# Preparation of Static Gas Mixtures of Anaesthetics by Various Techniques: Necessary Strategy and Equations

*FARKLI TEKNİKLERLE STATİK ANESTEZİK GAS KARIŞIMLARININ HAZIRLANMASI: GEREKLİ STRATEJİ VE DENKLEMLER*

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## ÖZET

*Anestetik maddelerin yol açtığı kirliliğin belirlenmesinde birçok analiz tekniği kullanılmaktadır. Ne var ki bu amaçla kullanılan cihazların tümü kullanıcı tarafından kalibre edilmeye ihtiyaç duyarlar. Tıbbi alanlarda yapılacak herhangi bir bilinmeyen analizi ancak bu maddelerin bilinen derişim/erdeki standartlarının elde bulunmasıyla mümkün olur. Bu makalede, herhangi bir kirlilik araştırma çalışmasının temel başlangıç noktası olan çok blleşenli standart gas karışımları hazırlama teknikleri gas ve sıvı anestetikler için detaylı bir şekilde tarif edilmiştir.*

Anahtar Kelimeler: Gas, Anestezikler

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## SUMMARY

*In the anaesthetic pollution studies various analysis techniques are used. Nevertheless all off those need to be calibrated and an unknown analysis from the medical environment is only carried out by using a collection of the required standard of the investigated substances. In this paper various standard mixture preparation techniques which are the mean starting point of any type of pollution study are described in detail for multi-component gas mixtures of gas and liquid anaesthetics.*

Key Words: Gas, Anaesthetics

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The analysis of gas mixtures is an application to which gas chromatography is well suited. The great advantage of the technique lies in the fact that on a single instrument may be analysed all the gases and vapours encountered in anaesthetic practice. But, the response of a gas chromatograph, like the other instruments, is not absolute and therefore, to be able to carry out quantitative work it must be calibrated against accurately prepared mixtures of known composition. Obtaining the required standards in wide spectrum is not always possible and is even frequently expensive for routine uses. For this reason several techniques have been devised to permit the analyst to make up his own calibration standards. In addition to the required accuracy, the preparation technique should be reproducible and preferably also simple, and applicable over a wide concentration range (1,2).

The methods being used for preparing mixtures with reasonable accuracy could broadly be classified into two main groups underlined dynamic and static preparation methods, respectively. The dynamic methods such as gas stream mixing, permeation, diffucsion, evaporation, etc. generate continuous flows of mixtures and are generally employed in the studies where large volumes of standards are needed. The static methods for producing standard gas mixtures are popular when relatively small volumes of mixtures are required at moderately high or low concentration level and have been of wide used in calibrating gas chromatographic instruments. The static methods involve introducing known masses or volumes of components into vessels of fixed dimensions such as cylinders (pressurised) or metal, glass and plastic containers (atmospheric). Of these, only metal cylinders are capable of storing mixtures at high pressures (2).

All types of mixtures described in this paper represent the preparations by static methods in cylinders (pressurised) and in glass sampling sbulbs, bags-in-the-bottles, plastic laminated aluminium bags, Tedlar® bags and glass syringes (at atmospheric pressure). The mixture preparation procedures and calculation of the final mixture concentrations for different techniques are given in detail.

## GRAVIMETRIC PREPARATION OF GAS MIXTURES IN CYLINDERS

Although pressurised static cylinder mixtures could be prepared on the basis of volume or pressure measurements (3). With these methods it is necessary to measure accurately the gas volumes or pressures and the temperatures. Furthermore, the compressibility factors at working pressures and temperatures must be known to perform the necessary corrections. These methods may therefore involve the use of expensive and generally cumbersome apparatus and despite this it is still not that easy to attain high accuracy (4), and tolerances of 5-20%, depending on the concentration level, are usually quoted (2).

In the gravimetric (weighing) method, which is extensively used in the preparation of accurate pressurised mixtures, the concentrations are determined from the mass of each component present in the cylinders irrespective of the temperature and pressure of the mixture. This technique represents the nearest practicable approach to an absolute method (4-7). Therefore, in industry and laboratory, the pressurised static mixtures are often prepared on the basis of mass weighing (gravimetric mixtures) in cylinders.

