Determination of the Iodine Value of Selected Oils: An Experiment Combining FTIR Spectroscopy with Iodometric Titrations

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Received July 14, 2000. Accepted October 17, 2000

Abstract: An experiment has been developed that combines the FTIR analysis of oils and fats with an iodometric titration. While it is most appropriate for a sophomore or junior laboratory for majors, it also makes a suitable group project or demonstration for nonscience majors. Integration of the experiment into an upper-level biochemistry laboratory is described.

In recent years people have become increasingly aware of the effects of diet on health. It is common knowledge that the average person's diet contains too high a percentage of fats, especially saturated fats. This increases the risk factors for cardiovascular disease. Lowering the amount of dietary cholesterol and saturated fats has become a goal for more and more people, including a significant number of college students. In science courses, students show substantial interest in experiments involving the isolation and analysis of fats. It is appropriate to introduce into the undergraduate laboratory curriculum analytical methods that can distinguish saturated from unsaturated fats.

Background

Fats and oils are more correctly called triglycerides [1]. Triglycerides contain three fatty acids esterified with glycerol. A representative structure is shown in Figure 1, which shows one stearoyl and two palmitoyl groups esterified with glycerol to form a saturated triglyceride. In contrast, unsaturated triglycerides contain a larger proportion of unsaturated fatty acids. The second structure in Figure 1 shows two molecules of oleic acid and one molecule of linoleic acid esterified with glycerol.

The degree of unsaturation of a triglyceride is expressed as its iodine value (IV) [2], which is defined as the number of grams of iodine absorbed by one hundred grams of fat. This definition exploits the ability of carbon–carbon double bonds to undergo addition of halogens. Consider the example triglyceride structures in Figure 1. The first structure has no double bonds, so its iodine value is zero. The second structure can add four moles of iodine per mole (882 g) of triglyceride. Calculation of its iodine value is a stoichiometric exercise that any freshman can do.

$$\text{iodine value} = \frac{4 \times 254 \text{ g } I_2 \times 100 \text{ g triglyceride}}{882 \text{ g triglyceride}} = 115$$

Saturated fats and some tropical oils typically have iodine values from 35 to 70, vegetable oils range from 80 to 150, and more highly unsaturated oils have values from 160 to 195 [3]. Consider a sample of vegetable oil with an iodine value of 135. If the average molecular weight of triglycerides in the oil sample is determined by separate methods to be 825, a similar calculation indicates the average number of double bonds present per molecule:

$$\frac{135 \text{ g } I_2 \times 825 \text{ g oil} \times 1 \text{ mol } I_2 \times 1 \text{ mol C=C}}{100 \text{ g oil} \times 1 \text{ "mol" oil} \times 254 \text{ g } I_2 \times 1 \text{ mol } I_2} =$$

4.38 mol C=C per mole of oil or 4.38 C=C per molecule

Because elemental iodine is not sufficiently reactive with carbon–carbon double bonds, determination of iodine value is typically done via the method developed by Wijs [4]. A weighed sample of oil is dissolved in an organic solvent and a measured amount of ICl is added. The solution is allowed to stand in the dark for 30 min. The excess ICl is converted to I_2 by addition of KI. The concentration of I_2 is determined by titration with standardized sodium thiosulfate. Synthesis of Wijs reagent involves handling gaseous Cl_2 [5], and many chemists will wish to avoid this. An alternative reagent, developed by Hanus, utilizes a solution of equimolar I_2 and Br_2 in glacial acetic acid [6]. The Hanus reagent gives iodine values that are comparable to the Wijs reagent, but the Hanus reagent is easier to prepare and more stable during storage. Strong and Koch have developed an undergraduate laboratory based on the Hanus method [7].

Instrumental Methods

Instrumental methods for the determination of iodine values are well documented. Arnold used transmission IR to analyze 25 different fats and oils with iodine values ranging from 31 to 142 [8]. A transmission spectrum of a 10% solution of oil in CCl_4 was run, and the olefinic absorbance was divided by the aliphatic absorbance to produce a ratio. This ratio was found to be proportional to the iodine value. More recently, van der Voort's group used FTIR to determine both iodine values and saponification numbers (SN) [9]. This method used attenuated total reflectance (ATR) sampling, and a computer program was written to integrate sample handling, spectrum acquisition, and numerical analysis of results.

