

6.2   EXPERIMENTAL

6.2.1   Determination of Fluorescein⁼ (II) in Plasma and Urine

Throughout this chapter concentrations and amounts are expressed in terms of the anhydrous fluorescein anion, Fluorescein⁼ (II) (see Figure 6.1). Calibration curves of relative fluorescent intensity (with reference to a standard of known concentration) versus concentration were constructed for concentrations of II ranging from 1 to 12 ng of II per ml in 10% v/v Beagle plasma, 1% v/v Beagle plasma and 0.01% v/v centrifuged (1000g for 15 minutes, Christ bench top centrifuge, type 0300) Beagle urine diluted in pH 12.0 Sorensens glycine buffer (Sorensen, 1909). Excitation and emission wavelengths of 486 and 516nm respectively were employed in an Aminco Bowman spectrofluorimeter (Type 4-8202, American Instrument Company Inc) using 10mm spectrophotofluorimeter cells (transmission matched) with lids (Type 6010, code colour green, Helma (England) Ltd). The standard reference solution contained a known concentration of II in the centre of the examined concentration range. Relative intensity, (RI), with reference to the standard solution was calculated according to the following equation

$$RI = \frac{Z}{Zs} \qquad \text{Equation 6.1.}$$

where Zs is the % transmission of the standard solution and Z the % transmission of the test solution.

6.2.2   The Pharmacokinetics of Fluorescein$^{=}$ (II)

This section describes the methods employed in the delineation of the intrinsic pharmacokinetics of II. It is possible when compounds have proven linear pharmacokinetics, to compute the amount of drug absorbed from an extravascular site, $[A]_{ex}^{\infty}$, using (Gibaldi and Perrier 1975a)

$$[A]_{ex}^{\infty} = \frac{[Do]_{iv} [AUC]_{ex}^{0\rightarrow\infty}}{[AUC]_{iv}^{0\rightarrow\infty}} \qquad \text{Equation 6.2}$$

where the amount absorbed at time, $t = \infty$, can be calculated by reference to the total areas under the plasma concentration, Cp, versus time profiles for extravascular, $[AUC]_{ex}^{0\rightarrow\infty}$, and iv bolus, $[AUC]_{iv}^{0\rightarrow\infty}$, administrations. The term $[Do]iv$ refers to the amount of drug administered as an intravenous bolus which provides an area, $[AUC]_{iv}^{0\rightarrow\infty}$, under its Cp versus time profile.

When compounds display non-linear pharmacokinetics however, Equation 6.2 does not hold and more sophisticated procedures are necessary in order to compute the amount absorbed. It is essential therefore to establish II's pharmacokinetics prior to its adminstration by extravascular routes. This is normally performed by administering the compound in a range of doses as intravenous solution boli. For a compound to have linear pharmacokinetics $[AUC]_{iv}^{0\rightarrow\infty}$ should be directly proportional to $[Do]iv$ (Notari, 1980a). Under these circumstances, Equation 6.2 holds and enables the computation of the amount absorbed from extravascular sites.

6.2.2.1 Preparation of intravenous solutions for injection

The water content of the batch of disodium fluorescein (analytical grade, Koch Light) used in these _in vivo_ experiments was determined as 21.6% w/w (ICI Pharmaceutical Division, Analytical Group, Mereside, Alderley Park). Aqueous solutions containing the desired dose of II were prepared in double distilled water and sterilised by positive pressure filtration through a 0.22μm pore size filter (Millipore (UK) Ltd) into a previously sterilised container. The concentration of the final solution was determined on the residual solution after sterile removal of the dose using the analytical technique documented in section 6.2.1 after appropriate dilution in pH 12 Sorensens glycine buffer.

6.2.2.2 Intravenous dose ranging study

Doses of fluorescein (II) ranging 0.43 to 123mg were administered as intravenous boli to a Beagle dog. The 12.6kg Beagle was anaesthetized using a Boyles' apparatus with O_2 at 500ml min^{-1}, N_2O at 2000ml min^{-1} and halothane (Fluorothane, ICI Ltd) at concentrations ranging 1.5 to 4.0% of the inspired oxygen. The right or left saphenous vein was cannulated using a 35cm indwelling iv cannula (Intravenous cannula set, 17g needle, internal diameter 0.60mm, Pink luer fitting, Type 200/500/030, Portex Ltd). This cannula had a void volume of 0.15ml. The potency of the cannula was maintained during the course of each experiment by filling the cannula with a heparin (The Boots Company Ltd., Nottingham) solution of

5 I.U. per ml in 0.9% w/v sodium chloride solution for injection B.P. (Polyfusor, The Boots Company Ltd. Nottingham). The first 1ml of blood taken at each sample time was discarded.

In experiments involving urine collection the bladder was catheterized using a 2.0mm x 50cm dog catheter with a Luer fitting (A Cox (Surgical) Ltd.).

In most experiments the dog was allowed to regain consciousness and initial blood and urine sample taken before the dose was administered as an intravenous aqueous solution bolus via the intravenous cannula. After dose administration the cannula was rinsed with at least 10 void volume changes of heparinized saline. The luer fitting of the cannula was further rinsed externally with 10ml of heparinized saline. Three ml blood samples were taken at regular time intervals and mixed in previously heparinized containers (Brunswick, LH 10 tubes, Sherwood Medical Ltd). At the end of the experiment after centrifugation (1000g for 15 minutes in Christ Centrifuge, type 03400), the plasma was removed and assayed for fluorescein (section 6.2.1). Urine samples (the bladder was emptied at each sampling time) were stored on ice during the experiment and subsequently assayed for fluorescein after centrifugation (1000g for 15 minutes in Christ Centrifuge, type 03400). The product of urine concentration and volume enabled construction of cumulative urinary excretion of II versus time curves. Data for plasma concentration versus time was subjected to kinetic analysis as detailed in section 6.3.2.

6.2.3.3 Intratracheal administration

After catheterization and cannulation as previously
described, halothane/N_2O anaesthesia was deepened by increasing
the inspired halothane concentration. A cuffed endotracheal
tube (110mm, Magill cuff, BOC Medical Supplies Ltd) was
introduced. Initial blood and urine samples were taken prior
to administration of II. One ml of sterilized fluorescein
aqueous solution containing intratracheal doses of II,
$[Do]_{it}$, ranging 8.2 to 30.5mg, were instilled into the lungs
of the 12.6kg Beagle used previously. Instillation was via a
sterile polythene tube inserted into the endotracheal tube
so that the end of the inner tube projected beyond the
termination of the endotracheal tube. The polythene and
endotracheal tube were removed and washed to estimate the
amount of II not administered to the Beagle's respiratory
tract. The animal was allowed to recover consciousness.
Blood samples were taken at various time intervals (see
section 6.3.3) and assayed for II as described in section
6.2.1.

6.3   <u>RESULTS AND DISCUSSION</u>

6.3.1   <u>Quantitative determination of fluorescein$^=$ (II) in plasma</u>
        <u>and urine</u>

        Calibration curves of relative intensity (Equation 6.1)

versus concentration of II in all plasma and urine dilutions

could not be distinguished from calibration curves obtained

in the absence of plasma or urine (Figure 6.2) provided the

instrument was first zeroed using the appropriate reference

sdlution without fluorescein$^=$. Thus for plasma concentrations

$\leqslant$ 10% v/v and urine concentrations $\leqslant$ 0.01% v/v in buffer

(pH 12), purification of body fluids to extract II was

unnecessary in that interference with the assay for II, was

proven to be negligible. Calibration curves for II of the

type shown in Figure 6.2 were rectilinear for concentrations

up to concentrations of 55ng per ml. The solid line shown

in Figure 6.2 is the best fit by linear regression to the

data for RI versus concentration of II in buffer alone.

Repetitions of the experiment in buffer alone enabled $\geqslant$ 5%

errors to be ascribed to concentrations $\leqslant$ 1.75ng per ml of

II. This limit was determined by the method of accuracy of

coefficients of the line of regression (Topping, 1962) using

data from an experiment performed in quintuplicate. More than

5% errors are incurred in concentrations determined for relative

intensity $\leqslant$ 0.3 (Equation 6.1). Thus an accurate, sensitive

assay procedure for II in the presence or absence of soluble

biological 'contaminants' was successfully developed.

6.3.2   The Pharmacokinetics of Fluorescein$^=$ (II)

The results of the iv dose ranging study are presented
as the discrete data points for plasma concentration, Cp,
versus time in Figures 6.3 to 6.8. Each of the experiments
provides apparently biexponential data where Cp at any time,
t, appears to be described by an equation of the form

$$Cp = Ae^{-\alpha t} + Be^{-\beta t} \qquad \text{Equation 6.3}$$

where A, B, α and β are constants in discrete experiments.
The area under the Cp versus time curve between time, t =
5 minutes and t = 60 minutes ($[AUC]_{iv}^{5 \to 60}$) was computed
trapezoidally (Gibaldi and Perrier, 1975b) for each individual
dose. The results are presented in Figure 6.9 and clearly
show that for iv bolus doses greater than 11.4mg the
pharmacokinetics may be approximated by a linear model. That
is, $[AUC]_{iv}^{5 \to 60}$ is directly proportional to dose when
$[Do]_{iv} \leqslant 11.4mg$.

6.3.2.1 Linear Pharmacokinetics

The simplest intravenous pharmacokinetic model which is capable
of producing an analytical expression for plasma concentration
as a function of time of the type shown by Equation 6.3 is
illustrated by Scheme 6.1.
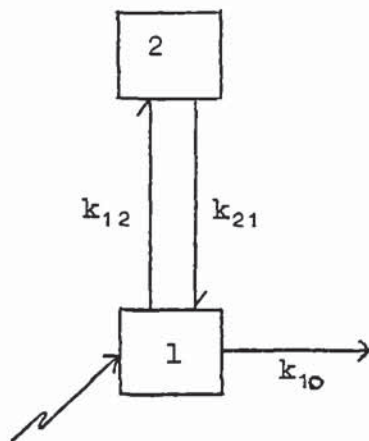
Scheme 6.1   Two compartment open body model

Drug is administered as a bolus to the sampled compartment
(1) and simultaneously distributed to a peripheral compartment
(2) and eliminated according to the apparent first-order rate
constants k_{12}, k_{21} and k_{10} . Equation 6.3 can be rewritten
according to Scheme 6.1 as (Gibaldi and Perrier; 1975c).

$$Cp = \frac{[Do]_{iv}}{V_1 (\alpha-\beta)} \left[(\alpha-k_{21})e^{-\alpha t} + (k_{21}-\beta) e^{-\beta t} \right]$$

$\alpha > \beta$ Equation 6.4.

where $[Do]$ iv is the iv dose and V_1 , the apparent volume of
compartment 1. The apparent first order rate constants α and
β are functions of k_{12}, k_{21} and k_{10} according to

$$\alpha = \frac{(k_{12} + k_{21} + k_{10}) + \sqrt{((k_{12} + k_{21} + k_{10})^2 - 4k_{10}k_{12})}}{2}$$

Equation 6.5

and

$$\beta = \frac{(k_{12} + k_{21} + k_{10}) - \sqrt{((k_{12} + k_{21} + k_{10})^2 - 4 k_{10} k_{12})}}{2}$$

Equation 6.6

The pharmacokinetics of II have been shown to be apparently linear when $[Do]_{iv} \leqslant 11.4$mg by the direct proportionality between $[AUC]_{iv}^{5 \to 60}$ and dose in Figure 6.9. Under these circumstances Scheme 6.1 and Equation 6.4 describe the Cp versus time data. Values for the apparent rate constants k_{12}, k_{21} and k_{10} (Scheme 6.1) should therefore be fluorescein= dependent constants, as should α, β (Equations 6.5 and 6.6) and V_1. Rearranging Equation 6.4 by dividing both sides by $[Do]_{iv}$ should enable normalised data to be expressed as Cp/ $[Do]_{iv}$ versus time when $[Do]_{iv} \leqslant 11.4$mg and the pharmacokinetics of II are linear. Thus

$$\frac{Cp}{[Do]_{iv}} = \frac{1}{V_1 (\alpha-\beta)} \left[(\alpha-k_{21})e^{-\alpha t} + (k_{21} - \beta) e^{-\beta t} \right].$$

Equation 6.7

Accordingly plasma concentrations determined by fluorescence after iv administration of II of doses less than or equal to 11.4mg were divided by dose in each case and thereby normalised. The results of this procedure are shown in Figure 6.10. This data was subjected to least mean squares non-linear regression analysis (Metzlar, 1969) based on Equation 6.7.

The computer was allowed to float values for k_{12}, k_{21}, k_{10} and V_1 in order to provide their 'best estimates' based upon minimised least mean square deviations of the theoretical curve from the experimentally determined values for $Cp/[Do]_{iv}$ versus time. The solid curve in Figure 6.10 shows the best fit determined by this procedure. The best estimates and standard deviations of the rate constants k_{12}, k_{21}, k_{10} (Scheme 6.1) and the apparent volume of the sampled compartment, V_1, are documented in Table 6.1.

The total area under the plasma concentration versus time profiles, $[AUC]_{iv}^{0 \to \infty}$, for $[Do]_{iv} \leq 11.4mg$ were calculated trapezoidally (Gibaldi and Perrier 1975b) from the raw data of Cp versus time for each administered dose. In order to perform this calculation it was necessary to ascribe values to Cp at $t = 0$ and $[AUC]_{iv}^{x \to \infty}$, for each of the doses (where $t = x$ was the final sampling time in each case). These values were computed according to

$$Cp_{t=0} = \frac{Do\ (\alpha - k_{21})}{V_1\ (\alpha - \beta)} + \frac{Do\ (k_{21} - \beta)}{V_1\ (\alpha - \beta)} \qquad \text{Equation 6.8}$$

and

$$[AUC]_{iv}^{x \to \infty} = \frac{Cp_{t=x}}{\beta} \qquad \text{Equation 6.9}$$

Values for α, β were calculated according to Equations 6.5 and 6.6. Values for the rate constants and V_1 were assigned according to their best estimates when $[Do]_{iv} \leq 11.4mg$ (Table 6.1). Equation 6.8 can be derived by taking Equation 6.4 to its limit at $t = 0$. The derivation of Equation 6.9 is shown below.

If Equation 6.4 is taken to its limit as $t \to \infty$ then because $\alpha > \beta$, the apparently monoexponential decay of C_p versus time (see Figure 6.10) is described by

$$C_p \to \frac{Do\ (k_{21} - \beta)}{V_1\ (\alpha - \beta)}\ e^{-\beta t} \qquad\qquad \text{Equation 6.10}$$

Integration of Equation 6.10 between $t = x$ and $t = \infty$ with respect to time and observing that

$$\frac{Do\ (k_{21} - \beta)}{V_1\ (\alpha - \beta)} = B \qquad \text{(compare Equations 6.3 and 6.4)}$$

gives

$$[AUC]_{iv}^{x\ \to\infty} = \int_{t\ =\ x}^{\infty} C_p\ dt$$

$$= B \int_{t\ =\ x}^{\infty} e^{-\beta\ t}\ dt$$

$$= B \left[\frac{e^{-\beta\infty}}{-\beta} - \frac{e^{-\beta\ (t\ =\ x)}}{-\beta} \right]$$

$$= \frac{Be^{-\beta\ (t\ =\ x)}}{\beta}$$

$$= \frac{C_p\ t\ =\ x}{\beta} \qquad\qquad \text{Equation 6.11}$$

Thus Equation 6.9 can be used to compute the terminal area of the iv plasma concentration versus time profile provided that the data at $t = x$ is apparently monoexponential. Given $C_{p_{t=0}}$ (Equation 6.8), the remaining area can be computed trapezoidally and $[AUC]_{iv}^{0\ \to\infty}$ determined by summation.

