

Non-invasive glucose monitoring

Mark A Arnold

Several recent reports claim success in measuring blood glucose non-invasively in human subjects with near-infrared spectroscopy. A critical examination of these published results suggests more fundamental research is needed to verify the validity of these claims. In addition, progress continues in assessing the utility of near-infrared spectroscopy as a non-invasive probe for continuous bioreactor monitoring during fermentation processes. Recent work demonstrates that five critical fermentation components, including glucose, may be measured simultaneously.

Address

Department of Chemistry, University of Iowa, Iowa City, Iowa 52242, USA

Current Opinion in Biotechnology 1996, 7:46–49

© Current Biology Ltd ISSN 0958-1669

Abbreviations

ANN artificial neural network

PLS partial least squares

Introduction

Non-invasive blood glucose sensing is a technique for measuring *in vivo* blood glucose concentrations without collecting a blood sample. Near-infrared spectroscopy has been proposed as a means for accomplishing such a measurement. The concept is to pass a band of near-infrared radiation through a vascular region of the body and then to extract the corresponding blood glucose concentration from the resulting spectral information. A major challenge is to collect spectral information with sufficiently high signal-to-noise ratios to discriminate weak glucose absorbances from the underlying spectral noise. In addition, sophisticated data-processing algorithms are required to selectively extract the glucose-dependent information in the presence of dominating signals from other more concentrated matrix components and from concomitant spectral variations.

Successful development of a non-invasive blood glucose monitor would represent a major advancement in the treatment and management of diabetes mellitus. Tight glycemic control is clearly needed to reduce the major complications of hyperglycemia [1] and frequent glucose monitoring is required before appropriate insulin levels can be administered to achieve a desired level of glycemia. The pain, messiness, and inconvenience associated with contemporary home glucose-monitoring technology are the major barriers to tight control of blood glucose; these would effectively be avoided by a reliable non-invasive blood glucose monitor.

The enormous potential healthcare benefits of non-invasive glucose sensing have stimulated considerable research and development efforts in this field of study. Unfortunately, the tremendous financial market for such a device has created an atmosphere of secrecy, particularly within the industrial setting. The proprietary nature of the work has resulted in limited amounts of published peer-reviewed information in the scientific literature. Many of the claims stated in the mass media and published in the patent literature are simply not supported by sound scientific data.

In this review, the feasibility and potential of non-invasive glucose monitoring are considered for clinical blood glucose measurements and bioreactor monitoring. In addition, published accounts of non-invasive blood glucose experiments are critically evaluated and the validity of their results are questioned. Evidently, more fundamental experiments are needed to characterize the influence of chemical and physical parameters on non-invasive approaches and to identify the instrumentation and computation specifications required for reliable and accurate measurements. Similar arguments apply to the future development of near-infrared chemical sensing technology for bioreactor monitoring.

Non-Invasive glucose monitoring in medicine

It is important to realize that no one has yet proven the analytical feasibility of measuring *in vivo* glucose levels by the non-invasive spectroscopic experiment described above. For the most part, the supporting documentation provided with claims in the patent literature [P1,P2*,P3,P4*,P5*,P6–P9] is severely lacking in terms of scientific rigor. Few relevant spectra are provided, no attempts are made to correlate spectral information with the spectroscopic properties of the glucose molecule, and essentially no effort is made to accurately predict *in vivo* glucose concentrations from independent sample measurements. Typically, judgements of measurement accuracy are completely omitted.

Perhaps the best documented attempts of non-invasive measurements have been presented in publications from research groups headed by Haaland [2] and Heise [3*,4,5]. Haaland and co-workers [2] published a preliminary evaluation of non-invasive glucose monitoring in which near-infrared spectra were collected across the fingertips of human volunteers during a meal-tolerance test. A total of 41 samples were collected, glucose values varying from 2.7 mM to 27.7 mM. The corresponding near-infrared spectra were collected over 875–1300 nm and an 11-factor partial least squares (PLS) calibration model was computed. Cross-validation analysis indicated

an average absolute error of 1.1 mM for glucose. Similar results have been reported recently by Jagemann *et al.* [6*] with measurements collected across finger tips over the 900–1200 nm spectral range. In this later work, artificial neural networks (ANNs) were judged superior to PLS regression in extracting the information on glucose.

