ABSTRACT

LING-KWEI TSENG. Optimization of the Uranine Wash-Off Method for Measuring Aerosol Concentrations. (Under the Direction of Dr. Russell W. Wiener)

In the fluorescence-washing technique, oleic acid particles tagged with uranine are washed out and analyzed fluorometrically. The possible sources of errors in the technique are evaluated in this study. First, the sensitivity of uranine fluorescence in different solutions is compared. The results indicate that uranine in distilled water with pH 10 buffer and in sodium hydroxide have high readings. Second, the interference of oleic acid in uranine solutions is investigated. The results indicate that there is no interference of oleic acid in distilled water and sodium hydroxide under our operating conditions. However, there is a significant quenching effect of oleic acid in ethanol. Third, the extraction ability of different solutions from glass fiber and Teflon filters is tested. The results indicate that distilled water and sodium hydroxide have high extraction ability. Fourth, based on the results above, distilled water is the best washing solution for inlet washing. Fifth, two commercial samplers, the Portable Indoor Particulate Samplers (PIPS) and Saturation monitors, have been calibrated in the test chamber. Sixth, some potential errors generated using this fluorescence-washing technique in practice are discussed. Seventh, a set of optimal operating conditions and a standard operating protocol are proposed.

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I. INTRODUCTION

Oleic acid particles, tagged with uranine produced from a vibrating orifice monodisperse aerosol generator (VOMAG), have been used as the basic aerosol standard for a long time (Willeke 1975, Tufto and Willeke 1982, Liu et al. 1984, Okazaki et al. 1987, Wiener 1987, Marple et al. 1987 and 1989, Wang and John 1988, VanOsdell et al. 1990, and Martinez et al. 1990). This method provides aerosols of high monodispersity and accurately known sizes. In this procedure, particles deposit on the collecting surface; then, they are washed out and analyzed fluorometrically. This fluorescence-washing technique provides a simple, rapid, inexpensive, and highly sensitive method to determine aerosol quantity. However, no investigator has thoroughly considered the sources of possible errors in the fluorescence-washing technique in detail and established an optimal operating procedure.

The purpose of this study is to evaluate the sources of possible errors in the fluorescence-washing technique and try to provide a set of optimal operating conditions which could increase the sensitivity and lower the errors of this method.

There are two important factors determining the sensitivity of uranine fluorescence in solutions. These factors are types of solutions and pH values. Distilled water, sodium hydroxide, and ethanol are the most frequent solutions used in fluorescence-washing technique, but the sensitivity and linear response of uranine fluorescence in these solutions were not checked in detail by any investigators. Moreover, pH value in uranine solutions plays an important role in the uranine fluorescence and should be investigated. In the sensitivity test section, the fluorescence intensity of uranine and linear relationship in distilled water, sodium hydroxide, and ethanol is compared. In addition the influence of pH value in uranine solutions is described.

When uranine is washed out from collecting surfaces, oleic acid also exists in the washing solutions. It is necessary to know if the oleic acid will produce interference in the uranine solutions. In the interference test section, the possible interference of oleic acid in uranine and distilled water, sodium hydroxide, and ethanol solutions is tested by using both the filter fluorometer and scanning fluorometer.

Filters are the most frequent surface used in collecting uranine and oleic acid particles. There exist two potential problems in filter wash. First, filters themselves probably will produce high background in washing

solutions which will bias the actual reading of uranine fluorescence. Second, these washing solutions probably could not extract all the uranine on the filters when uranine is covered by oleic acid. In the filter extraction test section, the background of glass fiber filters and Teflon filters in distilled water, sodium hydroxide, and ethanol is compared. Moreover, the extraction ability of distilled water, sodium hydroxide, and ethanol to oleic acid tagged with uranine on glass fiber filters and Teflon filters is compared.

Like filter wash, there exist two similar potential problems in the inlet wash. First, inlets could be made of any materials, and they may react with washing solutions which will produce high background reading. Second, these washing solutions may not extract all the uranine on the inlets when uranine is covered by oleic acid. In the inlet wash inference section, the best washing solution for inlet wash is proposed based on the results in the sensitivity test, interference test, and filter extraction test sections.

In the Portable Indoor Particulate Sampler (PIPS, MSP Corp., Minneapolis, MN) and Saturation Monitor (Pro-2, Lane Regional Air Pollution Authority, Springfield, OR) section, two commercial samplers, PIPS and Saturation Monitors, are calibrated in the test chamber by using the fluorescence-washing technique derived above. Oleic acid particles

tagged with uranine produced from VOMAG are collected on filters, impaction plates, and inlets in PIPS and Saturation Monitors. Particle sizes are calculated from VOMAG equations. The collected particles are washed out by distilled water and analyzed in the fluorometer. Hence, collection efficiency curves of the PIPS and the Saturation Monitors are obtained. Some potential problems are discussed when we apply the fluorescence-washing technique to calibrate a real sampler.

The federal regulation (Federal Reference Method, 40 CFR, Part 53, 1987) requires VOMAG and fluorescence-washing technique to be used for PM 10 analysis in wind tunnel tests. In the final section, a set of optimal operating conditions and a standard operating protocol is proposed.

II. BACKGROUND AND LITERATURE REVIEW

II.A. Principles of Fluorescence

When a molecule absorbs radiation, its energy level is increased. If part of this energy is converted to vibrational energy, the remainder, if radiated within 10^{-8} seconds, is emitted as light of lower energy (longer wavelength) than the absorbed energy. This property is called fluorescence. The shape of the excitation spectrum is that of the absorbance curve of the molecule. If the exciting light used is of a wavelength which is different from that of the absorption peak, a smaller portion of the light will be absorbed and proportionately less light will be emitted. However, the shape and location of the emission spectrum will not change. The fluorescence reading of a fluorometer is proportional to the concentration of the fluorescing molecule and the intensity of the exciting wavelength (Sequoia-Turner Corp., 755 Ravendale Drive, Mountain View, CA 94043).

Interference is a phenomenon where the real fluorescence intensity is increased or decreased by a molecule or some solvent present in the test alliquot. The

interference is called quenching in fluorometry if fluorescence intensity is decreased. There are two types of quenching, collisional quenching and static quenching. Collisional quenching involves a diffusion controlled interaction between an excited molecule and some quencher. Static quenching results from a complex formation between a potentially fluorescent molecule in the ground state and a quencher (Perkin-Elmer Corp., 1979).

Fluorescence is measured by fluorometers. Generally there are two types of fluorometers, filter fluorometers and spectrofluorometers. The major advantage of the filter fluorometer is that it permits a greater quantity of light to strike the sample which is desirable for trace analysis. The lack of selectivity of filters, in so far as obtaining a narrow wavelength is concerned, is their major disadvantage. On the contrary, a fluorescence intensity can be obtained at a specific excitation and an emission wavelength in a spectrofluorometer.

II.B. Uranine; Tracer

A tracer used in aerosol experiments should meet the requirements including low cost, nontoxicity, rapid analysis, and high sensitivity. Uranine meets all the requirements and has been used as a tracer since 1959 when Robinson et al. developed a meteorological tracer technique

using uranine dye. Since uranine is essentially insoluble in liquid particles, it exists within liquid particles as a precipitate (Liu and Agarwal 1974).

Uranine (Fluorescein Sodium) has molecular formula $C_{20}H_{10}Na_2O_5$ and molecular weight 376.27. It is a hydroscopic orange-red powder and freely soluble in water (Windholz, 1983). Uranine and water solutions form a deep-red color at a concentration of 10 percent changing to yellow-green in more dilute concentrations. Uranine and water solutions absorb blue light between the wavelengths of 440 and 520 nm and emit a brilliant yellow fluorescence between 510 and 590 nm (Robinson et al., 1959). Schulz et al. (1960) used a Photovolt Meter (Model 520M) to get a linear response between 0.1 µg/ml and 0.01 µg/ml and nonlinear response below 0.01 µg/ml in uranine and water solution. Burgess et al. (1961) used the same instrument with proper selection of light source and filters and detected concentration down to 0.0001 µg/ml in uranine and water solution. He was able to extend the linear relationship between 0.001 µg/ml and 2 µg/ml. Drabent et al. (1964) and Pant (1968) concluded that uranine aqueous solutions under 10 µg/ml have both the maximum intensity of absorption and emission when pH value is above 10 because only bivalent negative ions exist. However, the fluorescence is dependent on pH value when pH value is less than 10. The maximum excitation and emission wavelengths are 495 nm and 530 nm (Pant, 1968).

II.C. Fluorescence-Washing Technique

Since Berglund and Liu devised the vibrating orifice monodisperse aerosol generator (VOMAG) in 1973, a lot of different liquid particle solutions and washing solutions have been used to measure aerosol concentration by fluorometry. Liu and Agarwal (1974) observed aerosol deposition in turbulent flow by using olive oil liquid particles tagged with uranine. The solution of liquid particles is composed of olive oil, uranine, isopropanol, and distilled water. The particles are deposited on filters and glass pipes and then are washed out by distilled water. Turner and Hill (1975) used diotylphthalate (DOP) liquid tagged with uranine to calibrate an Anderson two-stage biological sampler. The solution of liquid particles is composed of DOP, uranine, and ethanol. The particles deposited on the glass fiber and aluminum foil and are washed out by ethanol. Three drops of 0.1 N sodium hydroxide were added to each cuvette containing washing solutions to adjust the pH and enhance fluorescence. Willeke (1975) found the characteristic of the slotted impactor by using oleic acid tagged with uranine. The solution used to generate liquid particles is composed of oleic acid, uranine, and isopropanol. The particles are collected on the surface of glass fiber filter and aluminum tape and then are extracted by distilled water. The pH

level of the washing solutions was stabilized at about 10.5 by adding three drops of buffer to each sample. These three papers presented above are the first publications which described the fluorescence-washing technique in detail.

Liu and Pui (1981) tested a new inlet by using DOP particles tagged with fluorescein, but Liu et al. (1984) assessed power air purifying respirators by using oleic acid particles tagged with uranine. The washing solutions which they used were 0.01 N sodium hydroxide. Tufto and Willeke (1982), Okazaki et al. (1987), Okazaki et al. (1987), and Wiener (1987) still used the same technique developed by Willeke (1975). They used distilled water to wash the deposited uranine out of the interior of inlets and added pH 10 Buffer to get the transmission efficiency. Chen et al. (1985 and 1988) used DOP liquid particles tagged with uranine to calibrate a virtual impactor and to test an aerosol generator connecting two virtual impactors in series. They selected pure isopropanol as washing solvent. In calibrating an impactor, Marple et al. (1987) also used the same technique developed by Willeke (1975), but Marple et al. used 0.001 N sodium hydroxide instead of distilled water as washing solvent. Wang and John (1988) calibrated the Berner impactor by using oleic acid particles tagged with uranine. They used 50% distilled water and 50% isopropanol as washing solution to extract uranine deposited. In all the references above investigators used a

Turner Filter Fluorometer (Model 110, Sequoia-Turner Corp., Mountain View, CA) to detect fluorescence intensity. VanOsdell et al. (1990) calibrated Personal Environmental Monitors (PEM) and Microenvironmental Exposure Monitors (MEM) by using oleic acid particles tagged with uranine. The particle solution is composed of oleic acid, uranine, and ethanol. They used 0.01 N sodium hydroxide to wash uranine deposited on filters, impaction plates, and bodies of 10 μm MEM, 10 μm PEM, and 2.5 μm PEM, but pure ethanol to extract uranine deposited on 2.5 μm MEM to prevent high background readings. An SLM Aminco Fluoro-colorimeter II (SLM Instruments Inc., Urbana, IL) was used in their experiments. Martinez et al. (1990) also used the same technique developed by Willeke (1975) to evaluate some biological samplers, including Andersen single-stage and two-stage cascade impactors, surface air sampler (SAS), and biotest reuter centrifugal. A Perkin-Elmer Spectrofluorometer (Model 650-40, Perkin-Elmer Corp., Nowalk, CT) was used in their experiments.

The federal regulation (Federal Reference Method, 40 CFR, Part 53, 1987) requires VOMAG and fluorescence-washing technique to be used for PM 10 analysis in wind tunnel tests. Monodisperse liquid particles of oleic acid tagged with uranine should be used.

Based on all these studies it is clear that a complete assessment of the uranine methodology is needed given the

many different techniques that are used to implement the method.

III. EXPERIMENTAL APPARATUS

III.A. Fluorometer

III.A.1. General Description

There are two types of fluorometers, filter fluorometers and spectrofluorometers. Filter fluorometers, such as the Aminco Fluoro-Colorimeter and Turner Filter Fluorometer, use a primary filter to select the desired excitation wavelength from the lamp source and a secondary filter that passes the sample fluorescence, but not the excitation wavelength, to the detector. A spectrofluorometer uses an excitation monochromator and an emission monochromator instead of a primary filter and a secondary filter. Both a filter fluorometer and a spectrofluorometer are designed with the detector perpendicular to the lamp beam. This configuration allows the analyst to adjust the background reading (blank) to zero.

The lamp source is usually a mercury or a xenon arc. The sample solution is exposed in a cell made of glass or quartz to the exciting radiation. The detector used is usually a high gain photomultiplier. The output of the

detector is displayed on the filter fluorometer by a meter. Spectrofluorometers use recorders.

III.A.2. SLM Aminco Fluoro-colorimeter II

The borosilicate cuvettes (Disposable Culture Tubes, Cat. No. 60825-538, VWR Scientific Inc., San Francisco, CA) are placed in a SLM Aminco Fluoro-colorimeter II (SLM Instruments Inc., Urbana, IL). This fluorometer is used for all experiments performed. The fluorometer operates by passing an ultra violet light through the primary filter. The UG-1 primary filter (bandpass) passes maximum excitation at 360 nm, but passes less than 1% transmission at 300 nm and from 420 to 670 nm. The KV 418 secondary filter (sharp cutoff) transmits light over 405 nm and has 50% transmission at 418 nm. Uranine is activated between 425 and 525 nm and emits between 475 and 650 nm. A photodetector can be used between 300 and 650 nm. The photomultiplier is set at 550 V and the fluorometer should be allowed to warm up for at least 30 minutes before use.

III.A.3. Perkin-Elmer Spectrofluorometer

A scanning spectrofluorometer (Model 650-40, Perkin-Elmer Corp., Norwalk, CT) has been used to measure the emission spectrum of uranine in distilled water, sodium

hydroxide, and ethanol and to measure possible interferences. The spectrofluorometer uses a Xenon lamp as the light source. Both the excitation and emission monochromators can pass the light in the range of 220 to 830 nm. The detector is a R928 photomultiplier detector used form 220 to 830 nm wavelength. The scanning spectrofluorometer has a pre-scan function with a speed of 960 nm/min. The spectrofluorometer should be allowed to warm up for at least 30 minutes before use.

III.B. Aerosol Generator

III.B.1. General Description

In Section IV.D, two commercial samplers, Portable Indoor Particulate Samplers (PIPS) and Saturation Monitors, are calibrated in the test chamber. Aerosol particles are generated using a vibrating orifice monodisperse aerosol generator (VOMAG, Model 3050, TSI Inc., St. Paul, MN). A solvent of high volatility containing a solute of low volatility is injected by a pressurized liquid feed into a tested air stream. A vibrating piezoelectric ceramic ring imparts a distorting frequency that helps cause the solution to shear into small equally sized droplets. The piezoelectric crystal is oscillated by a sine wave generated by a signal generator (Model 3010 Function Generator, B & K

precision, Chicago, IL) and is measured by a frequency counter (Model SM-2410, Heath Zenith Inc.,).

The solvent in the droplets evaporates so that a smaller liquid or solid (depending upon the solute being used) particle remains. This study uses ethanol (AAPER Alcohol and Chemical Co., Shelbyville, KY) as the solvent and oleic acid (Cat. No. A 195, Fisher Scientific Co., Fair Lawn, NJ) tagged with uranine (Sodium Fluorescein, Cat. No. A-833, Fisher Scientific Co., Pittsburgh, PA) for the solute. The resultant oleic acid and uranine particle is an oily liquid droplet.

Both dispersion and dilution air are adjusted by the metering valves and rotameters. The rotameter (Model RMB, Dwyer Instruments, Inc., Michigan City, IN) for dispersion air has a range of 0 to 2500 ml/min. The rotameter (Model RMB, Dwyer Instruments, Inc., Michigan City, IN) for dilution air has a range of 0 to 6 M^3/hr. Typical flow rates are 1500 ml/min for dispersion air and 6 M^3/hr for dilution air.

A high performance liquid chromatography (HPLC) programmable pump (Waters Model 590, Millipore Corporation, Milford, MA) replaced the syringe pump in the original generator system. This pump gives higher pressure delivery, maintains a constant flow, and permits operation for essentially as long a test as is desired. There is a filter (0.5 μm pore, 13 mm, No. FHLP 01300, Millipore Corporation,

Milford, MA) between the HPLC pump and the orifice assembly to prevent clogging during operation.

The VOMAG rests on top of a charge neutralizer (Model 3077, TSI Inc., St. Paul, MN). Droplets from the VOMAG are then sent down through a particle charge neutralizer and dispersed into the air. The neutralizer consists of an aluminum cylinder, 10.2 cm in diameter and 30.5 cm in length, enclosing a Kr^{85} radioactive gas with a strength of 10 mCi. The neutralizer, by removing the electrostatic charges on the droplets, helps prevent agglomeration of the aerosol and also inhibits electrical precipitation.

III.B.2 Solute, Solvent, and Washing Solution

III.B.2.a. Solute; Liquid

A solute could be a liquid or a solid. Liquid particles are commonly used above 1 μm because they do not bounce, and they are highly monodisperse, uniform, and spherical. A suitable liquid used for producing particles should have low volatility, nontoxicity, low cost, and no fluorescent or interference effect. Oleic acid and dioctylphthalate (DOP) have been the primary liquids used to create particles for a long time. Oleic acid is the only liquid used in our study. It has molecular formula $C_{18}H_{34}O_2$ and molecular weight 282.45. Oleic acid is a colorless or

nearly colorless liquid and practically insoluble in water, but soluble in alcohol. Oleic acid has a boiling point at 286°C (Windholz, 1983).

III.B.2.b. Solvent

A solvent used in VOMAG should have high volatility, low cost, and nontoxicity. The most important thing is that both liquid and tracer must be soluble in the solvent. Hence, ethanol and isopropanol are two primary choices. Ethanol is the only solvent in our study.

III.B.2.c. Washing Solution

Washing solutions play a very important role in the fluorescence-washing technique. They must have high extraction ability to wash uranine and liquid particles out of the collecting surface, but should not react with the surface to cause high background reading. Uranine in washing solutions should be stable and give a highly sensitive reading. Distilled water, sodium hydroxide, ethanol, and isopropanol are the most common washing solutions used. In the sensitivity test, interference test, and filter extraction test sections, distilled water, sodium hydroxide, and ethanol are used. In calibrating PIPS and Saturation Monitors, distilled water is the only washing

solution used.

III.B.3. Calculation of Particle Diameter

If the liquid feed rate, the disturbance frequency of VOMAG, and the ratio of uranine, oleic acid, and ethanol are specified, the resulting particle size can be calculated. The droplet diameter before vaporizing is:

$$D_d=[(6Q)/(\pi f)]^{1/3}*1000$$

where D_d is the droplet diameter before vaporizing in μm, Q is the liquid feed rate in ml/sec, and f is the disturbance frequency (Hz). However, uranine exists in the droplet as an impurity, so that the corrected diameter after vaporizing is:

$$D_{p,corrected}=(C+I)^{1/3}Dd$$

where $D_{p,corrected}$ is the corrected diameter in μm, C is the volumetric concentration of oleic acid in the oleic acid and ethanol solution, and I is the volumetric concentration of uranine in ethanol. Hence, the aerodynamic diameter of the resulting uranine and oleic acid particle can be calculated as:

$$D_a = (e_{avg})^{1/2} D_{p,corrected}$$

where D_a is the aerodynamic diameter in μm, and e_{avg} is the average density of uranine and oleic acid in solution in g/ml. Similarly, the optimal disturbance frequency can be calculated by reversing the calculation steps above if the desired aerodynamic diameter is specified.

