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Evaluation of Raman Spectroscopy for Determining *cis* and *trans* Isomers in Partially Hydrogenated Soybean Oil

G. L. Johnson,* R. M. Machado, K. G. Freidl, M. L. Achenbach, P. J. Clark, and S. K. Reidy

Air Products and Chemicals, Inc., 7201 Hamilton Boulevard, Allentown, Pennsylvania 18195-1501, U.S.A.

Abstract:

The determination of *trans* isomer content in partially hydrogenated vegetable oils is important in the characterization of fats and oils in the food industry. Current methods for characterization include gas chromatography and infrared spectroscopy of samples captured off-line. The determination of *trans* isomer content during the hydrogenation process has the potential to improve process productivity and quality control to meet the stringent requirements of oil producers. Raman spectroscopy was evaluated in comparison to infrared spectroscopy as a method for quantitatively determining the *cis* and *trans* isomer content in partially hydrogenated vegetable oils. Both infrared and Raman principal component regression (PCR) calibrations accurately modeled the GC values (AOCS Official Method Ce 1f-96) for *cis* and *trans* content. In addition, the *cis* and *trans* isomer content of canola oil could be determined after the results from the IR and Raman calibration methods for soybean oil were corrected for slope and offset. Raman spectroscopy possesses unique advantages and shows promise as a method for rapid in situ analysis.

Introduction

The determination of *trans* isomer content in partially hydrogenated vegetable oils is of interest in the food industry because it influences product texture. Evidence for cardiovascular health risks from *trans* fatty acids (TFA) have led to proposed federal regulations for labeling TFA content in food products and increased interest in process control to limit *trans* isomer formation.[1−3] Current methods for determining the *cis* and *trans* content of partially hydrogenated vegetable oils include gas chromatography and infrared spectroscopy of samples captured off-line. An in situ Fourier transform infrared (FT-IR) technique is currently being used at Air Products to monitor vegetable oils during laboratory hydrogenations.[4] Raman spectroscopy is another technique that is sensitive to molecular structure with some unique advantages for in situ sampling.[5] Previous investigations have explored its usefulness for authentication of edible oils and measurement of *cis/trans* isomer ratio.[6−8]

Although Raman spectroscopy was discovered in 1928, it was not commonly used until reliable, continuous output lasers became available. Consequently, it is not as well-known or as used as infrared spectroscopy even though it provides similar and oftentimes complementary information.[9] The *Official Methods and Recommended Practices of the American Oil Chemists' Society* lists methods using infrared spectroscopy for *trans* isomer determination, but none for Raman.

Raman spectroscopy is based on an observed frequency shift of light scattered from a sample. Since an intense source of monochromatic light is necessary, lasers are preferred. The Raman signal is approximately 1×10^{-6} of the total light scattered by the sample, so that efficient filters or multiple spectrographs are necessary to block the excitation frequency and observe the Raman signal. The frequency shift occurs when energy from an incident photon is transferred into an internal vibrational state of the molecule. Thus, a slightly lower-energy photon (Stokes-shifted) is emitted with the Rayleigh scattered light.[11] Samples that are opaque, photolabile, or fluorescent may present difficulties in obtaining Raman spectra. In many cases the proper choice of laser and sampling techniques may avoid these interferences.

Raman spectroscopy, like infrared spectroscopy, measures the energy of molecular vibrational states. Since the measurement mechanism is different, different vibrational modes may be observed; therefore, Raman and infrared spectroscopy are considered complementary techniques.[9−11] One major advantage of Raman spectroscopy is that there is virtually no sample preparation necessary. Direct contact is not necessary to obtain spectra of materials behind optically transparent materials, such as glass or sapphire. This is a major advantage since infrared spectroscopy is limited to a wavelength range 2.5−25 μm that requires special optical materials. These materials are usually expensive, and they can be sensitive to moisture and visible light.

* To whom correspondence should be addressed. Telephone: 610-481-8378. Fax: 610-481-6578. E-mail: johnsogl@apci.com.

(1) Mensink, R. P.; Katan, M. B. *N. Engl. J. Med.* **1990**, *323(7)*, 439.
(2) Judd, J. T.; Clevidence, B. A.; Muesing, R. A.; Wittes, J.; Sunkin, M. E.; Podczasy, J. J. *Am. J. Clin. Nutr.* **1994**, *59*, 861−868.
(3) *Fed. Regist.* **1999**, *64(221)*, 62745−62825.
(4) Machado, R.; Achenbach, M.; Gaumer-Freidl, K. Oral presentation given at the 1997 annual meeting of the American Oil Chemists Society, May 11−14, Seattle WA.
(5) Vickers, T. J.; Mann, C. K. In *Analytical Raman Spectroscopy*; Grasselli, J. G., Bulkin, B. J., Eds.; John Wiley and Sons: New York, 1991; pp 108−109.
(6) Marigheto, N. A.; Kemsley, E. K.; Defernez, M.; Wilson, R. H. *J. Am. Oil Chem. Soc.* **1998**, *75(8)*, 987−992.
(7) Bailey, G. F.; Horvat, R. J. *J. Am. Oil Chem. Soc.* **1972**, *49(8)*, 494−498.
(8) Sadeghi-Jorabchi, H.; Wilson, R. H.; Belton, P. S.; Edwards-Webb, J. D.; Coxon, D. T. *Spectrochim. Acta, Part A* **1991**, *47A(9−10)*, 1449−1458.
(9) Bulkin, B. J. In *Analytical Raman Spectroscopy*; Grasselli, J. G., Bulkin, B. J., Eds.; John Wiley and Sons: New York, 1991; pp 1−8.
(10) Bulkin, B. J. In *Analytical Raman Spectroscopy*; Grasselli, J. G., Bulkin, B. J., Eds.; John Wiley and Sons: New York, 1991; pp 9−12.
(11) Lin-Vien, D.; Colthup, N. B.; Fateley, W. G.; Grasselli, J. G. *The Handbook of Infrared and Raman Characteristic Frequencies of Organic Molecules*, Academic Press: San Diego, 1991; pp 1−7.

