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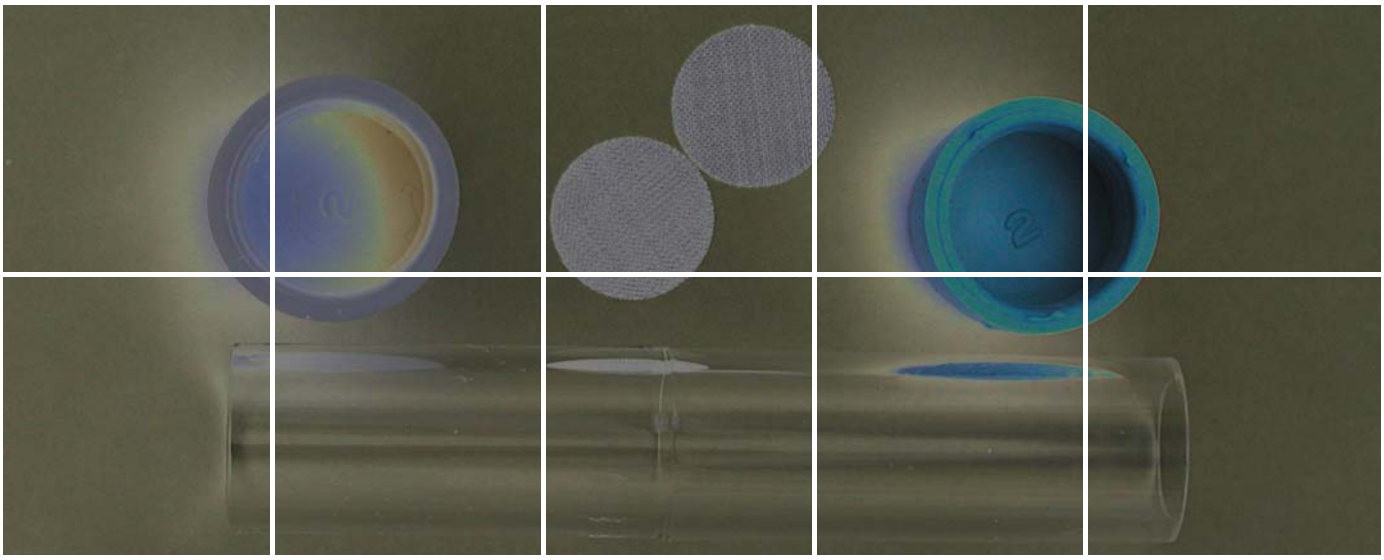

Diffusion Tubes for Ambient NO_2 Monitoring: Practical Guidance

for Laboratories and Users

Report to Defra and the Devolved Administrations

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Foreword

Palmes-type diffusion tubes are widely used in the UK for indicative measurement of ambient concentrations of nitrogen dioxide (NO_2) in the context of Local Air Quality Management. However, no UK or international standard method currently exists for diffusion tube preparation or analysis. Consequently, there is considerable variation in procedures among both supplying laboratories and end users. It is believed that this variation in methods and procedures is contributing to the observed variation in NO_2 diffusion tube performance, and that by reducing (as far as practicable) the inter-laboratory variation in methods, it will be possible to reduce inter-laboratory variation in results.

Therefore, Defra and the Devolved Administrations commissioned AEA Energy and Environment and Air Quality Consultants to set up and manage a Working Group on harmonisation of NO_2 diffusion tube preparation and analysis methods. This work was undertaken during 2006 and 2007 as part of the Defra contract RMP 2877, for Support to Local Authorities for Air Quality Management.

The following were agreed as the aims and objectives of the Working Group: "To investigate current practice in design, preparation and analysis of diffusion tube samplers for nitrogen dioxide, and to recommend changes as appropriate to improve in particular precision of measurement through harmonisation of preparation and analysis methods and tube design". The Working Group has therefore produced this document, which aims to provide practical guidance both for laboratories working with diffusion tubes, and those who make use of them – in particular, Local Authorities using diffusion tubes for Local Air Quality Management purposes. This Guidance attempts to harmonise the different steps in the UK diffusion tube methodology based on current knowledge of best practice.

It should be noted that this document does not constitute a formal standard method. However, in the absence of an international, European or UK standard method for diffusion tubes, this guidance is intended to form the basis of harmonisation of methods within the UK, until such time as a standard method is developed. Defra and the Devolved Administrations will expect laboratories to implement this guidance, for diffusion tubes used by Local Authorities for Local Air Quality Management, by the beginning of January 2009.

The Working Group included representation from laboratories, Local Authorities, experts in the field of diffusive sampling, and other stakeholders. **The following (in alphabetical order) actively participated in, and provided input to, the Working Group on Harmonisation of Diffusion Tubes:**

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This Guidance Document was compiled by AEA Energy & Environment and Air Quality Consultants from the findings of the Working Group and other sources.

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Glossary of terms used:

Accuracy - The degree of conformity of a measured or calculated value to its actual or specified value

Co-location study – in this context, a field trial in which diffusive samplers are exposed alongside an automatic analyser, in order to assess their precision and accuracy relative to the latter.

Colorimeter – a device used in chemical analysis, to determine the concentration of a particular substance in solution, by measuring how strongly the sample solution absorbs light of a particular wavelength.

Diffusive Sampler – A device which absorbs a gaseous or vapour phase pollutant directly from the surrounding air by means of diffusion, without actively drawing air through the sampler.

End cap – the plastic cap fixed on the closed end of a Palmes-type diffusion tube, which holds the absorbent-coated grids.

Extraction – in this context, the extraction into aqueous solution of the nitrite absorbed by the TEA in the diffusion tube. This is the first stage of the analytical process.

Grids – used here to refer to the small mesh grids used inside the diffusion tube, which are coated with the absorbent TEA.

Indicative monitoring technique – indicative monitoring techniques are those which provide an indication of air pollutant levels, but whose precision and accuracy are not as good as the automatic methods defined as reference techniques.

Membrane – a permeable membrane fixed across the open end of a Palmes tube: routinely used in sulphur dioxide tubes to prevent the ingress of particulate sulphate. Has been used experimentally in nitrogen dioxide tubes to reduce interference due to wind effects.

Mesh – used here to indicate a mesh fixed across the open end of a Palmes diffusion tube, used experimentally to minimise interference from wind effects (the term “mesh” is used to distinguish this from the “grids” above). This modification is not in routine use at present.

Palmes diffusion tube – type of passive sampler consisting of a tube, open at one end, with an absorbent at the other (closed) end for absorption of a specific pollutant from the surrounding air.

Passive Sampler, passive sampling techniques – methods which absorb the pollutant in question directly from the ambient air, and need no power supply.

Precision - the mutual agreement of a series of individual measurements or results, regardless of their accuracy.

Preparation method, preparation technique – used here to mean the method by which the tubes are prepared, most importantly the type of TEA solution used, and the means of applying it.

Reference method – a well-researched and validated method of making a particular measurement, with which other methods can be compared to test their precision, accuracy etc.

Spectrophotometer - A device for measuring intensity of light of a particular wavelength. In this context, used in chemical analysis of diffusion tubes.

TEA – Triethanolamine. The reagent used in Palmes-type diffusion tubes as an absorbent for ambient NO_2.

1 Introduction

As highlighted in the Foreword, Palmes-type diffusion tubes have been widely used in the UK for measuring ambient concentrations of nitrogen dioxide (NO_2) since their development in 1976 by Palmes *et al*[1]. Since then, many suppliers, analysts and end-users of diffusion tubes have introduced their own modifications in the preparation, analysis and exposure of the tubes, leading to considerable variation in procedures. QA/QC programmes[2] and other sources have highlighted considerable variation in NO_2 diffusion tube performance. It is believed that by harmonising the inter-laboratory variation in methods, it will be possible to reduce inter-laboratory variation in results.

Consequently, this Practical Guidance, (prepared on behalf of Defra and the Devolved Administrations) is intended to harmonise, as far as practicable, the methods used for Palmes-type NO_2 diffusion tube preparation, use, and analysis. This Guidance attempts to harmonise the different steps in the UK diffusion tube methodology based on current knowledge of best practice.

This Guidance is not intended to be a literature review on diffusion tubes: a comprehensive literature review was undertaken by CEH prior to the start of this Working Group[3]. Nor does it constitute a formal standard method. Rather, it aims to provide practical guidance both for laboratories working with diffusion tubes, and those who make use of them – in particular, Local Authorities using diffusion tubes for Local Air Quality Management purposes.

Various types of diffusive samplers are available, for a range of pollutants, and for indoor and outdoor use. The scope of this guidance is confined to Palmes-type NO_2 diffusion tubes, as used for indicative monitoring of outdoor air pollution.

This Practical Guidance focuses on the following important steps

- Tube components and preparation
- Exposure
- Extraction and analysis
- Data handling and use

Throughout this document, detailed instructions relating to each of these stages have been summarised in text boxes shaded grey. This is intended to help laboratories use this guidance for quick reference.

Grey boxes like this one show procedures to be followed by laboratories as part of the harmonised method.

Some aspects of the analytical procedures described in Section 4 differ, depending on whether the analysis is being carried out manually or using a fully automated system, in which the reagents are dispensed and mixed with the sample automatically. Therefore, in Section 4, guidance relating specifically to the manual method only is shown against a solid blue background like this, while guidance only applicable to the automated method is shown against a chequered yellow background , like this. All other guidance is applicable to both, and shown against a white background as normal.

2 Preparation of Palmes Diffusion Tubes

Diffusion tubes are passive samplers: they consist of small plastic tubes containing a chemical reagent to absorb the pollutant to be measured directly from the air (Figure 2-1). In the case of Palmes-type nitrogen dioxide diffusion tubes, the absorbent used is triethanolamine (TEA). Stainless-steel mesh grids at the closed end of the tube are coated with a water-based or acetone-based solution of this absorbent.

There are two main methods of applying TEA to the grids –

- by dipping them into a TEA/acetone solution, allowing to dry, and then assembling the tubes – or
- by first placing the grids into the caps, then pipetting a measured volume of TEA solution (which can be acetone-based or more commonly water-based) onto the grids, placing the tube on top, and finally putting the other end cap in place.

When preparing Palmes-type NO_2 diffusion tubes, attention to the following issues is essential (these are addressed in this section):

- Tube components
- Preparation of TEA solution
- Application of TEA solution onto grids
- Drying of impregnated/dipped grids
- Assembly

2.1 Tube Components

Figure 2-1 shows the typical components of a diffusion tube. These consist of an acrylic tube, two stainless steel grids and two caps.

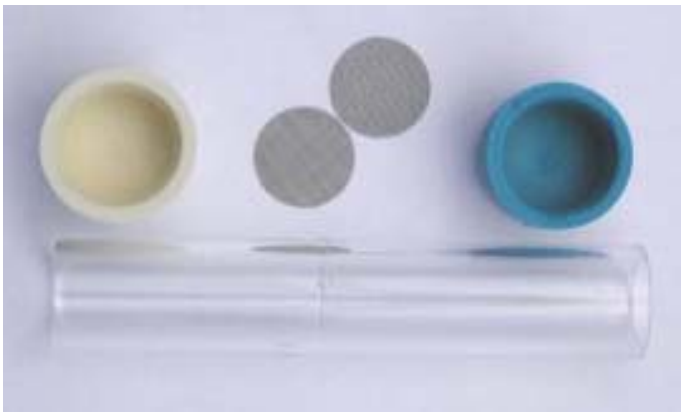

Figure 2-1 Diffusion Tube Components

2.1.1 Tube Material

Palmes type diffusion tubes should be made of clear or translucent colourless plastic. Tubes used in the UK are generally made from either acrylic or polypropylene (about 7.1 cm long). These materials have the disadvantage of being almost totally opaque to UV light. This blocking of UV light by the tube material is thought to result in reduced NO_2 photolysis in the tube[4,5], affecting the NO – NO_2 equilibrium within the tube and causing over-estimation of ambient NO_2 concentration.

There is insufficient evidence at present to harmonise tube material in favour of either acrylic or polypropylene. Further field trials will be necessary, to establish the effects of these tube materials and to test alternative tube materials which are more transparent to UV. Based on results of future trials, it may be possible to make recommendations on tube material in an updated version of this guidance.

In the mean time, either polypropylene or acrylic tubes may be used. However, all tubes used in any given survey should be of the same material, to avoid introducing any possible effects due to different materials.

Do not use any tubes which are cracked, damaged or have uneven ends which may prevent the end caps sealing properly. All tubes should have same dimension in terms of length and internal/external diameter.

2.1.2 Grids

Two grids should be used per tube. The grid material should be stainless steel. The mesh size of this grid should be 4x4 per mm^2. The diameter of each circular grid should be slightly larger than the inside diameter of the tube, so that they are fixed firmly in place when the tube is assembled and cannot fall out during exposure.

2.1.3 End Caps

End caps to be used on the end that holds the grids should be opaque flexible plastic of a dark colour. (Sunlight can degrade the nitrite complex formed when NO_2 combines with the TEA: dark opaque caps minimise this).

Coloured end caps may be re-used, but must be discarded when the colour begins to fade. Any cracked, discoloured or split caps *must* be discarded.

As far as possible, use end-caps of one colour only in any survey: different coloured caps may have different opacity, increasing the uncertainty of the results. Do not, therefore, alternate between different colours in different months. Alternating coloured labels should be used instead.

Obtain tubes and caps from the same supplier to ensure a correct fit. Note: it is advisable not to use the polypropylene caps (designed for use with polypropylene tubes) with acrylic tubes. The acrylic tubes are slightly larger in external diameter than polypropylene tubes. It is possible (though not proven) that this may occasionally cause the caps to split.

If obtaining diffusion tubes ready-made from a supplier, reject any that arrive with split end caps.

2.1.4 Cleaning Before (Re-)Use

Some laboratories use diffusion tube components only once, others re-use them many times.

Re-used components need to be effectively cleaned to remove all traces of nitrite from previous exposures. Failure to do this may result in high or variable nitrite content in both exposed and unexposed ("blank") tubes. Analysis of laboratory "blank" tubes with each batch (which is normal practice) allows the laboratory to check that cleaning procedures are adequate. If blanks remain consistently low, this indicates that cleaning of tube components is satisfactory. The French harmonised analytical protocol[6] recommends testing the effectiveness of the cleaning by submerging a few grids in the colorimetric reagent solution, and checking for any observable colour change.

- If tubes are to be used more than once, they should be cleaned before first use by the same method as before re-use.
- Tube components should be thoroughly cleaned using a suitable detergent. This may be done using an ultrasonic bath.
- Components should then be thoroughly rinsed in water.
- Final rinse should be in deionised water.
- Components should be thoroughly dried before use.

If the tube components are to be used only once, it is only necessary to clean them before use if there is the possibility that they may not be free from contamination.

2.1.5 Use of Membranes or Meshes at Open End of Tube

AEA Energy & Environment have been investigating the effects of applying a mesh or membrane at the open end of the tube[7,8]. These in theory would reduce the wind effects highlighted in Appendix 2, and could improve precision and accuracy. The use of a mesh or membrane at the open end of the

tube does indeed appear to improve precision and accuracy. Gerboles *et al*[9] show the improved precision and accuracy of diffusion tube results by introducing a membrane at the open end of the tube. However, membranes complicate the calculation of uptake rate, requiring wind speed, temperature and humidity data to get reliable results – all of which are not usually available at diffusion tube monitoring sites. This Working Group has concluded that membranes are not a viable option for diffusion tubes used in the context of Local Air Quality Monitoring.

