

Using FTIR-ATR Spectroscopy To Teach the Internal Standard Method

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The internal standard method is widely applied in quantitative analyses. However, it has been noted in this *Journal* that undergraduate analytical chemistry textbooks do not adequately teach students to apply the technique of internal standards (1). Out of seven undergraduate analytical chemistry texts that were examined (2–8), only two were found that provide an equation that can be used to quantify an analyte using an internal standard (2, 3). Both textbooks provide sample calculations using a single-point internal standardization. One goes on to describe the preparation of an internal standard calibration curve and provides a practice problem (3). Although almost no emphasis is placed in undergraduate chemistry textbooks on the preparation and use of an internal standard calibration curve, the technique is routinely applied to a variety of applications, such as quantifying proteins in gel electrophoresis (9), reaction monitoring (10), quantifying odor-causing compounds in foods (11), and quantifying nucleic acids in clinical diagnostics (12). It would be beneficial for students of either quantitative chemical analysis or instrumental analysis to perform an experiment that provides them with enough experience to apply the internal standard method to a wide variety of experiments.

Several experiments were found in this *Journal* in which students make an internal standard calibration curve (13–17). However, in these experiments, the concentration of the internal standard was constant and students could process the data without necessarily having a good knowledge of the internal standard method. Another experiment was published by Mathias et al. in this *Journal* that used an internal standard calibration curve in which the concentration of the internal standard was not constant throughout the experiment (18). In that experiment, the ethylene and vinyl acetate components of poly(ethylene-co-vinyl acetate) were quantified. Fourier transform infrared spectroscopy (FTIR) was used to collect spectra of thin films in the transmission mode. An ethylene peak was used as an internal standard to correct for the variation in film thickness. However, the internal standard calibration curve was not prepared in a way that gave a completely linear calibration curve. In a modified version of the Mathias experiment (18), poly(ethylene-co-vinyl acetate) samples were analyzed using Fourier transform infrared-attenuated total reflectance (FTIR-ATR) spectroscopy. An ethylene peak was again used as an internal standard to correct for differences in pressure applied to the sample on the ATR element. In addition, thermogravimetric analysis (TGA) was incorporated to more precisely quantify the sample components (19). However, the internal standard calculations were not corrected.

The experiment presented here uses the experiment in which the ethylene and vinyl acetate components of poly(ethylene-co-vinyl acetate) are quantified by ATR-FTIR spectroscopy as a

means of teaching students how to apply the internal standard method. The method of preparing the internal standard calibration curve is changed to give a linear calibration curve even though the concentration of the internal standard is not constant. Students gain valuable experience with FTIR-ATR spectroscopy, which is beneficial for students because the variety of samples that can be analyzed by FTIR spectroscopy is greatly increased by the use of an ATR accessory. Also, the use of an ATR accessory can speed up the analysis of liquids and solids because they can be analyzed with little or no sample preparation.

A review article describing the versatility of studying solids and liquids by FTIR-ATR spectroscopy was published in this *Journal* (20). The same authors describe an FTIR-ATR experiment for the undergraduate physical chemistry laboratory to investigate the adsorption of sulfate ions from solution onto TiO_2 particles (21). Other experiments that have been published in this *Journal* describe the use of FTIR-ATR spectroscopy for the quantitative analysis of ethanol in vodka (22), the measurement of trans fat content in cooking oils (23), quantifying the ortho, meta, and para ratios in brominated toluene (24), studying the kinetics of a polymerization reaction (25), and the use of ATR spectroscopy in the visible region in a copper sensor (26). In addition, many research articles have been published that use FTIR-ATR spectroscopy as a measurement tool. Examples include topics such as the adsorption of proteins onto substrates (27) and the formation of nanolayers (28, 29).

Experimental Procedure

Poly(ethylene-co-vinyl acetate) samples were purchased from the Aldrich Chemical Co. The samples contained 9%, 14%, 18%, 25%, 33%, and 40% vinyl acetate. Samples could be analyzed with no sample preparation, but the absorbance values were very low. Higher absorbance values were measured when the samples were melted and allowed to cool. Eight of the commercial sample beads were placed in a row on a piece of aluminum, which was then placed on top of an inverted 100 mL Pyrex beaker. The inverted beaker was placed on top of a hot plate and the hot plate was turned to high heat until the beads melted together. The sample was allowed to cool and was placed directly on the ATR element.

