Liquid Phase Quantitative Analyses Using Axsun's Targeted-Range Near-Infrared Spectrometers

APPLICATION NOTE

Introduction

Quantitative analysis using optical spectroscopy is based on the Beer-Lambert Law[1], which states that there is a linear relationship between how much light is absorbed by a sample and the product of the concentration of the absorbing species and its pathlength. Infrared quantitative analyses typically involve the measurement of many samples with varying concentrations, known as the training set. A calibration is calculated from these spectra. This may be a simple curve, or may involve a 'chemometric' procedure, whose basis is commonly principal components analysis[1]. From this calibration, the concentration of the substance in an unknown may be predicted. A calibration set is needed because the presence of component B can perturb the spectrum of component A, and these procedures account for the resulting non-linearity in spectral response. A chemical case where this non-linearity is most pronounced, and therefore analysis needs special care, is in hydrogen-bonded systems, for instance alcohols and water. This applications note describes two such systems: ethanol and water over a limited concentration range, and methanol and water over a range from pure water to pure methanol.

System 1: Ethanol in Water

Ethanol content is clearly the single most important parameter in alcoholic beverages. But ethanol is an important industrial chemical as well, and is used in a wide variety of industrial processes. Examples include: as a fuel and gasoline additive, as a solvent in the manufacture of varnishes and perfumes, in the preparation of essences and flavorings, in many medicines and drugs, and as a disinfectant. For many economic and regulatory reasons, convenient, accurate, and fast quantitative determination of ethanol in mixtures is important. With the increased importance of quality assurance in industries, NIR spectroscopy has become an important quantitative tool for solvent characterization, due to minimal required sample preparation, and fast data acquisition.[2]

Experimental

Mixtures of alcohols in water (0-10 wt % with increments of 0.5 wt % for ethanol and 0-100 wt % with increments of 5 wt % for methanol) were prepared. The spectra were measured at 2 nm spectral resolution, with 0.5 nm data point spacing, over the spectra region 1600-1800 nm, with the co-addition of 64 scans. A fiber-coupled holder accommodated a fixed 2.0 mm pathlength quartz liquid cell (Figure 7), as well as the input and output fibers of the NIR Analyzer. The temperature of the water-alcohol mixtures was not controlled for this study; the samples were measured at ambient temperature. For this reason, shifting and broadening of the O-H bands can occur. To prevent this, a thermostatted sample block could be used; alternatively, the measured temperature of the sample can be input as a second variable, and both the temperature and concentration can be predicted.

Data Analysis

The spectrometer was controlled by Axsun's ActiveX spectrometer control, hosted by GRAMS AI (Thermo Electron, Waltham, Mass). GRAMS AI Partial Least Squares multivariate statistical method (PLS) was calculated to predict the concentrations of alcohol in water. Two different models were created for each chemical system: the first using the spectra as collected, and the second after the spectra had been subjected to Savitsky-Golay second derivative processing, using a 21-point smooth. No other mathematical operations were performed on the spectra.

Qualitative Analysis

Figure 9 shows the near-infrared spectra of water-ethanol mixtures described above. The strong features in the 1650-1750 nm region consist of number of overlapping bands due to combinations of CH_2 and CH_3 symmetric stretching bands; these bands show a wavelength significant shift with an increase in the concentration of ethanol. In the water-ethanol mixture, the band at 1694 nm has been assigned as the first overtone of the CH_2 stretching modes. The band at 1732 nm is assigned as the first overtone of the CH_3 symmetric stretching modes.[2] Figure 10 shows the spectra in Figure 9, after they have been processed to their second derivatives.

