Sampling Polyhexamethylene Guanidine Aerosols Using Eosin Y-coated Glass Beads

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Fatalities caused by the use of polyhexamethylene guanidine (PHMG), a general-purpose chemical germicide used as a humidifier disinfectant in Korea, have raised concerns about exposure to biocide aerosols in indoor environments. A sampler capable of accumulating PHMG from aqueous aerosols was developed as an alternative to low-volume air samplers. This sampler was prepared by placing glass beads coated with 2-(2,4,5,7-tetrabromo-6-oxido-3-oxo-3H-xanthen-9-yl)benzoate (Eosin Y) in a custom-made plastic holder. Passive sampling rates, measured in a bench-top exposure chamber at two different aqueous PHMG aerosol generation rates, were found to be independent of the experimental conditions. This suggests that the capacity of the sampler to accumulate the PHMG aerosol was sufficient for the sampling duration tested. However, the passive sampling rate was 7.6×10^{-6} m^3/h for the sampler area of 22 cm^2. This rate is lower than the typical human breathing rate and inadequate for quantitative instrumental analyses at low concentrations in indoor air. A 30-fold enhancement of the sampling rate was achieved by forced convection using a commercial battery-operated fan at ≥2000 rpm. With this accelerated sampling rate, the sampler could be used to monitor time-integrated concentrations of PHMG aerosols in the air.

Keywords: Passive samplers, Exposure assessment, Colorimetry, Indoor air

Introduction

In 2011, the Korea Centers for Disease Control and Prevention announced, based on epidemiological investigations that fatal cases of severe respiratory distress were likely caused by the use of humidifier disinfectants.[1–4] The primary active ingredients of the humidifier disinfectants used were the guanidine-based biocides, polyhexamethylene guanidine (PHMG) and oligo(2-(2-ethoxy)ethoxyethyl)guanidinium chloride (PGH). Pulmonary inflammation in mice after direct instillation of PHMG to the lung also supported the inhalation toxicity of PHMG.[5]

Guanidine-based biocides such as PHMG are general-purpose chemical germicides used in a variety of applications including wet wipes, antibacterial sprays, and pool disinfectants.[6] These chemicals are often used as disinfectants in aqueous solution because of their high water solubility. Human inhalation of wet aerosols may be an important route of exposure, particularly when used in spray form. These chemicals may pose high risks to workers and consumers directly exposed to aqueous aerosols, as in the case of the humidifier disinfectants in Korea. Despite these risks, analytical methods for these chemicals, including ambient air sampling and instrumental analyses, remain under development. Like other water-soluble chemicals, aqueous or dry aerosols of PHMG and other guanidine-based biocides can be sampled using low-volume air samplers coupled with wet traps.[7] However, low-volume air samplers require air pumps that are noisy and require an electrical power source. Thus, passive or simple battery-operated samplers are desirable for many indoor uses.

In this study, we developed a simple sampling device that adsorbs PHMG as a potential alternative to low volume air samplers. The sampler consists of glass beads coated with 2-(2,4,5,7-tetrabromo-6-oxido-3-oxo-3H-xanthen-9-yl)benzoate) (Eosin Y), an anionic dye molecule whose visible light absorption changes in the presence of the guanidinium group. Prior to deployment, the passive sampling rate of the sampler was quantified in a chamber experiment with known concentrations of PHMG aerosols. Potential enhancement of the sampling rate was evaluated by increasing the turbulence near the surface of the sampler with a motorized fan.

Experimental

Aqueous PHMG-phosphate (25% w/w) was kindly provided by SK Chemicals (Seongnam, Republic of Korea) prior to its withdrawal following the official investigation by the Korea Centers for Disease Control and Prevention (the active ingredient of PHMG is no longer commercially available in Korea). This aqueous solution of PHMG was further diluted with deionized water to prepare external standards for quantification and all working solutions. Eosin Y and glycine (98.5%) were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Hydrochloric acid (35%) was purchased from Daejung Chemicals & Metals Co. Ltd (Siheung, Republic of Korea).

