Investigation of reagent distributions on glass fiber membrane filters used in air sampling†

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This project has arisen from the need to produce GFFs (glass fiber filters) bearing a thin and evenly distributed coating of a selected reagent in the equatorial plane for breakthrough studies. However, it has been discovered that today's two general techniques for coating GFFs (total immersion and application of reagent solution to GFFs) have usually produced unevenly distributed coatings of reagent in the equatorial plane. In addition, quantities of reagent on GFFs from commercial sources may vary widely in the same lot of coated GFFs. Consequences are variability in capacity of coated filters at the point of breakthrough and, perhaps, wasted reagent. Although today's reagent-coated filters may be satisfactory for routine air sampling, such filters may be unacceptable for precise breakthrough studies. Research has been conducted successfully to produce nearly evenly distributed coatings of reagents in the equatorial plane of GFFs by application of reagent solutions to the centers of GFFs which are resting on crisscrossing, fine, stainless-steel wire. Distributions of coatings have been determined by punching out twenty-one 5-mm circles from each GFF and analyzing each circle by flow-injection with a UV detector. Lowest achievable relative standard deviations of measurement (RSDs) for reagents in 5-mm circles have been 5 to 7%. Reagents studied have included 1-(2-pyridyl)piperazine (1-2PP), 2,4-dinitrophenylhydrazine (DNPH), and 1-(9-anthracenylmethyl)piperazine (MAP). Factors affecting the distribution of such coatings include choice of reagent and choice of solvent for the reagent solution.

Introduction

For several decades, reagent-coated glass fiber membrane filters (GFFs), including those which are 37 mm in diameter, have been employed for air sampling for reactive compounds. The reagents of interest will react with reactive air contaminants to form stable derivatives that can be measured in the laboratory.[1–21] Reactive air contaminants that can be stabilized with reagent-coated filters include isocyanates, aldehydes, and carboxylic acid anhydrides.

1-(2-Pyridyl)piperazine (1-2PP), acidified 2,4-dinitrophenylhydrazine (DNPH), and 1-(9-anthracenylmethyl)piperazine (MAP), are among the many reagents that have been employed for coating 37-mm GFFs to stabilize reactive air contaminants during active air sampling. See Fig. 1 for structures. Phosphoric acid is the usual acid present as a catalyst when GFFs are coated with DNPH, as shown in Fig. 1. Not only is 1-2PP used in Occupational Safety and Health Administration (OSHA) methods for at least eight different isocyanates,[1–6] it is also employed in a method for stabilizing acetic anhydride during air sampling.[11] Acidified DNPH is useful for stabilizing aldehydes (formaldehyde, valeraldehyde, crotonaldehyde, and glutaraldehyde) on GFFs during air sampling.[15–19] Both 1-2PP and DNPH are well-known reagents for coating GFFs.

Although MAP is less widely known than 1-2PP and DNPH, MAP is mentioned here because (1) this compound is synthesized in our laboratory as a very effective reagent for stabilizing isocyanates during air sampling, (2) NIOSH method 5525 for total isocyanates employs MAP as the reagent of choice for the coated GFFs,[10] and (3) a new project on breakthrough studies employing MAP-coated GFFs has been conceived. This new project requires that the GFFs have a thin

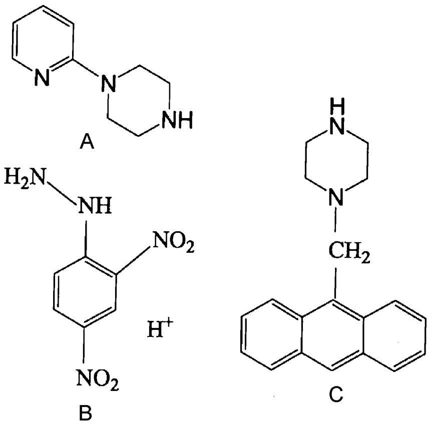

Fig. 1 Molecular structures for the reagents, 1-2PP (A), protonated DNPH (B), and MAP (C).

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but evenly distributed coating of MAP. The requirement for a thin coating stems from the fact that artifacts will develop during storage of MAP, artifacts that will raise the detection limits of isocyanate derivatives. Thus, the level of artifacts will be low when the level of MAP is low; consequently, the detection limits will be lower.

Other important reagents for stabilizing reactive compounds on GFFs during air sampling include 1-(2-methoxyphenyl)piperazine (2MP) and 9-(methylaminomethyl)-anthracene (MAMA).[9,30] Examination of the distributions of these reagents as well as other reagents on GFFs would be of interest. However, these distributions on GFFs have not been studied due to time constraints.

Consideration of this new project with MAP has led to the discovery that commercially available reagent-coated GFFs are generally characterized by uneven distributions of reagents in the equatorial plane. In addition, quantities of reagent on whole GFFs can vary widely from filter to filter in the same lot. Consequences of both (a) a variability in the degree of unevenness of reagent distribution in the equatorial plane and (b) a wide variability in the total quantity of reagent from GFF to GFF are variability in the capacity of the GFFs at the point of breakthrough and, perhaps, wasted reagent.

