VOLUME

1

Chemical Metrology Monograph Series

The Mala Khan

LECTURES ON

CALIBRATION OF LABORATORY EQUIPMENT

Mala • Anwar

The Mala Khan

LECTURES ON

CALIBRATION OF LABORATORY EQUIPMENT

Mala Khan
KM Mostafa Anwar

Instrumentation & Calibration Service Laboratory (ICSL)
En Route to the Ultimate Reference in Chemical Measurement

Bangladesh Council of Scientific & Industrial Research (BCSIR)

Ministry of Science and Technology

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LIST OF ABBREVIATIONS

AMBL	Abdul Monem Beverages Ltd.
APMP	Asia Pacific Metrology Programme
ASTM	American Society for Testing and Measurements
BAB	Bangladesh Accreditation Board
BCSIR	Bangladesh Council of Scientific & Industrial Research
BIPM	Bureau International des Poids et Mesures, Paris, France
BP	British Pharmacopoeia
CIPM	Comite International des Poids et Mesure
CMC	Calibration and Measurement Capabilities
CRM	Certified Reference Material
DRICM	Designated Reference Institute for Chemical Measurements
EC	Electrical Conductivity
EOQ	European Organization for Quality
EP	European Pharmacopoeia
EUROMET (EURAMET)	European Collaboration in Measurement Standards
EURACHEM	Network of Organisations in Europe dealing with chemical measurements
GoB	Government of the People's Republic of Bangladesh
ICSL	Instrumentation Calibration Service Laboratory BCSIR
ICT	Information and Communication Technology
IEC	International Electro-technical Commission
ILAC	International Laboratory Accreditation Cooperation
ILC	Inter-laboratory Comparison
IR	Infrared
ISO	International Standards Organization
JP	Japanese Pharmacopoeia
LQMS	Laboratory Quality Management System
MiC	Metrology in Chemistry
MoST	Ministry of Science & Technology
MRA	Mutual Recognition Arrangement
NIST	The National Institute of Standards and Technology USA
NMI	National Metrology Institute
OIML	nternational Organization of Legal Metrology
OQ	Operational Qualification
PQ	Performance Qualification
PSCS	PROTIPAALOCK Society for Culture and Science
PT	Proficiency Testing
PTB	Physikalisch-Technische Bundesanstalt Germany
RAM	Random Access Memory
ROM	Read Only Memory
QA	Quality Assurance
QC	Quality Control
QMS	Quality Management System
R&D	Research and Development
RM	Reference Materials
SI	International System of Units
SPS	Sanitary and Phytosanitary
SRM	Standard Reference Materials
TBT	Technical Barriers to Trade
TDS	Total Dissolved Solids
UV-VIS	Ultraviolate Visible

DEDICATED TO

All The Language Martyrs

who made their supreme sacrifice on 21 February 1952 the International Mother Language Day in Bangladesh as well as in the other parts of the globe for establishing the right to mother language

ABOUT THE AUTHORS

Dr. Mala Khan

Certified & registered with European Organization for Quality (EOQ) as Laboratory Quality Assurance Manger & EOQ Laboratory Assessor for ISO/IEC 17025 **Dr. Mala Khan** is a leading young scientist in Bangladesh. Throughout her career as a scientist, she has been a keen researcher particularly in the field of Chemical Metrology and Analytical Sciences. Mala Khan, the recipient of the Chancellor's Gold Medal from the Honorable President of the People's Republic of Bangladesh and Chancellor of the University of Asia Pacific. She is the first woman in Bangladesh working in the area of Chemical Metrology, Analytical Instrumentation and Laboratory Information Management System (LIMS). In recognition of her successful research in Chemical Metrology she was awarded Doctor of Philosophy (PhD) in Chemistry from American World University, California, USA. Currently she has been implementing a four years long development project funded by GoB for establishing the partnership based Chemical Metrology infrastructure for Bangladesh. In the process of implementation of the project she is striving for implementing the National Chemical Metrology system through leading, planning, designing, coordinating, implementing, evaluating, monitoring, controlling and reporting the activities for establishing the Accredited Instrumentation Calibration Service Laboratory (ICSL) to be the Designated Reference Institute (DRICM) for Chemical Measurements in Food, Drinks & Beverages and Pharmaceuticals. She is also a very good organizer in coordinating and conducting R&D and training courses and providing application support to the scientists and QC personnel in industries, scientific research institutes and universities for Instrumentation, Calibration and Chemical Metrology etc. From 2008 onwards she had been working with the regional/international metrology organization Asia Pacific Metrology Programme (APMP)/BIPM to take part to the technical-scientific activities of the international Technical Committee on Amount of Substance (APMP TCQM) and Committee on Developing Economies (APMP DEC). Besides working as a researcher and trainer, she has also authored a number of books and articles on Chemical Metrology and other areas of analytical sciences.

KM Mostafa Anwar

Born on 02 December 1967 KM Mostafa Anwar registered with & certified by European Organization for Quality as EOQ Laboratory Manager & EOQ Laboratory Assessor of ISO/IEC 17025, eceived his degree M. Sc. in Physics with First Class First from the University of Dhaka in 1989 is an Independent Expert in Standards, Metrology, Testing, Quality and Accreditation (SMTQ). In his more than 18 years scientific professional life Anwar served a number of public and private institutes including United Nations, namely, The Monthly Computer Jagat, Dhaka University of Engineering & Technology (DUET), Bangladesh Council of Scientific & Industrial Research (BCSIR); Ministry of Science and Technology and Plasma Plus+ Application & Research Laboratory, United Nations Industrial Development Organizatiuon (UNIDO). Being the expert team leader, in the period 2006-2011, Anwar served UNIDO as National Project Coordinator (QMS Component) & Laboratory Accreditation Expert for European Union-NOARD-GoB funded technical Assistance programmes: Bangladesh Quality Support Programme & Better Work and Stanadards (BEST) Programme and lead the team to strengthening Bangladesh national quality and conformity assessment infrastructuref for Standards, Accreditation, Metrology, Testing, Quality, Market Surveillance and Consumers' Rights Protection. During his tenure in 1998-2003 with Plasma Plus+ Application & Research Laboratory he held the position of Laboratory Manager of Plasma Plus+ and served as the Product Manager of AQ Chowdhury & Co. (Pvt.) Ltd. Anwar having areas of interest in analytical instrumentation and sciences, laboratory quality management systems LQMS accreditation, Metrology in Chemistry (MiC) with applications in pharmaceutical, water, analytical, environmental, industrial QAQC, science education and research is also a musician, actor, poet & journalis. He traveled many countries and received extensive training and working experience in the area of science and performing arts.

PREFACE

En route to establishing the Bangladesh national chemical metrology program under the project "Development of ISO/IEC 17025 Accredited Instrumentation & Calibration Service Laboratory for Chemical Measurement" with funding from the Government of Bangladesh (GOB) under the aegis of the Ministry of Science and Technology (MoST), Instrumentation & Calibration Service Laboratory (ICSL) - BCSIR took the initiative to formulate the nation's first training scheme on Chemical Metrology, Laboratory Quality Management System (LQMS), Laboratory Assessment & Accreditation as per ISO/IEC 17025, Proficiency Testing, Measurement Uncertainty, Metrological Traceability in Chemical Measurements, Method Validation, Analytical Instrumentation and Calibration of the Laboratory Equipment and the like.

Aiming to the goals mentioned, in the period 23 January- 02 February 2011, using solely the national resource for the first time in Bangladesh ICSL BCSIR launched the chemical metrology training scheme through successful conduction of the Training on Laboratory Quality Management & Assessment as per ISO/IEC 17025 for the staffs of Bangladesh Accreditation Board (BAB), Ministry of industries & Bangladesh Council of Scientific & Industrial Research (BCSIR), Ministry of Science and Technology.

In continuation to this action during the period 05-09 February 2012, ICSL-BCSIR imparted a five-days special training course on Laboratory Equipment Calibration for the QC personnel from the renown industry Abdul Monem Beverage Limited the manufacturer of the global brand soft drink Coca Cola in Bangladesh where Dr. Mala Khan delivered a series of lectures on the subject. This monograph is indeed the collection of the notes of those lectures brought under a single cover for the greater audience in the industries, academic and scientific communities. It would be worthy of mentioning that designing as well as conducting a comprehensive course on calibration of laboratory equipment is the first in its kind in Bangladesh. ICSL, BCSIR, Ministry of Science and Technology and Government of Bangladesh together may take this pride to have its national capacity built in this important area of scientific activities using solely the national resources.

Considering the above at the backdrop onn this very memorable moment of publishing this monograph, the Project Director and the principle author of this book is expressing her heartfelt gratitude to Arch. Yeafesh Osman, Honorable Sate-Minister, Ministry of Science and Technology for his continuous inspiration in implementing the project at ICSL under which this training scheme in Chemical Metrology has been designed, developed and launched for the benefit of the communities in industries, science and academia.

The Project Director & Principle Author is indebted especially to Prof. Dr. Al Mostafa, Chairman of BCSIR for his kind encouragement and executive support towards the

project as a guardian taking a lot of burden on his shoulder to keep the project on track.

The Project Director also takes the opportunity to express her humble respect to respected Md. Abdur Rob Howlader; Secretary; Ministry of Science and Technology, Mr. Dilip Kumar Boshak; Additional Secretary, Mr. Rabindranath Roy Chowdhury; Joint Secretary (Development), Mr. AKM Asaduzzaman; Joint Secretary (Admin) and all officers and staffs in the Ministry of Science and Technology for their great contributions in allocating funding as well as providing policy, executive and monitoring supports towards the project at ICSL BCSIR.

It is indeed an immense pleasure for the Project Director to pay her great respect to the management and participants from the M/S Abdul Monem Beverage Ltd who had eventually shown their keen interest in the late 2011 to receive this calibration training services from ICSL delivered successfully in the period 06-10 February 2012. Their continuous urge led ICSL to formulate this Calibration Training course as well as to publish this monograph.

The Principle author also takes the great opportunity to thank hereby Respected Md. Abdul Monem CIP, Chairman of Abdul Monem Group and Mr. Masud Ahmed, Member (Secretary); Planning Commission; Ministry of Planning who have been kind enough to be present as the Special Guests on the occasion of the Certificate Award Ceremony as well as the publication ceremony of this monograph held on 10 February 2012 at the newly built ICSL Auditorium at the end of the Calibration Training course.

The Project Director is indebted to the numerous known and unknown authors, organizations whose knowledge, works, tables, graphs, information and articles have been used in preparing this lecture notes. The authors are taking the opportunity to record their sincere apologies to all of them whose works have not been quoted duly in this work.

The heartfelt gratitude from the Project Director is also to all the colleagues and staffs in ICSL and BCSIR for their handwork in organizing and managing the training as well as the award giving and publication ceremony on 10 February 2012. At this point in time, the Project Director is not forgetting to appreciate the tremendous efforts by Mr. Syed Ashraf Hossain, Mr. Rafiqul Islam and Md. Touhid Ahmed of Utsha Printing Solutions in typesetting, designing and printing this monograph within a very short notice.

Last but not the least; the authors are indebted to their family members as they have never withdrawn their great supports from the back of the authors while the authors accomplishing such technical endeavor have taken off many invaluable moments from them.

Dhaka
28 Magh 1418/10 February 2012

Dr. Mala Khan
KM Mostafa Anwar

CHAPTER 1

CALIBRATION OF ANALYTICAL BALANCE

1. INTRODUCTION

The analytical balance is a precision instrument used for weighing masses upto, say, 4 decimal places (0.0001 g) in the common analytical or industrial QC laboratory.

Basic mass measurement technique of all other mass measuring devices is almost similar with slight variations in the technologies from different manufacturers.

Almost in all cases mass are measured indirectly from the transducer signal outputs produced due to the force exerted by the earth on the material under measurement. Like almost all other instrumental techniques, this method of analysis is also relative. The instrument registers a signal due to some physical or chemical property of the material under study. In this case, an analytical balance measures the output potential of a sensor which must be related to the force (w= mg mass times the acceleration due to gravity) exerted by the earth gravitation field on the matter under study by comparison against the signal generated due to the force exerted by the earth on the standard weight under the similar environmental conditions. Therefore the mass value displayed on the digital readout of an analytical balance must be calibrated in order to obtain accurate result.

A standard procedure for calibrating analytical balance (Range: 0-200 g) is to prepare a linear working curve known as a calibration curve by plotting the measured potentials as a function of the standard weights. In the modern digital analytical balance this calibration curve is automatically stored inside the memory of the system and the digital reading of the balance appears automatically based on this calibration curve while using the balance for measuring the weight of an unknown test sample.

All analytical balance needs to be calibrated on a regular basis. Due to frequent use of the balance and as time passes by the balance loses its calibration and becomes unstable or the system response drifts. One must consult the instruction manual prior to start any operation and calibration of any particular balance which are more and more sensitive and sophisticated in these days.

Prior to start a complete calibration process of any analytical balance one must remember that it is necessary to use several Standard Weights with nominal values: 1 mg, 100 mg, 500 mg, 1 g, 10 g, 50 g, 100 g, 200 g etc. having metrological traceability to SI Unit at BIPM through unbroken chain of comparability. But using at least 5 (five) Standard Weights is strongly recommended to bracket the masses usually are being measured at one's premises using any particular balance. Having Standard Weights for nominal mass values: 1 mg, 100 mg, 500 mg, 1 g, 10 g, 50 g, 100 g, 200 g etc. would be the best practice to cover the whole range of the balance for analyzing mass within e.g. 0 - 200 g. This type of full calibration is to be done typically annually.

But normally on daily basis it is not necessary to do the complete calibration as mentioned above. Using two nominal Standard Weights (Traceable to SI Unit at BIPM through, say, NIST or other national metrology institute or designated reference institute) to bracket the everyday mass values would be sufficient. Furthermore, it is essential to check daily the performance: drift or variations, say, at the mid-point the calibration curve using any standard weight (and or combination of weights) or any suitable artifact having mass falling at the middle of the curve. It is required to calculate the drift, to record it as well as to check whether this drift of the calibration curve is within the acceptable tolerance limits or not.

As stated above the sensor/transducer outputs of the sensor in voltage are recorded inside the memory. Based on its calibration, the equipment converts a given voltage to a corresponding mass value. As the balance is used, the signal from the transducer drifts slightly, changing the voltage. Because the voltage from this transducer is so small, a slight change produces a significant error in the mass measurements. Regular calibration eliminates this error. Specific calibration methods depend on its technology varying from manufacturer to manufacturer, but they have some basic steps in common. It is strongly recommended to consult the specific Operation Manual prior to start operating the balance which may dictate the variations in the operations of the system form that particular manufacturer. In this note one may find only a basic guidance for calibration of an analytical balance typically used in any analytical laboratory. Calibration of balances for scientific and industrial purposes are would not be much different from the process discussed here.

For verification of other balances used in trade and commerce are to also to be done as per the regulatory: legal metrology requirements. One should also check other requirements to be meet for the regulatory body (under legal metrology), the accreditation body (AB) and or certification body (CB) and or the client of the laboratory services.

It is worthwhile to note that in this document the calibration of weights (used in scientific, industrial, trade and commerce) are not covered in this document. The calibration of weighing balance has been discussed.

2. ENVIRONMENTAL REQUIREMENTS

2.1 The temperature in any laboratory must be maintained at 23°C ± 3°C if the uncertainties in mass measurements are declared to be less then or equal to ± 0.0006 % (± 6.10^{-6}*M).

2.2 Weighing instruments must be calibrated at ambient temperatures, say, at 24 °C ± 4°C.

2.3 In laboratory while conducting mass measurement using e.g. micro, semi-micro and or analytical balance, the measurement should be done at ambient temperatures, say, at 24°C ± 4°C.

2.4 No temperature control is necessary when quoting uncertainties more than ± 0.0006 % (± 6.10^{-6}*M).

2.5 In the industry floor, no temperature control is required unless otherwise there is any specific requirements for industrial process control.

3. BUOYANCY CORRECTION [APPLICABLE FOR CALIBRATION OF WEIGHTS]

3.1. In case of weights calibration or measuring mass of unknown sample with an uncertainty of less than ± 0.0001% (± 6.10^{-6}*M), the density of the weights must be determined and a buoyancy correction must be applied.

3.2. In case of mass measurements with uncertainty less than ± 0.0001% (± 6.10^{-6}*M), the density of the weights must be determined and a buoyancy correction must be applied.

3.3. In case of weights calibration, where an accuracy of ± 0.0001% (± 6.10^{-6}*M) or more is quoted, the density of the weights may be assumed and a buoyancy correction must be applied.

3.4. In case of weights calibration, no buoyancy correction needs to be applied when quoting uncertainties of more than ± 0.0006% (± 6.10^{-6}*M).

3.5. Again, In case of weights calibration, when cast iron weights are calibrated, laboratories may not quote uncertainties of less than 0.001 % (± 6.10^{-5}*M).

4. A GENERAL NOTE FOR CALIBRATION OF ANY BALANCE - MINIMUM TESTS

Following are the minimum tests which must be carried out when calibrating a weighing instrument unless the user or has requested a partial calibration. Whether the balance is partially or fully calibrated that must be recorded and reported in the calibration certificate/report with appropriate marking. Laboratory may also carry out any number of additional tests using various test loads/weights.

4.1 Repeatability (or precision) test

- Take at least five readings using (where practical) a single standard weight (or other suitable artifact) practically as close to half the capacity of the weighing instrument.

- Where the capacity of the weighing instrument exceeds 30 kg, the number of readings may be reduced to a minimum of three.
- Report at least the nominal load applied and the standard deviation of the repeated weighing.