Figure 6.11 shows a plot of $[AUC]_{iv}^{0\ \to\infty}$ versus dose for $[Do]_{iv} \leqslant 11.4mg$. Figure 6.11 may be adequately described by the rectilinear relationship

$$[AUC]_{iv}^{0 \to \infty} = 6.0 \ (ml^{-1} min) \cdot [Do]_{iv} \ (\mu g) + 0.24 \ (\mu g \ ml^{-1} min)$$

$$[AUC]_{iv}^{0 \to \infty} \leqslant 70 \mu g \ ml^{-1} min \quad \text{Equation 6.12.}$$

Thus, provided $[AUC]_{iv}^{0 \to \infty} \leqslant 70\mu g \ ml^{-1} min$, the iv pharmacokinetics of II in this Beagle dog were apparently linear.

The maximum plasma concentration attained with $[Do]_{iv} = 11.4mg$, that is $Cp_{t \simeq 0}$ computed according to Equation 6.8 was $7.5\mu g$ of II per ml of plasma. A plasma concentration, $Cp = 7.5\mu g \ ml^{-1}$ was therefore chosen as a threshold concentration beneath which linear pharmacokinetics were assumed.

Given this definition of linear pharmacokinetics then under circumstances when doses of II are administered extravascularly, provided Cp at any time t after administration $\leqslant 7.5\mu g$ per ml, then the amount absorbed can be determined from Equation 6.2.

6.3.2.2   Non-Linear Pharmacokinetics

It is clear from Figure 6.9 that no single Scheme 6.1 with first oder rate constants is capable of describing the intrinsic pharmacokinetics of II at all iv doses. If it were, then $[AUC]_{iv}^{0 \to \infty}$ would be a rectilinear function of intravenous dose over the whole range of doses studied (Notari, 1980a). At doses $> 11.4mg$ however the $[AUC]_{iv}^{5 \to 60}$ versus $[Do]_{iv}$ data in Figure 6.9 shows a clear deviation from the linear kinetics observed when $[Do]_{iv} \leqslant 11.4mg$ (Figure 6.11). Such a non-proportionality is typical of a compound with saturable elimination kinetics (Notari, 1980b). A more complex model must therefore be postulated in order to describe the apparent dose-dependent kinetics of II when $[Do]_{iv} > 11.4mg$.

-171-

Figure 6.12 shows the cumulative amount of II eliminated
in the urine as a function of time after an iv bolus of 18mg
(see also Figure 6.7 for the corresponding plasma concentration
versus time profile). Estimated values for the renal clearance
of II, Cl_r, were calculated from (Gibaldi and Perrier, 1975d)

$$Cl_r = \frac{U (x \to y)}{[AUC]\, x \to y \atop iv} \qquad \text{Equation 6.13}$$

where $U (x \to y)$ is the total amount of II excreted in the urine
during the time interval $t = x$ to $t = y$ and $[AUC]_{iv}^{x \to y}$ is the
area under the plasma concentration versus time profile during
the same interval . The estimated values for Cl_r at various
time intervals after $[Do]_{iv} = 18mg$ are presented in Table 6.2.
Renal clearance in all cases was greater than literature values
for glomerular filtration rate in 10 to 25kg mongrel dogs
$(30 \pm 2ml\ min^{-1})$ and tend to Rosenblatt's reported values for
renal blood flow of $176 \pm 32ml^{-1}$. The renal clearance
variations presented in Table 6.2 are probably due to
difficulties encountered in complete withdrawal of urine at a
given time after administration. Clearance values greater than
glomerular filtration rate may be taken as evidence for active
tubular secretion (Notari, 1980a). This observation is directly
supported by Figure 6.13 which shows the effect of halothane
anaesthesia upon plasma concentration versus time profiles
for II. This figure shows data collected from a conscious dog
(1 occasion) and the same animal maintained under halothane
anaesthesia (mean of 2 experiments) after an iv bolus of II =
0.43mg. Anaesthesia is known to reduce renal blood flow
significantly (Bonvalet et al. 1977), and would be expected to
produce a greater change in the elimination kinetics of an

actively secreted compound than would be the case when a compound
was eliminated only by simple filtration. These observations
in the Beagle would indicate that elimination of II in the
urine is primarily by active tubular secretion. The remaining
fraction of II to be eliminated could be by metabolism and
biliary secretion (Webb et al, 1962). Fluorescence has been
observed in the gall bladder of Beagle dogs after administration
of aerosols of I (Calderwood et al, 1974). Biliary secretion
of II in rats was reported to be via a non-specific anion
carrier mechanism (Hanson, 1952) probably similar to the
non-specific mechanism involved in its active tubular secretion
in the kidney (Mudge and Weiner, 1963).

An appropriate model to describe the dose dependent
kinetics of II when $[Do]_{iv} > 11.4$mg must therefore show at
least one capacity-limited elimination process from the central
compartment. In Scheme 6.1 the elimination process is
represented by the apparent first order constant, k_{10}. If
this process is saturable then Equation 6.4 will only hold
when $[Do]_{iv} \leq 11.4$mg and Cp at any time $t \leq 7.5 \mu g \ ml^{-1}$
(Section 6.3.2.1). Irrespective of dose, the rate of change of
the amount of drug in the central sampled compartment (1)
must be given by

$$\frac{d X_1}{dt} = \text{rate in } - \text{rate out} \quad \text{Equation 6.14.}$$

Provided the distribution process to the peripheral compartment
is apparently first order (Scheme 6.1; k_{12} and k_{21}) then the
rate out due to distribution must be given by the products
$k_{21} X_2$ and $k_{12} X_1$ respectively (where X_1 and X_2 are the amounts
of II in the sampled (1) and peripheral compartment (2)).

The elimination process, which also contributes to the rate of output from the central compartment should be described by a term at least as complex as the Michaelis-Menten term, $Vm\, X_1 /(Km + X_1)$ where Vm is the maximum rate of elimination and Km, the amount of drug in the central compartment at which the rate of elimination equals $Vm/2$ (Gibaldi and Perrier, 1975e). In enzyme kinetics X_1 and Km would more conventionally be written as concentrations. This assumes for simplicity, that the elimination process of II is only by a single saturable route. If this is assumed then Equation 6.14 can be rewritten as

$$\frac{d\, X_1}{dt} = -k_{12}\, X_1 + k_{21}\, X_2 - \frac{Vm\, X_1}{(Km + X_1)} \qquad \text{Equation 6.15}$$

Although Equation 6.15 can be integrated with respect to time, the resultant equation cannot be solved explicitly for X_1 at any time t (cf Equation 6.4). However, at low doses (producing low values for X_1), where $Km \gg X_1$, the Michaelis-Menten term simplifies to $Vm\, X_1/Km$. Under these circumstances Vm/Km provides an apparent first order rate constant, k_{10} such that the final term in Equation 6.15 could be rewritten as $k_{10}\, X_1$. If the pharmacokinetics of II are correctly described by Equation 6.15, the estimates for k_{10} found in section 6.3.2.1 would more correctly be termed estimates for Vm/Km (see Table 6.1 for $[Do]_{iv} \leqslant 11.4mg$). At very high doses when X_1 is much larger than Km, the Michaelis-Menten term will tend to Vm, an apparent zero-order constant, k_o, such that Equation 6.14 will become

$$\frac{d\, X_1}{dt} = = k_{12}\, X_1 + k_{21}\, X_1 - ko \qquad \text{Equation 6.16}$$

$$X_1 \gg Km.$$

In this limiting case, after an initial distributive phase, the fall in plasma concentration should be rectilinear with respect to time until the plasma concentration falls below a saturation value. The plasma concentration versus time profile after a 123mg iv bolus (Figure 6.14) however, provides no evidence that elimination from the sampled compartment ever tends to complete saturation. Moreover, all intravenous experiments described in this chapter provide Cp versus time profiles which apparently conform to Equation 6.4. In the absence of complete saturation it is difficult to estimate $ko = Vm$ (Equations 6.15 and 6.16).

Although further intravenous studies may enable II's kinetics to be completely delineated at all doses, thus was felt to be beyond the scope of this Chapter whose primary purpose was to describe the development of an experimental system capable of providing information on the systemic availability of a compound administered in various inhalation aerosols.

Because all iv experiments described in this Chapter provided data consistent with Equation 6.4, it proved possible to ascribe an apparent value of k_{10} (Scheme 6.1), a first order elimination rate constant for II at $123 \gg [Do]_{iv} > 11.4mg$. At $[Do]_{iv} > 11.4mg$ the pharmacokinetics of II however, have been proven to be non-linear. Thus, although the data for each individual iv experiment is apparently described by Equation 6.4, the elimination constant, k_{10}, should be a dose-dependent apparent first order rate constant when $[Do]_{iv} > 11.4mg$. Under these circumstances if the first order distribution rate constants k_{12} and k_{21} (Scheme 6.1) are held constant at their

best estimates for $[Do]_{iv} \leqslant 11.4mg$ (Table 6.1) then the

apparent values for k_{10} would be expected to all with increasing

$[Do]_{iv} > 11.4mg$. An equation describing the rate of change

of X_1 with respect to time is given by dX_1/dt = rate in -

rate out as

$$\frac{dX_1}{dt} = k_{21} X_2 - k_{12} X_1 - k_{10} X_1 \qquad \text{Equation 6.17}$$

Dividing both sides of Equation 6.17 by the volume of the

central compartment, V_1, and observing that $Cp = X_1/V_1$ gives

$$\frac{dCp}{dt} = k_{21} \frac{X_2}{V_1} - k_{12} Cp - k_{10} Cp \qquad \text{Equation 6.18}$$

An analog computer programme was designed to generate Cp versus

time profiles according to Equation 6.18 (Appendix 4). Data

for Cp versus time profiles of II at $[Do]_{iv} > 11.4mg$ were

plotted on a linear basis. Values for k_{12} k_{21} and V_1 were

held constant at their mean values as documented in Table 6.1

for $[Do]_{iv} \geqslant 11.4mg$. The programme described in Appendix 4

was established on an EAI 180 analog computer connected to a

Bryans 26000 X Y recorder. The circuitry enabled the

generation and recording of Cp versus time curves according

to Equation 6.18. Values for k_{10} were varied in order to produce

curves of Cp versus time profiles to provide good fits of the

data obtained from individual iv experiments for $[Do]_{iv} > 11.4mg$.

Thus distribution and the apparent volume of the sampled

compartment were assumed to be dose independent. A comparison

of Equation 6.17 and 6.15 reveals (if Scheme 6.1 is assumed to

hold at all doses) that as $[Do]_{iv}$ is increased the apparent

values of k_{10} (Equation 6.17) should decrease as the rate of

elimination ($Vm X_1/(Km + X_1)$, Equation 6.15) increases non-

linearly with increasing X_1.

The 'best' fits obtained as presented as the solid profiles of Cp versus time in Figures 6.6 to 6.8. Table 6.3 documents the values of k_1 as a function of $[Do]_{iv}$ and clearly displays the decreasing values of this apparent first-order constant with increasing iv dose .

6.3.2.3 Intratracheal Instillation

In order for II to be a suitable model compound for studying the systemic availability of aerosols, this compound must be shown to be absorbed from the respiratory tract. Aqueous solutions of II were therefore introduced into the respiratory tract. Ensuing plasma concentration versus time profiles were determined. These profiles are shown in Figure 6.15 to 6.18. The maximum plasma concentrations of II (1.1 to 3.3µg ml^{-1}) following these administrations were always substantially less than 7.5µg ml^{-1}. The pharmacokinetics of II after its administration were therefore assumed linear according to Equation 6.7 with the variables k_{12}, k_{21}, k_{10} and V_1 as documented in Table 6.1. Equation 6.2 was used therefore to determine the amount absorbed after intratracheal administration from the area under the plasma concentration versus time profile following intratracheal administration of II, $[AUC]_{it}^{0 \to \infty}$. The bioavailable fraction, Bf, of the administered it dose, $[Do]_{it}$, was then determined from

$$Bf = \frac{[AUC]_{it}^{0 - \infty}}{[Do]_{it}} \qquad \text{Equation 6.19}$$

Log-linear plots of plasma concentration versus time following intratracheal administration provide apparently monoexponential data when $t \geqslant 200$mins.

A typical example is shown as Figure 6.19 for $[Do]_{it} = 28.2$mg. From plasma concentration versus time profiles following iv administrations for $[Do]_{iv} \leq 11.4$mg, β was determined to be 0.039 ± 0.11 min^{-1}, giving a half life ($t_{0.5}$) of 17.9 ± 4.9 min. The values for the terminal log-linear slopes estimated from least mean square regression of the apparently monoexponential portion of the data (Table 6.4) following intratracheal instillation are $< \beta$ and fall outside this range. Byron and Notari (1976) have observed that the terminal slopes of log-linear plots of plasma concentration versus time after extravascular administration, S^1, will only tend to β estimated after iv administration if the input from the depot is some three times faster than output from the whole body. Similarly, S^1 will only approach ka, a first order absorption rate constant for drug input, if $S^1/\beta < 0.5$. The results obtained following intratracheal administration reveal that S^1 (Figure 6.19) should provide an estimate for first-order absorption rate constants following i.t. administration if it is valid to assume that input from the depot is first order. There is some tentative evidence therefore that values for S^1 (Table 6.4) are in fact estimates for input rate constants after i.t. administration. If this is the case then input is possibly rate determining. Such rate determination would be expected to become more and more apparent if fluorescein was administered in formulations with properties likely to delay its release prior to absorption. Under these circumstances, when II is administered in aerosol form, and absorption is slow, the bioavailability of II may be expected to fall due

to parallel mucociliary clearance to the gastrointestinal tract (Byron, 1980).

It was necessary to calculate the values of S^1 (see Figure 6.19) for all of the intratracheal instillations performed in order to be able to calculate the terminal areas under the Cp versus time profiles following each dose. In a similar fashion to that described in the derivation of Equation 6.9 the area under the curve of the terminal mono-exponential portion of data can be determined from

$$[AUC]_{it}^{x \to \infty} = \frac{Cp_{t=x}}{S^1} \qquad \text{Equation 6.20}$$

Bioavailable fractions were therefore calculated in a step_wise fashion $[AUC]_{it}$ following i.t administration up to and including the last sampling time were calculated trapezoidally. The final portion (from the last sampling time to time∞) was estimated from Equation 6.20 using the appropriate value of S^1 from Table 6.4 and $Cp_{t=x}$ from figures 6.15 to 6.18. The calculated bioavailable fractions for the various values of $[Do]_{it}$ are presented in Table 6.5. These results indicate that II is well absorbed from the respiratory tract.

It is unlikely that administration of aerosols of I would results in plasma concentration versus time profiles which are described by non-linear pharmacokinetics. This can be illustrated by considering the likely deposited dose from an administered monodisperse aerosol of I. Cuddihy et al (1974) have shown that the particle size for optimum pulmonary deposition in Beagle dogs would have an aerodynamic diameter of 2 to 3μm. However, the expected aerodynamic growth ratio of dry particles to equilibrium droplets, assuming complete

hygroscopic growth in the respiratory tract, is 2.77. It
would thus be expected that approximately 30% of a monodisperse
aerosol of 1μm dry particles of I would deposit in the
pulmonary region. Assuming an aerosol concentration, N^1 of
1×10^6 particles per ml, the mass of I per ml of inspired
air will be

$$\frac{4}{3} \; r^3 . d_p \; N^1 = 0.0008 \text{mg per ml.}$$

The reported tidal volume in resting Beagles is $204 \overset{+}{-} 2$ml
and the respiratory rate $23 \overset{+}{-} 2$ respirations per minute
(Park et al, 1970). Thus the expected deposited dose, assuming
30% deposition, would be approximately 1mg per minute. Without
extended administration times, it is thus unlikely that aerosols
of I would result in plasma concentration versus time profiles
which are described by non-linear pharmacokinetics .

Thus II is a suitable model compound for studying the systemic
availability of aerosols. It should therefore be possible to
investigate differences in systemic availability of different
particle sizes of fluorescein aerosols under constant
physiological conditions. Control of physiological parameters
such as tidal volume, respiratory rate and breathing pattern
(for example, the breath holding manouvre) may be achieved by
the use of intermittent positive breathing apparatus. Thus the
effect of physiological parameters on the systemic availability
of fluorescein aerosols could be investigated.

