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RECENT ADVANCES IN PHARMACEUTICAL SCIENCES

Volume - 6

Chief Editor
Dr. Chhavi Singla

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Contents

Chapter - 1
Posology

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Chapter - 1

Posology

Usha Kiranmai Gondrala

Abstract

The dose of a drug cannot be fixed rigidly because various factors like age, weight, gender, disease condition etc. must be taken into consideration. The official doses mentioned in pharmacopoeia are represented as average range of quty. Suitable for adults administered orally within 24hrs. When other routes are considered, the relevant appropriate doses are given. Thus, Posology is taken into consideration which is a branch of medical science dealing with the doses of drugs that are administered to the patients to show necessary pharmacological action, taking into account several factors. Doses of drugs are calculated using various formulas based on age, weight and body surface area. It is the responsibility of the pharmacist to calculate the appropriate dose individually to each and every patient so that patient compliance and satisfaction are easily achieved.

Keywords: Dose, Factors, Calculation

Introduction

Posology is defines as branch of medical science which deals with doses of drugs, factors affecting dosage, methods of calculation of doses, measurement of dosage forms etc. It is the term derived from Greek words i.e., “posos” means “how much” and “logos” means “science”. Thus, the other name for posology is “The Science of Dosage”.

Terminology

1) **Dose:** Dose of a drug may be defined as the quantity of drug which is “enough but not, too much”. The optimum dose of drug to produce the desired therapeutic effect varies from one person to other person. The doses of drugs are usually expressed in the form of range.

The dose may be expressed as

Single dose: The amount of drug taken one time.

Divided dose: The amount of drug is taken two or more times in a day dependingn characteristics of drug, type and severity of diseases.

2) **Dose frequency:** It is also called as "Dosage regimen or Scheduling of drugs". The pharmacopoeial doses represent the average range of quantities suitable for adults when administered by mouth.

Ex: Aspirin 0.3 to 1 g

Often, for the drugs which needed to be administered over 24hrs, they are needed to be given as divided doses.

Ex: Griseofulvin 1g daily, in divided doses

Calculation of doses

In Pharmacopoeias, the average adult dose or the usual adult dose for official drugs are prescribed. The dose calculation varies with age, weight, gender, surface area, disease condition etc of the patient.

For calculating the doses for children, the following methods are used.

a) According to age

1) Young's formula

$$\text{Child dose} = \frac{\text{Age (years) x Adult dose}}{\text{Age +12}}$$

This formula is used widely to calculate the doses for children of 2 to 12 years.

2) Dilling's formula

$$\text{Child dose} = \frac{\text{Age (years) x Adult dose}}{20}$$

This formula is used widely to calculate the doses for children of 4 to 20 years.

3) Cowling's formula

$$\text{Child dose} = \frac{\text{Age at next birthday (years) x Adult dose}}{24}$$

(Or)

$$\text{Child dose} = \frac{\text{1+Age (years) x Adult dose}}{24}$$

4) Fried's formula

$$\text{Child dose} = \frac{\text{Age (months) x Adult dose}}{150}$$

This formula is used widely to calculate the doses for babies under 2 years of age.

5) Bastedo's formula

$$\text{Child dose} = \frac{\text{3+Age (years) x Adult dose}}{30}$$

6) Gaubin's formula

According to this formula, the fraction of adult dose for infants, children of various ages and for the aged person is as under.

Age under (years)	Parts of adult dose
1	1/12
1-2	1/8
2-3	1/6
3-4	1/4
4-7	1/3
7-14	1/2
14-20	2/3
21-60	Full adult dose
60-70	4/5
70-80	3/4
Over 90	1/2

b) According to body weight

$$\text{Clark's formula} = \frac{\text{Weight (pounds/lbs) x adult dose}}{150}$$

Or

$$\text{Clark's formula} = \frac{\text{Weight (kgs) x adult dose}}{70}$$

The average weight of an adult is taken as 150lbs/70 kgs.

c) According to the body surface area

$$\text{Child dose} = \frac{\text{Body surface area of child x Adult dose}}{\text{Body surface area of Adult}}$$

Or

$$\text{Log } S = 0.425 \log W + 0.725 \log H + 1.8564$$

Where, S is surface area; W is weight in kg; H is height in cm

Calculation of dose according to body surface area is widely used in two types of patient groups i.e., chemotherapy receiving cancer patients and obese patients. In general, the adult body surface area is considered as $1.73m^2$ or $1.8\ m^2$.

Factors influencing or affecting the dose and therapeutic action of drugs

List of factors include are:

1) Age
2) Body weight and surface area
3) Gender
4) Route of administration
5) Time of administration
6) Frequency of administration
7) Tolerance
8) Addiction
9) Drug combinations
10) Idiosyncrasy
11) Hypersensitivity
12) Mental conditions
13) Metabolic disturbances
14) Environmental factors
15) Presence of disease
16) Accumulation
17) Habituation

1) Age

The dose varies considerably with the age of the patient. Children do not react to all drugs in the same fashion as the adults and with a few exception,

drugs are more active and more toxic in children than in adults. Since, New born infant have relatively high total body water content, low body fat content, immature renal and hepatic functions, altered protein binding and alteration in gastric acidity.

In special cases, like certain metabolic pathways such as oxidation and conjugation in children may exceed the adult values. So, requires high doses of drug than adult.

Ex: In case of theophylline

Children ca also tolerate relatively larger amounts of belladonna, digitalis and ethanol where as elderly patients are sensitive to some drugs such as hypnotics and tranquilizers which may produce confusion states in them.

2) Body weight and surface area

Generally recommended adult dose are based on a normal body weight of 70 kg. But such a dose will be too less for a muscular person of 100kg and too large for a weak person of about 50kg.

The relative proportion of muscular and adipose tissue in the individual may be sufficient to alter the distribution and clearance of a drug from the body. Since a dose for children is usually calculated on body weight basis i.e., mg/kg body weight.

The dose calculations for obese patients and malnourished children are done on the basis of body weight.

Many physiological factors such as plasma volume, oxygen consumption and requirement of body fluid, electrolytes, calories and glomerular filtration are proportional to the surface area of body.

Ex: Anti-cancer drug Methotrexate is administered on mg per sq.. of body surface.

3) Gender

Generally females require lesser dose than males because of their lesser weight and more fat and also they are more responsive to the effects of certain drugs than males.

Ex: Morphine and barbiturates may produce more excitement before sedation in women.

Drugs should be given very carefully during menstruation, pregnancy and lactation.

- Strong purgatives such as aloes should be avoided during menstruation.
- Drugs which stimulate the uterine smooth muscle like purgatives, anti-malarial drugs and ergot alkaloids are contraindicated drugs during pregnancy which may lead to abortion and miscarriage.
- Drugs like alcohol, barbiturates, anaesthetic gases, narcotic and non-narcotic analgesics etc. which on administration to mother are capable of crossing the placenta and affecting the foetus.
- During lactation, drugs such as anti histaminics, morphine and tetracyclines which are excreted in milk should be avoided or be given very cautiously to the mothers who are breast feeding the babies.

4) Route of administration

In general, the rate and degree of absorption increases in the following order:

oral → sub cutaneous → intra muscular →intra venous

The dose o a drug given may vary according to the dosage form and route of administration used. Drugs administered i.v enter the blood stream directly hence require lesser dose than s.c dose which in turn is smaller than the oral dose.

Ex: For ergotamine i.v- 0.25mg; i.m- 1mg; oral- 2 to 5mg

5) Time of administration

It is very important that drugs are rapidly absorbed from the empty stomach. An amount of drug i.e., effective when taken before a mersal may be ineffective if administered during or after meals. On the other hand, irritating drugs are better tolerated if administered after meals which will dilute the drugs concentration and reduce gastric irritation. Ex: iron, arsenic, cod liver oil etc. should always be given o full stomach.

Ferrous sulphate if administered in between meals is more effective than the effect produced by the same dose administered after meals.

Larger doses of hypnotics are always required during day time than at night.

Several physiological functions are altered during bed rest as compared to the upright position, including reduction in gastric emptying rate, increase in cardiac output and renal flow. It changes absorption, distribution and elimination of drug.

6) Frequency of administration

Drugs having short plasma life gets rapidly excreted from blood to maintain steady state plasma concentration. It requires frequent dosing. Controlled drug delivery systems are developed to reduce the frequency of administration.

7) Tolerance

Tolerance occurs after a prolonged use of drug. It results in diminution of responsiveness. The consequence of which is an increase in dose requirement.

Ex: Chronic administration of narcotic drugs like morphine, pethidine, caffeine and nicotine etc.

Rabbits can tolerate large doses of atropine due to quick destruction of the drug by enzyme atropine esterase present in their blood.

Some children can tolerate large doses of arsenic, calomel and belladonna.

Tolerance is classified into 4 types:

a) Natural tolerance: This is common in children and can tolerate large doses of calomel and belladonna.

b) Acquired tolerance: This develops over along period of usage of drug where the patient acquires an ability to take large doses of certain drugs like opium, cocaine, alcohol, caffeine etc., which in normal persons would produce harmful or fatal effects.

c) Tachyphylaxis: It is also known as "Acute tolerance". It has been observed that when certain drugs are administered by repeatedly at short intervals, the cell receptors get blocked up and pharmacological response to that particular drug decreased. The decreased response cannot be revered by increasing the dose. But if the administration of the drug is stopped for a long time and administered after being discontinued then the initial effect of the drug can be reobserved. This phenomena is known as "Tachyphylaxis".

Ex: Ephedrine, Amphetamine, Cocaine and Nitrites behave in this way.

d) Cross tolerance: Here, the use of a drug prolonged period may establish tolerance for other drugs of same category.

Thus, chronic alcoholics become less sensitive to large quantities of alcohol and more resistant to the action of ether than ordinary individual.

8) Addiction

It is the state of psychic and physical drug dependence. An undue craving to get the drug or a psychic dependence on the drug is known as "Addiction". It is developed after taking a drug for sometime in an individual. "withdrawal syndrome" occurs on stopping which may even endanger the life.

Ex: Alcohol, Cocaine, Tobacco etc.

9) Drug combinations

The combination of two or more drugs also influence the amount of a drug needed to produce a desire effect. The effects produced by combination of drugs is of 4 types:

a) Synergism: When two or more drugs given together results in a total effect greater than the sum of their independent effects.

Ex:

i) Codeine and Aspirin

ii) Adrenaline and Procaine, this combination increases the duration of action of procaine.

iii) Penicillin with Streptomycin, here the average dose of each drug is reduced as a result of which it gives a greater margin of safety.

b) Additive effect: When two or more drugs given together results in a total effect equal to the sum of their individual effects.

Ex: Ephedrine and Aminophylline in the treatment of Bronchial Asthma

c) Potentiation: In this case, when two or more drugs are administered to an individual, the combined effect produced is greater than the sum of their individual effects.

Here, one drug potentiates the action of other drug.

Ex:

i) Ephedrine and Adrenaline, a better broncho dilation action observed.

ii) Adrenaline increases the duration of action of procaine and reduces its toxicity.

d) Antagonism: When two or more drugs are given together produce opposite effect to each other on the same physiological system.

Ex: Adrenaline and Acetylcholine. Adrenaline is a vasoconstrictor whereas acetylcholine is a vasodilator. They neutralize the effect of another due to antagonism.

10) Idiosyncrasy

It is defined as a genetically determined abnormal or unusual response to a drug, that occurs in small proportion of individuals. This occurs with a very small dose of drug.

Ex:

i) Few mg of aspirin may produce gastric hemorrhage.

ii) Small doses of quinine may produce ringing in the ear.

11) Hypersensitivity

It is an allergic reaction to a drug and is different from either the expected pharmacological response or toxic reaction to the drug.

This is due to frequent or indiscriminate use of drugs like antibiotics, vitamins and especially proteinous substances.

Most common allergic effects are skin rashes, edema, anaphylactic shock, bronchospasm, serum sickness syndrome etc.

Ex: Penicillin, Sulfonamide, Phenacetin etc.

12) Mental conditions

Temperament as also influence or effects on the response to a drug.

Ex: Neurotic patients respond with low doses of CNS stimulants.

13) Metabolic disturbances

Changes in water electrolyte balance, acid base balance, body temperature and physiological factor may modify the effect of drugs.

Ex:

i) Salicylates reduce body temperature only in case an individual has rise in body temperature. They have no antipyretic effect if the body temperature is normal.

ii) the absorption of iron from GIT is maximum if the individual has an Iron deficiency anemia.

14) Environmental factors

Drugs like hypnotics if administered during day time shows diminishing effect while in darkness they behave as sedatives. These are more effective at night.

The amount of barbiturate required to produce sleep during day time is much higher than the dose required to produce sleep at night.

Alcohol is better tolerated in cool environments than in summer.

15) Presence of disease or pathological condition

Ex:

i) Barbiturates and chlorpromazine may produce usually prolonged effect in patients having liver cirrhosis.

ii) Streptomycin is excreted mainly by the kidney and not given to renal failure patients.

iii) During fever a patient can tolerate high doses of anti-pyretic than a normal person.

16) Accumulation

The drugs like digitalis, emetine and heavy metals are slowly excreted and may built up a sufficient high concentration in the body and produce toxic symptoms if they are repeatedly administered for a long time. This is due to accumulative effect of the drugs.

The cumulative effects are usually produced by slow excretion, degradation and rapid absorption of drugs. This effect is sometimes desirable in drugs like Phenobarbitone in the treatment of epilepsy.

17) Habituation

When repeated use of dug or agent leads to production of emotional or psychological dependence rather than compulsion, the condition is known as "Habituation".

Ex: use of tea, coffee, tobacco, chewing of betel nut, tranquilizers etc.

When such agents are withdrawn, the person can carry on his routine work. Here, there is no physical dependence. Hence, it can be easily tackled.

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Chapter - 2
Technical Aspects of Analytical Chemistry in Analysis of Soil

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Chapter - 2

Technical Aspects of Analytical Chemistry in Analysis of Soil

VR. Mageshen and K. Aswitha

Abstract

Analytical chemistry is a branch of chemistry that deals with separation, identification and determination of component in a sample. It can be performed qualitatively and quantitatively. We can measure qualitatively the presence of components and quantitatively the amount of such components. As soils are highly heterogenous, analytical techniques must be employed to find out the toxic substances present in the soil. The quantity of such toxic component is more important rather than is quality. Quantitatively, the chemical or physico-chemical property of substance is used for analyzing. The chemical method involves volumetric, gravimetric and gas analysis and the physico-chemical analysis is performed through instrumentation. The instrumental methods include the chromatographic techniques to separate the components, magnetic methods to study the physical, chemical and biological properties of matter, thermal methods to study the thermal stability of the elements, electrochemical methods to study the pH and EC, Spectral methods to identify the quantity of element present. The choice of method is based on the analyte we are going to measure. The instrumental methods are more accurate when compared to the chemical methods. We can also able to analyze a large number of samples in shorter period of time.

Keywords: quantitative, qualitative, heterogenous, chemical, instrumentation, accurate

Introduction

- Analytical chemistry is a scientific discipline used to study the chemical composition, structure and behavior of matter.
- The purpose of chemical analysis is to gather and interpret chemical information that will be of value to society in a wide range of contexts.

Classification of chemical analytical methods

Based on the quantity of sample required for analysis,

- Macro (> 25 mg).
- Semi-micro (10-15 mg).
- Micro (3-5 mg).
- Sub micro (1-3 mg).
- Ultra-micro (< 1 mg).

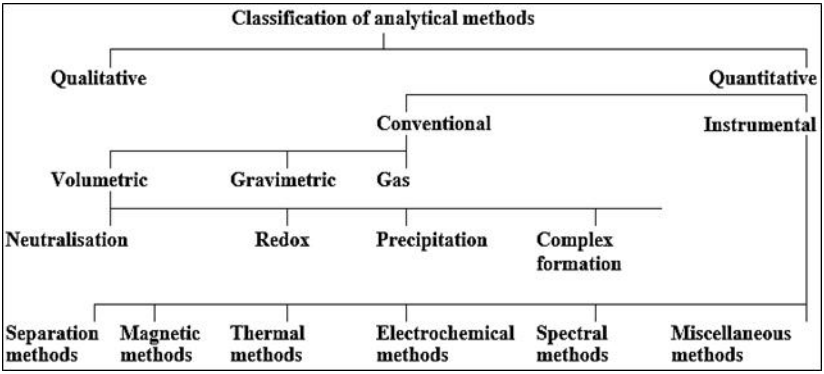
Based on the concentration of the element or constituent in the sample to be analysed,

- Major (%, few tens).
- Minor (less than 1%).
- Trace (1-100 ppm).
- ultra-trace (sub ppm).

Qualitative and quantitative analysis

An element, species or compound that is the subject of analysis is known as an analyte. The remainder of the material or sample of which the analyte(s) form(s) a part is known as the matrix.

- Qualitative analysis is the identification of elements, species and/or compounds present in a sample.



- Quantitative analysis is the determination of the absolute or relative amounts of elements, species or compounds present in a sample.
- Structural analysis is the determination of the spatial arrangement of atoms in an element or molecule or the identification of characteristic groups of atoms (functional groups).

Accuracy and Precision in analytical chemistry

Accuracy: It is the degree of agreement between the measured value and true value or by simply measuring its nearest value to the true value

Precision: The degree of measurement between reliable measurements of same quality which should be near to the true value that is repeatability of result.

"Good precision does not assure good accuracy in any analysis, the accuracy is required".

Conventional methods

Volumetric method: It includes all the titration reaction. It involves addition of reactant to a solution being analyzed until some equivalence point is reached. We can use different type of indicators to reach equivalence point. E.g.: Potentiometric titrations.

Types of volumetric methods

- **Neutralization reaction:** It is used to find out the concentration of acid or base in a solution. The amount of acid present in the solution can be determined by adding a solution of standard base and vice versa. E.g. Acid-base reactions.
- **Redox reaction:** The oxidant can be estimated by adding reductant in redox titrations or vice versa. E.g. Iodimetry and iodometry. It is also known as oxidation-reduction reaction.
- **Precipitation reaction:** In this titration, there will be formation of low solubility compound or the titrant will tend to precipitate. E.g. Argentometric titration involving chloride
- **Complex formation:** The titrant forms complexes with cations in this type of titrations. The complex may be soluble or insoluble. E.g. Complexometric titrations involving calcium and magnesium

Gravimetric method: It involves loss in weight for the determination of material present in the sample. E.g. Sulphate estimation in irrigation water.

Gas method: It can be used to determine relative amount of compounds present in a mixture by passing a vaporized sample in a gas stream. E.g. Gas chromatography.

Instrumental methods

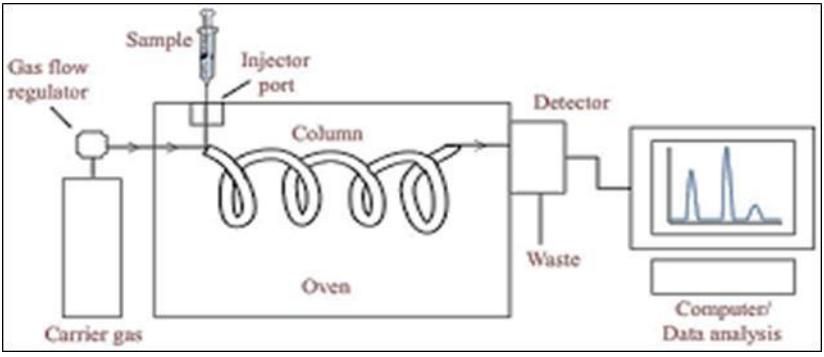
1) Separation methods-Chromatography

Gas chromatography (GC) is a common type of chromatography used in analytical chemistry for separating and analyzing compounds that can be vaporized without decomposition. Separation of mixtures in microgram quantities by passage of the vaporized sample in a gas stream through a column containing a stationary liquid or solid phase; components migrate at different rates due to differences in boiling point, solubility or adsorption. It is used to detect thermally stable compounds (residue analysis) upto 400 ^{o}C (Sliepcevich and Gelosa, 2009) [7].

Components of gas chromatography

- Gas Assembly
- Injection port
- Column
- Oven
- Detector

Instrumentation and working procedure



- A sample is injected into a heating block by syringe where the compound is vaporised and carried as vapour along with the carrier gas into the column inlet.
- Solutes are adsorbed at the head of the column by the stationary phase and the solute travels at its own rate through the column in proportion to its value of K_d.
- Solutes are eluted in increasing order of their partition coefficient K_d and then enter the detector.

- Here they register a series of signals resulting from concentration changes and different rate of elution.
- On the recorder, they appear as a plot of time against composition of the carrier gas stream.

High performance liquid chromatography

High-performance liquid chromatography (HPLC; formerly referred to as high-pressure liquid chromatography), is a technique in analytic chemistry used to separate the components in a mixture, to identify each component, and to quantify each component (Meyer, 2013) [5]. It is mainly used to detect thermally unstable compounds (Botanicals). It relies on pumps to pass a pressurized liquid solvent containing the sample mixture through a column filled with a solid adsorbent material. It is also known as high precision liquid chromatography.

In HPLC the stationary phase is a solid and mobile phase is liquid

Components of HPLC

- Mobile Phase
- Pumping system
- Injection port
- Column
- Detector

Mobile phase

It is very important. It is selected based on the charge of the compounds. Since the mobile phase governs solute- stationary phase interaction, its choice may range from organic solvents with low polarity such as hexane to those with high polarity such as methanol and water. The chemical purity if the solvent is important factor. It should be free of particulates which could clog the filters and columns.

Pumping system

Fluid flow is essential for any HPLC separation which is accomplished with high pressure pump. High pressure is needed to overcome the resistance to fluid flow arising from the micro porous stationary phase packed in a narrow borer column. Slower the solvent flow rate, the better the separation. A typical flow rate for analytical separation is 0.5 to 2.0 ml/min. The solvent gradient can be formed of two ways by low-and high-pressure gradient system. In low pressure gradient system, the solvents are mixed before

pressurized by the pump. This is quite simple and inexpensive. In high pressure gradient system the solvents are mixed after being pressurized by the pump. It uses two or three pumps for pressurizing each solvent separately.

Injector

The injector is located between the column and the high-pressure solvent pump. Injection may be made using a syringe at high pressure, under septum injection, stop flow condition or using a sample loop. For the best performance, the sample is to be placed at the head of the column in a narrow band. Septum injection device is similar to the familiar syringe injection using high pressure syringe. The drawback in the device is that the septum must be compatible with the solvent and to withstand high back pressure. The mobile phase is momentarily stopped during the injection made onto the column in case of stop flow injection. This method is quite in expensive and absolutely reliable because the dead volume is zero. Loop injection is by far the best also most widely used. This requires a multiple port, rotary switching value. The solute mixture is first loaded into a fixed volume loop. The sample is thus flushed on the column by the solvent without interruption of flow to the column. By changing the size of sample loop, sample volume can be selected.

