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Review

Spectroscopic and Clinical Aspects of Noninvasive Glucose Measurements

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Frequent determination of glucose concentrations in diabetic patients is an important tool for diabetes management. This requires repetitive lancing and finger bleeding. Use of noninvasive (NI) detection techniques offers several advantages, such as the absence of pain and exposure to sharp objects and biohazard materials, the potential for increased frequency of testing, and hence, tighter control of the glucose concentrations, and the potential for a closed-loop system including a monitor and an insulin pump. These potential advantages have led to considerable interest in the commercialization of NI glucose monitoring devices. Review of the scientific, patent, and commercial literature indicates that the spectroscopic basis for NI determination of glucose is not yet well established, and attempts at commercialization may be several steps ahead of our understanding the origin and characteristics of an in vivo glucose-specific or glucose-related signal. Several technologies have potential for leading to viable measuring devices, but most of the data are based on in vitro experimentation. Because of the technical complexity of in vivo glucose measurements, this review aims at discussing the gap between the established need and current technology limitations.

Interest in monitoring the glucose concentrations of diabetic patients has increased since the publication of the Diabetes Control and Complications Trials report showing that tight control of blood glucose concentrations, by frequent testing and concomitant adjustment of insulin doses, decreases long-term complications resulting from diabetes *(1)*. A computer simulation based on the Diabetes Control and Complications Trials results estimates an additional 5 years of life, 8 years of sight, 6 years free from kidney disease, and 6 years free from amputations for a diabetic following the tight control vs the standard regimen *(2)*. Noninvasive (NI)[1] monitoring of glucose has been of particular interest because of the pain associated with invasive self-monitoring. Ease of use and reduction of pain can encourage more frequent testing and hence tighter control of the glucose concentration. Patient care needs and the commercial importance of NI glucose monitoring has led to a flurry of "research" by entrepreneurial and commercial concerns that has been published mainly in patent literature. Patents are generally granted on the basis of novelty and potential usefulness, with less regard for accuracy and precision data or experimental design. A large number of NI glucose patents lack scientific rigor and some may be based on wrong or unproven assumptions. Several recent reviews have discussed the importance of NI glucose testing and report on attempts at its measurement *(3–7)*. Despite the dearth of information published in peer-reviewed journals, I will review the different optical methods for NI glucose measurements, physical principles, expected sources of error, and their potential impact on clinical decisions. This review will emphasize the theoretical principles and merits of each technique and the reported error limits.

Self-monitoring of blood glucose concentrations has advanced over the past few years. Glucose values determined by home meters correlate well with laboratory results. Because of the importance of precision and accuracy of self-monitoring blood glucose devices, guidelines for the performance of these devices were recommended in 1987 by the American Diabetes Association *(8)*. These goals and performance characteristics of current meters are important as a yardstick for establishing the potential performance of NI technologies. The limits of detection and quantification, the standard deviation of the measurement, the accuracy, and the total error of an NI measurement need to correlate with self-monitoring devices and with measurements in the laboratory. American Diabetes

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[1] Nonstandard abbreviations: NI; noninvasive detection; OGTT, oral glucose tolerance test; PLS, partial least squares; ANN, artificial neural network; IR, infrared; PA, photoacoustic; SEP, standard error of prediction; SEC, standard error of calibration; and LOD, lowest limit of detection.

Association guidelines for home glucose monitors state that values should fall within 15% of the readings obtained by a reference method. The goal set for future self-monitoring blood glucose meters is to achieve a variability (system plus user) of less than ± 10% *(8)*. Evaluation of commercial devices shows that not all meters fulfill this consensus goal *(9)*. The major error component is user error, which includes the volume of the blood droplet, the accuracy with which the blood drop is placed on the pad, hematocrit effects on serum volume, timing, and the effect of temperature on the signal-generating reaction. An NI measurement can potentially eliminate some of these effects but may have a different set of error sources.

An NI body glucose monitoring device is defined in this review as a device that comes in contact with, or remotely senses, a human body part, without protrusion through membranes or sampling a body fluid for analysis external to the body part. Thus implantable devices *(10)* and methods dependent on glucose diffusion through the skin *(11)* will not be discussed. For the purpose of this review, only optical methods in the visible and infrared spectral regions will be discussed.

An NI glucose monitor processes optical signals transmitted through or reflected by the stratum corneum, dermis and epidermis layers, subcutaneous tissue, interstitial fluid, and blood vessels (both arterial and venous blood), which represent independent compartments. Each of these compartments may have different optical properties, concentrations of interferents, and concentrations of glucose. An NI-determined glucose value may represent an average value of different glucose concentrations, and correlation between an NI glucose measurement and a blood glucose measurement may vary by body site, depending on the differences in tissue and vascular properties of each part. Once an NI detection technology is proven to produce clinically significant data, new goals for NI systems need to be established based on how they will affect clinical decisions and the added value to patient care. A trade-off between accuracy and ease of use, or between precision and potential for frequent testing may need to be made.

NI glucose monitors can be configured as bedside monitors for point-of-care testing, portable personal monitors, and closed-loop insulin pump/glucose monitors. Portable personal glucose monitors will have the most impact on patient care. Their use will increase the frequency of testing and lead to tighter diabetes control. They will require a rugged detection system that is not affected by environmental factors, robust algorithms, rigorous but easy-to-perform calibration routines, and miniaturized electronics and optics.

