Pharmaceutical Quality Management

MISCELLANEOUS TESTS

By:

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WEIGHT PER MILLILITRE:

- The weight per millilitre of a liquid is the weight in g of 1 ml of a liquid when weighed in air at 20°C, unless otherwise specified in the monograph.
- The weight per millilitre is determined by dividing the weight in air, expressed in g, of the quantity of liquid that fills a pycnometer at the specified temperature by the capacity, expressed in ml, of the pycnometer at the same temperature. The capacity of the pycnometer is ascertained from the weight in air, expressed in g, of the quantity of water required to fill the pycnometer at that temperature. The weight of a litre of water at specified temperatures when weighed against brass weights in air of density 0.0012 g per ml is given in thetable. Ordinary deviations in the density of air from the above value, here taken as the mean, do not affect the result of a determination in the significant figures prescribed for Pharmacopoeial substances.

Temperature	Weight of a litre of water
°C	g
20	997.18
25	996.02
30	994.62

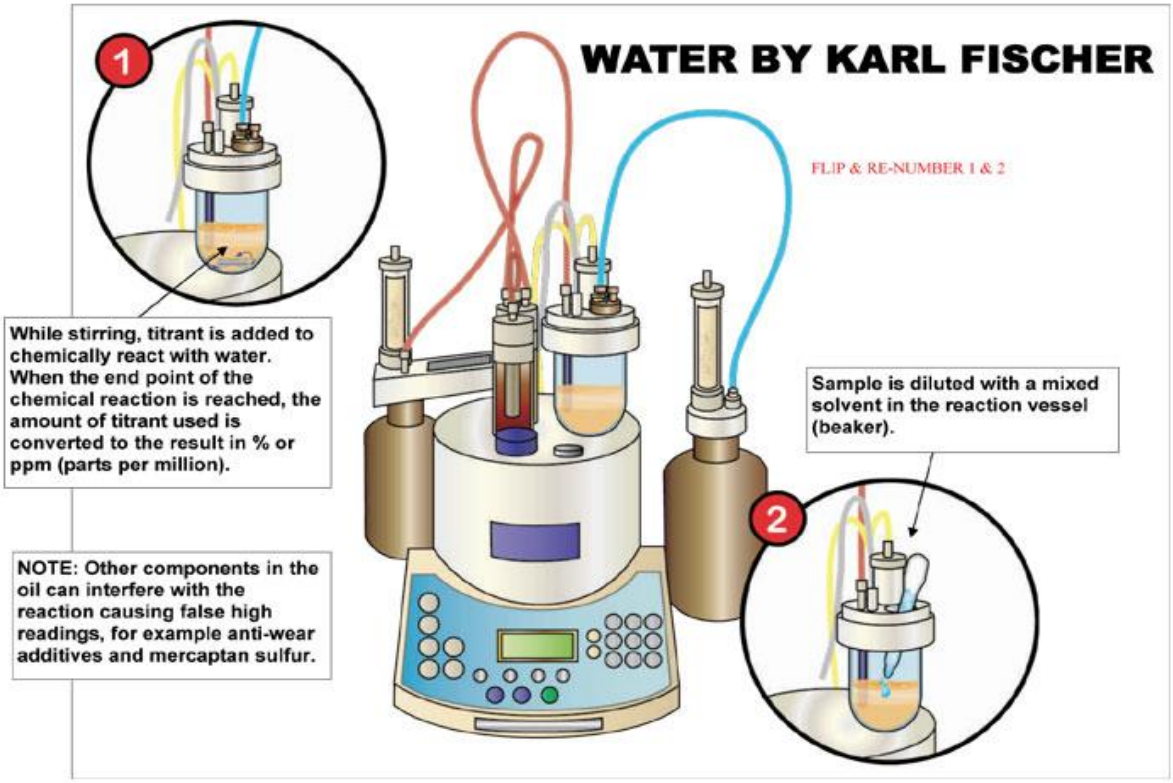
WATER/MOISTURE CONTENT:

- Presence of moisture influences chemical stability, crystal structure, powder flow, compaction lubricity, dissolution rate, and polymer film permeability in solid dosage forms and lead to growth of microorganisms, change in thixotropy in semi-solid dosage forms.
- Moreover, unit operations obviously depending on the amount and state of water present are also influenced by it. Therefore, moisture influences the properties of individual active ingredients and excipients, and it is essential to characterize the effect of moisture on these individual components. This article lay emphasis on determination of moisture by various methods and illustrates the changes induced by moisture on several product and process attributes
- Water content determination is mandatory for many materials used in the manufacturing of medicines. Karl Fischer (KF) titration is the long-standing standard method for this analysis prescribed by the leading Pharmacopoeias, like the European (Ph.Eur.), the United States (USP) and the Japanese (JP).

