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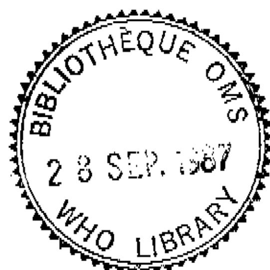

SELF-INSTRUCTION SHEETS FOR USERS OF LABORATORY INSTRUMENTS IN INTERMEDIATE HOSPITAL LABORATORIES

by

Mr P.M.G. Broughton
Deputy Director
WHO Collaborating Centre for Research and Reference Services
Wolfson Research Laboratories
Queen Elizabeth Medical Centre
Edgbaston, Birmingham, UK,

Dr S. Gharpure
Consultant Clinical Pathologist
Matunga, Bombay, India,

and

Dr D.A. Vazquez R. Olazabal
Scientist
Health Laboratory Technology
WHO, Geneva, Switzerland

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INTRODUCTION

This document is part of a series of documents prepared by the Health Laboratory Technology Unit on Preventive Maintenance and Repair of Basic Laboratory Equipment. This particular issue concerns the maintenance and repair of flame emission photometers and colorimeters. It is addressed to laboratory technicians working at intermediate hospital laboratories. In most countries, these laboratories are attached to a hospital between primary health care and central levels. They may have between 100 to 300 beds and serve a population of 100 000 to 300 000 inhabitants. The equipment includes routine instruments, two of which are flame emission spectrometers (flame photometers) and colorimeters (spectrophotometers).

I. FLAME EMISSION SPECTROMETERS (FLAME PHOTOMETERS)

Flame emission spectrometers are used in clinical laboratories to estimate concentrations of sodium, potassium and lithium ions in serum or plasma and other biological fluids.

In flame spectrometry, the light emitted by the ions that are excited in a flame is measured. In dilute solution the concentration of the ion is proportional to the intensity of light emission. Sodium produces a yellow flame with an emission wavelength of 589 nm, potassium a violet flame with an emission wavelength of 766 nm, and lithium has an emission wavelength of 671 nm.

It should be noted that some instruments which are suitable for sodium and potassium estimation may not be sufficiently sensitive to measure the small serum lithium concentrations present in patients treated with this drug. The manufacturer's specification should therefore be studied carefully to see whether the instrument is capable of measuring lithium by the method provided.[1]

Although the principles of all flame spectrometers are the same, details of their construction may vary. We shall here consider only one type.

1. Basic parts

- Atomizer sometimes called the nebuliser
- Burner
- Filters (separate ones for sodium, potassium and lithium)
- Photocell or other type of detector
- Meter or other device for readout

NOTE: Every specimen from a patient is potentially infectious and great care must therefore be taken with all specimens in order to minimize the risk from hepatitis, AIDS, etc.

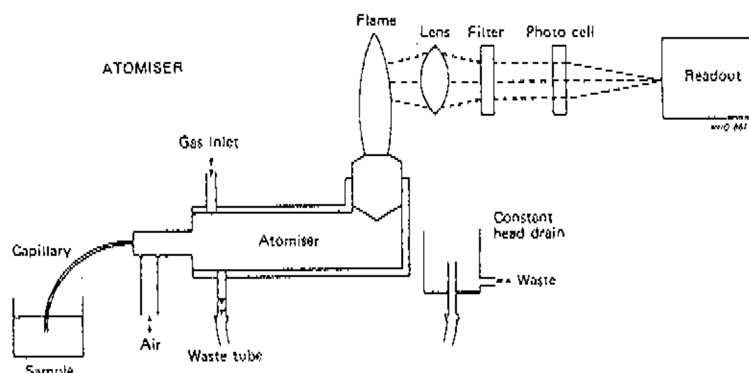

Fig. 1
SCHEMATIC LAYOUT OF A FLAME EMISSION SPECTROPHOTOMETER

[1] Varley et al. Practical Clinical Biochemistry. 5th ed. London : William Heinemann Medical Books Ltd, 1976, p. 330 (Vol. 2).

2. Other facilities needed

1. Air compressor - usually supplied by the instrument manufacturer. This has a gauge to regulate the air to a constant pressure around 12 lbs/sq inch (0,843683 kg/cm^2).

