Environ. Sci. Technol. **1998**, *32,* 1147−1152

Indoor Air Pollution and Sick Building Syndrome. Monitoring Aerosol Protein as a Measure of Bioaerosols

SIMON K. PORUTHOOR, PURNENDU K. DASGUPTA,* AND ZHANG GENFA

Department of Chemistry and Biochemistry, Texas Tech University, Lubbock, Texas 79409-1061

Excess aerosol protein concentration is suggested to be a diagnostic marker for biological causes of impaired air quality of a building. A simple inexpensive instrument has been developed to measure aerosol protein concentration. Aerosol particles are collected by allowing injected steam to condense upon them, thus resulting in their growth, followed by cooling and impaction. The liquid stream, bearing dissolved and suspended aerosol constituents, is put through a silica preconcentration column that is eluted subsequently with dilute H_3PO_4. The eluite stream is reacted with acidic Coomassie blue G; the color of the protein-dye adduct is detected by optical absorbance at ∼600 nm. Optimization efforts have been carried out to choose the best stationary phase for protein preconcentration and reagent composition. In liquid-phase experiments, bovine serum albumin (BSA) displays a limit of detection (LOD) of 16 ng. In aerosol experiments, there is some increased noise and the LOD deteriorates by ∼2×. The system collects aerosol particles with 99.5+% efficiency. The LOD for a 300 L (30 min at 10 L/min) air sample is thus equivalent to a concentration of ∼100 ng/m^3 BSA. The system is mounted on a single two-tier standard laboratory cart and has the potential to be an effective transportable monitoring device for airborne biological contamination. Detectable protein concentrations were found in an apartment dwelling setting during cooking activities and in a hotel known to have a fungal contamination problem.

Perspective

Indoor pollution is of concern. The average person spends far more time indoors than outside. Poor outdoor air quality is rarely the source for a significant indoor air pollution problem. In the wake of energy conservation measures that result in fewer exchanges of fresh air per unit time, a contaminant may be generated in the system due to some malfunctioning device and then concentrated (*1*). Concerns in regard to human health effects from indoor air pollution are steadily increasing. While pinpointing the source may not be simple, assigning culpability for indoor pollution is relatively easy. As a result, indoor air pollution issues have already resulted in a disproportionately large number of lawsuits; this situation is unlikely to change in the future. A recent database search of the key words *indoor air pollution* revealed that nearly half of the papers on the topic were of a legal nature! The market for diagnosing and treating indoor air pollution problems is rising steeply. By the year 2000, the indoor air quality market is projected to be $3.6 billion, of which more than an 11% share is expected for analytical and consulting services (*2*).

Though the indoor pollution can be caused by multitude of contaminants, case histories indicate that acute indoor pollution problems, so-called "sick building syndromes", are as often due to "bioaerosols" (*3*) (viral/fungal/bacterial matter in airborne particle form), as from nonbiological sources. It needs to be recognized that the so-called "sick building syndrome" represents the tip of the iceberg. At least 10 times as often, buildings are operating with an unhealthy indoor air environment, its occupants are not functioning optimally. Public awareness on health effects of indoor air pollution has never been higher. New standards for indoor air quality have been proposed. The biggest concern lies with the children: the General Accounting Office reports that severe indoor air quality problems exist in 15 000 U.S. schools, affecting nearly 9 million children (*4*).

A major impediment toward rapid treatment of indoor air pollution problems in a technically sound manner is to first make the basic distinction as to whether the problem is primarily chemical or biological in nature. If, for example, it is diagnosed to be chemical, portable gas chromatograph-mass spectrometers can be brought on site to unambiguously characterize unusual amounts of any species present. However, it is not completely straightforward; unusual concentrations of certain VOCs may originate also from fungal or microbiological sources (*5*). It would be better to first decide if a biological problem exists.

Rapid verification of the occurrence of a biological contamination problem is not simple. While immunoassays (*6*) and Limulus bioassay (*7*) have been used to shed light on the problem and the polymerase chain reaction (*8*) has been used to improve sensitivity, the typical approach utilizes culture and microscopy (*9*). This generally involves filter or impactor sampling, both inside and outside a building. The filters/impactor stages are then individually cultured. A substantially larger growth of an organism in the indoor sample then prompts efforts to find that organism. This requires several days, and a negative result is inconclusive: the pathogen/allergen may not be viable when sampled or after prolonged air passage. Also, it is now recognized that stressed organisms often will not grow in culture media that have otherwise been specifically designed for growing healthy organisms of the same type (*3*). Further, there is no "universal culture medium" that will promote the growth of all types of organisms. There is a consensus that the ability to rapidly determine the existence of a biological problem will be a major step toward treating sick buildings (*10*).