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Recent advances in Raman technology, including charged coupled device (CCD) array detectors, fiber optic probes, and high-throughput holographic and Fourier transform spectrometers, have made Raman spectroscopy attractive for on-line monitoring of industrial processes. A wide variety of in situ probes have become available for use with optical fibers for sampling at locations remote from the instrument. Since Raman spectrometers can operate at visible to near-infrared frequencies, low attenuation optical fibers are available to extend the distance between an instrument and process probes to >100 m. Process infrared instruments use either short runs of optical fibers or periscope-like assemblies to interface with an in situ probe. Optical fibers for infrared wavelengths are expensive and have low throughput, so that only short (about 1 m) runs are practical. Consequently, an IR instrument must be close to the system that is being characterized. Another advantage Raman spectrometers possess over infrared analyzers is the ease with which they are multiplexed. The output of a laser may be split into several (4−8) source fibers, and the signal fibers may be arranged to project each probe signal onto separate areas of an array detector, such as a CCD. This arrangement can provide truly simultaneous measurements at multiple sample sites. In contrast, most infrared instruments, including process-oriented systems, are capable of interfacing with only one sample probe. The objective of this research was to demonstrate the feasibility of using Raman spectroscopy as an in situ probe for qualitative and quantitative determination of *cis/trans* isomer ratios during hydrogenation of vegetable oils.

Experimental Section

Seven hydrogenation tests were performed with refined and bleached (RB) soybean oil obtained from a commercial oil refiner. Three Engelhard catalysts were used in these experiments: (1) E-479, a selective catalyst with 25% Ni, (2) Nysosel 325, a selective catalyst with 22% Ni, and (3) E-428, a nonselective, general-purpose catalyst with 22% Ni. For catalyst handling purposes, the active metal was encapsulated in tallow. The process conditions for these experiments were chosen to reflect typical operating parameters used in the fats and edible oils industry. Agitator speed was 1000 rpm and the temperature was 180 °C for runs 1, 4, 6, and 7 and 140 °C for runs 2, 3, and 5. The H2 pressure was 2 bar for runs 1, 2, 3, and 5 and 0.5 bar for runs 4, 6, and 7. The catalyst was Nysosel 325 for runs 1 and 2, E-479D for runs 3, 4, and 7, and E-428 for runs 5 and 6.

Hydrogenations were conducted in the Mettler Toledo RC1/HP60, a high-pressure reaction calorimeter that is equipped with a gas-induction draft tube agitation system.[12] Hydrogen gas was supplied to the RC1/HP60 by the Büchi Pressflow gas controller.[13]

Each hydrogenation experiment began by charging 1200 g (1300 mL) of RB soybean oil into the reaction calorimeter. The headspace of the reactor was purged twice with nitrogen and twice with hydrogen. The soybean oil was heated to the desired reaction temperature and held isothermally at either 140 or 180 °C. When the set temperature was reached, the catalyst was added. The E-428 or Nysosel 325 catalyst was charged to the reactor to yield a final nickel content of 0.018% Ni, while the E-479 catalyst was charged to yield a final nickel content of 0.021% Ni. The reactor was then sealed and pressurized with hydrogen to 7.25 psig or 29 psig. The agitation rate was then set to 1000 rpm. As determined by batch adsorption uptake experiments, this agitation rate corresponded to a gas/liquid mass transfer coefficient, k_{la}, of 0.013 s^{-1}.[14,15] Hydrogen was delivered on demand at constant pressure, and the reactor temperature was maintained within ±1 °C throughout the reaction. Partially hydrogenated samples were removed from the reactor through a sampling tube consisting of a 0.5-μm sintered metal filter immersed in the reactor. The sampling intervals were determined by percent conversion based on the hydrogen uptake indicated by the Büchi pressflow gas controller. Up to 10 samples of partially hydrogenated soybean oil were obtained during each experiment and stored in glass vials under a pad of nitrogen. Each reaction was run to completion, i.e., until an exotherm less than 0.2 W was monitored.

