PHARMACOGNOSY PRACTICE II

IODINE VALUE
PEROXIDE VALUE
ACID VALUE
SAPONIFICATION VALUE

- Among the drugs containing lipids, the most important one for pharmacognosy are fixed oils.
- The pharmaceutical value of a particular oil or fat depends upon its physical (refractive index, optical rotation, viscosity etc.) and chemical (iodine, acid, saponification value etc.) characteristics.
- The evaluation methods of this characteristics are registered in pharmacopoeias.
- In this experiment we will determine some of these parameters:

IODINE VALUE:

The iodine value of a substance is the weight of iodine absorbed by 100 parts by weight of substance.

- Iodine value indicates the degree of unsaturation of the oil. This value provides an estimation of purity and quality of the oil. If the oil is really pure, it helps to recognize the oil.

- Increased iodine values means increased unsaturation.

- The iodine index is assigned to the drugs containing the "long chain fatty acid".

Procedure:

Transfer an accurately weighed 0.2 ml of sunflower oil, into a flask **(A g)**

+ CCl_4 (15 ml) → + ICl (5 ml)
(Iodine monochloride)

insert the stopper soaked with KI in the vessel.

Allow to stand for 30 min protected from light

↓

+ KI (5 ml) + Water (100 ml)

↓

+ starch TS (1 ml) ... **indicator**

↓

Titration with 0,1 N $Na_2S_2O_3$ until the blue color discharged

• Perform a blank test at the same time with the same quantities of the same reagents and in the same manner.

Blank Test:

CCl_4 (15 ml) → + ICl (5 ml)
(Iodine monochloride)

insert the stopper soaked with KI in the vessel.

Allow to stand for 30 min protected from light

↓

+ KI (5 ml) + Water (100 ml)

↓

+ starch TS (1 ml) ... **indicator**

↓

Titration with 0,1 N $Na_2S_2O_3$ until the blue color discharged

Weight of 0.2 ml sunflower oil = A g
Volume of 0.1 N $Na_2S_2O_3$ consumed by the actual test = a ml
Volume of 0.1 N $Na_2S_2O_3$ consumed by the blank test = b ml

$$I.V. = \frac{(b-a) \times 0,01269 \times 100}{A\ (g)}$$

Principle:

$$R_1\text{-}\overset{H}{\overset{|}{C}}=\overset{H}{\overset{|}{C}}\text{-}R_2\text{-}COOH + ICl \longrightarrow R_1\text{-}\underset{I}{\underset{|}{\overset{H}{\overset{|}{C}}}}-\underset{Cl}{\underset{|}{\overset{H}{\overset{|}{C}}}}\text{-}R_2\text{-}COOH + ICl$$

(Unsaturated fatty acid)

the remaining unreacted ICl

$$ICl + KI \longrightarrow KCl + I_2^o$$

(it forms ICl to molecular iodine)

(the liberated I_2^o is titrated with $Na_2S_2O_3$)
(the liberated iodine is susceptible to oxidation by light.)

$$I_2^o + 2\ S_2O_3^{=} \longrightarrow$$

Starch + Water $\longrightarrow$ Amylose + Amylopectin

(Starch is used as the indicator for this reaction so that the liberated iodine will react with amylose to give **blue-purple colored product**)

1 N	1000 ml $Na_2S_2O_3$	Equivalent Weight of Iodine=126.91 g
0.1 N	1 ml $Na_2S_2O_3$	Equivalent Weight of Iodine=0.01269 g

0.1 N	1 ml $Na_2S_2O_3$	Equivalent Weight of Iodine=0.01269 g
0.1 N	(b-a) ml $Na_2S_2O_3$	Equivalent Weight of Iodine= (b-a) x 0.01269 g

X= (b-a) x 0.01269 g iodine

For A g oil	(b-a) x 0.01269 g iodine
For 100 g oil	Y

$$Y = \frac{(b-a) \times 0.01269 \times 100}{A}$$

<u>Peroxide value:</u>

The number that expresses, in milliequivalents (meq) of oxygen, the quantity of peroxide contained in 1000 g of the oil.

- The bitter taste of an oil is significantly related with the oil stability expressed by the peroxide value. Oils are degraded during storage or extraction due to the following reasons:

- Oxygen,
- Temperature,
- Moisture,
- The surface of the oil in contact with the air,
- Light,
- Absence or presence of antioxidants

- The peroxide value is a suitable parameter for measuring the deterioration of quality over time.

Procedure:

Place about 5 g of the rancid oil, accurately weighed, in a flask **(A g)**

+ Glacial acetic acid: Chloroform (3:1) (30 ml) →

+ saturated KI solution (0.5 ml) →

Shake for exactly 1 minute

+

water (30 ml)

+

starch TS (1ml) **indicator**

↓

Titrate with 0.1 N $Na_2S_2O_3$ until the blue color is discharged

Blank Test:

Glacial acetic acid: Chloroform (3:1) (30 ml) → + saturated KI solution (0.5 ml) → Shake for exactly 1 minute + water (30 ml) + starch TS (1ml) **indicator**

