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Noninvasive Blood and Tissue Analysis: Raman Spectroscopy, One Perspective for Monitoring of Glucose and Beyond

Joseph Chaiken, PhD[1] and Charles M. Peterson, MD, MBA[2]

Abstract

Noninvasive in vivo blood and tissue analysis remains a challenge to medical technology epitomized by the ongoing quest to replace fingerstick self-monitoring of blood glucose. Recent developments warrant comment on near-term prospects for using Raman spectroscopy to meet that challenge. These developments combined with 20 years of experimentation with noninvasive blood and tissue analysis suggest that it may be possible and practical to perform noninvasive in vivo glucose analysis with improvements in (1) the enabling technologies for making Raman measurements and (2) an underlying anatomical–physiological model of how in vivo spectroscopic measurements are made and interpreted. We review the substantial progress made toward meeting the challenge and the personal, public health, and economic implications of these ongoing efforts.

Keywords

diabetes, glucose, noninvasive, PVOH, PV[O]H, vital sign monitoring

Prologue

The advent of (1) glycated hemoglobin (Hgb) and (2) self-monitoring of blood glucose provided two independent means by which the relationship of ambient glucose levels could be related to short-, and long-term, outcomes in diabetes mellitus. It soon became apparent that the goal of normal physiologic glucose levels could not be achieved until systems of continuous surveillance and closed-loop feedback were developed that could mimic normal physiologic responses.[1-5] The goal of continuous blood glucose measurement that is simple, feasible, and available to all persons with diabetes, regardless of socioeconomic status or geographic location, has remained a major challenge for the intervening 45 years.

Enter Raman Spectroscopy

The initial step to achieve that goal with Raman spectroscopy was to determine if a Raman feature associated with in vivo plasma glucose exists, and, if so, it could be associated with a specific volume of blood or plasma. To our knowledge the first published results of attempting to measure blood glucose noninvasively using Raman spectroscopy in vivo in the capillaries of human fingertips appeared in 2000. Two additional independent data sets from the same group were published over the next two years with empirical calibration using Hemocue fingerstick measurements as reference. These results allowed (1) an estimation of concentrations and (2) partially normalized raw spectra to improve calibration across test subjects.[6,7]

Two studies from two different groups working independently confirming a Raman signal and a concentration effect with glucose, with different methodologies were published consecutively in 2005.[8,9] The sizes of the cohorts were relatively small, leaving many questions, including ultimate accuracy and precision for future studies. An important difference between the two groups involved their basic approaches to associating the glucose signal with either blood and/or interstitial fluid. One group relied primarily on chemometric analysis of spectra that contain contributions from both interstitial fluid and blood-borne glucose. The other approach relied on physical manipulations of the process for collecting spectra, that is, to produce a reproducible change in the blood content of a tissue, for example, skin, and then to isolate the spectrum of blood from the changed, that is, "modulated,"

[1]Department of Chemistry, Center for Science and Technology, Syracuse University, Syracuse, New York, USA
[2]MarquiSci, Potomac, MD, USA

Corresponding Author:
Joseph Chaiken, PhD, Department of Chemistry, 1-014 Center for Science and Technology, Syracuse University, Syracuse, New York, NY 13244-4100, USA.
Email: jchaiken@syr.edu

spectrum by subtraction, that is, "tissue modulation." Both groups indicated the presence of a potentially useful Raman signal for glucose in their spectra.

The initial rapid advancement of biomedical applications of Raman spectroscopy was built upon the (1) expansion of chemometric analysis beginning in the early 1990s and (2) appearance in the late 1990s of lasers, optical filters, and multichannel detectors capable of shot noise-limited performance. Coupling improved commercial off-the-shelf (COTS) positioners, sensors, custom embedded software, and fiber and free space optics, and instruments could be designed to more clearly establish the phenomenology of probing skin tissue with near-infrared (NIR) laser light.[10] Improvements in lasers, filters, detectors, and other enabling technologies continue. Thus, having documented a useable glucose Raman signal, we continue to explore blood and tissue analysis.[11-13]

The 2005 glucose publications[8,9] suggested two major problems. First, the modulated approach was better at measuring low glucose levels than high glucose levels. A more complete algorithm is needed to account for the contribution of glucose in interstitial fluid to the modulated spectra. People with diabetes who tend to have high blood glucose levels also tend to have high interstitial fluid glucose levels.[14-16] Algorithm development needs to include the relative amounts of blood, that is, plasma and interstitial fluid, their relative mobility, and relative glucose content, or even well-managed tissue modulation and subtraction could produce less than optimum blood glucose concentration measurements.

