

Fourier transform near infrared spectroscopy: its place among quantitative near infrared instruments and its utility for qualitative analysis

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Introduction

Interferometry has made an impact on much of spectroscopic instrumentation. Fourier transform spectroscopy has taken over the mid-infrared because with the availability of dependable interferometers, lasers to track the mirror motion, fast Fourier transforms, fast low cost computers and it was possible to make use of the multiplex (Fellgett) advantage and the throughput (Jacquinot) advantage. This was particularly applicable to get high resolution, excellent wavelength reproduction and to conserve signal intensity.

In the near infrared (NIR) region of the spectrum, there was not perceived to be a problem of spectroscopic resolution since the quantitative analysis done on solids and liquids was based mostly on wide band data. Also, bright sources in the NIR and sensitive detectors made conservation of signal less of an issue than it had been in mid-infrared. The key to success in quantitative NIR analysis using empirical statistically based expressions has been excellent wavelength reproduction and the ability to discriminate between small differences in intensity. One early concern regarding quantitative Fourier transform (FT) NIR was that since the detector was bombarded by so many photons, the ability to discriminate between small differences might be beyond the data registers available in the digitization process.

Several changes have come about since the first two decades of modern NIR spectroscopy where converted mid-infrared interferometers were only rarely used in the NIR range. The cost of more bits in the A/D converter has come down. Low cost, high speed computers are available. An interest has developed in the analytical use of narrow band NIR data in the petroleum and chemical industries where the reproduction of band shape may be of concern. Another development is that the classical spectroscopy instrument manufacturers have realized the market potential of NIR as complimentary to their traditional area and have entered the field. For a more detailed discussion of FT-NIR instrumentation refer to the review chapter by Wetzel.¹

Low cost and industrially rugged FT-IR benches were introduced approximately a decade ago. The author of this paper has had the opportunity to compare in his laboratory a Bomem MB-155 equipped with a tungsten source and a PbSe detector with results from a homemade acousto-optic tunable filter spectrometer (TFS) and with a commercial grating monochromator instrument, the Technicon I/A 500. The results were reported in the Cleveland FACSS meeting in 1990.² In that report, the quantitative NIR data was all obtained at Kansas State University but the *a priori* theoretical consideration and sample calculations presented were contributed by Buijs and Baudais of Bomem. Some of the illustrations from that presentation are included here.

Theoretical considerations

FT-NIR offers several advantages. These include: sensitivity in relation to throughput and resolution, wavelength accuracy and precision, ordinate accuracy and precision and resolution capability. Figure 1 illustrates the projected increase in signal-to-noise (S/N) of a dispersive system with an increase in throughput as an orderly progression. With an interferometer, the S/N is greater and the graph is steeper but a flattened maximum (saturation) is projected when the limit of the dynamic range of the A/D converter is reached. This would occur at different signal-to-noise levels for broad range and narrow range detectors as shown in the diagram. The result is that the A/D dynamic range limitation negates efforts to increase signal-to-noise beyond that level by increasing throughput.

Although resolution is not often an issue in NIR, and the nominal bandpass (band width at half height) on most grating monochromator instruments tends to be of the order of 10 nanometers throughout the range. A modest estimate of resolution for an FT-NIR is 4 cm^{-1} or 0.4 nm at 1000 nm . This is equivalent to 1.6 nm at the 2000 nm region. These bandpass values for FT-NIR at either wavelength are considerably greater than typically used on a grating monochromator operating with fairly large entrance and exit slits to allow reasonable throughput.

There are a number of reasons why the wavelength accuracy and precision are an inherent problem on dispersive systems. In the case of the interferometer, this depends on the distance of the movement of the mirror and on the assurance of the mirror position. Since the mirror is tracked with a laser running parallel to infrared radiation, the mirror position is verified at each movement and any deviation from this produces a "tilt" error message which avoids getting an erroneous reading. Note that the estimated uncertainty in optical frequency, $\Delta\nu$, for FT-NIR is $\pm 0.00125 \text{ cm}^{-1}$ at $10,000 \text{ cm}^{-1}$ compared to $\pm 5 \text{ cm}^{-1}$ for a typical dispersive system at the same frequency.