Column

HPLC columns form the heart of any HPLC system. Its careful design and correct choice of the type and quality of its packing material are essential for good separation. Stainless steel columns of open tubular, smooth internal walls, free from all surface defects are employed to contain the column packing materials used in analytical systems. Gaps in the packing material can result in loss of column efficiently due to channeling. The primary function of the stationary phase in a separation depends largely on its surface characteristics. Three forms of stationary packing materials are available based on a rigid soil structure (Khopkar, 2015) [3]. Micro porous supports in which micro pores ramify through the particles which are micro pores generally 5-10 micrometer in diameter. Pellicular supports in which porous particles are coated onto an inert solid core such as glass bead of about 40 micrometer in diameter. Bonded phases in which the stationary phase is chemically bonded onto an inert support such as silica.

Detectors

The HPLC detectors provided electrical output signals proportional to the column effluent concentrations. Detector monitors component elution

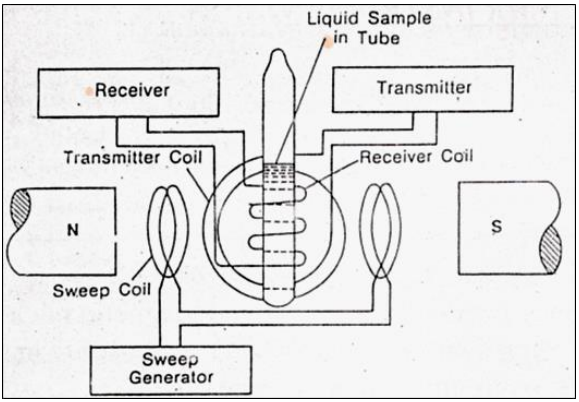
and the electric signals provide quantification. An ideal universal detector must give a rapid electrical response signal to all analysts, have analytical response signal to electric noise ratio of 2 and analytical response signal being linear against the concentration. Though number of detectors like refractive index, flame ionization and polarographic are used, only spectro photometric, fluorescence, electrochemical detectors are of major value in pesticide residue analysis. Spectrophotometric detector is capable of measuring the absorption of either Visible (VIS) or ultra violet (UV) light through the column effluent. UV absorption is widely used since UV absorbing species are more common in organic chemistry. Fluorescence detectors are extremely valuable for HPLC because of their sensitivity but this technique is limited by the fact that relatively few compounds fluoresce. Electrochemical detectors where a flow cell is fitted with two electrodes and a potential is applied on the molecule of the analyse when flown through the flow cell. The molecule undergo either oxidation or reduction, resulting in a current flow between the two electrodes.

2) Magnetic methods

Nuclear magnetic resonance

It is a technique which exploits the magnetic properties of certain nuclei to study physical, chemical, and biological properties of matter. Nuclear magnetic resonance involves the interaction between an oscillating magnetic field of electro-magnetic radiation and the magnetic energy of hydrogen nucleus or some other type of nuclei when these are placed in an external static magnetic field (James, 2012) [2]. The sample absorbs electromagnetic radiations in radio wave region at different frequencies since absorption depends on the type of proton or nuclei present in the sample.

Instrumentation and working procedure



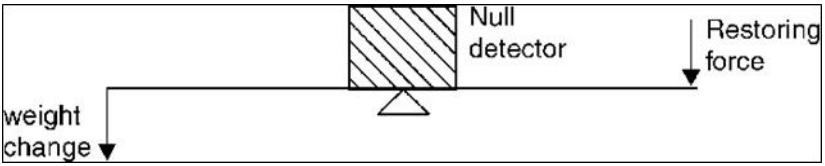
- The sample under investigation is taken in a sample tube and it is placed in between the poles of magnet.
- The magnet should give homogeneous magnetic field i.e.; the strength and direction of the magnetic field should be constant over longer periods.
- The strength of the field should be very high at least 20,000 gaus.
- A radio-frequency source is made to fall on the sample.
- It can be done by feeding energy into the coil wound round the sample.
- Source installed is perpendicular to the applied magnetic field and transmits radio waves of fixed frequency such as 60,100,200 or 300 MHz.
- A signal is detected if the nuclei in the sample resonates with the source, i.e. some energy is required to flip the proton.
- Energy is transferred from source to detector coil via nuclei.
- The output is fed to strip chart recorder after amplification.

3) Thermal methods

Thermogravimetric analysis (TGA)

It measures change in weight of sample with relation to change in temperature. There is change in weight of the sample due to kinetic reactions. There may be weight gain or weight loss in the system. The weight loss is mainly due to decomposition, evaporation, reduction and desorption. The weight gain may be due to oxidation and adsorption or absorption. It is helpful in detecting the thermal stability of minerals. (Lothenbach *et al*., 2016) [4].

Instrument and working procedure



It works on null balance principle. The amount of current applied is proportional to the weight loss or weight gain. At zero r null position there will be equal amount of light sun shine on the two photodiodes. If the balance moves out of null position an uneual amount of light sun shine on the two photodiodes.

4) Electrochemical methods

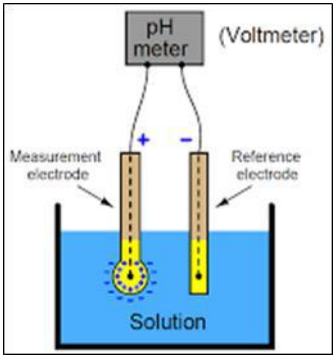
pH

The pH is defined as the negative logarithm of hydrogen ion concentration or simply the log of reciprocal of the hydrogen ion concentration.

$pH = -\log_{10}(H^+)$

Instrumentation and working procedure

A glass electrode in contact with H^+ ions of the solution acquires an electric potential which depends on the concentration of H^+ ions in the soil solution. This is measured potentiometrically against some reference electrode which is usually a calomel electrode. The potential difference between glass electrode and calomel electrode is expressed in pH units



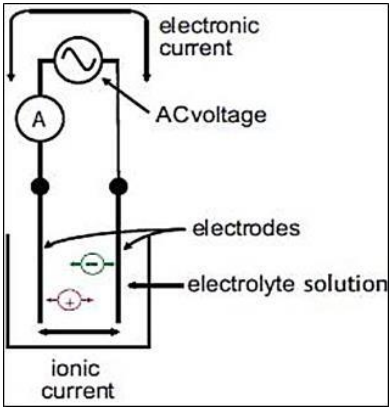
Immerse the electrodes into the beaker containing soil water suspension. Record the meter reading both in supernatant solution and in suspension.

Conductivity meter

The electrical conductivity (EC) measurement gives the total amount of soluble salts present in the soil and expressed as dS m^{-1}. As the amount of soluble salts in the solution increases, the electrical conductivity also increases. This electrical conductivity is measured in terms of resistance offered to the flow of current using conductivity bridge.

Instrumentation and working procedure

Use the same soil water suspension used for measuring pH for the estimation of EC. Stir the contents and allow the soil to settle for 15 minutes. Wash the electrodes carefully and immerse them into soil solution. Read the EC in the digital EC meter.



5) Spectral methods

Spectroscopy

Spectroscopy is defined as the study of interaction of electromagnetic radiations with matter.

Two types of spectroscopy

1) Emission spectroscopy
2) Absorption spectroscopy

Emission spectroscopy

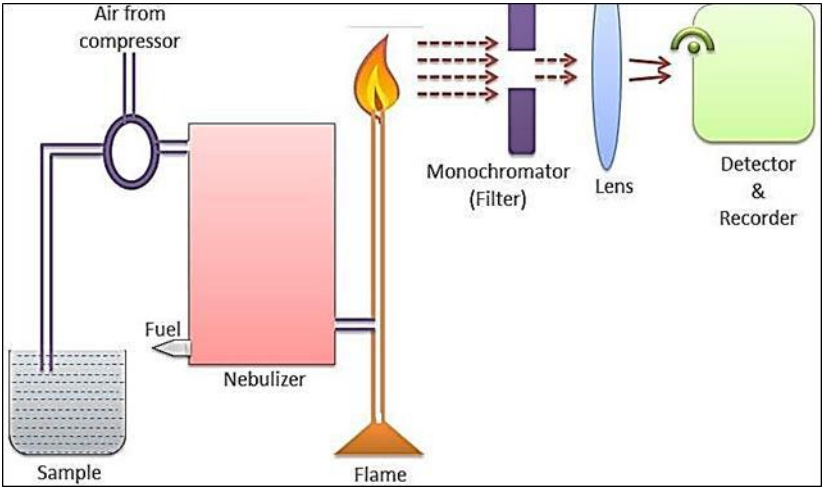
Samples when introduced into the flame of atoms get excited by absorbing energy and radiation emitted by excited sample or atoms. It is related to atoms whereas absorption spectroscopy related to molecules. Hence this method is used to determine elemental metal analysis

Flame photometer

Flame photometry is a process where in emission of radiation by neutral atoms are measured. The neutral atoms are obtained by introduction of sample into flame.

Instrumentation and working principle

When a solution of metallic salt is sprayed as fine droplets into a flame, Due to heat of the flame the droplets dry leaving a fine residue of salt. This fine residue converts into neutral atoms. Due to thermal energy of the flame, the atoms get excited and there return to ground state. In this process of returning to ground state, excited atoms emit radiation of specific wavelength. The wavelength of radiation emitted is specific for every element.



The specificity of wavelength of light emitted indicates the element and makes it a qualitative aspect. While the intensity of radiation, depends on the concentration of element. This makes it a quantitative aspect. This process is applicable only for few elements of group IA and group IIa (like Li, Na, K, Ca and Mg) are only analyzed.

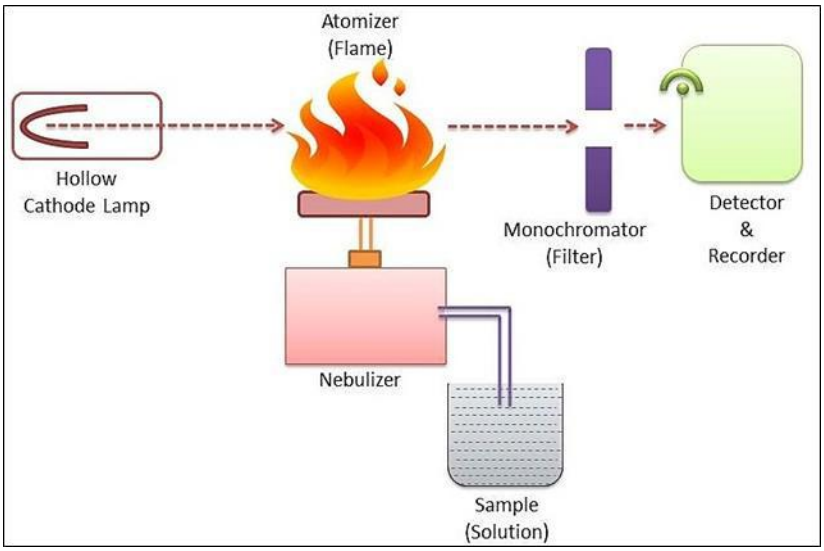
Element	Specific Wavelength (nm)	Colour
Sodium (Na)	589	Yellow
Calcium (Ca)	622	Orange
Lithium (Li)	670	Red
Potassium (K)	767	Violet/lilac

Atomic absorption spectroscopy

It is a technique which studies absorption of electromagnetic radiation in relationship to molecular structure.

Instrumentation and working procedure

When a beam of electromagnetic radiation is passed through a substance, the absorption of radiation would bring about increase in energy of the molecule. It leads to electronic excitations where electron jumps to higher energy levels. A particular wavelength that a given molecule can absorb depends upon the change in vibrational or rotational or electronic states.

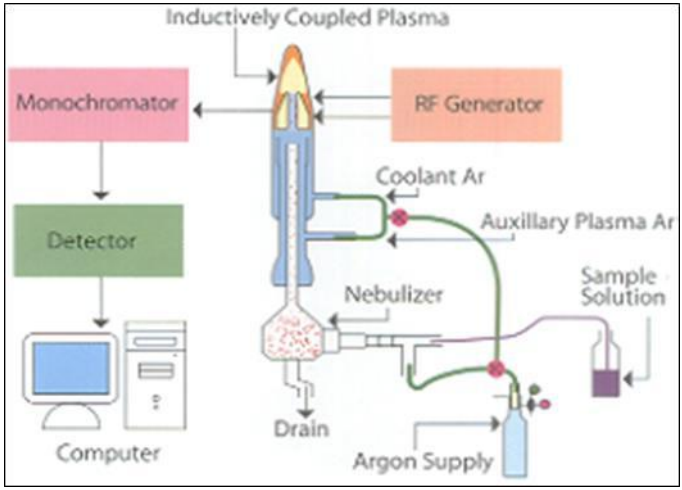


Inductively coupled plasma atomic emission spectroscopy

ICP-AES is an analytical technique used for the detection of chemical elements. It is a type of emission spectroscopy that uses the inductively coupled plasma to produce excited atoms and ions that emit electromagnetic radiation at wavelengths characteristic of a particular element. It is a flame technique with a flame temperature in a range from 6000 to 10000 K. The intensity of this emission is indicative of the concentration of the element within the sample (Moore, 2012) [6].

Instrumentation and working procedure

- An inductively coupled plasma spectrometer is a tool for trace detection of metals in solution, in which a liquid sample is injected into argon gas plasma contained by a strong magnetic field.
- The elements in the sample become excited and the electrons emit energy at a characteristic wavelength as they return to ground state.
- The emitted light is then measured by optical spectrometry.

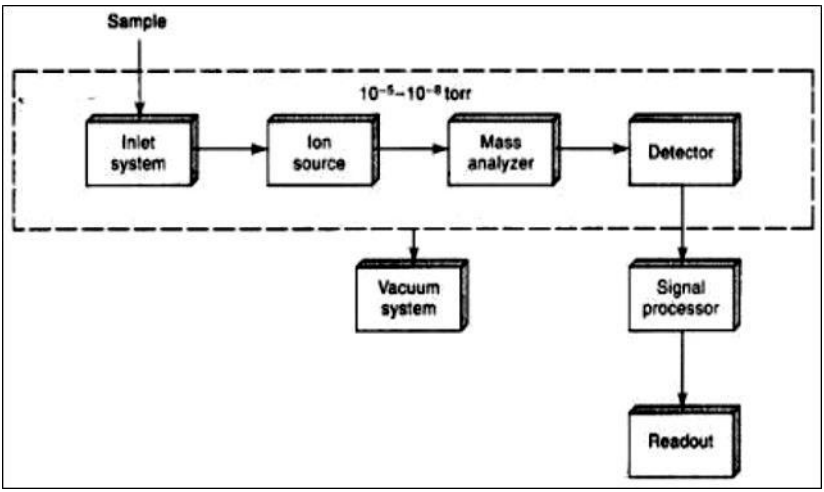


Mass spectrometer

Mass spectrometry is a powerful analytical technique used to quantify known materials, to identify unknown compounds within a sample, and to elucidate the structure and chemical properties of different molecules (Ammann, 2007) [1]. The complete process involves the conversion of the sample into gaseous ions, with or without fragmentation, which are then characterized by their mass to charge ratios (m/z) and relative abundances.

Instrumentation and working procedure

- Element to be identified and quantified is converted in to gaseous from by suitable means.
- This gaseous phase is bombarded with a beam of rapidly moving electron which produce positive ions.



- These positive ions are accelerated under the influence of higher electric field.
- These accelerated positive ion are subjected to electromagnetic field.as a result ions are deflected according to their mass and ionic charges.
- All these deflected ions are detected using suitable detector.

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Chapter - 3
Physicochemical Analysis of Herbal Drugs

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Chapter - 3

Physicochemical Analysis of Herbal Drugs

Milan Hait

Abstract

Medicinal plants and their bioactive molecules are always in demand and are a central point of research. To date, herbs have remained useful not only as remedy for different diseases that affect humans and animals, but also as good starting points for the discovery of bioactive molecules for drug development. Therapeutic efficacy of medicinal plants depends upon the quality and quantity of chemical constituents. Herbal materials, herbal preparations and finished herbal products are very complex. This can make the identification and quantification of herbal medicines very difficult and the detection of adulteration is very challenging. It should be emphasized that the identification of herbal medicines using markers, and quantification of marker substances in herbal medicines are not in themselves sufficient to guarantee the quality of herbal medicines. Quality control must cover all steps of their production and must be complemented by good agricultural and collection practices (GACP) and good manufacturing practices (GMP). Physicochemical Analysis in plants gives valuable information and help to assess the quality of herbal drugs.

Keywords: plant materials, herbal drug, physico-chemical analysis, quality control

1. Introduction

Herbal medicines are at great demand globally for primary healthcare due to their higher safety margins and cost effectiveness. Quality control of herbal medicines generates a lot of problems. So first and foremost task is the selection of the right kind of plant material which is therapeutically efficacious compounds. Herbal medicines are being manufactured on large scale where manufacturers face many problems such as low-quality raw material, lack of authentication of raw material, non-availability of standards, lack of proper standardization methodologies of single drugs and formulations and lack of quality control parameters. At the same time

consumers prefer to choose products with established standards. So it is a prime need to standardize Ayurvedic preparations to guarantee their purity, safety, potency and efficacy. Herbal products represent a number of unique problems related to quality which are further complicated by the use of combination of herbal ingredients being used in traditional practice. Therefore, in case of herbal drugs and products the standardization should encompass entire field of study from cultivation of medicinal plant to its clinical application. WHO involves in standardization and quality control of herbal crude drugs to monitor the physicochemical evaluation of crude drugs covering the aspects of selection and handling of crude material, safety, efficacy, stability assessment of finished product, documentation of safety and risk based on experience, provision of product information to consumer and product promotion (Rich, 2000; Tanna *et al*., 2011, Das *et al.*, 2011). Despite the modern techniques, identification of plant drugs by Physicochemical study is also more reliable like pharmacognostic studies. The physicochemical parameters are necessary for confirmation of the identity and determination of quality and purity of crude drugs (Fatehalrahman *et al*., 2018).

Some plant-based drugs have been used for centuries and for some like cardiac glycosides, there is no alternative conventional medicine. Therefore, medicinal plants and their bioactive molecules are always in demand and are a central point of research. As a result, there is a recent (WHO, 2011) surge in the demand for herbal medicine. To date, herbs have remained useful not only as remedy for different diseases that affect humans and animals, but also as good starting points for the discovery of bioactive molecules for drug development. The scientific exploitation of herbs used ethnomedicinally for curing of diseases resulted in the identification of a wide range of compounds that have been developed as new therapies for cancer, hypertension, diabetes and as anti-infectives etc. (Harvey, 2008). The study of plant drugs from the physocochemical stand point would include the study of the percentage of total ash, acid-insoluble, water soluble and sulphated ash, loss on drying, extractive values, foaming index, swelling index, crude fiber content, hemolytic activity, foreign organic matter and bitterness value and the chemistry of the constituents especially of those which may be used in therapeutics (Gupta *et al.*, 2009; Evan, 1996).

Authentication and standardization are prerequisite steps while considering source materials for herbal formulation in any system of medicine (Ahmad *et al*., 2009). For developing drug standardization, the quality of base material used for formulating the herbal products is a

prerequisite. Since the materials used in herbal drugs are traded mostly as roots, bark, twigs, flowers, leaves, and fruits and seeds, visible authentication of the material used is difficult and has led to a high level of adulteration. Standardization of natural products is a complex task due to their heterogeneous composition, which is in the form of whole plant, plant parts or extracts obtained there of. To ensure quality reproduction of herbal products, proper control of starting material is utmost essential. Thus, in recent years there has been a rapid increase in the standardization of selected medicinal plants of potential therapeutic significance. The misuse of herbal medicine or natural products starts with wrong identification. The most common error is one common vernacular name is given to two or more entirely different species (Peter and De, 2002). All these problems can be solved by pharmacognostic studies including physocochemical analysis of medicinal plants. According to WHO (WHO, 1996; WHO, 1992), standardization and quality control of herbals is the process involved in the physicochemical evaluation of crude drug covering aspects, such as selection and handling of crude material, safety, efficacy and stability assessment of finished product, documentation of safety and risk based on experience, provision of product information to consumer and product promotion. Methods of standardization should take into consideration all aspects that contribute to the quality of the herbal drugs (Mukherjee, 2002; Agarwal and Paridhavi, 2007; Annanomous, 2004).

2. Moisture contents

Moisture content (MC) is the quantity of water contained in the plant materials. It is used in a wide range of scientific and technical areas. An inevitable component of crude drugs is moisture, which must be eliminated as far as practicable. The preparation of crude drugs from the harvested plant drug involves cleaning or garbling to remove soil or other extraneous material, followed by drying, which plays a very important role in the quality as well as the purity of the material. The objectives of drying fresh material are:

- To aid in preservation.
- To “fix” the constituents, that is, to check enzymatic or hydrolytic reactions that might alter the chemical composition of the drug.
- To facilitate subsequent comminution (grinding into a powder) and
- To reduce their weight and bulk.

The crude plant materials are generally dried and stored as voucher specimens, as well as for extraction and isolation purposes. They are not

usually stored as such, though, according to the API (Ayurvedic Pharmacopeia of India), many quality control parameters have been determined using the raw plant material, without drying it. The moisture content of a plant material is important from a stability perspective. The moisture content affects the following parameters:

- Microbial growth, which may be bacterial or fungal growth and is deleterious to the drug.
- Enzymatic activity inside the plant cells may be a threat to the stability of the compounds.
- On drying, the bulk of the material also gets reduced, which helps in the storage of the plant material.
- After drying, further comminution and size reduction becomes easier for the drug.

The presence of excess water in raw material facilitates the growth of microorganisms that can reduce the quality of the raw material. The moisture content of raw material is determined by the following methods, as stated by WHO. An excess of water in herbal materials will encourage microbial growth, the presence of fungi or insects, and deterioration following hydrolysis. Limits for water content should therefore be set for every given herbal material. This is especially important for materials that absorb moisture easily or deteriorate quickly in the presence of water. The moisture content is determined by several methods. Moisture content is determined by the subtracting the dry weight form the initial weight. Among them, the WHO has prescribed two methods (Mukherjee, 2019).

The official compendia, such as the Indian Pharmacopeia and the Ayurvedic Pharmacopeia of India, have given guidelines on the optimum moisture content for official crude drugs. The moisture content in a crude drug may be determined by different methods, such as the Karl Fischer (KF) titration method, the toluene distillation method (Azeotropic distillation method), and the determination of the loss on drying.

There are many methods for determination of moisture content. Primary methods used are loss on drying and Karl fischer titration.

A) Loss on drying (LD)

Gravimetric method

In this method, a wet sample is weighed on a balance, placed in an oven, and heated until the end of the drying period, i.e., until the sample reaches equilibrium. The weight loss is the moisture content of the sample. In

moisture balances, this process is sped up by superheating the sample by a variety of means, including infrared, halogen, and microwaves (Mermelstein, 2009).

Determination of loss on drying

Procedure

i) Weigh about 1.5 mg of the powdered drug in to weighed flat and thin porcelain dish.

ii) Dry in the oven at 100 °C or 105 °C, until two consecutive weighing do not differ by more than 0.5 mg.

iii) Cool in desiccators and weigh. The loss in weight is usually recorded as moisture.

Moisture content (w) $= \frac{M_w}{M_s} \times 100$

Where w = moisture content of the crude drug in percent, M_w = mass of water in the sample, and M_s = mass of bone-dry solid in the sample.