Results from a 2006 study by Targa[8] show promising results from diffusion tubes with meshes covering the open end. These appear to reduce wind effects without unduly complicating the calculation of uptake rate. It is envisaged that such a modification could be incorporated into the harmonised method in the future. **However, further investigation and optimisation is required, so the use of meshes at the open end of the tube is not being introduced at the present time, and does not form part of the present Guidance.**

2.2 Quality of Chemicals, Glassware and Water

The quality of chemicals, glassware and water is important in order to ensure good results. The quality should be **at least:**

- Grade 'B' glassware for all volumetric flasks, pipettes and measuring cylinders.
- 'AR' grade or equivalent, for all reagents
- 18 M Ω deionised water, for making up all solutions, and for final rinsings of glassware and in cleaning tube components.

2.3 Calibration of Balances

Detailed guidance on calibration of laboratory balances is well documented elsewhere and is outside the scope of this Practical Guidance. However, balances should be calibrated at least once a year by a certified external organisation, to nationally traceable standards. Regular checks using standard weights should also be carried out on a daily basis (or on each day of use if less frequent).

2.4 Routine Checking and Calibration of Pipettes

Various procedures outlined in this Guidance require small volumes of liquid to be accurately dispensed using a pipette. The precision and accuracy of pipettes is therefore vital, and it is important that all pipettes are regularly checked to ensure they are dispensing the correct volume.

A simple gravimetric procedure is given below, for routine daily checking of pipettes. This is based upon dispensing a measured volume of water using the pipette, weighing the volume of water delivered, and then calculating the mean mass/volume ratio. This ratio is then compared to the theoretical density of water at the ambient temperature. This can be done using any accurately calibrated laboratory balance reading to 0.1 mg (0.0001 g), some deionised water and a small container. In the example below we have assumed that a micropipette is to be used to measure a volume of 50 µL.

1. Fill a clean beaker with deionized water.
2. Place a small beaker or weighing dish, (which should already contain a few mL of deionized water, to reduce uncertainties due to evaporation) on the balance.
3. Zero the balance using the tare control.
4. Rinse the micropipette tip 2 to 3 times with the water, and dispense 50 µL into the container on the balance.
5. Record the mass, zero the balance and deliver a second 50 µL aliquot of water into the container. Record the mass.
6. Repeat this process 5 times, recording the mass of each aliquot.
7. Calculate the mean and standard deviation of the measured masses of the 5 aliquots.
8. Calculate the mean mass/volume ratio and compare this with the theoretical density of water *at the ambient temperature of the laboratory.* This gives a measure of the accuracy of the pipette. In

the case of the micropipettes used to prepare calibration standards and dispense the reagents, the accuracy should be within +/- 1.0%.

9. Calculate the coefficient of variation (CoV) of the 5 measurements (this is the standard deviation as a percentage of the mean). This is a measure of the precision of the pipette.

If this is significantly larger than the pipette specification (usually +/-0.50% or better for 50 µL micropipettes and +/-0.15% for larger volume pipettes) the pipette may require attention, or there could be a source of uncertainty such as a hand-warming effect. If the latter, the pipette can be allowed to reach equilibrium temperature in the hand before starting: but the time required for this will depend on both the pipette and the operator.

Carry out the procedure as quickly as possible (although not at the expense of accuracy), to avoid introducing uncertainties due to temperature fluctuations and evaporation.

For the purposes of routine daily checks prior to use of the micropipette, a small number of aliquots (e.g. five as above) would be sufficient. For a more rigorous calibration - for example on a quarterly basis - or to test a new pipette, a larger number (upto 20) would be advisable.

2.5 TEA Solution

This solution should be freshly prepared on the day that it is to be used. TEA is very viscous and can be difficult to handle when cold. It is easier to handle if allowed to reach room temperature before use. The exact percentage of TEA is not critical: it is only necessary to ensure that it is **nominally** 50% (or 20%) TEA v/v. The following instructions make 50 mL of TEA solution: the quantities can be adjusted to make smaller or larger volumes as required.

Wear eye protection, especially when handling acetone.

50% TEA in Acetone (for use in the dipping method only)
Take a 50 mL volumetric flask. Using a measuring cylinder, measure 25 mL of TEA and pour this into the volumetric flask. Rinse the measuring cylinder with around 10 mL of acetone, and pour the rinsings into the volumetric flask. Repeat, ensuring that all the TEA goes into the solution. Mix well. Fill the volumetric flask accurately up to 50 mL with acetone. Mix well. The solution is ready to be used.

20% TEA in H_2O (for use in the pipetting method only)
Take a 50 mL volumetric flask. Using a measuring cylinder, measure 10 mL of TEA. Pour the TEA into the volumetric flask. Rinse the measuring cylinder with around 10mL deionised H_2O, and pour the rinsings into the volumetric flask. Repeat, ensuring that all the TEA goes into the solution. Mix well. Fill the volumetric flask accurately up to 50 mL with deionised water. Mix well. The solution is ready to be used.

(An acceptable alternative is to measure the TEA by weight and calculate the required weight of acetone or water to be added, from the densities of TEA and acetone or water.)

2.6 Application of TEA onto grids, drying and assembly

Field trials carried out for this Working Group in 2007, involving diffusion tubes prepared and analysed by eight laboratories[10], indicated that either of the following **two** preparation methods can be used:

- 50% solution of TEA in acetone, grids dipped into solution and dried before tube assembly.
- 20% solution of TEA in deionised water, solution pipetted onto grids already placed in the end cap.

No significant difference was found in precision or accuracy between tubes prepared and analysed by any given laboratory using these two methods. Inter-laboratory differences for tubes prepared using the same method were greater than differences between tubes prepared by the same laboratory using different methods.

(The field trial also tested tubes prepared by dipping the grids in a 50% solution of TEA in water: these however gave worse results to the methods above in terms of both precision and accuracy.)

There is sufficient evidence from previous research by other workers[11,12,13] that preparing diffusion tubes using a 50% solution of TEA in water, pipetted onto the grids, gives worse performance (worse precision[11,13] and significantly lower results[11,12]) than either of the above two methods, and so should be avoided.

Some laboratories add a small amount of surfactant (such as Brij 35) when preparing the 20% TEA/water solution for use in the pipetting method. There is currently no strong evidence for or against using such surfactants: laboratories may use them if they wish.

2.6.1 Dipping/soaking Method (50% TEA, 50% Acetone only)

Place the required number of grids (two per tube) in a clean beaker with freshly prepared 50% TEA/acetone solution. Mix well (using a clean stirring rod or a magnetic stirrer) for at least one minute to ensure the grids are fully and evenly coated. If there are a large number of grids in the beaker, it will require longer (upto 5 minutes), and will benefit from use of a magnetic stirrer. Remove grids using clean tweezers or forceps (not fingers!), and place them on an absorbent surface in a well-ventilated area to dry: this should only take a few minutes as the acetone will quickly evaporate. ***Do not use any heat sources near acetone-based solution, as acetone is flammable and volatile.***

Wear laboratory gloves and handle the grids with clean tweezers only: do not touch them with your fingers as this may contaminate them with nitrite.

Assembly of tubes should be carried out straight after drying the grids to avoid contamination. Place two impregnated grids into an opaque plastic end-cap (using tweezers). Push a tube into the cap, on top of the grids. Apply another cap at the other end of the tube – this second cap (which is usually clear or white in colour) is the cap that is removed during exposure.

2.6.2 Pipetting Method (20% TEA, 80% Water only)

Take the dark opaque coloured plastic end caps. Place two clean, dry grids inside each one. Using a micropipette, apply 50 microlitres (μL) of the 20% TEA/water solution onto the grids in the caps. Spread the solution over the grids as evenly as possible. It may be possible to improve distribution of the TEA solution by spreading the drop with the tip of the pipette, or rotating the grids slightly in the cap.

Some laboratories using this method allow the grids to dry for a few minutes before adding the tube and other cap. There is no clear evidence whether this is beneficial: however, it is important that

(i) the tube should be pressed into the cap gently: if this is done too forcefully, the grids may be compressed, forcing the TEA solution out onto the tube walls.

(ii) the tubes are not handled roughly, or inverted immediately, as the solution may dribble down the inside of the tube walls.

Note: it is important to pipette the solution onto the grids in the end caps only - *not* into fully-assembled tubes, as this increases the risk of contaminating the inner walls of the tube with TEA solution.

2.7 Storage

Tubes and batches of tubes should be labelled appropriately, and enough laboratory blanks should be stored within the laboratory to measure any possible contamination during preparation.

The date of preparation, method of preparation, and the expiry date (4 months after preparation) should be clearly identified for each tube batch. If preparation/components differ from the standard, the end user should be clearly notified.

The use of plastic containers and/or sealable clean plastic bags is essential in order to avoid contamination during transportation.

Unexposed tubes should be stored in a sealed plastic container, in a refrigerator. If a refrigerator is not available, they should be stored in a cool dark place without temperature fluctuations.

It is important that transport blanks (tubes which are not to be exposed and are left capped at all times) are included in any survey. Transport blanks should also be kept in a sealed plastic container as above.

Tubes should be exposed and analysed within 4 months of preparation.

Box 2-1 Summary of Diffusion Tube Preparation Instructions

1. The tube material should be colourless clear or translucent plastic, either acrylic or polypropylene.
2. The grids used to hold the TEA solution should made of stainless steel, mesh size 4x4 per mm^2.
3. Use two grids per tube.
4. End caps should be of flexible, dark-coloured opaque plastic. Get the caps and tubes from the same supplier to ensure the caps fit the tubes correctly.
5. If tube components are to be used more than once, they should be cleaned before first use by the same method as before re-use. Clean components using a suitable surfactant, (an ultrasonic bath can be used), and rinse thoroughly in deionised water (at least 18 M Ω).
6. Glassware used throughout should be at least Grade B.
7. Always use tweezers and laboratory gloves when preparing tubes: never touch them with your fingers.
8. The TEA solution should be either 50% v/v TEA/acetone, or 20% v/v TEA/water, prepared as in section 2.5.
9. If using acetone, wear suitable eye protection. Do not use any heat sources near acetone, as it is volatile and flammable.
10. The TEA solution should be applied to the grids by either
    - Dipping (50% TEA /acetone only) or
    - Pipetting (20% TEA/water only)
11. The method of applying the TEA solution onto the grids is as follows:

    i) Dipping: submerge them in the 50% TEA/acetone solution, stirring well and leaving for at least one minute (upto 5 minutes may be required for large numbers of grids). Remove using tweezers and place on an absorbent surface to dry. Assemble tubes as soon as grids are dry.

    ii) Pipetting: place two clean, dry disks into the coloured end cap. Using a micropipette, apply 50 μL of the 20%TEA/water solution onto the grids in the caps. Spread as evenly as possible. Grids may be left to dry for a few minutes before *gently* adding tube and other cap. Please take care when assembling tubes to make sure that the liquid stays on the grid and does not run off to the wall of the tube.

12. Store prepared tubes in sealed plastic containers or sealable bags, preferably in a refrigerator or otherwise in a cool dark place. Reserve some tubes for use as laboratory blanks.
13. Use and analyse the tubes within 4 months of preparation.
14. Include travel blanks when sending tubes for deployment.

3 Use of Palmes-Type Diffusion Tubes

Diffusion tubes are categorised as an "indicative" monitoring technique. This refers to a technique with relatively high uncertainty, in the case of diffusion tubes quoted as ± 25%. By contrast, the chemiluminescence method, used in most automatic ambient monitoring apparatus for NO_2, is defined as the reference method for this pollutant. There is a CEN standard for the chemiluminescent method[14] (EN 14211:2005 "Ambient air quality - Standard method for the measurement of the concentration of nitrogen dioxide and nitrogen monoxide by chemiluminescence") and its uncertainty is typically quoted as ± 15%.

This section covers the exposure side of diffusion tube monitoring. This includes:

- When and where to use diffusion tubes,
- Site selection,
- Exposing diffusion tubes and
- Co-location with an automatic analyser.

3.1 When And Where To Use Diffusion Tubes

Diffusion tubes are particularly useful:

- when simple, indicative techniques will suffice;
- to give an indication of longer-term average NO_2 concentrations;
- for indicative comparison with Limit Values and AQS Objectives based on the annual mean;
- for highlighting areas of high NO_2 concentration; and
- where installation of an automatic analyser is not feasible.

They are useful for identifying areas of high NO_2 concentration, particularly when dealing with sources such as traffic emissions, which do not change much from day to day. They are less useful for monitoring ambient concentrations around specific emission sources such as industrial plant, as they cannot identify short-term fluctuations in NO_2 such as may result from fluctuations in wind direction.

3.2 Selecting Diffusion Tube Sites

The selection of sites will of course depend on the objectives of the monitoring programme. However, for Local Air Quality Management (LAQM) purposes, sites should be located in areas where there is *relevant public exposure*. For other monitoring programmes, the reasons may vary (e.g. occupational health, highest concentration, investigation of background concentrations.) **Safety should be an important consideration when siting tubes at height or near to roads.**

The immediate area around the sampler location must be open, allowing free circulation of air around the tube. Ideally, samplers would be placed at breathing height, but in order to reduce theft of tubes, it is recommended that tubes are placed at a height of 2-4 m. Concentrations of NO_2 typically decrease with height above street level, so tubes placed some metres above street level may under-estimate the actual concentrations to which the public are exposed. As far as is practical, all tubes within any given monitoring programme should be placed at similar heights.

Many sites will be categorised as "kerbside", "roadside" or "urban background", and more detailed guidance is given below.

3.2.1 Roadside And Kerbside Sites

Roadside and kerbside sites often reflect the maximum concentration of NO_2 to which people may be regularly exposed, even if only for short periods, close to a busy main road. The road with maximum traffic flow within the area may not produce the highest ambient concentrations if it is situated in an open area; for instance a dual carriageway. Higher concentrations may be observed at a less busy road with tall buildings on either side (the street canyon effect), for instance in a town centre. In

general, unless data from other sources exist, local knowledge will be required to select the most appropriate sites.

Kerbside sites should be within **1 m of the kerb**, and are usually fixed to street furniture. **Roadside** diffusion tubes should be sited between ***1 and 5 m from the kerb edge***, and mounted ideally either on a lamp post or road sign on the pavement, or (with an appropriate fixing – see below) on the face of a building adjoining the pavement. Avoid locations where the tubes are likely to be affected by turbulence from passing fast traffic, as this may cause them to over-estimate the NO_2 concentration. Measurements from roadside and kerbside sites will only be representative over a very small area, as NO_2 concentrations close to sources vary considerably, even over short distances.

In some contexts Intermediate or ***"Near-Road"*** sites may also be relevant: these are sites at which air quality is affected by a nearby major road, despite being more than 5 m away, and therefore not technically falling into the "Roadside" category above. The same siting considerations apply.

3.2.2 Urban Centre, Urban Background, and Suburban (i.e. urban non-road) Sites

At distances of more than 50 m from a busy road, it is anticipated that NO_2 concentrations will have been diluted to the local urban background concentration. Hence, measurements made in this type of location are likely to be representative of a fairly large area, and can be reliably compared with similar locations in other urban areas.