Apart from mentioning the high pressure apparatus and some basic equations, there is not much on the strategy about the mixture preparations in the literature (2-4). This is perhaps because of the simplicity of the stoichiometric calculations. But, the whole preparation procedure is not as easy as using a simple mole fraction equation, particularly for an analyst who is just a beginner the field. When a dilution is needed for low concentration preparations the calculations become even more complex. Lack of knowledge about the necessary strategy causes loss of time, introduces additional errors and decreases the reliability of the prepared mixtures.

In fact, calculation of the final composition of a mixture from the added weight of the components is very easy. But, one should always remember that the mixtures are generally needed for a particular purpose and the concentrations of the prepared mixtures are desired to be close to the needed values. In order to prepare a mixture of a given concentration, a series of calculations is needed, primarily which allows to know the amount of each component to be added into the container. A mixture of, let it be 1% concentration, can be prepared at any pressure from 1 to 150 bar (or more). Hence, the total mixture pressure determines the amount of the final mixture or vice versa and is neded for the calculations. Therefore, it could be said that the first thing to decide is the total cylinder pressure and this has to be fixed before starting the preparation. This important point has not been mentioned even in international standard procedures (6,7). The total pressure of the mixture determines the volume of the final mixture and also affects the magnitude of the weighing errors, particularly, when working with the gases of low density (e.g. He, $H_2$). If the final mixture pressure is too low, the small amounts of the substances put in will reflect significant errors associated with the composition of the final mixture. Therefore, an optimum final mixture pressure should always be kept in mind.

Once the total pressure of the mixture is fixed, the total number of moles of the components present in the container may roughly be calculated using the following Equation 1..

$$PtVc\text{-}ZmixntRT \qquad \text{(eq1)}$$

where

Pt-total mixture pressure (atm),
Vc-volume of the container (cylinder) (Litre),
T-mixture (room) temperature (°C),
nt-total number of moles (mole),
Zmix" compressibility factor for a mixture,
R-ideal gas constant (Litre. atmmole"'.K"')

Cylinder volume (Vc), and laboratory temperature (T), must be measured carefully but the compressibility factor (Zmix) is not needed to be known precisely. At lower pressures (e.g. 1 to 35 bar) the compressibility factors for the known gases such- as He, $H_2$, $N_2$, $O_2$, Ar are between 0.98 and 1.01 (8) and their mixtures may be taken as close to those values by a good approximation. Making this assumption has no negative effect on the final mixture concentrations since each component will be precisely weighed in later steps. However, it only effects the difference between planned and obtained concentration values. Hence, the total number of moles of the components could be calculated from the Equation 1 as given below:

$$n_t = \frac{P_t V_c}{RT}$$

If the total number of moles of the components is roughly known, the mass of any component to be added into cylinders to form a mixture at a desired concentration C (mole%) could be calculated from the following Equation 2.

$$gr_A = nt. \frac{C_A}{100} .M_A$$

$$gr_B = n_t. \frac{C_B}{100} .M_B \qquad \text{(eq 2)}$$

$$gr_C = nt. \frac{C_C}{100} .M_C$$

In the equations,

g'A.g"B.g'c°"""*the mass to be added into cylinder (g),

CA,CB,CC "-final concentration of each component (mole%)

MA.MB,MC "-molecular weight of the components (g/mole).

If the determined mass of each component is successfully introduced into the cylinder, the final concentrations will be close to the desired values. To do this the calculated mass of the minor component is introduced into an evaluated and tarred cylinder and the exact quantity is determined by weight difference (grA). Then the major component is introduced and cylinder is weighed again and the exact mass of the major component is calculated (grB). From the weighed amounts of the components, the exact concentration of the mixture (C,%) can be calculated by using the Equation 3.

$$C,\% = \frac{gr_A/M_A}{gr_A/M_A + gr_B/M_B} .100 \qquad \text{(eq 3)}$$

The above procedure can be applied to prepare any gravimetric mixture in pressurised cylinders. The required components are introduced in the known masses in the liquid or gaseous state to obtain moderate concentrations. In the cases in which low concentration mixtures are needed (e.g. ppm levels), weighing the amount of the minor components accurately is often difficult and sometimes impossible for the same balance is usually used in cylinder and component weighing. To overcome this difficulty two procedures could be applied for the preparation of low concentrations.

1. single or multiple dilution of a stock cylinder mixture,
2. introducing the minor components (gas or liquid) into evacuated cylinders via a sealed septum.