Analytical Methods

Developing a spectroscopic method requires either standards with known concentrations or a primary method that is modeled with chemometrics. Spectroscopic models are developed because a primary method is either too slow to provide results useful for process control or unsuitable for on-line analysis with in situ sampling. In this case the primary method used to determine saturation, *cis* isomer, and *trans* isomer values was AOCS official method Ce 1f-96.[16] This method specifies gas chromatography (GC) analysis for methyl ester derivatives of fatty acids. The methyl ester derivatives were obtained by reaction with methanol/BF_3 according to AOCS official method Ce 2-66.[16] The derivatives were diluted to approximately 10 wt % in hexane before injection into the GC column. A Restek Rtx-2330 capillary column (60 m × 0.25 mm i.d., 0.2 μm film thickness, S/N 142416) was operated at 160 °C with helium carrier gas flowing at 0.7 mL/min. A Hewlett-Packard 7673 autosampler was used to inject 1 μL of sample into an injector at 250 °C. The flame ionization detector was operated at 250 °C. The values obtained from the GC analysis, listed in Table 1, were used to calibrate the infrared and Raman spectroscopic methods.

Infrared spectra were obtained at 4 cm^{-1} resolution with a Spectra-Tech Contact Sampler horizontal ATR accessory in a Nicolet Instruments, Inc. 510 FT-IR with a liquid nitrogen-cooled MCT-A detector. A ZnSe crystal with a 45°

(12) *RC1e Users Manual*; ASI Applied Systems, Inc., a Mettler Toledo Company, 8223 Cloverleaf Drive, Suite 120, Millersville, MD 21108, telephone: 410-987-3222, 1997.
(13) *Users Manual*; Buchi AG, CH-8610 Uster/Switzerland, PO Box, telephone: 01/941 20 61, 1997.
(14) Dietrich, E.; Mathieu, C.; Delmas, H.; Jenck, J. *Chem. Eng. Sci.* **1992**, *47(13/14)*.
(15) Puri, P. S.; deMan, J. M. *Can. Inst. Food Sci. Technol. J.* **1977**, *10(1)*, 53−55.
(16) *Official Methods and Recommended Practices of the AOCS*, 5th ed., 1st printing; Firestone, D., Ed.; American Oil Chemists' Society: Champaign, Illinois, 1998.

Table 1: **Saturation, *cis* isomer, and *trans* isomer values of soybean oil determined by GC (AOCS Standard Method Ce 1f-96)[a]**

sample no.	type	% H_2 uptake	% saturation	% *cis*	% *trans*
1	C	0	14.578	84.117	0.727
2	C	10.8	14.556	78.767	5.552
3	C	31	14.661	67.816	16.26
4	V	50.4	20.464	46.709	31.892
5	C	100	75.373	8.605	15.624
6	C	0	14.703	83.668	0.527
7	C	10.5	14.864	79.007	4.403
8	V	29.4	15.019	69.444	14.417
9	C	49.8	19.589	53.275	26.164
10	C	100	79.48	10.391	9.736
11	C	0	14.651	83.767	0.517
12	V	10	14.767	80.146	4.017
13	C	28.6	14.966	71.944	12.068
14	V	47.7	17.559	58.728	22.777
15	C	100	65.314	21.509	12.718
16	C	9.4	14.601	76.136	7.806
17	C	17.7	14.786	71.856	11.915
18	C	26.4	14.626	64.144	19.761
19	C	35.2	14.806	56.648	27.164
20	C	43.6	17.433	46.346	35.358
21	C	52.3	25.246	26.025	47.827
22	V	61.3	37.67	17.237	44.139
23	C	69.9	51.253	12.824	34.958
24	V	90	68.447	7.209	22.906
25	V	0	14.721	84.013	0.513
26	C	12.2	17.514	79.135	2.364
27	C	19.4	18.482	74.401	6.163
28	C	29.4	21.52	66.895	10.559
29	C	48.9	28.446	54.271	16.365
30	V	100	72.182	18.127	9.299
31	C	100	97.357	0.272	1.629
32	V	100	97.991	0.467	0.854

[a] The dashed lines separate data from each hydrogenation. Type designates how each sample was used: C = member of calibration set and V = member of validation set.

incident angle was used for all measurements. The samples were preheated to 60 °C in a water bath before sampling. The crystal was mounted in a heated plate that was held at 60 °C, which was sufficient to keep all the samples liquid. The ATR crystal was cleaned with methanol and lens tissue after each sample.

Raman spectra were obtained at 4 cm^{-1} resolution with a Nicolet FT-Raman accessory on a Nicolet Instruments, Inc. Magna 750 using a diode-pumped Nd:YAG laser at 1064 nm, InGaAs detector, and CaF_2 beam splitter. The solid samples were preheated at 100 °C in an oven to liquefy them. The laser power, 2 W, was sufficient to keep all the samples liquid in the path of the beam. The samples were contained in sealed glass vials under a nitrogen pad. The laser was directed through a hole in the center of an off-axis parabolic mirror and onto the sample. The scattered light from the sample was collected by the mirror and collimated into the interferometer. A high-efficiency holographic notch filter

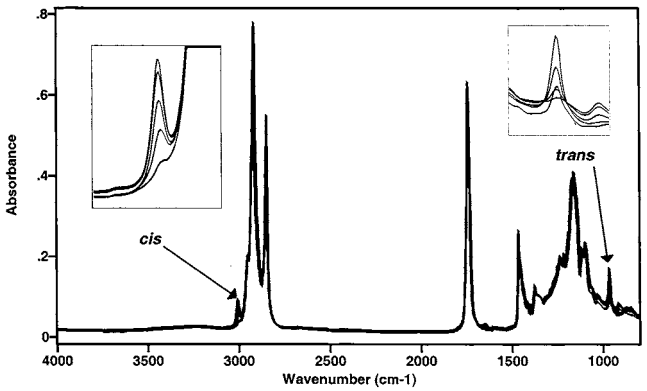

Figure 1. **Infrared spectra of hydrogenation reaction intermediates. There are five infrared spectra overlapped on the same scale in this figure. The absorbance bands assigned to *cis* and *trans* isomers are marked.**

with a bandwidth of ±100 cm^{-1} blocked the Rayleigh-scattered laser frequency.