Urban background sites must be located:

- >50 m from any major source of NO_2, such as multi-storey car parks;
- >30 m from any very busy road (> 30,000 vehicles per day). The old NO_2 Network required urban background sites to be at least 50 m from any busy road;
- >20 m from a busy road (10,000 – 30,000 vehicles per day) or from any medium sized sources, e.g. petrol stations or ventilation outlets from catering establishments;
- >10 m from any main road. (Quiet roads, for example within residential estates, are acceptable); and
- >5 m from anywhere where vehicles may stop with their engines idling.

Examples of typical urban background sites are on lampposts or street signs in quiet residential areas, schools or other public buildings, either close to the town centre or in suburbs bordered by a busy arterial road. When street furniture is used, even on quiet roads, the sampler must be more than 1 m from the kerb.

3.2.3 Detailed Siting Of The Sampler

Diffusion tubes must be held vertically with the open end downwards during sampling (Figure 3-1). Generally, a permanent clip (e.g. Terry clip or plastic clip) is mounted so that the tubes can be changed easily (see Figure 3-2). The clip and spacer (see below) may be simply mounted at the monitoring site with PVC tape, double sided tape, or cable tie as appropriate.

It is important that the open end of the tube is exposed to free circulation of air. Also, certain surfaces may act as absorbers for NO_2 leading to a thin layer of reduced atmospheric concentrations immediately adjacent to the surface. For these reasons tubes must not be fixed directly to walls etc., even when the objective is to monitor at a building façade. A spacer block of at least 5 cm must be used between the surface and the tube, as indicated in Figure 3-2. A small block of wood or plastic can be used as the spacer. The open end of the tube must be located below the lower surface of the spacer, as shown in Figure 3-2. Ideally the tube with spacer block should be mounted on some projection 0.5 - 1 m horizontal distance from the face of the building, or on a drainpipe or similar structure. Avoid placing diffusion tubes in any form of recess. Some examples of how diffusion tubes can be fixed in place are shown in Figures 3-2 and 3-3.

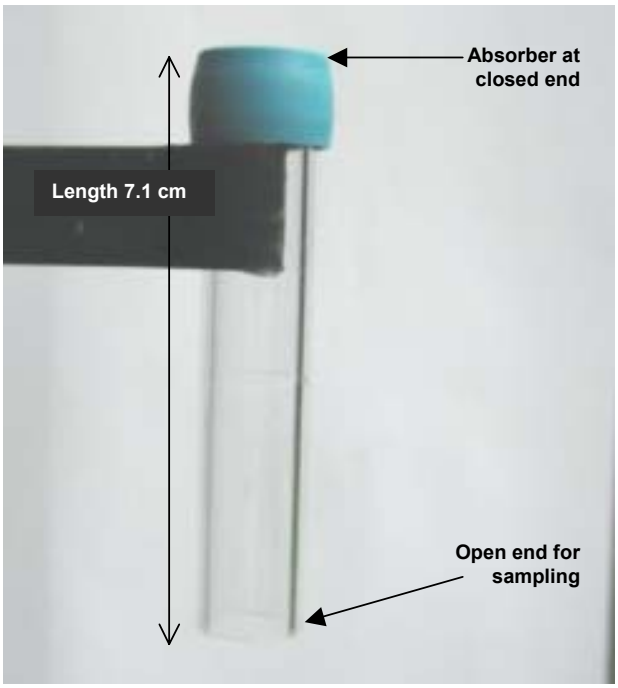

Figure 3-1 Palmes diffusion tube

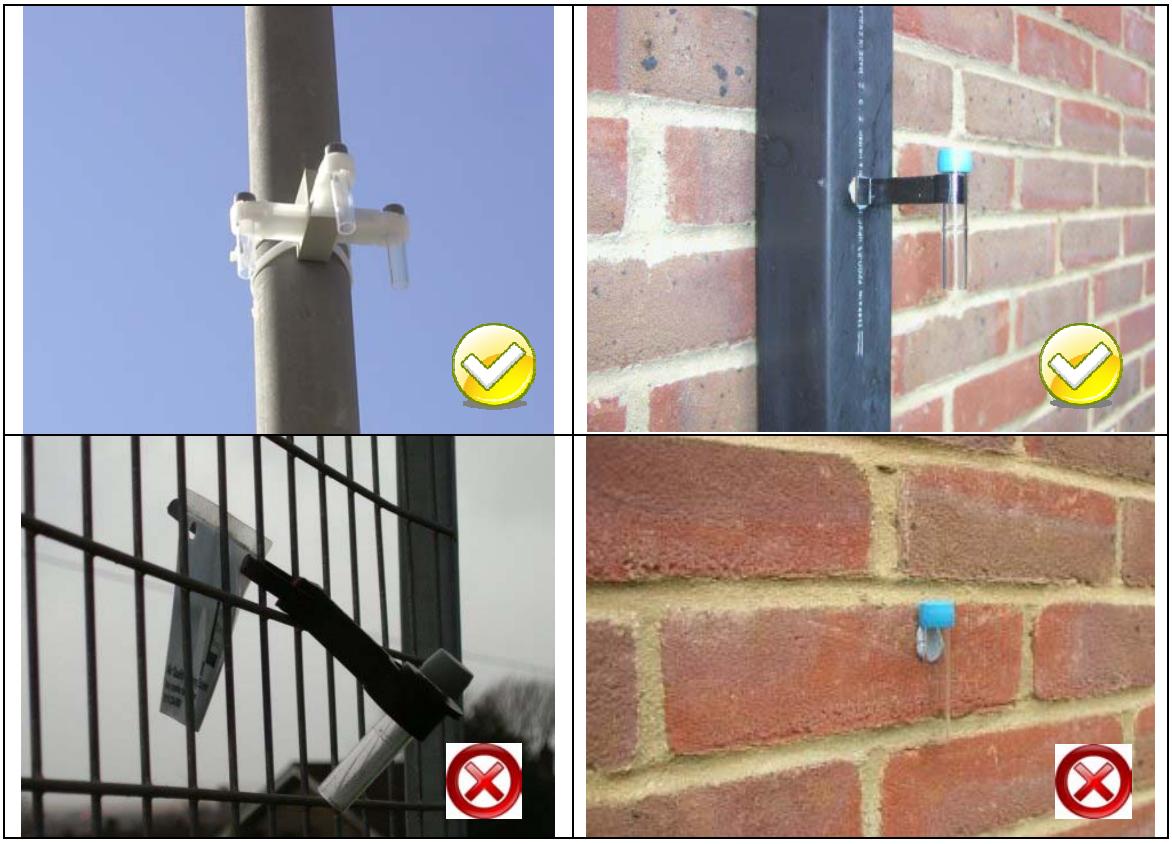

Figure 3-2 Examples of right and wrong ways to expose diffusion tubes

Although it is important to place diffusion tubes where there is free circulation of air around the tube, the opposite extreme should also be avoided, i.e. areas of higher than usual turbulence. For this reason, the tube should not be located on the corner of a building. Care must be taken to avoid any very localised sources, or sinks of NO_2, or disturbances to the airflow. For example, close proximity (less than 10 m) to the following must be avoided:

- heater flues (particularly low level balanced flues);
- Bushes or trees overhanging or surrounding the tube location;
- air conditioning outlets;
- extractor vents; or
- underground ventilation shafts.

The site should be open to the sky, with no overhanging vegetation or buildings.

3.2.4 Use of Boxes or Shelters

It is not common practice in the UK to expose diffusion tubes inside any kind of box or shelter, although such shelters are used in some other European countries. If shelters are used, they should be used at all sites in the survey (including any co-located with automatic analysers). The findings of any such co-location studies, in terms of diffusion tube precision and accuracy compared to the automatic analyser, may not be applicable to cases where shelters are not used.

3.2.5 Duration Of Monitoring Programme

The duration of the monitoring programme will of course depend on the objectives of the monitoring. However, for the purposes of LAQM[15] it is recommended that NO_2 diffusion tube monitoring is carried out for a full year, as the objective is usually assessment against the AQS Objective for the annual mean.

Nitrogen dioxide concentrations often show seasonal variation, so it is recommended that *all* surveys should be carried out for a minimum of six months, comprising three summer and three winter months, e.g. January to June inclusive or July to December inclusive.

Individual exposures should ideally be 2-4 weeks (no longer than 5 weeks and no shorter than 1 week). If a co-location study is carried out to assess the accuracy of the results, the exposure periods used for this should be the same as those used for the rest of the survey.

3.3 Instructions for Exposing Diffusion Tubes

In addition to following the guidance on siting in the previous section, it is important that the exposure of the samples is carried out within appropriate quality assurance (QA). This section covers the important issues on deployment, exposure and collection. The following procedures should be followed:-

- Remove tubes from the refrigerator on the day that they are to be put out, and ensure each one is clearly labelled with a unique identification number (if this hasn't already been done by the supplying laboratory). The labelling must be weatherproof (i.e waterproof labels or permanent pen).
- Take tubes to the site in a sealable plastic bag or plastic container. Travel blanks, where applicable, should be identified and their code numbers noted on the exposure details form provided by the laboratory.
- At each site, select a tube. Record its identification number, and the site at which it is to be exposed, on the exposure details form.
- With the absorbent (coloured) end cap uppermost, remove the bottom end cap (usually white or clear in colour) and clip/place the tube into the holder. Ensure the tube is positioned vertically with its open end downwards (Figure 3-3)
- Record the date and time of the start of the exposure period on the exposure details form, and make a note of any site irregularities (for example building/road works, traffic diversions).
- Keep the end caps in the bag/container, for use when the exposure period is completed.

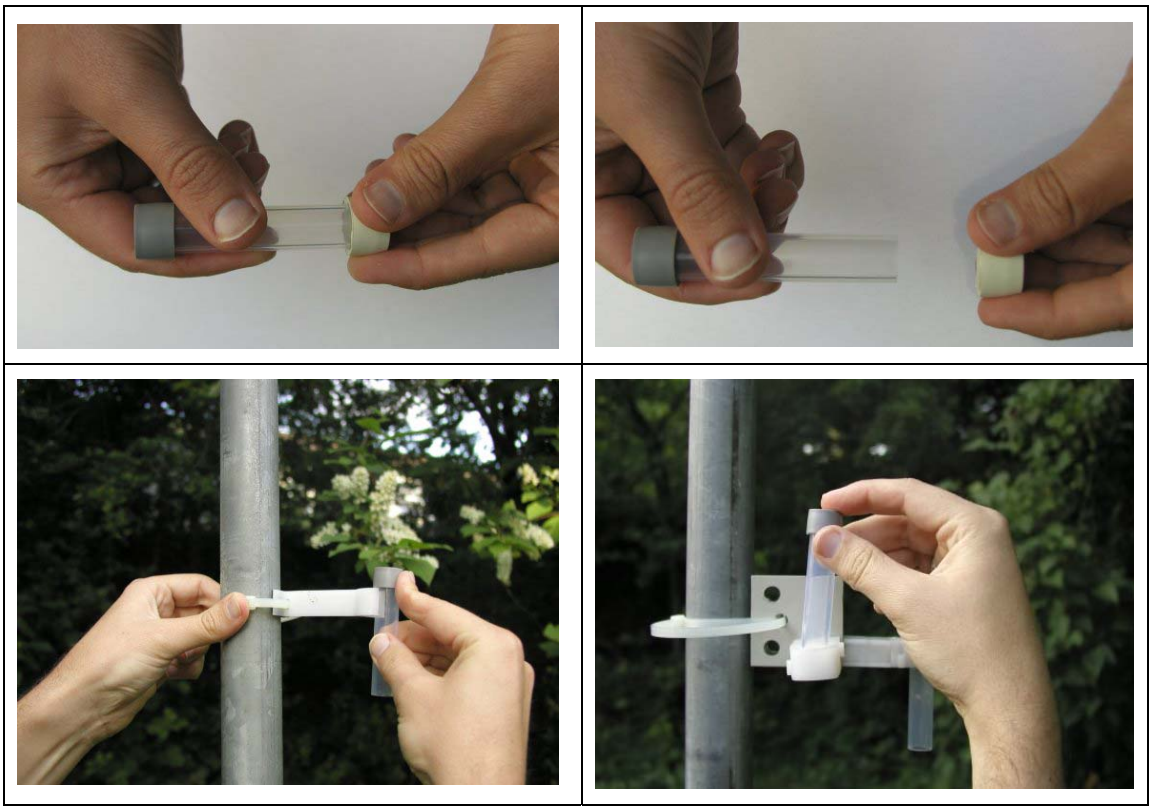

Figure 3-3 Deployment of diffusion tubes

On the appropriate date, the samplers will need to be changed and a new batch of tubes identified for exposure. The following procedures should be followed:-

- Transport the new batch of unexposed tubes to site, together with the end caps from the last batch, any travel blanks as appropriate, and exposure details forms for both batches.
- At each site, remove the exposed tube from the sample holder and replace the end cap tightly.
- Record the time and date of the end of the exposure period on the exposure details form, against the appropriate tube number.
- Make a note on the form of any site irregularities (building/road works, traffic diversions), also anything which might affect, or even invalidate, the tube's results (for example tube found on the ground, insects, dirt, or liquid inside the tube).
- Select a new tube for exposure. Remove its end cap and place it open end down in the holder, as above. Record tube identity details, date and time. Tubes that are damaged or have splits in the end-caps should not be used.
- Keep the tubes in a sealed container, in a cool place (a fridge is best) until they can be returned to the laboratory for analysis, which should happen as soon as possible.
- Ensure that the tubes are used and analysed within 4 months of preparation.

Tubes should always be capped securely after exposure; any tubes returned uncapped to the laboratory should be rejected. When visiting sites, it is recommended that the operator takes some spare tube end caps, also some spare mounting clips and spacer blocks to replace any missing or damaged.

More precise results will be obtained by using groups of three or more tubes together, rather than single tubes alone. However, this has to be balanced against the additional cost, and multiple tube

exposure is not essential if good precision is demonstrated, except in the case of co-location studies (see below) when it is advisable to expose tubes at least in triplicate.

3.4 Co-Location With Automatic Analysers

Diffusion tubes have become widely used by Local Authorities for air quality monitoring. However, as explained in Appendix 2, diffusion tube measurements may exhibit substantial under- or over-estimation compared to the reference method. This is due to factors affecting the performance of diffusion tubes in the field – for example wind-induced shortening of the effective diffusive path length – that (unlike most of the issues dealt with in this guidance) are not related to the laboratory's preparation or analysis of the tubes.

Clearly, any such under- or over-estimation is a problem in any situation where diffusion tube results are to be compared with air quality standards or objectives. Furthermore, diffusion tubes analysed by different laboratories may exhibit very different accuracy results, even when the tube preparation technique, tube materials, and analytical techniques are broadly the same. The reasons for this are still not fully understood.

As a result, Defra's Technical Guidance LAQM.TG(03)[15] recommends that Local Authorities making use of nitrogen dioxide diffusion tubes in their Review and Assessment should carry out their own investigation of diffusion tube accuracy (referred to as "bias" in TG(03)), then apply an adjustment factor to the annual mean if required.

