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Journal of Chromatography A, 710 (1995) 175–180

JOURNAL OF
CHROMATOGRAPHY A

Laboratory validation of a diffusive sampler for the determination of glutaraldehyde in air

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Abstract

A diffusive sampling method has been validated for determination of glutaraldehyde in air. The sampler consists of a filter impregnated with 2,4-dinitrophenylhydrazine, mounted in a polypropylene housing. The uptake rate was determined to be 11.8 ml/min, with a relative standard deviation of 13%. The effect of glutaraldehyde concentration, sampling time and relative humidity on uptake rate was undetectable or small. The temperature effect was significant, about 1.5%/°C. The samplers are stable for at least two weeks at 22°C. The detection limit is about 0.03 mg/m^3 for a 15-min sample.

1. Introduction

Glutaraldehyde is used in the drug and polymer industry, as a fixative for tissues, as a tanning agent in the leather industry and as a cold sterilizer for medical equipment. The substance is irritating to the respiratory tract, eyes and skin and can cause allergic contact dermatitis [1]. Occupational exposure limits are low because of health effects. The current Swedish ceiling value as well as the ceiling value of the American Conference of Governmental Industrial Hygienists (ACGIH) is 0.8 mg/m^3 [2,3].

Glutaraldehyde in air can be sampled by passing the air through a liquid absorber, but impingers or gas wash bottles makes personal sampling laborious. A solid sorbent would be preferable for field measurements.

For pumped sampling of glutaraldehyde, various adsorbents, such as alumina [4] or XAD-4 [1], have been used but the samples are not stable and have to be analysed the same day. The use of 2,4-dinitrophenylhydrazine (DNPH) as chemosorbent gives a stable derivative that allows selective and sensitive analysis with HPLC and UV detection. For pumped sampling of glutaraldehyde, DNPH-coated XAD-2 [5] or DNPH-coated glass-fiber filters have been used [1].

In recent years, diffusive sampling has become important as an efficient alternative to pumped sampling in occupational hygiene [6]. The theory of diffusive sampling is described by Fick's first law, i.e.

$$m/t = DA(C - C_0)/L$$

where m = mass collected on the sorbent (ng); t = sampling time (s); D = diffusion coefficient (cm^2/s); A = cross sectional area of the opening of the monitor (cm^2); C = external concentration (ng/cm^3 = mg/m^3); C_0 = concentration of the analyte above the surface of the sorbent (ng/

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SSDI 0021-9673(95)00047-X

$cm^3 = mg/m^3$); L = length of the diffusive zone of the monitor (cm). With an ideal adsorbent or a chemosorbent with no reverse reaction the assumption can be made that $C_0 = 0$, which reduces the Fick's law relationship to:

$$m/t = DAC/L = SC$$

where S = sampling rate, DA/L (cm^3/s). The diffusion coefficient (D) is a physical parameter of the sampled analyte and independent of the sampler construction whereas A and L are parameters associated with the sampler construction and independent of analyte. The sampling rate can be theoretically calculated from the diffusion coefficient and the geometry of the sampler, but these theoretical values often differ from measured values. An experimentally determined sampling rate is calculated after analysis of diffusive samplers exposed in atmospheres of known concentration.

The most important protocol describing how to test a diffusive sampling method has been proposed by the European Committee for Standardisation (CEN) [7]. This protocol describes how tests should be performed to examine effects on sampling rate from parameters such as sampling time, concentration, relative humidity, temperature, storage, wind velocity, etc. These tests must be performed under laboratory conditions with accurate control of the above-mentioned parameters.

We have previously reported the development of a diffusive sampler, designed to contain a reagent-coated filter for the sampling of reactive compounds [8]. The sampler, with a DNPH-coated filter, has been validated for formaldehyde [9]. It has also been validated for sampling of primary and secondary amines with 1-naphthyl isothiocyanate-impregnated filter [10,11]. We have now validated the sampler for the determination of glutaraldehyde with the use of DNPH-impregnated filters.