Bioaerosols include airborne bacteria, vira, fungal spores, pollens, plant/insect fragments, etc. Above a threshold concentration, many of these species can elicit pathogenic and allergenic effects. Indoor bioaerosols are currently being investigated with the goal of discovering a cause for ongoing disease or discomfort, (*11, 12*) to connect specific health effects with specific biocomponents, (*13*) and to increase the understanding of the ecology and physiology of the source organisms and the fate of their airborne effluents (*14*). Whether it is dead tissue or a living organism, bioaerosols have a greater protein content than the ambient background of dominantly inorganic aerosol. It is our hypothesis therefore that a method to quantify total airborne protein

* Corresponding author fax: 742-1289; e-mail: sandyd@pop.ttu.edu.

S0013-936X(97)01005-5 CCC: $15.00
Published on Web 03/12/1998

has the potential to be an effective way of monitoring bioaerosol contamination.

An experimental approach to accomplish this is described. The method was applied to air monitoring in a typical home environment and a suspect sick building; these results are also discussed.

Experimental Section

Reagents and Chemicals. Except as stated, all reagents used were of analytical grade and were procured either from Mallinckrodt or Baker. Distilled deionized water was used for making all solutions. Stock protein solutions were prepared at a concentration of 10 mg/mL and were stored frozen in polyethylene vials in the dark. Immediately before measurement, the frozen sample was thawed and diluted to the desired concentration with water. Thawed samples were stored in the refrigerator for a maximum period of 48 h before being discarded. The stock dye reagent was made by dissolving 1 g of Coomassie Brilliant Blue G-250 (CBG, U.S. Biochemical Corporation) in 50 mL ethanol to which 50 mL concentrated phosphoric acid (85%) was added. The working dye reagent was prepared fresh from stock every week by mixing 6 mL of the stock solution with 197 mL of 15 M H_3PO_4 and 97 mL of ethanol and making up to 1 L with water.

Aerosol Generation. Particle collection characteristics were determined with laboratory generated proteinaceous aerosol. Dilute aqueous solutions of bovine serum albumin (BSA) in the 10−25 mg/L concentration range were nebulized, using a Venturi type nebulizer with particle free "zero" air (model 737 Pure Air Generator, AADCO, Clearwater, FL) flowing at 3 L (STP) min^{-1}. The generated aerosol was dried in-line, by dilution with "zero" air at a flow rate of 30 L (STP) min^{-1}, with a total residence time en route of about 2 min in three sequential chambers of 22 L volume each. The concentration of the generated primary aerosol is dependent on the concentration of nebulizing solution and is further controlled by dilution with "zero" air. The size of the dry aerosol is dependent on the concentration of the nebulized solution. The aerosol size distribution is not affected by diluting the dried aerosol with further amounts of air. The aerosol size distribution was measured near the inlet of the aerosol collection system with a laser based optical particle counter (model A2212-.01-115-1, Met-One, Grant's Pass, OR).

Particle Collection. A vapor-condensation-based aerosol collection system (VCACS) developed in this laboratory has been previously reported (*15*). The presently used system has been modified from the original design and is schematically represented in Figure 1. Steam is generated by pumping water (1.0−2.0 mL/min) through a glass tube (120 cm long, 2 mm i.d.) that is coiled directly on a rod-shaped heater. To provide better heat transfer, the glass tube is filled with 1 mm diameter glass beads. The steam thus generated is then introduced to the aerosol flow and led to a mixing chamber. The aerosol inlet and mixing chamber designs are the same as those previously reported (*15*), except for an order of magnitude increase in the volume of the mixing chamber (∼2 L in the present design). The effluent from the mixing chamber is introduced to an inertial air−liquid separator (Figure 1, inset a) through an impactor device. The impactor is made by coiling a glass tube (120 cm × 0.5 cm i.d.) on a metal support of elliptical cross-section (1.2 cm × 3.3 cm) and flattening the glass tube on each of the broad sides of the support by pressing against a flat plate while the glass is heated above its softening temperature. The flow profile through this impactor resembles the flow profile through the stainless steel maze used in the previous design. The mixing chamber, impactor, the air−liquid separator and other downstream components (vide infra, see also Figure 1) are all mounted inside a circular acrylic jacket (∼12 cm diameter) and air cooled by the forced draft from a fan (80 ft^3 min^{-1}) placed at the bottom of the acrylic housing. The liquid effluent from the air−liquid separator is aspirated out by a peristaltic pump and is sent to the liquid-phase protein analyzer system. Further details of the particle collection system is given elsewhere (*16*).

The air sample is drawn through the system by an air pump (at 10 L/min) placed downstream to the air−liquid separator. The aerosol flow is controlled by a mass flow controller (FC-280, Tylan General, Los Angeles, CA) placed between the air pump and the air−liquid separator. To protect the mass flow controller from excess moisture in the effluent gas flow, the gaseous effluent from the air−liquid separator is processed through a water coalescing system and a heater (2 W power resistor placed inside a glass tube), prior to the mass flow controller. The water coalescer consists of a coiled aluminum tube (7.5 mm i.d. × 1 cm diameter × ∼3.75 m) intermittently filled with short filter plugs made out of kitchen pot scrubber material, ScotchBrite, 3M company) followed by a second air−liquid separator.

