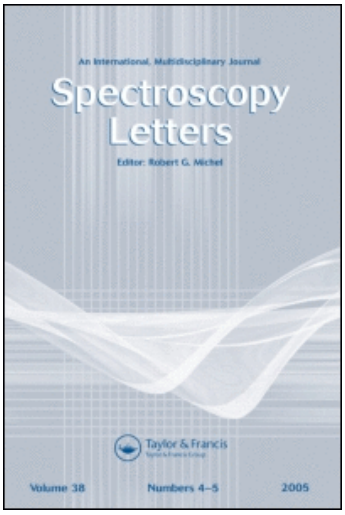

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A New FTIR Method for the Analysis of Low Levels of FFA in Refined Edible Oils

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Abstract: This paper summarizes the application of stoichiometric analytical approaches to quantitative IR analysis and describes the development of a rapid and sensitive Fourier transform infrared (FTIR) method using such an approach for the determination of low levels (<0.005%) of free fatty acids (FFA) in refined edible oils. The method simply involves mixing the sample with methanol containing 2 g/L sodium carbodiimide (NaHNCN) on a vortex mixer for 30 s to convert the FFA to their salts, centrifuging the sample to separate the methanol phase containing the FFA salts from the oil, recording the FTIR spectrum of the upper methanol layer in a 100-μm CaF_2 transmission flow cell, and ratioing this spectrum against that of the NaHNCN/methanol solution. The concentration of FFA salts is determined from the resulting differential spectrum by measurement of the $\nu(COO^-)$ absorbance at 1573 cm^{-1} relative to a reference wavelength of 1820 cm^{-1}. A calibration spanning the range 0–0.1% FFA (expressed as oleic acid) was devised by gravimetric addition of a defined, pure fatty acid to an acid-free oil. Validation of the method by standard addition of palmitic acid to a variety of oils yielded an overall standard error of $< \pm 0.001\%$ FFA. Comparison of triplicate FTIR and IUPAC titrimetric analyses of oils spiked with palmitic acid demonstrated that this FTIR method was more sensitive, accurate, and reproducible than the titration procedure, the latter having a significant positive bias of ~0.02%. Solvent/oil consumption in the FTIR method is 2 mL/10 g versus 150 mL/20 g for the titrimetric procedure. The FTIR method developed is particularly well suited for the determination of the low levels

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of FFA in refined oils but can readily be adapted with a simple adjustment of the oil/methanol ratio to cover FFA levels of up to 4.0%.

Keywords: Edible oils, FFA, FTIR, sodium carbodiimide, sodium hydrogen cyanamide

INTRODUCTION

The application of chemometric techniques has represented a major advance in quantitative IR analysis. In particular, partial-least-squares regression (PLS) has been of widespread utility[1] as it provides a powerful means of extracting quantitative spectral information related to component(s) of interest from the spectra of complex samples by mathematically modeling partially overlapping bands or other sources of spectral interference. However, as the complexity of the system increases, so does the difficulty of developing reliable PLS calibration models, and we have encountered diverse circumstances where PLS proved to be unsatisfactory as a calibration technique. Consequently, an alternative approach to quantitative IR analysis was developed based on the use of a reagent that reacts stoichiometrically with the component(s) of interest to produce a readily measurable IR signal. Furthermore, by combining this "signal transduction" approach with differential spectroscopy, quantitation can often be achieved via a simple univariate calibration.

Table 1 illustrates a number of successful applications of this approach. In the first case, difficulties were encountered in developing a robust PLS calibration model for determination of the peroxide value (PV) of edible oils. PV is a measure of hydroperoxides (ROOH), the primary oxidation products in edible oils, but Fourier transform infrared (FTIR) quantitation of these species was complicated by both spectral overlap and hydrogen-bonding interactions with a large variety of secondary oxidation products and other components that may be present in edible oils.[2] These problems were overcome by taking advantage of the rapid stoichiometric reaction of hydroperoxides with triphenylphosphine (TPP) to form triphenylphosphine oxide (TPPO), which has an isolated and intense absorption band that allowed for the accurate determination of PV down to <1.0 mequivalents ROOH/kg oil.[3]

The next three examples in Table 1 concern the analysis of new and used lubricating oils, which is beset by even more complex matrix effects than those encountered in oxidized edible oils. In the case of moisture analysis,[4] signal transduction was carried out by stoichiometrically converting dimethoxypropane through its reaction with water into acetone, a strong IR absorber, providing an alternative to the problematic Karl Fischer titration procedure. For the determination of acid number (AN) and base number (BN) in lubricants, there was the additional complication of the "structural specificity" of IR analysis as opposed to the "chemical specificity"

