NIR–special vista in observation of living systems

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Introduction

The 21st century will be the era of intensive developments in biosciences and their sub-sciences even because of their inter- and multidisciplinary character will combine and influence all parts of our life. The NIR spectroscopy provides a means for rapid reagentless measurement of analysis in biological materials and provides also a means for insight of chemical, morphological, or even thermal changes in living systems.

This paper will review the main trends in the application of NIR spectroscopy in:

- Detection, follow-up and control of biochemical reactions.
- Biotechnology, fermentation, bio-processing.
- Investigation the tissues and animal *in vivo*.
- Plant physiology and protection.
- The trends in instrument development and application of new imaging also mapping methods will be shortly discussed.

Biochemistry

The biochemical changes of living systems can be characterised by primary molecular processes or by description of their interactions with different chemical constituents in the system. In the following only some examples will be given to demonstrate the potential applications of NIR spectroscopy in the observation of both types of processes mentioned above.

Protein structure and denaturation

Mayazawa and co-workers[1] studied the molecular conformation of silk fibroin. They have followed the changes of secondary structure of fibroin based on the amide I bands. The α-helix to β-sheet conformational transition was observed near 2170 nm.

Two dimensional NIR correlation spectroscopic studies were carried out by Murayama *et al.*[2] in order to investigate the acid-induced structural changes and hydration of human serum albumin. It was pointed out that NIR make us enable to study the interactions between water and protein molecules which drastically change as function of pH and also strongly change the rearrangement of protein structure.

The effects of heat denaturation on human serum albumin and whey milk protein structure were investigated by Wu *et al.*[3] and by Czarnik-Matusewicz *et al.*[4]. It was confirmed that the NIR spectroscopy can be used as a conformation-sensitive monitor for thermally induced unfolding of proteins in aqueous solutions. These unfolding changes are strongly associated with different strength of hydrogen bonded interactions between C=O and N–H group.

Polymer–water interactions

The majority of biochemical processes are running in strongly hydrated conditions so the processes can be followed by the measurement the status of water.

Grant and co-workers[5] were investigated a glucoseaminoglycan-polypeptide interaction using NIR technique. It was suggested that complexation between two interacting polymers are influenced by water and by the formation of hydrogen-bonded water the complex was stabilized.

Hydrogen-bonding may result in the formation of water bridges between the interacting polymers. It has been confirmed by Grant *et al.*[6] that such artificial "surfaces" supporting also the cell adhesion is therefore paralleled by their ability to hold structured water.

Redox state of proteins

The redox status of specific proteins play essential role in the physiological processes.

According to Hoshi *et al.*[7] the redox state of cytochrome oxidase enzymes was measured by NIR. They have developed an algorithm where their model was independent from the concentration of oxygenated hemoglobin. Myoglobin is an important intracellular protein in cardiac and skeletal muscle. It is involved in the intracellular transport of oxygen from the cell membrane to the mitochondria (where oxidative phosphorylation takes place).

Schenkman *et al.*[8] developed a NIR method which was allowed the accurate prediction of myoglobin oxygen saturation in the presence of hemoglobin from either transmission or reflectance spectra.

According to Shaw *et al.*[9] the different metalloproteins can be detected in the NIR spectra. Deoxyhemoglobin and oxyhemoglobin and their oxygen saturation can be detected from NIR spectra. The absorption bands at 760 nm of deoxyhemoglobin and at 920 nm of oxyhemoglobin can be used for monitoring the oxygenation of blood and these are the basis for imaging regional variations of blood circulation.

Biotechnology and fermentation

At-line, on-line control

Antibiotic fermentation process was controlled by Hammond[10] using robot based auto sampler, flow cells and remote fibre optics. The products were controlled by the measurement of lipid components in the broth.

According to Tamburini *et al.*[11] NIR-based fermentation monitoring system was developed for measurement the concentration of biomass, growth substrates and metabolic products in industrial-scale fermentation producing starter cultures for salami processing. Glucose, lactic acid, acetic acid and biomass concentration were measured in the 700–1800 nm region using in-line method (steam-sterilisable probe, immersed directly into culture fluid).

The fermentation process of exopolysaccharide, lactic acid and lactose production was investigated recently by Macedo *et al.*[12] Lactic acid bacteria (*Lactobacillus rhamnosus* RW-9595M) produced exopolysaccharides, lactic acid and lactose which components were detected in different region of spectra. The average accuracies of measurements were between 2–12% CV.