2. Source of stable electric power for the flame spectrometer and for the compressor.

3. Gas - city gas, butane or propane as specified by the manufacturer. For sodium and potassium estimation a low intensity flame is needed at about 1500 to 2000 °C. Different gases with a higher temperature flame may be needed for lithium. In this case the manufacturer's recommendation should be followed.

4. Good quality distilled water - used as a blank, in the preparation of standards and for diluting samples. When sprayed in the flame spectrometer it should give a colourless flame and almost no deflection on the galvanometer.

5. Volumetric flasks (50 ml, 100 ml and 1000 ml capacity) for dilution of samples and preparation of standards.

6. Volumetric pipettes (0,5 ml, 1 ml, 5 ml and 10 ml) for dilution of samples and standards.

3. Installation of flame emission spectrometer

1. Check the packing case for signs of damage. If any damage is suspected inform the transporter/insurance agent/supplier before unpacking.

2. If the packing case shows no sign of damage, open it.

3. Check for any signs of damage. If the instrument is damaged or any parts missing, inform transporter/insurance agent/supplier.

4. Look for the instruction manual, which should be in the packing case.

5. Read the instruction manual and follow it exactly.

6. If the instruction manual is missing, demand a copy from supplier.

7. Remove the flame spectrometer from its packing and wipe it clean with a cloth. Assemble the various parts carefully.

8. Keep the instrument away from any heat source, which may damage it.

9. Install the instrument on a vibration-free bench away from direct sunlight. External light falling on photocell will vitiate the readings. Place the air compressor on the floor and not on the same bench as the flame spectrometer. Connect it to a stable source of electrical power.

10. The instrument should be housed in a dustproof room with no draughts, which may disturb the flame.

11. Check the operating voltage of the flame spectrometer and the air compressor as given on either the panel or the label on the instrument. Check the power supply voltage. The two must be the same.

12. Connect the flame spectrometer and the air compressor to the mains line.

13. Blow through all the external tubings with either air or water.

14. Connect the necessary length of flexible tubing to the drain, ensuring that it leads progressively downwards to the disposal point.

15. Connect the compressor to the atomizer by a thick walled tube.

16. Connect the source of gas supply by a thick rubber tubing to the atomizer. Check the tubing for any leakage. There is a DANGER OF EXPLOSION if there is a leakage.

17. Some instruments have a locking device for the meter, to protect it during shipment or when not in use. Unlock this device.

18. Never use the flame spectrometer without a filter in place.

19. Check the performance of the instrument, especially the flame source and the photocell, by keeping a log book recording the galvanometer readings of standards. Any deviation from the average past readings suggests malfunction of the instrument.

4. To start the instrument

1. Insert the appropriate optical filter.

2. After checking that the gas cylinder (if any) is adequately full, turn the gas supply on and light the flame.

3. Turn on the air supply and adjust the air control to give correct air pressure reading on the gauge.

4. Aspirate distilled water either directly from a cup or through a plastic capillary attached to the atomizer.

5. Adjust the gas control to produce the desired shape and size of flame.

6. Set the meter to zero against the blank solution.

7. Dilute samples and standards. Stopper the flasks and mix thoroughly. Do not allow solutions to come in contact with skin, as human sweat will contaminate solutions and cause high sodium readings.

8. Set the highest standard at a suitable reading (e.g. 100) using the sensitivity control to adjust the meter reading. Follow the instructions. Check, and if necessary, adjust this reading.

9. Take readings of standards and samples. It is advisable to recheck the standards regularly during a run of samples.

10. Plot a calibration curve. Any change in this (e.g. from linear to a curve) is an indication of malfunction.

5. To switch the instrument off

1. Aspirate distilled water for several minutes to flush the instrument.

2. Turn off the gas.

3. Wait for extinction of the flame.

4. Turn off the air.

5. Switch off the instrument.

6. Wait for the instrument to cool. Cover it with a plastic cover to keep off the dust.

6. Daily preventive maintenance

1. WARNING: Turn off and disconnect the mains line cord when making internal adjustments, replacements or repairs in order to prevent injury to yourself or damage to the equipment.