Note

- A very useful from of dish for the determination of moisture is a thin flat porcelain dish. If a platinum dish is available it may be used.
- The burning of the powder should proceed slowly and the material must not be allowed to catch fire or to give off smoke as dense fumes.
- The desiccation method is useful for materials that melt to a sticky mass at elevated temperatures.
- The most common methods for the determination of moisture is heat the drug till one gets constant weight at 100 °C, as is done for digitalis. However, many substance loose other volatile constituent, or some of their constituent undergo change with consequent loss of weight at a temperature of 100 °C, other method are used for material of these types.
- If the solvent is anhydrous, water may remain absorbed in it leading to false results. Hence, it is advisable to saturate the solvent with water before use (Khandelwal, 2010; Ahamed *et al*., 2013).

B) Karl fischer method

In this method, the amount of water in a sample is determined directly

by titration with Karl Fischer reagent, which consists of iodide, sulfur dioxide, a base, and a solvent system. There are two titration approaches: Coulometric titration for samples with very low moisture content (<0.03%) and volumetric titration for samples with moisture contents ≥0.03%. In coulometric titration, the sample is added to the reagent, and electrolytic oxidation causes the production of iodine. The amount of iodine produced is proportional to the electricity (number of coulombs) used, which in turn is proportional to the amount of water in the sample. In volumetric titration, a sample is added to a moisture-free solvent and titrated with the reagent, whose titer (mg of water/mL of reagent) is known. The titration endpoint, determined by a constant-current polarization voltage method, represents the moisture content of the sample (Mermelstein 2009).

C) Azeotropic volumetric method/toluene distillation mthod

Dean-Stark apparatus is used for the determination of moisture content by azeotropic volumetric method. Organic solvent like benzene, toluene or xylene is used for distillation. Organic solvent farms a binary azeotropic mixture with water (present in the crude drug). When drug is heated with one of the organic solvent, the solvent and water will distil over as a mixture. Waters being heavier than organic solvents, collect at the bottom of the graduated tube of Dean-Stark apparatus. When the level of the solvent reaches the side-arms it flow back to the receiver and thus continuous distillation with same volume of the solvent take place. The volume of water can be read directly from the graduated tube (Fig.1).

If the solvent is anhydrous, water may remain absorbed in it leading to false result. Hence, it is advisable to saturate the solvent with water before use.

Procedure

i) Take 100 gram of powdered drug in the flask.

ii) Add 100 ml of toluene.

iii) Boil for about 1 hour.

iv) Allow to cool and measure the volume of water in the receiver.

v) Repeat until there is no further increase in this volume and calculate the moisture content of the sample.

The moisture content may be calculated using the following formula after the experiment:

Moisture content = $\frac{\mathbf{100}\ (\boldsymbol{n_1 - n})}{\boldsymbol{w}}$

Where w is the weight in g of the material being examined; n the number of mL of water obtained in the first distillation and n_1 the total number of mL of water obtained in both distillations (Khandelwal, 2010; Mukherjee, 2019).

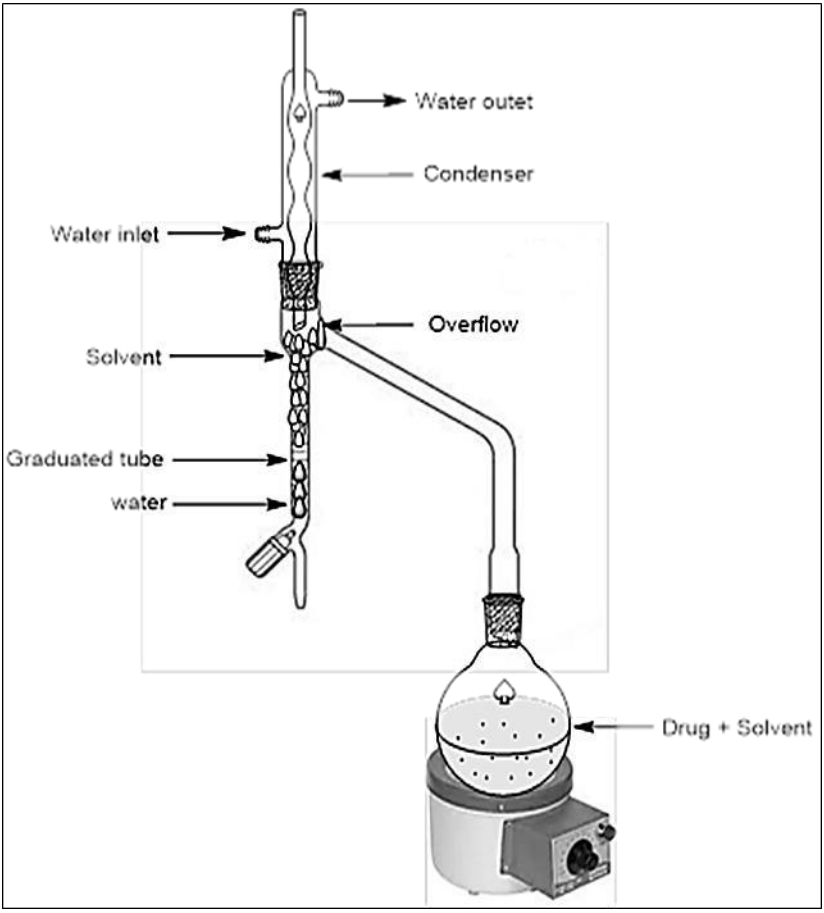

Fig 1: Dean-Stark apparatus for determination of moisture content by Azeotropic method

3. Extractive values

Various plant species would obviously have various chemical profiles. Chemical present in plant material could be dissolved in different solvent for the purpose of various analysis. Extractive values are used for evaluation of crude drugs when they cannot be estimated by any other method. Extractive values by various solvents are used to assess quality, purity and to detect adulteration due to exhausted and incorrectly processed drugs. It is useful for

the evaluation and gives an idea about the nature of the chemical constituents present in a crude drug. Useful also for the estimation constituents extracted with the solvent used for extraction. It is employed for material for which as yet no suitable chemical or biological assay exists.

The measurement of extractive values determines the amount of the active constituents in a given amount of plant material when extracted with a particular solvent. The extractions of any crude drug with a particular solvent yield a solution con taining different phytoconstituents. The compositions of these phytoconstituents depend upon the nature of the drug and the solvent used. They also give an indication of whether the crude drug is exhausted or not. The standard extractive values monitored for medicinal plants include extractions with a series of solvents based on their polarity; these may include alcohol and water-soluble extractive values, the petroleum ether-soluble extractive value, the ethylacetate-soluble extractive value, and the acetone-soluble extractive value (Chanda, 2014; Mukherjee, 2019).

Importance:

- Useful for the evaluation of a crude drug.
- Gives idea about the nature of the chemical constituents present in a crude drug.
- Useful for the estimation constituents extracted with the solvent used for extraction.
- Employed for material for which as yet no suitable chemical or biological assay exists.

Methods

I. Cold maceration

II. Hot extraction

Cold maceration

i) Determination of water-soluble extractive

10-gram sample (coarse powder form) was macerated with 100 ml of distilled water in a closed flask, for 24 hours, shaking frequently. Solution was filtered and 25 ml of filtrate was evaporated in a tarred flat bottom shallow dish, further dried at 100^{o}C and weighed. The percentage of water-soluble extractive was calculated with reference to the air-dried sample (coarse powder form).

ii) Determination of Alcohol-Soluble extractive

10 gram of air dried coarse powder form sample was soaked with 100 ml of alcohol in a closed flask for 24 hours with frequently shaking. It was filtered rapidly, taking precaution against loss of alcohol. 25 ml of filtrate was then evaporated in a tarred flat bottom shallow dish, dried at 100ºC and weighed. The percentage of alcohol soluble extractive was calculated with reference to the air dried coarse powder samples.

Calculation

25ml of alcoholic extract gives = x gram of residue.

100ml of alcoholic extract gives = 4gram of residue.

Since, 5gm of air dried drug gives 4x gram of alcohol (90%) soluble residue.

100gm of air dried drug gives 80x gram of the alcohol (90%) solute residue.

Alcohol (90%) soluble extractive value of the sample=80x%.

iii) Determination of methanol-soluble extractive

10 gram of air dried coarse powder form sample was soaked with 100 ml of MeOH in a closed flask for 24 hours with frequently shaking. It was filtered rapidly, taking precaution against loss of MeOH. 25 ml of filtrate was then evaporated in a tarred flat bottom shallow dish, dried at 100ºC and weighed. The percentage of MeOH soluble extractive was calculated with reference to the air-dried coarse powder drug samples.

iv) Determination of benzene-soluble extractive

10 gram of air dried coarse powder form drug sample was soaked with 100 ml of benzene in a closed flask for 24 hours with frequently shaking. It was filtered rapidly, taking precaution against loss of benzene. 25 ml of filtrate was then evaporated in a tarred flat bottom shallow dish, dried at 100 ºC and weighed. The percentage of benzene soluble extractive was calculated with reference to the air-dried coarse powder drug samples.

v) Determination of Pet. ether-soluble extractive

10 gram of air dried coarse powder form sample was soaked with 100 ml of petroleum ether in a closed flask for 24 hours with frequently shaking. It was filtered rapidly, taking precaution against loss of petroleum ether 25 ml of filtrate was then evaporated in a tarred flat bottom shallow dish, dried at 100 ºC and weighed. The percentage of petroleum ether soluble extractive

was calculated with reference to the air-dried coarse powder drug samples.

vi) Determination of chloroform-soluble extractive

10 gram of air dried coarse powder form sample was soaked with 100 ml of chloroform in a closed flask for 24 hours with frequently shaking. It was filtered rapidly, taking precaution against loss of chloroform 25 ml of filtrate was then evaporated in a tarred flat bottom shallow dish, dried at 100 ^{o}C and weighed. The percentage of chloroform soluble extractive was calculated with reference to the air-dried coarse powder drug samples.

vii) Determination of acetone-soluble extractive

10 gram of air dried coarse powder form sample was soaked with 100 ml of acetone in a closed flask for 24 hours with frequently shaking. It was filtered rapidly, taking precaution against loss of acetone 25 ml of filtrate was then evaporated in a tarred flat bottom shallow dish, dried at 100^{o}C and weighed. The percentage of acetone soluble extractive was calculated with reference to the air-dried coarse powder drug samples.

viii) Determination of hexane-soluble extractive

10 gram of air dried coarse powder form sample was soaked with 100 ml of hexane in a closed flask for 24 hours with frequently shaking. It was filtered rapidly, taking precaution against loss of hexane 25 ml of filtrate was then evaporated in a tarred flat bottom shallow dish, dried at 100^{o}C and weighed. The percentage of hexane soluble extractive was calculated with reference to the air-dried coarse powder drug samples.

Hot maceration

Determination of extractive by hot extraction methods

Procedure

i) Weight about 4 gm of the coarsely powdered drug in a weighing bottle and transfer it to a dry 250 ml conical flask.

ii) Fill a 100 ml graduated flask to the delivery mark with the solvent (90% alcohol). Wash out the weighing bottle and pour the washing, together with the remainder of the solvent in to the conical flask.

iii) Cork the flask and shake it well and allow to stands for one hour.

iv) Attach a reflux condenser to the flask and boil gently for one hour.

v) Cool and weigh.

vi) Readjust to the original total weigh with the solvent (water/alcohol/any other solvent) used for extraction.

vii) Shake well and filter rapidly through a dry filter.

viii) Filter in to 50 ml cylinder. when sufficient filtrate has collected, transfer 25 ml of the filtrate to a weighed, thin porcelain dish, as used for the ash value determination.

ix) Evaporate to dryness on a water bath and complete the drying in an oven at 105 ºC for 6 hour.

x) Cool in a desiccator for 30 minutes and weigh immediately.

xi) Calculate the percentage w/w of extractive with reference to the air-dried drug.

Calculation

25 ml of alcoholic extract gives = x gram of residue.

100 ml of alcoholic extract gives = 4gram of residue.

Since, 5 gms of air-dried drug gives 4x gram of alcohol (90%) soluble residue.

100 gms of air-dried drug gives 80x gram of the alcohol (90%) solute residue.

Alcohol (90%) soluble extractive value of the sample=80x% (Khandelwal, 2010; Ahamed *et al*., 2013; Trivedi *et al*., 2012).

4. Ash values

The ash content of a crude drug is generally taken to be the residue remaining after incineration. It usually represents the inorganic salts naturally occurring in the drug and adhering to it, but it may also include inorganic matter added for the purpose of adulteration. There is a considerable difference within narrow limits in the case of some individual drugs. Hence, an ash determination furnishes a basis for judging the identity and cleanliness of a drug and gives information relative to its adulteration with inorganic matter. Ash standards have been established for a number of official drugs. Usually, these standards give a maximum limit on the total ash or on the acid-insoluble ash permitted (Mukherjee, 2019).

The residue remaining after incineration (Ignition) is similarly known as the ash content of the drugs. It contains generally inorganic salts of carbonates, phosphates, silicates of Na, K, Ca and Mg etc. Ash value is the parameter to identify or purity of the crude drug.

Types of ash value: It is several types, which are as follows-

i) Total ash value

ii) Acid insoluble ash value

iii) Water soluble ash value

iv) Sulfated ash value

i) Total ash value (TAV)

The residue after incineration of sample at high temperature (500-600 ^{o}C) is called ash. The sample is subjected to a 500-600 ^{o}C and then the ash content is determined. During incineration (burning process) all organic compounds decompose there ions and pass in the form of gases, whereas the mineral elements remain in form of ash.

Procedure

Take 2 gram dry sample (powder form) in a previously weighed crucible. Heat it on hot plate for about 30 minutes, till the sample is sufficiently charred and turned black. Replace the lid of the crucible and keep it in muffle furnace. Allow the temperature to raises up to 600^{o}C and keep it constant for 2 hours. Remove the crucible on cooling and transfer to desiccator directly, cool and weigh immediately. Find out the weight of ash, obtained per 2 gram of sample, and calculate the ash content as percent of dry matter.

Calculation

Weight of the empty dish = x

Weight of the drug taken = y

Weight of the dish + ash (after complete the incineration) = z

Weight of the ash = (z-x) gram.

“y” gram of the crude drug gives (z-x) g of the ash

Since 100 gram of crude drug gives 100/y (z-x) gram of the ash

Total Ash value of the sample =100 (z-x)/y %

ii) Acid insoluble ash value (AIAV)

Acid-insoluble ash is an indication of the portion of the ash that is insoluble in dilute hydrochloric acid. It indicates mainly the silica and sand-like substances present in the ash. The acid-insoluble ash is determined by washing the ash with 2 M HCl and then placing the ash on a Gooch crucible, washing with water, and filtering with ashless filter paper. Then, the filter paper is burnt off and the weight of the carbon-free mass is taken for

estimation. The acid-insoluble ash, compared with the total ash, gives an estimate of the contaminating materials in the crude drug.

Procedure

Proceed as per the step mentioned in the procedure for determination of total ash value of a crude drug.

Further:

i) Using 25 ml dilute HCl acid, wash the ash from the dish used for total ash in to a 100 ml beaker.

ii) Place mere gauze over a Bunsen burner and boil for 5 min.

iii) Filter through an 'Ashless' filter paper, wash the residue twice with hot water.

iv) Ignite a crucible in the flame, cool and weigh.

v) Put the filter paper and residue together in to the crucible; heat gently until vapor cease to evalved and then more strongly until all carbon has been removed.

vi) Cool in a desiccator.

vii) Weigh the residue and calculate acid insoluble ash of the crude drug with reference to the air-dried sample of the crude drug.

Calculation

Similar to previous experiments

Weight of residue = "a'' gram

"y" gram of the air-dried drug gives "a" of acid-insoluble ash

Since 100 gram of the air dried drug gives 100×a/y gram of the acid insoluble ash.

Acid insoluble ash value of the sample =100×a/y %

Acid-insoluble ash value of a crude drug is all ways less then Total Ash value of the same drug.

iii) Water soluble ash value (WSAV)

Water-soluble ash is a determinant of whether the substance was previously extracted, that is, for detecting exhausted drugs. For doing this, first extract the ash by boiling it in water. After that, the residue is to be filtered by ashless filter paper. After burning, the ash, which is cooled and weighed. The difference between the weight of the total ash and the residue remaining after washing with water is known as the water-soluble ash.

Procedure

The ash was boiled for 5 minutes with 25 ml of distilled water. Insoluble matter was collected in ashlesss filter paper and washed with hot water, ignited and weighed. Weight of the insoluble matter was subtracted from the weight of ash. The difference in weight represents the water-soluble ash. Percentage of water-soluble ash was calculated with reference to the air-dried drug.

Calculation

Same as previous experiment

Weight of residue = "a'' gram

"y" gram of the air-dried drug gives "a" of water-soluble ash

Since 100 gram of the air dried drug gives 100× a / y gram of the water-soluble ash.

Water-soluble ash value of the sample =100×a/y %.

iv) Sulfated ash value (SAV)

The sulfated ash value test uses a procedure to determine the residual substance not volatilized from a sample when the sample is ignited in the presence of H_2SO_4. The test is usually used for measuring the content of inorganic impurities in an organic substance.

Procedure

Take 1-2 gram of substance in accurately weight crucible, ignite gently at first until the substance is thoroughly charred. Cool, moisture the residue with 1 ml of H_2SO_4, heat gently until white fumes are no longer evolved and ignite at 800 ^oC + 25 ^oC until black particles have disappeared. Allow the crucible to cool, and a few drop of $H_2SO_4$and heat. Ignite as before and allow to cool and weights. Repeat the operation until two successive weighing do not differ by more than 0.5mg (Khandelwal, 2010; Ahamed *et al*., 2013; Indian Pharmacopoeia, 2014).

5. Foaming index

Saponins give persistent foam when shaken with water. Hence, plant material/extracts containing saponins is evaluated by foaming ability in term of foaming index. Several medicinal plants contain saponin and, therefore, they produce foam during shaking of an aqueous decoction. The saponins are high-molecular-weight-containing phytoconstituents, having detergent activity. Saponins are mostly characterized based on their frothing property.

Medicinal plants of different groups, especially those derived from the families Caryophyllaceae, Araliaceae, Sapindaceae, Primulaceae and Dioscoreaceae, contain saponins. As per WHO (1998), the foaming index is described as the ability to form foam from an aqueous decoction of a plant sample and its extracts. To get a reliable result for the foaming index, it is essential to standardize the experimental conditions. A drug containing saponins has the capability to form froth, which depends upon the nature of the drug and/or the quantity of saponins present. This parameter also provides useful information and helps in the quality control of the drug (Chidambaram and Aruna, 2013).

Procedure

i) Take 1 gram of coarse powder of the plant material in a 500 ml conical flask.

ii) Add 100 ml boiling water and maintain moderate boiling for 30 minutes.

iii) Cool and filter.

iv) Collect the filtrate/decoction in a 100 ml volumetric flask and adjust the volume to 100ml by adding sufficient water.

v) Pour the decoction in to 10 stoppered test tubes (height 16 cm, diameter 16 mm) as 1 ml, 2 ml, 3 ml, etc. up to 10 ml.

vi) Adjust the volume of liquid in each test tube to 10 ml by adding sufficient quantity of water and stopper the tubes.

vii) Shake test tube in a length wise motion for 15 second (two shakes per second).

viii) Allow test tubes to stand for 15 min and measure the height of the foam.

Assessment

If the height of the foam in every tube is less than 1cm it mean foaming index is less than 100. If the height of the foam is more than 1 cm in every test tube; the foaming index is over 1000. In this case, repeat the experiment using a new series of dilution of the decoction in order to get a result.

If the height of foam is 1 cm in any tube, the volume of the plant material decoction in this tube (a) is used for determination of the foaming index using the following formula:

Foaming index =100/a

Where,

A = volume in ml of the decoction in the test tube showing 1 cm foam height.

Note: if the test tube showing 1cm foam height is first or second in the series, prepare an intermediate dilution in a similar manner to obtain precise result.

6. Swelling index

The swelling index is expressed as the volume of the solution (water or any other swelling agent) that is absorbed to swell 1 g of plant sample under specified conditions. The uses of solution are varied for individual plant samples (either whole, cut, or pulverized). The WHO Guideline 1998 has specified the swelling properties of several plant materials having specific therapeutic potential, including those plants containing gum and mucilage, pectin or hemicelluloses.

The term swelling factor gives an idea about the mucilage content of the drugs, hence it is useful in the evaluation of crude drugs containing mucilage.

Procedure

i) Take 1 gram of the seed in a 25 ml stoppered cylinder

ii) Add water up to 25 ml marking.

iii) Shake occasionally during 23 hours.

iv) Keep aside for one hour.

v) Measure the volume occupied by the swollen seeds e.g. Swelling factor of the Isabgol seed is not less than 10 ml.

7. Crude fiber content

It is necessary to assess the food used by humans or animals, specifically a determination of the extent of the ingestion of the food and its nutritional content absorption and maintenance in the body. A quantification of fecal output is a quick method for a digestibility study. Crude fiber determination is mainly performed to quantify the proportion of the food that cannot be digested by humans or animals. Crude fibers are generally those organic materials present in food or fodder that remain insoluble even when the material is treated with chemicals at a specific temperature and concentration.

The crude fiber determination helps in identifying the quantity of adulterants present in any drug sample, food, or fodder. Determination of the crude fiber assists in distinguishing the soluble and insoluble parts, which are acquired from the parts of plants and plant cells (Morales *et al*., 1999, Mukherjee, 2019).

Determination of crude fiber content

Take about 2 g of the drug sample, weigh accurately, and extract with ether. Then, add 200 mL of 1.25% sulfuric acid and boil the whole mixture for about 30 min in a 500-mL flask under reflux. Filter the mixture and wash the residue with water until it is acid free. Rinse the residue back to the flask and add 200 mL of boiling 1.25% sodium hydroxide solution and again boil under reflux for 30 min. Filter the liquid quickly and wash the residue with boiling water until neutral. Dry at 110°C to constant weight and incinerate to constant weight. The weight of the crude drug will be represented by the difference between the weight of the residue and weight of the incinerated residue. It will be expressed as a percentage of the original weight of the material. The crude fiber is calculated on a percent-dry basis as described below.

$$\% \text{ Crude fiber (dry basis)} = \frac{\boldsymbol{dry\ resdue\ (g) - ignited\ residue\ (g)}}{\boldsymbol{Sample\ (g)}} \times 100$$

8. Hemolytic activity

Many plants contain chemical substances, such as saponins, that have a hemolytic effect on human erythrocytes. It has been reported that erythrocytes obtained from different blood types have different stability. However, the effects of many plants on the red blood cell membrane are positive and even it may show some serious adverse effects, such as hemolytic anemia. However, to avoid unwanted reaction and side effects, many commonly used herbal plants are evaluated for their potential hemolytic activity (Zohra and Fawzia, 2014). Plants having medicinal value contain saponins as a key component, imparting frothing and detergent properties. Saponins have the ability to produce changes in the erythrocyte membranes when added to a blood suspension, causing hemolysis and resulting in hemoglobin diffusion in the surrounding media (Mukherjee, 2002). Medicinal plants and their bioactive constituents can destroy the membrane of an erythrocyte. It has been observed that hemolytic activity has a relationship directly proportional to the chain length and the presence of branches or cyclic groups makes the surfactant less hemolytic in nature.