III.C. Test Chamber

A cubical test chamber measuring 183 cm on each side was erected within the EPA Aerosol Test Facility wind tunnel. The walls of the wind tunnel formed the top, bottom, and two sides of the chamber. The section of the wind tunnel chosen included an entry door that was used to access the interior of the chamber. Temporary framing was used to form the other two sides of the chamber. In order to prevent any significant pressure difference between the chamber and its surroundings, a 61-cm square HEPA filter was installed in one wall to permit clean air exchange as required by the samplers. All seams were taped to prevent air entry except through the filter.

The test aerosol entered from the center of the chamber top. A 40 cm diameter fan was positioned 1.5 m directly below the aerosol entry point to provide mixing in the chamber. It was operated at 370 rpm for particles below 5

μm, and at 520 rpm for larger particles.

The samplers were positioned between 5 and 20 cm above the chamber floor at various points on a rough circle about 120 cm in diameter around the fan. Figure 1 and 2 give the overviews of the wind tunnel and the chamber layout.

III.D. Samplers

III.D.1. Portable Indoor Particulate Sampler (PIPS)

The particulate sampling system consists of three major assemblies, each contained in its own compartment: 1) the PIPS which contained an active size removal system in addition to the particle filter; 2) the Environmental Monitoring Services Incorporation (EMSI, Esotemic Systems Inc., Newbury Park, CA) sampling pump and integral motor; 3) the electronics for controlling the pump flow-rate and measuring elapsed time of pump operation.

The PIPS consists of three sections, each of which is made of aluminum and anodized: 1) an inlet-nozzle section, 2) an impactor plate, and 3) an exit section.

The aerosol-laden sample airstream enters through four holes and passes through each of ten nozzles located on the inlet section's upstream surface. The inlet section is available with one of two jet nozzle sizes, one with jets sized to give a 10 μm cut (gold-anodized), and the other

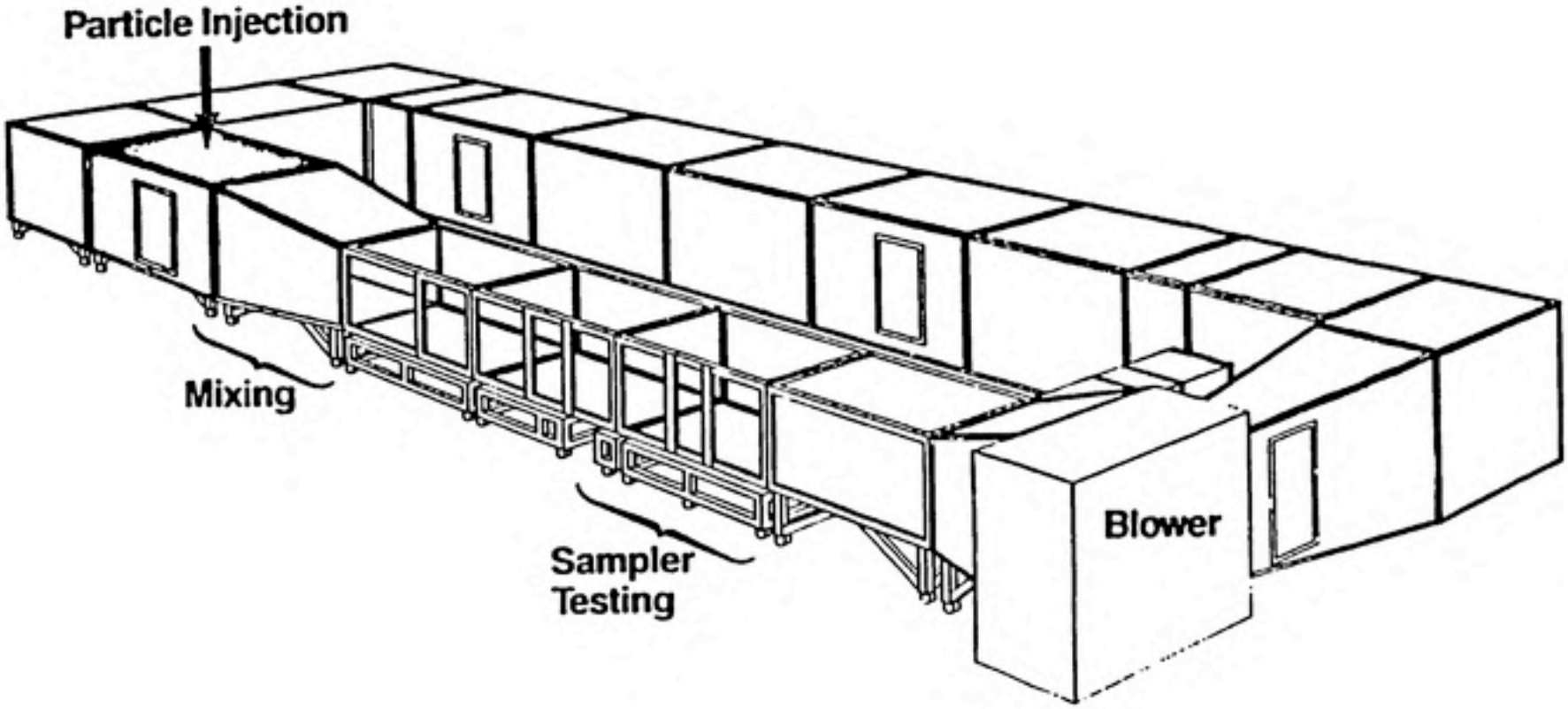

Figure 1. EPA Aerosol Test Facility

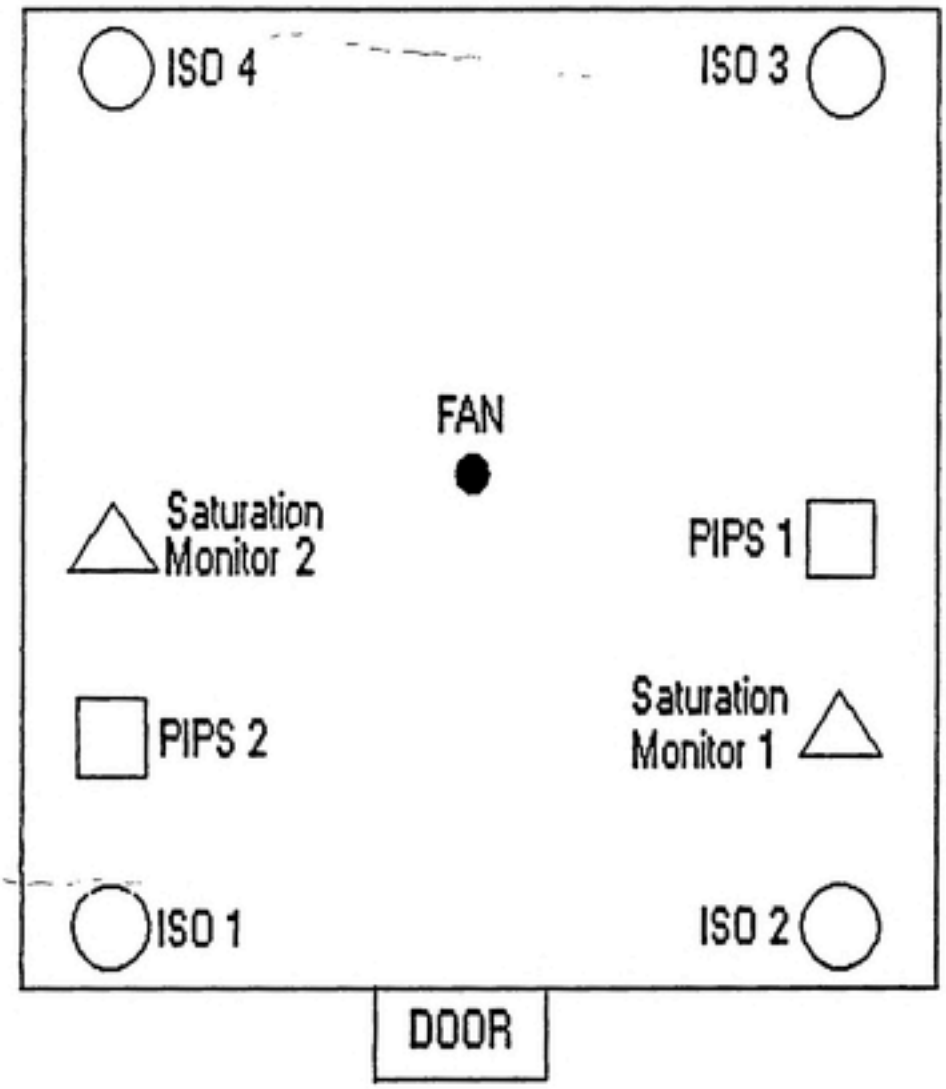

Figure 2.--Overview of the Chamber Layout

with jets sized for a 2.5 μm cut (silver anodized).

Figure 3 shows the PIPS assembly. Underneath the PIPS nozzle plate is the impactor plate which is fit deeply into the inlet suction. A Teflon filter (37 mm, Prod. R2PJ037, Gelman Scientific Inc., Ann Arbor, MI) in a round plastic frame is held directly under the impactor plate. Under the Teflon filter is a screen which rests on the bottom piece of the PIPS assembly. This bottom piece acts not only as the bottom support for the PIPS, but also as the exit plenum.

The impactor plate for the PIPS is a stainless-steel sintered annulus permanently mounted on an annular skirt. The pores of the sintered annulus are a nominal size of 10 μm. After passing through the nozzle impactor size selector, the sample airstream and remaining particles enter the hole in the annular impactor plate and then are deposited on the filter.

The sampling pump used with the PIPS is an EMSI pump. The pump and its associated flow control and elapsed time counting electronics are housed in a rigid, light alloy case. The noise level is extremely low.

III.D.2. Saturation Monitor

The Saturation Monitor is made of plastic. It can be subdivided into four major sections: 1) the inlet section; 2) the inertial impaction section; 3) the upstream section

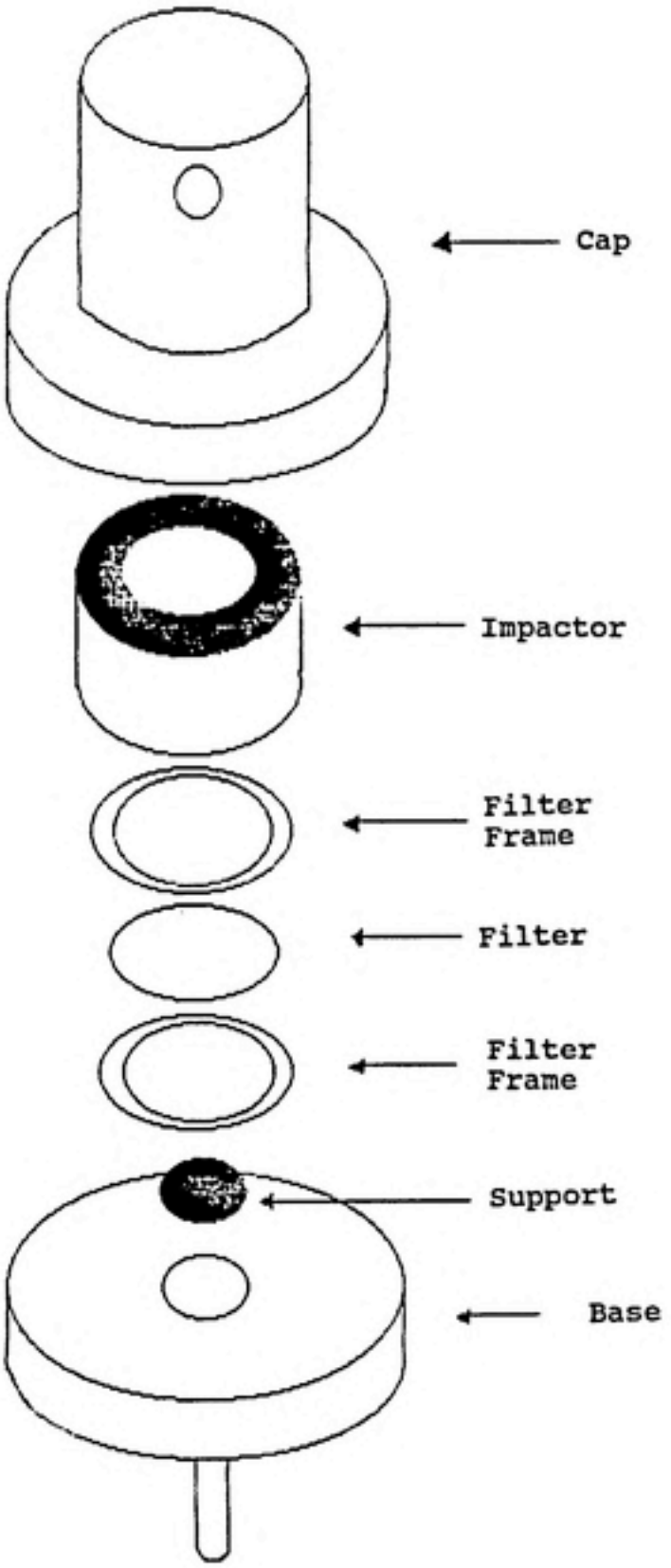

Figure 3. Portable Indoor Particulate Sampler (PIPS)

of the filter holder; and 4) the downstream section of the filter holder. Figure 4 shows the Saturation Monitor assembly.

The pump draws air through the inlet and then through the impactor. The inertial impactor section consists of two major components, a nozzle and an impaction plate. Inside the impactor, air is accelerated through a converging inlet and cylindrical throat. The sample airstream next encounters the impaction plate, a plastic disk with annular tracks. This plate is held by three slender cylinders. The space between the cylinders allows the sample airstream passage around the plate.

After passing through the impactor section, the sample airstream enters the upstream section of the filter holder. In addition to providing the upstream filter support, this section allows the airstream to redevelop fully after the flow disturbance caused by the impactor section and to deposit uniformly its particles on the filter.

A glass fiber filter (47mm, Type A/E, Gelman Scientific Inc., Ann Arbor, MI) is used as the particle collection medium. The downstream side of the filter is supported by a drain disk. An anti-twist ring is put over the filter to hold the filter in place.

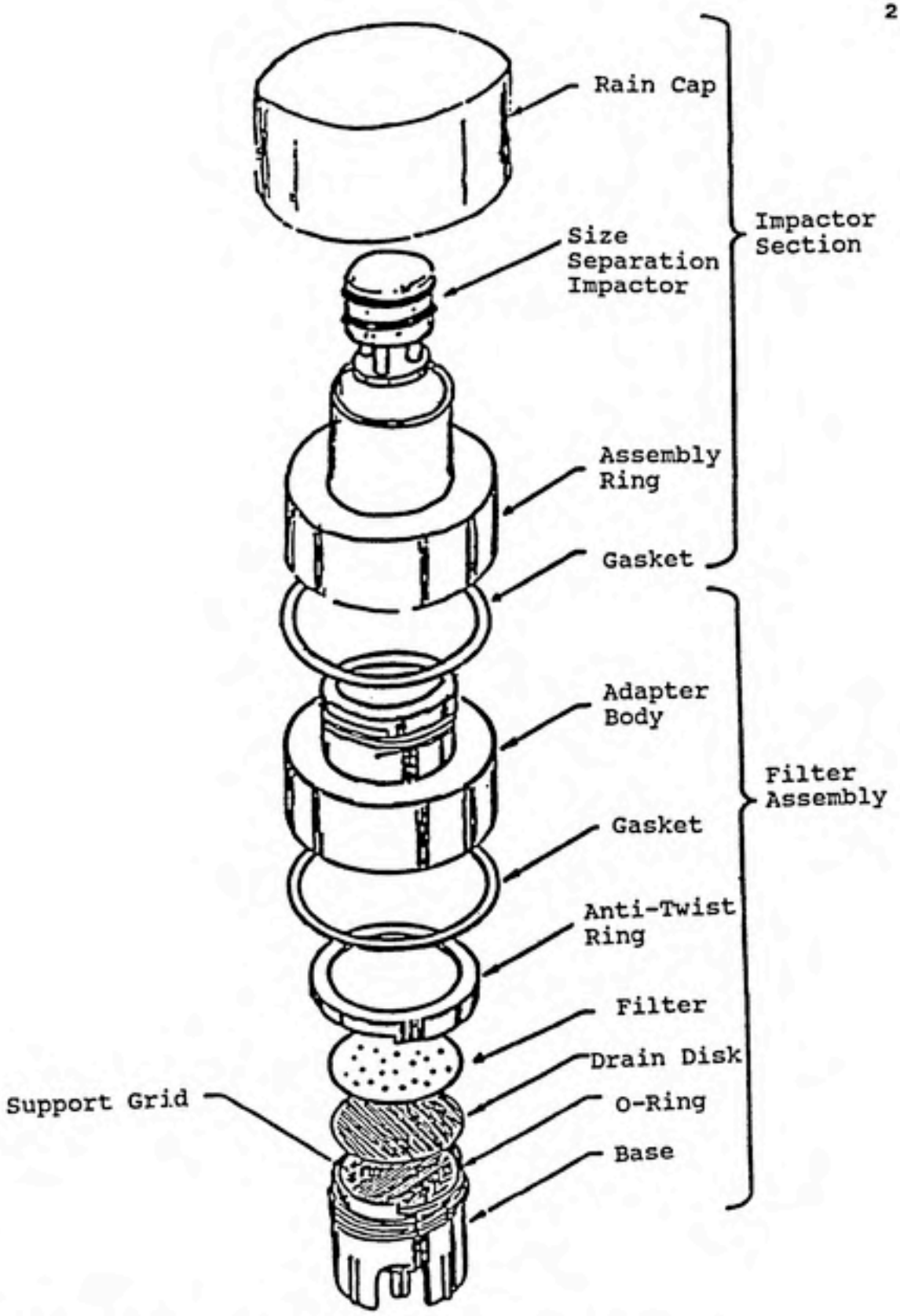

Figure 4. Saturation Monitor (From "Quality Assurance Project Plan for Portable PM-10 Sampling." (Unpublished), EPA Region 10, 1200 6th Ave., Seattle, WA 98101)

IV. METHODS AND PROCEDURES

IV.A. Sensitivity Test

IV.A.1. Test Summary

In the Calibration Curve Section, first the optimal amount of buffer in uranine and distilled water solution is determined. Then, the calibration curves of uranine in different solutions are obtained. In the Sensitivity Comparison Section, the same amount of uranine and distilled water solution is delivered into several different pH solutions and the sensitivity is compared. The following are lists of the step by step procedures used in performing these experiments.

IV.A.2. Calibration Curve

IV.A.2.a. Uranine and Distilled Water Solution

IV.A.2.a.i. PH Value Test

(1) Prepare 100 ml distilled water in eight different

flasks.

(2) Deliver 0.1, 0.2, 0.3, 0.4, 0.5, 0.8, 1, and 2 ml pH 10 buffer (Cat. No. SB116-1, Fisher Scientific Co., Pittsburgh, PA) into each 100 ml distilled water. Shake for 10 minutes.

(3) Analyze using pH meter (pH/Temp Meter, Model-6719, Jenco Electron, Co. LTD).

IV.A.2.a.ii. Determination of Amount of Buffer in Uranine and Distilled Water Solution

(1) Prepare 2, 1, 0.5, 0.2, 0.1, 0.05, 0.02, 0.01, and 0.005 µg/ml uranine and distilled water solutions.

(2) Pipet 3.3 ml each concentration solution into 6 different small cuvettes.

(3) Add 40, 50, 60, 75, and 100 µl pH 10 Buffer into five cuvettes separately. Leave one cuvette as blank. Keep these cuvettes in the dark for 15 minutes.