This should be done by a co-location study: that is, by exposing diffusion tubes alongside an automatic chemiluminescence analyser, and comparing the results of the two techniques. The co-location study should be of at least nine months' duration, with diffusion tubes exposed in triplicate at a suitable automatic monitoring site. When carrying out a co-location study:-

- Diffusion tubes should be placed within 1 m of the automatic analyser inlet, but care should be taken that they do not block the inlet in any way (Figure 3-4).
- If the co-location study is being carried out at a roadside monitoring station, ensure that the tubes are the same distance from the road as the analyser inlet.
- The co-located diffusion tubes should be exposed in triplicate (i.e. groups of three) if possible. Ideally tubes should be spaced at least 10 cm apart.
- The duration of the study should be at least 9 months.
- Exclude data from any months when the automatic analyser does not achieve at least 90% data capture.
- It is of paramount importance to ensure that the data from the analyser are of good quality, so good QA/QC procedures must be applied to the automatic monitoring.
- The co-located diffusion tubes should be prepared, handled and analysed in exactly the same way as those from the other (non co-located) monitoring sites in the survey. Exposure periods should be the same, to within +/- 2 days.

Details of how to calculate a bias adjustment factor are given in the Technical Guidance[15] LAQM.TG(03), so have not been reproduced here.

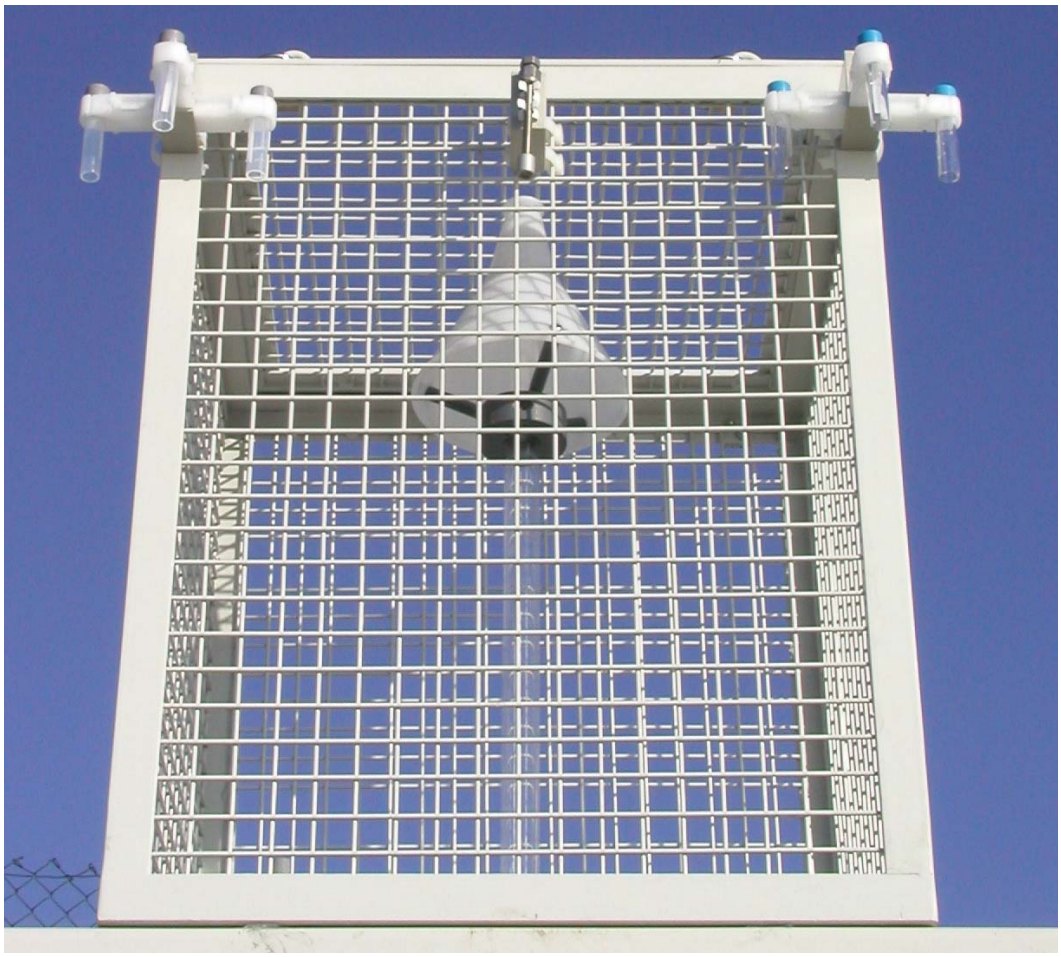

Figure 3-4 Example of how diffusion tubes can be fixed at an automatic monitoring site.

4 Analysis of NO_2 Diffusion Tubes

Sections 2 and 3 respectively have covered the preparation and exposure of NO_2 diffusion tubes. This section covers the analytical aspects of this technique.

The principle of analysis of diffusion tubes using colorimetry is as follows:

1. A mixture of water and reagents* is added to the tubes, and they are agitated, so that the nitrite collected on the grids is dissolved in aqueous solution.
2. The reagents (referred to here as "colour reagents") react with the nitrite in solution to form a purple compound. The intensity of the purple coloration can be measured using spectrophotometry. By calibration using a set of standard nitrite solutions it is possible to accurately measure the concentration of nitrite present in the sample.
3. The concentration of nitrite is used to calculate the mass of nitrite collected by the tube during its exposure. From this, the average ambient concentration of NO_2 during the exposure period can be calculated.

** in the manual method (see below). In the automated method, extraction is done using water alone, and the reagents are added subsequently.*

The analytical procedure for Palmes-type NO_2 diffusion tubes therefore consists of the following key stages:

- Purchase/preparation of stock standard
- Preparation of colour reagents and calibration standard solutions
- Calibration of the spectrophotometer or colorimeter – a full calibration should be carried out at least once per month, with a linearity check (at least four points including zero) at the start of each analysis run.
- Extraction of nitrite from grids of exposed tubes into aqueous solution
- Analysis of solution by spectrophotometry, to determine the concentration of nitrite
- Determination of the total mass of nitrite collected by the tube, and finally calculation of the average ambient concentration of NO_2.

There are two main methods by which analysis is usually carried out:

i) The majority of UK analysts use the **manual** method, in which the colour reagents are added manually to the tubes using a pipette: the intensity of the purple coloration is then measured using a spectrophotometer or colorimeter.

ii) A small number of UK analysts use the **automated** method. This involves extraction with water. Then, an aliquot of the resulting sample solution is drawn through an automatic analyser, in which the reagents are dispensed automatically. The sample then passes through mixing coils, and the intensity of the purple coloration is measured. This method has the advantage that the reagent volumes can be very accurately and consistently dispensed, and the reaction time and mixing is identical for all samples.

The key difference is that in the manual method the reagents are added manually, whereas in the automatic method they are dispensed automatically once the sample is in the analyser. There are inevitably some differences in procedures between the manual and automatic methods. Therefore, for clarity in this document:

- Guidance applicable to **both** the manual and automatic methods is shown against a **white** background, as normal.
- Guidance applicable only to the **manual** method is shown against a solid **blue** background, like this.
- Guidance applicable only to the **automatic** method is shown against a chequered **yellow** background, like this.

Note: ion chromatography may also be used for analysis of diffusion tubes, although at present only one UK laboratory does so. This is perfectly acceptable and indeed has some advantages over colorimetric methods (such as lower detection limits). However, ion chromatography falls outside the scope of the current guidance.

There is potential for significant imprecision and inaccuracy to be introduced during the analysis of diffusion tubes, if each stage is not carried out according to the correct procedures. This includes the accurate preparation of standards and colour reagents, which is an important part of the process.

The analytical technique presented in this document aims to harmonise the approach to diffusion tube analysis, based on current knowledge of best practise. The approach is based largely on the original Palmes *et al* (1976)[1] and Atkins *et al* (1986)[16] methods. It is important that no shortcuts or modifications are introduced, as this could introduce inter-laboratory variability.

Tubes should be exposed and analysed within the lifetime of the batch. It is recommended that the tubes should be exposed and analysed within four months of preparation.

Analysis of tubes should be performed in manageably sized batches, in order to maintain consistency throughout. QA/QC procedures must be in place, to ensure that every step of the analysis procedure is working correctly. These are described in this section.

4.1 Preparation of standards

As with many other analysis techniques, a set of standard solutions of accurately known concentrations is necessary in order to accurately calculate the mass of nitrite extracted from the grids of the exposed diffusion tubes.

The starting point is to purchase or prepare a "stock standard solution" of high concentration, from which the set of calibration standard solutions are subsequently prepared when required, by dilution.

Standard nitrite solutions are available commercially, and these may well be the most reliable and convenient option for many laboratories. *Commercially available standards are strongly recommended to cut down analytical time and reduce laboratory variability.* It is recommended that any laboratory experiencing difficulty in achieving satisfactory analytical results – which will be evident through participation in the Workplace Analysis Scheme for Proficiency (WASP Scheme) for this analysis - should consider using a bought-in standard, to eliminate the possibility of errors due to inaccurate standard preparation. Box 4.1 gives the specification for ready-made standards.

Box 4-1 Specification for Commercially Available Stock Standard Nitrite Solution

The specification for bought-in standard nitrite solution is as follows:

- Concentration of nitrite should be 1.000 g/L (= 1000 µg/mL or 1.000 mg/mL) in water. (In practice, sodium nitrite is usually used but it is the concentration of nitrite ion that is important).
- Solution should as a minimum be certified. Ion chromatography standards, guaranteed to 1%, would be suitable.
- Traceable standards to Standard Reference Materials (from NIST or equivalent) would be even better but it is recognised that the expense may not be justifiable in many cases.

Commercially available standards are the recommended option, but can be expensive (approximately £70 for 500 mL at the time of writing). Therefore, it is also acceptable to prepare standard solutions in-house, and the methodology for preparation of these is set out in this section. Necessary apparatus is listed in Box 4-2.

Box 4-2 Apparatus necessary for preparing colour reagents and standards

- **Accurate** laboratory balance, **regularly calibrated** using traceable standard masses.
- Burette, 25 mL capacity.
- Conical flasks, 500 mL capacity
- Vortex mixer – small test tube size or alternative equipment used to ensure a vigorous agitation of the tubes during extraction.
- UV/Vis spectrophotometer or colorimeter
- Pipettes – disposable tip, adjustable or fixed volume as appropriate to make up the calibration standards. A micropipette capable of dispensing 50 µL will be required.
- Glass Petri dish to contain the sodium nitrite during the drying and cooling cycle.
- Oven, to dry the nitrite used to make the stock standard solution. Temperature should be set to 102 ± 2 °C, and checked using a thermocouple gauge or accurate thermometer annually, and a record kept of the test results.
- Desiccator, used to store the Petri dish of sodium nitrite while it is cooling. This should contain self-indicating silica gel or other functional desiccant.

All ovens and heat sources used throughout must be electric rather than gas, because gas combustion generates oxides of nitrogen.

4.1.1 Preparation of Stock Standard Solution

For laboratories that prefer to prepare their own stock standard solution, the following section gives a suggested method for the preparation of the stock standard sodium nitrite solution.

The purity of the solid sodium nitrite to be used must be at least 99%: this should be specified on the label or supplier's fact sheet.

Box 4-3 Preparation Of Stock Standard Solution, 1000 µg/mL (1mg/mL or 1g/L) nitrite.

1. The solid sodium nitrite must be of analytical quality, with minimum purity 99%

2. It is not necessary to correct for the impurity of the sodium nitrite as the error introduced by this will be insignificant, providing the above condition is met.

3. The sodium nitrite must be dried before preparation of the solution. Place 1.7 – 2.0 g of sodium nitrite in a Petri dish and dry in an oven at 102 ± 2 °C for 1 – 2 hours. Using gloves, remove the Petri dish and contents and place it in a desiccator, with the lid on, to cool down. Allow the sodium nitrite to cool for between 30 minutes to 1 hour before use.

4. The mass required to prepare a 1 L solution of strength 1000 µg nitrite /mL (1 mg/mL) is :

   1 x 68.995/46.006 = 1.500 g

   (46.006 g being the molar mass of NO_2^- and 68.995 g being the molar mass of $NaNO_2$.)

4. Weigh out 1.500 ± 0.001 g of the dried sodium nitrite ($NaNO_2$) into a **glass** beaker or weighing bottle. ***(Glass should be used rather than plastic, as electrostatic effects can introduce errors in the weighing process)***.

5. It is normal to discard any unused dried sodium nitrite rather than returning it to the original bottle, to avoid the risk of contamination.

6. Put 100 –800 mL analytical grade deionised water (at least 18 M Ω) into the 1 L volumetric flask; transfer all the sodium nitrite by washing, and make the solution up to the 1 L mark using analytical grade deionised water. Shake well. The concentration of nitrite ion (NO_2^-) in the resulting stock standard solution will be 1.000 g NO_2^- per litre.

7. The quantities above can be scaled up or down if a larger or smaller volume is required.

It is recommended to replace the solution every 6±3 months – i.e. at least every 9 months. The stock solution is usually stable for at least a year if stored in a refrigerator at 4 ± 4 °C. However the stock solution is normally removed from the refrigerator the evening before use, and allowed to attain laboratory temperature, hence the recommendation to replace at a shorter interval.

4.1.2 Testing of New Standard Solution

Any new batch of stock standard solution, whether purchased or prepared in-house, must be tested by comparison with the previous batch of solution that it is intended to replace.. This is done by preparing a set of calibration standards and running a calibration curve (see below) for each solution, and comparing the results.

The results for the "new" and "old" solution should not differ by more than 2%. If they do,

1. the procedure should first be repeated, to rule out any errors, for example in dilution of the solution to make the calibration standards.
2. If the discrepancy remains, both "new" and "old" standard solutions should be compared with an independent "quality control" standard – see section 4.1.3 below.
3. The analyst must establish whether either the "new" solution is faulty (very unlikely with a commercial standard but not impossible) or (more likely) the "old" solution has deteriorated.

Note: it is also possible to test the concentration of a new batch of standard solution by titration. However, this requires high precision, in order to ensure high precision of the ultimate measurement results. This *optional* titration procedure is provided in Appendix 3: however, it does not form part of the harmonised method.

4.1.3 Preparation and use of Quality Control Standard Solution

This is a *separate* standard solution used as a test for quality control purposes. It can be made up as in 4.1.1 above, using sodium nitrite from a different source (i.e. a different manufacturer, or a different batch from the same manufacturer). QC standard solutions, suitable for this purpose, are supplied six-monthly by AEA Energy & Environment to all laboratories that participate in the diffusion tube QA/QC scheme. It is recommended that these are used.

This QC standard solution must be compared to the stock standard solution by running a calibration curve (see below) with each of the two solutions. The results should be identical. The QC standard should be replaced half way through the life of the main standard, rather than at the same time.

When a new QC standard is to be used for the first time it should be compared to the old standard that it replaces (by running calibration curves with each solution) and a record kept of the results.

4.2 Preparing Calibration Standards

In order to calibrate the spectrophotometer prior to the analysis of diffusion tubes (see section 4.6), a range of nitrite calibration standards is necessary. These are prepared by dilution of the stock standard solution (of concentration 1000 µg/mL as purchased or as prepared in Box 4.3 above).

