# AN EVALUATION OF THE 37 MILLIMETER CASSETTE FOR SAMPLING AN AIRBORNE PHARMACEUTICAL DUST

by

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ABSTRACT

Mark Wendell Wood. **An Evaluation of the 37 mm Cassette for Sampling an Airborne Pharmaceutical Dust.** (Under the direction of Michael R. Flynn, Sc.D., CIH )

The use of the 37 mm cassette for sampling total dust in industrial hygiene is well established. This method is used to make full shift and short-term exposure measurements. Environmental levels of ranitidine hydrochloride are typically in the ug/$m^3$ range. This necessitates taking short-term samples at flows markedly higher than the usual flow of 2 LPM. The results of these measures should be comparable. If not, then a bias exists in the method of collection in this flow range.

An evalution of the cassette to sample airborne ranitidine was completed by comparing paired samples with respect to concentration obtained and particle size sampled. Pairs were run at 2 and 4 liters per minute.

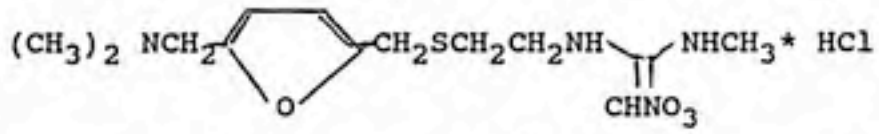
No significant difference in concentration was found in pairs taken in the Fette and dispensing areas. The particle size distributions sampled at 2 and 4 liters per minute were very similar. Thus, no bias was detected in the sampling method between 2 and 4 LPM. Full shift and short-term measures are comparable.

Analysis of the cassette indicates that significant quantities of ranitidine are depositing on the wall. In the Fette room more ranitidine deposited at low flow than at high flow. This indicates a possible residence time phenomenon is responsible. Data from the dispensing area indicates wall deposition is equal between 2 and 4 LPM. Possible explanations include sedimentation, impaction and electrostatic effects.

# SECTION ONE: INTRODUCTION

# CHAPTER ONE: LITERATURE REVIEW AND OBJECTIVES

Ranitidine hydrochloride is a pale, yellow substance used as the active ingredient in Zantac$^{R}$ tablets and injection. Chemically, it is identified as N[2-[[[5-[(dimethylamino)methyl]-2-furanyl]methyl]thio]ethyl]-N-methyl-2-nitro-1,1 ethenediamine hydrochloride. Its molecular structure is:



The empirical formula is ($C_{13}H_{22}N_4O_3S$,HCl). Ranitidine hydrochloride has a formula weight of 350.87 atomic mass units. Its packed bulk density is 0.74g/cc. As a salt, its vapor pressure is insignificant.

As a treatment for duodenal ulcers, ranitidine's mode of action is as a histamine $H_2$-receptor antagonist. The manufacture of Zantac tablets involves a combination of unit processes. The first is the dispensing of raw materials. Dispensing in the Zantac suite is done by using a "Matsui". Essentially, this is a large metal hopper attached to a vacuum source. Bulk ranitidine, magnesium stearate and microcrystalline cellulose (trade name is Avicel) are taken from large plastic barrels and evacuated into the hopper. One can observe dust being generated during dispensing. Then, the mixture is sieved using a Russell sieve. This creates the proper particle size for compressing the tablets. The mixture is then

transferred to the "buls cube". This is a container in which the components are mixed thoroughly.

From the dispensing station the buls cube is taken to the mixing room. It is here that the raw materials are churned by rotating the buls cube continuously. No personnel are permitted in the mixing room during the process.

After the components are mixed the hopper is moved to the Fette, or tablet compression room. The Fette machine forms tablets by compressing the powdered mixture using a piston. The raw material is emptied into the Fette by placing the buls cube above the machine and allowing gravity to feed the materials into it. The process is similar to using a funnel to load oil into an automobile. Because the process is energy intensive, the production of tablets creates an airborne dust. This is evidenced by a heavy layer of dust coating the machine after a batch is produced. After the tablets are made they are coated (hardened) in the coating room. The final step before packaging is inspection. The tablets are placed on a conveyor belt and are inspected by two to three persons. Figure 1.1 is a flowchart outlining the manufacture of ranitidine tablets.

Occupational exposure to ranitidine is major concern to Glaxo, Inc.. Although the compound has been shown to

THE MANUFACTURING OF RANITIDINE HYDROCHLORIDE TABLETS

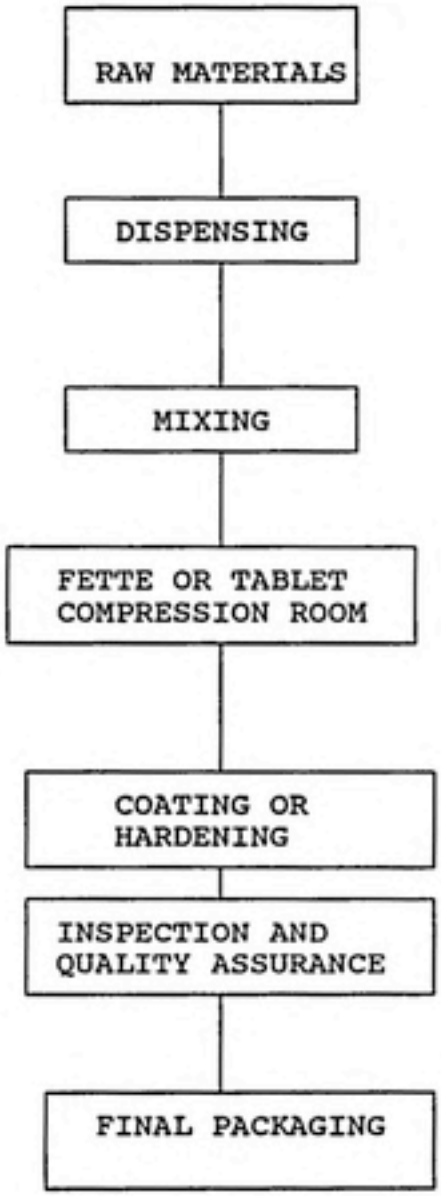



Figure 1.1: Flowchart outlining the manufacture of Ranitidine Hydrochloride Tablets. The highest exposures are expected in the Fette and dispensing areas.

be non-carcinogenic/tumorigenic in mice and rats with oral doses up to 2,000 mg/kg day, rare cases of hypersensitivity reactions (e.g. brochospasm) have been seen (11). Slight dermal irritation has also been observed (11).

The potential for exposure to ranitidine is mainly in the dispensing and Fette rooms, although, the coating room also presents concern. Based upon previous data collected by Glaxo it is suspected that the greatest exposures are in the Fette room (5).

Assessment of exposure to Ranitidine has been done by using an open-face 37-mm disposable cassette. The collection medium is a Gelman type A/E glass fiber filter. No organic binders are contained in the filter (9). Chemical analysis has been performed using high performance liquid chromatography (HPLC). Because ranitidine is a proprietary compound, produced only by Glaxo Inc., a method of air sampling has not been evaluated. Therefore, it is necessary to objectively evaluate the current method of sampling and analysis.

## SAMPLING AIRBORNE DUST

Evaluation of occupational health hazards often necessitates assessment of a potential exposure to a

worker. This is done in many cases by an industrial hygienist. The manner in which this is done depends upon the chemical and physical characteristics of the contaminant. Also, the purpose of the sampling must be considered. For example, one may wish to estimate a worker's exposure to respirable particulate. In this case size selective sampling would be appropriate (4). Given the plethora of sampling techniques available to evaluate airborne dusts it is reasonable that the selecton of a method should satisfy the requirements of sound aerosol science and practical industrial hygiene. With this in mind, a method of sampling airborne ranitidine is evaluated. The method considers both the theory of sampling with cassettes and the practical limitations imposed by the manufacture of Zantac.

Traditionally, hygienists have sampled airborne dusts using a closed-face cassette, or filter holder. This practice is especially prevalent in governmental industrial hygiene (1). There exists, however, debate as to whether open or closed face cassettes provide the most reliable estimate of airborne dust concentration (13). Prior to a detailed discussion of the sampling efficiency of 37-mm cassettes a brief review of pertinent sampling mechanics is indicated.

## ISOKINETIC AND ANISOKINETIC SAMPLING

If a particular method of aerosol collection is to be accurate it is imperative that it be unbiased. That is, the sample should closely resemble the original aerosol in both concentration and size distribution (4).

The most effective way to attain accurate sampling is to maintain isokinetic sampling conditions. This demands that the sampler inlet be parallel to the gas streamlines. Additionally, the gas velocity of the sampler inlet must be identical to that of the gas approaching the inlet (13). Isokinetic sampling ensures that distortion of gas streamlines at the inlet does not occur.
This is illustated in Figure 1.2a.

If the free stream velocity is $U_o$ , the velocity of gas in the sampler inlet by U, and the angle of the inlet with respect to the streamlines is O, then isokinetic conditions exist when $U=U_o$ and $\theta=0$ (13).

When these conditions are not met the sample will be biased. If the sample inlet is not properly aligned the aerosol concentration is underestimated. This results because particles in the original gas stream have inertia too great to make the turn into the inlet. This is seen in Figure 1.2b.

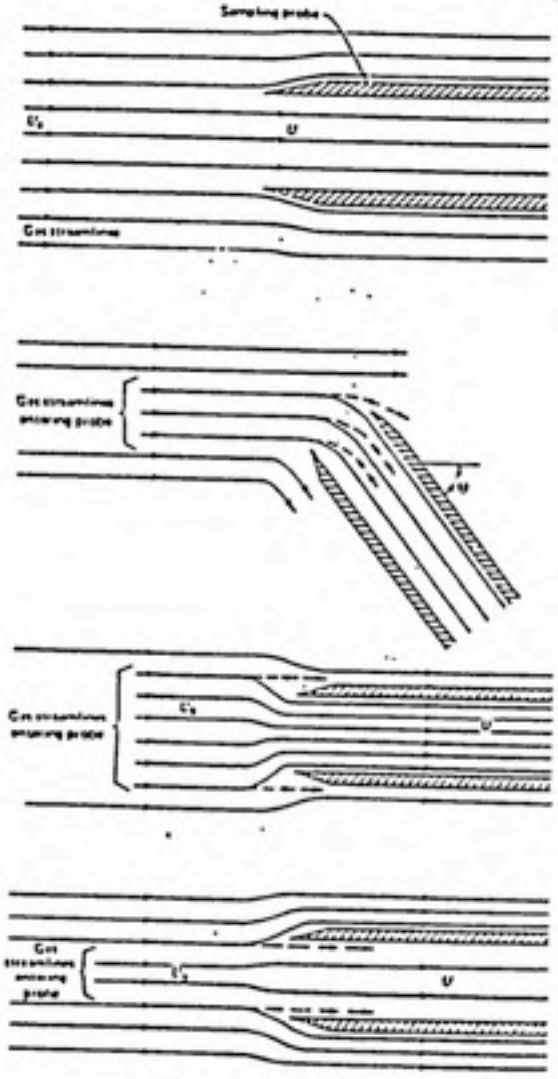



Figure 1.2: Illustration of isokinetic and anisokinetic sampling conditions. Adapted from ( ).

Figure 1.2a: Isokinetic sampling conditions present
1.2b: Sampling probe is misalligned
1.2c: Sampling flowrate is > free airstream
1.2d: Sampling flowrate is < free airstream

Durham and Lundgren (7) have investigated the relationship between misalignment of sampling inlets and aspiration efficiency. They found that collection efficiency drops markedly as the a probe is moved away from the airstream through angles of 15- 90 degrees. Additionally, it was found that aspiration of particles decreases as particle diameter increases.

If it happens that the sampling flowrate is greater than the isokinetic flowrate particles with significant inertia may not follow streamlines and enter the inlet. This can be see in Figure 1.2c. Therefore, samples collected under these conditions underestimate actual concentrations.

When the flowrate of the sampler is lower than the isokinetic flowrate the measured concentration will be greater than the actual concentration. This occurs because the streamlines diverge at the inlet and large particles which were not in the sampled gas volume travel into the inlet (13). This is illustrated in Figure 1.2d.

Much of the preceeding discussion is based upon sampling in flow systems. Usually, experiments testing the validity of such theory are completed in wind tunnels. The industrial environment, however, may not resemble these conditions. There are some instances in

which the hygienist may assume that he is sampling in a calm-air setting.

If there exists in the occupational environment low air turbulence and low air velocity then it is sometimes possible to assume the sample is taken from "still air". According to Davies, there are two errors associated with sampling from still air: an error due to the terminal settling velocity of a particle and one due to the particle's inertia (6). The terminal settling velocity of a particle is given by:

$$V = \tau g, \quad (1)$$

where $\tau$ is the relaxation time of a particle and is equal to:

$$\tau = (1/18)\, d^2/u, \quad (2)$$

, where d is the particle diameter and u is the kinematic viscosity. In equation (1) g is the acceleration due to gravity (980 cm/ $sec^2$).

If the sampling rate is low and the inlet is oriented upward, particles may settle out of the air and into the inlet. Thus, the measured concentration is an overestimate of the true concentration. In fact, the sampling error is considered infinite when the flowrate approaches zero. Conversely, if the orientation of the inlet is downward an underestimation of concentration occurs (13).

As a particle approaches the inlet of the sampler the influence of the sampler's flow field becomes more marked. As a result, the particle's velocity , and hence its inertia, is increased. As this occurs the stopping distance of the particle also increases. This quantity describes the distance which a particle with a given velocity in still air will travel under its own inertia before stopping. If the stopping distance is large relative to the dimensions of the inlet, the particle may travel across or away from the inlet and escape capture (13).

Davies, in 1968, established sampling criteria which sought to minimize the errors caused by settling and inertia. To minimize settling error it is recommended that the air velocity at the inlet be at least 25 times greater than the terminal settling velocity of the particle or:

$$U > 25V \quad (3)$$

where U is the air velocity at the inlet, and V is the terminal settling velocity of the particle. This equation may also be expressed as a function of the sampling flow:

$$D < 2/5\ (Q/\pi Tg)^{0.5} \quad (4)$$

where D is the diameter of the sampler inlet and Q is the sample flowrate. If we assume standard temperature and pressure this equation reduces to:

$$D < 4.1(Q^{0.5}/d) \quad (5)$$

where D is in centimeters , Q is flow in cc/sec and d is the aerodynamic diameter in microns. Standard pressure is 1 atmosphere, or 760 Torr. Standard temperature is $273^{o}$ Kelvin.

If the conditions of this equation are fulfilled then the error due to settling is negligible (13). Davies also derived an equation which ensures negligible error due to the inertial forces discussed above:

$$D > 0.062\ Q^{0.33}\ d^{0.67}, \text{ at STP} \quad (6)$$

D is in cm, Q in cc/sec and d in um. Combining the last two equations we have an upper and lower limit for the diameter of the inlet which minimizes the sampling error in still air (13):

$$0.062 * Q^{0.33} * d^{0.67} < D < 4.1 * Q^{0.5} * 1/d \quad (7)$$

If during still air sampling the inlet of the sampler is oriented horizontally to the sampling axis the error due to settling is negligible (13). Therefore, the right side of equation 7 is ignored. All samples in this study were taken horizontally. According to equation 7 the maximum diameters of particulate that may be sampled without inertial error in still air using 37 mm open face cassettes with flows of 2 and 4 LPM are 63.4um and 45.1um, respectively.

Marple and Liu give a simpler and far less restrictive relation for the establishment of still air conditions:

$$W < 0.002\ (D^2U/d^4)^{0.33} \quad (8)$$

where W is tbe wind velocity in cm/s, D is the probe diameter in cm, U is the entry velocity in cm/s, d is the aerodynamic particle diameter in cm. A restriction on equation 8 is that d must be less than $0.003*D^{0.2}*U^{0.09}$. It is noted that equation 8 seems to have a major flaw. If wind speed is equal to zero then D, U and d may have any value. The theoretical limits of this equation were not included in the reference cited. It is recommended that equation 8 not be used when W=0. Because of its simpler form this criterion is used in this paper. Compliance with the above equation permits the assumption of about 90% inlet efficiency for a specified particle size range (3).

The effect of low velocity winds on sampling dust in the industrial environment has been investigated by Ogden and Wood (19). These workers sampled benzene soluble material in coke-oven emissions using Casella personal samplers. The samplers were mounted on rotating arms to produce a relative windspeed of 2.6 m/s. They concluded that the mass of dust collected depended on the windspeed when large particles (>25um) were present. At very low

windspeeds the sampler was found to have collected all particles up to 15um. Although the sampler was not a cassette this example underscores the need to establish still air conditions in order to minimize sampling error. Raynor (21) also found that windspeed markedly effected the efficiency of sampling for filter holders.

In sampling for the evaluation of airborne dusts with cassettes parameters which receive primary consideration usually include flowrate, sampling time and the sensitivity of the analytical method used. Rarely, if ever, is the aspiration efficiency of the cassettes considered by Industrial Hygienists (8).

