CHAPTER THREE

RADIOLABELLING WITH GAMMA RADIONUCLIDES

CHAPTER 3 - RADIOLABELLING WITH GAMMA RADIONUCLIDES

3.1 INTRODUCTION

The incorporation of a γ -emitting label into an aerosol formulation permits detection and measurement of respiratory deposition patterns from outside the body. In the present study, three different approaches were used to incorporate a radiolabel into the aerosol formulation. The *in vivo* aerosol deposition patterns were detected by a gamma camera using radionuclides suitable for this purpose.

3.1.1 Selection of radionuclides

Fig. 3.1 illustrates the problems associated with selection of a radionuclide with a gamma energy high enough to provide good tissue penetration, but not so high that it may not be readily attenuated. When fitted with appropriate collimators, most gamma cameras will provide useful data over a wide energy range (50-400keV), but the optimum gamma energy range is 100-200 keV. The detection characteristics of the Anger-type gamma camera are such that spatial resolution is lost if very low energy radionuclides are used. Similarly, sensitivity is lost at the high end of the available energy range. Kelly (1981) has reviewed the range of radionuclides available for scintigraphic studies and Table 3.1 lists those most commonly used. Technetium-99m, which has ideal properties for gamma camera work (Gottschalk, 1969), was used almost exclusively in the present studies for labelling aerosols. The physical and biological half-lives of technetium-99m and the lack of α and β -radiation ensure a minimum radiation dose for studies of a few hours duration. The general properties, uses and preparation of this radionuclide are given in Appendix 3.1.

Fig 3.1

Selection of a Radionuclide with a Suitable Gamma Energy for Optimum Detection.

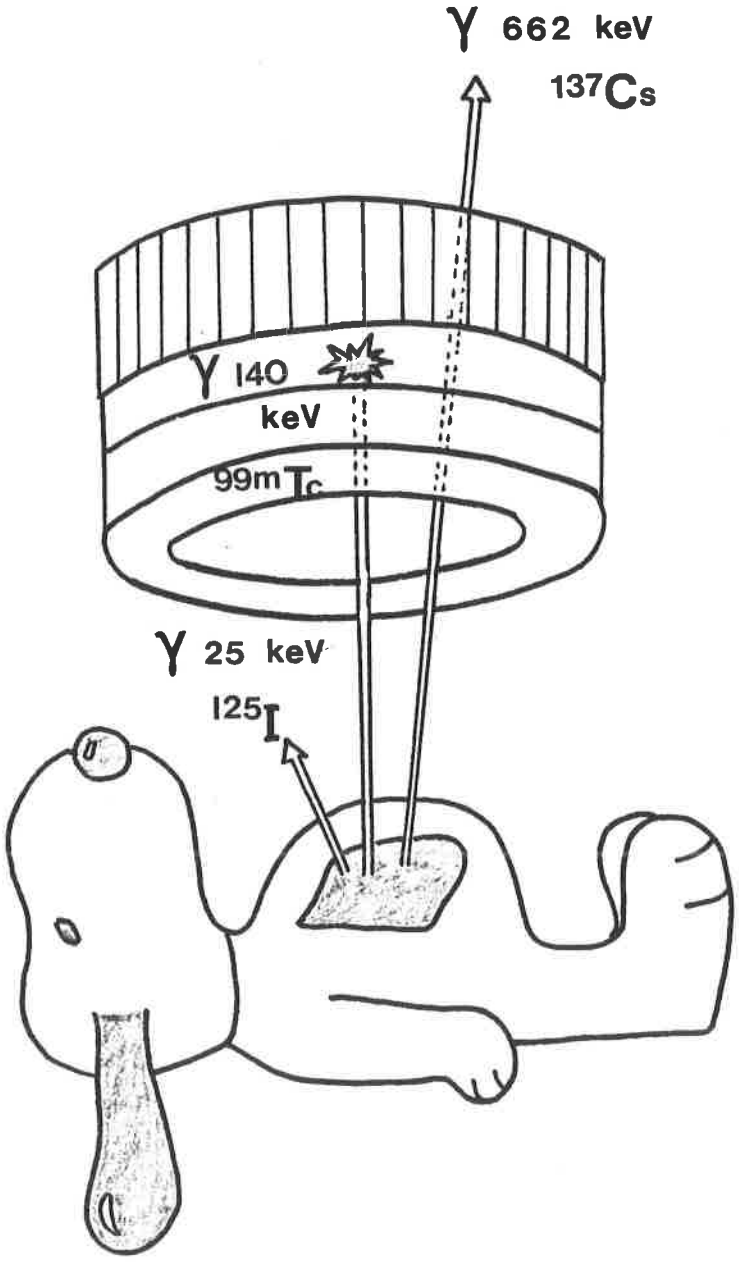


Table 3.1 Radionuclides Commonly Used for Imaging and their Physical Properties.

NUCLIDE	HALF-LIFE	GAMMA ENERGY (KeV)
Krypton-81m	13s	190
Indium-113m	1.7h	393
Technetium-99m	6.0h	140
Iodine-123	13h	159
Indium-111	2.8d	173
		247
Gallium-67	3.2d	93
		184
		297
		388
Xenon-133	5.3d	81
Iodine-131	8.1d	364
Selenium-75	120d	122
		136
		269
		281

3.1.2 Selection of radiolabelling methods

The γ-labelling work in the present study falls into three categories:

(i) Labelling of human serum albumin microspheres with ^{99m}Tc.

(ii) Establishing the validity of labelling the excipient oleic acid for salbutamol aerosol formulations.

(iii) Labelling salbutamol by indirect methods such as complexation and salt formation.

The initial *in vivo* studies used γ-labelled human serum albumin (HSA) millimicrospheres which were nebulised as a suspension in saline. The millimicrospheres were used to establish the biological variability of aerosol deposition in the animal models used. The advantages of HSA included the availability of kits containing ready-made millimicrospheres which were physiologically inert and a stated fine size for adequate lung deposition. Exploratory methods for preparation of larger size microspheres were undertaken to provide suitable aerosols to establish the particle size differentiation in the *in vivo* deposition patterns.

In this study the term 'microspheres' refers to particles of human serum albumin generally, but the term 'millimicrospheres' refers to the albumin particles generated from a kit (CIS (U.K.) Ltd. Kit No. TCK-9) which has a published particle size of 0.5μm.

The introduction of a γ-emitting label into aerosol formulations containing the most widely used bronchodilator drug salbutamol was the main objective of the present study. Indirect methods of studying regional aerosol deposition and drug distribution have used tritiated salbutamol (Walker

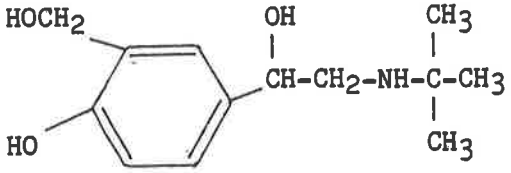
et al., 1972b), autoradiography (Barnes et al., 1982) and clinical measurement of respiratory functions (Godfrey et al., 1974; Ruffin et al., 1981). These studies have indicated that β -adrenoceptors probably lie chiefly in the smaller conducting airways. γ -radiolabelled bronchodilators may be employed in more direct methods of establishing receptor sites and metabolic pathways. The lack of published studies using this type of compound is due to the difficulty in γ -radiolabelling the drug molecules, because of their molecular chemistry. Fig. 3.2. shows the molecular structure of several bronchodilator drugs including salbutamol. The molecules contain only elements such as carbon, nitrogen and oxygen. Radionuclides of these elements are produced from a cyclotron and have half-lives which are too short for practical use in this case. (e.g. Carbon-11, $t_{\frac{1}{2}}$ = 20.5min.). The introduction of radionuclides of other elements into the drug molecule cannot be accomplished without changing the physical, chemical or biological properties of the drug.

As an alternative to labelling the drug molecule itself (either directly or indirectly) the radionuclide may be incorporated into an excipient present in the aerosol formulation. However, it must first be shown that the excipient is uniformly distributed amongst the drug particles in the aerosol cloud so that the in vivo deposition patterns are valid. This approach was considered for radiolabelling the aerosol delivered from a metered-dose inhaler containing salbutamol. The oleic acid present in this formulation as a surfactant may be labelled with radioactive iodine. (Lubran & Pearson, 1958).

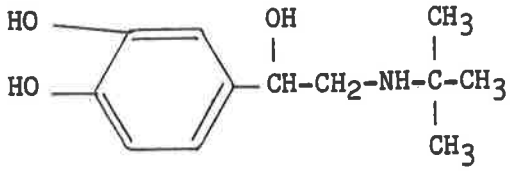
Metered-dose inhalers containing bronchodilator drugs are very widely used as an effective, selective and convenient treatment for asthma. However, few studies have, until recently, indicated techniques of use which should

Fig. 3.2 Molecular structure of bronchodilator drugs.

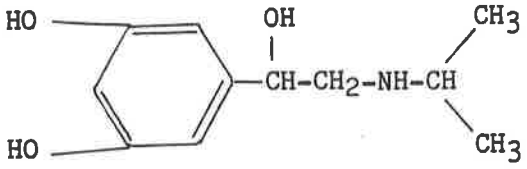
SALBUTAMOL



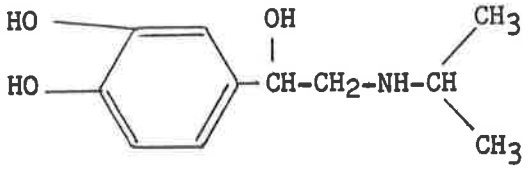
TERBUTALINE



ORCIPRENALINE



ISOPRENALINE



optimise lung deposition and hence clinical efficacy (Newman, 1983). The third approach to γ-radiolabelling in the present study was designed to produce an aerosol of salbutamol particles, radiolabelled with technetium-99m, from a metered-dose inhaler(MDI). Several methods for labelling salbutamol aerosol formulations were studied including cocrystallisation, chelation complex formation and salt formation.

MDI's were used primarily in the present study because of their dominance in aerosol therapy and because little published work describes the assessment of *in vivo* deposition of aerosols from these devices. This is particularly true for drug aerosols, where the particles can be hygroscopic. A recent study (Short *et al.*, 1981) describes the use of radiolabelled ipratropium bromide (Br^{77}) in MDI's and lung deposition studies with these in humans. A similar technique could not be used in the present study because of the lack of suitable cyclotron-produced radionuclides for salbutamol.

3.1.3 Materials

Indium-113m sterile generator, 50mCi, from Amersham International plc, Amersham, Bucks.

Tetraphenylarsonium chloride from Aldrich Chemical Co. Ltd., Gillingham, Dorset.

HSA millimicrospheres kits no's TCK-5S, TCK-9 and Technetium -99m sterile generator, 1 Ci, from CIS Ltd. Commisariat a L'Energie Atomique, Gif-sur-Yvette, France.

Stannous chloride, $SnCl_2.2H_20$, Analar grade. BDH Chemicals Ltd., Poole, England.

Priolene 6952 (oleic acid) from Unichema Chemicals Ltd., Bebington, Merseyside.

Human serum albumin and glutaraldehyde from Sigma Chemical Co., USA.

Propellants 11 and 12 from ICI, Macclesfield, Cheshire.

Salbutamol, B.P. B/N 82145 from Glaxo Operations (UK) Ltd.
Other chemicals used were laboratory grade.

3.2 HUMAN SERUM ALBUMIN

3.2.1 Introduction

The usual method of preparation of human serum albumin microspheres is based on the techniques of Zolle et al. (1970). This involves emulsification of aqueous albumin solution in a suitable oil and heat denaturation of the albumin particles. However, microspheres produced by this technique are too large for inhalation studies (usual range 10-150μm). In addition, time-consuming washing with organic solvents is required to remove the oil, and many process variables, which are difficult to control, affect the quality of the product. The same disadvantages are present in the technique published by Tomlinson et al. (1982), which uses glutaraldehyde instead of heat for protein denaturation.

Aldrich & Johnson, (1974) produced microspheres smaller than about 10μm by a spinning disc generator, using heated oil for denaturation. They manufactured particles of 3-4μm by this technique with the addition of an updraught of warm air to allow the airborne particles to dry before collection.

The spinning disc technique produces particles of a narrow size range, but the method is lengthy and difficult and the labelling process is tedious and inefficient. (Rhodes et al., 1971).

To avoid the lengthy washing processes, Przyborowski et al. (1982) heat denatured the microspheres by allowing them to settle under gravity in a 2 metre high oven, but the particles produced were too large for inhalation purposes.

Exploratory methods using the spinning disc generator and heated oil for denaturation were studied, in an attempt to produce microspheres of a mean particle size by weight of 5-6μm.

The manufactured kit containing ready-made HSA millimicrospheres was repeatedly used for the initial *in vivo* deposition studies. The kit (no. TCK-9, CIS Ltd.) contains ready-made particles of albumin in a sealed, sterile vial. The stannous chloride present in the kit reduces the technetium, which is introduced as sodium pertechnetate in a saline eluate from a generator. (see Appendix 3.1, for details). The reduced technetium readily complexes with the albumin, and a dispersing agent and antioxidant promote a stable suspension of labelled HSA. The manufacturers claim a particle size distribution having 90% less than 1μm and a shelf-life of 8 hours for the reconstituted, labelled product.

3.2.2 Methods

3.2.2.1 HSA Millimicrospheres

A single vial of millimicrospheres, containing 2mg albumin, was used for each *in vivo* experiment. 5ml of eluate (sodium pertechnetate $Na^{+99m}TcO_4^-$ in saline) from a technetium generator was added and the vial shaken vigorously for one minute. The resulting suspension was placed in the CIS nebuliser (see Chapter 2) for subsequent administration. The activity used in the final nebulised suspension was approximately 2mCi/ml and 10mCi/ml for the rabbit and dog experiments respectively.

Table 3.3 PMS light-scattering instrument measurements of particle size distribution of HSA millimicrospheres

Experiment	mass mean diameters (μm)	
	d_{gn}	d_{gw}
A	0.67	8.21
B	0.42	7.61
	0.39	6.53
	0.41	6.87
	0.40	6.10
	0.38	5.86
C	0.62	4.54
D	0.35	1.13
	0.35	1.15
	0.35	0.97
	0.35	1.33
E	0.35	3.66

Table 3.4 Measurement of free activity present in a single batch of large microspheres

Sample Number (5ml aliquots isotonic saline)	counts/sec. Ⓐ γ-activity	Ⓑ %
1	41408	20.1
2	41237	20.2
3	10678	5.2
4	3592	1.7
5	2799	1.3
filter and microspheres	106383	51.6%

Notes

Ⓐ The γ-activity values represent the activity measured in each 5ml aliquot of isotonic saline which was used to separately wash a single batch of HSA microspheres collected on a filter.

Ⓑ The results are expressed as a percentage of the total activity originally present in the batch.

The particle size distribution of this batch measured microscopically and by cascade impaction gave mean sizes of 5.6μm and 4.3μm respectively. The log-probability graph of these results is shown in Fig. 3.6.

3.2.4 Discussion

3.2.4.1 HSA millimicrospheres

The HSA kit is simple to use, and produces a firmly-labelled reproducible product, providing the particles are dispersed well and used within 8 hours of reconstitution (manufacturer's instructions).

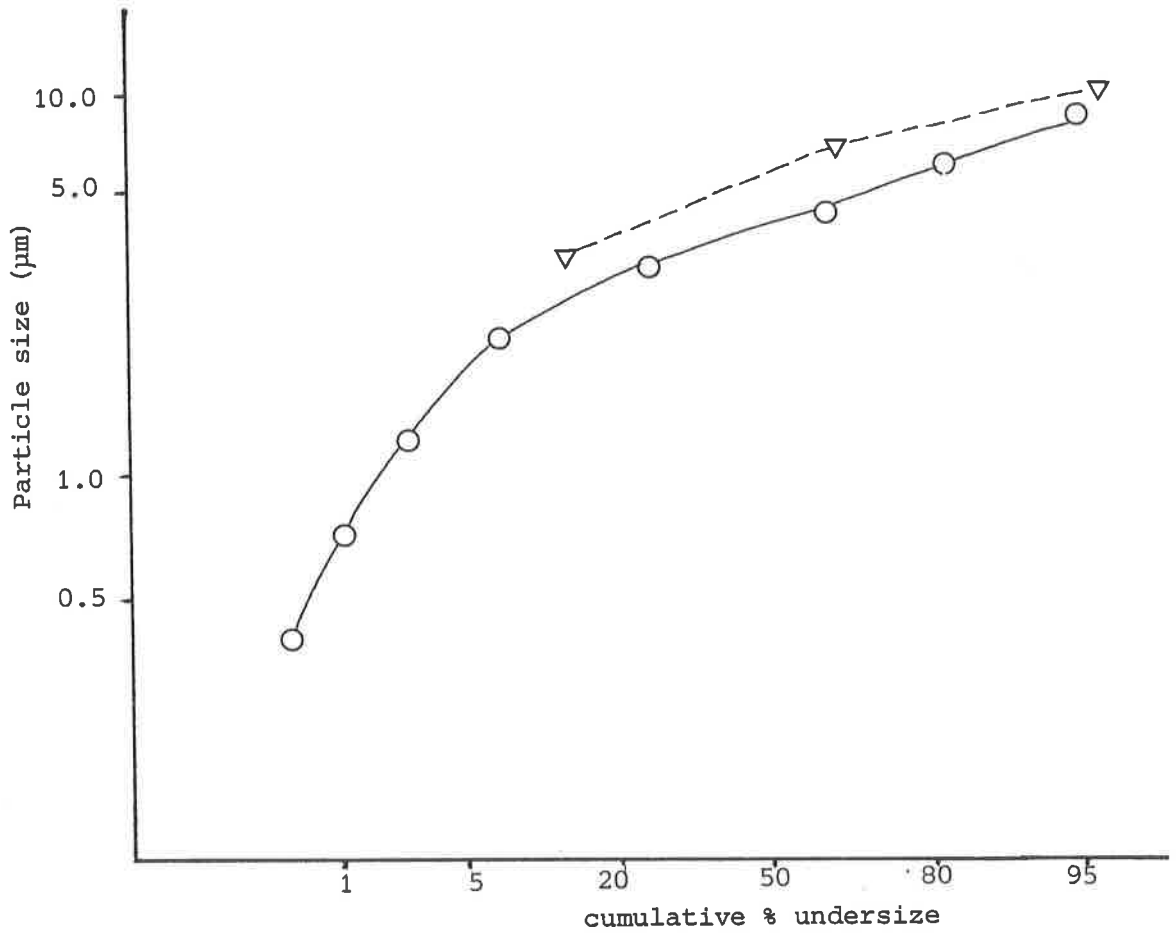
Table 3.5 compares the factors involved in particle size measurement by the manufacturers (CIS (UK) Ltd.) and the two methods employed in this study. The mean diameters do not agree because of differences in sampling methods and measurement of particle size by number and weight. The following points should be noted:-

1) The manufacturer's state the particle size range to include 90% less than 1μm. The mean particle size by weight may be much larger than the mean size by number if the remaining 10% of particles have diameters substantially greater than 1μm.

2) The published particle size is that of the millimicrospheres themselves, and does not allow for the fact that the nebulised aerosol consists of millimicrospheres contained in droplets of water.

3) The mean size by number measured by the PMS instrument (Table 3.3) is consistently <1μm, which agrees with the published figure.

Fig 3.6 Particle Size Distribution of HSA Microspheres Manufactured by Method D.



labelled nebulised suspension (msrd. by Andersen Sampler)
AMAD = 4.3µm, σg = 1.5

unlabelled microspheres (msrd. by microscope)
dgn = 5.6µm, σg = 1.4

Table 3.5 Comparison of factors involved in measuring the particle size of HSA millimicrospheres.

Method	Manufacturer CIS (UK) Ltd. -microscopy*	Andersen sampler -impaction	PMS instrument -light scattering
measured particle size (mean)	<1µm	2.2µm	1.1-8.2µm
particle size parameter	projected area diameter by number	mass median aerodynamic diameter	computed projected area diameter by mass
Sampling method	collection on filter	various	various
environmental conditions	unknown	ambient conditions -sampling airflow 30 l/min. -nebuliser airflow 8 l/min	ambient conditions -sampling airflow 8cc/min -nebuliser airflow 8 l/min.
measured particles/ droplets	particles	almost dry particles	droplets.

* microscopic method published in Villa et al., (1976)

4) The addition of a sampling airflow of approx. 30 litres/min. into the Andersen sampler probably contributes to the drying of the nebulised droplets. This would account for the consistent mean size with various sampling methods, in contrast to the PMS instrument results.

5) The large variation in results from the PMS instrument is probably due to degrees of drying of the nebulised droplets. For example, the mean sizes measured directly from the nebuliser, via 30cm length of tubing and via a 5 litre flask were 8.2, 4.5 and 1.1µm respectively.

Of course, one must remember that all these measurements were made under ambient conditions of temperature and humidity. At best, the results provide estimates of the particle size before inhalation. The mean size of particles or droplets available for *in vivo* deposition is unlikely to be the same as that which is measured *in vitro*, due to the high temperature and humidity encountered in the respiratory tract. This is a recognised limitation in all particle size measurements of volatile aerosols (Byron *et al.*, 1977; Davis & Bubb, 1978).

3.2.4.2 HSA microspheres

Microspheres greater than approx. 7µm are difficult to nebulise in sufficient numbers for efficient inhalation. However, large microspheres (eg. 5-6µm, narrow size range) were required to provide *in vivo* deposition patterns in contrast with the images obtained from the millimicrospheres. The batches of microspheres produced were not used for *in vivo* studies however, since the microspheres were in small numbers or a wide size range. The methods A-D in the flow chart (Fig. 3.3) outline some of the problems.

The main difficulties with these methods are as follows:-

1) The addition of stannous chloride to the initial formulation allows easy labelling by simple addition of sodium pertechnetate. However, the relative amounts of stannous chloride and albumin are important in controlling the possible hydrolysis of tin. In addition, the reduced ^{99m}Tc may undergo hydrolysis in aqueous solution. These factors probably account for the 50% activity losses when the albumin is washed.

2) The spinning disc generator produced relatively polydisperse aerosols (σ_g 1.4) due to fluctuations in air pressure and condition of the disc components. The mean size of the aerosols produced was not reproducible (variation $\pm$ 2μm) because of the process variables, and the stability of the disc was poor at high rotational speeds. The addition of stannous chloride and acetate buffer to the initial solution therefore produced an increase in particle size, since the disc rotational speed could not be increased to compensate for the increase in solution concentration. The problems of producing reproducible, monodisperse aerosols from a spinning disc generator are well known (Mitchell, 1981).

3) The method used for drying the generated droplets (Fig. 3.4) was inefficient, and large losses were encountered due to impaction of the droplets on the aspirator walls, and sedimentation losses. The volume and temperature of the drying chamber should be increased, or a more volatile solvent mixture used.

4) In methods A and D, the time taken for protein denaturation is dependent on the relative concentrations of albumin and glutaraldehyde. If the time interval was

too long, the microspheres were not formed before collection, but if the time interval was too short the protein denatured in the generation system and the liquid feed became blocked.

The combination of these disadvantages prevented the use of HSA microspheres in the in vivo studies, although the HSA millimicrospheres were used.

3.3 ESTABLISHING THE VALIDITY OF LABELLING THE EXCIPIENT, OLEIC ACID

3.3.1 Introduction

Before labelling the oleic acid with radioactive iodine, the distribution of the excipient amongst the drug particles in the aerosol cloud was established.

Ventolin inhaler contains oleic acid as a surfactant in the proportion 10% w/w of the drug weight. Measurement of the homogeneity of the two components in the aerosol cloud was accomplished using the Andersen Sampler (see Chapter 2). The sampler separates the aerosol cloud into eight aerodynamic size fractions by the technique of cascade inertial impaction (Andersen, 1966). If complete homogeneity of the two components existed in the aerosol cloud, the measured particle size distributions of salbutamol and oleic acid would be identical. In other words, the theoretical input ratio by weight of drug to oleic acid of 10:1, should be found on each stage of the Andersen Sampler.