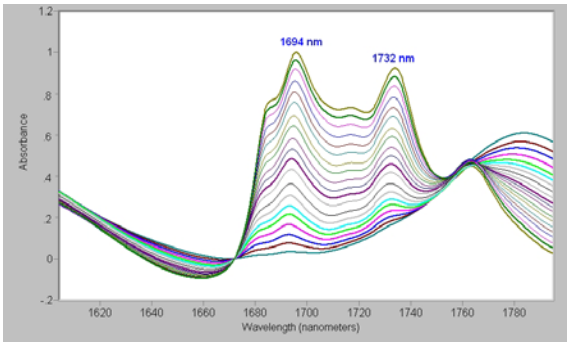

Figure 9. The near-infrared spectra of water-ethanol mixtures measured over ethanol concentration of 0-10 wt % with increments of 0.5 wt % at ambient temperature of 25^{0}C in the 1600-1800 nm region.

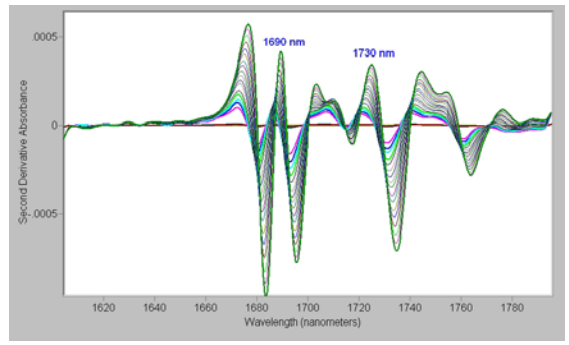

Figure 10. The second derivative near-infrared spectra of water-ethanol mixtures measured over ethanol concentration of 0-10 wt % with increments of 0.5 wt % at ambient temperature of 25^{0}C in the 1600-1800 nm region.

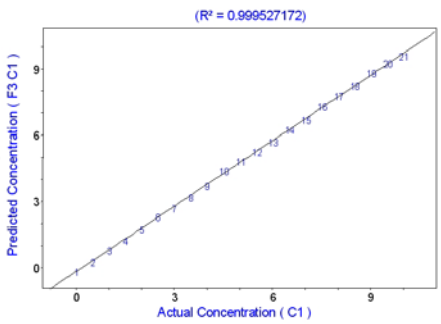

Figure 11. Fit between actual and predicted ethanol concentration for the water-ethanol mixtures with no spectral preprocessing.

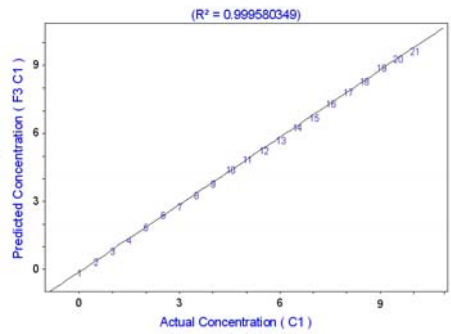

Figure 12. The fit between actual and predicted ethanol concentration for the water-ethanol mixtures with second derivative spectral preprocessing.

Quantitative Analysis

A linear prediction model, using partial least squares (PLS), was developed for the water-ethanol mixture. The results are presented as actual, predicted, and residual concentrations of ethanol in the water-ethanol mixtures, in Table 1. Figure 11 shows the fit attained for the actual versus predicted ethanol concentration. For this model, the accuracy (defined as Standard Error of Cross Validation or SECV) was 0.07%, and a correlation coefficient of 0.9995, where 1.0 is a perfect fit, was obtained. Table 1 also shows the results for the second derivative PLS, and the fit between the prepared volume % ethanol and the estimated volume % ethanol is shown in Figure 12. For this second derivative model, the SECV of the 0.06% with a correlation coefficient of 0.9996 was obtained, with no significant difference from the first model. These were our estimated sample-preparation errors. Therefore, using both of these models, we were able to obtain a very accurate determination of the proportion of ethanol in these water-ethanol mixtures.

Table 1. PLS results for actual vs. predicted concentrations of ethanol in water-ethanol mixtures, using spectra in the 1600-1800 nm range, first with no spectral preprocessing, and, second, using second derivative spectral preprocessing.