As is discussed above, sampling errors are generally of two sorts: inertial and gravitational. Fairchild (8) maintains that sampling errors , to some extent, depend on inlets being "thick or thin walled". Samplers are defined as thick walled if $D/l > 1.1$ ; $t/l > .05$. They are thin walled if $D/l < 1.1$ ; $t/l < .05$. Where D is the inlet outside diameter, l is the inlet inside diameter and t is the inlet wall thickness. A 37-mm cassette is a thick walled sampler. For an open face cassette $D/l=1.2$ and $t/l= .10$. These dimensions are illustrated in Figure 1.3.

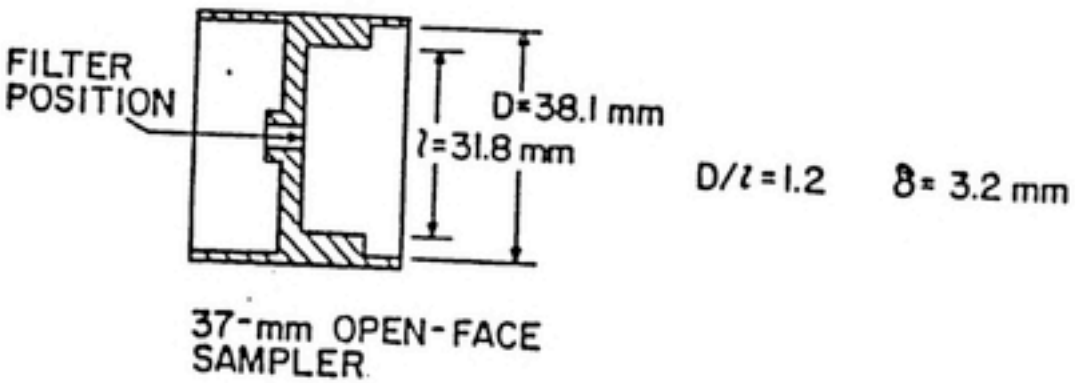



Figure 1.3: Dimensions of a typical 37mm sampling cassette. The cassette is considered a thick walled sampler. Adapted from Fairchild et al.

D: inlet outside diameter
l: inlet inside diameter
S: inlet wall thickness

Strictly speaking, thick walled inlets cannot achieve a collection efficiency of 100% One possible explanation for this is that particles have a tendency to rebound from the wall of the inlet (8). Levin et al (8) describe equations which predict the efficiency of thin walled samplers in significant wind. Fairchild et al (8) used these equations to predict the efficiency of 37-mm cassettes in still air. They maintain that with proper adjustment the equations may be applied to 37-mm cassettes and used as reasonable estimate of inlet efficiency.

Levin et al(8) describe the efficiency of the sampler as the aspiration coefficient, A:

$$A = c/c_o \qquad (9)$$

where c is the measured concentration and $c_o$ is the true concentration. If we consider the aspiration coefficient as a product of several components each contributing to the loss of aerosol at the inlet the above equation is rewritten as: $A = A_i * A_d * A_y$. Where A is the aspiration coefficient, $A_i$ is the component of A due to aerodynamic and inertial characteristics, $A_d$ is the component of loss due to wall deposition and $A_y$ is the component of loss due to particle rebound. $A_d$ and $A_y$ can only be estimated by experiment. Therefore, the authors limited themselves to the inertial component, $A_i$.

An empirical equation was developed which is based upon Stokes' number (STK) and the inverse of the isokinetic sampling parameter ($U/U_o$):

$$A_i = 1 + [(U/U_o)-1]\ B(STK, U/U_o) \qquad (10)$$

where $U/U_o$ is the inverse of the isokinetic sampling parameter (U is the velocity of gas in the inlet; $U_o$ is the velocity of the gas in the airstream). The Stokes' number is defined as:

$$STK = V*U_o/1*g \qquad (11)$$

where V is the terminal settling velocity of the particle, $U_o$ is the airstream velocity, g is the acceleration due to gravity and 1 is the inlet diameter.

$$B(STK, U/U_o) = 1-(1/[1+(2+0.62U/U_o)STK]) \qquad (12)$$

Substituting B into the aspiration equation we have:

$$A_i = 1+( (U/U_o)-1)[1-(1/[1+(2+.62U/U_o)STK])] \quad (?).$$

The above equation, when strictly applied, estimates inlet efficiency for thin walled inlets in significant winds. However, Fairchild et al (8) applied another version of the equation to the case of still air sampling with thick walled cassette samplers ( 37-mm cassettes ). They assumed that the STK could be replaced by another parameter, $K_u$. $K_u$ is the Stokes' number calculated by using the velocity at the sampling inlet rather than the

free airstream velocity (8). As a result, the aspiration equation reduces to:

$$A_i = 1-(0.62K_u/[0.05K_u+(1+0.62K_u)]) \quad (14)$$

Fairchild et al (8) used these equations to predict the efficiency of 37-mm cassettes sampling in still air. The cassette flowrate was 1.8 LPM. They showed that open face cassettes oversampled for increasing particle size. The authors maintain that the oversampling is not in gross violation of Davies' theory which predicts negligible error (8). It may be possible that significant rebounding of particulate into the inlet was occurring. This would be especially relavant using a thick walled sampler.

The authors also noted that as the open face cassette was moved away from directly facing the airstream in the wind tunnel the efficiency drops markedly. This is in good agreement with Durham and Lundgren (7). Additionally, isokinetic sampling theory predicts this result (13).

Moreover, the authors (8) found that wall deposition was significant. Using Eosin-Y-flourescent dye as an aerosol, the group states that wall losses averaged from

4% to 59% of total mass collected for open face cassettes.
The aerosol used ranged from 4 to 21um in size. There was an increase in wall deposition as size increased.

Buchan et al (3) also studied the efficiency of 37mm cassettes. Using a wind tunnel, samples with both open and closed face cassettes were taken while being compared to an isokinetic sampling probe upstream. The challenge aerosol was composed of polydisperse aluminum spheres ranging in size from 2.4 to 24um. They concluded that sampling efficienies of all cassettes decreased with increasing particle size. In the case of the open face cassette the authors submit that impaction of large particles on the walls of the inlet explains the decreased efficiency. Although this explanation is plausible the authors did not support their position by examining the atcual mass deposited on the walls of the inlet.

Beaulieu et al (1) also investigated the efficiency of the 37mm cassette. They found that particle size markedly effected the efficiency of the closed face sampler. Sampling in calm air, industrial conditions, the authors compared the results of sampling with open face cassettes to those obtained with closed face cassettes. Closed face casstte consistently

undersampled. It is proposed that large particles possess too much inertia as they approach the inlet. Therefore, they miss the inlet. Although this explanation is consistent with theories of isokinetic sampling the authors make no attempt to verify it by measuring the diameters of the particle which deposit in the cassette.

The authors (1) suggest the use of a 37-mm open face cassette for total dust sampling due to the biased sampling seen with the closed face cassette.

OBJECTIVES OF THE STUDY

The major objective of the study is to perform a field test of the 37mm open-face cassette in sampling ranitidine hydrochloride. This study should:

1. Provide confidence that samples taken at low flows, such as full shift personal samples, are comparable to high flow samples used in measurements of ceiling limits. If they are not comparable a sampling bias exists within this range of flowrates. The study does not measure accuracy. However, if paired samples run at very different flowrates are comparable it does suggest that both are sampling well.

2. The study should provide Glaxo with some environmental monitoring of ranitidine levels in suite air.

# SECTION TWO: EXPERIMENTAL METHODS

# CHAPTER TWO: CHEMICAL METHODS

The analysis of environmental samples for the detection of ranitidine has been described previously by Glaxo method AM0119-03. This method outlines the use of high performance liquid chromatography (HPLC) as the method of choice.

The use of chromatographic methods in industrial hygiene is widespread. The ability to resolve complex mixtures into their respective components is invaluable. However, it is believed that no separation is necessary in the case of monitoring for ranitidine. Therefore, a separate method of analysis is presented. It is based upon ultraviolet absorption of radiation by the ranitidine molecule. The furan ring system on the ranitidine molecule presents an excellent chromophore.

In order to be consistant with Glaxo's standard operating procedures the validation of this method follows the company's outline for the verfication of methods. This includes review of:

1. Specificity of the method
2. Linearity of the method
3. Precision of the method
4. Limit of detection
5. Analytical recovery from filter media

SPECIFICITY

If separation is not part of the method described one must be certain that all interferences are well characterized.It is well known that within the Zantac

characterized.It is well known that within the Zantac suite three components are present, possibly as dusts. These are magnesium Stearate, microcrystalline cellulose and ranitidine.

Each compound was scanned in the ultraviolet region for significant absorption. The spectra are presented in Figures 2.1, 2.2 and 2.3. No absorption was found in the uv range for either magnesium stearate or microcrystalline cellulose. The concentration of the solutions used were 50ug/ml. This level was chosen in order to duplicate suspected levels in the suite, although no quantitative justification is possible.

It is noted that the solubilty of these compounds (magnesium stearate and cellulose) in water is very poor. Therefore, it is important to filter the samples prior to analysis. It is possible that undissolved materials in the sample will scatter light and bias the analysis. This effect is commonly called turbidity. However, turbidity was not anticipated as no absorbance was found even without filtering the solutions.

In Figure 2.3 it is seen that ranitidine shows marked aborbance in the uv region. This is due to the ideal furan chromophore located on the molecule. In methanol the wavelength of maximum absorbance is noted as 322nm (10). However, as a practical and economical

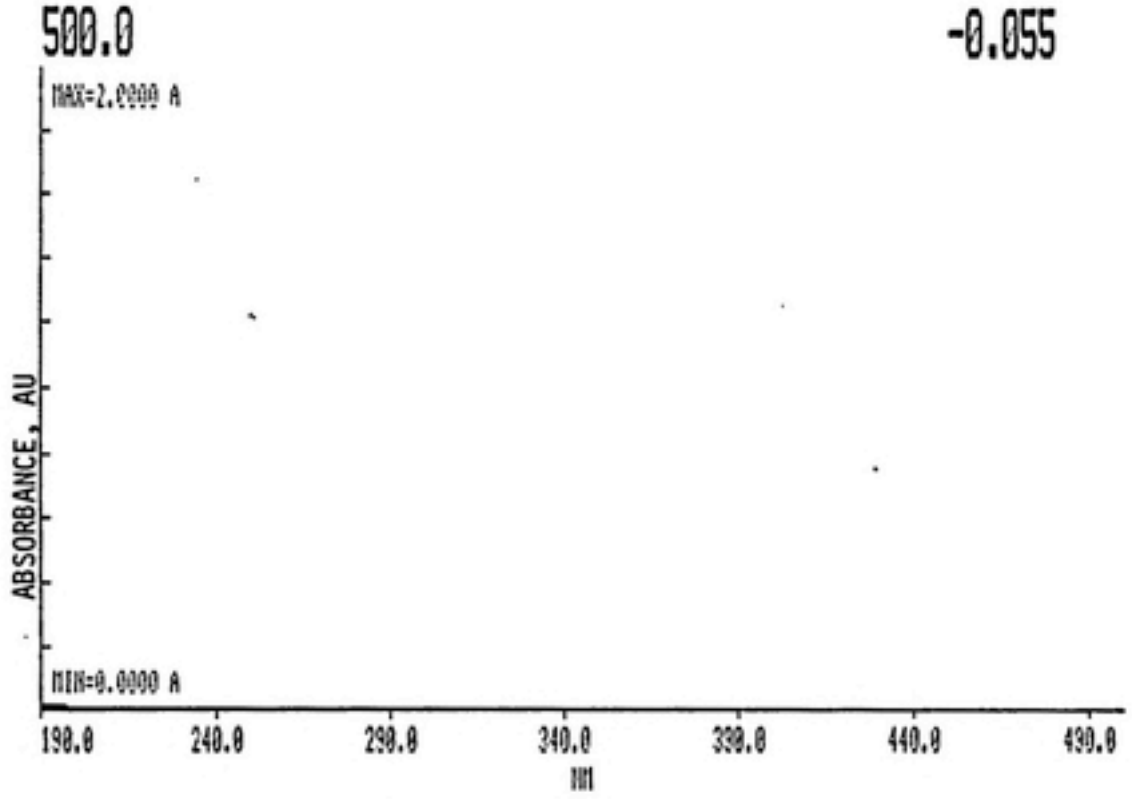



Figure 2.1: UV scan of magnesium stearate suspension. Note the lack of absorbaance in the uv range.

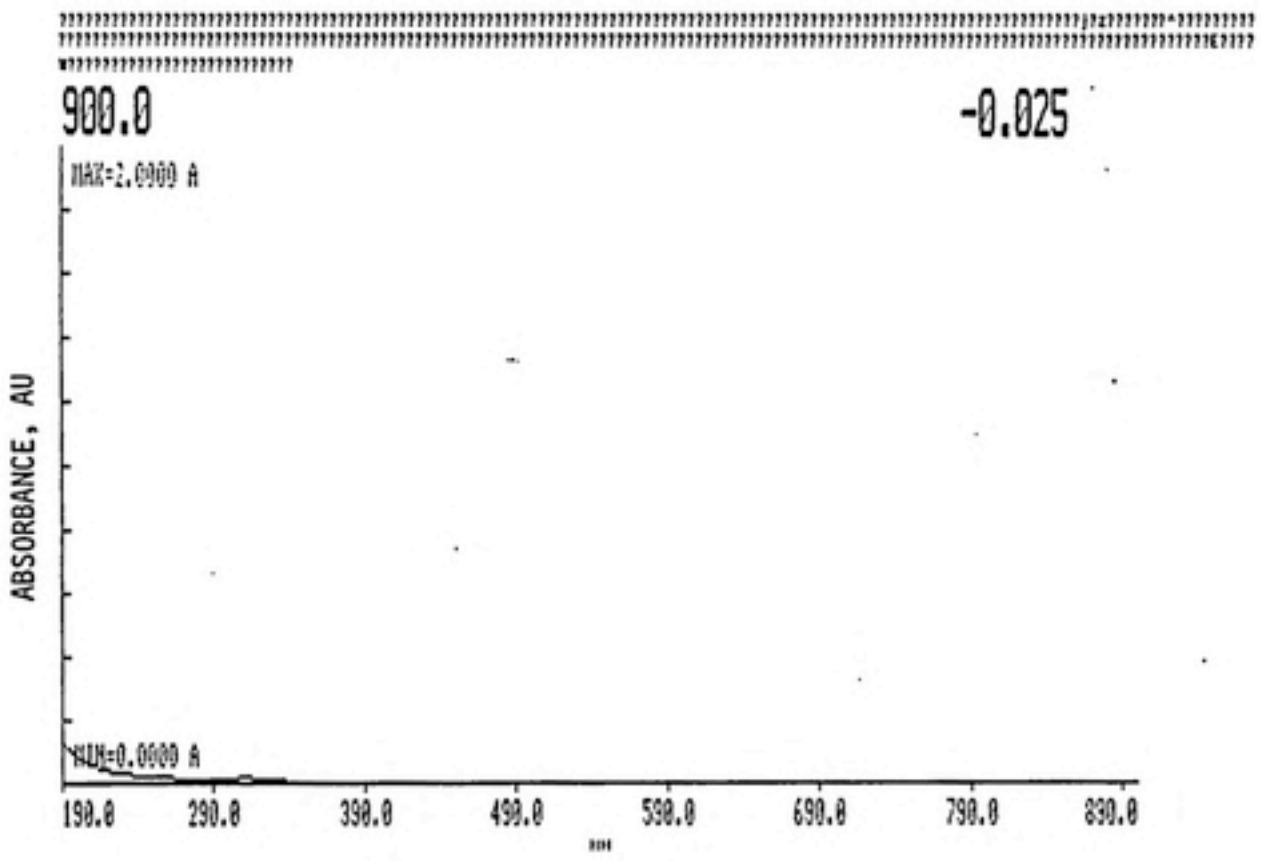



Figure 2.2: UV scan of microcrystalline cellulose (Avicel) suspension. Note the lack of absorbance in the uv range.