2. Experimental

2.1. Diffusive sampler

The diffusive sampler is shown in Fig. 1. The housing, measuring 60 × 30 × 5 mm, is made of polypropylene. The impregnated filter, 20 × 45 mm, is placed beneath a 2.9-mm thick screen of the same size. Within an area 20 × 20 mm, the screen has 112 holes with a diameter of 1.0 mm. The filter part beneath the holes is used for sampling (sampling filter) and the other half is used to quantitate the filter blank (control filter). The tape is marked into the two sections by a small ridge on the back of the screen plate. A sliding cover is used to seal the holes when the sampler is not in use. The sampler is available from GMD Systems (Hendersonville, PA, USA).

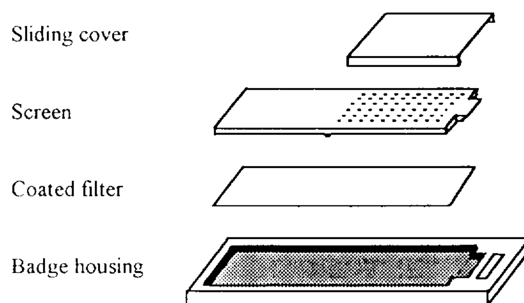

Fig. 1. Diffusive sampler for reactive compounds.

2.2. Chemicals

Solvents used for the HPLC analysis were acetonitrile (HPLC grade, Rathburn, Walkerburn, UK) and water (purified by use of Milli-RQ system, Millipore, Bedford, MA, USA). 2,4-Dinitrophenylhydrazine (DNPH) (Fluka p.a.) was recrystallized twice with 4 *M* HCl. For coating filters, phosphoric acid (Merck, p.a.), glycerol (May and Baker, p.a.), ethanol (99.99%) and acetonitrile (Rathburn, HPLC grade S) were used. For the dynamic generation, glutaraldehyde (TAAB, 24.8%, purified for electron spectroscopy, Reading, UK) was used. Glutaraldehyde–2,4-DNPH was prepared from DNPH, glutaraldehyde and concentrated HCl and recrystallized twice from ethanol [5].

2.3. Filters for diffusive sampling

A solution for coating the filters was made from 160 mg recrystallized DNPH, 0.3 ml con-

centrated phosphoric acid, 0.7 ml 20% glycerol in ethanol and 20 ml acetonitrile. Glass fiber filters (2 × 2 cm), were cut from round filters (Type AE, 0.3 μm pore size, diameter 25 mm, SKC, PA, USA). These were then dipped into the coating solution and allowed to dry on a glass surface. One filter was placed under the sampling part of the sampler and another under the control part.

2.4. Reference method

As a reference method, pumped sampling with a 13-mm glass-fiber filter impregnated with DNPH was used. This method has been described previously [12].

2.5. Generation of glutaraldehyde

The glutaraldehyde was diluted in water to obtain concentrations ranging from 6.4 to 32 mg/ml. These solutions were injected with a syringe pump (Carnegie Medicine, Stockholm, Sweden) into a glass nebulizer (J.E. Meinhard Assoc., CA, USA). The syringe pump flow varied from 2 to 10 μl/min and the air flow though the nebulizer was 0.9 l(N)/min. The aerosol from the nebulizer was mixed with air (5 l(N)/min) and evaporated in an evaporation chamber with an internal volume of about 0.5 l (Fig. 2.) [13]. The air mixture was then further diluted and transported to an exposure chamber that has been described earlier [12]. The air flow in the exposure chamber was 40 l/min. The air was controlled in respect of relative humidity and temperature. The wind velocity in the exposure chamber was 0.3 m/s for all experiments.

2.6. Sample analysis

The glutaraldehyde–DNPH was eluted from the filter by shaking for 1 min with 2.0 or 3.0 ml acetonitrile. A volume of 10 μl was injected into the liquid chromatograph. An HPLC system consisting of two Waters M-6000 A pumps, a Waters M-710 B auto sampler and a Shimadzu adsorbance detector was used. The system was controlled from a computer with a Waters Maxima data system which also served as the tool for evaluating the chromatograms. The column was a Cosmosil 5 NPE Waters 4.6 × 150 mm (Nacalai Tesque, Kyoto, Japan). The mobile phase was 80% acetonitrile in water and the flow-rate was 0.8 ml/min. The hydrazone was detected at 365 nm.

