

# The Determination of the Iodine Number of Lipids

## Introduction

The simplest lipids are the fatty acids. These are long chain hydrocarbons with carboxyl groups (COOH groups). We are interested in two groups of fatty acids: saturated and unsaturated fatty acids.

Saturated fatty acids are so called because each carbon atom in the chain holds all the possible hydrogen atoms it can. These lipids tend to be solids at room temperature. These are also the sorts of lipids found around organs in the human body, acting as cushions. The only bonds present between the carbon atoms are single bonds.

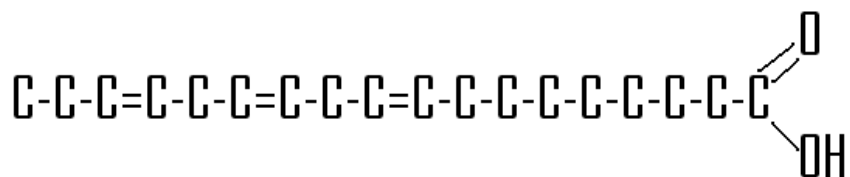
Unsaturated fatty acids do not have all the hydrogen atoms they can hold, for there are occasional carbon-carbon double bonds in addition to the single bonds between carbon atoms. These fatty acids tend to be liquids at room temperature and are the primary type of lipid found in skin deposits. Naturally occurring unsaturated fatty acids contain double bonds that are in the "cis" form and artificial unsaturated fatty acids contain double bonds that are in the "trans" form. The trans-fatty acids are found in oleo and margarine and have a high link with heart disease.

Below is a graphic of the two types of double bonds. The thicker black lines represent the rest of the fatty acyl molecule. The left graphic shows the hydrogen atoms on opposite planes across from each other in the double bond. This is the "trans" form. The right graphic shows the hydrogen atoms on the same side of the plane of the double bond. This is the "cis" form.



The "trans" form tends to be artificial, whereas the "cis" forms tend to be more "natural", i.e., they occur at greater frequency in nature. While there are more complex lipids, as we've discussed in lecture, they play no role in this experiment, so will be relinquished to the ethers for this discussion.

Before examining the unsaturated fatty acids there are three items we need to review. To do that we need a reference unsaturated fatty acid ( $\alpha$ -linolenic acid):



Note in the graphic that  $\alpha$ -linolenic acid contains three (3) double bonds. That renders this fatty acid a poly-unsaturated fatty acid (PUFA).

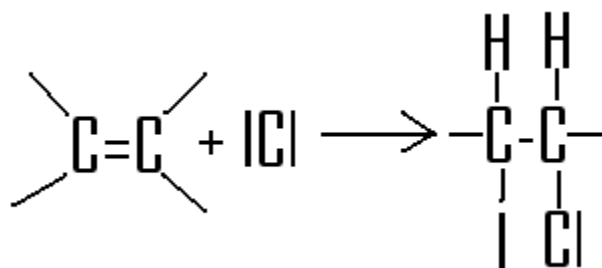
We can identify the characteristics of this fatty acid in one of the following three ways:

- 1) the "n-" nomenclature,
- 2) the " $\omega$ " (omega) system of nomenclature and
- 3) the "delta" system of nomenclature.

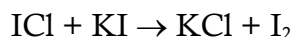
The "n-" system of nomenclature comes from going to the  $\text{CH}_3$  end of the molecule (the left end in this instance) and counting in from the end (end - [e + d] = n) to the carbon with the first double bond. That carbon is carbon # 3 in this example. We can refer to this fatty acid as an "n-3" fatty acid. The omega (" $\omega$ ") system is the same system, just another name, so we can refer to this fatty acid as an  $\omega 3$  fatty acid, as well. The " $\omega$ " comes from the Greek alphabet, where  $\alpha$  is the beginning (in this case, C #2) and  $\omega$  is the end – carbon # 18 by IUPAC, in this example.