A review of the literature reveals there is no widely accepted, standard technique for coating GFFs with reagents. One common technique is total immersion of the filters, withdrawing the filters and letting the filters dry.[8,9,15,22–24] Another common technique is application of a known volume of reagent solution to the filters. Usually, a support for the filters during application of the reagent solution is not described.[1–7,11,13,14,25] A clean, glass plate has served as a support for filters.[12,26,27] In other cases, filters have been supported by 30-mL beakers[16,19,28] and filter cassettes.[29] Sometimes, the technique of coating filters with reagents is not described.[10,30] Results in the present work show that both of these common techniques of coating GFFs can lead to uneven distributions of reagent in the equatorial plane. Needless to say, these results certainly are consistent with the reagent distributions found for commercially available reagent-coated GFFs.

Since it is difficult to investigate axial distributions of reagents on GFFs *per se*, the concept of axial distributions is studied by stacking three GFFs, adding three times the normal volume of reagent solution (for a single GFF) to the stack, and measuring the quantity of reagent on each GFF. It appears that the nature of equatorial distributions of reagents is more important than the nature of axial distributions when the reagent-coated GFFs are employed for reproducible and precise breakthrough studies.

Experimental

Materials

Glass fiber membrane filters, 37 mm in diameter, were obtained from Millipore Corp. and Gelman Sciences, Inc. (Pall Life Sciences, Pall Corp.). Quartz fiber membrane filters, 37 mm in diameter, were obtained from Gelman Sciences, Inc. (Pallflex membrane filters, Pall Life Sciences) and Whatman International Ltd (grade QM-A). GFFs coated with 1-2PP were obtained from SKC, Inc. (SKC catalogue number 225–9013) and Supelco ("ORBO™-80 Isocyanate Filter", catalogue number 20811). GFFs coated with acidified DNPH were obtained from SKC, Inc. ("Glutaraldehyde Sampler", catalogue number 225–9003). The frame of the drying rack for preparing reagent-coated filters, 39.4 cm wide × 39.4 cm long × 2.5 cm deep with legs 5.1 cm high, was constructed by welding stainless steel. Stainless-steel wire (McMaster–Carr, P. O. Box 94930, Cleveland, OH 44101), 0.25 mm in diameter, crisscrossed the frame in a parallel fashion to make squares 2 cm on a side.

MAP, 99 + %, mp = 156.0–158.5 °C, was synthesized and purified.[21] 1-2PP, 98%, and DNPH (97% and containing 30% water) were obtained from Aldrich Chemical Co. DNPH was recrystallized from 95% ethanol by Soxhlet extraction to give red needles, mp = 205.2–208.7 °C. Anthracene, 99.5%, and (±)-phenyl-1,2-ethanediol, 98.7%, were obtained from Chem Service, Inc. Acetonitrile (HPLC grade), methanol (Optima grade), *ortho*-phosphoric acid, 85%, and sodium chloride (certified ACS grade) were obtained from Fisher Scientific. Cyclohexane ("high purity solvent" suitable for GC) was obtained from Burdick & Jackson.

DNPH solutions. DNPH (0.57 g) was mixed with 0.63 mL of phosphoric acid and 25 mL of acetonitrile.[16] The cloudy mixture was filtered with a syringe filter containing a PTFE membrane to give a clear orange–red filtrate (DNPH concentration = 7 mg mL^{-1}). The DNPH solution containing 50% excess acid was prepared by the technique above except 0.94 mL of phosphoric acid was employed (DNPH concentration = 8 mg mL^{-1}).

Investigation of the weight stability of filters. Four 37-mm filters [two GFFs and two quartz fiber filters (QFFs)] were weighed to the nearest 0.1 mg on a six-place balance for initial measurements soon after removal from packaging, and were weighed again after each of the following steps: filters were placed into (1) a desiccator for 6 hours, (2) a 5-L closed container at 75% relative humidity (RH) for 19 hours [water (900 mL) and 500 g of NaCl were on the bottom of the container to make the RH constant at 75% at room temperature][31], and (3) an oven at 116 °C for 1.1 hours. Overall weight changes were calculated.

Minimum volume of liquid to wet a membrane filter (wettability). A series of GFFs of the same weight (to the nearest 0.1 mg) from the same lot was placed onto the drying rack at room temperature. Volumes of liquid in a 500-μL syringe were applied to the filters while the tip of the syringe needle was positioned 1 cm above the center of each filter. The volumes of liquid were changed by 15-μL increments until the minimum volume needed to wet 100% of the filter was found. The progress of wetting filters was perceived easily by visual observation. Evaporation of solvents from GFFs was always conducted in a well-ventilated laboratory.

Coating filters with reagents. A set of membrane filters that weighed the same to the nearest 0.1 mg was placed onto the drying rack in a manner in which no filter touched another.

A 500-μL or 1000-μL syringe was loaded with the desired volume of reagent solution. The tip of the syringe needle was positioned 1 cm above the center of each filter (grid side down), and the reagent solution was applied to the filter over a 2–3 second period. Each filter was allowed to dry for 1 hour or more on the drying rack.

For total immersion, a membrane filter was placed into 8 mL of reagent solution (MAP or DNPH solution) resting in a watch glass, 8.7 cm in diameter, for 45 seconds, withdrawn from the solution with a pair of forceps, drawn across the lip of a beaker to remove excess solution, and placed onto a horizontal glass plate (borosilicate glass), 20 × 20 cm, for drying.

