Preparing Accurate Standard Gas Mixtures of Volatile Substances at Low Concentration Levels

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Abstract: A simple approach for preparing standard gas mixtures of environmentally important volatile organic substances for gas chromatographic calibration is described. A liquid solution of the volatile substance of interest in a suitable solvent is prepared in a flask at known low concentration. Then, an easily measurable volume of this solution containing a very minute amount of the analyte is injected into a fixed-volume vessel (e.g., a glass sampling bulb) that has been flushed with a dilutent gas (e.g., air, N_2, He). The generated gas-phase concentration of the substance after evaporation is easily calculated. This method allows students to prepare standard gas-phase mixtures at very low concentrations by direct injection of extremely small amounts into solution using a reasonable size microsyringe.

Gas chromatography is well suited for the analysis of environmentally interesting, harmful, or toxic volatile organic substances. The response of a gas chromatograph, however, is not absolute; therefore, peak heights must be calibrated against accurately prepared mixtures of known composition in order to carry out quantitative work. Obtaining the required standards at the appropriate concentration ranges (ppm level), commercially, is not always possible and is often expensive. For this reason several techniques have been devised to permit analysts to make up their own calibration standards [1, 2]. The normal methods of producing standard gas mixtures are useful when relatively small volumes of these mixtures are required at high or moderately low concentration level. These methods involve introducing known masses or volumes of volatile liquid components into dilutent-gas-filled vessels of fixed dimensions, for instance metal, glass, or plastic containers at atmospheric pressure (The vessel is simply flushed by the dilutent gas.) [2, 3].

Preparing very low concentration (ppm level or below) standard gas mixtures of volatile liquids by direct injection of a small volume into a vessel is not reliable unless a vessel with a very large volume (5 L or more) or a syringe with a very small volume (0.1 µl or less) is used. A small volume syringe may compromise concentration accuracy due to the large propagated uncertainties associated with the small volume measurement. A similar dilution method is recommended and frequently used for pressurised gravimetric gas mixtures. A pressurised gas mixture is transferred into an evacuated cylinder and brought to the desired concentration by adding pure dilutent gas under pressure [4]. Nevertheless, no reliable method is described for atmospheric-pressure gas mixtures in any of the well-known sources. We describe below the details of a simple and novel preparation procedure.

Procedure

Caution: This entire procedure should be performed in a fume hood. Gloves and safety glasses should be worn at all times. Exercise care in the use of methanol (poisonous and flammable), benzene (carcinogenic), and dichloromethane (narcotic). Note: less hazardous solvents may be used to if suitable chromatographic conditions are established.

Example 1

We want to prepare a gaseous methanol/air mixture with a 10-ppm (v/v) gas-phase concentration using a 500-mL glass sampling bulb. The vapor volume, v_{vap}, in µL of methanol that must be present in the bulb is calculated from equation 1

$$v_{vap} = \frac{1000CV_{bulb}}{1,000,000} \tag{1}$$

where C is the desired gas phase concentration (in ppm), V_{bulb} is the bulb volume (in mL), and multiplication by 1000 converts from mL to µL. We need to determine the volume of liquid methanol that must be injected into the bulb to form 5.0 µl of vapor in the bulb volume. We first determine how much vapor volume is produced from 1 ml of methanol at the laboratory temperature and pressure by using the ideal gas equation (equation 2).