According to the relationship suggested by Buijs, at a particular spectral element:

$$S/N = \frac{1}{2}\sqrt{N} (S/N_0) (A_j/A_t)$$

where S/N is at the j th spectral element, N is the number of interferogram measurements, S/N_0 is the signal-to-noise at the centerburst, A_j is the spectral intensity at the j th spectral element and A_t is the total intensity of all the spectral elements. If we assume, for example, that the total spectral

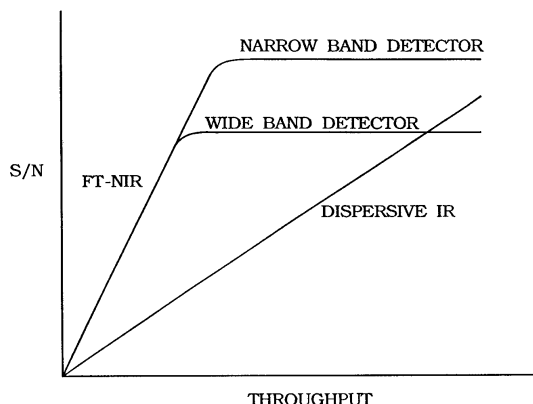


Figure 1. Predicted increase in signal-to-noise at increasing throughput for dispersive and FT-NIR instruments.

intensity for all spectral elements is 200,000 at 4000 cm^{-1} and the signal-to-noise at the centerburst is 10,000 peak to peak, if N , the number of interferogram measurements is estimated at 31,600 for 4 cm^{-1} resolution (assuming a double-sampled, double-sided interferogram) and if the spectral intensity, A , at 8000 cm^{-1} is equal to 100, then solving the suggested relationship, the signal-to-noise at 8000 cm^{-1} is equal to 444 peak to peak.

A characteristic of interferograms is that there is a large centerburst localized in a small region of the interferogram and there are small signals elsewhere. What this means in practice is that the narrow line precision is approximately 100 times better than baseline precision. Therefore, a 2% baseline shift results in only a 0.02% peak area change. For this reason, the precision and accuracy of the intensity value from the scanned data is excellent. An interferogram that has 10^4 points has only a few points at the centerburst region. Detailed spectral reproducibility is based on the entire set of 10^4 points. To summarize, the predictable FT-NIR advantages over typical dispersive NIR systems are wavelength accuracy and precision, signal intensity accuracy and precision and resolution capability.

Experimental

Previous quantitation results of 1991, using the Bomem MB-155 FT-NIR (Bomem/Hartmann and Braun Quebec, Canada), were obtained on optically difficult industrial waste samples. These samples contained dissolved material, were often present in two or three phases and solids were suspended in some of the liquid phases so they presented both a sampling and an optical challenge. A calibration was done for calorimetric values of BTUs per pound for potential incinerator feeds. Figure 2 shows the results of analyzing the same set of samples on three different instruments. For each instrument, a unique calibration was produced. Determination results based on these respective calibrations from the readings taken with the corresponding instruments are shown^{3,4} in comparison to bomb calorimeter data on the same specimens. Figure 3 shows comparative residual plots for the three different instruments involved.