Nakamichi *et al.*[13] used NIR methods for controlling glycolipid fermentation process. The concentration of mannosyl-erythritol lipid and soybean oil were measured successfully in a 100 hours batch fermentation broth.

Qualitative control, monitoring, biomass control

Sivertsson *et al.*[14] have been developed chemometric methods and spectral pre-treatments in order to recognize the qualitative changes, deviations and faults during fermentation in the early phase.

For fast glucose monitoring in a T cell culture system an automated on-line method was developed recently by Jung *et al.*[15] The system controls the glucose concentration in a flow cell positioning the sensor stepwise. The multivariate calibration model allowed to measure the concentration of glucose with 1% CV and other analytes as lactate, ammonia, glutamine and glutamate can be also measured in the system.

The NIR methods are sensitive tools to measure not only the composition of fermentation broths but also the "spectral signature" of biomass.

Vaidyanathan *et al.*[16] were carried out three experiments to investigate the validity of measuring biomass using NIR. He has been compared five representative microorganism (three filamentous fungi, one filamentous Gram positive bacteria and one Gram negative unicellular bacteria). It has been pointed out that the spectral signature of the five biomasses were essentially identical. The spectral signature correlated quantitatively with dry cell mass measurement. The biomass reflectance spectra remained unchanged at different culture ages. The results indicated those spectral regions which can be the basis for quantitative modelling of microbial biomass in bioprocesses using NIR, regardless of the microbial type and the culture age.

The morphology of microbial organisms is also influencing the possible application of NIR methods in the control of bioprocesses.

Despite the filamentous nature of biomass Vaidyanathan *et al.*[17] have been developed prediction model for determination of biomass with 5% CV and other analytes like substrates, total sugar and ammonium were detected parallel in culture broth. However the product (penicillin) and by-products (extracellular proteins) had to be monitored on the cell free fluids.

Animal science and biological response

The NIR spectroscopy has been used for long time in the quality control and evaluation of different biological tissues from animal origin and there are strong trends to measure and to evaluate the intact living organisms as is.

Animal tissue, biological response

The quality of meat tissues are influenced by breeding, sex, age of animal, feeding and also by post-slaughter conditions.

The tenderness of beef tissues were investigated by Rødbotten *et al.*[18] using NIR reflectance and Warner-Bratzler shear force methods during the post mortem period. Using a diode array detector (950–1700 nm) the tenderness was predicted with acceptable accuracy and it was confirmed that tenderness of beef tissues changed during ageing. The prediction models were able to classify the tissues based on their tenderness into two groups.

According to Cozzolino *et al.*[19] NIR and visible spectra of beef muscle tissues were sensitive enough to make differences between pasture feed and corn silage feed beef. The developed models classified 90% of pasture-fed tissues and 87% the corn silage-fed tissues correctly using PCR. The method is applicable for a rapid screening of tissue classification and support the objective measurement of authenticity and traceability.

The NIR spectroscopy and NIR microscopy was used recently by Baeten and Dardenne[20] for detection and quantify mammalian tissues in feedstuffs. These investigations targeted the application of non-destructive analytical methods as diagnostic tools in the detection of mad cow epidemic (BSE). The developed methods confirmed the potential of NIR to detect the meat and bone meal in fish meals.

According to Murray and co-workers[21] fish meal and meat/bone meals can be distinguished in the wavelength region around 1720 nm. They have developed modified PLS calibrations using the combined visible-NIR regions which detected the meat and bone meals in the fish meals with 0.85% SEC value.

Living animals *in vivo* tests

The Japanese research project on NIR for bio-monitoring in dairy production has been resulted a revolutionary breakthrough in the application of NIR methods for animal bio- and ecosystems.

Tsenkova[22] was conducted the survey for analysis of the biological objects on a dairy farm and investigations were carried out to determine interrelations between milk components and NIR spectra of other biological liquids like rumen juice and blood plasma. Intercorrelations between rumen juice spectra and milk composition (casein and fat) as well as between milk spectra and rumen juice composition (for acetic acid and propionic acid) have been pointed out in 1999. Blood plasma spectra and milk composition and the milk spectra and blood composition showed also statistically significant interrelations. So the simultaneous spectral data collection from different body liquids allowed to measure the condition of living animal *in vivo*.