3.3.2 Methods

The method of sampling metered-dose inhalers into the Andersen Sampler is described in detail in Chapter 2 (section 2.3.1). 40 shots of Ventolin Inhaler were fired into

the sampler, shaking the can between each actuation and firing four shots per minute at spaced intervals. Each stage was washed with methanol, and the resultant sample solution divided into two for separate salbutamol and oleic acid assays.

Salbutamol assay

The samples for salbutamol assay were suitably diluted and subjected to the usual colorimetric assay (see Chapter 2, section 2.3.1) either manually or by autoanalyser. The volumes and solvents were varied according to circumstances; aqueous methanol (50%) was used for samples intended for autoanalyser assay; and larger volumes were used when samples from several experiments were collected together to provide greater concentrations of oleic acid.

Oleic acid assay

The oleic acid samples were assayed by a gas chromatographic method which involved forming a methyl ester derivative and comparing the areas of peaks with a suitable internal standard. The derivatisation method is detailed below.

Methyl ester derivatisation method

The acid sample was placed in a 5 or 10ml round-bottomed flask and 3ml of 3M hydrochloric acid in dry methanol was added. Samples of 1mg or less of acid in 1ml methanol were used. The mixture was refluxed for approximately 10 minutes, and the solution taken to dryness on a rotary evaporator. The residue was redissolved in 1ml of a standard solution of pentacosane (c-25) in dichloromethane.

The reflux time, solvent and internal standard described in this method were all predetermined by experiment. The possible sources of error in the derivatisation method are:

(a) Inaccurate measurement of volumes by pipette and/or syringe.

(b) Incomplete drainage into the flask after refluxing.

(c) Incomplete evaporation (to remove all traces of HCl).

(d) Incomplete solution of residue in the solvent.

The completeness of reaction, reproducibility of results and sensitivity of the method were all determined by constructing calibration curves and calculating response factors for each sample.

The response factor Rf is equivalent to $\frac{1}{RWR}$ and is given by:-

$$RWR = \frac{\text{Area of sample peak}}{\text{Area of standard peak}} \times \frac{\text{weight of standard}}{\text{weight of sample.}}$$

The quantity of oleic acid in the sample is given by:-

$$\% \text{ oleic acid w/v} = \frac{\text{Area of sample peak}}{\text{Area of standard peak}} \times \frac{\text{Weight of standard}}{RWR} \times \frac{100}{1}$$

GC conditions used varied slightly with the use of different instruments, columns and times of experiment. In general, the conditions were:

Column:	2 metre, 3% OV210 or 5% OV210 (trifluoropropyl silicone).
Solvent for samples:	Dichloromethane
Column Temp:	170°-200°C
Carrier gas flow rates:	15 - 30L/min.
Sensitivity:	2 x 10 to 32 x 10 (At a sensitivity of 2 x 10, > 20µg 'oleic acid' can be accurately measured.
Internal standard:	Pentacosane C-25

The peak areas of the sample and internal standard were integrated automatically by the Hewlett-Packard computing system attached to the G.C. instrument.

The commercial form of oleic acid used in production of Ventolin inhaler is 'Priolene 6952', its specification is shown in Appendix 3.2.

Experiments carried out using the methyl esters of myristic palmitic, stearic and arachidic acids provide evidence to show that the subsidiary peaks present in the chromatogram are likely to be representative of C_{14}, C_{16} and C_{18} fatty acids. This is borne out by the retention times and relative sizes of the peaks. However, this means that measurement of the main peak area will only provide information on the quantity of C_{18} fatty acids present in that sample, which comprises about 80% of Priolene 6952 that will be present. Only 73.5% of the Priolene is actually oleic acid,

and this complicates the experiments since the quantities of sample involved in calculating response factors, for example, must be represented as the quantity of C_{18} fatty acid present and not the total Priolene content.

It was considered that these complications would not provide serious errors in the final analysis of results, since it is the ratio of oleic acid to drug in each sample which should remain constant, so that absolute values of oleic acid are not necessary. The composition of Priolene remains constant, since the same batch of Priolene was used throughout the studies, and the relative height of the C_{18} peak compared with the internal standard remains constant with time for a given sample - this was determined by experiment.

3.3.3 Results

Fig. 3.7 shows a typical GC chromatogram of a derivatised sample containing oleic acid.

Fig. 3.8 and Table 3.6 show the data produced for a calibration curve of weight of oleic acid (µg) vs ratio of peak areas.

Fig. 3.9 and Table 3.7 show the log-probability particle size distribution graph and the corresponding data from the Andersen sampler assays, respectively. The data are produced from a placebo metered-dose inhaler (i.e. an inhaler containing the standard quantities of oleic acid and propellants, but without salbutamol). The mass median aerodynamic diameter (MMAD) of the oleic acid particles is 1.1µm (σg 2.6).

Fig. 3.10 and Table 3.8 show similar data, but the samples were from an active (ie. containing salbutamol) metered-dose inhaler. The double particle size distributions of the

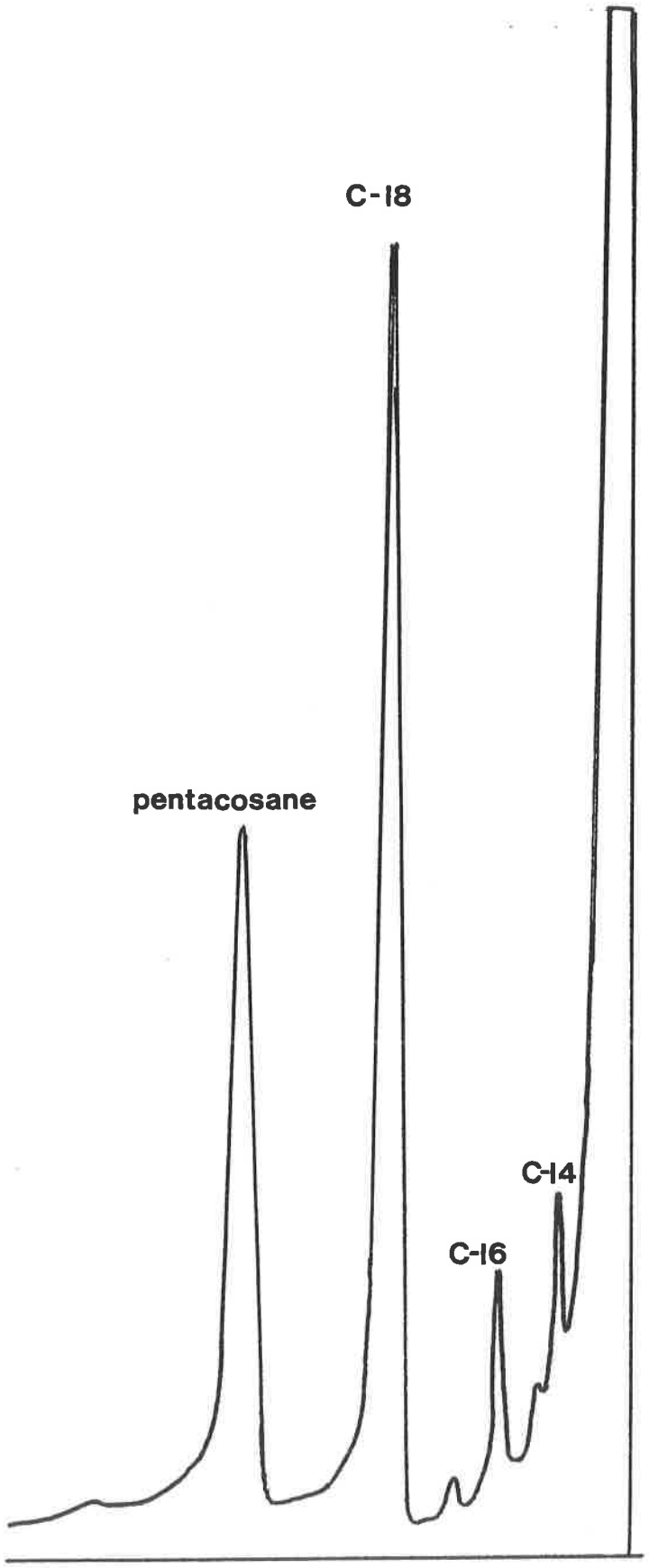


Fig 3.7

Typical GC Chromatogram of Derivatised Oleic Acid Sample.

Fig 3.8 Calibration Curve for Oleic Acid GC Assay.

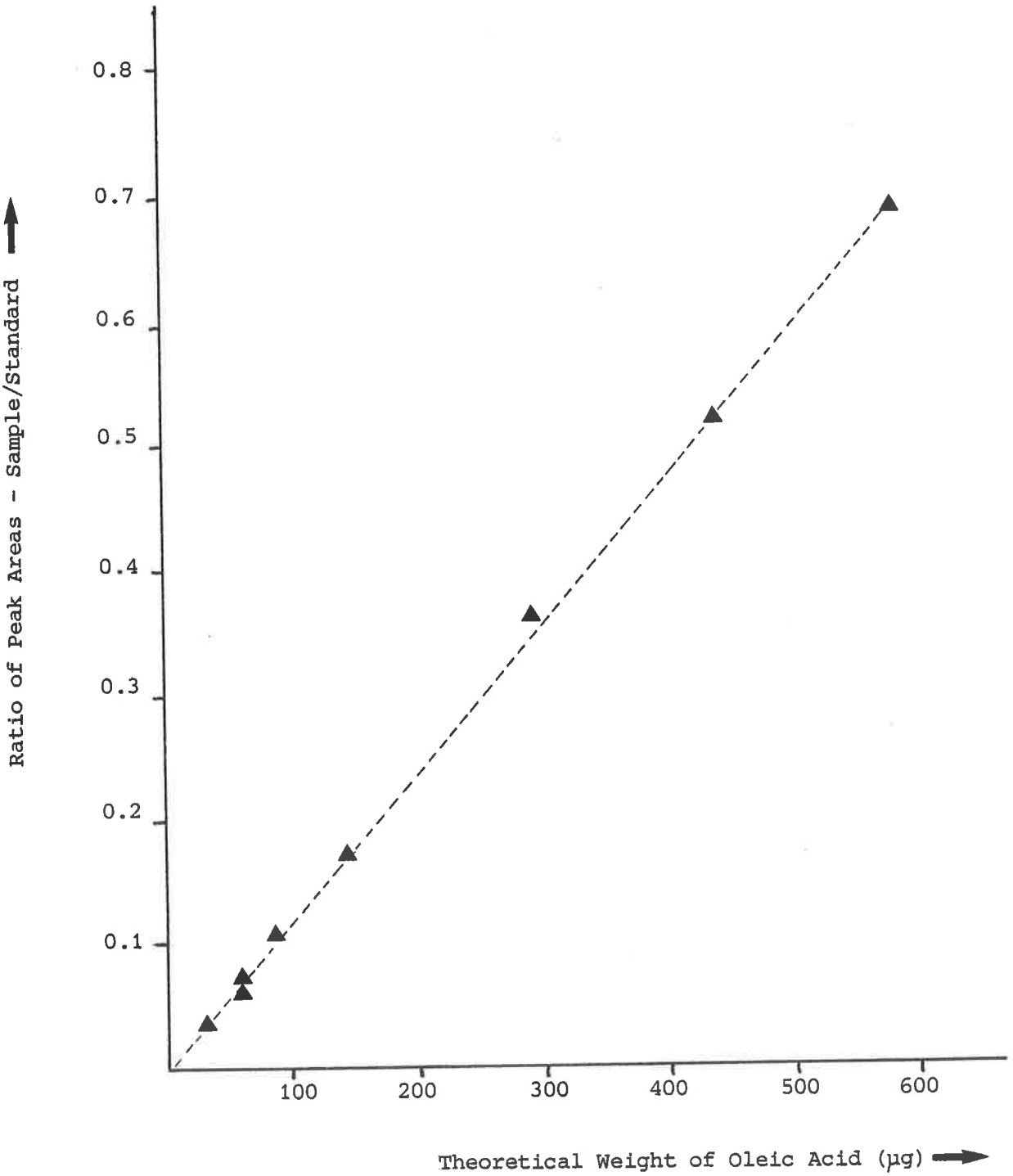


Table 3.6 Data for Calibration Curve for GC oleic acid assay (Fig. 3.8).

Vol. std. solution (µl)	theoretical wt. (µg)	Retention time (mins)	Area of peaks	Area %	Ratio peak areas
50	28.7	7.37 12.02	1618 51873	3.025 96.975	0.031
100	57.4	7.35 12.08	5697 90049	5.951 94.049	0.063
100	57.4	7.42 12.15	4638 67418	6.437 93.563	0.069
150	86.0	7.20 11.85	5794 56134	9.356 90.644	0.103
250	143.5	7.18 11.85	8865 53202	14.282 85.718	0.167
500	287.0	7.14 11.84	19302 54412	26.185 73.815	0.355
750	430.4	7.20 11.97	28287 54550	34.148 65.852	0.518
1000	573.8	7.17 11.94	37545 54277	40.579 58.663	0.692

Fig 3.9.

PARTICLE SIZE DISTRIBUTION OF OLEIC ACID FROM A PLACEBO INHALER.

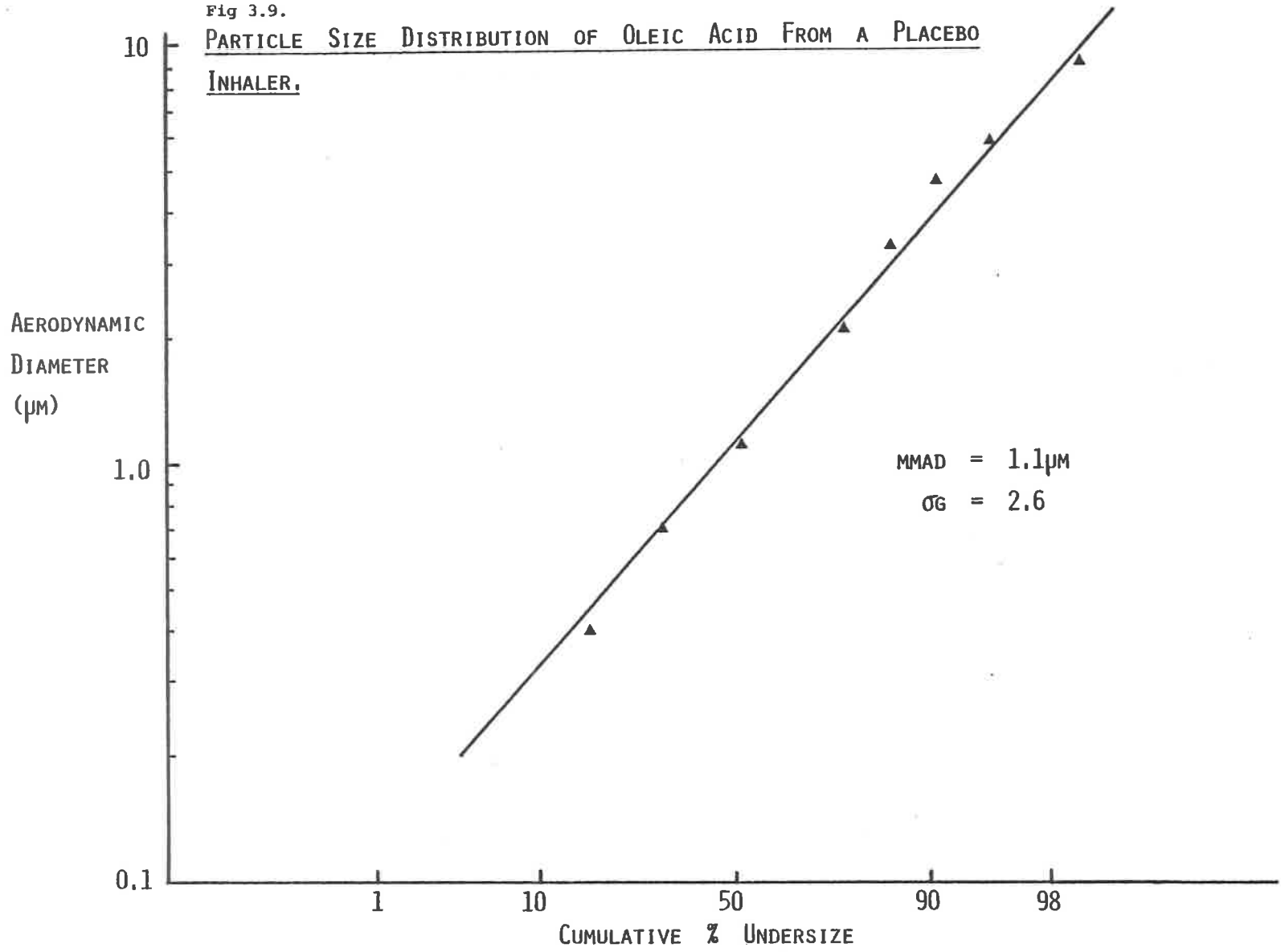


Table 3.7 Data for particle size distribution of oleic acid in a placebo inhaler (Fig. 3.9)

Impactor stage	µg oleic acid	%	% -throat	Cumulative % undersize
Throat	189.30	51.3		
0	2.06	0.6	1.1	98.8
1	6.21	1.7	3.5	95.3
2	8.46	2.3	4.7	90.6
3	10.76	2.9	6.0	84.6
4	15.37	4.2	8.5	76.1
5	44.51	12.1	24.7	51.4
6	35.70	9.7	19.8	31.6
7	26.81	7.3	14.9	16.7
Filter	29.98	8.1	16.7	
Total	369.16			

Fig 3.10

THE PARTICLE SIZE DISTRIBUTION OF SALBUTAMOL AND OLEIC ACID FROM VENTOLIN INHALER (N = 3).

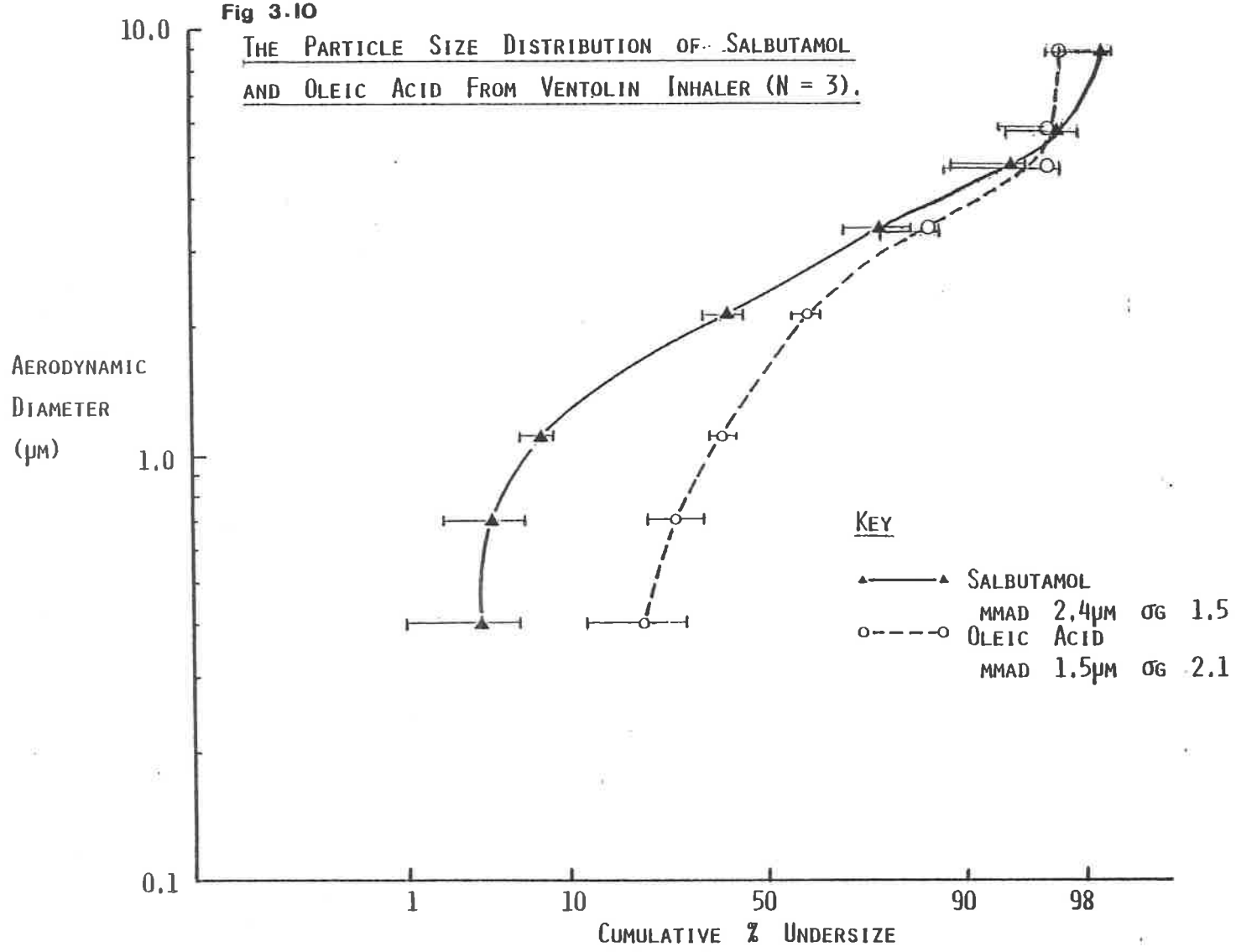


Table 3.8 Data for Fig. 3.10, the Particle Size Distribution of Salbutamol and Oleic Acid from Ventolin Inhaler (n=3).

ANDERSEN STAGE	SALBUTAMOL CUMULATIVE % UNDERSIZE		OLEIC ACID CUMULATIVE % UNDERSIZE	
	Median	Range	Median	Range
0	98.5	96.9-98.8	96.8	96.5-98.9
1	96.7	92.5-97.4	96.5	93.2-96.7
2	94.1	88.1-95.2	96.5	86.8-96.7
3	77.3	67.2-82.9	85.6	77.2-86.6
4	37.1	35.0-46.2	59.0	57.2-64.8
5	7.3	5.1- 8.6	39.2	35.0-42.4
6	3.8	1.4- 6.3	25.9	22.1-36.3
7	3.2	0.6- 6.0	18.2	11.5-33.7

salbutamol and oleic acid exhibit MMAD's of 2.4 and 1.5µm respectively (σ g 1.5 and 2.1 respectively).

Fig. 3.11 and Table 3.9 show the data of the weight ratio of drug: oleic acid vs particle size. It is clear that the measured values vary considerably from the theoretical ratio of 10:1 at most particle sizes. The differences in particle size distribution of the salbutamol and oleic acid are summarised in Table 3.10.

3.3.4 Discussion

The discrepancy between the actual and theoretical distributions of salbutamol and oleic acid in the aerosol cloud can be explained in the following way.

Calculation of the theoretical particle size distributions of salbutamol, oleic acid and propellant droplets in an aerosol from Ventolin Inhaler

Assuming:

(i) the oleic acid distribution in the placebo inhaler is log-normal,

(ii) all the propellant has evaporated from the droplets before collection in the impactor.

The wet droplet diameters of the original propellant mixture can be calculated from the particle size distribution of oleic acid from a placebo inhaler, using the following equation:-

$$d_w^3 = \frac{d_d^3 \rho_d}{C \rho_w}$$

where d_d = 1.1µm (MMAD oleic acid)

ρ_d = 0.89 gcm-3 (density of oleic acid)

ρ_w = 1.38 gcm-3 (density of propellant 11/12 mixture)

C = 0.0001294 (concentration of oleic acid ie. $\frac{2.64mg}{20.4g}$

Fig 3.11 Weight Ratio of Drug: Oleic Acid vs Particle Size of Aerosol Measured in an Andersen Sampler. (n=3, median and range values).