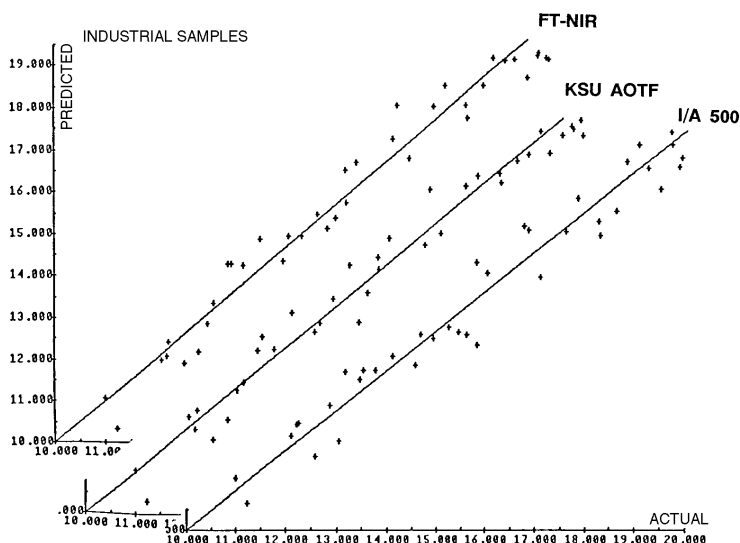


Figure 2. Predicted vs actual values for the calibration (SEC = 0.542) used on industrial waste samples with a range of 10.9–19.9 BTU/lb for three different NIR instruments.

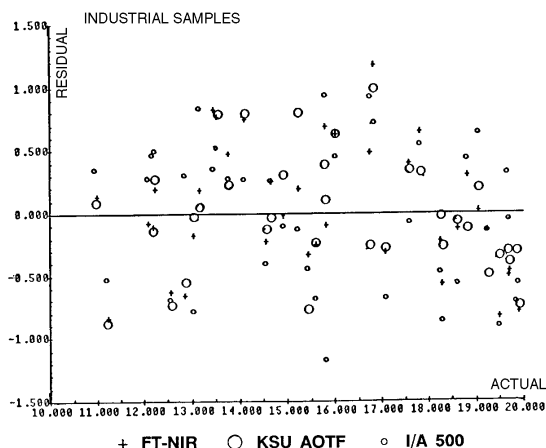


Figure 3. Residual plot for the calibration ($SEC = 0.542$) used on industrial waste samples with a range of 10.9–19.9 BTU/lb for three different NIR instruments.

Recently, in preparation for this conference, it was desirable to get additional new first hand quantitative data with a classical FT instrument. FT-NIR instruments designed specifically for rapid sorting are not of concern in this report. A series of experiments was done to challenge a Magna series (Nicolet Instruments, Madison, WI) FT instrument equipped with a CaF_2 beamsplitter, a special rapid response PbS detector, a quartz tungsten halogen source and a Nicolet fiber optic probe with a range up to 2200 nm. The fiber optic probe, used for all of the spectra obtained, consisted of a scrambled bundle of optical fibers. Some of the fibers conducted incident radiation to the specimen, the other fibers returned the reflected radiation to the detector. Referencing was done by scanning a ceramic disk housed in a special mount where the probe rested between sample probings. The reference spectrum was stored in the computer and recalled automatically for ratioing single beam scans to produce a transmittance or absorbance plot. Typically, in the work, 64 scans were coadded. No effort was made to emphasize speed in this series of experiments.

Liquid chemical mixtures were optically measured using the fiber optic probe against a 0.5 mm thick cell equipped with a quartz window and an aluminum reflective surface providing a 1 mm path. The same arrangement was used for quantitative data on a set of emulsions (cutting oil) of various percentages of oil. Polymer films were sprayed onto glass microscope slides or metal panels and cured at a prescribed temperature and time appropriate for the polymer. Polymer films coated on glass microscope slides were sampled with the dull side of kitchen grade aluminum foil used as a diffuse reflector behind the glass slide. Polymer coatings on phosphated steel were sampled similarly with the back reflection coming off of the inorganic layer on the steel underlying the polymer. Reflection off bone and back through a layer of soft tissue was used in the case of the human subject. Diffuse reflectance sampling of solids was done by placing the probe surface in direct contact with the solid material. In a special case, the probe was placed against a sealed glass bottle containing granular solid pharmaceutical concentrate from which a solution suitable for injection was to be prepared. In this instance, diffuse reflectance from the solid was collected through the glass.