All of the infrared and Raman spectra were obtained using Nicolet Omnic software. All spectroscopic manipulation and quantitative analysis was done with GRAMS/32 and PLS/IQ software from Galactic Industries, Inc.

Results and Discussion

Table 1 lists hydrogen uptake values, GC results for %*cis*, %*trans*, and %saturation for each soybean oil sample from the seven hydrogenation runs. Nine samples spanning the range of *cis*/*trans* values, marked "V" in Table 1, were selected to validate the spectroscopic methods, and their spectra were excluded from each calibration. The remaining samples, marked "C" in Table 1, were used to calibrate the IR and Raman methods.

Figure 1 shows infrared spectra of soybean oil samples from a single hydrogenation. Two absorbance bands change intensity during the hydrogenation. The first absorbance band, marked as "*cis*" in Figure 1, is assigned to the 3007 cm^{-1} C−H stretching vibration of the *cis* isomer. The second absorbance band at 966 cm^{-1}, marked as "*trans*" in Figure 1, is assigned to the out-of-plane C−H bending vibration of a *trans* olefin.[17] The relative intensities of these two absorbance bands have been used to determine the isomer ratio in partially hydrogenated vegetable oil.[18]

Figure 2 shows Raman spectra of soybean oil from a single hydrogenation test run. Three bands change in intensity during the hydrogenation. The band at 1658 cm^{-1}, marked "*cis*" in Figure 2, is assigned to the C═C stretching vibration of a *cis*-olefin. A second feature, marked "*cis*" in Figure 2, at 1266 cm^{-1} is assigned to the CH═CH symmetric rock of the *cis* isomer. The band marked "*trans*" at 1670 cm^{-1} is assigned to the C═C stretching vibration of a *trans*-olefin.[7,17]

A qualitative analysis of the spectra was performed to determine if the reaction coordinates could be monitored with Raman spectra as well as with IR. Qualitative tracking of

(17) Lin-Vien, D.; Colthup, N. B.; Fateley, W. G.; Grasselli, J. G. *The Handbook of Infrared and Raman Characteristic Frequencies of Organic Molecules*; Academic Press: San Diego, 1991; pp 73−81.
(18) van de Voort, F. R.; Ismail, A. A.; Sedman, J. *J. Am. Oil Chem. Soc* **1995**, *72(8)*, 873−80.

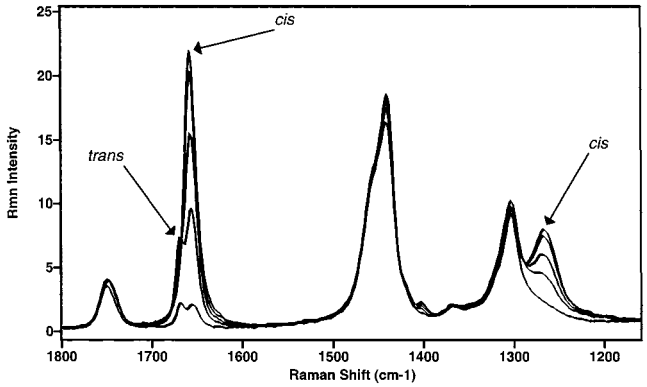

Figure 2. **Raman spectra of hydrogenation reaction intermediates. There are five Raman spectra overlapped on the same scale in this figure.**

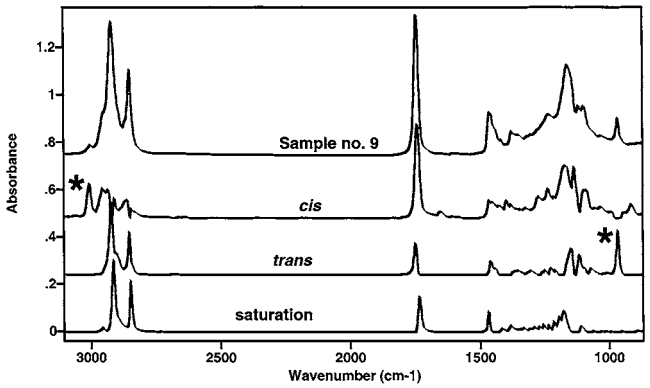

Figure 3. **Top trace is an infrared spectrum of a hydrogenation reaction intermediate at 40% hydrogen uptake. Bottom three traces are ITTFA model spectra obtained from the full set of IR spectra listed in Table 1. The model spectra exhibited features assigned to either *cis* isomer, *trans* isomer, or saturation.**

