Growth of near-infrared spectroscopy in pharmaceutical and medical sciences

Emil W. Ciurczak[a]

[a]Private Consultant, 77 Park Road, Golden's Bridge, NY 10526
phone: (914) 767-0720, fax: (914) 767-0173, email: emil@ciurczak.com

ABSTRACT

Near-Infrared Spectroscopy (NIRS) is used extensively in the health services industries: medical research, pharmaceutical production, and bioprocessing. NIRS is rugged, simple to operate, flexible, and relatively inexpensive. It may be used to monitor the progress biochemical reactions. It is used to control mixing, blending, drying, and coating in pharmaceutical production and is used for imaging and chemical determinations in living patients.

Keywords: near-infrared, pharmaceuticals, bioprocessing, medical

1. Introduction

Over the past decade, near-infrared spectroscopy (NIRS) has seen phenomenal growth in the health care industries. By health care industries, I mean medical research and patient care, bioprocessing (e.g., fermentation of antibiotics), and pharmaceutical development and process control. The near-infrared potion of the spectrum has been known since the year 1800, but little has been done within this region until recently.

With the advent of powerful minicomputers and software capable of extracting information from the complex and overlapping spectra generated by NIR instruments, NIR has become a widely used tool in the medical and pharmaceutical fields.

Near-infrared can measure the potency of an individual tablet in seconds, determine the biomass of a fermentation broth, or determine whether there is a tumor in a breast and what the potential of its being cancerous. The speed, precision, and accuracy of this vibrational spectroscopic method are unmatched by any other analytical technique on the market at the moment.

Biomedical Nanotechnology Architectures and Applications, Darryl J. Bornhop, et al., Editors,
Proceedings of SPIE Vol. 4626 (2002) © 2002 SPIE · 1605-7422/02/$15.00

2. History of Near Infrared

At the end of the eighteenth century, astronomers were investigating the nature of light. One problem encountered was the heat of sunlight when observing sunspots. This heat could damage the eyes of the astronomers. Sir William Herschel, discoverer of Neptune, designed an experiment to determine which of the "colours" of the spectrum conveyed the heat. He could thus use a filter to block that particular color when observing sunward.

Herschel wrapped a dark cloth around the bulb of a thermometer to cause it to absorb heat. He used a glass prism to split the light of the sun into its respective colors, causing them to shine on a tabletop. He then placed the thermometer bulb into each color, noting any rise in temperature. There was a very slight rise in each of the colors, but not enough to account for the heat from "pure" sunlight outside the lab.

When he broke for lunch, he placed the thermometer nest to the red light. When he returned, the mercury had risen significantly. He immediately surmised that there was another, unseen color present. He named it *infra* red or "next to" the red. This was the first wavelength region of the electromagnetic spectrum discovered aside from the visible.

Herschel reported his findings in 1800 (1), but mistakenly concluded that this *infrared* radiation was somehow different from light and referred to it as *radiant heat* and the *thermometrical spectrum.* [Ultraviolet was reported in 1801, but that is a topic for another paper.] It wasn't' until 1835 that Ampere used a thermocouple to demonstrate that NIR has the same characteristics as visible light. Thus, the concept of an extended spectrum was born.

Theoretically, Maxwell had formulated his four laws concerning the propagation of light and theorists such as Kirchoff, Stefan, and Wein laid the foundations for Max Plank's law of radiation in 1900. In practice, little was done during the nineteenth century following Herschel's and Ampere's observations. Experimentally, Fraunhofer used a new invention, the diffraction grating, to resolve the sodium "D" lines from a flame of a Bunsen burner in 1823, while Ampere recorded the atomic spectra of numerous elements by the 1860's. However, little was done with vibrational spectra throughout the century.

The first recorded use of NIR was in a publication by Abney and Festing (2) in 1881 where the spectra of organic liquids in the range of 1 to 1.2μm's were recorded on film. Inspired by their work, W.W. Coblentz constructed a spectrometer using a rock salt

prism and a sensitive thermopile connected to a mirror galvanometer to generate IR spectra (3). Coblentz would rotate the prism a fraction of a degree, leave the room until the thermopile equilibrated, then read the galvanometer via a telescope form the next room. This procedure required one day per spectrum, but produced first-rate spectra.

In 1905, Coblentz published a series of papers containing several hundred IR spectra in the wavelength region of 1 to 15μm. His major contribution was to show that no two compounds had the exact spectrum. His examples of isomers included 1- and 2-propanol. He also began to notice patterns in the spectra: e.g., all compounds containing an OH group had a peak in the same region (roughly 2.7μm).