The approach used here is to effectively prepare a set of "spiked tubes" containing known masses of nitrite. This is done by preparing a range of intermediate solutions of different strengths, then pipetting an identical small volume (50 µL) of each one, into a set of tubes. These tubes are then treated in the same way as real exposed samples: the same volume of reagent mix will be added to them, and they are analysed in the same way as exposed samples.

The range of concentrations required for calibration will depend on the likely amounts of nitrite captured by the tubes that are being analysed. This in turn will depend on the anticipated ambient NO_2 concentration at the monitoring location, and the length of the exposure periods. **The concentrations shown in Box 4-4 below are therefore examples and can be varied as required.**

Box 4-4 Preparation of Standard Solutions

Using a calibrated micropipette (50 – 2000 μL), measure small volumes of the stock standard solution into a set of 10 mL volumetric flasks. Dilute to 10mL using deionised water. The example below shows the dilutions for a range of solutions from 15 – 120 μg/mL:

A – 120 μg/mL prepared as follows: 1.2 mL of stock solution measured out using a calibrated micropipette, and made up to 10 mL in a volumetric flask
B – 90 μg/mL prepared as follows: 0.9 mL of stock solution measured out using a calibrated micropipette, and made up to 10 mL in a volumetric flask
C – 60 μg/mL prepared as follows: 0.6 mL of stock solution measured out using a calibrated micropipette, and made up to 10 mL in a volumetric flask
D – 30 μg/mL prepared as follows: 0.3 mL of stock solution measured out using a calibrated micropipette, and made up to 10 mL in a volumetric flask
E – 15 μg/mL prepared as follows: 0.15 mL of stock solution measured out using a calibrated micropipette, and made up to 10 mL in a volumetric flask
F - Blank. 0 mL of stock solution

Pipettes used must be of accuracy ± 1.00% and precision ± 0.50% or better. This must be checked daily as recommended in section 2.4. Accuracy of volumetric flasks must also be regularly checked.

Having prepared the series of diluted solutions, pipette 50 μL of each one into clean mounted tubes clearly labelled (i.e. Standard A, Standard B…etc.). These will contain the following total masses of nitrite (remember that these are examples and can be varied as required):

A – 6.0 μg of NO_2^-
B – 4.5 μg of NO_2^-
C – 3.0 μg of NO_2^-
D – 1.5 μg of NO_2^-
E – 0.75 μg of NO_2^-
F – 0.0 μg of NO_2^-

For the purpose of calibration, **these tubes should not contain grids or TEA** (to avoid introducing uncertainties related to extraction).

It is preferable to make up these calibration standards in tubes rather than cuvettes (to minimise the differences in handling of standards and samples). However, this is not essential and use of cuvettes is acceptable.

The tubes are then taken through the analytical process in the same way as exposed samples, by addition of reagent solution and analysis.

Note: it is also acceptable to carry out dilution by mass rather than by volume, if this is your normal laboratory procedure, provided an accurate balance is used. Please contact AEA if you wish to use an alternative method to prepare your calibration standards.

4.3 Colour reagent

4.3.1 Colour reagent

The analysis of diffusion tubes requires preparation and use of the following reagent solutions:

- N-1 (Naphthyl-1) Ethylene Diamine Dihydrochloride (often abbreviated to NEDA, or more correctly NEDD, which is the abbreviation used here)
- Sulphanilamide
- Orthophosphoric acid (concentrated, 85%)

Box 4-5 Safety Precautions Relating to Reagents

All these compounds are hazardous. NEDD in particular is harmful by ingestion, can cause serious damage to eyes, and is a skin irritant. Suitable personal protection, including goggles and gloves, must be worn when handling these reagents. Please refer to your reagent supplier's safety data sheet (supplied with the product) for the latest information and necessary precautions.

The analysis of diffusion tubes can be either done by manual or automatic method. In order to maintain the same chemistry between a manual and an automatic method, there are slight variations when preparing the colour reagents. If the automatic analyser is used as a manual spectrophotometer without injection of colour reagents or dilutions, follow the instructions for the manual method.

4.3.2 Colour reagent for MANUAL METHOD

The use of a pre-mixed colour reagent is recommended, as this should assist in decreasing laboratory inter-variability. There is a negligible reduction in sensitivity of the colour reaction caused by using a pre-mixed colour reagent[17]. Even at the low concentrations measured at rural locations the sensitivity of the method is not a problem.

The sulphanilamide:NEDD ratio adopted in this methodology is 1: 7 x 10^{-3} (i.e. 7 x 10^{-3} g of NEDD present per 1 g of sulphanilamide). This ratio has been found to be important for optimum colour formation[17].

Box 4-6 Preparation of Colour Reagents – MANUAL METHOD

1. In a 500mL flask, dissolve 10 ±0.01 g of sulphanilamide in 300 mL of deionised water, mixing it well. Add 25 mL of orthophosphoric acid carefully, and mix well again. Make up to 500 mL with deionised water. Store in a clearly labelled sealed dark glass bottle.
2. Dissolve 70 ± 1 mg of NEDD in about 300 mL of deionised water. Make up to 500 mL with deionised water. Store in a second clearly labelled sealed dark glass bottle.

Note 1: the two above preparations should now be kept separately. Immediately before use, the required quantity of mixed reagent should be prepared by mixing the above solutions in a 1:1 ratio. Once mixed, the colour reagents should be used the same day, not stored.

Note 2: the above procedure makes 500 mL of each solution, but if a different volume is required, the quantities of reagents indicated above can be adjusted accordingly.

4.3.3 Colour reagent for AUTOMATIC METHOD

If the automatic method is set up to introduce the reagents through the auto-analyser, it is recommended to follow the instructions in this section. This section has been written to follow the same principle as specified by Palmes *et al*[1] and Atkins *et al*[16]. The sample : reagent ratio therefore is assumed to be 1:2:2 (Sample:sulphanilamide solution:NEDD solution).

Depending on the automatic analyser set up, laboratories will either use pre-mixed or separate reagents. If using pre-mixed reagents, the ratio of volume sampled and pre-mixed reagent should be 1:4. On the other hand, if the reagents are used separately a 1:2:2 ratio of sample:sulphanilamide: NEDD should be used. The NEDD/sulphanilamide mass ratio adopted in this methodology remains at 0.7% (i.e. 7 x 10^{-3} g of NEDD present per 1g of sulphanilamide). This ratio has been found to be important for optimum colour formation[17]. The quantities and ratios specified are intended to maintain the optimum NEDD:sulphanilamide ratio above, and consistency with the manual method. **However, it is recognised that some flexibility may be required to allow for the set-up of different automatic analysers, so please contact AEA if you have any questions or difficulties**.

Box 4-7 Preparation of Colour Reagents for AUTOMATIC METHOD

1. In a 1L flask, dissolve 10 ±0.01 g of sulphanilamide in 300 mL of deionised water, mixing it well. Add 30mL of orthophosphoric acid carefully, and mix well again. Make up to 1L with deionised water. Store in a clearly labelled sealed dark glass bottle.

2. Dissolve 70 ± 1 mg of NEDD in about 300 mL of deionised water. Make up to 1L with deionised water. Store in a second clearly labelled sealed dark glass bottle.

Note 1: the two above preparations should now be stored separately. If mixed reagent is required, the required quantity of the two solutions should be mixed in a 1:1 ratio on the day they are to be used. Mixed reagent should be used the same day, and not stored.

Note 2: the above procedure makes 1L of each solution, but if a different volume is required, the quantities of reagents indicated above can be adjusted accordingly.

4.3.4 Storage of Colour Reagents

Once prepared, the reagents above should be stored as follows:

The sulphanilamide and orthophosphoric acid mixture should be stored in a clearly labelled dark glass bottle. It is advisable to keep it in a cool, dark place, although refrigeration is not necessary and it is not as sensitive to light as is NEDD. The shelf life under these conditions is 6 months.

The NEDD solution should be stored in a clearly labelled dark glass or black bottle, in a cool light-proof cupboard or refrigerator. NEDD must be stored in cool conditions and in particular protected from light (which will degrade it). Some laboratories store NEDD in a dark glass bottle wrapped in black PVC tape for added light protection. The shelf life under these conditions is 6 months.

The two above preparations should be kept separately, and only mixed immediately before use. Once mixed, the colour reagents should not be stored, but used within the same day.

4.4 Extraction method

The first stage of analysis involves extracting the absorbed nitrite from the TEA-coated grids into a solution, so that it is available to react with the reagents. This is done by pipetting solution into the tube, as described in detail in section 4.7. The tube must then be agitated so that the absorbed nitrite dissolves into solution. **Efficient extraction of all the nitrite from the grids is essential.**

The tubes should be agitated vigorously using *either* a **vortex mixer** for at least 15 seconds *or* alternatively a **vibrating tray**[17] for 10 – 30 minutes. If the latter is used, the tubes should be kept **vertical** to reduce the possibility of drops of solution being lost through the cap. Manual shaking is not recommended: it is not practicable for laboratories dealing with large numbers of tubes; also, it is difficult to ensure all samples are shaken efficiently and consistently. It is also not recommended due to the risk of repetitive strain injury. Figure 4.1 illustrates good and bad practice for tube extraction.

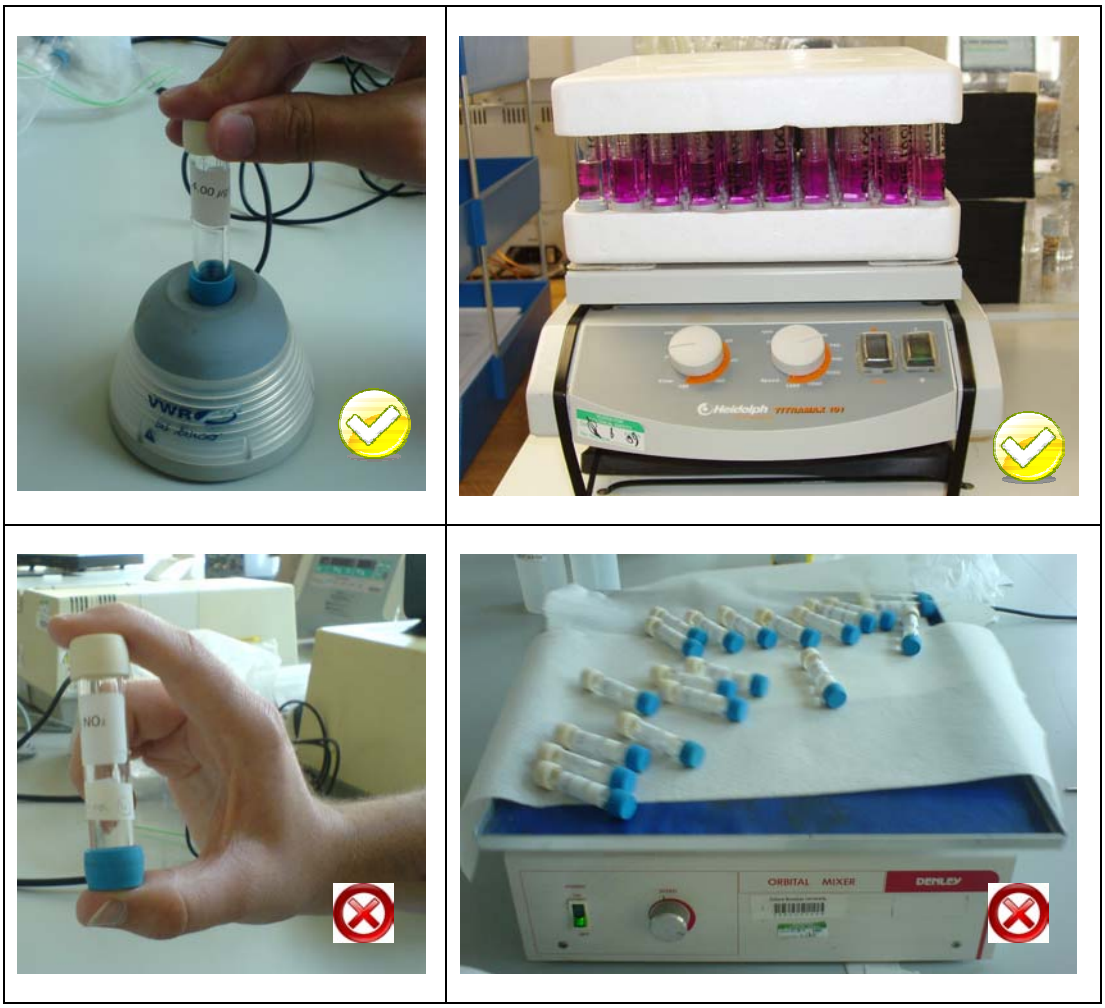

Figure 4-1 Extraction from diffusion tubes

750 rpm is suggested as a suitable vibration frequency setting. However this will vary from instrument to instrument, so should be optimised by the analyst. Tubes should be agitated vigorously, but not so violently as to cause risk of solution leakage.

4.5 Calibration of Spectrophotometer/Colorimeter

4.5.1 Full Calibration (Monthly)

A full calibration of the spectrophotometer or colorimeter must be carried out at least once a month. This should be based on at least four points if calibrating only upto 1 absorbance unit (AU), or at least six points if calibration is required beyond 1 AU. Points should include a zero standard.

This calibration is carried out using an appropriate range of calibration standards, prepared as in section 4.2 above. If diffusion tubes are used, they should not contain TEA.

The absorbance corresponding to each solution is recorded as shown in Table 4.1, and used to plot a calibration curve as shown in Figure 4.2. (Note: the analyser must be allowed sufficient warm-up time, as specified by the manufacturer, before use). ***The calibration standards must be taken through the analytical process in exactly the same way as samples and blank tubes.***

Table 4.1 Example of Calibration Standards and Calibration Curve (based on Manual Method).

Standard	Conc. of Calibration Standard (μg/mL) **C_s**	Mass of nitrite in tube (μg)* **m_t**	Nitrite conc.* in tube (μg/mL)** **C_t**	Absorbance at 542 nm
A	120	6.00	1.967	2.14
B	90	4.50	1.475	1.60
C	60	3.00	0.984	1.05
D	30	1.50	0.492	0.54
E	15	0.75	0.246	0.27
F - Zero	0	0.00	0.000	0.00

** **v** From pipetting 50 μl of each of the range of calibration standards into each tube.*
*** **v_x** Nitrite concentration calculated with a liquid volume of 3.05 mL (3.0 mL of extraction volume + 0.05mL of standard)*
The calibration standards shown here are examples: the range of concentrations prepared can be varied as appropriate.

The mass 'm_t'of nitrite in each tube is calculated as:

$$m_t(\mu g) = C_s\ (\mu g/mL) \times v\ (mL)$$

where:
- C_s = concentration of nitrite in the relevant standard solution – as prepared
- v = total volume of standard solution added, i.e. 0.05 mL (50 μL)

The concentration C_t of nitrite in each tube is calculated as:

$$C_t(\mu g/mL) = m_t(\mu g)/\ v_x\ (mL)$$

where:
- m_t = the total mass of nitrite in the tube
- v_x = total extraction volume *In this example the volume used was 3.05 mL (3.0 mL of extraction volume + 0.05 mL of standard). It is important to include the small (50 μL) volume of the standard solution, in addition to the extraction volume.*

The relevance of calculating the concentration, as well as the mass, of nitrite in the tube is that the volume of solution used in extraction of actual samples will not include the small (50 μL) volume of standard solution.