According to Purnomoadi *et al.*[23] the nutritional status and also the heat adaptation of cows can be predicted from blood, milk or urine based on measuring the urea N in blood by NIR.

Milk, blood, rumen juice and urine contain specific informations related to metabolic and health status of ruminants. Tsenkova and Atanassova[24] have been confirmed that the mastitis induced compositional changes of biofluids can be sensitively measured by NIR in two spectral regions.

The rumen fluid composition (acetic, propionic acid, ammonia N, other constituents) of living animals in cows were measured on-line recently using specially designed fibre optics by Turza *et al.*[25]

Insects, worms

In 1995 Jin *et al.*[26] have been reported that male, female and dead worm cocoons of silkworms can be distinguished based on their NIR spectra. In the 700–1235 nm region developed MLR models were able identify the sex of cocoons with 96% safety.

Internally insects-infested wheat samples were tested by NIR in order to determine the presence of grain weevil in the seed by Ridgway and Chambers[27] In the spectral region between 1200–1300 nm the infested and control kernels were distincted with subtraction the 1202–1300 nm spectral values. It was thought that loss of starch absorbed at 1202 nm due to insect feeding was detected.

Dowell *et al.*[28] have been developed NIR methods for detection of parasitized fly puparia. Results derived from a PLS analysis of NIR spectra showed that about 80–90% of parasitoids could be identified correctly. It was pointed out that parasitized and unparasitized puparia may have been due to moisture content and/or differences of chitin or lipid components.

Plant tissues, plant physiology

Seeds, separated plant parts like leaves, grasses, fruits, silage *etc.* were tested intensively during the last three decades. The next parts are discussing some interesting, non-conventional applications of NIR in the plant world.

Plant tissues

The age of plant tissues was predicted by Blakeney and Batten.[29] They have confirmed that both chronological and physiological age of tissue can be predicted based on NIR spectra. Same authors have been reported that NIR has a potential to monitor the crop energy reserves where the basis of monitoring is the change and fluxes of non-structural carbohydrates in plants.

Plenty of developed models are existing for measurement of kernel hardness (particle size index) using NIR spectra. Manley *et al.*[30] have developed an FT-NIR procedure recently to measure kernel hardness.

Maturity and maturation process of plant materials

Cotton fibre maturity was predicted by an automated NIR high volume tester according to Ghosh.[31] The degree of maturity in cotton can be described by the measurement of secondary cell wall thickening. Ghosh developed NIR models for prediction of microscopic maturity and also for cotton dye uptake.

The stages of maturation and their effect on melon and pineapple quality were investigated by Guthrie *et al.*[32] The models allowed to use the NIR as a "sweetness" grading for individual fruit.

Pawpaws (*Carica papaya*) in various states of maturity were tested using a simple, portable fiber-optic spectrometer in a 500–1000 nm wavelength range. The ripening process was followed sensitively in the 720–815 nm region where a dramatic falling in concentration of chlorophyll was pointed out by Greensill and Newman.[33] Based on their observations a four category maturity ranking system was developed.

Wheat seed maturation process was investigated by NIR methods in our laboratory (Gergely and Salgó).[34] It has been pointed out that the molecular changes running during maturation processes are strongly associated with the amount and status or character of water. The various metabolic events of maturation (source/sink effects of carbohydrates and amino acids, synthesis of proteins and polysaccharides) can be followed in different segments of relevant spectral regions.

The changes of the fine structure of storage proteins during maturation were cleared more detailed by Piot *et al.*[35] using confocal micro-Raman microscopy. They have detected a significant increase of α-helixes in the late phase of maturation while the amount of other conformations (β-structures and random coil) was decreased. They have postulated that the α-helical secondary structure of protein could be associated with hardness.

Viability and germination of seed

NIR spectroscopy provides an effective tool and technique to substitute the difficult biotests in the measuring the physiological status and reserve mobilisation of seed.

Radish seed viability was predicted using NIRS by Min *et al.*[36] recently. They have developed a PCA based algorithm to differentiate germinated and non-germinated seed.

The germination process was also followed by NIRS in wheat by our research group.[37] The most characteristic changes were observed in the form and status of water during the early phase of germination. In the first 24 hours the amount of "free" water was decreased and the seed swelling initiated the mobilisation processes of carbohydrates and amino acid pools.

Allosio *et al.*[38] have been developed a parallel factor analysis model for evaluation the barley germination (malt production) process. Their PARAFAC model described the chemical, biological as well as technological variations reflected by the NIR spectra in industrial environment.