Flow-injection analysis (FIA). The HPLC was a Waters HPLC component system including a Waters 600-MS System Controller and either a Waters 486 Tunable Absorbance Detector or a Shimadzu SPD-10AV VP UV-Vis Detector. Appropriate wavelengths for detection of reagents were selected from UV spectra of the reagents in solution. Solvents for these solutions were the same as those which made up the mobile phase for FIA.

FIA of 1-2PP samples. Twenty-one 5-mm circles punched from 1-2PP-coated GFFs were placed into separate vials containing 1.60 mL of 50 : 50 acetonitrile–water. The HPLC mobile phase was 50 : 50 acetonitrile–water at a flow rate of 1.00 mL min^{-1}, the detector wavelength was set at 242 nm, and the injection volume was 8.0 μL.

FIA of DNPH samples. Twenty-one 5-mm circles were placed into separate vials containing 3.00 mL of acetonitrile. The HPLC mobile phase was acetonitrile at a flow rate of 2.00 mL min^{-1}, the detector wavelength was set at 355 nm, and the injection volume was 8.0 μL.

FIA of MAP samples. Twenty-one 5-mm circles punched from MAP-coated GFFs were placed into separate vials containing 2.40 mL of acetonitrile. The HPLC mobile phase was acetonitrile at a flow rate of 2.00 mL min^{-1}, the detector wavelength was set at 366 nm, and the injection volume was 40.0 μL. During the course of analysis of about 100 MAP samples, symmetrical peaks of MAP slowly became skewed, and, later, distorted. Distorted peaks required the cleaning of the HPLC line by pumping nitric acid (∼8 M) through the system at 1 mL min^{-1} for about 12 minutes. Line cleaning was unnecessary for other reagents employed.

FIA of (+/−)-phenyl-1,2-ethanediol samples. Twenty-one 5-mm circles were placed into separate vials containing 1.60 mL of deionized water. The HPLC mobile phase was water at 2.00 mL min^{-1}, the detector was set at 207 nm, and the injection volume was 8.0 μL.

FIA of anthracene samples. Twenty-one 5-mm circles were placed into separate vials containing 3.00 mL of acetonitrile. The HPLC mobile phase consisted of methanol at a flow rate of 2.00 mL min^{-1}, the detector wavelength was set at 251 nm, and the injection volume was 8.0 μL.

Issue of filtering solutions before analysis. Obstruction of the HPLC line at a point following the auto-injector or in the detector caused by a build-up of glass fibers occasionally occurred in early work with unfiltered solutions; reversal of flow of mobile phase quickly removed the obstruction. Fortunately, obstructions caused by fibers in unfiltered solutions ceased to occur in later work. Therefore, filtration of solutions before analysis was unnecessary.

Determination of axial distributions of reagents. GFFs from Millipore Corp. of the same weight to the nearest 0.1 mg were placed into stacks of three. The stacks of GFFs were compressed for better contact by pressing down with (a) the outward side of a watch glass and (b) the outside bottom of a 50-mL beaker. Reagent solution in total volumes of 1275 to 1446 μL was applied by syringe when the tip of the syringe needle was positioned 1 cm above the center of a stack (each total volume was three times the normal volume for a single filter). The stacks of filters were allowed to dry at room temperature. Each whole filter from each stack was analyzed separately by FIA to determine the total quantity of reagent and to determine the axial distribution.

Results and discussion

Problems with commercially available reagent-coated filters

Twenty-one 5-mm circles that had been punched from a GFF coated with 1-2PP from SKC, Inc. gave rise to a relative standard deviation of measurement (RSD) of the quantities of 1-2PP of 30.8% upon analysis. See Fig. 2 for the quantities (expressed in μg). The largest quantity of 1-2PP found in a circle (28.0 μg) was three times the smallest quantity of 1-2PP found in another circle (9.30 μg). The eight starred circles accounted for 178 μg (51%) of the total of 348.5 μg found in the twenty-one circles. The uneven distribution of 1-2PP on a second GFF in the same lot from SKC, Inc. gave rise to an RSD of 30.7% for 1-2PP in twenty-one circles. Analysis of eight whole filters in the same lot from SKC, Inc. gave rise to

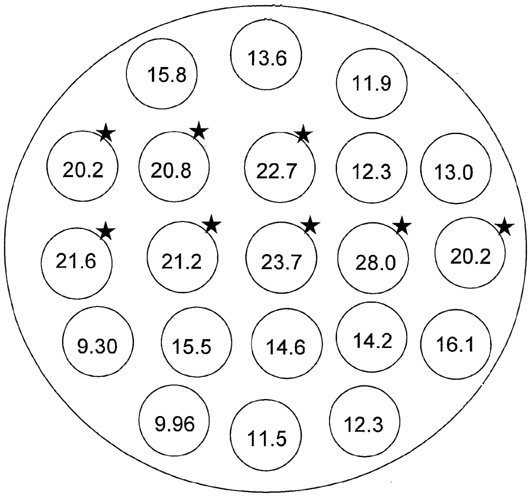

Fig. 2 Diagram of a 37-mm GFF coated with 1-(2-pyridyl)piperazine (1-2PP) from SKC, Inc. showing the quantities of 1-2PP found in the circles (expressed in μg). The eight starred circles contained the most 1-2PP and accounted for 51% of the total quantity of 1-2PP found in the twenty-one circles.

total quantities of 1-2PP ranging from 309 to 576 µg per filter (average = 383 µg; standard deviation = 85.9 µg).