Please note that the example in Table 4.1 above is only applicable to the manual method, as automatic analysers usually introduce a further dilution.

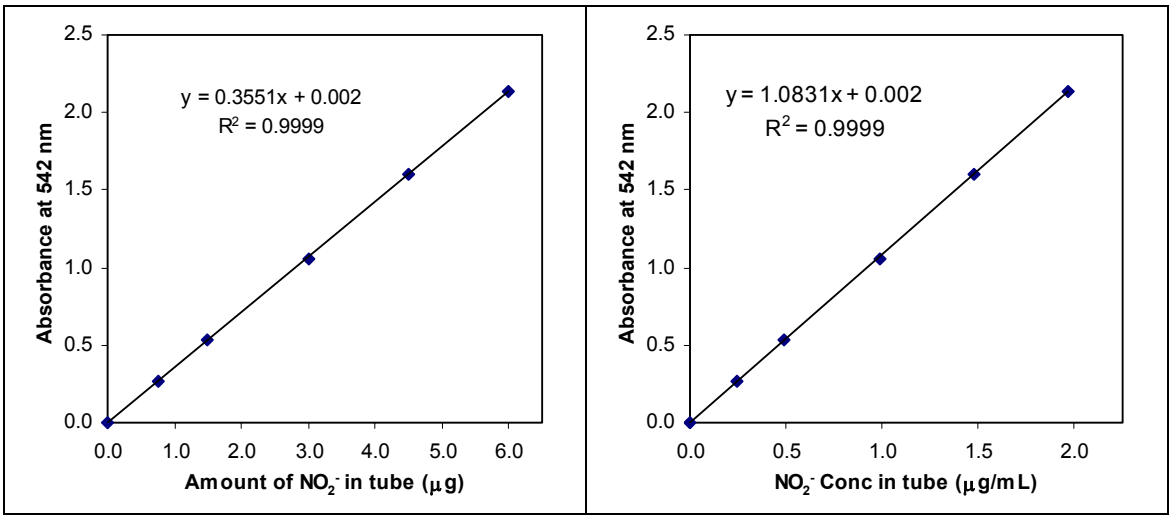

Figure 4-2 Example of a Calibration Curve

The calibration "curve" should in fact be linear. If it is not, this indicates that something may be wrong – either that the spectrophotometer or colorimeter is faulty, or else that the colour strength of the standard solutions used is outside the linear range. The value of r^2 must be at least 0.999 for linear fits upto at least 1.0 absorbance units (AU).

The zero calibration standard "F" in the example above is deionised water, with reagent solution added (as opposed to water alone). This is important in order to take account of any nitrite present in the added reagent.

The equation of best fit, which relates the instrument's response to the total mass of nitrite in the solution, is subsequently used to calculate the mass of nitrite in the samples.

A calibration curve, using a full set of standards, must be produced at least once per month. A minimum of four points should be included if working upto 1 absorbance unit: a larger number (e.g. six) will be required if the calibration range extends above 1 absorbance unit (AU), in order to carry out a full calibration.

It is important that the standards must cover the full range of samples likely to be encountered, and should go up to at least 1.0 absorbance unit, preferably to 1.5. *Any samples subsequently found to be outside the calibration range must be diluted **with colour reagent solution** and re-analysed - **it is not acceptable to extrapolate beyond the calibrated range.** See also section 4.8.1.*

Also, if a fresh batch of reagents is mixed during a day's run, a new calibration should be carried out using the new reagents.

4.5.2 Linearity check (daily)

In addition to the full calibration required at least once a month, a linearity check should be carried out at the start of every day in which analysis is to be carried out. (This may be daily, weekly or monthly, depending on how often the lab does diffusion tube analysis). This should be done, and the linearity checked, before the start of the analysis run. Three points (including zero) are usually sufficient for this, but again it is important that the standards selected cover the full range of samples likely to be encountered.

4.6 Analysis Schedule

It is important that the analysis schedule – i.e. the sequence in which samples are analysed - is designed to include the nitrite calibration standards, and to ensure that tubes are handled in manageably sized batches throughout the analysis.

By using small batches of tubes the analyst will ensure:

- All tubes are treated the same way
- Minimal loss of results if problems occur with instruments
- Instrument drifts can be easily corrected

The issue of batch size is most relevant in the case of the manual method, as automatic analysers -

- ensure that time between addition of reagents and measurement of absorbance is identical for all samples and
- typically incorporate software which allows a drift correction to be applied.

Optimum batch size will vary depending upon each laboratory's instrumentation and procedures and is thus at the analyst's discretion: however, it is suggested that for the manual method an appropriate batch size might be the number of tubes that can be analysed in around two hours.

Fresh nitrite calibration standards, prepared on the day of use, must be analysed at the beginning of each run as a linearity check (see section 4.5.2). A mid-range and zero standard must be analysed at intervals throughout the sequence, in order to identify and assess any problems (such as drift).

When dealing with large number of tubes, each individual tube should be treated the same way. In the case of the manual method this includes ensuring that the standing time, i.e. the interval allowed for

colour development between addition of the mixed colour reagent and analysis of the sample, is similar throughout the batch. Table 4.2 below shows an *example* of the beginning and end of such an analysis schedule. This shows minimum requirements in terms of standards.

Table 4.2 Example of Part of Analysis Schedule (manual method assumed).

1.	Nitrite Standard B	19.	Tube_02580	…	
2.	Nitrite Standard D	20.	Tube_02581	…	
3.	Zero Standard (F)	21.	Tube_02582	…	
4.	Tube_02565	22.	Tube_02583	…	
5.	Tube_02566	23.	Tube_02584	…	
6.	Tube_02567	24.	Nitrite Standard B	…	
7.	Tube_02568	25.	Zero Standard	…	
8.	Tube_02569	26.	Tube_02585	…	
9.	Tube_02570	27	Tube_02586	…	
10.	Tube_02571	28.	Tube_02587	…	
11.	Tube_02572	29	Tube_02588	…	
12.	Tube_02573	…	…	…	
13.	Tube_02574	…	…	…	
14.	Tube_02575	…	…	…	
15.	Tube_02576	…	…	…	
16.	Tube_02577	…	…	…	
17.	Tube_02578	…		…	Nitrite Standard B
18.	Tube_02579	…		…	Zero Standard

The above is an example, but important points are as follows:

- The analysis schedule should, as a minimum requirement, begin with a high, mid-range and zero standard and linearity should be checked.
- A high or mid-range nitrite standard, and a zero standard, should be analysed after every 20 samples as a minimum requirement.
- The schedule should finish with a high standard and zero standard.

4.7 Analysis

4.7.1 Manual Method

Switch on the spectrophotometer analyser and allow to warm up for the time specified by the manufacturer. Then follow the procedure in Box 4-8 below.

Box 4-8 Analysis Using the Manual Method

1. Take the exposed tube and wipe off any obvious dirt from the outside before removing the white end cap. Arrange the tubes in a test tube rack following the order for analysis. If tubes are not already labelled, label all samples clearly.

2. Remove the (usually white) cap at the "open" end of the tube, making sure not to remove the cap at the other end of the tube, which holds the grids. If there is any foreign material inside the tube, such as dirt or spider's web, hold the tube open-end down, and use tweezers or forceps to remove it. Do not touch the TEA-coated grids, or allow the material you are removing to contaminate these. Make a note of the affected tubes.

3. Add 3.0 mL of pre-mixed reagent solution to each tube. (Note: this volume may need to be larger, e.g. 4.0 mL - or smaller, e.g. 2.0 mL, depending on tube exposure duration and ambient NO_2 concentration). **The volume of pre-mixed reagent should be accurately and precisely dispensed using a pipette, calibrated to an accuracy of 1.00%. The total volume of liquid added to the tube is used in the calculation of the mass of nitrite, so it is important that it is accurately measured and recorded.** Replace the white plastic cap on the filled diffusion tube (sealing in the liquid) and agitate using a vortex mixer for at least 15 seconds or alternatively a vibrating tray shaker (with the tube in a vertical position) for 10-30 minutes.

4. Leave to stand to allow development of the colour for at least 10 minutes. Once the colour has developed, it is stable for upto 2 hours[17].

5. Transfer a volume of the sample solution to a 10 mm glass cell or cuvette for analysis using the spectrophotometer. The volume in the cuvette must be sufficient to ensure that the spectrophotometer beam passes through the solution, and not through the interface or the air above it.

6. The optimum wavelength (in the range 540 – 550 nm) should be determined by using the spectrophotometer's scan function to go through the wavelength range until the maximum response is obtained. Having determined the optimum wavelength, it is then essential that this wavelength setting be maintained throughout the calibration process and while analysing the full set of samples.

7. Take a reading as soon as the result is stable. The time between addition of reagents and taking the reading should be no less than 10 minutes, and no more than two hours.

A dual beam spectrophotometer should be used if possible, in preference to a single beam instrument. This is not essential, but has the advantage that a sample of the zero nitrite calibration standard can then be used to fill the cuvette in the reference beam.

4.7.2 Automatic method

Automatic analysers have the advantage that the colour reagents are dispensed automatically and mixed sequentially with the sample. Reagent ratios and reaction times are thus identical for all samples. Therefore, procedures are slightly different when using these instruments.

Laboratories that use an automatic analyser should follow the procedure outlined in this section. Switch on the automatic analyser and allow to warm up for the time specified by the manufacturer. Then follow the procedure in Box 4-9 below.

Box 4-9 Analysis Using An Automatic Analyser

1. Take the exposed tube and wipe off any obvious dirt from the outside before removing the white end cap. Arrange the tubes in a test tube rack following the order for analysis. If tubes are not already labelled, label all samples clearly.

2. Remove the (usually white) cap at the "open" end of the tube, making sure not to remove the cap at the other end of the tube, which holds the grids. If there is any foreign material inside the tube, such as dirt or spider's web, hold the tube open-end down, and use tweezers or forceps to remove it. Do not touch the TEA-coated grids, or allow the material you are removing to contaminate these. Make a note of the affected tubes.

3. Add 3.0 mL of deionised water (at least 18 MΩ) to the tube. (Note: this volume may need to be larger, e.g. 4.0 mL - or smaller, e.g. 2.0 mL depending on how long the tubes have been exposed for and whether the tubes have been exposed to high or low polluted areas). **The volume of water should be accurately dispensed using a pipette, calibrated to 1.00%: the total volume of liquid added to the tube is used in the calculation of the mass of nitrite, so it is important that it is accurately measured and recorded.** Replace the white plastic cap on the filled diffusion tube (sealing in the liquid) and agitate using a vortex mixer for at least 15 seconds or alternatively a vibrating tray shaker (with the tube in a vertical position) for at least 20 minutes.

4. Leave to stand for at least 10 minutes.

5. Transfer tubes to the autosampler, from where the extracted samples will, in turn, be sampled.

6. The pre-mixed colour reagent solution will be automatically dispensed through the different loops in the automatic analyser. This will be dispensed as *either* **1:4 ratio of sample:pre-mixed reagents** *or* **1:2:2 ratio of sample:sulphanilamide solution:NEDD solution**, if adding the latter reagents separately.

Determine the concentration of sodium nitrite present in the sample by reference to the calibration curve, see section 4.5

4.8 Calculation of Mass of Nitrite from Tube

Having followed the procedures in section 4.5, a calibration "curve" (usually very close to a straight line) will have been obtained, based on the spectrophotometer's response to a range of standard solutions. Using the equation of the "best fit" line as shown in Figure 4-2 above, the concentration of nitrite in each sample can be calculated.

Using the calibration curve previously prepared, as shown in Figure 4-2, obtain the nitrite concentration from the instrument response.

Table 4.3 below shows an example of calculation of the mass in microgrammes (µg) of nitrite from the instrument response in absorbance units. Using - **in this example** - the formula $y = 1.0831x + 0.002$ as obtained from Figure 4-2, the concentration in the analysed tube (µg/mL) can be obtained from the absorbance. With the concentration and the extraction volume, the amount of NO_2^- in the sampled tube is then calculated.

Table 4.3 Calculation of mass of nitrite: example using dummy data

Schedule order	Label	Instrument response (absorbance units)	Conc. in tube (μg/mL)*	μg NO_2^- in tube**
13.	Tube_02565	0.233	0.213	0.64
		Using Calibration curve →	*Conc⁺ x extractionVOL* →	
14.	Tube_02566	0.490	0.451	1.35
15.	Tube_02567	0.989	0.911	2.73
16.	Tube_02568	0.173	0.158	0.47
17.	Tube_02569	0.752	0.692	2.08

* From example calibration curve equation in Figure 4-2: y = 1.0831x+ 0.002 (y being the absorbance and x the NO_2^- concentration)
** Based on 3.0 mL extraction

The example calibration curve shown in Figure 4-2 is close to a straight line, and this will usually be the case. However, linearity should not be assumed. It is important to check the linearity of the curve**. In particular, if any samples give rise to responses above the calibrated range, linearity should not be assumed, as this can lead to under-estimation of the mass of nitrite. See section 4.8.1 below for advice on dealing with such samples.**

4.8.1 Dealing with Samples Above the Calibration Range

Tubes exposed at highly polluted sites – such as the kerbside of busy roads, where NO_2 concentrations might be as high as 70-100 μg m^{-3} – are likely to have absorbed higher masses of nitrite on their grids. This could potentially take the instrument response outside the calibrated range. Some analysts, who regularly have to analyse diffusion tubes from city centre sites, may often encounter this. However, for others it may be a rare occurrence.

If such tubes are regularly encountered (or known to be likely within a particular survey), the volume of mixed reagent added to the tubes at the extraction stage may be increased – for example upto 4.0 mL. (The upper limit is determined by the volume of the tube.)

However, if on analysis one or more samples is unexpectedly found to exceed the extent of the calibration curve, it will be necessary to either prepare stronger standards in order to draw another calibration curve, or additional dilution will be necessary within the tube. (The latter is the more usual course of action).

To carry out an additional dilution for an over-range sample, in the manual method remove a smaller volume of sample from the tube, using an accurately calibrated pipette, and mix with the same volume of pre-mixed colour reagent, e.g. 1.0 mL sample + 1.0 mL pre-mixed colour reagent = 1:1 or x 2 dilution. (Use reagent, not water). Users of automatic analysers would carry out the dilution within an autosampler cup, with deionised water. (Remember to take the dilution factor into account when working out the sample concentration from the absorbance measurements.)

4.8.2 Dealing with Instrument Drift during Analysis

By running standards at the beginning and end of the analysis run, and at intervals throughout , it is possible to identify any drift in the instrument's response. If significant drift (as a guideline, greater than 10%) is identified between the start and end of the run, the operator should be able to use the standard results to identify the point during the run at which the problem started, and/or to apply a correction. In the case of the manual method, the best course of action in the event of significant drift may be to re-run the entire batch. As highlighted in section 4.6 above, this is one of the reasons why batch size should not be too large.

Automatic analysers typically incorporate software which allows a drift correction to be applied, so re-running a batch is unlikely to be necessary.

However, in either case, excessive drift, step changes or repeated occurrence of drift should always be investigated. It can be an indication that the instrument requires attention.