4.2 Eccentric (or corner) test

- Place standard weights (or a suitable artifact) of between one quarter and one third of the weighing instrument's capacity alternately in the centre and in the various eccentric positions on the load receptor to determine the deviation (if any) caused by the off-centre loading.
- Report at least the nominal load applied and the maximum deviation from the centre, caused by the off-centre loading.
- Where a manufacturer of a weighing instrument specifies a particular load for this test, that load may be used.
- The eccentric test is not carried out in the case of a weighing instrument with a suspended load receptor.
- Where the load receptor is supported on more than four points, a load as close as practical to the capacity of the instrument divided by the number of supporting points minus one, is used.
- Where a load receptor has up to four points of support, the load must be placed in any position on the load receptor, at a distance of approximately two thirds of the distance from the centre to the edge of the load receptor.
- In the case of a square or rectangular load receptor, the distance from the centre to one of the corners is regarded as the radius.
- Where a load receptor has more that four supporting points, the surface area is divided by the number of supporting points and the load is placed in the centre of (or evenly spread over) each segment in turn.
- Where a load receptor is in the form of rails or tracks (e.g rail vehicle scales or overhead track scales) the load must be applied at the beginning, in the middle and at the end of the rail or track

4.3 Accuracy and linearity

Test the accuracy and linearity using at least five different test loads (excluding zero), approximately evenly spread over the weighing range of the instrument. Additional tests must be carried out in the case of

- multi-range or multi-interval weighing instruments,
- weighing instruments with multi-revolution indicators,
- weighing instruments with optically projected indication together with built-in unit weights to increase the capacity.

In the case of non-self-indicating weighing instruments, the accuracy of any traveling or sliding poises and proportional counterpoise weights must be tested. Report both the actual values of the loads applied and the resultant indication. Where the total of the actual errors or inaccuracies of the standard weights used is less than one quarter of the value of the smallest graduation or readability of the weighing instrument, the nominal values of the loads may be stated instead of the actual values.

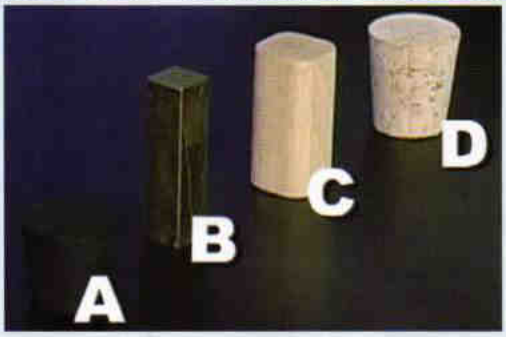
5. FREQUENCY OF CALIBRATION

- It is recommended to calibrate the Analytical Balance completely once in a year
- It is strongly recommended that prior to use at least once in a day one must check the calibration
- But if there is any problem with the response/voltage output of the sensor consultation with the qualified engineer is strongly recommended so that the calibration status of the equipment is restored within the acceptable limit.
- After any maintenance and servicing checking/verifying the performance of the balance as well as the calibration of the system is a MUST.

6. CHECKING THE PERFORMANCE OF AN ANALYTICAL BALANCE

It is recommended to verify the performance by checking the linearity of its response at the mid point of the calibration curve. This is done by measuring the masses of Standard Weight or combination of Standard Weights falling at the middle of the calibration curve as per the sequence below. At the mid-point of the calibration curve the linearity error is checked. If this linearity error is beyond manufacturer's specification then the balance must be repaired by the professional engineer.
Example is given here the balance having a range, say, 0-200 g then it is recommended to check the balance calibration via loading/weighing a number of Standards Weights together having total sum ~100 g or so. Bellow is the procedure of checking the performance of an analytical balance operating within range: 0 - 200 g.

Alternatively, one may use four different artifacts having different dimensions as well as made of different materials: rubber stopper, metal bar, wooden block and cork (see the figure) and consider them as A, B, C and D respectively.



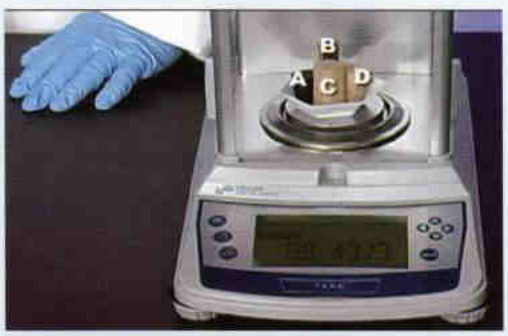
6.1 Take at least 4 (four) Standard Weights (total nominal mass ~ 200 g). Nominal masses of the Standard Weights are, e.g. A = 20 g, B = 80 g, C = 30 g and D = 70 g.

6.2 Place the weigh boat on to the tray and close the sliding glass door

6.3 Wait for stable reading

6.4 Press the TARE button

6.5 Place the four objects on the weigh boat. Weigh these four (4) Standard Weights or the four artifacts together. Record the combined mass of these four objects W_{ABCD} = (~A+B+C+D). This is called the full scale value.



6.6 Remove the four objects from the balance

6.7 TARE the balance if necessary

6.8 Weigh the first set of 2 Standard Weights or the two artifacts W_{AB} = (~A+B). Record the combined mass of the objects A & B.

Analytical Balance

Materials:
Analytical Balance
Weigh Boat
(A) rubber stopper
(B) metal bar
(C) wood block
(D) cork

A + B = 83.9690 g

Full Scale Value:
A + B + C + D = 99.43773 g

6.9 Similarly weigh the other 2 Standard Weights or the other two artifacts W_{CD} = (~C+D). Record the combined mass of these two objects.

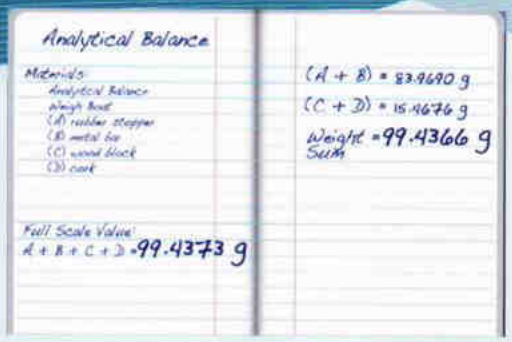
Analytical Balance

Materials:
Analytical Balance
Weigh Boat
(A) rubber stopper
(B) metal bar
(C) wood block
(D) cork

(A + B) = 83.9690 g
(C + D) = 15.4676 g

Full Scale Value:
A + B + C + D = 99.43773 g

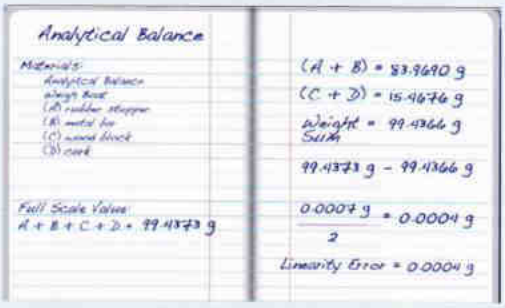
6.10 Calculate the Weight Sum $W_{AB+CD} = W_{AB} + W_{CD}$

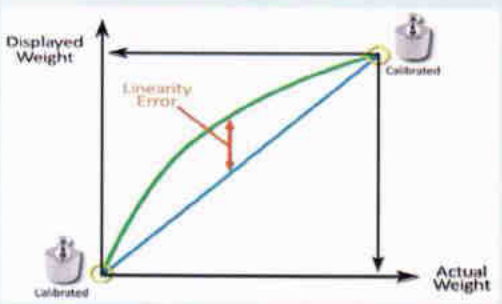


Note: Due to the nonlinearity at the mid point of the calibration curve the Full Scale Value (ex: 99.4373) and the Weight Sum (ex: 994366) are NOT equal. In ideal case, if there is no non-linearity these two values to be equal.

6.11 Calculate the Difference △: $(W_{AB}+W_{CD}) \sim (W_{ABCD})$.

6.12 Calculate the Linearity Error. As two measurements have been taken, this Difference (△) value to be divided by 2. This is the Linearity Error of the balance $\varepsilon_L = \triangle/2$





6.13 Check whether this Linearity Error is within the acceptable tolerance limits specified by the manufacture. If the Error is out of the specification, take appropriate action to repair the balance by a professional/certified engineer.

7. A TYPICAL OPERATING PROCEDURE FOR CALIBRATION OF AN ANALYTICAL BALANCE

Note: This is only an example for Model Mettler AE 160. For other model one must consult the manufacturer's operational manual.

10.1 The balance must be leveled first to assure the repeatable results.

10.2 Locate the air bubble on the balance. The leveling feet are typically at the back of the balance

10.3 Turning the leveling feet confirm the air bubble is at the centre of the level control

10.4 The balance is needed to be cleaned at all times. Take a soft brass and swipe up the balance tray and the surrounding area. Use lint free tissue to wipe out any debris on the tray/pan. [Caution: While measuring using hand gloves is strongly recommended]

10.5 Make sure that all the balance doors are closed.

10.6 Turn ON the balance

10.7 The warm up time 30 minutes is suggested optimal performance

10.8 When 0 appears press and hold the control bar, and wait to see the operationa MODE.

10.9 When CAL - - - - - appears on the display release the control bar

10.10 CAL 100 g flashes after a few seconds

10.11 Slowly move the Calibration lever on the lower side of the balance towards the rear

10.12 CAL with the dashed line will appear and then 100 g

10.13 When the display CAL 0 appears move the lever back to its original position

10.14 Dashes will flash followed by 0

10.15 Two prior calibrated weight 50 g and 100 g are suggested

10.16 Use forceps to handle weights to avoid oil or other contaminations adding extra mass to the weights from the hand of the operator

10.17 Open one of the doors and place the 50 g weight at the centre of the tray

10.18 Close the door and wait for the balance to be stabilized (Caution: keep the sliding doors closed during any measurement to avoid the air draft affecting the measurements)

10.19 The reading is stable when the instability disappears

10.20 The display should be within the limit of 0.0003 mg

10.21 Remove the 50 g weight

10.22 Place the 100 g weight

10.23 Close the door and allow the balance reading to be stabilized again

10.24 The balance reading should be within the limit of 0.0003 mg

10.25 Remove the 100 g weight

10.26 Allow the balance to come to it its original stabilized 0 g reading

10.27 REZERO by PRESSING 0

10.28 The calibration is completed

10.29 The Analytical Balance is ready for use

Caution: Using hand gloves and keeping the sliding door closed are recommended

CHAPTER 2

CALIBRATION OF VOLUMETRIC FLASK & BURETTE-PIPETTE

1. INTRODUCTION

Calibration is the process by which a stated measure such as the volume of a container is checked for accuracy. In general, measurements of mass can be determined more precisely and accurately than measurements of volume. Therefore, the mass of the liquid contained (To Contain TC) or dispensed (To Deliver TD) by the glassware will be measured and the corresponding volume calculated using the density of the liquid. However, a relatively small change in temperature causes a change in the liquid's volume and thus its density.

In precise work it is never safe to assume that the volume delivered by or contained in any volumetric instrument is exactly the amount indicated by the calibration mark. Instead, recalibration is usually performed by weighing the amount of water delivered by or contained in the volumetric apparatus. This mass is then converted to the desired volume using the tabulated density of Water:

Volume = mass / density ---------- (Eq. 1)

All volumetric apparatus should be either purchased with a Calibration Certificate traceable to NIST or calibrated by the competent analyst in the following manner.

1.2 Systematic Errors Affecting Volumetric Measurements

The volume occupied by a given mass of liquid varies with temperature, as does the volume of the device that holds the liquid. 20°C has been chosen as the normal temperature for calibration of much volumetric glassware.

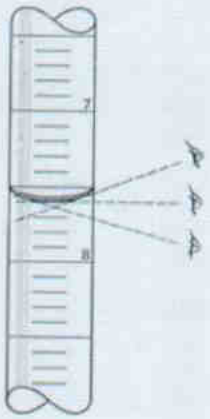
Glass is a fortunate choice for volumetric ware as it has a relatively small coefficient of thermal expansion; a glass vessel which holds 1.00000L at 15°C holds 1.00025 L at 25°C. If desired, the volume values (V) obtained at a temperature (t) can be corrected to 20°C by use of:

$$V_{20} = V\,[1 + 0.000025\,(20 - t)] \quad \text{---------- (Eq. 2)}$$

In most work, this correction is small enough it may be ignored.

However, the thermal expansion of the contained liquid is frequently of importance. Dilute aqueous solutions have a coefficient of thermal expansion of about 0.025%/°C. A liter of water at 15°C will occupy 1.002 L at 25°C. A correction for this expansion must frequently be applied during calibration procedures.

Parallax is another source of error when using volumetric ware. A correction for this expansion must frequently be applied during calibration procedures. Frequently, graduation marks encircle the apparatus to aid in this.



Readings which are either too high or too low will result otherwise.

1.3 Tips for Correct Use of Volumetric Glassware

1.3.1 Pipettes

The Pipette is used to transfer a volume of solution from one container to another. Most Volumetric Pipettes are calibrated To-Deliver (TD); with a certain amount of the liquid remaining in the tip and as a film along the inner barrel after delivery of the liquid. The liquid in the tip should not be blown-out. Pipettes of the

"blow-out" variety will usually have a ground glass ring at the top. And, drainage rates from the pipette must be carefully controlled so as to leave a uniform and reproducible film along the inner glass surface. Measuring Pipettes will be gradated in appropriate units. Once the pipette is cleaned and ready to use, make sure the outside of the tip is dry. Then rinse the pipette with the solution to be transferred. Insert the tip into the liquid to be used and draw enough of the liquid into the pipette to fill a small portion of the bulb. Hold the liquid in the bulb by placing your fore finger over the end of the stem.

Withdraw the pipette from the liquid and gently rotate it at an angle so as to wet all portions of the bulb. Drain out and discard the rinsing liquid. Repeat this once more. To fill the pipette, insert it vertically in the liquid, with the tip near the bottom of the container. Apply suction to draw the liquid above the graduation mark. Quickly place a fore finger over the end of the stem. Withdraw the pipette from the liquid and use a dry paper to wipe off the stem. Now place the tip of the pipette against the container from which the liquid has been withdrawn and drain the excess liquid such that the meniscus is at the graduation mark.

Move the pipette to the receiving container and allow the liquid to flow out (avoiding splashing) of the pipette freely. When most of the liquid has drained from the pipette, touch the tip to the wall of the container until the flow stops and for an additional count of 10.

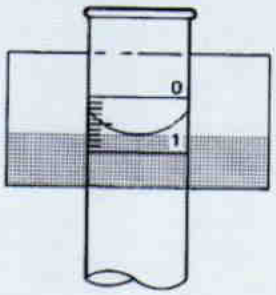
1.3.2 Volumetric Flasks

The Volumetric Flask is used to prepare Standard Solutions or in diluting a sample. Most of these flasks are calibrated To-Contain (TC) a given volume of liquid. When using a flask, the solution or solid to be diluted is added and solvent is added until the flask is about two-thirds full. It is important to rinse down any solid or liquid which has adhered to the neck. Swirl the solution until it is thoroughly mixed. Now add solvent until the meniscus is at the calibration mark. If any droplets of solvent adhere to the neck, use a piece of tissue to blot these out. Stopper the flask securely and invert the flask at least 10 times.

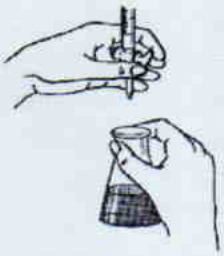
1.3.3 Burettes

The Burette is used to accurately deliver a variable amount of liquid. Fill the burette to above the zero mark and open the stopcock to fill the tip. Work air bubbles out of the tip by rapidly squirting the liquid through the tip or tapping the tip while solution is draining.

The initial burette reading is taken a few seconds, ten to twenty, after the drainage of liquid has ceased. The meniscus can be highlighted by holding a white piece of paper with a heavy black mark on it behind the burette.



Place the flask into which the liquid is to be drained on a white piece of paper. (This is done during a titration to help visualize color changes which occur during the titration.) The flask is swirled with the right-hand while the stopcock is manipulated with the left hand.



The burette should be opened and allowed to drain freely until near the point where liquid will no longer be added to the flask. Smaller additions are made as the end-point of the addition is neared. Allow a few seconds after closing the stopcock before making any readings. At the end-point, read the burette in a manner similar to that above.

As with pipettes, drainage rates must be controlled so as to provide a reproducible liquid film along the inner barrel of the burette.

Two or three rinsing with tap water, a moderate amount of agitation with a dilute detergent solution, several rinsing with tap water, and two or three rinsing with distilled water are generally sufficient if the glassware is emptied and cleaned immediately after use.

If needed, use a warm detergent solution (60-70°C). A burette or test tube brush can be used in the cleaning of burettes and the neck of volumetric flasks. Volumetric flasks can be filled with cleaning solution directly. Pipettes and burettes should be filled by inverting them and drawing the cleaning solution into the device with suction. Avoid getting cleaning solution in the stopcock.

Allow the warm cleaning solution to stand in the device for about 15 minutes; never longer than 20 minutes. Drain the cleaning solution and rinse thoroughly with tap water and finally 2-3 times with distilled water. Pipettes and burettes should be rinsed at least once with the solution with which they are to fill before use.

1.3.4 A General Calibration Procedure

As was noted above, volumetric glassware is calibrated by measuring the mass of Water that is Contained In (TC) or Delivered By (TD) the device.

To obtain an accurate mass measurement, buoyancy effects must be corrected for. The amount of air displaced by the standard weights of the balance is somewhat different than the amount of air displaced by the weighed water. This difference leads to different buoyancies for these objects; meaning the balance levels at a point other than when the two objects are of the same mass. This can be corrected for using:

$$m_{true} = m_{meas} + d_a \left((m_{meas}/ d) - (m_{meas}/ d_s) \right) \text{ ---------- (Eq.3)}$$

where,

d_s is the density of the standard weights (8.47 g/cm^3),

d_a is the density of air (= 0.0012 g/cm^3),

d is the density of the object being measured.

This mass data is then converted to volume data using the tabulated density of water (Table 1) at the temperature of calibration. (In very accurate work, the thermometer must also be calibrated as an incorrect temperature reading will lead to the use of an incorrect density for Water. This, in turn, will give an inaccurate volume calibration.)