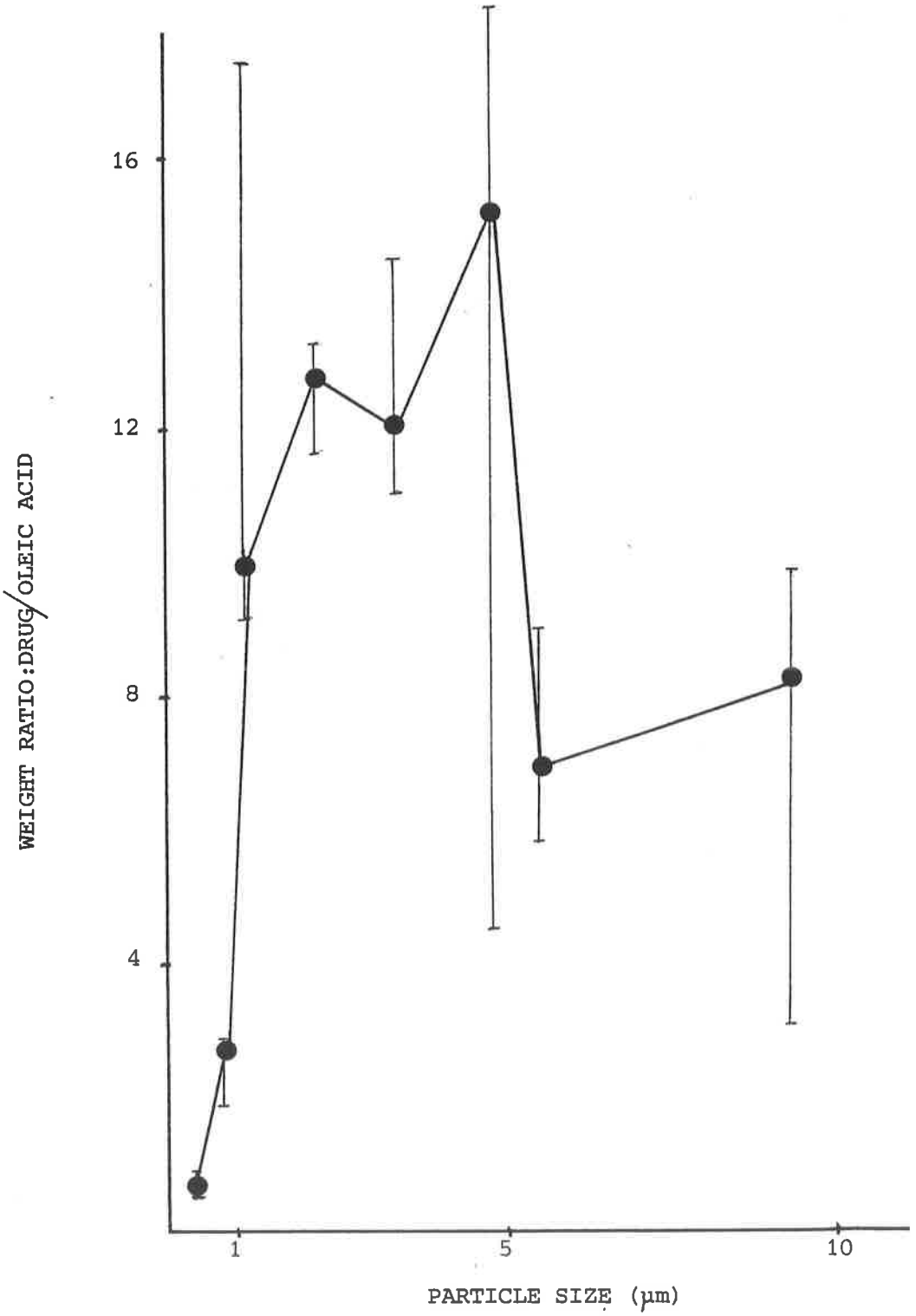


Table 3.9 Ratios of Weights of Oleic Acid and Salbutamol (see Fig 3.11).

Andersen Sampler	Median of Ratios n = 3	Range
0	8.47	3.18-10.0
1	6.97	5.89- 9.23
2	15.38	4.48-18.46
3	12.06	11.15-14.34
4	12.79	11.73-13.33
5	9.96	9.20-17.34
6	2.70	1.84- 2.79
7	0.66	0.58- 0.91

Table 3.10 Summary of Andersen Sampler Results

Particle Size Fraction (Represented by Stages of Andersen Sampler)		0-1.1μm (Stages 6, 7, 8F)	1.1 - 4.7μm (Stages 3, 4, 5)
Ventolin Inhaler	% of Total Salbutamol	5-8%	80-85%
	% of Total Oleic Acid	30-35%	45-55%
Placebo Inhaler	% of Total Oleic Acid	51%	39%

Using the MMAD and σg the log-probability graphs of size distribution can be plotted. (Fig. 3.12). The propellant droplet distribution by weight is therefore calculated.

To illustrate the differences in distributions in terms of numbers of droplets, the propellant droplet distribution by number is calculated using the Hatch-choate equation:-

$$\ln X_gN = \ln X_gW - 3.0 \ln^2 \sigma g$$

where X_gN and X_gW are mean diameters by number and weight, and σg is the geometric standard deviation.

The propellant droplet distribution by number is compared with the number distribution of salbutamol input into the aerosol, to calculate the relative quantities at any one size. (Fig. 3.13).

The graphs are plotted by calculating the diameters at 16% and 84% by the following relationships:

$$d_{16\%} = \frac{d_{50\%}}{\sigma g}$$

and $d_{84\%} = d_{50\%} \times \sigma g$

assuming log-normal distributions.

Fig 3. 12 Size distributions of oleic acid and propellants by weight from a placebo metered-dose inhaler.

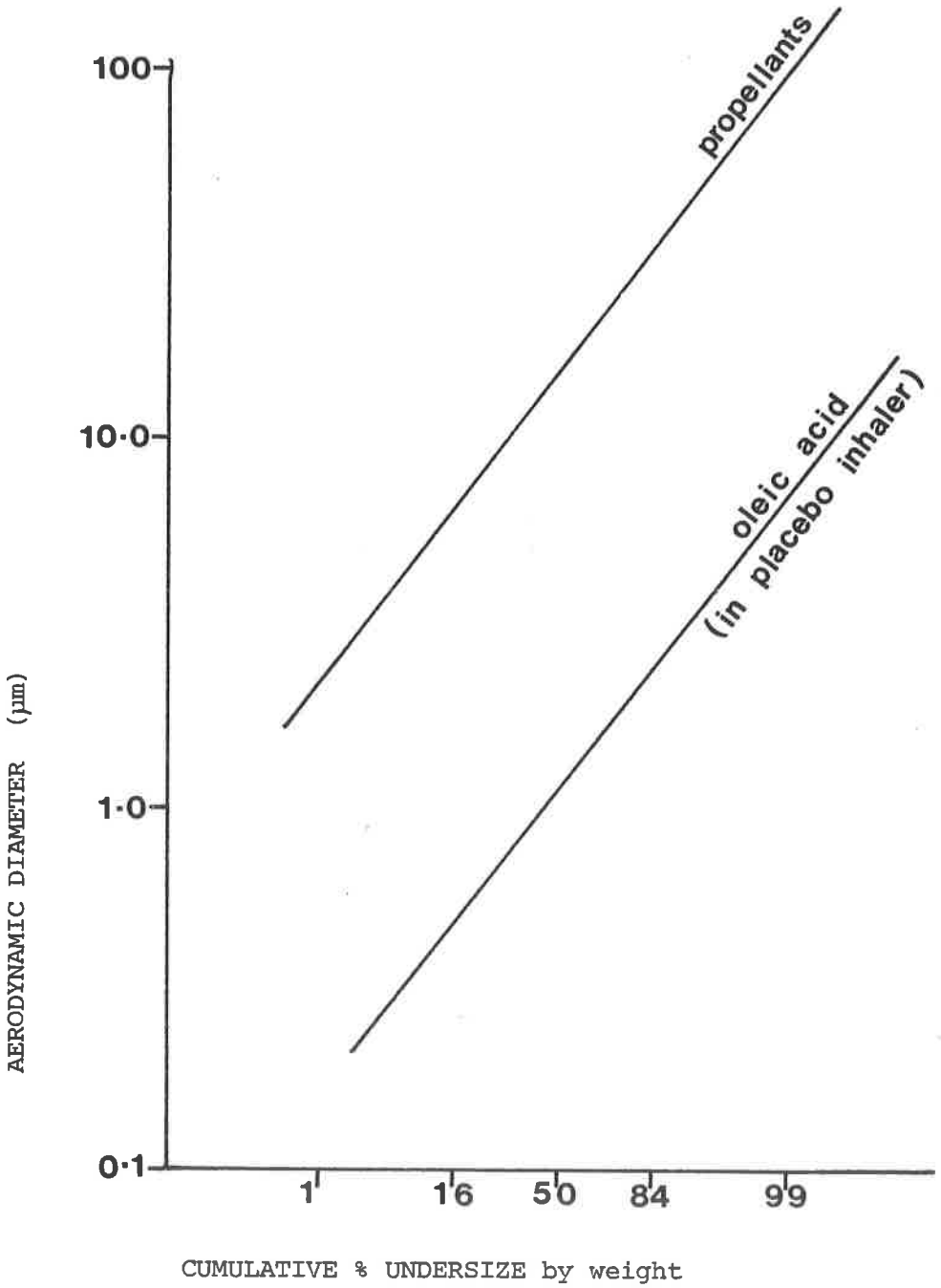
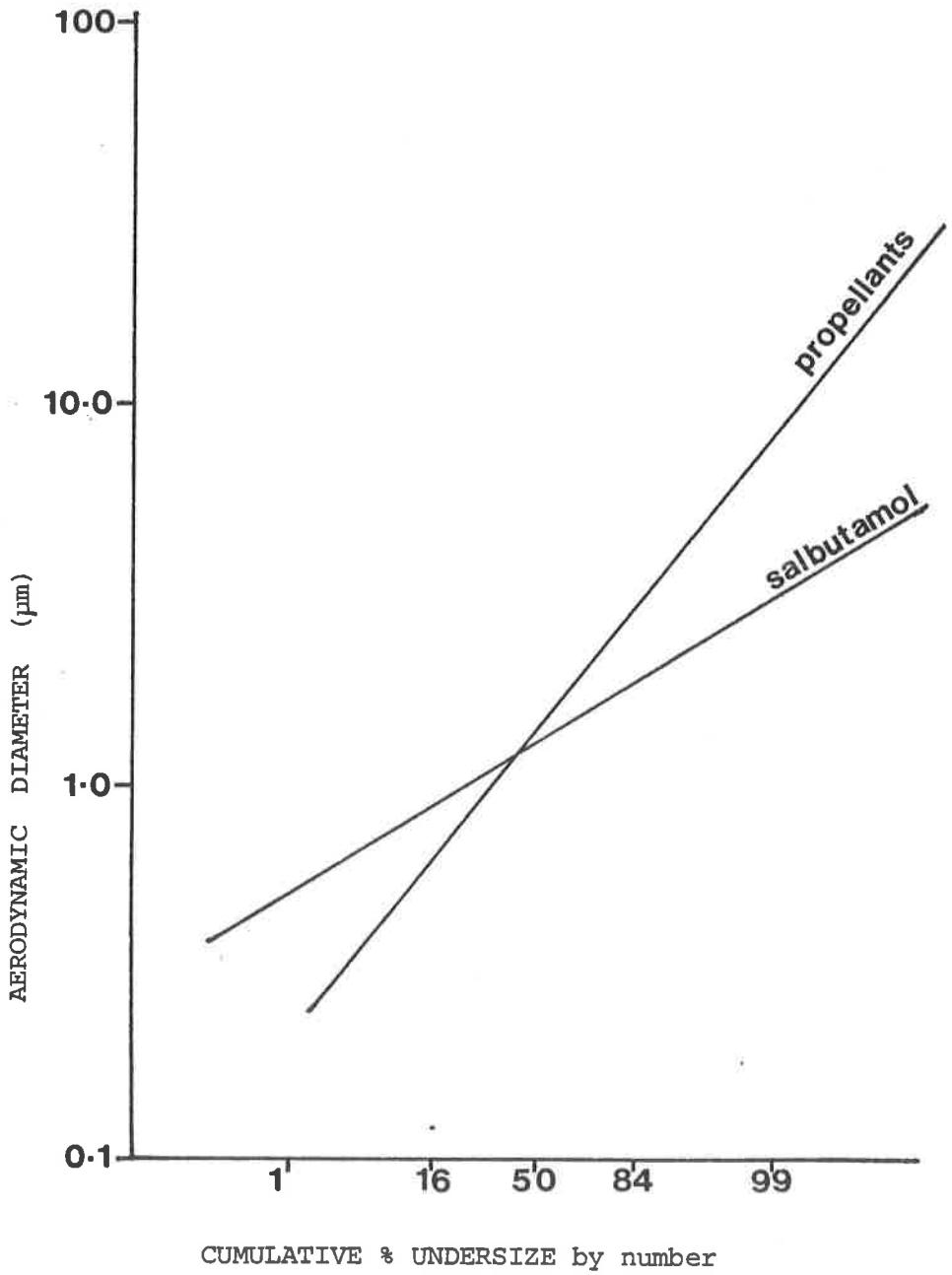


Fig 3.13 Size distributions of salbutamol and propellants by number.



The distribution parameters for each graph are:

	$d_{16\%}$	$d_{50\%}$	$d_{84\%}$	σ_g
oleic acid (placebo inhaler)	0.4	1.1	2.8	2.5
propellant droplets (by weight)	7.5	18.8	47.0	2.5
propellant droplets (by number)	0.5	1.2	3.0	2.5
micronised salbutamol (by number)	0.8	1.2	1.8	1.5

To compare the number distributions of propellant drops and salbutamol particles, the percentage number of drops below a given particle size is taken from the graphs:

	Propellant drops	Salbutamol particles
< 2μm	70%	89%
< 1μm	42%	33%
< 0.7μm	28%	9%
< 0.4μm	12%	0.35%

Calculations from the oleic acid distribution in the discharged spray show that there are far more discharged propellant droplets than drug particles and this ratio increases steeply below 1μm. It follows that the decreasing tendency for droplets to contain drug particles is enhanced as the droplet size decreases. Figs. 3.14 and 3.15 illustrate the probable behaviour of the discharged spray from Ventolin Inhaler. The previous calculations have shown that smaller particles are less likely to contain salbutamol than oleic acid. Since the oleic acid is in solution in the propellants, when the latter evaporate the smaller particles remaining are mostly droplets of oleic acid alone.

Fig 3.14 The Distribution of Oleic Acid in an Aerosol Spray from Ventolin Inhaler.

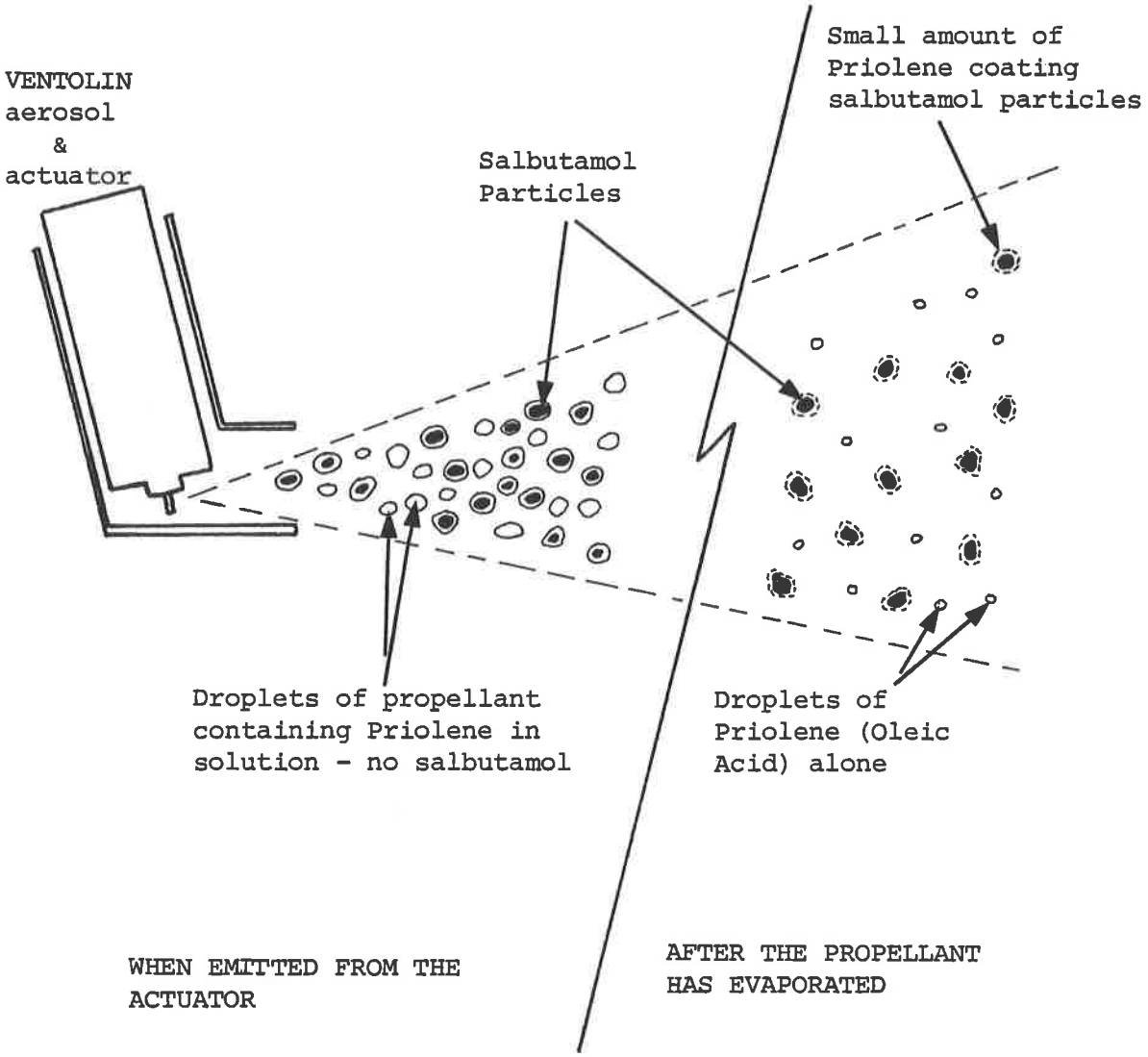
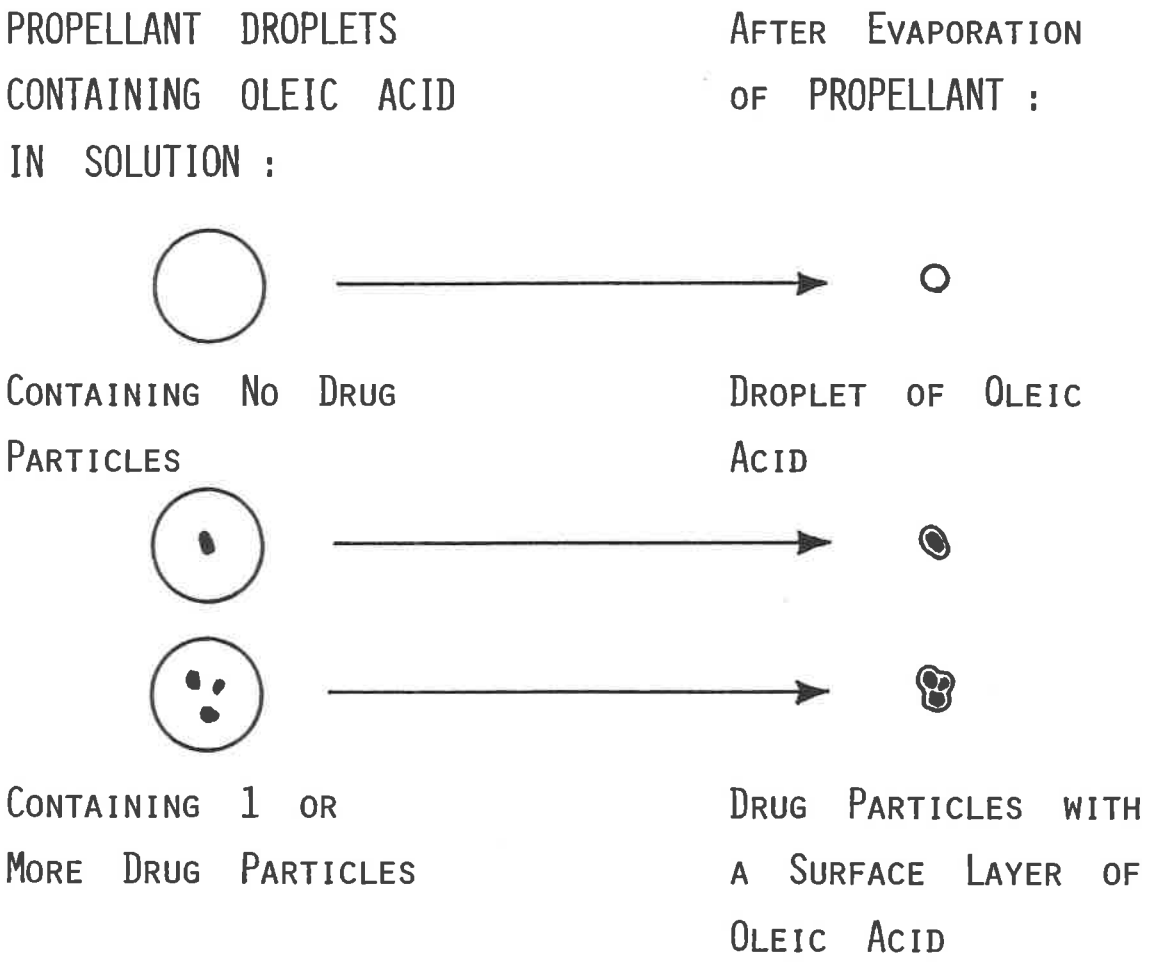


Fig 3.15. Details of behaviour of Ventolin aerosol spray.



3.4 LABELLING SALBUTAMOL

3.4.1 Introduction

Direct γ-labelling methods for the salbutamol base molecule are not possible without access to a cyclotron and availability of rapid synthetic methods, because of the molecular structure of the drug which contains no suitable elements. An alternative method was required which would introduce a suitable radionuclide into the salbutamol aerosol formulation indirectly, either by physical or chemical means. Therefore, methods of radiolabelling the drug particles such as cocrystallisation and chelation were studied. The aim was to introduce a label without significantly altering the physico-chemical properties of the drug particles, which may affect hygroscopic growth, regional deposition and clearance rates in the lung. The only published work on γ-labelling a bronchodilator drug has recently appeared (Short & Few, 1981). This method labels the bromide salt with cylcotron-produced Br-77. Salt formation was an additional exploratory technique for salbutamol labelling.

Apart from one study which used Indium-113m, the radionuclide used was Technetium-99m. The *in vivo* deposition experiments required an aerosol dose of two actuations from a metered-dose inhaler (see Chapter 4). However, the minimum quantity required in the can for proper valve metering was thirty times this amount. The losses in preparation, administration and radioactive decay meant that 10-20mCi of Tc-99m activity were required for each preparation of a single inhaler. The properties of technetium allowed safe handling of these quantities in repeated experiments. However, the use of technetium provided additional problems because of the very limited current knowledge of technetium chemistry. For this reason, chemical

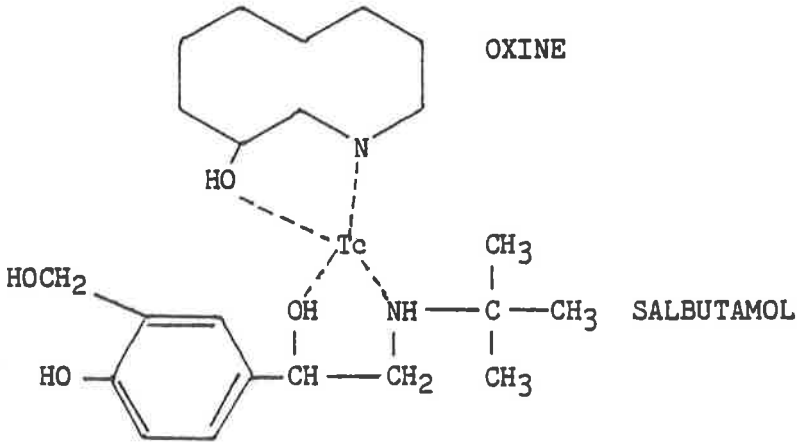
complexes which are commonly used in nuclear biomedical research were employed in the labelling work, such as Tc-oxine, Tc-DTPA (diethylenetriamine penta-acetic acid) and Tc-Ph4AsCl (tetraphenylarsonium chloride).