4.9 Calculating NO_2 Ambient Concentrations

Diffusion tube samplers operate on the principle of molecular diffusion, with molecules of a gas diffusing from a region of high concentration (open end of the sampler) to a region of low concentration (absorbent end of the sampler). Appendix 1 explains further detail on the theory behind molecular diffusion and the calculations behind the formulae in this section.

The ambient NO_2 concentration measured by the exposed tube can be calculated using the formula in Box 4-10 below:

Box 4-10 Calculation of ambient NO_2 concentration

$$C = \frac{1}{"s.rate"} \times \frac{m}{t}$$

where:

C	=	the concentration of NO_2 in the atmosphere ($\mu g\ m^{-3}$);
"s. rate"	=	the sampling rate of NO_2 ($m^3\ h^{-1}$);
m	=	mass of nitrite in tube (μg);
t	=	the time of exposure (h)

The sampling rate is calculated from the tube dimensions and the diffusion coefficient of NO_2 in air, and is treated as a constant:

$$Sampling\ rate = \frac{D_{12}\, a}{l}$$

where:

a	=	the cross sectional area of the tube;
l	=	the length of the tube
D_{12}	=	diffusion coefficient of gas 1 through gas 2 – in this case NO_2 through air.

Tube dimensions vary depending on the manufacturer, so it is important to calculate the sampling rate based upon the *actual dimensions of the tubes being used*. Dimensions of a representative sample of tubes should be checked regularly.

The sampling rate also depends on the diffusion coefficient D. Recent research, reported by Massman in 1998[18], suggests that the best estimate of D is 0.1361 $cm^2\ s^{-1}$ at STP, i.e. 0 Celsius (273 K) and 1 atmosphere (101.3 kPa). **However, D is temperature-dependent**, and for this purpose should be corrected to typical mean UK ambient temperatures. Based on Met Office data for the past 10 years, these are in the range 10-12 Celsius or 283-285 K. Using the temperature dependence relationship used by Massman[18] in which D is proportional to temperature to the power 1.81, and assuming a mean UK temperature of 11 Celsius or 284 K:

$$D_{284K} = D_{273K} \times (284/273)^{1.81} = 0.136\ cm^2\ s^{-1} \times (284/273)^{1.81} = 0.146\ cm^2\ s^{-1}$$

For tubes exposed in the UK, the sampling rate should be calculated assuming D = 0.146 $cm^2\ s^{-1}$. *(Note: it is not necessary to correct D for different temperatures each month: the UK mean should suffice in this context of indicative monitoring.)*

In addition, for comparison with EU Limit Values or AQS Objectives and reporting in Review and Assessment, the measured concentrations must be reported in mass units ($\mu g\ m^{-3}$) at a temperature of 293 K (20 Celsius) and pressure of 101.3 kPa. Therefore, a temperature correction should be applied to the measured concentration, as follows:

$$C_{293K} = C_{284K} \times (284/293)$$

- where C_{284K} is the measured concentration at assumed ambient temperature 284 K, and C_{293K} is the measured concentration corrected to 293 K.

This has the effect of reducing the reported concentration by about 3%.

The correction to 293 K can be incorporated into the calculation of ambient concentration:

$$C_{293} = (\frac{284}{293}) \times \frac{m}{"sampling\ rate" \times t} = \quad 0.969 \times \frac{m}{"sampling\ rate" \times t}$$

where:

C	=	the concentration of NO_2 in the atmosphere ($\mu g\ m^{-3}$);
m	=	mass of nitrite in tube (μg);
t	=	the time of exposure (h)

For the purposes of Review and Assessment, measured concentrations should be corrected to 293 K as above. When reporting the results, the analyst must make clear that this temperature correction has been incorporated, to avoid the possibility of the end-user correcting again.

Box 4-11 Example of how to calculate NO_2 concentrations in air

Suppose a diffusion tube with the above dimensions is analysed and found to contain a mass of 2.1 μg of nitrite. The duration of the exposure period was 28 days, or 672 hours. The sampling rate has been calculated as 68.8 x 10^{-6} m^3 h^{-1}, assuming an average exposure temperature of 284 K (11 Celsius). It is necessary to correct the reported concentration to a temperature of 293 K (20 Celsius) for comparison with the relevant AQS objective:

The average ambient concentration of NO_2 in the air during the sampling period is calculated as follows:

$$C_{293} = \left(\frac{284}{293}\right) \times \frac{1}{"s.rate"} \times \frac{m}{t}$$

$$C_{293} = \left(\frac{284}{293}\right) \times \frac{1}{68.8x10^{-6}} \times \frac{m}{t} = 0.969 \times 14{,}535 \times \frac{m}{t} = 0.969 \times 14{,}535 \times \frac{2.1}{672} = 44.0 \mu g m^{-3}$$

The measured average concentration of NO_2, corrected to reporting temperature of 293 K and rounded to the nearest integer, is 44 μg m^{-3}.

4.10 Problems and Troubleshooting

This section highlights some problems that laboratories sometimes encounter, and common causes.

As highlighted in Section 4.1, participation in an independent performance testing scheme such as WASP is recommended, as this gives participants a regular check on their analytical performance, and provides an opportunity for problems to be identified more quickly. In the WASP scheme, participants are required to analyse regular test samples (in this case spiked tubes) containing an unknown quantity of the analyte.

1. **Persistent under-estimation** of the mass of nitrite in the sample. This might be identified in the WASP scheme as the standardised result being consistently less than 1.0. Possible causes include: inefficient extraction (failure to get all the nitrite off the grid and into the sample solution), incorrect standard solution concentration, problems with the analyser.
2. **Persistent over-estimation** of the mass of nitrite in the sample. This can result from incorrect standard solution concentration.
3. **Occasional very low results (in actual exposed samples**). This may result from problems with tube preparation – grids not being properly coated with TEA, grid(s) having fallen out, leaking/split end caps allowing rain to get in.
4. **Occasional very high results (in actual exposed samples)** may result from contamination of the sample, and can also result from damaged end caps.
5. **Poor precision**. This can be identified in cases where replicate measurements are made (e.g. diffusion tubes exposed in triplicate, as in some co-location studies and in the Field Intercomparison operated on behalf of Defra as part of the centralised QA/QC activities). The coefficient of variation of triplicate diffusion tube measurements at the same site and over exactly the same period, would normally be expected to be within 10%, and certainly no more than 20%. *Occasional* cases of high CoV, and/or outlying values in triplicate measurements, are to be expected, and may sometimes be due to site-related factors outside the analyst's control. However, if the CoV of triplicate measurements is *consistently* greater than 10%, this indicates that the precision of the measurements is not as good as it might be, and if the CoV of triplicate measurements is frequently greater than 20%, there could be a problem. Possible causes of this include:
    - Variable extraction efficiency
    - Inconsistent coating of grids with TEA when preparing tubes
    - Problems with the analyser or its calibration

   Duplicate "spiked" samples have been incorporated into the WASP scheme as of April 2007, and these will provide an objective test of analytical precision, unaffected by site-related factors.
6. **Under-estimation of high samples.** This may result from non-linearity of the analyser response at high concentrations. It is important that the range of calibration standards used includes sufficiently high standards to cover the full range of samples likely to be encountered. It is important not to assume linearity above the calibrated range – this must be checked.

4.10.1 How to Monitor Extraction Efficiency

There is evidence from the Workplace Analysis Scheme for Proficiency (WASP) laboratory performance testing scheme (which is based on analysis of tubes doped with a known amount of nitrite) that some laboratories are not achieving complete extraction.

Participants in the WASP scheme can tell if they have a problem with this by keeping a close eye on their standardised result (that is, the ratio of the result obtained over the nominal mass of nitrite in the spiked tube). If this is consistently less than 1.0, the analytical procedures are under-estimating and a common cause of this is poor extraction.

It is therefore recommended that all laboratories carrying out diffusion tube analysis should participate in the Workplace Analysis Scheme for Proficiency (WASP Scheme) for this analysis.

4.11 Laboratory Accreditation

Laboratory accreditation, to ISO 17025, is recommended and encouraged. Also, each laboratory should fully document its own working procedures, to ensure consistency and reproducibility.

5 Data Handling and Interpretation

5.1.1 Use of Blank Tubes

Laboratory Blanks
When each batch of diffusion tubes is prepared (or purchased), a set of tubes should be reserved for use as laboratory blanks. These should be kept in sealed containers in a refrigerator, while the other tubes in the batch are distributed for exposure. The laboratory blanks are analysed with the exposed tubes, in order to provide a measure of nitrite concentration on unexposed tubes.

Current thinking is moving away from the recommendation that laboratory blank results should routinely be subtracted from exposed tube results. Rather, the main purpose of laboratory blanks is to serve as an indicator of any problems relating to the preparation of a particular batch of tubes: contamination within the laboratory, or inadequate cleaning of re-used components to remove all traces of nitrite. The laboratory blank results should be less than the limit of detection: if this is not the case, the reason should be investigated. **Laboratory blank results should not be routinely subtracted from the results of exposed tubes as a matter of course.**

Where it is deemed appropriate to subtract a laboratory blank value, the mass of nitrite on the blank tube should be extracted from the mass of nitrite on the exposed tube(s), prior to calculation of the ambient concentration as in Box 4-11.

Travel blanks
The purpose of travel (or “transport") blanks is to identify possible contamination of diffusion tubes while in transit or in storage by the end user. Travel blanks are sent out to the user with the tubes for exposure. They go everywhere the exposed tubes go, but are not themselves exposed. They are taken to the site when the tubes are put out, but returned to the end user’s refrigerator (in their sealed bag) for the duration of the exposure period. They are taken to the site again when the tubes are collected after exposure.

While in the past, travel blank results (like lab blank results) were often routinely subtracted from exposed tube results, current guidance is that these, like laboratory blanks, are better used as an indicator of contamination problems. Travel blank results, like laboratory blank results, should normally be less than the limit of detection. **Neither laboratory blank or travel blank results should be routinely subtracted from the results of exposed tubes as a matter of course.**

It is recommended that the end users should be encouraged to include travel blanks in their survey: laboratories supplying diffusion tubes should include them in quotations to potential clients.

5.1.2 When To Reject Diffusion Tube Results

Any tubes arriving from the supplier with split end caps, cracks, or other damage should not be exposed, but should be returned to the supplier. The same applies to any tubes containing large drops of liquid (a fine mist of condensation is not a problem).

After exposure, the following factors may cause a diffusion tube result to be unreliable, and should be recorded on the exposure sheet:

- Tube found on the ground at the end of the exposure period (reject, as it’s not possible to know when it fell from its fixing);
- Insects or spiders (and occasionally slugs) inside the tube.
- Dirt or other foreign object in the tube; or
- Bonfires etc. near the site, or unusual traffic activity.

Sometimes, a diffusion tube result may be much higher or lower than usual results from the site. The first step should be to check with the analyst, to ensure that the result has been correctly calculated and reported. Have details such as the exposure period been correctly reported?

Having ruled out calculation or reporting errors, it will be necessary to decide whether the value should be rejected. Some general guidelines are as follows:

- Low concentrations (3 μg m^{-3} or less) are rare at urban background or roadside sites in built up areas. If such a low concentration is measured at an urban site, where measured NO_2 concentrations are usually much higher, it is unlikely to be genuine, and more likely due to a faulty diffusion tube. (Of course, this does not apply at rural sites, where such low concentrations may well be typical).
- High concentrations: unless there is a reason why the result is likely to be spurious, it is best to err on the side of including high values rather than rejecting them.

In the case of insects or foreign objects in tubes, the analyst should use their judgement as to whether the tube result is likely to be invalid. Small spiders are not usually a problem unless the tube contains a large amount of web. Larger creatures, or slugs, can be difficult to remove and contaminate the tube. Deliberately inserted items (chewing gum, twigs) should usually lead to the result being treated as questionable.

5.1.3 Identifying Outlying Values From Triplets

Where groups of three or more diffusion tubes are exposed together, occasions may arise where one or more of the results differs substantially from the others, and may be considered suspect.

If four or more diffusion tubes are exposed together, it is possible to use a statistical test (such as Grubb's test or Dixon's test) to identify an outlying value. However, in the more usual case of triplicate exposures, these tests are not valid.

Instead, it is possible to use the following "common sense" approach to help identify and exclude an outlying value:

1. Calculate the coefficient of variation (CoV, sometimes also called the relative standard deviation) of all the triplets in your survey. This is the ratio of the standard deviation to the arithmetic mean, expressed as a percentage.
2. The CoV of the "suspect" triplet should be compared with those of the rest of the survey. If it is substantially higher, this may indicate a problem with one or more of the three results.
3. In some cases, there may be one result that is clearly much higher or lower than the other two, and this can confidently be rejected.

However, on other occasions there may be considerable "spread" in the three results, but with no clear outlier. From experience with Palmes type NO_2 diffusion tubes, a CoV of around 10% or less would be expected. A CoV of more than 20% would indicate that the precision of the triplet of results is relatively poor. In such cases, where it is not possible to identify any one value as an outlier, a judgement must be made on whether to accept or reject all three results.

5.1.4 Application Of Bias Adjustment Factors

Having calculated the bias adjustment factor (see LAQM.TG(03)[15]), this should be applied to the annual mean concentration.

Some points to remember when using bias adjustment factors are as follows: -

- Apply a bias adjustment factor to the *annual mean* NO_2 concentration only, not to individual monthly results. Performance of diffusion tubes can vary from month to month depending on meteorological and other factors, so it is not valid to adjust monthly values in this way.
- The performance of diffusion tubes can vary over time, so it is not valid to apply a bias adjustment factor obtained from a recent study to previous years' data.
- When using a bias adjustment factor in Review and Assessment reports, always report the unadjusted annual means as well. In the accompanying text, give details of how the bias adjustment factor was obtained.