Table 1. FTIR analyses based on the use of stoichiometric reactions for "signal transduction"

Analysis	Reagent	Stoichiometric reaction	FTIR measurement	Ref.
PV (>1.0 mequiv/kg) edible oils	Triphenylphosphine (TPP) in 1-hexanol	$ROOH + TPP \rightarrow ROH + TPPO$	542 cm^{-1} [X-sensitive ring-breathing vibration of phenyl groups in TPPO]	[3]
H_2O (<1000 ppm) lubricants	1,3-Dimethoxypropane (DMP) in isooctane	$H_2O + DMP \rightarrow CH_3COCH_3 + 2CH_3OH$	1717 cm^{-1} [ν(C=O) absorption of acetone]	[4]
AN (<0.1 mg KOH/g) lubricants	Potassium phthalimide (K^+Ph^-) in 1-propanol	$HA + K^+Ph^- \rightarrow Ph + A^-K^+$	1774 or 1727 cm^{-1} [ν(C=O) absorptions of phthalimide]	[5]
BN (<0.1 mg KOH/g) lubricants	Trifluoroacaetic acid (TFA) in 1-propanol	$B: + TFA \rightarrow TFA^-BH^+$	1679 cm^{-1} [$\nu(COO^-)$ absorption of trifluoroacetate]	[5]
FFA (>0.2% $C_{18:1}$) edible oils	Potassium phthalimide (K^+Ph^-) in 1-propanol	$RCOOH + K^+Ph^- \rightarrow RCOO^-K^+ + Ph$	1570 cm^{-1} [$\nu(COO^-)$ absorption of $RCOO^-$] *or* 1776 cm^{-1} [ν(C=O) absorption of phthalimide]	[6]
FFA (>0.002% $C_{18:1}$) refined edible oils	Sodium hydrogen cyanamide in methanol	$RCOOH + NaHNCN \rightarrow RCOO^- + H_2NCN$	1573 cm^{-1} in spectrum of methanol extract [$\nu(COO^-)$ absorption of $RCOO^-$]	This work

PV, peroxide value; H_2O, moisture; AN, acid number; BN, base number; FFA, free fatty acids; Ph, Phthalimide.

of the traditional titrimetric methods employed for these analyses. The latter methods directly measure a large variety of acids and bases, their response being dependent only on the pK_a of the acid or base in relation to the titrimetric end point. In contrast, prediction of AN or BN by direct IR analysis would require the development of calibrations that model all the species contributing to the acidic/basic characteristics of lubricating oils, many of which are undefined. This severe limitation was overcome by reacting all the acidic or all the basic species with, respectively, a basic or an acidic "signal-transducing" reagent. By subtracting the spectrum of the unreacted sample from that of the reacted sample, the spectral changes associated with the acid–base reaction were isolated, and the extent of conversion of the "signal-transducing" reagent could be directly measured to determine the AN or BN of the sample.[5]

The last two examples cited in Table 1 concern the application of similar concepts to the determination of the free fatty acid (FFA) content of edible oils, the first of which was described in a previous paper.[6] Among the oil quality parameters, FFA content is a crucial factor associated with the quality and economic value of edible oils, especially for unrefined high value oils such as olive oil. FFA content is also an important quality indicator in relation to oil processing and is used to assess deodorizer efficiency or as an indicator of frying oil quality.[7,8] FFA content is most commonly determined by titration of an oil, dissolved in neutralized ethanol or ethanol/diethyl ether, with a strong base to a phenolphthalein end point.[9,10] Although the standardized titrimetric methods are fairly sensitive, with limits of detection (expressed as percent oleic acid) of the order 0.03% being attainable, more sensitive methods would be useful for the analysis of refined, bleached and deodorized (RBD) oils, which tend to have FFA levels of $\leq$0.05%,[7] and could also provide an alternative means of monitoring secondary oxidation products[11] accumulating in an oil in the form of carboxylic acids. In recent years, a variety of approaches have been investigated as possible alternatives to the titrimetric methods employed to determine the FFA content of oils, including the use of flow injection systems,[12,13] pH metric, potentiometric and colorimetric[14–16] methods, and chromatographic procedures[17–20] as well as FTIR-based spectroscopic techniques.[21–29] Although many of these offer substantial benefits, in terms of speed of analysis, amenability to automation, and/or a reduction in the use of solvents and the attendant environmental problems and disposal costs, none of them provide a substantive gain in sensitivity over that attained with titrimetric methods. This paper describes a simple, robust FTIR method based on the concepts outlined above that is capable of measuring FFA levels as low as 0.005% in refined oils. Thus, the new methodology described in this paper exemplifies the means by which the sensitivity of IR analysis can be substantially enhanced under certain circumstances by the signal transduction/differential spectroscopy approach.