Likewise, Heise and co-workers [3*,4,5] used cross-validation techniques to generate three distinct models based on diffuse reflectance near-infrared spectra collected from mucous lip tissue. The first model was based on 133 spectra collected over 2 days from a single human subject. The second model consisted of 219 spectra collected over 2 weeks from an individual subject and the third model used spectra collected from 133 different individuals. In all cases, spectra covered a spectral range from 9000 cm⁻¹ to 5450 cm⁻¹ and 20-factor PLS models provided the best analytical performance. The computed standard error of prediction values were 2.6 mM, 3.0 mM, and 3.1 mM for the first, second and third models, respectively.

None of the above described experiments provides proof that what it is measuring is related to actual glucose concentration. The researchers cannot distinguish between direct detection of glucose and chance correlations within the data set. PLS and ANN are powerful techniques for correlating extremely small spectral variations with analyte concentration. Unfortunately, it is very easy to overmodel a system or, even worse, to build models on the basis of some other experimental variable that happens to correlate with variations in analyte concentration. Time is a particularly critical parameter in this regard. In the above experiments, the glucose level varies with time as spectra are collected. Many different experimental parameters are likely to change with time that may alter, albeit ever so slightly, magnitudes of the near-infrared signals. A common example is slight variations in room temperature, which can affect spectra by altering the absorption characteristics of water within the sample. Changes in room temperature may also alter instrument performance by changing alignment, detector response, or radiant power from the light source. The human-to-spectrometer interface is another experimental variable, fluctuations of which can significantly alter the spectral information. As the experiment continues, tissue at the location of the measurement may be altered slightly either by the pressure of the interface holding the tissue or by minute temperature changes caused from frequent handling. These physical changes might alter vascularity at the measurement sight and, therefore, create non-glucose specific spectral variations within the data set.

The major point is that tiny variations of any number of critical experimental parameters can create a combination of spectral variations that correlate with glucose concentration. The PLS and ANN algorithms will 'lock-in' on such correlations and provide reasonable predictions, particularly from the 'leave-one-out' type cross-validation

method used in the above experiments. It is possible to get models with prediction errors of the order of those specified in the work of Haaland's group [2], Heise's group [3*,4,5], or Jagemann's group [6*] by randomly mixing glucose concentrations with respect to individual spectra. Statistical analysis of calibration performance is insufficient to prove that non-invasive calibration models are based on glucose-specific information. The analytical and clinical chemistry communities demand more direct evidence before being convinced that near-infrared methods are capable of providing reliable and accurate measurements of *in situ* physiological glucose levels.

Additional proof can be obtained by analyzing spectral loadings to confirm that chemical information is actually being incorporated into the calibration model. The spectral range that provides the best models must correspond to absorption bands known to be associated with either glucose or a major source of interference. In addition, the large number of model factors needed to provide optimum prediction capabilities must be justified. In the work by Heise *et al.* [3*], 20 factors are needed for optimum prediction of results. This large number of factors seems excessive and invokes skepticism in most analytical chemists that the results come from an overdetermined non-functional calibration model.

Issues of spectral range, number of factors, and structure of spectral loadings can best be established from fundamental experiments based on sample matrices of known composition. For example, the influence of protein ([7]; S Pan, H Chung, MA Arnold, GW Small, unpublished data) or temperature [8**] can be determined by designing experiments where such variables are examined in a controlled and systematic manner. Only through experiments of this nature will we be able to establish credible, or expected, values for important experimental parameters. For example, fundamental experiments are needed to establish quantitatively the relationship between number of model factors and critical experimental variables such as temperature, number of interferences, and magnitude of concentration variation of interfering species. Only after this relationship is firmly established can a 20-factor PLS model be accepted or rejected. Similar arguments can be made for other key parameters such as spectral range, spectral loadings, optical path length, and spectral resolution.

Most efforts to establish the utility of near-infrared spectroscopy for clinical measurements have centered on statistical analysis of calibration models corresponding to measurements of glucose in matrices as simple as phosphate buffer or as complex as whole blood ([7,8**,9–15,16*]; S Pan, H Chung, MA Arnold, GW Small, unpublished data). When combined, these studies strongly suggest that near-infrared spectroscopic analysis can extract glucose information from complex biological matrices. More effort must now be placed on character-

izing the fundamental nature of such measurements in order to rigorously extend the technique to non-invasive measurements. Without a solid foundation of fundamental knowledge, non-invasive near-infrared measurements will never be accepted.