2. Aspirate distilled water preferably with added nonionic detergent for a couple of minutes after each day's run. Some manufacturers advocate injecting distilled water with a needle and syringing through the capillary attached to the atomizer.

3. Clean any spilled material from the instrument and wipe the surface dry with a cloth. Rinse all flasks and pipettes with distilled water after use.

7. Weekly preventive maintenance

1. Remove the atomizer from the instrument and flush water through it.

2. Clean the capillary with a thin wire to remove small clots and/or dried serum, and aspirate water again.

3. Without removing them, clean lenses and any mirrors with a soft cloth or soft brush to remove dust and carbon particles.

8. Periodic preventive maintenance

1. Clean the surface of the photocell with a soft cloth.

2. Remove the combustion chamber according to the instructions given in the manual. Clean the chimney and the burner with water.

9. Trouble shooting - diagnosis and remedial measures

	Symptom	Diagnosis	Remedy
1.	Blank reads high and shows coloured flame	Contamination	Change distilled water. Aspirate distilled water until flame is colourless.
2.	Varying reading	Excessive vibration	Provide a shock proof base.
		Air supply unit	The tube connecting the compressor may be blocked by overlying objects. Remove them.
		Blocked atomizer	Clean it with a jet of water.
		Low gas pressure	The cylinder may be almost empty. Change it.
		Contaminated burner	Remove, wash and dry the burner.
		Photocell fatigue	Turn the instrument off. If the problem continues change the photocell.
3.	No meter light or deflection	Faulty connection to mains power	Check line cord, fuse and plugs. Replace if necessary.
4.	No deflection or reading	Blocked atomizer	Clean atomizer with jet of water.
		Poor connection to photocell or meter	Check connections.
		Faulty photocell	Change the photocell.
		(1) Compressor not working (2) No air supply	Check air line and compressor. Tube connecting the compressor to the instrument may be blocked; remove the blockage.

	Symptom	Diagnosis	Remedy
5.	Samples read uniformly low	The concentration of standard solution is too high (e.g. evaporation)	Prepare fresh standards as described in the method.
		Sample constituent concentration is too low	Properly dilute the sample.
6.	The flame shows separate small cones and cannot be adjusted to produce large cones	Low gas pressure	Check gas pressure and change the the cylinder if necessary.
7.	Samples and/or standards read high	Contamination	Check distilled water. Change the standards. Redilute samples.
8.	The flame shows no large single cone, but very small separate cones	Blocked atomizer	Clear the blockage with fresh distilled water.

For major repairs to the instrument contact the manufacturer or his representative. Always follow the manufacturer's instructions and use his recommended spares.

10. Recommended spare parts

1. Bulbs
2. Fuses
3. Atomizer
4. Photocell
5. Capillaries
6. Capillary cleaning wire
7. Gas cylinder

II. COLORIMETERS (SPECTROPHOTOMETERS)

1. Colorimetry

Colorimeters are used for measuring the concentration of analytes in coloured solution. Sometimes the analyte itself is coloured (e.g. haemoglobin) but more often it is reacted with reagent(s) to produce a coloured solution. They can also be used to measure the turbidity of particulate matter suspended in solution as a measure of concentration.

The components, method of use, sources of error and maintenance of these instruments vary widely, depending on their complexity. We shall consider two types of instrument:

(a) Simple colorimeters used for determining concentration in the visible region of the spectrum.

(b) Spectrophotometers provided with a source of monochromatic light and usable in both the visible and near ultra-violet region of the spectrum (down to about 340 nm). These instruments can be used to determine concentration, to measure absorbance at specified wavelengths, and to record the absorption spectrum.

In this document we deal with colorimeters. Most of the problems which arise with these instruments also apply to spectrophotometers, but in addition, these require special and more detailed checking procedures which are not relevant for colorimeters. These additional checks will be described in a subsequent document.