EXPERIMENTAL

Reagents and Standard Methods

Sodium hydrogen cyanamide (NaHNCN, 99+%), palmitic acid (99%), and anhydrous methanol (MeOH) were obtained from Aldrich (St. Louis, MO, USA) and were all of analytical grade. Refined edible oils were purchased locally or obtained from Canamera Foods (Toronto, ON, Canada). The reagent solution employed in the FTIR FFA analysis was prepared by dissolving NaHNCN in anhydrous MeOH (2 g/L). This solution was allowed to stand for ~4 days, or until the $\nu(C{\equiv}N)$ band at 2100 cm^{-1} completely disappeared, before use.

Instrumentation

The FTIR spectrometer used for this study was a Bomem WorkIR (Bomem, Quebec, PQ, Canada) equipped with a DTGS detector and purged with dry air using a Balston dryer (Balston, Lexington, MA, USA). The sample-handling accessory was a valved 100-μm CaF_2 transmission flow cell (Dwight Analytical, Toronto, ON, Canada). Samples were aspirated into the cell under vacuum, and the cell was flushed clean after each sample with 1 mL of methanol. All spectra were collected by co-adding 32 scans at a resolution of 8 cm^{-1} and a gain of 1.0. The spectrometer was controlled by an IBM-compatible Pentium 150-MHz PC running under proprietary Windows-based UMPIRE (Universal Method Platform for InfraRed Evaluation) software (Thermal-Lube, Pointe-Claire, PQ, Canada). This software provides programming capabilities so that repetitive operations can be performed in a specified sequence and designated spectral data collected and processed through a calibration equation, thereby automating the analysis to provide direct output of FFA data.

Preparation of Calibration Standards

A series of 12 standards covering a range of 0–0.1% FFA was prepared by gravimetric addition of palmitic acid to a refined and deodorized soybean oil after it had been run though an activated silica gel column to remove any traces of FFA and other oxygenated compounds. The FFA contents of the standards were expressed in terms of % oleic acid.

Sample Preparation

Ten grams (±0.001 g) of each oil sample or standard was weighed on an analytical balance into a tared 15-mL clinical centrifuge tube. To the tube

containing the oil, 2 mL of the NaHNCN reagent solution was added using a calibrated re-pipette. The tubes were capped, shaken on a vortex mixer for 30 s, and then centrifuged for 5 min at 6000 rpm ($\sim 5000 \times g$) to ensure consistent separation between the oil and methanol phases.

Analytical Protocol

Approximately 1 mL of the MeOH/NaHNCN reagent was loaded into the transmission flow cell and its single-beam spectrum recorded to serve as the background spectrum. A new background spectrum was collected in the same manner after every 20 samples or 1 hr, whichever occurred first. For both samples and calibration standards, 1 mL of the upper methanol layer formed after centrifugation was loaded into the cell, and its single-beam spectrum was recorded and ratioed against the MeOH/NaHNCN background spectrum. The peak height of the carboxylate band at 1573 cm^{-1} was then measured relative to an invariant baseline point at 1820 cm^{-1}. The overall sample preparation procedure and analytical protocol is illustrated in Fig. 1.

Calibration and Validation

The calibration standards were taken through the analytical protocol described above, and a calibration equation for the prediction of FFA content was derived by plotting the concentrations of the standards (% oleic acid) versus carboxylate peak height. The reproducibility and accuracy of the FTIR method were assessed by standard addition, spiking three different acid-free oils (canola, soybean, and sunflower) with known amounts of palmitic acid (w/w). These oils were each analyzed in triplicate, on different days, by both the IUPAC titrimetric method[9] and the FTIR method to allow a direct comparison of their performance. A comparative analysis of locally purchased refined oils (soybean, sunflower, peanut, and corn oils and a commercial oil blend) was also carried out.