Research Methodologies for NI Glucose Measurements

Two research methodologies are used for NI glucose measurements. An empirical approach involves the following steps: (*a*) collect NI signals from nondiabetic individuals and diabetic patients while performing an oral glucose tolerance test (OGTT) or a glucose challenge test; (*b*) simultaneously measure blood glucose concentrations by an invasive method; and (*c*) compute models based on the correlation between measured glucose values and NI optical signals. This approach does not measure the effect of other metabolites and interferences, biological noise, or variability in instrument-body interface, but attempts to compute-out these noise contributions. The number of variables and the complexity of data analysis necessitates the use of multivariate chemometric techniques such as principal component analysis, partial least squares (PLS), or artificial neural network (ANN) methods *(12, 13)*. Special criteria are needed to avoid overfitting data and to eliminate outliers without affecting the fidelity of data presentation.

A second approach is the physical model approach. This methods involves the following steps: (*a*) measurement of a glucose optical signal in a simple matrix; (*b*) progressive increases in the complexity of the matrix to mimic human tissues; (*c*) demonstration of accuracy and precision at each step; and (*d*) correlation of the data with a mathematical model for light propagation in tissue. Finally, the detection system and the measurement method are applied to body parts. The in vivo signals are again correlated with the invasive data by use of chemometric techniques. This stepwise approach allows for identification of noise components so that strategies may be derived to minimize their contribution to the signal before the use of chemometric techniques.

Calibration of NI Glucose Measurements

ANALYTICAL CALIBRATION

Analytical calibration, defined as the determination of the concentration of the analyte from a calibration curve generated by calibrators and standards, is difficult for NI glucose measurements. There are numerous sources of error that can affect the measurement, which need to be either eliminated or compensated for by a calibration method. Some of these error sources that can affect the measurement, but cannot be easily incorporated into the calibration are detector positioning error, temperature and cardiac pulse effect, motion, mechanical pressure of the test device, hydration state, sweat, blood volume, and hematocrit change.

Several tissue-simulating phantoms have been proposed as calibration systems. Some of these phantoms are suspensions of lipids or polystyrene particles in solutions having different concentrations of glucose. A phantom containing fat and glucose solution has been used to mimic tissue glucose absorption in the 2000–2500 nm range *(14)*. Glucose concentrations used in these studies have generally been higher than the physiological range. Because of the nonspecificity of the signal measured by several technologies (as will be discussed later), developing tissue-simulating phantoms as analytical standards for NI glucose determination in tissue is a challenging

goal. An NI device that is analytically calibrated can be considered as universally calibrated; i.e., can be used with any patient.

CLINICAL CALIBRATION

In vitro and in vivo measurements are performed on a fasting subject at time intervals during an OGTT, meal-tolerance test, or a glucose clamp procedure. These methods offer a concentration range over which the glucose signal can be monitored. Data from an OGTT can be used to establish an NI instrument response as glucose concentration for an individual is changing. Data that are generated during the test period are used to predict glucose concentrations in subsequent NI measurements. Because the response of an NI instrument may embody non-glucose-related physiological effects, relying on clinical calibration based on the correlation of OGTT data with NI instrument response leads to a calibration curve that is unique to the individual tested. This calibration curve may need to be periodically updated, by use of an invasive test.

Use of an OGTT or a meal-tolerance test for calibration leads to a series of measurements that are sequential in time. Spurious drift and time-dependent artifacts can influence the results from multivariate calibrations when randomized sampling cannot be performed *(15)*. Thus, the temporal distribution of signal and noise may lead to erroneous glucose correlation. Furthermore, Arnold et al. *(16)* have challenged the validity of several in vivo blood glucose measurements based on spectra collected in a time-dependent manner. Chance temporal correlation between assigned glucose concentrations and some uncontrolled experimental parameter have been suggested as responsible for the apparent functionality of some of these models.

DATA PRESENTATION

In addition to the use of statistical methods to present the performance of NI glucose measurements, the Clarke error grid has also been used for data presentation. The Clark error grid analysis offers a quick estimation of the accuracy of the measurement compared with a reference method. Data from the test device are plotted against the results of a reference method. A scatter diagram is established and divided into A, B, C, D, and E zones *(17, 18)*. Data points falling in zone A represent acceptable performance, whereas those in zones C, D, and E represent unacceptable performance. A personal glucose monitor is considered to have acceptable performance in the hands of a user if >95% of the data points on the scatter graph fall in A zone and 0% fall in the C, D, and E zones *(18)*. This will be approximately equivalent to a total error of 20% at blood glucose concentrations >3.5 mmol/L. A constant error (i.e., not a percentage) is used below this concentration. Data points in the C, D, and E zones represent progressively increasing deviation between the home glucose monitor and the reference method. This increased inaccuracy may lead to the wrong type of intervention.

Principles of Glucose Tissue Measurements

NEAR-IR ABSORPTION OF GLUCOSE IN AQUEOUS MEDIA

Water, the major component of biological tissues, has a simple infrared (IR) spectrum and a rich combination and overtone spectrum that extends into the near-IR. The assignment of the near-IR absorption bands for water has been established *(19)*. The intensity of the near-IR absorption bands for water is sensitive to solute concentration and temperature *(20–22)*. It decreases as solute concentration increases because of change in the molar ratio of water. This is referred to as water displacement. The physical properties of aqueous solutions have been determined from the temperature dependence of the intensity of the 1400 nm band *(23, 24)*. A similar temperature sensitivity of the near-IR absorption of water is observed in tissue *(25)*.