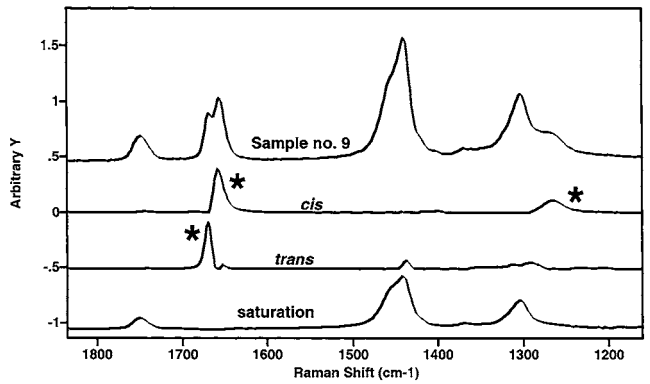

Figure 4. **Top trace is a Raman spectrum of a hydrogenation reaction intermediate at 40% hydrogen uptake. Bottom three traces are ITTFA model spectra obtained from the full set of Raman spectra listed in Table 2. The model spectra exhibited features assigned to either *cis* isomer, *trans* isomer, or saturation.**

hydrogenation products with a ReactIR FT-IR and an in situ ATR probe has already been reported.[4] The qualitative analysis was performed by an iterative target transformation factor analysis (ITTFA), a chemometric method based on principal component analysis (PCA).[19] This technique can model the components of the system that change in a linearly independent manner and extract their relative intensity versus time profiles. An ITTFA was performed on both sets of IR and Raman spectra. The frequency range for ITTFA was limited to 3102−872 cm^{-1} for the IR spectra and 1834−1161 cm^{-1} for the Raman spectra.

PCA factors, scores, and eigenvalues were obtained from singular value decomposition of the data covariance matrix for both sets of IR and Raman spectra. An F-test was performed on the reduced eigenvalues of each set of PCA factors to determine the optimum number of factors necessary to model the IR and Raman spectra. The alpha function calculated from the *F*-ratios of the reduced eigenvalues was greater than 99% significance for the first three principal components in the PCA of the IR spectra. The alpha function was greater than 95% significance for the first three principal components in the PCA of the Raman spectra. Therefore, three principal components were necessary and sufficient to reproduce any individual spectrum in either set of spectra.

Since the models are derived from linear combinations of PCA factors, their intensities are arbitrary. Therefore, each model was divided by its root sum of squares (RSS) intensity to provide a common scale for comparison. The scores of the models are obtained by linear regression of each model against the corresponding set of spectra. Each score of an RSS normalized ITTFA model represents its weighting in the corresponding spectrum. The scores may be considered a relative measure of the contribution of each ITTFA model to any given spectrum in the data set.

The three ITTFA models obtained from the full set of IR spectra are shown with a representative infrared spectrum in Figure 3. The model labeled "*cis*" exhibited the feature, marked with an asterisk, assigned to *cis* isomer in Figure 1. The model labeled "*trans*" had a band at 966 cm^{-1}, marked with an asterisk, that was assigned to *trans* isomer in Figure 1. The model labeled "saturation" was indicative of an aliphatic compound.

In comparison, the three ITTFA models obtained from the full set of Raman spectra are shown with a representative Raman spectrum in Figure 4. The model labeled "*cis*" exhibited bands, marked with an asterisk, assigned to *cis* isomer in Figure 2. The model labeled "*trans*" had the 1670 cm^{-1} feature assigned to *trans* isomer in Figure 2. The model labeled "saturation" had bands indicative of aliphatic esters.

Figure 5 shows the GC %*cis* isomer values and the scores of the IR and Raman ITTFA models labeled "*cis*" in Figures 3 and 4 versus sample number (Table 1). The ITTFA scores were scaled to the GC values to provide a common ordinate for comparison. Both sets of ITTFA model scores agree with the trends in the GC values for *cis* isomer, indicating that Raman spectra can be used to track the progress of *cis* isomer in a hydrogenation reaction as well as infrared techniques.

The GC %*trans* isomer values are compared in Figure 6 to the scores obtained from the IR and Raman ITTFA models labeled "*trans*." The ordinate values are the sample numbers from Table 1. The ITTFA scores were scaled to the GC values to aid comparison. Once again, the plot shows that

(19) Liang, X.; Andrews, J. E.; deHaseth, J. A. *Anal. Chem.* **1996**, *68(2)*, 378−385.

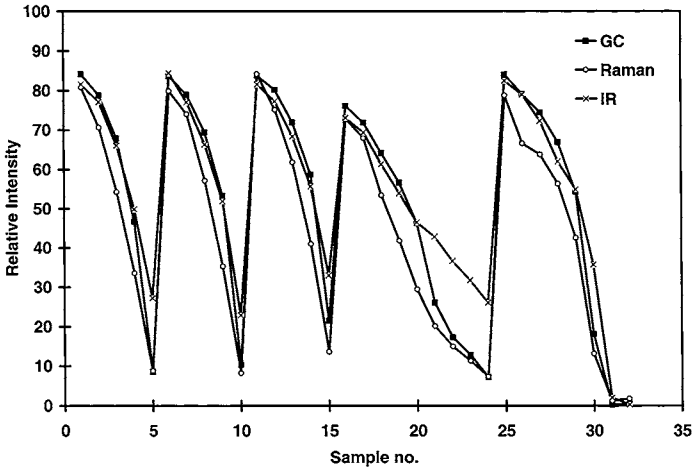

Figure 5. **Scores obtained from IR and Raman *cis* ITTFA models versus GC values as a function of sample number from Table 2. In these plots the scores were scaled to the GC values for a better visual comparison.**