Not of direct importance at the time, A.A. Michelson published a paper wherein he describes a dual-beam interferometer (4). Since his purpose was to attempt to determine the speed of light through the "ether" of space, the work was not immediately noted a s potentially a spectroscopy work. Only in the 1970's did commercial instruments containing an interferometer become available. Using the mathematical formulae of M. Fourier to deconvolve the pattern buried within the iterferrogram was the instrument available. The new Fourier Transform IR, or FT-IR, took vibrational spectroscopy to a new level. The speed and resolution now available was a vast improvement over the good, but slow, grating based instruments.

Little was done with IR (and NIR) in the first half of the twentieth century. The first quantitative NIR measurement occurred in 1912 (5). F.E. Fowle determined the atmospheric moisture level at Mount Wilson observatory. In 1938, Ellis and Bath reported the analysis of moisture in gelatin (6). During the 1940s, Barchwitz (7) used NIR to analyze fuels while Barr and Harp (7) generated spectra of vegetable oils for publication.

While Harry Ellis of ICI used NIR to characterize polymers and, later, to measure the thickness of polymer films in the 1940s and some workers experimented with NIR for organic compounds and rubbers, by 1970 only about 50 papers on NIR had been published.

With the advent of scanning instruments with computer control, the number of papers on NIR results has increased geometrically. There is even a journal (Journal of Near-Infrared Spectroscopy), a newsletter (NIR News), and two awards (the Hirschfeld Award at PittCon and Achievements in NIR Award at Eastern Analytical Symposium) devoted to the theory and applications of NIR.

3. Hardware and Software

The major reason for NIR becoming a popular tool in health related fields are the tremendous improvements in available equipment in the last decade or so. Another factor is the cost of equipment has, like computers, fallen in recent years. The modern sources are rugged, usually tungsten-halogen bulbs with a life in excess of one thousand hours. Compared with the glow-bars and other sources for the midrange infrared, NIR sources are far more powerful and long-lasting. The durability and long life of modern sources has aided the application in industrial settings. Longer life translates to less maintenance.

During the 1930s, lead sulfide (PbS) was introduced as a semiconductor for detection of IR light. World War II spurred IR research for heat-sensing in battle conditions. This led to cheap, sensitive, and low-noise detectors being readily available in numerous configurations. This detector's range is 1100 to 2500nm and that was considered "the" NIR for many years. With the addition of cheap silicon detectors (originally designed for visible spectrometers), the near-infrared region was expanded to what may be called the "near, near-infrared" or, as championed by Tony Davies, the "Hirschfeld" region, named after a brilliant pioneer in NIR.

Newer additions including indium arsenide (InAs), indium gallium arsenide (InGaAs), germanium (Ge), to name a few, have both extended the range of wavelengths and increased sensitivities. Low noise (often in the ppm range) and high sensitivity allow the experimenter to detect lower and lower concentrations of analyte. In some cases, the detection limits rival ultraviolet spectroscopy.

Since an NIR spectrum consists of broad, overlapping peaks, it almost needs a computer to make sense out of it. Thus, NIR spectrometers were wedded to computers in the 1970s, before any other type of instrument. The early algorithms were, by today's standards, primitive. But, so were the computers. Most suppliers of equipment supplied a multiple linear regression (MLR) program, a multi-wavelength version of Beer's law. The limits of this programming were seen in the limits of types of samples able to be analyzed: grains, polymers, and chemicals. Complex mixtures and rapidly changing reactions were not very easily monitored.

With the introduction of faster computers with larger memory capacities, programming kept apace. New algorithms such as Partial Least Squares (PLS), Principle Components Analysis (PCA), and, now, Neural Networks (NN). The new programs can take the entire spectrum into consideration and quantify small changes

and account for spectral shifts and other results of physical changes that are not the phenomenon being studied. One important improvement in the software has been the realization by the instrument manufacturers and third-party software writers that any program used for an FDA related project must be validated as per 21 CFR 11 guidelines.

With instrument manufacturers selling pieces of their equipment (monochromators, fiber optic probes, etc.) and software writers doing a better job of following FDA guidelines, more work is being started in the US. Previously, most biotech and pharmaceutical process innovations originated in Europe and Japan. Now, there is an upsurge in the US, as well.

