Published in final edited form as:

Trends Analyt Chem. 2015 January 1; 64: 100–108. doi:10.1016/j.trac.2014.09.005.

Emerging trends in optical sensing of glycemic markers for diabetes monitoring

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Abstract

In the past decade, considerable attention has been focused on the measurement of glycemic markers, such as glycated hemoglobin and glycated albumin, that provide retrospective indices of average glucose levels in the bloodstream. While these biomarkers have been regularly used to monitor long-term glucose control in established diabetics, they have also gained traction in diabetic screening. Detection of such glycemic markers is challenging, especially in a point-of-care setting, due to the stringent requirements for sensitivity and robustness. A number of non-separation based measurement strategies were recently proposed, including photonic tools that are well suited to reagent-free marker quantitation. Here, we critically review these methods while focusing on vibrational spectroscopic methods, which offer highly specific molecular fingerprinting capability. We examine the underlying principles and the utility of these approaches as reagentless assays capable of multiplexed detection of glycemic markers and also the challenges in their eventual use in the clinic.

Keywords

Diabetes; Diabetes monitoring; Fructosamine; Glycemic marker; HbA1c; Optical sensing; Photonics; Point of care; Raman scattering; Spectroscopy

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1. Introduction

Disorders of glucose homeostasis, including Type I and II and gestational diabetes, represent a leading cause of morbidity and mortality worldwide. Diabetes presents a significant healthcare, economic and societal challenge, as evidenced by its prevalence in nearly 382 million people across the globe – with an expected increase to 592 million diabetic patients by 2035 [1]. Unfortunately, at this time, diabetes has no well-established cure, but must be treated with regular insulin injections and other related medications based on careful monitoring of blood-glucose levels. Maintenance of glycemic control is critical to the patient's quality of life and to avoid serious secondary complications, such as microvascular and macrovascular changes that may result in diabetic neuropathy, nephropathy and retinopathy. Thus, direct quantification of blood-glucose values remains the “gold standard” for diagnosis and monitoring of diabetes. A number of research groups, including our own laboratory, have been engaged in developing a fully non-invasive, continuous, glucose-monitoring system that can eliminate the inconvenience associated with frequent finger pricks while the patient can readily recognize and predict trends of blood-glucose changes [2]. Despite promising preliminary results, such a method for *in-vivo* glucose tracking in a completely label-free manner has yet to be clinically validated.

In this setting, significant attention has been focused –from both research and clinical standpoints – on the measurement of glycated proteins for monitoring long-term glycemic control in diabetics. These long-term glycemic markers reflect the average value of blood-glucose concentration with the time span of the glycemic history being unique to the biomarker and provide vital information to complement the blood-glucose measurements. These biomarkers, notably glycated hemoglobin (HbA1c) and glycated albumin (GA, fructosamine) [3], provide retrospective indices of integrated blood-glucose values over extended periods of time, with intrinsic half-lives of ~60–90 days and 14–21 days, respectively. Unlike measurement of fasting plasma glucose (FPG), HbA1c and fructosamine do not require an overnight fast, are not affected by short-term lifestyle changes, and show significantly less variability within individuals [4]. Recent studies showed that elevated concentrations of these biomarkers strongly correlate with the presence of diabetic complications [5], such as kidney damage, cardiovascular problems and retinopathy [6] – often predicting the presence of these conditions better than FPG [7]. While affinity chromatography, high-performance liquid chromatography (HPLC) and immunoassays have been employed for analyzing glycemic control, especially for HbA1c, they often suffer from specificity limitations, as the presence of hemoglobin variants, as well as uremia and ethanol ingestion, can result in erroneous readings. Moreover, there is no existing “gold standard” for fructosamine measurement because of the lack of suitability of existing approaches for routine clinical laboratory application. As a consequence, there is an unmet need for sensitive, specific determination of these biomarkers, preferably in a reagent-free, easy manner.

This need has led to a surge of emerging techniques that exploit an array of chemical and optical contrast mechanisms. Of these, the photonic and direct spectroscopic routes appear to be the most promising, as they could offer simultaneous determination of both biomarkers, without necessitating significant sample preparation. Based on recent research

articles in this area, we discuss the emerging optical approaches, especially those employing direct spectroscopic routes to glycemic marker determination. More importantly, we examine in detail the outstanding properties of these sensing mechanisms, their future prospects and research opportunities, while critically analyzing the potential challenges on the path to use in the clinic.

2. Markers of glycemia

Since the first results from the comprehensive Diabetes Control and Complications Trial (DCCT) regarding the importance of complementary biomarkers for diabetes screening and monitoring, opening up and development of this domain has been of great significance both to care for diabetic patients and to assist in unraveling the cause of diabetic complications. While a number of such biomarkers of glycemic variability have been proposed, HbA1c and fructosamine have exhibited stronger correlations with diabetes complications, macroangiopathies and microangiopathies across a larger cross-section of studies. In this review, our discussion therefore focuses on the detection and the quantitation of these markers, which are formed by non-enzymatic glycation of proteins *in vivo* and, as such, are also relevant to the research thrust in advanced glycation end products (AGEs).