➢ European Pharmacopoeia requirements for Karl Fischer titration

- Ph.Eur. specifies KF titration to measure the water content of many solvents, chemicals and other substances. Method A the direct titration of water and in Method B the indirect method of back titration. In practice, the direct method A is easier to carry out and widely used since the development of the highly reactive HYDRANAL reagents:

- *"Method A. Introduce into the titration vessel methanol R, or the solvent indicated in the monograph or recommended by the supplier of the titrant. Where applicable for the apparatus used, eliminate residual water from the measurement cell or carry out a pre-titration. Introduce the substance to be examined rapidly and carry out the titration, stirring for the necessary extraction time."*



➢ Techniques

1. **Volumetric Karl Fischer technique**
2. **Coulometric Karl Fischer technique**

ALKALINITY OF GLASS:

"Alkalinity is a measure of the ability of a solution to neutralize acids to the equivalence point of carbonate or bicarbonate"

- In the natural environment *carbonate* alkalinity tends to make up most of the total alkalinity due to the common occurrence and dissolution of carbonate rocks and presence of carbon dioxide in the atmosphere. Other common natural components that can contribute to alkalinity include *borate, hydroxide, phosphate, silicate, nitrate, dissolved ammonia, the conjugate bases of some organic acids and sulfide.*
- Alkalinity is usually given in the unit ***mEq/L (milliequivalent per liter).***
- In all glass, the sodium and potassium oxides are hygroscopic; therefore, the surface of the glass absorbs moisture from the air. The absorbed moisture and exposure to carbon dioxide causes the NaO_2 or NaOH and KO_2 or KOH to convert to sodium or potassium carbonate.
- Both $NaCO_2$ and KCO_2 are extremely hygroscopic. In water, especially salt water, the Na and K carbonates in unstable glass may leach out, leaving only fragile, porous hydrated silica (SiO_2) network. This causes the glass to craze, crack, flake, and pit, and gives the surface of the glass a frosty appearance.

❖ *Effect of Alkalinity of Glasses on Pharmaceutical Products*

a. Effect on vaccines

b. Effect on parenteral products

c. Effect on solutions

a) Effect on vaccines

- Vaccines made were tested periodically for stability of pH and of potency. The acetone-treated cultures prepared in buffered saline solutions retained potency beyond 30 months of storage at 0 to 5 ℃. Similar vaccines in unbuffered saline solutions lost potency coincident with increase of alkalinity.
- Vaccines packaged in United States Pharmacopeia borosilicate glass vials retained potency and pH stability, whereas those soda-lime glass vials were less stable due to occurrence of alkalinity.

b) Effect on Parenteral Products

- The scanning electron micrographs showed surprising differences in the appearance of the surface regions. “Sulfur treatment” of ampoules was associated with a pitting of the surface and the presence of sodium sulfate crystals. The sulfur treatment of vials altered the glass surface in a characteristically different manner. This is due to alkalinity.
- The dissimilarity between the surface appearances was attributed to the method of sulfur treatment. Ampoules exposed to sulfuric acid solutions at room temperature did not show the pitting associated with the sulfur treatment.

c) Effect on Solutions

- Glass Container make solution contained more alkaline
- Alkalinity of a solution is the capacity of it to react with a strong acid (usually sulfuric acid H2SO4) to a predetermined pH. The alkalinity of a solution is usually made up of carbonate, bicarbonate, and hydroxides.

❖ *Test of Alkalinity of Glass:*

Test Procedure for limits of alkalinity of whole Glass Container:

- Take sufficient containers, not less than 3 from each batch, so that the total volume of water to be tested is not less than 250 ml. Rinse the containers thoroughly with distilled water and complete the rinsing with redistilled water.
- Fill each container to 90% of its overflow capacity with the redistilled water or above. Cover the unsealed containers with crimped pieces of new tin foil wash thoroughly with acetone.
- Place the containers on the rack in autoclave and close the door securely, leaving the vent open.
- Heat until steam issues vigorously from the vent and continue heating for 10 min. close the vent adjust the heating so that the temperature rises 1 ℃ /min until it reaches 121 ℃, taking 20 to 25 min to reach that temperature. Keep the temperature at 121 ℃ ± 0.5 ℃ for one hour.

- At the end of that period decrease the supply of heat and cool at the rate of 0.5 ℃ per min, until the internal pressure is equal to the atmospheric pressure.
- The time to cool from 121 ℃ – 100 ℃ should be from 40 to 50 min.
- Open the autoclave take out the containers and allow to cool them to 25 ℃, transfer 100 ml of water from each container add 5 drops of methyl red solution, and titrate with 0.01 N sulphuric acid.
- The time elapsing between opening the autoclave and titrating should not exceed 60 minutes. Carry out blank test on 100 ml of water from the same lot, and make the necessary correction.
- The quantity of 0.01N sulphuric acid used for containers with a capacity of up to 100 ml should be, not more than 1.5 ml and for containers of capacity greater than 100 ml, not more than 0.5 ml.