The fundamental IR absorption bands of glucose have been reported in solid pellets and in solution *(26–30)*. The strongest bands that can generate intense combinations and overtones are the broad OH stretch at 3550 cm^{-1} and the C—H stretch vibrations at 2961 and 2947 cm^{-1}. Possible combination bands are a second OH overtone band at 939 nm (3νOH) and a second harmonic CH overtone band at 1126 nm (3νCH). A first OH overtone band can be assigned at 1408 nm (2νOH). The 1536 nm band can be assigned as an OH and CH combination band (νOH + νCH). The 1688 nm band is assigned as a CH overtone band (2νCH). Other bands at >2000 nm are possibly combinations of a CH stretch and a CCH, OCH deformation at 2261 nm (νCH + νCCH, OCH) and 2326 nm (νCH + ν CCH, OCH). The presence of the CCH, OCH ring deformation component confers some glucose specificity on these bands. The calculated near-IR overtone and combination spectra of glucose overlap with several (more intense) combinations and overtone bands of water and fat and hemoglobin electronic absorption bands, as shown in Table 1 *(31)*. These are the major potential interferences with the NI determination of glucose. Near-IR (2000–2500 nm) spectrophotometric determination of glucose has been achieved in aqueous media *(32–38)*.

Optical Properties of Tissues

The optical techniques that have been pursued by various groups in the past few years are classified in Table 2. The near-IR spectral region is commonly proposed or used in all reported technologies. The 600-1100 nm region of the spectrum represents a window between the hemoglobin and visible absorption bands and water IR absorption *(39–42)*. Light can penetrate deep enough into the tissue to allow a spectral measurement or a therapeutic procedure. This spectral region is used for oxygen saturation, pulse oximetry, and laser-Doppler flow measurements *(41–46)*.

Table 1. Near-IR bands for components of solid tissue demonstrating potential interferences with NI glucose detection.

Glucose[a]	Water[b]	Hemoglobin[b]	Fat[b]	Protein[b]
714	749			
		760 (E)		
			770	
		805 (E)		
		820 (E)		
	880			
939		910	920	910
	980			
1126	1211	1020 (E)	1040	1020
1408	1450			
1536				
1688				
	1787			
	1934			
2261			2299[c]	2174[d]
2326			2342[c]	2288[d]

[a] Calculated from glucose fundamental vibrations.
[b] "E" indicates an electronic absorption band; others are vibration overtone and combination bands *(19, 31)*.
[c] From Burmeister et al. *(14)*.
[d] From Pan et al. *(38)*.

NEAR-IR TISSUE OPTICAL PROPERTIES

The transport equation, and the diffusion theory approximation to this equation *(47–51)*, describes the path of photons through human tissue. It expresses light propagation in tissues by a set of spectroscopic properties; the absorption coefficient μ_a, the scattering coefficient μ_s, the refractive index of the cells and the interstitial fluid; and the anisotropy factor g (the average cosine of the angle at which a photon is scattered). Another set of properties are the transport properties, such as the reduced scattering coefficient μ_s', where $\mu_s' = \mu_s[1 - g]$. The absorption coefficient μ_a equals the absorbance per unit path length, 2.303 ϵC cm^{-1}, where ϵ is the molar absorptivity and C is the molar concentration. The scattering coefficient $\mu_s = \sigma\rho$ where σ is the scattering cross-section and ρ is the number density of the particle. It has the same unit as μ_a (cm^{-1}) and is equivalent to the product of an absorptivity caused by scattering and the concentration of the scattering centers.

Attenuation of light in tissue is described, according to light transport theory, by the effective attenuation coefficient μ_{eff}, i.e.:

$$I = I_0 e^{-\mu_{eff} l}$$

Where:

$$\mu_{eff} = \sqrt{(3\mu_a(\mu_a + \mu_s'))} = \sqrt{(3\mu_a[\mu_a + \mu_s(1 - g])}$$

An exact solution of the light transport equation in turbid media can be modeled by following the path of each individual photon and calculating the probability of scattering or absorption in a series of steps, using the Monte Carlo simulation *(49–50)*. This modeling is used to study the path of photons in tissues and is widely used for optimization of photodynamic therapy, improvement in pulse oximetry, laser-Doppler flowmetry, and optical mammography; all have very important clinical utility. Several recent volumes of the proceedings of the Society of Photo-optical Instrumentation Engineers have covered these topics. Methods that are used for measuring the optical properties of tissues (μ_s, μ_a, and g) include transmission, diffuse and localized reflectance, and frequency domain measurements *(52–65)*.

EFFECT OF GLUCOSE ON ABSORPTION PROPERTIES OF TISSUES

Glucose can affect the measured transmitted or reflected signal by absorption of light at the overtone and combination band wavelengths. Light absorption can be expressed as $I = I_0 e^{-\mu_a l}$, where l is the effective path length in the medium, and μ_a (2.303ϵC) is the absorption coefficient. Changes in glucose concentration can influence the measured μ_a of tissue through changes in absorption corresponding to water displacement (absorption decreases as glucose concentration increases) or changes in its intrinsic absorption (absorption increases as glucose concentration increases). Changes in μ_a because of water displacement are nonspecific, and analytes with higher molecular weights will displace more water than does glucose. Changes in the temperature and hydration status of the body may affect water absorption bands and act as

Table 2. Methods proposed for NI glucose detection in tissue.