Glass beads (2.5–2.85 mm diameter) were physically coated with Eosin Y for sampling PHMG aerosols. In a Petri dish, 25 g of the glass beads were submerged in 5 mL of Eosin Y aqueous solution (0.05% w/w). The solution was evaporated overnight in a fume hood to physically coat the glass beads with Eosin Y. The concentration of the coated Eosin Y was 0.085 mg/g and the coating thickness was approximately 0.1 μm. Ten grams of the coated glass beads were placed in the sampler (Figure 1(a)). The basic sampler unit used for passive sampling consisted of the Eosin Y-coated glass beads and the sampler holder. To increase the sampling rate, a small motorized fan operated at 12 V was attached to the sampler to generate forced convection around the sampler.

PHMG-phosphate aerosols were generated using a commercial nebulizer (Family Silver Aerosol System; Philips, Eindhoven, The Netherlands). The PHMG-phosphate solution (2.5 or 5.0 g/L) was introduced into the nebulizer using a syringe pump at the desired rate to ensure that the nebulizer contained sufficient volume of PHMG-phosphate solution (Figure 1(b)). Fresh air flowed through the nebulizer at 2.0 L/min from a gas cylinder to generate wet aerosols of PHMG. The relative humidity was 60–65% and the temperature was maintained at 25–28 °C in the chamber. The size distribution of aqueous aerosol particles was analyzed using a 7-stage cascade impactor (135 mini MOUDI^{TM}; MSP Co., Shoreview, MN, USA). The mass median aerodynamic diameters and geometric standard deviations at generation rate of 2.5 and 5.0 mg/L were 0.83 and 0.90 μm and 2.01 and 1.88, respectively.

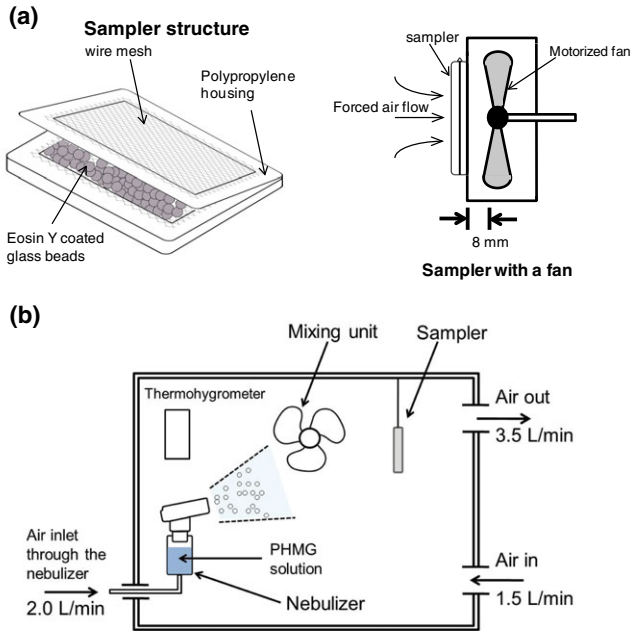

Figure 1. Schematic designs of (a) the Eosin Y-coated glass bead sampler and (b) the laboratory chamber used for quantification of the sampling rate.

To determine the absorption of PHMG aerosols by Eosin Y, the glass beads were removed from the sampler and placed in a vial containing 10 mL deionized water. The Eosin Y coating was re-dissolved into deionized water using a sonicator (28 kHz, 50 W) for 20 min. A 5 mL aliquot of the re-dissolved solution was diluted with 5 mL of deionized water and then analyzed. The concentration of PHMG in the solution was quantified using a colorimetric method, as follows.[7,8] Ten milliliters of 50 mM glycine buffer solution (pH = 3.6 adjusted with concentrated HCl) was added to the prepared aqueous solution of PHMG with Eosin Y. The mixture was vortexed briefly and held for 5–10 min at room temperature for color development. The absorbance of the mixture was then measured at 549 nm using a DR/4000U UV/Vis spectrophotometer (Hach Co., Loveland, CO, USA). The spectrophotometric method has a narrow range of linear concentration dependency (0.1–5 mg/L, see Figure 2). The method detection limit (MDL) was derived using the error distribution method.[9] The lowest external standard (0.125 mg/L) was analyzed seven times using the procedure described above and the MDL was estimated by multiplying the standard deviation of the measurements by the corresponding t value for the sample size (3.143). The results satisfied the criterion of the method and the estimated MDL was 0.10 mg/L in the solution, which corresponds to 2.0 μg in mass. The concentration of PHMG in the extract was quantified using a calibration curve obtained using external standards with at least six different concentrations over a linear range of 0.125–1.25 mg/L (Figure 2); samples that exceeded this concentration range were diluted before analysis. The coefficient of linear regression (r^2) was at least 0.99 for all analyses.