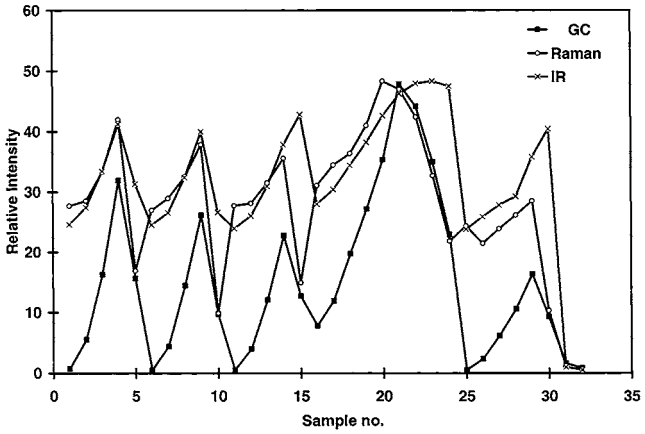

Figure 6. **Scores obtained from IR and Raman *trans* ITTFA models versus GC values as a function of sample number from Table 2. In these plots the scores were scaled to the GC values for a better visual comparison.**

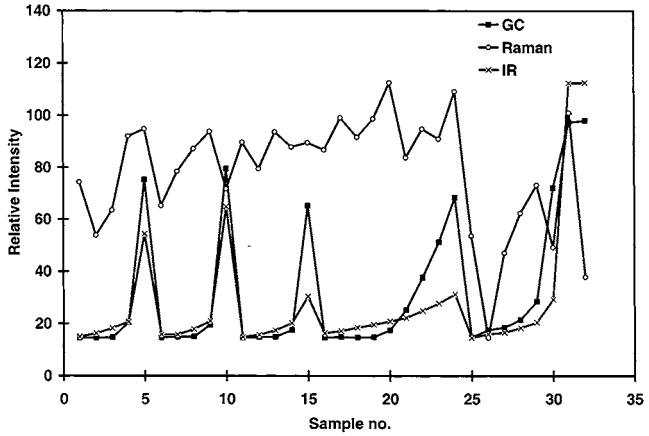

Figure 7. **Scores obtained from IR and Raman saturation ITTFA models versus GC values as a function of sample number from Table 2. In these plots the scores were scaled to the GC values for a better visual comparison.**

the ITTFA model scores from either Raman or infrared spectra may be used to follow *trans* isomer during a hydrogenation reaction. The apparent bias observed in both the IR and Raman ITTFA model scores may be attributed to components in the data that are collinear with the *trans* moiety. The spectral features of components whose concentrations have a linear relationship cannot be isolated into separate models.

The scores obtained from the IR and Raman ITTFA models labeled "saturation" in Figures 3 and 4 are compared to corresponding GC saturation values in Figure 7. The ITTFA scores were scaled to the GC values to aid comparison. The ITTFA model scores obtained from infrared spectra showed reasonable agreement with the trends in the GC saturation values, while the scores of the Raman ITTFA model labeled "saturation" in Figure 4 did not correlate well to the GC saturation values. The "saturation" Raman ITTFA model may be better assigned to a relatively constant component throughout the spectra. The insensitivity of the Raman ITTFA model to the saturated component of the samples is probably due to leaving the C−H stretching region, 2700−3100 cm^{-1}, out of the computation of the model. Although this region was measured in the FT-Raman spectra, many process instruments use near-infrared lasers at 785 nm in combination with CCD detectors that exhibit a large drop in sensitivity above 2500 cm^{-1}. The narrower frequency region was selected in anticipation of using this type of system for online analysis.

Principal component regression (PCR) was selected as the quantitative method because of its similarity to the formalism of the ITTFA calculations.[20] PCR calibrations were computed using the *cis* and *trans* isomer values from the GC primary method and the IR and Raman spectra. Saturation was not included as a component in the calibrations since it was a dependent variable of the isomer values and its inclusion would result in overfitting and biasing the method. The spectra were pretreated by taking the first derivative to remove baseline offsets. Then the spectra were mean-centered, i.e. the average of the calibration set was subtracted from each spectrum, placing the variation in the data about a common origin. The frequency regions 4000−2720 cm^{-1} and 1873−866 cm^{-1} were selected for calibration of the infrared spectra, and 1776−1167 cm^{-1} was selected for calibration of the Raman spectra. The optimum number

(20) Fredericks, P. M.; Lee, J. B.; Osborn, P. R.; Swinkels, D. A. J. *Appl. Spectrosc.* **1985**, *39*, 303.