RESULTS AND DISCUSSION

The various approaches that have been investigated for the determination of FFA content in edible oils by FTIR spectroscopy were reviewed in a previous paper[6] and the limitations of each approach discussed. In that paper, these limitations were addressed by the development of a new FTIR method based on previous work on AN determination in lubricants, which employed the mild base potassium phthalimide as a signal-transducing reagent in conjunction with differential spectroscopy to circumvent matrix effects.[6] Although the phthalimide FTIR procedure is both more accurate

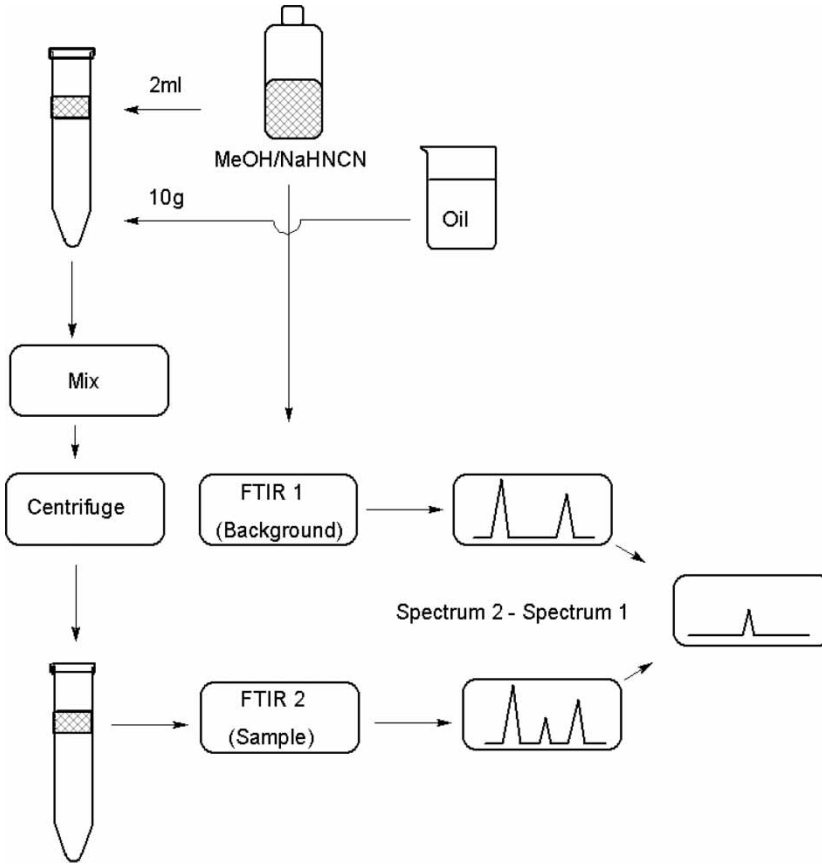

Figure 1. Schematic diagram of the sample preparation and analytical protocol.

and reproducible than conventional titration, it is ultimately not more sensitive per se. This is largely due to the use of propanol as a solvent and polarity enhancer for the reaction, effectively diluting the COO^- IR signal. A means by which sensitivity could be improved would be to treat the oil with methanol containing a base that is immiscible with the oil to facilitate the acid–base reaction as well as concentrate the FFA salts in the methanol layer.[14] Such a procedure would have the additional advantage of minimizing matrix effects by partitioning out the spectral contribution of the oil. For highly accurate analyses, a weak base would be required to avoid any saponification of the oil, and one could use either the spectral changes associated with the loss of the base or formation of FFA salts as a basis for quantitation. Examination of a range of reagents led to the consideration of the sodium salt of carbodiimide (NaHNCN), which has a strong $\nu(C{\equiv}N)$ absorption at $2100\,cm^{-1}$, is readily soluble in methanol, and is capable of converting