Liquid-Phase Protein Analyzer System. The liquid-phase measurement system is shown schematically in Figure 2. Liquid pumping was carried out with a multichannel peristaltic pump (Minipuls 2, Gilson). The concentrator columns CC (50 mm long × 4 mm i.d., ∼ 630 μL volume) are packed with 120−170 mesh silica gel (Sigma) or ion-exchange resins with porous polyvinylidene fluoride frits on either side. Before use, the columns were precleaned with water and dilute H_3PO_4.

Two such concentrator columns are connected through a dual stack eight port injection valve V (Dionex) to allow sample preconcentration on one column while the other is being eluted. For liquid-phase calibration, the concentrator columns are loaded at a constant flow rate (1.6 mL/min) for a fixed period (typically 5 min, amounting to a volume of 8 mL being preconcentrated) with aqueous protein standards. During measurement, the liquid effluent from the VCACS is concentrated in a similar manner, (typically for 30 min for the measurement of ambient aerosol protein). After loading is completed, the adsorbed protein is eluted with 135 mM H_3PO_4 flowing at a rate of 0.8 mL/min. The eluted protein-bearing stream then merges at a tee with the working CBG reagent solution, flowing at a rate of 0.4 mL/min. Following a PTFE reactor, made by weaving a 0.7 mm i.d. × 600 mm tube in a serpentine I fashion (*17*), that results in a reaction time of 2 min, the streamflows through an optical absorbance detector. The detector is either a commercial HPLC type detector (SpectroFlow 757, Kratos Analytical Instruments) or a homemade absorbance detector using a light-emitting diode (LED) as a light source (605 nm, Stanley Electric, Tokyo, Japan, P/N HAA5566X) and a flow cell with a path length of 8 mm (*18*). The detector output was acquired with an integrator (model 3394A, Hewlett-Packard) or by a personal computer equipped with an A/D card.

Results and Discussion

Choice of a Protein Measurement Method. There are a variety of absorbance- and fluorescence-based techniques for protein asays, including, for example, biuret chemistry, the use of Folin reagent (Lowry method), dye binding approaches, and fluorescence detection after derivatization with fluorescamine. These methods, respectively, produce LODs in the range 400−2000, 2−16, 0.4−4, and 0.04−0.8 μg/mL (*19*). Direct UV detection is also commonly used but a chromatographic separation step is essential.

The biuret method involves the quantitation of a purple color (540 nm) formed by the chelation of Cu^{2+} with peptide nitrogen atoms in an alkaline solution; it is relatively insensitive. In the Lowry method, Cu^{+} is produced by proteins in alkaline solution from Cu^{2+}; Cu^{+} catalyzes the reduction of phosphomolybdate/phosphotungstate anions