Finally, this volume data is corrected to the standard temperature of 20°C. This can be accomplished using the thermal expansion coefficient of Water; 0.00025/°C:

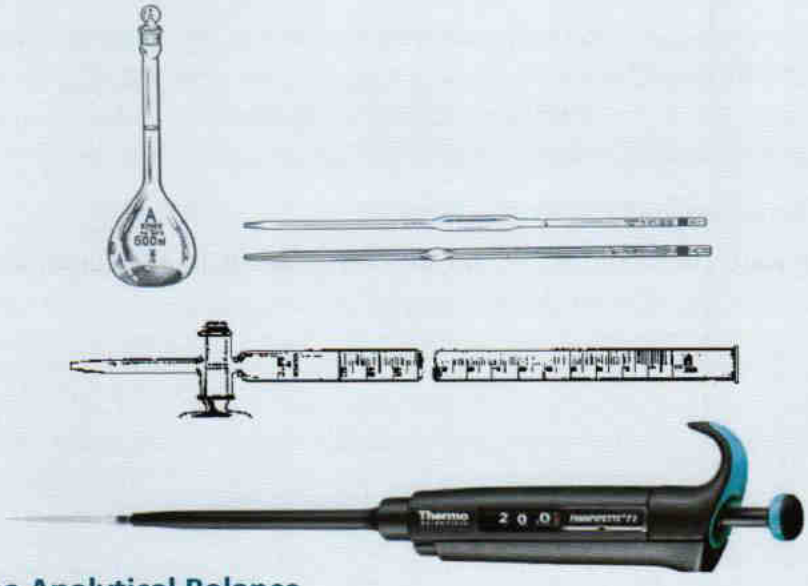
$$V_{20} = V [1 + 0.00025 (20 - t)] \text{ ----------(Eq.4)}$$

1.3.5 Cleaning Volumetric Glassware

Cleaning of volumetric glassware is necessary to not only remove any contaminants, but to ensure its accurate use. The film of water which adheres to the inner glass wall of a container as it is emptied must be uniform.

1.4 To Contain vs To Deliver

Volumetric glassware is calibrated either to contain (TC) or to deliver (TD) the stated volume. Beakers and graduated cylinders are generally calibrated to contain (TC) while most pipettes and burettes are calibrated to deliver (TD).



1.5 The Analytical Balance

The basic measuring device in the laboratory is the analytical balance. The accuracy of the counterweights inside the balance is much better than one part per thousand and the balances are serviced and calibrated at regular intervals to ensure their accuracy.

In the most accurate work two corrections are required. One is to correct for difference between an object weighed in air and the same object weighed in vacuum. According Archimedes' principle an object is buoyed up by a force equal to the weight of air it displaces. Second, is the fact that glass expands with increasing temperature, so the volume of a container also increases. By convention, volumetric glassware is always calibrated at 20°C. Since the temperature at which the calibration is done may be somewhat different there is a small correction for the cubic coefficient of expansion of glass. Fortunately the correction is very small within a few degrees of 20°C and can be neglected in ordinary work.

1.6 Volumetric Glassware

Volumetric glassware is calibrated at a specific temperature, usually 20°C, but quite often it is used to deliver or contain volumes at a different temperature. The temperature variations make it necessary to adjust samples and/or standards to the calibration temperature before measurement, or to apply temperature

corrections to the volumes measured. Glassware that is designed to deliver specific volumes also may have specific drain time associated with its calibrated volume and must be scrupulously cleaned to drain properly. Therefore it can be seen that variances in volumetric measurements can be a major, if not the chief, source of error in an analytical laboratory.

There are three general methods commonly employed to calibrate glassware:

1. Direct, absolute calibration
2. Indirect, absolute calibration
3. Relative calibration

1.6.1 Direct Calibration

A volume of water delivered by a burette or contained in a volumetric flask is obtained directly from the weight of the water and its density (at the particular temperature). For example, if at 25°C, a 20.00 mL pipette delivered 19.970 g of water then the volume delivered at 25°C would be 19.970 g x 1.0040 mL/g = 20.05 mL. At 20°C the volume would be 19.970g x 1.0037 mL/g = 20.04 mL. (See Table 1 for temperature specific values for water density)

1.6.2 Indirect Calibration

Volumetric glassware can be calibrated by comparison of the mass of water it contains or delivers at a particular temperature with that of another vessel which had been calibrated directly. The volumes are directly related to the masses of water. This method is convenient if many pieces of glassware are to be calibrated.

1.6.3 Relative Calibration

It is often necessary to know only the volumetric relationship between two items of glassware without knowing the absolute volume of either. The situation arises, for example, in taking an aliquot portion of a solution. Suppose that it is desired to titrate one-fifth of an unknown sample, the unknown sample might be dissolved and diluted to volume in a 250 mL volumetric flak. A 50 mL pipette would then be used to withdraw an aliquot for titration.

For the calculation in this analysis, it would be necessary to know the exact volume of the flask or the pipette, but it would not be necessary to know the exact volume of the flask or the pipette, but it would be required that the pipette deliver exactly one-fifth of the contents of the flask. The method used for the relative calibration in this case would be to discharge the pipette five times into the flask and marking the level of the meniscus on the flask.

1.7 In Conclusion

The use of calibrated glassware is of great importance in a chemical laboratory in order to ensure that any volumes measured are known with a certain degree of accuracy.

In this procedure mainly the gravimetric principle has been applied for the calibration of volumetric flask typically used by an analytical chemist. In order to avoid introducing Systematic Errors into our measurements, each volumetric flask must be properly calibrated. And, to reduce the Random Errors inherent when using this glassware, the proper use must be understood. The quality of the measurements obtained from the volumetric glassware depends heavily on the care taken in calibrating and in using them. This procedure provides a method for calibrating volumetric flask used in the laboratory.

This procedure also may be used for calibrating graduated pipettes and burettes.

2. OPERATING PROCEDURE FOR CALIBRATION OF VOLUMETRIC FLASK

2.1 Procedure Outline

In this gravimetric principle the weight of deionized water contained in the flask or pipette is used to determine the actual volume contained in the glassware. Water weight is corrected for density differences due to temperature. The temperature of the room should be maintained within 24±4°C.

2.2 Requires Equipment & Supplies

Analytical balance
Thermometer, 0-150°C
Deionized water

2.3 Documentation Control & Record

Using a copy of the attached data sheet titled "Volumetric Flask Calibration", write the date and flask identification information in the appropriate blanks. Data from each of the below sections should also be recorded on this form. The "Calibration Due Date" shall be 3 months from the current date. Maintain the data sheet in the same laboratory as the flask. After the next calibration, sheets should be transferred to and maintained in a file in the lab record archive for a period of at least 3 years.

2.4 Procedure

2.4.1 Perform a full calibration check on the balance to be used according to an approved standard operating procedure (SOP) (Ref: The Chapter 1 on Calibration of Analytical Balance in this monograph). Record the results on the data sheet. During a full calibration of an analytical balance one should

utilize at least five standard weights in the range of the expected weight of the water plus the flask. Performance check should be done daily to verify the linearity error of the calibration curve at the middle of the curve using several Standard Weights or artifacts as stated in the approved SOP.

2.4.2 Start Volumetric Flask Calibration.

2.4.3 Record the serial number of the flask.

2.4.4 If it does not already exist, add a serial number to the flask in permanent ink or by attaching a label.

2.4.5 Wash, dry, weigh, and record the mass of the volumetric flask W_{flask} to be calibrated.

- For volumes of 100 mL or less, the balance used must be capable of measuring in increments of 0.001 g.
- For volumes greater than 100 mL, the balance used must be capable of measuring in increments of 0.1 g.

2.4.6 Fill the volumetric flask to the mark with deionized water.

2.4.7 Wipe dry the outside of the flask and then weigh. Record the weight of the flask with the water in it ($W_{flask + water}$).

2.4.8 Measure and record the water temperature (T).

2.4.9 Using Table 1, look up and record the density of the water corresponding to the temperature recorded in step 2.4.8.

2.4.10 Calculate the expected mass of the flask at the experimental temperature (W_{TX}) as well as the volume of the flask V_{flask} as follows:

$$V_{flask} = \frac{W_{flask+water} - W_{flask}}{\rho_{water}}$$

where:

V_{flask} is the volume of the flask (in mL);

$W_{flask + water}$ is the weight of the water recorded in step 2.4.7 (in g);

W_{flask} is the weight of the flask recorded in step 2.4.5 (in g); and

ρ_{water} is the density of water at the temperature recorded in step 2.4. 8 (in g/mL)

2.4.11 Repeat steps 2.4.6 through 2.4.10 for at least five runs.

2.4.12 Calculate and record the average, standard deviation (σ), and twice the standard deviation (2σ) of the flask volume based on the runs.

2.4.13 Identify any aliquots for which the volume is greater than 2σ from the average value. If any aliquots are greater than 2σ from the average value repeat the procedure.

2.4.14 Calculate and record the flask tolerance based on the following formula

Tolerance = Listed Pipette Volume - Average Volume

2.4.15 Ensure that the tolerance is within the limits of a Class B flask as listed in Table 2.

2.4.16 Calculate and record flask precision based on the following formula

$$\text{Precision}(\%) = \frac{\sigma}{\text{Average Volume}} \times 100$$

2.4.17 Sign and date the data sheet. Maintain the sheet in accordance with the established laboratory procedure.

2.5 Origin of Uncertainties

- Weight of water
- Density of water
- Error in sighting the level of the water
- Error in temperature reading

2.6 Consult the References

- Lecture note on the Calibration and performance check of laboratory analytical balance.
- Lecture note on Calibration of laboratory thermometers.
- ASTM E288-94. Standard speciation for laboratory glass volumetric flasks.

Table 1. Density of pure water for various temperatures.

Temperature (C)	density (g mL^{-1})		Temperature (C)	density (g mL^{-1})
16.0	0.99897		23.5	0.99745
16.5	0.99889		24.0	0.99732
17.0	0.99880		24.5	0.99720
17.5	0.99871		25.0	0.99707
18.0	0.99862		25.5	0.99694
18.5	0.99853		26.0	0.99681
19.0	0.99843		26.5	0.99668
19.5	0.99833		27.0	0.99654
20.0	0.99823		27.5	0.99640
20.5	0.99812		28.0	0.99626
21.0	0.99802		28.5	0.99612
21.5	0.99791		29.0	0.99597
22.0	0.99780		29.5	0.99582
22.5	0.99768		30.0	0.99567
23.0	0.99757			

Table 2. Required tolerances of volumetric flasks (ASTM E-288-94).

Capacity (mL)	Tolerances (± mL)	
	Class A	Class B
5	0.02	0.04
10	0.02	0.04
25	0.03	0.06
50	0.05	0.10
100	0.08	0.16
200	0.10	0.20
250	0.12	0.24
500	0.20	0.40
1000	0.30	0.60
2500	0.50	1.00

Volumetric Flask Calibration

Date: ..... Calibration Due Date: .....

Flask Identification

Manufacturer: ..... Volume: ..... mL

Serial Number: ..... Laboratory Used In: .....

Calibration Check of Balance

Balance Manufacturer: ..... Weight Set Serial Number:.....

Balance Serial Number: ..... Laboratory: .....

Balance Calibration Data

Weight (g)	Reading (g)	Weight (g)	Reading (g)
2000.0		20.0	
200.0		10.0	
150.0		5.00	
100.0		2.00	
50.0		1.00	

Balance Performance Check

Balance Linearity Error ε_L: ..... Tolerance Limit of Linearity Error: .....

Calibration of Flask

Step 2.4.5 - Mass of Empty Flask: .....g

Run #	Step 2.4.7 (g)	Step 2.4.8 (°C)	Step 2.4.9 (g/mL)	Step 2.4.10 (mL)
1				
2				
3				
4				
5				
6				
7				
8				

Average Volume: ..... mL

Tolerance: .....mL

Precision: .....%

ASTM Classification: .....

Analyst: ....
Signature Date

Review: ....
Laboratory Supervisor Date

3. OPERATING PROCEDURE FOR CALIBRATION OF BURETTE

3.1 Burette or Pipette Calibration Procedure

3.1.1 Record the temperature in the laboratory

3.1.2 Wash the supplied burette thoroughly such that no drops of distilled water are left on the internal surface of the burette.

3.1.3 If droplets of water are still observed to adhere to the inner surface of the burette after delivering deionised water, the burette must be considered as still dirty and the cleaning process repeated.

3.1.4 Allow the burette to drain for some time.

3.1.5 Clean and dry a small flask using paper towels and a stream of warm air. Handle the flask using the paper towels since fingerprints and sebaceous oils may cause the calibration process to be imprecise.

3.1.6 Fit the flask with a stopper.

3.1.7 Weigh the flask on an analytical balance to the nearest 0.1 mg.

3.1.8 Using a transfer pipette, rinse the burette with distilled water at room temperature

3.1.9 Drain the burette completely into a beaker.

3.1.10 Place the burette into the burette clamp.

3.1.11 Ensure that the clamp is mounted tightly and is level.

3.1.12 Using a funnel, re-fill the burette was with deionised distilled temperature equilibrated water.

3.1.13 Ensure that the burette is not over-filled. (The burette must be filled to slightly past the 0.00 mL mark.)

3.1.14 Carefully wipe off any solution spilled on the outside of the burette.

3.1.15 Open the stopcock and drain the burette to the 0.00 mL mark.

3.1.16 Ensure that no air bubbles are present in the burette or in the stopcock tip. If any are present, the burette must be re-drained slightly to force out the air bubbles and re-filled.

3.1.17 Touch the beaker to the tip of the burette to remove any adhering drops of water.

3.1.18 Allow the burette to stand for 5 minutes.

3.1.19 If the level of the burette has changed, more water must be added slowly, to reach the 0.00 mL mark.

3.1.20 Place the flask under the burette.

3.1.21 Open the stopcock and slowly transfer 10 mL into the flask

3.1.22 Touch the tip of the burette to the wall of the flask.

3.1.23 Allow the flask to stand for 30 seconds such that the film of liquid on the walls of the burette to drain.

3.1.24 Stopper the flask in order to prevent evaporation.

3.1.25 Record the apparent volume of liquid extracted from the burette to the nearest 0.01 mL.

3.1.26 Weigh the flask to the nearest 0.1 mg.

3.1.27 Repeat the procedure in 10 mL increments, all the way to 50 mL.

3.1.28 Refill the burette was re-filled and repeat the entire procedure for a further 2 times.

3.1.29 Record your results onto the tables provided in Section 3.2 below. Refer to the definitions included at the end of Section 4.0 prior to recording your results.

3.1.30 Estimate the Standard Deviation, Precision, and Absolute and Relative Error of these results.

3.1.31 Comment on the significance of these results

3.2 Data Reduction and Analysis for calibration of burette and pipette

Water temperature: .....°C

Density of water (from the table bellow): ..... gm/L

Example: Density of Water & Corrected Volume

Temperature (°C)	Density (g/mL)	Corrected volume of 1 g of water at 20(°C)* (m/L)
19	0.9984082	1.0027
20	0.9982071	1.0029
21	0.9979955	1.0031
22	0.9977735	1.0033
23	0.9975415	1.0035
24	0.9972995	1.0038
25	0.9970497	1.0040
26	0.9967867	1.0042

Interval 0 - 10 ml

Final Reading (mL)			
Initial Reading (mL)			
Apparent Volume (mL)			
Mass			
Actual Volume (mL)			
Correction			

Average Correction for interval 0-10 mL: .....
Consequently, the burette delivers ----------mL less than indicated by the burette reading.

Interval 10 - 20 mL

Final Reading (mL)			
Initial Reading (mL)			
Apparent Volume (mL)			
Mass			
Actual Volume (mL)			
Correction			

Average Correction for interval 10-20mL : .....
Consequently, the burette delivers ----------mL less than indicated by the burette reading.

Interval 20 - 30 ml

Final Reading (mL)			
Initial Reading (mL)			
Apparent Volume (mL)			
Mass			
Actual Volume (mL)			
Correction			

Average Correction for interval 20-30mL:
Consequently, the burette delivers ----------mL less than indicated by the burette reading.

Interval 30-40mL

Final Reading (mL)			
Initial Reading (mL)			
Apparent Volume (mL)			
Mass			
Actual Volume (mL)			
Correction			

Average Correction for interval 30-40 mL: .....
Consequently, the burette delivers ----------mL less than indicated by the burette reading.

Interval 40-50mL

Final Reading (mL)			
Initial Reading (mL)			
Apparent Volume (mL)			
Mass			
Actual Volume (mL)			
Correction			

Average Correction for interval 40-50mL: .....
Consequently, the burette delivers ----------mL less than indicated by the burette reading.

Total average correction over the 0-50ml range = ... mL

4.0 DEFINITIONS & FORMULAE

The **apparent volume** is calculated by finding the difference between the finaland initial volumes read from the burette.

The **mass volume** is calculated by weighing the flask after adding each 10 mL increment by means of an electronic balance

The **actual volume** is calculated by simple proportion using the density of water 23°C as shown:

Example: 1 g of water weighs 0.9975415 g
Thus Y g of water weighs ? g

Actual volume of water delivered = (0.9975415 x Y)/1 = Z mL

The **correction factor** is calculated by finding the difference between the actual volume and the apparent volume in each case.

5.0 FURTHER DATA MANIPULATION

5.1 Calculation of Standard Deviation:

$$\sigma = \sqrt{\frac{\sum [x - \bar{x}]^2}{n - 1}}$$

5.2 Calculation of Precision:

$$\text{Precision CV (\%)} = \frac{\sigma}{V_{average}} \times 100$$

CHAPTER 3

CALIBRATION OF pH METER

1. INTRODUCTION

Most instrumental methods of analysis are relative. Instrument registers a signal due to some physical or chemical property of the solution. For example, pH meter measures the electrode potential which must be related to the $[H^+]$ or $[OH^-]$ of the solution by comparison against known $[H^+]$ or $[OH^-]$ standard buffers. In other words, the pH meter must be calibrated in order to obtain accurate results. A standard procedure for calibrating pH meters is to prepare a linear working curve known as a calibration curve by plotting the measured potential as a function of pH or pOH. In the modern digital pH meter this calibration curve is automatically stored inside the memory of the meter and the reading of the meter appears automatically based on this calibration curve while using the meter for measuring the pH of an unknown solution.

All pH meter need to be calibrated on regular basis; due to frequent use of the meter and as time passes by the meter loses its calibration. You must consult the instruction manual prior to start any operation and calibration.