A single GFF coated with 1-2PP from Supelco exhibited a distribution having an RSD of 19.9%. Total quantities of 1-2PP found on twelve other coated GFFs from Supelco ranged from 779 to 1145 µg per filter (average = 959 µg; standard deviation = 106 µg).

Twenty-one 5-mm circles from each of four GFFs coated with acidified DNPH (SKC, Inc.) gave rise to RSDs of 11.6, 14.1, 14.6 and 20.4%. Total quantities of DNPH on twelve other filters in the same lot ranged from 1.89 to 2.63 mg (average = 2.28 mg; standard deviation = 0.27 mg).

Equatorial distributions of selected reagents following coating by total immersion and drying on a glass plate

FIA of twenty-one 5-mm circles from each of two GFFs from Millipore Corp. that had been coated with MAP by total immersion of the GFFs in an acetonitrile solution of MAP at 2.40 mg mL^{-1} gave rise to RSDs of 8.6 and 21.5%. Consequently, preparation of nearly even coatings of MAP on GFFs by total immersion appears to be unreliable due to a high variability in the type of equatorial distribution.

By the same technique, two GFFs from Millipore Corp. that had been coated with acidified DNPH (without excess acid) gave rise to RSDs of 11.1 and 12.3% for quantities of DNPH in twenty-one 5-mm circles. Excessive phosphoric acid (1.5 times the stoichiometric quantity) seemed to cause no significant change in the RSDs (10.8 and 14.3%).

Equatorial distributions of MAP after applications of MAP solution to GFFs on a glass plate

FIA of twenty-one 5-mm circles from each of four GFFs from Millipore that had been coated with an acetonitrile solution of MAP (2.04 mg mL^{-1}) while resting on a glass plate gave rise to RSDs ranging from 12.9 to 14.5% (volumes of MAP solution applied ranged from 469 to 529 µL). Thus, this technique appears to be consistent in producing MAP coatings on GFFs, which are characterized by RSDs between 10 and 15% for twenty-one 5-mm circles.

Selection of a brand of 37-mm membrane GFF for nearly even reagent coating in the equatorial plane

Since 37-mm GFFs from different manufacturers have different physical characteristics, effort was made to identify a brand of GFF that exhibited three favorable characteristics for producing nearly even distributions of reagents. These favorable characteristics were (a) weight stability during the course of changing humidity, (b) consistent or nearly consistent weight from filter to filter in the same lot due to manufacture, and (c) minimum volume of solvent or solution required to wet the filter 100%. GFFs from Millipore Corp. were found to be superior to GFFs from Gelman Sciences, Inc. in all three categories. The weight changes of a representative GFF from Millipore Corp. were quite tolerable with an overall weight loss of only 0.102 mg (102 µg) due to changes in humidity, while the overall weight loss of a representative GFF from Gelman Sciences was almost five times larger (0.490 mg) due to changes in humidity.

Table 1 Wettability of glass fiber filters[a]

Liquid	Minimum volume required to obtain a 100% wet 37-mm GFF/µL Millipore GFF	 Gelman sciences GFF
Acetonitrile	365 ± 5	445 ± 5
Methanol	380 ± 5	480 ± 5
Cyclohexane	415 ± 5	530 ± 5
DNPH, acetonitrile with phosphoric acid	385 ± 5	430 ± 10

[a] Weight range of Millipore GFFs was 77.82–77.86 mg. Weight range of Gelman GFFs was 75.3–76.3 mg.

Weight variability from filter to filter in the same lot from Millipore Corp. (due to manufacture) was relatively small in order that one could identify a dozen or more GFFs of the same weight (to the nearest 0.1 mg) in the same lot very quickly. The average weight of 150 Millipore GFFs (Lot R6CN51890) was 80.3 mg with weights ranging from 79.1 to 81.5 mg soon after removal from the packaging (standard deviation = 0.80 mg). On the other hand, GFFs from Gelman Sciences, Inc. exhibited such a wide variability in weights in the same lot that one would find it time consuming to identify a dozen or more GFFs of the same weight (to the nearest 0.1 mg). The average weight of 400 Gelman GFFs (Lot 31725) was 70.0 mg with weights ranging from 63.1 to 76.9 mg soon after removal from packaging (standard deviation = 4.39 mg).

Results of testing in the third category (wettability) are shown in Table 1. Since GFFs from Millipore Corp. required significantly smaller minimum volumes for complete wetting than the volumes GFFs from Gelman Sciences, Inc. required, the GFFs from Millipore Corp. were less absorbent.