↓

Titrate with 0.1 N $Na_2S_2O_3$ until the blue color is discharced

Weight of ~5 g rancid oil = A g
Volume of 0,1 N $Na_2S_2O_3$ consumed by the actual test = a ml
Volume of 0,1 N $Na_2S_2O_3$ consumed by the blank test = b ml

$$P.V. = \frac{(a-b) \times N \times 1000}{A\ (g)}$$

N = exact normality of the $Na_2S_2O_3$ solution (0.1 N)

Principle:

The peroxide value is determined by measuring the amount of iodine which is formed by the reaction of peroxides (formed in rancid fat or oil) with iodide ion in acidic solutions. The iodine liberated is titrated with $Na_2S_2O_3$

$$O_2 + 2\,I + 2\,H \longrightarrow I_2^o + 2\,OH^-$$

$$I_2^o + 2\,S_2O_3^= \longrightarrow 2\,I^- + S_4O_6^=$$

1 N	1L	$Na_2S_2O_3.5.H_2O$	124 g
0.1 N	1L	$Na_2S_2O_3.5.H_2O$	12.4 g

Weigh 12.4 g of $Na_2S_2O_3.5.H_2O$ and make up to the 1L mark with distilled water.

Acid Value:

Acid value is the number of mg KOH required to neutralize the free acids in 1 g of the oil.

This test is used for measuring the free fatty acids especially in fats, fixed oils, waxes, resins and similar substances.

High content of free fatty acids in oils affect the quality of them an they are not in compliance with the Pharmacopoeia.

Procedure:

Weigh 10 ml sunflower oil in a flask **(A g)** + ethanol R : ether (1:1) (50 ml) + **Phenolphthalein TS (5 ml)** → **Titrate with 0.1 N (micro burette should be used for analysis) until the solution remains faintly pink**

PHENOLPHTHALEIN is an indicator of acids (COLORLESS) and **bases (PINK).**

Weigh 1 g of phenolphthalein and make up to the 100 mL mark with ethanol.

Weight of 10 ml sunflower oil = A g
Volume of 0,1 N KOH consumed by the test = a ml

$$\boxed{\textbf{A.V.} = \frac{\textbf{a x 0.00561 x 1000}}{\textbf{A (g)}}}$$

1 N	1000 ml KOH	56.1 g
0.1 N	1 ml KOH	0.00561 g
0.1 N	a ml KOH	X g

X= a x 0.00561 x 1000 mg

For A g oil	a x 0.00561 x 1000 mg KOH
For 1 g oil	Y mg KOH

$$Y = \frac{a \times 0.00561 \times 1000}{A\ g}$$

Saponification Value:

Saponification value is the number of mg of KOH required to neutralize the free acids and saponify the esters contained in 1 g of the oil.

-This test is especially applied to fats, fixed oils, waxes, resins and balsams.

-It is a measure of the average molecular weight (or chain length) of all the fatty acids present. S.V. is an important parameter usually considered in the determination of oil quality and purity.

-If the saponification value is not in compliance with the Pharmacopoeia it means the oil is not pure.

- **What is saponification?** Saponification is the hydrolysis of fats or oils under basic conditions to afford glycerol and the salt of the corresponding fatty acid. Saponification literally means "soap making".

Procedure:

Weigh 2 ml of sunflower oil in a flask **(A g)** + Alcoholic KOH (25 ml)

for glycerin formation

Place the flask under reflux condenser for 30 minutes

(saponification)

+ phenolphtalein

indicator

Titrate with 0.5 N HCl until the endpoint point of titration observed as the disappearance of pink color to white color.

- Perform a blank test at the same time with the same quantities of the same reagents and in the same manner.

Blank Test:

Alcoholic KOH (25 ml) → + phenolphtalein

indicator

Titrate with 0.5 N HCl until the endpoint point of titration observed as the disappearance of pink color to white color.

The blank test allows the amount of reactive substance within the plain solvent to be determined and hence allows a determination of the error in future titration experiments using this solvent.

Weight of 10 ml sunflower oil = A g
Volume of 0.5 N HCl consumed by the actual test = a ml
Volume of 0.5 N HCl consumed by the blank test = b ml

$$S.V. = \frac{(b-a) \times 0.02805 \times 1000}{A\ (g)}$$

Principle:

For glyceride:

$$\begin{array}{l} CH_2 - O - COR_1 \\ | \\ CH - O - COR_2 \\ | \\ CH_2 - O - COR_3 \end{array} + 3\ KOH \longrightarrow \begin{array}{l} CH_2 - OH \\ | \\ CH - OH \\ | \\ CH_2 - OH \end{array} + \begin{array}{l} R_1\text{-}COOK \\ R_2\text{-}COOK \\ R_3\text{-}COOK \end{array}$$

(Fatty acid ester) (Glycerol) (soap)

For fatty acid:

$$R\text{-}COOH + KOH \longrightarrow R\text{-}COOK + H_2O$$

(ester and fatty acids)
(the excess 0.5 N KOH)
(0.5 N HCl)

1 N	1000 ml KOH	56.1 g
0.5 N	1 ml KOH	0.02805 g
0.5 N	(b-a) ml KOH	X g

$$X = (b-a) \times 0.02805 \times 1000 \text{ mg}$$

For saponification of A g oil	(b-a) x 0.02805 x 1000 mg KOH	(meq KOH = meq HCl)
For saponification of 1 g oil	Y mg KOH	

$$Y = \frac{(b-a) \times 0.02805 \times 1000 \text{ mg KOH}}{A \text{ g}}$$