Technology	Measurement/optical constant	Spectral region
Near-IR transmission and reflectance	Total transmission or diffuse reflectance of tissue or mucus membranes	600–2000 nm
Mechanical manipulation of tissues	Total transmission of tissue at different mechanical perturbations	1300–1700 nm
Near-IR Kromoscopy	Change in tissue absorption	600–1100 nm
Spatially resolved diffuse reflectance	Absorption and scattering coefficient, controlled pathlength	Selected near-IR wavelengths
Frequency domain measurements	Absorption and scattering coefficients, controlled pathlength and depth	600–1100 nm
Polarimetry measurements	Change in optical rotation	633 nm
Raman measurements	Raman shift, O—H and C—H fundamental vibrations	514, 647, and 830 nm
PA measurements	Absorption coefficients and thermal properties	1100–2100 nm

noise sources for an NI glucose measurement. The glucose μ_a in the near-IR is low and is much smaller than that of water. It is higher at longer wavelengths. However, its magnitude is too small to allow for direct absorption measurements at wavelengths <1400 nm. Attenuation of light (<1400 nm) in a small body part such as an average-sized finger varies in the range 3–4 absorbance units, and the expected change in absorbance because of a 5 mmol/L change in glucose concentration is $\sim 10^{-5}$ absorbance units.

EFFECT OF GLUCOSE ON TISSUE SCATTERING

Changes in the glucose concentration affect the intensity of light scattered by tissue. The reduced scattering coefficient of a tissue can be expressed in a function form as: $\mu_s' = f(\rho, a, n_{cells}/n_{medium})$, where ρ is the number density of scattering cells in the observation volume, a is the diameter of the cells, n_{cells} is their refractive index, and n_{medium} is the refractive index of interstitial fluid *(51)*.

Changes in the n_{medium} are not specific for a particular analyte and are affected by any change in the total concentration of solutes in blood and interstitial fluid. The calculated Δn_{water} as a function of the change in the concentration of several metabolites, as calculated from the slope of tabulated n values at different solute concentrations, is given in Table 3 *(66)*. During hyperglycemic episodes, the glucose concentration changes rapidly, whereas other analyte concentrations presumably change at a slower rate. It may be possible to relate $\delta\mu_s'$ to changes in glucose concentration over a short time span. The measured n_{water} decreases as temperature increases *(66)*. This affects n_{cells}/n_{medium} in tissue and presents a source of error in scattering measurements. Values of μ_s' are reported to decrease with increasing concentrations of glucose and other sugars in tissue-simulating phantoms because of their effect on n_{medium} *(53–65)*.

A recent Monte Carlo modeling of the effect of physiologic concentrations of glucose (5–30 mmol/L) on the diffuse reflectance or transmittance of tissue-simulating phantoms predicts very small changes in signal. The modeling is performed at 800 and 960 nm with water as the only absorber. The estimated $\delta\mu_s'$ is $<1 \times 10^{-3}$/mmol of glucose, which is much higher than the changes μ_a. Other physiological factors, such as changes in the water content, temperature, and protein concentrations are considered. The value of $\delta\mu_s'$ caused by an increase of 1 mmol/L in glucose concentration is equal to a 2×10^{-3} increase of water content, a 1×10^{-3} increase of protein concentration, or a 0.1 °C decrease in temperature *(67)*. In addition, the concentrations of pigments in the epidermis strongly influence light penetration in the near-IR *(68)*. The temperatures and body water content of an individual are tightly regulated, but differ between individuals. Although body temperature is regulated within a fraction of a degree, temperature at the extremities may vary by >1 °C. A temporary change in water content of 240 mL for a 75-kg individual can lead to an error equivalent to 2 mmol/L glucose. The reference range for albumin spans 20% of the mean concentration. This spread will affect the signals from different subjects with normal albumin concentrations. Hemoglobin has not been considered as an absorber in this simulation *(67)*. However, changes in its concentration will affect optical signals in the 600–900 nm range. Positioning errors and body interface effects seem to be the largest contributors to measurement errors.

Table 3. Refractive index changes associated with changes in concentration of tissue components.

Analyte	Concentration change	$\Delta n_{medium} \times 10^6$	Notes
Glucose	5.6 mmol/L	140.9	Fast
Creatine	0.3 mmol/L	6.6335	Slow
Lactic acid	1.3 mmol/L	9.906	Slow
Serum albumin	1.5 μmol/L	18.47	Slow
NaCl	1 mmol/L	10.1	Slow, except during dehydration

Optical Methods for Noninvasive Determination of Glucose

NEAR-IR TRANSMISSION AND REFLECTANCE

Robinson et al. *(69)*, Haaland et al. *(70)*, and Ward et al. *(71)* have attempted NI glucose detection in two overlapping regions, 750-1050 and 850-1300 nm, which encompass the 3νOH and 3νCH glucose overtone bands. Hiese and co-workers *(72–76)* and Marbach et al. *(77)* have reported a series of studies on the determination of glucose in oral mucosa in the 1111–1835 nm spectral range. This range encompasses bands corresponding to the 3νOH, 2νOH, νOH + νCH, and 2νCH glucose vibrational combinations. Jagemann et al. *(78)*, Muller et al. *(79)*, and Fischbacher et al. *(80)* have measured reflected light in the wavelength range 900-1200 nm through a fiber bundle touching the skin. Khalil and Malin *(81)* have proposed the use of reflected signals from four distinct near-IR spectral ranges: 1320–1340, 1440–1460, 1940–1960, and 1670–1690 and 2120–2280 nm. The 1320–1340 nm range is used for correcting for optical coupling and sample positioning errors. The 1440–1460 nm or 1940–1960 nm ranges are where the highly absorbing water bands lie. The 1670–1690 nm range (2νCH glucose overtone band) and the 2120–2280 nm range are where glucose combination bands νCH + νOH, CH are located. The correction method involves normalizing the signal to the shortest wavelength range, subtracting the normalized signal, and correlating the subtracted signals with glucose concentration. Brumeister et al. *(82)* have used the 1400–2000 nm range for NI determination of glucose with the tongue as the body site.