Technetium-99m oxine was prepared for three reasons:

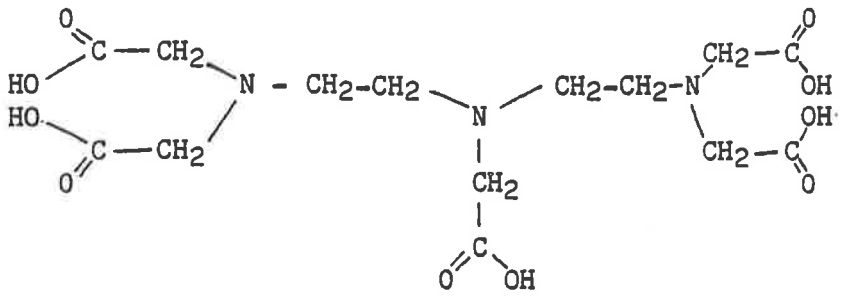
(a) the longer half-life of Technetium-99m compared with Indium-113m meant aerosol preparation used less activity and the emission properties of Tc-99m were advantageous for repeated imaging studies.

(b) Most technetium compounds used in nuclear medicine are water-soluble. Tc-oxine is lipophilic, and so likely to be soluble in the propellant mixtures in a MDI.

(c) The structure of oxine (8-hydroxy quinoline) suggests possible chelation complexes. A possible structure of a complex with salbutamol could be:



DTPA (diethylenetriamine pentaacetic acid) is a commonly used chelating agent in nuclear medicine. Simultaneous chelation with Tc-99m and salbutamol was considered as a possibility, since DTPA has 8 possible complexing sites. The structure of DTPA is:



3.4.2 Methods

3.4.2.1 General

The general method for preparation of metered-dose inhalers containing radiolabelled salbutamol suspensions is shown below. Particular problems in manufacture included:

(i) Limited time due to the use of short half-life radionuclides - this precluded lengthy processes such as drying or milling.

(ii) Small quantities of ingredients. The limitations on total activity used in each experiment prevented preparation of large size batches. For most preparations quantities of labelled products for single cans were required, and unlabelled suspensions for batches of 10 cans were the maximum used.

A mortar and pestle was used for mixing small volume suspensions and the small weights of ingredients were weighed on a simple pan balance (accuracy 0.05g).

(iii) Both propellants used for preparation were volatile at room temperature (B.p. 23.5°C and -30°C for propellants 11 and 12 respectively). However preparations of labelled products were carried out at room temperature (23 ± 1°C) and some evaporation of propellants inevitably resulted, although this was minimised by dispensing the propellants from vacuum flasks.

Details of the 'cold-fill' method of preparation used throughout the studies are shown in Fig. 3.16.

The validity of each radiolabel was established using the Andersen Sampler, as described in section 2.3.1. The activity particle size distribution of the radiolabel was measured using a well-counter. The same samples from each stage of the Andersen Sampler were assayed colorimetrically for salbutamol to establish its mass particle size distribution. It was assumed that if the mass and activity size distributions matched in each stage of the impactor, the drug and radiolabel were associated in the aerosol particles. The radionuclidic images produced in vivo would thus represent the in vivo mass deposition of drug particles.

Table 3.11 shows the details of the cocrystallisation and chelation methods for indirect salbutamol labelling.

3.4.2.2 Cocrystallization

Indium-113m oxine and salbutamol were cocrystallized from ethanol. When milled to a suitable particle size this could provide a uniform crystalline mixture for aerosol can preparation. The preparation of indium-113m oxine is

Fig. 3.16 Cold-fill method for manufacture of MDI's

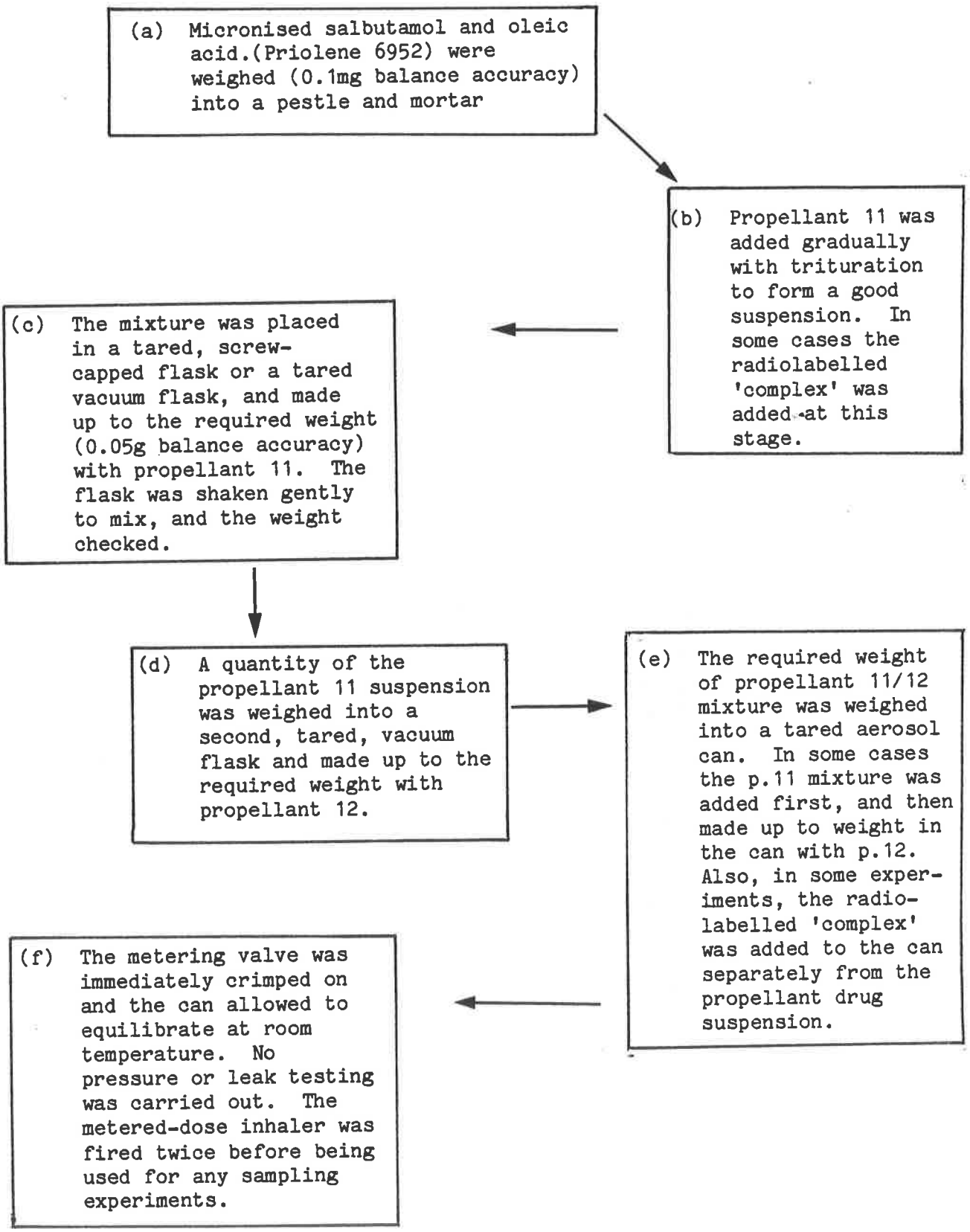


Table 3.11 Details of experiments from section 3.4.2.2 and 3.4.2.3

Method	Isotope	Preformulation and milling (a)	MDI formulation (b)	Cold-fill method (c) (p= propellant)	Assay (d)	Notes
Cocrystallisation section 3.4.2.2) Indium oxine	In-113m	0.7g salbutamol base 7ml ethanol 1ml Indium-oxine solution. Evaporate to dryness. Milling 14 hrs.	324mg In-salbutamol 32.4g oleic acid to 70g Propellant 11	40g p.11 suspension to 143g p.12. foll 20.4 ± 0.4g	Andersen Sampler 40 shots	Activity leaching test (see Section 3.4.2.2) 3 cans filled
Chelation (section 3.4.2.3) Technetium oxine Method A	Tc-99m		324mg salbutamol base 0.2ml Tc-oxine in ethanol 32.4mg oleic acid to 70g Propellant 11	40g p.11 suspension to 143g p.12 fill 20.4 ± 0.4g	Andersen Sampler 40 shots	Labelled solution added to p.11 suspension. 3 cans filled. Approx. activity used 1mCi
Chelation (section 3.4.2.3) Technetium oxine Method B	Tc-99m	0.7g salbutamol base 7ml ethanol 0.6ml Technetium-oxine in ethanol. evaporate to dryness Milling 14 hrs.	324mg Tc-oxine/ salbutamol 32.4mg oleic acid to 70g Propellant 11	40g p.11 suspension to 143g p.12 fill 20.4 ± 0.4g	Andersen Sampler 40 shots	3 cans filled. Approx. activity used 3 mCi
Chelation (section 3.4.2.3) Technetium oxine Placebo	Tc-99m		0.3ml Tc-oxine in ethanol 32.4mg oleic acid to 70g propellant 11	40g p.11 suspension to 143g p.12 fill 20.4 ± 0.4g	Andersen Sampler 30 shots	Approx. activity used 0.3 mCi
DTPA (section 3.4.2.3) Method A	Tc-99m		60mg Salbutamol base 1.0ml Tc-DTPA in aqueous ethanol to 57g Propellant 11	5.7g p.11 suspension to 20.4g p.12	Andersen Sampler 40 shots	1 can filled Approx. activity used 0.25mCi

Table 3.11 Details of experiments from section 3.4.2.2 and 3.4.2.3 Contd.

Method	Isotope	Preformulation and milling (a)	MDI formulation (b)	Cold-fill method (c) (p= propellant)	Assay (d)	Notes
DTPA (section 3.4.2.3) Method B	Tc-99m		60mg Salbutamol base 0.4ml Tc-DTPA in saline to 68.4g Propellant 11	5.7g p.11 suspension to 20.4g p.12	Andersen Sampler 30 shots	1 can filled Approx. activity used 6.3mCi

(a) Dissolve drug in warm ethanol, add solution of radiolabel, mix and evaporate to dryness using a rotary evaporator. Triturate dry powder with propellant 11 in a pestle and mortar to produce a dilute slurry. Mill the slurry for 14 hours in a ball-mill made from a 30ml amber glass, screw-capped bottle containing 3mm steatite balls.

(b) Details of quantities in propellant 11 suspension, method of manufacture Fig. 3.14 (a) - (c)

(c) See Fig. 3.14 for details of this method.

(d) For details of Andersen assay method see section 2.4.2.

detailed below, and is a modification of the method of Goodwin et al. (1978).

Preparation of 113m-Indium oxine

1) 6ml of $^{113m}InCl_3$ in HCl were evaporated to dryness. (Activity approx. 40mCi).
2) The residue was redissolved in 1ml of 0.3M acetate buffer, pH5.5, and 150µg of oxine (8-hydroxyquinoline) in absolute ethanol was added.
3) The solution was mixed on a vortex mixer for approximately one minute, then left to stand at room temperature for 15 minutes.
4) The resultant chelate was extracted in one volume in each of two 1.75ml extractions of chloroform and evaporated almost to dryness with an airstream. One drop of chloroform was left with the residue as this made it easier to redissolve in 1ml absolute ethanol.
5) The final solution of In-113m oxine in ethanol was then added to the solution of salbutamol base in ethanol and evaporated to dryness. (see Table 3.11).

Leaching of activity from the powder mixture was estimated by filtering the propellant 11 suspension and washing the filter with further aliquots of propellant 11. The results, taken at 0, 2, 5 and 22 hours after milling, are expressed as activity counts per minute per gram of powder, and the percentage activity remaining compared with a standard sample before washing.

Details of the preparation of a MDI from the Indium-Salbutamol mixture are shown in Table 3.11 and Fig. 3.16. The activity and particle size distributions of the discharged aerosol were estimated using the Andersen Sampler (sections 2.3.1 and 3.3.1).

3.4.2.3 Chelation complexes

The details of preparation of ^{99m}Tc-oxine from tin oxine are described below, from a method published by Subramanian (1976).

Preparation of ^{99m}Tc-oxine

^{99m}Tc-oxine was prepared from tin (II) oxine by a simple addition reaction. Method for preparation of tin (ii) oxine (SnOx):-

(a) 4g $SnCl_2 2H_2O$ was dissolved in 100ml 1N HCl.

(b) 4.1g 8-Hydroxy quinoline (oxine) was dissolved in 30ml 1N HCl and the solution was added to that from (a).

(c) 200ml chloroform was added and the mixture was vigorously stirred.

(d) 130ml of 35% sodium acetate solution was added to bring the pH to approx. 5.0 (checked with pH indicator paper).

(e) The chloroform layer was separated, washed with water and filtered using Whatman No.1 phase-separating paper. The chloroform was evaporated to dryness under reduced pressure.

(f) The crude product was recrystallised from ethanol and stored under nitrogen. (Approx. yield of crystallised product 5.2g).

Preparation of ^{99m}Tc-oxine:-

(i) 100mg SnOx were added to 20ml ethanol, stirred well for 20 minutes and filtered into a sterile evacuated vial (solution contains 1mg/ml).

(ii) 5ml sodium pertechnetate in saline (containing 5.1mCi) were placed in a conical centrifuge tube and 0.2ml of saturated SnOx solution from (i) was added. The mixture was vigorously stirred for 2-3 minutes and allowed to stand for 10 minutes.

(iii) 10ml chloroform was added, mixed thoroughly for 5 minutes and allowed to stand for 5 mins.

(iv) The chloroform layer was separated and evaporated off using filtered air and a heated water-bath (approx. 75°C).

(v) 1ml of ethanol was added and the residue redissolved. The resultant ethanolic solution of ^{99m}Tc-oxine (approx. 5 mCi/ml) was used to radiolabel MDI formulations.

The ^{99m}Tc-oxine is produced as an ethanolic solution, and is introduced into the aerosol formulation in two ways, as shown in Table 3.11. Preparation of Tc-oxine labelled placebo MDI is described similarly. The Andersen Sampler assay technique was used to assess the activity and particle size distributions (Section 2.3.1 and Table 3.11).

The technetium-DTPA chelation complex was introduced into MDI formulations in two ways, as described in Table 3.11. Assessment of the validity of the γ -radiolabel was carried out using the Andersen Sampler, as before.

3.4.2.4 Salt Formation

Three potentially useful salts of salbutamol were considered for radiolabelled aerosol formulations. Appendix 3.3 details the method for production of salbutamol hemiselenate and salbutamol iodate, as developed by I. Fellows (Chemistry Development Dept. Glaxo Group Research Ltd.). Both of these preparations were non-radioactive but the radionuclides envisaged for use were selenium-75 and iodine-123.

Although the production of salbutamol hemiselenate (^{75}Se) is feasible, the use of this isotope in aerosol production is undesirable except under strictly controlled circumstances. This is because of the danger of contamination due to the long half-life of 118 days and suggested long term retention in the liver and blood (Weissman et al., 1979). The potential advantages of this compound would be the likelihood of similar physico-chemical properties to salbutamol sulphate (which is used effectively in some inhalation aerosol products) and the length of time available for aerosol preparation. However, for safety reasons no radiolabelled preparations were made with Se-75.

The preparation of salbutamol iodate is relatively simple using iodic acid. However, iodine-123 is supplied as sodium iodide in weak alkaline solution with a very high specific activity and would require an additional oxidation step using only nanogram quantities. An electrochemical method (Toth, 1959) is the most feasible. However, the iodate preparation would have the following disadvantages:

(i) The efficiency of absorption and handling problems would require larger initial quantities.

(ii) Large quantities would be required for each batch due to the time taken in delivery and preparation. (Half-life of Iodine-123 is 13 hours). Frequently repeated batches would not be feasible because of the high cost of Iodine-123 and the single weekly deliveries.

(iii) Only sufficient isotope could be handled safely for one batch (20-30mCi), which would require milling of very small quantities (approx. 2mg) of salbutamol iodate.

For the above reasons no radiolabelled preparations were made with Iodine-123.

It is also possible that salbutamol pertechnetate could be formed under the right conditions. An aqueous solution containing salbutamol sulphate and sodium pertechnetate in equilibrium will contain salts including salbutamol pertechnetate. The salbutamol sulphate is in great excess so that most of the pertechnetate would be in the form of salbutamol pertechnetate. This interaction could be useful for aqueous nebulised solutions, so this type of formulation was tested in the usual way with the Andersen Sampler.

Formulation:-

0.1ml $NaTcO_4$ in isotonic saline (approx. 80µCi)

100mg salbutamol sulphate

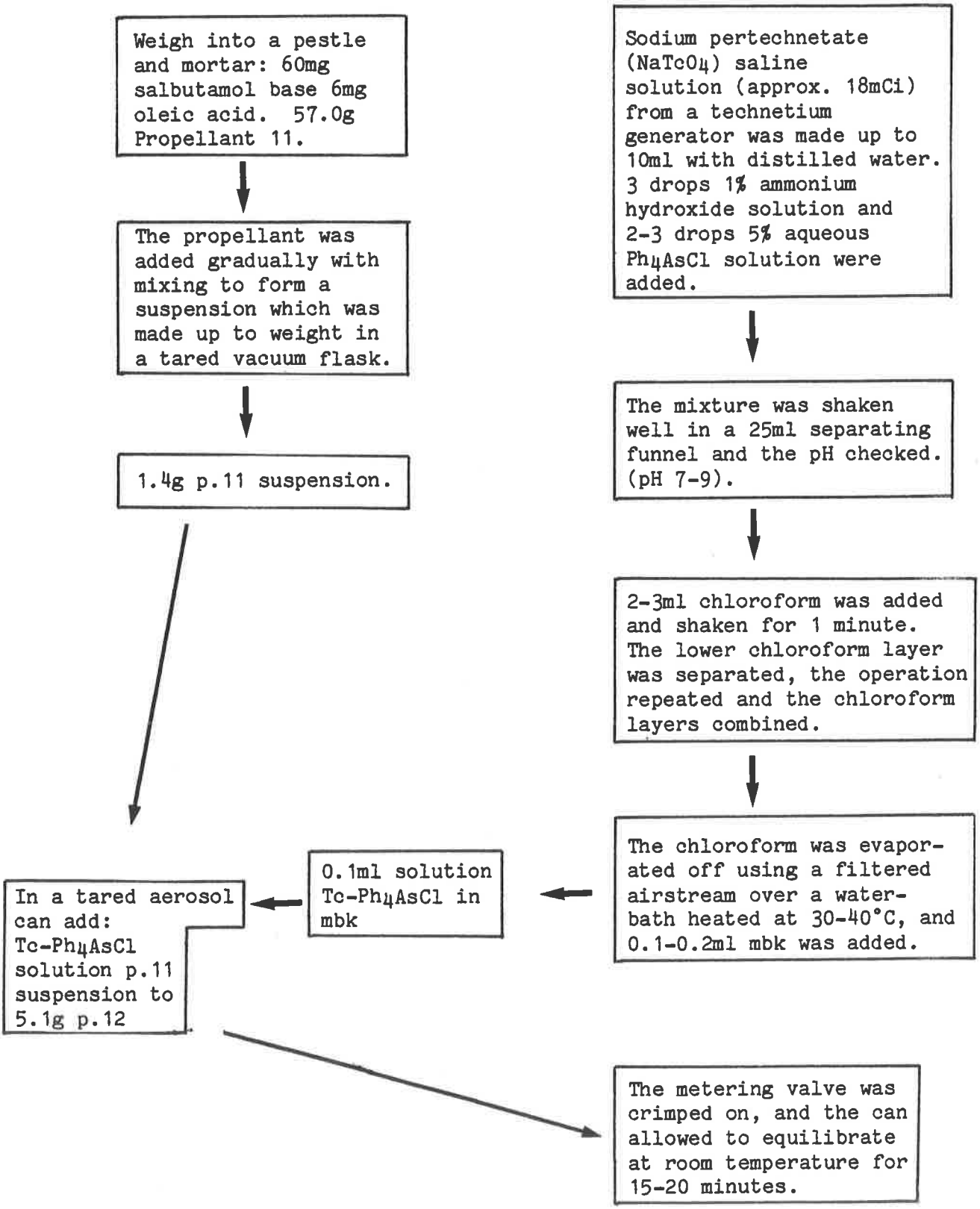
to 10ml distilled water.

The solution was placed in a Bennett twin-jet nebuliser (see Chapter 2) and the aerosol generated at 5l/min for 5 mins into the Andersen Sampler. Each impactor stage was washed with water and the samples were assayed for activity and mass particle size distributions.

3.4.2.5 Tetraphenylarsonium chloride complex with Technetium-99m (Tc-Ph_4AsCl).

Tc-Ph_4AsCl is a lipophilic technetium complex which has been used for labelling polystyrene aerosol particles (Few et al., 1970). Details of the modified method for addition of the complex to MDI formulations are described in Fig. 3.17. This method was used for preparing single cans of

Fig. 3.17 Preparation of MDI's containing a tetraphenylarsonium chloride complex.



high activity for in vivo experiments. The initial exploratory work was done using quantities for a ten can batch which included the addition of 3ml Tc-Ph_4AsCl complex in methyl isobutyl ketone (mbk) to 57.0g propellant 11 suspension.

The metered-dose inhalers were characterised using the Andersen Sampler as before. The initial results from this method appeared encouraging, so a subsequent series of preparations was undertaken to test the effect of various process variables on the nature of the labelled complex. The process variables investigated were:-

(a) relative concentrations of Tc-Ph_4AsCl and mbk in the labelled complex solution.

(b) the amount of shaking during separation and temperature of the water-bath used for evaporation.

(c) the order of addition of components to the tared can. The labelled complex can be added separately, mixed with the propellant 11 suspension, or mixed with the propellant 11 and 12 suspension.

(d) the number of shots in the can and the dose per shot.

(f) the presence of oleic acid in the formulation.

(g) the time interval between manufacture and sampling of the MDI.

(h) the reproducibility of results with respect to:-
- a single batch of 2 or 3 cans.
- repeated samples from 1 can.
- repeated assay measurements.

Details of these experiments are shown in Tables 3.12 and 3.13.

In addition to the quality control method using assays from the Andersen Sampler technique, several preparations were tested for dose/shot and shot weights.

The dose/shot method was as follows:-

(i) the MDI was shaken for at least 10 seconds.

(ii) a small valve stem actuator was fitted, and 1 shot fired into 15-20ml 50% aqueous ethanol in a measuring cylinder with the metering valve immersed.

(iii) The volume was recorded, and salbutamol content assayed by autoanalyser (see Chapter 2).

The shot weights were calculated from the loss of weight of can and actuator after each shot fired, weighing to 0.1mg.

Table 3.12 Details of experiments using Tc-Ph_4AsCl complex - section 3.4.2.5.

