Physical evaluation

D- Physical evaluation

The application of physical constants is applied to the **active principles** of drugs such as alkaloids, volatile oils, fixed oil..etc.

1- Solubility:

-We should determine solubility of active constituents in different solvents.

-Usually expressed in the following form:

1g is soluble in ….ml of water, … ml of alcohol, etc.

2- Specific gravity:

- Particularly of the fats and volatile oils.

-Weight of a given **volume of liquid** compared with the weight of equal **volume of water** at specific temperature and pressure in air.

- The specific gravity of anise oil is not less than 0.978 and not more than 0.988.
- **Specific gravity of water =1**
- **Most of V.O. is lighter than water, except V.O. of clove, Cinnamon (heavier than water).**

- Specific gravity is not absolutely constant,

<u>Because other factors affected it:</u>

- ✓ maturity of drug from which v.o. is prepared.
- ✓ Age of v.o.
- ✓ Method of preparation
- ✓ Purification

3- Optical rotation:

Optical activity is the ability of a chiral molecule (i.e. lacking microscopic mirror symmetry) to rotate the plane of plane-polarized light either;

- ✓ Dextro (+) rotary, rotate plane of polarized light to right
  (clock wise)
- ✓ Levo (-) rotary, rotate plane of polarized light to left
  (anti-clock wise)

- It is measured by polarimeter.

1-Automatic Digital Polarimeter

2-Hand-Held Polarimeter

Atropine ± zero
Pilocarpine +
Iso pilocarpine -

4- Refractive index:

- Particularly of the fixed and volatile oils (crystals, liquids).

It is a ratio of the velocity of light of a specified wavelength in air to its velocity in the examined substance.

- Depending upon purity, it's constant for a liquid and can be consider as one of **its standardization**.
- Refractive index of a compound varies with **the wave length of the incident light, temperature and pressure.**

- **RI measure by refractometer.**

-The RI of peppermint oil is not less than 1.4590 and not more than 1.4650 at 20 °C.

5- Congealing point:

- Particularly of fixed and volatile oils.
- The solidification range of fatty acids in olive oil is between 17 and 26°C.

6- Melting point:

- In case of pure chemicals or phytochemicals, melting points very sharp and constant.
- Since the crude drugs from animal or plant origin contain the mixed chemicals, they are described with certain range of melting point (i.e. melting point range for few crude drugs)

e.g. Drugs Melting Point (°C)

Colophony 75-85

Coca butter 30-33

Bees wax 52-65

7-Determination of extractive value:

(i) Determination of alcohol soluble extractive

(ii) Determination of water soluble extractive.

8- Volatile oil content:

Pharmaceutical significance of aromatic drugs is due to their odorous principal that is volatile oils such crude drugs are standardized on the **basis of their volatile contain**.

Drugs volatile oil content (% v/w)

Caraway Not less than 2.5

Lemon peel Not less than 2.5

Clove Not less than 15

Fennel Not less than 1.4

Cardamom seed Not less than 4.0

9- Viscosity:

Viscosity of a liquid is constant at a given temperature and is an index of its composition. Hence it can be used as a means of **standardizing liquid drugs**.

10 - UV:

UV light is importance in several cases, notably in detecting:

1- adulteration:

a- e.g. rhubarb, where **rhapontic rhubarb** can easily be distinguished from **genuine Chinese** or **Indian rhubarb** **by** its marked fluorescence.

Chinese and Indian rhubarb fluorescence brown, while rhubarb rhaponticum is bright blue.

b- Powdered ergot exhibits a strong reddish fluorescence and can be detected in wheat flour.

❖UV light is used for determining fluorescence and a play of colors in connection with certain extracts such as chlorophyll and the extracts of certain drugs (catechu, senna).

❖Many alkaloids show distinctive colors under this light, aconitine (light blue), berberine (yellow), emetine (orange).
An alkaloids such as quinine show fluorescence in acid solution even in day light, exhibits a much stronger fluorescence under the quartz lamb.

11- Moisture :

is normally present to the extent of **5-10%** in all dried drugs. An excess of moisture is considered as an **adulterant.**

Excessive moisture is considered an adulterant because:

a- **Added weight**.

b- Promote the **mold and bacterial growth** and subsequently to deterioration and spoilage of the drug.