FTIR spectra were collected with a Nicolet Protégé 460 spectrometer. The resolution was set to 8 cm^{-1} and 50 scans were averaged. The wavenumber range of the spectra was from 650 to 2000 cm^{-1} . One sample of each vinyl acetate concentration was analyzed. The ATR accessory used was the horizontal attenuated total reflectance (HATR) accessory from Pike Technologies. The HATR accessory contained a flat plate crystal

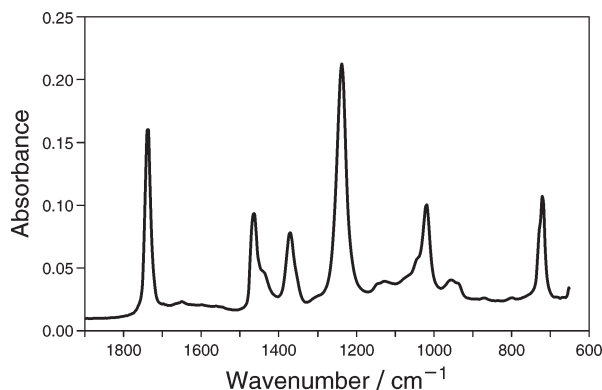


Figure 1. FTIR-ATR spectrum of a sample of poly(ethylene-co-vinyl acetate) that contains 18% vinyl acetate.

element made of zinc selenide. The angle of incidence of the radiation onto the element was 45° .

Absorbance data were used as the y axis of the calibration curve, similar to the Mathias experiment (18): the height of the C—O peak at 1020 cm^{-1} due to the presence of the vinyl acetate copolymer was divided by the height of the C—H peak at 720 cm^{-1} due to the presence of polyethylene. However, instead of plotting the percent vinyl acetate on the x axis, as was done in the Mathias experiment, the ratio of the percent vinyl acetate to percent polyethylene was plotted. Peak heights were measured using the “peak height” tool in the software of the FTIR spectrometer. However, good results can also be obtained by exporting the spectra as ASCII files from the FTIR spectrometer and using Excel to measure the peak heights. If this is done, a baseline can be obtained by averaging the absorbance values of the two “valleys” on either side of the peak of interest.

If an unknown sample is analyzed, two equations are needed to solve for the two unknowns: the concentrations of the two copolymers. A sample calculation is given in the supporting information.

Results and Discussion

Students learn that it is important to get good contact between the sample and the ATR element. The commercial sample beads are hard and little contact is obtained between the beads and the ATR element, even when pressure is applied. In addition, because the sampling element is 80 mm long by 10 mm wide and has 10 reflections, increasing the area of the sample on the element increases the measured absorbance values in the spectra. Melting some beads together provides a smooth sample surface and increases absorbance values by a factor of about 10 over spectra with the same number of beads that have not been melted. Increasing the absorbance values in the spectra increases the signal-to-noise ratio of the spectra. A spectrum of poly(ethylene-co-vinyl acetate) that contains 18% vinyl acetate sample is shown in Figure 1.

Students also observe the effect of the internal standard in correcting for varied contact between the sample and the ATR element. First, a calibration curve without an internal standard is prepared. A plot of the absorbance of the analyte (vinyl acetate) versus concentration of the analyte (percentage) is shown in Figure 2. Second, students make a calibration curve using the 720 cm^{-1} polyethylene peak as an internal standard. They begin

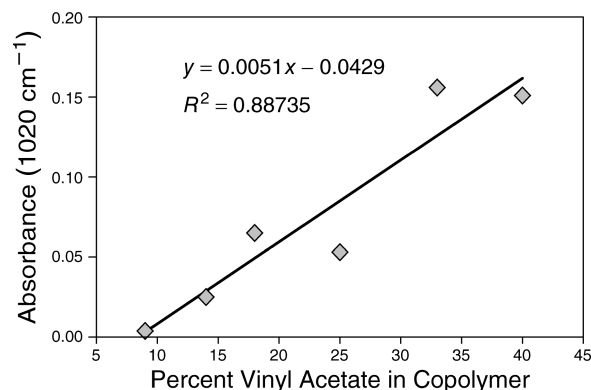


Figure 2. Calibration curve of absorbance of the vinyl acetate peak at 1020 cm^{-1} versus percentage of vinyl acetate in the copolymer.