## SINGLE OR MULTIPLE DILUTION OF A STOCK CYLINDER MIXTURE

If a dilution is preferred to reach a low concentration, a stock mixture of the components at total pressure Pt and at a reasonable concentration. CM, is prepared according to the procedure given above. \ITM>T-der to obtain a desired low concentration, let it be CA2 at a nt2 total mole number, the required mass of the minor component for the mixture CA2 is calculated from the following Equation 4.

$$grA_2 = n_{t2} . \frac{C_{A2}}{100} .M_A \qquad \text{(eq 4)}$$

If this mass is to be taken from the stock mixture CAL the question at this point will be "How much stock mixture (grAi) should be taken to obtain the needed net mass (grA2)?" To simplify the equations we shall re-call the stoichiometric meaning of CAL The mole per cent concentration, very broadly speaking, is the number of moles of that component present in 100 moles of the mixture.

Since the mixture contains the component A of concentration CAI (mole %), then 100 moles of the mixture contain the component A which is equal to the numerical value of the concentration. If the moles are converted into weight units,

100 moles mixture-

100 (MA.CAI/100+MB.CBI/100) g of mixture,

CAI mole minor component (Ai)-

CAI.MA g of component A.

In the equation, CBI and MB are the concentration and molecular weight of the major component (B) in the mixture CAI respectively and the parenthesis represents the Average Molecular Weight of the mixture (Mmix). Now calculation for the needed mass of the stock mixture (grAi) becomes a simple proportional Equation 5.

$$gr_{A1} = \frac{100\,(M_{mix}).n_{t2}.\frac{C_{A2}}{100}.M_A}{C_{A1}.M_A} \qquad \text{(eq 5)}$$

The mass grA1 taken from the stock mixture contains the needed amount grA2 to obtain a mixture at concentration CA2. Since the stock mixture contains the major component (B), the quantity taken above also contains this component and must be subtracted from the mass of the major component to be added into the cylinder. The mass of the major component in grA1 is calculated in the same way as below:

$$gr_{B1} = \frac{(100-C_{A1}).MB.gr_{A1}}{100\,(M_{mix})} \qquad \text{(eq 6)}$$

The mass of the major component grB2 present in the mixture CA2 can be calculated from the equation 2.4 by replacing CA2 by (100-CA2)- The mass of the net diluent gas to be added, therefore, is calculated as following:

$$gr_{final} = gr_{B2} - gr_{B1} \qquad \text{(eq 7)}$$

If the calculated mass (grA1) of the stock mixture CA1 is introduced into a cylinder and diluted with grfinal mass of the major component (diluent gas), a mixture which is very close to the concentration CA2 is obtained. The same procedure can be applied if more dilution is needed to prepare mixtures at desired low concentration levels by replacing the mixture number 1 with 2 and 2 with 3. It should be remembered that more dilution steps inevitably bring repeated weighing errors and decrease the reliability of the concentration values. Although it is difficult to obtain a round-figure concentration, values reasonably close to the planned concentrations may be obtained by this piocedure.

In the above calculations the stock mixture was assumed to be a two component mixture with one minor component. If the mixture contains more than one minor components, only one component's mixture can be prepared at a desired concentration at a time.

## INTRODUCING THE MINOR COMPONENTS VIA A SEALED SEPTUM

If the minor components are available in the liquid state, introducing them directly into cylinders in correct mass and without extensive air contamination is extremely difficult. Therefore, those components should be introduced under vacuum without atmospheric interference. Hill (3) described a method for the preparation of pressurised mixtures of liquid anaesthetics but the method was based on volume and pressure measurements, not on weighing. There is, however, an alternative technique which allows one to inject the measured amount via sealed septum. This method could be mentioned as well for the preparation of low-concentration mixtures in cylinders as an alternative to the single or multiple dilution technique.

In this method, a known mass of the minor component (gas or liquid) is introduced by a gas-tight syringe into an evacuated and tarred cylinder via a sealed septum which is mounted on the cylinder inlet as given in Figure 1. The preparation is completed by adding the precalculated mass of the major component into the cylinder and weighing the cylinder again. If the necessary precautions are taken before and during the preparations, an accurate mixture of two -or multiple-component mixtures at any concentration may be obtained by the injection method. The advantage of this method over the dilution method is that the time needed for the preparations is short, minor and major components could precisely be measured at once, therefore, the weighing error is smaller. More importantly, the preparation of multiple component mixtures at any desired concentrations could be prepared at once in one cylinder.