2. Principles of colorimetry

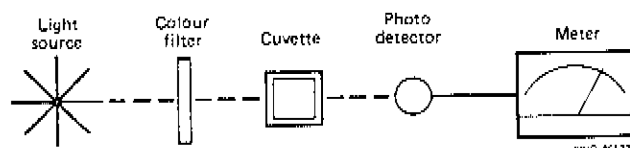

Fig. 2
SCHEMATIC LAYOUT OF A COLORIMETER

2.1 Components of a colorimeter

The main features of a typical instrument are illustrated in Fig. 2. The light source is usually a tungsten filament lamp which, when provided with a constant voltage, emits white light of constant intensity. The colour filter is used to select light of a colour which is absorbed by the solution. This colour is expressed in terms of the wavelength (in nanometers, nm) of the light, and for the visible spectrum extends from about 400 nm (violet) to 700 nm (red). The filter or wavelength used must be carefully selected so that the colour of the light it transmits is maximally absorbed by the solution. Thus a blue filter (transmitting light at 400-450 nm) is best for a red coloured solution which absorbs blue light. The efficiency of a filter depends on the band width of the wavelengths it transmits. Commonly used glass-gelatin filters (Fig. 3) have a wider wave-band (up to 50 nm) than interference types (about 10 nm).

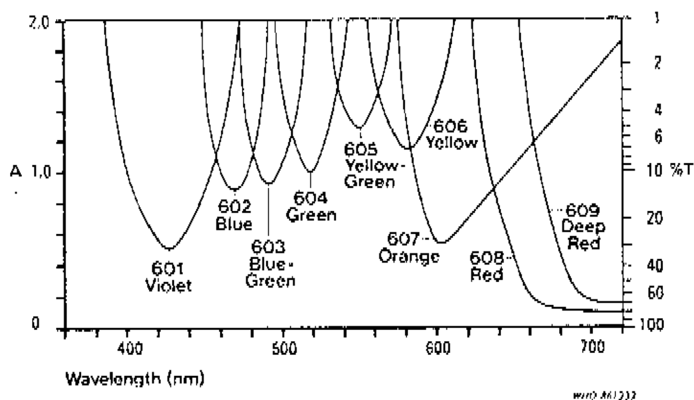

Fig. 3
TRANSMISSION CURVES OF SOME GLASS-GELATIN FILTERS
THE NUMBERS REFER TO THE MANUFACTURER'S (Ilford) CODE NUMBERS

The solution is contained in a cuvette (sometimes called a cell), usually made of glass or plastic, which may be either cylindrical or rectangular in shape. The internal width or diameter of the cuvette (known as the pathlength) is usually 1 cm.

Light which passes through the cuvette falls on a photodectector or photocell, where it generates a photoelectric current. This may be amplified before it is recorded on a readout device or meter. The reading of this meter is usually controlled by an adjustable potentiometer, sometimes referred to as the sensitivity control.

In addition to these components, lenses, mirrors, slits and a shutter are usually incorporated in the light path.

2.2 Relationship of light absorption to concentration

The photocell records the intensity of light transmitted by the solution. This is usually expressed as the transmittance (T) which is the ratio of the intensity of the transmitted light to that of the incident light falling on the cuvette. Thus at zero concentration T is 100% (because no light is absorbed) and at infinite concentration (when no light is transmitted) T will be 0%. According to the Beer Lambert law, concentration is logarithmically related to T, but it is more usual to express this law in the form:

$A = Kcl$ where

A is the absorbance (a term preferable to optical density or extinction) = $\log \frac{100}{\% T} = 2 - \log\% T$

K is a constant

c is the concentration

l is the pathlength of the cuvette

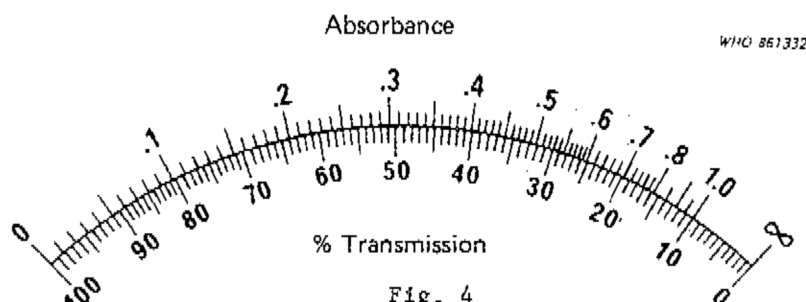

Fig. 4
A METER SHOWING BOTH ABSORBANCE AND % TRANSMISSION SCALES

Most meters are calibrated in absorbance units, usually on a logarithmic scale, with values ranging from zero (where T = 100%) to about 2 units, with a further scale mark for a value of infinity (where T = 0) as shown in Fig. 4. In these cases the absorbance reading should be directly proportional to concentration, and calibration will be linear. Sometimes the meter is also calibrated in % T (usually with linear gradations from 0-100), and if this scale is used the calibration curve will be logarithmic. It is usually best to ignore the transmittance scale and use only absorbance readings.

