

Anomalies in Near-infrared Spectroscopy

Emil W. Ciurczak

Purdue Pharma LP, Ardsley, NY, USA

1 INTRODUCTION

A*nom*a*ly, *n., pl. –lies*. **1.** a deviation from the common type, rule, arrangement, or form. **2.** someone or something abnormal, unusual, or irregular.

Ig*no*rant, *adj.* **1.** lacking in knowledge, education, or training. **2.** uninformed; unaware. **3.** showing lack of knowledge. **–ig*no*rance**, *n.* **–ig*no*rant*ly**, *adv.*¹

Whether or not something is anomalous can often depend on what *is* expected. If we are uninformed about some particular process, then surprises abound. To an average citizen in 1648, electricity would have been an anomalous occurrence! In the case of the infamous Wood's Anomaly, a strange occurrence, in retrospect, can be easily explained. The light energy striking a grating can be polarized at a particular angle (this depends on the blaze angle of the grating and wavelength of light) where the right- and left-polarized functions of the beam are split. One reflects off the grating at the expected angle while the other skims across the face, lost to the spectrometer. This phenomenon can cause some interesting effects and, until explained, was a true “anomaly”.

Since near-infrared (NIR) spectroscopy did not originate in a typical university setting, little was known about the technique by scientists with just standard academic training. Much of the early work in NIR was “empirical”. Most of what became “common knowledge” was based on experimental, rather than theoretical, knowledge. Typical instruments “evolved” in lieu of being designed from first principles.

Solid samples were the first to be assayed by NIR in an organized manner by the United States Department of Agriculture.^{2,3} Since wheat, soymeal, corn, and

other agricultural products were the first products to be tested, reflection spectroscopy was the mode of choice. In retrospect, numerous research articles and chapters have been written to explain the physics of diffuse reflection spectroscopy.^{4–6} One entire conference⁷ is devoted to the “art” of diffuse reflection, but it remains a nonexact science. Attempts were made to make the “art” more scientific from as early as 1931.^{8–10} However, workers in the NIR field still disagree as to which algorithm to use for diffuse reflectance spectra (to turn them into a useful form for quantitative analyses). This fact, alone, helps to explain why researchers have had “anomalous” experiences so often.

With the advent of better optics and quieter detectors, diffuse transmission is fast becoming popular. The same “anomalous” findings are present in diffuse transmission, due largely to scattering and other physical effects. Early workers in NIR often ground samples to a uniform, pre-ordained particle size range. It was often stated that there was no way to adjust the spectra for physical variations. This and other problems will be discussed in detail later.

While transmission measurements of liquids are no less prone to physical interferences than those in reflection, in the solid phase temperature effects are nearly non-observable. In liquid samples, either temperature variations must be accounted for or the sample must be thermostatted. The word “anomaly” implies that spectral occurrences happen to defy the laws of physics. Since NIR follows the same laws of physics as the other portions of the spectrum, this article should explain the causes and prevention of many seeming anomalies in NIR.

2 SOLID SAMPLES

Still the majority of NIR samples tested, solids were the first type of sample to be measured in large numbers.

Since theoretical help was scarce, early workers played with various angles of incidence to angles of measurement ratios. A 0° (normal to the surface) incident angle was decided by consensus during the “early years” of NIR. Measurement at an angle 45° to the normal was later to become the standard. The sample may or may not be pretreated and is then placed in the light beam, either in an open cup or within a sample container. It is the manner in which the sample is presented to the instrument that causes much of any error or “anomaly.”

2.1 Sample preparation

What is done to the sample prior to measurement is critical: grinding, sieving, drying, dissolving, and packing. All these actions can affect the final spectrum of a sample. Since it is necessary to present the sample in some manner, sample preparation is unavoidable.

2.1.1 Grinding

From the beginnings of NIR in the 1950s, samples have been ground to give a constant baseline for the spectra. It was known, for instance, that larger particle sizes resulted in larger baseline offsets. The spectrum baseline is sloped from a minimum offset at lower wavelengths to a maximum at the highest wavelength. This “scattering” effect increases for increasing particle size. It was seen that the early suggestion of using second derivatives¹¹ does not eliminate the particle size effect. In fact, when the distribution of sizes is great enough, spectral aberrations appear. This paper also demonstrates a linear response between the absorbance ($-\log 1/T$) and the reciprocal of the mean particle size.