Prior to start the pH Meter Calibration process your must remember that you have to have at least two solutions of known pH. But using three standard solutions is strongly recommended. Having pH Standard solutions for pH 4, pH 7 and pH 10 would be the best practice to cover the whole range of the meter for analyzing pH: 0-14. The pH meter electrode reads the weak electrical potential caused by the action of hydrogen ions in the solution. Based on its calibration, the meter converts a given voltage to a corresponding pH value. As the meter is used, the signal from the electrode drifts slightly, changing the voltage. Because the voltage from pH is so small, a slight change produces a significant error in the pH.

recommended so that the calibration status of the system as well as the sensor condition is restored within the acceptable limit.

- Recalibrate if the battery of the pH Meter is replaced.
- After any maintenance and repairmen checking/verifying the performance as well as the calibration is a MUST.

9. CHECKING THE PERFORMANCE

After calibration please check the pH of any fresh standard solution different from the solutions used for calibration. That means, avoid checking the pH Meter by measuring the pH of the same baseline calibration solutions. If the variation of the pH result is within the acceptable tolerance limit e.g. 0.1 then the performance is considered to be OK. Calibration solutions or baseline solutions once used please do not return them again back in the standard reagent container. You may use the disposable standards transfer pipettes. This is to avoid the contamination of the stock standard solution. Discard the solutions or buffer after use. While using the standards/buffers in any beaker it is strongly recommended to cover the same by aluminum foil. Direct exposure to air may change the buffer pH.

10. A TYPICAL OPERATING PROCEDURE FOR CALIBRATION OF A pH METER

Note: This is only an example of calibrating the OKTON pH Meter. For other model please consult the manufacturer's operational manual.

10.1 Take at least five clean glass/plastic containers that can hold sufficient buffer to immerse the pH probe. One container for rinsing de-ionized water, three for calibration buffer solutions and fourth one for standard solution (to run as an unknown one for verifying the performance of the pH meter after calibration).

10.2 Perform a visual inspection of the probe. There should not be any crack, any salt deposit or any other matter on the probe. If any clean it using moist cotton swab. Or use lint free tissue. DO NOT RUB, just soak.

10.3 Rinse the probe electrode in water and briefly shake off or wipe clean with lint free tissue.

10.4 Switch the meter ON.

10.5 Place the probe in the pH 7 Standard solution and allow the pH reading to settle.


Submerge electrode into the pH 7 buffer

10.6 Press the CAL button. " CAL" will flash briefly and then the pH reading will flash.

10.7 Press the HOLD/CON button. This will calibrate the meter to pH 7.

10.8 Rinse probe with water and shake or wipe clean with lint free tissue.

10.9 Place probe into pH 4 solution and allow the pH reading to settle.


Submerge electrode into the pH 4 buffer

10.10 Press the CAL button. " CAL" will flash briefly and then the pH reading will flash.

10.11 Press the HOLD/CON button. This will calibrate the meter to pH 4.

10.12 Rinse the probe with distilled water and shake or wipe clean with lint free tissue.


Rinse the electrode with distilled water and wipe clean with a Kimwipe

10.13 Place probe into pH 10 solution and allow the pH reading to settle.

10.14 Press the CAL button. "CAL" will flash briefly and then the pH reading will flash.

10.15 Press the HOLD/CON button. This will calibrate the meter to pH 10.

10.16 The pH meter is now calibrated.

10.17 Checking performance. Measure the pH of any other standard (known) solution as an unknown solution and verify whether the reading is within the acceptable tolerance limit e.g. 0.1 or not.

10.18 Submerge the pH Meter in the storage solution or distilled water with pH 7.

10.19 The pH Meter is now ready to measure the pH of other substances.

11. EXAMPLE OF SOME TECHNICAL FEATURES OF A TYPICAL BENCHTOP pH METER: EXAMPLE OF OAKTON

- Two line, dot matrix display provides clear instructions for setup and calibration.
- RS-232 output: sends data to printer or PC on programmed time intervals, when reading is stable, or manually.
- Calculates and displays slope/offset of electrode automatically; check electrode quality and conform to standard test method quality checks.

- Calibration alarm signals when meter needs recalibration-user-defined interval ensures regularly scheduled recalibration.
- Real-time clock stamps stored and calibration data with date and time-suitable for GLP documentation.
- Ion concentration mode offers direct display of ion readings in ppm, ppt, % or any other units of your choice (pH 2100).
- Use these meters to document large volumes of critical ISE (pH 2100) pH or mV data sets. Large dot matrix display shows pH, ion or mV reading plus temperature (°C). Meter sends measurements to a printer or PC with the touch of a button, or on timed intervals. Free software allows you to download data to your computer in a format easy to import into spreadsheet programs.
- Customize up to five pH calibration points, or select from four standard buffer sets. Meter displays last calibration date and time.
- Meter features a built-in clock with timer and alarm. Other features include memory of up to 50 readings with date and time, selectable manual or
- Automatic Temperature Compensation (ATC), analog recorder output and a selectable "Ready" function.
- Detachable electrode holder arm holds electrodes firmly in place. Includes instruction card that stores underneath meter for quick reference. 110/220 VAC.

Measures: pH, mV, Relative mV, Ion (Model: pH 2100), C, F
pH Range: 0.000 to 14.000
pH Resolution: 0.001/0.01
pH Accuracy: +/-0.002/0.01
pH Calibration: Up to 5 customizable calibration points, or USA, NIST, Bf1, or BF2 standard buffer sets
mV Range: +/-1850.0
mV Resolution: 0.1
mV Accuracy: +/-0.2
mV Calibration: offset up to +/-150
Ion Range (pH 2100): 0.000 to 19900
Ion Resolution (pH 2100): +/- least significant digit
Ion Accuracy (pH 2100): +/-0.5% of the reading
Temperature Range: -5 to 105C/23 to 221F
Temperature Resolution: 0.1C or F
Temperature Accuracy: +/-0.3C / +/-0.5F
Temperature Calibration: offset up to +/-5.0C/+/-9.0F
Dimensions, inches (cm): 9 x 7 x 2.4 (23 x 18 x 6)

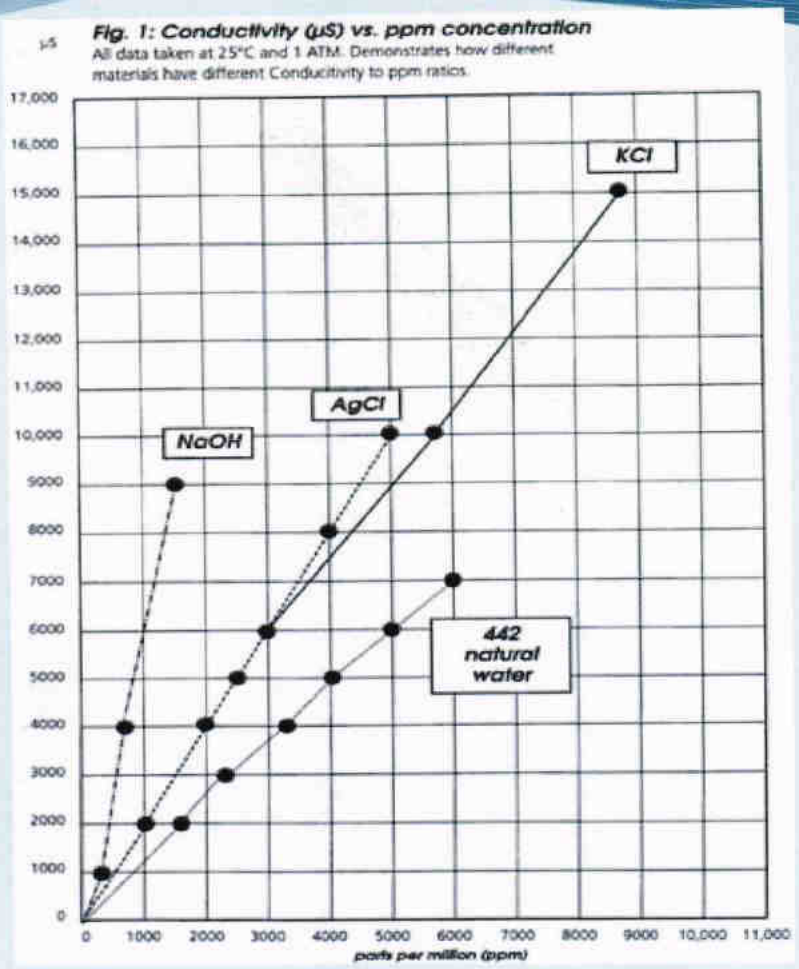


Fig. 1: Conductivity (µS) vs. ppm concentration
All data taken at 25°C and 1 ATM. Demonstrates how different materials have different Conductivity to ppm ratios.

A similar conclusion can be made for all types of dissolved solids. Most pre-formulated ppm TDS standard solutions are formulated with either sodium chloride (NaCl), potassium chloride (KCl) or the 442 (40% sodium sulfate, 40% sodium bicarbonate and 20% sodium chloride) natural water formulation.

In some cases, a KCl solution is made to a specific Conductivity value, and then the ppm values for NaCl, KCl and/or 442 formulation are referenced on the bottle giving the user the option to calibrate to any one of these. A Conductivity value is also usually given.

Conductivity/Resistivity Spectrum

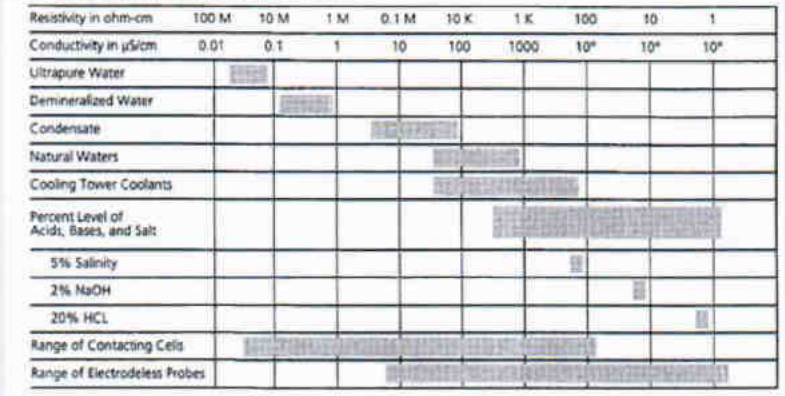


Table of Conductivity versus Concentration for Common Solutions

Conductivity (G) µSiem (µO/cm) at 25°C (77°F)

Weight %	ppm mg/litre	NaCl	NaOH	NH_4OH	NH_3	HCl	H_2SO_4	HNO_3	HF	SO_2	Acetic Acid
0.001	1	2.2	6.2	4.1	6.6	11.7	8.8	6.8	10	6.4	4.2
0.0003	3	6.5	8.3	8.3	12	5.0	6.1	20	30	18	7.4
0.001	10	21.4	61.1	17	27	116	85.6	67	99	54	15.10.00
0.003	30	64	182	31	49	340	251	199	290	150	30.6
0.01	100	210	603	58	84	1140	805	657	630	450	63
0.03	300	617	1780	102	150	3390	2180	1950	1490	1200	114
0.1	1000	1990	5820	189	275	11100	6350	6380	2420	3600	209
0.3	3000	5690	16900	329	465	32200	15800	18900	5100	7900	368
1.0	10000	17600	53200	490	810	103000	48500	60000	11700	17200	640
3.0	Rarely Used	48600	144000	790	1110	283000	141000	172000	34700	32700	1120
5.0		78300	223000	958	1115	432000	237000	275000	62000	42000	1230
10.0		140000	358000	1115	1120	709000	427000	498000	118000	61000	1530
20.0		226000	414000	968	4251	850000	709000	763000	232300	Sat	1600
30.0		Sat	292000	725	Sat	732000	828000	861000	390000	Sat	1405
40.0		Sat	191000	460	Sat	Sat	770000	820000	NA	Sat	1080
50.0		Sat	150000	285	Sat	Sat	620000	717000	NA	Sat	740
75.0		Sat	Sat	Sat	Sat	Sat	182000	340000	7.8 (0°C)	Sat	168
100.0		Sat	Sat	—	<1	Sat	10000	50000	4 (0°C)	<1	<1
Point of Maximum Solubility		26%	Abt 50%	13.6% (1 atm)	28% (1 atm)	37%	—	—	—	11.7% (1 atm)	
Point(s) of Maximum Conductivity		26%	15%	2.67%	5.5%	18.5%	31% 92.5%	31%	Abt 35%	11.7%	19%
Maximum Conductivity		244000	1120 412000	1120 (18°C)	(18°C)	830000 852000	139000	862000	NA	66000	1600

If your test solution's major dissolved solids components are the same as any of these, you may want to choose the pre-made formulation that best approximates your test solution. Generally NaCl is used for brines and the 442 formulation is used for general water and waste water, rinse water, boilers and cooling towers, lakes, streams and wells.

Alternatively, if the contents of the ppm standard calibration solution used for calibration are known and if there are figures such as Figure 1 or tables such as Tables 1 and 2 available, you can cross reference the calibration standard

solution's "Conductivity to ppm TDS" curve to the curves for other types of dissolved solids solutions. Other curves and tables are available in various reference books.

The previous discussion and references are based on standard conditions of temperature (25°C). When measuring Conductivity or TDS in non-standard conditions, corrections for temperature variations must be taken into account before determining the final values of Conductivity and TDS measurements. Otherwise the measurements will not be correct.

Meters with temperature compensation overcome this problem, because they incorporate temperature sensing elements and temperature compensating circuitry into the meter so that the value displayed is corrected to a standard temperature. If your meter does not have temperature compensation, you need to use a look-up tables or formulas to correct for the temperature effect, or to calibrate the meter using a calibration standard that has been brought to the same temperature as the test solution.

Specific calibration methods depend on the meter, but they have some basic steps in common. Please consult the Operation Manual prior to start operating the meter which may dictate the variations in the operations of the meter form that particular manufacturer.

3. STANDARD SOLUTIONS

Suitable conductivity standards are available commercially or these can be made up as required from Analytical Grade reagents with reference to the relevant physical tables. To calibrate each range one should have at least one solution for that range. Normally a TDS/Conductivity meter having four or five ranges as such four or five calibration standards are required for calibration.

3.1 Procedure for preparing general purpose conductivity standard:

3.1.1 Accurately weigh out 0.746 grammes of dried AR grade Potassium Chloride (KCl).

3.1.2 Dissolve in 1 litre of good quality deionised water. This produces a 0.01N KCl solution with a conductivity of 1413 μS @ 25°C.

3.1 Storage

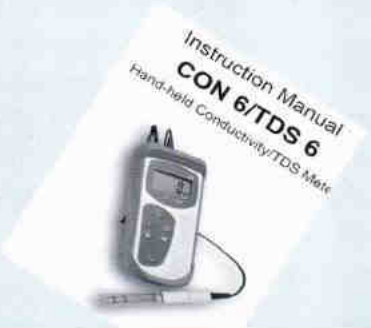
This solution must be stored in a plastic container and the air space should be kept to an absolute minimum. The shelf life of 1 week can be increased by storing below 4°C, but where any doubt exists about the viability of stored solution a fresh batch should be prepared.

			
84 µS/cm Standard	1413 µS/cm Standard	5000 µS/cm Standard	80,000 µS/cm Standard

4. TEMPERATURE CORRECTION

Because its temperature affects the voltage produced by the solution, you'll need to correct for the temperature. Most of the modern TDS/Conductivity meters perform this correction automatically, while others require that you enter the temperature into the meter using buttons or dials. Practically the temperature correction is automatic in the advanced meters available in the market having temperature compensating probe inside the electrode. Along with the TDS/Conductivity reading the reading of the temperature is also present.

For manual temperature correction, one MUST consult the Manual of the system supplied by the manufacturer. One should use the Standard solution for this correction. Rinse the electrode with water and dry it off, being careful not to rub hard enough to abrade the glass around the electrode. Submerge the electrode in the baseline standard solution, then turn the meter on and wait until the reading stabilizes. Once you set the meter to calibration mode, the more advanced meters detect the baseline solution and set the value themselves. If the meter doesn't do it automatically, you will be required to enter the value of the TDS/Conductivity manually. Confirm the Reading when prompted. The meter will associate the voltage being read with that particular standard.



5. MAINTENNACE OF A TDS/CONDUCTIVITY METER

After use rinse well the Conductivity/TDS Electrode using de-ionized water several times.

To make it dry just soak the probe using a lint free tissue paper but DO NOT rub them to avoid corrosion on the surface of the probe.

For long-term storage of the meter keep the electrode in the storage solution trapped inside a protective storage cap.

For short-time storage i.e. if you are using the meter again in the same day or in the next day it is NOT necessary to keep the electrode inside storage solution. In that case please keep the electrode submerged in the distilled water.

Please DO NOT KEEP SUBMERGED the electrode in any de-ionized water or any other acid or alkaline solution. Keeping in the neutral/natural water solution will help maintaining the sensor in a good and stable condition.

Storage solution is commercially available

6. FREQUENCY OF CALIBRATION

- It is recommended to calibrate the TDS/Conductivity meter prior to use at least once in a day. But if there is any problem with the response/voltage output of the sensor/electrode consultation with the qualified engineer is strongly recommended so that the calibration status of the system as well as the sensor condition is restored within the acceptable limit.
- Recalibrate if the battery of the pH Meter is replaced.
- After any maintenance and repairmen checking/verifying the performance as well as the calibration is a MUST.

7. CHECKING THE PERFORMANCE

After calibration please check the TDS/Conductivity of any fresh standard solution different from the solutions used for calibration. That means, avoid checking the TDS/Conductivity Meter by measuring the TDS/Conductivity of the same baseline calibration solutions. If the variation of the TDS/Conductivity result is within the acceptable tolerance limit e.g. 0.1 then the performance is considered to be OK. Calibration solutions or baseline solutions once used please do not return them again back in the standard reagent container. You may use the disposable standards transfer pipettes. This is to avoid the contamination of the stock standard solution. Discard the solutions after use. While using the standards in any beaker it is strongly recommended to cover the same by aluminum foil. Direct exposure to air may change the Standard Solution.

8. SAMPLE MEASUREMENT

Conductivity is a temperature dependent measurement. All substances have a conductivity coefficient which varies from 1%/°C to 3%/°C for most commonly occurring substances. The temperature coefficient defaults to 2%/°C, this being adequate for most routine determinations.

