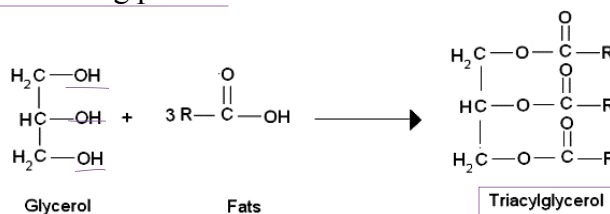


Experiment (6)

Determination of Fixed Oils from British Pharmacopoeia (BP)

Fixed oil is a triglyceride that can be extracted from vegetable origins, such as nuts or seeds of the botanical sources (e.g., almond, olive, sunflower.....etc.). Triglyceride is an ester derived from glycerol and three fatty acids. Fatty acid is a carboxylic acid with a long aliphatic chain, which is either saturated or unsaturated. The characteristics of oils are related to the properties of the fatty acids that they contain. The larger the number of carbon atoms, the higher the melting point, the larger the number of double bonds, the lower the melting point.



Physicochemical properties of oil like density, viscosity, boiling point, specific gravity, saponification value (SV), iodine value (IV), acid value (AV) and peroxide value (PV) were studied to evaluate the compositional quality of oils and their commercial importance.

I. Determination of Acid value (free fatty acids):

This value is a measure of the amount of free fatty acids which have been liberated by hydrolysis of triglycerides due to the action of moisture and temperature. It is a measure of hydrolytic rancidity which is used to determine the quality, age, edibility and suitability of oil for industrial use (Pharmaceutical oil must not have any acidity. Refined oils which referred to neutralized or low acidity oils are used in industry).

Acid value is a relative measure of hydrolytic rancidity because raw and crude oils are naturally hydrolyzed and consequently have a high acid value and it may be overestimated if other acid components are present in the system e.g., amino acids.

The acid value is defined as the number of milligrams of potassium hydroxide required to neutralize the free fatty acids present in one gram of oil.

The acid value is determined by directly titrating the oil with a standard solution of **potassium hydroxide** using **Phenolphthalein** as a color indicator.

- To calculate the Acid Value:

$$AV = (M * Mw * V) / W$$

Mw = molecular weight of potassium hydroxide, 56.11.

*V = the end point (mL)

M = molarity of the potassium hydroxide solution, 0.1.

W = weight of the sample taken (g)

II. Determination of Peroxide Value (Test of Rancidity):

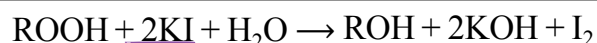
Fixed oils are odorless but some time it can emit a faint nutty aroma. When fixed oils are exposed to air they react with the oxygen or water vapor to form a series of breakdown products in stages; starting with primary oxidation products (Peroxides, Dienes, Free fatty acid), then secondary products (Carbonyls, Aldehydes, Trienes) and finally tertiary products. The short-chain acids are volatile and have unpleasant smells and tastes. This oxidation process is called **Rancidification**.

Oxidation progresses at different rates depending on many factors such as: storage temperature, light exposure, availability of oxygen, and the presence of moisture and

metals. Oils with a high degree of unsaturation are most susceptible to oxidation. The peroxide value is a parameter specifying the content of oxygen as peroxide in a substance. The peroxide value is a measure of the oxidation present. Therefore, it is useful for assessing the extent of oil spoilage and rancidity that occurred during storage.

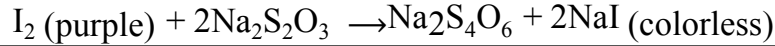
➤ The peroxide value:

is defined as the amount of peroxide oxygen per 1 kilogram of fat or oil. Traditionally this was expressed in units of milliequivalents (mEq). The peroxide value can be determined by measuring the amount of iodine which is formed by the reaction of peroxides with iodide ion.



The liberated iodine can be titrated with **sodium thiosulphate** ($Na_2S_2O_3$) using **starch solution** as an indicator (amylose forms a blue solution with iodine and is colorless where iodine is titrated).

للحفظ: $I_2 + \text{Starch} \rightarrow \text{Blue} \rightarrow Na_2S_2O_3 \rightarrow \text{Colorless}$
يعني: أزرق = لسه في يود، عديم اللون = خلس اليود.



POV of fresh oils are less than 10 mEq/Kg, when the PV is between 30-40 mEq/Kg a rancid taste is noticeable.

- Calculate the Peroxide Value:

$$\text{POV} = (\text{E.P} * \text{Normality of Na}_2\text{S}_2\text{O}_3 * 1000) / \text{W}$$

E.P = volume of sodium thiosulfate required to titrate sample (mL).