- **Moisture is usually determined in one of FOUR ways, the specific method often being stated in the drug monograph.**

1-Gravimetric method:

- If the drug contains **no volatile** material, a weighed sample is heated at 100°c to constant weight, the loss in weight being the water content.
- If **volatile** constituents are present, these must first be determined by the **volatile ether-extractive method** and their weight deduced from the **loss in weight by drying**, before the water content can be determined.

2-Volumetric (Toluene distillation) method:

It is applies in either of the above cases. The water is distilled from the drug with toluene in a continuous distillation apparatus. The water is caught in the trap and is determined by direct measurement of the volume.

3- Titrimetric (Karl Fischer) method:

It is applicable for **expensive drugs** and **chemicals containing small quantities of moisture**, It is based on the following reaction:

$$2H_2O + SO_2 + I_2 \rightarrow H_2SO_4 + 2HI$$

4-Determination of moisture by spectroscopic methods:

Water can absorb energy at various wavelength throughout the electromagnetic spectrum and this can be made a basis for the quantitative determination. Measurements can be made in both the **infrared** and **ultraviolet** regions. The **NMR** spectroscopy also employed for the determination of moisture in plant products.

12- Determination of ash:

The residue remaining after incineration is the ash content of drugs ,which simply represents **inorganic salts**, naturally occurring in drugs **or adhering** added to it as form adulteration.

Two types ash determine-

(i) Acid insoluble ash value.

(ii) Determination of water soluble ash.

Factors effect the amount and composition of ash:

- The part and age of the plant.
- Culture treatment: type of soil and elements present in it.
- The constitution of the ash varies with the time of collection.

In the Pharmacopeia a maximum of **2% of acid insoluble ash is permitted** unless otherwise stated in the monograph.

- **The total ash** and **acid insoluble ash** are determined by the official method.

- This consists of incinerating and weighting the total ash, then boiling **the total ash** with **dilute hydrochloric acid,** filtering, igniting and weighting the acid insoluble ash.

- **Acid insoluble ash** consists of **sand, silicates and other dirt** and is an indication of the amount of dirt present in the sample. **It is called foreign inorganic matter**.

13-Detection of foreign matters:

***Foreign organic matter** :

Refers to any **other** part of the plant or animals except that constituting the drug

*The **Permissible percentage** of foreign matter in a drug is usually specified in its official monograph**.**

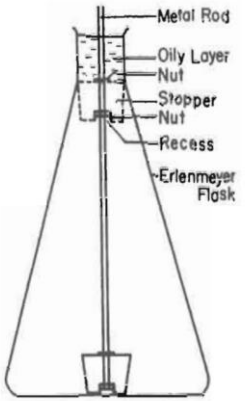
In quality control procedures the pharmacopoeias stated:

Drugs containing appreciable quantities of foreign organic matter, animal excreta, insects or mould should however be **rejected even** though the percentage of such substances be insufficient to cause the rejection of the drug.

Detection of filth:

1- Insects, insect parts, rodent hairs and feces may be separated from the drug by means of **liquids of varying specific gravity.**
Most drug material will float on chloroform, but rodent pellets will sink in this liquid.

2- Insect parts can be separated by boiling the drug with water, cooling and then vigorously stirring in a small amount of **mineral oil**.
The mineral oil rises to the top carrying with it the insect fragments. Floating strata may be separated by mean of **Wildman trap flask** and sinking strata by means of a percolator equipped with two corks at either end of the stem.
The recovered impurities are then identified microscopically.



19-2 figure

Wildman trap flask. Stopper on shaft is lifted up to neck of flask to trap off floating layer. [Adapted from (5), AOAC Method 945.75, Extraneous Materials in Products.]

14- Determination of Heavy Metals:

- Contamination by toxic metals can either be accidental or intentional.
- Contamination by heavy metals such as mercury, lead, copper, cadmium, and arsenic in herbal remedies can be attributed to many causes, including environmental pollution
- Determination of heavy metals is based on color reactions with special reagents such as thioacetamide or diethyldithiocarbamate, and the amount present is estimated by comparison with a standard.
- When the metals are present in trace quantities, in admixture, or when the analyses have to be quantitative. **The main method commonly used is** atomic absorption spectrophotometry (AAS).

15. Determination of Microbial Contaminants and Aflatoxins

- Medicinal plants may be associated with a broad variety of microbial contaminants, represented by bacteria, fungi, and viruses.
- Herbal drugs normally carry a number of bacteria and molds, often originating in the soil.
- Proliferation of microorganisms may result from failure to control the moisture levels of herbal medicines
- Poor methods of harvesting, cleaning, drying, handling, and storage may also cause additional contamination, as may be the case with *Escherichia coli* or *Salmonella* spp.