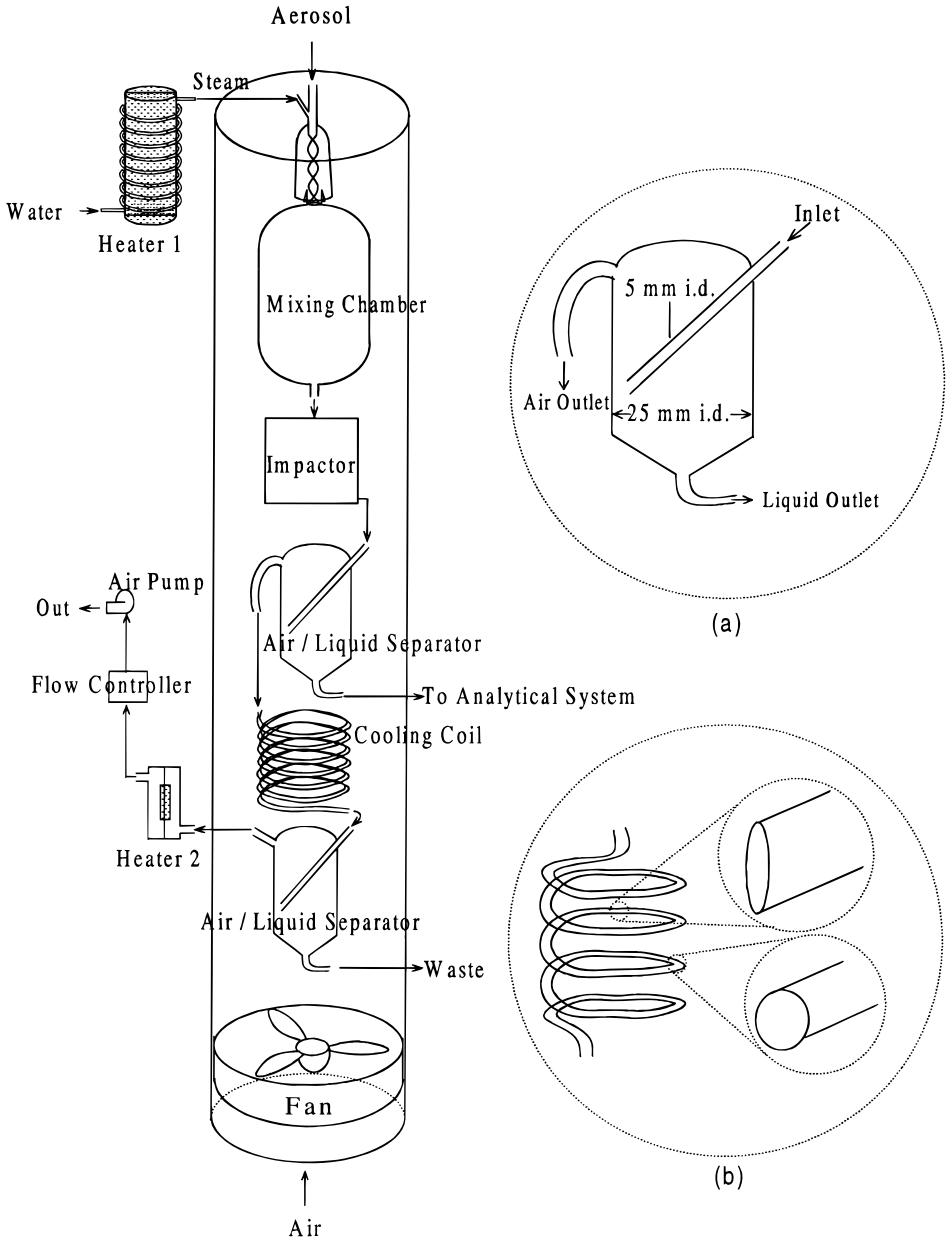

FIGURE 1. Vapor condensation aerosol collection system (VCACS). Insets a and b, respectively, show the details of the air/liquid separator and the flattened glass coil impactor.

by tyrosine. Heteropoly blue is formed and typically measured at 740−750 nm (*20*). The method is often difficult (*21*) and reportedly gives significantly lower values for certain protein matrixes (*22*−*24*), although the bicinchoninic acid modification of this has been reported to be less cumbersome (*25*). The Coomassie Blue-G (CBG) dye binding method utilizes an adduct formation of CBG with proteins resulting in a large shift in the absorption maximum. The unbound dye has an absorption maximum at ~470 nm, whereas the protein dye complex has an absorption maximum at ~595 nm. The exact wavelengths are dependent on the pH and the concentration of the dye. The fluorescence derivatization method with Fluorescamine is very sensitive. However, the reagent is very reactive with water and frequent calibration may be needed. Although the CBG dye binding method produces a LOD higher than that in the Fluorescamine method, it was judged to be adequate, at least in initial trials, for the present studies.

Coomassie Blue G Method for Protein Measurement: Adaptation to a Flow Analysis Method. The CBG binding method for protein assay was originally introduced by Bradford (*26*) and Sedmak et al. (*27*). One disadvantage of the technique is that the sensitivity to different proteins is not the same. A multitude of studies (*28*−*30*) have been conducted but no generally applicable model has emerged. Overall, the binding of the dye with proteins is considered to be due to combinations of ionic, van der Waals, hydrogen-bonding, and hydrophilic interactions (*30*). As such, the number of dye molecules attached to a protein is dependent on the type of protein as well as the dye, protein, and hydrogen ion concentrations. Experimental solutions to equalize the response to different proteins have been suggested (*27*, *30*, *31*). Read et al. (*30*) suggested very high dye concentrations both as a means of equalizing the response from different proteins and also to increase the sensitivity of the method. There are two problems with this approach. CBG is not very soluble in water and even though solutions of high concentrations can initially be made, aging of such a solution causes the dye to precipitate. Second, although a high dye concentration does increase the sensitivity (calibration slope)

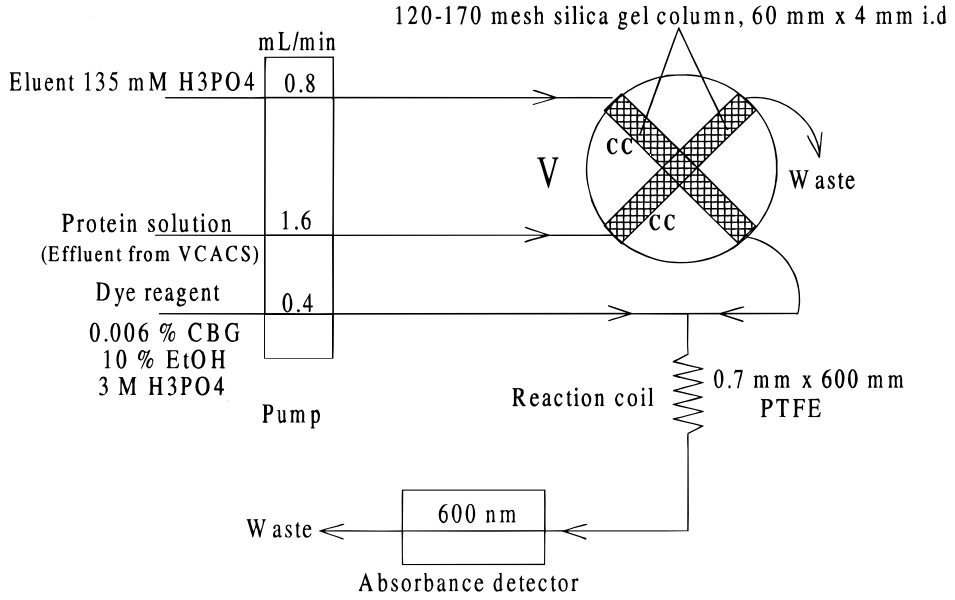

FIGURE 2. Analytical system for protein measurement. The liquid protein bearing effluent from the VCACS is concentrated on one concentrator column CC while the other one is being eluted.