FFAs to their carboxylate salts but not capable of saponifying triacylglycerols. In the first instance, the proportionate decrease in the intensity of the ν(C≡N) band appeared to be a very good measure of the amount of FFAs spiked into oils. Although this reaction worked, it was found that the reagent spectrum was unstable and that the band at 2100 cm^{-1} slowly disappeared over a period of ~4 days with the concomitant appearance of two new bands at 1650 and 1610 cm^{-1} (Fig. 2). Based on assignment of these two bands to C=N stretching and NH bending vibrations, respectively, the structural rearrangement from NaHN–C≡N → NaN=C=NH was postulated to be taking place in solution over time. This conversion was confirmed spectrally by dissolving NaHNCN in MeOD, whereby the band at 1610 cm^{-1} shifted about 100 cm^{-1} to lower frequency owing to hydrogen–deuterium exchange. This molecular rearrangement, which was established to be complete within 4 days after preparation of the MeOH/NaHNCN reagent, did not affect its ability to convert FFAs to their respective salts without causing oil saponification. The reactivity of the converted MeOH/NaHNCN solution as well as its spectral characteristics remained stable, with solutions kept at room temperature being used for up to two months without any apparent deterioration in their efficacy. On the other hand, the measurement of the ν(C≡N) band originally envisioned as a basis for quantitation is lost as a result of this transformation. However, measurements made using the carboxylate band of FFA salts at 1573 cm^{-1} were both reproducible and responsive to low FFA levels because of the concentration of the FFA salts in the methanol layer and the high extinction coefficient of the carboxylate band. It was found that optimum sensitivity and reproducibility were achieved with a 5:1 oil/methanol ratio (10 g oil plus 2 mL of "aged" MeOH/NaHNCN reagent mixed in a standard 15-mL clinical centrifuge

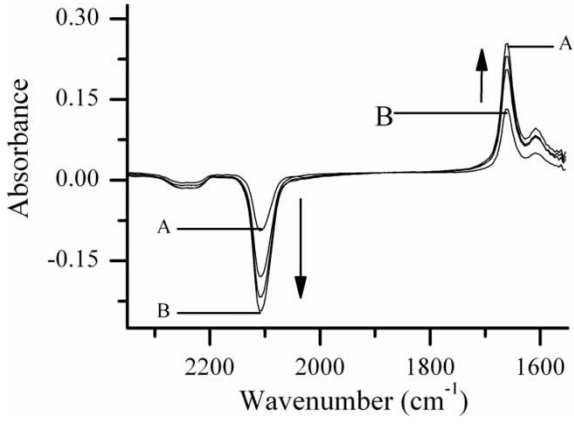

Figure 2. Differential spectra for sodium hydrogen cyanamide in methanol. The spectra show the decomposition of the reagent over time. A, one day; B, four days.

tube), producing consistent reactions and reproducible separations of the MeOH and oil phases when centrifuged for 5 min at 5000 × *g*. Oil stability with respect to saponification by the reagent was assessed by incubating oil samples with the reagent for 24 hr; this did not result in any measurable oil hydrolysis, but did lead to a minor displacement effect (~0.01% FFA) due to some additional oil migration into the methanol layer over extended periods of time.

Calibration

A calibration curve was developed by using a set of standards covering the range of 0.0 to 0.1% FFA, prepared by adding palmitic acid to acid-free soybean oil. Figure 3 illustrates the differential spectra obtained for these standards using the optimized analytical protocol outlined in Fig. 1. The carboxylate anion produced and extracted into the MeOH layer shows a rising absorbance at 1573 cm^{-1}; the other spectral features at 1650 cm^{-1} and the split band covering 1760–1700 cm^{-1} are the ν(C═N) absorption of the base and the ν(C═O) ester linkage absorption of a small amount of solubilized oil, respectively. A plot of the absorbance measured at 1573 cm^{-1}, referenced to a single-point baseline at 1820 cm^{-1}, versus % FFA (oleic acid) is presented in Fig. 4. Linear regression of the data obtained resulted in the following relationship:

$$\% \text{ FFA} = 0.70292\ A_{(1573/1820)} - 0.00883$$
$$R^2 = 0.9998; \quad SD = 6.706 \times 10^{-4} \tag{1}$$

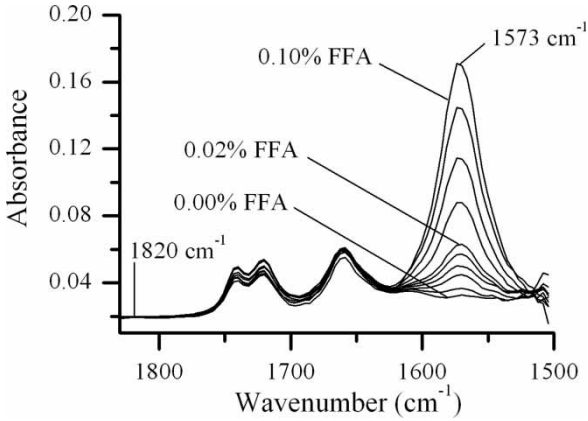

Figure 3. Differential spectra for soybean oil spiked with palmitic acid (0.0–0.1%) after carrying out the acid–base reaction. Spectra were recorded in a 100-μm cell at 8-cm^{-1} resolution.