(4) Analyze all samples fluorometrically using the SLM Aminco Fluoro-Colorimeter II.

IV.A.2.b. Calibration Procedure

(1) Prepare uranine and distilled water stock solution 100 µg/ml. Dilute it to 10 µg/ml.

(2) Dilute 10 µg/ml to 2, 0.5, 0.1, 0.02, and 0.005 µg/ml.

Dilute 10 µg/ml to 1, 0.2, 0.05, 0.01 µg/ml. Every new solution is shaken for 5 minutes and then put into the ultrasonic bath (Ultrasonic cleaner, Model B-52, Branson Co., Shelton, CT) for 5 minutes.

(3) Pipet 3.3 ml solutions into small cuvettes.

(4) Add 50 µl pH 10 buffer into each cuvette separately. Leave these cuvettes in the dark for 15 minutes.

(5) Analyze all samples fluorometrically using the SLM Aminco Fluoro-Colorimeter II.

(6) Repeat steps (1), (2), (3) and (5) using 0.001 N and 0.01 N NaOH Solutions. Repeat steps (1) to (5) using ethanol.

IV.A.3. Sensitivity Comparison

IV.A.3.a. Sensitivity Test

(1) Prepare uranine and distilled water stock solutions 100 µg/ml and 10 µg/ml.

(2) Prepare distilled water, 0.001 N NaOH, 0.01 N NaOH, 0.1 N NaOH, and ethanol solvents 100 ml in 5 different flasks.

(3) Deliver 1 ml of uranine and distilled water 100 µg/ml into 5 different solutions. Shake for 5 minutes. The real concentration in each flask is 0.99 µg/ml.

(4) Pipet 3.3 ml solutions into small cuvettes. Each

solution has 3 samples.

(5) Add 50 µl pH 10 buffer into cuvettes containing distilled water. Leave these cuvettes in the dark for 15 minutes.

(6) Analyze all samples fluorometrically using the SLM Aminco Fluoro-Colorimeter II.

(7) Repeat step (2).

(8) Deliver 0.1 ml of uranine and distilled water 10 µg/ml into distilled water, 0.001 N NaOH, 0.01 N NaOH, and 0.1 N NaOH solutions. Shake for 5 minutes. The real concentration in each flask is 0.01 µg/ml.

(9) Repeat steps (4) to (6).

IV.A.3.b. PH Value Test

(1) Prepare 7 different pH value sodium hydroxide solutions between concentration 0.001 N and 0.1 N.

(2) Prepare these 7 different pH NaOH solutions of 100 ml each in 7 different flasks.

(3) Prepare uranine and distilled water stock solution 100 µg/ml.

(4) Deliver 1 ml of uranine and distilled water 100 µg/ml into 7 different pH solutions. Shake for 5 minutes. The real concentration in each flask is 0.99 µg/ml.

(5) Pipet 3.3 ml solutions into small cuvettes. Each solution has 3 samples.

(6) Analyze all samples fluorometrically using the SLM Aminco Fluoro-Colorimeter II.

IV.B. Interference Test

IV.B.1. Test Summary

The interference of oleic acid in different uranine solutions is tested. In the Oleic Acid in Solution Test Section, a specified amount of oleic acid is added into different uranine solutions. Then, these solutions are analyzed with the SLM Aminco Fluoro-Colorimeter. In the Scanning Fluorometer Test Section, various uranine solutions are analyzed with the Perkin-Elmer Spectrofluorometer, specifically uranine, oleic acid, and ethanol solution.

IV.B.2. Oleic Acid in Solution Test

(1) Prepare an adequate amount of uranine and distilled water solutions for a concentration of 1 µg/ml and a concentration of 0.01 µg/ml.
(2) Deliver 10 µl oleic acid into 500 ml of 1 µg/ml uranine and distilled water. Shake 2 minutes, put in the ultrasonic bath for 10 minutes, and shake 2 minutes again.
(3) Pour about 20 ml solution in 25 ml beaker. Immerse the

pipet in the beaker and extract 3.3 ml solution into cuvettes.

(4) Add 50 µl pH 10 buffer in cuvettes. Leave these cuvettes in the dark for 15 minutes.

(5) Analyze cuvettes fluorometrically using the SLM Aminco Fluoro-Colorimeter II.

(6) Repeat steps (2) to (5) using 0.01 µg/ml solution.

(7) Repeat steps (1) to (3) and (5) using uranine and 0.001 N NaOH, uranine and 0.01 N NaOH, uranine and 0.1 N NaOH, and uranine and ethanol solution.

IV.B.3. Scanning Fluorometer Test

(1) Prepare 100 ml uranine and distilled water solution with 100 µg/ml.

(2) Prepare 100 ml of the five following solvents separately: distilled water, 0.001 N NaOH, 0.01 N NaOH, 0.1 N NaOH, and ethanol.

(3) Pipet 1 ml of 100 µg/ml uranine and distilled water into each solvent. The concentration is 0.99 µg/ml. Shake 10 minutes.

(4) Pipet 3.3 ml solution into small cuvettes. Add 50 µl pH 10 buffer into cuvettes with distilled water and with ethanol. Leave these cuvettes in the dark for 15 minutes.

(5) Analyze all samples fluorometrically with the Perkin-

Elmer Spectrofluorometer.

(6) Pipet 3.3 ml of the tested oleic acid, uranine, and ethanol solution in step (7) in IV.B.2. into small cuvettes. Add 50 μl pH 10 buffer and 0.1 N NaOH into cuvettes separately. Leave these cuvettes in the dark for 15 minutes.

(7) Analyze these cuvettes fluorometrically using the Perkin-Elmer Spectrofluorometer.

IV.C. Filter Extraction Test

IV.C.1. Test Summary

The purpose of the filter extraction test is to evaluate the background of different filters in various solutions and the extraction ability of these solutions. In the Pure Filter Background Test Section, the background of glass fiber filters and Teflon filters in different solutions is obtained. In the Uranine Extraction Test Section, a certain amount of uranine is delivered on filters and washed out by different solutions. In the Uranine and Oleic Acid Extraction Test Section, a certain amount of uranine and oleic acid is delivered on filters and washed out by different solutions.

IV.C.2. Pure Filter Background Test

(1) Put 15 glass fiber filters (47mm, Type A/E, Gelman Scientific Inc., Ann Arbor, MI) into fifteen 2 oz jars.

(2) Pour 20 ml of the five following solvents separately into three jars: distilled water, 0.001 N NaOH, 0.01 N NaOH, 0.1 N NaOH, and ethanol.

(3) Put these 15 jars in the ultrasonic bath for one hour.

(4) Take these 15 jars out of the ultrasonic bath. Pipet about 3.3 ml solution from each jar into each cuvette separately. Centrifuge (2600 RPM) these cuvettes for 10 minutes in a centrifuge (Model TJ-6, Beckman Instruments Inc., Palo Alto, CA).

(5) Analyze those cuvettes containing sodium hydroxide solutions using the SLM Aminco Fluoro-Colorimeter II.

(6) Add 50 µl pH 10 buffer solution into those cuvettes containing distilled water and containing ethanol. Leave these cuvettes in the dark for 20 minutes and analyze them using the SLM Aminco Fluoro-Colorimeter II.

(7) Repeat steps (1) to (6) using Teflon Filters (37mm, Prod. R2PJ037, Gelman Scientific Inc., Ann Arbor, MI).

IV.C.3. Uranine Extraction Test

(1) Prepare uranine and ethanol stock solution with 40 µg/ml.

(2) Put 12 Gelman glass fiber filters (47mm) on the edges of twelve 30 ml beakers.

(3) Deliver 0.5 ml stock solution to each filter. Let the filters dry for 30 minutes.

(4) Put these 12 filters into twelve 2 oz jars.

(5) Pour 20 ml of the four following solvents separately into three jars containing filters: distilled water, 0.001 N NaOH, 0.01 N NaOH, and ethanol.

(6) Pour 20 ml of the four following solvents separately into four jars: distilled water, 0.001 N NaoH, 0.01 N NaOH, and ethanol. Directly add 0.5 ml stock solution into these four jars. These four jars are used as blanks.

(7) Shake these samples gently for 15 minutes.

(8) Pipet about 3.3 ml of solution from each jar into each cuvette. Centrifuge these cuvettes for 10 minutes.

(9) Analyze those cuvettes containing sodium hydroxide solutions using the SLM Aminco Fluoro-Colorimeter II.

(10) Add 50 µl pH 10 buffer into those cuvettes containing distilled water and containing ethanol. Leave these cuvettes in the dark for 20 minutes and analyze them using the SLM Aminco Fluoro-Colorimeter II.

(11) Take these filters out and put them into twelve new jars containing 20 ml of distilled water, 0.001 N NaOH, 0,01 N NaOH, and ethanol separately. Put these jars in the ultrasonic bath for 30 minutes.

(12) Repeat steps (8) to (10).

(13) Take these filters out and put them into twelve new

jars again. Put these jars in the ultrasonic bath for 1 hour.

(14) Repeat steps (8) to (10).

IV.C.4. Uranine And Oleic Acid Extraction Test

(1) Prepare uranine, oleic acid, and ethanol stock solution with 40 μg/ml. The ratio of uranine and oleic acid is 0.05 g uranine to 1 ml oleic acid.

(2) Repeat steps (2) to (14) in IV.C.3.

IV.D. Portable Indoor Particulate Sampler (PIPS) and Saturation Monitor Determination

IV.D.1. Test Summary

This test is designed to verify the 2.5 and 10 μm cut-point Portable Indoor Particulate Sampler (PIPS) and to determine the Saturation Monitor, which was designed to provide a 10 μm cut point. The collection efficiency of PIPS was previously determined by Marple (1989). The 2.5 μm samplers were tested with monodisperse test particles between 1.5 and 3.5 μm, and the 10 μm samplers with particles between 6 and 25 μm aerodynamic diameter.

For each sampler, the sampler collection efficiency is determined as the mass of particulate material not on the

filter collected within the sampler divided by the total mass of particulate material that enters the sampler. Fractional mass penetration to the filter is therefore one minus the collection efficiency. The total mass entering the sampler is taken to be the mass on the filter plus the mass that collected on the interior surfaces of the sampler and the impaction disk. Thus, only particle behavior inside the samplers is evaluated during this test.

In overview, the test consisted of the following:

1. Generation of a monodisperse test aerosol in a chamber.
2. Operation of the samplers within that chamber long enough to obtain a suitable particle sample.
3. Analysis of the mass collected on the filter.
4. Analysis of the particulate mass collected within the sampler not on the filter by washing down the interior walls and the impaction stage.
5. Calculation of impactor efficiency as mass collected not on the filter divided by the total mass entering the sampler.

IV.D.2. Test Aerosol

The sampler collection efficiency for aerosols with aerodynamic diameters of 1.5, 2, 2.25, 2.5, 2.75, 3, 3.5, 6,

7, 9, 10, 10.5, 11, 13, 15, and 25 μm was determined during this study. The test aerosols are generated using a vibrating orifice monodisperse aerosol generator (VOMAG), and are composed of oleic acid tagged with uranine. As is required to make particles with a VOMAG, the feed solution is composed of a dilute solution of oleic acid and uranine in ethanol. After the primary particle is generated by the VOMAG, the ethanol evaporates and the desired test particle remains.

For particles less than 5 μm in diameter, a 10 μm orifice is used in the VOMAG. The feed rate is 0.1 ml/min at a VOMAG frequency of about 200 KHz. At 5 μm and above, a 20 μm orifice is used in the VOMAG at a frequency near 70 KHz and a feed rate of 0.165 ml/min.

According to the federal regulation (Federal Reference Method, 40 CFR, Part 53, 1987), multiplets (doublets and triplets) in a test particle atmosphere shall not exceed 10 percent. For particle sizes above 5 μm, the particle size uniformity and number of doublets and triplets are checked using an optical microscope (Model Labophot-Pol, Nikon Inc., Garden City, NY). These particles are collected on slides and the slide is examined. For particle sizes less than 5 μm, the particle size uniformity and number of doublets and triplets are checked by an aerodynamic particle sizer (APS, Model 3310, TSI Inc., St. Paul, MN).

IV.D.3. Sampler Operation

IV.D.3.a. Summary of Operation

All samplers are not tested with all particle sizes. The 2.5 μm cut-point PIPS are tested with particles from 1.5 to 3.5 μm aerodynamic diameter, while the 10 μm PIPS and Saturation Monitor tested with the particles larger than 5 μm in aerodynamic diameter.

Tests of the 10 μm cut-point samplers at particle sizes above 5 μm include the following:

1. Four 47 mm open-face filter samplers,
2. Two 10 μm PIPS operated with the EMSI pumps,
3. Two 10 μm Saturation Monitors operated with the EMSI pump.

The open-face filter samplers are used for checking aerosol uniformity and concentration in the chamber. For test particles smaller than 5 μm, two 2.5 μm cut-point PIPS replace two 10 μm cut-point samplers and two Saturation Monitors.

The procedures used to operate the PIPS and Saturation Monitor and to extract the fluorescent aerosol from the samplers are given in IV.D.3.b and c. The impactor stages are not greased for these tests because the liquid test

aerosols do not bounce.

We choose distilled water as the washing solution to eliminate possible reaction between the washing solvent and the sampler surface. Thus, the fluorescence background problem is minimized.

IV.D.3.b. Portable Indoor Particulate Sampler (PIPS) Standard Operating Procedure

(1) Refer to Figure 3 to see the components of the PIPS.
(2) Lay out all of the components except the filter on a surface that is free of contamination.
(3) With the base of the sampler flat on the plastic plate with holes, facing upward, use the forceps to place a pad onto the center of the base. Be sure that it is centered and flat.
(4) Load the Teflon filters into the round frames. Squeeze the top and bottom frame evenly until the two frames are jointed. During these procedures, the filter is to be touched only with clean tweezers.
(5) Place the round filter frame on top of the pad, carefully adjusting the frame so that it overlaps the pad and also fits onto the base securely. When it is securely in place, the frame will not move.
(6) Place the impactor on the top of the filter frame. If the impactor is properly installed, the impactor will

not move either.

(7) Keeping the base of the sampler still flat on the table, carefully attach the inlet to the base. Screw the inlet and the base evenly. Be sure that the inlet rests exactly on the base.

(8) Attach the PIPS to the EMSI pump line.

IV.D.3.c. Saturation Monitor Standard Operating Procedure

(1) Refer to Figure 4 to see the components of the Saturation Monitor.

(2) Lay out all of the components except the filter on a surface that is free of contamination.

(3) Insert the impactor into the assembly ring.

(4) With the base of the sampler flat on the plastic plate with holes, facing upward, use the forceps to place a glass fiber filter onto the center of the base. Be sure that it is centered.

(5) Place the anti-twist ring over the filter. Be sure that the anti-twist ring is tied with the base.

(6) Screw the adapter body into the base. Be sure that the anti-twist ring and the filter are in the proper position.

(7) Screw the assembly ring into the adapter body.

(8) Hold the sampler body upside down. Insert the body into the cap.

(9) Attach the Saturation Monitor to the mass flow meter (Model FM-361, Pat. No. 3938384, Tylan Corp., Carson, CA) and the EMSI pump line. After the pump has operated for at least 5 minutes, check the flow on the mass flow meter. Adjust the flow if necessary.

IV.D.4. Particle Mass Recovery

IV.D.4.a. Test Summary

The particle mass collected in each sampler is determined as the sum of the mass collected on the filter and the mass collected elsewhere in the sampler. The mass collected elsewhere is measured by washing the inside of the sampler, then determining the mass collected fluorometrically. The filter mass is determined by extracting the filter and using fluorometric analysis. The sampler penetration for a given particle size is determined by dividing the mass on the filter by the total sampler mass.

Detailed explanations of the procedures used to obtain the filter and sampler wash extracts are given in IV.D.4.b and c.

IV.D.4.b. Normal Filter Extraction and Interior Rinse Procedure for Portable Indoor Particulate Sampler

(PIPS)

(1) Place sampler on the table.

(2) Use plastic electrical tape to seal the inlet slits. Do not wrinkle the tape in order to prevent leaks.

(3) Remove the screws. Take the inlet out of the base and put it upside down on the table. Be careful not to contaminate the inlet.

(4) Using clean forceps, take the impactor out of the base and place it in a 32 oz. polystyrene disposable container, add 180 ml distilled water, cap and put in the ultrasonic bath for 30 minutes.

(5) Again using clean forceps, separate the section of the filter holding the frame and remove the Teflon filter.

(6) Insert the Teflon filter into a 2 oz. jar with the exposed side down, add 20 ml distilled water, cap and put in the ultrasonic bath for 30 minutes.

(7) Put 40 ml distilled water in a clean 2 oz. bottle. Hold the taped inlet upside-down. Dip a clean cotton swab (Food Lion Corp., Salisbury, NC) in the wash solution and swab out the underside of the inlet. Pour all the wash solution into the inlet through the small holes and shake for 2 minutes. Cut the head of the cotton swab and put it in this 2 oz. bottle. Drain the wash fluid into this bottle and put in the ultrasonic bath with the swab head for 20 minutes.

(8) Put 40 ml distilled water in a clean 2 oz. bottle. Use a clean pipet and rinse the inlet by rotating it with the fluid in each section that needs rinsing. Pour the remaining wash solution into the inlet through the small holes and shake for 2 minutes. Drain the wash fluid into this bottle completely.

(9) Repeat step (4) two or three times until all the uranine is washed out from the impactor.

(10) Pipet all sample solutions of 3.3 ml into small cuvettes. Add 50 µl pH 10 buffer into each cuvette and leave these cuvettes in the dark for 15 minutes.

(11) Analyze all the washing solutions using the SLM Aminco Fluoro-Colorimeter II.

IV.D.4.c. Normal Filter Extraction and Interior Rinse Procedure for Saturation Monitor

(1) Place the sampler on the table.

(2) Unscrew the adapter from the base. Place the body without the base upside down on the table. Using clean forceps, take the anti-twist ring out of the base and put it inside the upside-down body.

(3) Using clean forceps, insert the glass fiber filter into a 2 oz. jar with the exposed side down, add 20 ml distilled water, cap and put in the ultrasonic bath for 30 minutes.

(4) Remove the cap out of the body. Pull the impactor out of the assembly ring. Using clean forceps, place the impactor in a 4 oz. jar, add 80 ml distilled water, cap and put in the ultrasonic bath for 30 minutes.

(5) Put 40 ml distilled water in a clean 2 oz. bottle. Hold the body upside-down exactly above the cap. Dip a clean cotton swab in the wash solution and swab out the underside of the body. Let the excess washing solution flow into the cap. After swabbing the inside of the body, swab the inside of the cap. Cut the head of the cotton swab and put it in this 2 oz. bottle. Pour the wash fluid out of the cap into this bottle and put in the ultrasonic bath with the swab head for 20 minutes.

(6) Put 40 ml distilled water in a clean 2 oz. bottle. Use a clean pipet and rinse the body by turning it so that the fluid rinses each section. Let the excess wash solution flow into the cap. Rinse the cap after rinsing the body. Pour the wash solution completely out of the cap into the bottle.

(7) Repeat step (4) two or three times until all the uranine is washed out from the impactor.

(8) Repeat step (3) two times to make sure all the uranine washed out of the glass fiber filters.

(9) Pipet all sample solutions of 3.3 ml into small cuvettes. Add 50 µl pH 10 buffer into each cuvette and leave these cuvettes in the dark for 15 minutes.

(10) Analyze all the washing solutions using the SLM Aminco Fluoro-Colorimeter II.