Crystalline solids and powders were used with no sample preparation. Milling streams from the Kansas State University pilot flour mill were probed without further treatment after stirring the mixture in the sample bottle. Protein was determined by the Kjeldahl method to provide data

Table 1. Results of five component mixture analysis with FT-NIR.

Component	SEC	Range	Corr. Coef. (r)
Benzene	0.119	0–50	0.9999
Cyclohexane	0.147	0–20	0.9997
Octanol	0.284	0–40	0.995
Butylamine	0.181	0–28	0.998

for the local FT-NIR calibration and for verification tests. Whole wheat samples were prepared for FT-NIR fiber optic probe protein determination by grinding on a cyclotech grinder equipped with a 1 mm screen. Thorough mixing of the resulting heterogeneous granular sample was done prior to each probing. Calibration and verification was done with Kjeldahl data obtained in the KSU Grain Science routine analytical laboratory.

Synthetic mixtures of liquid organic compounds were made using an analytical balance to determine composition by weight. Industrial emulsions (cutting oil) in the oil concentration range of 1–30% were made by weight, mixing oil containing emulsifying agent with distilled water and shaking until a suspension was achieved.

Results and discussion

A spectrum obtained is shown (Figure 4) for paraffin oil in order to serve as a marker for the first C–H combination (extreme right). Next are the first C–H overtone, the second combination and the second overtone at the extreme left of the figure. With these spectral landmarks in mind, let us examine the baseline corrected spectrum of wheat gluten in Figure 5, where we observe particularly the characteristic amide groups at 4611 and at 4865 cm^{-1} . Those particular bands are absent in the spectra of both starch (Figure 6) and cellulose (Figure 7) where a strong band occurs at approximately 4762 cm^{-1} . A marked distinction is evident between the cellulose and the starch where a doublet occurs for cellulose at 4240 and 4388 cm^{-1} whereas for the starch, the main absorption is at 4388 cm^{-1} with a very minor shoulder next to it. From the baseline corrected spectrum in Figure 8 for wheat germ (known to be high in lipid) notice the carbohydrate band is

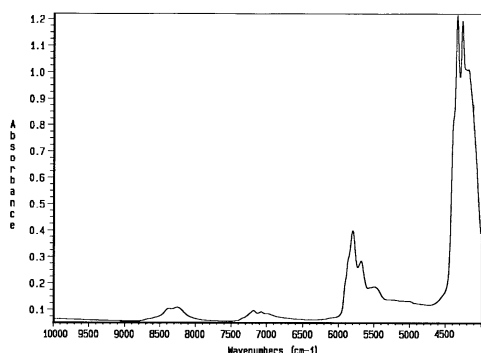


Figure 4. Spectrum of paraffin oil collected by FT-NIR.

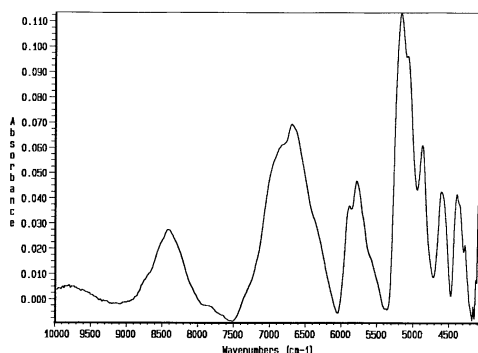


Figure 5. Baseline corrected wheat gluten spectrum.

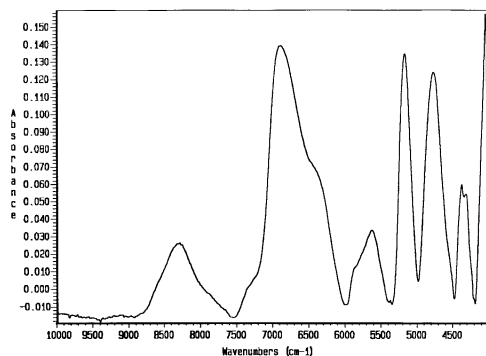


Figure 6. Baseline corrected wheat starch spectrum.