The glutaraldehyde hydrazone can exist as three different isomers (*cis–cis*, *cis–trans* and *trans–trans*). From the analysis, two of these can be seen with a major isomer of about 85–90% of the total area of the isomers (Fig. 3.). In a fresh standard solution the major isomer is about 90% or more of the total area, but decreases to about 85% after storage. As the relation between the peaks can alter, both have to be taken into account in the analysis. The extinction coefficient at 365 nm for different low molecular aliphatic dinitrophenylhydrazones are about the same which gives the assumption that the extinction coefficients of the glutaraldehyde–hydrazone isomers are equal. This assumption and the fact that two peaks have to be summed up can give some uncertainty to the method, but this is within the measured overall uncertainty of the method.

The analytical system detection limit of the main peak was about 70 pg (signal-to-noise ratio

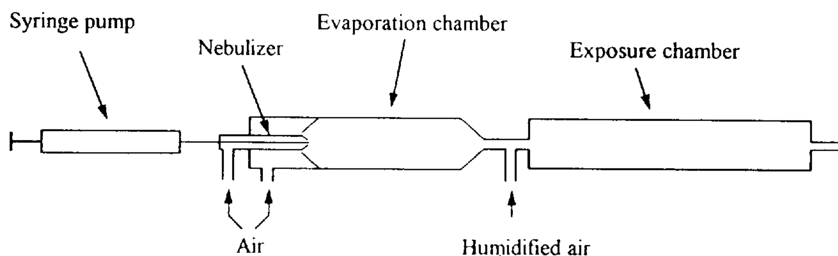

Fig. 2. Evaporation chamber with nebulizer for generation of gaseous glutaraldehyde.

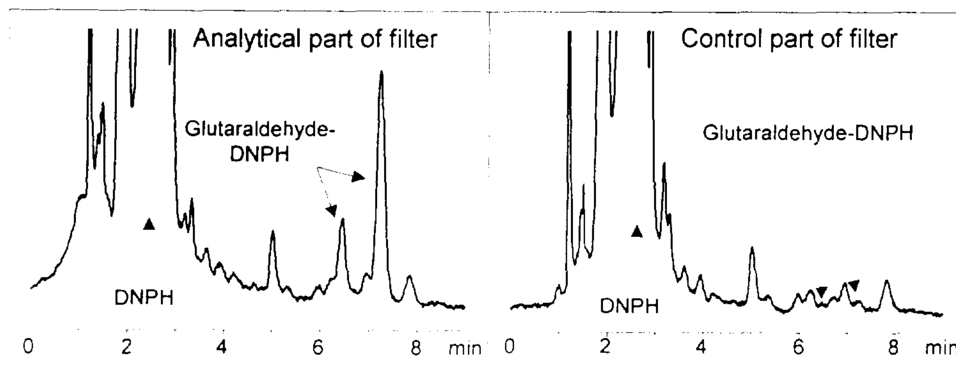

Fig. 3. HPLC chromatogram showing glutaraldehyde–DNPH from a diffusive sampler exposed to 0.08 mg/m^3 glutaraldehyde for 8 h. The concentration in the analytical part of the filter was 1.08 μg/ml and in the control part 0.053 μg/ml of glutaraldehyde–DNPH corresponding to 0.47 and 0.023 μg glutaraldehyde per sample, respectively.

3:1). However, because of isomers and impurities, the smallest detectable amount for the method was about 5 ng glutaraldehyde per sample.

3. Results and discussion

The diffusive sampler was validated according to the protocol proposed by European Committee for Standardisation (CEN) [7]. The effects of sampling time, concentration, relative humidity, temperature, zero exposure and storage were investigated. The effects of wind velocity and sampler orientation were previously studied in connection with validation of the sampler for formaldehyde [9]. In that study the uptake rate was constant with a wind velocity at the sampler face varying between 0.02 and 1.0 m/s. Most personal sampling conditions give wind velocities of about 0.1 m/s [6]. A slight increase in the uptake rate at high wind velocities was observed with the sampler in an orientation perpendicular to the air stream. The sampler has been compared with pumped sampling in office and home environments. These tests were performed without active ventilation and without any people present. The wind velocities were less than 0.02 m/s. The sampler performed well at these very low wind velocities, allowing static area sampling of indoor air [8,9]. As wind velocity and sampler orientation are parameters associated with the sampler and not the analyte, these effects were not studied further in this work.