V. RESULTS, DISCUSSION, AND CONCLUSIONS

V.A. Sensitivity Test

V.A.1. Calibration Curve

V.A.1.a. Determination of Amount of Buffer in Uranine and Distilled Water Solution

From Figure 5 the pH value increases sharply with the increase of pH 10 buffer but becomes stable when the ratio of buffer to distilled water reaches about 0.003. Hence, there is large buffer region to maintain the pH value at 10. If the ratio of buffer to distilled water is less than 0.003, the solution cannot maintain the pH value at 10. However, excess buffer solution will increase the volume of uranine and distilled water solution and lower the actual solution concentration, so that a balance point must be found. From Table 1, generally the uranine and distilled water solution with 50 μl buffer have the highest reading at every concentration, so that the 50 μl pH 10 buffer in 3.3 ml uranine and distilled water solution is specified. The specified ratio of buffer to distilled water is

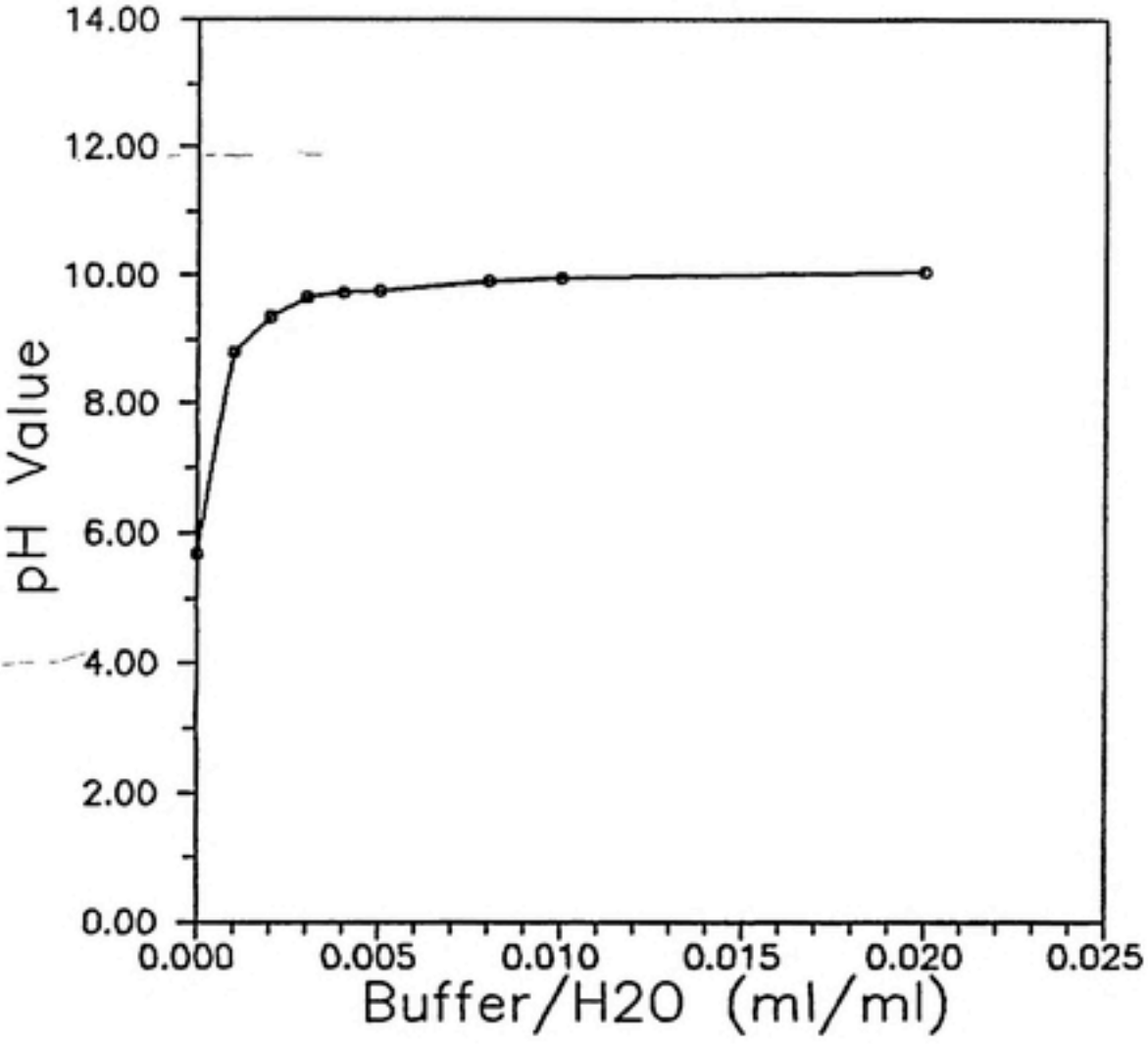

Figure 5. Ratio of Buffer to Distilled Water vs pH Value

Table 1. Fluorescence Intensity vs Amount of Buffer

Conc (µg/ml)	Pure Dis H2O	0.04 ml Buffer	0.05 ml Buffer	0.06 ml Buffer	0.075 ml Buffer	0.1 ml Buffer
2.000	4.1400	5.6400	5.6200	5.4500	5.5100	5.4700
1.000	1.9900	2.7100	2.7300	2.6600	2.6900	2.6600
0.500	1.0000	1.3400	1.3400	1.3300	1.3300	1.3100
0.200	0.3910	0.5360	0.5400	0.5260	0.5290	0.5260
0.100	0.1950	0.2660	0.2690	0.2570	0.2610	0.2610
0.050	0.0940	0.1290	0.1330	0.1250	0.1290	0.1270
0.020	0.0370	0.0502	0.0524	0.0470	0.0500	0.0500
0.010	0.0179	0.0232	0.0270	0.0222	0.0260	0.0246
0.005	0.0074	0.0112	0.0132	0.0081	0.0115	0.0122

approximately 0.015.

V.A.1.b. Calibration Curves

V.A.1.b.i. Uranine and Distilled Water Solution

From Figure 6, buffer dramatically increases the sensitivity of uranine and distilled water solution. This result is consistent with Drabent et al. (1964) and Pant's result (1968) because only bivalent negative ions exist in uranine and distilled water solution when the pH value is above 10. Therefore, uranine and distilled water solution with pH 10 buffer have both the maximum absorption and emission of fluorescence. There is a significant linear relation between fluorescence intensity and the concentration of the solution with a range of 0.005 μg/ml and 2 μg/ml. However, there is a poor linear relationship below 0.005 μg/ml.

In order to evaluate the accuracy of the SLM Aminco Fluoro-Colorimeter, we repeat the experiments above by continuously measuring the same sample ten times. The results are presented in Table 2. From Table 2, the accuracy above 0.005 μg/ml is high, but drops dramatically below 0.005 μg/ml. A possible way to increase the sensitivity and to reduce noise is to replace the primary filter. The UG-1 primary filter passes less than 1%

Table 2. Fluorometer Accuracy Test

	1 (µg/ml)	0.1 (µg/ml)	0.01 (µg/ml)	0.005 (µg/ml)	0.002 (µg/ml)	0.001 (µg/ml)
	2.72	0.257	0.0220	0.0099	0.0015	-0.0015
	2.71	0.256	0.0236	0.0101	0.0038	-0.0010
	2.70	0.255	0.0214	0.0092	0.0020	-0.0011
	2.70	0.255	0.0217	0.0089	0.0006	-0.0007
	2.71	0.256	0.0238	0.0084	0.0007	-0.0012
	2.70	0.255	0.0221	0.0082	0.0045	-0.0019
	2.70	0.255	0.0217	0.0085	0.0051	-0.0026
	2.70	0.256	0.0251	0.0094	0.0011	-0.0023
	2.69	0.254	0.0216	0.0095	0.0002	-0.0018
	2.69	0.255	0.0218	0.0096	0.0022	-0.0017
Mean	2.702	0.2554	0.02248	0.00917	0.00217	
Std Dev	0.0092	0.00084	0.00124	0.00065	0.00172	
Std/Mean	0.0034	0.0033	0.0552	0.0709	0.7952	

excitation light between 420 to 670 nm, whereas uranine and distilled water solution absorbs light between 440 to 520 nm. The secondary filter works well because it passes all emission light above 405 nm. The zero point reading in the fluorometer is between 0.002 µg/ml and 0.001 µg/ml.

V.A.1.b.ii. Uranine and Sodium Hydroxide Solution

Figure 7 and 8 show notable linear relationships between fluorescence intensity and the concentration of the solution with a range of 0.005 µg/ml and 2 µg/ml in both 0.001 N and 0.01 N sodium hydroxide solutions.

V.A.1.b.iii. Uranine and Ethanol Solution

From Figure 9, there is very poor sensitivity and a poor linear relationship of uranine and ethanol solution. Adding pH 10 buffer in ethanol significantly increases the sensitivity. There is a substantial linear relationship between fluorescence intensity and the concentration of the solution with a range of 0.005 µg/ml and 2 µg/ml. The chemical reactionship of uranine and ethanol solution with buffer is unknown. A reasonable explanation for the behavior of the buffered solution is that there is an increase of bivalent uranine ions, with the highest fluorescence existing in ethanol when the pH value is above

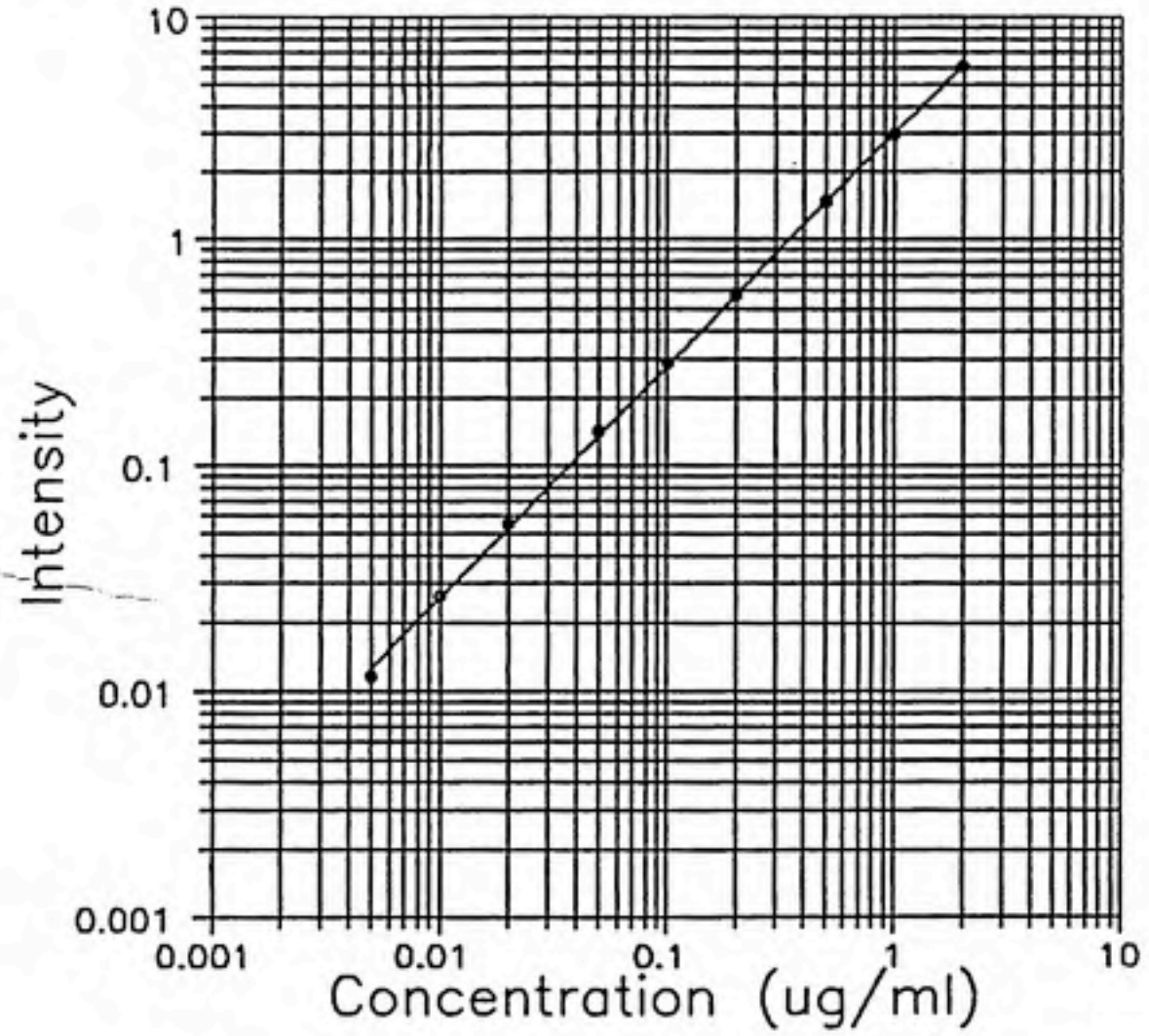

Figure 7. Calibration Curve of 0.001 N Sodium Hydroxide

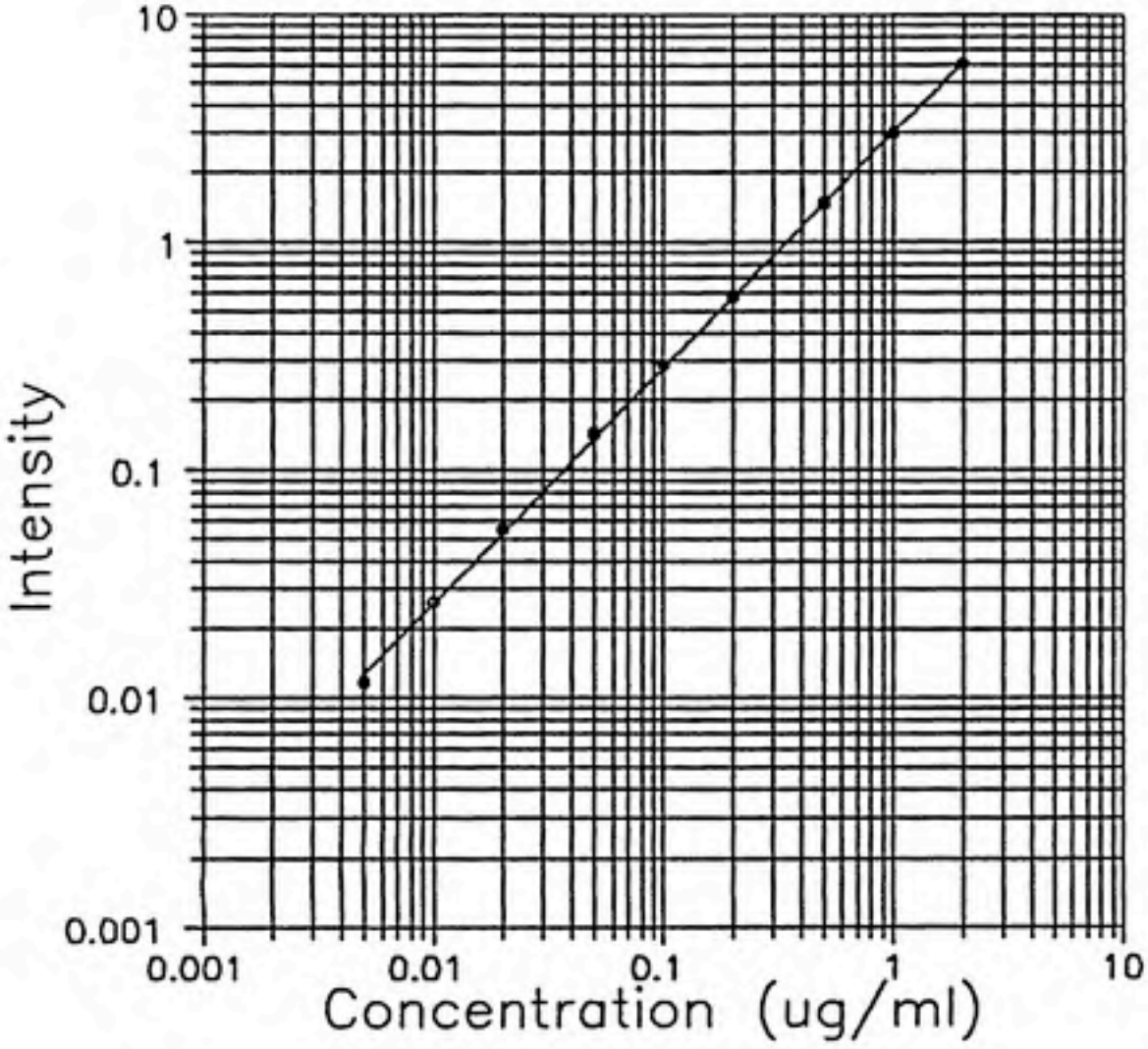

Figure 8. Calibration Curve of 0.01 N Sodium Hydroxide

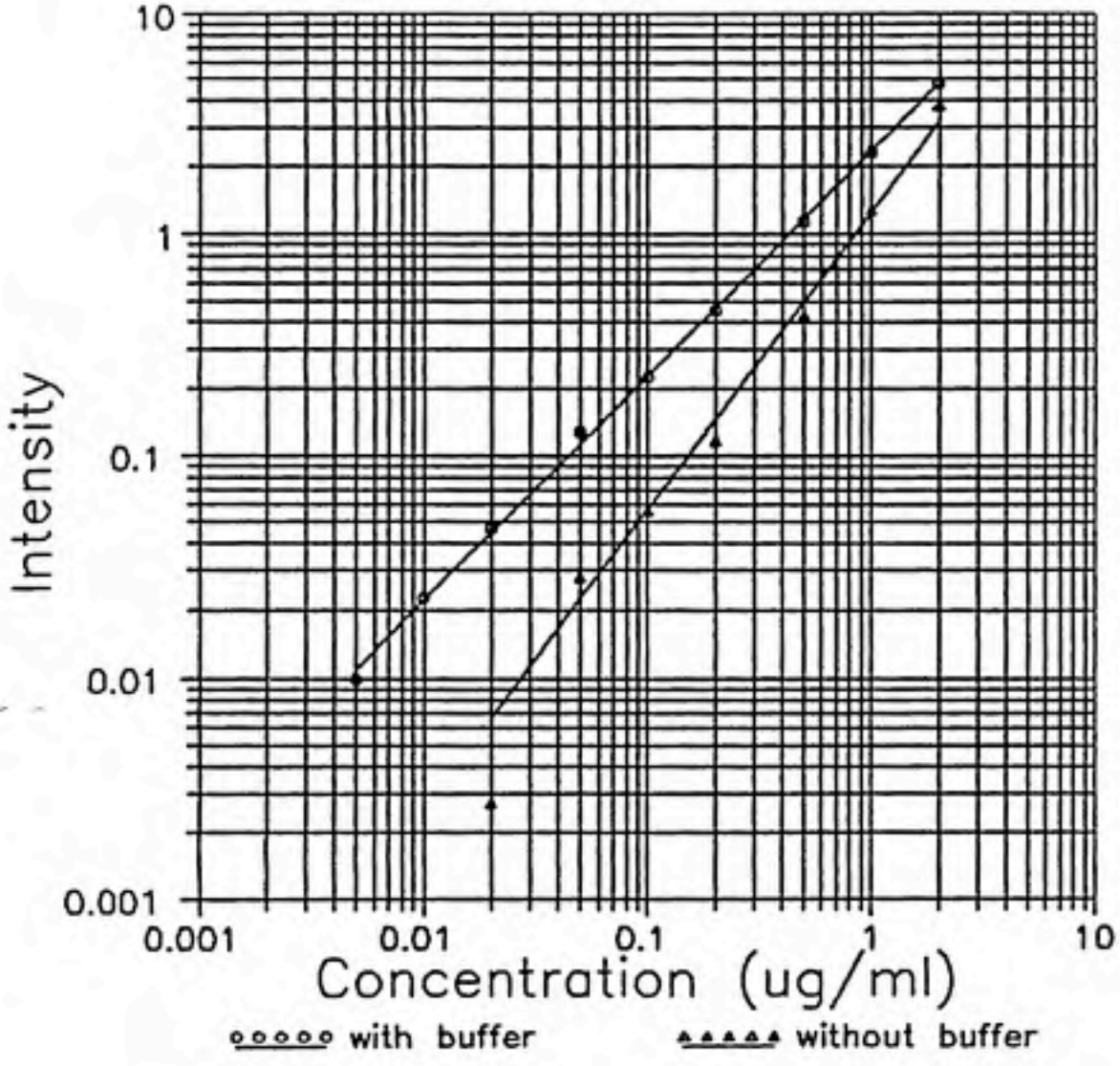

Figure 9. Calibration Curve of Ethanol

10.