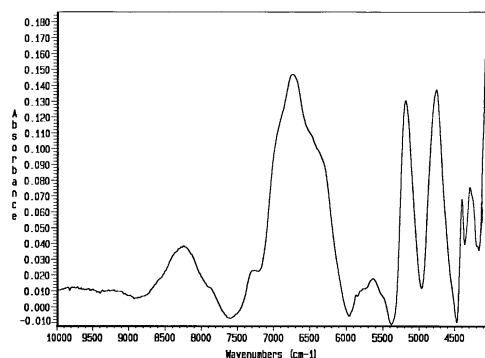


Figure 7. Baseline corrected cellulose spectrum.

diminished and bands at 5810 cm^{-1} are more pronounced and changes have occurred at 4388 cm^{-1} coincident with the difference in chemical composition. All of these powdered or granular solids were scanned by dipping the probe directly into the powder. In this case, the weight of the probe was used to achieve compression against the powder contained in a glass bottle.

One previously stated advantage of FT-NIR is that we have excellent reproduction of the frequency which allows us to perform spectral subtraction. This is illustrated by Figure 9, that is the spectrum produced by scanning a spectrum of Coffeemate™ regular and subtracting from it the spectrum of Coffeemate Lite™. The spectrum of the vegetable oil present shows the prevalent C–H bands that may be compared in wavelength to those in the paraffin oil in Figure 4. In a special case, spectra were obtained through a sealed glass bottle containing a sterile pharmaceutical preparation. Figure 10 shows the baseline corrected spectrum of the preparation. The spectrum of mannitol, known to be mixed with the drug, was subsequently subtracted (not shown) to enhance the spectral features of the active component. With the probe, it is possible to scan an Empore™ extraction disk (3M Minneapolis, MN). Figure 11 shows the absorption bands (4070 , 4677 , 6060 and 8825 cm^{-1}) from the spectrum of nitrobenzene revealed when the spectrum of a C-18 Empore™ disk was subtracted from the spectrum of a C-18 Empore™ extraction disk plus adsorbed nitrobenzene. The background spectrum includes hydrocarbon bands (4182 , 5682 , 5794 , 7087 and 8262 cm^{-1}) and a large band at 5261 cm^{-1} due to the silica substrate.

Quantitative analysis was attempted for a drug adsorbed onto Empore™ C-8 disks. This involved scanning a particular disk, drawing a solution with suction containing a pharmaceutical preparation through the solid phase extraction disk, allowing it to air dry and scanning the disk plus the adsorbed drug. Spectra produced by the spectral subtraction technique were obtained on a series of 30 solutions of known concentration on 30 prescanned disks and regressions were done. The resulting relationship indicated that spectral subtraction of solid phase extraction may potentially be used to semi-quantitatively determine material of very dilute aqueous solution (below detection limits). The practicality of this is compromised for the experiment reported because the size of the probe compared to the size of the disk does not allow full advantage to be taken of that concentration step. Although the sample is concentrated on the disk, the full disk cannot be measured with the present sized probe. This effort does illustrate that the use of spectral subtraction could come into play for quantitative analysis as well as for qualitative analysis.

Further quantitative experiments were done with a series of organic liquid mixtures. Five organic liquids, which had been used in our laboratory previously to test instruments designed

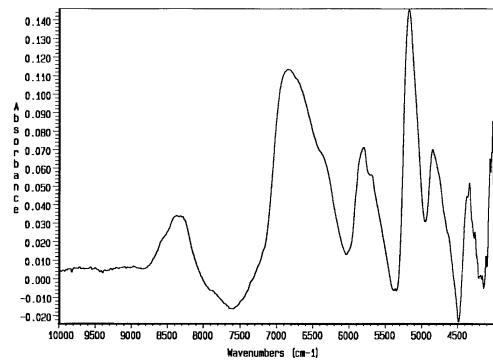


Figure 8. Baseline corrected wheat germ spectrum.