Experiment No. and study of variable	Can No.	MDI formulation (*)	Cold-fill method (*)	Contents of can	Sampling and Assay	Notes
(a) Concentration of Ph_4AsCl and mbk in labelled complex solution	1 2 3 4	120mg salbutamol base 12mg oleic acid to 34.2g P.11 to 122g P.12 fill 10.2g P11/12 suspn. per can	0.1 mbk Batch A 0.1ml-0.5ml mbk Batch A 0.1ml mbk Batch B 0.1ml-0.5ml mbk Batch B	100µg/.shot salbutamol 100 shots	2 shots fired to waste. 20 shots into Andersen sampler	Batch A complex 2 drops 5% Ph_4AsCl soln. Batch B complex 8 drops 5% Ph_4AsCl soln. assuming 1 drop = 0.1ml mbk Can 1 50mg/ml, 0.1ml Can 2 10mg/ml, 0.5ml Can 3 200mg/ml, 0.1ml Can 4 40mg/ml, 0.5ml
(b) amount of shaking during separation and temperature of water bath.	1 2 3 4	standard method same suspension for all 4 cans.	standard method	30µg/shot salbutamol	2 shots fired to waste. 40 shots into Andersen sampler	Vigorous shaking, temp. >40°C Vigorous shaking, temp. < 40°C gentle shaking, temp. >40°C gentle shaking, temp. < 40°C Actual water bath temps. were 30-38°C and 42-58°C, time taken for evaporation varied between 20-50 minutes.
(c) order of addition of components		as (a) for 100µg/ shot		30 or 100µg/shot		Details of these preparations shown in Table 3.7.
(d) dose/shot and number of shots in the can		standard method (Fig. 3.15) for 30µg/shot	standard method	60 or 100 shots per can		

(*) See Figs. 3.14 and 3.15 for details of these methods.

Table 3.12 Details of experiments using Tc-Ph_4AsCl complex - section 3.4.2.5. Contd.

(e) effect of solvent, mbk, alone		60mg salbutamol base 6mg oleic acid to 57.0g P.11	0.1ml mbk.to 1.7g P.11 suspn. to 6.1g P.12	60 shots 30µg/shot	8 shots fired to waste. 20 shots into Andersen sampler	
(f) effect of oleic acid in formulation		60mg salbutamol base to 17.1g P.11	0.1ml mbk/complex to 2.85g P.11 suspn. to 10.2g P.12	100 shots 100g/shot 2 cans filled	6 shots fired to waste. 20 shots into Andersen sampler.	
(g) time interval between manufacture and sampling		as (a)	standard method	100 shots 100µg/shot	2 shots fired to waste. 3 x 10 shots into Andersen sampler	Sampling at 3 diff. times after manuf. (i) 30 mins (ii) 3 hours (iii) 24 hours
(h) reproducibility of results	1	standard method	standard method	30µg/shot	Each can, 4 shots fired to waste. 40 shots into Andersen sampler.	Reproducibility of assay results tested by repeat assays of separate aliquots of samples in the auto-analyser. Samples from Can 1, Experiment (c) & (d) - see Table 3.7
	2 3	same P.11 suspn. & mbk/complex used.	3 cans filled			
	4	standard method	standard method	60 shots 100µg/shot	20 shots fired to waste. Andersen sampler shots 21-30 Shots 31-40 sampled separately.	
Placebo		64.8mg oleic acid 3ml mbk/complex to 140g P.11	40g P.11 soln. to 143g P.12	3 cans filled 200 shots	2 shots fired to waste. 30 shots into Andersen sampler	2 cans tested

(*) See Figs. 3.14 and 3.15 for details of these methods.

Table 3.13 Details of manufacture of MDI's

study (i) order of addition of components
(ii) dose/shot
(iii) number of shots in the can.

Can No.	Dose/shot	no. of shots	Components + mixture
1	30µg	60	mbk + complex + P.11 + P.12 (a)
2	100µg	60	mbk + complex + P.11 + P.12
3	100µg	60	(mbk/complex/P.11) + P.12
4	100µg	100	(mbk/complex/P.11) + P.12
5	100µg	60	(mbk/complex/P.11) + P.12 (b)
6	100µg	60	(mbk/complex/P.11/P.12)
7	100µg	100	(mbk/complex/P.11/P.12) (c)

(a) Can 1 represents the type of formulation used in all in vivo experiments.

(b) Same as can 3 but ratio of propellants 11:12 is 40:60 (usual ratio is 28:72)

(c) Duplicate results were obtained from 2 x 20 shot samples from the same can.

3.4.3 Results

Table 3.14 shows the results from each of the cocrystallisation and chelation methods shown in Table 3.11. The detailed results from each experiment are listed in Appendix 3.4, with graphs of size distributions and supplementary results shown in the main text, sections 3.4.3.1 and 3.4.3.2.

3.4.3.1 Cocrystallisation

Table 3.15 shows the results from leaching experiments performed on a single batch of cocrystallised indium oxine/salbutamol. Very little γ-activity leaches into the propellant 11 even after 22 hours ball-milling. This is probably due to the low solubility of indium oxine in fluorocarbons. Initial samples from the same batch, when filtered and the solid retained was washed separately with propellant 11 and water, 23.6% and 67.4% of the activity was lost, respectively.

Fig. 3.18 shows the activity and particle size distributions obtained from the Andersen Sampler assay. The results are summarised in Table 3.14.

3.4.3.2. Chelation complexes

Figs. 3.19 and 3.20 show the activity and particle size distributions of Tc-oxine/salbutamol MDI's prepared by Methods A and B. Fig. 3.21 shows the activity distribution of a placebo MDI containing Tc-oxine. These results are summarised in Table 3.14.

The size distributions of the Tc-DTPA/salbutamol formulations were assessed similarly using the Andersen Sampler. The size distributions are shown in Fig. 3.22 and the results summarised in Table 3.14.

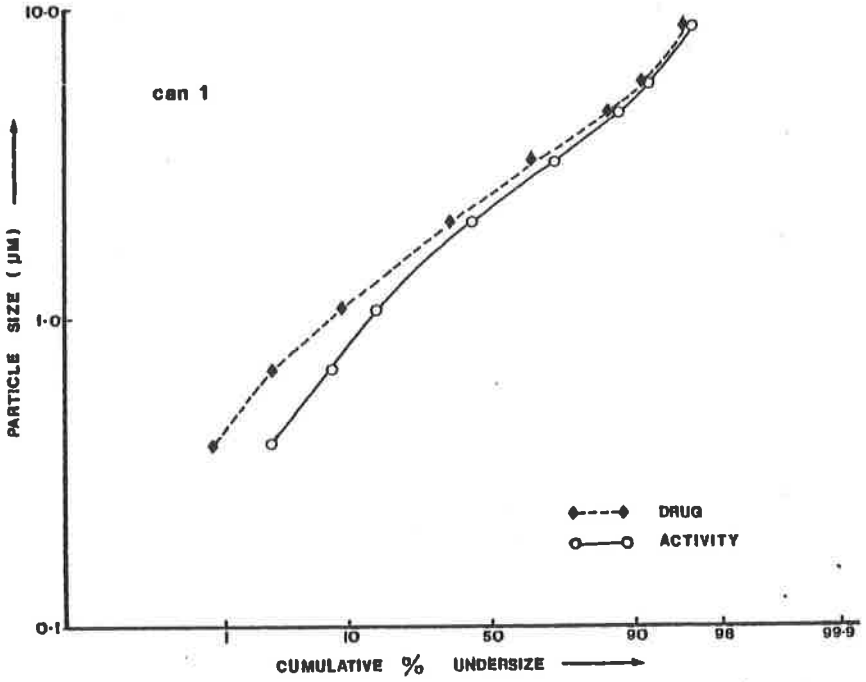
Table 3.14 Results from Cocrystallisation and chelation experiments (for methods see Table 3.11).

Method	Can No.	mass mean diameter (μm)	σg	activity mean diameter (μm)	σg	Appendix 3.4 Detailed Results	Graphs of size distributions
Cocrystallisation (section 3.4.2.2) Indium oxine	1	2.50	1.80	2.10	2.20	Table 3.4.1	Fig. 3.18
	2	2.40	1.85	2.05	2.15		
Chelation (Section 3.4.2.3) Technetium oxine Method A	1	1.65	1.51	1.55	1.92	Table 3.4.2	Fig. 3.19
	2	1.55	1.53	1.50	1.84		
	3	1.50	1.46	1.45	1.81		
	4	1.85	1.49	1.45	1.82		
Chelation (Section 3.4.2.3) Technetium oxine Method B	1	2.50	1.52	2.10	2.25	Table 3.4.3	Fig. 3.20
	2	3.00	1.61	2.30	2.44		
Chelation (Section 3.4.2.3) Technetium oxine Placebo	1	-	-	0.80	1.75	Table 3.4.4	Fig. 3.21
	2	-	-	0.80	1.65		
DTPA (Section 3.4.2.3) Method A		1.35	3.50	1.60	2.27	Table 3.4.5	Fig. 3.22
DTPA (Section 3.4.2.3) Method B		2.10	2.66	1.45	2.10	Table 3.4.5	Fig. 3.23

Table 3.15 Leaching of γ-activity from cocrystallised indium oxine/ salbutamol powder samples retained on a filter and washed with water or propellant

Sample (no. of hours milling)	Solvent	Counts per minute per gram x 10^{-4}	% of original activity remaining
Standard (initial)	-	145.7	100.0
0 hours	water	47.5	32.6
0 hours	Propellant 11	111.3	76.4
2 hours	Propellant 11	128.9	88.5
5 hours	Propellant 11	146.2	100.3
22 hours	Propellant 11	140.8	96.6

Fig 3.18 Activity and Mass Particle Size Distributions for MDI's Containing Salbutamol and Indium Oxine. (Data given in Table 3.4.1, Appendix 3.4).



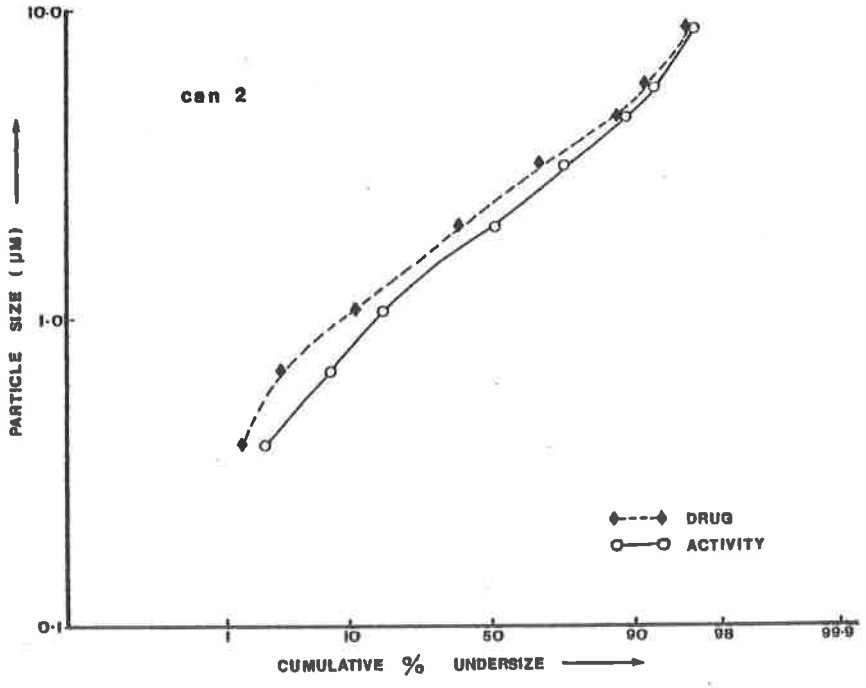


Fig. 3.19 Activity and Mass Particle Size Distributions for MDI's Containing Salbutamol and Technetium Oxine, Method A.
(Data given in Table 3.4.2, Appendix 3.4).

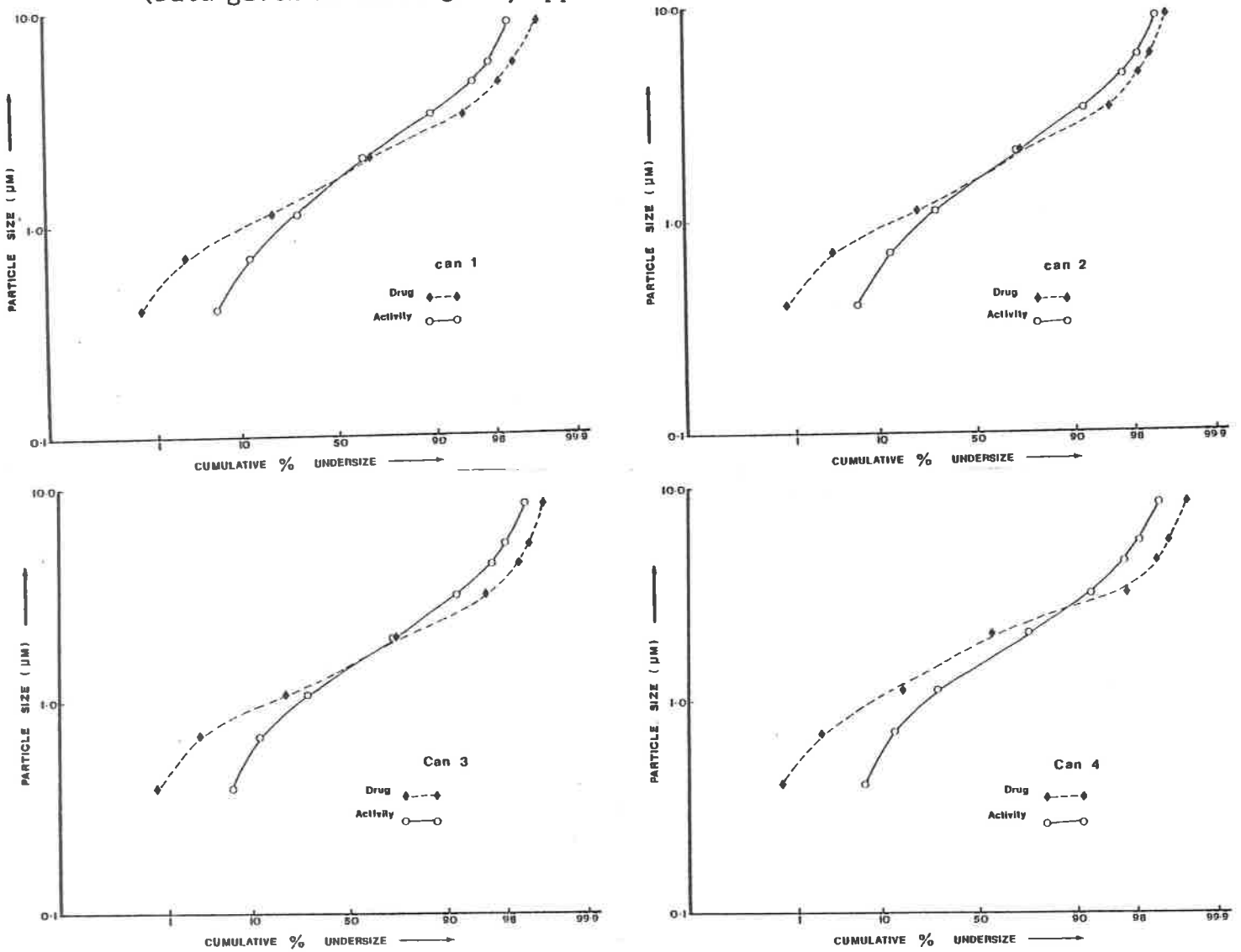


Fig 3.20 Activity and mass particle size distributions for MDI's containing salbutamol and technetium oxine, Method B. (Data given in Table 3.4.3., Appendix 3.4.)

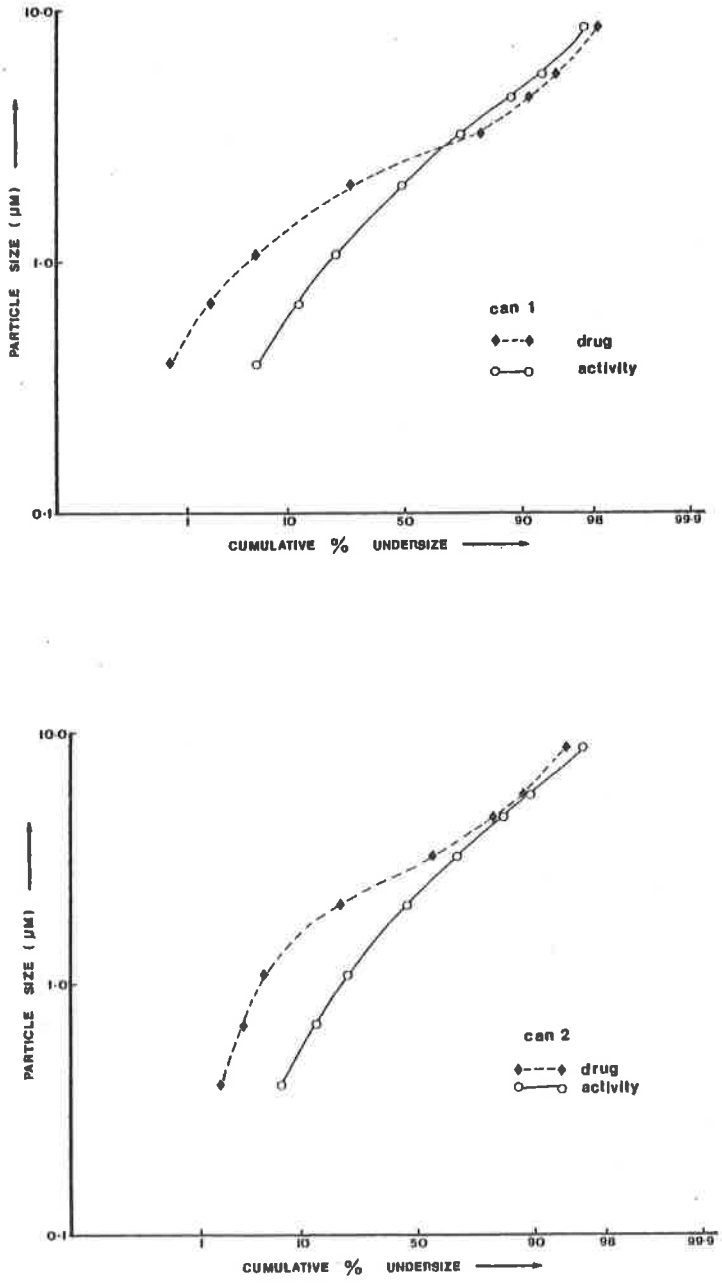


Fig 3.21 Activity Size Distributions for Placebo MDI Containing Technetium Oxine. (Data given in table 3.4.4, Appendix 3.4).

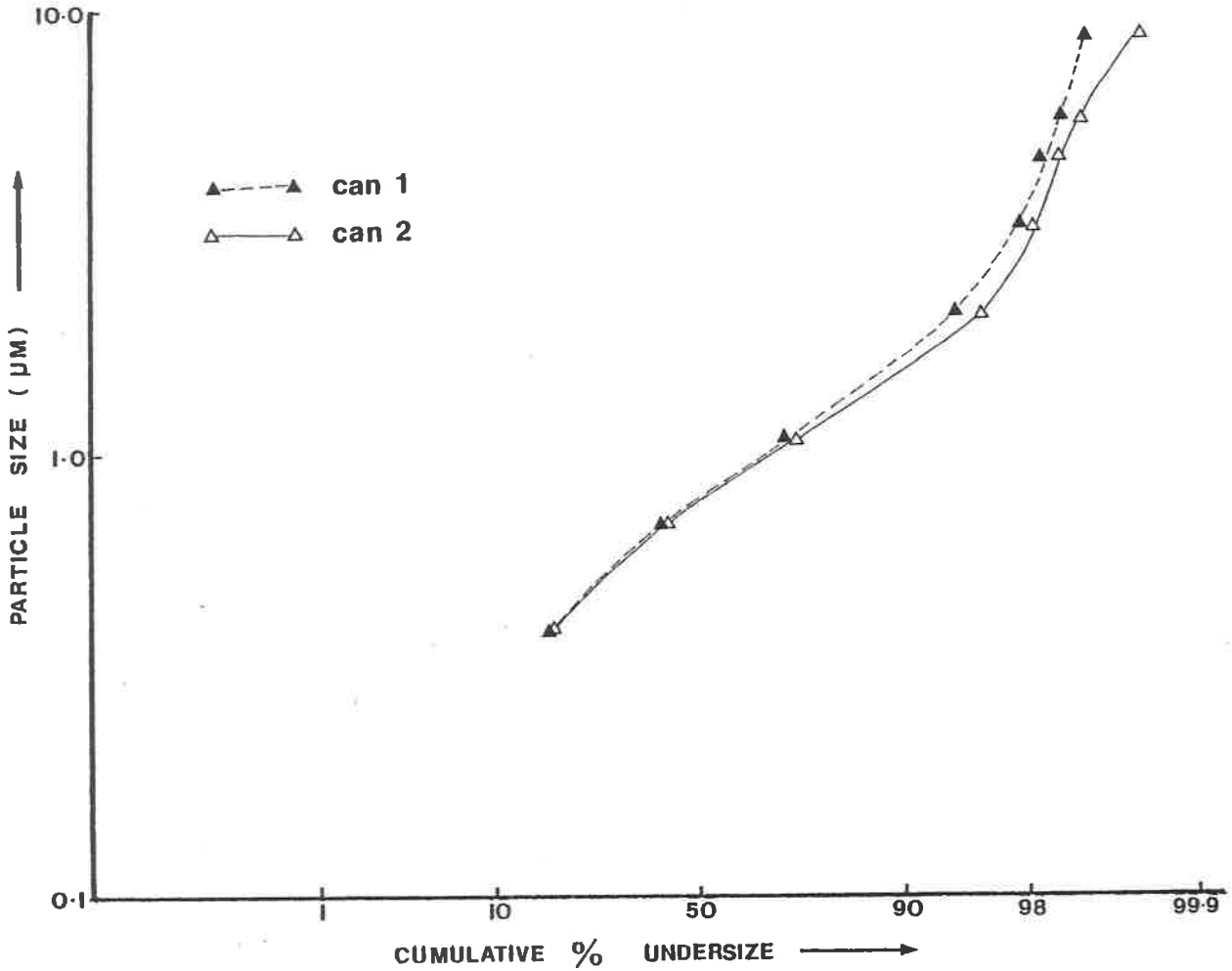
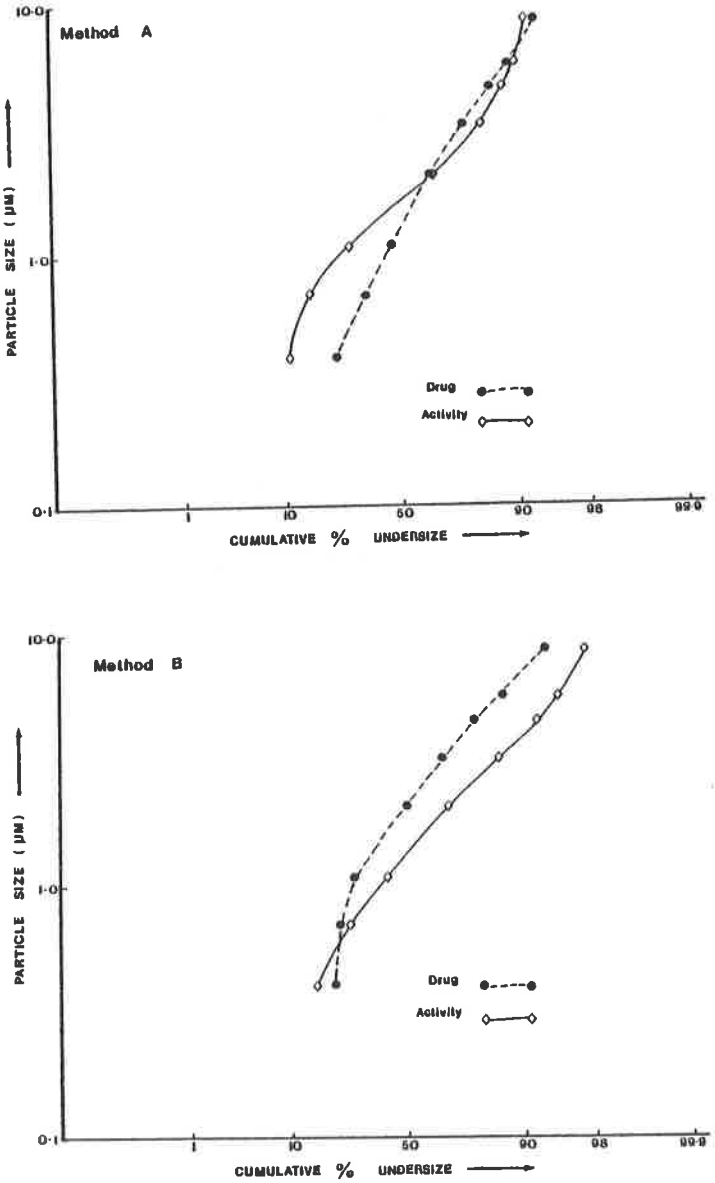


Fig. 3.22 Activity and Mass Particle Size Distributions for MDI's Containing Salbutamol and Tc-DTPA.
(Data given in Table 3.4.5, Appendix 3.4).