Rosenthal et al. *(83–88)* at Futrex Inc. have proposed using the spectral band in 600-1100 nm near-IR transmitted or reflected through a body part and analyzing the light by a set of filters and detectors. This spectral range encompasses the water spectrum and the 3νOH and 3νCH glucose overtone absorption bands.

Purdy and co-workers *(89, 90)* and Barnes et al. *(91)* at Biocontrol Inc. have used light at wavelengths >1100 nm falling on the forearm of a patient from light-emitting diodes. The reflected light is directed into a spectrometer and analyzed. The spectral range encompasses 3νCH, 2νOH, νCH + νOH, and the water bands.

mechanical manipulation of tissues

In a method developed by VivaScan Inc., measurements are made by altering the blood volume by changing pressure on a body part in a controlled way, while performing a near-IR transmission measurement in the 1300–1600 nm range. The spectral range encompasses 2νOH, νCH + νOH, and water absorption bands. Difference spectra are obtained by comparing spectra collected with different compressions within the exposed tissue samples *(92–95)*. Wavelength pairs in the 1300–1600 nm range are selected by use of an acousto-optic modulator, and measurements are taken at different compressions of a thin body part such as a web or an earlobe. This is considered as a hardware compensation for tissue contributions to the signal.

near-ir kromoscopic© measurements

Kromoscopy, developed by Optix Corp., is reported extensively in the patent and commercial literature *(96–101)*. It is claimed to have superior sensitivity compared with spectroscopic methods. Kromoscopy presumably has the potential for NI determination of glucose by using broad band light illumination, broad band overlapping filters, and multiple detectors; however, there is limited theoretical basis for the sensitivity improvement over photometric methods. The method reportedly is based on the ability of the eye to determine slight changes in color *(96–101)*; however, the effect of light scattering on Kromoscopic measurements, has not been discussed.

spatially-resolved diffuse reflectance R(r) measurements

In this technique, a narrow beam of light illuminates a restricted area on the surface of a sample or a body part, and the diffuse reflectance is measured at several distances from the illumination point *(52–57)*. This method is denoted as R(r) measurement. The value of μ_{eff} can be calculated from the data, and both μ_a and μ_s' values for tissue can be deduced *(55, 56)*. Changes in the values of μ_a and μ_s' can then be used to calculate the change in concentration of an analyte affecting the tissue optical properties *(102–104)*.

near-ir frequency domain reflectance measurements

Frequency-domain reflectance measurements use an optical system similar to that used for spatially resolved diffuse reflectance R(r), except that the light source and the detector are modulated at a high frequency. The difference in phase angle and modulation between injected and reflected beam is used to calculate μ_s' and μ_a of the tissue *(58–65, 105, 106)*.

optical activity and polarimetry

Polarimetry has been used for quantitative analysis of solutions of optically active (chiral) compounds such as glucose. When a plane polarized light beam is transmitted through a solution, its plane of polarization is rotated by an angle, α, which is related to the concentration of the optically active solute *(107–115)*.

The optical rotation of several chiral compounds at their physiological concentrations is given in Table 4. A 5.5 mmol/L change in glucose concentration yields the highest calculated short-term effect on α. It may be possible to detect hyperglycemic swings, assuming that changes in the concentrations of other optically active compounds occur over a longer time frame than that for a change in glucose concentration. Scattering is bound to depolarize the light and decrease the measured value of α.

raman scattering

Raman spectroscopy has been used mainly for vibration band assignment and qualitative analysis. Instrument complexity and difficulties with calibration delayed its acceptance as a quantitative analytical tool *(116, 117)*. The advent of holographic optical elements and charge-coupled device cameras has allowed the design of fast Raman spectrometers with which the spectrum is acquired over a wide range of wave numbers and averaged in ≤30 s. Chemometric methods have allowed ease of generating training sets for subsequent prediction of concentration.

Table 4. Optical rotation of chiral compounds at physiologic concentrations.

Compound	$[\alpha]_D^o$	Physiologic concentration	$10^4\ \alpha^o$ (1-cm path length)	Notes
Glucose	+52	5.6 mmol/L	+52	Blood
Glucuronic acid	+36.2	0.2 mmol/L	+1.348	Blood, negligible change during OGTT and long term
Glycogen	+197.6	1% in muscle <1 μmol/L in blood	+6	Short-term change
Hexosamine (glucosamine)	+47	4.6 mmol/L	+39	Long-term change >5 days
Lactic acid	−12	1.3 mmol/L	−1.44	Blood and muscle, day-to-day change
Cholesterol	−40	4–5 mmol/L	−60 to −80	Blood, long-term change

Long wavelength solid state lasers have allowed shifting the excitation wavelength to the near-IR, thus reducing the fluorescence background from biological samples. These advances in hardware and data analysis methods allow the measurement of the concentration of a weak Raman scatterer with a lowest limit of detection (LOD) comparable to other spectroscopic techniques *(118–130)*.