6.4   CONCLUSIONS

The data presented in this chapter forms a base enabling
the determination of systemic availability of II subsequent to
its administration in various inhalation aerosol formulations.
A sensitive assay for fluorescein= (II) in Beagle plasma and
urine was developed enabling estimation of concentrations of II
$\geqslant$ 20ng ml-^{1} with $<$ 5% error. The intrinsic pharmacokinetics
of II at iv. doses ranging 0.43 to 123mg are documented. The
results presented clearly indicate that the intrinsic pharmaco-
kinetics of II cannot be described by linear pharmacokinetics
at all values of Cp. However, for Cp $\leqslant$ 7.5ng per ml the data
may be adequately described by a two-compartment open body
model. It is suggested that non-linear pharmacokinetics occur
at higher i.v. doses due to the presence of saturable
elimination routes. Nevertheless, when II is administered
as an extravascular depot provided Cp $\leqslant$ 7.5ng per ml, the
existence of non-linear pharmacokinetics can be excluded and
the area under the plasma concentration versus time profiles
may be used as an indicator of available dose. Intratracheal
doses of II $\leqslant$ 30.5mg produced maximum plasma concentrations
$\leqslant$ 3.3ng per ml.

In order for II to be used as a model system for systemic
availability of aerosols, it was necessary to estimate the
magnitude of II's absorption from the respiratory tract.
There was an indication that the absorption of II from aqueous
intratracheal instillation may be rate determining relative
to its elimination from the body.

Good systemic availability $(Bf > 0.90)$ has been shown for
intratracheal instillations of 8.2mg to 30.5mg of II in aqueous
solution. It is unlikely that inhalation aerosol administration
of II will result in a need to delineate its more complex
non-linear kinetics at high doses. Fluorescein would thus
appear to be a suitable model compound for investigating the
systemic availability of drugs administered in inhalation
aerosols. It is envisaged that following administration of
characterised monodisperse aerosols of II the amount available
systemically may be determined from the ensuing plasma concen-
tration versus time profile. By comparison of the amount of
inhaled aerosol and that exhaled and retained in the apparatus
the bioavailable fraction of the dose retained in the
respiratory tract can be estimated. This can be investigated
as a function of formulation variables and variation in the
methods and modes of administration.

6.5   Tables

Table Captions

Table 6.1 Best estimates for the fitted parameters k_{12}, k_{21}, k_{10} and V_1 from non-linear least means square regression analysis after i.v. administration of II for $[Do]_{iv} \leqslant 11.4$mg.

Table 6.2 Estimates for renal clearance of an 18mg i.v. bolus of II according to Equation 6.13.

Table 6.3 k_{10} values estimated by analog computation of Cp versus time profiles according to Equation 6.18 for $[Do]_{iv} \geqslant 11.4$mg.

Table 6.4 Terminal slope, S^1, values following intratracheal administration, estimated by least mean square regression of the apparently monoexponential portions of the plasma concentration versus time profiles.

Table 6.5 Bioavailable fraction of II after intratracheal administration of an aqueous bolus, determined using Equation 6.19.

Table 6.1   Best estimates for the fitted parameters

k_{12} , k_{21} , k_{10} and V_1 from non-linear least mean square

regression analysis after iv administration of II for

$[Do]_{iv} \leqslant 11.4mg$

Parameter	Estimate	Standard Deviation
k_{12}	0.064 min^{-1}	0.023 min^{-1}
k_{21}	0.075 min^{-1}	0.028 min^{-1}
k_{10}	0.108 min^{-1}	0.016 min^{-1}
V_1	1.62 1.	0.26 1.

Correlation coefficient = 0.990 $(n = 30)$

Table 6.2 Estimates for renal clearance of an

18mg iv bolus of II according to Equation 6.13.

Time interval (mins)		Cl_r (ml min^{-1})
t = x	t = y	
6	11	60
11	16	109
16	22.5	132
22.5	54	160
54	80	200
80	102	152
102	140	152

Table 6.3 k_{10} values estimated by analog computation of Cp versus time profiles according to Equation 6.18 for $[Do]_{iv} \gg$ 11.4mg.

Dose (mg)	k_{10} (min^{-1})
123	0.030
33.6	0.055
18	0.070
13.95	0.090
13.6	0.098
11.4	0.107

Table 6.4 Terminal slope, S', values following intra-tracheal administration, estimated by least mean square regression of the apparently monoexponential portions of the plasma concentration versus time profiles.

β, for $[Do]_{iv} \gg$ 11.4mg = 0.0387 $\pm$ 0.0105 min^{-1}

Administered dose (mg)	S' (min^{-1})	S'/β
8.2	0.0090	0.23
19.0	0.0151	0.39
28.2	0.0059	0.15
30.5	0.0093	0.24

Table 6.5 Bioavailable fraction of II after intratracheal administration of an aqueous bolus, determined using Equation 6.19..

Administered dose (mg)	Bioavailable fraction
8.2	1.05
19.0	1.01
28.2	0.90
30.5	1.15

6.6   <u>FIGURES</u>

<u>Figure Captions</u>

<u>Figure 6.1</u> The chemical structure of Fluorescein disodium
(I) and Fluorescein$^=$ (II).

<u>Figure 6.2</u> Relative Intensity (Equation 6.1) versus
concentration for Fluorescein$^=$ (II) in plasma and urine.
Fluorescein in buffer (pH 12) (●); Fluorescein$^=$ with 10% v/v
plasma in buffer (o) Fluorescein$^=$ with 1% v/v plasma in buffer
(▲); Fluorescein$^=$ with 0.001% v/v urine in buffer (Δ). The
solid line is the best fit by linear regression to the data
for relative intensity versus concentration of II in buffer
alone.

<u>Figure 6.3</u> Plasma concentration versus time profiles for II
after iv administration as solution boli. $[Do]_{iv}$ was 0.43mg
(o) and 3.4mg (x). The curves are the 'best fits' according
to non-linear least mean square (lms) regression analysis
based on Equation 6.7 (section 6.3.2.1).

<u>Figure 6.4</u> Plasma concentration versus time profile of II
after iv administration as solution boli, $[Do]_{iv}$ was 8.2mg
(●) and 10.8mg (o). The curves are the 'best fits' according
to non-linear lms regression analysis based on Equation 6.7
(section 6.3.2.1).

<u>Figure 6.5</u> Plasma concentration versus time profile of II after
iv administration as solution bolus. $[Do]_{iv}$ was 11.4mg.
The curve is the 'best fit' according to non-linear lms regression

-189-

analysis based on Equation 6.7 (section 6.3.2.1)

Figure 6.6 Plasma concentration versus time profiles for II after iv administration as solution boli. $[Do]_{iv}$ was 13.6mg (●); 13.95mg (1st experiment) (x) and 13.95 (2nd experiment) (o). The curves are the 'best fits' obtained using an analog computer programme according to Scheme 6.1 (section 6.3.2.1)

Figure 6.7 Plasma concentration versus time profiles for II after iv administration as solution boli. $[Do]_{iv}$ was 18.0mg (●) and 33.0mg (o). The curves are the best fit obtained using an analog computer programme according to Scheme 6.1 (section 6.3.2.1).

Figure 6.8 Plasma concentration versus time profiles for II after iv administration as solution bolus. $[Do]_{iv}$ was 123mg (●). The curve is the best fit obtained using an analog computer programme according to Scheme 6.1 (section 6.3.2.1).

Figure 6.9 Area under iv plasma concentration $[AUC]_{iv}^{5 \to 60}$ versus time profiles (t = 5 to t = 60) versus dose, $[Do]_{iv}$.

Figure 6.10 Plasma concentration divided by dose $(Cp/[Do]_{iv})$ versus time for $[Do]_{iv} \le 11.4$mg (data from Figure 6.3 to 6.5) The curve is the 'best fit' according to non-linear lms regression analysis based on Equation 6.7.

Figure 6.11 Total area $[AUC]^{0 \to \infty}$ under plasma concentration versus time profile as a function of iv dose $[Do]_{iv}$ when $[Do]_{iv} \le 11.4$mg.

Figure 6.12 Cumulative amount of II excreted in urine after 18mg iv bolus.

Figure 6.13 Plasma concentration time profile after 0.43mg iv bolus to anaesthetised and conscious dog. Anaesthesia maintained throughout the experiment (o), dose administered to the conscious dog (•).

Figure 6.14 Plasma concentration of II (linear scale) versus time profile after administration of 123mg iv bolus. Experimentally determined concentrations (•), the curve is the 'best fit' obtained using an analog computer programme to model Scheme 6.1.

Figure 6.15 Plasma concentration versus time profile for II after $[Do]_{it}$ = 8.6mg intratracheal instillation. The curve illustrated shows the trapezoidal approximation used to calculate the area under the curve.

Figure 6.16 Plasma concentration versus time profile for II after $[Do]_{it}$ = 19.0mg intratracheal instillation. The curve illustrated shows the trapezoidal approximation used to determine the area under the curve.

Figure 6.17 Plasma concentration versus time profile for II after $[Do]_{it}$ = 28.2mg intratracheal instillation. The curve illustrated shows the trapezoidal approximation used to determine the area under the curve.

Figure 6.18 Plasma concentration versus time profile for II after $[Do]_{it}$ = 30.5mg intratracheal instillation. The curve illustrated shows the trapezoidal approximation used to determine the area under the curve.

Figure 6.19 Log-linear plot of plasma concentration versus time for II after intratracheal instillation of an aqueous bolus of 28.2mg. The terminal slope, S^1, determined by linear regression is illustrated by the solid line.
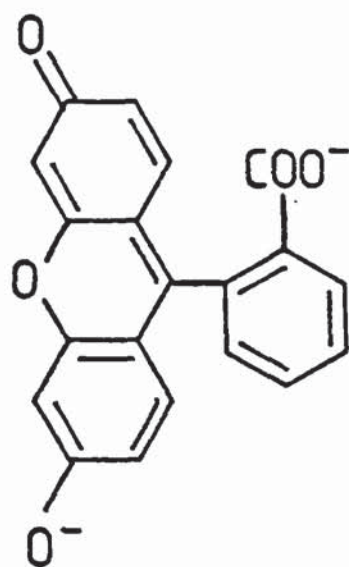
Figure 6.1 The chemical structure of Fluorescein disodium (I) and Fluorescein= (II)
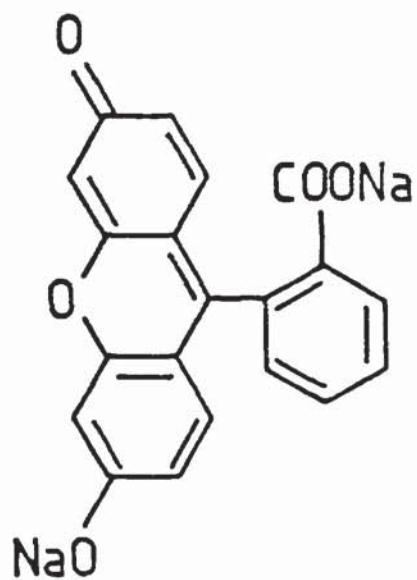
I