Effect of inhomogeneity of GFFs on the equatorial reagent distribution

Reports of inhomogeneity of GFFs have been published.[32,33] Indeed, weights of 5-mm circles from GFFs were found to be variable in this work. Individual weights of 5-mm circles from GFFs from Gelman Sciences, Inc. varied by as much as 360 µg or 31% (average weight of 5-mm circles was 1.18 mg). On the other hand, individual weights of 5-mm circles from GFFs from Millipore Corp. varied by as much as 150 µg or 12% (average weight of 5-mm circles was 1.31 mg). Tests were conducted to determine whether quantities of reagent on twenty-one 5-mm circles correlated with the weights of the 5-mm circles for each of ten reagent-coated GFFs. Two out of ten Pearson correlation coefficients were significantly different from zero (rho = 0.82 and 0.53); another eight of the ten had low (not statistically different from zero) correlation coefficients. The two significant correlation coefficients were found for two DNPH-coated GFFs from SKC, Inc.; the maximum weight variation of the 5-mm circles was 24% in each of the two cases. Consequently, it appears reasonable that choosing GFFs with a relatively small degree of inhomogeneity, such as GFFs from Millipore Corp., would reduce the frequency of significant correlations between quantities of reagent on 5-mm circles and the weights of the same circles.

Comparison of two model reagents in terms of capability to produce nearly even distributions in the equatorial plane

Two model reagents, (±)-phenyl-1,2-ethanediol and anthracene, were selected for study to illustrate the effect of hydrogen bonding by a reagent on the distribution in the equatorial plane of the GFF. The first model reagent (the diol cited above) was expected to engage in very significant hydrogen bonding with like molecules and with OH groups from other sources. Anthracene, however, was not expected to engage in significant hydrogen bonding. (±)-Phenyl-1,2-ethanediol and anthracene, exhibited widely different characteristics when applied to GFFs from Millipore Corp. (see Fig. 3). The curve for (±)-phenyl-1,2-ethanediol, which had been applied to the GFFs in acetonitrile solution at a concentration of 8.0 mg mL^{-1} (curve A), was characterized by large RSDs and a steep, positive slope. On the other hand, the curve for anthracene, which had been applied to the GFFs in acetonitrile solution at a concentration of 0.77 mg mL^{-1} (curve C), was characterized by smaller RSDs and a zero slope in the region of 420 to 445 μL of anthracene solution applied.

A proposed reason for the difference in curves A and C is significant hydrogen bonding of (±)-phenyl-1,2-ethanediol with like molecules and with hydroxyl groups on the surface of the GFFs. Curve B, which is based on application of anthracene in methanol solution at a concentration of 0.84 mg mL^{-1}, exhibits larger RSDs than curve C does. A proposed reason for the difference between curves B and C is hydrogen bonding of methanol molecules with other methanol molecules and with hydroxyl groups on the surfaces of the GFFs. Acetonitrile, on the other hand, is not expected to engage in significant hydrogen bonding (see curve C). The region of lowest RSDs in curve C (6% RSDs at 420 and 445 μL of anthracene solution) suggests that solution volumes in the range of 420 to 445 μL are the most appropriate for producing a nearly even distribution.

The RSDs in curves B and C of Fig. 3 are significantly different according to a one-way ANOVA model ($p < 0.028$). When the aberrant point in curve C is eliminated (12.8% RSD, 390 μL), the RSDs in curves B and C are significantly different according to a one-way ANOVA model ($p < 0.01$). Consequently, one should consider avoiding both solvents and reagents that are capable of significant hydrogen bonding for producing nearly evenly distributed coatings in the equatorial plane.

Application of 1-2PP to GFFs and QFFs

Application of 1-2PP in acetonitrile solution (4.0 mg mL^{-1}) to GFFs from Millipore Corp. gave rise to curve A in Fig. 4, which was characterized by large RSDs and a steep, positive slope. Because 1-2PP gave rise to a steep slope, 1-2PP was an example of a reagent that required precise weight measurements of the GFFs. Curve B was based on the application of 1-2PP in acetonitrile solution (4.0 mg mL^{-1}) to QFFs from Whatman International Ltd. Curve B was shifted to the right because the QFFs were more absorbent than the GFFs were. The Pearson correlation coefficients (R^2) for curves A and B were 0.96 and 0.99, respectively. The proposed reason for large RSDs for 1-2PP on both GFFs and QFFs in Fig. 4 is significant hydrogen bonding of 1-2PP molecules with other 1-2PP molecules and with hydroxyl groups on the surfaces of the GFFs and QFFs. A proposed reason for the steep, positive slopes for 1-2PP on GFFs and QFFs is that increases in volume of 1-2PP solution result in increases in the quantities of 1-2PP, and, hence, additional hydrogen bonding. The diamond at point C was based on application of 1-2PP in acetonitrile solution (4.0 mg mL^{-1}) to a QFF from Gelman Sciences, Inc. Thus, QFFs from Gelman Sciences, Inc. were more absorbent than QFFs from Whatman International Ltd. QFFs coated with 1-2PP were examined to determine whether