3.4.3.3 Salt formation

Fig. 3.23 shows the results obtained from an initial experiment, sampling an aqueous solution containing salbutamol sulphate and sodium pertechnetate from a nebuliser into the Andersen Sampler. The distribution parameters are summarised below:

Salbutamol mass distribution: dgw = 1.50μm σg = 1.60

Technetium activity distribution: d_{act} = 1.40μm σg = 1.65

3.4.3.4 Tetraphenylarsonium chloride complex with Technetium-99m (Tc-Ph_4AsCl)

The results from the initial experiments and studies of process variables using this complex are shown in Figs. 3.24 - 3.34 and summarised in Table 3.16. The data used to plot the activity and size distributions is shown in Appendix 3.15.

From the graphs it is clear which formulations produce a valid radiolabelled product. When the drug and activity size distributions match over the range measured in the Andersen Sampler, it may be assumed that the γ-radiolabel is homogenous with the drug in the aerosol cloud. Several of the graphs show good agreement between drug and activity distributions except at the extreme ends of the particle size range (e.g. Fig. 3.26, cans 2 and 4). However, it must be remembered that the probability scale introduces a bias in the appearance of the curve, and the apparent discrepancies represent a very small proportion of the overall distribution by weight.

Fig 3.23 Activity and Mass Particle Size Distributions from a Nebulised Solution Containing Salbutamol Sulphate and Sodium Pertechnetate.
(Data given in Table 3.4.6, Appendix 3.4).

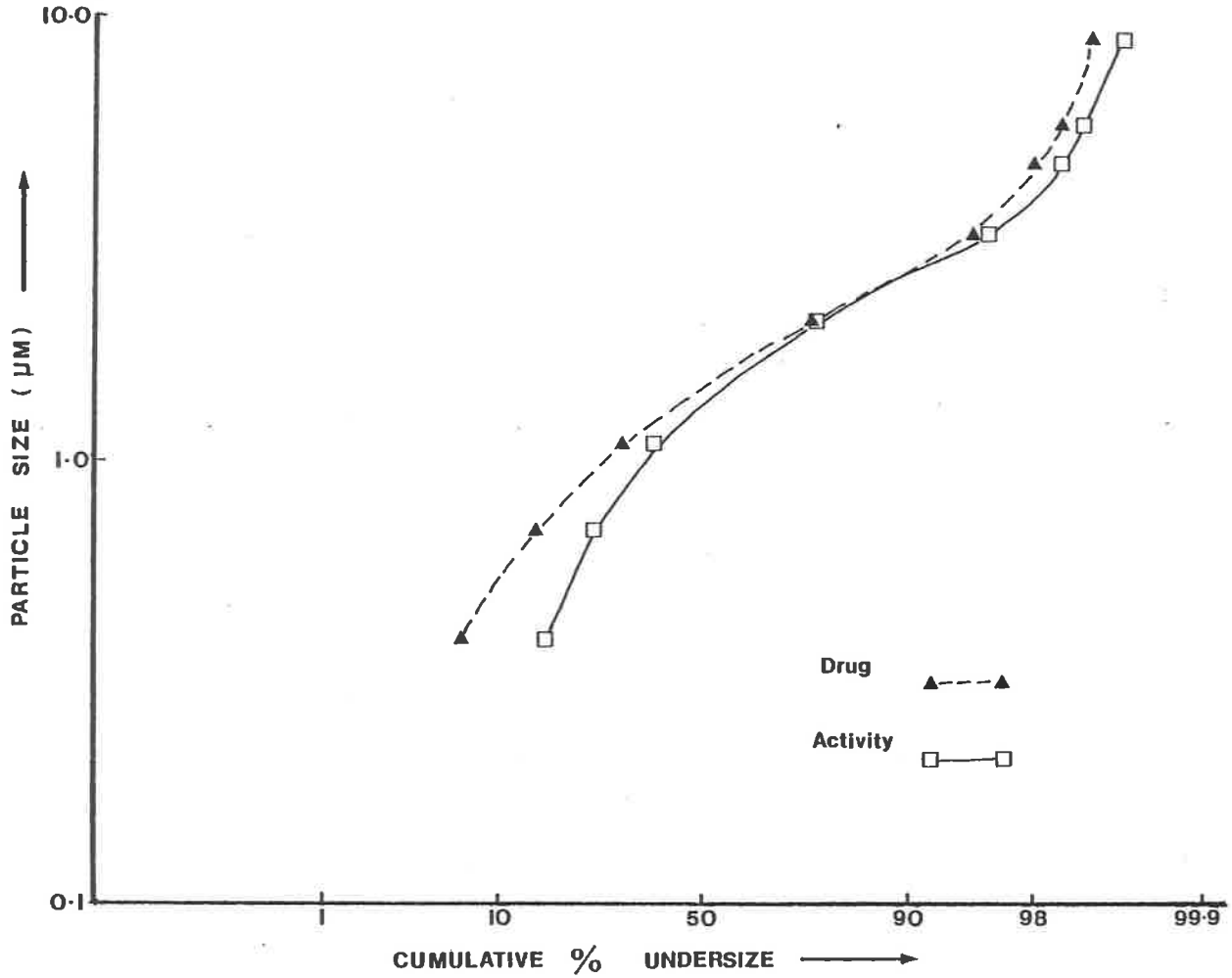


Table 3.16 Results from experiments using Tc-Ph_4AsCl complex (see Table 3.12)

Experiment	Can No.	mass mean diameter (μm)	σ g	activity mean diameter (μm)	σ g	Appendix 3.5 Results	Graphs of size distributions
Initial	1	1.95	1.67	2.00	1.70		
	2	2.10	1.67	2.20	1.80		
	3	1.60	1.63	1.65	1.69	Table 3.5.1	Fig. 3.24
	4	1.85	1.69	1.80	1.71		
(a)	1	3.00	1.36	3.20	1.60		
	2	2.30	1.65	1.95	1.72		
	3	3.10	1.60	2.95	1.91	Table 3.5.2	Fig. 3.25
	4	2.65	1.81	2.10	1.77		
(b)	1	2.30	1.48	2.10	1.62		
	2	2.50	1.49	2.45	1.56		
	3	2.20	1.53	2.05	1.62	Table 3.5.3	Fig. 3.26
	4	2.55	1.56	2.65	1.56		
(c) and	1	2.10	1.69	1.90	1.72		
(d)	2	2.85	1.51	3.00	1.52		
	3	2.75	1.50	2.30	1.75	Table 3.5.4	Fig. 3.27
	4	2.95	1.49	2.50	1.91		
	5	2.95	1.48	2.70	1.77		
	6	2.75	1.48	2.85	1.64	Table 3.5.5	Fig. 3.28
	7	2.7, 2.85	1.51, 1.51	2.65, 2.95	1.66, 1.62		
(e)		2.40	1.47	-	-	Table 3.5.6	Fig. 3.29
(f)	1	2.40	1.88	2.70	1.89		
	2	2.50	1.81	2.30	2.08	Table 3.5.7	Fig. 3.30
(g)	1	2.35	1.54	2.45	1.56		
	2	2.65	1.55	2.90	1.59	Table 3.5.8	Fig. 3.31
	3	2.70	1.93	2.80	2.89		
(h)	1	2.40	1.66	2.30	1.48		
	2	2.30	1.52	1.85	1.78	Table 3.5.9	Fig. 3.32
	3	2.30	1.56	2.15	1.63		
	4a	2.67	1.74	2.85	1.68		
	4b	2.40	1.86	2.10	1.89	Table 3.5.10	Fig. 3.33
	1 (c) & (d)	2.30	1.52	1.93	1.72		
Placebo	1	-	-	1.15	2.07		
	2	-	-	1.05	2.12	Table 3.5.11	Fig. 3.34

Fig. 3.24 Activity and Mass Particle Size Distributions for MDI's from Initial Experiments.
(Data given in Table 3.5.1, Appendix 3.5).

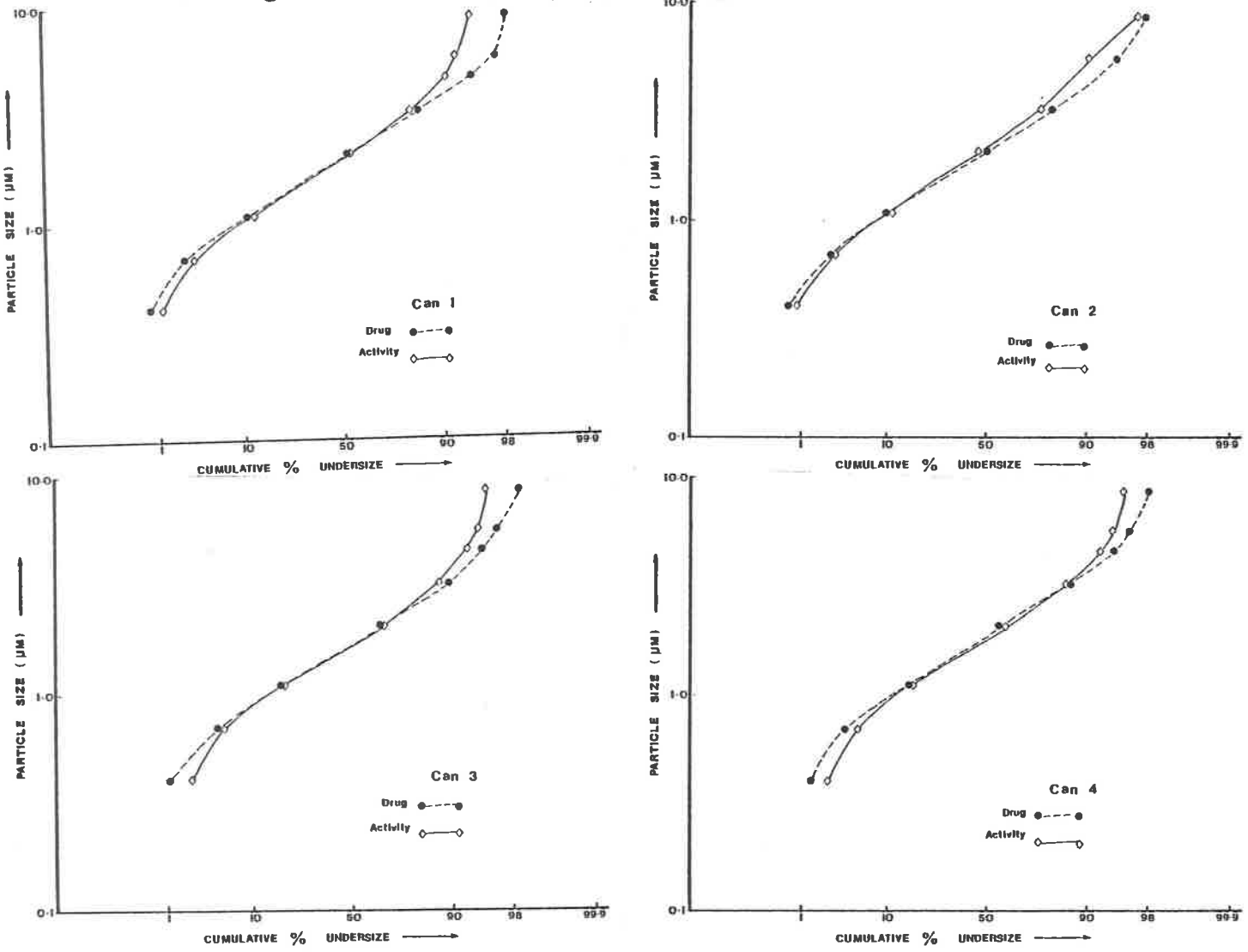


Fig. 3.25 Activity and Mass Particle Size Distributions for MDI's from Experiment (a) - A Study of Concentration of Ph_4AsCl and mbk in Labelled Complex Solution. (Data given in Table 3.5.2, Appendix 3.5).

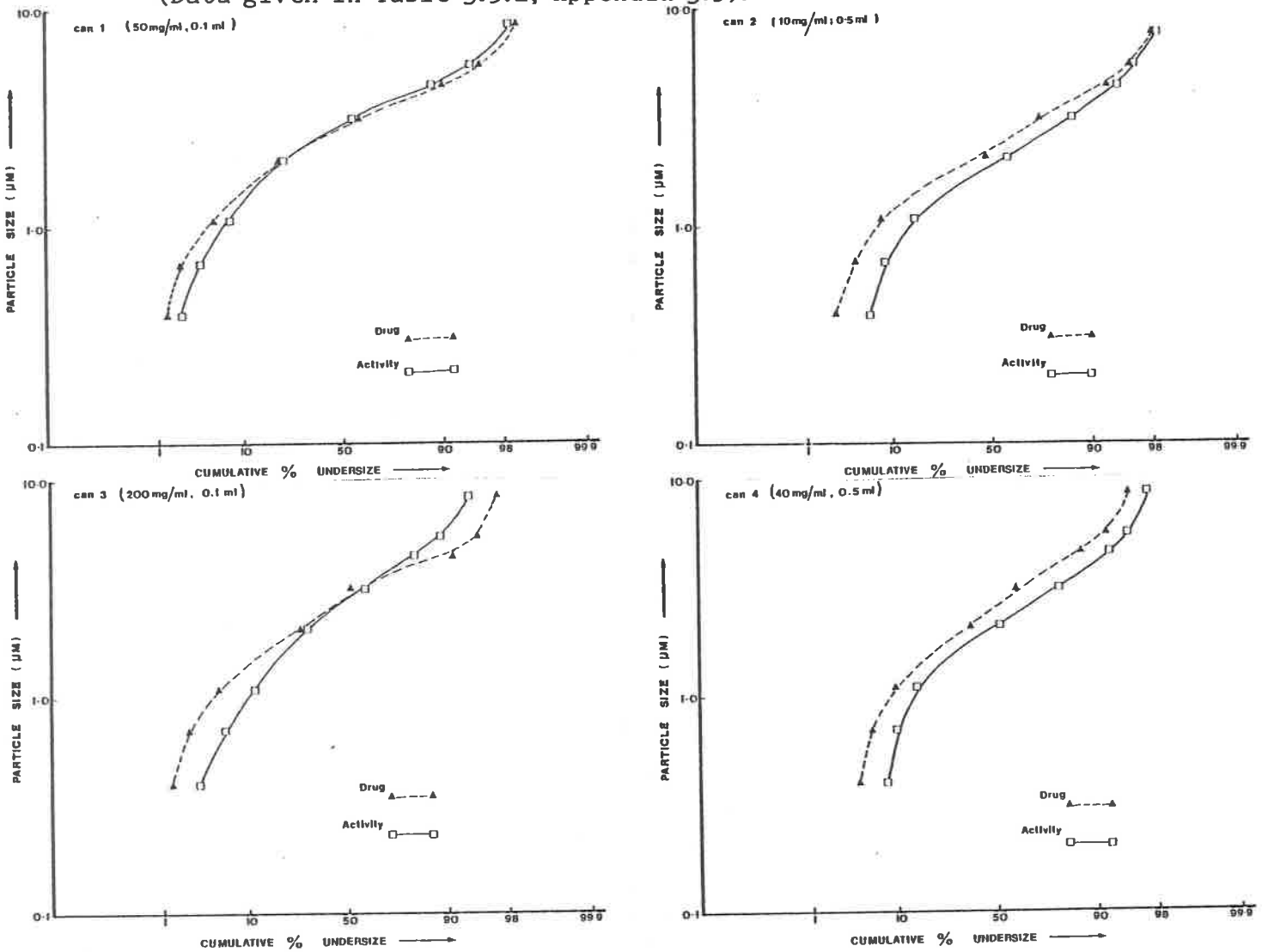


Fig. 3.26 Activity and Mass Particle Size Distributions for MDI's from Experiment (b) - A study of Shaking During Separation and Temperature of Water Bath. (Data given in Table 3.5.3, Appendix 3.5).

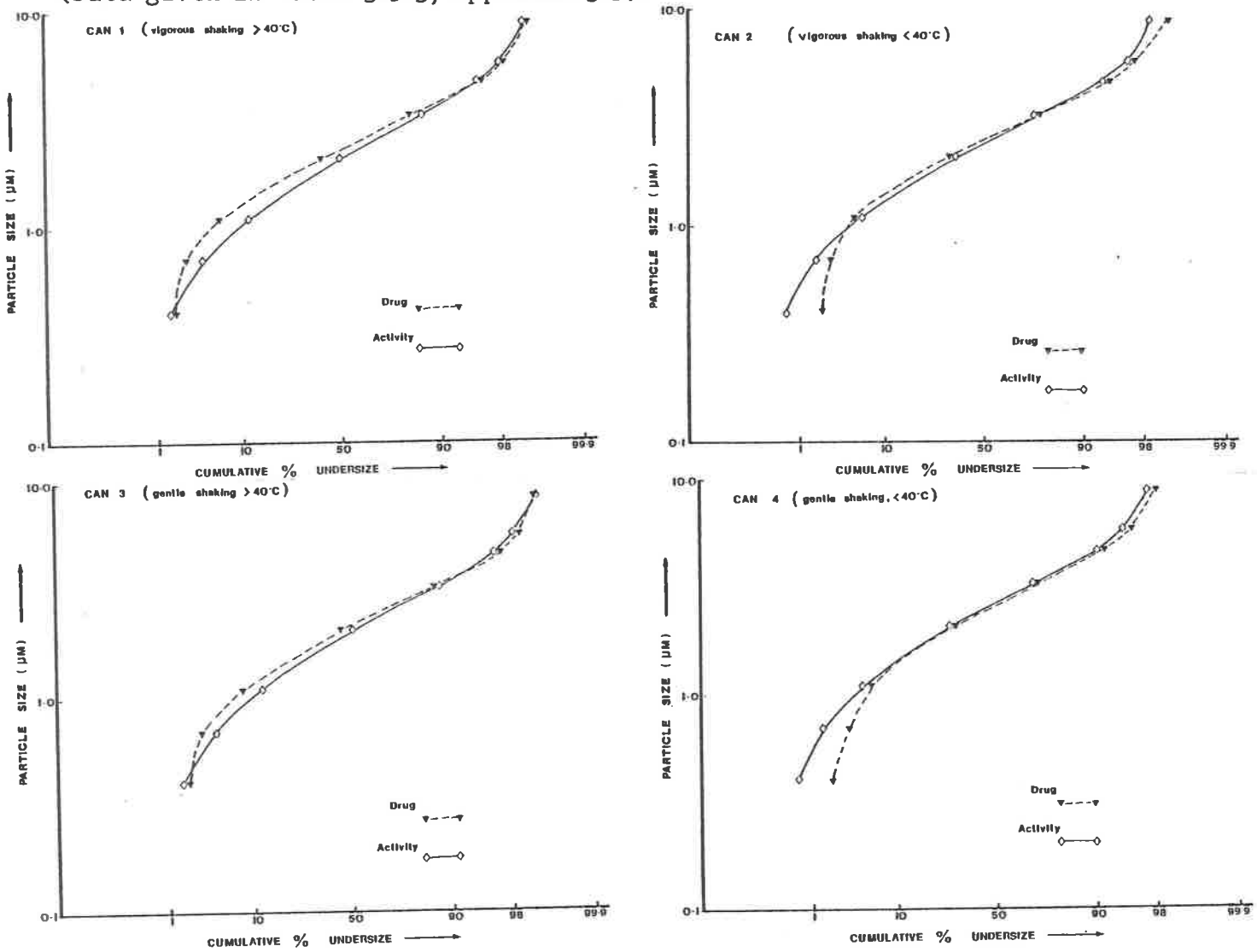


Fig. 3.27 Activity and Mass Particle Size Distributions for MDI's from Experiments (c) and (d) - A Study of Addition of Components, Dose/Shot and Number of Shots. (Data given in Table 3.5.4, Appendix 3.5).

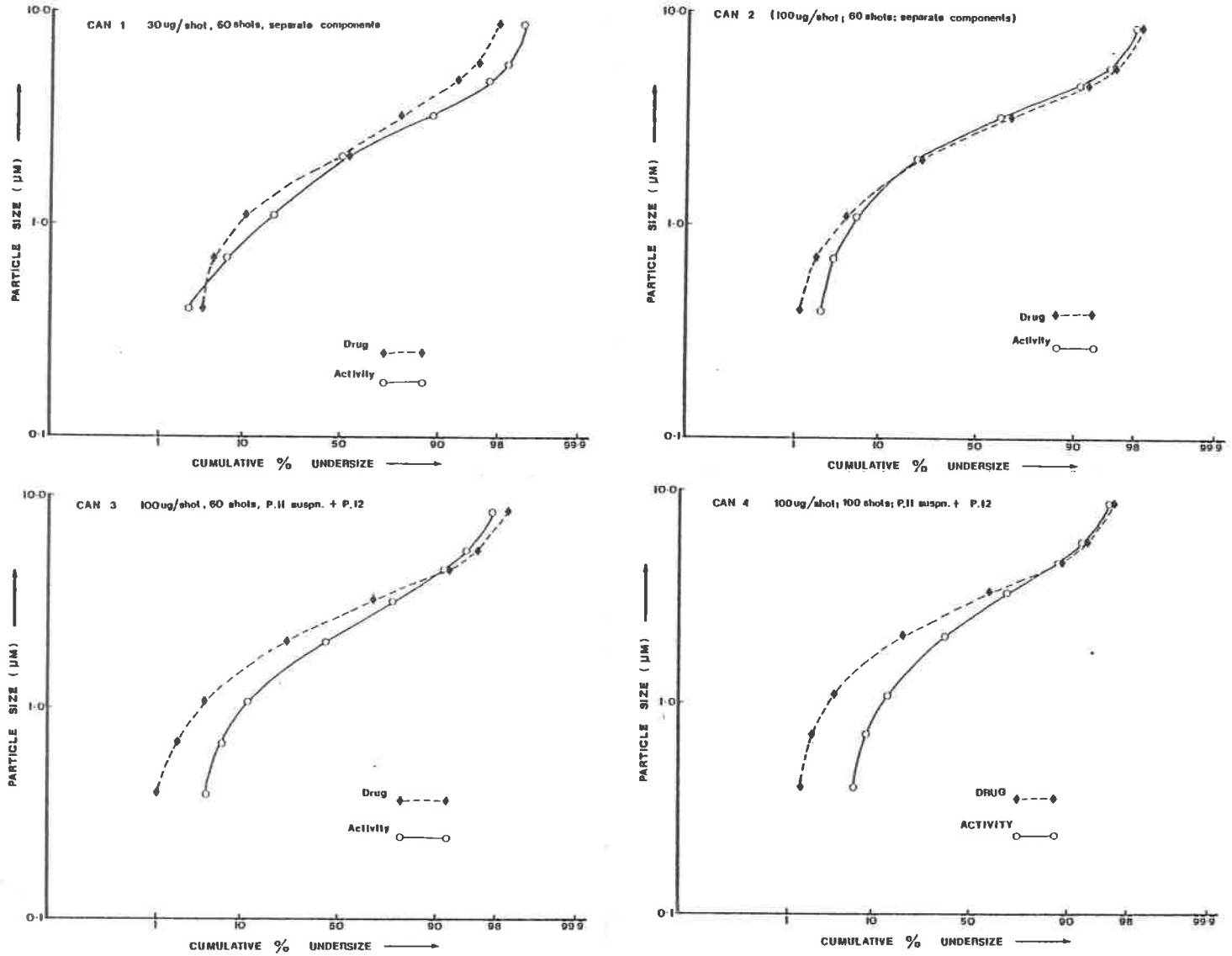


Fig 3.28 Activity and Mass Particle Size Distributions for MDI's from Experiments (c) and (d) - Continued.
(Data given in Table 3.5.5, Appendix 3.5).