It was also seen that, for larger particle sizes, the spectrum (exaggerated by derivatives) is skewed at several wavelengths. This “anomaly” is easily explained by considering the texture of the individual crystals. Sugar crystals have smooth, almost polished faces. When the particle size is large, these faces act as surfaces that give rise to specular reflection. The mixed mode of specular and diffusely reflecting media causes the spectra to take on strange shapes. Sieving or milling could take care of this problem.

When the baseline offset is due solely to particle size differences, a multiplicative scatter algorithm does a fair job in eliminating particle size differences. However, no one algorithm eliminates spectral variations between particle ranges of great difference (e.g., $1\mu\text{m}$ and $100\mu\text{m}$). If the samples to be analyzed have large variations in particle size, then grinding may be necessary. Some difficulties to be aware of are heating during grinding (can degrade sample or, upon cooling, may cause condensation of moisture),

segregation of materials (loss of active or matrix materials), or a change in crystalline form (through heating). Gentle grinding in a cooled grinder will solve most problems, but care is needed to reproduce grinding conditions (speed of grind, temperature, and length of grind).

2.1.2 Drying

Strange and wondrous things occur when a “wet” sample (more than several percent water or solvent, not bound in the crystal lattice) is dried by heat, vacuum, or both. There is a binding energy between solute and solvent associated with drying. That is, a given solvent (e.g., water) will evaporate at a different rate from the various ingredients within a complex solid matrix. Take, for example, a typical pharmaceutical blend of drug(s), lactose, starch, and two or three other organic materials. Add a portion of water, mix, and proceed to dry. If the “water” region of its NIR spectrum (around 1940nm) is observed during drying using a second derivative function, an interesting series of peaks and shoulders is seen. The multipeak or “udderlike” shape is seen to constantly change as the solvent evaporates at differing rates from each component (Figure 1a–c).

If an attempt is made to generate a predictive equation, poor correlation is seen using typical segment and gap sizes. Since water is the most easily measured analyte by NIR, this might almost seem anomalous. It is merely simple physics. What is needed is a way to measure all these peaks simultaneously. To accommodate the multiple water peaks, a derivative, using a large segment and gap (e.g., 40nm each), may be used to create a large, smooth water peak which does give a good correlation and prediction equation.

Another problem with hydration and drying is hysteresis: the different rates of adsorption and desorption of a solvent, e.g., water, by a solid material. Paper is a good example. If a curve of relative humidity (RH) versus weight of water per square inch of a paper sheet is plotted, a zero to 100% curve is convex, while the 100% to zero curve is concave. The consequence of this is that two samples of the same material, stored in the same RH chamber, could have different water contents. If a wet sheet were dried to 50% RH, and a dry sheet hydrated to 50% RH, there would be marked differences. This phenomenon should be investigated for any matrix/analyte to be analyzed for moisture content.

2.1.3 Dissolving

The act of dissolving a sample in a suitable solvent is not, in itself, very unusual. It must be understood that this act destroys all physical information about the original sample: particle size, crystalline form, position of moisture