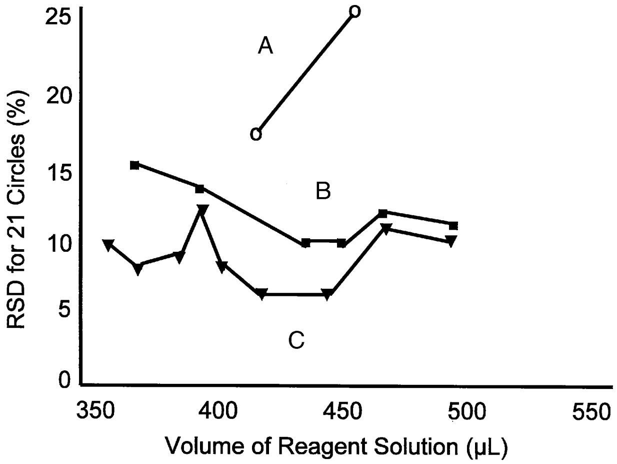

Fig. 3 Plots of RSD for quantities of reagent in twenty-one circles *versus* volume of reagent solution applied. Curve A is based on application of (±)-phenyl-1,2-ethanediol in acetonitrile solution to GFFs from Millipore Corp. Curves B and C are based on application of anthracene in methanol solution and in acetonitrile solution, respectively, to GFFs from Millipore Corp.

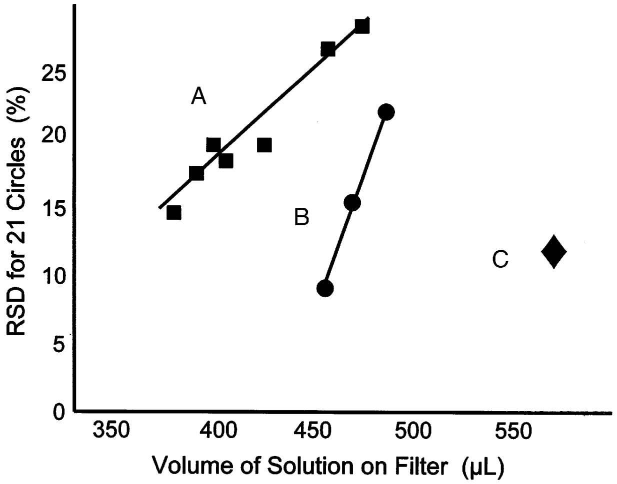

Fig. 4 Plots of RSD for quantities of 1-2PP in twenty-one circles *versus* volume of 1-2PP in acetonitrile solution applied. Curves A and B are based on application of 1-2PP solution to GFFs from Millipore Corp. and to QFFs from Whatman International Ltd, respectively. The diamond at point C is based on application of 1-2PP in acetonitrile solution to a single QFF from Gelman Sciences.

QFFs were advantageous over GFFs for giving rise to small RSDs.

Observed differences between 1-2PP and MAP in terms of RSDs

A curious phenomenon was observed between 1-2PP and MAP. While RSDs for 1-2PP increased from 15 to 27% with increasing volumes of solution applied to GFFs in Fig. 4, RSDs for MAP generally remained somewhat steady near values of about 6% with increasing volumes of solution applied (see Fig. 5). Both 1-2PP and MAP can undergo hydrogen bonding with like molecules and with hydroxyl groups on the surfaces of the GFFs.

Hypotheses for the major difference between 1-2PP and MAP in terms of RSDs are the following: (a) while the 1-2PP molecule bears three nitrogen atoms, which account for 26% of the molecular weight, the MAP molecule bears only two nitrogen atoms, which account for only 10% of the molecular weight (see Fig. 1); (b) the bulky anthracenyl group in the MAP molecule causes some steric hindrance, which may help deter hydrogen bonding, both among like molecules and with hydroxyl groups on the surfaces of GFFs. Since the actual reasons for the disparity have not been confirmed, additional experiments would be required. In addition, this disparity suggests that every reagent that is employed for coating filters should be tested for the capability to produce nearly evenly distributed coatings in the equatorial plane.

Comparison of solvents for application of MAP to GFFs

The solvent effect was quite apparent in the application of MAP in solution to GFFs (see Fig. 5). A cyclohexane solution of MAP (2.0 mg mL^{-1}), which gave rise to large RSDs for curve A, appeared to be unsuitable as a solvent in this case because cyclohexane is hydrophobic and the GFFs are hydrophilic. A methanol solution of MAP (2.0 mg mL^{-1}) and an acetonitrile solution of MAP (2.0 mg mL^{-1}) gave rise to curves B and C, respectively. As in the case with methanol as a solvent for anthracene, methanol as a solvent for MAP caused increases in RSD in comparison with acetonitrile, probably due to hydrogen bonding. Curve C, in which RSDs were relatively stable in the region of 390 to 560 μL, indicated that MAP is a reagent that does not require precise weight measurements of the GFFs. Increases in RSD beyond 560 μL in curve C suggested that larger volumes of MAP solution were excessive for the purpose of producing a nearly even distribution.

According to a one-way ANOVA model, curves A, B, and C in Fig. 5 are significantly different from each other ($p < 0.001$). By Bartlett's test for equality of RSDs, there are significant differences among RSDs in both curves B and C ($p < 0.001$). A t-test for slope (slope = 0.0108) for a linear regression of the points in curve C indicated that the slope is not significantly different from zero ($p = 0.09$). Consequently, there is not a significant trend for curve C.