As near-infrared spectroscopy continues to be developed for blood glucose measurements, it is critical to assess model accuracy and precision on the basis of clinically acceptable criteria. Clarke *et al.* [17] have proposed an error grid analysis for the purpose of evaluating any method of blood glucose analysis. The Clarke error grid is constructed by plotting blood glucose values from the measurement device relative to the actual blood glucose concentration. A grid of five regions (labeled A, B, C, D, and E) is superimposed on this scatter plot. These regions correspond to different levels of correctness of clinical decisions based on the analytical measurement. Measurements in the 'A' region are ideal and correspond to the proper clinical action being taken, given the measured value. Analogously, the 'E' region corresponds to the worst-case scenario, where the clinical action indicated by the measured value is exactly opposite that required from a physiological standpoint. The Clarke error grid is a standard method for judging measurement performance and a maximum standard error of 0.8 mM is allowed for a method to be classified in the 'A' range.

Non-invasive glucose monitoring for bioreactors

Another area where non-invasive near-infrared spectroscopic sensing of glucose is beginning to make an impact is bioreactor monitoring ([18–21,22*]; M He, D Lorr, NS Wang, abstract #116b, American Institute for Chemical Engineers National Meeting, St Louis, Missouri, USA, November 11th, 1993). The concept is essentially the same as for clinical measurements. Here, the near-infrared spectrum is collected non-invasively during the fermentation process and the corresponding concentration of glucose in the growth medium is determined. It is envisioned that spectra can be collected through a fiber-optic probe directed toward an optical window in the reactor. Diffuse reflectance spectra can be collected, from which analyte information is obtained.

Over the years, many workers have attempted to use biosensors to measure critical medium components such as glucose, lactate and glutamine. Biosensors are electrochemical devices with enzymes immobilized at the surface of the electrochemical transducer. In general, these attempts have failed because of limited enzyme stability, poor transducer reliability within the demanding environment of the reactor, and complications associated with sterilization. Conceptually, a non-invasive monitor with near-infrared spectroscopy can eliminate these classical problems with biosensor technology.

Much of the research reported for non-invasive blood glucose monitoring has direct implications in bioreactor monitoring applications. Issues pertaining to sample temperature [8**], for example, are the same, regardless of the application. In addition to measuring glucose, this near-infrared approach has the potential to measure many components within these reactors. In this regard, the simultaneous measurement of glucose and glutamine [18], as well as glutamine and asparagine [19], has been demonstrated recently. Furthermore, we [22*] have recently used a single near-infrared spectrum to obtain concentrations of glucose, glutamine, ammonia-nitrogen, lactate and glutamate when in a mixture.

Conclusions

The development of near-infrared spectroscopy for clinical and biotechnological applications is just beginning. Further advances in instrumentation and computer-based data analysis algorithms will enhance current analytical capabilities and propel the progress of this exciting technology of non-invasive analysis to practical applications. Once realized, dedicated spectrometers will be designed to control and optimize the systems of interest (i.e. the human body and/or bioreactors) on the basis of continuous *in situ* glucose concentrations. These advances will be based on sound fundamental investigations aimed at understanding near-infrared spectrometric measurements from a basic chemistry standpoint.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
 - of outstanding interest
1. Diabetes control and complications trial research group: **The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus.** *N Engl J Med* 1993, **329**:977–986.
 2. Robinson MR, Eaton RP, Haaland DM, Koepf GW, Thomas EV, Stallard BR, Robinson PL: **Non-invasive glucose monitoring in diabetic patients: a preliminary evaluation.** *Clin Chem* 1992, **38**:1618–1621.
 3. Heise HM, Marbach R, Koschinsky TH, Gries FA: **Non-invasive blood glucose sensors based on near-infrared spectroscopy.** *Artif Org* 1994, **18**:439–447.
- This paper details results from a non-invasive experiment where near-infrared spectra are collected as diffuse reflectance spectra from the lips of human volunteers. Typical data analysis methods are illustrated and the calibration models are judged on the basis of a 'leave-one-out' type cross-validation scheme.
4. Marbach R, Koschinsky TH, Gries FA, Heise HM: **Non-invasive blood glucose assay by near-infrared diffuse reflectance spectroscopy of the human inner lip.** *Appl Spectrosc* 1993, **47**:875–881.
 5. Heise HM, Marbach R: **Effect of data pretreatment on the non-invasive blood glucose measurement by diffuse reflectance NIR spectroscopy.** *Proc Soc Photoinstrum Eng* 1994, **2089**:114–115.
 6. Jagemann KU, Fischbacher C, Danzer K, Müller UA, Mertes B: **Application of near-infrared spectroscopy for non-invasive determination of blood/tissue glucose using neural networks.** *Z Physik Chem* 1995, **191**:179–190.