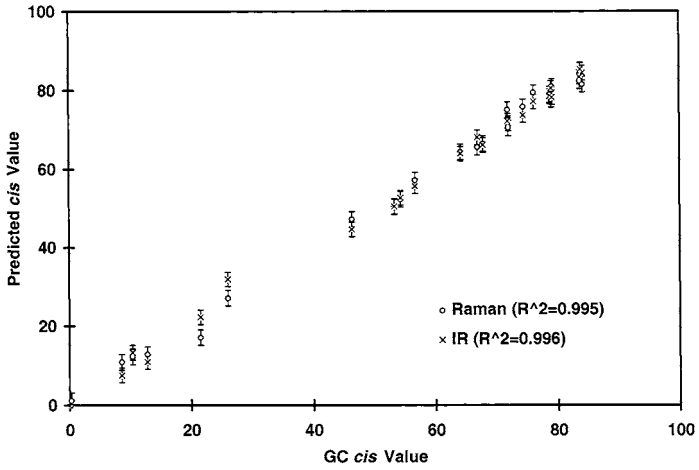

Figure 8. **Principal component regression self-prediction results for *cis* isomer in soybean oil. These results were obtained by submitting the calibration spectra to the model as unknowns. The error bars represent ±1 standard error of calibration (SEC).**

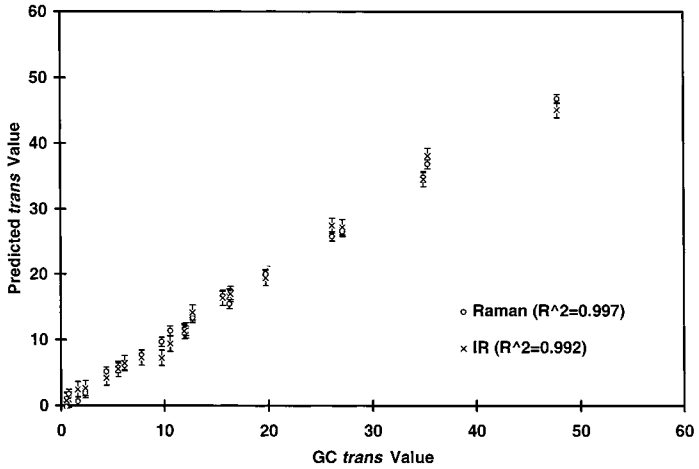

Figure 9. **Principal component regression self-prediction results for *trans* isomer in soybean oil. These results were obtained by submitting the calibration spectra to the model as unknowns. The error bars represent ±1 standard error of calibration (SEC).**

of PCR factors for each calibration was determined from the prediction residual error sum of squares (PRESS) cross-validation calculations, in which one spectrum was left out of each test.

Self-validation plots of the GC isomer values against PCR-predicted isomer values were obtained by using the IR and Raman calibration spectra as unknowns (Figures 8 and 9). The correlation coefficients, R^2, between the predicted and primary method values are shown in the figures. The error bars indicate ±1 standard error of calibration (SEC). The SEC is determined by:

$$\mathrm{SEC} = \sqrt{\sum (G_C - P_C)^2/(N_C - 1)} \qquad (1)$$

where G_C = GC isomer value, P_C = predicted isomer value, and N_C = number of calibration samples. The SEC for the *cis* isomer was 1.85 for IR and 1.98 for Raman. The SEC for the *trans* isomer was 1.16 for IR and 0.71 for Raman. The self-validation test indicates that infrared and Raman spectra produce nearly equivalent calibrations.

The results of using the PCR models created with the IR and Raman spectra to predict the *cis* and *trans* isomer values of the validation samples are shown in Table 2. The SEP is determined by:

$$\mathrm{SEP} = \sqrt{\sum (G_V - P_V)^2/N_V} \qquad (2)$$

where G_V = GC isomer value, P_V = predicted isomer value, and N_V = number of validation samples. The correlation coefficient, R^2, and standard error of prediction (SEP) between the predicted and primary method values for each isomer are also shown in Table 2. The SEPs indicate that the IR calibrations were slightly better at predicting the isomer values than the Raman data. This discrepancy may be due to a combination of leaving out part of the spectral region that contains isomer information in the Raman calibration as well as the lower signal-to-noise ratio inherent in FT-Raman versus FT-IR.

Generating a multivariate calibration can be an expensive and time-consuming process because of the efforts to obtain samples and obtain primary analytical results. One way to save resources is to transfer a calibration from one process to a chemically related system. Canola oil was selected to test whether the PCR calibrations could be extended to different types of vegetable oil. The primary GC method (AOCS official method Ce 1f-96) was applied to five samples

Table 2: **Comparison of *cis* and *trans* values predicted by PCR calibrations of infrared and Raman spectra of validation samples to primary method**

sample no.	GC: %*cis*	IR: *cis* (PCR)	Raman: *cis* (PCR)	GC: %*trans*	IR: *trans* (PCR)	Raman: *trans* (PCR)
4	46.709	46.9972	40.1373	31.892	33.5294	37.6232
8	69.444	68.6788	67.2781	14.417	14.0575	14.5966
12	80.146	81.3394	80.6465	4.017	3.6640	3.6088
14	58.728	55.7896	56.7650	22.777	23.0702	21.7235
22	17.237	20.0013	15.6523	44.139	42.7836	44.3308
24	7.209	3.9215	8.2883	22.906	22.2557	21.8317
25	84.013	84.0814	86.4228	0.513	1.4406	−0.5160
30	18.127	24.3728	20.4213	9.299	10.0066	7.7954
32	0.467	−1.3367	8.7034	0.854	2.2748	0.9029
	R^2	0.9915	0.9851	R^2	0.9961	0.9846
	SEP:	2.8175	3.8668	SEP:	0.9810	2.0728