Although ATR is the more modern technique, we choose transmission IR for this undergraduate experiment. ATR

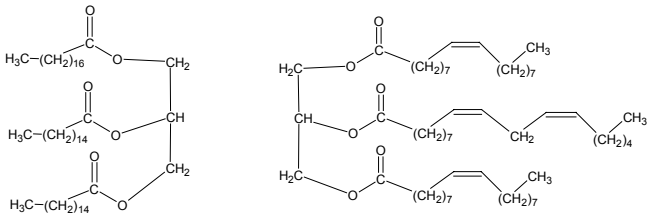

Figure 1. A saturated fat (left) and an unsaturated oil.

Table 1. Typical Iodine Values for Selected Vegetable Oils

Oil Analyzed	Iodine values determined by	
	IR	Hanus method
Almond	117 ± 5	97 ± 6
Corn	150 ± 18	112 ± 6
Olive	86 ± 19	93 ± 10

analysis required 128 scans versus only 4 scans for transmission spectra; fewer samples could be analyzed by ATR in a single laboratory period. Also, switching between transmission and ATR accessories involves significant effort, and optical alignment changes in the sample compartment can make it difficult to obtain reproducible spectra. Still, the effort (and the expense of the ATR accessory) should be justified if both IV and SN values are to be determined in a single period.

Results

We have combined the estimation of iodine values via FTIR with the classical iodometric determination described in reference 7. The experiment provides a straightforward introduction to sampling and transmission IR spectroscopy. It connects general, analytical, and organic chemistry via identification of simple (alkene and ester) functional groups and stoichiometric calculations. When commercial samples of vegetable oils and fats were provided to the students, a single period was sufficient for both the Hanus method and FTIR analysis as described in the experimental procedure.

Iodine values obtained by the Hanus method were compared to values predicted from the FTIR analyses. The agreement between Hanus iodine values and IR estimates was good, with the more competent students reporting relative errors within 10%. These results compare favorably with van der Voort's results [9].

In a worst-case scenario (Table 1), we ran the experiment during a sophomore organic laboratory where students had not yet had qualitative analysis. Magnetic stirring was not used. Tolerable results were obtained; the larger differences between IR and Hanus iodine values were traceable to two artifacts. First, titrant is added to a two-phase solution and good mixing is essential (magnetic stirring is recommended); otherwise, iodine remaining in the $CHCl_3$ layer is not immediately neutralized by thiosulfate. Second, if the oils have a substantial color, students will overshoot the endpoint when they mistake the color for excess iodine.

Estimating iodine values from IR absorbances was straightforward. A calibration curve was prepared by the instructor using methyl stearate (IV = 0), methyl oleate (IV = 83), methyl linoleate (IV = 173), and mixtures of 50% methyl stearate/methyl oleate (IV =41) and 50% methyl oleate/methyl linoleate (IV = 129) for the standards. Linear regression yielded the formula below for iodine value; the formula from Arnold's group is provided for comparison [8]

$$\text{IV} = 740\ (A_{\text{olefin}}/A_{\text{alkane}}) - 2$$

$$R = 0.91$$

$$\text{IV} = 495\ (A_{\text{olefin}}/A_{\text{alkane}}) - 31$$

$$R = 0.98 \text{ (ref 8)}$$

where A_{olefin} is the absorbance of the olefinic (alkene) C–H stretch above 3000 cm^{-1} and A_{alkane} is the absorbance of the saturated (alkane) C–H stretch near 2950 cm^{-1}.

Experimental Procedure

Hanus solution is a corrosive mixture of iodine and bromine in concentrated acetic acid. It should be kept in the hood and dispensed from an automatic-delivery pipet if possible. Spills should be neutralized immediately with 10% sodium bisulfite solution. Carbon tetrachloride is a suspected carcinogen and should be handled only in small amounts under the hood. Analytical reagents used were purchased from Fisher Scientific or Aldrich Chemical and were of reagent grade purity. Methyl ester standards were purchased from Sigma Chemical, refrigerated during storage, and used without further purification. Vegetable oils were obtained from local retail outlets or existing chemical stock. FTIR analysis was performed on a Nicolet Magna 550 optical bench; spectra were acquired, annotated, and analyzed using OMNIC version 3.0 software.