V.A.1.b.iv. Overall Comparison

The calibration curves of distilled water with buffer, 0.001 N sodium hydroxide, 0.01 N sodium hydroxide, and ethanol with buffer are put on at the same time in Figure 10. From Figure 10 it seems that distilled water with buffer, 0.001 N sodium hydroxide, and 0.01 N sodium hydroxide have nearly the same sensitivity; however, ethanol with buffer has a lower sensitivity than other solutions.

V.A.2. Sensitivity Comparison

V.A.2.a. Sensitivity Test

From Table 3 at concentration 0.99 μg/ml, 0.01 N sodium hydroxide has the highest sensitivity. At the same concentration, distilled water with buffer, 0.001 N sodium hydroxide, and 0.1 N sodium hydroxide have high sensitivity. Ethanol with buffer has only fair sensitivity. Pure distilled water and ethanol have poor sensitivity. From Table 4, at concentration, 0.01 μg/ml distilled water has the highest sensitivity and 0.001 N, 0.01 N, and 0.1 N sodium hydroxide have high sensitivity. Pure distilled water has poor sensitivity.

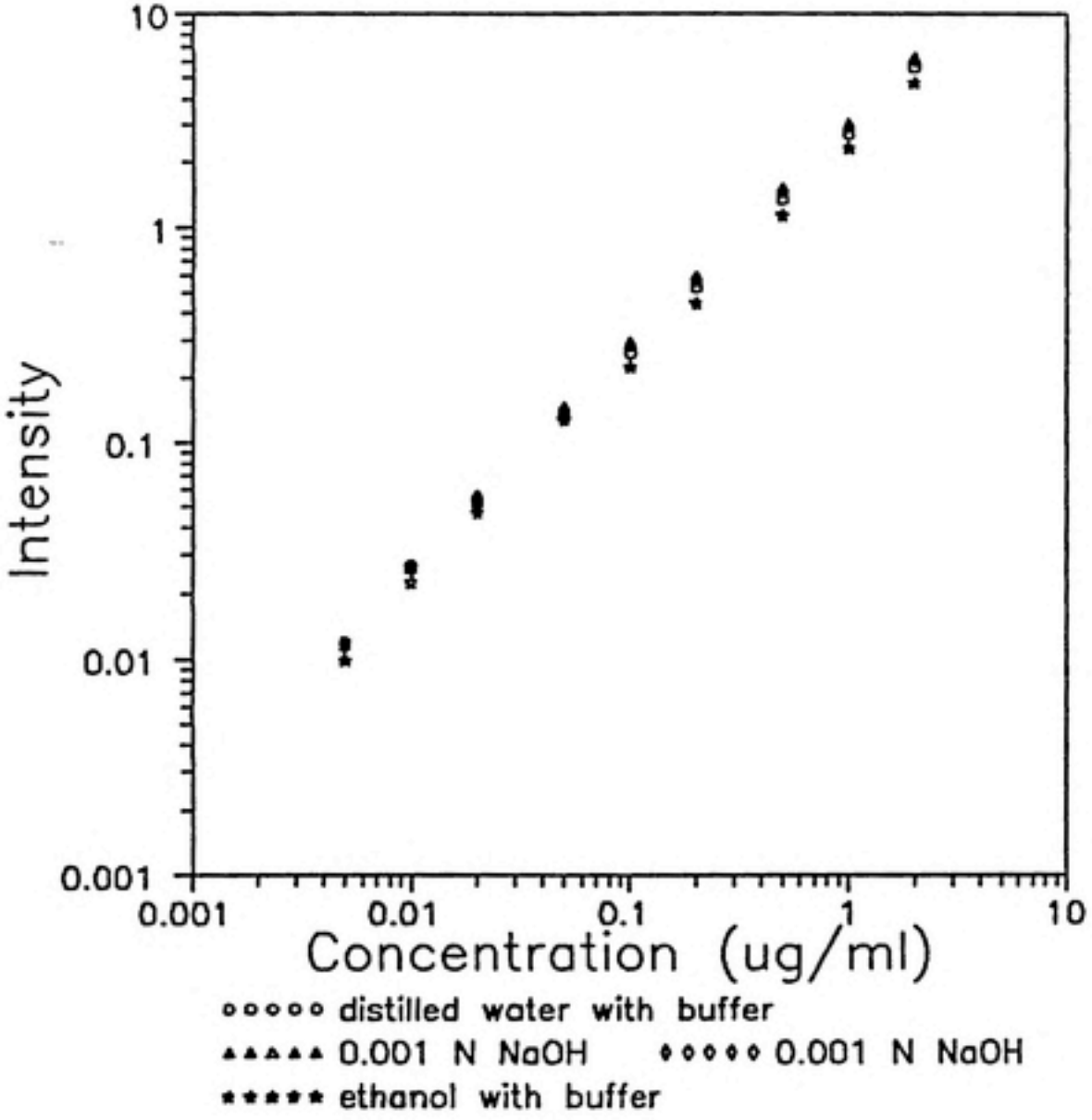

Figure 10. Comparison of Calibration Curves

Table 3. Comparison of Sensitivity at High Concentration

Sample No	Dis H2O with Buffer	Dis H2O without Buffer	0.001 N NaOH	0.01 N NaOH	0.1 N NaOH	Ethanol with Buffer	Ethanol without Buffer
1	2.83	2.03	2.79	3.10	2.82	2.32	1.39
2	2.75	1.99	2.81	3.08	2.78	2.30	1.35
3	2.82	2.03	2.81	3.15	2.85	2.35	1.42

Table 4. Comparison of Sensitivity at Low Concentration

Sample No	Dis H2O with Buffer	Dis H2O without Buffer	0.001 N NaOH	0.01 N NaOH	0.1 N NaOH
1	0.0239	0.0154	0.0201	0.0199	0.0187
2	0.0225	0.0136	0.0199	0.0212	0.0187
3	0.0227	0.0144	0.0199	0.0205	0.0187

V.A.2.b. PH Value Test

From Figure 11 there is only 3% fluctuation of fluorescence intensity between pH value 10 and 12. Hence, pH value above 10 has no effect on fluorescence intensity.

V.A.3. Conclusions of Sensitivity Test

a. The addition of pH 10 buffer changes the pH value of solutions and significantly increases the sensitivity of both uranine and distilled water and uranine and ethanol solutions.
b. There is a substantial linear relationship between fluorescence intensity and the concentration of the solution when the concentration of the solution is higher than 0.005 μg/ml in distilled water with buffer, 0.001 N sodium hydroxide, 0.01 N sodium hydroxide, 0.1 N sodium hydroxide, and ethanol with buffer. There is a very poor linear relationship in these solutions when the concentration is lower than 0.005 μg/ml.
c. The order of sensitivity:

[Distilled water with buffer
0.001 N sodium hydroxide
0.01 N sodium hydroxide
0.1 N sodium hydroxide] > [Ethanol with pH 10 Buffer]

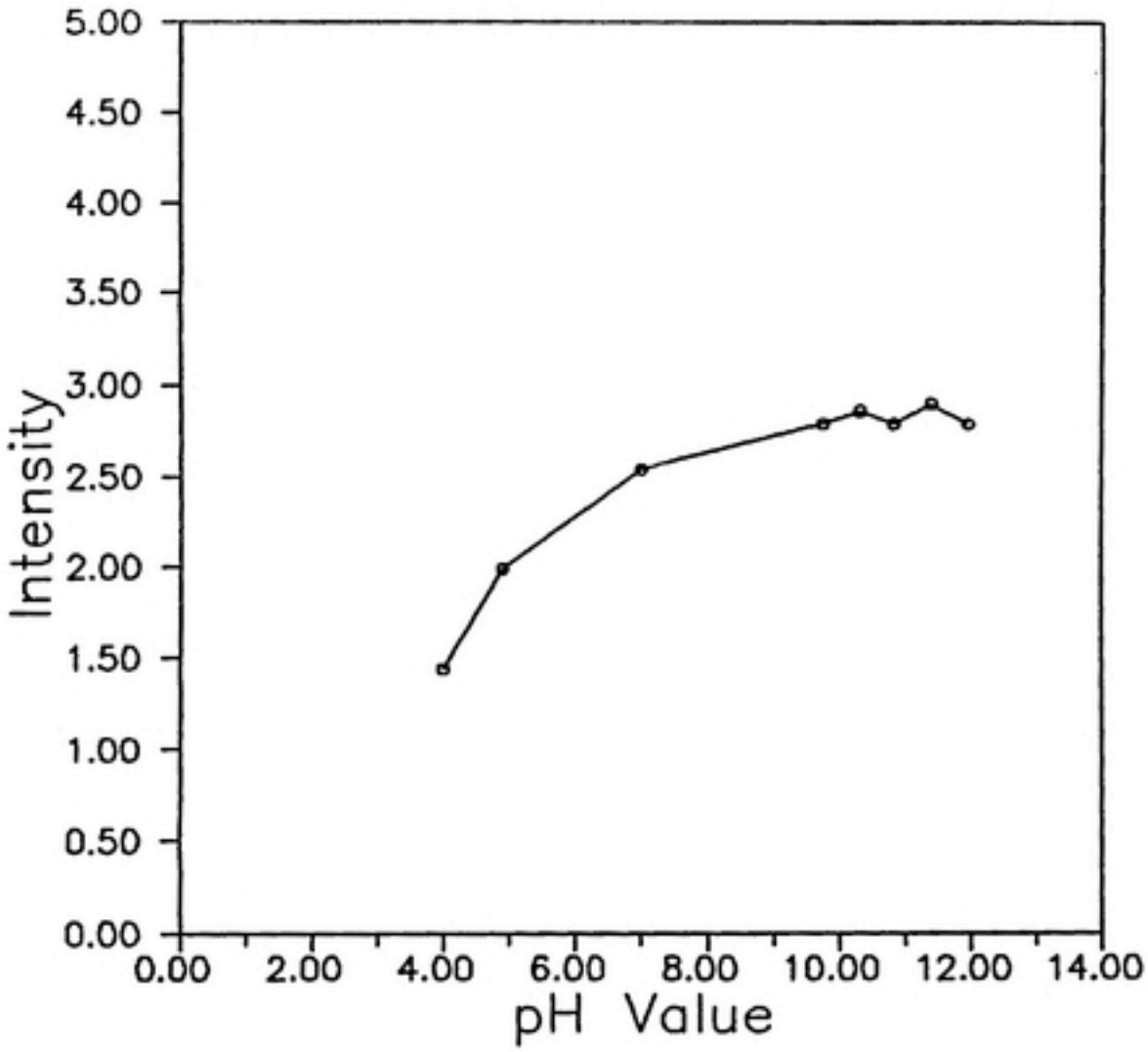

Figure 11. Fluorescence Intensity vs pH Value

> [Distilled water] > [Ethanol]

d. There is no significant effect of pH value on fluorescence intensity when the pH value of the solution is above 10.

V.B. Interference Test

V.B.1. Oleic Acid in Solution Test

V.B.1.a. Interference Test of Oleic Acid in Distilled Water

From Table 5, there is no interference of oleic acid in uranine and distilled water solution at concentration 1 μg/ml and 0.01 μg/ml because oleic acid and distilled water are totally immiscible.

V.B.1.b. Interference Test of Oleic Acid in Sodium Hydroxide

V.B.1.b.i. 0.001 N Sodium Hydroxide

From Table 6, there is no interference of oleic acid in uranine and 0.001 N sodium hydroxide solution at concentration 1 μg/ml, but there is a small fluctuation at concentration 0.01 μg/ml. From Table 2, the error of SLM Aminco Fluoro-Colorimeter II at concentration 0.01 μg/ml is

Table 5. Interference Test of Oleic Acid in Distilled Water with Buffer

Sample Conc (μg/ml)	Sample Volume (ml)	Oleic acid Add Vol (μl)	SLM Reading	Conc From Calibration (μg/ml)
1	---	0	2.88	1.055
1	500	10	2.85	1.044
0.01	---	0	0.0278	0.011
0.01	500	10	0.0284	0.011
0.01*	---	0	0.0270	0.011
0.01*	500	10	0.0269	0.011

Note:

A. *: Repeat the same experiment once by using the same stock solution

B. Conc from calibration=Concentration calculated from calibration curve of distilled water with buffer:

$$LOG(Reading)=1.016091*LOG(Conc)+0.435761$$

Table 6. Interference Test of Oleic Acid in 0.001 N Sodium Hydroxide

Sample Conc (μg/ml)	Sample Volume (ml)	Oleic acid Add Vol (μl)	SLM Reading	Conc From Calibration (μg/ml)
1	---	0	3.06	0.993
1	500	10	3.09	1.003
0.01	---	0	0.0249	0.010
0.01	500	10	0.0269	0.010
# 0.01	500	10	0.0249	0.010
0.01*	---	0	0.0237	0.009
# 0.01*	---	0	0.0239	0.009
0.01*	500	10	0.0260	0.010
# 0.01*	500	10	0.0246	0.010

Note:

A. *: Repeat the same experiment once by using the same stock solution.
B. #: Second sample taken from the same flask.
C. Conc from calibration=Concentration calculated from calibration curve of 0.001 N sodium hydroxide:

LOG(Reading)=1.039163*LOG(Conc)+0.488755

about 5 to 6%. In the real washing operation, the ratio of oleic acid to sodium hydroxide is approximately $2*10^{-7}$ at concentration 0.01 µg/ml. Thus, we should deliver only 0.1 µl oleic acid in 500 ml of 0.01 µg/ml uranine and 0.001 N sodium hydroxide solution instead of delivering 10 µl oleic acid, so that there will be no interference of oleic acid at concentration 0.01 µg/ml.

V.B.1.b.ii. 0.01 N Sodium Hydroxide

From Table 7, there is no interference of oleic acid in uranine and 0.01 N sodium hydroxide solution at concentration 1 µg/ml, but there is a small fluctuation at concentration 0.01 µg/ml. However, this small fluctuation is within the error of the fluorometer used. Again, we should deliver only 0.1 µl oleic acid in 500 ml of 0.01 µg/ml uranine and 0.01 N sodium hydroxide solution instead of delivering 10 µl oleic acid, so that there will be no interference of oleic acid at concentration 0.01 µg/ml.

V.B.1.b.iii. 0.1 N Sodium Hydroxide

From Table 8, there is no interference of oleic acid in uranine and 0.1 N sodium hydroxide solution at concentration 1 µg/ml, but there is a small fluctuation at concentration 0.01 µg/ml. The fluctuation exists because of excess oleic

Table 7. Interference Test of Oleic Acid in 0.01 N Sodium Hydroxide

Sample Conc (µg/ml)	Sample Volume (ml)	Oleic acid Add Vol (µl)	SLM Reading	Conc From Calibration (µg/ml)
1	---	0	3.11	1.027
1	500	10	3.14	1.037
# 1	500	10	3.15	1.040
0.01	---	0	0.0255	0.010
0.01	500	10	0.0245	0.010
# 0.01	500	10	0.0254	0.010
• 0.01	500	10	0.0240	0.010

Note:

A. #: Second sample taken from the same flask.
B. •: Third sample taken from the same flask.
C. Conc from calibration=Concentration calculated from calibration curve of 0.01 N sodium hydroxide:

LOG(Reading)=1.034539*LOG(Conc)+0.480797

Table 8. Interference Test of Oleic Acid in 0.1 N Sodium Hydroxide

Sample Conc (µg/ml)	Sample Volume (ml)	Oleic acid Add Vol (µl)	SLM Reading
1	---	0	3.02
1	500	10	3.00
# 1	500	10	3.01
0.01	---	0	0.0214
0.01	500	10	0.0235
# 0.01	500	10	0.0222
• 0.01	500	10	0.0214

Note:
A. #: Second sample taken from the same flask.
B. •: Third sample taken from the same flask.

acid. Although oleic acid strongly reacts with 0.1 N sodium hydroxide, the normal amount of oleic acid in 500 ml of 0.01 μg/ml uranine and sodium hydroxide is only 0.1 μl. Therefore, there should be no interference of oleic acid in 0.1 N sodium hydroxide.

A qualitative observation is also performed when excess oleic acid reacts with excess sodium hydroxide and produces white salt precipitation. The reactivity increases as the concentration of sodium hydroxide increases. As a result, 0.001 N sodium hydroxide has the slowest reaction, whereas 0.1 N sodium hydroxide has the fastest and strongest reaction. However, the amount of oleic acid in the washing operation is extremely small, so that there should be no interference phenomenon.

V.B.1.c. Interference Test of Oleic Acid in Ethanol

From Table 9, there is a significant quenching phenomenon of oleic acid in uranine and ethanol solution at concentration 1 μg/ml. The reading of the quenching solution is only approximately 15% of the reading of the original solution. This phenomenon will be discussed in detail in section V.B.2.b.

V.B.2. Scanning Fluorometer Test

Table 9. Interference Test of Oleic Acid in Ethanol

Sample Conc (μg/ml)	Sample Volume (ml)	Oleic acid Add Vol (μl)	SLM Reading	Conc From Calibration (μg/ml)
1	---	0	1.360	1.061
1	500	10	0.113	0.166
# 1	500	10	0.109	0.161
• 1	500	10	0.111	0.164
▲ 1	500	10	0.109	0.161
1*	---	0	1.310	1.032
1*	500	10	0.103	0.155
# 1*	500	10	0.106	0.158
• 1*	500	10	0.103	0.155
▲ 1*	500	10	0.100	0.151

Note:

A. *: Repeat the same experiment once by using the same stock solution.
B. #: Second sample taken from the same flask.
C. •: Third sample taken from the same flask.
D. ▲: Fourth sample taken from the same flask.
E. Conc from calibration=Concentration calculated from calibration curve of ethanol with buffer.

LOG(Reading)=1.340227*LOG(Conc)+0.099186

V.B.2.a. Fluorescence Intensity in Different Solutions

Table 10 shows that uranine in distilled water with pH 10 buffer, 0.001 N sodium hydroxide, 0.01 N sodium hydroxide, and 0.1 N sodium hydroxide has the same maximum excitation wavelength, the same maximum emission wavelength, and a high sensitive reading. Uranine in distilled water and ethanol has the same maximum excitation wavelength and maximum emission wavelength, but the reading of uranine and distilled water solution is much higher than that of uranine and ethanol solution. The uranine and ethanol with buffer solution has distinct maximum excitation and emission wavelength and a high reading. This sensitivity comparison is consistent with that found in Table 3 when the concentration is approximately 1 μg/ml.