	No Preprocessing		Second Derivative Preprocessing	
Actual Concentration	Predicted Concentration	Residual Concentration	Predicted Concentration	Residual Concentration
0.00	0.05	0.05	0.02	0.02
0.50	0.50	0.00	0.49	-0.01
1.00	1.01	0.01	0.99	-0.01
1.50	1.47	-0.03	1.47	-0.03
2.00	2.00	0.00	2.03	0.03
2.50	2.54	0.04	2.56	0.06
3.00	2.92	-0.08	2.95	-0.05
3.50	3.40	-0.10	3.46	-0.04
4.00	3.96	-0.04	3.97	-0.03
4.50	4.65	0.15	4.61	0.11
5.00	5.09	0.09	5.06	0.06
5.50	5.50	0.00	5.47	-0.03
6.00	5.94	-0.06	5.92	-0.08
6.50	6.52	0.02	6.49	-0.01
7.00	6.94	-0.06	6.90	-0.10
7.50	7.55	0.05	7.54	0.04
8.00	8.01	0.01	8.01	0.01
8.50	8.51	0.01	8.52	0.02
9.00	9.08	0.08	9.09	0.09
9.50	9.51	0.01	9.51	0.01
10.00	9.85	-0.15	9.86	-0.14

Spectrometer Non-Linearity

Spectrometers must themselves have a linear optical response to perform quantitative analyses. For a variety of reasons, this response will always deviate from linearity at some absorbance value. In many spectrometers this is around 2 or 2.5 absorbance units, and the reasons for this can depend on the spectrometer technology employed. Linearity is generally a function of the dynamic range of the spectrometer. In diode array spectrometers, this is a function of the well depth, the dark current and the A/D converter used, while in Fourier transform spectrometers, it depends on the A/D converter and 'hidden' parameters governing apodization and phase correction.

'Stray light' will prevent a band from approaching zero transmission as the concentration or pathlength of the species increases. Stray light can be actual light falling on the detector that has not passed through the sample, or poorly compensated 'dark current' in the detector itself. This effect can be seen in the following synthetic spectra examples. Figure 1 shows the expected increase in absorbance of a band as pathlength increases. This example shows a band with 3AU absorbance at the longest pathlength. Figure 2 shows the effect on the same system, with the same pathlengths, if there is a 1% stray light contribution. The maximum absorbance that can now be observed is only 2AU, and the band shape appears perturbed. This spectrometer will not perform well in quantitative analysis. At low absorbance values the calibration curve (in this case absorbance versus pathlength) starts to deviate significantly form linearity above 1AU (Figure 3), and exhibits severe curvature as 2AU is approached (Figure 4). From this synthetic data it can be seen that proper design of a spectrometer to minimize 'stray light' effects is critical to good quantitative analyses.

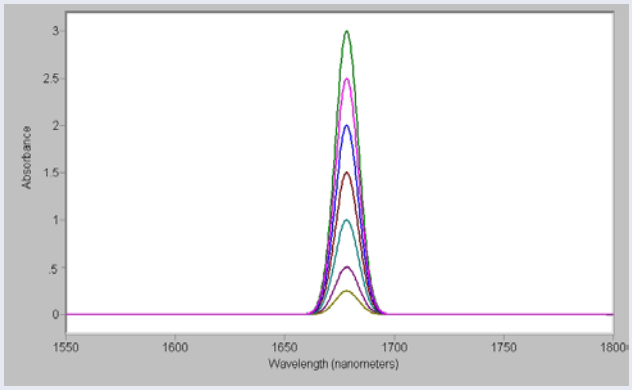

Figure 1. Synthetic spectra with no 'stray light' showing spectra at 3.0 AU.