$$V_{vap} = \frac{nRT}{P}\frac{m_{liq}}{M}\frac{RT}{P} = \frac{d_{liq}V_{liq}}{M}\frac{RT}{P} \tag{2}$$

m is the mass of the liquid, M is its molecular weight, T is the temperature, P is the pressure, and d_{liq} is the density of the liquid. For a liquid volume of 1ml, equation 2 becomes

$$V_{vap} = \frac{d_{liq}}{M}\frac{RT}{P}\times 1000 \tag{3}$$

V_{vap} is now the vapor volume in ml produced from 1 ml of liquid; d_{liq} is the density of methanol (0.79 g/mL); M is the molecular weight of methanol (32.04 g mol^{-1}); R, the ideal gas constant, is 0.08206 L atm mol^{-1} K^{-1}; T is the temperature in the laboratory (291K); and P is the pressure in the laboratory (0.992 atm). Using equation 3 with the given values, V_{vap} is 593.5 mL of vapor per mL of liquid. The amount of pure

liquid analyte, v_{liq}, needed to yield a concentration of 10 ppm in a 500-mL bulb is

$$v_{liq} = \frac{v_{vap}}{V_{vap}} \quad (4)$$

or 0.0084 µL. By substitution of equations 1 and 3 into equation 4,

$$v_{liq} = \frac{CV_{bulb}}{1{,}000{,}000}\frac{MP}{d_{liq}RT} \quad (5)$$

Note that the conversion factors of 1000 for ml per L (from *R*) and mL per µL cancel. (A previous study [5] showed that vapors of volatile substances (e.g., liquid anaesthetics) act approximately as ideal gases in common laboratory conditions and so the error due to this assumption is negligible; therefore, equation 5 is valid for calculations of the vapor volume of any volatile liquid substance at atmospheric pressure.)

We now know to prepare a methanol solution in a suitable solvent that contains the calculated amount (from equation 4) of methanol (0.0084 µl) in an easily measurable volume of the solution (in this case 1 µl) and inject this solution into the bulb to produce a gas-phase methanol concentration of 10 ppm. We choose a volume for the prepared stock methanol solution (for instance, 50 mL). We want a 1-µl aliquot of this solution to contain 0.0084 of methanol. The volume of pure liquid methanol that should be added into the flask, V_{liq}, (= 420 µL) can be calculated from equation 6 where V_{sol} is the volume of the prepared solution (50 mL), and 1000 is the conversion factor from mL to µL.

$$V_{liq} = V_{sol.}v_{liq.}1000 \quad (6)$$

The calculations above are an example of the type of solutions that can be prepared in this manner. If lower concentration gas mixtures are needed, the bulb volume can be increased, the volume of the prepared solution can be increased, or the amount of analyte added to the solution can be lowered. The volume of the pure liquid analyte, V_{liq}, that must be added to the stock solution, V_{sol} to produce the required gas-phase concentration, *C*, of any liquid analyte in a vessel of fixed volume, V_{bulb}, at any *P* and *T* by injection of a chosen µL volume, V_{inj}, of the stock solution may be calculated by using the general equation below.

$$V_{liq} = \frac{CV_{bulb}}{1000}\frac{MP}{d_{liq}RT}\frac{V_{sol}}{V_{inj}} \quad (7)$$

Example 2

A student needs to prepare a gas mixture of methanol (d = 0.79 g mL^{-1}, M = 32.04 g mol^{-1}) with a concentration of 10 ppm using a 500 mL sampling vessel. The student decides to make a 50 mL stock solution and wants to use a syringe capable of injecting 1 µl of solution into the vessel. How many microliters of pure liquid methanol should be added to make up the 50 ml stock solution at P = 0.992 atm and T = 291 K?

$$V_{liq} = \frac{10\ (500\ \text{mL})(1000\ \mu\text{L mL}^{-1})(32.04\ \text{g mol}^{-1})}{1{,}000{,}000(0.790\ \text{g mL}^{-1})(0.08206\ \text{L atm mol}^{-1}\ \text{K}^{-1})}$$

$$\times\ V_{liq} = \frac{(0.992\ \text{atm})(50\ \text{mL})(1000\ \mu\text{L mL}^{-1})}{(1000\ \text{mL L}^{-1})(291\ \text{K})(1\ \mu\text{L})} = 421\ \mu\text{L}$$

If the student injects 1 µL of stock solution in to the vessel, he will obtain a 10 ppm gas mixture concentration, if he injects 2 µL of stock solution in to the same vessel he will obtain 20 ppm gas mixture concentration and so on.