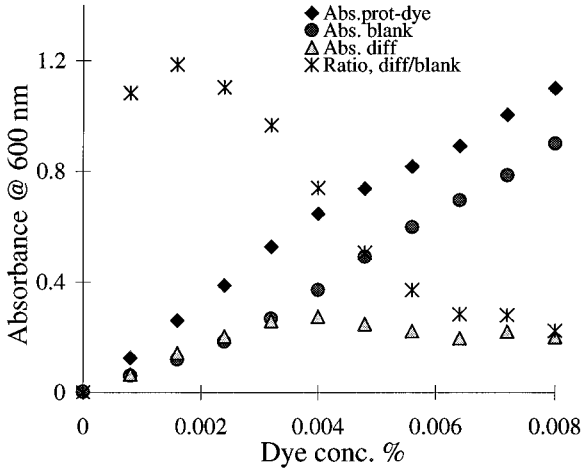

FIGURE 3. Absorbance of protein solution and blank at different dye concentrations. (◆) Absorbance of protein solution, (●) blank absorbance, (▲) difference between blank and protein solution, and (*) ratio of absorbance difference over blank.

of the method, the concomitant increase in the value of the reagent blank and its absolute variability is such that little gain is made in the attainable LOD. Since the blank noise is typically proportional to the value of the blank itself, a better method is to optimize the value of $\Delta S/S_b$ rather than that of ΔS, where ΔS is the difference in the signal between the blank and the analyte and S_b is blank signal. Results from such an optimization study are shown in Figure 3. On the basis of these results, the reagent concentrations reported in the Experimental Section were chosen. Detailed data are not shown but the linearity of response is also much better at lower rather than higher dye concentration, especially at low concentrations of protein.

The solubility of CBG can be increased by adding acid or ethanol. We chose phosphoric acid because of its compatibility with most proteins. Increasing acidity reduces the blank value; unfortunately, it also decreases the response to proteins. Addition of large amounts of ethanol causes an increase in the blank *and* a decrease in the response. In short, increasing either acid or alcohol content reduces response while increasing dye solubility. Some significant concentrations of acid and/or alcohol must be present otherwise even if a solution could be initially prepared, the dye later falls out of solution. Thus, the best attainable sensitivities may not lead to a stable and reproducible analysis system. To a degree, this limitation is partially corrected in the present flow analysis system. The eluent flow rate is set at twice that of the dye reagent, allowing enough alcohol and acid in the working reagent for it to be stable (>1 month), yet allowing reasonably high sensitivity at the final concentrations attained after dilution with the eluent. Shift in calibration attributed to this working reagent is <2% over an 1 month period. Due to the relatively short reaction time (2 min) prior to detection, the dye does not significantly precipitate. Nevertheless, over a period of time with actual analysis of the protein being conducted, the slow buildup of the dye on the tubing wall is visible. Apparently, the protein-dye adduct precipitates more easily than the dye itself. This precipitation not only occurs on the conduit walls, the same happens on the detector cell windows as well. As a result, the detector background increases over a period of time, necessitating periodic washing (at least once a day) of the conduits and the cell with ethanol.

As an aid to keep the proteins in solution and thereby to reduce the loss of proteins on to walls of a container, addition of Triton-X-100 (TX100) has been suggested earlier (*30*). We have observed that TX100 does indeed help reduce the adhesion of proteins on the conduit walls. However, when tested with the present measurement scheme, a solution of 0.01% Triton-X gives a signal corresponding to 25 ng of BSA; we chose not to use TX100 for the measurements reported here. Nevertheless, TX100 may be of value if more aggressive steps such as cell lysis at an elevated temperature is attempted (vide infra).

Choice of Stationary Phase for Protein Preconcentration. For the determination of low analyte concentrations, preconcentration is essential. Since proteins are typically charged positively in acid solutions, we investigated ion exchanger sorbents including strong acid type cation exchangers (Dowex 50Wx2, Dowex 50Wx8, Nafion beads), gel and macroreticular weak acid type cation exchangers (Amberlite CG-50, Amberlite IRC-50), bifunctional exchangers/ion retardation resins (Biorad AG-11-A8), nonfunctional macroreticular adsorbents (Amberlite XAD-2), and silica. Initial testing was done with small columns of ~50 μL of volume each by loading ~15 column volumes of a 2 μg/mL