The mass balance equation for PHMG in a well-mixed sampling chamber is as follows:

$$V\frac{dC}{dt} = -QC - RC + E \quad (1)$$

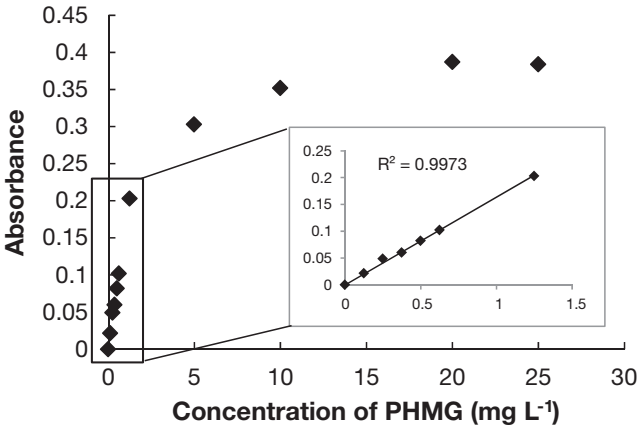

Figure 2. Change in absorbance with concentration of PHMG. External standards with PHMG concentrations of 0.125–1.25 mg/L were used for linear regression because absorbance linearly increased with PHMG concentrations below 2 mg/L (box).

where V is the volume of the chamber (m^3), C is the concentration of PHMG in the air (mg/m^3), t is the sampling time (h), Q is the volumetric flow rate of the air (m^3/h), R is the sampling rate (m^3/h), and E is the generation rate of the PHMG aerosols (mg/h). Aerosol generation was terminated at t_1 and the samplers were removed for analysis at t_2 to conform to safety precautions. The analytical solutions of Eq. (1) for the concentrations of PHMG in the chamber (mg/m^3) are given by:

$$C = \frac{E}{Q+R}\left[1-\exp\left(-\frac{Q+R}{V}t\right)\right], \quad t \le t_1 \tag{2}$$

$$C = C_{t=t_1}\exp\left(-\frac{Q+R}{V}t\right), \quad t > t_1 \tag{3}$$

and the total sampled mass (M, mg) by the sampler is given by:

$$M = \int_0^{t_1} RC\,dt + \int_{t_1}^{t_2} RC\,dt = \frac{ER}{Q+R}\left[t_1 + \frac{V}{Q+R}\exp\left(-\frac{Q+R}{V}t_1\right) - \frac{V}{Q+R}\right] + \frac{ERV}{(Q+R)^2}\left[1-\exp\left(-\frac{Q+R}{V}t_1\right)\right]\left[\exp\left(-\frac{Q+R}{V}t_1\right) - \exp\left(-\frac{Q+R}{V}t_2\right)\right] \tag{4}$$

The only unknown variable in Eq. (4) is the sampling rate R. The sampled mass (M) was determined using the concentration of PHMG in the sampler. With the obtained value of M and the other variables controlled in the experiment, the sampling rate (R) was obtained from Eq. (4).

Passive sampling rates for PHMG were calculated for sampling times (t_1) of 5, 8, 10, and 15 h. The time between t_1 and t_2 was 1.5 h. Triplicate measurements were used to determine the sampling rates at two aerosol generation rates (10.25 and 20.5 mg/h). For the sampler with a motorized fan, the sampling rate was obtained for a sampling time of 5 h and an aerosol generation rate of 10.25 mg/h.

Results and Discussion

Spectroscopic Analysis of Eosin Y from the Glass Bead Coating. Figure 3 shows differences in absorbance of Eosin Y in the glycine buffer solution (22.5 mg/L) with and without PHMG. The absorption spectrum of freshly dissolved Eosin Y did not differ from that of the re-dissolved solution after coating onto the glass beads (Figure 2(a) and (b)). Slight differences in absorbance are likely due to differences in the concentration of Eosin Y. The absorption band near 520 nm broadened and shifted slightly to a higher wavelength in the presence of PHMG (Figure 2(c) and (d)). As suggested by an earlier study,[8] interactions between the guanidinium group in PHMG and the carboxylate group in Eosin Y explains this shift. Thus, coating and dissolution of Eosin Y do not appear to change the binding interactions between Eosin Y and PHMG or the corresponding spectroscopic shift of Eosin Y in the glycine buffer solution.