Second, movement of red blood cells (RBCs) during the tissue modulation process was correlated with spurious/outlier glucose measurements. RBCs have the largest scattering coefficients of all objects in skin resulting in uncompensated effects of turbidity.[16] "Turbidity" refers to "cloudiness" of a material, affecting every aspect of the measurement process. We can model the propagation of incoming light to probe the tissue, to the creation of either "spectroscopic signals" or "physical optics signals" along the way, to the propagation of those signals to the detection system. Compensating for variable turbidity means being able to assign a relative plasma volume and red blood cell volume to a volume of, for example, skin containing capillaries and blood, when the RBCs move as during a cardiac pulse, applied external pressure, or respiratory effort.

For the non-specialist we note that upon shining a single color of any light into any tissue, either light of the same color (EE) or light of a different color (IE) comes back out later. Spectroscopic signals (IE) contain the molecule-specific information, that is Raman signals or in certain cases fluorescence. Both are composed of wavelength-shifted light. Physical optics (EE) refers to either Rayleigh or Mie scattering, that is, elastic scattering that only changes the direction of light and comprises only light leaving the tissue at the incoming laser wavelength. The effects of elastic (physical, EE) and inelastic (spectroscopic, IE) scattering on the propagation and production of light in vivo must be included simultaneously in any quantitative model to relate measured inelastic signals to chemical information, for example, the concentration of glucose. Analysis using radiation transfer equation simulations and experiments led to the creation of the plasma volume hematocrit algorithm (PV[O]H) and the plasma volume hematocrit device (PVOH).[17-22]

An anatomical–physiological model of in vivo spectroscopic measurements reduces the number of factors needed to obtain desired information from the available measurements. In this case PV[O]H assumes skin has only three components or "phases" by volume: plasma, RBCs, and static tissue. Static tissue deforms under external pressure while blood moves. Furthermore, knowing the volume of two of the components allows calculation of the volume of the third. PV[O]H simultaneously accounts for the variation in RBC and plasma content by measuring *two* output signals produced by a single probing laser, that is, the EE and IE, as described above.

Moreover, PV[O]H employs measurement optics that only collect outbound light that has been elastically scattered at most *only one time*. In this limit the EE and IE signals are connected to the plasma volume and RBC volume in a bilinear fashion that can be calibrated with traceability to a Food and Drug Administration (FDA)-approved gold standard. PV[O]H gives the relative amounts of plasma and RBCs in the probed volume providing a denominator, that is, plasma volume, to calculate concentrations using spectroscopic signals coming from the same volume, for example, glucose Raman signals.

Raman signals appear as small peaks superimposed of a broad background of fluorescence. Despite significant effort, Raman features cannot be separated from this emission, and a few "baseline," that is, fluorescence subtraction recipes exist. The fluorescence is so much stronger than Raman that the shot noise associated with the fluorescence is sometimes greater than the size of the Raman features and noise can be smoothed with loss of spectral resolution, but it cannot be subtracted. PV[O]H can employ the combined Raman and fluorescence without being separated because these signals represent a sampling of the entire probed volume. The high precision and temporal bandwidth of PV[O]H-based devices follows directly from the strength of the fluorescence.

We note in this year, the 60th anniversary of the first demonstration of the laser, that Raman signals can only be observed with laser excitation and not with, for example, light-emitting diode (LED) excitation. All noninvasive in vivo tissue probing with NIR lasers produce fluorescence in addition to Raman signals.[23] Biological "autofluorescence" has multiple origins and it "photobleaches," that is, decreases monotonically over time with continued irradiation with NIR light. We assume that multiple photochemical pathways exist that remove fluorescent molecules from the probed volume of tissue although overall, the photobleaching process is poorly understood. We do know that plasma and RBCs are replaced in the laser-probed volume with every cardiac pulse, and then are diluted in 4 L of circulating blood, each minute. So, if plasma and RBCs bleach, likely much time is required to show accumulated effects.

But the static tissues are irradiated continuously so that after about five minutes of probing with our IRB-allowed laser power, about 50 mW CW at 830 nm, the static tissue autofluorescence decreases by about 25% approaching a steady state. Until steady state is established, the proportion of observed fluorescence and Raman associated with the cardiac pulse, that is, plasma increases. Thus, an improved understanding of the underlying science provides a previously unanticipated way to enhance plasma-bound fluorescence and Raman signals relative to those of the static tissues. It is possible that a better understanding of autofluorescence photobleaching will contribute to research into so-called "photobiomodulation".[24]

The EE and the fluorescence part of the IE are very large raw *emission* signals that can be measured with high precision and accuracy, and for IE emission, there is essentially no background. On the other hand, absorption of light is the basis for pulse oximetry, hemoglobinometry, and related noninvasive approaches to measuring Hct. Measuring absorption amounts to measuring a small change in a large background signal. Absorption spectroscopy at best can provide a detection limit for an analyte in the micromolar concentration range, whereas fluorescence routinely allows nanomolar analysis and better.[25] For this reason, PVOH allows continuous measurement of Hct variation with unprecedented precision.