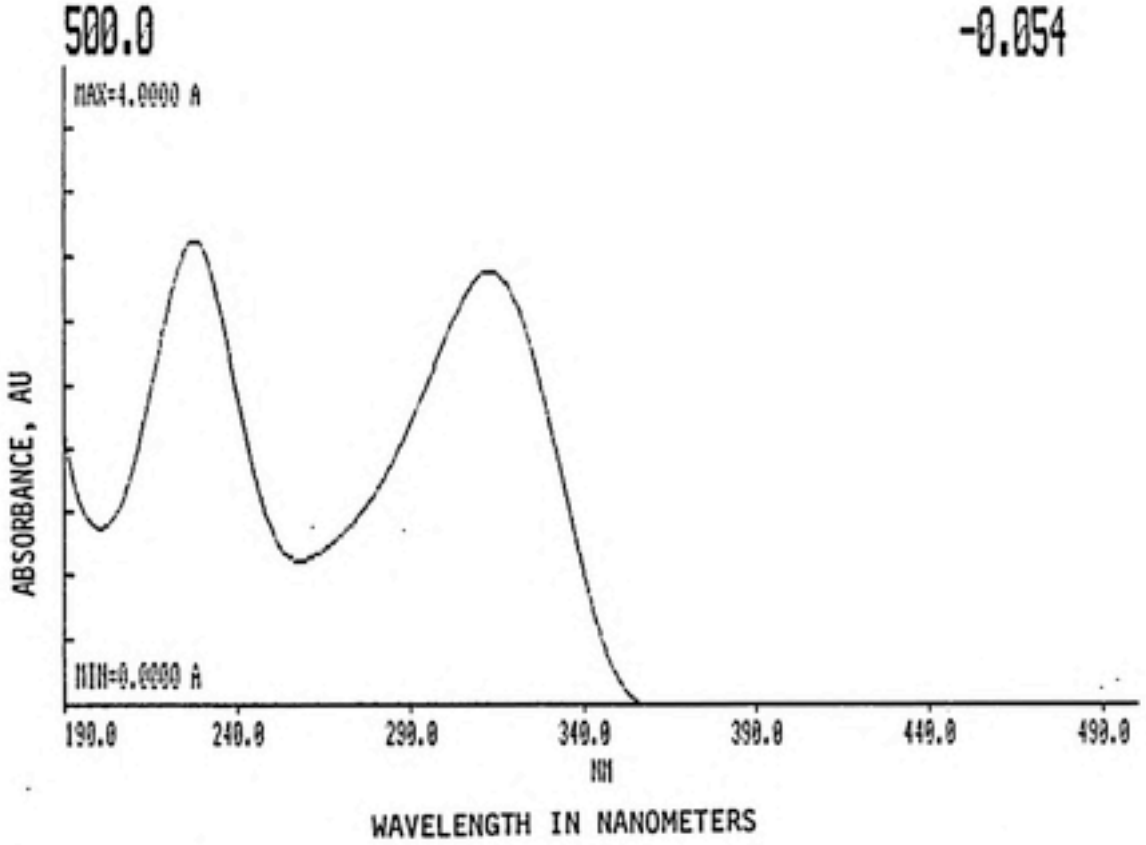



Figure 2.3: UV scan of Ranitidine Hydrochloride. Note the marked absorbance in the uv range. The concentration is 50ug/ml and the solvent is deionized water.

alternative distilled water is used in the uv method. Therefore, solvent effects must be investigated.

Generally speaking, if the pH of a solvent is lowered the wavelength of maximum aborption for a given chromophore may decrease (23). An example of this is the common indicator phenol red. In basic solution the absorption maximum is about 558nm. In acidic solution (lower pH) the maximum is reduced to 433nm. These shifts are primarily caused by shifts in the position of chemical equilibria in the absorbing species (23).

Due to this change in solvent an investigation into a possible shift in wavelength of maximum absorption was made. A solution of ranitidine was scanned for absorbance at wavelengths in the region of interest. The data are presented in Table 2.1 and in Figure 2.4.

Table 2.1: Absorbance of ranitidine in distilled water (50ug/ml)

| WAVELENGTH | ABSORBANCE | WAVELENGTH | ABSORBANCE |
|---|---|---|---|
| 300 | 2.2723 | 316 | 2.6944 |
| 302 | 2.3748 | 317 | 2.6695 |
| 303 | 2.4236 | 318 | 2.6346 |
| 304 | 2.4685 | 319 | 2.6065 |
| 305 | 2.5115 | 320 | 2.5663 |
| 306 | 2.5570 | 321 | 2.5155 |
| 307 | 2.5923 | 322 | 2.4626 |
| 308 | 2.6245 | 323 | 2.4005 |
| 309 | 2.6518 | 324 | 2.3330 |
| 310 | 2.6783 | 325 | 2.2608 |
| 311 | 2.6950 | 326 | 2.1846 |
| 312 | 2.7018 | 327 | 2.1028 |

Table 2.1 continued

| | | | |
|---|---|---|---|
| 313 | 2.7104 | 328 | 2.0216 |
| *314 | 2.7110 | 329 | 1.9301 |
| 315 | 2.7040 | | |

* WAVELENGTH OF MAXIMUM ABSORBANCE

Because of the shift in absorbance 314nm is the wavelength of choice in this analytical method.

LINEARITY

In the development of any analytical method a calibration curve covering the expected range of interest is usually constructed. According to Beer's law the absorbance of a compound is linear with respect to the concentration of analyte. This relation is usually true for dilute solutions. Accordingly, a curve was constructed over the range 0.2410ug/ml to 24.1ug/ml. This corresponds to absorbances in the range of 0.0187AU to 1.2961AU. It is noted that only three standards were used to construct the calibration curve seen in Figure 2.5. The software installed in the spectrophotometer's computer did not permit any more than three. Another curve is presented in Figure 2.6. This curve contains more standards and illustrates the linearity of the method well.

Fig 7

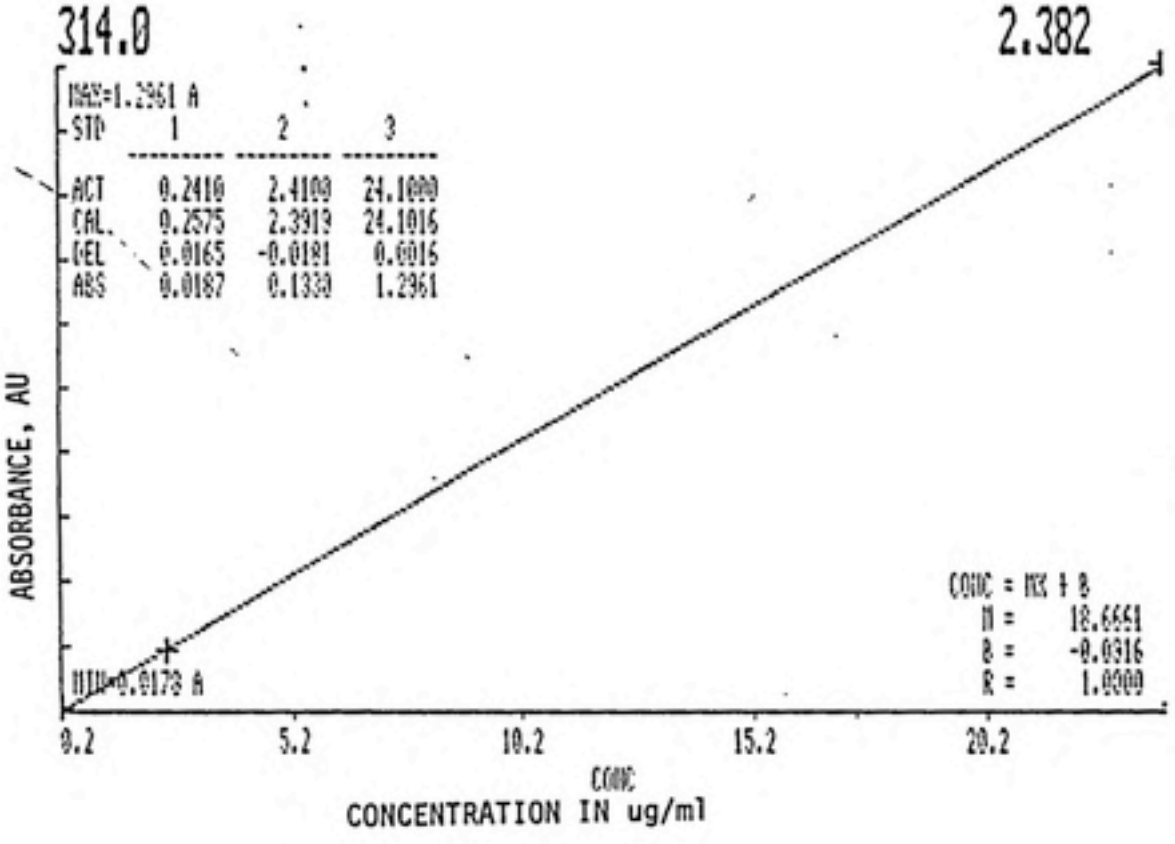



Figure 2.5: Three point calibration curve generated on Glaxo's Perkin-Elmer double-beam spectrophotometer which illustrates expected linear relationship between absorbance and concentration.

| X | Y | X | Y |
|---|---|---|---|
| 1.09000 | 0.04800 | 13.64000 | 0.55400 |
| 5.05500 | 0.22100 | 19.22000 | 0.79600 |
| 7.58400 | 0.33200 | 25.28400 | 1.11000 |

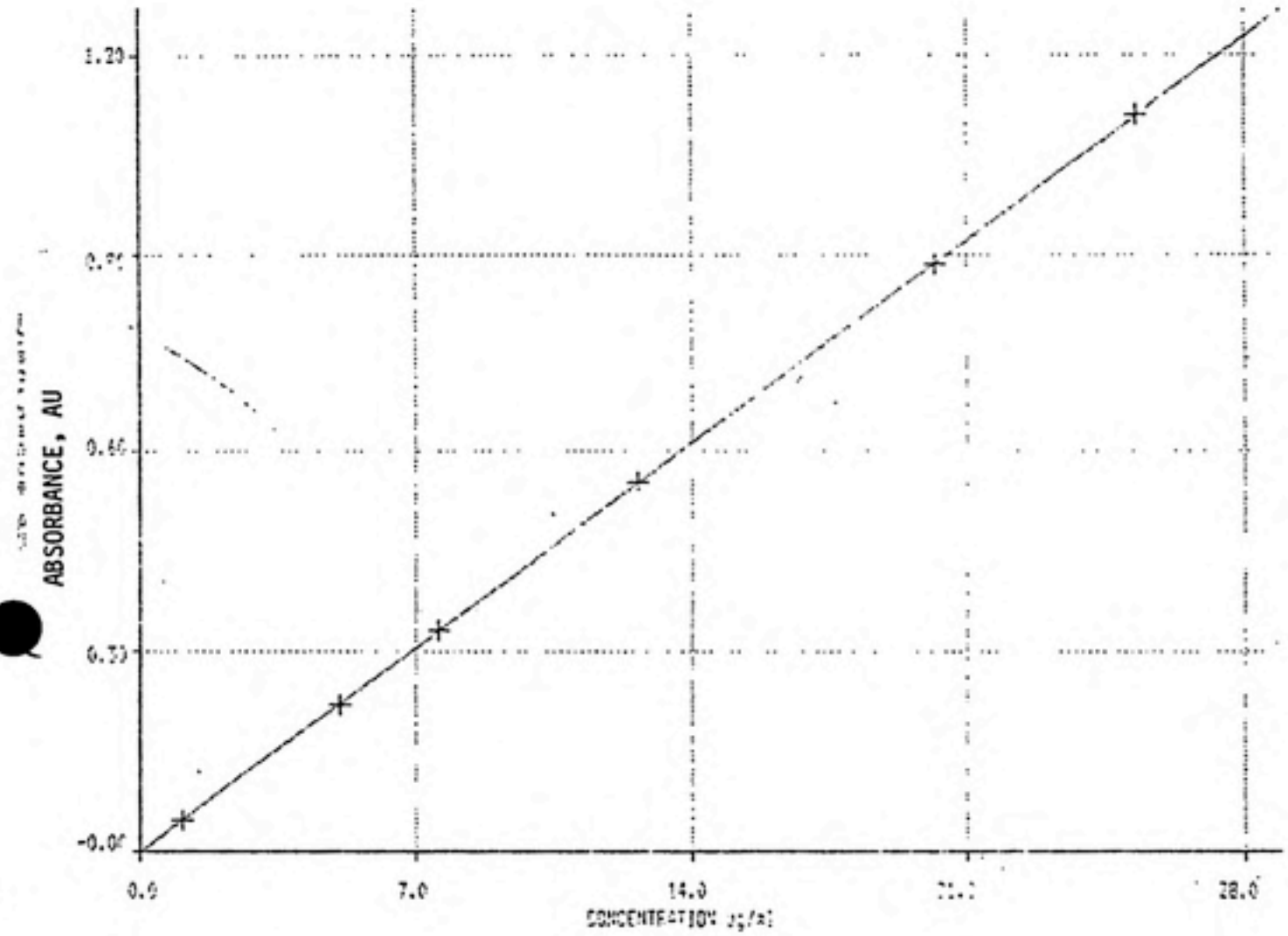



Figure 2.6: Six point calibration curve generated with raw absorbance data further illustrates linear relationship between absorbance and concentration.

The justification for using 0.2419 and 24.1ug/ml as a standard range follows from the instrument specifications (Appendix I ). The stated photometric accuracy is about +/- 0.005AU with 1.00AU as a maximum scale value. Therefore, a repeatable value, in ug/ml, was sought that gave an absorbance reading three times as large as the reported accuracy. An absorbance of 0.0187AU was obtained for a solution of 0.2419 ug/ml. 24.1ug/ml was chosen as the upper range limit because it corresponded to an absorbance reading of about 1.00 (1.296). This ensured that the information on photometric accuracy would apply to work completed with the calibration curve. Using a reading three times the photometric accuracy ensures that the limit of detection is not violated.

LIMIT OF DETECTION

The limit of detection can be defined as the concentration of analyte which gives a detector response two times as great as the noise (23). The measured noise at 314 nm was 0.0008AU. Twice this value is 0.0016AU, corresponding to a solution concentration of 0.024ug/ml. This is taken as the limit of detection. Given that filters are desorbed in 10ml of water after being collected in cassettes the limit of detection is approximately 0.2ug per filter (HPLC limit of detection is 1.0ug/filter).

SYSTEM PRECISION

A series of six replicate measurements were taken for each of three standard concentrations. The data and statistics are presented in Table 2.2.

Table 2.2: Assessment of system precision made by making replicate measurements on three standards.

| REPLICATE NUMBER | CONCENTRATION,ug/ml | |
|---|---|---|
| 1. | 24.0926 | |
| 2. | 24.0905 | |
| 3. | 24.0997 | Mean:24.098 |
| 4. | 24.0997 | Actual: 24.100 |
| 5. | 24.1047 | CV: 0.021% |
| 6. | 24.0994 | |

| REPLICATE NUMBER | CONCENTRATION,ug/ml | |
|---|---|---|
| 1. | 4.7550 | |
| 2. | 4.7547 | |
| 3. | 4.7556 | Mean: 4.756 |
| 4. | 4.7555 | Actual:4.810 |
| 5. | 4.7598 | CV: 0.042% |
| 6. | 4.7564 | |

| REPLICATE NUMBER | CONCENTRATION,ug/ml | |
|---|---|---|
| 1. | 2.3776 | |
| 2. | 2.3776 | |
| 3. | 2.3774 | Mean: 2.377 |
| 4. | 2.3785 | Actual: 2.410 |
| 5. | 2.3821 | CV: 0.168% |
| 6. | 2.3745 | |

In order to be acceptable, the coefficient of variation for each set of readings must not exceed 3.0%. These data are well within this Glaxo method guideline.

ANALYTICAL RECOVERY

In order to evaluate the recovery of active (ranitidine) from filter media a study was conducted in accordance with the guidelines suggested by NIOSH (18). These guidelines suggest that three levels of analyte be spiked onto media. The levels should cover the entire range of analyte that is likely to be found in sampling. Specifically, six filters are to be spiked at each of the levels. The recovery of ranitidine must be greater than 90%. The pooled coefficient of variation cannot exceed 0.07.

The three levels chosen were: 5, 50, and 500ug per filter. If we assume an 8 hour sample is taken at 2.0 liters per minute 0.96 cubic meter of air is sampled. This corresponds to levels in the suite of 5.2, 52, and 520 ug per cubic meter. This should cover the expected range of air levels of ranitidine in the suite. This assumption is based on data previously collected in the suite.

A stock solution of ranitidine was prepared and aliquots spiked onto the fliters. After allowing the filters to sit undisturbed for ten minutes they were

placed in a 125ml flask. Using a volumetric pipette 10ml of distilled water is added to the beaker. A cap is placed on the beaker's top. The beaker is shaken vigorously for one minute. The filter is then left to desorb for fifteen minutes. After fifteen minutes the solution is once again shaken for one minute. Then 2-3ml is transferred to a quartz cell that provides a 1cm pathlength. Another 1-cm quartz cell is filled with deionized water. This cell is placed in the reference cell holder. The concentration of the solution is read from the calibration curve stored in the computer, or from a graph created by the analyst. Because the Ranitidine is first desorbed in 10ml of solvent the resulting concentration is multiplied by 10 in order to obtain the micrograms of active on the filter.

In the analysis of environmental samples two procedural changes are noted. Because magnesium stearate and microcrystalline cellulose are present, and are insoluble, filter samples should be filtered prior to analysis. This is done by using a 0.45um Acrodisc filter attached to a disposable syringe. It is imperative that the filter and syringe not be reused. Additionally, the wall of the cassette holder must be rinsed in order to extract deposited ranitidine. This is done by gently washing the inside of the middle piece of the cassette with 10ml of solvent. A volumetric pipette should be

used. Generally, this rinse can be done over a 50ml beaker to avoid losses.