II

Figure 6.2 Relative Intensity (Equation 6.1) versus concentration for fluorescein= (II) in plasma and urine. Fluorescein in buffer (pH 12) (●), fluorescein= with 10% v/v plasma in buffer (o), fluorescein= with 1% v/v plasma in buffer (▲), fluorescein= with 0.01% v/v urine buffer (Δ). The solid line is the best fit by linear regression to the data for relative intensity versus concentration of II in buffer alone.
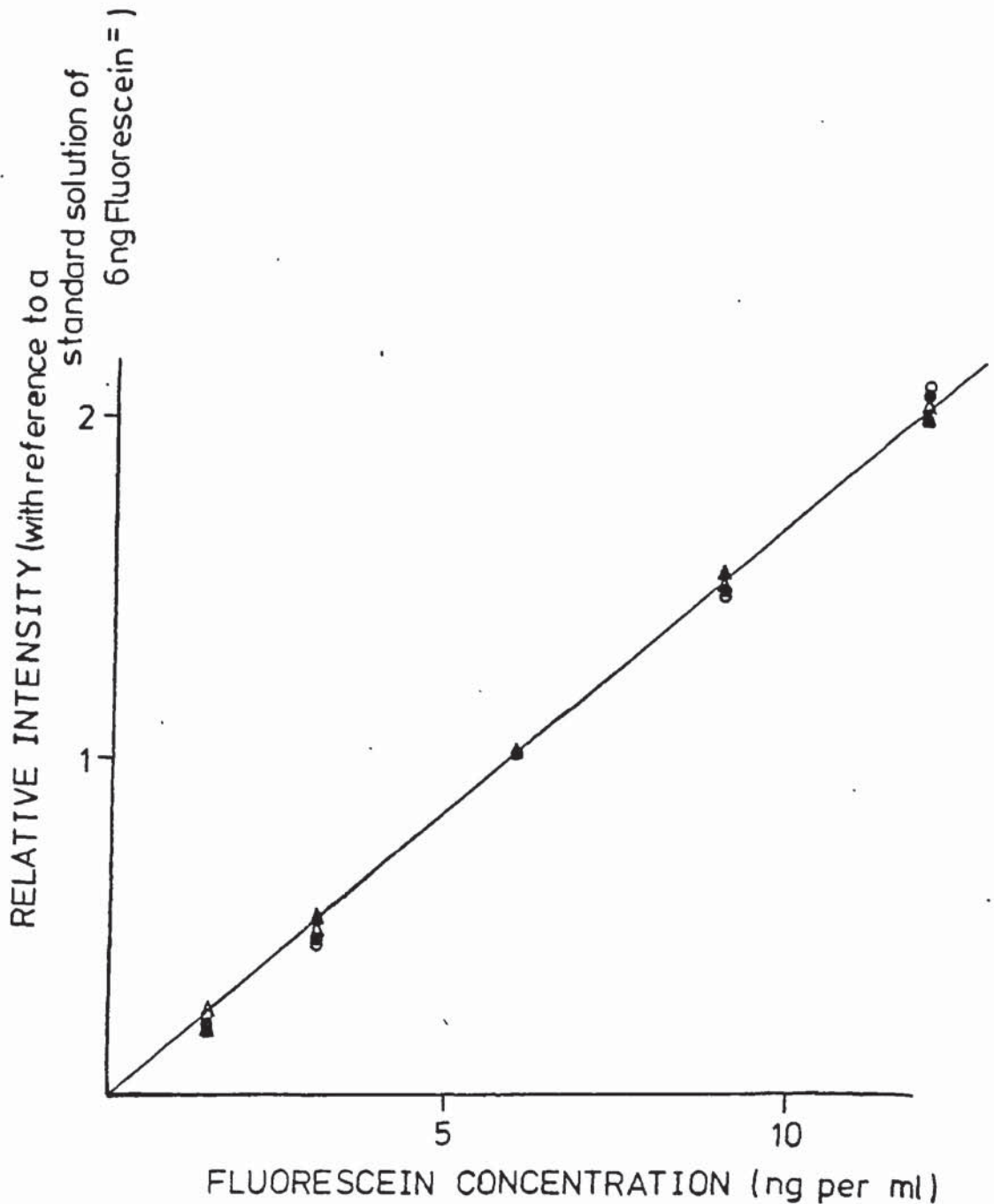
Figure 6.3 Plasma concentration versus time profiles for II after iv administration as solution boli $[Do]_{iv}$ was 0.43mg(o) and 3.4mg (x). The curves are the 'best fit' according to non-linear least mean square (lms) regression analysis based on Equation 6.7 (Section 6.3.2.1).
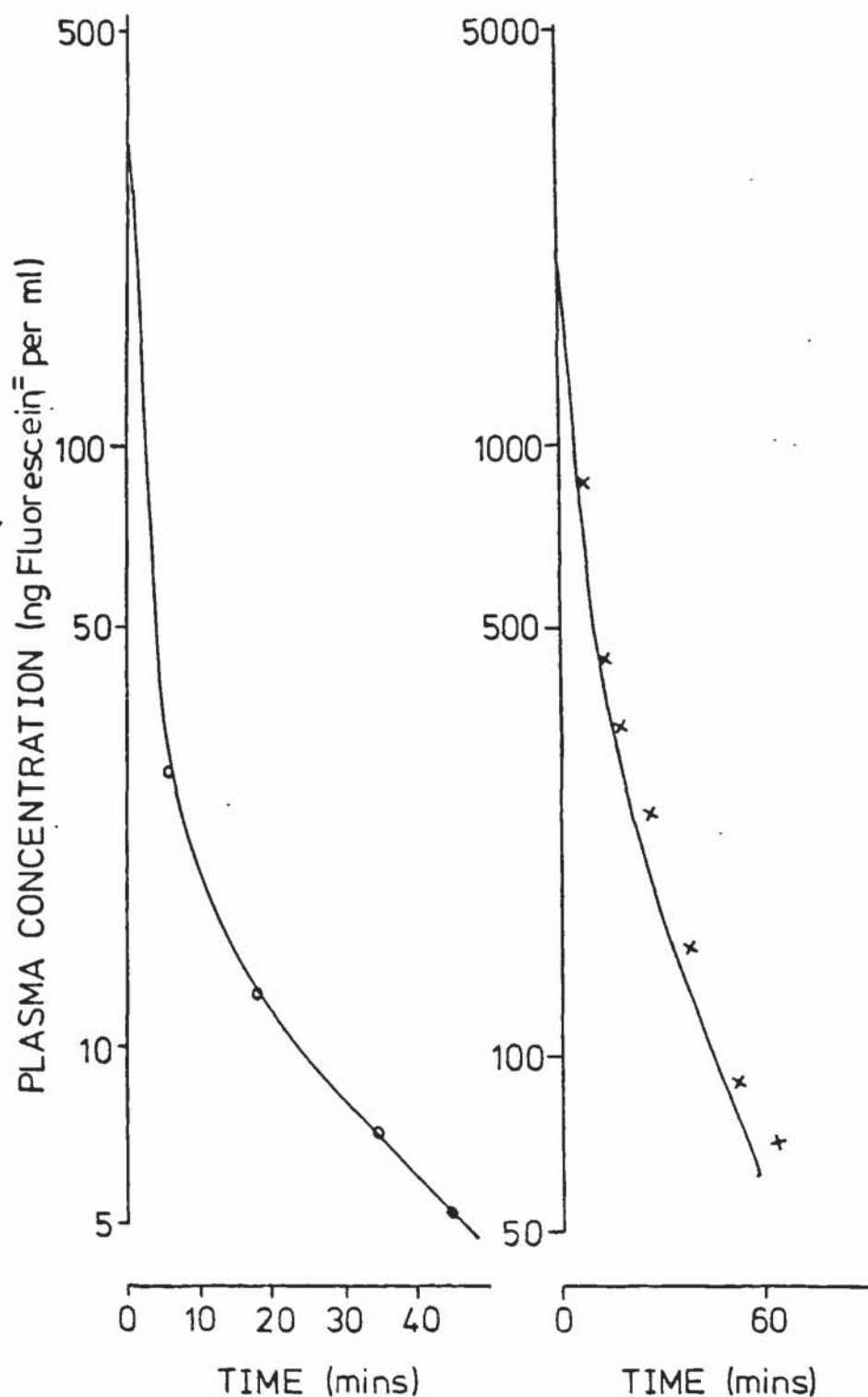
Figure 6.4 Plasma concentration versus time profiles for II after iv administration as solution boli. $[Do]_{iv}$ was 8.2mg (●) and 10.8mg (○). The curves are the 'best fits' according to non-linear lms regression analysis based on Equation 6.7 (section 6.3.2.1).
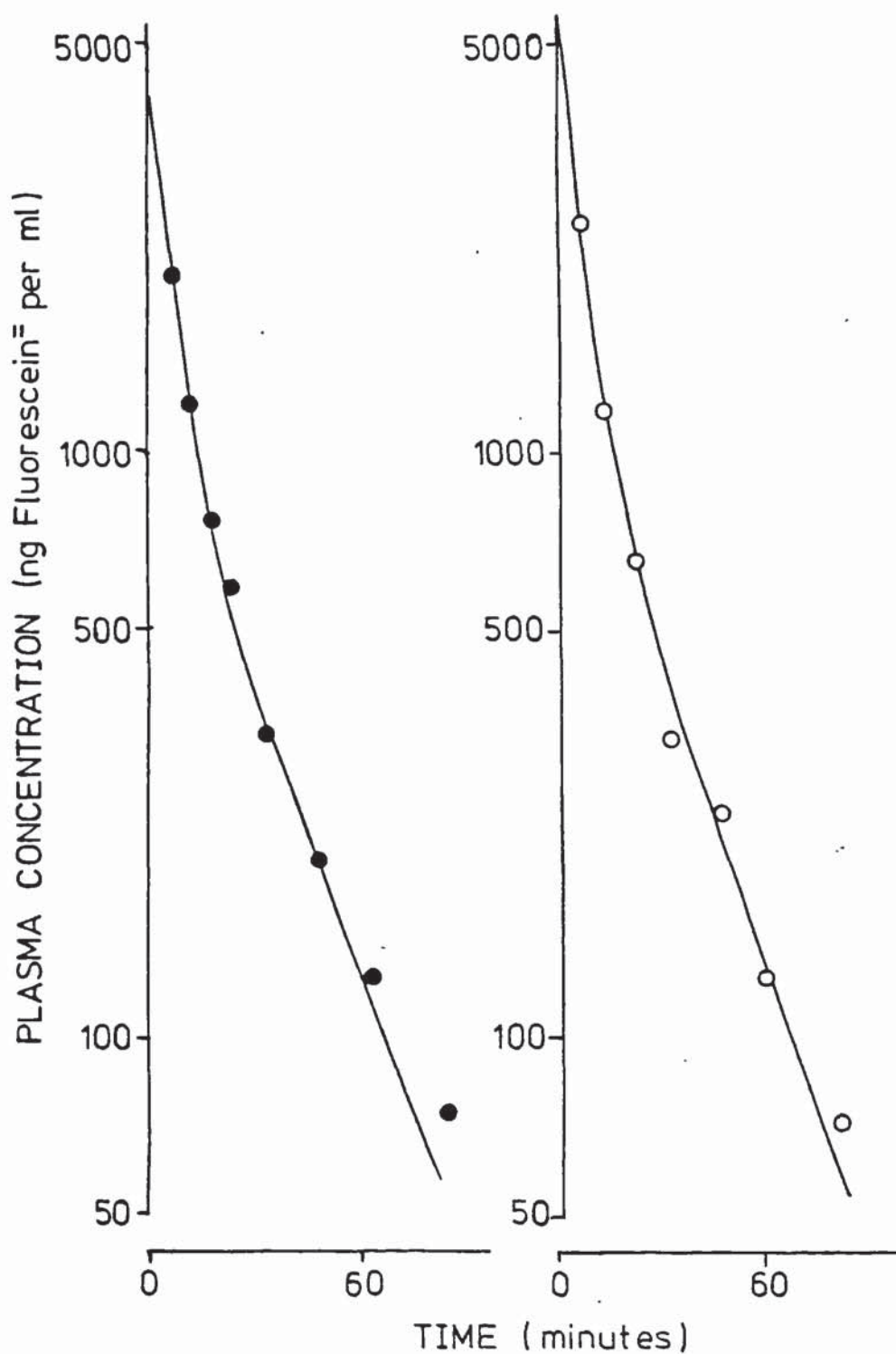
Figure. 6.5 Plasma concentration versus time profile for II after iv administration as solution bolus. $[Do]_{iv}$ was 11.4mg (x). The curve is the 'best fit' according to non-linear lms regression analysis based on Equation 6.7 (section 6.3.2.1).
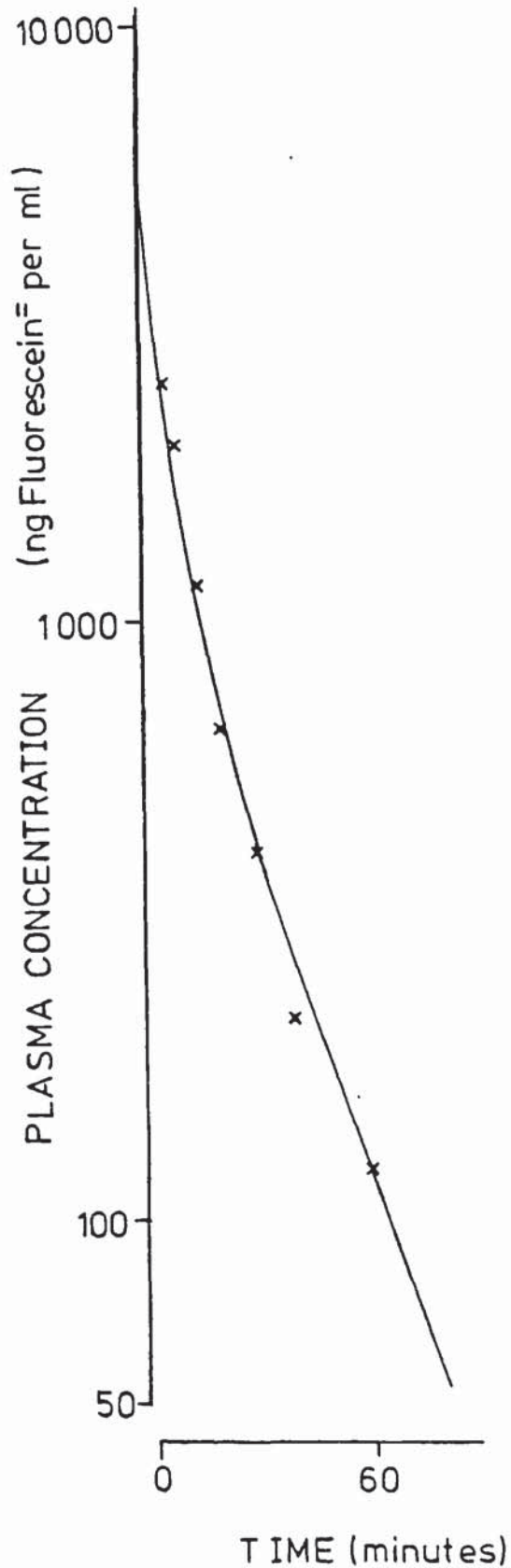
Figure 6.6 Plasma concentration versus time profiles for II after iv administration as solution bolus. $[Do]_{iv}$ was 13.6mg (●), 13.95mg (1st experiment (x) and 13.95mg iv bolus, 2nd experiment (O). The curves are the best fits obtained using an analog computer programme according to Scheme 6.1 (section 6.3.2.1).
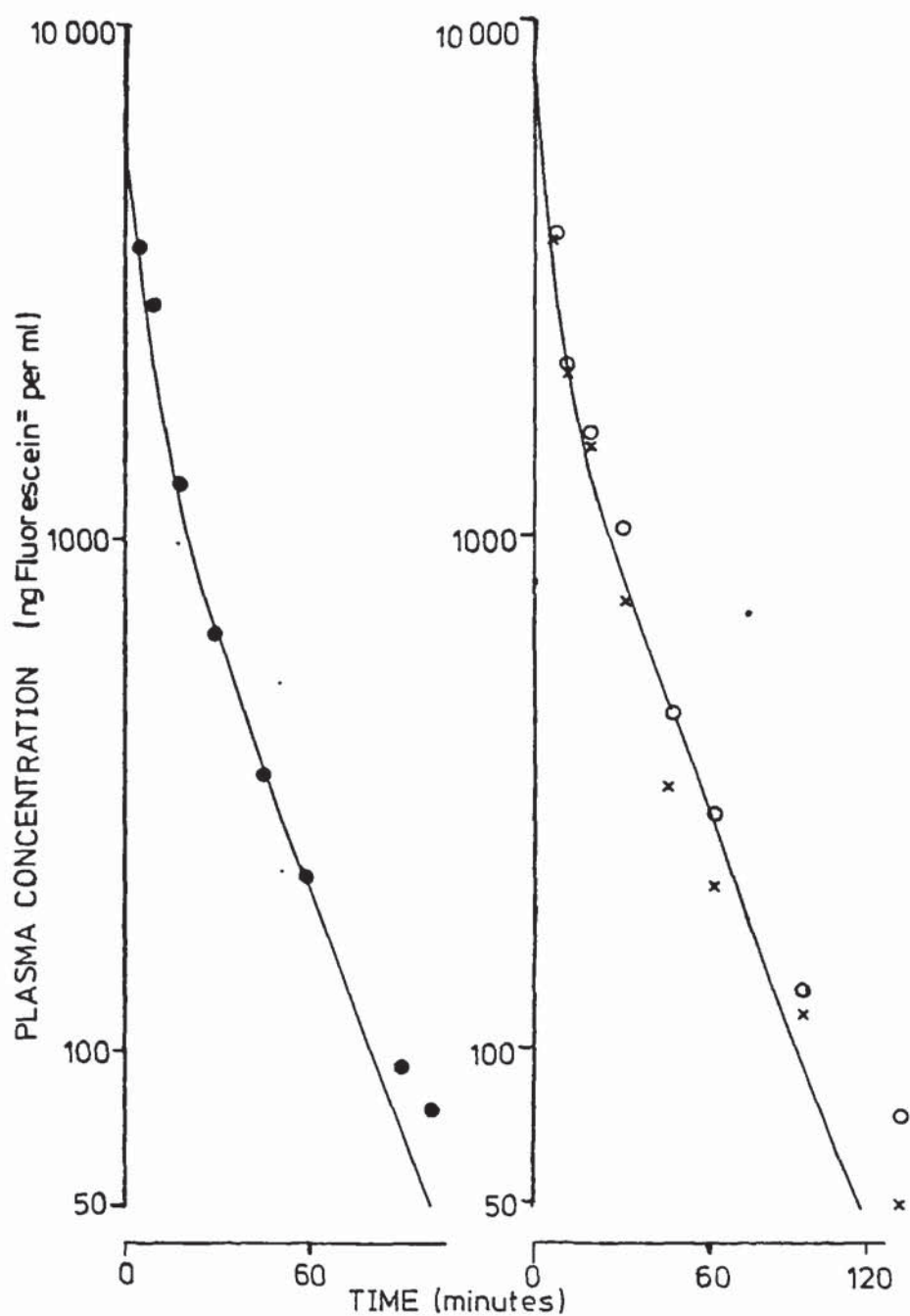
Figure 6.7 Plasma concentration versus time profile for II
after iv administration as solution boli. $[Do]_{iv}$ was 18.0mg
(●) and 33.0mg (O). The curves are the best fit obtained
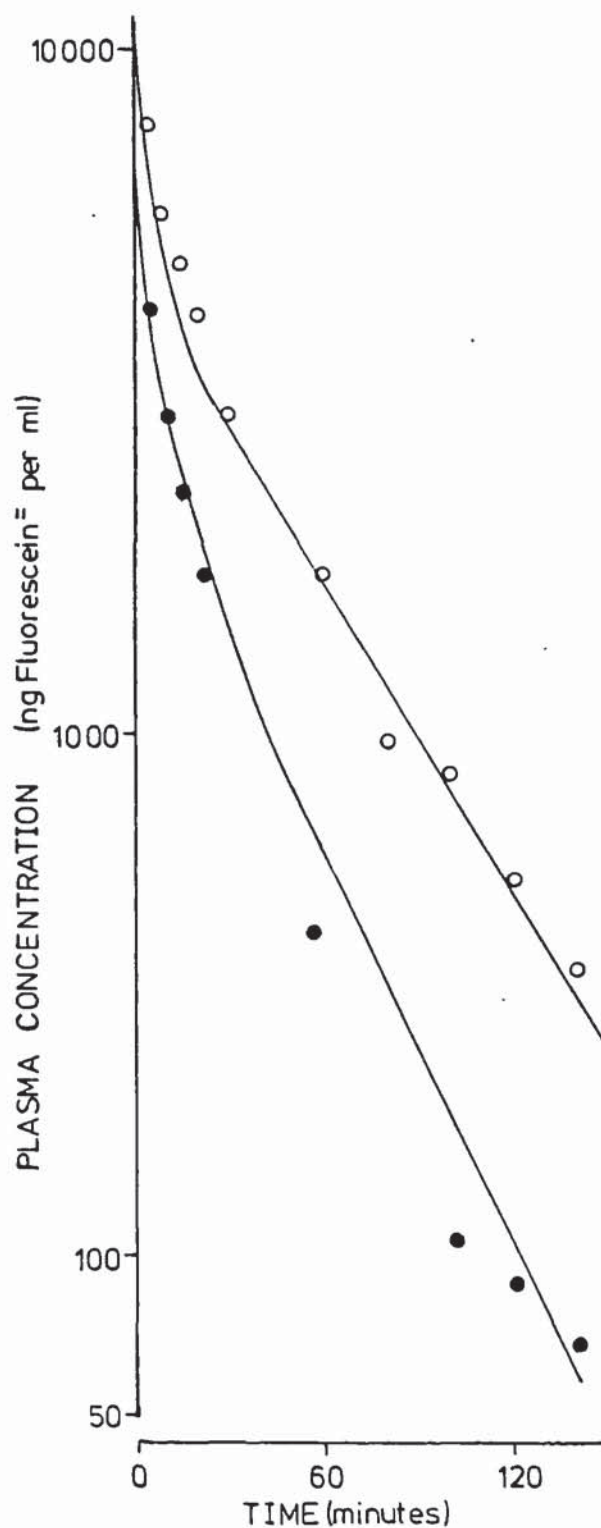using an analog computer programme according to Scheme 6.1
(section 6.3.2.1).