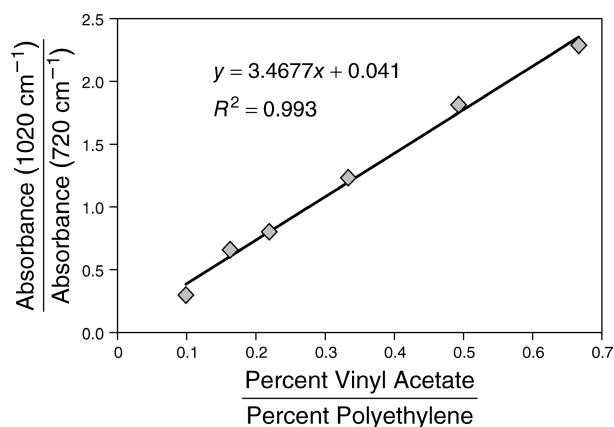


Figure 3. Calibration curve, using the same data as in Figure 2, when an internal standard is used.

with eq 1 (2):

$$\frac{\text{Analyte Signal}}{\text{Analyte Conc.}} = \text{Relative Response Factor} \left(\frac{\text{Internal Standard Signal}}{\text{Internal Standard Conc.}} \right) \quad (1)$$

which can be rearranged to

$$\frac{\text{Analyte Signal}}{\text{Internal Standard Signal}} = F \left(\frac{\text{Analyte Conc.}}{\text{Internal Standard Conc.}} \right) \quad (2)$$

Note that when eq 2 is used to make a calibration curve, the slope of the calibration curve equals the relative response factor, F , between the analyte and the internal standard. The experimental data in Figure 2 after the y axis has been converted into the signal ratio and the x axis has been converted into the concentration ratio is shown in Figure 3. Because the effect of varied sample contact with the ATR element has been corrected, the plot is more linear. Students who made a plot similar to Figure 3 were given the 25% standard as an unknown and got a result of 26%. It would also be possible to use commercial products made from poly(ethylene-co-vinyl acetate) for quantitative analysis.

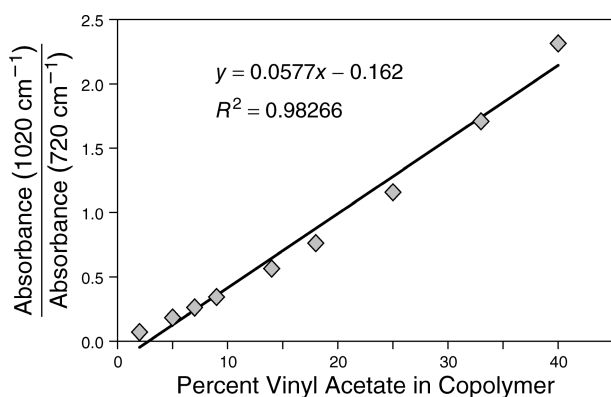


Figure 4. Theoretical data if the x axis is not a concentration ratio.

In the original form of this experiment, the x axis equals the percentage of vinyl acetate, rather than the ratio of the percentage of vinyl acetate to that of polyethylene. The x axis can equal the concentration of the analyte only if the concentration of internal standard is constant. Equation 2 is used to show the theoretical results when the x axis is the concentration of analyte, rather than the ratio of analyte concentration to internal standard concentration. The calibration curve is shown in Figure 4. The relative response factor used in eq 2 was the experimentally measured slope from Figure 3. Note that the curve will not be perfectly linear and the y intercept will not be exactly zero. Equation 2 shows that the signal ratio is dependent on the concentration of the internal standard. If the concentration of internal standard is not constant in the experiment, the calibration curve cannot be linear unless the x axis is a concentration ratio.

Hazards

Care must be taken when handling the hot samples. There is limited evidence of a carcinogenic effect with poly(ethylene-co-vinyl acetate).

Conclusions

This experiment can be used in analytical chemistry courses to teach students how to make an internal standard calibration curve, even when the concentration of the internal standard is not constant. Students also gain hands-on experience with an ATR accessory. Through minimal sample preparation, students learn the value of increasing the contact between a sample and an ATR element.

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Supporting Information Available

Instructor notes; experimental procedure for the students. This material is available via the Internet at <http://pubs.acs.org>.