The cassette rinse is treated as a separate sample. This is necessary as removing Ranitidine from the cassette is probably a good deal less quantitative than desorbing a filter. The error in extracting ranitidine from the wall may be significant. If included with the filter extract it may bias the entire sample. Treating them separately allows one to be confident that at least the filter extraction result is accurate.

Table 2.3 illustates the recovery obtained in the spiking of glass fiber filters.

Table 2.3 : Analytical recovery obtained by spiking known masses of active onto filters.

| AMOUNT SPIKED | AMOUNT RECOVERED |
|---|---|
| 5.2 | 5.1 |
| 5.2 | 5.1 |
| 5.2 | 5.2 |
| 5.2 | 5.0 |
| 5.2 | 5.3 |
| 5.2 | 5.1 |

mean recovery: 98.7% CV: 0.0199%

| AMOUNT SPIKED | AMOUNT RECOVERED |
|---|---|
| 49.8ug | 50.6 |
| 49.8 | 51.4 |
| 49.8 | 50.0 |
| 49.8 | 49.7 |
| 49.8 | 50.8 |
| 49.8 | 50.4 |

Mean recovery: 101.3% CV: 0.0109%

| AMOUNT SPIKED | AMOUNT RECOVERED |
|---|---|
| 498ug | 502 |
| 498 | 484 |
| 498 | 499 |
| 498 | 479 |
| 498 | 475 |
| 498 | 479 |

Mean recovery: 97.6% CV: 0.0209%

The pooled coefficient of variation for all three levels is 0.0175%. Bartlett's test of homogeneity was applied to test the hypothesis that no difference between the CVs exists at the 95% level. No such difference is found. Bartlett's test can be found in Appendix II.

Currently environmental samples are analyzed using HPLC (method AM011-03:Appendix III). Because of this, analytical recovery is reported for this method as well as for the UV method. Glass fiber filters are spiked in a similar manner as that described above. The results are illustrated in Table 2.4.

TABLE 2.4 : Analytical recovery from glass fiber filters using HPLC.

| AMOUNT SPIKED | AMOUNT RECOVERED |
|---|---|
| 1.01 | 1.01ug |
| 1.01 | 1.14 |
| 1.01 | 1.02 |
| 1.01 | 1.05 |
| 1.01 | 0.99 |
| 1.01 | 0.99 |

Mean recovery: 102.3% CV: 0.052%

| AMOUNT SPIKED | AMOUNT RECOVERED |
|---|---|
| 60.27ug | 62.86 |
| 60.27 | 64.20 |
| 60.27 | 63.67 |
| 60.27 | 63.67 |
| 60.27 | 64.06 |
| 60.27 | sample void |

Mean recovery: 104.6% CV: 0.0072%

| AMOUNT SPIKED | AMOUNT RECOVERED |
|---|---|
| 500.00ug | 504.32 |
| 500.00 | 506.75 |
| 500.00 | 498.18 |
| 500.00 | 492.89 |
| 500.00 | 519.92 |
| 500.00 | 520.21 |

Mean recovery: 101.3% CV:0.028%

Table 2.4 cont'd

| AMOUNT SPIKED | AMOUNT RECOVERED |
|---|---|
| 1000.00ug | 1005.40 |
| 1000.00 | 1018.10 |
| 1000.00 | 1009.59 |
| 1000.00 | 1019.99 |
| 1000.00 | 1017.14 |
| 1000.00 | 1008.66 |

Mean recovery: 101.3% CV: 0.054%

The hplc recovery results are very good (> 90%) However, Bartlett's test of homogeneity shows a significant difference in the coefficients of variation. In the test only three levels of spiking were considered. Two tests were run first with the 500ug level then with the 1000ug level. This may be due to the low range over which the method was calibrated by Glaxo. Recalibration was not done because as a standard method, AMO119-03 can only be revalidated by Glaxo's methods development department. Thus, a pooled coefficient of variation is not possible at this time.

# CHAPTER THREE: TESTING BIAS IN SAMPLING

Currently, the internal occupational exposure limit for ranitidine hydrochloride is 50ug/$M^3$. The 15 minute ceiling limit is also 50ug/$M^3$. Flows for personal sampling for total dust are typically about 2 LPM. Given the expected low levels of ranitidine in the suite air and the analytical detection limit of 1ug/filter (HPLC), a 15 minute sample taken at 2 LPM may not collect enough active on the filter to be detected. This necessitates taking ceiling measurements at high flowrates. The Alpha-one pumps used by Glaxo can to sample for 15 minutes at 4 LPM. However, it is necessary to ensure that samples taken at low flows are comparable, or are not biased. If a large difference is observed then the sampling technique as a whole must be questioned.

In order for a sampling method to be unbiased it should sample accurately both the true particle size distribution and concentration of the aerosol of interest (4). As was discussed earlier in this paper many sampling methods are validated in wind tunnels. This allows the isokinetic sampling of an aerosol. Because in the industrial environment free stream airflow patterns cannot be readily characterized certainty with respect to isokinetic sampling is not possible.

However, in some cases the wind speeds present are low enough that one may assume that they are not

interfering with the sampling of a particular aerosol. In this case one assumes that still air is being sampled. The wind speed which allows one to make this assumption is based upon particle size, probe diameter and sampling flowrate. Marple and Liu give the simple relation:

$$W < 0.002(D^2U/d^4)^{0.33} \qquad (8)$$

Where W is the maximum allowable windspeed that may be present along with probe diameter, D (cm), inlet velocity, U (cm/s) and aerodynamic particle size, (cm), for still air conditions to be assumed. If this condition is met the sampler should sample particles within the given size range with at least 90% efficiency (3).

The use of the 37mm cassette for sampling total dust in industrial conditions is well established (1). It is not the intention of this paper to revalidate this method. However, what is important is how one uses this method.

Air velocity measurements in the Fette room and dispensing areas (including the dispensing booth) were made with a TSI hot wire anemometer (VelociCalc;model 8350). The anemometer was calibrated at the factory. The air speed was about 15-20 feet per minute (7.6-10.2 cm/s) in both regions of the suite. If one considers an open face cassette sampling at 2 LPM (a frequently used

flowrate) and a particle size range of up to 50 um a maximum air speed of 7.67 cm/s may be present in order to assume still air conditions (from above equation). Particles smaller than 50um would allow higher airspeeds to be present. For example, a 10um particle present may be sampled using the still air assumption with a windspeed up to 71 cm/sec (140 FPM)

Sampling at 4 LPM the maximum allowable airspeed is 10.50 cm/s (for a 50um particle) 4 LPM is considered because it is highest flowrate that Glaxo's sampling pumps (alpha 1) can continuously draw over an extended sampling period. Marple and Liu (1) predict that because still air conditions are met both flowrates should sample the aerosol with excellent efficiency.

Earlier in this paper an equation is described that is used by Fairchild to estimate the inlet efficiency of the 37-mm cassette sampling in still air. Given that still air conditions have been established in the Fette and dispensing areas estimates of the sampling efficieny of the cassette sampling at 2 and 4 LPM are calcuated. Table 3.1 presents the estimates.

Table 3.1 : Calculated estimates of the inlet efficiency of an open face 37-mm cassette sampling at 2 and 4 LPM in still air.

| Flowrate: 2 LPM | | Flowrate: 4 LPM | |
|---|---|---|---|
| Particle Diameter,um | $A_i$ | Particle Diameter,um | $A_i$ |

Table 3.1 cont'd

| | | | |
|---|---|---|---|
| 0.5 | 99.9% | 0.5 | 99.9% |
| 1.0 | 99.9% | 1.0 | 99.9% |
| 5.0 | 99.9% | 5.0 | 99.9% |
| 10.0 | 99.9% | 10.0 | 99.9% |
| 30.0 | 99.7% | 30.0 | 99.6% |

Where $A_i$ is the aspiration efficiency of the inlet calculated using empirical equations of Levin et al (8). The equation describes only inertial forces. Gravitational settling is not considered. The estimates indicate that over the flowrate range of 2 to 4 LPM sampling efficiency should be very good up to 30um. Moreover, the efficiency of both 2 and 4 LPM samplers is about the same. Thus, there should be no bias in the sampling method within this range of flowrates.

Stan Roach (20) suggests an easy method of testing this hypothesis that the method is not biased over a given flowrate range. It involves drawing paired samples in the workplace. One sample is run at twice the flowrate of the other. If no bias exists in the method then, on average, there should exist no significant difference in the concentrations calculated. Additionally, the particle sizes sampled should, on average, be identical.

Paired samples (area samples) were collected in the Fette room by using Alpha-one personal sampling pumps (DuPont Corp.). The pumps were calibrated using a Mini

Buck Calibrator. This device is reported as being a primary standard. It operates using a soap bubble tube. The bubble is sensed by an infrared beam detector. This allows for very fast readings to be taken. During the calibration five readings are taken for each pump at a chosen flowrate. In order for a flowrate to be acceptable individual readings must not vary more than +/- 5% of each other. The pumps were calibrated before and immediately after use. If the before and after calibration varied by more than 5% the data were not accepted. Samples were taken for 4 to 5 hours. The flowrates used were 2 and 4LPM. They were chosen because they represent the most likely range of flowrates to be used in the future by Glaxo.

37-mm plastic cassettes (Gelman Sciences) were taped together and each was attached to its own pump. One sample is taken at 2 LPM and the other at 4 LPM. It is assumed that pairing the samples in this manner does not influence the sample results (12). Taping the samples together ensures that both samplers are sampling similar air. In order to minimize the effects of settling cassettes were oriented horizontally with respect to the sampling axis (13). In the Fette room samples were taken on a table approximately 10 feet from the Fette machine. The table is about 3.5 feet in height. All samples are area samples.

In the dispensing area one half of the sample pairs are taken from inside a laminar flow booth which surrounds the dispensing process (air velocity measurement shows windspeeds of 20 feet per minute). The sampling pumps were taped to the wall of the booth in an effort to avoid interfering with the dispensing of ranitidine. The other half are taken from outside the booth on a table about 10 feet from the dispensing operation. Eleven pairs of cassette samples were taken for the Fette room and ten pairs were taken in the dispensing area. Two samples from the dispensing area were discarded because of pump failure. All samples were analyzed using the uv-spec method previously described. Both the cassette wall and the filter were assayed for the presence of ranitidine.

If indeed no significant difference exists between sampling at 2 LPM and at 4 LPM then one might expect that the sizes of particulate being collected on the filter are also identical. The air samples taken for particle size anlysis were taken in the Fette room. This was done in order to limit the cost of the study. Samples were collected on 37mm nucleopore filters at flowrates of 2 and 4 LPM. Samples were not taped together because the objective was not to attempt to perform another pairing experiment. Rather, the experiment was designed to determine ,roughly, what sizes of particles were being

collected by the cassette at 2 and 4 LPM in the Fette area.

Five samples at each flowrate were collected in the Fette area. Two SEM stubs were prepared from each filter.

STATISTICAL ANALYSIS

Environmental sampling data are frequently observed to follow the lognormal distribution (16). As a result, statistical analysis which uses the normal distribution is applied to the logarithmically transformed data rather than the raw data itself. In this study it is assumed that the concentration data generated in the bias experiment follow the lognormal distribution. This assumption is tested by employing the Kolmogorov-Smirnov test. This test evaluates given sampling data for its fit to a specified distribution. In this case the logs of the data were tested for fit to the normal distribution.

A two-way fixed factor analysis of variance (ANOVA) was

completed to investigate possible interactions between flow and location of samples. If an interaction is found a bias exists in the pairing method itself. Therefore paired t-tests must be done for each area sampled. The data may not be pooled in order to complete the t-test.

After the distribution was tested and the ANOVA completed a paired t-test was run (95% confidence level). The null hypothesis was that no significant difference exists between the logs of concentrations observed at 2 LPM and at 4 LPM in each pair of samples. Paired t-tests were also run on concentrations considering filter mass alone. This was done in order to elucidate differences of active collecting on the filters rather than considering the overall mass found (wall included). The confidence level used was 95%.

# CHAPTER FOUR: PARTICLE SIZING OF RANITIDINE

Within the Zantac suite it is suspected that three air contaminants are present: ranitidine, magnesium stearate (MgSt) and microcrystalline cellulose (MCC). The two latter components are used as tablet lubricant and binder, respectively. Because each is dispensed in a similar fashion, as a powder, the contaminants are probably dusts.

In order to objectively evaluate the cassette sampling efficiency a particle size characterization of both bulk active and airborne materials is in order.

A method of sizing airborne particles of active must be able to distinguish between the active, MgSt and MCC. A method which is used commonly as a reference in sizing is electron microscopy. The method employed in this study is Scanning Electron Microscopy (SEM). SEM was used because of its ability to perform elemental analysis and for its superior resolution. It is noted that the use of SEM can be costly. However, given that a detailed particle size analysis has not been completed for ranitidine the possibility exists that some particles present in the suite air may be below the limit of resolution on a light microscope. Moreover, even if ranitidine could be readily distinguished morphologically from MgSt and MCC it is likely that some particles present may contain a combination of components.

Therefore, basing analysis on morpholoIgy could lead to significant error.

The method used to size Ranitidine particles is based on x-ray microanalysis. As electrons are focused onto the sample electrons on the sample surface are raised to an excited , or elevated energy state. When the electron leaves its lower energy orbit for a higher state a vacancy is created. Because atoms are more stable in the ground state an electron in higher energy orbital drops down to fill this new vacancy, thus returning the atom to the ground state. The difference in energy between the higher orbital position and the new lower position is released in the form of x-rays (15).

Because each element has a unique electronic structure the x-ray energy produced upon bombardment with electrons is characteristic to that element. In this manner it is possible to examine particles of suspected ranitidine and identify them by identfying their characteristic x-rays.

Samples of bulk ranitidine were prepared by lightly dipping a small camel's hair brush into some ranitidine powder. Then, lightly tapping the brush, ranitidine was deposited on an aluminum SEM stub. The stub had double-sticky tape on it. All preparation was done in a plastic

glove-bag filled with nitrogen. This was done to prevent contamination with ambient dust.

Then, using a Denton vacuum evaporation unit, a coating of carbon was applied to the sample. The sample was then analyzed with the x-ray microanalyzer. Recall that ranitidine contains sulfur and chlorine. These two atoms emit characteristic x-rays between 1.28 and 3.84 kiloelectron volts (KeV). An x-ray scan of a ranitidine particle is represented in Figure 4.1. The x-axis represents the energy of x-rays detected. The y-axis represents the number of counts per second detected. It is noted that the y-axis lacks units. Most frequently elemental analysis is completed for qualitative purposes only. Therefore, ordinate units are not as important as knowing which characteristic energy is being observed.

In Figure 4.1 it can be seen that both sulfur and chlorine can be readily detected. Carbon is used here as the coating in order to avoid the possible interferences that metal coatings may produce. The x-ray output of carbon is too low to elicit a response from the detector. However, carbon is a poor conductor of electrons. Therefore, as the electron beam strikes the surface, electrons tend to build- up, heating the sample. Destruction of the sample may occur quickly. It is, therefore, common practice to coat the sample with a

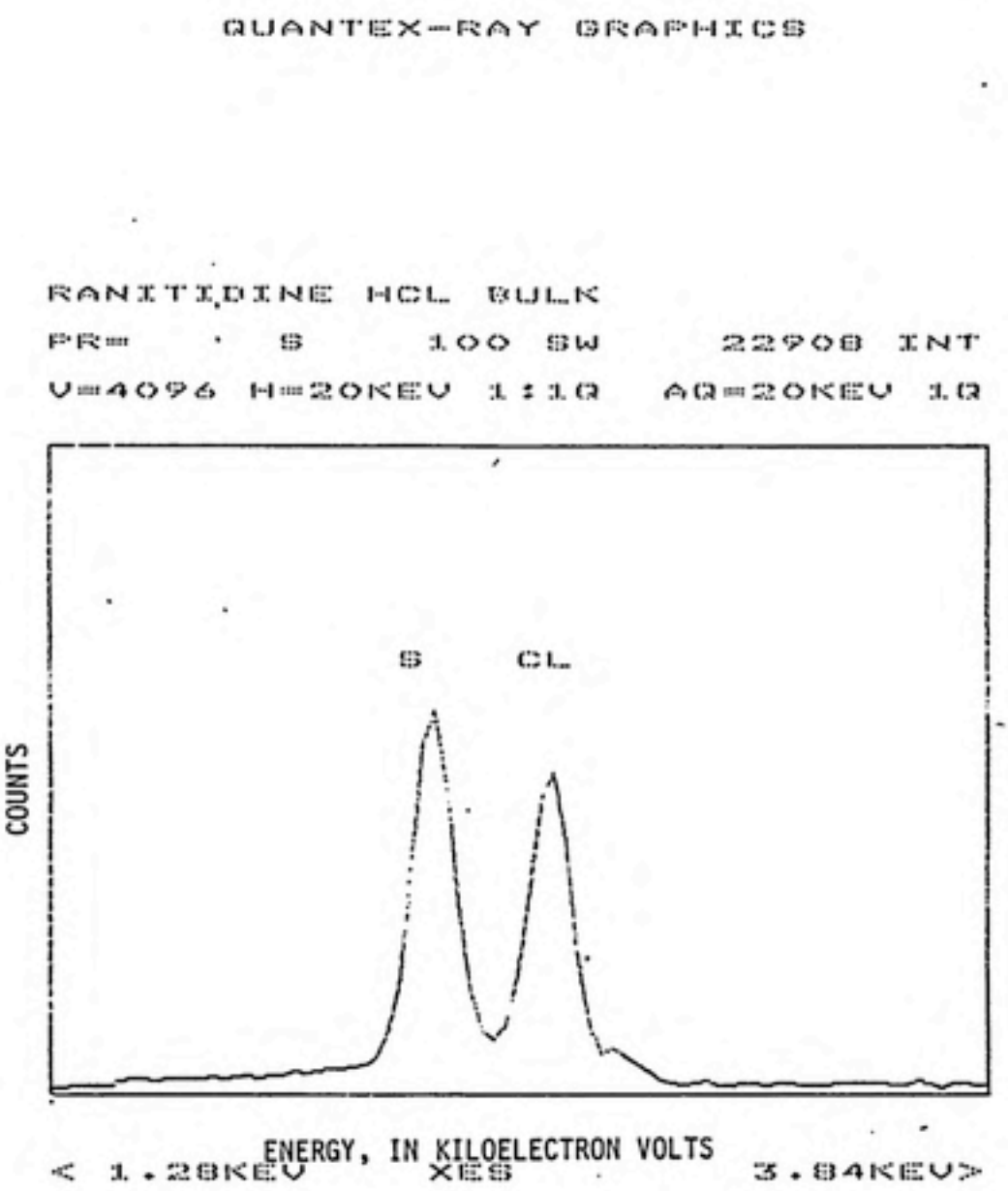



Figure 4.1: X-ray spectrum of Ranitidine Hydrochloride illustrates the presence of Sulfur and Chlorine as markers.