- In **Pharmacopeias**, as well as in the WHO guidelines reported the complete laboratory procedures investigating microbial contaminations **consists of determining** the total aerobic microbial count, the total fungal count, and the total *Enterobacteriaceae* count, together with tests for the presence of *Escherichia coli*, *Staphylococcus aureus*, *Shigella*, and *Pseudomonas aeruginosa* and *Salmonella* spp.
- The **European Pharmacopoeia** also specifies that *Shigella* and *Salmonella* spp. should be absent from herbal preparations. However it is not always these two pathogenic bacteria that cause clinical problems. For example, a fatal case of **listeriosis** was caused by contamination of alfalfa tablets with the Gram positive bacillus *Listeria monocytogenes*.

- The requirements for microbial contamination in the European Pharmacopoeia allow higher levels of microbial contamination in herbal remedies than in synthetic pharmaceuticals.
- The allowed contamination level may also depend on the method of processing of the drug. **For example, higher contamination levels are permitted if the final herbal preparation involves boiling with water.**

Untreated herbal material harvested under acceptable hygienic conditions:

- *Escherichia coli,* maximum 104 per gram
- mould propagules, maximum 105 per gram
- *Shigella*, **absence per gram** or ml.

Herbal materials that have been pretreated:

For herbal materials that have been pretreated (e.g. with boiling water as used for herbal teas and infusions) or that are used as topical dosage forms, **the limits are**:

- aerobic bacteria, maximum 107 per gram
- yeasts and moulds, maximum 104 per gram
- *Escherichia coli,* maximum 102 per gram
- Other enterobacteria, maximum 104 per gram
- *Clostridia*, **absence per** 1 gram
- *Salmonellae*, **absence** per 1 gram
- *Shigella*, **absence** per 1 gram.

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Other herbal materials for internal use:

For other herbal materials for internal use, the limits are:

- aerobic bacteria, maximum 105 per gram
- yeasts and moulds, maximum 103 per gram
- *Escherichia coli*, maximum 10 per gram
- Other enterobacteria, maximum 103 per gram
- *Clostridia*, **absence** per 1 gram
- *Salmonellae*, **absence** per 1 gram
- *Shigella*, **absence** per 1 gram.

Herbal medicines to which boiling water is added before use:

- aerobic bacteria, maximum 107 per gram
- yeasts and moulds, maximum 104 per gram
- *Escherichia coli,* maximum 10 per gram
- Other enterobacteria, maximum 103 per gram
- *Clostridia*, **absence** per 1 gram
- *Salmonellae*, **absence** per 1 gram
- *Shigella*, **absence** per 1 gram

- The presence of fungi should be carefully investigated and/or monitored
- **Aflatoxins** in herbal drugs can be dangerous to health even if they are absorbed in minute amounts. Aflatoxin producing fungi sometimes build up during storage.
- Procedures for the determination of aflatoxin contamination in herbal drugs are published by the WHO. After a thorough clean-up procedure, TLC is used for confirmation.
- Certain plant constituents are susceptible **to chemical transformation** by contaminating microorganisms.

16. Determination of Pesticide Residues:

- There are **no serious** reports of toxicity due to the presence of pesticides and fumigants.
- It is important that herbs and herbal products are free of these chemicals or at least are controlled for the absence of unsafe levels.
- Herbal drugs are liable to contain pesticide residues, which accumulate from agricultural practices, such as spraying, treatment of soils during cultivation, and administering of fumigants during storage.
- However, it may be desirable to test herbal drugs for broad groups in general, rather than for individual pesticides.

- Many pesticides contain chlorine in the molecule, which, for example, can be measured by **analysis of total organic chlorine**. In an analogous way, insecticides containing phosphate can be detected by measuring total organic phosphorus.

- Samples of herbal material are extracted by a standard procedure, **impurities are removed by partition and/or adsorption, and individual pesticides are measured by GC, MS, or GC/MS.**

17-Determination of Radioactive Contamination:

- The exposure can not be avoided because many naturally occurring sources including radio nucleotide occurring naturally in ground and atmosphere.
- Dangerous contamination may be consequence of a nuclear accidents

Health risk depend on:

1-Specific radio nucleotides.

2- Level of contamination.

3-Quntity of food.

4- The dose and duration of use of product use.