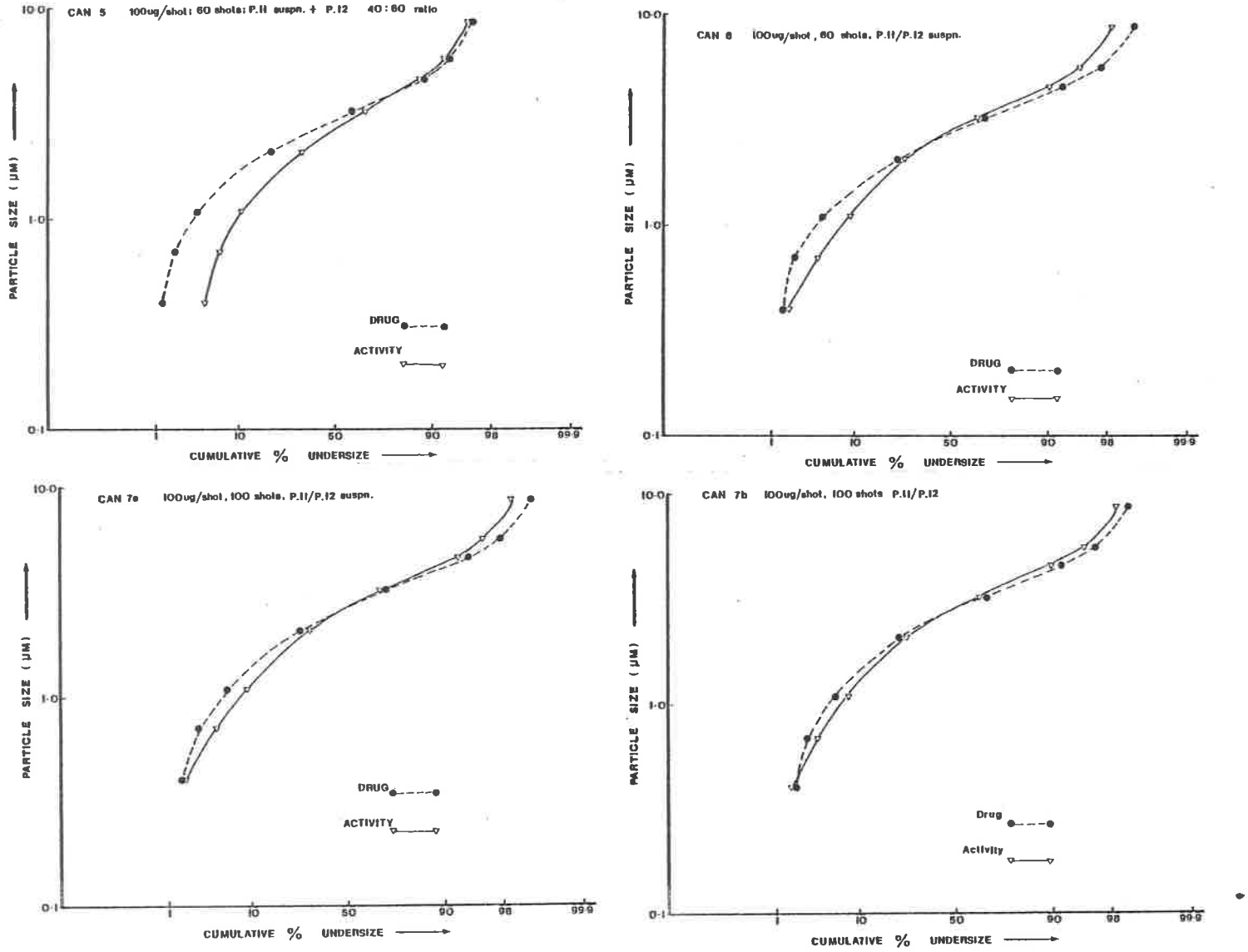


Fig 3.29 Particle Size Distribution for MDI from Experiment (e) Containing mbk Solvent but no Tc-Complex.
(Data given in Table 3.5.6, Appendix 3.5).

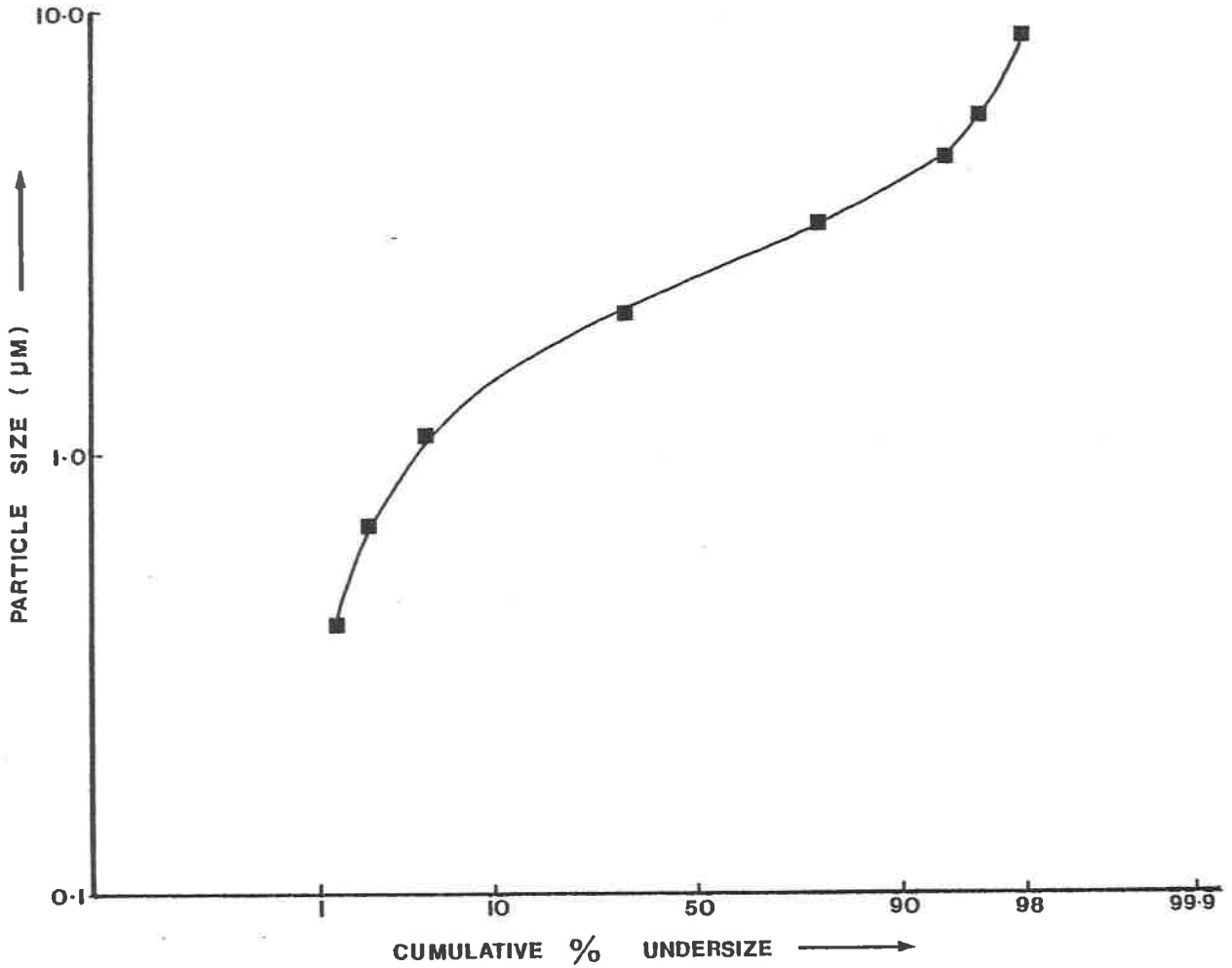
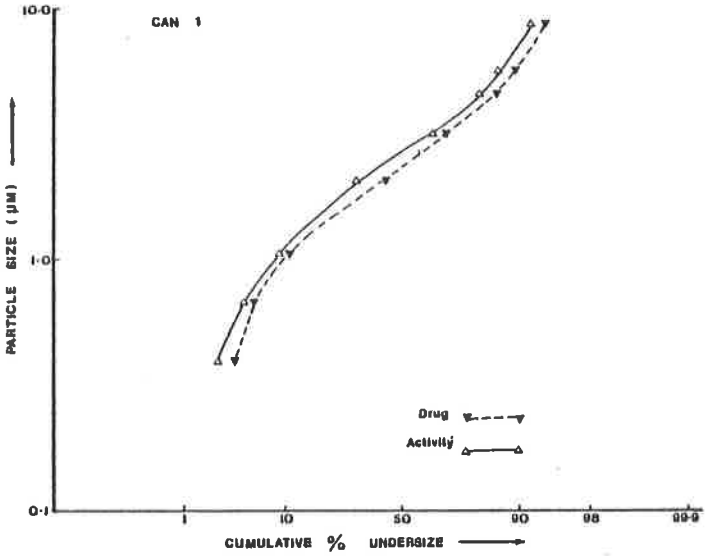


Fig 3.30 Activity and Mass Particle Size Distributions for MDI's from Experiment (f) - Containing no Oleic Acid. (Data given in Table 3.5.7, Appendix 3.5).



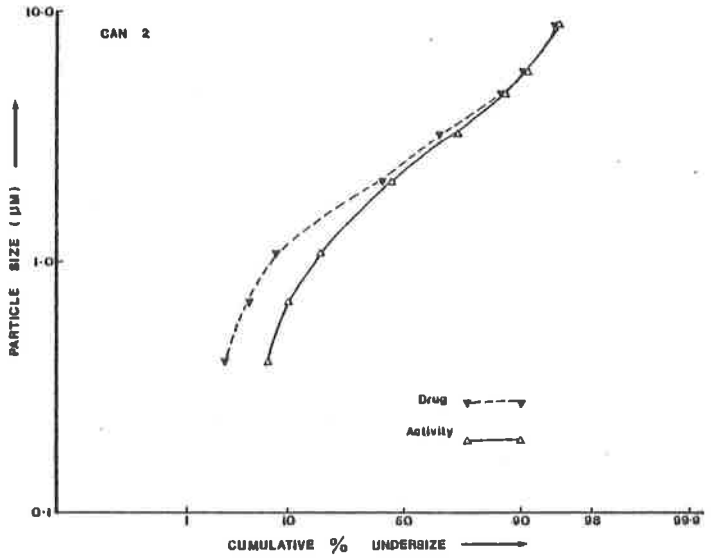


Fig 3.31 Activity and Mass Particle Size Distributions for MDI's from Experiment (g) - To Study the Effect of the Time Interval Between Manufacture and Sampling. (Data given in Table 3.5.8, Appendix 3.5).

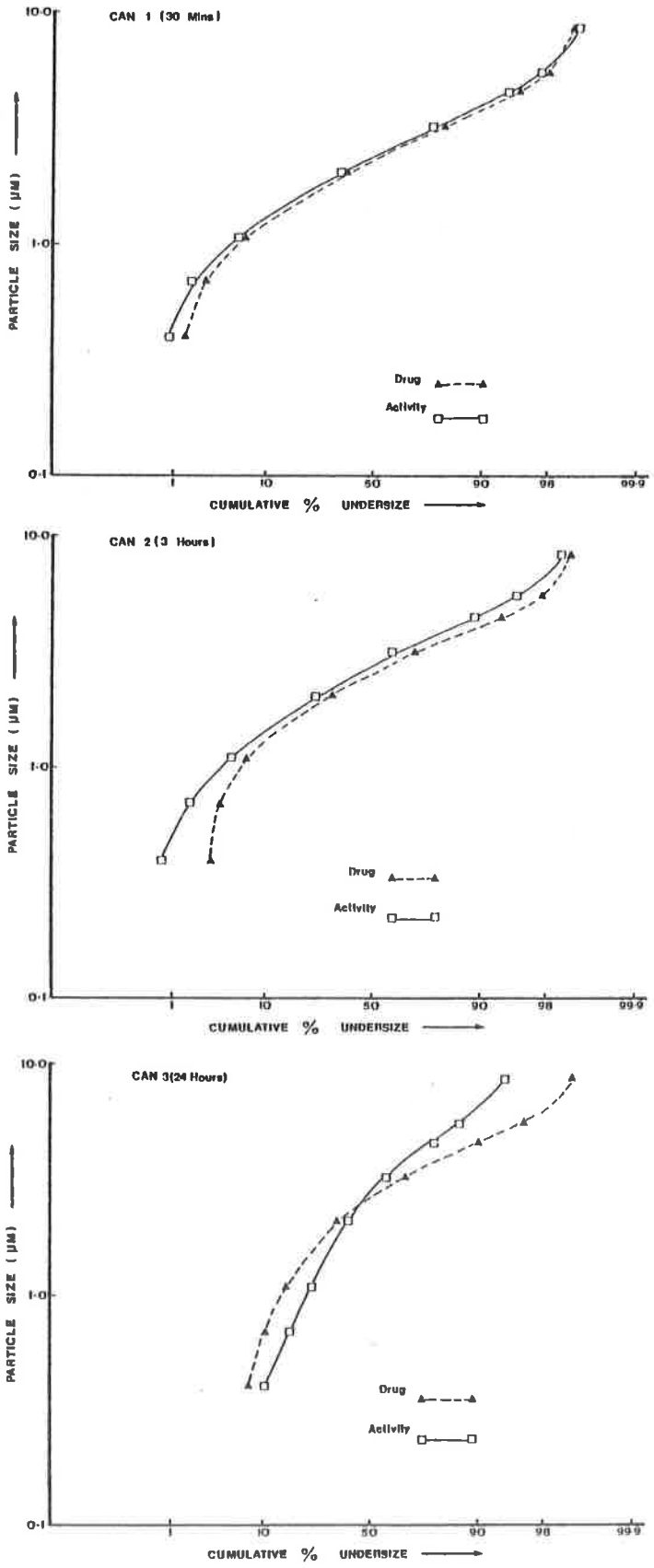


Fig 3.32 Activity and Mass Particle Size Distributions for MDI's from Experiment (h) - To Study the Reproducibility of Results, 3 Cans from the same Batch.
(Data given in Table 3.5.9, Appendix 3.5).

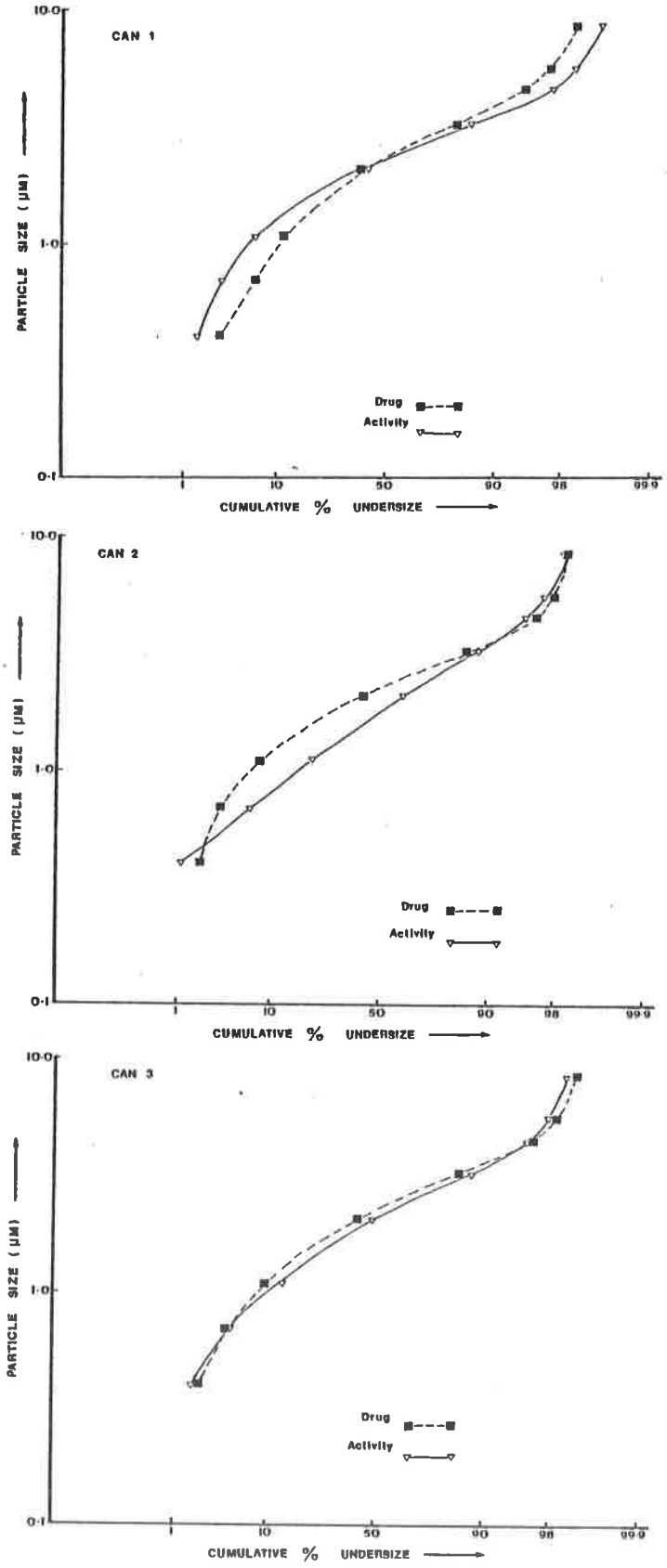


Fig 3.33 Activity and Mass Particle Size Distributions for MDI's from Experiment (h) - To Study the reproducibility of Results, from 1 Can and Assay Method.
(Data given in Table 3.5.10, Appendix 3.5).

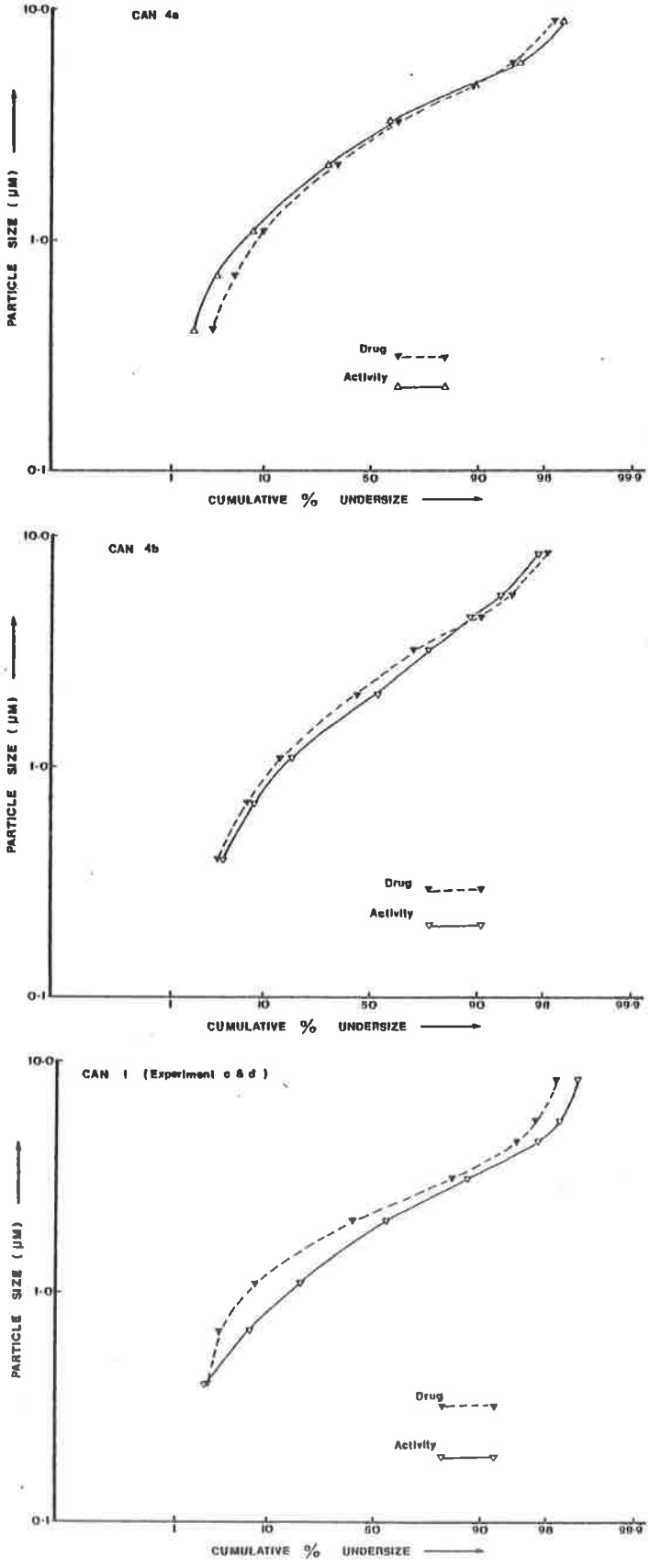


Fig 3.34 Activity Size Distributions for Placebo MDI Containing Tc-Ph_4AsC1 Complex. (Data given in Table 3.5.11, Appendix 3.5).