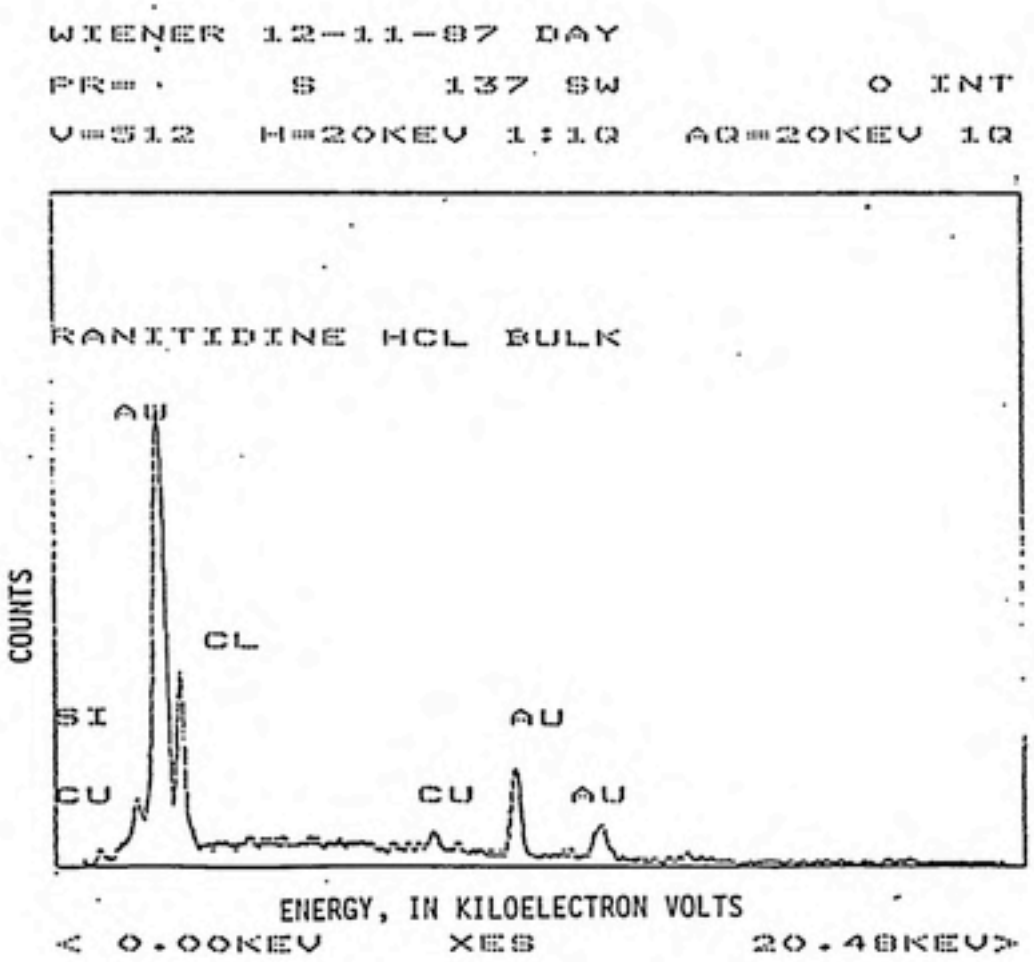



Figure 4.2: X-ray spectrum of ranitidine hydrochloride with gold coating. Chlorine is resolved, and hence, may be used as the marker in sizing. Note that gold masks sulfur. The scale has been expanded from that of Figure 4.1.

conducting, volatile metal. Examples of such metals are gold and palladium. By identifying Ranitidine in this manner one can distinguish active particle from others such as magnesium stearate and Microcrystalline Cellulose.

To size the particles a Au/Pd alloy was then vacuum evaporated onto the sample of bulk active. It was observed that gold and palladium overlap in their spectra with sulfur and chlorine. Because of this interference using the gold-palladium alloy coating was not possible.

The problem was solved by coating the sample with only gold. As seen in Figure 4.2 gold masks the sulfur peak. However, the chlorine peak is clearly resolved. Thus, we may identify ranitidine particles among other contaminants (which do not contain chlorine) using a gold coating.

Although the above method is useful, it is time consuming. This problem is abated by interfacing an audio jack with the x-ray detector. Consequently, when a scan "hits" a ranitidine particle very many clicks, or counts, can be heard. This is accomplished by placing a window on the chlorine peak energy. The computer automatically tries to match the scanned particle's spectrum to that of ranitidine. If the particle contains

chlorine then the operator will hear the counts. Alternatively, the analyst can view the spectrum and ascertain the presence of ranitidine. This method of analysis is still a bit tedious. However, it does permit the analyst to have reasonable certainty that most of the particles on the image are rRanitidine containing.

Particles of bulk ranitidine were sized using the above method of preparation. After being coated with gold the samples were placed into the SEM's specimen chamber. This chamber is always kept under vacuum. The vacuum is necessary to prevent electron interaction with air molecules. The magnitude of the vacuum is about $10^{-5}$ Torr. An image was created on the screen by scanning the specimen. A detailed treatment of scanning electron microscopy is beyond the scope of this paper. However, some important parameters are discussed below.

THE WORKING DISTANCE

The working distance used was in the range of 15-25mm. The working distance is the distance from the final lens to the surface of the specimen. This parameter is proportional to the depth of focus. Consequently, a large working distance results in good depth of focus. Because resolution is inversely

proportional to the depth of focus, short working distances give the best resolution (15).

MAGNIFICATION

The magnifications used were about 100, 200, 400, 1180 and 3500X. These magnifications allowed the analyst to cover a rather wide range of sizes, from particles > 20um to those smaller than the pores of the filter. It is admitted, however, that the actual choice of magnification is arbitrary. The resolution in SEM is generally equal to the electron beam diameter. For many insruments this is about five nanometers. This magnification was chosen because it permitted a wide range of particle sizes to be measured.

ACCELERATING VOLTAGE

The accelerating voltage used is about 20 KeV. This plays a crucial role in image production. Most SEMs can produce accelerating voltages of up to 30KeV. High accelerating voltages result in good resolution and deep penetration of the specimen. The accelerating voltage applied in this study is fairly high. This is done in order to penetrate the surface of the specimen deeply. Deep pentration enhances x-ray production. This aids in elemental analysis of Ranitidine particles. It is noted, however, that high accelerating voltages can induce excess charge build-up on the specimen. This can damage the sample quite readily (15). A photomicrograph of Ranitidine is presented in Figure 4.4. This particle was captured on a 25mm nucleopore filter (.2 um pore size) held in place by a 25mm plastic cassette. The flowrate was 4 liters per minute. Sampling time was about 3-4 hours.

After images of bulk Ranitidine were created they were stored in the SEM's computer in the form of a digital image. In this manner the image may be interfaced with a sizing instrument.

The instrument used to size the particles is a Zeiss Video-Plan Morphometry system. Basically, the system

operates by sizing particles (Feret's X-diameter) seen in the digital image stored on hard-disk. The operator produces a digital image on a television monitor. By using a magnetic screen interfaced with the Video-Plan TV screen the operator outlines the perimeter of particles to be sized with a magnetic pen/tracer. In order to size the particles one must enter an appropriate magnification factor into the Video-Plan. This is obtained from the magnification used to produce the digital image on the SEM.

The number of particles sized was 187. An attempt was made to size as many particles as was reasonable. The data were then reduced into intervals and plotted on a log-probability graph. A straight line is then drawn to connect the data points. If the data are lognormally distributed most of the points will fall on the line.

VALIDATION OF THE SEM METHOD

As is discussed above the samples are subjected to a very high vacuum in the sample chamber of the SEM. This vacuum is about $10^{-5}$ Torr. An effort was made to ensure that these extream conditions did not alter the integrity of the examined material. This was done by sizing the bulk material using light microscopy. A dry mounted slide of bulk Ranitidine was prepared in the same manner as the SEM samples. The slide used was a standard

haemocytometer. The Zeiss microscope was set at 200X. The haemocytometer enabled the analyst to avoid sizing particles more than one time. A total of 178 particles were sized. The results of the two methods of sizing are then compared. If no difference exists then it may be assumed that the distribution of sizes is not being changed by the vacuum of the SEM, or for that matter by the vacuum of the evaporation unit.

AIR SAMPLES FOR PARTICLE SIZE ANALYSIS

Air samples taken for particle size analysis were taken on 37mm nucleopore filters (pore size 0.2 um). After collection a small piece of filter was cut from the middle region of the filter and mounted on the SEM stub with double-sticky tape. The samples were then coated with gold and examined using the above method. Because the method of identifying particles of Ranitidine in the presence of other dusts is so tedious and quite costly only 117 particles were measured at 2 LPM and 151 were measured at 4 LPM. The results of the sizing are contained in chapter 6 of this paper.

# SECTION THREE: EXPERIMENTAL RESULTS

# CHAPTER FIVE: RESULTS OF BIAS EXPERIMENT

The concentrations obtained from pairing in the Fette room are presented in Table 5.1. Results are expressed in micrograms per cubic meter ($ug/M^3$). Table 5.2 presents the data collected in the dispensing area. Some samples are from inside the laminar flow booth. Tables 5.3 and 5.4 present the data broken down into mass of ranitidine found on filters and on the inside wall of the cassette. Additionally, the tables highlight the contribution made by the wall mass found to the total mass collected by the cassette and filter. Table 5.5 presents the air concentrations calculated by considering only the masses of ranitidine found on the filters.

TEST OF LOGNORMAL ASSUMPTION

The concentration data from both dispensing and Fette areas was log-transformed in a Systat file. The data were then standardized. The values were then compared to the standard normal distribution using the Kolmogorov-Smirnov (KS) one sample test. The results are presented below.

| Variable | Number | P-value |
|---|---|---|
| (high flow) 4 lpm | 19 | 0.440 |
| (low flow) 2 lpm | 19 | 0.690 |

The data indicate that the assumption of a lognormal distribution is solid. A P-value less than 0.05

Table 5.1: Results from pairing in the Fette area during second shift (FS). Samples are taken on a table about 10 feet from the Fette machine.

| Pair | Location | Flowrate | Concentration, ug/$M^3$ |
|---|---|---|---|
| 1 | FS | 2 lpm | 14.840 |
| | | 4 lpm | 20.670 |
| 2 | FS | 2 lpm | 17.170 |
| | | 4 lpm | 15.580 |
| 3 | FS | 2 lpm | 16.750 |
| | | 4 lpm | 23.534 |
| 4 | FS | 2 lpm | 44.272 |
| | | 4 lpm | 60.979 |
| 5 | FS | 2 lpm | 64.200 |
| | | 4 lpm | 84.313 |
| 6 | FS | 2 lpm | 102.750 |
| | | 4 lpm | 95.224 |
| 7 | FS | 2 lpm | 182.720 |
| | | 4 lpm | 127.010 |
| 8 | FS | 2 lpm | 30.287 |
| | | 4 lpm | 16.281 |
| 9 | FS | 2 lpm | 20.343 |
| | | 4 lpm | 29.755 |
| 10 | FS | 2 lpm | 28.495 |
| | | 4 lpm | 63.971 |
| 11 | FS | 2 lpm | 79.854 |
| | | 4 lpm | 30.999 |

Table 5.2: Results from pairing in the dispensing area during first shift. DB= samples taken from inside laminar flow booth in dispensing area;DT= samples taken from table about 5-7 feet outside the laminar flow booth.

| Pair | Location | Flowrate | Concentration, ug/$M^3$ |
|---|---|---|---|
| 12 | DB | 2 lpm | 33.50 |
| | | 4 lpm | 6.72 |
| 13 | DB | 2 lpm | 25.99 |
| | | 4 lpm | 16.55 |
| 14 | DB | 2 lpm | 35.47 |
| | | 4 lpm | 22.57 |
| 15 | DT | 2 lpm | 61.77 |
| | | 4 lpm | 50.73 |
| 16 | DT | 2 lpm | 37.73 |
| | | 4 lpm | 29.29 |
| 17 | DT | 2 lpm | 67.62 |
| | | 4 lpm | 71.72 |
| 18 | DT | 2 lpm | 85.60 |
| | | 4 lpm | 48.79 |
| 19 | DT | 2 lpm | 73.57 |
| | | 4 lpm | 48.79 |

Table 5.3: Masses of Ranitidine found on filters and on inside cassette wall. All were collected in the Fette area on a table about 10 feet from the Fette machine.

| Pair | Flowrate | Filter | Cassette | % of total from cassette |
|---|---|---|---|---|
| 1 | 2 lpm | 11.754ug | 1.503ug | 11.34% |
| | 4 lpm | 31.990 | 1.306 | 3.92 |
| 2 | 2 lpm | 4.224 | 7.812 | 64.91 |
| | 4 lpm | 13.012 | 9.985 | 43.40 |
| 3 | 2 lpm | 5.785 | 8.277 | 58.86 |
| | 4 lpm | 19.663 | 18.546 | 48.54 |
| 4 | 2 lpm | 20.578 | 14.657 | 41.60 |
| | 4 lpm | 82.084 | 18.923 | 18.73 |
| 5 | 2 lpm | 23.636 | 21.503 | 47.63 |
| | 4 lpm | 104.598 | 11.198 | 9.70 |
| 6 | 2 lpm | 50.799 | 18.897 | 27.11 |
| | 4 lpm | 108.812 | 18.436 | 14.50 |
| 7 | 2 lpm | 67.959 | 59.324 | 46.60 |
| | 4 lpm | 98.913 | 69.282 | 41.20 |
| 8 | 2 lpm | 5.141 | 13.640 | 73.00 |
| | 4 lpm | 12.960 | 7.750 | 37.42 |
| 9 | 2 lpm | 4.584 | 12.020 | 72.00 |
| | 4 lpm | 19.04 | 26.441 | 58.10 |
| 10 | 2 lpm | 7.390 | 14.870 | 66.80 |
| | 4 lpm | 34.500 | 63.069 | 64.60 |
| 11 | 2 lpm | 30.912 | 31.470 | 50.45 |
| | 4 lpm | 23.680 | 23.600 | 49.83 |

Table 5.4: Masses of Ranitidine found on filters and on inside cassette wall. All were collected in the dispensing area. Pairs 12-14 are from inside the laminar flow booth. Pairs 15-19 were taken on a table about 5-7 feet outside the booth.