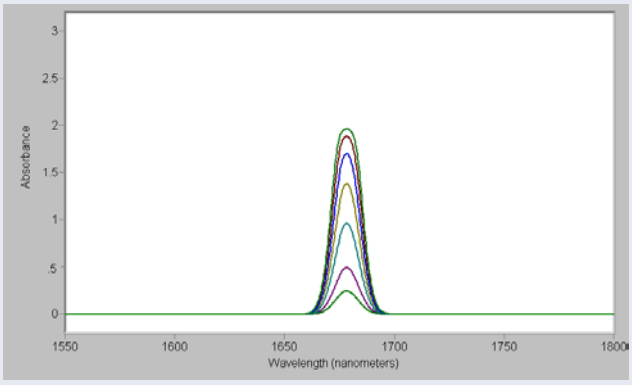

Figure 2. Synthetic spectra with 1% 'stray light': the maximum absorbance that can be reached is 2.0 AU.

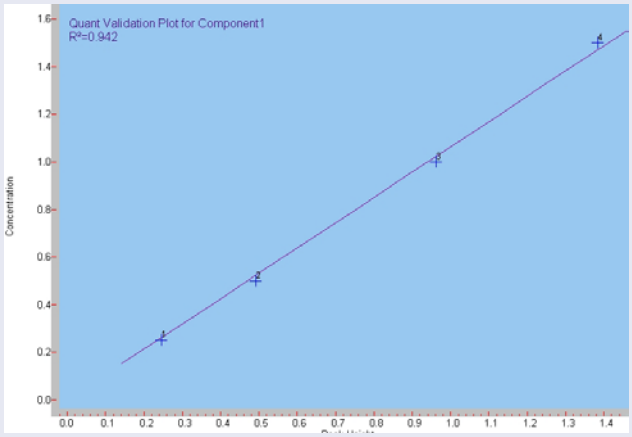

Figure 3. With 1% stray light, there is significant deviation from a linear plot at 1.5 AU.

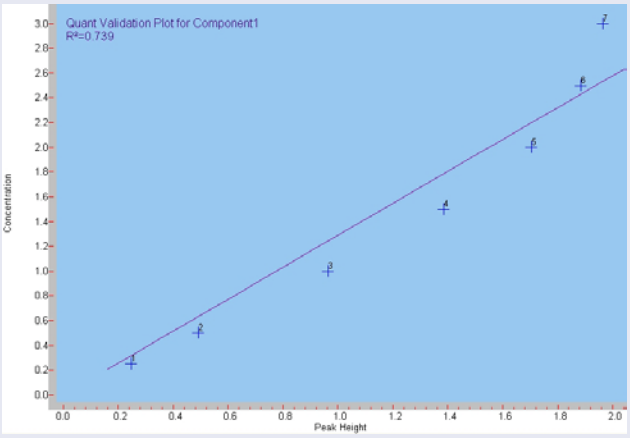

Figure 4. With 1% 'stray light' the calibration curve becomes very non-linear, and an absorbance limit is reached.

System 2: Methanol and Water

Methanol is another important industrial chemical, with myriad uses, ranging from feedstock to antifreeze, fuel, industrial solvent, as well as fine chemical and pharmaceutical precursor. Figure 13 shows the near-infrared spectra of water-methanol mixtures measured over a methanol concentration of 0-100 wt % (i.e., pure methanol to pure water), with increments of 5.0 wt %, at ambient temperature, in the 1600-1800 nm region. In Figure 14, the corresponding second derivative spectra are shown. The band at around 1700 nm is due to the first overtone of the CH_3 group stretching mode.

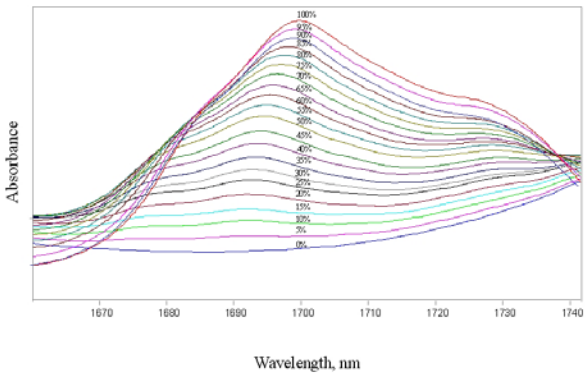

Figure 13. The near-infrared spectra of water-methanol mixtures, measured over methanol concentration of 0-100 wt %, with increments of 5.0 wt %, in the 1600-1800 nm region.