Equation 7 may also be used to back calculate. For example, suppose a student added 500 µL pure liquid methanol into the stock solution instead of 421 µL under the same conditions, but still wants to inject 1 µL of the stock solution into the same vessel. What will be the concentration of the gas mixture in ppm? Using equation 7, the concentration will be 11.86 (≈12) ppm. If the student wants a final concentration of 10 ppm using the same stock solution, how much stock solution must be added to the vessel? Again, equation 7 is used to calculate that 0.84 µL must be added (the student should realise that this is a difficult volume to dispense precisely).

Preparation of the Static Gas Mixtures

Using the calculations discussed above, begin by dissolving 421 µL of methanol in cyclohexane and toluene using 50 mL A-grade flasks containing 0.084 µL methanol per µL of solution. A 500-mL glass sampling bulb (J. Young Scientific Glassware Ltd., England) is flushed for several minutes with the dilutent gas (nitrogen) to clean the bulb, then the gas is turned off and the Teflon stopcocks closed. The required volume (e.g., 0.25 µL for 2.5 ppm, 0.5 µL for 5.0 ppm, etc. methanol concentration in the gas phase) of the liquid mixture is then injected into the clean bulb through the septum using 0.5 or 1.0 µl syringe. Following evaporation of the injected liquid (about 30 s for the most concentrated case), the mixture is allowed to rest for 30 min to insure proper mixing. The mixtures are introduced into the chromatographic system by means of a 1-mL capacity gas-tight syringe.

A Packard Model 439 gas chromatograph (Alltech, Netherlands) fitted with a FID detector and a 2-m ¼ in o.d. glass column coated with 10% Carbowax 20 M and 2% KOH on 80–100 mesh Chrom waw support (Alltech, Netherlands) is employed and operated isothermally at a temperature of 50 °C. Flow rates for the carrier gas, hydrogen, and air are measured at the outlet of the detector by using a soap bubble-flow meter. They are 30, 20, and 250 mL min^{-1}, respectively.

Discussion

Dissolving a minute amount of a volatile liquid in an organic solvent and injecting very small volumes of analyte using a reasonable size (1.0, 5.0 or 10.0 µL) microsyringe is a useful method for preparing gas-mixture standards. This permits the analyst to prepare a series of standard gas mixtures, which can be used to construct calibration graphs, at very low concentrations by adding increasing volumes of solution into dilutent-gas flushed containers. The amount of the volatile substance in the volume of injected solution depends on the concentration of the solution; therefore, preparing gaseous

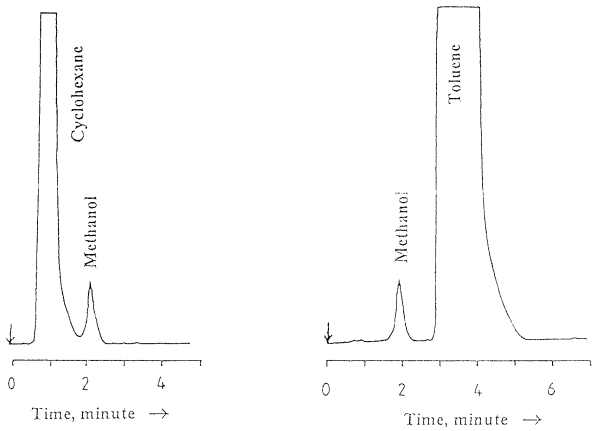

Figure 1. Chromatographic separation of gas mixtures of methanol: (a) in cyclohexane, (b) in toluene.