Raman spectroscopy offers several advantages for measurement of glucose. Raman bands are specific to glucose molecular structure, the fundamental vibrations are monitored in Raman spectra and thus are sharper and have less overlap compared with near-IR combination bands, and water has a low Raman cross-section as opposed to its high IR absorption. It is possible to detect glucose by monitoring the 2900 cm^{-1} C—H stretch band or the C—O and C—C stretch Raman bands at 900-1200 cm^{-1}, which represents a finger-print for glucose *(118).*

near-IR photoacoustic spectroscopy

Photoacoustic (PA) measurement is an alternative detection technology for near-IR light interaction with tissues. PA is used to detect weak absorbance in liquids and gases *(131)*. The tissue is excited at a wavelength that is absorbed by glucose molecules by pulses of a 1000–1800 nm near-IR laser light. Subsequent optical absorption causes microscopic localized heating. The increase in temperature causes rapid thermal expansion, which generates an ultrasound pressure wave detectable by a hydrophone or a piezoelectric device in the cuvette or located at the skin surface *(131–137)*. The magnitude of a pulsed PA signal, *P*, is related to the absorption coefficient of solution by:

$$P = K(\mu_a \beta \sqrt{\nu})/C_p$$

where μ_a is the optical absorption coefficient, β is the thermal expansion coefficient, ν is the sound velocity, C_p is the specific heat of the solution, and *K* is a proportionality constant that is related to the bulk modulus of the medium. The C_p of a solution decreases, whereas the acoustic velocity increases with increasing glucose concentration. At a glucose absorption wavelength, change in the PA signal are the result of changes in μ_a, ν, and C_p. This multiplicative effect increases the PA signal as a function of concentration. The speed of sound and the C_p values change as the total solute concentration changes. When excited in the near-IR, PA detects the absorption caused by the overtones of O—H and C—H bond vibrations of glucose and other analytes; this absorption is subsequently converted into an acoustic pulse. PA measurements have some sensitivity advantages over other near-IR detection methods because a PA detector collects all generated signals in a volume of the tissue and detects signals generated at wavelengths longer than the range of silicon or gallium arsenide detectors.

In Vitro and ex Vivo Results

Glucose has been determined in vitro and ex vivo at concentrations close to its physiological range by several of the techniques proposed for its NI determination.

near-IR reflectance and transmittance measurements

Near-IR spectrophotometric determination of glucose has been achieved in aqueous solutions and serum-like matrices; by the use of IR absorption bands in the 2000–2500 nm range [standard error of prediction (SEP), 0.35 mmol/L]. Digital filtering is used as a preprocessing step before the PLS calculation to remove spectral features not associated with glucose *(32–38)*. The 2270 nm wavelength provides the lowest standard error of calibration (SEC), at 0.24 mmol/L.

near-IR frequency-domain reflectance measurements

The change in phase and attenuation of a modulated laser beam has been measured at different glucose concentrations in tissue-simulating phantoms *(61–62)*. μ_a remains unchanged, whereas μ_s' decreases with increasing glucose concentration. The decrease in μ_s' is attributed to changes in n_{medium} caused by dissolution of glucose. From these measurements, the fractional change in μ_s' of tissue is estimated as ranging between -1×10^{-3} and -5×10^{-4} per mmol/L of glucose. This estimation assumes that only changes in glucose concentration affect n_{medium} and do not affect cell size. A 1 °C change in temperature is estimated to decrease the μ_s' of the lipid emulsion by a value that is four- to eightfold higher than a change caused by an increase of 1 mmol/L of glucose *(61–62)*. Similar results are reported using glucose, sucrose, or mannitol *(60–63)*. Because $\delta\mu_s \gg \delta\mu_a$ at these wavelengths, it easier to measure $\delta\mu_s$ in scattering solution and relate it to the concentration of weakly absorbing solutes.

optical activity and polarimetry

Ex vivo measured glucose concentrations show reasonable correlation with blood glucose concentrations *(107, 108)*. The improved sensitivity of polarimeters allows measurements in small path lengths and at lower glucose concentrations *(110–114)*. Glucose has been determined in vitro in the 5.6–33 mmol/L range in cell growth media (SEC = 0.3 mmol/L; SEP = 0.47 mmol/L) and ex vivo in bovine ocular fluid (SEC = 1.25; SEP = 1.13 mmol/L) *(112, 113)*.

Raman scattering

Glucose concentration can be estimated in vitro by use of Raman bands at 900-1150 and 2850–3000 cm^{-1} down to 0.7 mmol/L in water and to 7.2 mmol/L in bioreactor material by use of laser excitation and PLS analysis *(119)*. The glucose concentration also has been determined in fluorescent serum and plasma samples down to 2.5 mmol/L by monitoring the anti-Stokes Raman band at

Table 5. LOD of glucose in aqueous solutions, using Raman spectroscopy, demonstrating potential for detection close to physiologic concentrations.

Glucose samples (laser line, nm)	LOD (blank + 3 SD)	Error estimate
Glucose, lactic acid, urea solution (514.5 nm)[a]	Lowest concentration used, 7.8 mmol/L	SD of concentration residuals, 1.15 mmol/L
Analytical curve (water, 514.5 nm)[b]	0.7 mmol/L	
Analytical curve (bioreactor media, 514.5 nm)[b]	7.2 mmol/L	
Analytical curve (bovine serum albumin solution, 785 nm)[b]	1.7 mmol/L	
Serum, anti-stokes Raman at 1130 cm^{-1} (514.5 nm)[c]	2.9 mmol/L	

[a] From Goetz et al. *(123)*.
[b] From Xu et al. *(119)*.
[c] From Dou et al. *(125)*.