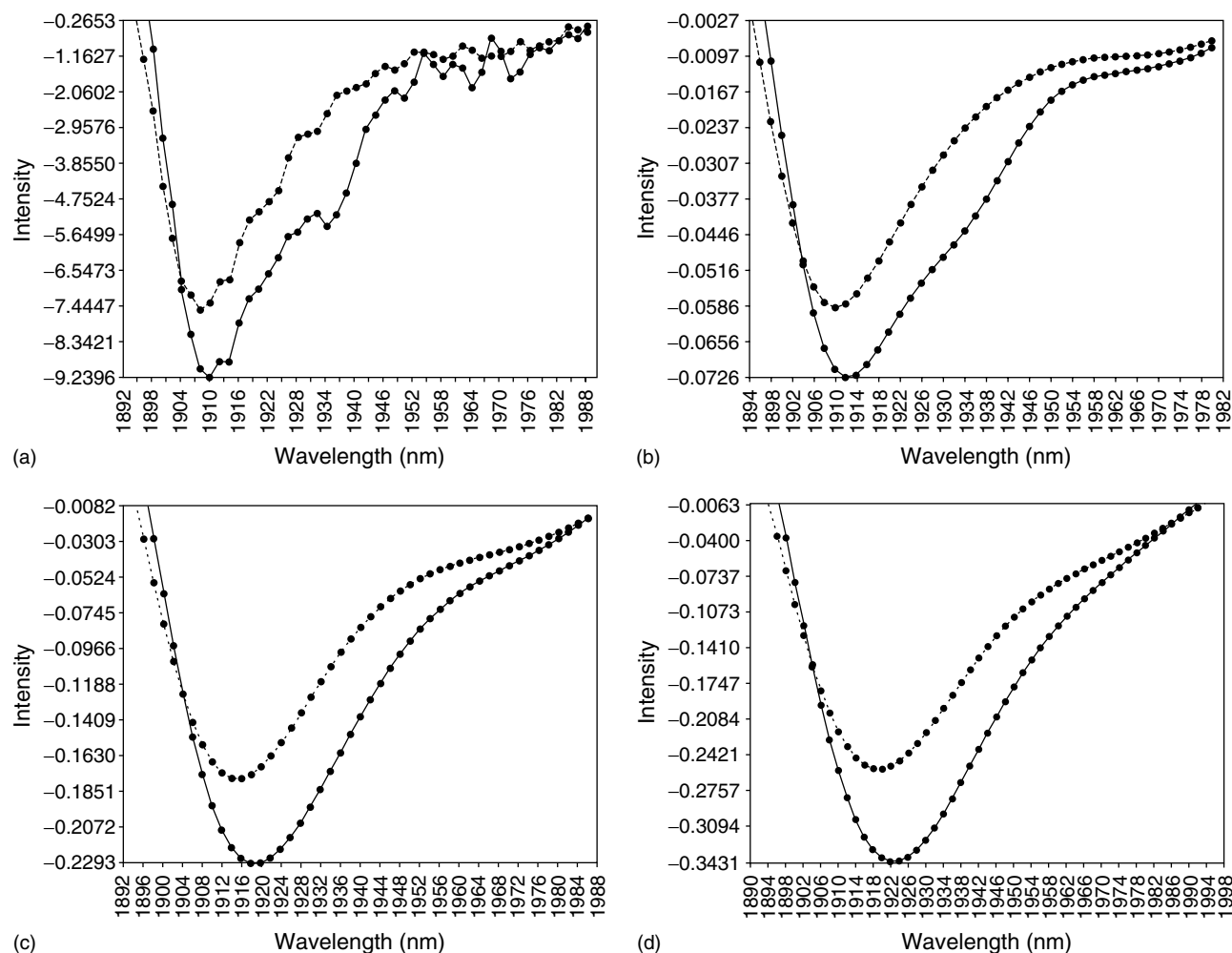


Figure 1. Combination O–H band region of a typical pharmaceutical mixture: wet (15% water) and dry (5% water) using a second derivative with a: (a) 2-nm segment; (b) 10-nm segment; (c) 20-nm segment; (d) 30-nm segment.

(in crystal lattice or adsorbed). There is also the possibility of reaction with the solvent or trace impurities therein. The analyst should understand the chemistry of the samples and use the highest purity solvents. If impurities are present, anomalous readings can take place. Reactions, wherein the analyte degrades, can occur. Acids or bases can cause some of the analyte to acquire a charge through reaction or dissociation.

For example when studying the spectra of solutions of a weak acid (e.g. salicylic acid), several problems occur. With the first molecules to dissolve, there is complete dissociation and the resultant spectra reflect that. As more material dissolves, the effect of the acid dissociation constant, K_a , is to allow two spectrally distinct species to exist simultaneously. As more material is dissolved, dimers of both associated and dissociated material form. As many as six combinations can exist in solution. The hydrogen bonding effects, seen as shifts in the spectra, are substantial and give (analytically) terrible spectra. In this case, making

the solution acid with a strong mineral acid suppresses the dissociation of the organic acid and gives spectra suitable for analysis.

Similar results are seen with weak bases and salts of weak acids or bases. With a weak base, the analyst could either suppress ionization or make the medium acidic to make the conversion total. When a salt dissolves in a pure solvent, usually water, the ionic strength of the solution will change with the addition of more analyte. The constantly changing environment will cause spectral shifting. The overall ionic strength can be made roughly constant (as in ion-selective electrode readings) with a strongly dissociating, neutral salt, such as sodium chloride, added to the solvent prior to spectral measurement.