| Pair | Flowrate | Filter | Cassette | % of total from cassette |
|---|---|---|---|---|
| 12 | 2 lpm | 10.95ug | 5.840ug | 34.78% |
| | 4 lpm | 2.280 | 4.570 | 66.72 |
| 13 | 2 lpm | 9.130 | 4.570 | 33.58 |
| | 4 lpm | 11.410 | 6.850 | 37.51 |
| 14 | 2 lpm | 13.700 | 10.270 | 42.85 |
| | 4 lpm | 5.710 | 18.260 | 76.17 |
| 15 | 2 lpm | 12.560 | 20.550 | 62.070 |
| | 4 lpm | 25.110 | 34.250 | 57.70 |
| 16 | 2 lpm | 8.900 | 13.700 | 60.62 |
| | 4 lpm | 18.260 | 15.98 | 46.67 |
| 17 | 2 lpm | 21.230 | 18.260 | 46.24 |
| | 4 lpm | 38.810 | 29.680 | 43.33 |
| 18 | 2 lpm | 18.260 | 29.680 | 61.91 |
| | 4 lpm | 29.680 | 25.110 | 45.83 |
| 19 | 2 lpm | 22.830 | 17.120 | 42.85 |
| | 4 lpm | 29.680 | 25.110 | 45.83 |

Table 5.5: Concentrations obtained when considering only mass of active found on the filters.

| Pair | Location | Flowrate | Concentration |
|---|---|---|---|
| 1 | FS | 2 lpm | 13.16ug/$M^3$ |
| | | 4 lpm | 19.85 |
| 2 | FS | 2 lpm | 6.03 |
| | | 4 lpm | 8.81 |
| 3 | FS | 2 lpm | 6.89 |
| | | 4 lpm | 12.11 |
| 4 | FS | 2 lpm | 25.85 |
| | | 4 lpm | 49.55 |
| 5 | FS | 2 lpm | 33.62 |
| | | 4 lpm | 76.16 |
| 6 | FS | 2 lpm | 74.89 |
| | | 4 lpm | 81.43 |
| 7 | FS | 2 lpm | 97.55 |
| | | 4 lpm | 74.69 |
| 8 | FS | 2 lpm | 8.29 |
| | | 4 lpm | 10.18 |
| 9 | FS | 2 lpm | 5.61 |
| | | 4 lpm | 12.46 |
| 10 | FS | 2 lpm | 9.45 |
| | | 4 lpm | 22.62 |
| 11 | FS | 2 lpm | 39.56 |
| | | 4 lpm | 15.53 |

Table 5.5 continued

| Pair | Location | Flowrate | Concentration, ug/$M^3$ |
|---|---|---|---|
| 12 | DB | 2 lpm | 21.86 |
| | | 4 lpm | 2.23 |
| 13 | DB | 2 lpm | 17.32 |
| | | 4 lpm | 10.34 |
| 14 | DB | 2 lpm | 26.62 |
| | | 4 lpm | 5.36 |
| 15 | DT | 2 lpm | 23.43 |
| | | 4 lpm | 21.46 |
| 16 | DT | 2 lpm | 14.86 |
| | | 4 lpm | 30.48 |
| 17 | DT | 2 lpm | 36.35 |
| | | 4 lpm | 40.63 |
| 18 | DT | 2 lpm | 32.61 |
| | | 4 lpm | 26.42 |
| 19 | DT | 2 lpm | 42.04 |
| | | 4 lpm | 26.43 |

indicates a significant difference between the samples and the null hypothesis that the data follow the lognormal distribution. Probability plots of the transformed data are contained in Appendix III.

ANALYSIS OF VARIANCE

The analysis of variance showed no interaction between flows and location. However, a nearly significant difference was found when considering concentrations calculated with only the mass of ranitidine on the filter. The P-value in this case was 0.06. A value less than 0.05 is considered significant. Therefore, the mass of ranitidine deposited on the wall of the cassette should be considered in the calculation of concentration.

PAIRED T-TESTS ON CONCENTRATION DATA (WALL AND FILTER)

The pairs of samples taken in the Fette and dispensing areas were evaluated using a paired t-test on the log-transformed data.

$H_o$: There is no difference, at the 95% confidence level, between the concentrations observed in pairs at 2 and 4 lpm.

$H_a$: A significant difference does exist. Therefore, results obtained at either flowrate should be suspected as being biased.

Observed P-value= 0.1627 (two tailed)

A P-value of less than 0.05 causes the null hypothesis to be rejected. Thus, no significant difference is noted. A two tailed test is used because one might expect random differences between pairs to be positive and negative.

The data obtained from extracting ranitidine off the wall of the cassette is shown in Tables 5.3 and 5.4. When considering the results obtained in the Fette room it seems that much more Ranitidine is depositing on the cassette in the case of low flow than high flow. The average ratio of the percentage contribution to total mass at 2 lpm to the contribution at 4 lpm within each pair is 1.9. This indicates that about twice as much ranitidine is depositing (on a percentage basis) on the wall of the cassette during low flow in the Fette room when one considers individual pairs.

When considering wall deposition in samples taken in the dispensing area the situation is different. The ratio of the average percentage contribution is 0.97. Thus, in the dispensing area, wall deposition is occurring at an equal level comparing pairs at 2 and 4 LPM when one considers total ranitidine recovered from the sample.

Considering both areas the ratio of the low flow contribution to the high flow contribution is 1.27. This indicates that, on average, about 25% more ranitidine is collected on the 2 LPM samples than on the 4 LPM samples (total ranitidine considered).

TEST OF LOGNORMAL ASSUMPTION IN FILTER CONCENTRATION DATA

Traditionally, analysts consider only the amount of material collected on the filter mounted in the cassette. Data analysis was also performed on the concentrations of Ranitidine calculated when considering only the mass of active found on the filter. These data are present in Table 5.5.

The KS test was applied to the data and the results were as follows:

| Variable | Number | P-value |
|---|---|---|
| Conc at high flow | 19 | 0.996 |
| Conc at low flow | 19 | 0.976 |

The filter concentrations can be assumed to follow the lognormal distribution. Plots illustrating the conformity of the samples to the standard normal distribution can be seen in Appendix III.

PAIRED T-TESTS ON THE FILTER CONCENTRATION DATA

A paired t-test was run on the log-transformed data.

$H_o$: No significant difference exists,at the 95% confidence level, between concentrations of active

found in pairs collected at 2 and 4 LPM when considering only filter active.

$H_a$: A significant difference does exist at the 95% level.

Observed P-value: 0.918 (two tailed)

A P-value of less than 0.05 is cause to accept the null hypothesis. Therefore, we do not reject in this case.

These results indicate that the concentrations obtained at 2 and 4 LPM, when considering only filter active are not different. Therefore, we might expect that the particle sizes of Ranitidine impacting on the filter are similar. Confirming this presents another method of determining sampling bias. The results of the particle sizing are presented in the next section of this paper.

# CHAPTER SIX: RESULTS OF PARTICLE SIZING

The results of the bulk sizing are encouraging. Sizing by using the SEM was validated quite well using the optical technique. The bulk particle size data obtained using the SEM is found in Table 6.1. Because data followed the log-normal distribution the Hatch-Choate equation was used to transform count median diameters to mass median diameters.

Table 6.1: Bulk particle size data obtained using Scanning Electron Microscopy.

| Interval,um | Number | Cumulative percentage |
|---|---|---|
| 0.4-1.0 | 2 | 1.07 |
| 1-2.0 | 19 | 11.23 |
| 2-3.0 | 32 | 28.34 |
| 3-4.0 | 34 | 46.52 |
| 4-5.0 | 24 | 59.35 |
| 5-10.0 | 52 | 87.16 |
| 10-15.0 | 12 | 93.58 |
| 15-20.0 | 5 | 96.25 |
| 20-25.0 | 3 | 97.85 |
| 25-30.0 | 2 | 98.92 |
| >30 | 2 | 99.99 |
| | Total: 187 particles | |

A log-probability plot of the data is presented in Figure 6.1. This was constructed by plotting the upper limit of the class interval against the cummulative percentage less than that class. A line was drawn between the calculated geometric mean and the 84th percentile point calculated with the geometric standard deviation. It can be seen that the data do fall on a straight line. Thus, the assuption of lognormality is sound (22). The geometric mean is 4.60. The geometric

standard deviation is 2.10. The mass median diameter is 24.15um.

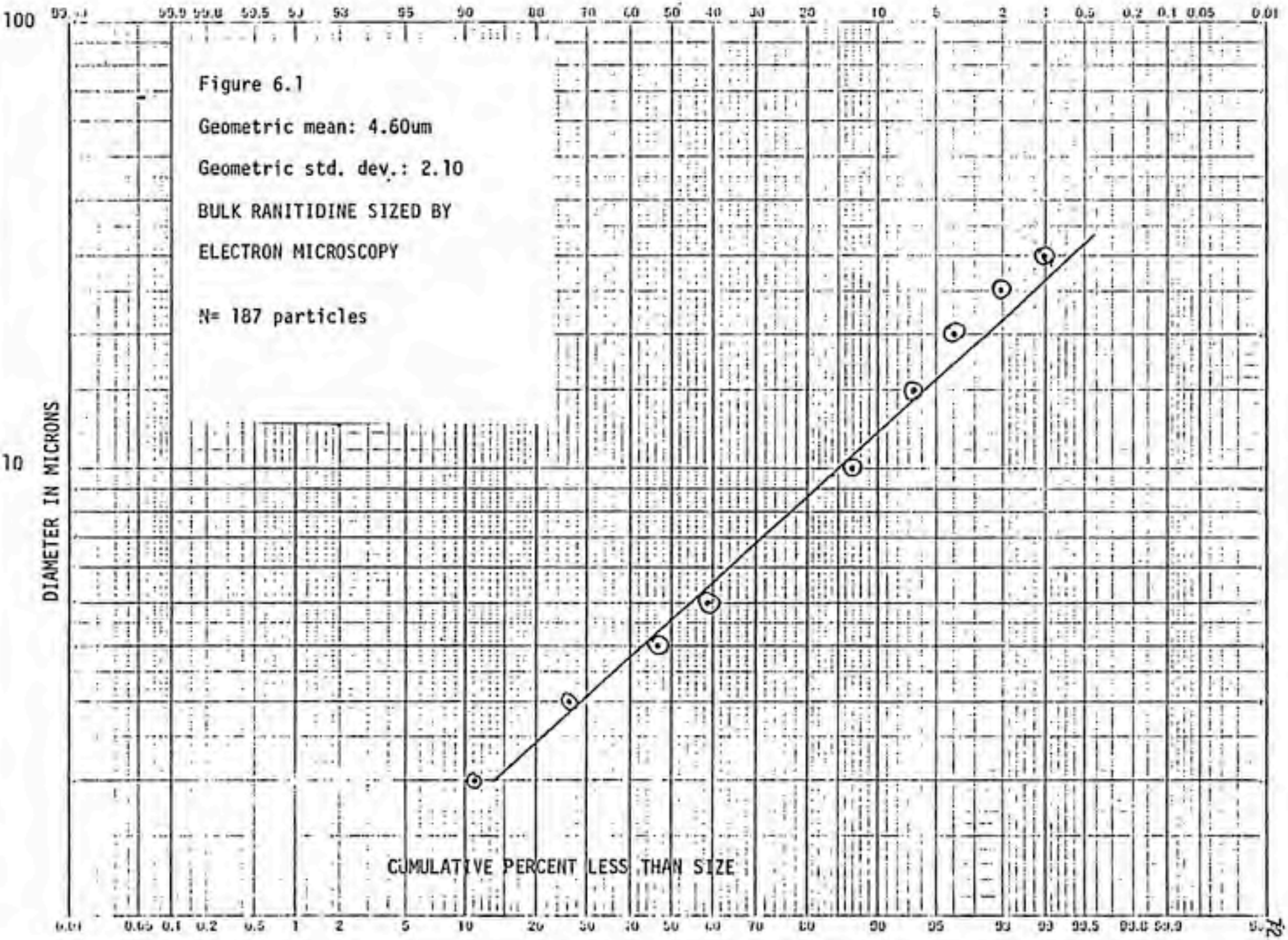


Figure 6.1
Geometric mean: 4.60um
Geometric std. dev.: 2.10
BULK RANITIDINE SIZED BY
ELECTRON MICROSCOPY
N= 187 particles
100
10
DIAMETER IN MICRONS
99.9 99.8 99.5 99 98 95 90 80 70 60 50 40 30 20 10 5 2 1 0.5 0.2 0.1 0.05 0.01
0.01 0.05 0.1 0.2 0.5 1 2 5 10 20 30 40 50 60 70 80 90 95 98 99 99.5 99.8 99.9
CUMULATIVE PERCENT LESS THAN SIZE

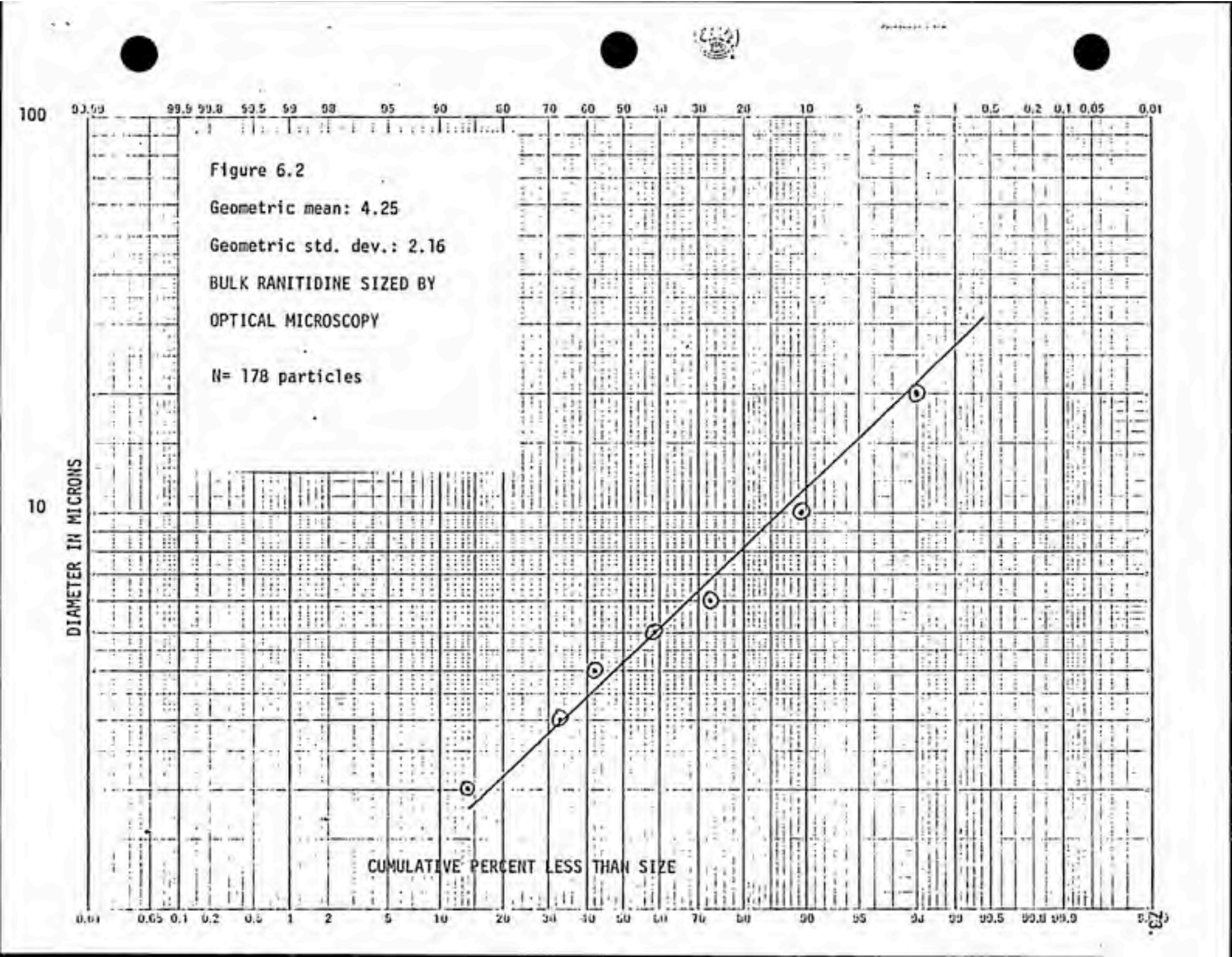


Figure 6.2
Geometric mean: 4.25
Geometric std. dev.: 2.16
BULK RANITIDINE SIZED BY
OPTICAL MICROSCOPY
N= 178 particles
DIAMETER IN MICRONS
100
10
99.99 99.9 99.8 99.5 99 98 95 90 80 70 60 50 40 30 20 10 5 2 1 0.5 0.2 0.1 0.05 0.01
0.01 0.05 0.1 0.2 0.5 1 2 5 10 20 30 40 50 60 70 80 90 95 98 99 99.5 99.8 99.9
CUMULATIVE PERCENT LESS THAN SIZE