Passive Sampling Rates. To confirm that Eosin Y coating of the glass beads was required for effective sampling, bare glass beads without Eosin Y coating were tested in a preliminary study under the same experimental conditions. The sampled masses of PHMG after 3 and 10 h sampling periods were below the MDL. Thus, physical adsorption onto the glass beads is not a dominant process and specific interaction between Eosin Y and PHMG is necessary for sampling.

Figure 4 shows the PHMG concentration in the test chamber over time modeled using Eqs. (2) and (3) for a sampling time (t_1) of 10 h, a termination time (t_2) of 11.5 h, a generation rate

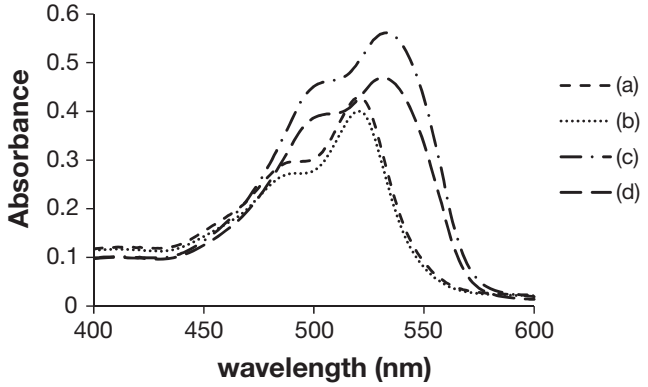

Figure 3. Absorption spectra of aqueous solutions containing (a) Eosin Y, (b) re-dissolved Eosin Y from the glass bead coating, (c) Eosin Y with PHMG, and (d) re-dissolved Eosin Y with PHMG.

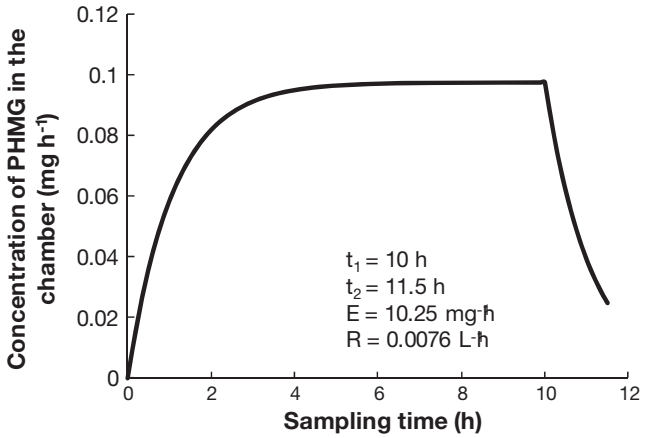

Figure 4. Example calculation of the PHMG concentration in the test chamber over time when the sampling time (t_1) is 10 h, the termination time (t_2) is 11.5 h, the generation rate (E) is 10.25 mg/h, and the sampling rate (R) is 0.0076 L/h.

(E) of 10.25 mg/h,, and a sampling rate (R) of 0.0076 L/h, as an example. The sampled mass is the product of the area under the curve and the constant sampling rate.

Passive sampling rates for PHMG for two different generation rates and various sampling durations are listed in Table 1. The passive sampling rate was 7.6 (±2.7) × 10^{-6} m^3/h and was independent of sampling time, generation rate, and the concentration of PHMG aerosols in the chamber. These results imply that the reactive surfaces of the sampler were not saturated with PHMG during the sampling period; hence, Eosin Y-coated glass beads were found to be a suitable for sampling the concentration ranges and time periods investigated. Adsorption of wet aerosols containing PHMG on the surfaces of the test chamber may have reduced the concentration of PHMG calculated using Eqs. (2) and (3). However, losses due to adsorption were likely insignificant because passive sampling rates were invariant regardless of the aerosol generation rate or sampling time and no significant adsorption onto the bare glass beads was observed.

A typical diffusion coefficient for aerosols with an aerodynamic diameter of about 0.83 μm in air is estimated as 1.2 × 10^{-7} m^2/h.[10] The surface area of the sampler (Figure 1(a)) is approximately 0.0022 m^2 and thus the corresponding mass transfer coefficient is 3.5 × 10^{-3} m/h. If deposition of PHMG aerosols onto the sampler was limited by diffusion in the mass transfer boundary layer, the corresponding thickness of the air boundary layer would be 34 μm. This value agrees well with the estimated thickness of the air boundary layer for the rough surface of latex paint,[11] suggesting that the sampling rate of the sampler in passive sampling mode can be explained by diffusion of aerosols through the air boundary layer.