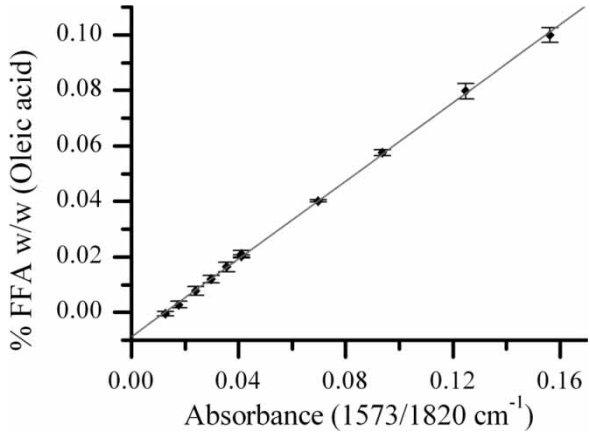

Figure 4. Calibration curve for % FFA in oil obtained from the differential spectra in Fig. 3. The % FFA is expressed as % oleic acid (w/w). Error bar amplitude indicates mean $\pm$ SD of three replicates.

The regression SD implies that FFA levels in the order of $1/1000$ of a percent may be measurable by this technique.

Validation

Validation and comparison of the FTIR method relative to the IUPAC titrimetric method were carried out by analyzing three acid-free oils (soybean, canola and sunflower) spiked with palmitic acid. Figures 5A and 5B present comparative plots for triplicate analyses of these oils by FTIR spectroscopy and the IUPAC titration procedure, respectively. The corresponding regression equations are:

$$\mathrm{FTIR_{FFA}} = 1.004\ \mathrm{FFA} + 4.4 \times 10^{-4}$$
$$R^2 = 0.9998; \quad \mathrm{SD} = 7 \times 10^{-4} \tag{2}$$
$$\mathrm{IUPAC_{FFA}} = 1.047\ \mathrm{FFA} + 2.3 \times 10^{-2}$$
$$R^2 = 0.9887; \quad \mathrm{SD} = 5 \times 10^{-3} \tag{3}$$

These results clearly indicate that the FTIR method tracks standard addition very well, with a slope and an intercept very close to unity and zero, respectively, with an overall error of about $<0.001\%$. The titrimetric plot clearly shows greater variability. Table 2 presents the data in terms of the relative mean difference (MD) and standard deviation of the differences (SDD) for both accuracy ($_a$) and reproducibility ($_r$). The $\mathrm{MD_a}$ of 0.025% for the titrimetric method indicates a significant bias relative to standard

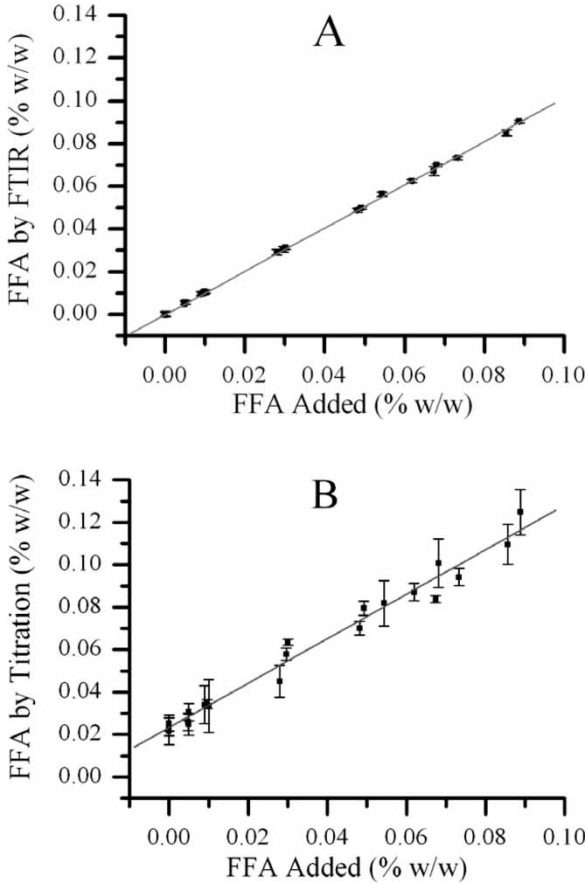

Figure 5. A plot of % FFA determined by the FTIR method (A) and % FFA determined by the titrimetric method (B) against the spiked amount. Error bar amplitude indicates mean $\pm$ SD of three replicates.