2.3 Deviation from Beer Lambert law

With colorimetry the analytical procedure is greatly simplified if the Beer Lambert law is obeyed, i.e. a graph of concentration against absorbance is linear. However, the calibration curve MUST NEVER be assumed to be linear as it may be influenced by several factors:

(a) Calibration is more likely to be linear with monochromatic light (i.e. light of a single wavelength). Linearity tends to decrease as the band width of light increases.

(b) The response of the photocell may not be linear at all wavelengths.

(c) Some coloured solutions may give a linear calibration on one instrument but not on another. Linearity and other aspects of performance must therefore be judged for each instrument in the circumstances in which it is used.

3. Installation

3.1 Unpack the instrument carefully and assemble it according to the manufacturer's instructions. Make sure that the operating manual is supplied.

3.2 Set the instrument up on a level and stable bench where it will be free from vibration and not in direct sunlight. The laboratory environment should be free of dust, as well as fumes and smoke (including tobacco smoke).

3.3 Always handle optical components, such as the lamp, filters, cuvettes, lenses, etc, by the base or sides, so that fingerprints or other marks are not made on optical surfaces.

3.4 Check that filters are clean and not cracked or marked. They should be identified by a manufacturer's number or by the wavelength of maximum transmission. Store spare filters in a dust-free container and ensure that they cannot be broken or scratched.

3.5 Clean cuvettes by soaking for a few hours in a mild detergent or dilute solution of wetting agent. Excessive concentrations should be avoided as these may cause a film to be deposited on the cell surface. If necessary clean the inside surfaces of the cuvette with a swab of cotton wool. Rinse with distilled water and invert to dry. Store cuvettes in a dust-free container and ensure that they cannot be scratched or broken by contact.

3.6 The cuvette holder (or cell carriage) may either accomodate one cuvette only, or it may have spaces for 2-4 cuvettes, which can be positioned, in turn, in the light path by sliding the holder. In this case, ensure that the holder slides smoothly and locates positively and in a reproducible position.

3.7 If a flow through cuvette is provided it must be located in the cuvette holder in a fixed immovable position and connected to a suitable source of suction and/or drainage.

3.8 Ensure that all lenses, mirrors and other optical components are free of dust and finger prints. If necessary, clean with tissue or a soft cloth. If any optical unit is sealed, DO NOT dismantle it to clean it.

3.9 Connect the instrument to a suitable stabilized electric power supply, taking great CARE to make sure that the colour-coded wires are connected by a suitable plug to the correct mains terminals. DO NOT switch on the instrument without first ensuring that there is a filter in position: failure to observe this may result in damage to the photocell and meter. DO NOT leave the instrument on with the photocell exposed to light for longer than necessary. As a precaution it is advisable to block the light path when the instrument is not in use, e.g. by closing the shutter or inserting the cuvette cover in the holder in place of a cuvette.

3.10 The optical alignment of the system should now be checked to ensure that the maximum amount of light reaches the photocell after passing through the cuvette. Reflection from internal surfaces produces unwanted stray light which bypasses the cuvette and causes photometric errors.

With a water-filled cuvette and a suitable filter in position set the meter to a mid-scale reading (roughly 0,3 absorbance or 50% T). Now make minor movements or manipulations of each optical component in turn (lens, mirrors, filters, cuvette, etc.) to see whether those affect the reading. If necessary make small adjustments so that the maximum transmission (i.e. minimum absorbance reading) is obtained.

The lamp positioning is probably the most important factor. Usually the lamp is prefocused, but minor adjustments in both horizontal and vertical planes may be possible. To make these adjustments easier, it is possible with some instruments to place a white card immediately in front of the photocell, if necessary after first removing the cuvette holder. A clear image of the lamp filament can usually be seen on the card. If this image is distorted or not vertical, minor adjustments should be made until the best image is obtained.