4. Medical Applications

One compelling reason that NIR is so popular is the benign radiation used. Near-infrared light is less energetic than visible light and less easily absorbed than infrared (by 10 to 1000 times less.) This greatly lowered absorbance also allows deep penetration of tissues, organs, and blood vessels. With proper software and hardware, it is almost like having a non-radioactive x-ray machine.

Blood glucose is the one application most sought-after. With the rise in diabetes at near-epidemic levels, especially among children, the non-intrusive nature of NIR is needed. Control of blood sugar levels by frequent testing is strongly recommended by physicians. The problem with current methods is the need to draw blood. This is painful and inconvenient and often leads to non-compliance. The continuously high blood sugar can lead to glaucoma and blindness, loss of extremities, heart and kidney failure and numerous other serious consequences. The company that eventually produces an accurate, easily used, non-invasive blood glucose monitor will have performed a service akin to the discovery of the first polio vaccine.

Measuring blood oxygen is currently possible and widely performed. As early as World War II, devices attached to the earlobes of pilots measured blood oxygen levels. Every hospital patient is given a finger-covering device to measure heartbeat and blood oxygen. The short-wave NIR radiation used with visible light measures the ratio of oxygenated to deoxygenated hemoglobin, the pathlength changes of the arteries and capillaries are accounted for by the visible wavelength.

This use of NIR is quite useful to determine the amount of tissue damage after trauma or burns. The level of necrosis may be determined after skin grafts and the health of the tissues monitored. The perfusion and oxygenation may be determined

objectively, used to support the decision to administer HBO and determine the number of treatments needed.

Urine may be analyzed for glucose, protein, urea, and creatinine concentrations. Synovial fluid, drawn from a patient's knees, can be used to diagnose arthritis. In vivo chemistry is often performed with the aid of fluorescent probes (8) attached to various species within the body. Uses include enzyme amplification, membrane probes, and DNA probes. The markers are used to determine proteases, lipases, alkaline phosphatase, and peroxidase, as some examples.

Cancer research has been helped by NIR. PAP smears have been examined for abnormal cells and non-X-ray based mammograms may be conducted using NIR. In fact, many organs have been imaged by NIR, avoiding potentially harmful X-rays.

Some of the most interesting work has been done with newborns, and in some cases, fetuses. One of the most important is the measurement of cerebral oxygenation and blood volume in preterm infants during apnea. It is believed (9) that decreased cerebral perfusion induces cerebral ischemia and worsens the brain damage in neonatal bacterial meningitis. Simply following blood oxygen levels in premature infants (in oxygen tents) allows the level of oxygen to be kept at an optimum level.

New uses for non-intrusive methods are being discovered continuously. The imagination of the clinician is the only limit that appears to shape the speed at which the new uses are attempted.

5. Pharmaceutical Applications

Using methodology derived from food technology, the pharmaceutical industry has not changed its approach to manufacturing solid dosage forms (tablets and capsules) for forty years. It still could take up to 180 days between the delivery of the raw materials to the loading dock to the time that a product is shipped. The majority of that time is waiting for an analysis result. It was assumed (rightly) that real-time analysis results could result in extreme timesaving and product quality.

The timesaving comes from **not** having to wait for analysis results to come back from the quality control lab. While waiting to perform the next operation, the granulation or mixture may be "bumped" from its place in the schedule and have to wait until a time slot opens to continue production.

Over the last fifteen years, many applications in the pharmaceutical field have been published, and more importantly, been instituted by the companies. The application categories are loosely termed “qualitative and quantitative.”

By qualitative, we mean that the identity of a substance, mixture, or solid dosage form is determined, using an algorithm. The identity is based on a series of spectra, generated previously from materials for which the chemistry is “known.” Since the NIR reflectance and transmission spectra are affected by physical as well as chemical properties, this technique may be used to “qualify” the “goodness” of a raw material as well.

This “goodness” is actually based on the combination of particle size, moisture, polymorphic form, degree of crystallinity, etc. when comparing a spectrum of an unknown to a group of previously accepted materials. Using methods such as Principle Components Analysis (PCA), subtle variations in spectra may be used to discriminate between “good,” “questionable,” and “bad” materials.

This qualitative process may be used on final dosage forms, as well. In-process granulations may be checked for uniformity and dryness before proceeding to the final tablet compression or capsule filling stage.

Clinical trials are performed on all new drug products for proof of efficacy. The product is packaged along with a set of placebos, tablets or capsules of the same size, shape, and color. These placebos have only inert ingredients and no pharmacologically active materials. The patient response to the actual drug substance is then compared with thes placebos in a “blinded’ fashion. That is, only the drug manufacturer knows which patient is receiving which material at any step of the procedure. This prevents a doctor from influencing the patient, even subconsciously.