Figure 6.8 Plasma concentration versus time profiles for II after iv administration as solution bolus $[Do]_{iv}$ was 123mg (●). The curve is the best fit obtained using an analog computer programme according to Scheme 6.1 (section 6.3.2.1).
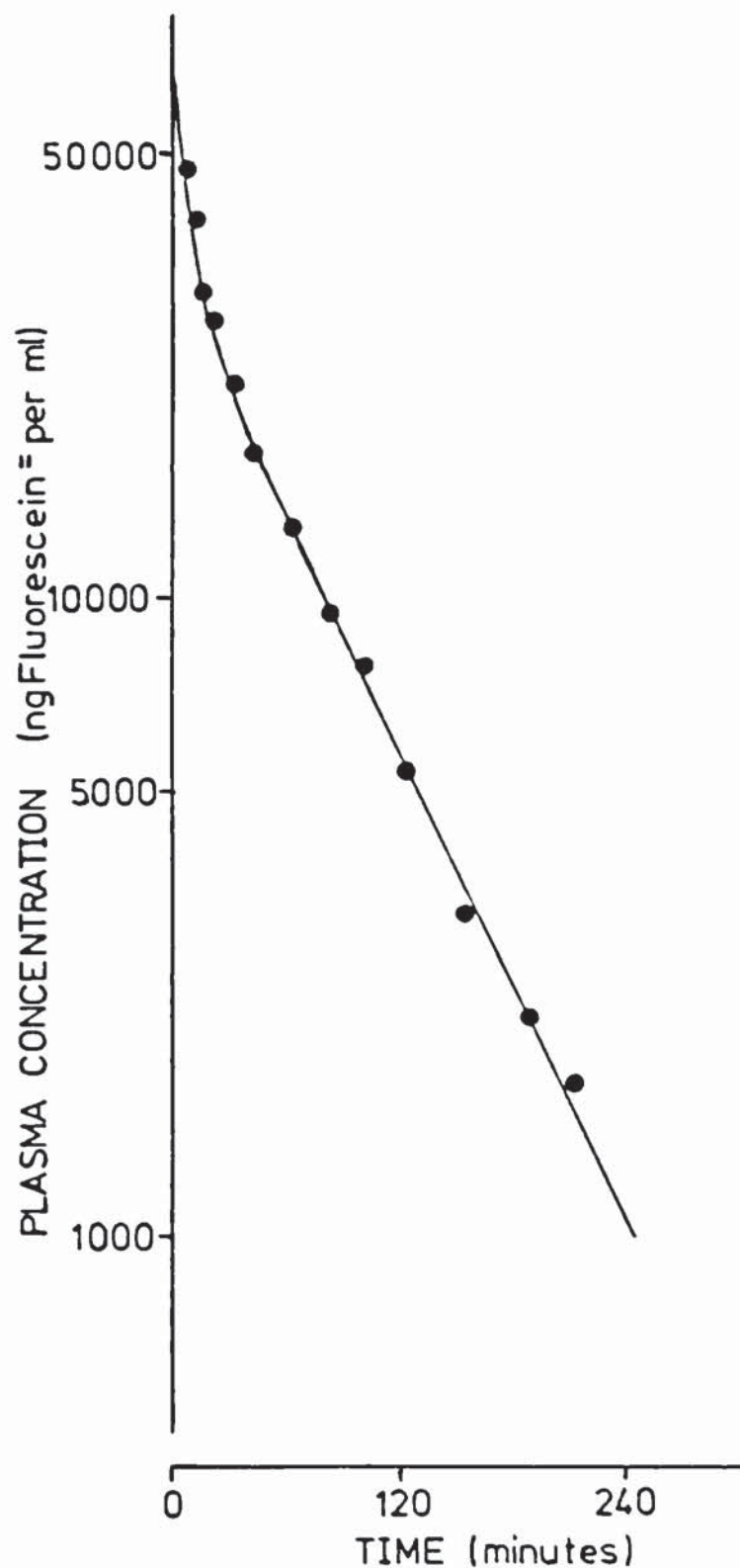
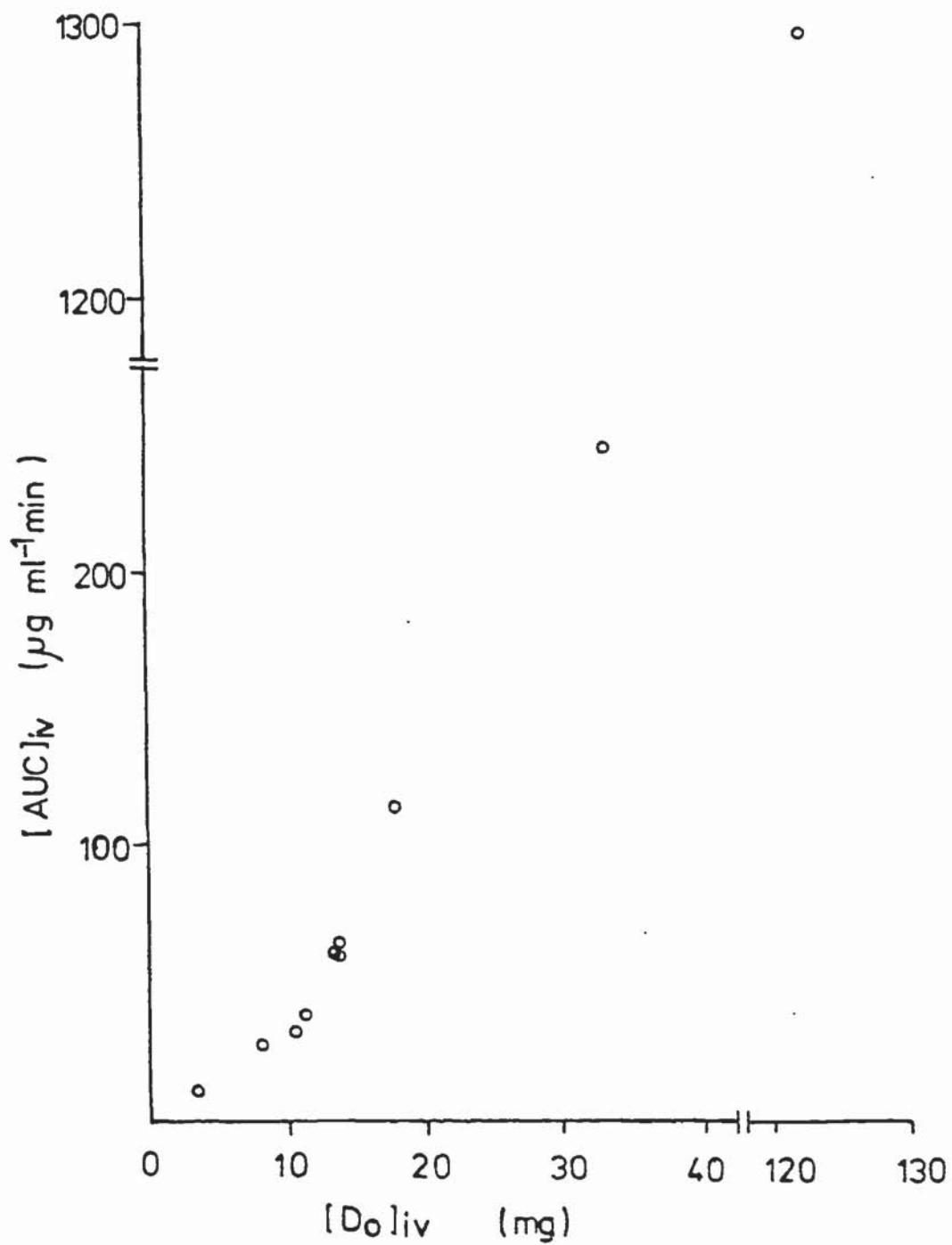Figure 6.9 Area under iv plasma concentration $[AUC]_{iv}^{5 \to 60}$ versus time profiles (t = 5 to t = 60) versus dose $[Do]_{iv}$

Figure 6.10 Plasma concentration divided by dose $(Cp/[Do]_{iv})$ versus time for $[Do]_{iv} \leq 11.4mg$ (data from Figure 6.3 to 6.5). The curve is the 'best fit' according to non-linear least mean square regression analysis based on Equation 6.7.