1130 cm^{-1} *(129)*. The use of this Raman band at higher energy than that of the excitation laser beam eliminates the red-shifted fluorescence background. The reported in vitro LOD values are summarized in Table 5. Some LOD values are close to the glucose physiological range.

NEAR-IR PA SPECTROSCOPY

The concentration of glucose in vitro has been measured in a flow cell in the 1.7–33 mmol/L range. Pulsed laser wavelengths centered at 1440, 1550, and 1680 nm have been used *(131)*. The use of pulsed solid-state lasers at 780, 830, 1300, and 1550 nm has also been reported. Linear response curves between the pulsed PA signal and glucose concentration have shown the same slope in the presence of fixed concentration of cholesterol, sodium chloride, and human serum albumin. Analytical curves in aqueous solutions indicate that the PA signal that is excited at 1680 nm varies linearly with glucose concentration with a slope of $\approx 3.6 \times 10^{-2}$ per mmol/L of glucose and that the intercept varies with the concentration of the interfering compound added *(137)*.

In Vivo Studies and Results

NEAR-IR TRANSMISSION AND REFLECTANCE

A summary of the published in vivo glucose data is given in Table 6. Robinson et al. *(69)*, Haaland et al. *(70)*, and Ward et al. *(71)* have reported data on three subjects with 1.1 mmol/L $<$ SEC $<$ 2.1 mmol/L, depending on the spectrometer configuration and chemometric model used. The data represent a single data set; a calibration set for one patient on 1 day, and are not used to predict glucose values on other days. All of these authors have noted that the baseline shifts during measurements and use derivatives to reduce this shift. None of these authors, however, indicates which wavelengths are necessary or whether additional wavelengths can improve SEC.

Hiese and co-workers *(72–76)* and Marbach et al. *(77)* have reported a correlation between the measured reflected signal of oral mucosa in the 1111–1835 nm range and blood glucose concentration . The lowest SEP reported was 2.1 mmol/L. The medium through which light was transmitted and reflected differed from the skin and tissue used in other studies. This method has a potential lag time between the measurements of glucose concentrations in blood and saliva, and saliva components and residual food in the mouth present sources of interference.

Jagemann et al. *(87)* have measured transmission in the 900-1200 nm range. The data for 11 test persons were analyzed using PLS or an ANN. A Clark error grid presentation shows that, depending on the type of calculation used, 59–65% of the data points fall in the A zone, 25–30% fall in the B zone, and ~10% fall in the D zone.

Brumeister et al. *(82)* recently studied near-IR transmission through the tongue of five type I diabetics over 39

Table 6. Estimation errors in NI glucose measurements on human subjects by near-IR transmission and reflectance methods.

Spectral range, nm	Type of test	Estimation error
875–1300[a]	Meal-tolerance, across fingertip 41 subjects, 11-factor PLS	SEC; 1.1 mmol/L
1111–1835[b]	Diffuse reflectance spectra, lip mucous membrane, 133 spectra, 2 days, one subject, 20-factor PLS	SEP; 2.6 mmol/L
	219 spectra, 2 weeks, one subject, 20-factor PLS	SEP; 3.0 mmol/L
	133 individuals, 20-factor PLS	SEP: 3.1 mmol/L
900–1200[c]	Meal-tolerance, across fingertip, ANN	Root mean SEP; 1–3 mmol/L, depending on the number of factors
1414–2000[d]	Transmittance across tongue, five individuals, five samples per day over 39 days, individual calibration	SEP >3 mmol/L

[a] References *(69–71)*.
[b] References *(72–77)*.
[c] Jagemann et al. *(78)*.
[d] Brumeister et al. *(82)*.

days, performing five measurements per day. They used this procedure to reduce contamination of the data set with temporal effects. PLS calibration models were generated for the different individuals, and models were used to predict blind sample concentrations (every fifth measurement) or the later data (SEP >3 mmol/L). Concentration correlation plots were superimposed on the Clark error grid for a single subject calibration model. Approximately 50% of the 39 predicted glucose values fell in the A zone, 37% fell in the B zone, 5% fell in the C zone, and 7% fell in the D zone.

No clinical performance data have been reported on the methods of Rosenthal and et al. *(83–88)*. The selected number of wavelengths was limited compared with the full spectrum used in other studies *(69–71)*. Clinical data are needed to compare the result of using this wavelength range and measurement method with those data published by other groups *(69–80)*.

The method proposed by Purdy and co-workers *(89*, *90)* and Barnes et al. *(91)* is the only in vivo glucose monitoring method that has clinical data submitted for regulatory approval. However, except for news in trade publication and company press releases, no published clinical data are available to allow the merits of this technology to be evaluated. Nor have any clinical data been published on the proposed method of mechanical manipulation of tissue *(92–95)*.