Conductivity readings varying with temperature may be due to the substances under test having a coefficient other than the typical value of 2%/°C. To eliminate this variation it is necessary to maintain all samples at the reference temperature by use of a thermostatic water bath or equivalent. In some meter, adjustment may be made by selecting % on the secondary display via the Mode key and then using the keys to the required value. Or one should consult the Operating Manual of the particular meter.

After calibration the measurement of samples is carried out by immersing the probe in the samples, allowing the reading to stabilise and recording the result. The probe should be rinsed in deionised water between each sample to avoid contamination, shaken to remove internal droplets, and the outside wiped (not rubbed, just soaked) using lint free tissue prior to immersion in the next sample.

9. A TYPICAL PROCEDURE TO CALIBRATE A TDS/CONDUCTIVITY METER

Note: This is only an example of calibrating the OAKTON TDS/Conductivity Meter. For other model please consult the manufacturer's operational manual.

9.1 For a full calibration i.e. calibration for all ranges of the meter you have to take Standards for all ranges. If the meter is having five ranges then take at least five Standards and some extra two or three Standards for checking performance of the meter after calibration.

9.2 For three point (say, for three ranges: 0-100, 101-1000, 1001-10000 µS/cm), take at least five clean glass/plastic containers that can hold sufficient Standards to immerse the TDS/Conductivity probe. One container containing the rinsing de-ionized water, three containing calibration solutions and one contains a Standard solution having TDS/Conductivity around the mid range (this one would be running as unknown for verifying the performance).

9.3 Perform a visual inspection of the probe. There should not be any crack, any salt deposit or any other matter on the probe. If any clean it using moist cotton swab. Or use lint free tissue. DO NOT RUB, just soak.

9.4 Rinse the probe electrode in water and briefly shake off or wipe clean with lint free tissue.

9.5 Switch the meter ON.

9.6 Place the probe in the Standard solution: 84 ?S/cm and allow the TDS/Conductivity reading to settle.

9.7 Press the CAL button. " CAL" will flash briefly and then the TDS/Conductivity reading will flash.

9.8 Press the HOLD/CON button. This will calibrate the meter to 84 µS/cm.

9.9 Rinse probe with water and shake or wipe clean with lint free tissue.

9.10 Place probe into Standard solution: 1413 µS/cm and allow the Meter reading to settle.

9.11 Press the CAL button. " CAL" will flash briefly and then the TDS/Conductivity reading will flash.

9.12 Press the HOLD/CON button. This will calibrate the meter to 1413 µS/cm.

9.13 Rinse the probe with distilled water and shake or wipe clean with lint free tissue.

9.14 Place probe into Standard 5000 µS/cm solution and allow the TDS/Conductivity reading to settle.

9.15 Press the CAL button. "CAL" will flash briefly and then the TDS/Conductivity reading will flash.

9.16 Press the HOLD/CON button. This will calibrate the meter to Conductivity 5000 µS/cm.

9.17 The TDS/Conductivity meter is now calibrated.

9.18 Checking performance. Measure the Conductivity of any other standard (known) solution as an unknown solution and verify whether the reading is within the acceptable tolerance limit e.g. 0.1 or not.

9.19 Submerge the Probe in the storage solution or distilled water.

9.20 The TDS/Conductivity Meter is now ready to measure the TDS/Conductivity of other substances.

10 SPECIFIC CALIBRATION EXAMPLE FOR OAKTON TDS/CONDUCTIVITY METER MODEL CON6/TDS6

10.1 Important Information on Meter Calibration

This Model OAKTON CON 6 has five measuring ranges. One can calibrate one point in each of the measuring ranges (up to five points). If some one is measuring values in more than one range, it is recommended that one should

make sure to calibrate each of the ranges one is measuring. The following table lists the corresponding conductivity and TDS ranges. One should calibrate each range using a solution that falls between the values in the "recommended calibration solution range" column.

Conductivity Range	Recommended Calibration Solution Range
0.00 → 20.00 µS	6.00 to 17.00 µS
0.0 → 200.0 µS	60.0 to 170.0 µS
0 → 2000 µS	600 to 1700 µS
0.00 → 20.00 mS	6.00 to 17.00 mS
0.0 → 200.0 mS	60.0 to 170.0 mS

TDS Range	Recommended Calibration Solution Range
0.00 → 10.00 ppm	3.00 to 8.50 ppm
10.0 → 100.0 ppm	30.0 to 85.0 ppm
100 → 1000 ppm	300 to 850 ppn
1.00 → 10.00 ppt	3.00 to 8.50 ppt
10.0 → 200 ppt	30.0 to 170 ppt

When some recalibrate the meter, old calibrations are replaced on a range basis. For example, if someone previously calibrated the conductivity meter at 1413 µS in the 0 to 2000 µS range and if this meter is now recalibrated at 1500 µS (also in the 0 to 2000 µS range), the meter will replace the old calibration data (1413 µS) in that range. But the meter will retain all previous calibration data in other ranges.

To completely recalibrate the meter, or when someone use a replacement probe, it is best to clear all calibration data. To erase all the old conductivity or TDS calibration data completely, see the instruction manual containing Section for "Restore Factory Default Values".

10.2 Preparing the Meter for Calibration

- Before starting calibration, make sure that you are in the correct measurement mode.
- For best results, select a standard value close to the sample value you are measuring.
- Alternatively use a calibration solution value that is approximately 2/3 the full-scale value of the measurement range you plan to use. For example, in the 0 to 2000 µS conductivity range, use a 1413 µS solution for calibration.
- Calibrate to all measurement ranges to ensure the highest accuracy throughout all measurement range.
- Note that CON 6/ TDS 6 will not accept calibration values less than 40 µS/cm (20 ppm).
- All new calibration values will automatically override existing data.

■ If you are measuring in solutions with Conductivity lower than 100 µS/cm or TDS lower than 50 ppm, calibrate the meter at least once a week to get good accuracy.

■ If you are measuring in the mid ranges and you wash the probe in deionized water and store it dry, calibrate the meter once a month.

■ If you take measurements at extreme temperatures, calibrate at least once a week.

■ Ensure that you use new Conductivity standard solutions or sachets during calibration.

■ Do not reuse standard solutions as it may be contaminated and affect the calibration and accuracy of measurements.

■ Use fresh calibration solution each time you calibrate your meter.

■ Store solutions in a dry and cool environment if possible.

■ Always rinse the probe with either deionized water or rinse solution before and after each calibration/sample measurement to avoid cross-contamination.

■ For any specific instruction or note , example below, please consult the Instruction Manual.

NOTE: These meters are factory set to a temperature coefficient of 2.1% per °C. For most applications this will provide good results.
To set the temperature coefficient to different value, see Section 6.5 – Temperature Coefficient.
Also, see Addendum 3 - Calculating Temperature Coefficient to determine the appropriate temperature coefficient for your solution.

NOTE: The factory default value for normalization temperature is 25 °C. If you need to normalize to a value other than 25 °C, see Section 6.6 – Normalization Temperature.

10.3 Selection of Automatic or Manual Calibration

■ This meter is capable of performing either automatic (only CON 6) or manual calibration.

■ In the automatic calibration mode, the meter (only CON 6) automatically detects

and verifies the appropriate known calibration standards solutions being calibrated before accepting these particular calibration standards as one of its calibration values in a specific measurement range.

■ This automatic calibration mode frees you from cumbersome calibration procedure.

■ The known calibration standards used for automatic calibration are:

Temperature Coefficient (tc °C)	Calibration Standards (Range)
25 °C	1. 84 µS (for 0 – 200 µS/cm) 2. 1413 µS (for 0 – 2000 µS/cm) 3. 12.88 mS (for 0.00 – 20.00 mS/cm) 4. 111.8 mS (for 0.0 – 200.0 mS/cm)
20 °C	1. 76 µS (for 0 – 200 µS/cm) 2. 1278 µS (for 0 – 2000 µS/cm) 3. 11.67 mS (for 0.00 – 20.00 mS/cm) 4. 102.1 mS (for 0.0 – 200.0 mS/cm)

■ In the manual calibration, non-standard calibration values can be used for calibration. You can manually input the appropriate values as your desired calibration standards in each specific range. This is useful when you have a customized calibration standard specifically unique for your application.

■ To select Automatic or Manual Calibration settings, refer to Instruction Manual Section 6.3 - Automatic Calibration for more information.

10.4 Automatic Calibration

■ In the Automatic Calibration mode, the meter is capable of accepting either single-point or up to 4 points for multi-point calibration with maximum of 1 point per specific measurement range.

10.4.1 Press MODE key and select CONDUCTIVITY mode

10.4.2 Rinse the probe thoroughly with de-ionized water or a rinse solution, then rinse with a small amount of calibration standard. [Note: For Automatic Calibration you must use one of the calibration standards listed in the table above]

10.4.3 Dip the probe into the calibration standard. Immerse the probe tip beyond

the upper steel band. Stir the probe gently to create a homogeneous sample. Allow time for the reading to stabilize.

10.4.4 Press CAL key to enter conductivity calibration mode. The [CA] indicator will appear for 1.5 seconds, and a value will appear flashing. [NOTE: To exit calibration without confirmation, press CAL key again to go back to measurement mode.]

10.4.5 Wait for the value to stabilize and press ENTER key. The calibration standard value will appear for 3 seconds. If the calibration is successfully performed, a [donE] will be displayed for about 3 seconds, and the meter returns to measurement mode.

10.4.6 To perform the next point calibration in the multi-point calibration, repeat step 10.4.1 to 10.4.5 again until all points have been calibrated if necessary.

10.4.7 The meter is now calibrated. Please check the performance by measuring the Conductivity value of any Standard solution running as an unknown one.

10.4.8 The Meter is ready for use.

IMPORTANT NOTES:

1. *Meter allows a tolerance range of ±40% of its calibration standard. An error message "**Err 1**" will be displayed for 3 seconds if you attempt to calibrate with a solution whose value is outside the tolerance range.*
   For instance: For 1413 µS conductivity calibration standard, 40% tolerance is from 848 µS to 1978 µS.
2. *If the temperature (t °C) of the conductivity calibration solution is below 0 °C or above 50 °C (0°C < t °C > 50 °C), an error message "**Err 2**" will be displayed when performing the auto calibration, and meter will return to measurement mode.*
3. *All new calibration data will over-ride existing stored calibration data for each measuring range calibrated.*
4. *It is important to use new conductivity calibration standards.*
5. *Low conductivity standard solution (less than 20 µS /cm) cannot be available easily. Such low conductivity standard will be contaminated as soon as it is exposed to the air therefore exercise caution during calibration in the first measurement range (0.00 to 20.0 µS /cm).*

11 GOOD PRACTICE GUIDELINES

- The presence of particulate matter in the sample can lead to unstable and non-reproducible results. If necessary filter, or allow the particles to settle prior to immersion.
- Ensure no air bubbles are trapped in the measuring cell. Gentle agitation of the cell should ensure that bubbles are purged.
- The entire plate area must be immersed in the solution under test. The slots in the side of the sensor should be below the surface.
- It is advisable to clean the sensor if contamination is evident. This should be approached in a progressive manner, beginning with deionised water and progressing to other solvents or a soft air brush if the deposits persist. The carbon plates can be damaged and should not come into contact with anything which is likely to abrade their surface.
- The temperature coefficient is very dependent on the solution being measured and its concentration level. The effect of temperature change on conductivity can be very significant, and if the temperature coefficient is not known it is wise to measure all samples at the same temperature.
- The TDS mode displays results which have been calculated from the measurement of conductivity. The salinity mode assumes that the major constituent responsible for the conductivity of the solution is sodium chloride. If significant quantities of other conductive species are present then the displayed results could be inaccurate. Likewise, the TDS mode assumes some knowledge of the electrolyte balance of the analyte. The EC ratio parameter found in the set up routine allows selection of a factor suitable for the solution under test. Most analysers which do not offer this option use a default value of 0.6.

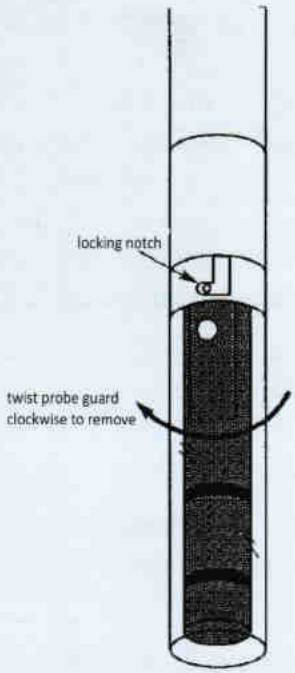
12 PROBE CARE AND MAINTENANCE

- Keep the conductivity probe clean.
- Rinse the probe twice, and gently swirl it while you take readings. For best accuracy, soak a dry probe for at least 5 to 10 minutes or longer before calibration.
- Rinse the probe with deionized or distilled water before storing.

- Never scratch the bands with a hard substance.
- Do not strike the probe against any hard surface.
- Do not make continuous contact with your solutions. Readings will rise over a period of time while you soak your probe.
- Do not immerse the probe in oily solutions.
- Clean the electrode thoroughly by stirring it in a mild detergent bath or isopropyl alcohol.
- Wipe the probe with a soft lint free tissue paper.
- Rinse thoroughly in tap water and then in deionized water.
- Recalibrate the meter after cleaning the probe.
- Some times conductivity probe comes with a removable probe guard to make cleaning easy.
- To remove probe guard:

a. Grip yellow probe guard and twist clockwise. The locking notch will release.

b. Slide probe guard off end of probe. [Note: Remember to re-attach the probe guard prior to taking readings. Failure to do so could result in erroneous readings.]



13 EXAMPLE OF TECHNICAL FEATURES OF A TYPICAL TDS/CONDUCTIVITY METER: OAKTON MODEL CON 6/TDS 6

SPECIFICATIONS	DESCRIPTIONS	CON 6	TDS 6
Conductivity Range	0 to 20.00, 200.0, 2000 µS/cm; 0 to 20.00, 200.0 mS/cm	•	
Resolution	0.01, 0.1, 1 µS/cm; 0.01, 0.1 ,S/cm		
Accuracy	±1% F.S.		
TDS Range	0 to 10.00, 10.0 to 100.0, 100 to 1000 ppm; 1.00 to 10.00, 10.00 to 100.0, Up to 200 ppt depending on the TDS factor setting.		•
Resolution	0.01, 0.1, 1 ppm; 0.01, 0.1 ppt	•	•
Accuracy	±1% F.S.	•	•
Temperature Range	-10.0 to 110.0 °C	•	•
Resolution/Accuracy	0.1 °C / ⊥ 0.5 for °C	•	•
Cell Constant	0.1, 1.0, 10.0 (selectable)	•	•
Temperature Compensation	Automatic / Manual (from 0 to 50 °C)	•	•
Temperature Coefficient	0.0 to 3.0% / °C	•	•
Normalization Temperature	20.0 °C and 25.0 °C (selectable)	•	•
Conductivity to TDS Conversion factor	0.4 to 1.0		•
Number of calibration points	5: Maximum 1 per range	•	•

CHAPTER 5

CALIBRATION OF UV-VIS SPECTROSCOPY SYSTEM

1. INTRODUCTION

Absorption Spectroscopic methods of analysis rank among the most widespread and powerful tools for quantitative analysis. The use of a spectrophotometer to determine the extent of absorption of various wavelengths of visible light by a given solution is commonly known as colorimetry. This method is used to determine concentrations of various chemicals which can give colours either directly or after addition of some other chemicals.

As an example, in the analysis of phosphate, a reaction with orthophosphate is made, to form the highly coloured molybdenum blue compound. The light absorption of this compound can then be measured in a spectrophotometer. Some compounds absorb light in other than the visible range of the spectrum. For example, nitrates absorb radiation of 220 nm wave length in the UV region.

Many of these were inorganic minerals, but several important organic dyes were also known. These included the crimson pigment, kermesic acid, the blue dye, indigo, and the yellow saffron pigment, crocetin. A rare dibromo-indigo derivative, punicin, was used to color the robes of the royal and wealthy. The deep orange hydrocarbon carotene is widely distributed in plants, but is not sufficiently stable to be used as permanent pigment, other than for food coloring. A common feature of all these colored compounds, displayed below (**Fig 3**) , is a system of **extensively conjugated pi-electrons**.

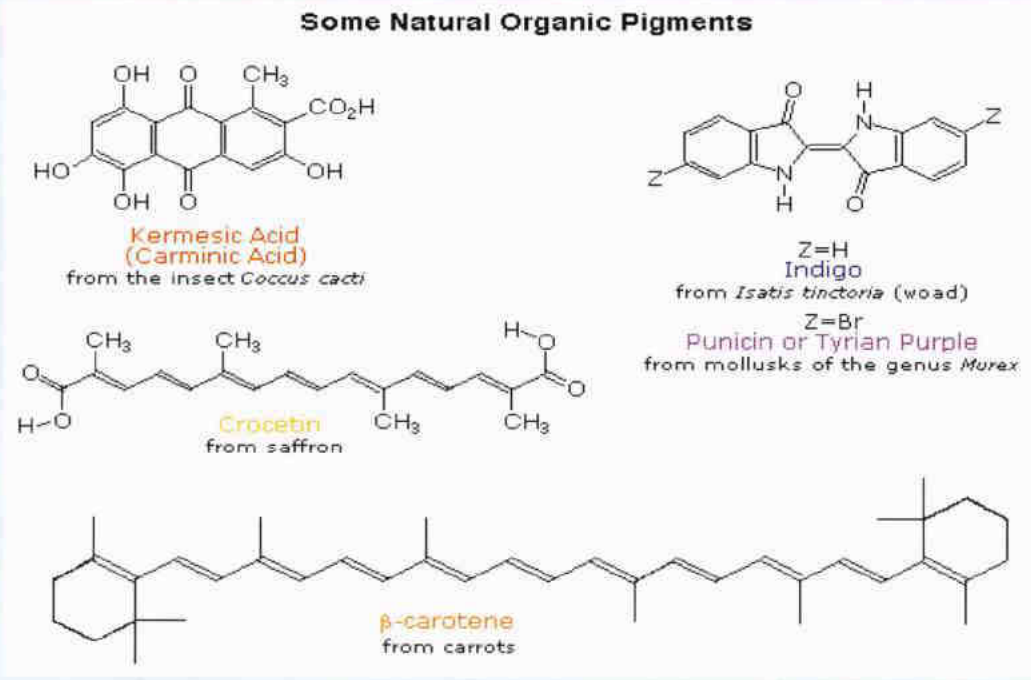


Fig 3: Natural Organic Pigments or colored compounds

2.1 The Electromagnetic Spectrum

The visible spectrum constitutes but a small part of the total radiation spectrum. Most of the radiation that surrounds us cannot be seen, but can be detected by dedicated sensing instruments. This **electromagnetic spectrum** ranges from very short wavelengths (including gamma and x-rays) to very long wavelengths (including microwaves and broadcast radio waves). The following chart displays many of the important regions of this spectrum, and demonstrates the inverse relationship between wavelength and frequency (shown in the top equation below the chart).