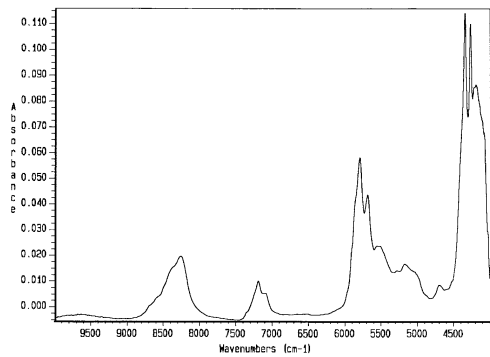


Figure 9. Subtraction result of Coffeemate Lite™ from regular Coffeemate™.

and built at Kansas State University, were composed of benzene, cyclohexane, octanol and butylamine in a paraffin oil petroleum base. The results for the five component mixture are shown in Table 1. These compare favorably with previous experiments in our laboratory using different mixtures of these same analytes in the same concentration range. Similar types of test mixtures previously used in our laboratory gave results on these respective compounds that were categorized as follows:

	Benzene	Cyclohexane	Octanol	Butylamine
AOTF	0	+	0	+
Dispersive	0	+	—	+

(+ = greater value, — = lesser value, 0 = nearly the same).

The results with the five component mixture are indicative of the optical performance of the instrument and of the ability of the detector to discriminate between small differences in intensity on that axis as well. This mixture was deliberately chosen for the lack of overlap among major absorption bands so that spectral resolution would not be a problem. They were also chosen for a certain degree of mutual solubility over a fairly large range of concentrations. In fact, we had “stacked the deck” initially by choosing these types of mixtures in order to increase the probability of quantitative success of the first FT instrument test that we did in the past.

Quantitative response of emulsions was done and a calibration was prepared vs composition by weight. The performance was comparable to results we have reported by a dispersive system on a similar set of synthetic samples.⁵ That calibration was successfully transferred to industrial samples collected from an aluminum transmission housing machining operation and compared to the conventional method used in that industry.

In routine usage, diffuse reflectance is very popular for NIR and the fiber optic probe eliminated the sample cup while allowing the use of this technique. All of the solid samples were tested by gently lowering the probe into a bottle of non-compressed solid sample allowing the weight of the probe to provide some compression on its own. Precision of resampling is a factor in solid sampling with a probe just as repack error is of concern with a closed sample cup. A certain amount of care is required but successful results were obtained. Using wheat flour milling streams, in the range of 7.8–15.7% protein, a standard error of calibration of 0.26 and standard error of performance of 0.33 resulted from three wavelength multiple linear regression equations for

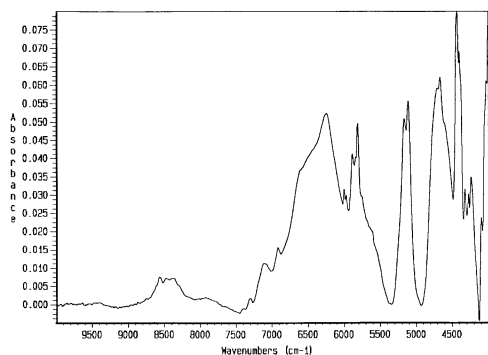


Figure 10. Baseline corrected spectrum of a pharmaceutical preparation collected through a glass bottle.