A paper similar to [3*], detailing results that pertain to a non-invasive near-infrared experiment in humans. ANNs are used to generate calibration models. The procedures and prediction errors are essentially the same as those in [3*].

7. Marquardt LA, Arnold MA, Small GW: **Near infrared spectroscopic measurement of glucose in a protein matrix.** *Anal Chem* 1993, 65:3271-3278.
 8. Hazen KH, Arnold MA, Small GW: **Temperature insensitive near-infrared spectroscopic measurement of glucose in aqueous solutions.** *Appl Spectrosc* 1994, 48:477-483.
- Offers a general procedure for reducing the effects of temperature on near-infrared chemical analysis. The temperature sensitivity of the underlying water absorption bands can dramatically impact near-infrared spectra. This paper demonstrates the effectiveness of using digital Fourier filtering to remove baseline variations caused by temperature variations of several degrees.
9. Arnold MA: **New developments and clinical impact of non-invasive monitoring.** *Clinical Laboratory Automation, Robotics and Knowledge Optimization*. Edited by Kost GJ. New York: John Wiley and Sons; 1995:631-647.
 10. Arnold MA, Small GW: **Determination of physiological levels of glucose in an aqueous matrix with digitally filtered Fourier transform near-infrared spectra.** *Anal Chem* 1990, 62:1457-1464.
 11. Small GW, Arnold MA, Marquardt LA: **Strategies for coupling digital filtering with partial least-squares regression: application to the determination of glucose in plasma by Fourier transform near-infrared spectroscopy.** *Anal Chem* 1993, 65:3279-3289.
 12. Haaland DM, Robinson MR, Koepf GW, Thomas EV, Eaton RP: **Reagentless near-infrared determination of glucose in whole blood using multivariate calibration.** *Appl Spectrosc* 1992, 46:1575-1578.
 13. Janatsch G, Kruse-Jarres JD: **Multivariate calibration for assays in clinical chemistry using attenuated total reflection infrared spectra of human blood plasma.** *Anal Chem* 1989, 61:2016-2023.
 14. Hall JW, Pollard A: **Near-infrared spectroscopic determination of serum total proteins, albumin, globulins, and urea.** *Clin Biochem* 1993, 26:483-490.
 15. Peuchant E, Salles C, Jensen R: **Determination of serum cholesterol by near-infrared reflectance spectrometry.** *Anal Chem* 1987, 59:1816-1819.
 16. Sodickson LA, Block MJ: **Kromoscopic analysis: a possible alternative to spectroscopic analysis for non-invasive measurement of analytes *in vivo*.** *Clin Chem* 1994, 40:1838-1844.
- This paper describes a novel, and patented (see [P9]), approach to non-invasive glucose sensing based on collecting spectral information from a variety of complementary detector elements. The advantage is the ability to operate each detection under extremely high signal-to-noise ratio conditions. The key issue is the availability of glucose-specific information in the spectral region being probed.
17. Clarke WL, Cox D, Gonder-Frederick LA, Carter W, Pohl SL: **Evaluating clinical accuracy of systems for self-monitoring of blood glucose.** *Diabetes Care* 1987, 10:622-627.
 18. Chung H, Arnold MA, Rhiel M, Murhammer DW: **Simultaneous measurement of glucose and glutamine in aqueous solutions by near infrared spectroscopy.** *Appl Biochem Biotechnol* 1995, 50:109-126.
 19. Zhou X, Arnold MA, Chung H, Rhiel M, Murhammer DW: **Selective measurement of glutamine and asparagine in aqueous media by near infrared spectroscopy.** In *Biosensor and Chemical Sensor Technology: Process Monitoring and Control*. Edited by Rogers KR, Mulchandani A, Zhou W. Washington, DC: American Chemical Society; 1995:116-132.
 20. Vaccari G, Dosi E, Campi AL, González-Vara YR, Matteuzzi D, Mantovani G: **A near-infrared spectroscopy technique for the**