Traditional physical sampling of blood followed by centrifugation to directly measure Hct is limited to a precision of at least ± 2 with most of the uncertainty resulting from sample preparation/handling.[26] Implementing Twersky's algorithm combining absorption at two wavelengths produces measurements of either Hct or Hgb concentration with a standard deviation of 8.5%.[27-29] PVOH routinely measures physiological variations of Hct of 0.25% so the standard deviation of the measurements must be better than that. Our current estimate indicates that PVOH can measure a change of Hct of ± 0.07 on a Hct of 28 in 3 s.[30] Furthermore, if one chooses to exclude the possibility of obtaining Raman information, then the entire PVOH unit can be implemented as a one laser-2 single-channel detector unit, as small and light as any pulse oximeter.[21,22] And, if one can implement PV[O]H using LEDs instead of lasers, with currently available COTS LEDs, and using only fluorescence as the IE, we may see *wearable* PVOH units.

The current standard of care for self-monitoring of blood glucose maintains that glucose itself must be referenced to plasma since this is the tissue in which glucose changes first, in response to any number of cues or perturbations from homeostasis. Enhancement of plasma-borne fluorescence by bleaching the surrounding tissues has led to the capacity to measure plasma volume and Hct with unprecedented sensitivity, accuracy, and precision.[21] The quality of Raman spectra for physiologic analytics is directly related to the quality of the available lasers, that is, spectral bandwidth. But lasers afford other advantages as well.

The use of a laser allows more efficient spatial and temporal focusing of the probing light relative to an LED. In designing probing optics to achieve EE and IE detection in the single scattering regime, a requirement to implement the PV[O]H, the probing naturally selects the outermost capillaries relative to the tissue surface. The activities of the capillaries reflect the homeostasis and condition of local tissues, but also systemic physiology. Consider the role of capillaries in thermoregulation in mammals or in the redistribution of intravascular fluids in response to hemorrhage.[31,32] This is because Hct is a strong function of the internal diameters (<125 μm) of the vasculature.[33] Oximetry and other related photoplethysmographic devices mostly employ transillumination, which simultaneously probe near-surface blood and blood in the deeper vascular plexus, possibly masking a physiological response of the peripheral vasculature.[34,35]

Advancing Physiological Monitoring—The Two-Channel PVOH Without Raman

The two-detector PVOH itself[21,22] is finding an initial application in detecting bleeding and fluid shifts in persons following civilian or military trauma with minimal external injury[36] and continues to be developed for plasma volume normalization for noninvasive glucose monitoring inter alia by Raman spectroscopy. Studies calibrating PVOH with reference to an FDA-approved monitor of Hct for people undergoing hemodialysis are ongoing.[21] The granularity, high temporal bandwidth relative to physiology, and the deterministic connection of Hct to other vital signs suggests that PVOH will provide the raw data needed for integrating all other vital signs into a single coherent picture of physiology in real time.

Simple experiments show that exerting/flexing of a muscle (group) recruits blood from another location in the cardiovascular system, causing a PVOH response. Simultaneously, the PVOH independently responds to changing Hgb oxygenation because the quantum yield for emission of IE for oxygenated Hgb is different from that of deoxygenated Hgb. Thus, the PVOH response to holding one's breath and sitting quietly is qualitatively and quantitatively different from that to holding one's breath and flexing one's abdominal muscles. Observations like these open the possibility of analytics and algorithm development with continuous, multidevice data in a manner that has not been done before. The potential utility of an "expanded" PVOH-linked vital sign device for military use and the current pandemic is discussed in the context of COVID-19 below.

Additional Potential Modifications in the COVID Pandemic

Persons with diabetes appear no more likely to contract COVID-19 than people without diabetes, but they appear to

Table 1. Vital Signs/Variables for Which There Is Preliminary Published Data[38-43] That Could Result From a New PVOH Configuration Along With Technical Comments of Feasibility and Differences From Currently Used Technologies.