Table 3: **Results of predicting *cis* and *trans* isomer values for canola oil with IR and Raman PCR calibrations for soybean oil[a]**

GC: %*cis*	IR: *cis* (PCR)	Raman: *cis* (PCR)	GC: %*trans*	IR: *trans* (PCR)	Raman: *trans* (PCR)
88.5230	74.6077	86.7950	2.5580	0.0153	−2.2811
86.4820	72.7236	78.3012	4.1180	1.7371	0.3935
80.7480	67.4804	75.4809	9.1250	7.2566	5.7664
73.9560	60.7380	69.4952	15.3280	14.3691	13.4085
68.1670	55.3343	65.1848	20.3360	20.4886	18.9945
R^2	0.9998	0.9174	R^2	0.9996	0.9987
SEP[b]	13.4041	5.0288	SEP[b]	1.8203	3.2879

[a] Correlation coefficients and SEP values are shown at bottom of table. [b] Standard error of prediction of PCR predictions with no applied corrections.

Table 4: **Corrected IR and Raman predictions for *cis* and *trans* isomer values predicted from PCR calibrations**

GC: %*cis*	IR: *cis* (corrected)	Raman: *cis* (corrected)	GC: %*trans*	IR: *trans* (corrected)	Raman: *trans* (corrected)
88.5230	88.4443	91.1038	2.5580	2.6634	2.2397
86.4820	86.4623	82.7655	4.1180	4.1634	4.4981
80.7480	80.9466	79.9969	9.1250	8.9717	9.0349
73.9560	73.8537	74.1207	15.3280	15.1678	15.4877
68.1670	68.1691	69.8892	20.3360	20.4988	20.2045
R^2	0.9998	0.9174	R^2	0.9996	0.9987
slope:	1.0520	0.9817	slope:	0.8712	0.8444
intercept:	9.9586	5.8977	intercept:	2.6501	4.1658
SEP[b]	0.1063	2.1923	SEP[b]	0.1333	0.2436

[a] The initial predictions from Table 4 were fit to the GC values with a linear regression. Slope, intercept, and correlation coefficient, R^2, obtained from fit are shown at bottom of table with new SEP values. [b] Standard error of prediction after linear correction was applied to PCA predictions.

of canola oil obtained from a partial hydrogenation. The infrared and Raman spectra of the canola oil samples were obtained and used to predict the *cis* and *trans* isomer values by applying the soybean oil PCR calibrations. The PCR results are compared to the GC values for *cis* and *trans* isomer values of canola oil in Table 3. The soybean oil-based PCR calibrations do not appear to be directly applicable to canola oil, based on the large SEP values shown in Table 3. While the predicted values were offset from the expected quantities, there was still a strong linear response, based on the R^2 values listed in Table 3. This linear correlation suggests that a correction based on a linear regression between the predicted values and the GC values could be applied to the results of the PCR predictions. The slopes and intercepts obtained from the two least-squares linear regressions of the GC values to the IR and Raman PCR-predicted values are shown in Table 4. The results of applying the slope and intercept to correct the PCR predictions are also shown in Table 4. Application of the linear corrections greatly improved the SEP for both calibrations, indicating that the soybean oil-based PCR calibration could be used for canola oil after correcting with slope and intercept values. In this case, the canola oil samples became validation standards for correcting bias in the calibration.

While the PCR calibrations can be transferred from soybean to canola oil with slope and offset adjustments, this is not recommended for routine use. Special care must be taken to ensure that the method can handle nonlinear responses in the spectral data. While additional factors can account for nonlinear terms in the PCR calibration for soybean oil, the offset and slope corrections may only apply to a narrow range of *cis* or *trans* values from canola oil. The set of samples used to produce the slope and offset corrections for calibration transfer should fully span the expected range of concentrations. This type of correction is not a substitute for fully calibrating for a new product;

however, it could save time in getting a method online until a new calibration set is developed and validated.

Conclusions and Future Work

The results of this study show that IR and Raman spectra can be used to quantify the *cis*/*trans* isomer content in partially hydrogenated soybean oil. Using a primary GC standard as reference, iterative target transformation analysis (ITTFA) of IR and Raman spectra and principal component regression (PCR) calibrations accurately modeled the GC values (AOCS Official Method Ce 1f-96) for *cis* and *trans* content. Calibrations based on partially hydrogenated soybean oil can be transferred to canola oil with slope and offset adjustments, and while this is not recommended for routine use, it could save time in getting a process control method online until new calibration standards are developed and validated. The ease of using fiber optics in a manufacturing environment makes the potential industrial use of in situ Raman spectroscopy possible for the improvement of the process productivity and quality control of oil hydrogenation.

Our plans for future work include comparing in situ measurements obtained with the ReactIR system to data obtained with a Kaiser Optical Systems Holoprobe instrument with a fiber-optic-coupled immersion probe.

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