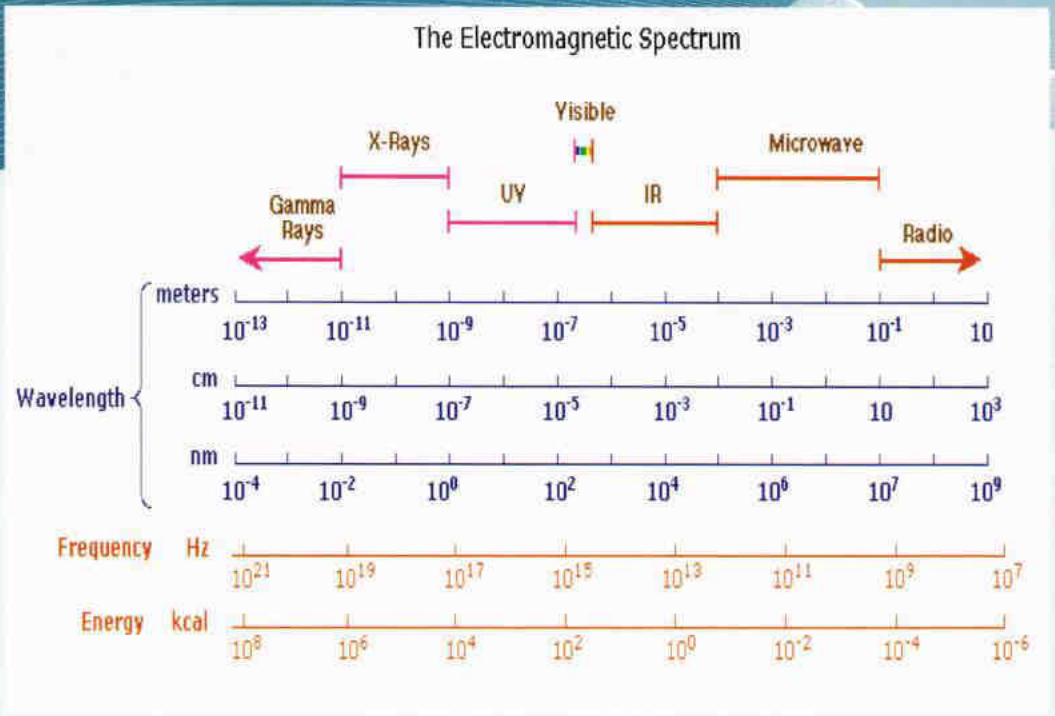


Fig 4: Electromagnetic Spectrum beyond the visible range

The energy associated with a given segment of the spectrum is proportional to its frequency. The bottom equation describes this relationship, which provides the energy carried by a photon of a given wavelength of radiation.

$V = c / \lambda$ where, V = frequency, λ = wavelength, c = velocity of light ($c = 3*10^{10}$ cm/sec)

$\Delta E = h V$ where, E = Energy, V = frequency, h= Plank's constant ($h = 6.6*10^{-27}$ erg-sec)

To obtain specific frequency, wavelength and energy values these relations are used.

2.3 UV-Visible Absorption Spectra

To understand why some compounds are colored and others are not, and to determine the relationship of conjugation to color, we must make accurate measurements of light absorption at different wavelengths in and near the visible part of the spectrum. Commercial optical spectrometers enable such experiments to be conducted with ease, and usually survey both the near ultraviolet and visible portions of the spectrum.

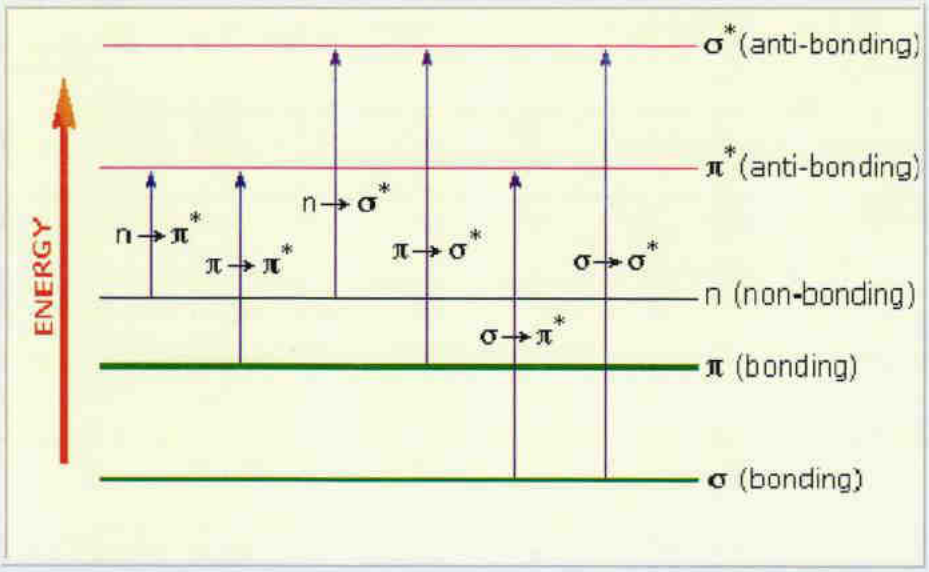


Fig 5: π and **σ**-electron transitions among the quantum mechanical energy levels

The visible region of the spectrum comprises photon energies of 36 to 72 kcal/mole, and the near ultraviolet region, out to 200 nm, extends this energy range to 143 kcal/mole. Ultraviolet radiation having wavelengths less than 200 nm is difficult to handle, and is seldom used as a routine tool for structural analysis.

The energies noted above are sufficient to promote or excite a molecular electron to a higher energy orbital. Consequently, absorption spectroscopy carried out in this region is sometimes called "electronic spectroscopy". A diagram (**Fig 5**) showing the various kinds of electronic excitation that may occur in organic molecules is shown on the left. Of the six transitions outlined, only the two lowest energy ones (left-most, colored blue) are achieved by the energies available in the 200 to 800 nm spectrum. As a rule, energetically favored electron promotion will be from the **highest occupied molecular orbital (HOMO)** to the **lowest unoccupied molecular orbital (LUMO)**, and the resulting species is called an **excited state**.

When sample molecules are exposed to light having an energy that matches a possible electronic transition within the molecule, some of the light energy will be absorbed as the electron is promoted to a higher energy orbital. An optical spectrometer records the wavelengths at which absorption occurs, together with the degree of absorption at each wavelength. The resulting spectrum is presented as a graph of absorbance (A) versus wavelength, as in the isoprene spectrum shown below. Since isoprene is colorless, it does not absorb in the visible part of the spectrum and this region is not displayed on the graph. **Absorbance** usually ranges from 0 (no absorption) to 2 (99% absorption), and is precisely defined in context with spectrometer operation.

Because the absorbance of a sample will be proportional to the number of absorbing molecules in the spectrometer light beam (e.g. their molar concentration in the sample tube), it is necessary to correct the absorbance value for this and other operational factors if the spectra of different compounds are to be compared in a meaningful way. The corrected absorption value is called "molar absorptivity", and is particularly useful when comparing the spectra of different compounds and determining the relative strength of light absorbing functions (chromophores).

Molar absorptivity (ε) is defined as:

Molar Absorptivity, $\varepsilon = A / CL$

Where,

A= Absorbance,

C = sample concentration in moles/liter

L = length of light path through the sample in cm

If the isoprene spectrum on the right was obtained from a dilute hexane solution ($C = 4 * 10^{-5}$ moles per liter) in a 1 cm sample cuvette, a simple calculation using the above formula indicates a molar absorptivity of 20,000 at the maximum absorption wavelength. Indeed the entire vertical absorbance scale may be changed to a molar absorptivity scale once this information about the sample is in hand.

Chromophore	Example	Excitation	λ_{max} nm	ε	Solvent
C=C	Ethene	$\pi \rightarrow \pi^*$	171	15,000	hexane
C≡C	1-Hexyne	$\pi \rightarrow \pi^*$	180	10,000	hexane
C=O	Ethanal	$n \rightarrow \pi^*$ $\pi \rightarrow \pi^*$	290 180	15 10,000	hexane hexane
N=O	Nitromethane	$n \rightarrow \pi^*$ $\pi \rightarrow \pi^*$	275 200	17 5,000	ethanol ethanol
C-X X=Br X=I	Methyl bromide Methyl Iodide	$n \rightarrow \sigma^*$ $n \rightarrow \sigma^*$	205 255	200 360	hexane hexane

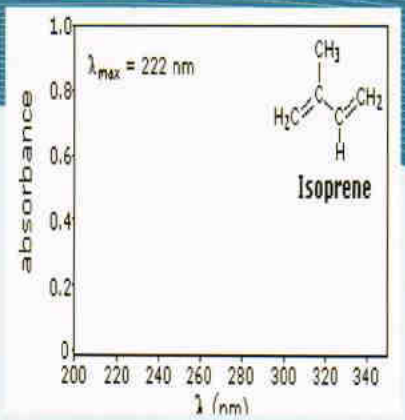


Fig 6: Wavelengths of the energies absorbed or emitted due transitions of the bond electrons and the corresponding molar absorption coefficients (showing different bond energies C=C, N=O, C-X, X-Br, X-I)

From the chart above it should be clear that the only molecular moieties likely to absorb light in the 200 to 800 nm region are pi-electron functions and hetero atoms having non-bonding valence-shell electron pairs. Such light absorbing groups are referred to as **chromophores**. A list of some simple chromophores and their light absorption characteristics is provided on the left above. The oxygen non-bonding electrons in alcohols and ethers do not give rise to absorption above 160 nm. Consequently, pure alcohol and ether solvents may be used for spectroscopic studies.

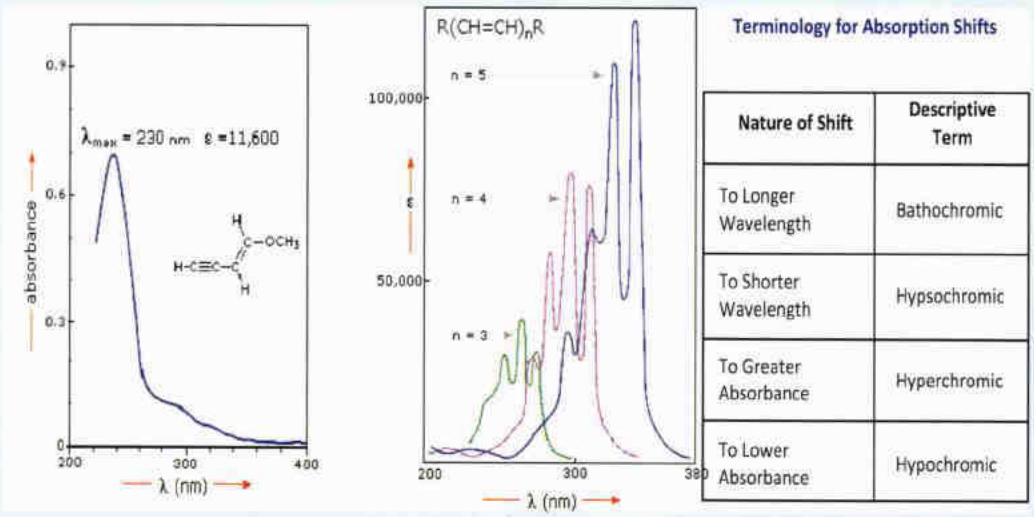
The presence of chromophores in a molecule is best documented by UV-Visible spectroscopy, but the failure of most instruments to provide absorption data for wavelengths below 200 nm makes the detection of isolated chromophores problematic. Fortunately, conjugation generally moves the absorption maxima to longer wavelengths, as in the case of isoprene, so conjugation becomes the major structural feature identified by this technique.

Molar absorptivities may be very large for strongly absorbing chromophores (>10,000) and very small if absorption is weak (10 to 100). The magnitude of ε reflects both the size of the chromophore and the probability that light of a given wavelength will be absorbed when it strikes the chromophore.

3.4 The Importance of Conjugation

A comparison of the absorption spectrum of 1-pentene, λ_{max} = 178 nm, with that of isoprene (above) clearly demonstrates the importance of chromophore conjugation. Further evidence of this effect is shown below. The spectrum on the left illustrates that conjugation of double and triple bonds also shifts the absorption maximum to longer wavelengths. From the polyene spectra displayed in the center diagram, it is clear that each additional double bond in the conjugated pi-electron system shifts the absorption maximum about 30 nm in the same direction. Also, the molar absorptivity (ε) roughly doubles with each new conjugated double bond. Spectroscopists use the terms defined in the table on the right when describing shifts in absorption. Thus, extending conjugation generally results in bathochromic and hyperchromic shifts in absorption.

The appearance of several absorption peaks or shoulders for a given chromophore is common for highly conjugated systems, and is often solvent dependent. This fine structure reflects not only the different conformations such systems may assume, but also electronic transitions between the different vibrational energy levels possible for each electronic state. Vibrational fine structure of this kind is most pronounced in vapor phase spectra, and is increasingly broadened and obscured in solution as the solvent is changed from hexane to methanol.



Nature of Shift	Descriptive Term
To Longer Wavelength	Bathochromic
To Shorter Wavelength	Hypsochromic
To Greater Absorbance	Hyperchromic
To Lower Absorbance	Hypochromic

Fig 7: Solvent effects or shift of wavelength due to solvents

To understand why conjugation should cause bathochromic shifts in the absorption maxima of chromophores, we need to look at the relative energy levels of the pi-orbitals. When two double bonds are conjugated, the four p-atomic orbitals combine to generate four pi-molecular orbitals (two are bonding and two are antibonding). This can only understand properly after applying some quantum mechanical treatments which is beyond the limit of this article. In a similar manner, the three double bonds of a conjugated triene create six pi-molecular orbitals, half bonding and half antibonding. The energetically most favorable $\pi \rightarrow \pi^*$ excitation occurs from the highest energy bonding pi-orbital (**HOMO**) to the lowest energy antibonding pi-orbital (**LUMO**).

following diagram illustrates this excitation for an isolated double bond (only two pi-orbitals) and, on clicking the diagram, for a conjugated diene and triene. In each case the HOMO is colored blue and the LUMO is colored magenta. Increased conjugation brings the HOMO and LUMO orbitals closer together. The energy (ΔE) required to effect the electron promotion is therefore less, and the wavelength that provides this energy is increased correspondingly (remember $\lambda = h \bullet c/\Delta E$).

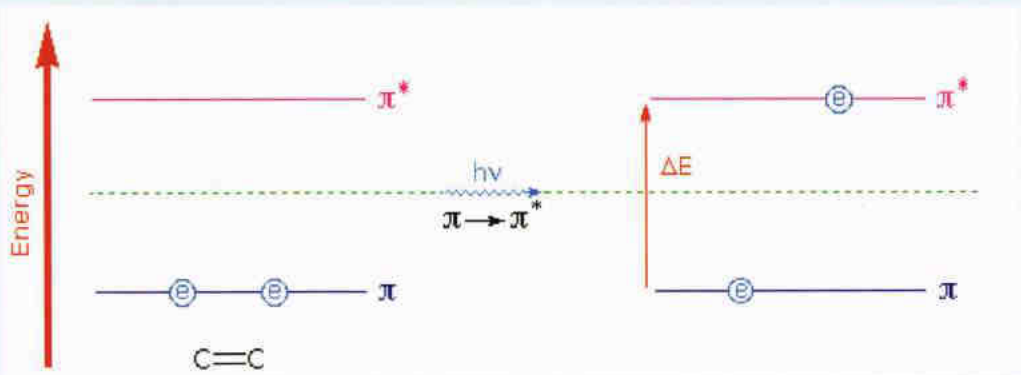


Fig 8: Examples of $\pi \rightarrow \pi^*$ Excitation

Many other kinds of conjugated pi-electron systems act as chromophores and absorb light in the 200 to 800 nm region. These include unsaturated aldehydes and ketones and aromatic ring compounds. A few examples are displayed below (**Fig 9**) . The spectrum of the unsaturated ketone (on the left) illustrates

the advantage of a logarithmic display of molar absorptivity. The $\pi \rightarrow \pi^*$ absorption located at 242 nm is very strong, with an $\varepsilon = 18{,}000$. The weak $n \rightarrow \pi^*$ absorption near 300 nm has an $\varepsilon = 100$.