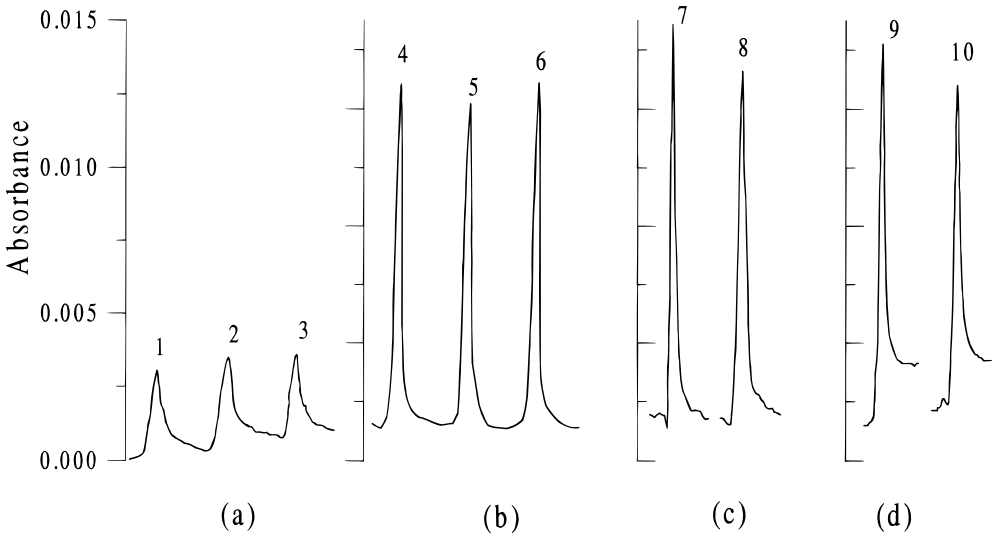

FIGURE 4. Representative system output. (a) Blank; peaks 1−3 are from loading 8 mL blank each, (b) 50 ng/mL BSA standard; peaks 4−6 are from loading 8 mL solution each, (c) system output in an apartment dwelling, during cooking; peaks 7 and 8 are from 700 and 650 L air sample, (d) system output in a hotel room known to have fungal contamination; peaks 9 and 10 are from 450 and 420 L air sampled inside the contaminated room.

TABLE 1. Relative Response (Calibration Slopes) for Different Proteins

protein	specific color yield (% of response for BSA)
lysozyme	30
pepsin	48
α-chymotrypsinogen A	64
fibrinogen	140
cytochrome *c*	130
BSA	100

bovine serum albumin (BSA) to a total amount of 1.4 μg with subsequent elution and detection using the analytical system of Figure 2. The relative peak heights obtained were 8.0 (empty column), 9.5 (Bio-Rad AG-11-A8), 11.5 (Amberlite XAD-2), 17.8 (Dowex 50Wx8), 19.6 (Amberlite CG-50), 21.4 (Nafion), 26.9 (Dowex 50Wx2), 35.6 (Amberlite IRC-50), and 37.2 (silica). Whether the test protein was not fully taken up by or eluted out from the other supports, it is clear that the silica and the IRC-50 preconcentration columns produce higher signals than the other columns and these sorbents are comparable to each other. Further experiments showed that silica is preferred to IRC-50, it requires less equilibration time during startup and shows less memory effects when there are large changes in protein concentration. Silica was henceforth used.

Further experiments also showed that not only BSA but other protein samples listed in Table 1 were also efficiently preconcentrated on silica and are completely eluted by modest concentrations (135 mM) of H_3PO_4. Normally, loss of silica from a packed bed is of concern; however, over a month-long period of use, no significant changes in the packed bed or any gradual peak broadening was observed. In any case, it is simple and inexpensive to make new silica filled precolumns.

The present procedure calls for concentrating the protein on the column, eluting it, and reacting the same with the dye. Two other alternatives that would have potentially led to a lower background detection scheme were explored. (a) The dye was loaded on to the column first, and then attempts were made to preconcentrate the protein on the column and to elute the complex subsequently. The dye is apparently bound to silica in a manner that it is not available for interacting with the proteins. No uptake of the proteins was observed. (b) Attempts were made to form the dye-protein adduct first and preconcentrate this on the column. No uptake was observed in the strongly acidic medium necessary to keep the dye in solution.

Liquid-Phase Calibration. Initial system calibration was carried out with aqueous BSA solutions. A typical set of blank and analyte responses are shown in Figure 4, panels a and b. A linear response behavior ($r^2 = 0.9947$) was observed between 0 and 0.8 μg/mL (8 mL sample preconcentrated) and the data could be fit to a quadratic equation (multiple $r^2 = 0.9983$) for the entire tested concentration range (0−1.4 μg/mL). The within-day relative standard deviation (rsd) of response is <1.5%. The corresponding value for disparate days is better than 5%. The liquid-phase detection limit, calculated as the signal equivalent to 3× the standard deviation of the blank, above the blank, was determined to be 16 ng for BSA. Responses from other proteins were measured, and in agreement with previous reports (*27*, *30*, *31*), the response per unit mass for different proteins was found to be different. These are listed in Table 1 with the response slope for BSA being arbitrarily normalized to 100. It is obvious that unless the nature of the proteins present in a given sample is known, an *exact* quantitation of proteins will not be possible. However, in most natural protein mixtures, the basic amino acid content, the building blocks responsible for interacting with the dye, is in the range 10−17 mol % with a sharp maximum at 13 mol % (*29*). Except in a truly unusual scenario, it should be possible to determine whether the overall protein concentration in a given indoor environment is significantly higher than the corresponding control (e.g., outdoor air sampled at the same location).