Published data for near-IR Kromoscopic measurements show an uncontrolled meal-tolerance test on one subject of unknown diabetic status *(97)*. Data are presented as a plot of the signal difference from two pairs of detectors. The signal difference changed immediately after a meal and returned to near its original value 2 hours after the meal.

spatially-resolved diffuse reflectance R(r) measurements

A glucose clamp experiment has been conducted in conjunction with an R(r) measurement *(102)*. The optical probe was affixed to the patient's abdomen, and μ_s' was estimated at 650 nm. The blood glucose was held at 5 mmol/L, and then step changes in its concentration were induced between 5 and 14 mmol/L. The drift in μ_s' independent of the glucose concentration prevented statistical analysis. $\delta\mu_s'$ was used to track the blood glucose concentration in 30 out of 41 diabetic patients studied *(102)*. $\delta\mu_s'$ was reported for three subjects as -2×10^{-3}, -3.4×10^{-3}, and -1.1×10^{-3}/mmol/L, respectively. Changes in μ_s' cannot be considered exclusively as resulting from changes in n_{medium} caused by increased blood glucose concentrations. The possibility that other physiological processes contribute to $\delta\mu_s'$ still exists *(102*, *103)*.

near-IR frequency domain reflectance measurements

μ_s' and μ_a in tissues have been determined in vivo by use of a frequency-modulated 805 nm laser. The optical probe was placed on the thigh of a nondiabetic subject during an OGTT test *(60*, *105*, *106)*. The results were similar to those reported by R(r) measurement *(102*, *103)*.

polarimetry and raman spectroscopy

No human clinical data have been published on the use of polarimetry or Raman spectroscopy to determine glucose concentrations in vivo.

near-IR PA spectroscopy

Initial clinical data show a change in signal with glucose concentration in a meal-tolerance test. However, no statistical data analysis has been presented to show its advantage over a near-IR transmission or reflectance measurement *(134–137)*.

other reported in vivo methods

An IR emission technique based on measurement of the fundamental absorption bands of glucose at 9.1–10.5 μm as they affect the intensity of the tissue black body radiation has been proposed *(138)*. The body emits IR energy, which can be used as the light source that is absorbed by glucose at 10 μm. In the proposed method, the surface of the skin is cooled to eliminate its absorptive effect, and the emission from the subcutaneous layer is detected. In vivo clinical calibration data on several type I diabetics have been reported *(139)*. A linear response between in vivo detected glucose and blood glucose values has also been reported. Multiple linear least squares fit to the calibration data yield an SD of 1.4 mmol/L. No prediction data have been published. Measurement of the IR emission from the tympanic membrane by use of a filter at 10.5 μm and the measurement of the difference in the signal to that of a known glucose concentration has been proposed. The data in the patent example indicate that the signal and the glucose concentration in a meal-tolerance test have been tracked *(140)*. A totally different technique, which is based on body thermal effects, has been proposed by Cho and Hoizgreve *(141)*. They claim that accurate measurements of changes in body temperature yield good NI glucose calibration plots. These IR emission methods are quite interesting because they require simpler instruments.

Conclusion, Predictions, and Future Directions

There have been several serious experimental attempts to develop NI measurements of glucose in biological tissues, and some correlation does exist between blood glucose values and measured optical signal. None of the NI experiments reviewed provides proof that the measured signal is related to the actual blood glucose concentration. The only indication is the existence of a correlation with the change in glucose concentration during the experiment. The application of chemometric techniques to extract glucose concentration from noisy data sets compensates for lack of specificity. To date, there has been no differentiation between direct detection of glucose and

chance correlation within the data set *(35)*. Controlling temporal effects on the measurements is an important step toward minimizing the potential of chance correlation *(82)*. The reported SEP values are mostly ≅3 mmol/L. A Clark error grid presentation shows performance that is not acceptable for home glucose meters *(17, 18)*. The accuracy is especially poor in the 3–6 mmol/L glucose concentration range. The performance of the reported methods also falls short of the American Diabetes Association recommendations for home glucose monitors.

The qualitative tracking between the signal and the glucose concentration can be seen in the scattering coefficient values. The magnitude of the change in signal as a function of glucose concentration is very small, and drifts in instrument or physiological conditions are quite large. The need for more sensitive and stable scattering instrumentation is quite obvious. The source of signal tracking in different test subjects is not clear, and no statistical analysis equivalent to that utilized on transmission methods has been performed on the scattering data.

Polarimetry and Raman spectroscopy show adequate detection sensitivity in vitro, but in vivo applications have not been shown. Polarimetry requires a body part with low scattering, such as the cornea; appropriate calibration; and understanding of the lag time between the glucose concentrations in blood and in aqueous humor *(108)*. Corneal rotation, corneal birefringence, and eye motion artifacts are potential sources of error in polarimetric ocular measurements *(6, 107, 108)*. Some issues need to be resolved before Raman spectral measurements can be used in vivo. These include the scattering effect of tissues, potential broadening of the Raman bands, fluorescence background, the dermatological effects of laser excitation, and ways to calibrate the signal vs blood glucose concentration. The eye has been suggested as the measurement site to avoid variable tissue background fluorescence *(120, 121)*. Low laser power is required to prevent eye injury, but this may lead to undetectable Raman signals. PA measurements track glucose concentration in vivo and have good sensitivity in vitro.

It is possible to measure changes in the glucose concentration during a hyperglycemic swing, using current instrumentation. Chemometric or ANN methods are necessary for data analysis, and it is important to guard against overfitting. Calibration has, thus far, relied on individual clinical calibration with an invasive reference method. Statistical analysis of calibration performance thus far has been insufficient to prove that NI calibration models derived from the reviewed spectroscopic techniques are based on glucose-specific information. Unless the physical effects producing the input data are understood, the numerous factors needed to optimize the prediction of results raise the concern that these results may come from an overdetermined nonfunctional calibration model.

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