Box 5-1 Choice of Bias Adjustment Factors

One frequently asked question is whether it is better to use a locally-derived bias adjustment factor, or one based on results from several studies at different sites. The answer to this question depends on several factors, and this issue is dealt with as an FAQ on the Review and Assessment Helpdesk site, at http://www.uwe.ac.uk/aqm/review/ . The following text is taken directly from the FAQ on this web site, with the permission of Defra:

"The most important factors to be considered when deciding which bias adjustment factor to use are the following:

- *Tube exposure time (1 week, 2 weeks, 1 month)*
- *Length of the monitoring study*
- *QA/QC of the chemiluminescence analyser*
- *QA/QC of diffusion tubes*
- *Siting of the co-location study*
- *Siting of other tubes in the survey*

Local Authorities using diffusion tubes as part of their Review and Assessment are advised to report both the adjustment factor from their local study, and the "national" bias adjustment factor. However, the decision of which to use will depend upon a number of factors that will need to be considered. At the end of the day it will be up to each Local Authority to take account of these factors and set out the reasons for the choice made. Specific factors that should be addressed are:

Cases Where the Locally Obtained Bias Adjustment Factor May be More Representative:

- *Where the diffusion tube exposure periods are weekly or fortnightly (or anything other than monthly – the national database of co-location results only covers monthly exposure.)*
- *If the co-location site is unusual in some way: for example, affected by specific large NO_x sources other than road traffic, such as local industrial processes. (This is a strong indication in favour of using a locally-derived factor).*
- *For tubes exposed in a similar setting to the co-location site (open/sheltered, height…)*
- *Where the duration of the whole diffusion tube study is less than one year, especially if it is less than 9 months (when adjustment is best made for a matched time period, rather than using an annual factor).*
- *Where the Review and Assessment Helpdesk spreadsheet contains data from few (i.e. less than five) other studies using the same laboratory and preparation technique – although the local result can be added to the national values to derive a new national value (see below).*
- *Where the co-location study is spread across more than one calendar year, e.g. October 2003 to September 2004 – especially where there is evidence of different national adjustment factors for different calendar years.*
- *For co-location sites with good precision for the diffusion tubes and with high quality chemiluminescence results, i.e. to national AURN standards.*

Cases Where the Combined Bias Adjustment Factor May be More Representative:

- *Where the survey consists of tubes exposed over a range of settings, which differ from the co-location site, e.g. the co-location site is in a very exposed setting and the tubes being assessed are on a building façade in a canyon-like street.*
- *Where the co-location study is for less than 9 months, although the diffusion tube monitoring is for a longer period.*
- *Where the automatic analyser has been operated using local, rather than national, QA/QC procedures.*
- *Where data capture from the automatic analyser is less than 90%, or there have been problems with data quality*
- *For co-location sites with poor precision. "*

It has been reported that diffusion tube performance varies between roadside and urban background locations, with diffusion tubes at roadside locations under-estimating compared to those at urban background locations[18]. There are theoretical reasons why this might be the case, relating to the photochemistry of oxides of nitrogen and ozone in the presence of sunlight. While this effect is not believed to be large, nevertheless it would be a wise precaution to take this into account in the choice

of site for a co-location study, i.e. if the majority of monitoring sites in a given survey are (for example) roadside, it would be preferable to use a roadside site for the co-location study, if available.

It is not necessary for every Local Authority to do its own co-location study, and it is recognised that it would not be feasible for every LA to do this. Instead, results from studies carried out by other Local Authorities, or the tube supplier/analyst, can be used. A database of bias adjustment factors is available on the Review and Assessment Helpdesk website at http://www.uwe.ac.uk/aqm/review. As explained in Box 5-1, there are some cases when it is advisable to use a locally-derived bias adjustment factor alone, and others when it is more appropriate to use a combined bias adjustment factor which takes account of similar studies carried out elsewhere in the country.

Before using the results of a co-location study carried out by another organisation, it is important to ensure that:–

- the tubes are identical, being prepared and analysed by the same laboratory, using the same materials and techniques;
- the exposure period is the same as in your own survey; and
- the duration of the co-location study is at least 9 months.

For Local Authorities with their own co-location studies, a spreadsheet is available on the "LAQM tools" section of the Air Quality Archive at http://www.airquality.co.uk/archive/laqm/laqm.php, which can be used to calculate the precision and accuracy of co-located diffusion tubes. The spreadsheet provides a bias adjustment factor (with a 95% confidence interval as an estimate of its uncertainty).

Finally, although the chemiluminescence analyser is defined as the reference technique for NO_2, it should be remembered that it, too, has a certain amount of uncertainty associated with the results: typically around 10-15% for the annual mean (although at present, Local Authorities are not required to take this into account in Review and Assessment).

6 Summary Checklist

The following checklist summarises the stages covered by this Practical Guidance, which should be followed when preparing, using and analysing diffusion tubes:

- ✓ **All glassware, reagents and water to meet the specification stated**
- ✓ **Balances and pipettes to be calibrated, and checked regularly as specified.**
- ✓ **Diffusion tubes to be prepared using one of the two harmonised methods (50% TEA / 50% acetone, grids dipped in solution *or* 20% TEA /80% water, 50 µL of solution pipetted onto grid).**
- ✓ **Follow the instructions on diffusion tube storage, handling and use (and ensuring these are communicated to end-users).**
- ✓ **Purchase or prepare the standard nitrite solution as specified.**
- ✓ **Prepare the reagent solutions as specified.**
- ✓ **Carry out a full calibration monthly, comprising four to six points including zero, and covering the full range over which samples are likely to be encountered.**
- ✓ **Carry out a linearity check (comprising at least 3 points and again covering the full range likely to be encountered) at the start of each day on which analysis is to be done.**
- ✓ **Follow an appropriate analysis schedule.**
- ✓ **Ensure mass of nitrite and ambient concentration are correctly calculated.**
- ✓ **Ensure that working procedures are fully documented. Laboratory accreditation is encouraged.**
- ✓ **Correct use of laboratory and transport blanks.**

Finally, it is not the intention of the Working Group to stifle innovation. If, now or in the future, you would like to use a variation to the above method, which you consider represents an improvement, please contact the authors to discuss the implications.

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Appendices

Appendix 1

How Diffusion Tubes Work

Diffusion tubes are a type of passive sampler; that is, they absorb the pollutant to be monitored directly from the surrounding air and need no power supply. Passive samplers are easy to use and relatively inexpensive, so they can be deployed in large numbers over a wide area, giving good spatial coverage. This has made them a popular choice for Local Authorities, who often use diffusive samplers to complement more expensive automatic monitoring techniques, or at locations where it would not be feasible to install an automatic monitor.

It should be noted that diffusion tubes have two limitations. Firstly, they are an *indicative* monitoring technique. Whilst ideal for screening surveys, or for identifying locations where NO_2 concentrations are highest, they do not provide the same level of accuracy as automatic monitoring techniques. Secondly, as the exposure period is typically several weeks, the results cannot be compared with air quality standards and objectives based on shorter averaging periods such as hourly means.

The development and use of passive samplers originated in the field of occupational exposure monitoring. However, diffusion sampling techniques have been further developed and tested, and now are widely used for ambient air quality monitoring, where concentrations are generally much lower. NO_2 diffusion samplers are designed either as a badge, or tube configuration. In the UK, most Local Authorities use Palmes-type diffusion tubes. These consist of a small plastic tube, approximately 7 cm long, as shown in Figure 3-1. During sampling, one end is open and the other closed. The closed end contains an absorbent for the gaseous species to be monitored, in this case NO_2. The exposed tubes are analysed using a colorimetric or spectrophotometric technique, or alternatively ion chromatography.

Diffusion tube samplers operate on the principle of molecular diffusion, with molecules of a gas diffusing from a region of high concentration (open end of the sampler) to a region of low concentration (absorbent end of the sampler).

The movement of molecules of gas (1) through gas (2) is described by Fick's law, which states that the flux is proportional to the concentration gradient:

$$J = - D_{12} \frac{dC}{dz}$$

where:

J	=	the flux of gas (1) through gas (2) across unit area in the z direction;
C	=	the concentration of gas (1) in gas (2);
z	=	the diffusion path; and
D_{12}	=	the constant of proportionality, i.e. the molecular diffusion coefficient of gas (1) in gas (2), with dimensions of length^2 time^{-1}.

For a tube of area a and length l then Q , the quantity of gas transferred along the tube in time t, is given by:

$$Q = \frac{D_{12} \, (C_1 - C_0) \, a \, t}{l}$$

where: C_0 and C_1 are the gas concentrations at the absorbent end and open end of the tube respectively.

In a diffusion tube, the concentration of gas (1) is maintained at zero (by an efficient absorbent) at the closed end of the tube (i.e. C_0 = zero) and the concentration C_1 is the average concentration of the gas (1) at the open end of the tube over the period of exposure. Hence:

$$C = \frac{Q\,l}{D_{12}\,a\,t}$$

For the gas monitored, the diffusion coefficient must be determined, or obtained from the literature. The area and length of the tube are determined by measurement.

The above expression can also be expressed in terms of the mass of nitrite collected on the tube – which is determined by spectrophotometric analysis as described above:

$$C = \frac{ml}{D_{12}\,at}$$

where:

m	=	the quantity of the gas absorbed over the period of exposure;
a	=	the cross sectional area of the tube;
t	=	the time of exposure; and
l	=	the length of the tube
D_{12}	=	diffusion coefficient of gas 1 through gas 2 – in this case NO_2 through air.

In practice, the value

$$\frac{D_{12}\,a}{l}$$

- which depends on the tube dimensions and the value of D_{12}, is treated as constant and termed "uptake rate" or "sampling rate", and the concentration of NO_2 can be calculated as:

$$C = \frac{1}{\text{"}s.rate\text{"}} x \frac{m}{t}$$

The area and length of the tube are determined by measurement. Typical tube dimensions are length = 7.1cm (0.071m), cross-sectional area = 9.3 x 10^{-5} m^2, although these vary and it is important that they are checked.

The sampling rate also depends on the diffusion coefficient D. Recent research, reported by Massman in 1998[18], suggests that the best estimate of D is 0.1361 $cm^2\ s^{-1}$ at STP, i.e. 0 Celsius (273 K) and 1 atmosphere (101.3 kPa**). However, D is temperature-dependent**, and for this purpose should be corrected to typical mean UK ambient temperatures. These are in the range 10-12 Celsius or 283-285 K. Using the temperature dependence relationship used by Massman[18] in which D is proportional to temperature to the power 1.81, and assuming a mean UK temperature of 11 Celsius or 284 K:

$$D_{284K} = D_{273K} \times (284/273)^{1.81} = 0.136\ cm^2\ s^{-1} \times (284/273)^{1.81} = 0.146\ cm^2\ s^{-1}$$

For tubes exposed in the UK, the sampling rate should be calculated assuming D = 0.146 $cm^2\ s^{-1}$. *(Note: it is not necessary to correct D for different temperatures each month: the UK mean should suffice in this context of indicative monitoring.)*

In addition, for comparison with EU Limit Values or AQS Objectives and reporting in Review and Assessment, the measured concentrations must be reported in mass units ($\mu g\ m^{-3}$) at a temperature of 293 K (20 Celsius) and pressure of 101.3 kPa. Therefore, a temperature correction should be applied to the measured concentration, as follows:

$$C_{293K} = C_{284K} \times (284/293)$$

- where C_{284K} is the measured concentration at assumed ambient temperature 284 K, and C_{293K} is the measured concentration corrected to 293 K.

This has the effect of reducing the reported concentration by about 3%.

The correction to 293 K can be incorporated into the calculation of ambient concentration:

$$C_{293} = \left(\frac{284}{293}\right) \times \frac{m}{\text{"sampling rate"} \times t} = 0.969 \times \frac{m}{\text{"sampling rate"} \times t}$$

where:

C	=	the concentration of NO_2 in the atmosphere ($\mu g\ m^{-3}$);
m	=	mass of nitrite in tube (μg);
t	=	the time of exposure (h)

For the purposes of Review and Assessment, measured concentrations should be corrected to 293 K as above. When reporting the results, the analyst must make clear that this temperature correction has been incorporated, to avoid the possibility of the end-user correcting again.

Appendix 2

Factors Affecting Diffusion Tube Performance in the Field

NO_2 diffusion tubes are an *indicative* monitoring technique: although ideal for screening studies and for identifying areas of high concentration, they do not offer the same precision and accuracy as the automatic chemiluminescence analyser (which is defined by the European Union as the reference method of measurement for this pollutant). In particular, NO_2 diffusion tubes are affected by several mechanisms, which may cause them to exhibit positive bias (over-read), or negative bias (under-read) relative to the reference technique.

Over-read may be attributed to the individual and combined effect of three interfering factors:

- the shortening of the diffusive path length, by turbulence at the open end of the tube caused by wind[A1,A2]
- blocking of UV light by the tube material, resulting in reduced NO_2 photolysis in the tube[A3]; or
- the interfering effects of peroxyacetyl nitrate (PAN)[A1], a pollutant associated with vehicle emissions.

Some factors known to cause under-read are as follows:

- Increasing exposure period. It has been reported that the average of four consecutive one-week, or two consecutive two-week exposures is systematically greater than one four-week exposure[A3,A4,]. This is considered to be due to the degradation of the absorbed nitrite over time[A4].
- Insufficient extraction of nitrite from the grids.
- The photochemical degradation of the triethanolamine-nitrite complex by light. This has been largely minimised, by the widespread use of opaque diffusive end caps.
- In the specific case of tubes prepared using a 50% v/v solution of TEA in water, it has been reported that there may be a mechanism reducing NO_2 uptake, resulting in negative bias[A5]. Such tubes are no longer widely used in the Network. Tubes prepared using other methods (10% or 20% v/v solution of TEA in water, 50% solution of TEA in acetone) appear not to be affected.

Of these factors, those causing positive bias are usually the most difficult to eliminate, and positive bias is more common than negative (although the latter is certainly not rare).

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Appendix 3

Test titration for standard nitrite solution (optional)

1. Put approximately 150 mL of 0.25 M sulphuric acid into a 500 mL conical flask.
2. Add 10.0 mL of 0.020 M potassium permanganate solution.
3. Pipette 20.0 mL of the stock solution into the conical flask
4. Allow the flask to stand for 10 – 15 minutes in a dark place e.g. cupboard.
5. Remove the flask from the dark place; add 3 $\pm$ 0.5 g potassium iodide.
6. Add a few drops of starch indicator.
7. Titrate with 0.010 M sodium thiosulphate ($Na_2S_2O_3$) solution until the dark colour just disappears. Record the volume of titrant in mL.
8. Repeat steps 2-7 above for another 4 aliquots of the stock standard.
9. Calculate the concentration of the solution being tested, **in g/L,** using the following equation –

Concentration NO_2^- = ($2x10^{-4}$ – (titre x $2x10^{-6}$)) x 5750

Calculate the mean of the 5 tests, the standard deviation and the coefficient of variation.

An alternative method is described by Vogel *et al*: this involves reaction of the nitrite with cerium (IV) sulphate, then titrating the excess Ce (IV) with a standard iron (ii) solution using ferroin as an indicator. See (reference)

Appendix 4

Factors Affecting Colour Formation Reaction

Effect of pH

Laboratory investigations carried by AEA Energy & Environment and Oxford Brookes University (Targa *et al*, 2007) carried out for this Working Group investigated the pH dependency of the colour formation reaction. The diazo coupling reaction (which leads to the formation of the purple coloration) is known to be sensitive to pH, and was previously thought to be decreased at pH > 2. The current study, on the basis of recent data and that from a study in 1971, *(Truesdale, V - Investigation of pH dependency of the colour formation during diazo coupling reaction. Oxford Brookes University. Unpublished investigation, 1971)* indicates that in fact the reaction *begins* to decrease at pH 2.0. To ensure the pH is well within the correct range, pH should be kept below 1.7-2.0. In practice, the quantity of acid added by most labs is sufficient to achieve this: pH of the reagent solution does not appear to be a problem.

Effect of presence of TEA

TEA is basic (pH ~ 9), and it has been postulated that systematic errors could occur due to the presence of TEA in samples, if it is not also included in standards. Laboratory investigations carried out for this Working Group tested this effect: varying amounts of a 50% TEA/water solution were added to a 20 mL volume of 3% orthophosphoric acid (H_3PO_4) solution. (The purpose of adding orthophosphoric acid to the sulphanilamide solution is to keep the pH low). This study concluded that the optimum v/v ratio of TEA / H_3PO_4 should be 1.0, to ensure the pH remains below 2.0. The quantity of acid specified in this Guidance is sufficient to achieve this. Consequently, there is no requirement to add TEA to standards.