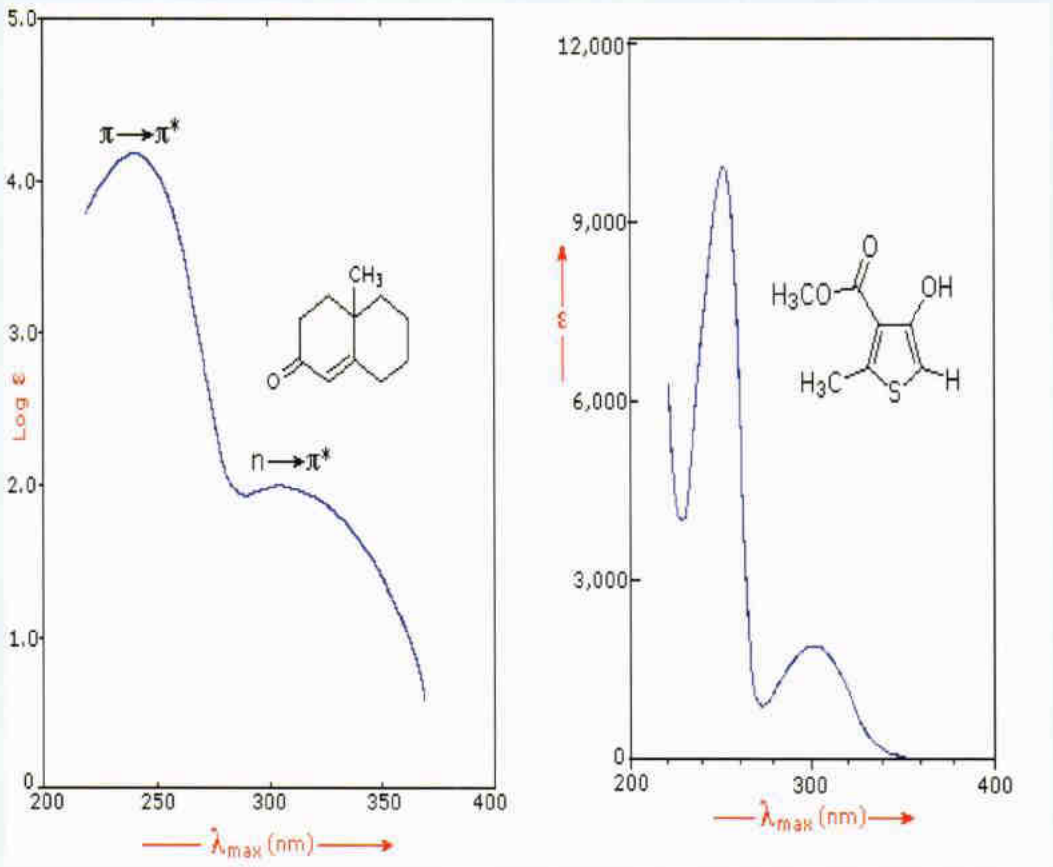


Fig 9: Absorptions for aromatic rings of benzene and other aromatic compounds

Benzene exhibits very strong light absorption near 180 nm (ε > 65,000), weaker absorption at 200 nm (ε = 8,000) and a group of much weaker bands at 254 nm (ε = 240). Only the last group of absorptions are completely displayed because of the 200 nm cut-off characteristic of most spectrophotometers. The added conjugation in naphthalene, anthracene and tetracene causes bathochromic shifts of these absorption bands, as displayed in the chart on the left below. All the absorptions do not shift by the same amount, so for anthracene (green shaded box) and tetracene (blue shaded box) the weak absorption is obscured by stronger bands that have experienced a greater red shift. As might be expected from their spectra, naphthalene and anthracene are colorless, but tetracene is orange.

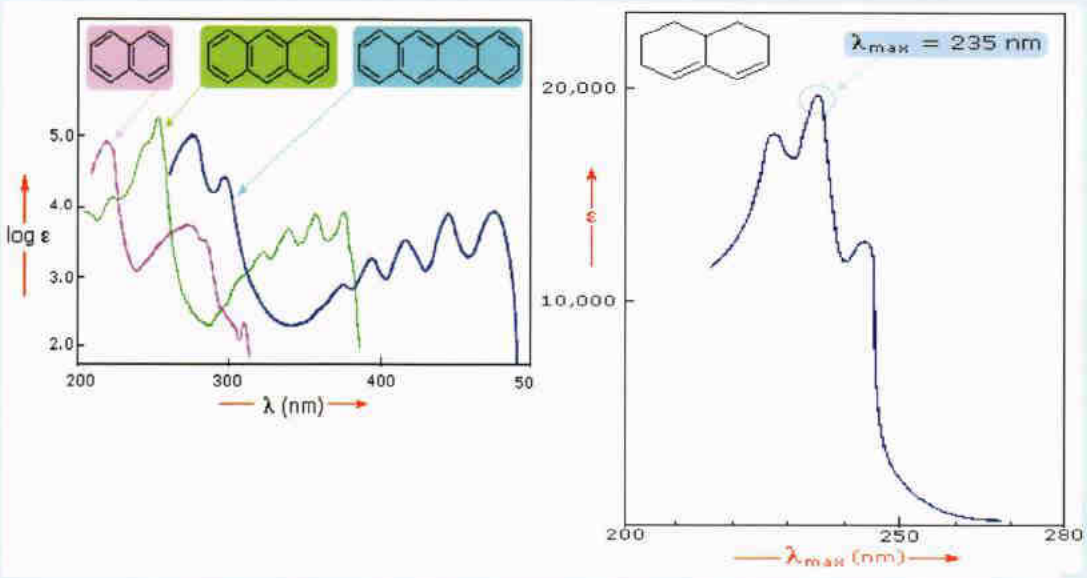


Fig 10: Spectra of bicyclic diene and isoprene

The spectrum of the bicyclic diene (above right) shows some vibrational fine structure, but in general is similar in appearance to that of isoprene, shown above. Closer inspection discloses that the absorption maximum of the more highly substituted diene has moved to a longer wavelength by about 15 nm. This "substituent effect" is general for dienes and trienes, and is even more pronounced for enone chromophores.

1. SIMPLE QUANTIFICATION IN UV-VIS SPECTROSCOPY

Absorption Spectroscopic methods of analysis are based upon the fact that compounds ABSORB light radiation of a specific wavelength. In the analysis, the amount of light radiation absorbed by a sample is measured. The light absorption is directly related to the concentration of the colored compound in the sample.

The wavelength (λ) of Maximum Absorption is known for different compounds. For example, the coloured compound formed for analysis of Phosphate (molybdenum blue) has maximum light absorption at λ = 640 nm. Conversely, a minimum amount of light is transmitted through the compound at λ = 640 nm. This is shown schematically in **Fig 11.**

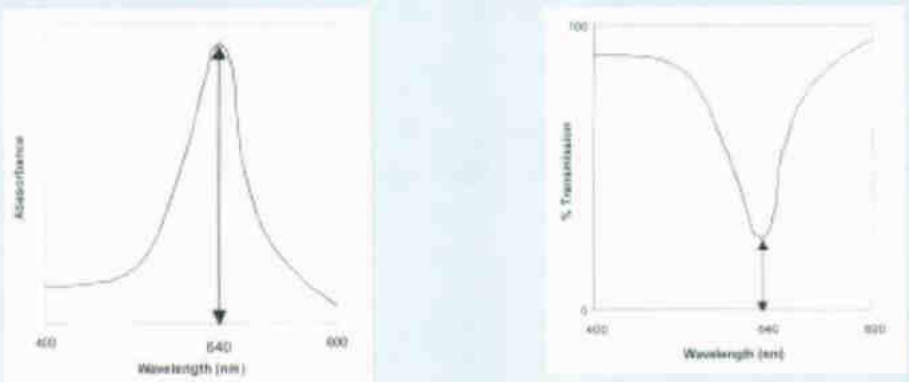


Fig 11: Light Absorption and Transmission by Phosphate-molybdenum blue compound. Schematic diagram showing maximum light absorption (and minimum light transmission) at λ = 640 nm.

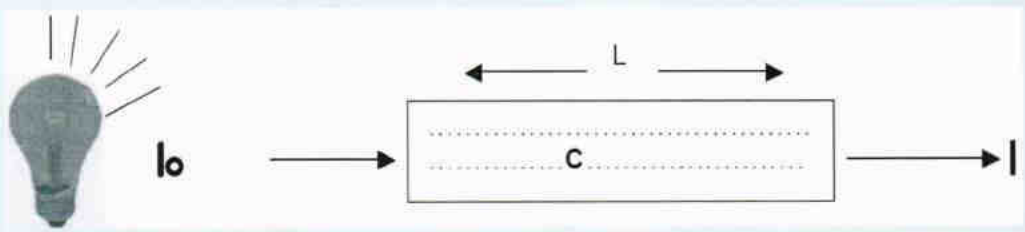


Fig 12: Light energy of Intensity 'I_0' passes through a sample with concentration '**C**'. Some light energy is absorbed by the sample. The amount of light energy exiting the sample has Intensity '**I**'

Then the famous ***Beer-Lambert Law*** sates that:

Light Absorbance **(A) = log (I_0 / I)= KCL**

Or

Light Transmission **(T) = I_0 / I = 10^{-KCL}**

Where,

- **I_0** = Light Intensity entering a sample
- **I** = Light Intensity exiting a sample is
- **C=** The concentration of analyte in sample
- **L=** The length of the light path in glass sample cuvette
- **K=** a constant for a particular solution and wave length

Plots of absorbance (A) and transmission (T) versus concentration (C) of the analyte in sample according to the above equations is shown in **Fig 13**.

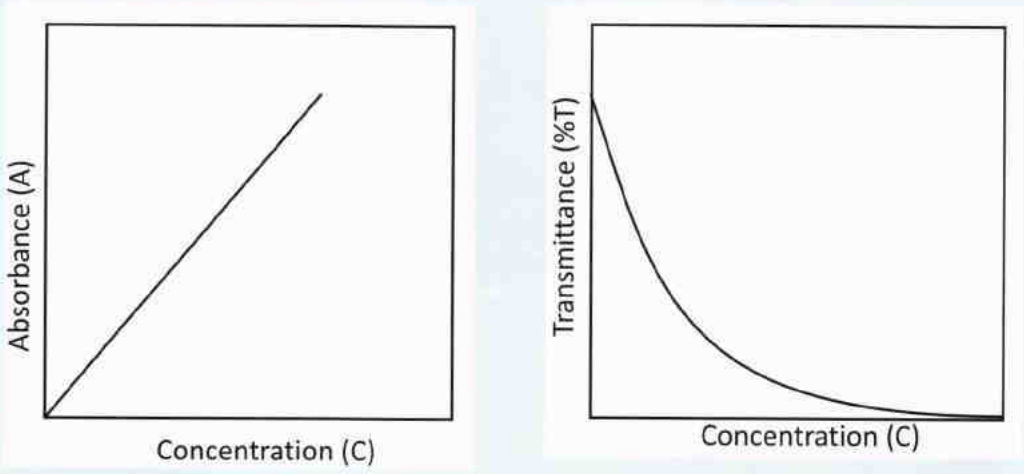


Fig 13: Beer-Lambert Law relates the amount of light Absorbance (A) by a solution to the Concentration (C) of a compound in solution and the length of light path L.

From the Equation and the graph it is clear that:

- If Concentration (C) increases, light Absorption (A) increases, linearly .
- If Concentration (C) increases, light Transmission (T) decreases, exponentially

1. THE BASIC COMPONENTS OF THE SPECTROMETER

All spectrophotometer instruments designed to measure the absorption of radiant energy have the basic components as follows (**Fig 14**):

- a stable source of radiant energy (Light);
- a wavelength selector to isolate a desired wavelength from the source (filter or monochromator);
- transparent container (cuvette) for the sample and the blank;
- a radiation detector (phototube) to convert the radiant energy received to a measurable signal; and a readout device that displays the signal from the detector.

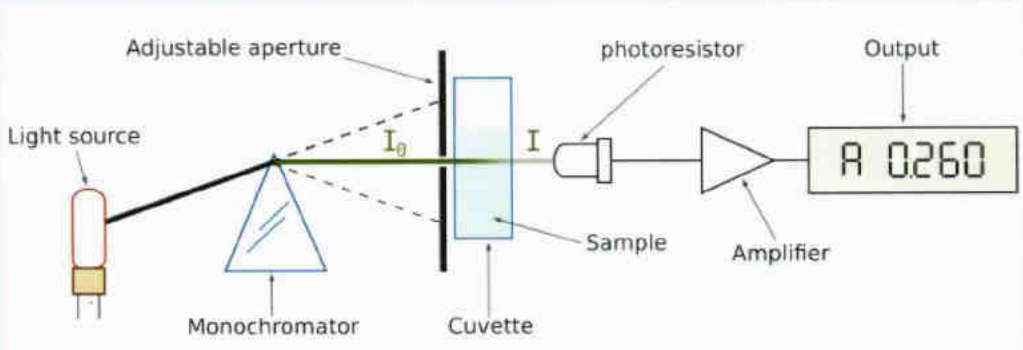


Fig 14: Components of a spectrophotometer

The energy source is to provide a stable source of light radiation, whereas the wavelength selector permits separation of radiation of the desired wavelength from other radiation. Light radiation passes through a glass container with sample. The detector measures the energy after it has passed through the sample. The readout device calculates the amount of light absorbed by the sample displays the signal from the detector as absorbance or transmission.

The spectrophotometers which are used for such measurements may vary from simple and relatively inexpensive colorimeters to highly sophisticated and expensive instruments that automatically scan the ability of a solution to absorb radiation over a wide range of wavelengths and record the results of these measurements.

One instrument cannot be used to measure absorbance at all wavelengths because a given energy source and energy detector is suitable for use over only a limited range of wavelengths.

True linearity between absorbance and concentration according to Beer-Lambert Law requires the use of monochromatic light. In addition, a narrow band of light ensures a greater selectivity since substance with absorption peaks in other close by wavelengths are less likely to interfere. Further, it increases sensitivity as there is a greatest change in absorbance per increment of change in concentration. Both filters and monochromators are used to restrict the radiation wavelength. Photometers make use of filters, which function by absorbing large portions of the spectrum while transmitting relatively limited wavelength regions. Spectrophotometers are instruments equipped with monochromators that permit the continuous variation and selection of waveiength.

The effective bandwidth of a monochromator that is satisfactory for most applications is about from 1 to 5 nm. The sample containers, cells or cuvettes, must be fabricated from material that is transparent to radiation in the spectral region of interest. The commonly used materials for different wave length regions are:

- Quartz or fused silica: UV(190 nm) to 20000 nm (2 mm) in IR (Infrared)
- Silicate glass: Above 350 nm to 20000 nm (2 mm) in I R (infrared)
- Plastic: 400-700 nm in visible region
- Polished NaCI or AgCI: Wavelengths longer than 20000 nm (2 mm)

Cuvettes or cells are provided in pairs that have been carefully matched to make possible the transmission through the solvent and the sample. Accurate spectrophotometric analysis requires the use of good quality, matched cells. These should be regularly checked against one another to detect differences that can arise from scratches, etching and wear. The most common cell path for UV-visible region is **L=1 cm**. For reasons of economy, cylindrical cells are frequently used. Care must be taken to duplicate the position of such cells with respect to the light path; otherwise, variations in path length and in reflection losses will introduce errors.

1. GENERAL MEASUREMENT PROCEDURES

As explained above, the ***Beer-Lambert Law*** forms the basis of the measurement procedure. The amount of light radiation absorbed by a compound is directly related to the concentration (C) of the compound.

The general measurement procedure consists of 5 steps:

- Step-1: Prepare samples to make coloured compound
- Step-2: Make series of standard solutions of known concentrations and treat them in the same manner as the sample for making coloured compounds
- Step-3: Set spectrophotometer to l of maximum light absorption
- Step-4: Measure light absorbance of standards

Step-5: Plot standard curve: Absorbance vs. Concentration, as shown in **Fig 15**. Once the standard plot is made, it is simple to find the concentration of an unknown sample:

- Measure the absorption of the unknown, and from the standard plot, read the related concentration (**Fig 16**).

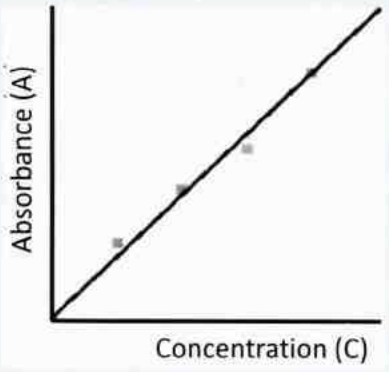


Fig 15: Plot of the standard calibration curve: showing the linear relation between light absorption (A) and concentration (C) of the Standards

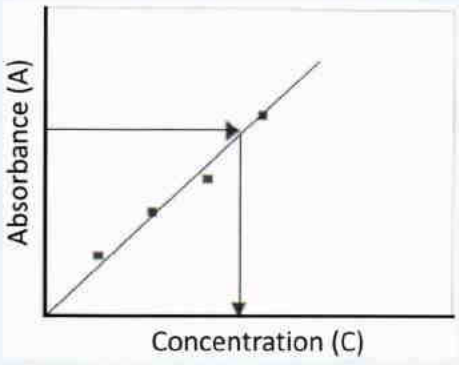


Fig 16: Finding the concentration of an unknown sample from the calibration curve produced by the standard

Due to the fact that the overall composition of the sample is seldom the same as that of the calibration standard, in some cases, the absorption characteristics of the two may differ. Where such discrepancy is suspected, the standard addition approach may be used, Here, a known amount of analyte is added to a second aliquot of the sample. The difference in absorbance is used to calculate the analyte concentration of the sample as illustrated in the example below.

1. A SIMPLE EXAMPLE OF ANALYSIS

A 25 mL sample after treatment with reagents to generate colour for measurement of phosphate yielded absorbance of 0.428. Addition of 1.00 mL of a solution containing 5.0 µg phosphorus to a second 25 mL aliquot and development of colour resulted in an absorbance of 0.517. Calculate µg phosphorus in each mL of sample.

Solution:

Correct absorbance for dilution:

- Corrected absorbance = 0.517 (26.0/25.0) = 0.538
- Absorbance caused by 5 µg phosphorus = 0.538 - 0.428 = 0.110

Therefore, amount of phosphorus in the 25 mL sample = (5.0/0.11) * 0.428 (µg) = 19.5 µg

- Or The Concentration C= 19.5/25 µg/mL = 0.7781 µg/mL = 0.8 µg/mL
- Or C = 0.8 ppm
-

2. ENVIRONMENTAL REQUIREMENTS

- The temperature in the laboratory must be maintained at 24 °C ± 4 °C and the Relative Humidity must not exceed 65%.
- Before running analysis warm up the system for at least 30 minutes.

7 SOP FOR OPERATIONAL QUALIFICATION/PERFORMANCE QUALIFICATION (OQ/PQ)

Note: This is only an example *for Shimadzu Model* 1601 PC. For other model one must consult the manufacturer's operational manual.