Determination of hemolytic activity

The hemolytic activity of saponin-containing plant materials or drugs is evaluated by comparison with saponin R (reference standard). The hemolytic activity of saponin R is 1000 units/g. To determine the hemolytic activity, an equal volume of plant material (serial dilution) is mixed with a suspension containing erythrocytes. The lowest concentration at which com-plete hemolysis occurs is considered to be the hemolytic index (HI) of the plant material or drug. A similar test is performed simultaneously using saponin R. This procedure is applied for all medicinal plants containing saponin, but the results may vary (Mukherjee, 2002). The HI of a plant material is described as the 2% (v/v) of a blood sample containing erythrocytes that is hemolyzed by 1 g of saponin or tested plant sample.

The hemolytic activity of a plant should be determined by using human red blood cells. Human erythrocytes from healthy individuals are collected in tubes containing EDTA (anticoagulant). The erythrocytes are harvested by centrifugation for 10 min at 2000× g at 20°C. The supernatant is discarded and the pellet is washed thrice with PBS. To the pellet, PBS should be added to yield a 10% (v/v) erythrocytes/PBS suspension. The 10% suspension is diluted in PBS (1:10). From each suspension, 0.1 mL is added in triplicate to 100 mL of a different dilution series of catechu dye (or fluconazole and amphotericin B) in the same buffer in Eppendorf tubes. Total hemolysis should be achieved with 1% Triton X-100. The tubes should be incubated for 1 h at 37°C and then centrifuged for 10 min at 2000 × g at 20°C. From the supernatant fluid, 150 mL is transferred to a flat-bottomed microtiter plate, and the absorbance is measured spectrophotometrically at 450 nm. The hemolysis percentage should be calculated by the following equation:

$$\% \text{ of Hemolysis} = \frac{A\,450\ of\ test\ compound\ treated\ sample - A450\ of\ buffer\ sample}{a\,450\ of\ 1\%\ Triton\ X{-}100\ treated\ sample - A450\ of\ buffer\ treated\ sample}$$

9. Foreign organic matter

Foreign organic matter means the material consisting of any one or all of the following substances:

a) Material not collected from the original plant source.

b) Insect, moulds or other animal contamination.

c) Parts of the organ or organs from which the drug is derived other than the parts named in the definition and description.

d) Any other organ than those named in the definition and description.

Procedure

i) Weigh 100 to 500 of the sample (or the quantity specified in the monograph of the drug).

ii) Spread the sample on a white tile or a glass plate uniformly without overlapping.

iii) Inspect the sample with naked eyes or by means of a lens (5x or above).

iv) Separate the foreign organic matter (mention above) manually.

v) After complete separation, weigh the matter and determine % w/w present in the sample.

10. Bitterness value

Botanicals having a strong bitter taste are used therapeutically as appetizing agents. The bitterness results from the stimulation of gastric juice in the gastrointestinal tract. As per WHO, the bitterness of a plant is evaluated by comparing the thresh-old bitter concentration of the plant material with quinine hydrochloride solution. Bitterness is believed to be responsible for the flow of saliva, gastric juices, and bile and, thus, enhances the function of the digestive system. Bitter extracts or sub-stances are also known to act on the cardiovascular system by decreasing the heart rate and cardiac stroke volume (Olivier and van Wyk, 2013). As per WHO, the content of bitter substances can also be quantified chemically.

The bitterness value is presented in terms of unit equivalent of bitterness in a 2000 mL solution containing 1 g of quinine hydrochloride. For extraction of herbal material, potable drinking water is used and also mouthwash is required.

Bitter tonics, e.g. Chirata, Quassia, Piccrorhiza etc; which stimulate subsection in the GI tract especially of gastric juice are used therapeutically as appetizers. Bitter constituent present in such drug are responsible for the activity, which may be more than one with different degree of bitterness hence before going the chemical determination, total bitterness by taste is first measured.

Principle

There hold bitter concentration of an extract of the plant material compared with that of a dilute solution of Quinine hydrochloride. The bitterness value is determined in units equivalent to the bitterness of a solution of 1kg Quinine hydrochloride in 2 litre of water.

As described by the WHO (1998), the bitterness value of a specific sample can be analyzed as follows:

Quinine hydrochloride (0.1 g) is dissolved in sufficient potable drinking water to yield a final volume of solution of 100 mL. Further, the solutions (5 mL) are diluted with potable drinking water (500 mL). This solution contains 0.01 mg/mL of quinine hydrochloride. Nine test tubes are used for the serial dilution. Safe drinking water is used to wash the mouth properly. After that, 10 cc of the most diluted solution is tested and remains in the mouth for 30 s. If the bitter sensation fades after 30 s, then the solution is spit out, followed by a waiting period of 1 min. After proper washing of the mouth, the next highest concentration of solution is tested for 10 min. After the first series of testing, the mouth is properly rinsed with potable drinking water and 10 min is allowed to pass until the next series is tested. The threshold of bitterness is described as the lowest dilution concentration at which the sample provokes a bitter taste after 30 s. It is assumed that if the solution in tube no. 5 (containing 5 mL of ST in 10 mL) gives a bitter sensation, then the threshold of bitter concentration should be tested using the solutions of tubes 1-4. If the solution of tube no. 5 does not give a bitter sensation, then the threshold bitter concentration should be determined by testing the solutions in tubes 6-10.

$$\text{Bitterness value (in gm)} = 2000\ \frac{2000 \times \textit{concentration of quinine hydrochloride R (mg)}}{\textit{concentration of the stok solution (mg/mL)} \times \textit{volume of stock solution (ml)}}$$

Note:

- Safe drinking water should be used for the extraction and for the mouth wash after each tasting.
- Some person should taste both, test sample and standard sample of Quinine hydrochloride.
- Middle part of the upper surface of the test sample should be used for this determination.
- In each test series, the lowest concentric should be used for determination.
- This test should not be carried out until the identity of the plant material has been confirmed (Khandelwal, 2010).

11. Conclusion

In almost all traditional system of medicine, the quality control aspect has been covered by careful observation of skillful persons. However, in modern concept, it requires necessary changes in their approach by way of

quality control in terms of development of modern methodologies. Thus, today quality assurance is a thrust area for the evaluation of traditionally used medicinal plants and herbal formulations. It is necessary to explore the parameters related to standardization to be carried out in different batches to set the limit for the reference standards for the quality control and quality assurance of Herbal drugs. The micro and macro standards obtained here can be identifying parameters to substantiate and authenticate the drug. The total ash value, extractive values will be helpful in identification and authentication of the plant material along with the microscopic method, which is the cheapest method to establish the correct identification of the source material, and determination of microorganisms of great importance, because medicinal plant materials normally carry a great number of bacteria and moulds, often originating in soil. Criteria for the selection of reference substances and quality control of herbal medicines should take into account that various ingredients may have different levels of influence on the final quality, safety and efficacy. It provide information on moisture content, ash content, volatile matter content, ash, fixed carbon etc. Ash is the inorganic residue remaining after water and organic matter have been removed by heating, which provides a measure of total amount of minerals with in the food. Minerals are not destroyed by heating and they have a low volatility as compared to other food components. Total ash may vary with in wide limits for specimen of genuine drugs due to variable natural or physiological ash. Ashes give us an idea of the mineral matter contained in a plant. Measuring it is important, because mineral matter may be the cause of a pharmacological effect. So, physicochemical analysis of herbal drugs is an important issue of quality control aspects of natural products.

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Chapter - 4
A Review on Analytical Methods for Estimation of Mometasone Furoate and Terbinafine Hydrochloride in Pharmaceutical Dosage Form

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Chapter - 4

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Abstract

Fungal infection of the skin are also known as "mycoses". They are common and generally mild. However, in very sick or otherwise immune suppressed people, fungi can sometimes cause severe disease. The combination of Mometasone furoate and Terbinafine Hydrochloride is available as Creams and Gels formulation for topical use in treatment of various types of skin infections.it minimizes symptoms of inflammation such as redness, swelling, and itching by acting against infection-causing bacteria's. Mometasone furoate (9α, 21-Dichloro-11β-hydroxyl-16α-methyl-3, 20-dioxopregna-1, 4-dien-17-yl furan-2-carboxylate) is reported in latest pharmacopoeias such as IP 2018 and USP 2004. Terbinafine Hydrochloride [(*E*)-*N*, 6, 6-trimethyl-*N*-(naphthalen-1-ylmethyl) hept-2-en-4-yn-1-amine; hydrochloride] is reported in latest pharmacopoeias such as IP 2018. This Review focuses on recent development in analytical method development for Mometasone Furoate and Terbinafine Hydrochloride. It provides information about different analytical method development like UV spectrophotometry, HPLC, HPTLC, and stability indicating Assay methods reported for Mometasone Furoate and Terbinafine Hydrochloride for individual and other drug combination.

Keywords: mometasone furoate, terbinafine hydrochloride, analytical method, UV spectrometry, HPLC, HPTLC

Introduction

Mometasone furoate (9α, 21-Dichloro-11β-hydroxyl-16α-methyl-3, 20-dioxopregna-1, 4-dien-17-yl furan-2-carboxylate) is reported in latest pharmacopoeias such as IP 2018 and USP 2004. Mometasone Furoate is a Glucocorticoid drug that can be used for the treatment of asthma, rhinitis, and certain skin condition. Mechanism of action of Mometasone Furoate is Anti-

inflammatory, antipruritic, and vasoconstrictive properties. Corticosteroids are thought to act by the induction to phospholipase A2 inhibitory proteins, collectively called lipocortins. It is postulated that these proteins control the biosynthesis of potent mediators of inflammation. The indication of Mometasone Furoate is used in redness, itching, inflammation.

Terbinafine Hydrochloride [(*E*)-*N*, 6, 6-trimethyl-*N*-(naphthalen-1-ylmethyl) hept-2-en-4-yn-1-amine; hydrochloride] is reported in latest pharmacopoeias such as IP 2018. Terbinafine hydrochloride is a synthetic allylamine antifungal. It is highly lipophilic in nature and tends to accumulate in skin, nails, and fatty tissues. Mechanism of action of Terbinafine Hydrochloride is

Terbinafine inhibits the enzyme squalene monooxygenase, preventing the conversion of squalene to 2, 3-oxydosqualene, a step in the synthesis of ergosterol. This inhibition leads to decreased ergosterol, which would normally be incorporated into the cell wall, and accumulation of squalene.

The indication of Terbinafine Hydrochloride is used in Ringworm of body, foot, and groin.

Physical and chemical property

Mometasone Furoate is White powder. IUPAC name is (9α, 21-Dichloro-11β-hydroxyl-16$^{\alpha}$ methyl-3, 20-dioxopregna-1, 4-dien-17-yl furan-2-carboxylate). Molecular formula of Mometasone Furoate is $C_{27}H_{30}C_{l2}O_6$. Molecular weight is 521.4 g/mol. It is freely soluble in acetone and dichloro methane, slightly soluble in methanol, Insoluble in water.

Terbinafine Hydrochloride is White fine crystalline. IUPAC name is (E)-N,6,6-trimethyl-N-(naphthalen-1-ylmethyl) hept-2-en-4-yn-1-amine; hydrochloride. Molecular formula of Terbinafine Hydrochloride is $C_{21}H_{25}N$. Molecular weight is 327.9 g/mol. It is freely soluble in dehydrated alcohol and in methanol, slightly soluble in acetone, very slightly or slightly soluble in water.

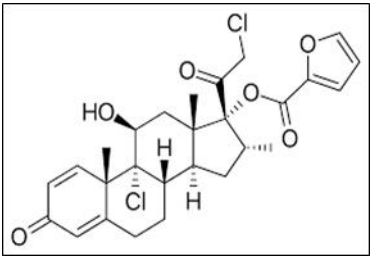

Fig 1: Chemical structure of Mometasone Furoate

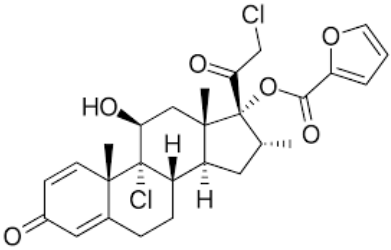

Fig 2: Chemical structure of Terbinafine Hydrochloride

Analytical method development

Analytical method development and validation assume fundamental part in the drug discovery, drug advancement and assembling the pharmaceutical products. It includes identification of the purity and toxicity of a drug substance. Analytical method development is the process of selecting an accurate assay procedure to determine the composition of a formulation. It is the process of proving that an analytical method is acceptable for use in laboratory to measure the concentration of subsequent samples Analytical methods should be used within GMP and GLP environments and must be developed using the protocols and acceptance criteria set out in the ICH guidelines Q2(R1). Analytical method development and Validation play important roles in the discovery, development, and manufacture of pharmaceuticals. However, UV Spectrophotometry, RP –HPLC, HPTLC, Stability indicating RP-HPLC methods, reported for Mometasone Furoate and Terbinafine Hydrochloride individual and along with other drugs.

Table 1: Official Methods for assessment of Mometasone Furoate

Sr. No.	Official In	Method	Description	Ref. No.
1.	IP 2018	High performance liquid Chromatographic Method	**Column:** 25cm × 4.6mm packed with octadecylsilane bonded to porous silica (5 µm). **Mobile phase:** A mixture of 50 volume of acetonitrile and 50 volume of water. **Wavelength:** 254nm. **Flow rate:** 1ml per minute. **Injection volume:** 20µl.	5
		Thin layer Chromatographic Method	**Stationery Phase:** Silica gel GF254 **Mobile Phase:** Water: Methanol: Ether: Dichloromethane (1.2: 8: 15 :77 %v/v/v/v) **Wavelength:** 254 nm	

2.	USP 2004	Chromatographic Methods	**Column:** 25 cm × 4.6 mm packed with octadecylsilane bonded to porous silica (5 µm). **Mobile phase:** Methanol: Water (65:35 %v/v) **Wavelength: 254nm.** **Flow rate:** 1.7 ml/min **Relative Retention Time:** **MF:** 1 min	6

Table 2: Reported methods for assessment of Mometasone Furoate

Sr. No.	Method	Description	Ref. No.
1.	Set-up of a method using LC-UV to assay Mometasone Furoate in pharmaceutical dosage forms	**Solvent:** Methanol: Water (80:20 %v/v) **Wavelength:** 248 nm **Retention time:** 4 min **Linearity:** 1 - 20 µg/ml	7
2.	Simultaneous estimation of Mometasone Furoate and salicylic acid in topical formulation by UV-Visible spectrophotometry	**Solvent:** Methanol **Wavelength:** 300 nm (zero absorbance of MF) 250 nm (both drugs showing absorbance's) **Linearity:** MF-1-10 µg/ml SALI-5-50 µg/ml	8
3.	Simultaneous estimation of mupirocin and Mometasone Furoate in pharmaceutical dosage form by Q-ABSORPTION RATIO METHOD.	**Solvent:** Methanol **Wavelength:** 226 nm (isoabsorptive point) 220 nm (max of mupirocin) **Linearity:** 5-25µg/ml for both MF and MUPI	9
4.	Spectrophotometric Method for simultaneous estimation of formoterol fumarate and Mometasone Furoate in respicaps.	**Solvent:** Methanol **Wavelength:** FF: 210-220 nm MF: 242-252 nm **Linearity:** FF: 3-18 µg/ml MF: 6-21 µg/ml	10
5.	High-performance liquid chromatographic analysis of Mometasone Furoate and its degradation products. Application to *in vitro* degradation studies	**Column:** Beckman C_8 column **Wavelength:** 248nm **Linearity:** 0.2-100µg/ml **Solvent:** Dichloro methane with dexamethasone 21- acetate **flow rate:** 1.5 ml/min	11
6.	Validated Stability-Indicating Methods for Determination of	**Column:** Silica Gel 60 F_{254} **Wavelength:** 250nm **Linearity:** 0.5-5 µg/ml	12

	Mometasone Furoate in Presence of its Alkaline Degradation Product.	**Mobile Phase:** Hexane: chloroform: methanol: acetonitrile (6:6:1:0.3%v/v/v/v)	
7.	Simultaneous estimation of Mometasone Furoate and Formoterol fumarate by HPLC METHOD in rotacaps original.	**Column:** C18 (250 mm x 4.6 mm, 5 µm) **Mobile Phase:** Methanol: Water (pH 3.5) (85:15%v/v) **Wavelength:** 225 nm **Retention Time:** MF-5.217 min FOR-8.65 min **Flow Rate:** 0.8 mL/min **Linearity:** MOM-33.33-299.97 µg/ml FOR-1-9 µg/ml	13
8.	Development and validation of analytical method for simultaneous estimation of Mometasone Furoate and fusidic acid in pharmaceutical dosage form by RP-HPLC.	**Column:** C18 (150 mm x 4.6 mm, 5 µm) **Mobile Phase:** 10 mm ammonium formate in water pH 5.5: Methanol (65:35 %v/v) **Wavelength:** 240 nm **Retention Time:** FUS-4.325 min MOM-8.109 min **Flow Rate:** 1 mL/min **Linearity:** MF-5-15 µg/ml FUS-100-300 µg/ml	14
9.	Development of analytical method and validation for Simultaneous estimation of clotrimazole, Mometasone Furoate and fusidic acid in pharmaceutical cream formulation by RP-HPLC method.	**Column:** C8 (250 mm x 4.6 mm, 5 µm) **Mobile Phase:** Acetonitrile: Methanol: Water: Glacial Acetic Acid (60:10:30:0.1 %v/v/v/v) adjust pH 3.5 by 10% orthophosphoric acid **Wavelength:** 240 nm **Retention time:** CLO: 1.7 min MF: 5.4 min FUS: 8.2 min Flow Rate: 1.5 mL/min **Linearity:** CLO: 48-112 µg/ml MF: 4.8-11.2 µg/ml FUS: 96-224 µg/ml	15
10.	Method development and validation for estimation of clotrimazole, fusidic acid and Mometasone Furoate in cream by RP-HPLC.	**Column:** C18 (250mm x 4.6mm, 5µm) **Mobile Phase:** 0.05M potassium dihydrogen phosphate (pH 6) using sodium hydroxide: Acetonitrile (60:40 %v/v) **Wavelength:** 230 nm **Retention time** CLO-4.277 min FUS-3.103 min	16

<table>
<tr><td></td><td></td><td>MF-5.707 min</td><td></td></tr>
<tr><td>11.</td><td>Validated Stability-Indicating Methods for Determination of Mometasone Furoate in Presence of its Alkaline Degradation Product by HPTLC</td><td>Stationary Phase: Silica gel 60F254
Mobile Phase: Toluene: Ethyl acetate: Glacial acetic acid (8: 4: 0.1 %v/v/v)
Wavelength: 250 nm
Rf Value:
MF: 0.36
MF DEG PRODUCT: 0.66
Linearity:
0.5-5 µg/ml</td><td>17</td></tr>
<tr><td>12.</td><td>Stability indicating HPLC method for simultaneous estimation of Mometasone Furoate and formoterol fumarate in combined dosage form.</td><td>Column: C18 (25 mm x 4.6 mm, 5 µm)
Mobile Phase: Acetonitrile: 0.05 M orthophosphoric acid: methanol (60:30:10 %v/v/v), pH 3
Wavelength: 248 nm
Retention Time: FOR 1.68 min
MF 7.17 min
Flow Rate: 1 mL/min
Linearity:
FOR: 3-9 µg/ml
MF: 100-300 µg/ml Stability results: (for FF)
<table>
<tr><th>Conditions</th><th>% Degradation</th></tr>
<tr><td>Acidic degradation (0.1 N Hcl, 30min)</td><td>1.41</td></tr>
<tr><td>Alkaline degradation (0.1N NaOH, 30min</td><td>0.84</td></tr>
<tr><td>Oxidative degradation (3% H2O2, 24 hours)</td><td>13.95</td></tr>
<tr><td>Photolytic degradation (UV Light, 1 week)</td><td>9.82</td></tr>
<tr><td>Thermolytic degradation (100 ºC, 1 week)</td><td>9.81</td></tr>
<tr><td>Neutral degradation (3 hours)</td><td>1.01</td></tr>
</table>
Stability results: (for MOM)
<table>
<tr><th>Conditions</th><th>% Degradation</th></tr>
<tr><td>Acidic degradation (0.1 N HCl, 30min)</td><td>25.30</td></tr>
<tr><td>Alkaline degradation (0.1N NaOH, 30min)</td><td>14.41</td></tr>
<tr><td>Oxidative degradation (3% H2O2, 24 hours)</td><td>0.67</td></tr>
</table></td><td>18</td></tr>
</table>

		Photolytic degradation (UV Light, 1 week) 26.04 Thermolytic degradation (100ºC, 1 week) 12.93 Neutral Degradation (3 hours) 0.31	
13.	Development and Validation of RP-HPLC-PDA Method for Estimation of Mometasone Furoate, Salicylic Acid, Methyl Paraben and Propyl Paraben in Combined Topical Formulation by Using Design of Experiment.	**Column:** C18 (250 mm x 4.6 mm, 5 µm). **Mobile Phase:** Acetonitrile: 0.1% acetic acid (60:40 %v/v). **Wavelength:** 264 nm. **Flow Rate:** 0.8 mL/min **Linearity:** MF: 1-10 µg/ml SALI: 35-350 µg/ml MP: 2-20 µg/ml PP: 0.2-2 µg/ml	19
14.	Development and validation of analytical method for simultaneous estimation of Mometasone Furoate, hydroquinone and Tretinoin in topical Formulation by RP-HPLC.	**Column:** C18 (250 mm x 4.6 mm, 5 µm) **Mobile Phase:** Acetonitrile: Methanol (90:10%v/v) **Wavelength**:266 nm **Flow rate:**0.5 mL/min **Linearity:** MF: 5-25µg/ml HYQ: 50-250 µg/ml TRE: 1-5 µg/ml	20

Table 3: Official Methods for assessment of Terbinafine Hydrochloride

Sr. No.	Official	Method	Description	Ref. No.
1.	IP 2018	Chromatographic Method	**Column:** 15cm x 3.0mm, packed with octadecylsilane bonded to porous silica (5mm). **Mobile phase:** A. 70 volumes of solution A and 30 volumes of buffer solution. B. 950 volumes of solution A and 50 volumes of buffer solution. **Wavelength:** 280nm. **Flow Rate:** 0.8ml/min. **Injection volume:** 20µL.	25