When a flowthrough cuvette is used its position may require careful adjustment. Once this has been done, it is best to leave the cuvette in situ and not interchange it frequently with other cuvettes.

4. Operation

4.1 Check dark current

Switch on the instrument and set the meter to read infinity on the absorbance scale (0%T) with the shutter closed or the light path blocked so that no light falls on the photocell. This setting should be checked each time the instrument is switched on.

4.2 Check stability of readings

With a water-filled cuvette in position, set the absorbance reading to a convenient reading such as 0,100. Repeat this reading at 5 minute intervals until it is steady (i.e. changes by less than 0,005 units in 5 minutes). In routine use, never take readings before this warm up period is complete and readings are stable (usually 5-30 minutes).

4.3 Use of cuvettes

After a cuvette is filled with solution, wipe the outside clean and dry, and check that it contains no bubbles or particles. Handle it by the top or sides and not by the optically-transmitting surfaces. After inserting the cuvette in its holder, check that minor movements do not affect readings.

4.4 Matching of cuvettes

Whenever a new cuvette is introduced (e.g. as a replacement for a broken one), it must be matched with the others in the set. This set usually comprises one cuvette containing water or a reagent blank and one or more others containing solutions of the samples, standards, etc. It is essential that all cuvettes in a set give the same reading when filled with the same solution. This should be checked by filling all cuvettes with a suitable clear stable coloured solution [copper sulfate, oxyhaemoglobin, potassium chromate, etc ($CuSO_4$, HbO_2, K_2CrO_4, etc.)]. Set the first cuvette at a convenient absorbance reading (e.g. 0,100). Now read each of the others in turn. Repeat all readings and note any which differ consistently by 0,005 units or more. These differences may be due to several causes:

(a) Dirt or finger marks on the cuvette. Clean the cuvette (paragraph 3.5) and repeat the test.

(b) Scratches or imperfections on the optical surface. With cylindrical-shaped cuvettes, this error can sometimes be overcome by rotating the cuvette slowly in its holder and noting the position (if any) where the readings match. This position should be marked and the cuvette always used with the mark in the correct position.

(c) Variations in pathlength. This effect is only apparent with light-absorbing solutions and not with water (see Beer Lambert law, above).

Any cuvette which after cleaning cannot be matched with others in the set should be discarded.

4.5 Solution volume required

Check the volume of solution required by adding increasing volumes to a cuvette and noting the amount necessary to produce a constant reading. In routine practice it is advisable to use more than the minimum volume, but it is not usually necessary to fill the cuvette to the top.

4.6 Carryover

Conventional cuvettes are usually rinsed with the next solution before a reading is made in order to avoid errors due to carryover. With flowthrough types, the suction arrangement may not remove all the first solution, and it is then necessary to rinse with the second solution, and flush it out, before taking a reading on a further aliquot. It is advisable to check the efficiency of the suction and rinsing process to ensure that errors due to carryover are avoided.

4.7 External illumination

With a filled cuvette in position check that the reading is not affected by variations in external illumination (e.g. daylight) from above the instrument. If this occurs, the instrument should either be moved or the external light excluded by closing the lid or blocking off the top of the cuvette holder.

4.8 Selection of filter and checking linearity

(1) If possible use the type of filter specified in the method. If this filter is not provided, select a similar one from those available. Choose one which gives the largest absorbance reading with the solutions to be measured, and which gives the most linear calibration curve (see Fig. 5).

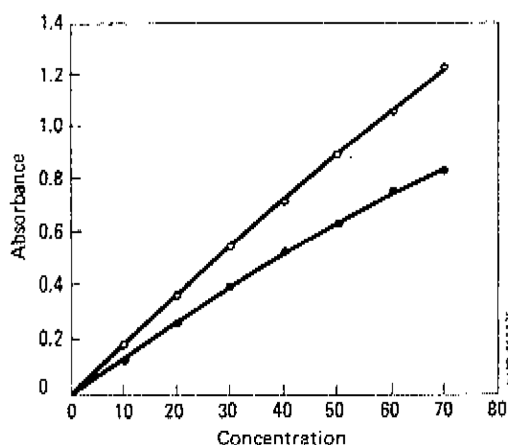

Fig. 5
CALIBRATION CURVES WITH TWO DIFFERENT FILTERS

(2) Analyse one mid-range standard solution and a reagent blank by the given method. Measure the absorbance of these two solutions against a water blank, using each filter in turn. Note the readings. Select one or two filters which give the largest difference between test and reagent blank readings, and if possible the lowest reagent blank reading.