Variable	Current status	Noninvasive PVOH
Temperature	Handheld device at various times, epidermal	Resting probe on one or two sites, continuous, epidermal
Pulse	Photoplethysmography, in person or sensor (Doppler)	Continuous probe using light source
Blood pressure	Intermittent manometer	Continuous mean arterial pressure via light signal from small capillaries
Respiratory rate	In person, chest strap	Continuous via light signal changes detected by PVOH/FFT with each breath in small capillaries
Hematocrit	Blood sample/lab, or hemoglobin concentration, ie, SpHb as a surrogate	Noninvasive continuous per PVOH
Plasma volume	Blood sample lab	Noninvasive continuous per PVOH
Oxygen saturation	Colorimetrically in capillaries (pulse oximetry, ie, SpO_2)	Adapt to current probe configuration, PVOH/FFT
pH	Blood sample to laboratory	Adapt Raman spectroscopy as previously published to existing configuration, continuous
Glucose	Blood sample bedside or lab	Adapt tissue-modulated Raman spectroscopy as previously described with improved enabling technologies, continuous
Blood bacterial detection and enumeration	Blood sample to lab, culture	Light sizing detection of particles as previously published, continuous
Blood viral load	Blood sample to lab, culture	Light sizing of particles to be developed, continuous
Ankle/brachial index	Dedicated technician	Light signal detection using second probe currently on PVOH continuous

FFT, fast Fourier transform; PVOH, plasma volume hematocrit device.

be twice as likely to die from complications.[37] Ongoing research suggests that diabetes is one of the most common comorbidities among people infected with COVID-19; however, its exact prevalence remains unclear. People on dialysis comprise a large cohort with a diagnosis of diabetes (up to 50%) along with hypertension, cardiovascular disease, and kidney failure. Peripheral vascular disease is a bane for people with diabetes and would seem to be a worthwhile target for PVOH in assessing present condition and the efficacy of treatments.

Current practice clusters these patients in "units" that are space constrained either as self-standing or in hospital facilities with a minority treated in the home environment. Current recommendations for care of these patients and others with COVID-19 infection include general guidelines of personal hygiene, limited physical interaction with others, seeking medical care from home, minimizing risk in public, and make work as safe as possible. At present there are few technical solutions to facilitate isolation of cases at home or protect medical personnel, especially in clustered units such as dialysis, emergency care, or intensive care.

An unmet fundamental *clinical* need across the full spectrum of medical diagnostic and treatment needs is the capacity to continuously measure and quantify, noninvasively and without expensive and cumbersome instrumentation, the presence of multiple critical variables of process and composition of blood. COVID-19 with its protean manifestations in multiple organ systems and rapidity of onset highlights the need for real-time monitoring with the absence of invasive blood sampling. Such an approach requires enough sensitivity, precision, and temporal granularity to diagnose and treat interrupted or unusual blood supply along with other clinically important variables. Such sensing must take place without labs and must be telemedicine-ready from the outset.

A further need includes the ability to communicate the results over short (Bluetooth) or longer (Wi-Fi) distances as has currently been developed for PVOH[21,22] along with algorithms for interpreting large (continuous) data sets. The system also must be capable of communicating the information over a friendly user interface such as our concurrent configuration that allows user settings for surveillance and alerts in response to absolute levels and/or changes over time. Because of its sensitivity to many, if not all, indications associated with dialysis, respiratory stress, with diabetes comorbidity, PVOH may be uniquely suited to vital sign monitoring in a COVID-19 environment.

At this point, there appears to exist an actual Raman signal from plasma-borne glucose, and its variation with physiology can be monitored. Current efforts are now focused on including glucose in the PVOH platform.

Table 1 includes the vital signs/variables for which there is preliminary published data[38-43] that could result from a new PVOH configuration along with technical comments of feasibility and differences from currently used technologies.

Elastic scattering and fluorescence/Raman activity of a tissue, turbid medium, object, or material can be used for imaging fingertips[10] or spinal cords[42] and other applications with a PVOH. In validating the basic PV[O]H in various venues from quartz spheres to bacteria we have shown that bacteria, and possibly viruses and perhaps even microclots can be sensed before they become a problem. Recently PVOH with Raman was able to image and measure the pH of cerebrospinal fluid in vivo and perimortem in rat and pig models.[42,43]

In summary, the journey that began with a search for an approach to noninvasive blood glucose monitoring technologies has now come full circle to providing the raw data to stimulate an era of noninvasive analytics that may benefit not only those with diabetes but many others as well.

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ORCID iD

Joseph Chaiken https://orcid.org/0000-0002-7715-3852

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