Measurement of Laboratory Generated Protein Aerosols. The aerosol generation system was reasonably stable, the rsd of 1 min cumulative particle counter readings taken every 5 min over a period of 6 h was 2.4%. The concentration of generated protein aerosol was calculated by collecting the aerosol on a 0.22 μm pore size PTFE filter at a fixed flow rate over an extended period of time. The collected protein was extracted with acid and measured by the same CBG binding assay. To minimize potential errors due to temporal variations in aerosol generation, the filter sampling was carried out in an isokinetic fashion simultaneously with the VCACS operation. In different experiments, the BSA aerosol concentrations were varied in the range 26−85 μg/m^3. On the

basis of the optical counter data, the aerosol mass median diameter varied in the range 0.4−0.6 μm. The collection efficiency of the VCACS was calculated to be >99.5% by comparing total protein collected by VCACS with that collected on the PTFE filter, consistent with earlier detailed performance evaluation of the system with inorganic aerosols (*16*). As previously reported, collection of larger particles is expected to be at least as efficient (*15*). In terms of analytical slope, precision, blank values, and reproducibility, measurement with generated aerosol showed essentially the same performance characteristics as that observed with the direct injection of liquid-phase standards except that system noise was higher for the absolute LOD to be ~30 ng BSA. On the basis of the $S/N = 3$ criterion, the LOD for BSA aerosol is calculated to be 100 ng/m^3 for a 300 L air sample (10 L/min, 30 min).

The coupling of the VCACS with the online protein preconcentrator/analyzer was straightforward. The only requirement is that the VCACS liquid effluent flow rate must be under the maximum permissible sample loading rate of the protein analyzer (2.5 mL/min). In general, when ambient temperature and humidity are relatively constant within the duration of a experiment (true for the majority of indoor sampling situations), the VCACS effluent flow rate is nearly constant. If ambient temperature and humidity variations are expected, slightly lowering the liquid aspiration rate for injection into the protein analyzer effectively avoids introduction of air bubbles to the analytical system. Since by far the vast majority of the VCACS effluent is still injected, there is very little effect on the accuracy of the results.

Air Sampling Applications. The above system was used to measure the protein content of the outdoor and indoor aerosols in a number of different situations. For outdoor air just outside the laboratory and indoor air in the laboratory measured on several occasions (10 L/min, 30 min), the response signal was not statistically different from the blank. We assumed therefore that the protein concentrations in these samples are below the measurement capabilities of the system. The instrument was then transported to a typical apartment dwelling for a small family and used to continuously monitor the air around the living room/kitchen area. The protein concentration remained at or below the instrument LOD except sometimes during cooking, when the instrument indicated the presence of protein aerosols at 700 ng/m^3, well above the instrument LOD. The instrument output is shown in Figure 4c. It is of interest to note that in their assessment on sources of urban contemporary carbon aerosol, Hildemann et al. (*32*) concluded that some 17% originate from meat cooking.

The instrument was subsequently taken to a luxury hotel in a major metropolitan city in Texas where a biological contamination problem due to fungal growth was known to exist. Previously, the nature of the problem was diagnosed by aerosol collection with an impactor followed by culturing and microscopic examination for identification. The present instrument did indicate significantly higher protein content of the ambient indoor air than the blank, in the range of 900−1100 ng/m^3. Representative instrument output is shown in Figure 4d.

Future Work. The measurement of aerosol protein content with in situ field deployable instrumentation has been shown to be viable. It may be premature to conclude whether excess protein content can be used to diagnose a biological origin of a sick building syndrome; however, initial results are certainly encouraging. The present system likely does not measure or is very inefficient in measuring the protein contained *in tact* cells, vira, or bacteria. To measure the protein contained therein, it would be necessary to implement an on-line hydrolytic or proteolytic cleavage, possibly at an elevated temperature and potentially in the presence of an enzyme or a surfactant that promotes such lysis. At that point, fluorometric methods can be incorporated to further improve the intrinsic sensitivity of the method. In principle, it should ultimately be possible to hydrolyze a major fraction of the collected protein to amino acids and thence obtain an amino acid signature profile of the collected material.

Acknowledgments

We gratefully acknowledge the support of this work by the State of Texas Advanced Technology Program, Texas Higher Education Coordinating Board.