EVALUATION OF OINTMENTS

- *Ointments are semisolid dosage forms in which one or more drug substances are dissolved or dispersed or emulsified in a suitable ointment base and are meant for application on skin or mucous membrane where it exhibit local or systemic effects.*
- ***The different methods of evaluation of ointments are***

I. Physical methods

- **A. Physical appearance**
- **B. Particle size determination**
- **C. Weight variation test**
- **D. Test of rate of absorption**
- **E. Test of non-irritancy**
- **F. Test of rate of penetration**
- **G. Test of rate of drug release**
- **H. Test of rheological properties**
- **I. Test of content uniformity**

II. Microbiological methods

- **A. Test of microbial content**
- **B. Test of preservative efficacy**

❖ Physical Methods:

A.Physical appearance

The main characteristics need to be checked are

- Cracking of creams/Ointments (separation of oil and water)
- Development of granular and lumpy appearance
- Marked change in viscosity
- Crystal growth
- Microbial contamination

B.Particle size determination

- Dilute a suitable quantity of preparation with equal volume of glycerol or liquid paraffin, as specified
- Mount on a glass slide and examine under light microscope
- Count the number of particles with diameter above or below than that specified in monograph
- Compare the percentage with official limits

C.Weight variation test

- Applies to those products in which labeled net weight is not more than 150g
- Select 10 filled containers, remove the label, clean and weigh individually
- Remove the contents by cutting the containers and wash with suitable solvent
- Dry and again weigh each empty container together with its corresponding part, take difference as weight of contents.

- The average net weight of contents of 10 containers should not be less than the labeled amount
- The net weight of contents of any single container should not be less than 90% of the labeled amount (for ≤ 60g)
- And not less than 95% of the labeled amount (60-150g)
- If this requirement is not met repeat this procedures taking additional 20 containers
- The average net weight of contents of 30 containers should not be less than labeled amount

D.Test of rate of absorption

- The ointment should be evaluated for the rate of absorption of drug into the blood stream. This test can be done in-vivo only.
- The ointment should be applied over a definite area of the skin by rubbing.
- At regular intervals of time, serum and urine samples should be analyzed for the quantity of drug absorbed .
- The rate of absorption i.e., the amount of drug absorbed per unit time should be more.

E.Test of non-irritancy

- The bases used in the formulation of ointments may cause irritation or allergic reactions.
- Non-irritancy of the preparation is evaluated by patch test.
- In this test 24 human volunteers are selected.
- Definite quantity of ointment is applied under occlusion daily on the back of fore arm for 21 days.
- Daily the type of pharmacological action observed is noted.

- No visible reaction or erythema or intense erythema with edema and vesicular erosion should occur.
- A good ointment base shows no visible reaction.

F. Test of rate of penetration

- The rate of penetration of a semisolid dosage form is crucial in the onset and duration of action of the drug.
- Weighed quantity of the preparation should be applied over selected area of the skin for a definite period of time.
- Then the preparation left over is collected and weighed.
- The difference between the initial and the final weights of the preparation gives the amount of preparation penetrated through the skin and this when divided by the area and time period of application gives the rate of penetration of the preparation.
- The test should be repeated twice or thrice.
- This test can also be performed ex-vivo on Franz cell.

G. Test of rate of drug release

- To assess the rate of release of medicament from ointment is evaluated by applying dissolution studies. These studies can be conducted by dialysis bag dissolution or by using vertical diffusion cell.

H. Test of rheological properties

- The viscosity of the preparation should be such that the product can be easily removed from the container and easily applied to the skin.
- Using cone and plate viscometer the viscosity of the preparation is determined.

I. Test of content uniformity

The following procedure should be followed for testing tube uniformity of semisolid topical dosage forms:

- 1. Carefully remove or cut off the bottom tube seal and make a vertical cut up the face of the tube. Then carefully cut the tube around the upper rim and pry open the two ‘‘flaps’’ to expose the semisolid.
- 2. At the batch release and/or designated stability time point remove and test 0.25- to 1.0-g samples from the top, middle, and bottom of a tube. If assay values for those tests are within 90.0% to 110.0% of the labeled amount of active drug, and the relative standard deviation (RSD) is not more than 6%, then the results are acceptable.
- 3. If at least 1 value of the testing described above is outside 90.0% to 110.0% of the labeled amount of drug and none is outside 85.0% to 115.0% and/or the RSD is more than 6%, then test an additional 3 randomly sampled tubes using top, middle, and bottom samples as described. Not more than 3 of the 12 determinations should be outside the range of 90% to 110.0% of the labeled amount of drug, none should be outside 85.0% to 115.0%, and the RSD should not be not more than 7%.
- 4. For very small tubes (e.g., 5 g or less), test only top and bottom samples, and all values should be within the range of 90.0% to 110.0% of the labeled amount of drug.

❖ Microbiological methods

A. Test of preservative efficacy

- Using pour plate technique the number of micro-organisms initially present in the preparation are determined.
- Solutions of different samples of the preparation are made and mixed with Tryptone Azolectin (TAT) broth separately.
- All cultures of the micro-organisms are added into each mixture, under aseptic conditions. All mixtures are incubated.
- The number of micro-organisms in each sample are counted on 7^{th}, 14^{th} ,21^{st} , and 28^{th} days of inoculation.

Microbial limits:

- On 14^{th} day, the number of vegetative cells should not be more than 0.1% of initial concentration.
- On 28^{th} day, the number of organisms should be below or equal to initial concentration.