W = weight of the sample taken for the test (g).

Note: Normality of $\text{S}_2\text{O}_3^{2-}$ = Molarity of $\text{Na}_2\text{S}_2\text{O}_3$

✓ Saponification value:

- The Saponification Value is the number of mg of potassium hydroxide required to neutralize the free acids and saponify the esters contained in 1.0 g of the substance.

Saponification is the process of breaking down a neutral fat into glycerol and fatty acid salt by treatment with strong base. Saponification value is a measure of the average molecular weight of the triglyceride in a sample. The smaller the SV the larger the average molecular weight of the triglyceride present. The long chain fatty acid found in fats have low SV because they have a relatively fewer number of carboxylic functional groups per unit mass of the fat and therefore high molecular weight.

✓ Iodine value:

Unsaturated fatty acids can be converted into saturated by the process of hydrogenation. Depending upon the degree of unsaturation, the fatty acids can combine with oxygen or halogens to form saturated fatty acids. The iodine value gives a measure of the degree of unsaturation of a lipid. An increase in IV indicates high susceptibility of lipid to oxidative rancidity due to high degree of unsaturation.

- The Iodine Value represents the number of grams of iodine absorbed by 100 g of the substance.

أهم نقطة للحل:
رقم الصابون يعطي فكرة عن متوسط الوزن الجزيئي للـ triglycerides في العينة.
العلامة عكسية:
SV ↓ → Average molecular weight ↑
SV ↑ → Average molecular weight ↓
لماذا؟
إذا كانت الأجزاء الدهنية طويلة السلسلة:
• عدد مجموعات COOH لكل وحدة كتلة يكون أقل.
• وبالتالي يكون الوزن الجزيئي أكبر.
• لذلك يكون SV منخفضاً.

ملاحظة إضافية:
Long chain → High MW → Lower COOH per gram → Less KOH required → Low SV
Short chain → Low MW → More carboxylic acid per gram → More KOH → High SV

من المادة و تمثل عدد غرامات اليود التي تمتصها 100 Iodine Value 100
الفكرة بسيطة:
تحفظ العلاقة:
Iodine Value ↑ → Unsaturation ↑ → Oxidative rancidity ↑
بمعنى:
كلما زادت الروابط المزدوجة C=C في الدهون → زادت قدرتها على امتصاص اليود → زادت Iodine Value → وصارت أكثر قابلية للتأكسد والتزنخ.
أهم جملة للحل:
Iodine value = grams of iodine absorbed by 100 g of substance.

إدرا ذات القيمة	الفكرة الأساسية	شو يستخدم؟	شو يقيس؟	القيمة
↑ Free fatty acids	KOH تحتاج لمعادلة الـ FFA في 1 g oil	KOH	كمية Free fatty acids	Acid Value (AV)
↑ Oxidation / rancidity	الـ peroxide بحرقه، ويعدن تقاس الـ KOH بالـ thiosulfate	KI ثم $\text{Na}_2\text{S}_2\text{O}_3$	كمية Peroxide / oxygen / peroxides	Peroxide Value (PV)
↑ Unsaturation	اليود يتفاعل مع الروابط المزدوجة	Iodine	Degree of unsaturation	Iodine Value (IV)
↑ عدد الـ triglycerides الأصغر / ↓ average MW	تفسير triglycerides إلى glycerol + fatty acid salts	KOH	مقدار KOH اللازم لتسبيل 1 g fat/oil	Saponification Value (SV)

Practical work:

✓ ***Fixed oil examples:***

Arachis oil, Caster oil, Olive oil, Corn oil, Palm oil ...etc.

✓ ***Determination of AV***

1. Dissolve about 10.0 g of oil, accurately weighed, in 50 mL of a mixture of equal volumes of alcohol and ether (which has been neutralized to phenolphthalein with 0.1 M potassium hydroxide or 0.1 M sodium hydroxide) contained in a flask.
2. Titrate with 0.1 M potassium hydroxide using 0.5 mL of phenolphthalein until the solution remains faintly pink after shaking for 30 s.

✓ ***Determination of PV***

1. Place about 5 g of oil, accurately weighed, in a 250-mL conical flask fitted with a ground- glass stopper.
2. Add 30 mL of a mixture of glacial acetic acid and chloroform (3:2) to the flask and shake to dissolve and add 0.5 mL of saturated potassium iodide solution.
3. Shake for exactly 1 min.
4. Add 30 mL of water. Titrate with 0.01 M sodium thiosulfate, adding the titrant slowly with continuous shaking, until the yellow color is almost discharged.
5. Add 5 mL of starch, and continue the titration, shaking vigorously, until the blue color is discharged.