control of fermentation processes: an application to lactic acid fermentation. *Biotechnol Bioeng* 1994, 43:913-917.

21. Tenhunen J, Sjöholm K, Pietilä K, Home S: **Determination of fermentable sugars and nitrogenous compounds in wort by near and mid infrared spectroscopy.** *J Inst Brew* 1994, 100:11-15.
22. Chung H, Arnold MA, Rhiel M, Murhammer DW: **Simultaneous measurements of glucose, glutamine, ammonia, lactate and glutamate in aqueous solutions by near-infrared spectroscopy.** *Appl Spectrosc* 1996, in press.

The simultaneous measurement of five components in aqueous solutions is demonstrated. Near-infrared spectra are collected from a series of solutions composed of varying levels of glucose, glutamine, glutamate, lactate and ammonia-nitrogen. Multivariate calibration models are generated for each analyte and the ability of each model to accurately predict analyte concentrations from an independent set of prediction spectra is illustrated. Critical issues of spectral range and co-variance between analyte species are discussed.

Patents

- of special interest
 - of outstanding interest
- P1. Rosenthal RD, Paynter LN, Mackie LH: **Noninvasive measurement of blood glucose.** 4/2/92 US 5,086,229.
 - P2. Purdy DL, Wiggins RL, Castro P: **Noninvasive determination of analyte concentration using noncontinuous radiation.** 1/11/94 US 5,360,004.
- This patent describes a spectrometer designed to collect diffuse reflectance near-infrared spectra from mammalian subjects. An optical filter is used to remove the wavelengths of light adsorbed by water, thereby reducing internal heating of the tissue matrix. Resulting spectra cover the overtone and combination spectral ranges; however, no data are presented to indicate glucose can be measured accurately using this device
- P3. Barnes RH, Brasch JW: **Non-invasive determination of glucose concentration in the body of patients.** 10/12/91 US 5,070,874.
 - P4. Mendelson Y, Harjunmaa H, Yi W, Gross BD: **Blood constituent determination based on differential spectral analysis.** 13/12/94 US 5,372,135.
- Discloses a method for measuring glucose in blood from differential absorbance spectra. The principal feature is the method used to obtain differential spectra. According to the patent, differential spectra are obtained by comparing spectra collected with different volumes of blood within the exposed tissue sample. Blood volume is altered between spectra by increasing the pressure across the sample, thereby physically displacing blood from the optical path. Although this method promises superior spectral data by providing a sample-matched background spectrum, no details are provided to demonstrate the analytical advantages of this approach.
- P5. Robinson MR, Ward KJ, Eaton RP, Haaland DM: **Method of and apparatus for determining the similarity of a biological analyte from a model constructed from known biological fluids.** 4/12/90 US 4,975,581.
- The concept of kromoscopy (see [16*]) is detailed and the instrumental requirements for the measurement outlined. Kromoscopy is described by analogy to the processes thought to be involved in human vision. The primary advantage of this approach is the high signal-to-noise ratios obtained from striking the detectors with non-dispersed radiation. ANNs are proposed for generating non-invasive glucose calibration models. No experimental results are presented, however, to prove the ability to measure glucose by this method.
- P6. Tarr RV, Staffes PG: **Non-invasive blood glucose measurement system and method using stimulated Raman spectroscopy.** 14/9/93 US 5,243,983.
 - P7. Schlager KJ: **Non-invasive near infrared measurement of blood analyte concentrations.** 21/11/89 US 4,882,492.
 - P8. Hutchison DP: **Personal glucose monitor.** 20/2/90 US 4,901,728.
 - P9. Block MJ: **Noninvasive testing.** 14/8/94 US 5,321,265.