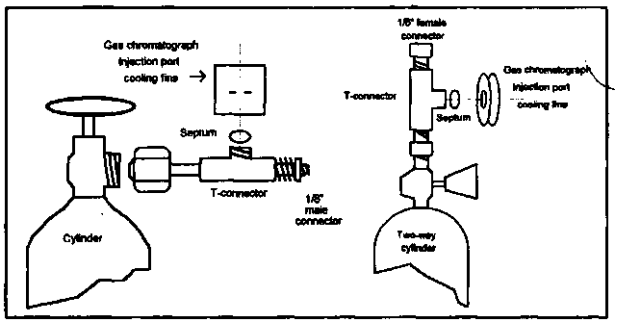


Figure 1. Sealed septum injection ports on cylinders used in the preparations

Equations 1 and 2 can be used in the calculations of the masses of the components to be added into cylinders before the preparations. Since the concentration of the minor component is low, compressibility factor (Zmix) could be assumed equal to the compressibility factor of the pure gas (about 1.00).

In the subsequent step, the calculated mass of the liquid is taken into a gas-tight glass syringe and precisely weighed by using a sensitive balance. Then, the syringe is inserted into the septum of a tarred and evacuated cylinder and allowed to be sucked by the cylinder's vacuum. As soon as the liquid introduction is completed, the syringe is weighed again and the exact quantity is calculated. At last, the major component is added and the cylinder and contents are weighed. The concentration of the mixture inside of the cylinder could be calculated by using Equation 3.

Weighing the minor component in a syringe can not be applied if the component is in the gaseous state due to the remarkable buoyancy effect and weighing errors. Instead of weighing the calculated needed mass of the gaseous component by using Equation 2 is converted to volume units by using Equation 1 at atmospheric pressure and temperature. A precisely measured volume of the gas is injected into an evacuated cylinder and preparation is completed by adding the major component into the cylinder. The accuracy of this technique depends on the atmospheric pressure, temperature and volume measurements. If the necessary care is taken this technique could also produce a reliable low concentration mixture without any dilution step.

## DIRECT INJECTION METHODS (INTO A CONTAINER)

Pressurised gas mixtures offer accurate and stable mixtures in relatively large amounts for long term needs, but they are sometimes less suitable for daily use in the laboratory, since a special high-pressure filling apparatus and rather complex procedures are involved. The mixtures of liquid anaesthetics for the gas chomatographic calibration purpoes could be prepared in glass sampling bulbs (J.Young Scientific Glassware Ltd., England), TedlarTM bags (K.A.D. Detection. Sys.Ltd., England), bag-in-the-bottles and polymeric material laminated aluminium bags (wine containers) at per cent concentration levels according to the static preparation methods. The glass sampling bulb preparation procedure suggested for the liquid anaesthetics by Gray (9) could be modified as well as for the preparation of permanent gas mixtures. In the case of low concentration preparations (e.g. ppm level), direct injection would not be reliable unless a micro-syringe with very small volume is employed, or else a container with a large volume is used. Whereas alternatively, the solution method may be benefited for even trace

concentrations by direct injection using a reasonable micro-syringe. The densities of liquid anaesthetics are necessary to calculate the final concentration of the mixtures. The density-temperature equations of the common liquid anaesthetics are available in the literature (10,11).

## PREPARATIONS OF THE MIXTURE OF LIQUID ANAESTHETICS BY DIRECT INFECTION

The glass sampling bulbs are flushed for several minutes prior to the preparation with the required dilunet gas (Helium, nitrogen or air) and then the gas is turned off and the Teflon stopcocks are closed. This procedure provides an atmospheric pressure inside the bulb.

A Tedlar bag or bag-in-the-bottle or plastic laminated aluminium bag is filled with the diluent gas (helium or nitrogen) and evacuated several times by a vacuum pump to clean the containers before the preparation of each mixture. Then diluent gas is filled into the container, the pressure within the container is allowed to reach the atmospheric pressure and finally, the valve of the container is closed.