2.2 Sample presentation

The manner in which the sample is presented to the spectrometer is often the source of most irregularities.

Since diffuse reflection spectroscopy is such an “art”, reproducibility is as important as finding the correct arrangement for analysis. Choosing the correct sample container is not a trivial part of the experiment. Indeed, sample presentation is one of the most critical portions of the analysis, often the major source of “anomalous” readings! Virtually every purveyor of NIR equipment offers a range of sample cups, containers, fiber optic-based equipment, etc., to make the sample presentation as reproducible as possible.

Unfortunately, most analysts become deeply involved in the mathematical treatments used in NIR and overlook the physical presentation of the sample. The advantage of NIR equipment designed for NIR is the plethora of accessories available for sampling.

2.2.1 “As-is”

Where possible, “as-is” is the best mode of measuring any sample. Large surfaces (e.g., paper, and walls of reactors, fabric) are quite easily measured in this manner. Fiber optics has made it possible to direct the light on the sample rather than bringing the sample to the spectrometer. The rapid acceptance of fiber optic probes led to several spectral problems.

In powders, the packing density strongly affects the penetration of the incident light and the resulting absorbances. The probe, in contact with powder, can pack it to different densities. With larger-sized samples, surface texture and angle of presentation are critical. Physical effects, in general, have a strong influence on the final spectrum; thus, sample handling should be minimized and be made very reproducible.

The fiber optics themselves can be the source of anomalous behavior. Bends in the cable can cause attenuation of portions of the spectrum; breaks of some fibers (in multi-fiber bundles) can affect the point where the beam concentrates on a sample as well as the intensity of that light.

2.2.2 Sample cups

Sample cups, one of the earliest conveyances of powdered or granular materials, are a potential source of error. Too little material in the cup and cavitation occurs, too much and there is a packing density effect. Simply filling a cup with a weighed amount of material can bring a potential 5% error down to less than 1%.

To account for texture differences, rotating cup holders have been developed by instrument manufacturers. Some materials (e.g., oriented polymers) can have a polarizing effect on the spectra. Rotating the sample during multiple scans and averaging these scans (32 or 64 are the common number averaged) can obviate these polarization

problems. Not averaging scans with such samples invites an “anomaly.”

When the particle size of a powdered sample varies, the surface scattering effects can be ameliorated by the rotation and repacking of the sample cell. These, combined with spectral averaging, can reduce the physically induced effects to a minimum.

Another potential source of error in using a sample cup is the possible “wicking” effect of the liquid in a “wet” solid sample (where several percent of a solvent or oil is present). For example, peanut butter tends to pool its oil at the window when compressed in a sample cup. This leads to erroneously high values for the oil content.

2.2.3 Sample transports

The manner of introducing the sample to the sample beam is not trivial. Some manufacturers have developed sample holders that contain 40–50 g of material. These are then moved across the sample beam to average the spectra. This is primarily used for nonhomogeneous samples where there is a fair amount of sample available and/or large particle sizes (e.g., wheat, soymeal, and corn).

Sample holding devices exist where the sample is rotated in the beam, allowing multiple scans to be averaged. This helps overcome surface irregularities and polarizing effects as well as averaging a larger sample size. Cells also exist for slurries and semisolids; heated cells, stirred cells, and inverted cells are available for almost any sample type.

3 LIQUID SAMPLES

Liquid samples, especially when clear and colorless, can lead to a false sense of security. When strange or anomalous readings are seen for this type of sample, the analyst is more surprised than when analyzing solid samples. When analyzing liquid samples, temperature and concentration “effects” are far more pronounced than in solid samples. Even a clear sample can have microscopic solid particles present. A Tyndall effect will cause loss of light across the spectrum and will vary with wavelength and amount of suspended solids present.

The absorbance value and wavelength of any particular absorption peak is directly affected by temperature and slightly by pressure. The refractive index (RI) is affected by both temperature and concentration (of either analyte or matrix salts). This RI effect is greatest when using a fiber optic probe. This is due to the convergence or divergence of the incident light (and reflected light in a transfection probe) and is exacerbated by the inherently lower intensity of light emerging from the fiber probe. The light emerging

from a fiber probe is collimated, as a rule, either in air or water. When the matrix is other than the medium for which the probe is optimized, there is a loss of energy from diverging light rays. The result of this divergence is that the angle of acceptance of the returning fiber is not met by a fraction of the emitted light. As the RI changes, a larger and larger portion of the light is “lost” in this manner. The result is an increasing baseline absorbance. This offset can easily be corrected by a first or second derivative.