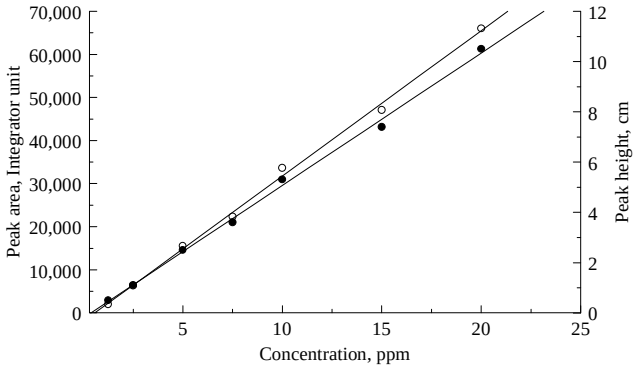

Figure 2. Calibration graph for methanol mixtures prepared in cyclohexane: (a) peak areas (○), R^2 = 0.9992; Y = 3373.10 X – 1951.24; the statistical limit of detection is 0.42 PPM, (b) peak heights (●), R^2 = 0.9998; Y = 0.5267 X – 0.1947; the statistical limit of detection is 0.63 PPM. The dilutent gas is nitrogen.

samples containing extremely small amounts of substance, which would be impossible to measure directly using a micro-syringe or a four-place analytical balance (due to its volatility), may be prepared. The effect of atmospheric pressure changes on the solvent evaporation is usually negligible, but environmental temperature changes more significantly effect the final concentration. A loss of 1 mL of solvent from a 50 mL stock solution produces a 2% error in the final concentration. The stock solution should be leveled before use. Still, the loss of the analyte by evaporation can not be compensated for; therefore, care must be taken during the preparation, storing, and injection of the stock solution.

Three methods of preparing the solutions that depend on the solvent impurities and their effect on the chromatogram can be employed.

1) A stock solution of a volatile substance is prepared in a flask. Increasing volumes of this solution is injected into the container by means of a microsyringe. In this case, the peaks for the solvent impurities increase parallel to the peak of the analysed substance. This procedure is the most economical of the three.
2) Different amounts of the substance are added to a series of solvent-filled flasks of the same volume. The same amount of solution from each flask is injected into the container. This method leaves the peaks of the solvent impurities unchanged on the chromatograms.
3) A stock solution is prepared in a flask at low concentration. Following each injection, more substance is added and the concentration calculated. Each injection dispenses the same volume into the container. This procedure, like method 2, keeps the ratio of the solvent impurities constant on the chromatogram. The concentration changes due to the amount of solution used by previous injections are negligible.

The solutions are not reused.

The selection of the solvent must be carefully considered. It must completely dissolve the volatile liquid, be pure, and be well-separated chromatographically from the analyte. The retention time of the solvent can be either shorter or longer than that of the analysed substance (see Figure 1). If the solvent and substance peaks are very close, the peak for the substance inevitably appears on the tail of the solvent peak, especially at low concentrations. If there is reason to use a solvent with a longer retention time than the analyte, consider that the shorter the retention time of the solvent, the shorter the total analysis time. If the solvent contains impurities, the chromatographic pattern of the solvent alone must be obtained. If impurity and analyte peaks overlap on the chromatogram and this cannot be eliminated by adjusting the chromatographic conditions, one of the other methods listed above should be chosen. Also, any suitable organic solvent may be used and the chromatographic conditions adjusted to obtain the best separation or the solvent may be chosen to work well under the specific chromatographic conditions desired.

One of the main disadvantages of this method is that calculation of the concentrations is based on the ideal gas assumption. This assumption does not, however, produce much error because the concentrations are very low.

We have used this method for three years for environmental pollution studies for several analytes, particularly lightweight hydrocarbons [6], in our instrumental analysis laboratory for postgraduate teaching students. Very accurate calibration graphs were produced. An example is shown in Figure 2.

Although a sample-storing procedure has been described for volatile solutions [7], the highly volatile nature of the analytes and solvents used prevents keeping the solutions for long time periods. Storing the solutions in the refrigerator may be helpful if it is not possible to prepare fresh standards each day. Particularly for trace preparations, however, freshly prepared standards are highly recommended.

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