Following the above procedures, the required volume of the liquid anaesthetic is injected into the containers by using micro syringes. Following the evaporation of the injected liquid, which is complete in about 30 seconds for the most concentrated case, the gases are mixed by rapidly rotating the bulb in the nads or squeezing the flexible containers. Then the mixtures are allowed to rest for homogenisation for at least 5 minutes for smaller containers and 10 minutes for the larger ones. The concentration of the mixtures are calculated from Equation 8.

$$C_{Anaest,\%} = \frac{\mu l_{added} . d_{Anaest} . V_{Anaest}}{1000 . M_{Anaest} . V_C} .100 \quad (eq\ 8)$$

In the equation,

CAnaest,%-final concentration of the anaesthetic inside the container (%,v/v),
uUdded-added volume of liquid anaesthetic into the container (ul),
dAnaest-liquid density of the anaesthetic (g/ml),
VAnaesrspecific volume of the anaesthetic vapour calculated from ideal gas equation (ml vapour/mole liquid),
MAnaest-molecular weight of the liquid anaesthetic (g/mole),
Vc-volume of the container (ml),
1000-conversion factor from ul to ml.

## MIXTURES OF PERMANENT GASES

Gray's method (9) was initially developed and commonly employed to produce static mixtures of known concentrations of volatile liquids in glass sampling bulbs. But the same method may be used in the preparation of standard permanent gas mixtures of known concentrations with small modifications. For this purposes, the bulb is flushed with the diluent gas and Teflon stopcocks are closed. Then one arm of the bulb is connected to a vacuum pump via a flexible tube and evacuated by opening the Teflon stopcock. Following this, the stopcock is closed and the required volume of gas is introduced into the evacuated bulb by means of a gas-tight syringe. Subsequently the pressure inside the bulb is balanced to atmospheric pressure with the diluent gas from a diluent gas-filled gas-tight syringe by suction of the bulb's low pressure. It should be noted that near the atmospheric pressure, as the pressure inside the bulb approaches atmospheric pressure, the rate of effusion of gas from the syringe is greatly reduced and correct balancing takes time. Incomplete balancing during the preparation step will produce a calibration line which is below the calibration point of 100% and effect the accuracy of the calibration at higher concentrations. As the turbelent flow of the gas entering the vessel was sufficient, no further time is needed for the mixing.

Percentage concentrations for the prepared gas mixtures are calculated from Equation 9 by the ratio of the gas and the volume of the container.

$$C_{Gas,\%} = \frac{V_{Gas}}{V_C} .100 \quad (eq\ 9)$$

In the equation,

Coas. %-concentration of the mixture inside the container (%, v/v),
VGas-volume of the minor component (ml),
Vc-volume of the container (ml),
100-conversion factor for % concentration

## SOLUTION METHOD FOR THE LIQUID ANAESTHETICS

Preparing low-concentration mixtures of liquid anaesthetics by the direct injection method is not likely to be reliable unless a container with a large volume or a syringe with a very small volume is used. Even using a small volume syringe may not help to attain a reasonably accurate mixture because a big error in the volume measurements is to be expected. Dissolving the liquid anaesthetic in a solvent (e.g. methanol or dichloromethane) would be helpful in the measuring of very small volumes of anaesthetic by a reasonable micro-syringe. The amount of the anaesthetic in the volume taken depends on the concentration of the solution prepared. Therefore, the amount of the anaesthetic in the prepared solution could be lowered for a trace preparation, and taking a reasonable amount of solution with a micro-syringe will make it possible to obtain a small amount of liquid anaesthetic which

would be impossible to measure directly by a microsyringe.

In the selection of the solvent the criteria must be considered that the solvent must completely dissolve the liquid anaesthetics and must be pure and chromatographically well separated from the other components. For this purpose, the solvents, which completely mixed with the liquid anaesthetics, such as ethyl alcohol, acetone, dichloromethane, chloroform, toluene, and acetonitrile could be used in the solution method (12). A calculated volume of the prepared solution which contains the required amount of liquid anaesthetic is injected into the glass bulb or other types of containers. The final concentration of the anaesthetic in the mixture was calculated from Equation 10.

$$CAna\text{«}rt,ppm = \frac{Csoi\text{-}filAddad\text{-}dAnaMt\text{-}VAnaMt'}{1000.MAnaMt.Vc} \cdot 10^{6} \quad (eq\ '$$

In the equation,

CAnaMt.ppm-final concentration of the anaesthetic in the container (ppm, v/v),

Csor concentration of the volatile anaesthetic in the solution (ml/ml),

plAdded-added volume of the solution into the container (yl),

d/west-liquid density of the anaesthetic (g/ml),

VAnaasrspecific volume of the anaesthetic vapour calculated from ideal gas equation (ml vapour/mole liquid),

MAnaMt-molecular weight of the liquid anaesthetic (g/mole),

Vc-volume of the container (ml),

$10^{6}$-conversion factor for ppm.