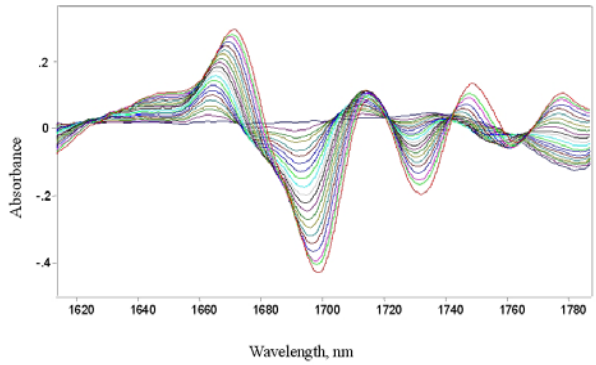

Figure 14. The second derivative near-infrared spectra of water-methanol mixtures measured over methanol concentration of 0-100 wt %, with increments of 5.0 wt %, in the 1600-1800 nm region.

The same PLS approaches were used for the methanol-water mixtures as the ethanol-water mixtures, including both original and second derivative spectra. The results for actual, predicted and residual concentrations are shown in Table 2. Figure 15 shows the fit attained for the actual vs. predicted methanol concentration for the original spectra model. For this model, the accuracy (SECV) of the 0.77% with a correlation coefficient of 0.99928 (1.0 is a perfect fit) was obtained. The fit using second derivative spectra is shown in Figure 16. For this model, the SECV of the 0.80% with a correlation coefficient of 0.99930 was obtained, a slight improvement. Normally, it would be unusual to construct a calibration for this vast concentration range (0 – 100%), especially for hydrogen-bonded species where significant deviations from linearity are to be expected. Nonetheless, this calibration worked well. If the system under study were confined to narrow concentration ranges, as in the ethanol case, then a much improved SECV would be possible.

Table 2. PLS results for actual vs. predicted concentrations of water-methanol mixture in the 1600-1800 nm spectral range, using no spectral preprocessing and using second derivative spectral preprocessing.

	No Preprocessing		Second Derivative Preprocessing	
Actual Concentration	Predicted Concentration	Residual Concentration	Predicted Concentration	Residual Concentration
0.00	-1.80	-1.80	-0.80	-0.80
5.00	5.90	0.90	6.00	1.00
10.00	11.20	1.20	11.20	1.20
15.00	14.40	-0.60	14.30	-0.70
20.00	20.50	0.50	20.10	0.10
25.00	24.40	-0.60	24.30	-0.70
30.00	29.50	-0.50	29.30	-0.70
35.00	35.00	0.00	34.70	-0.30
40.00	39.60	-0.40	39.60	-0.40
45.00	45.40	0.40	45.20	0.20
50.00	50.60	0.60	50.80	0.80
55.00	54.60	-0.40	55.00	0.00
60.00	59.90	-0.10	60.30	0.30
65.00	65.10	0.10	65.20	0.20
70.00	70.20	0.20	70.20	0.20
75.00	74.80	-0.20	74.90	-0.10
80.00	79.80	-0.20	79.80	-0.20
85.00	84.90	-0.10	84.70	-0.30
90.00	91.50	1.50	90.90	0.90
95.00	93.20	-1.80	93.20	-1.80
100.00	98.2	-1.80	101.90	1.90