The delta ( $\Delta$ ) system of nomenclature of unsaturated fatty acids is equally as simple: the first number is the number of carbon atoms in the hydrocarbon chain. The number after the colon is the number of double bonds in the whole molecule between carbon atoms. The numbers superscripted with the delta ( $\Delta$ ) sign tell you the location of the double bonds, e.g.,  $\Delta 9, 12, 15$ : the double bonds are between carbons 9 and 10, 12 and 13, and 15 and 16, where we only identify the carbons by the lowest number in the double bonds. Hence, we can use this nomenclature method to identify our example fatty acid in this manner:  $18:3^{\Delta 9,12,15}$ . 18 carbons are in  $\alpha$ -linolenic acid; it contains 3 double (unsaturated bonds) located between the carbons as described, above.

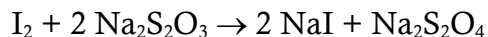
One of the most interesting reactions involving PUFA's is that of the "Hanus reaction". In this reaction, iodine monochloride (or the monobromide) reacts with the unsaturated bonds to produce a di-halogenated single bond, of which one carbon has bound an atom of iodine:



After the reaction is complete, the amount of iodine that has reacted is determined by adding a solution of potassium iodide to the reaction product. This causes the remaining unreacted ICl to form molecular iodine:



The I<sub>2</sub> is then titrated with a standard solution of sodium thiosulfate as performed in the redox titration in CHEM 121:



Using starch as the indicator, again, and watching for the identical endpoint.

Concurrently, one runs a blank alongside the sample, i.e., a sample that contains everything in identical amounts, volume and concentration EXCEPT the fatty acid, so that the iodine number can be calculated.

The iodine number measures the degree of unsaturation of fatty acids – this reaction does NOT occur with saturated fatty acids, at all. The higher the iodine number is, the more unsaturated the fat. As a general rule, if the iodine number is in the range of 0-70, then you're looking at a "fat"; if the iodine number is greater than 70, you're looking at an oil.

To calculate the iodine number using the method described, below, all one need do is obtain the volume of Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> used in the titration of both the sample and the blank and plug them into the following formula:

$$Iodine\ Number = \frac{(mL\ 0.1\ N\ Na_2S_2O_3\ Blank - mL\ 0.1\ N\ Na_2S_2O_3\ Test) (12.7) (100)}{(1000) (0.2)}$$

Where "mL 0.1 N Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> Blank" = the volume of sodium thiosulfate required to titrate the blank solution,

Where "mL 0.1 N Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> Test" = the volume of sodium thiosulfate required to titrate the test solution that contained either solid fat or oil as the sample,

Where "12.7" is the amount of grams of iodine contained in one liter of 0.1 N iodine,

Where "0.2" is the amount of fat taken in grams (for your calculations, use your values in its place), and,

Where "100" converts the sample to per 100 grams of fat.

### Materials

The lipids to be used for this experiment are as follows: linoleic acid,  $\gamma$ -linolenic acid, olive oil, canola oil, sunflower seed oil.

The solvents to be used in this experiment are as follows: Chloroform, Ethanol.

The remainder of the materials include 0.1 N Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>, 10% KI, 0.2 N ICl, disposable pipets, 1% starch indicator, and buret and clamp with ring stand.