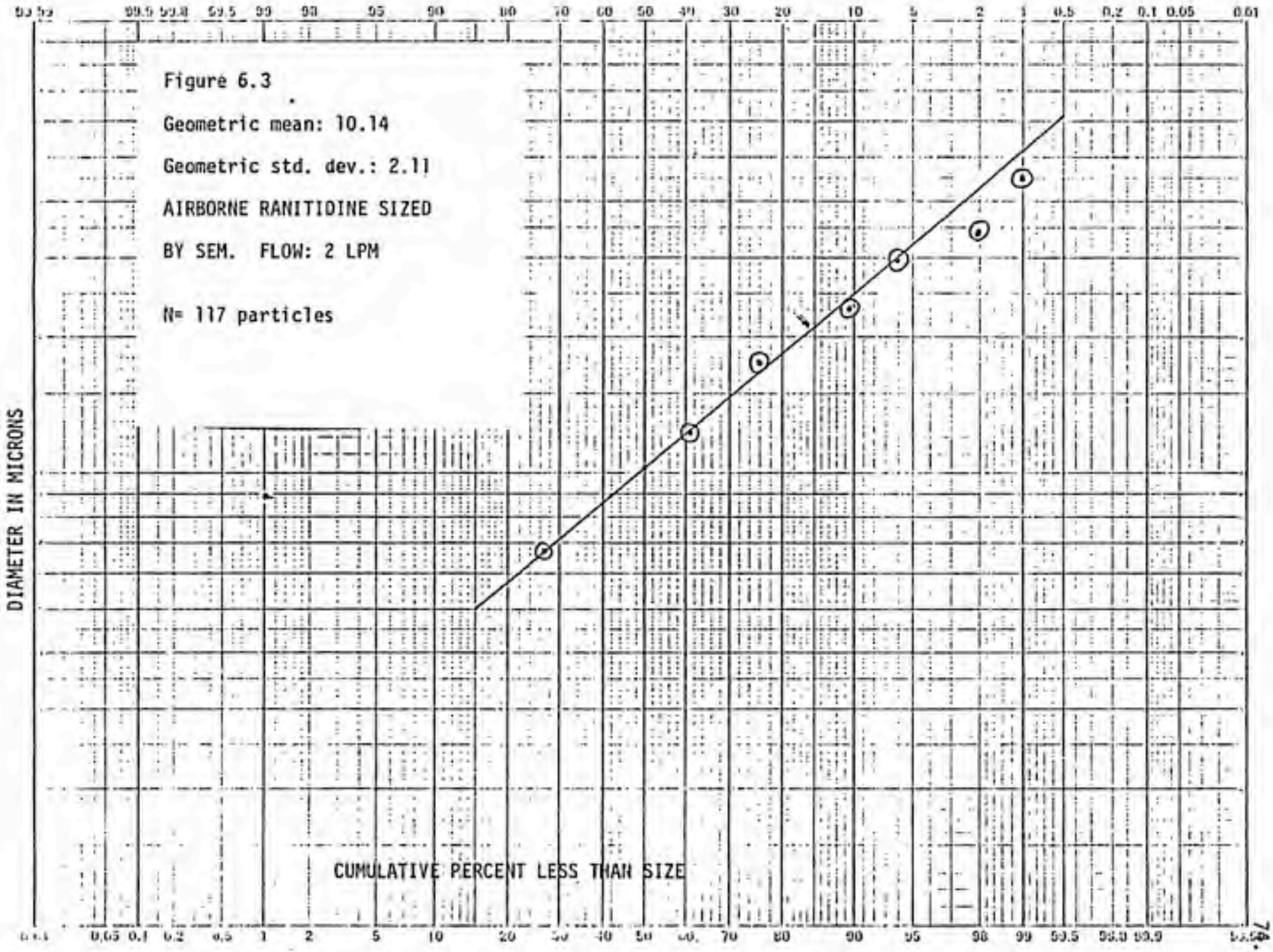


Figure 6.3
Geometric mean: 10.14
Geometric std. dev.: 2.11
AIRBORNE RANITIDINE SIZED
BY SEM. FLOW: 2 LPM
N= 117 particles
100
10
DIAMETER IN MICRONS
99.9 99.8 99.5 99 98 95 90 80 70 60 50 40 30 20 10 5 2 1 0.5 0.2 0.1 0.05 0.01
0.05 0.1 0.2 0.5 1 2 5 10 20 30 40 50 60 70 80 90 95 98 99 99.5 99.8 99.9
CUMULATIVE PERCENT LESS THAN SIZE

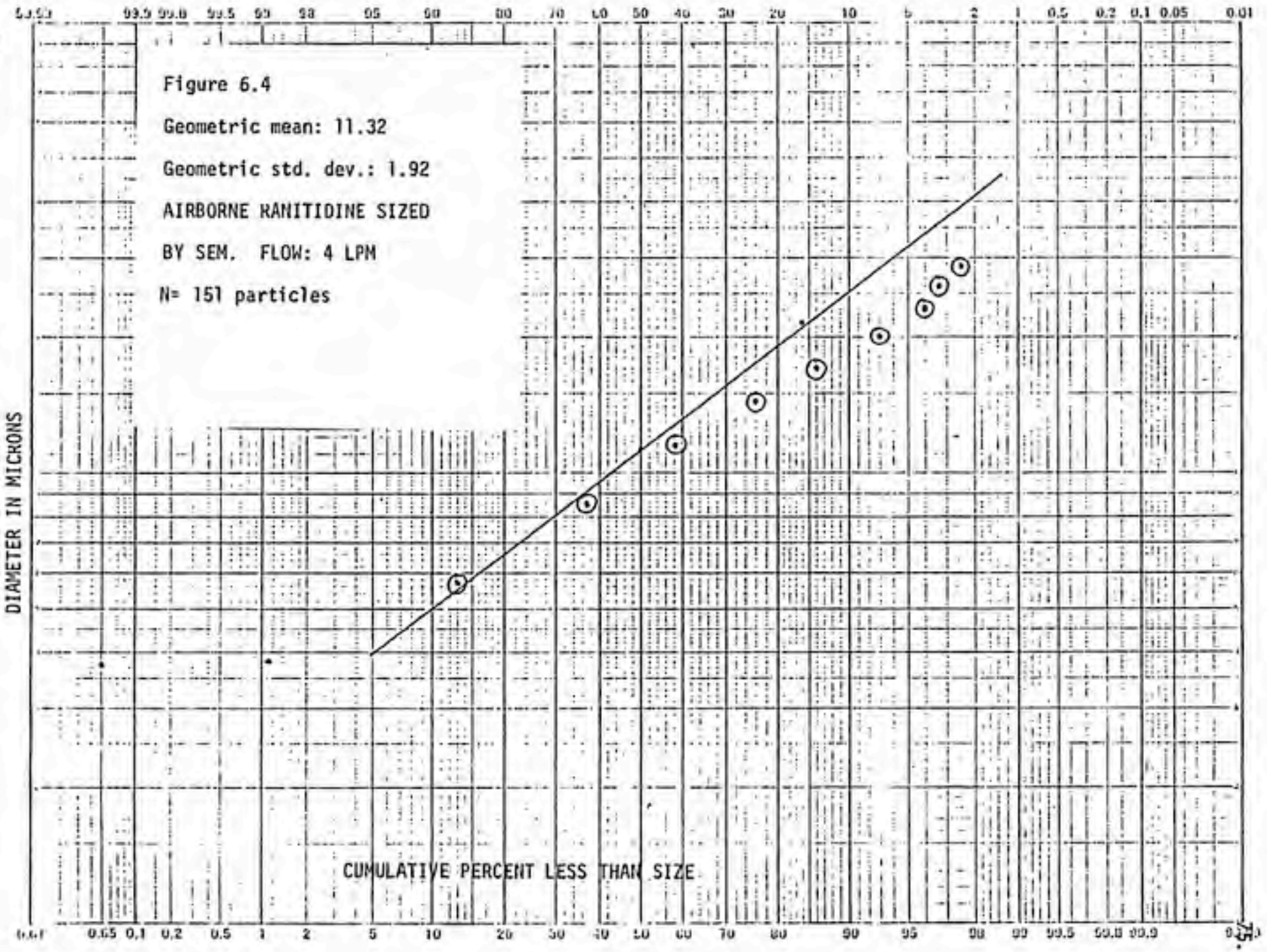


Figure 6.4
Geometric mean: 11.32
Geometric std. dev.: 1.92
AIRBORNE RANITIDINE SIZED
BY SEM. FLOW: 4 LPM
N= 151 particles
100
10
DIAMETER IN MICRONS
CUMULATIVE PERCENT LESS THAN SIZE

The results of sizing using the optical microscope are seen in Table 6.2.

Table 6.2: Bulk particle size data obtained using optical microscopy.

| Interval,um | Number | Cumulative percentage |
|---|---|---|
| 0.4-1.0 | 9 | 5.61 |
| 1-2.0 | 16 | 14.05 |
| 2-3.0 | 31 | 31.47 |
| 3-4.0 | 17 | 41.02 |
| 4-5.0 | 33 | 59.56 |
| 5-6.0 | 23 | 72.48 |
| 6-10.0 | 30 | 89.33 |
| 10-20.0 | 16 | 98.33 |
| 20-30.0 | 3 | 100.02 |
| | Total: 178 particles | |

The data are also presented in Figure 6.2. These data also fit well to a straight line. The geometric mean is 4.25um and the geometric standard deviation is 2.16. The mass median diameter is 25.13um.

RESULTS OF PARTICLE SIZING FROM AIR SAMPLES

The results of sizing Ranitidine particles at 2 LPM are shown in Table 6.3.

Table 6.3: Results of particle sizing taking samples at 2 LPM in the Fette room.

| Interval,um | Number | Cumulative percentage |
|---|---|---|
| 1.28-6.74 | 31 | 26.50 |
| 6.74-12.21 | 40 | 60.68 |
| 12.21-17.65 | 18 | 76.07 |
| 17.67-23.12 | 16 | 89.74 |
| 23.12-28.58 | 5 | 94.02 |
| 28.58-34.04 | 5 | 98.29 |
| 34.04-39.50 | 0 | 98.29 |
| 39.50-44.96 | 1 | 99.15 |
| 44.96-50.42 | 0 | 99.15 |
| 50.42-55.88 | 1 | 100.00 |
| | Total: 117 particles | |

The log-probability plot is presented in Figure 6.3. The geometric mean is 10.14um and a geometric standard deviation of 2.11. Using the Hatch-Choate relation the mass median diameter is 55.7um.

The results of sizing using a flowrate of 4 LPM in the Fette room are in Table 6.4.

Table 6.4: Results of particle sizing taking samples at 4 LPM in the Fette room.

| Interval,um | Number | Cumulative percentage |
|---|---|---|
| 2.86-5.72 | 20 | 13.25 |
| 5.72-8.57 | 36 | 37.09 |
| 8.57-11.44 | 33 | 58.94 |
| 11.44-14.30 | 26 | 76.16 |
| 14.30-17.16 | 15 | 86.09 |
| 17.16-20.03 | 10 | 92.72 |
| 20.03-22.89 | 5 | 96.03 |
| 22.89-25.75 | 1 | 96.69 |
| 25.75-28.61 | 1 | 97.35 |
| 28.61-31.47 | 4 | 100.00 |
| | Total: 151 particles | |

The data are also presented in Figure 6.4. The geometric mean is 11.32. The geometric standard deviation is 1.92. The mass median diameter is calculated to be 40.9um.

A Kolmogorov-Smirnov test indicates that a significant difference in the shape of the distributions at 2 and 4 LPM exists. However, this is probably due to the differing geometric standard deviations. The differing GSDs may be a result of the small number of particles measured. A two sided t-test reveals no significant difference between the medians at the 90% level. Although the KS test may call the t-test's reliabilty into question Figures 6.3 and 6.4 indicate that the distributions are not strikingly different. Therefore, the results of the t-test are accepted.

# CHAPTER SEVEN: DISCUSSION OF RESULTS

ANALYTICAL METHODS

The development of an alternate analytical method (uv-spec) was completed primarily to expedite the analysis of samples during the study. In order to use the HPLC method (AM0119-03) a good deal of planning was necessary. In order to use the HPLC at Glaxo 2-3 days notice needed to be given. Additionally, 2-3 days notice was needed to take samples in the Zantac suite.

Moreover, the Lab Automation System (LAS) used to calculate the results of analyses takes up to 24 additional hours to print out a report. Using the new method allowed rapid analysis with excellent accuracy.

The uv-spec method does, however, have a major shortcoming. The spectrophotometers at Glaxo do not have an automatic injection system as do the HPLCs. This injection system allows the analyst to load up to 40 samples in the system. The samples are injected and analyzed automatically. This reduces the man-hours needed to analyze a large number of samples. However, for the relatively small number of samples in the present study preparation of mobile phase and checking the system suitablity entails a significant amount of time.

Given that the industrial hygiene personnel do not have a dedicated technician or instrumentation the

current method of analysis (HPLC) is probably the better of the two methods.

As methods of analysis both the uv-spec and HPLC methods show very good analytical recovery. The variabilty encountered in the HPLC recovery tests can probably be explained by the author's inexperience with the technique. The HPLC method can, therefore, be used as an analytical

method in evaluating exposure to ranitidine hydrochloride.

The limits of detection are also comparable for both methods of analysis.

PARTICLE SIZING METHOD

The SEM method of sizing bulk and airborne ranitidine has distinct advantages and disadvantages. The method allows one to differentially size ranitidine particles in the presence of other contaminants. Although effective, the method is also quite tedious, and it is very expensive.

However, if SEM is used in the future to size ranitidine the validation of the method is done. Optical analysis showed that the vacuum of the SEM specimen chamber did not alter the size distribution of bulk material.

The results of the sizing do illustrate an interesting point. The size distribution of bulk active is markedly different from that found in air samples taken in the Fette room. The bulk material shows a count median diameter of 4.60um. The count median diameter of airborne active is between 10.14 and 11.32um. This difference is attributed to agglomeration.

Figure 7.1 is a photomicrograph of ranitidine taken from Fette room air. Note the presence of agglomeration. Because the Fette machine operates by compressing tablets, or agglomerating material, the presence of agglomeration is not surprizing. This data suggests that the particle size distributions of each unit process

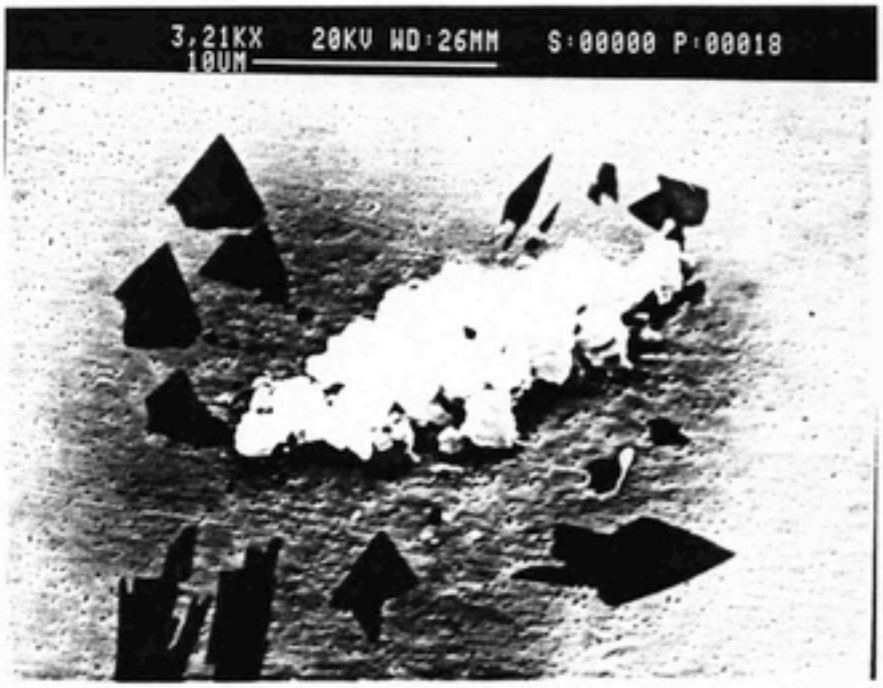



Figure 7.1: Photomicrogragh of Ranitidine HCl. Note the agglomeration. Magnification: 3,210X

within the suite may be very characteristic of the particular operation.

The comparison of particle sizes taken at 2 and 4 LPM in the Fette area is encouraging. Because no signficant difference in air concentrations was found when considering only filter masses of active no difference in particle sizes was expected. In fact, none was found. The geometric mean for the samples taken at 2 LPM was 10.14um. The geometric mean at 4 LPM was 11.32um. The geometric standard deviations did vary a bit: 2.11 at 2 LPM and 1.92 at 4LPM. This resulted in the mass median diameters being different: 55.7um at 2 LPM and 40.9um at 4 LPM. The difference in GSD is explained by the low number of particles measured. However, a two sided t-test showed no significant difference in the medians of the two distributions at the 90% level. This indicates that no size bias exists over the flowrate range of 2 to 4 LPM.

As is mentioned in the results a Kolmogorov-Smirnov test did reveal a sigificant difference in the shape of the distributions found at 2 and 4 LPM. However, when the distributions are plotted on log-probability paper they are very similar.