addition considering its SDD_a, while the FTIR results are basically an order of magnitude better in terms of both accuracy and reproducibility with no significant bias. The apparent titrimetric overdetermination relative to the FTIR method may be due to systematic errors associated with a slower acid–base reaction owing to the lower solvent polarity, the substantive volume of solvent used, and CO_2 absorption, as well as variability contributed by the visual end point determination. Table 3 presents comparative triplicate FTIR and titrimetric data obtained for locally purchased samples of refined oils. The two methods correlate ($R^2 = 0.83$), showing a similar trend as the standard addition data, with the titrimetric procedure predicting higher values individually as well as an overall mean bias of $\sim$0.02% and yielding poorer reproducibility.

Table 2. Mean difference (MD) and standard deviation of the differences (SDD) for accuracy ($_a$) for the titrimetric and FTIR procedures vs. gravimetric addition and for reproducibility ($_r$) for duplicate analyses carried out by titrimetric and FTIR analyses, respectively

Statistic	FTIR	Titration
MD_a	5.84×10^{-4}	2.50×10^{-2}
SDD_a	7.00×10^{-4}	5.10×10^{-3}
MD_r	4.56×10^{-4}	3.65×10^{-3}
SDD_r	9.47×10^{-4}	9.86×10^{-3}

FTIR, Fourier transform infrared.

The carbodiimide FTIR method was further examined in relation to expanding its general utility by changing its oil:reagent ratio. By decreasing the oil:reagent ratio from 5:1 to 1:4, while maintaining the same carbodiimide concentration, a calibration covering a range of 0–4% FFA was developed which produced the following calibration linear regression equation:

$$\% \text{ FFA} = 18.994A_{(1573/1820)} - 0.03598$$
$$R^2 = 0.9999; \quad \text{SD} = 0.0206 \tag{4}$$

Thus simply by changing oil:reagent ratio, semirefined and crude oils can also be analyzed accurately using the same basic analytical protocol outlined in Fig. 1. As such, the method developed provides a simple FTIR-based analytical procedure for the measurement of both very low and moderate levels of fatty acids in oils. Aside from providing excellent sensitivity and reproducibility, it overcomes many of the limitations associated with conventional titrimetric and potentiometric methods. There is no need to use an FTIR

Table 3. Results of triplicate analyses of locally purchased oil samples by the IUPAC reference (titrimetric) method and the FTIR method

	Titrimetric method		FTIR method	
Oil type	Mean (%w/w)	SD	Mean (%w/w)	SD
Peanut oil	0.050	5.39×10^{-3}	0.030	7.03×10^{-5}
Sunflower oil	0.041	6.40×10^{-3}	0.021	9.43×10^{-4}
Corn oil	0.053	3.50×10^{-3}	0.024	4.39×10^{-4}
Mixed oil	0.061	7.15×10^{-3}	0.032	8.28×10^{-4}
Soybean oil	0.045	3.62×10^{-3}	0.020	1.62×10^{-4}
Overall mean	0.050	—	0.026	—

FTIR, Fourier transform infrared.

spectrometer for this analysis per se, given that measurements at only two wavelengths are required and a simple dual-wavelength filter instrument would suffice. In either case, the method is readily amenable to automation.

CONCLUSIONS

Our work on FTIR analysis of lubricating oils led us to develop a novel approach for the determination of acidity in nonaqueous systems based on the combined use of signal transduction via a stoichiometric reaction and differential spectroscopy. In subsequent work, we demonstrated the suitability of this approach for the quantitation of FFAs at levels of $>0.2\%$ oleic acid by employing the same reagent as used for the determination of acidity in lubricating oils. In the current study, the use of sodium carbodiimide and a modified procedure has allowed the analysis to be extended down to FFA levels as low as 0.001%, which would not be measurable by direct measurement of FFA absorptions in the IR spectra of oils. With a simple adjustment in the reagent/oil ratio, the method becomes scalable and the analysis of semirefined and crude oils containing up to 4.0% FFA is possible. The strength of the method is its sensitivity, allowing for the possibility of using FFAs as an indicator of lipid oxidation and to more accurately monitor the refining processes carried out on edible oils.

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