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7.1 Test Overview for OQ/PQ

Following is a list of tests pertaining to the verification of the UV-1601PC as per JP & EP/BP

Sl.No.	Test Name	Test Purpose	Initial & Date
1	System initialization Test	Verify Current ROM Checksum Version	
2	Baseline Stability.	Verify stability of baseline.	
3	Wavelength Accuracy Test.	Verify wavelength accuracy using internal D2 Lamp.	
4	Wavelength Repeatability Check	Verify wavelength repeatability using internal D2 Lamp.	
5	Wavelength Repeatability check	Verify wavelength repeatability using standard didymium filter.	
6	Control of Absorbance	Checking specific absorbance of $K_2Cr_2O_7$ peaks at 235, 257, 313 & 350 nm.	
7	Limit of Stray Light	Checking stray light analyzing absorbance of (12g /L) potassium Chloride KCl solution.	
8	Checking Resolution of the system.	Checking resolution of the system using 0.02% (v/v) toluene in hexane solution	
9	Second Derivative Spectrophotometry	Checking second derivative spectrophotometry functionality to find a small negative extremum between to big extrema at 261 nm and 268 nm. using 0.02% (v/v) toluene in methanol solution	

Materials Used:

a. Standard Didymium Filter traceable to NIST or NMI
b. 0.005 M Sulfuric acid solution
c. 61.8 mg/L $K_2Cr_2O_7$ in 0.005 M sulfuric acid solution
d. 12 g/L KCL solution in distilled water
e. 0.02% (v/v) toluene in hexane solution
f. 0.02% (v/v) toluene in methanol solution

7.2 Performance Testing

7.2.1 Test 1:

Test Name:	System Initialization Test.
Purpose:	Verify Current ROM Checksum Version.
Method:	1) Remove all sample cuvettes from the sample holder and depress the **[POWER]** switch. 2) The instrument will perform an initialization sequence detailing the following self checks: **[LSI Initialize] / [ROM Memory Check] / [RAM Memory Check] / [Filter Motor Initialize] / [Light Motor Initialize] / [Scan motor Initialize] / [WI Lamp Energy Check] / [Wavelength origin 1 search] / [D2 Lamp energy Check] / [Wavelength origin 2 search] / [Standby...]** 3) Successful completion of this test results in the instrument being ready for use.
Acceptance Criteria:	Version [___] Checksum [___].
Parameter Values for Verification:	Version [___] Checksum [___].

Conformity/Non-conformity Report:

7.2.2 Test 2:

Test Name:	Baseline Stability
Purpose:	Verifying stability of baseline.
Method:	1) Remove cuvettes from the sample compartment and close the cover. 2) Allow instrument to remain on for approx **30** minutes for D2 lamp to reach maximum stability. 3) select **"Spectrum"** from **"Acquire Mode"** menu. 4) Select **"Parameters"** from **"Configure"** menu. 5) set the **Measuring** mode to ⊙ **Abs.** 6) Set the **Recording Range** to Low [0.0] To High [0.01]. 7) Set the **Wavenumber Range(nm)** to **Start** [1100] To **End** [190] nm. 8) Set the **Scan Speed** to ⊙ **Slow**. 9) Set the **Sampling Interval (nm)** to ⊙ **Auto** 10) Click on **OK** Button on **Spectrum Parameters** Window. 11) Click on **Baseline** Button to correct Baseline. 12) Click on **Start** Button to scan for spectrum. [Wait until Completion of scanning from 1100 to 190 nm]. 13) Write the file name and click on **[Save]**. 14) Select **Peak Pick...** from **Manipulate** menu. 15) On **Peak Pick** window select **Graphics Plot** from **Output** menu to print the spectrum and data.
Acceptance Criteria:	Baseline noise = 0 ± 0.002 Abs.
Parameter Values for Verification:	Actual Baseline noise: ± ____.

Conformity/Non-conformity Report:

7.2.3 Test 3:

Test Name:	Wavelength Accuracy Test
Purpose:	Verify wavelength accuracy using internal D2 Lamp.
Method:	1) Remove cuvettes from the sample compartment and close the cover. 2) select "**Spectrum**" from "**Acquire Mode**" menu. 3) Select "**Parameters**" from "**Configure**" menu. 4) set the **Measuring** mode to **Energy**. 5) Set the **Recording Range** to Low [1.0] To End [150.0]. 6) Set the **Wavenumber Range (nm)** to **Start** [490] To **End** [480] nm. 7) Set the **Scan Speed** to **Slow**. 8) Set the **Sampling Interval (nm)** to **Auto**. 9) Click on **Energy...** Button. 10) Set **Lamp** to **D2**. 11) Set **SiPD Gain** to **2**. 12) Click on **OK** Button. 13) Click on **OK** Button on **Spectrum Parameters** Window. 14) Click on **Baseline** Button to correct Baseline. 15) Click on **Start** Button to scan for spectrum. [Wait until Completion of scanning from 490 to 480 nm]. 16) Write the file name and click on [**Save**]. 17) Select **Peak Pick** from **Manipulate** menu. 18) On **Peak Pick** window select **Graphics Plot** from **Output** menu to print the spectrum and data.
Acceptance criteria:	The expected peak will found at any point between 483.0 nm to 489.0 nm.
Parameter Values for Verification:	Actual peak found at wavelength of ___nm.

Conformity/Non-conformity Report:

7.2.4 Test 4:

Test Name:	Wavelength Repeatability Test
Purpose:	Verify wavelength repeatability using internal D2 Lamp.
Method:	1) Remove cuvettes from the sample compartment and close the cover. 2) select "**Spectrum**" from "**Acquire Mode**" menu. 3) Select "**Parameters**" from "**Configure**" menu. 4) set the **Measuring** mode to ⊙ **Energy**. 5) Set the **Recording Range** to Low [1.0] To End [150.0]. 6) Set the **Wavelength Range (nm)** to **Start** [490] To **End** [480] nm. 7) Set the **Scan Speed** to ⊙ **Slow**. 8) Set the **Sampling Interval (nm)** to ⊙ **Auto**. 9) Click on **Energy...** Button. 10) Set **Lamp** to ⊙ **D2**. 11) Set **SiPD Gain** to ⊙ **2**. 12) Click on **OK** Button. 13) Click on **OK** Button on **Spectrum Parameters** Window. 14) Click on **Baseline** Button to correct Baseline. 15) Click on **Start** Button to scan for spectrum. [Wait until completion of scanning from 490 to 480 nm]. 16) Write the file name and click on [**Save**]. 17) Select **Point Pick** from **Manipulate** menu. 18) On Pick Point window select **Graphics Plot** from **Output** menu to print the spectrum and data. 19) Repeat the above steps from [14] to [18] three times to record 1st, 2nd and 3rd Peak. 20) Calculate max. Difference.
Acceptance criteria:	Wavelength difference should be max. +/- 0.1nm
Parameter Values for Verification:	Standard peak [_____]nm 1st peak [_____]nm 2nd peak [_____]nm 3rd peak [_____]nm Max. Difference [_____]nm.

Conformity/Non-conformity Report:

7.2.5 Test 5:

Test Name:	Wavelength Repeatability Test
Purpose:	Verify wavelength repeatability standard didymium filter.
Method:	1) Remove cuvettes from the sample compartment and close the cover. 2) select "**Spectrum**" from "**Acquire Mode**" menu. 3) Select "**Parameters**" from "**Configure**" menu. 4) set the **Measuring** mode to ⊙ **%T**. 5) Set the **Recording Range** to Low [0.0] To End [150.0]. 6) Set the **Wavenumber Range(nm)** to **Start** [600] To **End** [500] nm. 7) Set the **Scan Speed** to ⊙ **Slow**. 8) Set the **Sampling Interval (nm)** to ⊙ **Auto**. 9) Click on **OK** Button on **Spectrum Parameters** Window. 10) Click on **Baseline** Button to correct Baseline. 11) Insert a didymium filter in the sample side of the compartment. 11) Close the cover. 12) Click on **Start** Button to scan for spectrum. [Wait until Completion of scanning from 600 to 500 nm]. 13) Write the file name and click on [**Save**]. 14) Select **Point Pick...** from **Manipulate** menu. 15) Type **568.0** to **points 1** text box. 16) Click on **OK** Button. 17) On Peak Pick window select **Graphics Plot** from **Output** menu to print the spectrum and data. 18) Remove the didymium filter from the cell compartment. 19) Repeat the above steps from **[10]** to **[16].** 20) Convert the %T difference into wavelength difference by the relationship: 2.5% dispersion of the measured value corresponds to +/- 0.1 wavelength repeatability. R (nm) = 0.1* (%T1 ~ T2)/2.5
Acceptance criteria:	The result will be R < [+/-0.1] nm.
Parameter Values for Verification:	The value calculated [_____]nm.

Conformity/Non-conformity Report:

7.2.6 Test 6:

Test Name:	**Control of Absorbance**
Purpose:	Checking specific absorbance of $K_2Cr_2O_7$ peaks at 235, 257, 313 & 350 nm.
Method:	Spectrum mode: Wave length range (nm) : **400** to **190** Scan speed : **Slow** Sampling interval (nm) : **Auto** Measuring Mode : **Absorbance** 1)Place 0.005M H_2SO_4 Sol^n in both the cuvettes inside the sample compartment. 2)Click [Baseline] for baseline correction. 3)Wait for completion of baseline correction. 4)Take out the H_2SO_4 Sol^n from the sample cuvette. 5)Take $K_2Cr_2O_7$ Sol^n with sample cuvette and place this $K_2Cr_2O_7$ Sol^n in the sample holder.(Cuvette in the reference sample holder kept as it is) 6) Click [START] 7)Wait for completion of the acquisition. 8) Perform →Point Click. 9) Record the absorbance Values at 235, 257, 313 & 350nm. 10)Calculate Specific Abs A_λ for wavlength λ : $A_\lambda = (abs_\lambda * 10000)/61.8$ mL/mg-cm 11)Tabulate the results and compare with the tolerances specified in the EP.

Acceptance criteria:	**Wavelength (nm)**	**Expected Specific Abs. A_λ mL/mg-cm**	**Tolerance limit**
	235	124.54	122.9-126.2
	257	144.86	142.8-145.7
	313	48.62	47.0-50.3
	350	107.56	104.9-108.2

Parameter Values for Verification:	**Wavelength (nm)**	**Measured A_λ mL/mg-cm**	**Complies (YES/NO)**
	235		
	257		
	313		
	350		

Conformity/Non-conformity Report:

Wavelength (nm)	Measured Absorbance (abs)	Expected Specific Abs: $A_{\lambda\,exp}$ ml/mg-cm	Tolerance limit	Measured A_λ	Complies (YES/NO)
235		124.54	122.9-126.2		
257		144.86	142.8-145.7		
313		48.62	47.0-50.3		
350		107.56	104.9-108.2		

A_λ =(abs*10000)/61.8 =

7.2.7 Test 7:

Test Name:	**Limit of Stray Light**
Purpose:	Checking stray light analyzing absorbance of (12g/L) potassium Chloride (KCl) solution
Method:	Spectrum mode: Wave length range (nm) : **220** to **190** Scan speed : **Slow** Sampling interval (nm) : **Auto** Measuring Mode : **Absorbance** 1) Take distilled water in both the sample and reference cuvettes and place in the holders. 2) Click on [**Baseline**] 3) Wait for completion of base line correction. 4) Take out the cuvettes from the sample holder. 5) Place 12 g/L KCl solution in this holder (sample) 6) Click [**START**], Wait for completion of the scan. 7) From manipulation menu **Point Pick** 8) Type **200** in **Point 1 Box** and press **[OK]** 9) Record absorbance at 200 nm and take a print out selecting **Graphics Plot** from **Output menu** on Peak Pick window
Acceptance criteria:	Absorbance at 200 nm > 2.0
Parameter Values for Verification:	Measured absorbance 200 nm: [_____]

Conformity/Non-conformity Report:

7.2.8 Test 8:

Test Name:	**Checking Resolution of the system.**
Purpose:	Checking resolution of the system using 0.02%V/V toluene in hexane solution
Method:	From **Spectrum mode** set **Parameters**: Wave length range (nm) : **275** to **260** Scan speed : **Slow** Sampling interval (nm) : **0.5 (minimum)** Measuring Mode : **Abs** 1) Take Hexane in both the cuvettes and click (Baseline). 2) Takeout the hexane from the sample cuvette. 3) Place 0.02%v/v toluene in hexane solution in the sample cuvette (please keep hexane as the reference as it is) in the reference holder). 4) Click [Start] to collect the 0.02% toluene in hexane solution keeping hexane as reference. 5) From Manipulation→Point pick. Determine abs value of the maximum peak around 269 nm (a_{269}) and minimum peak around 266 nm (a_{266}) 6) Calculate the Ratio of absorbances R =(a_{269})/(a_{266}) and ratio should be ≥ 1.48
Acceptance criteria:	The minimum ratio of the absorbance at the maximum around 269 nm to that at the minimum around 266 nm should be at least 1.48
Parameter Values For Verification:	Ratio observed $R_{269/266}$ =

Conformity/Non-conformity Report:

7.2.9 Test 9:

Test Name:	**Second Derivative Spectrophotometry**
Purpose:	Checking second derivative spectrophotometry functionality to find a small negative extrimum between to big extrema at 261 nm and 268 nm.
Method:	Spectrum mode: Wave length range (nm) : **275** to **260** Scan speed : **Slow** Sampling interval (nm) : **Auto** Measuring Mode : **Abs** 1) Run **Baseline** keeping methanol in both reference and sample cuvettes. 2) collect 0.2% (v/v) toluene in methanol placing the solution in sample cuvette by pressing [**Start**]. 3) From Manipulate → Transforms Source: **Default** Destination: **1** Order: **2nd** Delta-lamda: **2.00** (lowest) Scaling factor: **1.00** Click [**OK**] → Enter file name. 4) Click [**OK**]. 5) Presentation → Radar and Zoom the portion of the graph. 6) From Manipulate → Peak Pick 7) On Peak Pick window from Options → Find Valleys 8) Observe a small negative extrimum located between two large negative extrema at 261 nm and 268 nm 9) From Output → Graphics Plot to have a prinout
Acceptance criteria:	A small negative extremum should be observed (around 265 nm) between two large negative extrima at 261 nm and 268 nm.
Parameter Values For Verification:	A small negative extremum at λ= nm observed

Conformity/Non-conformity Report:

7.3 Preventive Maintenance (PM)

7.3.1 Preventive Maintenance Information:

a. Follow the instructions in System Manuals and supplied documentation.

b. Warm up at least 30 min before running sample.

c. Maintain Relative Humidity 45% ~ 80% and T within 20°C ~ 30°C. Record in equipment log-book.

d. Avoid vibration.

e. Use UPS with built-in voltage stabilizer to provide stable power supply. Avoid using common ac mains supply of analytical instrument and that of heavy duty and or heating devices e.g. AC/Dehumidifier/Oven/Furnace/shaker/mixing device.

f. For maintenance only Manufacturer's authorized service personnel is allowed.

g. Annual Maintenance Contract (AMC) with the Manufacture's lational agent .

Next Performance Checking (PQ) Date:_____
Note: Recommended frequency of checking is quarterly and or after maintenance work/servicing whichever comes earlier.

7.4 Degree of Criticality:

Nil

7.5 Critical component (s):

Cuvette should be handled properly to avoid scratch on the light ente ing side.

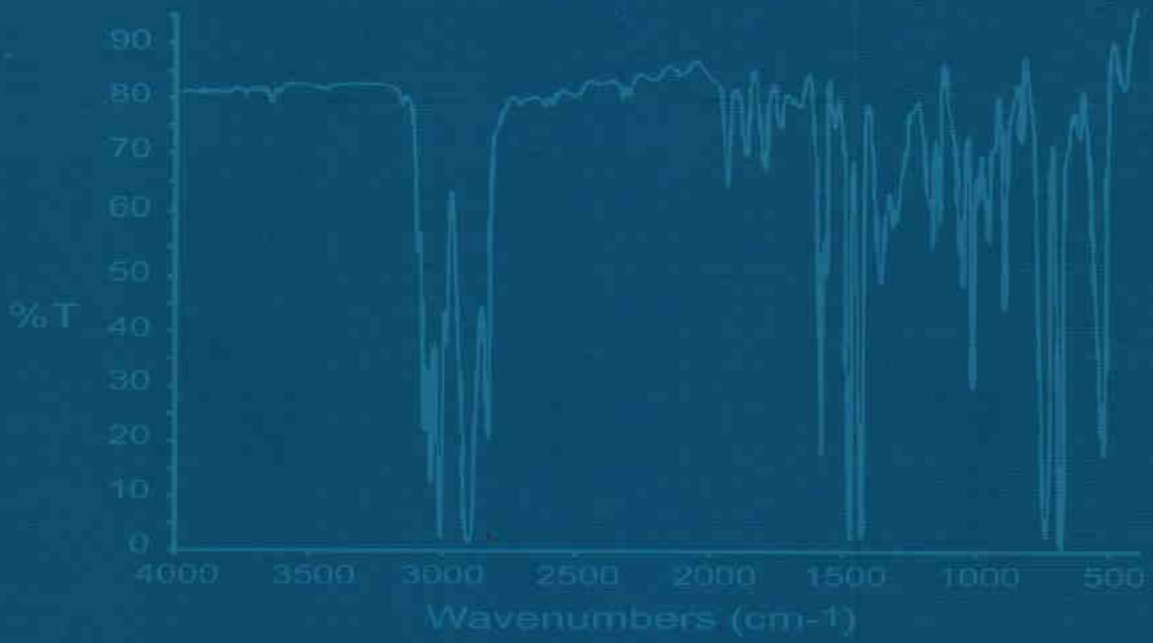
Avoid spilling over corrosive reagent/fume generation inside the sample chamber.

7.6 System Certification

Studied data has determined that the system described in this document **meets/does not meet** all criteria outlined in this Operational Qualification/ Performance Quality (OQ/PQ) report. The exceptional conditions have been identified and documentation as non-conformity report in the respective section.

The system is **Ready/Not-ready** for specified usage

Report Prepared By:	**Abdul Monem Beverages Ltd. (AMBL), Comilla**	
	Name: _____ Title: Calibration Engineer AMBL	Date:_____
Laboratory Technical Manager's Authorizations:		
Name: _____ Title: _____ Site: _____		Date:_____



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