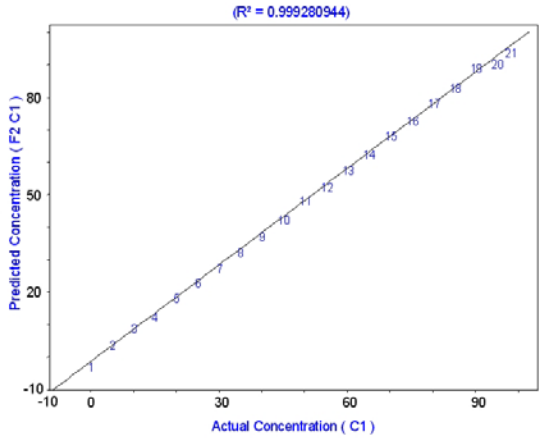

Figure 15. Fit between actual and predicted methanol concentration for water-methanol mixture with no spectral preprocessing.

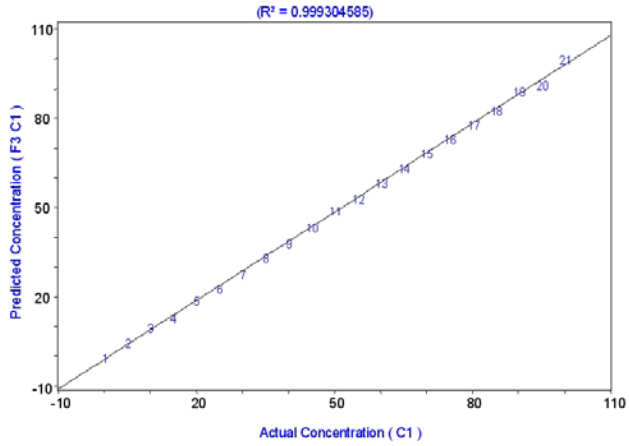

Figure 16. The fit between actual and predicted methanol concentration for water-methanol mixture with second derivative spectral preprocessing.

Axsun's Spectrometer Linearity

The combination of a very bright source, cooled, targeted-cut-off detectors and the widest possible dynamic range (24 bits in a single scan), enable Axsun spectrometers to perform linearly to 4 absorbance units. This was then verified experimentally using NIST-traceable neutral density filters, and a Lambert Law plot using a pure substance with varying pathlengths.

Spectra of neutral density filters, having 0.03, 0.5, 1.2 and 2.4 Optical Density (OD), were measured in transmission in the 1550-1800 nm range using Axsun's NIR Analyzer (Figure 5), at 2 nm resolution. These NIST-traceable filters were purchased from Avian Technologies (Wilmington, OH). A neutral density filter has a constant optical density (or absorbance) across a wide range. A plot of measured versus actual optical density is shown in Figure 6, and it can be seen that this plot is linear, with a correlation co-efficient of 0.99993, or as close to unity as could be expected.

Figure 5. Axsun's NIR Analyzer

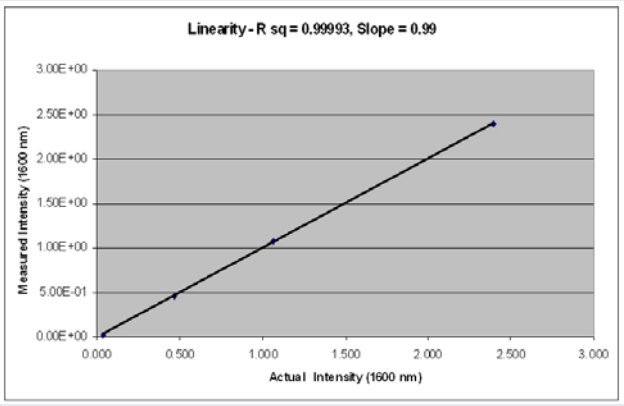

Figure 6. Plot of absorbance linearity for Axsun Analyzer using ND filters of 0.03, 0.5, 1.2 and 2.4 OD (actual intensity vs. measured intensity).

Figure 7. The fiber-optic coupled cell holder, and typical liquid cells.