Fig. 6 presents a diagram of the distribution of MAP in twenty-one circles from a GFF from Millipore Corp., which gave rise to the first point in curve C (solvent = acetonitrile) in Fig. 5 (RSD = 5.30%). The eight starred circles contained larger quantities of MAP than the other circles did and accounted for 68.4 μg or 40.0% of the total of 171.0 μg of MAP found in the twenty-one circles. Note that all eight starred circles are located near the edge of the GFF; the apparent reason is that when solvent (acetonitrile) evaporates from the edge during drying, additional MAP solution travels to the edge by capillary action, thereby causing an increase in quantity of MAP near the edge. The difference between the largest quantity found in a circle (8.86 μg) and the smallest quantity found in another circle on the entire filter (7.46 μg) was only 1.40 μg. Examination of the nine other GFFs for curve C revealed that there was a major tendency for all of the 5-mm circles bearing the largest quantities of MAP to be located near the edges of the GFFs (quantities in approximately the 60th percentile). The number of 5-mm circles

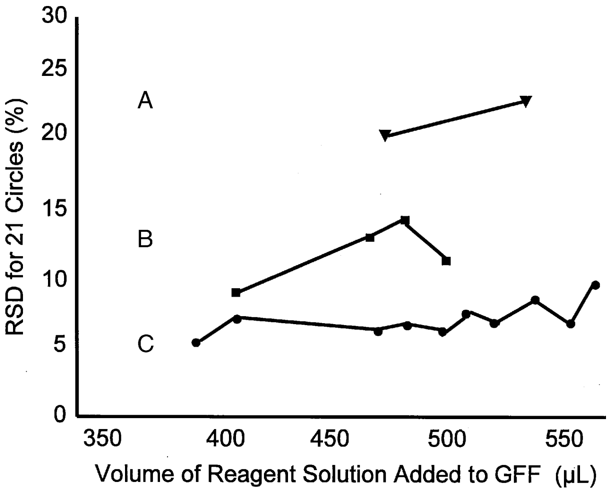

Fig. 5 Plots of RSD for quantities of MAP in twenty-one circles *versus* volume of MAP solution applied. Curves A, B, and C are based on cyclohexane, methanol, and acetonitrile as solvents for MAP during application of solution to GFFs.

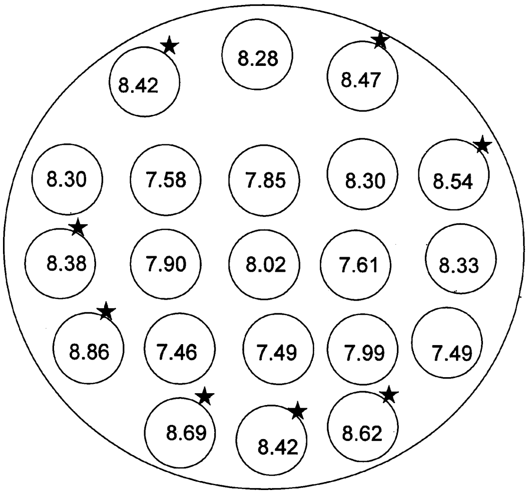

Fig. 6 Diagram of a GFF filter from Millipore Corp. showing the quantities of MAP found (in μg) in the twenty-one 5-mm circles. This GFF gave rise to the first point for Curve C in Fig. 5 with RSD = 5.30%. The eight starred circles contained the largest quantities of MAP.

bearing the largest quantities of MAP, which were located near edges of GFFs varied, however (from three to eight circles).

Effect of the quantity of phosphoric acid on the distribution of DNPH on GFFs from Millipore Corp.

When the DNPH solution (in acetonitrile) contained the stoichiometric quantity of phosphoric acid as catalyst, the RSDs for quantities of DNPH in twenty-one 5-mm circles remained in the 5–7% range (see curve B in Fig. 7). However, when 1.5 times the stoichiometric quantity of phosphoric acid was present, the RSDs were generally larger (see curve A in Fig. 7). The reason for the major dip in curve A at 435 μL of DNPH solution added is unclear (RSD = 6.5% for 435 μL of solution applied). According to a one-way ANOVA model, curve A is significantly different from curve B (p = 0.0002). When the major dip in curve A is removed, curve A is significantly different from curve B, also (p < 0.0001). A proposed reason for the acid effect is that excessive acid protonates hydroxyl groups on the surfaces of the GFFs; consequently, positive charges on the surfaces of the GFFs repel positively charged DNPH molecules, causing disorder in the equatorial distribution of DNPH. Note that excessive phosphoric acid did not cause a significant change in RSDs in a different technique [the technique involving total immersion and drying of the GFFs on a glass plate (see above)].

Investigation of axial distributions of selected reagents on GFFs from Millipore Corp.

Since it would be difficult to measure a reagent or compound at different depths of a coated GFF, a decision was made to apply about three times the normal volume of solution for a single GFF to a stack of three GFFs with each GFF having the same weight to the nearest 0.1 mg. Thus, the stack of three GFFs served as a model for a single GFF, and analysis of the separate GFFs (as whole filters) provided useful information on the axial distribution of a compound in a single filter. Although axial distributions of reagents on GFFs may be of major interest, the primary goal of this work was to conduct investigations on distributions of reagents in the equatorial plane.