Table 4: Reported methods for assessment of Terbinafine Hydrochloride

SR. No.	Method	Description	Ref No.
1.	Development and validation of the UV-spectrophotometric method for determination of Terbinafine hydrochloride in bulk and in formulation	**Solvent:** Distilled water **Wavelength:** 283nm **Linearity:** 5-30 µg/ml	26
2.	Simultaneous determination of Terbinafine HCL and triamcinolone acetonide by UV derivative spectrophotometry and spectrodensitometry.	**Solvent:** Methanol, Dichloromethane, Ether **Wavelength:** First order derivative-297nm Radio spectra of first derivative at 298nm.	27
3.	High Performance Liquid Chromatography Method for the determination of Terbinafine Hydrochloride in Semi Solids Dosage form.	**Column:** C_{18} **Mobile phase:** Methanol, acetonitrile (60:40 v/v) with (0.15% triethylamine and 0.15% phosphoric acid) pH-7, 8, 9. **Wavelength:** 224nm. **Flow Rate:** 0.4ml/min.	28
4.	Determining Terbinafine HCL in plasma and saline using HPLC.	**Column:** Symmetry shield RP_{18} (5µm) 4.6 x 100mm **Mobile phase:** Water, Phosphoric acid and Triethylamine (pH-3.0) with acetonitrile (63: 35 v/v) **Flow Rate:** 1.1mL/min **Retention time:** Terbinafine-6.30 Butenafine-8.88 **Linearity:** 1-25 µg/ml	29
5.	RP-HPLC method development and validation for the estimation of antifungal drug Terbinafine HCL in bulk and pharmaceutical dosage form.	**Column**: INERTSIL ODS 50 x 4.6mm 5 µm **Mobile phase:** potassium dihydrogen phosphate and acetonitrile (65:35 v/v) **Wavelength:** 220 nm (UV detector) **Flow rate:** 1.5 ml/min **Linearity:** 50-150 µg/ml	30
6.	A Stability Indicating High Performance Liquid Chromatographic Assay (HPLC) for the Determination of Terbinafine Hydrochloride in Bulk Drug Substance (Photodiode array detector)	**Column:** Intersil: L1ODS (150×4.6mm) particle size 5 µm **Mobile phase:** Methanol-acetonitrile (60:40, v/v) with (0.15% triethylamine and 0.15% phosphoric acid) pH=7.68 **Wavelength:** 224 nm **Flow rate:** 0.4 ml/minute **Condition** | **Result of degradation**	31

<table>
<tr><td></td><td></td><td><table>
<tr><td>Acidic hydrolysis</td><td>27%</td></tr>
<tr><td>Basic hydrolysis</td><td>44%</td></tr>
<tr><td>Oxidation</td><td>50%</td></tr>
<tr><td>Thermal degradation</td><td>20%</td></tr>
<tr><td>Photo degradation</td><td>No degradation</td></tr>
</table></td><td></td></tr>
<tr><td>7.</td><td>Stability-Indicating RP-HPLC method for analysis of Terbinafine Hydrochloride in bulk and in tablet dosage form.</td><td>Column: Neosphere C18 (250 x 4.6 mm, 5µm).
Mobile phase: Methanol: 0.5% Triethanolamine.
Wavelength: 250 nm.
Flow rate: 1.2 ml/minute.<table>
<tr><td>Degradation condition</td><td>% degradation</td></tr>
<tr><td>Hydrolytic degradation</td><td>No degradation</td></tr>
<tr><td>Acidic hydrolysis</td><td>17% degradation with 1 N HCl</td></tr>
<tr><td>Basic hydrolysis</td><td>No degradation</td></tr>
<tr><td>Oxidative degradation</td><td>No degradation</td></tr>
<tr><td>Thermal degradation</td><td>No degradation</td></tr>
<tr><td>Photo degradation</td><td>5 to 7% degradation when exposed to both wavelengths</td></tr>
</table></td><td>32</td></tr>
<tr><td>8.</td><td>A validated HPTLC method for determination of Terbinafine Hydrochloride in pharmaceutical solid dosage form.</td><td>Stationary Phase: Silica gel 60 F254 TLC plates.
Mobile Phase: Acetonitrile: 1, 4 dioxin: Hexane: Acetic acid (1:1:8:0.1) (v/v/v/v).
Wavelength: 282 nm
Rf Value: 0.45
Linearity:
500-4500ng/spot</td><td>33</td></tr>
<tr><td>9</td><td>Stability-Indicating Method Development and Validation of Itraconazole And Terbinafine Hcl in Bulk and Pharmaceutical Tablet Dosage Form.</td><td>Column: C18 (250 mm×4.6 mm, 5 µ).
Mobile Phase: Acetonitrile and 0.1% TEA in the ratio (90:10).
Wavelength: 225 nm.
Retention Time: Itraconazole: 3.464.
Terbinafine
HCl: 8.705
Flow Rate: 1.2 ml/min
Stability results: for Itraconazole<table>
<tr><th>Conditions</th><th>% Degradation</th></tr>
<tr><td>Acid degradation</td><td>17.2%</td></tr>
<tr><td>Base degradation</td><td>4.3%</td></tr>
</table></td><td>34</td></tr>
</table>

		Water hydrolysis degradation: 0.05% Thermal degradation: 0.1% Oxidative degradation: 16.00% Stability results: for Terbinafine hydrochloride **Conditions** / **% Degradation** Acid degradation: 0.1% Base degradation: 0.7% Water hydrolysis degradation: 0.4% Thermal degradation: 0.09% Oxidative degradation: 12.0%	

Conclusion

This article gives an idea about improved activity of Mometasone Furoate and Terbinafine Hydrochloride from other drugs. The presented review provides information about the various methods available in the literature for the determination of Mometasone Furoate and Terbinafine Hydrochloride. The different analytical methods are reported for the individual and other combination like UV Spectroscopy, HPLC, and HPTLC. This article also present with Pharmacological action, chemical structure, solubility, etc. of Mometasone Furoate and Terbinafine Hydrochloride. This Article also suggest that reported methods for Mometasone Furoate and Terbinafine Hydrochloride for individual and other combinations. This review will help in future to develop the analytical method for this combination and also gives the knowledge about its characteristics of both drugs.

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Chapter - 5
Comparative Studies on Nutritional Profile of Unripen Fruits of *Carica papaya* L. Collected from Two Different Locations

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Chapter - 5

Comparative Studies on Nutritional Profile of Unripen Fruits of *Carica papaya* L. Collected from Two Different Locations

Mannikandaselvi S, Bhuvaneswari K and Kalaivani P.

Abstract

The food eats nurtures body. To function properly, the body needs a variety of macro and micronutrients. Several factors can influence the nutritional quality of crops, either directly or indirectly. Soil factors like pH, available nutrients, texture, organic matter content, and soil-water relationships; weather and climatic factors like temperature, rainfall, and light intensity; crop and cultivar; postharvest handling and storage; and fertilizer applications and cultural practices are just a few of them. The economic and ecological backdrop for the hunt for more nutrient efficient plants is a scarcity of resources and the environmental challenges of intensive agriculture. With this in mind, let's have a look at the nutritional status. The current study focused on the nutritional profile of unripe *Carica papaya* L. fruits obtained from two separate locations. Thanjavur sample has more water, proteins, and carbohydrates than Thiruvarur sample in this investigation. Thiruvarur sample performed better in terms of ash, lipids, and fibre than Thanjavur sample. *C. papaya* from Thanjavur had a high mineral content. The Thanjavur sample extract exhibited the strongest free radical scavenging activity of 49.2% at 250 µg/mL than Thiruvarur sample extract 45.8% at 250 µg/mL, when compared to BHT (Thanjavur sample was 43.7% at 100 µg/mL and Thiruvarur sample was 42.6% at 100 µg/mL). Environmental pollution and storage were found to play a significant effect in determining the quality of herbal products.

Keywords: nutritional value, environment, *Carica papaya*

Introduction

The economic and ecological backdrop for the search for more nutrient-efficient plants is a scarcity of resources and intensive agriculture's environmental issues. Fruits are at the top of our food pyramid because of

their nutritious importance. Fruits have a lot of vitamins and a mineral in them. Taking fruit every day boosts our energy levels (Tyler *et al*., 2017). Obesity, osteoporosis, iron insufficiency, and dental cavities are all reduced by consuming a healthy diet. A poor diet can cause an energy imbalance and raise the risk of becoming overweight or obese (Najeeb *et al.,* 2016). Lung, oesophagus, stomach, colorectal, and prostate cancers can all be exacerbated by a bad diet. Fruits contain phytochemicals as well as vitamins and minerals that act as antioxidants, phytoestrogens, anti-inflammatory agents, and other protective mechanisms, (Zhang *et al.,* 2015). Poor nutrition and unhealthy diets are among the leading causes of chronic diseases around the world. In 2014, 462 million adults in the world were underweight, with 1.9 billion being overweight or obese. In 2016, 155 million children under the age of five were expected to be stunted, with 41 million being overweight or obese (Khan *et al*., 2019). Undernutrition is responsible for almost 45 percent of mortality among children under the age of five. Low-and middle-income people of the countries are the most affected. At the same time, childhood obesity and obesity are on the rise in these same countries. Malnutrition is especially dangerous for women, infants, children, and adolescents. Poverty increases the risk of and severity of malnutrition (Martins *et al*., 2011). Nutrient availability is directly related to agricultural productivity and production. Nutrient application is necessary for high crop yields to be sustained. The phrase "nutrient availability" has been defined and used in a variety of contexts. The major goals of modern agriculture are to maximize and maintain agricultural production. Nutrient deficit for crop production is one of the primary issues impeding the growth of commercially successful agriculture. Changes in sunshine exposure are linked to changes in phytochemical substances, ascorbic acid, and carotenoids concentrations. Fruits that are exposed to the sun have higher phenolics and vitamin C than those that are shaded (Bui *et al*., 2019). Total phenolics in tomato plants exposed to higher sunlight accumulated by twofold. Carotenoids and vitamin C levels were additionally higher in these plants (Muzolf-Panek *et al*., 2017). As a result, radiation interception is crucial for generating antioxidant-rich commodities. The impact of cultural practices on nutrition has been studied in an exceedingly range of analysis. Strawberries cultivated in plastic mulch had additional antioxidants than those cultivated in open beds. Reduced vitamin C is joined to high element levels. Adding compost as a soil supplement significantly accumulated vitamin. Vitamin C accumulation is reciprocally connected with rain (Padayatty and Levine, 2016). Some studies have found that organic product accumulate further antioxidants and vitamins than conventionally big commodities. The fruit development stage

includes a pleasant impact on total matter capability. In tomatoes and peppers, the whole matter capability can increase as carotenoids and vitamin C accumulate throughout ripening. Total anthocyanin can increase throughout ripening altogether berries (Spinardi *et al.*, 2019). However, the matter capability peaks in numerous species early in development. Throughout blueberry ripening, anthocyanins accumulate whereas phenolic acids decrease. Carotenoids increase throughout development in pepper, tomato, mango, and genus Prunus species (Khoo *et al*., 2011). In product among that anthocyanins or chlorophylls dominate, carotenoids typically drop throughout development. Tissue damage greatly affects total matter concentration. Cell disruption exacerbates the turnover of phenolic compounds. Eliminating cellular compartmentalization triggers the chemical reaction of pre-existing phenolics by PPOs and can increase peroxide, providing the co-substrate for POD-mediated degradation. Wounding put together alters phenolic biogenesis (Guan *et al*., 2019). In lettuce, cutting elicited essential organic compound ammonia lyase accumulation of chlorogenic acid. Careful handling to attenuate physical damage is usually recommended to chop back matter loss. Refrigeration slows the deterioration of vitamin C; in broccoli, losses once seven days of storage were zero at 0^0C but fifty-six at 20 ^{o}C. With the exception of broccoli and bananas, most products lose visual quality before necessary matter capability is lost. Aliphatic compound induces the buildup of the bitter iso-coumarin 6-methoxymellein. The biogenesis is triggered by elicitors like UV or gas. Postharvest UV-C and gas exposure increased resveratrol accumulation in grapes (Hasan and Bae, 2017). Elicitation and accumulation of matter compounds put together occur in numerous fruits. Several factors can directly or indirectly have a control on the organic process quality of crops. Among these unit soil factors, such as pH, out there nutrients, texture, organic matter content, and soil-water relationships; weather and environmental condition factors, yet as temperature, rainfall, and lightweight intensity; the crop and cultivar; postharvest handling and storage; and chemical applications and cultural practices. Earlier analysis on the organic process standing of crops big with either chemical or organic fertilizers is peopled (Hammed *et al*., 2019). Keeping this in mind, let's take a look at the organic process standing among the present study, which might be conducted by a study on the organic process profile of unripened fruits of *Carica papaya* L. fruits collected from two totally different locations.

Materials and methods

Collection of plant material and sample preparation

Carica papaya L. fruits were collected in two different regions and were homogeneously harvested. Fruits were collected in the Thiruvarur and Thanjavur districts. During the harvest season, from November to January, sampling was done. The samples were packed in plastic bags and put on dry ice before being transported to the laboratory and held at 80 °C for further processing. Analyses were carried out on the peel and pulp samples of the fruit. The fruits were rinsed with tap water, split into components, then homogenised with a mortar and pestle before being powdered in liquid nitrogen as a preventative measure. The powdered sample for the fruit component was placed in test tubes and kept at 20 °C until analysis.

Preparation of extract

The aqueous extract was made by weighing out (20 g) of the milled powder of samples, soaking them in 100 mL of distilled water in a conical flask, rapidly agitating with a glass rod for efficient extraction, and then allowing them to cool at room temperature. After filtering the mixture with Whatman 42 filter paper, it was centrifuged for 20 minutes.

Moisture content

In a tared evaporating plate, accurately weighed around 100 g of plant powder (without pre-drying). The weighted plant powder samples were placed in the tared evaporating dish, dried for 5 hours at 105 °C, and weighed. The drying and weighing were carried out at one-hour intervals until the difference between two successive weighings was less than 0.25 percent. When the material attained a consistent weight, it was desiccated for 30 minutes. The moisture content was calculated as a percentage.

Determination of total ash

In a crucible, around 1g of plant powder was precisely weighed. The sample was burned at a temperature of not more than 450 °C for 4 to 5 hours, then allowed to cool at ambient temperature in a desiccator before being weighed until it reached a steady weight.

Determination of total alkaloids

In a separating funnel, the plant powder was treated with 0.1N HCl, and the resulting aqueous acidified layer was partitioned with chloroform. The chloroform layer was removed, and the aqueous layer was alkaline pH-basified with ammonium hydroxide and chloroform partitioned in a

separating funnel. The water layer was removed, and the chloroform layer was evaporated, yielding total alkaloids that were verified with Dragendorff's reagent. Weighed, dried, and computed residues.

Determination of total phenolics

A solution containing 1 mL of extract with a defined concentration (1 mg/mL) and 3 mL of distilled water was mixed with Folin-Ciocalteu reagent (0.5 mL). After 3 minutes, 0.5 mL of 2 percent sodium carbonate solution was added to the solution. The mixture was incubated for 90 minutes, and the absorbance at 760 nm was measured.

Determination of total flavonoids

A diluted sample of 1 mL was mixed with 1 mL of a 2 percent aluminium trichloride methanol solution. The absorbance of the reaction mixture was measured at 430 nm with a Shimadzu UVmin-1240 UV-Vis spectrophotometer after 15 minutes at room temperature.

Estimation of total carbohydrate

4 mL anthrone reagent was cautiously added to 1 mL standard and to test solution. After that, the tubes were heated in a 50 °C oven for ten minutes. Tubes were then boiled in a water bath for 5 minutes. Allowed for a 15-minute cool after heating. The coloured solution's absorbance was determined at 620nm.

Estimation of total protein

4.5mL of alkaline $CuSO_4$ reagent is added to the sample and incubated for 10 minutes at room temperature. Folin's phenol reagent (0.5mL) is also added. The contents were well mixed, and after 15 minutes, the blue colour developed was measured at 640 nm.

Estimation of total fat

Using a mortar and pestle, the sample was homogenised in cooled diethyl ether. The supernatant was obtained after centrifuging the homogenate. The residues were extracted five times with cooled diethyl ether and then stored at 60 °C in a hot air oven. The weight of the dried sample was recorded after it was placed in a desiccator to bring it to room temperature. The weight of total fat in the tissue was calculated from the difference in weight.

Estimation of energy value

$$\text{Calorific Value in Kilocalories} = 4(\%\ \text{carbohydrates}) + 4(\%\ \text{proteins}) + 9(\%\ \text{Fats})$$

Estimation of total crude fibre

To remove fat, 2g of the extracted material is ground with ether. 2g of dried material was heated in 200ml of sulphuric acid for 30 minutes with bumping chips after extraction with ether. For 30 minutes, boiled 200ml sodium hydroxide solution. Filtered and washed three times with 50ml water and 25ml alcohol after filtering and washing with 25ml boiling 1.25 percent sulfuric acid. Removed the residue and placed it in an ash dish (Pre-weighed dish W1). Dried the residue for 2hr at 130±2 °C. Cooled the dish in a desiccator and weighed (W2). Ignited for 30 min at 600 ±15 °C. Cooled in a desiccator and reweighed (W3).

Calculation

$$\text{Crude fibre in \%} = \frac{\text{Loss in weight on ignition (W2 - W1)} - \text{(W3 - W1)}}{\text{Weight of the sample}} \text{X100}$$

Estimation of vitamin C

The material was weighed at 5 g, and 100 ml of EDTA/TCA (2:1) extraction solution was mixed and agitated for 30 minutes. This was centrifuged for about 20 minutes at 3000 rpm, and the extracting solution was added to make up to 100 ml. To obtain a dark end point, 20 ml of the extract was pipetted with 1 percent starch indicator and titrated against a 20 percent $CuSO_4$ solution.

Estimation of vitamin E

1.5 mL of plant extract was combined with 1.5 mL of ethanol. 1.5 mL xylene was added, and the mixture was centrifuged. xylene layer added to 1.0 ml of dipyridyl reagent. At 460 nm, 1.5 mL of the combination was measured. Then, added 0.33 ml of ferric chloride solution, mixed and set the wavelength to 520 nm and 1.5 min after mixing, read the absorbance (520) of the test solution and standard against the blank.

Calculation

$$\text{Tocopherol} = \frac{\text{(Reading of unknown at 520 nm} - \text{Reading at 460 nm)}}{\text{Reading of control at 520 nm}} \times 0.2 \times 10$$

Determination of total elements by X-ray fluorescence spectroscopy

The XRF measurement equipment includes a multichannel analyzer ORTEC®, a semiconductor detector Si/Li (beryllium window thickness = 0.25 mm, Beryllium window diameter = 5 mm), and a radioactive source of radiation 238Pu (A=370 MBq, E = 12-22 keV, $T_{½}$ = 86.4 years) made by AMERSHAM in form of planar disk source.

Antioxidant capacity assay

5 mL of a 0.004 percent methanol solution of DPPH, 50 microliters of various concentrations of the extract dissolved in methanol were added. The absorbance was measured at 517nm after a 30-minute incubation period at room temperature (6800 UV-VIS spectrophotometer).

Results and Discussion

Environmental change's impact on food systems and health will be determined by a number of environmental, behavioural, and economic factors. To begin with, the scale of environmental change will be determined by the existing level and trends of various environmental stressors, as well as mitigation measures adopted by both individual countries and the global community as a whole. Second, the consequences of environmental change will be determined by the development and implementation of adaptation mechanisms. Changing agricultural production practices and crop kinds planted in some places that are less vulnerable to particular environmental stressors are examples of this. Finally, markets are critical in the distribution of food between production and consuming sites. Fourth, food prices have an impact on consumer behaviour, with some foods being far more sensitive to price fluctuations than others. Finally, due to price responsiveness and differences in pre-existing dietary patterns, the impact of changing food availability on nutrition and health is expected to differ between countries and demographic groups (Tyler *et al*., 2017). Predicting the effects of environmental changes on diets and health thus necessitates a thorough understanding of the multiple interactions and feedback loops among different actors and processes, as well as data on environmental, social, and economic factors. Previous study has primarily been one-way, focusing on particular steps in the pathways connecting the environment, diet, and health. Climate change's effects on staple crops have dominated studies on the effects of environmental change on food production (Parrya *et al*., 2004), but the effects on other foods and other environmental stressors have received less attention. A few researches have combined environmental change, agriculture, markets, nutrition, and health, with a focus on important staple crops and/or meat. These researches have given us a better understanding of the potential scope of environmental change's impact on the food system, but the nutritionally essential fruit has remained largely unstudied. Fruits and vegetables have the potential to play a critical role in future healthy population diets due to their unique nutritional properties, importance for public health, and relatively low environmental footprint (Tyler *et al*., 2017). Papayas are high in antioxidant elements such as carotenes, vitamin C, and

flavonoids, as well as B vitamins, folate, and pantothenic acid, as well as fibre. These nutrients work together to improve cardiovascular health while also protecting against colon cancer. Papaya also contains papain, a digestive enzyme that, like bromelain, a similar enzyme found in pineapple, is used to treat sports injuries, various types of damage, and allergies. Clinical experiments and epidemiological studies have recently discovered an inverse relationship between the consumption of fruits and vegetables and the onset of chronic diseases, the world's most common causes of mortality (Bode and Dong, 2015). The antioxidant capabilities of several chemicals have been attributed to this protective function, which coordinate and balance the body system to protect tissues and fluids from harm by reactive oxygen species (ROS) or free radicals. Antioxidants play a vital role in the food industry, in addition to their health and nutritional benefits. Throughout the oxidative process, these chemicals limit free radical proliferation, preserving food quality and shelf life during handling and storage (Lourenço *et al.*, 2019). Any substance taken to give nutritional support for the body is referred to as food. It's mainly made from plants or animals and it's high in vital elements like carbohydrates, lipids, proteins, vitamins, and minerals. Solids, water, and other substances are present in all diets. The moisture in a material is made up of all the components that evaporate when heated and cause the sample to lose weight. A balance determines the weight, which is then translated into the moisture content. Measuring water activity in relation to moisture content is another key metric for food quality and stability. The amount of water in most foods impacts how they taste, feel, and seem. It is one of the most essential methods for determining the quality of food. The shelf life of a product is determined by its water content at the time of packaging as well as the rate of moisture accumulation during storage, a process known as sorption isotherm analysis. Thanjavur sample had a greater water content than Thiruvarur sample (Table 1).

Ash values, especially in powder form, are useful in establishing the quality and purity of crude pharmaceuticals. The goal of ashing vegetable products is to get rid of any organic debris that might interfere with an analytical assessment. Ash, which is mostly made up of salty, inorganic substances, is one of the components in the proximate analysis of biological materials. Metal salts are vital for processes that require ions like Na^+, K^+ and Ca^{2+}. It also has trace minerals in it. Thiruvarur sample had a higher ash value than Thanjavur sample (Table 1). Protein is an essential component of our diet and is necessary for the growth and maintenance of all forms of bodily tissue, including muscle. They're advertised as aiding muscular growth, aiding metabolism (helping with weight loss), assisting in reaching

peak physical performance, boosting energy, and combating the ageing process. Adults should not consume more than twice the recommended daily protein consumption, according to the Department of Health (55.5g for men and 45g for women). Protein is the most satiating macronutrient, followed by Carbohydrates, and finally fat, which is the least satiating. After high-protein diets, this satiating effect is most noticeable. Fullness was much higher after a 60 percent protein meal than after a 19 percent protein meal, according to a visual analogue scale (VAS), which is a classic instrument for measuring subjective appetite and satiety. Increased secretion of satiety hormones (GIP, GLP-1), reduced orexigenic hormone secretion (ghrelin), the increased thermic effect of food, and protein-induced changes in gluconeogenesis to improve glucose homeostasis are some of the potential mechanisms that account for weight loss associated with high-protein diets. When deciding to follow a high-protein diet, there are a few things to keep in mind. Thanjavur sample had a higher protein value than Thiruvarur sample (Table 1). Carbohydrates provide a variety of functions in living organisms, including supplying energy. Carbohydrate byproducts play a role in the immune system, blood coagulation, and reproduction. Carbohydrates should make up 45-65 percent of a person's overall calorie intake on a daily basis. Increased fibre consumption has also been associated to a lower incidence of metabolic syndrome, a set of characteristics that raises the risk of heart disease and diabetes.