3.1 Sample preparation

While there are a large number of attachments available for most sample types, there is sometimes a necessity to do some sample preparation. The keystone to whichever method is used to prepare a sample for scanning is reproducibility. Performing the same functions in the same order for the same duration assures limited variability. It is easy to forget that, in many samples, the matrix and physical changes account for the majority of variation between spectra. Since the matrix is usually a higher proportion of a sample than the analyte, care must be taken to understand the matrix parameters as well as the analyte(s) of interest.

3.1.1 Degassing

When a fluid (liquid or semisolid) contains entrained gases, it tends to act as a highly scattering material. Materials such as highly viscous polymers can easily entrain bubbles when being poured. In order not to have to use algorithms to account for the “scattering”, degassing might be the preferred route. Applying a mild vacuum can rid the sample of air bubbles, but care must be taken to avoid foaming. (Allowing bubbles to rise naturally or discounting them could lead to a changing sample through settling or evaporation. This could be a source of “anomalous” behavior.)

Exactly the same time, temperature, and air pressure should be used on every sample to avoid nonreproducible loss of volatiles. Solvent loss must also be considered to avoid concentration of solids.

3.1.2 Filtration

When a sample needs filtration, many of the problems encountered in degassing must be obviated. Vacuum filtration is a source of evaporation and a real possibility exists for one or more components to have an affinity to the filtration medium. If vacuum is used, the strength of vacuum and time under reduced pressure must be constant. Affinity of particulate matter to the analyte must also be considered. There are cases where the analyte was soluble,

but was diminished after filtration. In such cases, it may be preferable to contend with scatter corrections in lieu of filtration.

Various species, both analyte and interferent, may be adsorbed on the filter matrix. Thus, there is a possibility of either diminishing or enhancing the analyte signal by filtering the solution.

3.1.3 Dilution

While not used often in NIR analyses, the act of dilution is so commonplace in standard “wet” chemical tests that its effects can easily be overlooked. In typical chemical analyses, where the analyte in question is the only species reacting or being registered, the physical effects associated with dilution may be overlooked, in large part. Dilution with a solvent different from the one already present will cause temperature changes (causing volume changes, concentration changes, etc.). Even diluting an aqueous solution with water can cause large changes in physicochemical properties: viscosity, RI, density, and ionic strength.

Before evaluating an equation, it should be determined that linearity is preserved when diluting either a sample or standard. This may seem like an obvious step, but chemists, especially novices to vibrational spectroscopy and chemometrics, may not appreciate the potential for error when the matrix is changed.

3.2 Sample presentation

The manner in which the sample is presented, treated, and stored is most critical. Since NIR is often more affected by the physical component(s) of the sample than variations in chemical composition, these must be either stabilized or accounted for in the calibration step. The chance of specular reflection when diffuse reflection was intended could cause strange effects. Maintaining a constant temperature (or accounting for temperature variations via the software), constant pathlength, constant pressure, and assuring constant matrix compositions would be ideal. This is not always possible; thus, the effect of each parameter should be understood and either corrected for or eliminated.

Heated cells and fixed-pathlength cells and probes are in common use now. Measurements of these parameters should be taken and recorded wherever possible.

3.2.1 Temperature effects

Since NIR is a vibrational spectroscopy technique, temperature does make a difference. The obvious change in the spectra will be peak shifting and/or broadening due

to a different energy distribution of the vibrational modes, especially for hydrogen-bonded species. There may also be enhanced absorbances or decreased absorbances. All these changes are at the fundamental vibrational levels: altering the vibrational frequencies or varying the distribution of energies around the mean.

The secondary effects are due to changes in the matrix. As the (usually liquid or gaseous) matrix changes temperature, the volume changes (Boyle's law). If there is a fixed volume container or cell, then the pressure will change with changing temperature. In either case, there will be spectral consequences.

3.2.2 Physical/chemical effects

While NIR spectra originate at the molecular level, it has been seen that macro effects such as particle size can strongly influence the spectra also. Hydrogen bonding affects both absorption maxima and peak intensities; solvent polarity and even solid-matrix group polarities will cause peak shifting of the analyte spectrum. These effects may be considered chemical in nature. Purely physical changes also may give widely varying results in NIR analyses.