A second test was performed using a pure compound (isopropyl acetate), which exhibited a single strong band in the region of interest, at different pathlengths. Isopropyl acetate was contained in quartz cuvette cells with pathlengths of 1, 2, 5, 10 and 20 mm. A fiber-coupled holder accommodated the liquid cells (Figure 7), as well as the input and output fibers of the NIR Analyzer. The spectra were measured at 2 nm spectral resolution, over the spectral region 1555-1800 nm. 50 scans were co-added to give each final spectrum. Light was delivered to the cuvette holder via a single-mode fiber and 5.5 mm diameter collimator, and returned to the single-element InGaAs detector via a second collimator and 600 micron diameter multimode fiber. The same cells and samples were used to measure the spectra on a modern, commercial, FT-NIR spectrometer.

The heights of the band in the spectrum at each pathlength were calculated, and are shown in Figure 8. The band heights at the shortest pathlength were identical for the FT-NIR and Axsun Analyzer. This was taken as the theoretical, and was scaled with pathlength to indicate the theoretical height at each pathlength. The results show that the Axsun Analyzer is linear to almost 4 AU. After that it measures less than the theoretical, consistent with extremely low levels of 'stray light' in the experiment. However, the commercial FT-NIR shows an opposite deviation at much lower absorbance levels, starting at just over 2 AU, most likely due to limited dynamic range in the centerburst region coupled with phase correction and apodization effects.

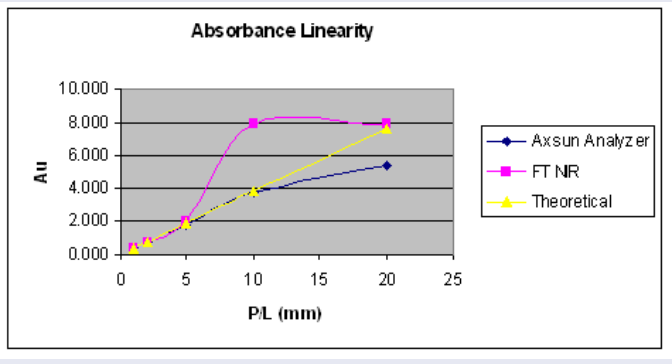

Figure 8. Plot of absorbance linearity for Axsun Analyzer, FT NIR spectrometer and theoretical values (transmission cell pathlength vs. absorbance units).

Conclusions

Axsun's NIR spectrum analyzers provide extremely accurate, high-speed data acquisition with reproducible spectral output, for real-time measurements at high spectral resolution. With these features, the Axsun NIR Analyzer supports qualitative and quantitative analysis of liquid-phase systems. All organic solvents have very specific overtone bands in the first carbon-hydrogen stretching region, from 1600 to 1800 nm and this targeted range is sufficient for these analyses.

Designed for process applications that require analysis of the first overtone CH and/or first overtone OH spectral range, Axsun's near-infrared micro-optical spectrometers feature a rugged, stable, and versatile platform, ideal for in-line process applications. Offering non-destructive, no sample preparation, process-based NIR measurements, Axsun's low cost, easy to use, miniature analyzers are well-suited for industrial QA/QC.

References

1. See, e.g., James H. Duckworth, "Spectroscopic Quantitative Analysis" in "Applied Spectroscopy: A Compact Reference for Practitioners," Jerry Workman, Jr. and Art Springsteen, Eds., Academic Press, 1998.

2. Sonja Engelhard, Hans-Gerd Löhmannsröben, Frank Schael, "Quantifying Ethanol Content Beer Using Interpretive Near-Infrared Spectroscopy," Appl. Spectroscopy, **58**, 1141-1264 (2004).

3. Daisuke Adachi, Yukiteru Katsumoto, Harumi Satol, Yukihiro Ozaki, "Near-Infrared Spectroscopic Study of Interaction Between Methyl Group and Water in Water-Methanol Mixtures," Appl. Spectroscopy, **56**, 357-361 (2002).

4. See, e.g., ASTM E 1655, Standard practices for infrared Multivariate Quantitative Analysis", ASTM, West Conshohocken, PA, 2000.

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