High blood pressure, high insulin levels, excess weight (particularly around the abdomen), high triglyceride levels, and low HDL (good) cholesterol are some of these risk factors. A higher fibre intake, according to several studies, may provide protection against this illness (Buttar *et al*., 2005). Thanjavur sample has more water content, proteins, and carbohydrates than Thiruvarur sample in this investigation. Thiruvarur sample performed better in terms of ash, lipids and fibre than Thanjavur sample.

Table 1: Comparison of basic components of *Carica papaya*

Component (100g)	Thanjavur Sample	Thiruvarur Sample
Moisture	88.83%	82.24%
Ash	0.61 mg	0.75 mg
Proteins	0.7 g	0.6 g
Lipids	0.2 g	1.1 g
Carbohydrates	6.2 g	5.6 g
Fiber	0.9 g	1.1 g

Energy	29.4 kcal	38.3 kcal

Table 2: Comparison of the vitamins of *Carica papaya* L.

Component (100g)	Thanjavur Sample	Thiruvarur Sample
Vitamin C	51.6 mg	47.4 mg
Vitamin E	867 IU	854 IU

Table 3: Comparison of the Organic Phytoconstituents of *Carica papaya* L.

Organic Phytoconstituents (100g)	Thanjavur sample	Thiruvarur sample
Total alkaloids	3.91±1.59µg/g	3.87±2.10µg/g
Total flavonoids	47.44±8.90µg/g	38.97±2.34µg/g
Total phenols	11.32±8.90µg/g	10.68±8.90µg/g

Vitamin C can be found in a wide range of foods. The finest sources of ascorbic acid are fruits, vegetables, and organ meats; muscle, meats, and most seeds do not contain large amounts of ascorbic acid. It is critical to have a thorough understanding of the nutritional worth of fruits and vegetables, as well as the content of vitamin C estimates, in order to make better use of them as human food. Ascorbic acid is oxidised reversibly to create L-dehydroascorbic acid (DHA), which has biological function as well. Because DHA is quickly converted to AA in the human body, measuring both AA and DHA in fruits and vegetables is necessary to determine vitamin C activity (Abeysuriya *et al*., 2020).

Vitamin C (ascorbic acid), one of the vitamins, is an important ingredient for the body's regular metabolic activity. Vitamin C is easily oxidised, and it relies on this property for the majority of its *in vivo* functions. Because the human body is incapable of producing ascorbic acid, it must be received solely through the food. In humans, a vitamin C shortage causes scurvy, a condition characterised by bleeding, joint discomfort, and tiredness. It helps to decrease blood pressure and cholesterol. A sufficient intake of vitamin C has been demonstrated in numerous studies to be useful in lowering the risk of cancer of the breast, cervix, colon, rectum, lung, mouth, prostate and stomach development. The human body needs be kept saturated with vitamin C in order to maintain excellent health and prevent the common cold. The concentration of ascorbic acid varies depending on factors such as temperature and preservation time (Joseph Hubert Yamdeu Galani, 2017). When fat is oxidised and free radical reactions propagate, vitamin E is a powerful chain-breaking antioxidant that limits the creation of reactive oxygen species molecules. Vitamin E appears to decrease platelet aggregation through the inhibition of protein kinase C and also supports

membrane repair by limiting the development of oxidised phospholipids that might possibly interfere with membrane fusion processes. The amount of vitamin C and E in fruits collected in Thanjavur is higher than in Thiruvarur (Table 2).

Table 3 shows the total alkaloids, phenols, and flavonoids based on the absorbance values of the plant extract. Alkaloids, flavonoids, and phenols were found to be abundant in the extract. The amounts of alkaloids [(3.91± 1.59µg) µg ARC/g], total phenols [(11.32± 8.90) µg GAE/g] and flavonoids [(47.44 ± 8.90) µg QE/g] contents were in the Thanjavur sample extract. The amounts of alkaloids [(3.87± 2.10µg) µg ARC/g], total phenols [(10.68± 8.90) µg GAE/g] and flavonoids [(38.97 ± 2.34) µg QE/g] contents were in the Thiruvarur sample extract. Thanjavur sample has more total alkaloids, phenols and flavonoids than Thiruvarur sample in this investigation. These phytoconstituents have a variety of therapeutic properties, including anti-mutagenic, anti-carcinogenic, low-density lipoprotein prevention and cardioprotective actions. Minerals and trace elements are inorganic substances found in nature that make up around 4% of the total mass of the human body. Its components are used as materials and regulators in a variety of biological processes that contribute to the formation of body structures and are essential for good health.

The inorganic elements can be quantified using a variety of experimental techniques once the organic matrix has been removed. Instrumental approaches typically provide increased analysis speed, automation, and high precision and accuracy. There are a variety of other analytical procedures for minerals that can produce comparable results. For macro elements calcium and potassium measurement in food samples such as fruits, vegetables, and cumin spice, non-destructive energy-dispersive X-ray fluorescence (ED-XRF) spectroscopy was performed (Kalcsits, 2016). *C. papaya* from Thanjavur had a high mineral content (Figure 1 and 2).

Several studies, clinical trials, and epidemiological studies have recently indicated that fruits and vegetables contain bioactive substances with antioxidant and antibacterial properties, including phenolic compounds, carotenoids, and vitamins (Liu, 2013). These have been found to aid in the prevention of cardiovascular disease, atherosclerosis, and the reduction of the risk of some malignancies, among other health benefits. Natural antioxidant consumption has been linked to a lower risk of cancer and heart disease, prompting more research into the antioxidant content of fruits and vegetables. Harvesting at the right time ensures a high-quality fruit with long-term preservation potential. Phenols, also known as polyphenols, are

secondary metabolites found in all plants and plant products. Plants' total antioxidant capabilities are aided by phenolic chemicals. In general, phenolic substances have antioxidant activity via inactivating lipid free radicals and inhibiting hydroperoxide breakdown into free radicals. The initial blue/purple solution will turn yellow due to the production of diphenyl picryl hydrazine if the extract has the ability to scavenge the DPPH free radical (İlhami Gülçin *et al*., 2010). This reaction is used to determine how well an extract can scavenge free radicals.

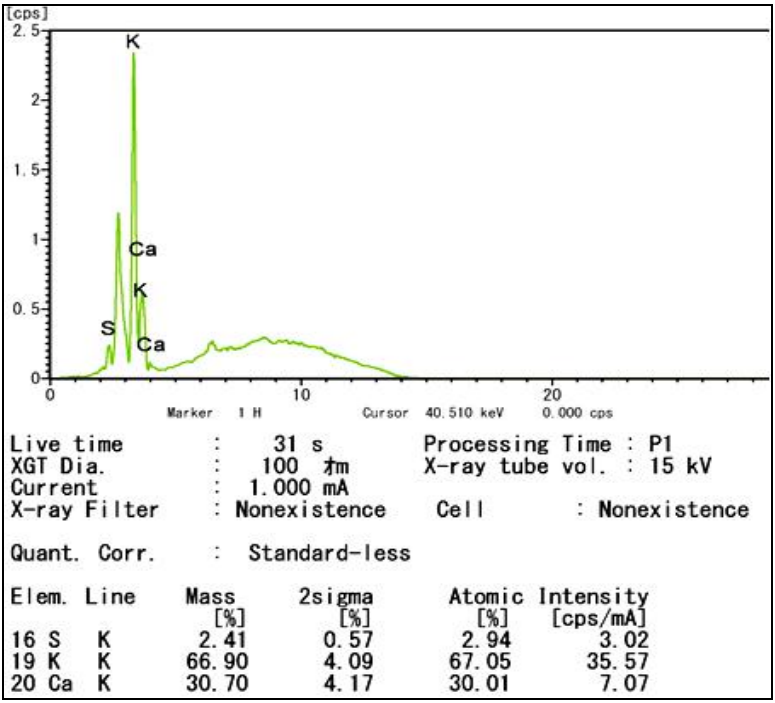

Fig 1: Minerals in *C. papaya* L. collected from Thiruvarur

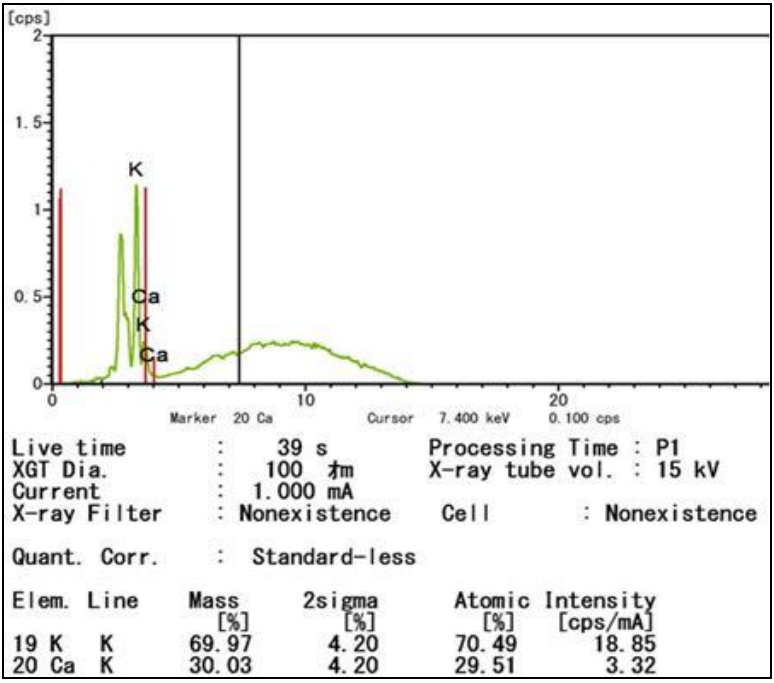

Fig 2: Minerals in *C. papaya* L. collected from Thanjavur

Table 4: Comparison of free radical-scavenging capacity of the extract

S. No.	Concentration (µg/mL)	DPPH free radical scavenging activity (%)	
		Thanjavur Sample	Thiruvarur Sample
1.	250	49.2±4.1	45.8±1.0
2.	200	42.2±2.3	41.1±1.7
3.	150	31.9±1.8	28.7±1.1
4.	100	26.5±3.1	25.6±1.4
5.	50	19.2±2.6	17.4±1.5
6.	BHT (100µg)	43.7±1.3	42.6±9.1

Values are mean ± S.D. (n=3)

Many harmful pathophysiological processes, such as cancer, diabetes, cardiovascular and neurological illnesses, are linked to the accumulation of free radicals, according to substantial evidence. A free radical is an atom or molecule that has one or more unpaired electrons, making it unstable. In healthy human cells, this unstable radical has a tendency to become stable by electron pairing with biological macromolecules such as proteins, lipids, and DNA, resulting in protein and DNA damage (Rahman *et al.,* 2015). Due to weaker cellular antioxidant defence systems, such radical-caused cell

damage may become more common. Antioxidant defence mechanisms exist in all biological systems to eliminate damaged molecules, however they can be ineffective. As a result, antioxidants must be consumed in order to protect cells from free radical damage. Antioxidants are chemicals that supply electrons to damaged cells in order to prevent and stabilise damage caused by free radicals. Antioxidants also convert free radicals into waste by-products that the body eliminates. Antioxidant-rich fruits and vegetables have been shown to reduce the risk of a variety of diseases caused by free radicals (Lobo *et al*., 2010). The presence of phytochemicals such as polyphenols, carotenoids, vitamin E, and vitamin C contribute to these health advantages. *C. papaya* fruit extract has a high antioxidant activity. The DPPH technique has shown that the aqueous extract is more efficient than the reference standard antioxidant BHT. The ability of DPPH, a persistent free radical, to decolorize in the presence of antioxidants is used in the DPPH antioxidant assay. The odd electron in the DPPH radical is responsible for the absorbance at 517 nm as well as the visible deep purple colour. When DPPH absorbs an electron from an antioxidant chemical, it decolorizes, which can be quantified quantitatively using changes in absorbance. The fruit extract from Thanjavur exhibited a significant dose-dependent inhibition of DPPH activity than the fruit extract from Thiruvarur. The DPPH method was used to test the free radical scavenging activity (DPPH) of unripe fruits of *C. papaya* L., and the findings are shown in Table 4 as percent SCV (µg dry weight extract/mL). The Thanjavur sample extract exhibited the strongest free radical scavenging activity of 49.2% at 250 µg/mL than Thiruvarur sample extract 45.8% at 250 µg/mL, when compared to BHT (Thanjavur sample was 43.7% at 100 µg/mL and Thiruvarur sample was 42.6% at 100 µg/mL).

Conclusion

According to the findings, environmental pollution and storage had a significant impact on the quality of herbal products. The fruit harvested from Thanjavur was found to be superior to the sample from Thiruvarur. This is because environmental contamination and sample storage played a significant effect in determining the sample's quality. The components responsible for the antioxidative activity, on the other hand, are yet unknown. As a result, more research is needed to isolate and identify the antioxidant components found in the plant extract. Furthermore, prior to clinical implementation, this extract's *in vivo* antioxidant activity must be determined.

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Chapter - 6
Osmotic Drug Delivery Systems a Review

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Chapter - 6

Osmotic Drug Delivery Systems a Review

Sanjana Gaikwad, Manish Lonare, Jay Sharma, Namrata Mane, Swati Gaikwad and Mayuri Deshmukh

Abstract

Oral drug delivery is the most predominant drug delivery system and for chronic diseases and dose related issues dose attainment osmotic drug delivery systems play key role in reduction of repeated administration the osmotic delivery system consists of an osmotic core containing drug, an osmogen surrounded by a semipermeable membrane with or without an aperture. A system with constant internal volume delivers a volume of saturated solution equal to the volume of solvent uptake at any given time interval. Excess solid present inside a system ensures a constant delivery rate of solute. These parameters of osmotic drug delivery may prove beneficial in designing of dosage form for modification in drug release of various drugs who have limitations in conventional form.

Keyword: osmotic drug delivery, components, formulation, design and operation, types

1.1 Introduction

Novel drug delivery system is the prime area for industrial as well as academic research**.** Recently massive advances are in progress befitting the area of controlled drug delivery system. This in part is due to evolving disciplines of biopharmaceutics, pharmacokinetics and pharmacodynamics. In a typical therapeutic regimen, the drug dose and the dosing interval are optimized to attain required therapeutic window, drug efficacy and minimum toxic side effects. It is fact that daily dosing of two or more dosage units greatly affects the compliance by patient [1]. Hence, the primary objective of system is to deliver a pharmacologically active agent in predetermined, predictable and reproducible manner and maintain drug concentration within therapeutic window, this would improve patient's compliance to dosage regimen. It would also improve the efficacy with simultaneous reduction in toxic side effects [2].

Several technologies have been governed to get through the control of the systemic delivery of drug viz. extended drug delivery system, targeted

drug delivery system, pulsatile drug delivery system etc, Majority of these drug delivery systems fall in category of matrix, reservoir and osmotic system. The matrix type embeds drug in polymer matrix and release takes occurs via partitioning of drug polymer matrix and release medium. While in reservoir systems the drug core is surrounded or coated with rate controlling membrane. There are several factors like pH, food and many physiological factors that affects the drug release from conventional controlled drug delivery system. This systems depends on osmotic pressure for delivery of drugs. However, drug is released independent of pH and other physiological parameters [3].

The obvious advantage of osmotic pressure-based delivery systems is the near ideal zero order release pattern of the drug candidate, which is due to the fact that osmotic pressure is a colligative property i.e. it depends number of solute species [4] (neutral molecule or ionic species). In 1974, Theeuwes and Higuchi [5] used this principle of osmotic pressure to formulate new generation of controlled drug delivery systems. The various systems of osmotic delivery are classified according to route of administration [13]. (Fig 1.1).

These systems have some features in common but some of the features are exclusive to the design. The following chart takes an account of the same.

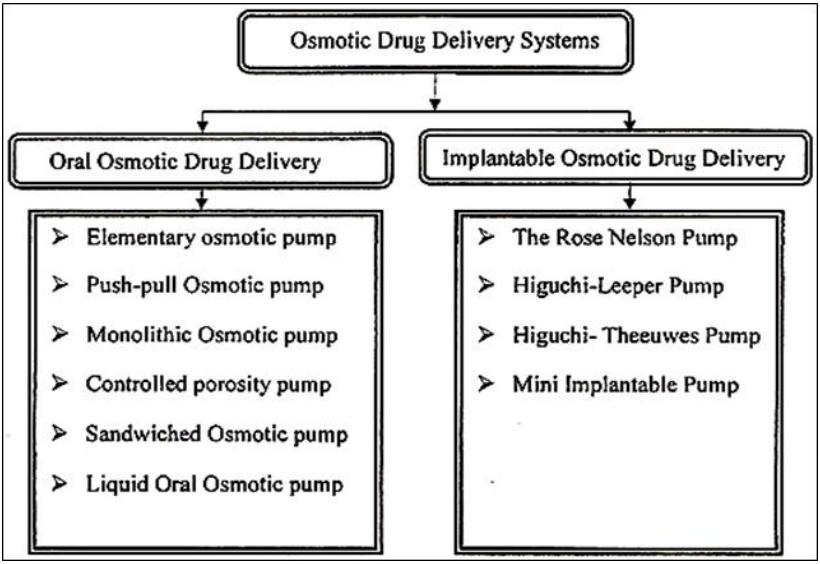

Fig 1.1: Classification of osmotic drug delivery systems

Table 1.1: Comparative account of oral osmotic drug delivery systems

Sr. No.	Osmotic System	Number of components	Mechanism
1	Elementary Osmotic Pump (EOP)	Drug, osmogen, semipermeable membrane (Three).	The water penetrates inside the dosage form at the determined rate from the fluid permeability of the membrane and osmotic pressure of core formulation. A saturated solution of drug formed within the core, will be dispense at a controlled rate from the delivery orifice at the membrane.
2	Push-pull osmotic pump	drug, osmogen, polymeric osmotic agent, semipermeable membrane (Three to four).	In aqueous environment, polymeric osmotic layer swells and pushes the drug layer, and thus releasing drug in the form of fine dispersion via the orifice.
3	Controlled-porosity osmotic pump	Drug, osmogen, semipermeable membrane with water soluble additives (Three to five).	Water-soluble additives dissolve when they come in contact with water, resulting in an *in-situ* formation of a microporous membrane. The resulting membrane is substantially permeable to both water and dissolved solutes and the mechanism of drug release is osmosis.
4	Sandwiched Osmotic pump	Drug, semipermeable membrane, push layer (Three)	The middle push layer swells and drug is released from delivery orifices.
5	Liquid oral osmotic pump	Liquid drug, push layer, semipermeable membrane (Three).	Water permeates across the rate controlling membrane and activates the osmotic layer. A hydrostatic pressure formed inside the system will force the liquid formulation to break gelatine capsule shell at the delivery orifice.
6	Rose-Nelson pump	Rigid housing, water chamber, salt chamber, drug chamber, elastic diaphragm, membrane (Five).	The difference in osmotic pressure across the membrane moves water from the water chamber into the salt chamber. Water flow enhances the volume of the salt chamber, which distends the latex diaphragm separating this salt and drug chamber, thereby pumping drug out of the device.
7	Higuchi-Leeper pump	Rigid housing, salt chamber, drug chamber, elastic diaphragm, membrane (Five).	The device is activated by the water imbibed by the surrounding environment.
8	Higuchi-Theeuwes pump	Salt chamber, drug chamber, elastic diaphragm, membrane (Three).	The device is loaded with drug prior to use. When the device is placed in aqueous environment, release of drug follows a time course set by the salt used in the salt chamber and the permeability of the outer membrane casing.

9	Mini implantable pump	Drug, reservoir, osmotic agent, impermeable membrane (Three to four).	An additional component called flow moderator is inserted into the body of the osmotic pump after filling. The expansion of the osmotic layer results in the development of hydrostatic pressure inside the system and the drug release from the orifice.

The figures, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9 give schematic presentation of design and/operation of each of the osmotic delivery systems.