Literature Cited

(1) Tate, N. *Sick Building Syndrome*; New Horizon Press: New Jersey, 1994; pp 71−76.
(2) Lighthart, B.; Mohr, A. J., Eds. *Atmospheric Microbial Aerosols*; Chapman & Hall: New York, 1994.
(3) *Indoor Pollution News*; July 25, 1994; p 199.
(4) *Federal Register*, 29 CFR parts 1910, 1915, 1926, and 1928, April 5, 1994; U.S. General Accounting Office: *School Facilities. Condition of America's Schools*; GAO/HEHS-95-61, February, 1995.
(5) Bayer, C. W.; Crow, S. *Proc. ASHRAE*, **1993**, p 165; Börhesson, T.; Stöllman, U.; Adamek, P.; Kaspersson, A. *Cereal Chem.* **1989**, *66*, 300; Kostiainen, R. *Atmos. Environ.* **1995**, *29*, 693.
(6) Dorner, J. W., Blankenship, P. D., Cole, R. J. *J. AOAC Int.* **1993**, *76*, 637.
(7) Milton, D. K., Feldman, H. A., Neuberg, D. S., Bruckner R. J., Greaves, I. A. *Environ. Res.* **1992**, *57*, 212.
(8) Palmer, C. J, Tsai, Y. L., Paszkokolva, C., Mayer, C., Sangermano, L. R. *Appl. Environ. Microbiol.* **1993**, *59*, 3618.
(9) Muilenberg, M. L. *Immunol. Allergy Clin. North Am.* **1989**, *9*, 245.
(10) Spooner, C. M. *Clin. Chest. Med.* **1992**, *13*, 179; Harrison, J.; Pickering, C. A. C., Faragher, E. A. B. *Resp. Med.* **1992**, *86*, 225; Roe, F. J. C. *Indoor Environ.* **1994**, *3*, 191.
(11) Burge, H. A. *Bioaerosols*; Lewis: Ann Arbor, 1995; pp 1−23.
(12) Croft, W. A., Jarvis, B. B., Yatawara, C. S. *Atmos. Environ.* **1986**, *20*, 549.
(13) Chapman, M. D., Heymann, P. W., Wilkins, S. R., Brown, M. J., Platts-Mills, T. A. E. *J. Allergy Clin. Immunol.* **1987**, *80*, 184.
(14) Wood, R. A., Laheri, A. N., Eggleston, P. A. *Clin. Exp. Allergy* **1993**, *23*, 733.
(15) Simon, P. K.; Dasgupta, P. K. *Anal. Chem.* **1995**, *67*, 71; Simon, P. K.; Dasgupta, P. K. *Environ. Sci. Technol.* **1995**, *29*, 1534.
(16) Poruthoor, S. K.; Dasgupta, P. K *Anal. Chim. Acta* (in press).
(17) Curtis, M. A.; Shahwan, G. J. *LC-GC.* **1988**, *6*, 158−64.
(18) Dasgupta, P. K.; Bellamy, H. S.; Liu, H.; Lopez, J. L.; Loree, E. L., Morris, K.; Petersen, K.; Mir, K. A. *Talanta* **1993**, *40*, 53−74.
(19) Harris, D. A. Spectrometric assays. In *Spectrometry and Spectrofluorimetry*; Harris, D. A.; Bashford, Eds.; IRL Press: Washington DC, 1987.
(20) Lowry, O. H., Rosebrough, N. J., Farr, A. L., Randall, R. J. *J. Biol. Chem.* **1951**, *193*, 265.
(21) Peterson, G. L. *Anal. Biochem.* **1977**, *83*, 346.
(22) Jenzano, J. W., Hogan, S. L., Noyes, C. M., Featherstone, G. L., Lundblad, R. L. *Anal. Biochem.* **1986**, *159*, 370.
(23) Levine, M. J., Elison, S. A. *Arch. Oral Biol.* **1973**, *18*, 1859.
(24) Oppenheim, F. G., Hay, D. L. *Biochemistry* **1971**, *10*, 4233.
(25) Smith, P. K. Krohn, R. I., Hermanson, G. T., Mallia, A. K., Gartner, F. H., Provensano, M. D., Fujimoto, E. K., Goeke, N. M., Olson, B. J., Klenk. D. C. *Anal. Biochem.* **1985**, *150*, 76.
(26) Bradford, M. M. *Anal. Biochem.* **1976**, *72*, 248.
(27) Sedmak, J. J., Grossberg, S. E. *Anal. Biochem.* **1977**, *79*, 544.
(28) Wei, Y. J., Li, K., Tong, S. Y. *Talanta* **1997**, *44*, 923.
(29) Tal, M., Silberstein, A., Nusser, E. *J. Biol. Chem.* **1985**, *260*, 9976.
(30) Read, S. M., Northcote, D. H. *Anal. Biochem.* **1981**, *116*, 53.
(31) Loffler, B. M., Kunze, H. *Anal. Biochem.* **1989**, *177*, 100.
(32) Hildemann, L. M.; Klinedinst, D. B.; Klouda, G. A.; Currie, L. A.; Cass, G. R. *Environ. Sci. Technol.* **1994**, *28*, 8, *1585*.

Received for review November 17, 1997. Revised manuscript received January 22, 1998. Accepted February 3, 1998.

ES9710058