(3) Now analyse a series of at least five standard solutions, spanning the concentration range of the given method, together with a reagent blank. With the chosen filter in position, measure the absorbance of these solutions against a water blank, and plot the readings on a graph.

If the calibration curve is linear, perform additional analyses with stronger standards in order to determine the upper limit of linearity, which ideally should not be less than an absorbance of 1,0. If the calibration curve is not a straight line, or if it curves at readings below 1,0, try another similar filter. Choose one filter which gives the most linear response over the desired concentration range.

(4) In routine practice, all readings must lie within the linear part of the calibration curve. With most instruments, the most accurate and precise readings are in the range 0,2-0,7 absorbance units. Readings of less than 0,1 or more than 1,0 absorbance units are less reliable, and critical measurements on patients should, if possible, be made within these limits. If the reading is more than 1,0, or outside the linear range of the method, the analysis should be repeated using a dilution of the sample. DO NOT take readings outside the limits of linearity of the method.

(5) Check the stability of the colour by making repeated readings of one solution for about 30 minutes after preparation. If the colour is unstable, readings must always be made within the time over which the colour intensity is stable.

(6) The linearity experiment should be repeated at regular intervals (e.g. monthly) and ALWAYS done after replacing the lamp, photocell or filter.

5. Preventive maintenance

5.1 Always follow the manufacturer's recommended maintenance schedule. Keep records of readings of standards and reagent blanks, which may be useful indications of slowly deteriorating performance. Records should also be kept of the date when any parts were replaced, or other adjustments made.

5.2 Always clean the instrument after use, paying particular attention to any spillages within or on the surface of the instrument. When the instrument is not in use, turn it off, allow it to cool and cover it to exclude dust.

5.3 Do not leave dirty cuvettes in the instrument after use. Wash them with distilled water and allow to drain before storing them in a dust-free container. If cuvettes become greasy or contaminated with protein after prolonged use, soak them overnight in a detergent solution. Do not use strong alkalis or chromic acid.

5.4 Do not allow filters to become hot by, for example, leaving them exposed to the lamp for long periods. Inspect them regularly for clarity and if necessary clean them with a soft cloth to remove dust. Always leave a filter in position so that the photocell is not damaged when the lamp is turned on. Store spare filters in a dust-free container.

5.5 Turn off the lamp after use, as it has a finite life. Do not place objects on top of the colorimeter as these may impede air flow and cause overheating.

5.6 At regular intervals, clean the other optical components on which dust may have accumulated. If any adjustments are made, check the optical alignment as described in paragraph 3.10.

5.7 The front surface or window of the photocell should be inspected periodically in case dust or chemical deposits have accumulated. Clean the surface with a soft cloth. Do not dismantle the photocell housing unless the photocell is to be replaced.

6. Repairs and replacements

6.1 As a minimum it is recommended that the laboratory should keep several spare lamps, fuses and cuvettes, and perhaps a couple of photocells. (NOTE: photocells will deteriorate if stored in hot or humid conditions, or in a contaminated atmosphere, particularly mercury.) The number of other spare parts to be kept depends on the type of instrument and their availability locally when required.

6.2 Keep a supply of spare fuses of the type specified by the manufacturer. Replace when necessary.

6.3 Lamps slowly deteriorate during use and the glass envelope darkens, resulting in loss of light output and poor performance. Replace such lamps before they fail completely, using the type supplied or recommended by the manufacturer. If this is not available, great care is needed in selecting a suitable alternative. After replacing the lamp, check its alignment (paragraph 3.10) and the linearity of the calibration curve (paragraph 4.8).