Pressure. Pressure is, as would be expected, most critical in vapor systems. The pressure on a solid would not be of much consequence unless it was high enough to change the polymorphic form of any crystalline material or melt it outright. Because of the change in scattering coefficient, the diffuse reflection spectrum of a powder is greatly affected by the packing density. Both the apparent surface area and depth of penetration of the radiation in a diffuse reflection measurement are changed under pressure.

Liquids would not change, per se, under pressure, but the cell pathlengths (especially in a flowcell) could be changed by hydraulic pressure. Since metal tubing will not expand with internal pressure increases, there is a tendency to forget that silica or quartz windows are flexible and will bend under pressure. The effect is a longer pathlength and higher absorbance reading with higher pressure.

Flow rates. In a flowing system, usually a process stream, the speed at which a solution or slurry flows past the sensor may well affect the spectral shape. Laminar-type flows can cause gradient effects across the width of a tube. This is not so serious for transmission measurements, since the spectrum will represent the entire sample stream. For reflection measurements, this effect could severely alter the analytical value depending upon the depth of penetration of the probe, speed of the fluid through the pipe, composition and diameter of the pipe, temperature, and viscosity of the material.

These various effects may give nonlinear results or, unless the flow can be precisely controlled, non-reproducible values. Often, the analyst will work on the chemical composition of the matrix, varying compositions to satisfy an experimental design, when it is merely the flow characteristics of the sample that need definition. The pressure fluctuations in the sample will also cause distensions of the observation window. The resultant pathlength variations will also seem like constituent variations.

Refractive index. The RI can strongly affect a NIR spectrum. The refraction of the light beam will vary with RI and will cause baseline shifts between spectra. The shifts are due to decreases or increases in light intensity reaching the detector directly or the return fiber optic.

RI changes are usually due to either temperature or concentration changes. If the composition remains somewhat constant (controlled reaction), then temperature changes may account for baseline shifts. (In fact, this shift may be used to measure temperature.¹²) In both cases, composition and temperature variation, the use of derivatives will obviate most of the variation. Simple software programs, such as multiplicative scatter correction or normalization, may also work in this case. In all cases, the RI should be accounted for when liquid samples are involved.

4 CONCLUSIONS

What are perceived as anomalies are often quite easily explained by simple chemistry: organic and physical. Good sample practices (a term meant to mimic good manufacturing practices) will assure that most of the problems outlined above will not be encountered in NIR analyses. If the process is fully understood, and all precautions are taken, there will be no anomalies to deal with.

ABBREVIATIONS AND ACRONYMS

RH Relative Humidity

REFERENCES

1. 'Random House Webster's Dictionary', 3rd edition, Ballantine Books, New York (1996).
2. I. Ben-Gera and K. Norris, *Isr. J. Agr. Res.*, **18**, 125 (1968).
3. I. Ben-Gera and K. Norris, *J. Food Sci.*, **33**, 64 (1968).
4. G. Mie, *Ann. Physik*, **25**, 377 (1908).
5. J.M. Olinger and P.R. Griffiths, 'Theory of Diffuse Reflectance in the NIR Region', in "Handbook of Near-infrared Analysis", eds D.A. Burns and E.W. Ciurczak, Marcel Dekker, Inc., New York, 13–36 (1992).

6. A.M.C. Davies and H. Martens, "Near-infrared Spectroscopy: Bridging the Gap between Data Analysis and NIR Applications", eds K.I. Hildrum, T. Isaksson, T. Naes and A. Tandberg, Ellis Horwood, New York, 41–46 (1992).
7. International Diffuse Reflectance Conference, Chambersburg, PA (even numbered years).
8. P. Kubelka and F. Munk, *Z. Tech. Physik.*, **12**, 593 (1931).
9. N.T. Melamed, *J. Appl. Phys.*, **34**, 560 (1931).
10. P. Kubelka, *J. Opt. Soc. Am.*, **38**, 448 (1948).
11. E.W. Ciurczak, R.P. Torlini and M.P. Demkowitz, *Spectroscopy*, **1**(7), 36 (1986).
12. E.W. Ciurczak and D.E. Honigs, 'The Measurement of the Refractive Index of Liquids via NIRS Utilizing a Single Fiber Optic Probe', PittCon, New York (1990).