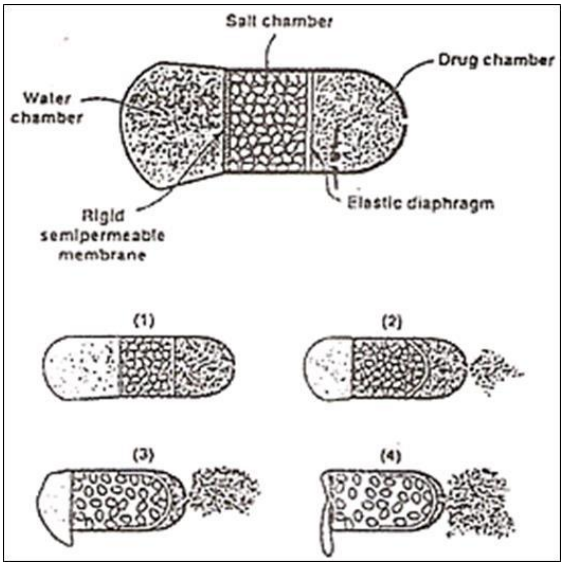

Fig 1.2: Schematic presentation of Rose Nelson osmotic pump

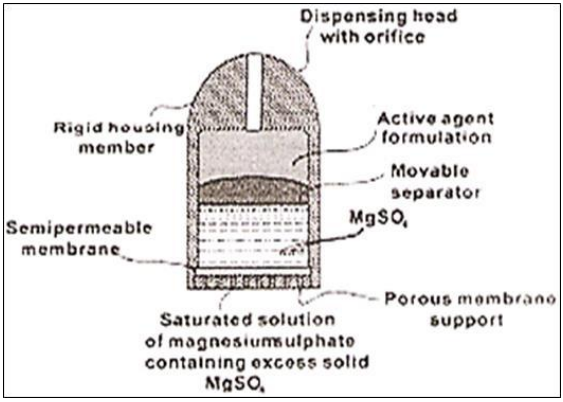

Fig 1.3: Schematic presentation of Higuchi Leeper osmotic pump

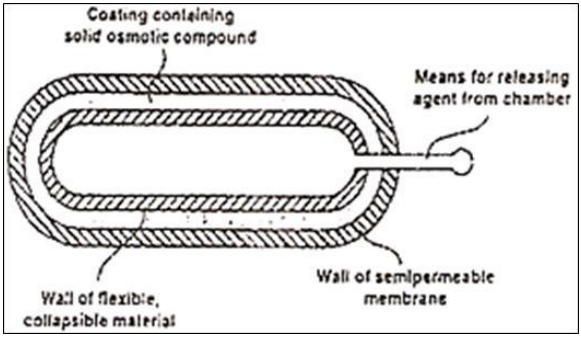

Fig 1.4: Schematic presentation of Higuchi Theeuwes osmotic pump

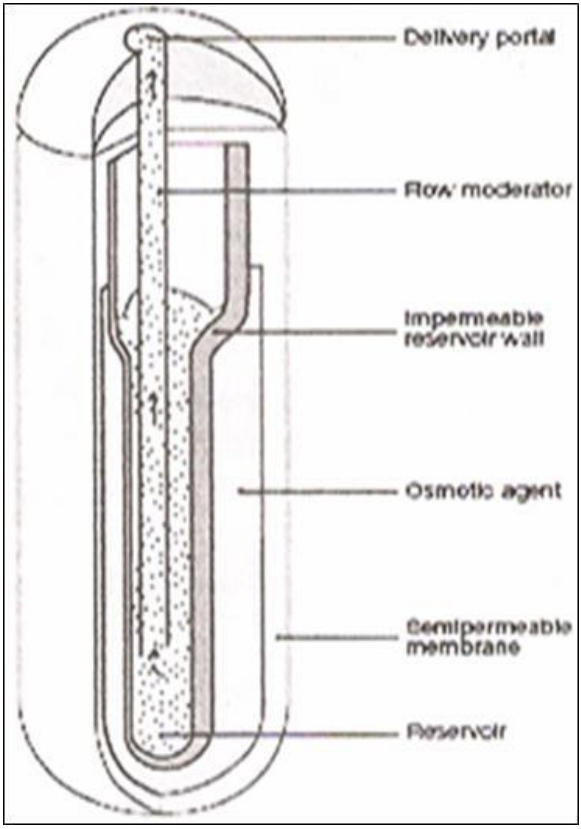

Fig 1.5: Schematic presentation of Implantable mini osmotic pump

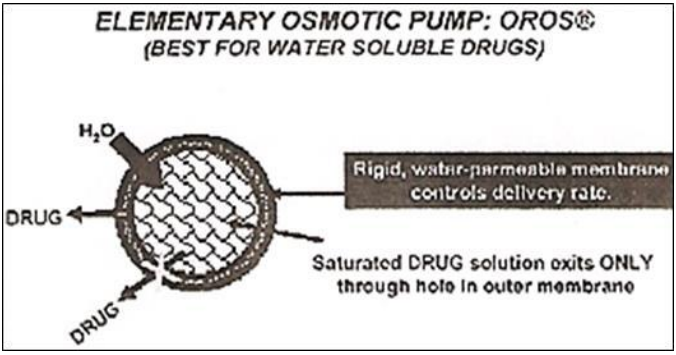

Fig 1.6: Schematic presentation of Elementary osmotic pump

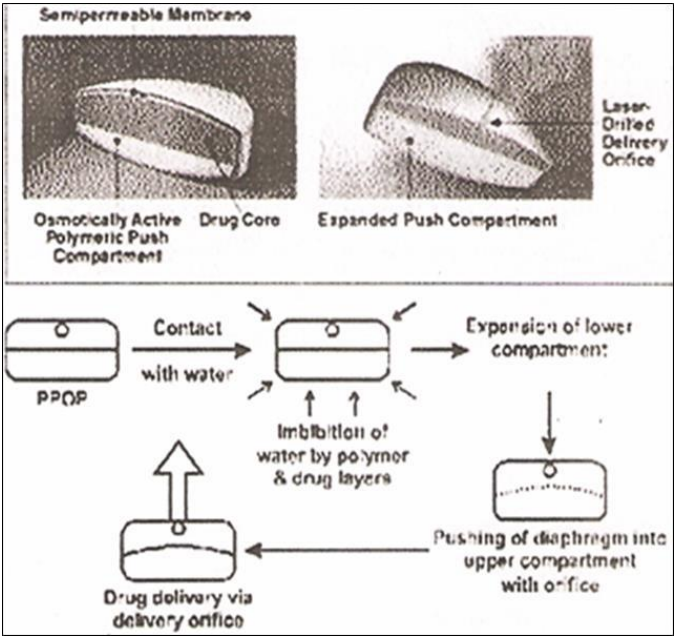

Fig 1.7: Schematic presentation of Push-pull osmotic pump and its mechanism of operation

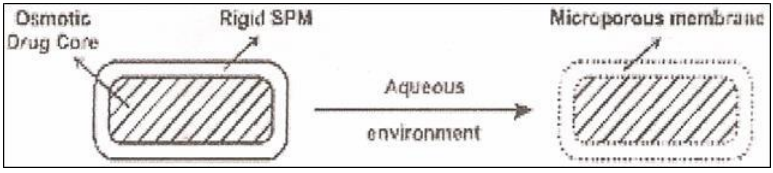

Fig 1.8: Schematic presentation of Controlled porpsity osmotic pump

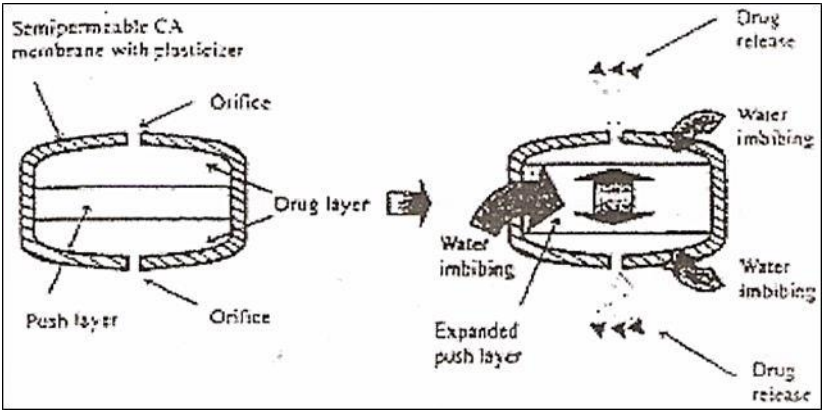

Fig 1.9: Schematic presentation of Sandwich osmotic pump

1.2 Factors affecting the design of osmotic drug delivery system

Following parameters play important role in osmotic drug delivery,

- Size of delivery orifice.
- Solubility of drug candidate.
- Osmotic pressure within the core.

1) Size of delivery orifice

Typically, a diameter of about 0.2 mm through a membrane thickness of 0.2 mm is needed to maintain a delivery rate of order 10 mg/h for water soluble compounds [22]. The minimum cross-sectional area can he estimated from the following equation.

$$S_{min} = 5\left[\left(\frac{L}{P_{max}}\right)\mu\left(\frac{dV}{dt}\right)\right]^{1/2}$$

Where

dV/dt = Volume of flux by an orifice.

L = Length of orifice.

μ = Viscosity of solution flowing through the orifice.

P_{max} = Maximum hydrostatic pressure differences across the membrane.

The maximum cross-sectional area of the orifice is obtained by specifying that the release rate must be smaller than a fraction f of the zero order pumping rates and is defined by following equation:

$$S_{max} = \frac{M_{tz} f L}{D_s C_s}$$

Where,

M_{tz} = The amount of the drug is delivered in zero order fashion.

D_s = The drug diffusion coefficient within the permeating solvent.

In practice, fraction smaller than 0.025 generally is necessary for minimum diffusion contributions [23].

Methods to create a delivery orifice in osmotic tablet coating include mechanical drilling [24], laser drilling [25], use of an apparatus with slid able punches [26], indentation that is not covered in coating process [6] and use of leachable substances in the coating.

2) Solubility of drug candidate

Drug candidate possessing aqueous solubility in the range of 20-200mg/ml [19] are suitable for osmotic drug delivery system. However, solubility can be modulated by using one or more of the following approaches.

- **Alternative salt form:** Change in salt form of drug may change solubility, e.g. Oxprenolol hydrochloride salt is too soluble to maintain a saturated solution and hence, zero order delivery for the anticipated delivery life of dosage form [31]. Succinate salt is found to have optimum solubility, and suitable for extended release up to 24 h.
- **Co-compression of drug with excipients:** Many excipients can be used to determine the solubility of drugs with different mechanisms like saturation solubility [34], pH dependent solubility. e.g. organic acids, buffering agent etc.
- **Crystal habit modifiers:** Distinct crystal forms of the drug have distinct solubility [33]. Hence, the excipients which may change crystal habit of the drug can be used to modulate solubility.
- **Cyclodextrin derivatives:** Increase solubility by forming complexes with poorly soluble drugs [30].
- **Effervescent mixtures:** Creates pressure in the osmotic system and ultimately control the release rate. e.g. Mixtures of citric acid and sodium bicarbonate [29].
- **Resin modulation approach:** Ion-exchange resin methods are commonly used to modify the solubility of drugs [32]. Some of the resins used in osmotic systems are poly (4-vinyl pyridine), pentaerythritol, citric and adipic acids.

- **Swellable polymers:** Provide uniform swelling rate and release drug at constant rate e.g. vinyl acetate copolymer. Polyethylene oxide has uniformity swelling rate which causes constant rate drug release [27].
- **Wicking agents:** Enhance the surface area of drug with the incoming aqueous fluids. e.g. colloidal silicon dioxide, SLS, etc. Ensotrol technology applies this principle to deliver drugs via osmotic mechanism [28].

3) Osmotic pressure

The osmotic pressure expressed in equation 10 directly affects the release rate. To attend a zero-order rate of release rate, it is important to keep constant osmotic pressure by keeping a saturated solute solution. Mostly, the generated osmotic pressure may not be enough to achieve the desired driving force. In such case, other osmotic agents are added that enhance osmotic pressure [6].

1.3 Formulation of osmotic drug delivery system

The osmotic delivery system consists of an osmotic core containing drug, an osmogen surrounded by a semipermeable membrane with or without an aperture. A system with constant internal volume delivers a volume of saturated solution equal to the volume of solvent uptake at any given time interval. Excess solid present inside a system ensures a constant delivery rate of solute. The rate of delivery most of times follow the zero-order kinetics and decreases when the solution concentration falls below saturation. The solute delivery rate from the system is controlled by solvent influx through the semipermeable membrane.

The osmotic delivery system includes following formulation ingredients

- Drug candidate.
- Osmotic agent.
- Semipermeable membrane (coating polymer).
- Wicking agent.
- Solubilizing agent.
- Coating solvent.
- Flux regulating agents.
- Plasticizer.
- Pore forming agents.

1) **Drug:** The kinetics of osmotic release is directly related to solubility of drug within core table. Both highly soluble and poorly soluble drug are not good candidate for osmotic delivery [3].

2) **Osmotic agent (osmogen):** Ionic compounds consisting salts, sugar and hydrophilic polymers are used as osmogens (Fig No. 1.10). Following table (Table No. 1.2) enlists various osmogens with osmotic pressure values of their saturated solutions.

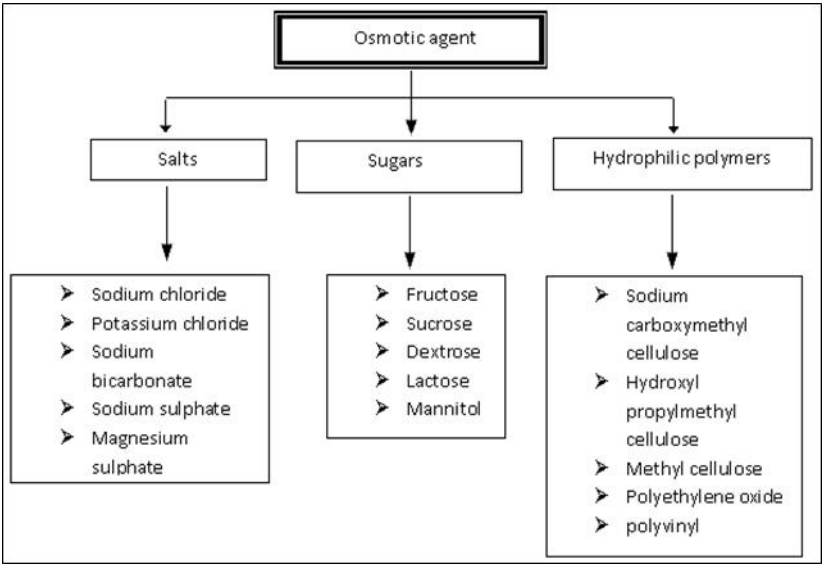

Fig 1.10: Classification of osmotic agents

Table 1.2: Osmotic pressure values of saturated solutions of osmogens [7]

Sr. No.	Solute or Mixture	Osmotic pressure (atm)
1.	Sodium phosphate monobasic	28
2.	Sodium phosphate dibasic	31
3.	Sodium phosphate tribasic	36
4.	Mannitol	38
5.	Potassium sulphate	39
6.	Dextrose	82
7.	Mannitol-lactose	130
8.	Sucrose	150
9.	Mannitol-sucrose	170
10.	Dextrose-sucrose	190
11.	Lactose-dextrose	225
12.	Mannitol-dextrose	225

13.	Potassium chloride	245
14.	Lactose- sucrose	250
15.	Fructose	355
16.	Sodium chloride	356
17.	Mannitol-fructose	415
18.	Sucrose-fructose	430
19.	Dextrose-fructose	450
20.	Lactose-fructose	500

4) Semipermeable membrane (coating polymer)

The polymeric membrane important for osmotic delivery formulation and should have following characteristics [9].

- It should be stable with the outer as well as the inner environment of the device.
- Should be rigid for maintaining dimensional integrity.
- Should be impermeable to the contents of dispenser to prevent get through of osmogen by diffusion.

Numerous polymers are currently available to form semipermeable membrane. One of its type includes cellulosic polymers such as cellulose ethers, cellulose esters and cellulose ester-ethers. The cellulosic polymers have a degree of substitution (DS) of 0 to 3 on the anhydroglucose unit. The DS is number of hydroxyl groups present on the anhydroglucose unit being replaced by substituting group. Examples of this group include cellulose acylate, cellulose deacetylase, cellulose triacetate, cellulose acetate, cellulose diacetate, and mono, di and tri cellulose alkynylation.

Cellulose acetate is available in different grades, such as cellulose acetate (DS of 1 to 2, an acetyl content of 21 to 35%) or cellulose acetate (acetyl content of 32 to 39.8%). Other forms of cellulose polymers with a more specific substitution are cellulose propionate with DS of 1.8, a propyl content of 39.2 to 45% and a hydroxyl contact of 13 to15% and a butyrate content of 34 to 39%. Moreover, the semipermeable membrane may consist of mixture of cellulose acetates, alkoxylates or acrylates with different degrees of substitution. Generally, in osmotic pumps, the semipermeable membrane must be 200-300 microns thick to withstand the pressure within the device [6].

Additional semipermeable membrane forming polymers are from the group consisting of acetaldehyde dimethyl cellulose acetate, cellulose acetate, cellulose acetate ethyl carbamate, cellulose dimethyl amino acetate,

semipermeable polyamides, semipermeable polyurethanes or semipermeable sulfonated polystyrenes. Semipermeable cross-linked selectively permeable polymers formed by co-precipitation of poly anion and a poly cation also can be used for these purpose [10]. Other polymer meterials such as lightly cross-linked polystyrene derivative, semipermeable cross-inked poly (sodium styrene sulfonated) and semipermeable poly (Viny benzyl triethyl ammonium chloride) may be considered [11].

Selection criteria for membrane polymer [12]

- The material whose reflection coefficient as close as possible to one.
- Relatively high-water permeability for maintaining water flux (Table No. 2).
- Sufficient wet strength and good dimensional stability is suitable as membrane.
- Biocompatibility and biodegradability.

Table 1.3: List of semipermeable membranes with their water vapour transmission rate (WVTR)

Sr. No.	Polymer Membrane	WVTR (g/100 m^2/24h/mm thick)
1.	Polyvinyl chloride (extruded)	6-15
2.	Poly vinyl chloride (cast)	10-20
3.	Polyurethane	31-50
4.	Cellulose acetate	40-75
5.	Cellulose acetate butyrate	50
6.	Methyl cellulose	70
7.	Ethyl cellulose	75
8.	Polyvinyl alcohol	100

4) Wicking agent

These are either swellable or non swellable porous materials with ability to draw water also known as physisorption. Physisorption is the form of absorption in which the solvent molecules can loosely adhere to surfaces of the wicking agent via Vander Waals interactions between the surface of the wicking agent and the absorbed molecule. The main role of the wicking agent is to carry water to the surfaces inside the core of the tablet and create channels or a network with increased surface area. For bioactive agents with low solubility in water, the wicking agent aids in the delivery of partially solubilized bioactive agent through the passageway in the semipermeable coating. Materials, which suitably act as wicking agents include colloidal

silicon dioxide, kaolin, titanium dioxide, alumina, niacin amide, sodium lauryl sulfate, low molecular weight poly (vinyl Pyrrolidone), m-pyrol, bentonite.

5) Solubilizing agent

Non swellable solubilizing agents are classified in to three groups:

- Substance which inhibit crystal formation of the drugs or which cause complexation with the drugs (e.g. PVP, PEG 8000, α, β, γ - cyclodextrin).
- A high HLB micelle-forming surfactant, particularly anionic surfactants (e.g. Tween 20, 60 and 80, poly oxyethylene or poly ethylene containing surfactants and other long chain anionic surfactants such as Sodium lauryl sulfate).
- Citrate esters and their combinations with anionic surfactants (e.g. alkyl esters particularly triethyl citrate).

6) Coating solvent

These are the solvents used for dissolution coating membrane polymer. They include inert inorganic and organic solvents that do not adversely harm the core, wall and other materials. The typical solvents are methylene chloride, acetone, methanol, ethanol, isopropyl alcohol, butyl alcohol, ethyl acetate, cyclohexane, carbon tetrachloride, water, etc. Moreover the solvent mixtures such as acetone-methanol (80:20), acetone-ethanol (80:20), acetone-water (90: 10), methylene chloride-methanol (79:21), methylene chloride-methanol-water (75:22:3) can also be used.

7) Flux-regulating agent

Flux regulating agents are added to the coating membrane to assist regulation of fluid permeability of flux through the membrane. They also increase the flexibility and porosity of the membrane [8]. The examples are both hydrophilic and hydrophobic polymers.

Example:

i) Hydrophilic polymers viz Polyethylene glycols, polyhydric alcohols.

ii) Hydrophobic polymer viz diethyl phthalate.

8) Plasticizer

Lower temperature of the second order phase transition of the coating membrane or the elastic modulus of the membrane also increase the workability, flexibility and permeability of the fluids. For this, a plasticizer

or mixtures of plasticizer (0.001-50 parts per 100 parts of membrane) are incorporated in the membrane. They comprise of Phthalates (dibenzyl, dibutyl), triacetin, alkyl adipates, citrates and glycolates. They maintain flexibility of coating membrane.

9) Pore forming agent

Particularly used in the osmotic pumps developed for poorly water-soluble drugs or for the development of controlled porosity or multi particulate osmotic pumps. They form channels for transport of fluids. They cause in situ formation of micro porous membrane due to their leaching during the operation of the system. The pores formation in the membrane be achieved by gas formation due to chemical reaction within the coating polymer solutions which results in voids p in the film of membrane. Yet another technique involves volatilization of component of polymer solution before application or during application of core mass resulting in the creation of polymer foams serving as the porous wall. The pore formers ideally should be non-toxic.

The examples include,

Organic: Carbohydrates viz glucose, fructose, mannose, lactose, sorbitol, mannitol.

Inorganic: Alkaline metal salt viz such as sodium chloride, sodium bromide, potassium chloride. Alkaline earth metal viz such as calcium chloride, calcium nitrate.

1.4 Advantages and limitations of osmotic delivery system

Advantages

Osmotic drug delivery system for oral and parenteral has many distinct and practical utility than others. The following advantages have contributed to the popularity of osmotic drug delivery system:

- Zero order delivery [35], is crucial for chronic treatment.
- Its attainable delivery rate is great enough than the rate that can be attained with diffusion-based systems. This is especially important for cases where there is consumption of larger dosage of drugs [2].
- Delivery rate is independent of pH and outside agitation (advantage over normal enteric coated tablet) [36].
- Delivery of drugs takes place in solution form, which is ready for absorption. Thus this is an *in-situ* prepared liquid dosage form.

- Delivery rate *in vitro* can be accurately predicted since the system is well described by relevant equations and the delivery rate in GI tract is equal to *in vitro* delivery rate. A high degree of *in-vitro* and *in vivo* correlation.
- Possibility of design for drugs with wide range of solubilities.
- Can be used in early stages of drug research, such as drug screening, animal toxicology and pharmacology and initial clinical testing.

Limitations

- Need for special equipment for making an orifice in the system.
- Risk of film defects, may result in dose dumping.
- Variation in residence time of the system in the body with the gastric motility and food intake.
- Risk of gastric irritation, ulcer due releasing of saturated solution of drug.

1.5 Technologies for osmotic delivery of drugs

1) OROS-CT

OROS-CT is used as a once or twice a day formulation for targeted delivery of drugs to the colon. The OROS-CT can be a single osmotic agent or it can be comprised of as many as five to six push pull osmotic units tilled in a hard gelatin capsule.

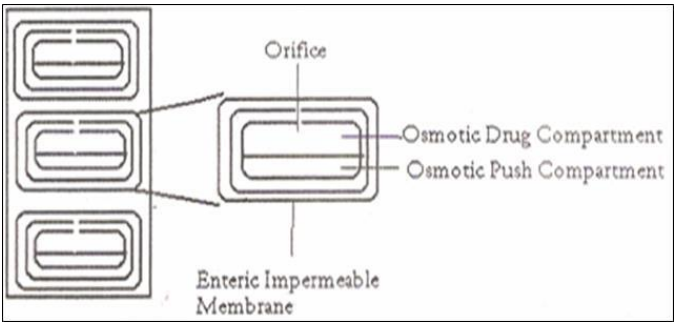

Fig 1.11: Schematic presentation of OROS-CT technology

When in contact with the gastric fluids, gelatin capsule gets dissolved and the enteric coating does not allow entry of fluids from stomach to the system as the system enters into the small intestine the enteric coating dissolves and water is imbibed into the core leading the push compartment to

swell. The flowable gel is formed in the drug compartment is forced out of the orifice at a controlled rate, a rate of water transport across the semipermeable membrane.

2) Concerta-XL

Concerta XL is new technology in osmotic drug delivery system. Push-pull design comprises push layer of osmotic agent having swellable polymer and pull layer contains drug with osmotic agent. Also there is immediate release layer of methylphenidate over Semipermeable membrane. So, Concerta provides quick initial 30% release of methylphenidate after administration and releases remaining 70% drug at predetermined rate. Concerta-XL is been very promising treatment for attention-deficit hyperactivity syndrome.

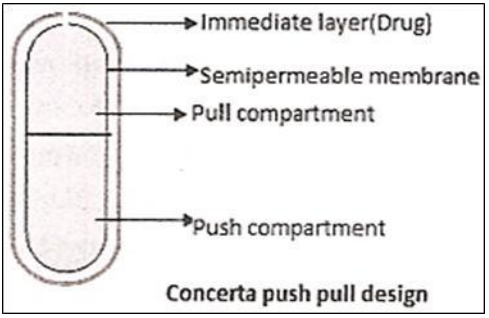

Fig 1.12: Schematic presentation of Concentra-XL technology

The following table (Table No. 1.4) enlists a few commercially available osmotic pump delivery systems.

Keywords

SEOP: Self-emulsified elementary osmotic pump.

DOEOP: Drug overcoated elementary osmotic pump.