6.4 Scratched or damaged cuvettes should be replaced immediately by others of the same type from the same manufacturer. Conventional test tubes are not usually a satisfactory substitute for cylindrical cuvettes, as they are rarely optically matched. If cuvettes from another manufacturer must be used, it is best to keep these as a separate set, as different types may not be optically matched. Any new cuvette must be optically matched with others in the set (paragraph 4.4).

6.5 Replacement of the photocell is necessary only occasionally. The type recommended by the manufacturer should be used, and his instructions followed. After replacing the photocell, check the optical alignment (paragraph 3.10) and the linearity of the calibration curve (paragraph 4.8).

6.6 Replacement of the meter, sensitivity potentiometer or transformer is rarely necessary. The type recommended by the manufacturer should be used and his instructions followed.

7. Trouble shooting and remedial measures

In colorimetry errors may arise from either the instrument or the chemical method and technique used (e.g. pipetting errors, reagent deterioration, etc.). Some common instrumental faults are listed below, but when attempting to remedy any of these it must always be remembered that SIMPLE FAULTS ARE THE COMMONEST ONES. Always use your eyes, and your previous experience, before telephoning the manufacturer or reaching for a screwdriver.

7.1 No reading with water blank

Check whether the lamp is on.

(a) If not - check switches and terminals.
- check lamp positioning in socket.
- replace fuse.
- replace lamp.

(b) If the lamp is illuminated:
- check whether the meter is locked or stuck.
- check for loose photocell connections.
- meter failure is unlikely, but if everything else fails, replace it.

7.2 Drift in water blank reading

(a) Insufficient warm-up time.

(b) Lamp failing or overheating:
- Inspect it (see paragraph 6.3) and if necessary replace.

(c) Failing photocell (e.g. overheating or overexposure)
- Switch off and allow the photocell to rest. If the problem persists, replace the photocell.

7.3 Water blank reading fluctuates and cannot be set to zero

(a) Mains voltage variations
- Examine light intensity.

(b) Faulty potentiometer
- Rotate the knob slowly. If the response is erratic, the slide wire may be dirty or worn in some places but not others. Clean the slide wire carefully.
If the problem persists or recurs consider replacing the potentiomenter according to the manufacturer's instructions.

7.4 Reagent blank reads less than water blank

(a) Cuvettes incorrectly positioned:
- Try interchanging them.

(b) Dirty water blank cuvette:

- Clean it (paragraph 3.5).

(c) Check solutions for turbidity.

7.5 Daily standard readings variable

(a) Fault in method or technique.

(b) Incorrect, dirty or misaligned filter:
- Check and, if necessary, clean it.

(c) Positioning of cuvettes variable:
- Check movement of cuvette holder (paragraph 3.6).

(d) Dirty or mismatched cuvettes:
- Inspect and, if necessary, clean (paragraph 3.5).
- Check matching (paragraph 4.4).

(e) Change in linearity:
- Check. For possible causes see paragraph 7.6.

(f) If the lamp or photocell is failing, deterioration is more likely to be progressive than to vary randomly, and there should be other signs of failure (see paragraphs 7.2, 7.6). If necessary, replace.

7.6 A previously linear calibration curve becomes non-linear

(a) Fault in method, reagents or technique.

(b) Misaligned optics:

- Check and, if necessary, adjust (paragraph 3.10).

(c) Stray light due to dust accumulation on the optical system:
- Clean and adjust (paragraph 3.10).

(d) Deterioration of filter:
- Inspect and, if necessary, clean or replace.

(e) Lamp failing:
- Inspect and, if necessary, replace (paragraphs 6.3, 3.10).

(f) Photocell failing:
- Check and, if necessary, replace (paragraph 6.5).

7.7 Errors arising in the sample

Whenever faults arise it is advisable to first check the appearance of the solution in the cuvette. Possible sources of error include:

(a) Unstable colour.

(b) Test and standard solutions are of (slightly) different colours, or one may be fluorescent:
- Check for the presence of interfering compounds (e.g. drugs) in the sample.

(c) Turbidity, which sometimes develops slowly:
- Lipaemic sample. Consider repeating the test after removing lipids from the sample, or measuring a sample blank and subtracting this value from the sample reading.

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