For FTIR analysis, about 50 mg (recorded to 0.001 g) of oil was weighed into a clean, dry vial. Sufficient CCl_4 was added to bring the mass of the solution to 10 to 20 times the mass of the sample [10]. The vial was capped and the solution swirled until the oil dissolved and the solution was drawn into a 1-mL Luer-tip glass syringe. The syringe was used to fill a Perkin-Elmer demountable IR cell with a 0.5-mm spacer between two 25 mm × 4-mm rectangular NaCl windows. A transmission spectrum (4 scans, 4000 to 400 cm^{-1}) was obtained and stored on disk. The maximum absorbance of any olefinic C–H absorption bands (between 3000 and 3050 cm^{-1}) was recorded along with the aliphatic C–H band (between 2900 and 2075 cm^{-1}).

Hanus iodine solution was prepared by dissolving 26.4 g of I_2 in 1200 mL of glacial acetic acid with warming, then cooling, adding 17.1 g Br_2 and diluting to 2.000 L in a volumetric flask [7]. Sodium thiosulfate (25.00 g) was dissolved in 1.000 L of water and standardized [11] against KIO_3. A 25.00-mL aliquot of Hanus solution was treated with 20 mL of 3 M HCl and 10 g of solid KI to convert unreacted IBr to I_2. The iodine content of the Hanus solution was determined by titration with thiosulfate using 2% starch indicator.

For best results, classical analysis of the oils were done in duplicate. Approximately 250 to 300 mg of oil was weighed into a clean, dry Erlenmeyer flask. The mass was recorded to the nearest milligram. The oil was dissolved in 15 to 20 mL of $CHCl_3$ and 20.00 to 25.00 mL of Hanus solution was added via volumetric pipette. The flask was stoppered and allowed to stand in the dark for 45 min. After reaction, each oil sample was treated with 20 mL of 3 M HCl and 10 g of solid KI and titrated with thiosulfate. A magnetic stirrer was used to mix the solutions during the titration; the optimal speed was as high as possible without causing splashing. Rapid addition of titrant is possible early in the titration, as the color of excess iodine fades from brown to orange to yellow. Addition of 1 mL of 2% starch indicator just before the endpoint in reached ensures a sharp endpoint. The relationship below was used to calculate the iodine value from titration of the oil (equivalent to the equation in reference 6):

IV = $[V_f - V_i]$ (for the Hanus solution) – $[V_f - V_i]$ (for the Hanus solution plus lipid) × M × 126.9 m^{-1}, where M is the molarity of

thiosulfate titrant, [$V_f - V_i$] is the final burette reading minus the initial burette reading for the iodometric titration, *m* is the mass in milligrams of the oil analyzed, and IV is the iodine value of the fat or oil.

Conclusion

This experiment is quite adaptable to several undergraduate chemistry courses. It would work quite well in a combined quantitative and instrumental analysis laboratory. Students could make and standardize their own thiosulfate solutions, perform the iodometric titrations on the oils and finish the FTIR analysis in four- five hours.

When we originally developed this experiment for our biochemistry laboratory, we ran a three-week sequence of experiments. During the first week, students isolated lipids from a variety of nuts and grains, then characterized the oils via TLC [12]. During the second week, students determined SN and IV via FTIR; they also saponified their triglycerides and converted them to fatty-acid methyl esters (FAMEs). A strong finish to the sequence of experiments resulted during the third week when students analyzed the FAMEs via GCMS as described by Rubinson [13].

The FTIR estimation of iodine value (as a group experiment or a demonstration) is an excellent addition either to a basic chemistry laboratory for students majoring outside the sciences or to a sophomore organic laboratory. We have modified and expanded an experiment for nonscience majors from the text by Richardson [14]. In one laboratory period, a sample of meat is dehydrated and defatted with organic solvent. The second period begins with the FTIR analysis of the residual fat, and the exercise concludes with an analysis of the students' results and a discussion of the differences between saturated and unsaturated fats. The experiment generated a substantial amount of enthusiasm among nonscience majors. On several occasions students have decided to change their eating habits as a result of their observations in this laboratory!

Acknowledgment. Appreciation is expressed to the National Science Foundation (NSF-ILI grant # 96-50908), to Elon College for the matching funds, and to the reviewers for their comments.

References and Notes

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