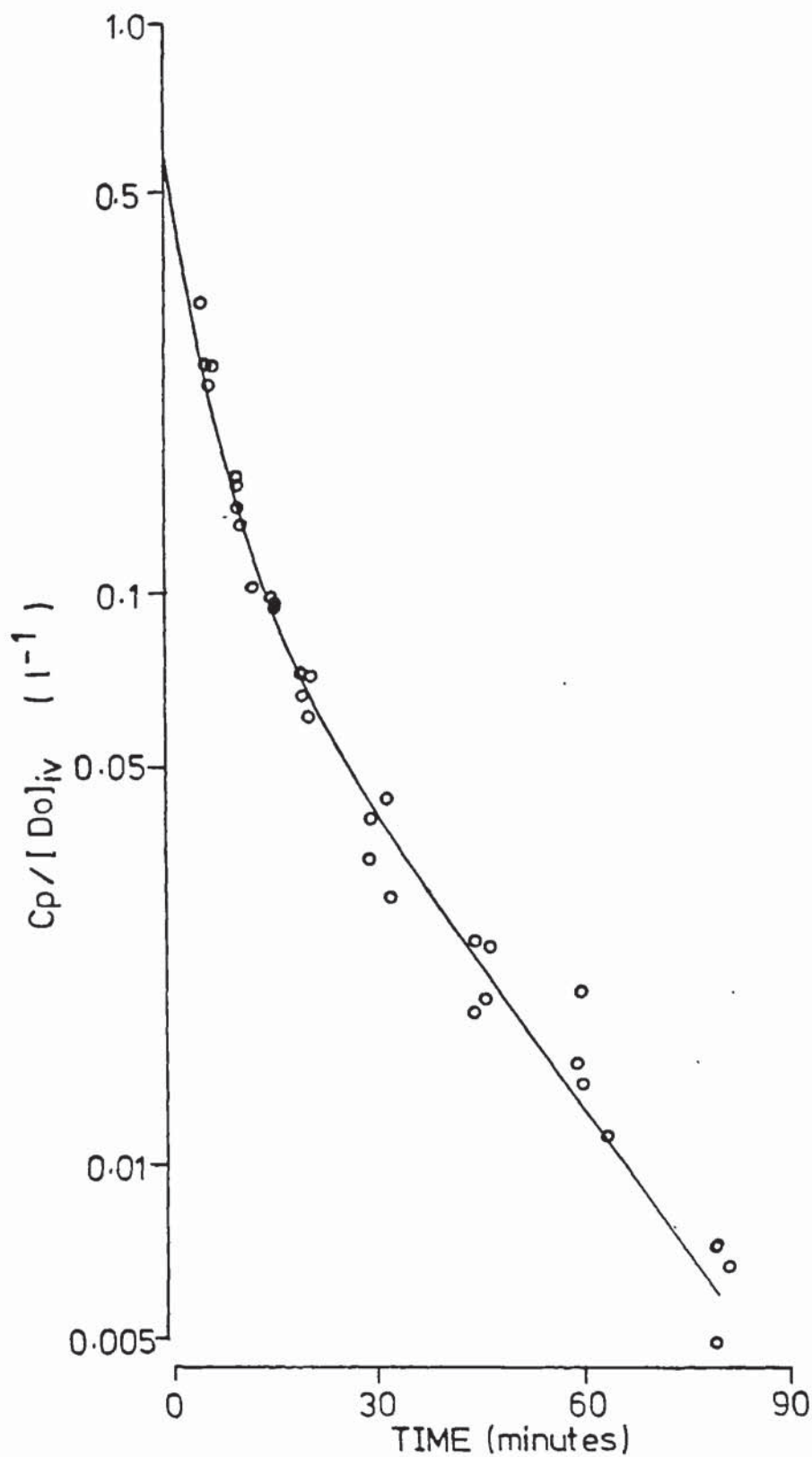Figure 6.11 Total area, $[AUC]_{iv}^{0 \to \infty}$ under plasma concentration versus time profiles as a function of iv dose, $[Do]_{iv}$ when $[Do]_{iv} \leqslant 11.4mg$.
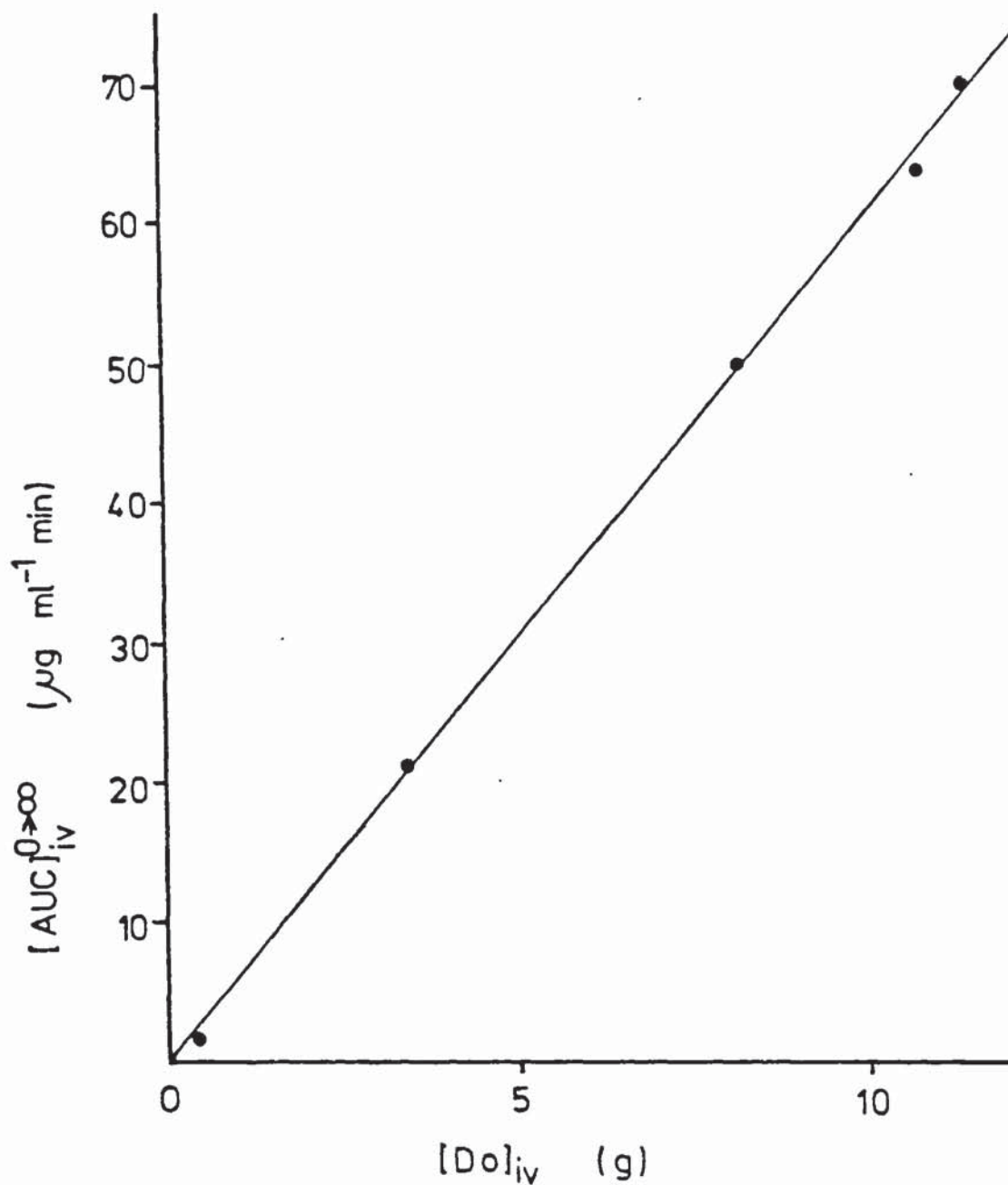
Figure 6.12 Cumulative amount of II excreted in urine after 18mg iv bolus.
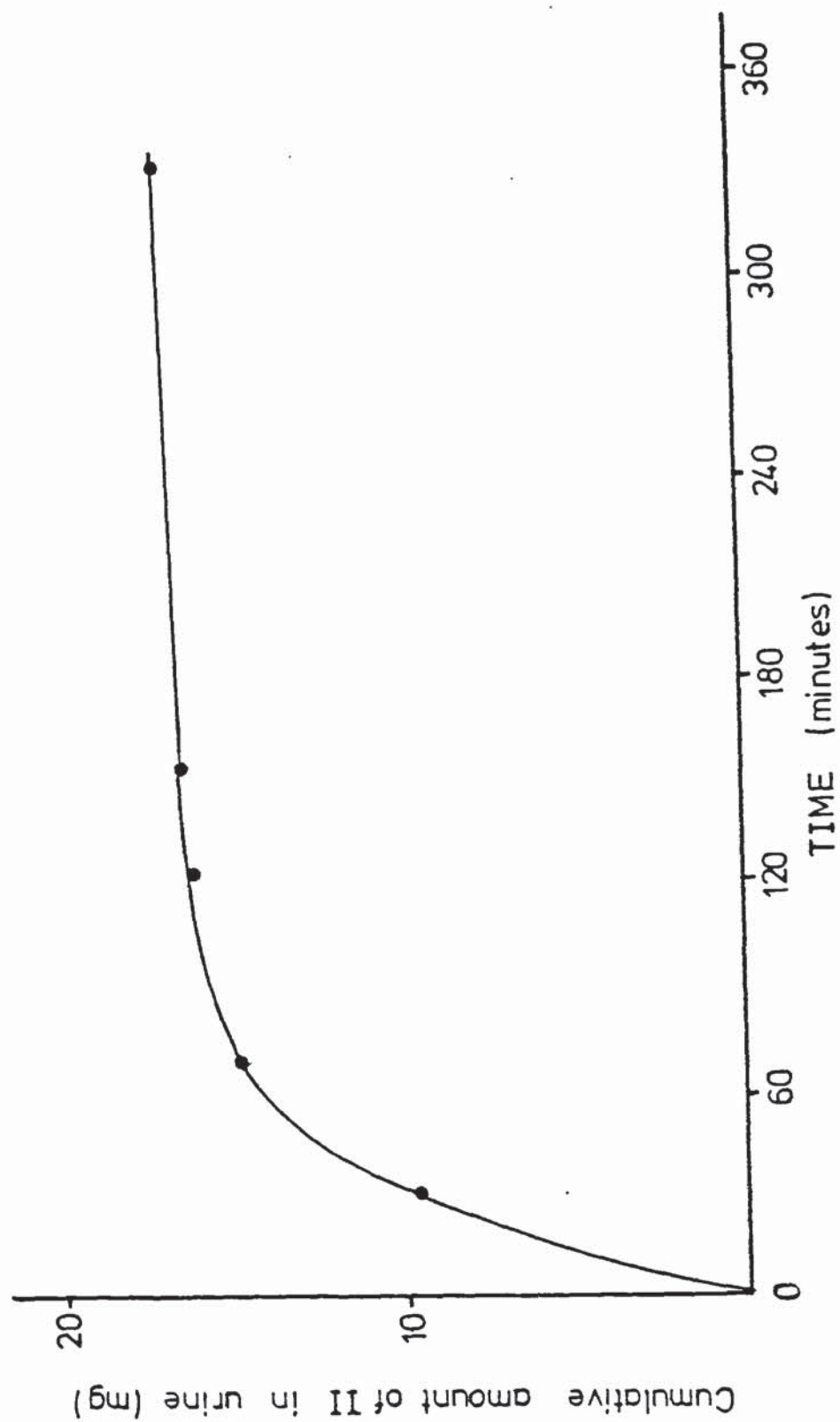
Figure 6.13 Plasma concentration time profile after 0.43mg iv bolus to anaesthetised and conscious dog, o anaesthesia maintained throughout the experiment, ● dose administered to the conscious dog.
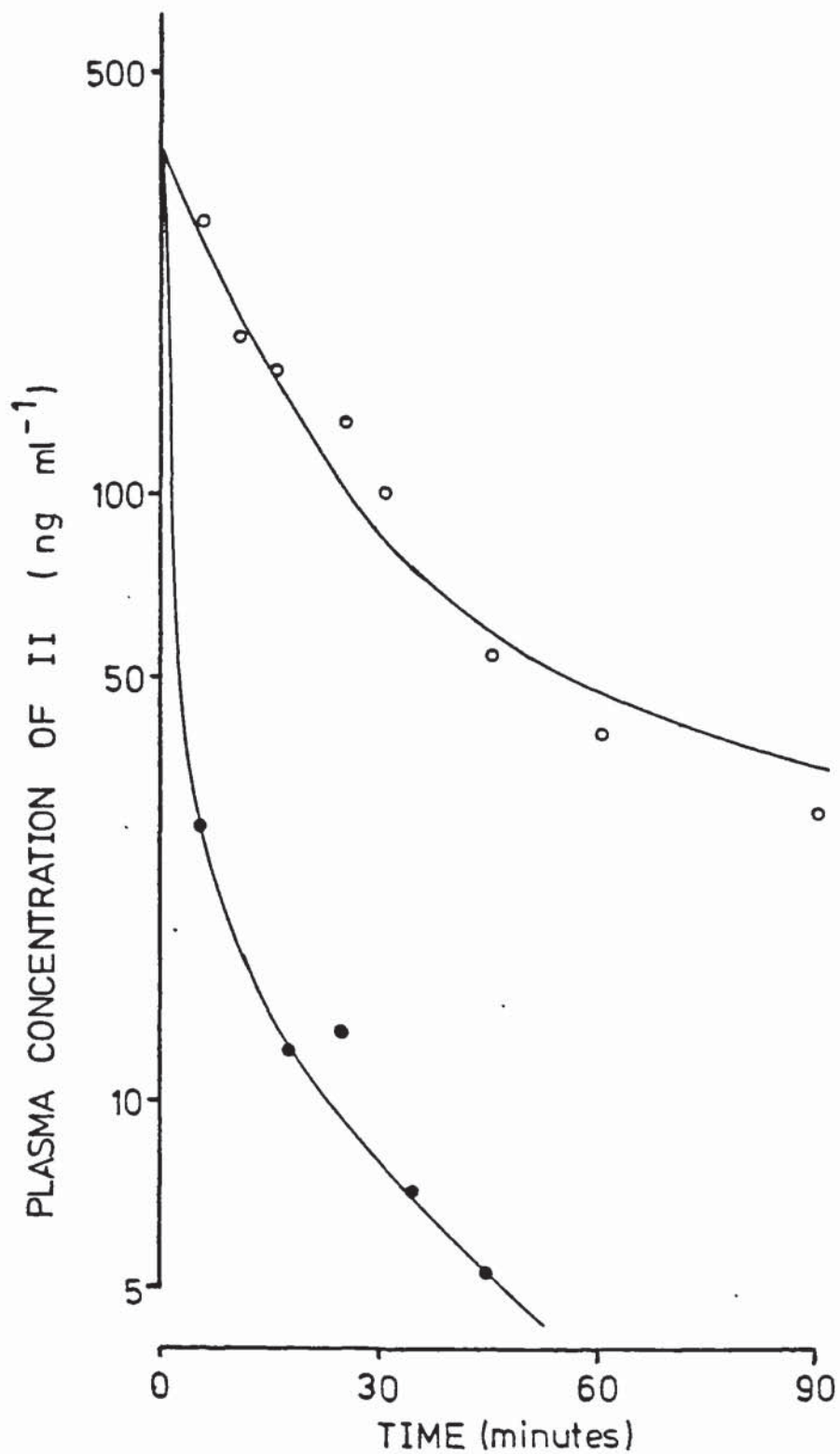
<u>Figure 6.14</u> Plasma concentration of II (linear scale) versus
time profile after administration of 123mg iv bolus.
experimentally determined concentrations, the curve is the
best fit obtained using an analog computer programme to
model Scheme 6.1.

Figure 6.15 Plasma concentration versus time profile for II
$[Do]_{it}$ = 8.6mg intratracheal instillation. The curve illus-
trated shows the trapezoidal approximation used to calculate
the area under the curve.

Figure 6.16 Plasma concentration versus time profile for II after $[Do]_{it}$ = 19.0mg intratracheal instillation. The curve illustrated shows the trapezoidal approximations used to determine the area under the curve.

Figure 6.17 Plasma concentration versus time profile for II
after $[Do]_{it} = 28.2$mg intratracheal instillation. The curve
illustrated shows the trapezoidal approximations used to
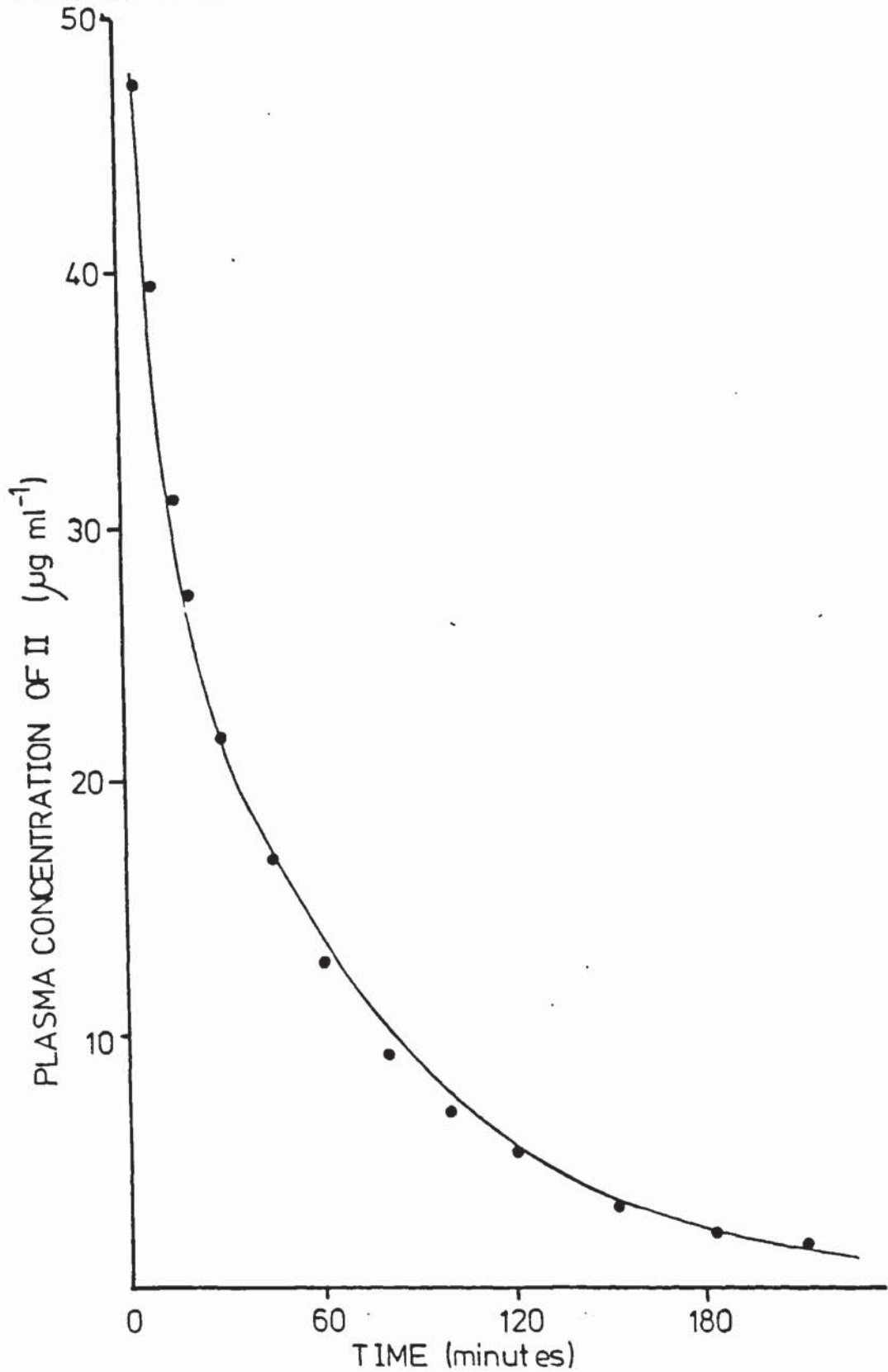determine the area under the curve.