The sampler is manufactured with a cellulosic material filter tape. This original tape contained some contaminants that made the detection limit too high for short-time sampling of low levels of glutaraldehyde. The use of a glass-fiber filter material gave detection limits that were close to the analytical detection limit. In this study we used our own coated glass-fiber filters in all the experiments.

The concentration in the exposure chamber was calculated from the amount delivered through the nebulizer and the total air flow. The concentration was confirmed by the reference method. The experimentally determined values were generally within ± 10% of the calculated values. In all experiments the calculated value was taken as the true value of the delivered concentration.

The effect of sampling time, concentration and relative humidity (RH) was investigated by a three-factor factorial design. The individual results from each experiment are shown in Table 1. The statistical analysis was performed with the use of multiple regression [14]. This analysis gives information on the influence of the different parameters on sampling rate. As can be seen in Table 2, there is a small positive influence by time and a small negative influence by concen-

Table 1
Sampling rate of diffusive sampler at various glutaraldehyde concentrations, sampling times and relative humidities

Time (min)	Concenration (mg/m^3)	RH (ml/min)	Uptake	RSD	n
15	0.16	20%	11.8	13%	6
17	0.16	80%	12.1	6%	6
434	0.08	20%	13.5	4%	6
15	1.6	20%	11.6	3%	6
17	1.6	80%	9.3	8%	6
479	1.6	20%	10.6	3%	6
481	1.6	80%	13.5	2%	6
288	0.84	50%	12.7	1%	6
Mean			11.8	13%	48

Face velocity 0.3 m s^{-1}. RH = relative humidity, RSD = relative standard deviation, n = number of determinations.

tration. These effects give deviations less then 10% from the mean. There was no influence by relative humidity. The mean was 11.8 ml/min, with a relative standard deviation of 13%. This gives a relative overall uncertainty of 26%, calculated according to the European Standard EN 482 [15].

With a diffusion coefficient of 0.0718 cm^2/s, calculated according to Hirschfelder et al. [16], the theoretically calculated uptake rate is 13.1 ml/min. This deviation from the measured value by only 11% is well within accepted limits.

The hydrazone derivative stability was evaluated in a zero exposure test. Glutaraldehyde exposure for 30 min was followed by a zero concentration (clean air) exposure for 7.5 h.

As Table 3 shows, the result obtained from the test was 11.6 ml/min. This shows that there is no decomposition of the glutaraldehyde hydrazone.

Table 2
Multiple regression analysis of the influence of sampling time, concentration and relative humidity

Variable	Parameter estimate (ml/min)	Standard error	
Intercept (uptake rate)	11.8	0.42	
Time	$3.9 \cdot 10^{-3}$	$8.4 \cdot 10^{-4}$	S
Concentration	−1.1	0.25	S
RH	$6.0 \cdot 10^{-3}$	$6.3 \cdot 10^{-3}$	NS

S = significant; NS = not significant.

The temperature tests showed a significantly lower uptake rate (8.6 ml/min) at 12°C, and a significantly higher uptake rate (13.5 ml/min) at 40°C (Table 3). The effect is about 1.5% per degree Celsius. A temperature dependence of $T^{1.75}$ on the diffusion coefficient from temperature is given by Fuller et. al. [17]. This diffusion coefficient variation of about 0.6% per degree can only partly explain the temperature effect on uptake rate. Possibly there is also a reaction rate effect.

Two storage tests were performed, one with the filter cut into two pieces and one with the filter uncut (2 × 4 cm). This was done in order to detect possible hydrazone migration on the filter. The filters were stored for 14 days at 22°C. No difference between the two experiments could be seen which shows that there is no migration within the filter (Table 3). The storage tests also confirm the assumption based on the zero exposure test that there is no decomposition of the hydrazone.