Wood structure, characteristics and ecology

A non-traditional application of NIR spectroscopy was reported by Tsuchikawa.[39] The biological material with special cellular structure like wood was investigated by NIR using special approach. New optical models were developed to clarify the behaviour of light propagation in wood. These new optical concepts allowed to measure physical and chemical characteristics from spectroscopic data.

According to Gindl *et al.*[40] other physical characteristics like density, bending strength and compressive strength can also be predicted from NIR spectra of wood samples (radial wood surfaces).

A unique biological system was observed and evaluated by Foley *et al.*[41] using NIR as tool. They have investigated why koalas and other ringtail possums eat leaves from certain eucalyptus trees. Investigations were pointed out a new group of natural plant toxins called sideroxylonals can be present in foliage. These compounds are potential antifeedants against koalas. When these were present in the foliage at concentration of more than 15 mg g^{-1} leaf were refused to eat any leaf at all. It means koalas selected trees based on the level of toxic compounds. NIR calibration was developed to measure the sideroxylonal in eucalyptus leaves and a population of trees were tested whether they are producing low, marginal or enough palatability leaves to support possums.

Instruments and new techniques in observation of biosystems

The conventional NIR/NIT test methods and techniques can be used wide-ranging in the observation of living systems but recently were published some very dedicated and unique methodologies which are able to follow the biochemical and physiological processes with enhanced sensitivity and which are operating with samples having extreme big structural and compositional variations.

New technical approaches

An electronically tuned Ti:sapphire laser system was applied to measure the transmission spectra of the whole human hand in which the bands due to hemoglobin and lipid were identified by Sato *et al.*[42] The oxygenation of hemoglobin was detected in the wavelength region between 700–1000 nm.

A new measurement concept was developed by Tsuchikawa *et al.*[43] using the so-called time of flight (TOF) NIR in the investigation of fruits. This system combines a tunable laser and a NIR photoelectronic multiplier and the time-resolved profile of transmitted output power can be measured sensitively in nanoseconds.

Imaging and mapping

The imaging spectroscopy (NIR, FT-NIR or FT-IR) define the spatial relationship between physical and compositional information for a sample yielding information on structure and function of the material measured. The imaging spectroscopy using visible, NIR and fluorimetry can be applied in the plant science to measure the *in vivo* effects of fungal pathogens, detection of plant diseases and nutrient status, changes of water or even the quality testing of flower bulbs according to Workman.[44] The formation and localisation of LDL plaques in cardiovascular system was also determined *in vivo* by NIR imaging using an intravascular fiber-optic catheter according to Cassis *et al.*[45]

The development of hyperspherical imaging will be resulted a revolutionary progress in the investigation of biological objects too. The results is a three dimensional data set where the X and Y axes represent the spatial information and Z axis represents the reflectance at the selected NIR wavelengths according to Lewis *et al*.[46] This exciting new technology is capable of providing insight into the structure, composition and function of biological objects. The hyperspherical imaging will be probably used not only for monitoring the biological objects but also for monitoring biosystems and/or ecosystems.

Hand-held instruments

There is a trend to develop simple, low cost and portable instruments to measure the key chemical constituents in different biological systems. McClure[47,48] reviewed some of hand-held spectrometers which were used for detection of nicotine (N-meter), vanillin and moisture (V-meter), chlorophyll and moisture (TW-meter) and protein in plant tissue (G-meter). He has been made a futuristic concept to develop a “nutrient pencil” that allows to measure the need of fertilisers and analysing the tissue of growing plants objectively. This concepts prefers to control and to improve the agricultural production and the improvement the sustainability of food production.

We are expecting that the first hand-held "personal pencil types" spectrometers will control "clinical" types of parameters, or composition of human body constituents and the NIR spectroscopy will play important role in the monitoring and in prevention of diseases.

Conclusion

The following future trends can be imagined in the development of NIR methods in order to observe easier the biological systems. Sophisticated sample cells and sample preparation methods will be developed in order to measure the biosamples with higher sensitivity. Both high resolution, sensitive and expensive as well as dedicated, hand-held and cheap instruments and detectors will be developed to fulfil the demands the market directly and/or the demand of R&D sector. The imaging and mapping methods will be expanded including the macro-, bio- and ecosystems. The innovation and application of airborne instruments and sensors will be accelerated.

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