1000-conversion factor from jil to ml.

The highly volatile nature of the liquid anaesthetics and the organic solvents used prevents the solutions being kept for long time for further use. Storing them in the fridge may help if they are not prepared fresh each day, but freshly prepared mixtures are recommended, particularly for trace preparations. An eminent sample keeping procedure has also been described for volatile solutions (13).

## STANDARD PREPARATION BY SYRINGE DILUTION METHOD

. The syringe dilution technique is often employed in gas chromatographic analysis to produce the needed standard calibration mixtures withoud using complex procedures. This technique is regarded as less reliable because of inaccuracies in sample handling, such as volume errors, adsorption on glass surfaces or leaky syringes. A significant improvement could be achieved by using the internal standard method, as suggested by Engimaier (14), but it does not seem to have any real davantages over the other static gas mixture preparation techniques described earlier in this paper.

Folmer (1) described a syringe dilution method which could be used in the preparation of the standard calibration mixtures. A stock mixture of an anaesthetic at a higher concentration is prepared. Before making any dilution, an all-glass (preferably gas-tight) syringe is flushed with the stock mixture to expel the remaining air inside the syringe by repetitive filling and emptying. Following the flushing, the syringe is filled with the gas mixture and the gas chromatograph's sample loop is flushed with the half of the mixture. Just before making the subsequent injection, the syringe was filled to its original voule with air or another diluent gas (the first dilution) and sample loop is flushed with half of the mixture. In the later step, the syringe is again filled with diluent gas to its original volume (the second dilution). This procedure is repeated up to a concentration value for which a reliable and measurable peak quantity is obtained. Since one set of data produce a single peak area value foor each concentration, the same procedure could be carefully applied to get more than one set of calibration data for each component. The injected concentration in each dilution step was calculated from the Equation 11.

$$C_{injected} = \left( \frac{C_{original}}{2^{n}} \right) \qquad (eq\ 11)$$

In the equation,

Cinjactad-concentration of the injected mixture,

Coronal-concentration of the original mixture,

n-number of the dilution (1,2,3,...).

## CONCLUSIONS

With the improvement of gas analysis techniques, in particular of gas chromatography, the accuracy of measurements is frequently limited by the accuracy of the calibration mixtures. However preparation of the pressurised gravimetric mixtures represents the nearest practicable approach to an absolute preparation method: the level of accuracy depends on the purity of the components and on weighing errors including buoyancy changes on the cylinders (4).

Absolute corrections for the buoyancy effect involve complex and tedious calculations and in most situations are not necessary. The common method employed for the buoyancy correction is to weigh an identical "control" cylinder each time whenever the mixture cylinder is weighed. Increases in the control cylinder weight are substracted from the sample cylinder weight. This method seems reliable when cylinders, made of light material, and a sensitive balance are used.

An essential feature of any process for preparing a standard gas mixture is that the various components are thoroughly mixed. With static systems the time required for mixing depends on the size and the shape of the storage vessel, on diffusion characteristics and on the turbulence induced on mixing the components.

Generally no mixing problem is encountered with the mixtures prepared at atmospheric pressure, but under high pressure conditions, particularly in pressurised cylinders, the mixing time becomes an important parameter. Therefore, the prepared cylinder mixtures should be kept on its side at leasd 24 hours before using for any purposes.

In the glass sampling bulb preparations, the initial atmospheric pressure in the bulb is increased by introducing the liquid anaesthetic into the bulb because of bulb's smaller volume and their remarkably high vapour pressures. But this increase is not significant for about 33% concentration is needed to reach their vapour pressure values at atmospheric pressure. As the volume of the bulbs is relatively small the number of the gas samples to be taken, at reasonable injection volume, is not unlimited. When the number of the samples taken from the bulb is increased the pressure and the bulb sucks in atmospheric air from the needle insertion points.

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