V.B.2.b. Fluorescence Intensity in Ethanol

Because there is a substantial quenching phenomenon of oleic acid in uranine and ethanol solution, further examination is performed by using a scanning fluorometer. Table 11 shows that oleic acid in uranine and ethanol solution will quench a sizable amount of fluorescence, but the maximum excitation and emission wavelength is the same as that of uranine and ethanol solution without buffer. If we add buffer in the quenching solution again, the maximum

Table 10. Scanning Fluorometer Test

Solvent	Max Excite Wavelength (nm)	Max Emit Wavelength (nm)	Scanning Reading
H_2O with buffer	484	514	1123
H_2O	464	512	130
0.001 N NaOH	484	514	1212
0.01 N NaOH	484	513	1345
0.1 N NaOH	484	513	1195
Ethanol with buffer	492	522	1082
Ethanol	464	515	61.4

Table 11. Quenching Test in Oleic Acid and Ethanol Solution

Solvent	Max Excite Wavelength (nm)	Max Emit Wavelength (nm)	Scanning Reading
Ethanol	464	516	61.8
Ethanol with buffer	492	522	1073
Ethanol with 0.1 N NaOH	492	521	1078
Ethanol+10 µl oleic acid	463	515	2.38
*Ethanol+10 µl oleic acid +buffer	492	522	1061
*Ethanol+10 µl oleic acid +0.1 N NaOH	492	522	1084

Note:* After ethanol and 10 µl oleic acid react, add buffer or 0.1 N NaOH again.

excitation and emission wavelength will shift to 492 nm and 522 nm, the same as that of uranine and ethanol solution originally with buffer. In addition, the fluorescence of the quenching solution becomes the same as that of uranine and ethanol solution originally with buffer. The 0.1 N sodium hydroxide has the same effect as pH 10 buffer. The pH values of the uranine and ethanol solution before and after oleic acid is added are 7.33 and 5.50. The chemical mechanism for this quenching effect is complex and unknown. A possible explanation for this phenomenon is that the added oleic acid lowers the pH value of the uranine and ethanol solution. The number of monovalent uranine ions is decreased. When the pH value is above 10, bivalent uranine ions with highest fluorescence form, and the quenching effect disappears. The quenching phenomenon only exists in ethanol but not in distilled water or in sodium hydroxide because only oleic acid is soluble in an ethanol solution and could change the pH value of ethanol solution.

V.B.3. Conclusions of Interference Test

a. There is no interference of oleic acid in uranine and distilled water solution.
b. There is no interference of oleic acid in uranine and sodium hydroxide aqueous solution under our operating conditions.

c. There is a significant quenching phenomenon of oleic acid in uranine and ethanol solution. However, alkaline uranine and ethanol solution with a pH value greater than 10 may inhibit the quenching effect.

V.C. Filter Extraction Test

V.C.1. Pure Filter Background Test

V.C.1.a. Pure Solvent Background

Table 12 shows that there is no fluorescence reading for distilled water, 0.001 N sodium hydroxide, 0.01 N sodium hydroxide, 0.1 N sodium hydroxide, and ethanol measured with a fluorometer. This result is the same with the distilled water and ethanol both containing pH 10 buffer.

V.C.1.b. Glass Fiber Filter

Table 13 there are background readings of glass fiber filters in both distilled water and ethanol solutions containing buffer. However, there is no background reading of glass fiber filters in 0.001 N, 0.01 N, and 0.1 N sodium hydroxide aqueous solutions.

V.C.1.c. Teflon Filter

Table 12. Pure Solvent Background

Solvent	SLM Reading	PH Value
H_2O with buffer	Negative	9.95
H_2O	Negative	4.89
0.001 N NaOH	Negative	9.98
0.01 N NaOH	Negative	11.44
0.1 N NaOH	Negative	12.04
Ethanol with buffer	Negative	11.75
Ethanol	Negative	7.33

Table 13. Background Reading for Glass Fiber filters

(1) General Comparison

Sample No	Dis H2O with Buffer	0.001 N NaOH	0.01 N NaOH	0.1 N NaOH	Ethanol with Buffer
1	0.0030	Negative	Negative	Negative	0.0040
2	0.0035	Negative	Negative	Negative	0.0047
3	0.0016	Negative	Negative	Negative	0.0067

(2) Distilled Water with Buffer

Sample No	SLM Reading	Conc From Calibration (μg/ml)	Solution Volume (ml)	Fake Weight (μg)
1	0.0030	0.0012	20	0.025
2	0.0035	0.0014	20	0.029
3	0.0016	0.0007	20	0.013

Note: Conc from calibration=Concentration calculated from calibration curve of distilled water with buffer

LOG(Reading)=1.016091*LOG(Conc)+0.435761

(3) Ethanol with Buffer

Sample No	SLM Reading	Conc From Calibration (μg/ml)	Solution Volume (ml)	Fake Weight (μg)
1	0.0040	0.0018	20	0.037
2	0.0047	0.0022	20	0.043
3	0.0067	0.0031	20	0.061

Note: Conc from calibration=Concentration calculated from calibration curve of ethanol with buffer

LOG(Reading)=1.340227*LOG(Conc)+0.099186

From Table 14 there is no background reading of Teflon filters in distilled water with buffer, 0.001 N sodium hydroxide, 0.01 N sodium hydroxide, and 0.1 N sodium hydroxide solutions. However, there is some background reading of Teflon filters in ethanol solution with buffer.

V.C.2. Uranine Extraction Test

Table 15 to 18 demonstrates that there is nearly no uranine extracted in the third distilled water, 0.001 N sodium hydroxide, and 0.01 N sodium hydroxide wash, but there is a very small amount of uranine left in the ethanol wash. The results of the first wash reveals that the extraction ability of distilled water, 0.001 N sodium hydroxide, and 0.01 N sodium hydroxide is nearly the same, although the extraction ability of distilled water is slightly higher than that of 0.001 N sodium hydroxide. In addition, extraction ability of 0.001 N sodium hydroxide is also slightly higher than that of 0.01 N sodium hydroxide. The extraction ability of ethanol is apparently lower than that of distilled water, 0.001 N sodium hydroxide, and 0.01 N sodium hydroxide solutions. From qualitative observation it is known that the solubility of uranine in solvents is expressed by the following order:

H_2O > 0.001 N > 0.01 N > 0.1 N > ethanol

Table 14. Background Reading for Teflon filters

(1) General Comparison

Sample No	Dis H2O with Buffer	0.001 N NaOH	0.01 N NaOH	0.1 N NaOH	Ethanol with Buffer
1	Negative	Negative	Negative	Negative	0.0075
2	Negative	Negative	Negative	Negative	0.0079
3	Negative	Negative	Negative	Negative	0.0089

(2) Ethanol with Buffer

Sample No	SLM Reading	Conc From Calibration (μg/ml)	Solution Volume (ml)	Fake Weight (μg)
1	0.0075	0.0034	20	0.069
2	0.0079	0.0036	20	0.072
3	0.0089	0.0041	20	0.081

Note: Conc from calibration=Concentration calculated from calibration curve of ethanol with buffer

LOG(Reading)=1.340227*LOG(Conc)+0.099186

Table 15. Uranine on Glass Fiber Filters Extracted by Distilled Water

(1) First Wash

Sample No	SLM Reading	Conc From Calibration (μg/ml)	Solution Volume (ml)	Uranine Weight (μg)	Weight Percent (%)
1	2.91	1.0658	20	21.317	97.20
2	2.86	1.0478	20	20.956	97.38
3	2.91	1.0658	20	21.317	96.87
* Ref	2.97	1.0875	20.5	22.293	---

(2) Second Wash

Sample No	SLM Reading	Conc From Calibration (μg/ml)	Solution Volume (ml)	Uranine Weight (μg)	Weight Percent (%)
1	0.0789	0.0306	20	0.612	2.79
2	0.0725	0.0282	20	0.563	2.62
3	0.0890	0.0344	20	0.689	3.13

(3) Third Wash

Sample No	SLM Reading	Conc From Calibration (μg/ml)	Solution Volume (ml)	Uranine Weight (μg)	Weight Percent (%)
1	0.0003	0.0001	20	0.003	0.01
2	Negative	0.0000	20	0.000	0.00
3	Negative	0.0000	20	0.000	0.00

(4) Total

Sample No	Uranine Weight (μg)
1	21.931
2	21.519
3	22.005
* Ref	22.293

Note:

A. *: Directly deliver 0.5 ml of uranine and ethanol into 20 ml distilled water.

B. Conc from calibration=Concentration calculated from calibration curve of distilled water with buffer:

$$LOG(Reading)=1.016091*LOG(Conc)+0.435761$$

Table 16. Uranine on Glass Fiber Filters Extracted by 0.001 N Sodium Hydroxide

(1) First Wash

Sample No	SLM Reading	Conc From Calibration (µg/ml)	Solution Volume (ml)	Uranine Weight (µg)	Weight Percent (%)
1	2.99	0.9714	20	19.429	96.72
2	3.03	0.9839	20	19.679	96.38
3	2.96	0.9620	20	19.241	96.34
* Ref	2.93	0.9527	20.5	19.530	---

(2) Second Wash

Sample No	SLM Reading	Conc From Calibration (µg/ml)	Solution Volume (ml)	Uranine Weight (µg)	Weight Percent (%)
1	0.0887	0.0329	20	0.658	3.28
2	0.1000	0.0369	20	0.739	3.62
3	0.0990	0.0366	20	0.731	3.66

(3) Third Wash

Sample No	SLM Reading	Conc From Calibration (µg/ml)	Solution Volume (ml)	Uranine Weight (µg)	Weight Percent (%)
1	Negative	0.0000	20	0.000	0.00
2	Negative	0.0000	20	0.000	0.00
3	Negative	0.0000	20	0.000	0.00

(4) Total

Sample No	Uranine Weight (µg)
1	20.087
2	20.417
3	19.972
* Ref	19.530

Note:

A. *: Directly deliver 0.5 ml of uranine and ethanol into 20 ml 0.001 N sodium hydroxide.

B. Conc from calibration=Concentration calculated from calibration curve of 0.001 N sodium hydroxide:

LOG(Reading)=1.039163*LOG(Conc)+0.488755

Table 17. Uranine on Glass Fiber Filters Extracted by 0.01 N Sodium Hydroxide

(1) First Wash

Sample No	SLM Reading	Conc From Calibration (μg/ml)	Solution Volume (ml)	Uranine Weight (μg)	Weight Percent (%)
1	3.06	1.0110	20	20.220	95.97
2	3.00	0.9919	20	19.837	96.03
3	3.06	1.0110	20	20.220	96.22
* Ref	2.98	0.9855	20.5	20.202	---

(2) Second Wash

Sample No	SLM Reading	Conc From Calibration (μg/ml)	Solution Volume (ml)	Uranine Weight (μg)	Weight Percent (%)
1	0.112	0.0413	20	0.827	3.92
2	0.111	0.0410	20	0.819	3.97
3	0.105	0.0388	20	0.777	3.69

(3) Third Wash

Sample No	SLM Reading	Conc From Calibration (μg/ml)	Solution Volume (ml)	Uranine Weight (μg)	Weight Percent (%)
1	0.0027	0.0011	20	0.023	0.11
2	Negative	0.0000	20	0.000	0.00
3	0.0022	0.0009	20	0.019	0.09

(4) Total

Sample No	Uranine Weight (μg)
1	21.069
2	20.656
3	21.015
* Ref	20.202

Note:

A. *: Directly deliver 0.5 ml of uranine and ethanol into 20 ml 0.01 N sodium hydroxide.

B. Conc from calibration=Concentration calculated from calibration curve of 0.001 N sodium hydroxide:

LOG(Reading)=1.034539*LOG(Conc)+0.480797

Table 18. Uranine on Glass Fiber Filters Extracted by Ethanol

(1) First Wash

Sample No	SLM Reading	Conc From Calibration (μg/ml)	Solution Volume (ml)	Uranine Weight (μg)	Weight Percent (%)
1	2.34	0.9903	20	19.805	93.68
2	2.33	0.9861	20	19.722	93.28
3	2.36	0.9986	20	19.972	93.54
* Ref	2.27	0.9610	20.5	19.701	---

(2) Second Wash

Sample No	SLM Reading	Conc From Calibration (μg/ml)	Solution Volume (ml)	Uranine Weight (μg)	Weight Percent (%)
1	0.147	0.0646	20	1.292	6.11
2	0.155	0.0681	20	1.361	6.44
3	0.153	0.0672	20	1.344	6.29

(3) Third Wash

Sample No	SLM Reading	Conc From Calibration (μg/ml)	Solution Volume (ml)	Uranine Weight (μg)	Weight Percent (%)
1	0.0047	0.0022	20	0.043	0.20
2	0.0064	0.0029	20	0.059	0.28
3	0.0038	0.0018	20	0.035	0.16

(4) Total

Sample No	Uranine Weight (μg)
1	21.141
2	21.142
3	21.351
* Ref	19.701

Note:

A. *: Directly deliver 0.5 ml of uranine and ethanol into 20 ml ethanol.

B. Conc from calibration=Concentration calculated from calibration curve of ethanol:

LOG(Reading)=1.340227*LOG(Conc)+0.099186

This order is the same as the extraction ability, so that the solvent extraction ability for pure uranine primarily depends on the solubility of uranine in that solvent.

V.C.3. Uranine and Oleic Acid Extraction Test

V.C.3.a. Glass Fiber Filter

From Table 19 to 22 we find that there is a very small amount of uranine left in the third washing steps of all the solvents. If we compare Table 19 to 22 and Table 15 to 18, we find that because uranine may be covered by a layer of oleic acid, the extraction ability of all the solvents is lowered, but not significantly so. However, the extraction ability of distilled water, 0.001 N sodium hydroxide, and 0.01 N sodium hydroxide is still higher than that of ethanol. This result implies that although ethanol is completely miscible with oleic acid, its extraction ability still depends on solubility of uranine.

A typical chamber test in section IV.D is illustrated in Table 23. The sampled glass fiber filters of isokinetic samplers are placed into small jars containing 20 ml of distilled water and in an ultrasonic bath for 30 minutes. We repeat the same step 3 times. Table 23 reveals that two ultrasonic washes can wash out nearly all the uranine on the glass fiber filters by using distilled water as solvent.

Table 19. Uranine and Oleic Acid on Glass Fiber Filters Extracted by Distilled Water

(1) First Wash

Sample No	SLM Reading	Conc From Calibration (μg/ml)	Solution Volume (ml)	Uranine Weight (μg)	Weight Percent (%)
1	2.96	1.0838	20	21.677	96.13
2	2.98	1.0911	20	21.821	97.30
3	3.01	1.1019	20	22.037	95.92
* Ref	2.91	1.0658	20.5	21.849	---

(2) Second Wash

Sample No	SLM Reading	Conc From Calibration (μg/ml)	Solution Volume (ml)	Uranine Weight (μg)	Weight Percent (%)
1	0.108	0.0417	20	0.833	3.70
2	0.078	0.0303	20	0.605	2.70
3	0.119	0.0458	20	0.917	3.99

(3) Third Wash

Sample No	SLM Reading	Conc From Calibration (μg/ml)	Solution Volume (ml)	Uranine Weight (μg)	Weight Percent (%)
1	0.0049	0.0020	20	0.040	0.18
2	Negative	0.0000	20	0.000	0.00
3	0.0026	0.0011	20	0.021	0.09

(4) Total

Sample No	Uranine Weight (μg)
1	22.550
2	22.426
3	22.976
* Ref	21.849

Note:

A. *: Directly deliver 0.5 ml of uranine, oleic acid, and ethanol into 20 ml distilled water.

B. Conc from calibration=Concentration calculated from calibration curve of distilled water with buffer:

LOG(Reading)=1.016091*LOG(Conc)+0.435761

Table 20. Uranine and Oleic Acid on Glass Fiber Filters Extracted by 0.001 N Sodium Hydroxide

(1) First Wash

Sample No	SLM Reading	Conc From Calibration (μg/ml)	Solution Volume (ml)	Uranine Weight (μg)	Weight Percent (%)
1	3.08	0.9995	20	19.991	96.76
2	3.20	1.0370	20	20.740	96.27
3	3.16	1.0245	20	20.490	95.72
* Ref	3.09	1.0027	20.5	20.555	---

(2) Second Wash

Sample No	SLM Reading	Conc From Calibration (μg/ml)	Solution Volume (ml)	Uranine Weight (μg)	Weight Percent (%)
1	0.0904	0.0335	20	0.670	3.24
2	0.1070	0.0394	20	0.788	3.66
3	0.1220	0.0447	20	0.894	4.18

(3) Third Wash

Sample No	SLM Reading	Conc From Calibration (μg/ml)	Solution Volume (ml)	Uranine Weight (μg)	Weight Percent (%)
1	Negative	0.0000	20	0.000	0.00
2	0.0017	0.0007	20	0.015	0.07
3	0.0025	0.0011	20	0.021	0.10

(4) Total

Sample No	Uranine Weight (μg)
1	20.661
2	21.543
3	21.406
* Ref	20.555

Note:

A. *: Directly deliver 0.5 ml of uranine, oleic acid, and ethanol into 20 ml 0.001 N sodium hydroxide.

B. Conc from calibration=Concentration calculated from calibration curve of 0.001 N sodium hydroxide:

$$LOG(Reading)=1.039163*LOG(Conc)+0.488755$$

Table 21. Uranine and Oleic Acid on Glass Fiber Filters Extracted by 0.01 N Sodium Hydroxide

(1) First Wash

Sample No	SLM Reading	Conc From Calibration (µg/ml)	Solution Volume (ml)	Uranine Weight (µg)	Weight Percent (%)
1	3.27	1.0780	20	21.560	95.64
2	3.26	1.0748	20	21.497	95.75
3	3.25	1.0716	20	21.433	96.01
* Ref	3.14	1.0366	20.5	21.249	---

(2) Second Wash

Sample No	SLM Reading	Conc From Calibration (µg/ml)	Solution Volume (ml)	Uranine Weight (µg)	Weight Percent (%)
1	0.130	0.0477	20	0.955	4.23
2	0.126	0.0463	20	0.926	4.13
3	0.121	0.0445	20	0.891	3.99

(3) Third Wash

Sample No	SLM Reading	Conc From Calibration (µg/ml)	Solution Volume (ml)	Uranine Weight (µg)	Weight Percent (%)
1	0.0033	0.0014	20	0.027	0.12
2	0.0033	0.0014	20	0.027	0.12
3	Negative	0.0000	20	0.000	0.00

(4) Total

Sample No	Uranine Weight (µg)
1	22.542
2	22.450
3	22.323
* Ref	21.249

Note:

A. *: Directly deliver 0.5 ml of uranine, oleic acid, and ethanol into 20 ml 0.01 N sodium hydroxide.

B. Conc from calibration=Concentration calculated from calibration curve of 0.001 N sodium hydroxide:

$$LOG(Reading)=1.034539*LOG(Conc)+0.480797$$

Table 22. Uranine and Oleic Acid on Glass Fiber Filters Extracted by Ethanol

(1) First Wash

Sample No	SLM Reading	Conc From Calibration (μg/ml)	Solution Volume (ml)	Uranine Weight (μg)	Weight Percent (%)
1	2.31	0.9777	20	19.555	92.85
2	2.39	1.0111	20	20.223	93.82
3	2.36	0.9986	20	19.972	91.82
* Ref	2.33	0.9861	20.5	20.215	---

(2) Second Wash

Sample No	SLM Reading	Conc From Calibration (μg/ml)	Solution Volume (ml)	Uranine Weight (μg)	Weight Percent (%)
1	0.168	0.0737	20	1.474	7.00
2	0.148	0.0650	20	1.301	6.03
3	0.197	0.0862	20	1.725	7.93

(3) Third Wash

Sample No	SLM Reading	Conc From Calibration (μg/ml)	Solution Volume (ml)	Uranine Weight (μg)	Weight Percent (%)
1	0.0035	0.0016	20	0.032	0.15
2	0.0034	0.0016	20	0.031	0.15
3	0.0059	0.0027	20	0.054	0.25

(4) Total

Sample No	Uranine Weight (μg)
1	21.061
2	21.555
3	21.751
* Ref	20.215

Note:

A. *: Directly deliver 0.5 ml of uranine, oleic acid, and ethanol into 20 ml ethanol.

B. Conc from calibration=Concentration calculated from calibration curve of ethanol:

LOG(Reading)=1.340227*LOG(Conc)+0.099186

Table 23. An Example of Glass Fiber Filters Extracted by Distilled Water

(1) First Wash

Sample No	SLM Reading	Conc From Calibration (μg/ml)	Solution Volume (ml)	Uranine Weight (μg)
1	2.85	1.0442	20	20.884
2	2.98	1.0911	20	21.821
3	3.04	1.1127	20	22.253
4	3.05	1.1163	20	21.325

(2) Second Wash

Sample No	SLM Reading	Conc From Calibration (μg/ml)	Solution Volume (ml)	Uranine Weight (μg)
1	0.1060	0.0409	20	0.818
2	0.1090	0.0421	20	0.841
3	0.0578	0.0225	20	0.451
4	0.0127	0.0051	20	0.101

(3) Third Wash

Sample No	SLM Reading	Conc From Calibration (μg/ml)	Solution Volume (ml)	Uranine Weight (μg)
1	Negative	0.0000	20	0.000
2	0.0018	0.0007	20	0.015
3	Negative	0.0000	20	0.000
4	0.0033	0.0013	20	0.027

(4) Total

Sample No	Uranine Weight (μg)
1	21.702
2	22.677
3	22.704
4	22.454

Note: Conc from calibration=Concentration calculated from calibration curve of distilled water with buffer:

$$LOG(Reading)=1.016091*LOG(Conc)+0.435761$$

From previous experiments 0.001 N sodium hydroxide and 0.01 N sodium hydroxide aqueous solutions should have the same performance as distilled water.