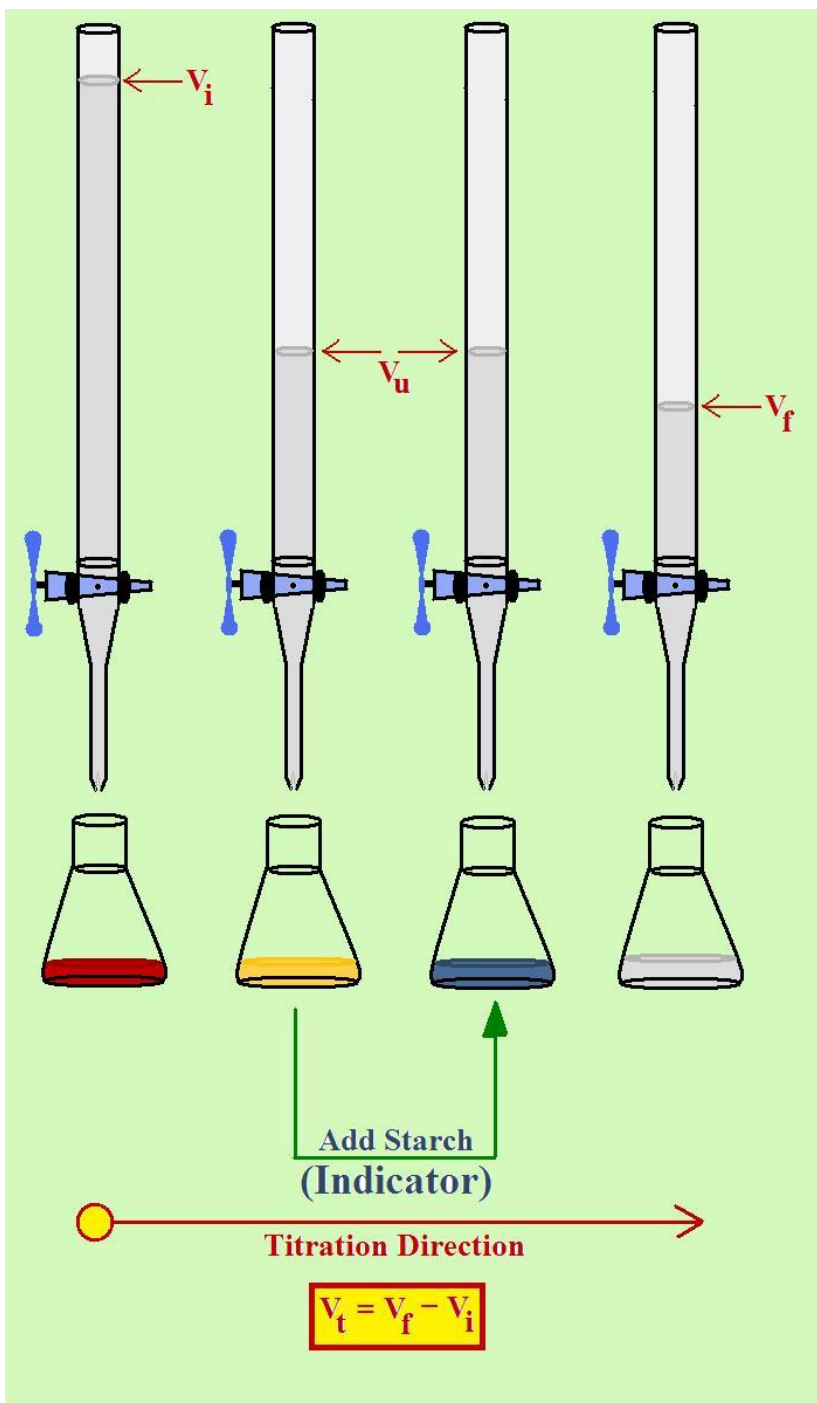
## Method

Record all data in the table, below. Each sample (except the blank, of course) requires 0.2 g of each fat (or as close as possible to 0.2 g) per flask. Each student will utilize the fat assigned and two different solvents: one blank of each trial and one test sample of each trial.

Dissolve the lipid in 10 mL of the solvent as best as possible (remember no lipid in the blank sample). Once the fat sample is as dissolved as it gets, add 25 mL of the Hanus reagent (0.2 N ICl). Seal the flasks, shake well and place in a darkened area for 30 minutes.

During this 30 minute time period, set up your buret and ring stand and buret clamp so that it has 0.1N sodium thiosulfate in it and is ready to go when your samples have completed reacting. Record the volume of thiosulfate in your buret in the data table, below.