However, the passive sampling rate was relatively low to ensure that the time-weighted average concentration of PHMG in ambient air could be quantified. For example, the indoor concentration of PHMG has been estimated to be approximately 0.05 mg/m^3 based on the typical use scenario of PHMG as a humidifier disinfectant.[3,7] If passive sampling was conducted at this air concentration for 10 h, the mass of PHMG sampled would only be 0.0038 μg and the corresponding concentration in aqueous solution (following the experimental protocol used in this study) would be 0.00038 mg/L. This value is much lower than the MDL (0.1 mg/L) by a factor of 260 and therefore, enrichment would be required to allow analytical quantification. The sampling rate could be linearly increased by increasing the surface area of the sampler or by using more sensitive instrumental analysis such as matrix-assisted laser desorption/ionization time-of-flight mass spectrometry[12] or high-performance liquid chromatography–mass spectrometry.[13,14] However, passive sampling alone would be insufficient for precise quantification of PHMG unless the ambient concentration was very high, as was the case in the test chamber used in this study and in theoretical predictions using the film diffusion model.

Table 1. Passive sampling rates for PHMG using the Eosin Y-coated glass bead sampler for various generation rates and sampling times.

	Sampling rate, R (×10^{-6} m^3/h)	
Sampling time (h)	E = 10.25 mg/h	E = 20.5 mg/h
5	10.1 ± 1.7	7.8 ± 2.0
8	7.8 ± 1.1	7.9 ± 2.1
10	7.8 ± 2.2	5.7 ± 1.1
15	6.0 ± 1.6	7.5 ± 1.7
Average	7.6 ± 2.7	

E is the generation rate of PHMG aerosols.

Increasing the sampling rate for PHMG was evaluated using a motorized fan (Figure 1(a)). Figure 5 shows increases in the sampling rate compared to the passive sampling rate (R/$R_{passive}$). The sampling rate was greatly increased (~30-fold) by increasing fan rpm. The change was more significant at lower rpm with no meaningful increase observed above 2000 rpm. These results confirm that the overall mass transfer of PHMG aerosols is dominated by diffusion through the stagnant air layer near the surface of the sampler in the passive sampling mode and at lower rpm consistent with other passive air sampling studies.[15] The invariance of the sampling rate at higher rpm may be explained by (1) limitations in decreasing the diffusion boundary layer thickness through air agitation and/or (2) binding of PHMG with the Eosin Y coating becoming rate limiting. With an increase in the sampler size, forced convection is expected to make this sampler feasible for sampling PHMG aerosols at ambient concentrations.

Conclusion

In conclusion, a new sampler for reactive biocide aerosols of PHMG was developed using a specific reaction between the guanidinium group of PHMG and Eosin Y, as an alternative to conventional low-volume air samplers. The sampler can be used to conduct time-integrated passive sampling to monitor the concentration of PHMG aerosols. The sampling rate can be enhanced approximately 30-fold using a small motorized fan, making the sampler more feasible for application in indoor environments.

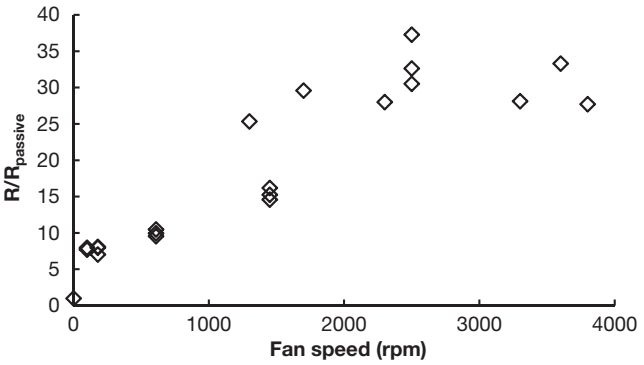

Figure 5. Enhancements in the sampling rates with increasing rotation speed of the fan on the sampler. The increase in sampling rate is presented as a ratio of sampling rate to that of passive sampling ($R_{passive}$).

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