4. Conclusions

The diffusive sampler tested in the study has been designed for use with reagent-coated filter

Table 3
Tests on zero exposure, temperature and storage

	Time (min)	Concentration (mg/m^3)	RH	Uptake rate (ml/min)	RSD	n
Zero exposure[a]	30	1.6	20%	11.6	6%	5
Exposure at 12°C[b]	200	1.6	50%	8.6	2%	6
Exposure at 40°C[b]	250	1.6	50%	13.5	3%	6
Storage, filter uncut[c]	240	1.6	50%	12.7	3%	6
Storage, filter cut[c]	240	1.6	50%	12.9	1%	5

[a] Exposure for 30 min followed by a zero concentration (clean air) exposure for 7.5 h.
[b] Calculated against normalized volume (22°C, 1013 bar).
[c] Storage at 22°C for 14 days.

tape. For sampling of glutaraldehyde, 2,4-dinitrophenylhydrazine is used. The determined sampling rate was 11.8 ml/min, with a standard deviation of 13%. The influence of concentration, time and humidity on uptake rate was undetectable or small. The effect of temperature was significant, and about 1.5%/°C. No decomposition could be seen in the storage test. The sampler can be used for short-time sampling (15 min) with a sensitivity of about 0.05 mg/m^3 as well as for 8 h sampling.

The sampler has been shown to perform well at extremely low wind velocities which makes the method suitable both for static and for personal monitoring.

Acknowledgement

The authors wish to thank the European Commission, Measurement and Testing Programme (BCR) for financial support.

References

[1] OSHA Analytical Methods Manual: U.S. Department of Labour, Occupational Safety and Health Administration; OSHA Analytical Laboratory: Method 64: Glutaraldehyde. American Conference of Governmental Industrial Hygienists (ACGIH): Cincinnati, OH, ISBN: 0-936712-66-X, ACGIH, Salt Lake City, UT, 1985.
[2] NSBOSH Occupational Exposure Limits, Ordinance AFS 1993: 3, National Swedish Board of Occupational Safety and Health, Stockholm (in Swedish).
[3] Threshold Limit Values for Chemical Substances in the Work Environment, American Conference of Governmental Industrial Hygienists, Cincinnati, OH, 1993.
[4] W. Wliszczak, F. Meisinger and G. Kainz, Microchim. Acta, II (1977) 139.
[5] K. Andersson, C. Hallgren, J.-O. Levin and C.-A. Nilsson, Chemosphere, 10(3) (1981) 275.
[6] A. Berlin, R.H. Brown and K.J. Saunders, (Editors): Diffusive Sampling — an Alternative Approach to Workplace Air Monitoring, Royal Society of Chemistry, London, 1987.
[7] Requirements and Test Methods for Diffusive Samplers for the Determination of Gases and Vapors, prEN 838, European Committee for Standardisation (CEN), Brussels, 1992.
[8] J.-O. Levin and R. Lindahl, Analyst, 119 (1994) 79.
[9] J.-O. Levin, R. Lindahl and K. Andersson, Environ. Technol. Lett., 9 (1988) 1423.
[10] J.-O. Levin, R. Lindahl, K. Andersson and C. Hallgren, Chemosphere, 18(11–12) (1989) 2121.
[11] R. Lindahl, J.-O. Levin and K. Andersson, J. Chromatogr., 643 (1993) 35.
[12] J.-O. Levin, R. Lindahl and K. Andersson, Environ. Sci. Technol., 20(12) (1986) 1273.
[13] A. Gustavsson and O. Nygren, Spectrochim. Acta, 42B(7) (1987) 883.
[14] R. Carlsson, Design and Optimization in Organic Synthesis (Data Handling in Science and Technology, Vol. 8), Elsevier, Amsterdam, 1992.
[15] General Requirements for the Performance of Procedures for Workplace Measurements, EN 482, European Committee for Standardisation (CEN), Brussels, 1993.
[16] J.O. Hirschfelder, R.B. Bird and E.L. Spotz, Trans. Am. Soc. Mech. Eng., 71 (1949) 921.
[17] E. Fuller, P. Schettler and C. Giddings, Ind. Eng. Chem., 58(5) (1966) 19.