Generally, two continuous ultrasonic washes can wash out nearly all uranine and oleic acid particles deposited on the glass fiber filters by using distilled water , 0.001 N sodium hydroxide, or 0.01 N sodium hydroxide.

V.C.3.b. Teflon Filter

Because Teflon filters do not have flat surfaces, it is impossible to deliver uranine and oleic acid directly on their surfaces. A typical chamber test in section IV.D is shown in Table 24. The sampled Teflon Filters of Portable Indoor Particulate Samplers (PIPS) are put into small jars containing 20 ml of distilled water and in an ultrasonic bath for 30 minutes. We repeat the same step twice. Table 24 shows that by using distilled water, one ultrasonic wash will wash out all the uranine on Teflon filters. Distilled water cannot penetrate Teflon filters; consequently, Teflon filters always float face down on the water. However, distilled water may have better extraction ability for Teflon filters than for glass fiber filters because uranine and oleic acid particles deposit only on the surface of Teflon filters, whereas some uranine and oleic acid particles deeply penetrate Glass fiber filters. Again, the

Table 24. An Example of Teflon Filters Extracted by Distilled Water

(1) First Wash

Sample No	SLM Reading	Conc From Calibration (μg/ml)	Solution Volume (ml)	Uranine Weight (μg)
1	1.53	0.5661	20	11.322
2	1.70	0.6280	20	12.559

(2) Second Wash

Sample No	SLM Reading	Conc From Calibration (μg/ml)	Solution Volume (ml)	Uranine Weight (μg)
1	Negative	0	20	0
2	Negative	0	20	0

(3) Total

Sample No	Uranine Weight (μg)
1	11.322
2	12.559

Note: Conc from calibration=Concentration calculated from calibration curve of distilled water with buffer:

LOG(Reading)=1.016091*LOG(Conc)+0.435761

extraction ability depends on the solubility; thus, 0.001 N and 0.01 N sodium hydroxide aqueous solutions should have the same performance as distilled water.

Generally, one ultrasonic wash using distilled water, 0.001 N sodium hydroxide, or 0.01 N sodium hydroxide can wash out all uranine and oleic acid particles deposited on Teflon filters.

V.C.4. Conclusions of Filter Extraction Test

a. The extraction ability of a solvent depends on the solubility of uranine in that solvent. Hence, distilled water, 0.001 N sodium hydroxide, and 0.01 N sodium hydroxide have high extraction ability. However, ethanol has low extraction ability.
b. Distilled water is a suitable solvent for a Teflon filter wash because it has high extraction ability and no background reading. However, distilled water may not be an optimal choice for a glass fiber filter wash because it does have some background reading.
c. Both 0.001 N and 0.01 N sodium hydroxide aqueous solutions are suitable solvents for both Teflon filter and glass fiber filter washes because these solutions have high extraction ability and no background reading.
d. Ethanol is not a suitable solvent for both Teflon filter and glass fiber filter washes because it has a fair

extraction ability and high background reading with both filters.

e. Extracting uranine from Teflon filters is easier than from glass fiber filters.

V.D. Inference of Inlet Wash

Sampler inlets are made of many materials (e.g., aluminum, stainless steel, plastic, etc.). Because sodium hydroxide is a very strong solvent, it may react with many inlet surfaces. For example, in inorganic chemistry, aluminum strongly reacts with sodium hydroxide and the solution darkens. This reaction will interfere with the fluorescence measurement. VanOsdell et al. (1990) described high background problems when they calibrated PEM and MEM made of aluminum by using 0.01 sodium hydroxide. This problem is particularly serious when the concentration of uranine and oleic acid particles collected is low. Other materials may be eroded by sodium hydroxide. Some materials may also react with ethanol.

From the discussion of the filter extraction test, it is known that the ability of a solvent to extract uranine and oleic acid from a filter surface depends on the solubility of uranine in that solvent. Distilled water has a high extraction ability and will not react with common inlet surface. Thus, distilled water is the best solvent

and the only solvent suitable for inlet wash.

V.E. Portable Indoor Particulate Sampler (PIPS) and Saturation Monitor Determination

V.E.1. Results and Discussion

The results of the 10 μm cut-point PIPS efficiency tests are presented in Table 25 and Figure 12. After the experiments, the EMSI pumps and the mass flow meters were calibrated by a root meter (Model 3M125 CTR, Dresser Industries Inc., Houston, TX) and the results are set forth in Table 27. Table 27 shows that both real flow rates of EMSI pumps are higher than the expected 10 l/m flow. The flow errors of PIPS No 1 and PIPS No 2 are about 3% and 6%. From Figure 12 the estimated cut-points of PIPS No 1 and No 2 are 10.0 μm and 10.2 μm respectively. The estimated cut-point of PIPS from Marple's data is about 9.3 μm. Although the measured cut-points of PIPS are higher than Marple's measured cut-point, the measured collection efficiency of 10 μm cut-point PIPS is still very close to that of Marple's data.

The results of the 2.5 μm cut-point PIPS efficiency tests are presented in Table 26 and Figure 13. Because the pumps used are the same as 10 μm cut-point PIPS used, both real flow rates are higher than the expected 10 l/m flow.

Table 25. Collection Efficiency of 10 μm PIPS

Aerodynamic Diameter (μm)	Collection Efficiency (%) PIPS No 1	PIPS No 2	Marple's Data
6.05	9.7	5.8	---
6.96	10.0	8.8	---
7.91	---	---	17.9
8.93	23.8	24.0	---
9.78	38.8	38.3	---
9.85	---	---	67.7
10.12	---	---	68.9
10.37	---	---	80.9
10.43	68.8	65.6	---
11.08	87.4	85.9	---
11.70	---	---	94.8
13.09	96.7	95.2	---
15.12	98.5	98.3	---
25.33	99.7	100.0	---

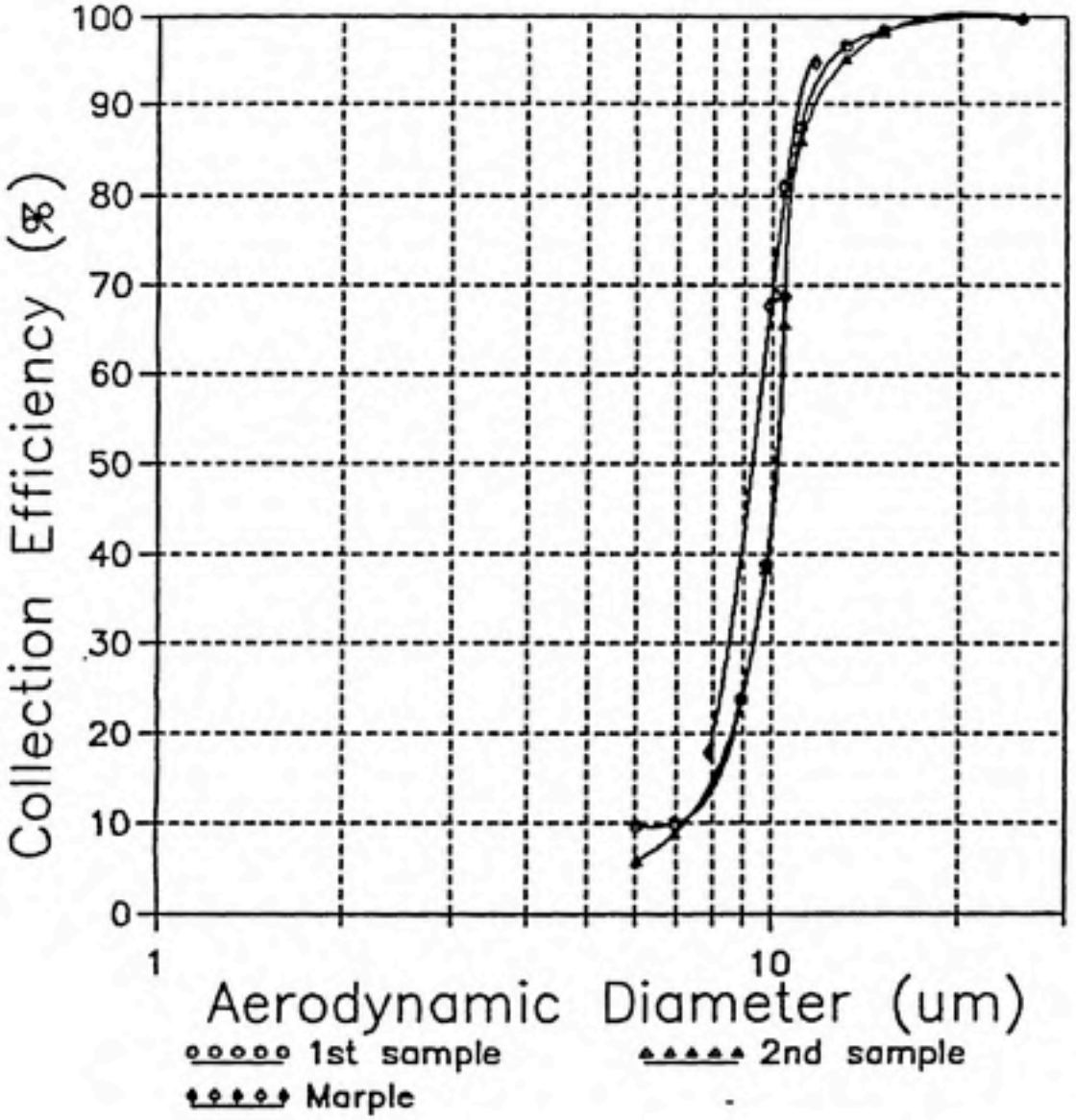

Figure 12. Collection Efficiency of 10 μm PIPS

Table 26. Collection Efficiency of 2.5 μm PIPS

Aerodynamic Diameter (μm)	Collection Efficiency (%) PIPS No 1	PIPS No 2	Marple's Data EMSI Pump	Vaccum Pump
1.46	---	---	8.5	---
1.47	---	---	---	10.8
1.49	1.7	6.3	---	---
1.94	---	---	---	23.4
2.03	7.5	7.2	---	---
2.27	18.6	19.8	---	---
2.29	---	---	---	54.3
2.31	---	---	40.7	---
2.45	---	---	47.5	---
2.50	---	---	62.0	---
2.51	---	---	---	80.3
2.54	24.9	24.8	---	---
2.55	---	---	70.6	---
2.72	---	---	84.6	---
2.82	53.2	52.7	---	---
2.97	---	---	---	97.7
3.02	---	---	96.5	---
3.06	91.9	92.4	---	---
3.59	100.0	100.0	---	---

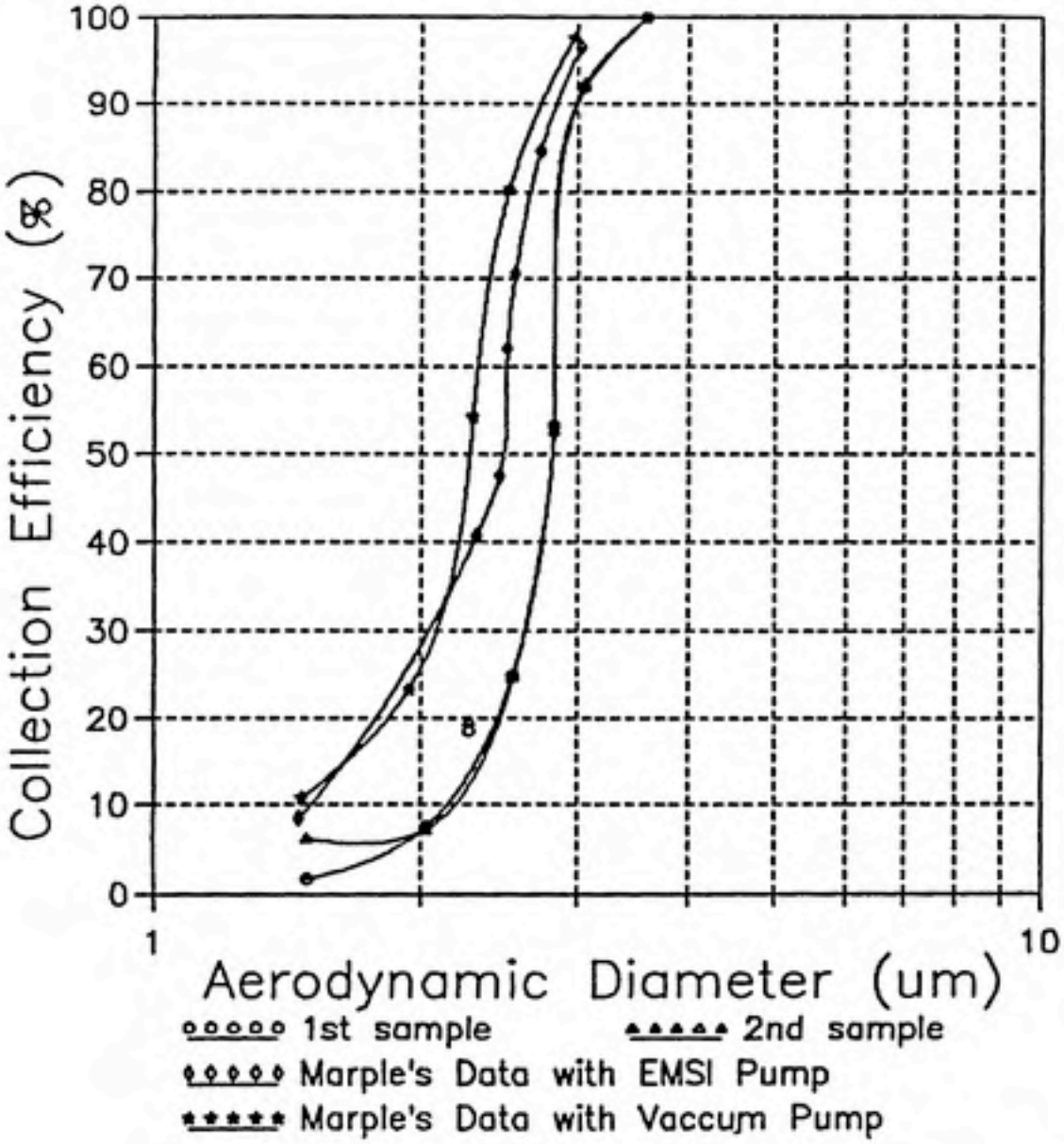

Figure 13. Collection Efficiency of 2.5 μm PIPS

Table 27. Calibration of the EMSI Pumps and Mass Flow Meters

	Flow on Mass Flow Meter (l/min)	Flow Measured by Root meter (l/min)
PIPS No 1	----	10.31
PIPS No 2	----	10.64
Saturation Monitor No 1	5.00	5.13
Saturation Monitor No 2	5.02	5.65

From Figure 13 both estimated cut-points of PIPS No 1 and No 2 are about 2.8 μm. The estimated cut-points of PIPS from Marple's data are 2.25 μm by using the vacuum line and 2.5 μm by using the EMSI pump. The collection efficiency of 2.5 μm cut-point PIPS has larger errors than that of 10 μm PIPS. This phenomenon will be discussed later. Again, although the measured cut-points of PIPS are higher than Marple's measured cut-points, the measured collection efficiency of 2.5 μm cut-point PIPS is still relatively close to that of Marple's data.

The results of the 10 μm cut-point Saturation Monitor efficiency tests are presented in Table 28 and Figure 14. Table 27 shows that both real flow rates are higher than the displayed flow rates on mass flow meters. The flow errors of Saturation Monitor No 1 and No 2 are about 2.6% and 13%. From Figure 14 the estimated cut-points of Saturation Monitor No 1 and No 2 are 13.0 μm and 12.7 μm respectively. The collection efficiency of Saturation No 2 is higher than that of Saturation Monitor No 1 because Saturation Monitor No 2 has higher flow rates. From the analysis above we know that the cut-point of the Saturation Monitor is around 13 μm. There is a significant difference between the measured cut-point and the manufacture's claimed cut-point of the Saturation Monitor.

The collection efficiency of PIPS and Saturation Monitors can be expressed as the following:

Table 28. Collection Efficiency of 10 μm Saturation Monitor

Aerodynamic Diameter (μm)	Collection Efficiency (%) Saturation Monitor No 1	Saturation Monitor No 2
6.05	11.8	11.6
6.96	14.1	20.5
8.93	21.2	21.4
9.78	23.6	24.8
10.43	30.3	31.5
11.08	33.6	37.6
13.09	53.3	55.7
15.12	77.6	80.7
25.33	99.9	98.6

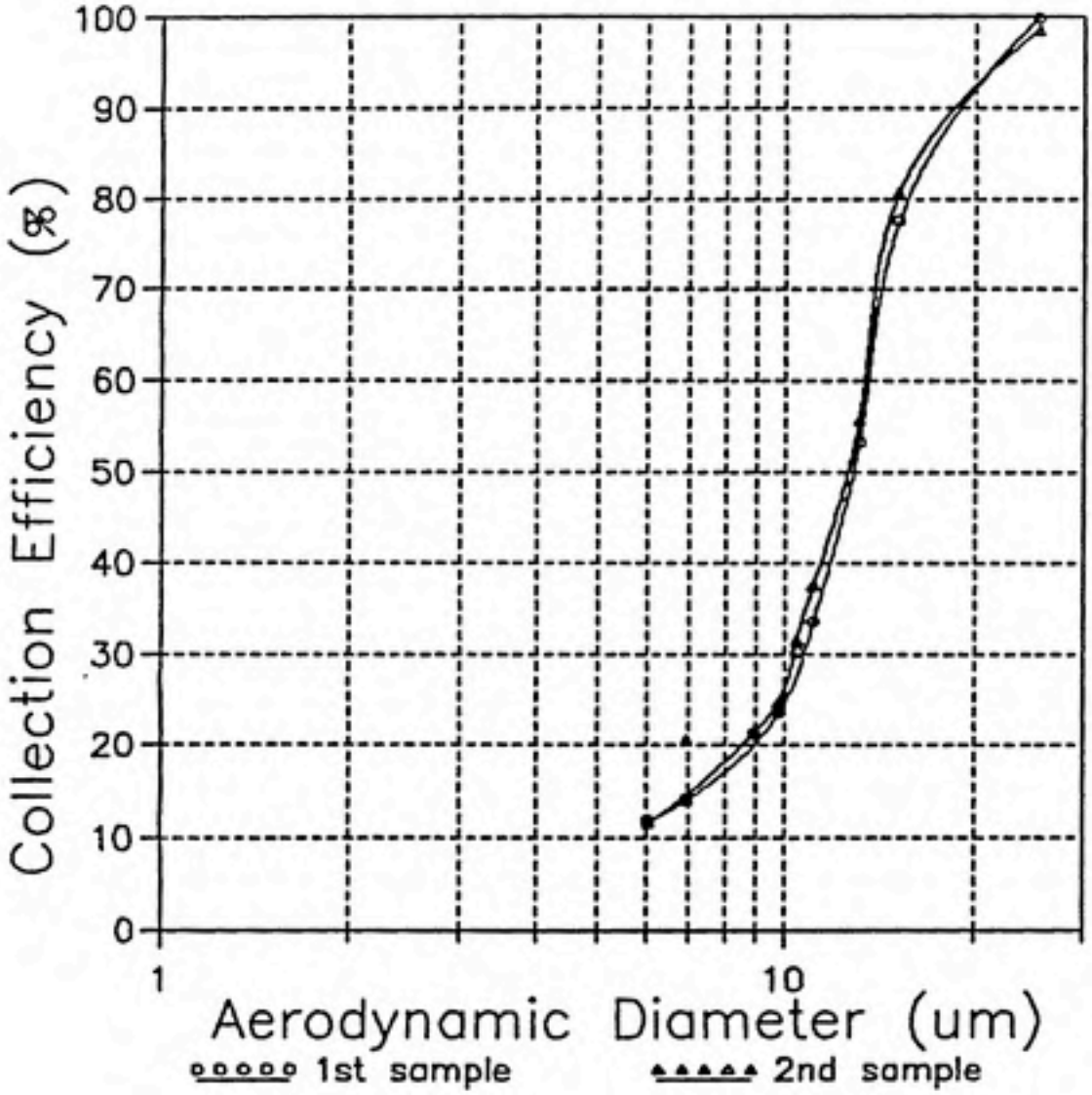

Figure 14. Collection Efficiency of 10 μm Saturation Monitor

$$\epsilon=(U_i+U_b)/(U_i+U_b+U_f)$$

In this equation, ϵ is the collection efficiency, U_i is the uranine deposited on the impaction plate in μg, U_b is the uranine deposited on the body in μg, and U_f is the uranine deposited on the filter in μg. U_f will increase with a decrease in the particle size, whereas U_i and U_b will decrease with a decrease in the particle size. From the section V.C, the background of glass fiber filters and Teflon filters in distilled water is extremely low. In addition distilled water can wash out all the uranine on these filters. Hence, U_f can be measured accurately. The accuracy of collection efficiency primarily depends on the accuracy of the total amount of uranine collected on the impaction plate and on the body.