After the 30 minute reaction period has passed, add 10 mL of 10% KI with mixing to the resulting solution/mixture/colloid. Immediately begin titrating the samples with the thiosulfate. Titrate the samples to a yellow straw color as you did in the redox titration in CHEM 121, then add 5 mL of the 1% starch solution to your solution. It will turn a dark purple-black in color (See graphic, above) – if it turns clear when adding the starch solution, you titrated too far – better to add the starch sooner than later.



Now titrate the solution a drop at a time from your buret until the dark solution turns transparent/clear, i.e., the dark purple-black is gone and it looks like clear water. THAT'S the titration end-point. Record the volume of thiosulfate to get to this point in the data table, below.

Repeat the titration process with your other three samples and record your data in the data table, below.

### Data Recording

	Trial 1		Trial 2	
Lipid				
Solvent				
	Blank	Test Sample	Blank	Test Sample
Mass of Flask and Lipid (g)				
Mass of Flask (g)				
Mass of Lipid (g)				
Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> Normality				
Final Volume Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> (mL)				
Initial Volume Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> (mL)				
Volume used Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> (mL)				
Iodine Number (Calculated)				
Iodine Number (Theoretical)				
% Error				

## Results

Based on your results, what do you conclude about your lipid?

Based upon your results, what do you conclude about the solvents you used in this experiment?

## References

1. Atkins, R.C. and Carey, F.A.: Organic Chemistry: A Brief Course. (McGraw-Hill: New York). Pp. 455-456. © 1997.
2. Blicq, D.: [http://xnet.rrc.mb.ca/davidb/new\\_page\\_18.htm](http://xnet.rrc.mb.ca/davidb/new_page_18.htm), 25 October 2007.
3. Carman, F.S., III: <http://www.wnc.edu/%7Ecarman/km220lex/week6.pdf>, 25 October 2007.
4. Hanus Method, AOAC 920.158 (ISO 3961:1996 Animal and vegetable fats and oils - - Determination of iodine value).
5. \_\_\_\_\_: [http://www.pianetachimica.it/olimpiadi/26icho/26\\_practical\\_IChO.pdf](http://www.pianetachimica.it/olimpiadi/26icho/26_practical_IChO.pdf), 25 October 2007.
6. \_\_\_\_\_: [http://www.personal.psu.edu/faculty/w/x/wxm15/Online/Biochem\\_unit/adv\\_lipid\\_notes.htm](http://www.personal.psu.edu/faculty/w/x/wxm15/Online/Biochem_unit/adv_lipid_notes.htm), 25 October 2007.
7. \_\_\_\_\_: <http://www.vetsci.psu.edu/coursedesc/vsc497a/09lipids.htm>, 25 October 2007.

8. Kleiner, I.S. and Dotti, L.B.: Laboratory Instructions in Biochemistry. (The C.V. Mosby Co: Saint Louis). Pp. 37-38. ©1962.
9. Matsunaga, N.: <http://phoenix.liu.edu/~nmatsuna/che4x/e8lipids.pdf>, 25 October 2007.
10. Nicholson, L.M., Professor of Chemistry, Fort Hays State University, Hays, KS: personal communication, 14 March 2008.
11. Plummer, D.T.: An Introduction to Practical Biochemistry. (McGraw-Hill: London). Pp. 186-187. ©1971.
12. Pritham, G.H.: Anderson's Laboratory Experiments in Biochemistry. (The C.V. Mosby Company: Saint Louis). Pp. 59-60. ©1968.
13. Vedde, J.: <http://folk.uio.no/johnv/26thIChO/problems/p1.html>, 25 October 2007.
14. Wiese, T.J.: <http://www.fhsu.edu/chemistry/twiese/360/lipids.htm>, 25 October 2007.

This page generated 14 March 2008, 1527 hours PDT.  
Updated 18 January 2010, 0824 hours, PST.