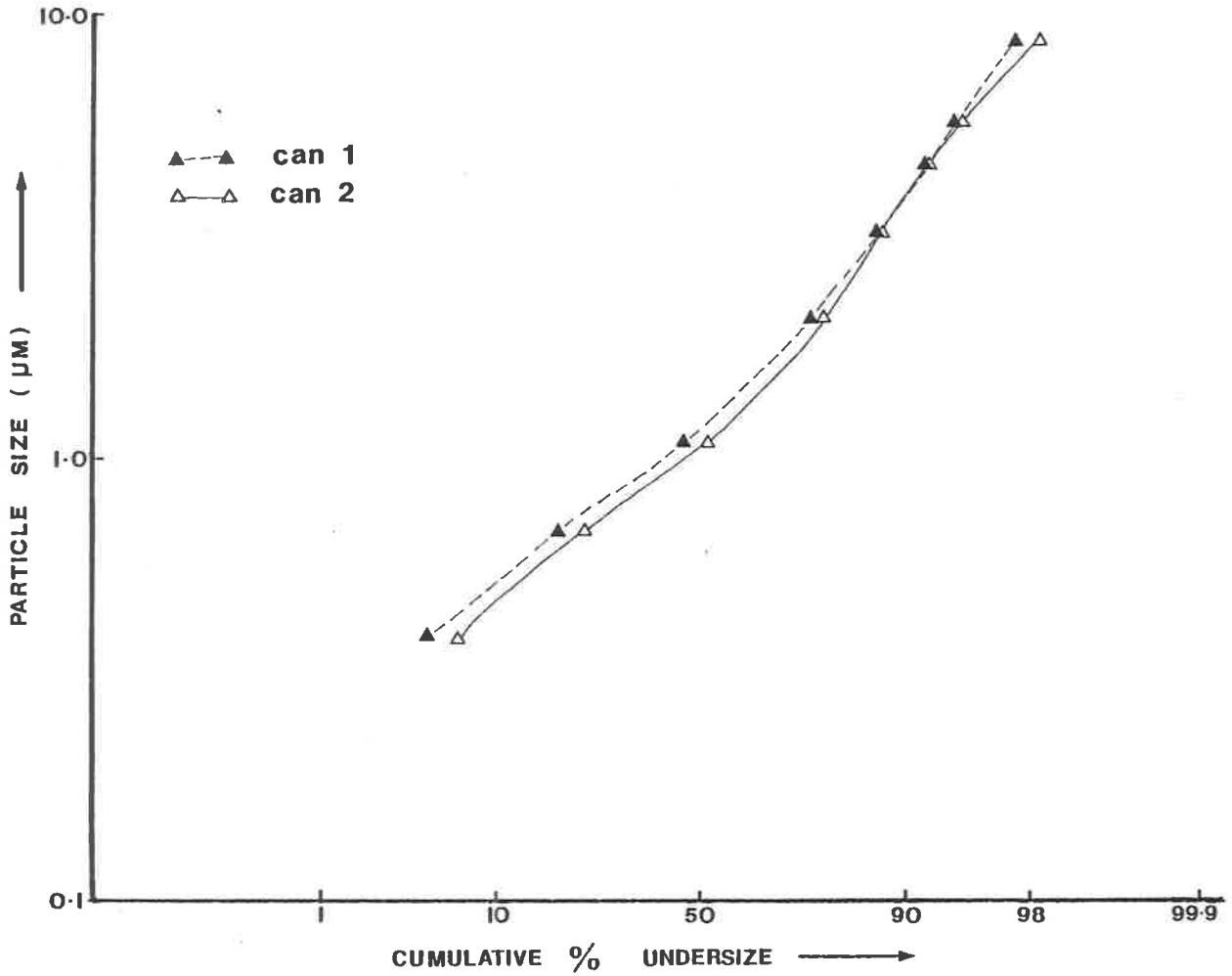


Fig. 3.35 and Table 3.17 show the dose/shot and shot weight data obtained from several of the experimental formulations. The range of shot weight values confirm normal valve functioning and the dose/shot data show correct quantities for 30µg/shot and 100µg/shot formulations.

The low dose/shot figure of 5.5µg (range 1.7-14.6µg) in experiment (h) (Table 3.17) for shot numbers 45-55 reflects the normal reduction in dose/shot in the last few doses from an inhaler.

3.4.4 Discussion

The particle size distribution of salbutamol in a Ventolin Inhaler is measured reproducibly by the Andersen Sampler (see Chapter 2). The mass mean aerodynamic diameter (± S.D.) and geometric standard deviation (G.S.D.) for micronised salbutamol in Ventolin Inhaler are 2.43µm (1.07) and σg 1.56 (1.09) (n=12) respectively. The variation in these size distribution parameters is likely to increase for laboratory scale manufacturing processes (particularly mixing by pestle and mortar) and preparation of single can batches as with the radiolabelled products. Nevertheless, in the reproducibility studies carried out on the Tc-Ph_4AsCl/salbutamol preparation, the distribution parameters for salbutamol were comparable to those measured in the standard Ventolin product (MMAD 2.40 (1.12), σg 1.64 (1.01) n=6). The corresponding activity distribution parameters for these samples were: AMAD 2.20 (1.13), σg 1.70 (1.03) (n = 6). The salbutamol assay methods are also reproducible (see Table 3.16), so that any large differences in distributions as demonstrated by the log-probability plots of results from the Andersen Sampler, are probably due to differences in preparation or formulation. The major criterion used for determining the validity of a radiolabel

Fig 3.35 Dose Delivery of Salbutamol from Experiment (c&d) Cans 1 and 2.

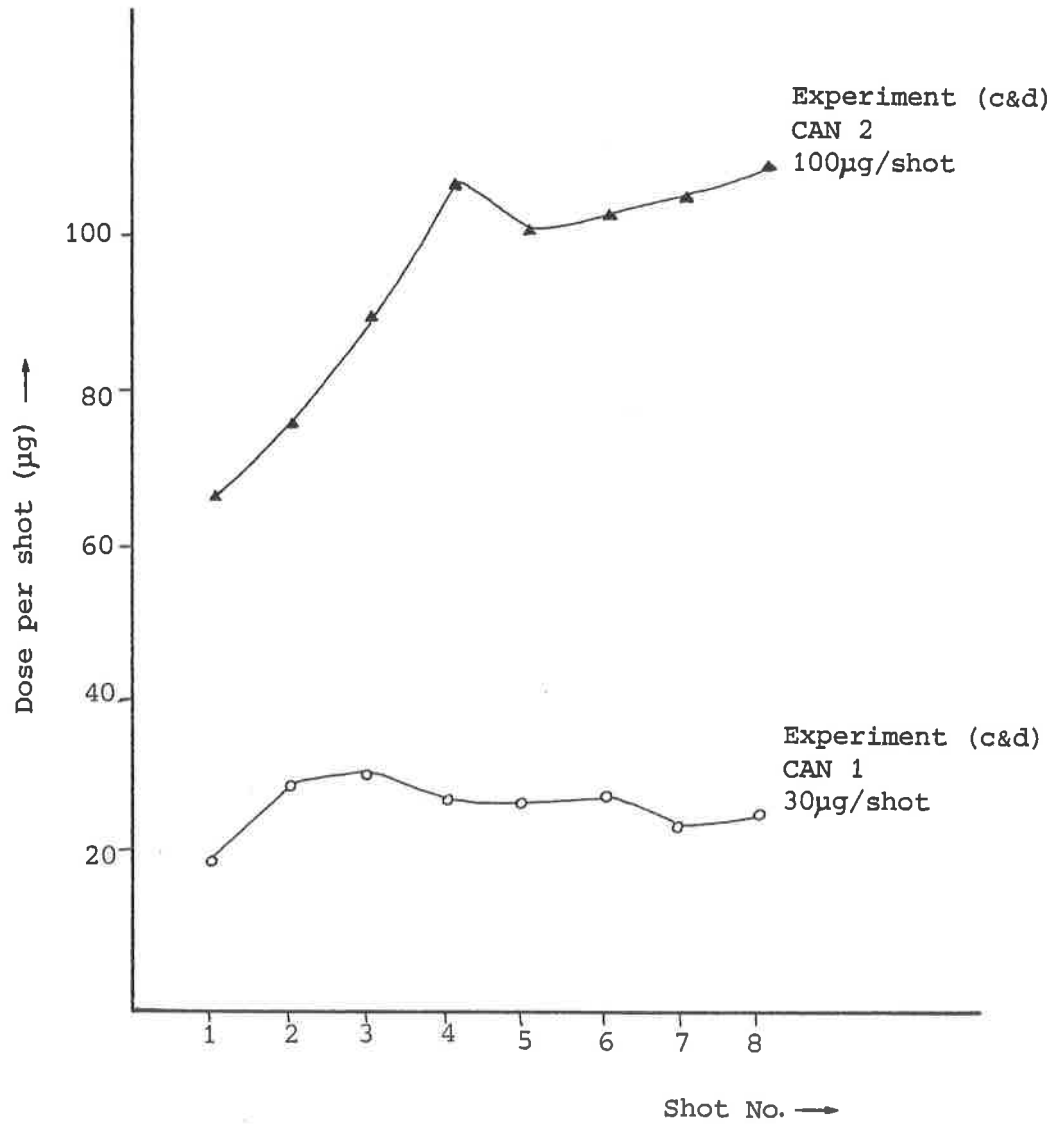


Table 3.17 Dose delivery of salbutamol and shot weight data for various experimental formulations containing Tc-Ph_4AsCl complex

Sample (see Tables 3.6, 3.7)	shot no.s	Dose/shot µg	S.D. & range	Shot weight (mg)	S.D. & range
Experiment (h) can 1 30µg/shot, 60 shots	1-4	23.3	5.78 (14.5-30.2)		
	45-55	5.5	3.62 (1.7-14.6)		
Experiment (c & d) can 1 30µg/shot, 60 shots	1-8	26.4	3.04 (19.6-30.1)		
Experiment (c & d) can 2 100µg/shot, 60 shots	1-8	95.4	15.17 (75.9-109.9)		
	9-28			89.1	0.75 (88.0-90.8)
Experiment (c & d) can 3 100µg/shot, 60 shots	9-28			89.7	1.12 (88.4-91.7)
Experiment (c & d) can 4 100µg/shot, 100 shots	1-30			90.5	1.13 (88.6-92.9)
Experiment (c & d) can 7 100µg/shot, 100 shots	1-24			89.9	1.13 (88.2-92.2)
	25-44	96.1	13.17 (56.1-113.1)		

used in this work is the similarity between the size distribution of salbutamol and the activity distribution of the technetium -99m (or Indium-113m). For convenience, the mean diameters and the geometric standard deviations (ie. the polydispersity) of the distributions have been compared. The variation in results of the labelled reproducibility studies suggest that a difference in mean diameter of 0.6μm and σg 0.3 would lie within the normal range of results. A range of mean sizes for radiolabelled preparations of 2.4 $\pm$ 0.3μm (allowing for small scale manufacturing errors), shows that the preparations are similar to Ventolin Inhaler in particle size distribution.

Table 3.18 summarises the results produced from the preliminary labelled salbutamol preparations. The last three columns in this table indicate whether the results comply with the criteria defined above, to test the validity of the radiolabel.

The table clearly shows why the initial formulations were rejected; either the differences in activity and mass distributions were larger than the defined limits, or the mean size of salbutamol lay outside the usual range. Several additional points should be noted in summarising the results:

(1) The 'chelation' complexes of Tc-oxine and Tc-DTPA were not successful, and chelation with the salbutamol molecule is regarded as unlikely because of the spatial arrangement of the nitrogen and hydroxyl groups; the tertiary butyl group would prevent the formation of complexes.

Table 3.18 Summary of results from first γ-labelled salbutamol preparations and application of criteria for validity

Sample		Mass mean diameter (μm)	σg	Activity mean diameter (μm)	σg	Difference in mean diameters ≤ 0.6μm	Difference in σg ≤ 0.3	Salbutamol mean diameter 2.4 + 0.3μm
In-oxine	1	2.50	1.80	2.10	2.20	✓	X	✓
	2	2.40	1.85	2.05	2.15	✓	✓	✓
Tc-oxine Method A	1	1.65	1.51	1.55	1.92	✓	X	X
	2	1.55	1.53	1.50	1.84	✓	X	X
	3	1.50	1.46	1.45	1.81	✓	X	X
	4	1.85	1.49	1.45	1.82	✓	X	X
Tc-oxine Method B	1	2.50	1.52	2.10	2.25	✓	X	✓
	2	3.00	1.61	2.30	2.44	X	X	X
Tc-oxine Placebo	1	-	-	0.8	1.75			
	2	-	-	0.8	1.65			
Tc-DTPA	1	1.35	3.50	1.60	2.27	✓	X	X
	2	2.10	2.66	1.45	2.44	X	✓	✓
TcO_4^- salt		1.50	1.60	1.40	1.65	✓	✓	X

(2) The preparation of Tc-oxine/salbutamol by Method A probably forms a salbutamol solution within the can due to the solubility of salbutamol base in ethanol. The MMAD's for salbutamol in solution with ethanol in MDI's are 1.25, 1.08 and 1.0μm as measured in previous experiments. However, Bell et al. (1973) found an isoprenaline solution type MDI produced a coarser aerosol than a suspension type using micronised drug.

(3) The results from Tc-oxine/salbutamol Method A also illustrate the care needed in interpretation. The mean diameters of the activity and salbutamol mass distributions agree very well, but the radiolabel is unsuitable because the slope of the log-probability plot (σg) is very different. In fact the distribution curves cross, and there appears to be a larger percentage of activity in the smaller sizes.

(4) The Tc-DTPA formulation is clearly not suitable for radiolabelling salbutamol metered-dose inhalers. The introduction of a water-soluble complex into the inhaler containing fluorocarbon propellants is unsuccessful.

(5) The aqueous solution of salbutamol salts for use in nebulisers appears to be a simple and efficient radiolabelling method. More results are required, however, to confirm this view.

(6) The Tc-Ph4AsCl/salbutamol formulation is regarded as the most suitable method which has been found in this work for radiolabelling salbutamol in suspension in metered-dose inhalers. The dual distributions generally match very well (see Table 3.19) and the mean sizes of salbutamol are similar to the standard Ventolin product. The theoretical basis for this labelling method is assumed to be as follows:

Table 3.19 Summary of results from Tc-Ph_4AsCl/salbutamol preparations

Sample		Mass mean diameter (μm)	σg	Activity mean diameter (μm)	σg	Difference in mean diameter < 0.6μm	Difference in σg < 0.3	Salbutamol mean diameter 2.4 ± 0.3μm
Initials	1	1.95	1.67	2.00	1.70	✓	✓	X
	2	2.10	1.67	2.20	1.80	✓	✓	✓
	3	1.60	1.63	1.65	1.69	✓	✓	X
	4	1.85	1.69	1.80	1.71	✓	✓	X
experiment (a)	1	3.00	1.36	3.20	1.60	✓	✓	X
	2	2.30	1.65	1.95	1.72	✓	✓	✓
	3	3.10	1.60	2.95	1.91	✓	X	X
	4	2.65	1.81	2.10	1.77	✓	✓	✓
experiment (b)	1	2.30	1.48	2.10	1.62	✓	✓	
	2	2.50	1.49	2.45	1.56	✓	✓	
	3	2.20	1.53	2.05	1.62	✓	✓	
	4	2.55	1.56	2.65	1.56	✓	✓	
experiment (c) & (d)	1	2.10	1.69	1.90	1.72	✓	✓	✓
	2	2.85	1.51	3.00	1.52	✓	✓	X
	3	2.75	1.50	2.30	1.75	✓	✓	X
	4	2.95	1.49	2.50	1.91	✓	X	X
	5	2.95	1.48	2.70	1.77	✓	✓	X
	6	2.75	1.48	2.85	1.64	✓	✓	X
	7	2.70, 2.85	1.51, 1.51	2.65, 2.95	1.66, 1.62	✓	✓	X ✓
experiment (e)		2.40	1.47	-	-			✓
experiment (f)	1	2.40	1.88	2.70	1.89	✓	✓	✓
	2	2.50	1.81	2.30	2.08	✓	✓	✓
experiment (g)	1	2.35	1.54	2.45	1.56	✓	✓	✓
	2	2.65	1.55	2.90	1.59	✓	✓	✓
	3	2.70	1.93	2.80	2.89	✓	X	✓
experiment (h)	1	2.40	1.66	2.30	1.48	✓	✓	✓
	2	2.30	1.52	1.85	1.78	✓	✓	✓
	3	2.30	1.56	2.15	1.63	✓	✓	✓
	4	2.67	1.74	2.85	1.68	✓	✓	✓
	4	2.40	1.86	2.10	1.89	✓	✓	✓
	1 c&d	2.30	1.52	1.93	1.72	✓	✓	✓

The introduction of Ph_4AsCl produces the pertechnetate ion as a salt with a weak base (Ph_4As^+). In the presence of salbutamol (also a weak base), the salbutamol pertechnetate salt may also be formed. The equations are:

$$Na^+TcO_4^- + Ph_4As^+Cl \xrightarrow{pH\ 7\text{-}9} Ph_4As^+TcO_4^- + Na^+Cl^-$$

$$Ph_4As.TcO_4 + \text{salbutamol base} \longrightarrow \text{Salbutamol}.TcO_4 + Ph_4As.OH^-$$

The problems with this formulation include:

(a) the apparent variation in results in certain circumstances due to possible precipitation of the labelled complex in the propellant mixture in an inhaler (see Chapter 4).

(b) the presence of a large molecule (Ph_4AsCl) and its solvent (methyl isobutyl ketone) in the formulation which may be toxic. These components make the MDI dissimilar to the Ventolin Inhaler formulation and unsuitable for human administration. In addition, because of the lack of knowledge of technetium chemistry, the complete chemical reactions involved are not known. An extensive study of the process variables was undertaken, in order better to understand the requirements for producing a reproducible aerosol.

(7) The results of examining the optimum concentrations of Ph_4AsCl and mbk in the formulation suggest that low concentrations of the complex and slightly higher volumes (0.5ml) of mbk are required in each preparation. The highest concentration of Ph_4AsCl produces an activity distribution with a similar mean size to salbutamol but more polydisperse. The increase in mean

size of salbutamol for cans 1 & 3 in this study also indicates some instability in the suspension. Further studies are required to establish the nature of the apparent coarsening of the drug distribution in this experiment. It is likely that the Tc-Ph_4AsCl complex precipitates out of mbk solution if the concentration is too high. This mechanism would be encouraged by addition of highly volatile propellants which would rapidly cool the solution.

(8) The changes in water-bath temperature and shaking of the chloroform/water mixture during extraction make little difference to the final product. All subsequent preparations, including those for in vivo experiments were standardised, using gentle shaking for extraction and with a water bath temperature between 33 and 38°C.

(9) The order of addition of components does not seem to make much difference, although the mean sizes of salbutamol are slightly higher than normal. This may be due to a relatively poor dispersion resulting from the crude pestle and mortar mixing method. Also the formulation used the concentrations of Ph_4AsCl and mbk as in can 1, experiment (a) which also produced a slightly high mean size. Can 5 exp.(c) and (d) produced very similar results although the relative quantities of propellants was altered. Published work (Newman et al., 1982a) suggests that lowering the vapour pressure in the inhaler (as in the experiment), decreases lung deposition, thereby inferring an increase in particle size. More experiments are required to determine the extent of the difference in vapour pressure which would influence particle size. However,

the experiments suggest that any small differences in propellant ratio, caused by evaporation during preparation, will have little effect on the particle size distribution.

(10) The addition of the solvent itself (exp.(e)) has no apparent effect on the salbutamol size distribution.

(11) The removal of oleic acid from the formulation (exp.(f)) has no apparent effect on the dual size distributions.

(12) The time of sampling experiments (g) indicate that sampling up to 3 hours after manufacture provides representative results. After 24 hours, the Tc-Ph_4AsCl complex shows some instability, evidenced by the increase in polydispersity of the activity distribution. This could indicate dissociation of the pertechnetate ion from the salbutamol particles.

(13) The reproducibility of results is as good as the standard Ventolin product, both within one can and between cans of the same batch.

(14) The results from placebo preparations which contain the Tc-Ph_4AsCl complex alone show a marked decrease in mean activity diameter. This indicates that there is some positive interaction between the labelled complex and salbutamol in the active preparations.

(15) The shot weights measured appear to have a slight downward trend in the experiments measured, although the values of 90 ± 2mg are well within normal limits.

The dose/shot measurements vary slightly in the series measured, and the first few shots from a can show low relative values. The actual quantities measured vary from can to can, since the activity used in the preparation of each can varied.

In studying these results in general, it must be borne in mind that the variations in results are relatively small. In terms of measurable lung dose, or imaging of in vivo deposition sites the differences between aerosols with mean diameters of 2.5 and 3.0μm for example, would probably be negligible. However, the detailed studies have shown the number of process variables involved in a radiolabelled preparation, and how the process may be optimised to produce a stable, reproducible product suitable for in vivo studies.

3.4.5 General Discussion

Three different approaches were used for γ-radiolabelling aerosols in the present study. Human serum albumin millimicrospheres radiolabelled with technetium were easily prepared and used to provide good lung images in the initial in vivo work. The second approach studied the homogeneity of oleic acid and salbutamol in a metered-dose aerosol, with a view to radiolabelling the excipient for in vivo studies of drug formulations. Finally, several methods of γ-radiolabelling salbutamol itself were explored, and a single technique used in the subsequent in vivo work with metered-dose inhalers.

The use of human serum albumin millimicrospheres had several advantages for the initial in vivo work. The HSA kits were readily available, easily radiolabelled, and the particles were a suitable size for efficient inhalation and deposition. HSA millimicrospheres were chosen because they are used routinely in diagnostic nuclear medicine to produce

excellent gamma camera lung images. In the present study the preparations of HSA were used to establish the inherent biological variability in the in vivo deposition patterns measured in beagle dogs and rabbits. Well-defined lung images were obtained with relatively slow clearance rates. The administration and imaging techniques used with the animal models were established using the HSA millimicrospheres kits. However, aerosols with a larger mean diameter were required to determine the differences in lung deposition patterns with differing particle sizes. Several batches of HSA microspheres were prepared with a mean diameter of 5-6μm, but the concentration of aerosol particles was low and the size distributions were not reproducible. No in vivo studies were carried out using the larger microspheres.

The first approach to radiolabelling aerosol formulations containing salbutamol was the labelling of an excipient in the metered-dose inhaler. Oleic acid is present as surfactant in MDI's containing salbutamol and may be labelled using radioactive iodine. The relative size distributions of the drug and oleic acid were measured in the Andersen Sampler to establish the degree of their association (Malton et al., 1982b). Approximately 30% of the oleic acid was shown to form separate droplets in the metered-dose aerosol which were smaller than most of the drug particles. The results show that a propellant-soluble surfactant cannot be used for valid labelling of both small and large drug particles. Clearly it is also unwise to assume a uniform surfactant-drug ratio for small and large drug particles in the aerosol. This has implications in the study of retardation of hygroscopic particle growth in humid airways due to surfactant on the particles (Martonen et al., 1982). The present study is the first to establish the degree of association between drug and surfactant in a metered-dose inhaler.

An alternative approach for producing γ-radiolabelled aerosols for *in vivo* studies would be to use a 'model system' employing an alternative material that could be more easily radiolabelled than salbutamol. However, although a model material may be easier to label it is questionable if a material with physicochemical and biological properties similar to salbutamol could be found. The relevance of the results to the administration of drug aerosols would be diminished unless this could be achieved.

Properties such as the crystalline structure and hardness of the material will influence the micronisation process used to grind the powder to particle sizes suitable for inhalation. The solubility and density of the micronised material will affect its suspension properties in propellants. Particle properties such as shape, surface area, hygroscopicity and particle charge may in turn influence their behaviour in an aerosol cloud. Similar *in vivo*/*in vitro* behaviour to a salbutamol formulation will therefore be difficult to achieve using a model material. The use of an inert model system will also preclude the option of any clinical efficacy studies with the labelled material.

Administration of inert Teflon particles from MDI's has been studied recently by Newman and coworkers (Newman *et al.*, 1981a; Newman *et al.*, 1981b). Although the particle mass median aerodynamic diameter (MMAD) of 3.2μm of the suspensions was approximately the same as that discharged from bronchodilator MDI's (Hiller *et al.*, 1978a), the spherical shape and monodispersity of the particles bore little resemblance to the irregular shaped drug crystals of polydisperse size distribution in therapeutic aerosols. Teflon particles will not exhibit the hygroscopic growth and possible dissolution of particles at the high humidities experienced in the respiratory tract. Such growth has been shown to be significant in bronchodilator aerosols (Hiller

et al., 1980d) and may modify the sites of deposition. The many disadvantages accompanying the use of 'model' materials for in vivo studies therefore lead to the present use of γ -radiolabelled salbutamol in MDI's for the in vivo/ in vitro correlations. The third approach to the radiolabelling in the present study was direct labelling of the salbutamol in spite of the difficulties due to the simplicity of the drug molecule.

Measurements of both the drug and activity distributions with the Andersen Sampler (Andersen, 1966) enabled simultaneous assessment of the aerodynamic behaviour of labelled and non-labelled particles in vitro. The validity of each labelling method was determined by matching the two distributions. It was found that the earlier methods of cocrystallisation and chelation produced a substantial portion of unlabelled drug particles. The method finally chosen for the majority of in vivo work using γ-radiolabelled aerosols in metered-dose inhalers employed a complex of technetium -99m and tetraphenylarsonium chloride. The number of different techniques which were studied for γ -labelling salbutamol underline the difficulties in introducing a radiolabel into an aerosol formulation containing fluorocarbon propellants under pressure and bronchodilator drug with a fairly simple molecular structure.