At the same time, when the particle size decreases, the aerosol concentration in the test chamber will also decrease because the oleic acid and uranine in the particle solution decrease. This phenomenon becomes serious when particle size is below 5 μm. The uranine collected on the impaction plate and on the body will approach the background reading. In addition, from section V.A.1.b.i, the accuracy of the SLM Aminco Fluoro-Colorimeter drops dramatically below 0.005 $\mu g/ml$. Hence, that decrease in particle size will cause errors.

In our washing procedure, although distilled water is

used instead of sodium hydroxide, a very low background reading still occurs for the impaction plate. Moreover, in order to wash out the uranine deposited on the body, cotton swabs are used. These cotton swabs are cut and put in an ultrasonic bath for 20 minutes to ensure washing out all the uranine in the swabs. The average background reading of the swabs in distilled water is approximately 0.007. This procedure also increases the background of body wash.

From Figure 12 and 14 the collection efficiency of 10 μm cut-point PIPS and Saturation Monitors produces some errors at 6.05 and 6.96 μm particle sizes because of the reasons elaborated above. In calibrating 2.5 μm cut-point PIPS, aerosol concentration is extremely low. Uranine collected on the body approaches the background reading with particle sizes below 2.5 μm. At particle sizes 1.49 and 2.03 μm, both the uranine collected on the body and impaction plate approaches background reading, and large errors occur. To reduce the errors, the sampling time has to be increased, so that the uranine collected on the body and plate will be much greater than the background.

V.E.2. Conclusions

a. Two tested 10 μm cut-point PIPS have cut-points of 10.0 μm and 10.2 μm.
b. The tested 2.5 μm cut-point PIPS has a cut-point of 2.8

μm.

c. Two tested Saturation Monitors have cut-points of 13.0 μm and 12.7 μm.

d. The error of collection efficiency will increase with a decrease in the particle size. The error in calibrating the 2.5 μm cut-point sampler is much higher than that in calibrating the 10 μm cut-point sampler.

VI. SUMMARY AND RECOMMENDATIONS

VI.A. Summary and Optimal Washing Solutions

In the Sensitivity Test Section, the fluorescence intensity of uranine solutions and the linear relationship between fluorescence intensity and the concentration of solutions is compared. The order of sensitivity of uranine solutions can be expressed as:

[Distilled water with buffer
0.001 N sodium hydroxide
0.01 N sodium hydroxide
0.1 N sodium hydroxide] > [Ethanol with pH 10 Buffer]

> [Distilled water] > [Ethanol]

In the Interference Test Section, the interference of oleic acid in different uranine solutions is tested. There is no interference of oleic acid in uranine and distilled water or uranine and sodium hydroxide solutions under our operating conditions. There is a significant quenching effect of oleic acid in uranine and ethanol solution.

However, adding pH 10 buffer in uranine and ethanol solution can prevent this quenching effect.

In the Filter Extraction Section, the ability of different washing solutions to extract oleic acid and uranine is investigated. The extraction ability of a solution depends on the solubility of uranine in that solution. Hence, distilled water, 0.001 N sodium hydroxide, and 0.01 N sodium hydroxide have high extraction ability. However, ethanol has low extraction ability.

The results discussed above can be summarized as:

	Sensitivity	Interference Effect	Extraction Ability
Distilled Water	Fair	No	High
Distilled Water with pH 10 Buffer	High	No	High
0.001 N Sodium Hydroxide	High	No	High
0.01 N Sodium Hydroxide	High	No	High
0.1 N Sodium Hydroxide	High	No	Fair
Ethanol	Low	Yes	Low
Ethanol with pH 10 Buffer	Fair	No	Low

The sensitivity of uranine solutions, the interference of oleic acid in uranine solutions, and the extraction ability of washing solutions determine the optimal washing solutions in the fluorescence-washing technique. Therefore, distilled water with pH 10 buffer, 0.001 N sodium hydroxide, and 0.01

N sodium hydroxide are the best choices.

In the Filter Extraction Section, the background readings of glass fiber filters and Teflon filters in different pure solvents are also checked. There are no background readings using glass fiber filters and Teflon filters in both 0.001 N and 0.01 N sodium hydroxide solutions. There is no background reading with Teflon filters but a very low background reading with glass fiber filters in distilled water. There are high background readings for both glass fiber filters and Teflon filters in ethanol.

In the Inference of Inlet Wash Section, we state that because sodium hydroxide either strongly reacts with aluminum and the solution darkens or erodes other materials, distilled water is the best choice for inlet wash. The recommended uses of distilled water, 0.001 N sodium hydroxide, and 0.01 N sodium hydroxide can be summarized as:

	Glass Fiber Filter Wash	Teflon Filter Wash	Inlet Wash
Distilled Water with pH 10 Buffer	Not Recommend	Recommend	Recommend
0.001 N Sodium Hydroxide	Recommend	Recommend	Not Recommend
0.01 N Sodium Hydroxide	Recommend	Recommend	Not Recommend

VI.B. Recommendations

1. The UG-1 primary filter used in the SLM Aminco Fluoro-Colorimeter II can only pass 1% of the incident light which uranine solutions absorb. Other filters with appropriate range should replace the UG-1 Filter.
2. The background readings of glass fiber filters and Teflon filters in distilled water and sodium hydroxide are only applied to those filters made by Gelman Scientific Incorporated. The background readings of glass fiber filters and Teflon filters of other brands or the background readings of other kinds of filters should be checked before used. The background reading of the inlet or impaction plate in distilled water should also be checked before used.

VI.C. Standard Operating Protocol

VI.C.1. Operating the SLM Aminco Fluoro-Colorimeter II

(1) Turn on the SLM Aminco Fluoro-Colorimeter II (ON/OFF switch located on rear panel).

(2) Activate the lamp by momentarily lifting up the Lamp Starter Switch located on the rear panel. Release the switch when the translucent dot on the lamp cap brightens.

(3) Set the high voltage to 550 V:

(a) Press DATA key to turn on the high voltage.

(b) Press HV key and the status displays 250 V.

(c) Increase to 550 V by pressing the increase CHANGE key.

(d) Press GAIN key.

(e) Press DATA and SINGLE keys to turn off the high voltage.

(5) Allow 30 minutes for the fluorometer to stabilize.

(6) Offset the dark current:

(a) Press DATA and SINGLE keys to turn on the high voltage.

(b) Press OFFSET key to subtract the dark current.

(c) Press DATA and SINGLE keys to turn off the high voltage.

(7) Take off the cell adapter cap. Hold the upper edge of the test cuvette. Use a tissue to wipe away any dust on the outer surface of the cuvette. Insert the cuvette into the cell adapter. Replace the cell adapter cap.

(8) Press DATA and SINGLE keys to get the reading.

(9) Press DATA and SINGLE keys to turn off the high voltage.

(10) Take off the cell adapter cap and remove the test cuvette. Replace the cell adapter cap.

(11) Repeat steps (7) to (10) for the next test cuvette.

(12) Repeat step (6) after a series of test samples.

VI.C.2. Measuring the Fluorescence

(1) Use a clean tissue to hold a pipet. Put a rubber head on the top of the pipet. With the other hand, hold a cuvette with a tissue. Pipet about 3.3 ml washing solution into cuvettes (the levels of the washing solution in cuvettes reach the sides of the tube stand).

(2) Follow step (3) if the cuvettes contain 0.001 N sodium hydroxide or 0.01 N sodium hydroxide. Follow steps (4) and (5) if the cuvettes contain distilled water.

(3) Analyze the cuvettes in step (1) fluorometrically following the steps in VI.C.1.

(4) Add 50 µl pH 10 buffer into the cuvettes in step (1). Leave these cuvettes in the dark for 20 minutes.

(5) Analyze these cuvettes fluorometrically following the steps in VI.C.1.

VI.C.3. Preparing the Calibration Curves

(1) Weigh 0.1 g uranine on a weighing plate using the balance.

(2) Put the weighing plate containing uranine in a funnel. This funnel pours directly into a 1000 ml flask. Squeeze distilled water, 0.001 sodium hydroxide, or 0.01 N sodium hydroxide from washing bottles to wash all the uranine on the plate into the 1000 ml flask. Prepare 1000 ml of the stock solution at a concentration of 100 µg/ml. Stir the stock solution for 30 minutes.

(3) Dilute 100 ml of 100 µg/ml with solvent to yield 1000 ml of 10 µg/ml. Stir the new solution for 5 minutes.

(4) Dilute 20 ml of 10 µg/ml to yield 100 ml of 2 µg/ml. Dilute 25 ml of 2 µg/ml to yield 100 ml of 0.5 µg/ml. Dilute 20 ml of 0.5 µg/ml to yield 100 ml of 0.1 µg/ml. Dilute 20 ml of 0.1 µg/ml to yield 100 ml of 0.02 µg/ml. Dilute 25 ml of 0.02 µg/ml to yield 100 ml of 0.005 µg/ml. Stir each new solution for 5 minutes.

(5) Dilute 10 ml of 10 µg/ml to yield 100 ml of 1 µg/ml. Dilute 20 ml of 1 µg/ml to yield 100 ml of 0.2 µg/ml. Dilute 25 ml of 0.2 µg/ml to yield 100 ml of 0.05 µg/ml. Dilute 20 ml of 0.05 µg/ml to yield 100 ml of 0.01 µg/ml. Stir each new solution for 5 minutes.

(6) Analyze the solutions following the steps in VI.C.2.

VI.C.4. Filter wash

VI.C.4.a. Gelman Glass Fiber Filter Wash

(1) Use clean forceps to put the tested glass fiber filters, which have oleic acid and uranine on their surfaces, in 2 oz. jars.

(2) Pour 20 ml of 0.001 N sodium hydroxide or 0.01 N sodium hydroxide into each jar. Cap these jars.

(3) Put these jars in the ultrasonic bath for 30 minutes. These jars contain the first washing solutions.

(4) Use clean forceps to take these filters out and put them into other clean jars. Pour 20 ml of 0.001 N sodium hydroxide or 0.01 N sodium hydroxide into each jar. Cap these jars and put them in the ultrasonic bath for 30 minutes. These jars contain the second washing solutions.

(5) Pipet the washing solutions in steps (3) and (4) into cuvettes following step (1) in VI.C.2.

(6) Centrifuge (2600 RPM) these cuvettes for 10 minutes.

(7) Analyze these washing solutions following step (3) in VI.C.2.

VI.C.4.b. Gelman Teflon Filter Wash

(1) Use clean forceps to put the tested Teflon filters, which have oleic acid and uranine on their surfaces, in 2 oz. jars.

(2) Pour 20 ml of distilled water, 0.001 N sodium hydroxide or 0.01 N sodium hydroxide into each jar. Cap these jars.

(3) Put these jars in the ultrasonic bath for 30 minutes.

(4) Pipet these washing solutions into cuvettes following step (1) in VI.C.2.

(5) Centrifuge (2600 RPM) these cuvettes for 10 minutes.

(6) Analyze these washing solutions following steps (2) to (5) in VI.C.2.

VI.C.4.c. Other Kinds of Filter Wash

VI.C.4.c.i. Background Test

(1) Use clean forceps to put nine blank filters in nine different 2 oz. jars.
(2) Pour 20 ml of distilled water into three jars. Pour 20 ml of 0.001 N sodium hydroxide into three jars. Pour 20 ml of 0.01 N sodium hydroxide into three jars.
(3) Put these jars in the ultrasonic bath for 1 hour.
(4) Pipet these solutions into cuvettes following step (1) in VI.C.2.
(5) Centrifuge (2600 RPM) these cuvettes for 10 minutes.
(6) Analyze these washing solutions following steps (2) to (5) in VI.C.2.
(7) Select the solution with the lowest background reading as the washing solution.

VI.C.4.c.ii. Filter Wash Test

(1) Use clean forceps to put the tested filters, which have oleic acid and uranine on their surfaces, in 2 oz. jars.
(2) Pour 20 ml of the washing solution selected in VI.C.4.c.i into each jar. Cap these jars.
(3) Put these jars in the ultrasonic bath for 30 minutes. These jars contain the first washing solutions.

(4) Use clean forceps to take these filters out and put them into other clean jars. Pour 20 ml of the washing solution selected in VI.C.4.c.i into each jar. Cap these jars and put them in the ultrasonic bath for 30 minutes. These jars contain the second washing solutions.

(5) Again, use clean forceps to take these filters out and put them into other clean jars. Pour 20 ml of the washing solution selected in VI.C.4.c.i into each jar. Cap these jars and put them in the ultrasonic bath for 30 minutes. These jars contain the third washing solutions.

(6) Pipet the first, second, and third washing solutions produced in steps (3), (4), and (5) into cuvettes following step (1) in VI.C.2.

(7) Centrifuge (2600 RPM) these cuvettes for 10 minutes.

(8) Analyze these washing solutions following steps (2) to (5) in VI.C.2.

(9) Decide how many times the washes are needed:

(a) If the readings of the second washing solutions are negative, these filters need one 30 minute ultrasonic wash. Follow steps (1) to (3) and (6) to (8) for these filter washes.

(b) If the readings of the third washing solutions are much higher than 0.005, these filters need three sequential ultrasonic washes. Follow steps (1) to

(8) for these filter washes.

(c) Other than the conditions (a) and (b), these filters need two sequential ultrasonic washes. Follow steps (1) to (4) and (6) to (8) for these filter washes.

VI.C.5. Impaction Plate Wash

(1) Get the background of the impaction plate before tested:
   (a) Put the impaction plate into a suitably sized jar.
   (b) Pour an adequate amount of distilled water into the jar and let the distilled water completely cover the impaction plate.
   (c) Cap this jar and put it in the ultrasonic bath for 30 minutes.
   (d) Analyze this solution following steps (1), (4) and (5) in VI.C.2.
   (e) Usually the background of the impaction plate should be less than 0.005. If the background is much higher than 0.005, repeat steps (a) to (d) again.
(2) Take the impaction plate out of the jar. Let it dry completely.
(3) Set this impaction plate in a sampler that collects particles.
(4) Use a clean forceps to take the tested impaction plate out of the sampler after the collection test.
(5) Repeat steps (a) to (d) in (1).

(6) If the reading of the washing solution is much higher than 0.005, use a clean forceps to take the impaction plate out and put it in a new jar. Repeat step (5) until the reading of the washing solution is less than or around 0.005.

(7) Repeat steps (2) to (6) for the next collection test.

VI.C.6. Inlet Wash

VI.C.6.a. Inlet with Holes

(1) Get the inside background of the inlet before being tested:

(a) Use tape to seal the inlet slits. Do not wrinkle the tape. Wrinkling the tape will cause leaks.

(b) Pour an adequate amount of distilled water into a jar.

(c) Hold the taped inlet upside-down.

(d) Dip a clean swab into the jar and swab out the underside of the inlet.

(e) Pour all the solution left in the jar into the inlet through the small holes and shake for 2 minutes.

(f) Cut the head of the cotton swab and put the head into this jar. Drain the washing fluid into this jar and put it in the ultrasonic bath for 20 minutes.

(g) Analyze this solution following steps (1), (4), and (5) in VI.C.2.

(h) Usually the background of the inlet body should be less than 0.01. If the background is much higher than 0.01, repeat steps (a) to (g) again.

(2) Take the tape off the inlet. Let the inlet dry completely.

(3) Set this inlet on a sampler that collects particles.

(4) Take the inlet out of the sampler base after the collection test.

(5) Repeat steps (a) to (g) in (1).

(6) Put the same amount of distilled water in a jar. Use a clean pipet and rinse the inlet by rotating it with the fluid in each section that needs rinsing. Pour the remaining washing solution into the inlet through the small holes and shake for 2 minutes. Completely drain the washing fluid into this jar. (7) Analyze this washing solution following steps (1), (4), and (5) in VI.C.2.

VI.C.6.b. Inlet without Holes

(1) Get the inside background of the inlet before being tested:

(a) Pour an adequate amount of distilled water into a jar.

(b) Hold the inlet upside-down exactly above another jar.

(c) Dip a clean swab into the jar and swab out the underside of the inlet. Let excess solution flow into the jars under the inlet.

(d) Pour all the solution into one jar. Cut the head of the cotton swab and put the head in this jar. Put this jar in the ultrasonic bath for 20 minutes.

(e) Analyze this solution following steps (1), (4), and (5) in VI.C.2.

(f) Usually the background of the inlet body should be less than 0.01. If the background is much higher than 0.01, repeat steps (a) to (e) again.

(2) Let the inlet dry completely.

(3) Set this inlet on a sampler that collects particles.

(4) Take the inlet out of the sampler base after the collection test.

(5) Repeat steps (a) to (e) in (1).

(6) Put the same amount of distilled water in a jar. Use a clean pipet and rinse the inlet by rotating it with the fluid in each section that needs rinsing. Pour the remaining washing solution into the inlet through the small holes and shake for 2 minutes. Completely drain the washing fluid into this jar.

(7) Analyze this washing solution following steps (1), (4), and (5) in VI.C.2.

VI.C.7. Caution

(1) Ensure that the high voltage of the SLM Aminco Fluoro-Colorimeter II is off (DATA key is off) whenever changing the cuvettes. This helps prevent PMT fatigue.

(2) The background of distilled water with buffer, 0.001 N sodium hydroxide, and 0.01 N sodium hydroxide must be checked before being used. The background reading should be negative. Otherwise, these solutions are contaminated and should be replaced.

(3) Do not directly touch the pipet body or the cuvette body except with a tissue.

(4) Fluorometry is a very sensitive technique. Very little contamination will cause large experimental errors. Whenever your hands touch the uranine, wash them immediately. Wearing gloves all the time is a wise choice.

(5) All equipment that touches uranine solutions should be washed with water before put into detergent solution. Do not directly put used equipment into detergent solution.

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