Figure 6.18 Plasma concentration versus time profile for II after $[Do]_{it} \simeq 30.5mg$ intratracheal instillation. The curve illustrated shows the trapezoidal approximations used to determine the area under the curve.
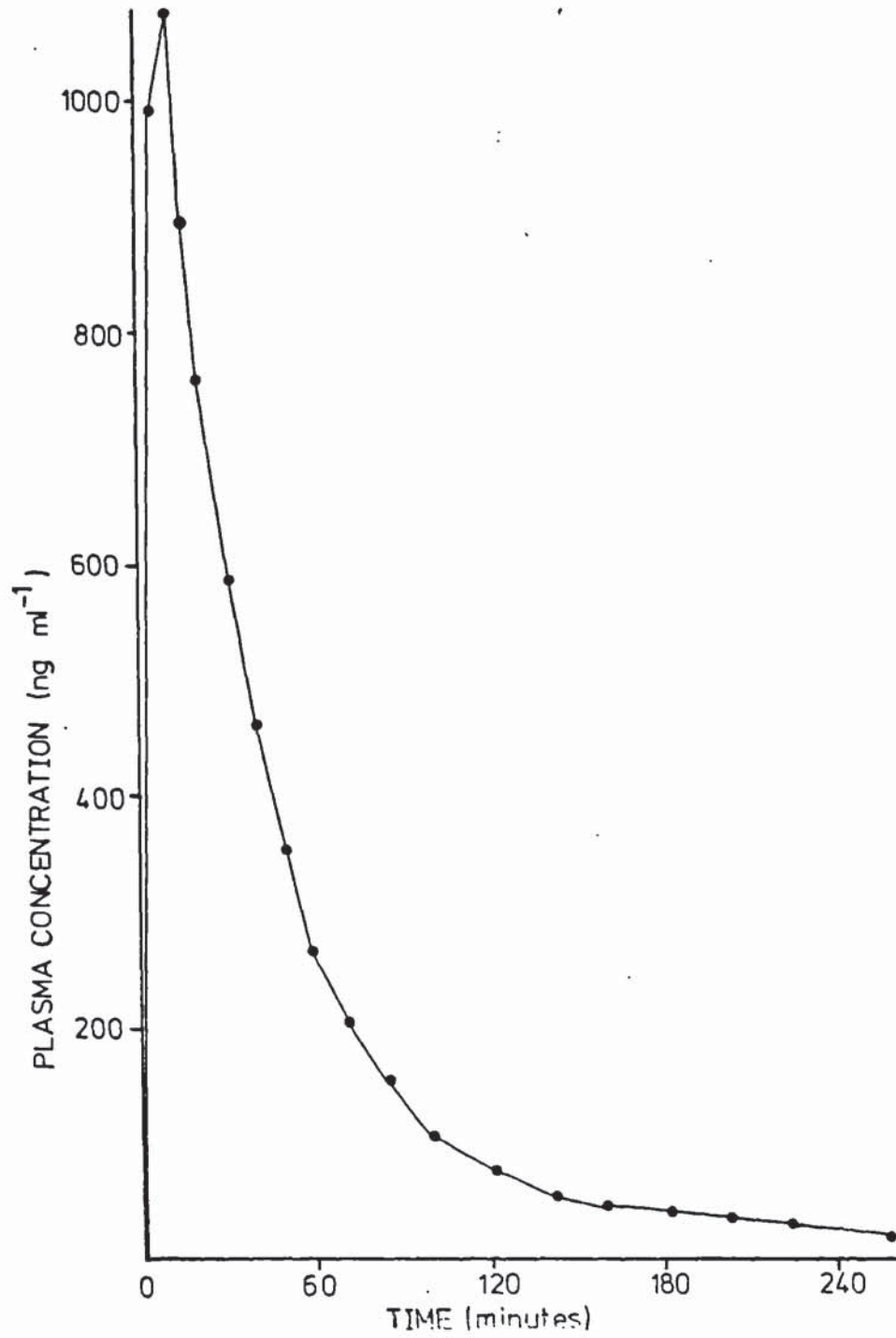
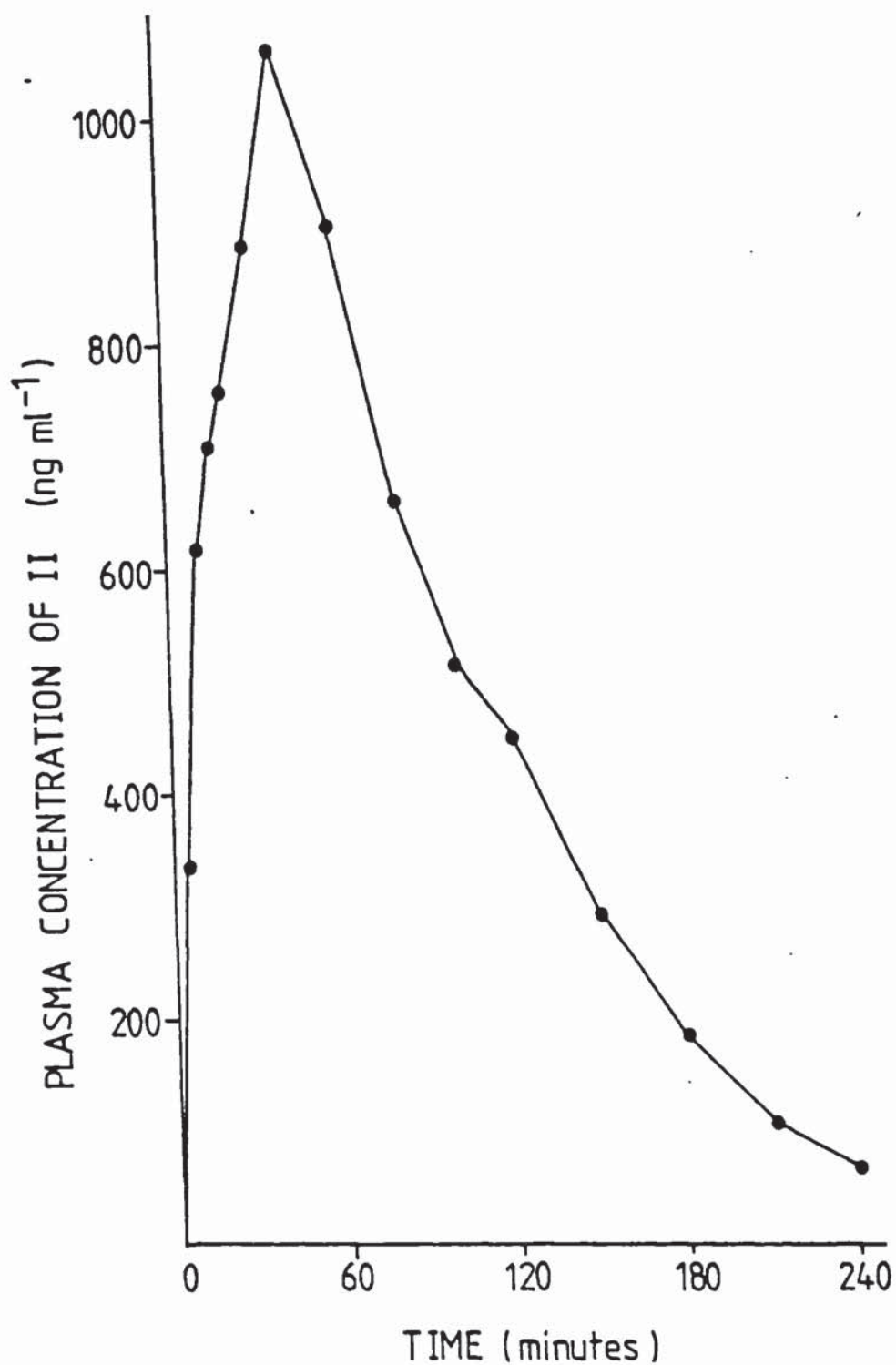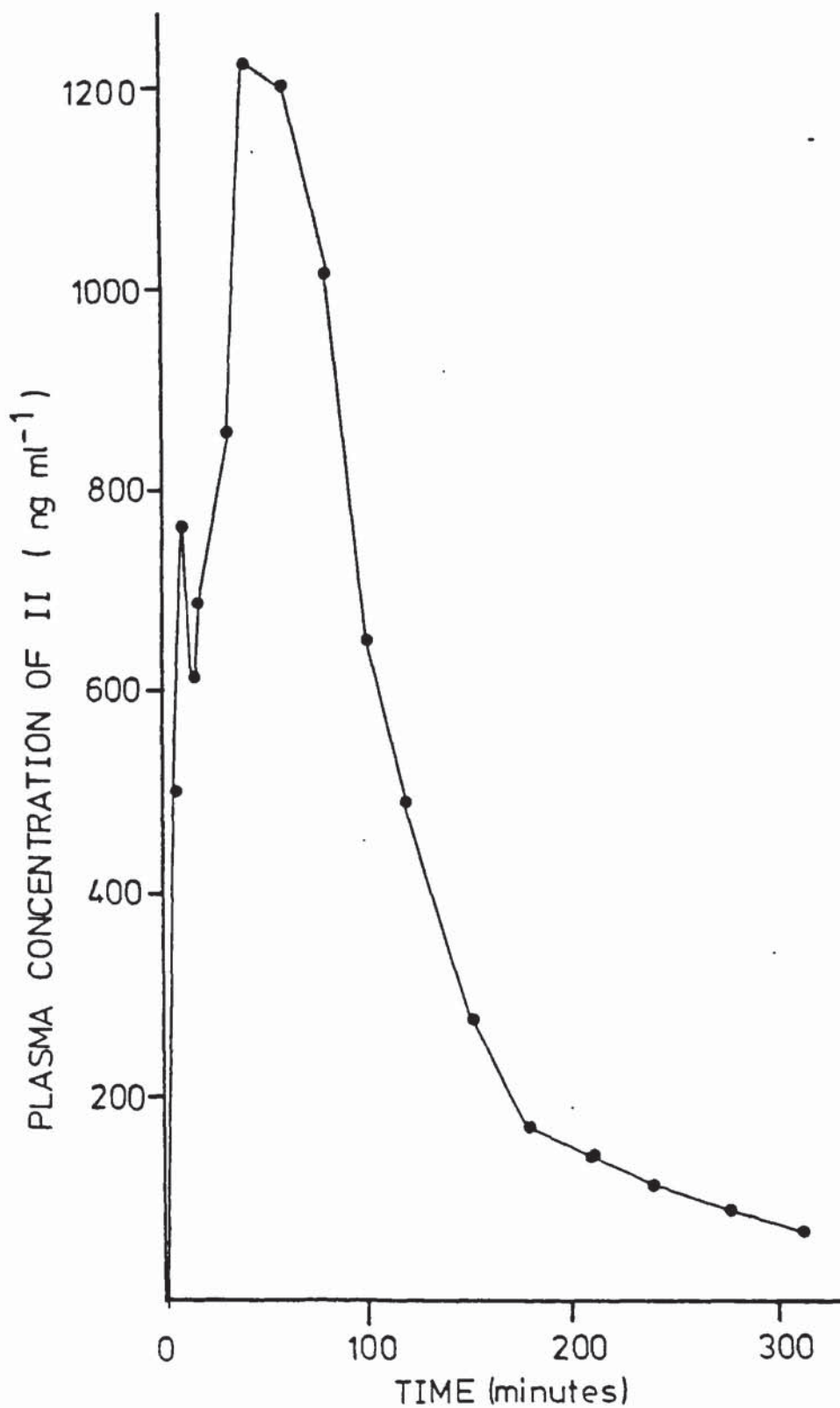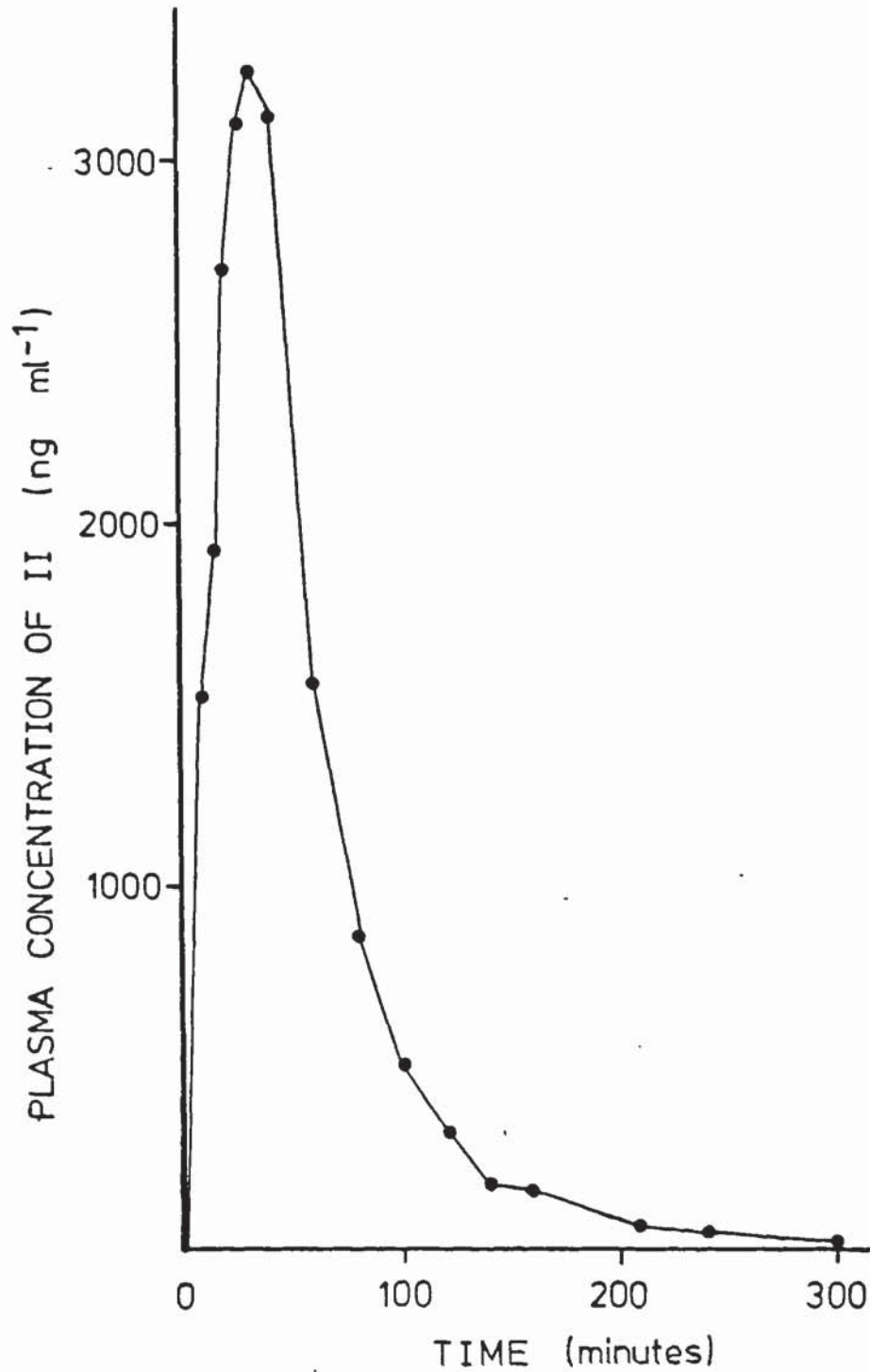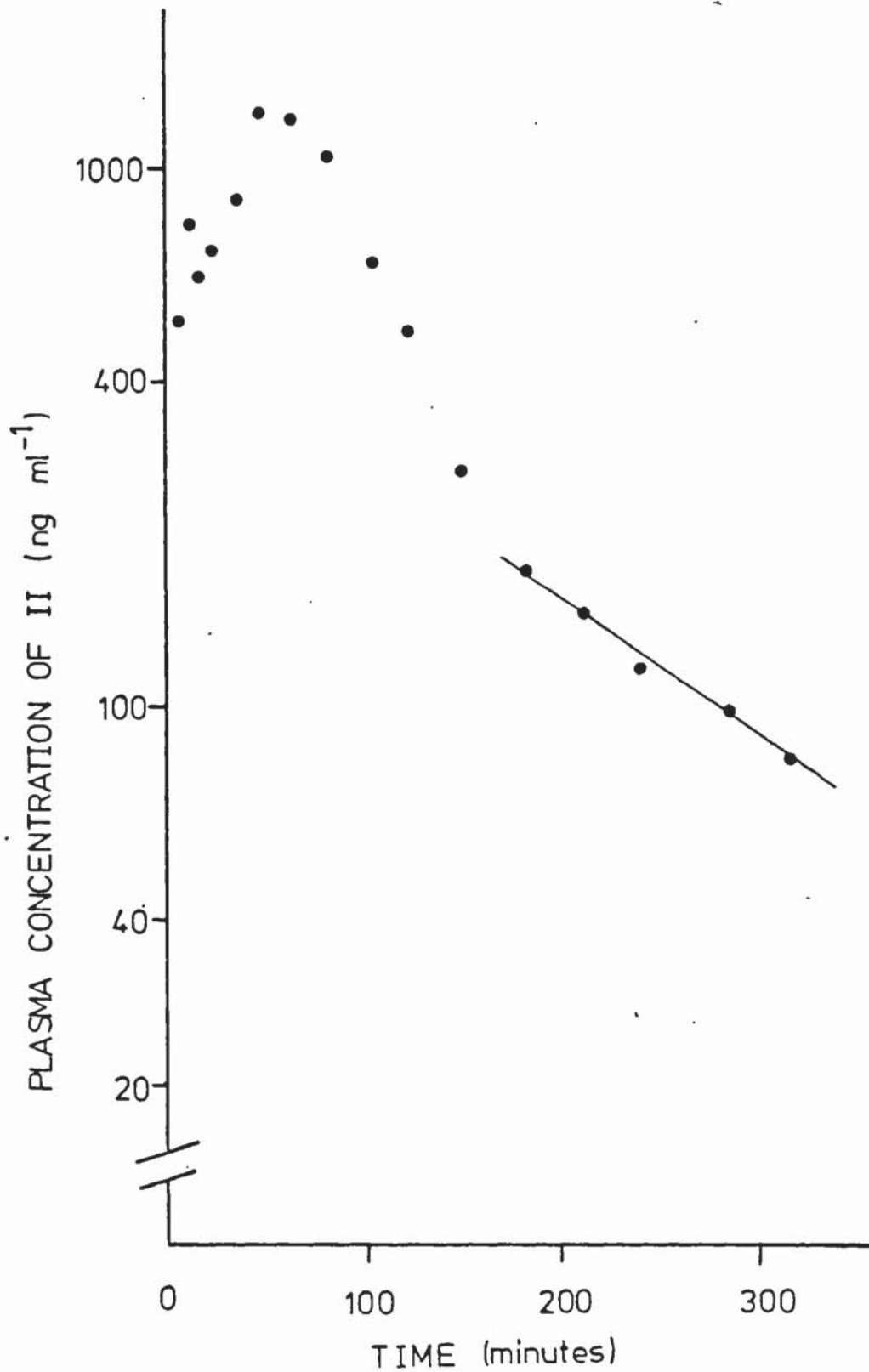Figure 6.19 Log-Linear plot of plasma concentration versus time for II after intratracheal instillation of an aqueous bolus of 28.2mg. The terminal slope S', determined by linear regression is illustrated by the solid line.