NIR may be used to determine that the correct dose is in the correct position on the blister pack, through the plastic, without disturbing the dose. This allows the clinical supplies people to perform a 100% examination of the patient doses, obviating mistakes.

Quantitative simply means attaching a value to a spectrum: for instance, % active, mean particle size, thickness, or concentration. While quantitative measurements were the first used in NIR work, they are often the most subtle in pharmaceutical applications.

If, for instance, an analyst wanted to use NIR transmission to measure the amount of drug in a solid dosage form, he would face several non-traditional problems. The maximum absorbance value would be in the 4-6 AU range. This has traditionally been taught to be well outside the range where linear spectroscopy could be performed. The

Beer-Bouger-Lambert law states that the optimum for spectroscopic analyses is between 0.2 and 1.0 AU. Further, the law is based on the non-absorbance of the matrix; the NIR spectrum is often mostly the matrix. It also states that there should be no interaction between the solvent (matrix) and the analyte; the typical sample in NIR has numerous wavelengths where hydrogen bonding is apparent, causing spectral shifts of varying magnitudes.

The advent of extremely low noise detectors, consistent sources, and powerful new algorithms allow the analyst to generate useful equations where previously he would have had to resort to wet chemical methods. It is possible, with some of the newer equipment to either measure tablets linearly at an astounding rate or even "see" multiple in blister packs, both qualitatively (is it the correct material?) and quantitatively (is there the correct amount or drug?)

In short, the standard paradigm for spectroscopic analyses is different for NIR. The FDA and a number of committees are addressing that issue at the moment. A group, Near-Infrared Validation Working Group (NIRVWoG) recently sent a series of recommendations to the United States Pharmacopoeia (USP). These will be published in the spring 2002 Pharmacopoeial Forum for discussion. In addition, the FDA has begun an advisory committee to the FDA, called the Process Analysis Technology Advisory Committee. The author is in the Process Validation Working Group under this committee. Anyone attempting to use NIT in the pharmaceutical field is advised to follow the progress of these committees.

Other quantitative uses include, moisture levels, coating thickness, and, potentially, degradation products. Degree of polymorphic change due to processing may also be quantified. Placing NIR monitors throughout the process line to monitor the quality of the product is a goal of advanced companies and is being strongly encouraged by the FDA.

The goal is to produce an excellent product, reduce waste, and save large amounts of money. For instance, one large company saw that it took up to 180 days to take raw materials and process them into a finished product, shipped to a distributor. Most of this time was due to waiting for results, then rescheduling the in-process materials for the next step. By monitoring the entire process, the company was able to bring manufacturing time down to fourteen days!

6. Biotech Applications

By biotech, I am referring to, mainly, fermentation of various materials: antibiotics, vaccines, *E. coli* based materials, alcohol, etc. The strength of NIR in this case is the ability to measure *in situ*. In many applications, it is not only inconvenient, but also hazardous for the operator to sample the broth during and after fermentation. If a reaction is being performed anaerobically, the CO_2 atmosphere can be compromised prematurely by opening a port to sample the contents of a vessel.

Since water is only a minor inconvenience to NIR, probes located throughout a reaction vessel can follow the reaction in "real time." When a reactor is first charged, or loaded with the starting materials, an initial reading may be taken to ensure that the nutrients, pH, etc. are correct. As the batch ferments or reacts, real time samples are measured without need of intrusion into the reactor.

Not only can the biomass be determined, but all ingredients such as ammonium salts, oils, etc. that would have to have been assayed by wet chemical methods. Figure 1 shows a typical bioreactor using on-line NIR monitoring to follow and adjust the reaction. The NIR readings allow the computer-controlled operation to make subtle adjustments in feedstock additions, temperature, pH, etc. throughout the run with no outside intervention necessary.

The ability to monitor bioprocesses in real time simultaneously lowers the cost and increases the yield of the end product. In short, biotech is no different from any other manufacturing process. Constant monitoring ensures quality products at the lowest possible cost.

ACKOWLEDGEMENTS

I wish to thank Jim Drennen of Duquesne University for permission to use his mixing study slides and data. Gary Ritchie provided results (previously presented) of the analysis of tablets by transmission NIR. I would also like to thank Ramesh Raghavachari for asking me to write a chapter on this subject and for the opportunity to present this paper at the SPIE meeting.

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