PPOP: Push-pull osmotic pump.

COER: Controlled-onset extended-release.

CPOP: Controlled porosity osmotic pump.

SCOT: Single composition osmotic tablet.

EOP: Elementary osmotic pump.

PSOP: Push-stick osmotic pump.

Table 1.4: Commercialy available osmotic pump delivery systems

Sr. No.	Product	Active ingredient	Type	Developer
Cardiovascular diseases				
1.	UT-15C	Treprostinil diethanolamine	SEOP	United Therapeutics
2.	LCP-Lerc	Lercanidipine	DOEOP	Recordati
3.	Cardura CR	Doxazosin mesylate	PPOP	Pfizer
4.	Covera HS	Verapamil hydrochloride	COER	Pfizer
5.	Tiamate Dilacor XR	Diltiazem hydrochloride	CPOP	Aventis
6.	Procardia XL	Nifedipine	PPOP	Pfizer
Metabolic disorders				
7.	Fortamet	Metformin hydrochloride	SCOT	Andrx
8.	Altoprev	Lovastatin	EOP	Andrx
9.	Glucotrol	Glipizide	PPOP	Alza
Nervous and neuronal disorders				
10.	Concerta	Methylphenidate hydrochloride	PSOP	Alza
11.	Halifax XR	Venlafaxine hydrochloride	EOP	Phoenix
12.	Tegretol XL	Carbamazepine	SEOP	Novartis
Respiratory and seasonal disorders				
13.	Volmax	Albuterol	EOP	GSK
14.	Acutrim	Phenylpropanolamine	DOEOP	Alza
Gastrointestinal disorders				
15.	Osmoran 300	Ranitidine hydrochloride	DOEOP	Osmotica

Conclusion

The osmotic drug delivery is a useful drug delivery for any crucial cases where in osmotic pressure plays important role. Expanding pressure inside the system from water imbibition makes the medication discharge of drug at a controlled rate from the framework irrespective of environmental factors with reduced side effect profile with wide use of different molecules. Further, with the disclosure of more up to date and powerful medications by the biotechnology business, the need to convey such rate constant drug delivery system unquestionably will make ready for osmotic delivery systems to assume a key role. The products and patents available till date proves its effectiveness and success rate.

Acknowledgement

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Chapter - 7
Clinical Evaluation of Diabetic Complication: Nephropathy

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Chapter - 7

Clinical Evaluation of Diabetic Complication: Nephropathy

Dr. Ladli Kishore

Abstracts

Diabetes mellitus is a common non-communicable disease which is classified as a metabolic disorder. Chronic diabetes is associated with one of complication like nephropathy defined as the loss of functions of kidney associated with nephrotic syndrome, glomerulosclerosis, type IV renal tubular acidosis, persistent albuminuria, declining glomerular filtration rate (GFR); also is associated with risk factors such as high blood glucose, elevated cholesterol levels and proteinuria with the residual renal functions. A variety of factors contribute to the renal damage in diabetes.

According to advance biochemical evaluation clinically, the human population worldwide appears to be in the midst of an epidemic of diabetes and steps have been taken for the understanding diabetes and its long-term complications. Simple biochemical investigations were served to identify diabetes which assist in its management and facilitate the early recognition of complications. This Chapter discusses some of the various relevant biochemical tests to identification of diabetes complication through metabolic profile, Kidney function test, serum electrolyte test, Glycosylated haemoglobin test and enzymatic test.

Keywords: diabetes, nephropathy, biochemical, hyperglycaemia, oxidative stress

Introduction

Diabetes has an ancient origin. Sushruta, father of Indian Medicine, diagnosed diabetes mellitus as early as 1000 B.C. The graph of complications of diabetes-related mortality is rising unabated. Diabetic nephropathy (DN) is a major cause of end stage of renal disease (ESRD) and it affects 30% of patients with type 1 diabetes mellitus (DM) and 20% of patients with type 2 DM. Much of the attention for the treatment of diabetes revolves around its long-term complications. Pathophysiology of these diseases has led to the research in different new avenues to identify and develop novel therapies to combat the diabetic plague.

An advancing of recent medicine and clinical research work is rapidly becomes the ever-expanding field and shifting to investigating biochemical data for proper diagnosis and prognosis studies. Some of the biochemical and functional abnormalities found in long-standing poorly controlled of diseases. Simple biochemical investigations were highlight in chapter to identify diabetes which assist in its diagnosis, management and concerned to facilitate the early recognition of complications.

The chronic complications of diabetes one such of development of diabetic nephropathy disorder can be confirm and identify by different types of biochemical test using blood and urine specimens.

Collection of specimen sample

Initially Fasting Blood sample (FBS) of specimens. After that serum and plasma can be separate in a cold centrifuge at 2000 rpm for 10 minute. Then also collect urine sample of specimens who were 24 hours with proper access to drinking water and food.

The blood and urine sample of specimens are used to estimate with following parameters for detection of nephropathy condition in diabetic cases on certain day depending upon the period of biochemical studies.

Assessments of biochemical test

Metabolic profile

Fasting blood glucose level: Fasting blood sample can be estimate using a blood glucose kit (GOD/POD) method.

Lipid profile: There is most widely used parameter up to today. The serum samples are used to analyse and can be estimate to observe lipid profile such as serum cholesterol, serum triglyceride and HDL cholesterol level.

Total cholesterol: Total cholesterol can be estimate by one of scientific Chod-PAP method by using kit. It is based on the principle that cholesterol in the presence of cholesterol oxidase forms H_2O_2. This H_2O_2 reacts with 4-aminoantipyrine to form red quinine in the presence of peroxidase.

Serum triglyceride: Serum triglyceride is estimate by enzymatic colorimetric method by using kit. It is based on the principle that triglycerides in the presence of lipoprotein lipase and glycerol kinase form glycerol-3 phosphate. It forms H_2O_2 which reacts with 4-amino antipyrine to form red quinine.

High density lipoprotein: HDL cholesterol is estimate by Chod-PAP method using kit. It is based on the principle that HDL is separate from

plasma by precipitating with phosphor-tungstic acid and magnesium chloride. After centrifugation, the cholesterol in the HDL is assay with enzymatic cholesterol method using cholesterol esterase, cholesterol oxidase, peroxidase and chromogen 4-amino antipyrine/phenol.

Kidney functions test

Blood urea nitrogen: BUN is estimate by modified Berthelot method using kit. It is based on the principle that Urease hydrolases urea to ammonia and CO_2. The ammonia formed further reacts with a phenolic chromogen and hypochlorite to form a green colored complex. Intensity of the color formed is directly proportional to the amount of urea present in the sample. The readings can be measure using the Clinical analyser.

Serum creatinine: The serum creatinine values is estimate using commercially available kit based on modified Jaffe's kinetic method. It is based on the principle that picric acid reacts with creatinine to form an orange colored complex with the alkaline picrate. The intensity of color form at the fixed time that is directly proportional to the percentage of creatinine present in the sample. The readings can be observed using the Clinical analyser.

Albumin level in urine: The level of albumin in urine (UAE) is estimate using commercially available kit based on the BCG (Bromo Cresol Green). It is based on the principle that albumin in a buffer solution reacts with the anionic bromocresol green with a dye binding reaction to give a proportionate green color which is measured at 628 nm. The final color is found to be stable for 10 minutes. The readings are taken on a Clinical analyser.

Total protein in urine: Reagent A is prepare by adding 2% w/v of Na_2CO_3 in 0.1N NaOH. Reagent B is prepare by adding 0.5% w/v $CuSO_4.5H_2O$ in 1% w/v potassium tartrate. Reagent C is the alkaline $CuSO_4$ solution i.e. 50ml of Reagent A is mixed with 1ml of Reagent B. Reagent D are carbonate-copper solution which is prepare by the same method as Reagent C except for omission of NaOH. Reagent E is 2-fold diluted Folin-ciocalteu phenol reagent. To 0.2 ml of urine samples, 1 ml of Reagent C then added, mixed well and allow standing for 10 minutes at room temperature. 0.1 ml of Reagent E is then adds very rapidly and mix within a second. After 30 minutes, the absorbance is seen in a spectrophotometer at wavelength of 750 nm. These in turn are check against a standard solution of crystalline bovine serum albumin.

Urea clearance: Urea clearance is estimate by modified berthelot method using kit.

Calculations

Urea clearance, UrCl = U × V/P

Where

U= Concentration of drug in urine (mg/100ml).

V= Concentration of urine (ml/min).

P= Concentration of drug in serum (mg/100ml).

Creatinine clearance: A Creatinine clearance value is estimate using commercially available kit based on modified Jaffe's kinetic method.

Calculations

Creatinine clearance, CrCl = U × V/P.

Where

U = Concentration of drug in urine (mg/100ml).

V= Concentration of urine (ml/min).

P= Concentration of drug in serum (mg/100ml).

Serum electrolytes test: Measurement of sodium, potassium, chloride, calcium and magnesium in serum is assay and can be estimate according to spectrophotometric method respectively.

Serum sodium: Reagents 1 is standard sodium chloride solution prepare by dissolving 763 mg sodium chloride in 100 ml distilled water which becomes equivalent to 300 mg of sodium. Reagent 2 is uranyl magnesium acetate solution is prepared by dissolving 8 g uranyl acetate and 30 g magnesium acetate in 150 ml of distilled water. Then 30 ml of acetic acid is added and boiled for 2 minutes. After cooling, volume is made upto 200 ml with distilled water. This then transfer to a 1 litre volumetric flask and volume are made up to the mark with 800 ml of absolute alcohol. It is then stored in an amber bottle and 1 ml of 1% w/w sodium chloride are add to it. It is mixed well and allows standing for several days until the precipitate of sodium, magnesium and uranyl acetate are form. The resulting supernatant fluid was used. Reagent 3 is 1% v/v aqueous solution of acetic acid and Reagent 4 is 20% w/v aqueous solution of potassium ferrocyanide. After the preparation of all the reagents, 3 test tubes namely for standard, unknown and blank are taken and the following table can be representing as below:

Table 1: Sampling of Solution for Estimation of Serum Sodium

	Standard	Unknown	Blank
Reagent 2	5 ml	5 ml	5 ml
Reagent 1	0.1 ml	-	-
Serum	-	0.1 ml	-
Distilled water	-	-	0.1 ml
It is allow to stand for 5 min, shake well for 30 sec and centrifuge for 1 minutes 2 ml each of the supernatant fluid are transfer to 100 ml volumetric flasks and continue as below:			
Supernatant fluid to (100 ml) volumetric flask	2 ml	2 ml	2 ml
Reagent 3	About 80 ml	About 80 ml	About 80 ml
Reagent 4	2 ml	2 ml	2 ml
Reagent 3 make up to	100 ml	100 ml	100 ml

Then measure sample within 10 min in a colorimeter at wavelength 480 nm/blue filter.

Calculations

Serum sodium = (absorbance of unknown/absorbance of standard) × 300 mg per 100 ml.

Serum potassium: For Reagent 1, the two solutions A and B have to prepare. Solution A is sodium cobalt nitrite solution which is prepare using 25 g cobaltous nitrite (potassium free), 12.5 ml glacial acetic acid and volume make upto 50 ml with distilled water. Solution B is prepared by using 129 g of sodium nitrite dissolve in 180 ml of distilled water. Then both solutions A & B is mix together. Air has to draw rapidly through the mixture until all nitric oxide gas is removing (from 3 to 6 hours) and then store in a refrigerator at 4 ^{o}C. The solutions remain stable for 4 weeks and then filter before use. Reagent 2 is preparing using 11.2 ml sulphuric acid and volume is make up to 100 ml with distilled water and the concentration are approximately 4N. Reagent 3 is potassium permanganate 0.2 N solutions which have to prepare using a normal solution of potassium permanganate (0.3161 g of potassium permanganate per litre of distilled water) and dilute to make 0.2 N solutions. Reagent 4 is sodium oxalate 0.01 N solutions which then prepare by diluting 0.1 N (6.7 g of sodium oxalate per litre of distilled water) with 5 ml of sulphuric acid. After the preparation of all 4 reagents, 1 ml of serum is transfer into a 15 ml centrifuge tube. Then add 2 ml of reagent dropwise, mix thoroughly and allows standing for 45 minutes, Then 2 ml of distilled water is dissolve and contents have it mix and allow

centrifuging for 30 min at 1400 R.P.M. After centrifugation the supernatant is carefully remove and add 5 ml of distilled water and re-centrifuge for 5 minutes. This procedure can be repeats for 3 times and then supernatant fluid can be removed in the last wash. Then add left reagent 3 and 1 ml of reagent 2. The contents of the tube is mix once and heat in a boiling water bath until no further color change is visible, preferably within 1 and half mins. Then add 2 ml of reagent 4 to decolorize the mixture completely. Finally titrate with reagent 3 until the definite pink color formation that determines the presence of excess oxalate.

Calculations

$$\frac{\text{ml solution of unknown}}{\text{ml solution of known}} \times 100 = \text{mEq. Potassium per litre}$$

Serum chloride: Reagent 1 is mercuric nitrate solution which can be preparing by dissolving 2.9 g mercuric nitrate in 100 ml of distilled water. Then add 20 ml of 2N nitric acid and make volume up to a litre with distilled water. Reagent 2 diphenyl carbazone indicators which can be prepare by dissolving 100 mg diphenyl carbazone in 100 ml of 95% ethanol and then finally place in the refrigerator. Reagent 3 standard chloride solution which is prepare by dissolving 585 mg sodium chloride dried at 120^{o}C in distilled water and make volume upto 1000 ml. After the preparation of all the three reagents, 1.8 ml of distilled water is mix with 0.2 ml of serum and adds 0.06 ml of indicator and then titrates with mercuric nitrate solution using a micro burette. The first drop of excess mercuric nitrate solution will change in an intense violet blue color which showing the end point with protein free filtrate solution. For standard, 2 ml of standard sodium chloride can be preferred for titration.

Calculations

$$\frac{\text{ml of mercuric nitrate solution required for the unknown}}{\text{ml of mercuric nitrate solution required for the known}} \times 100 = \text{mEq. chloride per litre}$$

Serum calcium: In a centrifuge tube mix 2 ml of distilled water with 2 ml of serum and then 1 ml of Reagent 1 (ammonium oxalate saturated aqueous solution) and allow standing for 30 to 40 minutes. Then centrifuge at 5000 r.p.m. for 5 min and further remove supernatant fluid. Then mix the solution well and recentrifuge for 5 mins. Then remove supernatant fluid and wash repeatedly. Thereafter add 2 ml of Reagent 3 (sulphuric acid N solution) and the tube should place in a boiling water bath for 1 minute.

Then titrate with reagent 4 {potassium permanganate, 0.1 N solution (3.16 g potassium permanganate, distilled water to 1 litre)} using microburette until a faint trace of pink persisted. For standard, add 0.1 ml of reagent 2 (ammonium hydroxide 2% aqueous solution) and mix with 2 ml of reagent 3 in a centrifuge tube. Then heat the mixture in a boiling bath for 1 min and finally titrate with reagent 4 as a blank.

Calculations

Calcium in serum = (ml of unknown-ml of blank) × 10 mg of calcium per 100 ml of serum

Serum magnesium **(Denis' method):** First measure 2 ml of serum into a centrifuge tube and then add 1 ml of 30% w/v ammonium oxalate solution with stirring. The centrifuge is allowing standing overnight and then transfers 3 ml of supernatant fluid into another 15 ml tube. Then add 0.5 ml of Reagent 1 (ammonium phosphate 50g, ammonia 5 ml, distilled water to 1 litre), do stirring and then add Reagent 2 (ammonia Sp. gr. 0.880) drop wise, allow to stand overnight and then centrifuge. The supernatant have to siphon off and washing tube with 5 ml of Reagent 2 diluted in the ratio of 1:3 and centrifugation. This procedure is repeat twice. Washing repeat with using 5 ml of reagent 3 (ethanol 750 ml, ammonia 10 ml, distilled water to 1 litre) and then allowing to stand the residue in a warm place to volatilize the ammonia. To the residue, add 1 ml of reagent 4 (ammonium molybdate 25 g, sulphuric acid 10 N solution 500 ml, distilled water to 1 litre) and set aside for a few minutes. Add 0.4 ml of reagent and immediately make volume up to 10 ml with distilled water. Mix final sample and allow standing for 5 minutes. Take a reading in a colorimeter against the standard. For the standard, add 1 ml of reagent 4, 3ml of reagent 6 (phosphate 10 ml, distilled water to 1 litre) and 2 ml of distilled water. Then mix reagent 5 (1,2,4-aminonaphthosulphonic acid 0.5 g, sodium bisulphate 15% 200 ml) and make volume up to 10 ml with distilled water. Then measure sample in a colorimeter at wavelength 450 nm.

Calculations

$$\frac{\text{Reading of standard}}{\text{Reading of unknown}} \times 0.03 \times \frac{100}{1.2} = \text{mg of magnesium per 100 ml serum}$$

Glycosylated hemoglobin: Glycosylated hemoglobin level can be estimate by ion exchange resin method using kit. Glycosylated hemoglobin (GHb) is defined operationally as the fast fraction hemoglobins HbA1 (Hb A1a, A1b, A1c) which elute first during column chromatography (Karpen *et*

al., 1982). The non-glycosylated haemoglobin, which consists of the bulk of haemoglobin, has been designated HbAo. A hemolysed preparation of whole blood is mixed continuously for 5 minutes with a weakly binding cation-exchange resin. The labile fraction is eliminated during the hemolysate preparation and during the binding of this resin. During mixing, the HbAo binds to the ion exchange resin leaving GHb free in the supernatant. The percentage of GHb is determined by measuring absorbance of GHb fraction and total hemoglobin (THb) fraction. The ratio of the absorbance of the GHb and THb of the control and the sample is used to calculate the percent glycosylated hemoglobin in the sample.

Procedure: The estimation of glycosylated hemoglobin involved the following steps:

a) Hemolysate preparation

Dispense 0.5 ml lysing reagent into the tubes label as control (C) & test (T). Then 0.1ml of the reconstitute control and add well-mix blood sample into the appropriately label tubes. These mix until complete lysis should evident and allow standing for 5 minutes.

b) Glycosylated hemoglobin (GHb) separation

Remove the cap from the ion-exchange resin tubes and label as control and test. Then add 0.1 ml of the hemolysate into the appropriately label ion exchange resin tubes. Insert resin separator into each tube so that the rubber sleeve should approximately 1 cm above the liquid level of the resin suspension. Then mix contents of the tubes on a vortex mixer continuously for 5 minutes. Allow resin to settle and then push resin separator into the tubes until the packing resin firmly. Pour each supernatant directly into a cuvette and measure absorbance at 415 nm against distilled water.

c) Total Hemoglobin (THb) fraction

Dispense 5.0 ml of distilled water into tubes label as control and test. Then add 0.02 ml of hemolysate into the appropriately label tube and mix well. The absorbance can be read at 415 nm against distilled water.

Calculations

$$\text{GHb in \%} = \frac{\text{Ratio of test}}{\text{Ratio of control}} \times 10 \text{ (value of control)}$$

Antioxidant level in specimen sample

There are chances for free radicals to induce hyperglycemia; which

impairs the endogenous antioxidant (include both enzymatic and non-enzymatic pathways) defense system in patients with diabetes in other hand; also counterbalance toxic reactive oxygen species (ROS). Common antioxidant enzymes superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione reductase (GRx) are the important endogenous antioxidant defense systems, their relationship to several pathophysiological processes can be estimate by following parameter.

Estimation of malondialdehyde (MDA): Lipid peroxidation is estimate in terms of thiobarbituric acid reactive species (TBARS), using malondialdehyde (MDA) as standard. Mix 1 ml of homogenized renal tissue in 2 ml of normal saline with 24% TCA and centrifuge at 2,000 rpm for 20 mins. To 2 ml of protein-free supernatant, add 1 ml of fresh TBA (0.67%) reagent, mix thoroughly and heat at 95° C for 1 hour in a water bath. Cool the suspension at room temperature, centrifuge at 2000 rpm for 10 mins and then take pink colored supernatant for spectroscopic measurement at 532 nm for the assay of MDA. Lipid peroxide is expressed in terms of nM of MDA /mg of renal tissue. Since 99% TBARS is malondialdehyde (MDA), so TBARS concentrations of the samples is calculate using the extinction co-efficient of MDA, which is $1.56 \times 10^5\ M^{-1}\ cm^{-1}$.

Calculations

Equation obtained from standard curve of lipid peroxidation:

$Y = 0.00098X - 1.0083$

Where,

Y= Absorbance of test sample.

X= Concentration of malondialdehyde in test sample.

$X = Y + 1.0083/0.00098$.

Unit: nmol/L

Estimation of catalase (CAT): The enzyme catalyzes the conversion of H_2O_2 into water. 3 ml of CAT reaction solution contained 100 mM phosphate buffer (pH 7.0), 30 mM H_2O_2, and 0.1ml of enzyme extract. The reaction is initiate by adding the enzyme extract. Note a changes in absorbance of the reaction solution at 240 nm after every 30 s. One unit CAT activity is defined as an absorbance change of 0.01 unit/min.

Calculations

Catalase = log A_1/log A_2 x 2297.3 (factor) x dilution factor

Where

A_1 = Initial absorbance.

A_2 = Final absorbance.

Units: µmol of H_2O_2/min/mg

Estimation of superoxide dismutase (SOD): An indirect method of inhibiting auto-oxidation of epinephrine to its adrenochrome is used to assay superoxide dismutase activity. Auto-oxidation of epinephrine is initiate by adding 1ml of Fenton reagent. To a mixture of epinephrine ($3x10^{-4}$ M), Na_2CO_3 (10^{-3}M), EDTA (10^{-4}M) and 1.0ml of deionised water, add fenton reagent. Read the auto oxidation in a spectrophotometer at 480 nm every 30 sec for 5 minutes. Plot a graph of absorbance against time for each and the initial rate of auto-oxidation. One unit of superoxide dismutase activity is defined as the concentration of the enzyme (mg protein/ml) in the homogenate that causes 50% reduction in the auto-oxidation of epinephrine. Superoxide dismutase activity is subsequently calculate for each sample.

Calculations

SOD = {0.25 – (X x 50 x 100)}

Where

X = final absorbance - initial absorbance.

Units: EU/dl

Estimation of glutathione-s-transferase (GST): GST activity can be determine by measuring the increase in absorbance at 340 nm using 1-chloro-2,4-dinitrobenzene as the substrate. In a 3 ml reaction mixture the final concentration contained 97 mM potassium phosphate, 0.97 mM ethylene diamine tetra acetic acid, 2 mM glutathione reduces, 1.0 mM 1-chloro-2,4-dinitrobenzene, 3.2% v/v ethanol and 0.0075-0.015-unit glutathione-S-transferase.

Calculations

Units/ml enzyme = {ΔA340nm/min Test-ΔA340nm/min Blank) (3.0)(df)}/(9.6)(0.10)

Where

3.0 = Total volume (in milliliters) of assay.

df = Dilution factor.

9.6 = Millimolar extinction coefficient of glutathione-1-chloro-2,4-dinitrobenzene conjugate at 340 nm.

0.10 = Volume (in milliliter) of enzyme.

References

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