Although the sizing results do support the contention that no bias exists in the method in the

flowrate range of 2 to 4 LPM it should be noted that a possible bias exists in the method of collection for size analysis. It has been shown that Nucleopore filters carry a high charge when sampling (17). Because of this it is possible that some particles which carry a charge were differentially attracted to the surface of the filter or repelled to the wall of the cassette. It is assumed that samples run at 2 and 4 LPM experienced the same charging on the filters. Thus, any difference in size distribution would be due to sampling bias present also during sampling with non-charging glass fiber filters (17).

The results of the bias experiment were excellent. They indicate that no bias exists between cassettes sampling at 2 and 4 LPM. This allows Glaxo to choose a flowrate in this range and be confident that the concentrations obtained are consistent with those obtained at other flows within this range.

Currently, the personal exposure limit to ranitidine is 50ug/$M^3$. Likewise, the ceiling limit is 50ug/$M^3$. The analytical method used by Glaxo has a limit of detection of 1 ug of active per filter. Because ranitidine is present in suite air at low concentrations a large quantity of air must be drawn in order to assess the 15 minute ceiling limit. The Alpha-one pumps used by Glaxo have an upper flowrate range of 5 LPM. However, the pump may not be able to sustain this flow for more than a few minutes (this was not experimentally determined). It will, however, sustain a flow of 4 lpm. During sampling for ceiling exposure a flow greater than 2 LPM is needed to accumulate enough active to detect.

During shift sampling (10 hours) a more reasonable, and widely used, flow is 2 LPM. The present study confirms that samples taken at a high flow and samples taken at a low flow are comparable. That is, they sample with the same degree of accuracy. If it is found that sampling at 4 LPM does not provide enough active for

analysis two suggestions are made. It may be possible for Glaxo's chemists to revise the HPLC method so that the limit of detection is lowered enough to detect active well below 1ug per filter. Another suggestion is that Glaxo purchase a high volume sampler. Gilian Instrument Corporation (Wayne, N.J.) markets the Aircon 520 series pump. This pump draws between 2 and 20 LPM samples through 37mm cassettes. It should be noted that the current literature does not validate the use of the 37mm cassette at such high flowrates. A paired analysis with a lower flow pump such as is completed in this study could evaluate bias at such high flowrates.

LIMITATIONS

The accuracy of the method was not evaluated. The evaluation of accuracy is typically done in an aerosol chamber. It is difficult, if not impossible, to mimic field conditions in a chamber. Factors such as particle size, electrostatics and cross winds are not controlled easily. The accuracy of the open face 37-mm cassette sampling in calm air has been characterized (1,8). It is also noted that the use of this method to sample total dust is well established in industrial hygiene. As a practical matter it is unlikely that one could question Glaxo's use of this method. Indeed, it is the method of collection used by compliance agencies to sample total dust.

Because of logistical problems personal sampling was not possible during this study. As a result one must consider the application of the method to personal sampling with caution. The area samples taken in the suite were taken from still air. It may be possible that persons wearing the cassettes would produce air movement by their walking that would violate still air conditions. However, currently the workers in the suite are in full containment air suits. Personal sampling would most likely be done in the suit. Therefore, air currents due to walking are probably negligible.

Perhaps the most surprising and important discovery in the study is that a significant anount of ranitidine is depositing on the wall of the cassette during sampling.

Recall that in the Fette room the average ratio of the percentage contribution made by the wall deposits to the total mass found within each pair is 1.9. In the dispensing area this value is 0.97. The deposition of Ranitidine on the wall of the cassette can be eplained by sedimentation, inertial and electrostatic effects.

SEDIMENTATION EFFECTS

The particle size analysis of air samples in the Fette room revealed a count median diameters of 10.14 and 11.32um. The mass median diameters are significantly larger: 55um at 2 LPM and 41um at 4 LPM. It may be that some settling of particles onto the wall of the cassette is occurring during sampling. This explanation is plausible for large particles. Consider a 50um particle being sampled by a cassette at 4 LPM. If we assume that the velocity of the particle is equal to the velocity of the sampler air stream it takes 0.36 second for the particle to travel from the plane of the inlet to the filter. The settling velocity of the particle is calculated to be 5.5 cm/sec.

If we consider particles that are already close to the wall (remember, the orientation of the samplers is horizontal) say, one half the distance to the wall (1.9 cm;see Figure 1.3) it takes this 50um particle 0.29 second to fall to the wall of the cassette.

Clearly, this mechanism does not account for all wall deposition. Particles smaller than this 50um particle will probably not reside in the cassette long enough to settle out. In this case it is more plausible that impaction is taking place.

IMPACTION EFFECTS

In Figure 1.3 it can be seen that the 37mm cassette contains a "lip", or abrupt constriction. The cassette diameter changes from 38.1mm to 31.8mm. It may be that some particles possess too much inertia to follow air stream lines around this lip. The result is that particles impact on the wall of the cassette.

As was discussed earlier in this paper Fairchild et al observed significant wall deposition in his study of the 37mm open face cassette. Moreover, he observed that wall deposition increased with increasing particle size. This suggests impaction. Large particles would possess more inertia than small particles and would, therefore, impact more frequently.

The count median diameters found in this study indicate that both high and low flow cassettes had similar particles impacting on the filters. Because of this one may tend to expect that the size and amount of particles found on the wall would also be similar (assuming that the cassettes sampled a similar aerosol). If particles possess more inertia at higher velocities then one might conversly expect more deposition in the cassettes taken at high flow. Although the deposition in the dispensing area was equal between flows it was not equal in the Fette room (more was seen at low flow) It may well be that impaction accounts for only part of the deposition. Electrostatic effects may play an important role.

A POSSIBLE ELECTROSTATIC MECHANISM

Several researchers have investigated the effects of electrostatics on aspiration efficiencies of airborne dust samplers (2,14,17,). These studies indicate that when a charged aerosol is sampled with an inlet constructed of good insulator, such as the polystyrene plastic used in field sampling cassettes, a reduction in efficiency can occur.

In the Fette room conditions are suitable for aerosol charging. The relative humidity is low. This prevents the build-up of moisture on particles. The moisture acts as a conductor of electrons and prevents the build-up of charge on the particle. In the absence of moisture a particle may build a charge until its saturation point is reached. At this point the particle has a net positive or negative charge.

The Fette operation itself may act to produce charged particles. As the machine compressess material to form tablets it is possible that shear forces act to cause tribo-electrification. Tribo-electrification imparts a static charge to particles when they come into contact with other particles. This form of charging is common (22). Moreover, when tablets are ejected from the machine they are in very close contact with each other. This may produce additional charging.

Polystyrene cassettes can develop electrostatic fields. It has been shown that plastic cassttes can carry a charge of -300 volts/cm upon being taken out of the manufacturer's box (14). The combination of a highly charged aerosol and charged cassette has been shown to affect sampling (14). It may be that positivly charged Ranitidine particles are being attracted to the wall of the cassette that is negatively charged.

Liu et al (17) offer a possible mechanism for electrostatic deposition to the wall of the cassette. If one considers the change in number concentration (dN) of Ranitidine through a short length of tube (dL) , or cassette, the deposition ot charged particles on the walls because of surface electric field, E, can be written as:

$$Q\ dN = -EZN*pi*D_t\ dL \qquad (15)$$

where Q is the flowrate, Z is the particle electical mobility, N is the concentration of particles and $D_t$ is the diffusion constant. Integration of the equation yields an expression thatdescribes the aerosol penetration and its relation to the electric field of the cassette:

$$P = \exp(-AZE/Q) \qquad (16)$$

where A is the surface area of the inside of the cassette and P is the penetration of the aerosol. The fraction

that deposits on the wall of the cassette is expressed by:

$$1-P \text{ or } 1- \exp(-AZE/Q) \quad (17)$$

If we assume that the electrostatic conditions are the same for low and high flows then it can be seen that the fraction of Ranitidine that deposits on the wall increases as flowrate decreases. This is a result of the increased residence time for a particle in the electric field of the cassette. In fact, within pairs, more ranitidine was found on low flow cassettes in the Fette room.

However, in the dispensing area this phenomenon was not observed. Assuming that the process of dispensing does not involve charging aerosol, impaction mechanisms may play the dominant role in the dispensing area.

It should be noted that although much ranitidine was found on the walls of the cassettes the author's ability to recover active from the wall quantitativly is not established. Therefore, any conclusions or speculation based on this information must be considered with caution.

Although much of the preceeding discussion is speculative the deposition of ranitidine is clearly a fact. It is apparent that very complex mechanisms account for the deposition. Many of these possible

mechanisms of error in sampling have been considered individually. Currently, there exists no comprehensive analytic treatment of the sampling errors obtained when using the 37mm cassette in the field. A complete description should entail estimates of settling, impaction and possible electrostatic errors.

# CHAPTER EIGHT: CONCLUSIONS AND RECOMMENDATIONS

dusts (which do not contain chlorine) has been developed using Scanning Electron Microscopy and X-ray microanalysis.

6. Bulk ranitidine has been sized by optical and electron microscopy and is found to have a count median diameter of 4.60um by SEM and 4.25um by the optical method.

7. Airborne ranitidine was sized and found to have a geometric mean of 10.14um at a flow of 2 LPM and 11.32um at 4 LPM.

## RECOMMENDATIONS FOR FUTURE WORK

1. A comprehensive particle size analysis should be completed at each unit process in the suite. This would include using impactors (personal and area) and optical/ electron microscopy. This may allow further insight into possible mechanisms of deposition on the wall of the cassette.

2. A method should be found to evaluate the accuracy of the 37mm cassette for sampling Ranitidine. Frequently, such studies are conducted in wind tunnels and aerosol chambers. Caution is needed in such studies. This study suggests that factors such as particle size and electrostatic effects may play an important role in the sampling of Ranitidine. Experiments in the laboratory may not reproduce the environmental conditions which effect particle size and electrostatic fields.

3. A study could be designed to evaluate possible electrostatic effects. Measurement of electric fields generated by the cassettes could be made using an Electrostatic Fieldmeter (14). Additionally, paired samples may be taken with half being coated with an anti-static agent and half being untreated. A comparison of the samples may indicate the presence of an electrostatic field problem.

4. Finally, a study must be performed that accurately describes the ability of Glaxo to recover ranitidine from the wall of the cassette.

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APPENDIX I

The Spectrophotometer is available in both a Standard and a High Performance version. Performance specifications apply to both except as noted below.

Lambda 4C is operated by one of the Perkin-Elmer Series 7000 Professional Computers equipped with a hard disk, color graphics, an IEEE interface, and one of the three Lambda 4C Software packages. Each software package includes two parts: an INSTRMT part for spectrophotometer setup and data collection, and a CUV-3 part for post-acquisition data processing. The three software operating packages differ in the number of CUV-3 functions they provide.

# SPECIFICATIONS

| | |
|---|---|
| Principle | Double-beam, ratio recording, UV/Visible Spectrophotometer. Microcomputer control via a Perkin-Elmer Series 7000 Professional Computer. Compatible with various printers and plotters with an RS232C interface. |
| Optics | All reflecting optics with holographic grating monochromator. High Performance version includes pre-monochromator. |
| Wavelength Range | 190-900 nm |
| Stray Light | <0.02% at 220, 340, 370 nm. Measured at 2 nm SWB and Response of 5.<br><br><0.0005% at 220, 340, 370 nm with High Performance version. |
| Wavelength Accuracy | ±0.3 nm |
| Wavelength Repeatability | ±0.1 nm |
| Spectral Bandwidth | 0.25, 1, 2, 4 nm at 656.1 nm selected via computer. |
| Photometric Range | -2.000 to 4.000A. |

**SPECIFICATIONS (cont):**

| | |
|---|---|
| Photometric Accuracy | ± 0.005A at 1A measured with NBS 930 filters. |
| Photometric Repeatability | ± 0.001A at 1A measured with NBS 930 filters. |
| Zero Stability (Drift) | <0.0005A/hr. after warm up at 340 nm, 4 nm SBW, 6 response time. |
| Noise | <0.0003A at 500 nm, 0 A, 4 nm SBW, 6 response time. |
| Baseline Flatness | ± 0.002A, 120 nm/min., 4 nm SBW, 5 response time, from 200-850 nm after Background Correction. |
| Response | All modes of operation include "soft" key selection of response. The magnitude of response is selected by "soft" keys labeled 1-7 with 1 having the least amount of filtering and 7 having the maximum filtering. |
| Lamp | Automatic source change. Manual selection of UV lamp. |
| Ordinate Scale Expansion | -2.000 to 4.000 A in 0.001A increments<br>0 to 200%T in 0.1%T increments<br>0.000 to 9999 in concentration. |
| Menu Items | Scan, Time Drive, Concentration. Accessory Software programs allow for additional methods. |
| Scan Speed | 5, 20, 60, 100, 300, 480, 750 and x2 (multiplier) nm/min. |
| Dimensions of Optical Unit | Width: 65 cm (25 1/2 inches)<br>Depth: 56 cm (22 inches)<br>Height 20.5 cm (8 inches) |
| Weight of Optical Unit | 34 kg (75 pounds) |

APPENDIX II

Bartlett's test for homogeneity of CVs is applied in order to test the feasibility of "pooling the coefficients of variation" for any set of 18 generated samples (i.e., 6 at each of the 0.5, 1, and 2 times OSHA standard level). The following equation for chi-squared, with two degrees of freedom, was used:

$$\text{chi-squared} = \frac{f \ln (\overline{CV}_2)^2 - \sum_{i=1}^{3} f_i \ln (\overline{CV}_{2i})^2}{1 + \frac{1}{3(3-1)} \left[ \left( \sum_{i=1}^{3} \frac{1}{f_i} \right) - \frac{1}{f} \right]}$$



where:

$\overline{CV}_2$ = pooled coefficient of variation of 18 generated samples

$\overline{CV}_{2i}$ = coefficient of variation of six generated samples at the $i^{th}$ level

$f_i$ = degrees of freedom associated with $(\overline{CV}_{2i})^2$ and equal to number of observations at the $i^{th}$ level minus one.

$f = \sum_{i=1}^{3} f_i$

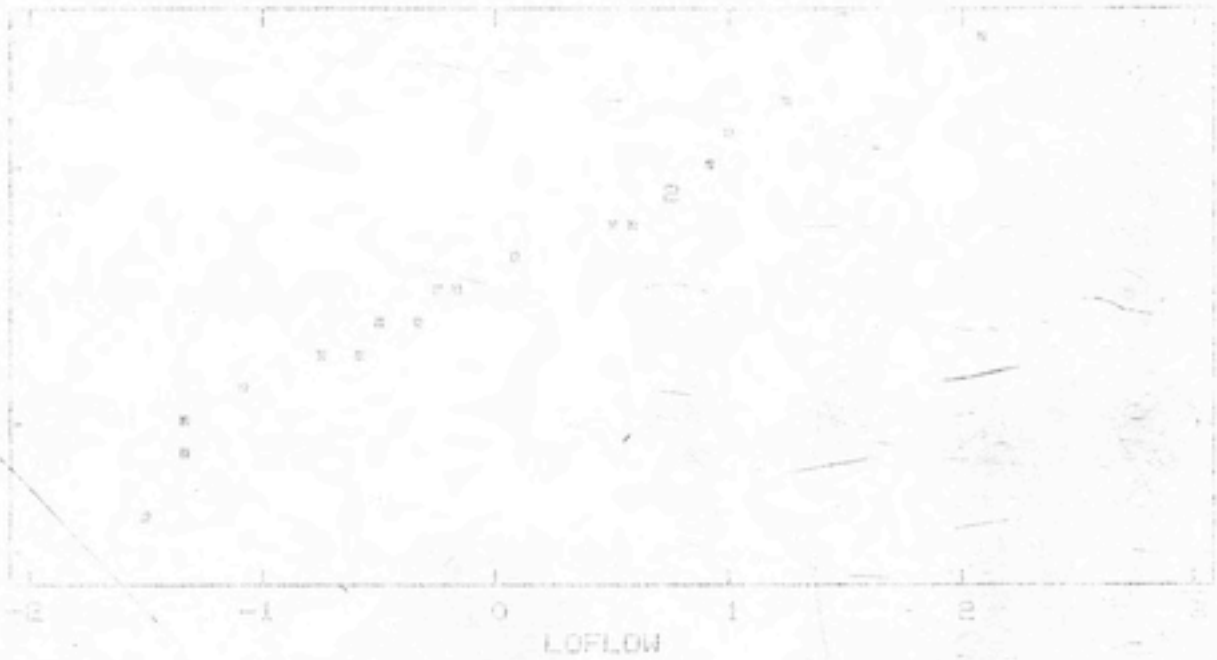
To pass Bartlett's test at the 1% significance level, chi-squared must be less than or equal to 9.21 (chi-squared has two degrees of freedom).

APPENDIX III

NORMAL PROBABILITY PLOT, N = 19

EXPECTED VALUE

NORMAL PROBABILITY PLOTS N = 19




NORMAL PROBABILITY PLOT, N=
EXPECTED
VALUE
-2
-1
0
1
2
STANDARDIZED


EXPECTED VALUE
NORMAL PROBABILITY PLOT, N = 10
2
1
0
-1
-2
-3
-2
-1
0
1
2
FH/FLOW