Chapter 7   DISCUSSION AND SUGGESTED FUTURE WORK

In these studies an attempt has been made to quantify the
effects of relative humidity on inhalation aerosols. A technique
for calculating the change in aerodynamic diameter of particles
containing a single water-soluble compound from the physico-
chemical characteristics of aqueous solutions of the compound
has been described. The results for sodium cromoglycate,
isoprenaline sulphate, and isoprenaline hydrochloride and
salbutamol sulphate indicate that there are significant deviations
from 'ideal' solution behaviour. This was most marked for
disodium cromoglycate which undergoes a phase transition from
solution to mixed solution and mesophase. This phase change is
indicated by a plateau in water activity. The concentration
at which this phase change occurs has been shown to be temperature
dependent (Cox et al, 1971). Furthermore the water activity
at the plateau is higher than the value for isotonic saline
at 25^0 C whereas the converse is true at 37^0 C. In the light of
this observation it is advisable that determinations of iso-
osmotic equivalents should be made at physiological temperatures.

The most exact expression for calculating aerodynamic
growth ratios takes into account the increased vapour pressure
above curved surfaces, the Kelvin effect. For the compounds
examined the over estimation in growth ratio if the Kelvin
effect is neglected is less than 5% for particles initially
greater than $1 \mu m$ in diameter. As submicron particles are
unlikely to occur in appreciable quantities in most currently
employed inhalation aerosols reasonable estimates for the

aerodynamic growth ratios may be obtained neglecting the Kelvin effect.

The magnitude of the effect of condensation growth on the site and amount of deposition may be determined by the use of computer models for deposition. The results obtained in this study indicate that for different salts of the same compound the site and amount of deposition may occur even if the initial particle size distribution of the dry particles is the same because of differing physicochemical properties. This type of study may be useful in preformulation studies to identify potential problems of poor pulmonary and systemic availability of pharmaceutical aerosols. (Gonda and Byron, 1978).

For more complex inhalation aerosol formulations comprising of water-soluble and insoluble components the behaviour of the aerosol under high humidity conditions cannot be predicted from the physicochemical measurements for pure drug alone. In this instance direct measurement of size changes with varying humidity is desirable. The cascade impactor is a useful sizing tool for polyphasic aerosols, in that by chemical or radio-chemical assay of the deposits it is possible to examine changes in the size distribution of the species of interest i.e. drug.

The experiments described in Chapter 4 have confirmed the observations of other researchers (Rao and Whitby, 1977a, 1977b, Cushing et al, 1979) that a suitable coating on the collection surface is necessary in order to obtain collection efficiency curves that approach 100% collection efficiency. Even with the most efficient slide coating there was some evidence of a decline in collection efficiency after reaching

a maximum value approaching 100%, presumably through rebound
and re-entrainment of the larger particles. In the light of these
observations it would be wise to avoid overburdening of the .
cascade impactor and only collect amounts in the order of the
loading limits suggested by Mercer (1966).

Appreciable differences were observed between the experimental
calibration performed at operating flow rates and the calibration
supplied by the manufacturer. These observations indicate the
necessity of calibration at the operating flow rates.

In practise it is difficult to achieve the degree of
temperature control required to maintain the relative humidity
experienced within the respiratory tract. For example in order
to obtain the growth ratio of a single water-soluble compound
with an accuracy of 5% at 99.5% relative humidity would require
temperature control within $\pm$ 0.015^0 C at 37^0 C (Gonda et al,
1981). Correspondingly, studies on the effect of relative
humidity on the particle size of aerosol are performed at sub-
physiological humidities. The temperature in the controlled
temperature and humidity cabinet used in these studies could
be controlled to $\pm$ 0.05^0 C at 37^0 C. The major problem in the
use of this apparatus arises from the addition of carrier gas
bringing aerosol into the equilibrium chamber perturbing the
previously established humidity. However good correlation was
observed between the observed growth ratio for fluorescein
disodium aerosols and that predicted from physicochemical
measurements of aqueous fluorescein disodium solutions. A
more constant humidity level could be achieved by the use of a

continuous mixing system and a reduction in carrier gas entering the equilibrium chamber.

It may be possible to retard condensation growth by the inclusion of water-insoluble films on aerosols. Surface active films have been found to retard droplet evaporation rates by several orders of magnitude (Snead and Zung, 1968; Eisner et al, 1960). The compounds used to potentially retard condensation growth on pharmaceutical aerosols must be carefully considered on toxicological grounds. The lungs are coated with an natural surfactant, lecithin, which may prove satisfactory from both a physical and toxicological view point in preventing or retarding condensation growth. The effect of humidity on coated particles could be examined using a controlled temperature and humidity apparatus. Differences in growth ratios will however only be observed if the coat retards the rate of condensation growth by at least an order of magnitude.

Using the data presented in chapter six the systemic availability of fluorescein disodium subsequent to its administration in various inhalation aerosol formulations may be estimated There is some evidence that the rate of absorption may vary with the site of deposition (Schanker, 1978), it would therefore be expected that the sytemic availability of fluorescein disodium effectively coated with a condensation retarding agent will differ from the untreated dry powder.

Appendix 1

Water activity of solutions used to calibrate the vapour pressure osmometer.

Sodium Chloride

Concentration mol kg^{-1}	Water activity at 25° C	Reference	Water activity at 37 C	Reference
0.0064	.9997757	1	-	
0.0256	.9991204	1	-	
0.0576	.9980498	1	-	
0.1	.996646	2	.9966 4(9)	
0.2	.993360	2	.9933 6(6)	
0.3	.99009	2	.9900 9(5)	linear interpolation
0.4	.98682	2	.9868 (15)	between data from
0.5	.98355	2	.9835 (3)	references 2 and 3 (50° C
0.6	.98025	2	.9802 (2)	data)
0.7	.97692	2	.976(8 8)	
0.8	.97359	2	.9735 (3)	
0.9	.97023	2	.970 (1 6)	
1.0	.96686	2	.9667	3
2.0	.9316	2	.9312	3
3.0	.8932	2	.8926	3
4.0	.8515	2	.8510	3
5.0	.8068	2	.8068	3
6.0	.7598	2	.7606	3

Potassium Chloride

Concentration (mol kg^{-1})	Water activity at 25° C	Reference
0.1	0.996668	2
0.2	0.993443	2
0.3	0.99025	2
0.4	0.98709	2
0.5	0.98078	2
0.6	0.98078	2

Calcium Chloride

Concentration (mol kg^{-1})	Water activity at 25° C	Reference
0.1	0.99540	2
0.2	0.99073	2
0.3	0.98590	2
0.4	0.98086	2

<u>Sucrose</u>

Concentration (mol kg^{-1})	Water activity at 25° C	Reference
0.1	0.99819	2
0.2	0.99635	2
0.3	0.99449	2
0.4	0.99260	2
0.5	0.99068	2
0.6	0.98874	2
0.7	0.98676	2
0.8	0.98475	2
0.9	0.98272	2
1.0	0.98065	2

<u>References</u>

1. Robinson and Stoke 1959a
2. Robinson and Stoke 1959b
3. Gibbard et al 1974

Appendix 2

Calibration curves for the Knauer vapour pressure osmometer Percentage full scale deflection versus negative natural logarithm of water activity

Temperature	Sensitivity setting	Slope	Intercept	Correlation coefficient
25	2	337	+ 1.29	0.998
25	16	3329	- 0.8	0.998
25	32	6754	- 0.4	0.998
25	64	14506	- 1.2	0.999
37	2	412	+ 1.8	0.999
37	32	8434	- 1.5	0.999

APPENDIX 3 DESCRIPTION OF DABRA 79

Proportional Power Controller

Proportional controllers are those which deliver a variable fraction
of their maximum power output. The actual amount of power depends upon
an error voltage - the larger the error, the greater the power output.
The power output corresponding to zero error voltage can usually be adjusted
over a range from 1 to 99% of full power, though the ideal state would be
near 50%.

The magnitude of the error voltage is compared with a ramp (sawtooth)·
waveform. In the case of the L121 zero voltage switch, which is the control
element in this design, the ramp waveform started about 1 volt (referenced
to mains NEUTRAL), and increases to about 6 volts before being reset to 1
volt and starting again. The time taken for one ramp cycle determines the
resolution of the power control - when the error voltage is less than the
ramp voltage, the power is on, and when the error voltage is greater, the
power is off. With a one second ramp cycle time, the smallest amount of
power that can be let through is one half mains cycle, the largest amount
being 99 half cycles out of the 100 occurring in that second. The other
alternatives are that the power is fully off, or fully on - so no control
is possible.

External components determine the gain properties of the operational
amplifier that is used to produce the error voltage, and the frequency of
the ramp oscillator. These components, together with a detector for turning
the parameter to be controlled into a suitable current or voltage, make up
the control part of the circuit. The two other parts of the circuit are
the power supply for the zero voltage switch, comprising one power resistor
as a mains dropper and two smoothing capacitors, and the triac protection
which comprises a R-C network which limits the maximum rate of rise of
voltage on the triac terminals. The triac itself, is a semiconductor switch
which, once triggered, stays on until the current passing through it drops
below the holding current. Since mains power is alternating, the current
should change polarity every 20 milliseconds or so, and this turns the
triac off. The R-C network is also intended to make sure that the triac does
turn off. The zero voltage switch will then turn the triac on again while
the voltage is close to zero in accordance with the error signal/ramp
voltages. This technique reduces the amount of radio frequency interference
reflected into the mains supply, and also reduces the amount of switching

work that the triac has to do- giving a lower power dissipation and better reliability.

In this design, a thermistor is used to detect temperature in such a way that an error voltage varying from 1 volt at 70°C to 6 volts at about 28°C. This determines the range of the controller. It is important to realise that an alteration in the temperature will produce an alteration in the output power. This will tend to bring the temperature back towards its control value, but an alteration in the amount of power required to maintain a given temperature - say a draught - will alter the control setting required to maintain the desired temperature. It is only possible to adjust the range between fully on and fully off by adjusting the gain of the operational amplifier. A high gain will give a narrow range, but any drift in component values will be accentuated as well. If in doubt, - use a thermometer!

WARNING: This device is not isolated from the mains. Thermistor connected to mains NEUTRAL. Heater power output is mains LINE and switched NEUTRAL.

Analog computer circuitry to model two-compartment open body model
(Scheme 6.1)
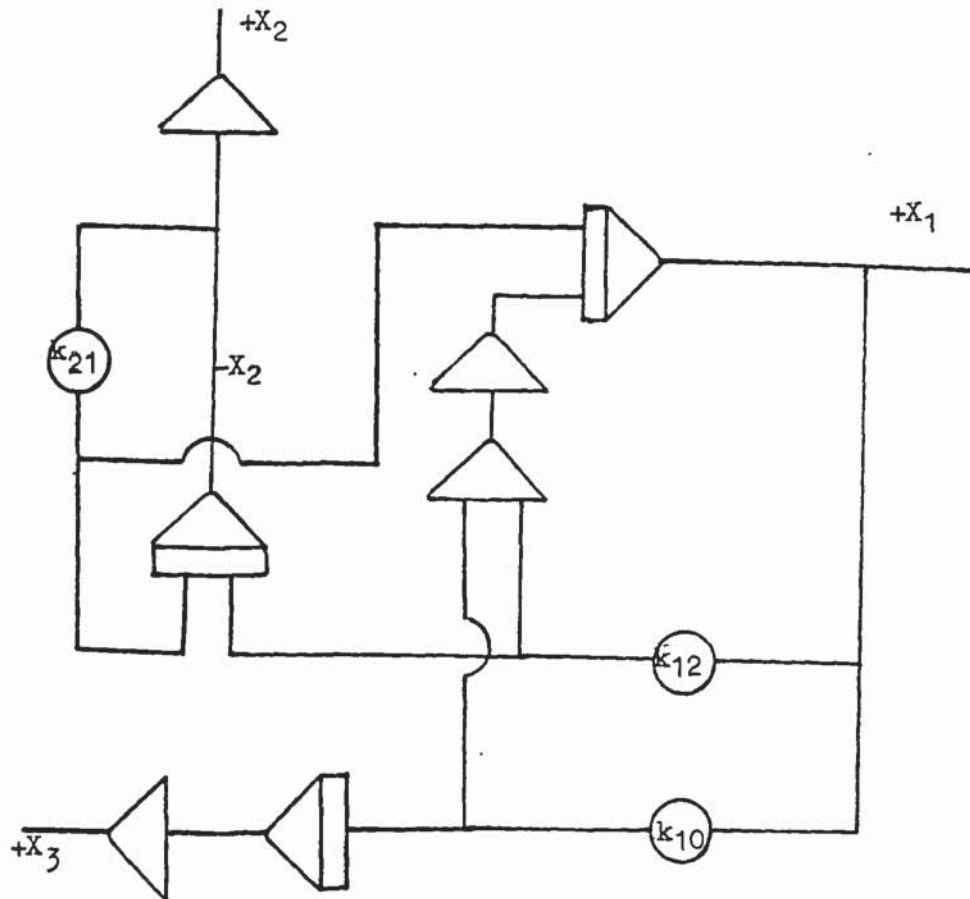
○   Potentiometer

△   Operational Amplifier

⬠   Integrator

Appendix 5   Publications

C.V. Groom, I. Gonda and F J T Fildes
'Prediction of equilibrium aerodynamic diameters of inhalation aerosols'
2nd Int. Conf. Pharm. Technol., APGI, Paris 5 p 124-131, 1980

C .V. Groom and I. Gonda
'Equilibrium diameters of inhalation aerosol droplets'
J. Pharm. Pharmacol. 32 (Suppl) 1P, 1980

C.V. Groom and I. Gonda
'Cascade impaction: the performance of different collection surfaces'
J. Pharm. Pharmacol. 32 (Suppl) 93P, 1980

I, Gonda and C.V. Groom
A data inversion method for sizing using a calibrated cascade impactor
in International symposium on Deposition and clearance of Aerosols in the
Human Respiratory Tract' GAF and IGAeM, Bad Gleichenberg, Austria, 22/23
May, 1981.

I. Gonda, JB Kayes, C.V. Groom and FJT Fildes
'Characterisation of hygroscopic inhalation aerosols'
to be presented at Particle sizing conference, Loughborough September,
1981.

A.R. Clark, PR Byron and C.V. Groom
'Fluorescein pharmacokinetics in the beagle'
to be presented at British Pharmaceutical Conference September 1981.