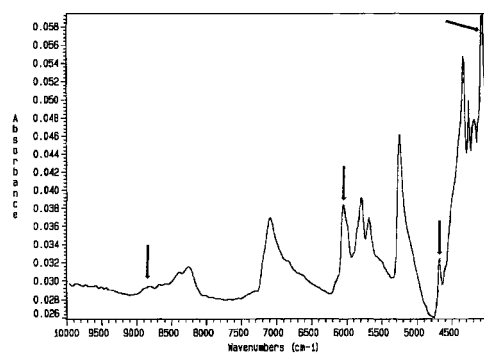


Figure 11. Spectrum resulting from the subtraction of an extraction disk from an extraction disk plus adsorbed nitrobenzene. Nitrobenzene bands are marked with arrows.

protein. A 0.24 value was produced using four PLS factors. These results were only slightly higher than those from a conventional filter or grating instrument used in our laboratory for these samples.

Ground whole wheat protein determinations resulted in an *SEC* of 0.28 when averaging was used for three replicate probings of the same sample. Calculating the *SEC* using individual probes resulted in a higher error of 0.39. It was apparent from this result that there was a considerable sampling error. The probe had a sampling diameter of 2–3 mm which is considerably less than would be used with a routine diffuse reflectance instrument. In a routine diffuse reflectance instrument with a sample cup, a field lens is put in the system to deliberately spread out the radiation to accommodate any localized heterogeneity. To test the sampling error, a single homogeneous flour stream sample was probed 20 times. There was stirring of the sample in between each probing. Standard deviation with a homogeneous sample was 0.11 for an 11% protein sample. In contrast, a heterogeneous wheat sample of approximately 11% protein probed 20 times gave a standard deviation of 0.16. The increased standard deviation is readily explained in terms of sampling heterogeneous material. This explains why averaging the results of three replicate probings gave a standard error of 0.26 whereas individual probes had a standard error of 0.39.

The probe was used on-line for a one time clinical experiment. The sample was blood pumped by the heart of a cardiac rehab patient (the author). Reference data for blood glucose was obtained from a photometrically read colorimetric test strip response to a drop of blood from the commonly used “finger stick” method. The probe was mounted on a helmet with a carved device rigged to hold the probe against the forehead of the patient walking on a treadmill. The experiment here involved monitoring blood glucose while exercise was used to change the glucose level. Sugar was administered to the patient one hour in advance which brought the glucose level to the starting point of 197 as determined by the colorimetric finger stick method. As the patient exercised on the treadmill over a period of 60 minutes, a finger stick reading was taken by the nurse every 10 minutes. The lower range during experimentation was 149. FT-NIR scans were run once per minute with the probe functioning in a double transmission mode. For each finger stick colorimetric reading, the assumption was made that the three adjacent scans could use the finger stick method as valid for regression purposes.

For the nearly 50 unit range of glucose, a *SEP* of five units resulted from a three wavelength calibration expression involving all of the data points. Predicting the 60 FT-NIR values from the resulting calibration and comparing the interpolated values from the 10 minute finger stick colorimetric glucose values gave reasonable agreement except, in instances where the probe obviously lost contact with the forehead. This impromptu seizing of the opportunity to apply the probe equipped FT-NIR to a custom one patient blood glucose calibration should be considered as merely a demonstration of potential applicability, not as a mature scientific procedure.

Summary

The fiber optic probe used had a small sample area and a limited solid angle of collection. Quantitative results with homogeneous solid samples were excellent. For heterogeneous samples, averaging of replicate probing was required with the geometry and dimensions of the probe that was used. It is safe to say that FT-NIR is here to stay in quantitative, as well as qualitative, applications. Quantitatively it has performed well for five component liquid test mixtures, for emulsions and for granular solids. Spectral subtraction with FT-NIR is useful. In the case of assessing solid phase extraction (adsorbed material) it provides a qualitative tool and the possibility of future semi-quantitative capability. On-line monitoring of a pump fluid works with an appropriate time constant. However, transient monitoring is difficult compared to random wavelength access or rapid scanning acousto-optic TFS, where analytical speed has been emphasized while maintaining wavelength reproduction for spectral subtraction.

Acknowledgements

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