In all experiments with MAP, anthracene and DNPH, the greatest relative quantity of compound was found on the top filter, and the smallest relative quantity was found on the middle filter (see Table 2). Of interest is the fact that methanol as solvent increased the relative quantity of anthracene (a model reagent) on the middle filter in comparison with acetonitrile as solvent.

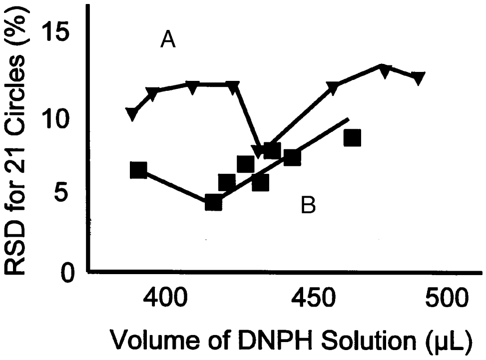

Fig. 7 Plot of RSD for quantities of DNPH in twenty-one 5-mm circles *versus* volume of DNPH solution applied to GFFs. Curve A is based on DNPH solution which contained 1.5 times the stoichiometric quantity of phosphoric acid as catalyst. Curve B is based on DNPH solution that contained the stoichiometric quantity of phosphoric acid.

Conclusions for equatorial distributions of reagents on GFFs

The proposed technique of employing the drying rack and applying a selected volume of reagent solution to the GFF appears to be the best available for producing a nearly even distribution in the equatorial plane. Since physical characteristics of the GFF depend on the manufacturer, a single brand of GFF should be employed. Choice of the solvent for the reagent to be applied to the GFF can be important. Volume of reagent solution applied to the GFF can be important. The effect of significant hydrogen bonding by the solvent with the reagent can lead to an uneven distribution of reagent in the equatorial plane. The effect of significant hydrogen bonding by the reagent with like molecules in solution and with OH groups on the surface of the GFF can lead to an uneven distribution of reagent in the equatorial plane. Excessive phosphoric acid (as catalyst) can cause an uneven distribution of DNPH in the equatorial plane.

Experiments with additional reagents on GFFs should be performed to gain a better understanding of the effects of chemical structure on equatorial distribution.

Conclusions for axial distributions of reagents on GFFs

Use of acetonitrile as reagent solvent can lead to a relatively light distribution of reagent in the center of the filter. Larger relative distributions appear to be on the top and bottom sides of the single conceptual GFF due to evaporation of solvent at

Table 2 Axial distributions of compounds in stacks of glass fiber filters

			Distribution on filters (%)		
Compound	Support	Volume of solution applied/μL	Top	Middle	Bottom
MAP in acetonitrile	Drying rack	1446	49.2	13.1	37.7
MAP in acetonitrile	Glass plate	1446	51.5	15.2	33.3
Anthracene in acetonitrile	Drying rack	1275	45.5	15.0	39.5
Anthracene in methanol	Drying rack	1275	38.4	28.1	33.5
DNPH without excess acid	Drying rack	1290	45.8	17.7	36.5
DNPH with 50% excess acid	Drying rack	1290	46.4	16.7	36.9

surfaces and movement of additional reagent solution to the surfaces due to capillary action. Choice of solvent for application of reagent solution can affect the axial distribution.

Recommendation for producing reagent coatings on GFFs with nearly even distributions

Depending on the reagent, one may find the need to weigh GFFs precisely (to the nearest 0.1 mg), and to measure volumes of reagent solutions precisely (to the nearest 3 μL). In such a case, a plot of RSD of quantities of reagent in twenty-one circles *versus* volume of reagent solution applied will have a steep, positive slope; one example is 1-2PP. In other cases, one may find the requirements of precise measurements can be relaxed; one example is MAP, which gives rise to a slope of zero over a wide range of volumes of reagent solution applied. The following seven steps are recommended for producing nearly even distributions of selected reagents in the equatorial plane of 37-mm GFFs in the course of testing other reagents: (1) determine the minimum volume of reagent solution required to wet a GFF of known weight (to the nearest 0.1 mg) on the drying rack. Place five or six GFFs of the same weight onto the drying rack in order that the GFFs are not touching each other. Add a known volume of reagent solution to a GFF by syringe with the syringe needle tip positioned 1 cm above the center of the filter. Increase or decrease the total volume of reagent solution by 15-μL increments, and add these volumes of reagent solution to other GFFs. The wet portion of a GFF can be distinguished easily from a dry portion by visual inspection; (2) place eight or more GFFs of the same weight onto the drying rack. With a syringe, add reagent solution to the center of each GFF by the same technique described in step 1. The volume of solution added is the minimum volume needed to wet the GFF; (3) increase the volume of reagent solution in 15- or 20-μL increments, and add reagent solution to other GFFs; (4) punch out twenty-one 5-mm circles from each reagent-coated GFF, analyze by FIA, and calculate the RSD for the twenty-one quantities of reagent; (5) construct a plot of RSD of quantities of reagent in twenty-one 5-mm circles *versus* the volume of reagent solution applied; (6) determine the volume or range of volumes of reagent solution that gives rise to the smallest RSDs for the GFFs of a particular weight; (7) coat GFFs with reagents by a procedure consistent with the information derived from step 6.

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