HbA1c is the major glycohemoglobin species in human blood [8] and has been employed for close to three decades for long-term assessment of glycemic control in diabetic patients. The American Diabetes Association (ADA) recently added HbA1c to screening for prediabetes (5.7% HbA1c 6.4%) and diabetes (HbA1c 6.5%) [9]. Glycated hemoglobin is produced from the multistep condensation reaction of glucose with a hemoglobin amine moiety [10]. The formation process is initiated by the condensation of a free primary amine on hemoglobin with the glucose carbonyl resulting in the formation of an unstable Schiff base, which may dissociate or undergo an Amadori rearrangement to form the final ketoamine, a conversion that is irreversible. HbA1c is formed by the specific reaction of glucose with the amino-terminal valine in one or both of the hemoglobin (Hb) β -chains, as shown in Fig. 1. The HbA1c value is, thus, directly proportional to the mean concentration of glucose in the bloodstream over the preceding two-month period, which is a direct function of the lifespan of erythrocytes. HbA1c is traditionally reported as a percentage of total hemoglobin; the range of HbA1c values seen in practice is 4–20%. It has been determined empirically that each percentage point increase in HbA1c concentration roughly corresponds to a 2 mM rise in mean blood-glucose level [11]. Driven by the pre-analytical stability of HbA1c and the ability to provide a measure of chronic rather than acute dysglycemia, HbA1c testing has become an integral part of routine clinical check-ups when assessing glycemic control in patients with diabetes mellitus. In addition to offering an estimate of the risk of microvascular and macrovascular complications and the risk of severe hypoglycemia, HbA1c measurements allow the setting of appropriate population and individual targets – and, by extension, enable comparison of efficacy of old and new treatments [12].

In selected diabetic patients, there is also a clinical need for biomarker measurements that are more sensitive than HbA1c to shorter-term alteration in average blood-glucose levels. Fructosamine has received increased attention as an intermediate indicator of glycemic

status because of its shorter half-life compared to hemoglobin [13]. It is important to recognize that fructosamine is not a specific molecule but refers to the aggregate of all serum proteins without distinction. Nevertheless, because all glycated serum proteins are fructosamines and albumin is the most abundant serum protein, measurement of fructosamine was previously considered to be an indirect measure of glycated albumin. The wisdom of this was questioned recently, spurring the development of more specific glycated albumin assays [14]. In contrast to HbA1c measurements that have been employed extensively in clinical laboratories as an adjunct to blood-glucose determinations, glycated albumin has remained an “underestimated marker of diabetes” [13]. Its recent importance and support in the clinical community stems from the profound impact of erythrocyte-lifespan variability on HbA1c levels, which, in turn, renders the glycated hemoglobin determinations ineffective in patients with hematological disorders (e.g., sickle cell anemia and autoimmune hemolytic anemia). Indeed, even with an accurate HbA1c measurement, the red blood count (RBC) lifespan issue means that the correct value will be clinically misleading, as detailed in one of our prior studies where both a method interference and an RBC lifespan issue was found to confound the true concentration values [15].

While further clinical studies are necessary to define which biomarker works best for specific classes of patients (since it is clear that none of the markers can provide the complete picture in all diabetic patients), measurement of glycated albumin has the potential to provide more accurate results in patients with certain hemoglobin variants and in patients whose RBC lifespans are altered, and to help implement therapy by providing a shorter term response. In the light of the growing recognition of the limitations of HbA1c and the lack of clinically available methods for glycated albumin estimation, development of robust, sensitive glycated albumin assays is extremely desirable in order to fill the gap in the present diagnostic landscape.

3. Determination methods of glycemic markers

The current diagnostic assays for HbA1c determination can be mainly segmented into methods employing charge differences and those using structural characteristics. The former class includes ion-exchange chromatography that works on the exchange of charged ions between a mobile phase and charged ligands covalently attached to the stationary phase. Since the glycated hemoglobin components are less positively charged at neutral pH and bind less strongly to the negatively charged resin, they elute before the unglycated fraction. The other important method in this class is electrophoresis, which commonly employs an electric potential across a support medium to separate the fast hemoglobins towards the cathodic side. Electrophoresis is rarely used these days in clinical practice, primarily due to its lack of precision with respect to other available methods and its labor-intensive nature. The principal drawbacks of these charge-based separation techniques are the sensitivity to sample storage conditions, environmental factors (temperature and pH) and interference from clinical factors, such as uremia and ethanol ingestion. Approaches that differentiate HbA1c based on structural characteristics signatures include affinity binding/ chromatography and immunoassay. Boronate-affinity chromatography based on the esterification reaction between cis-diols of glucose and boronic acid has been extensively used as a separation technique for HbA1c from the Hb solution. Affinity binding/

chromatography has the advantage of being able to separate other glycosylated components (besides HbA1c) that cannot be readily separated based on charge differences. This method is also more robust with respect to variations in temperature and pH in relation to ion-exchange techniques. Over the past two decades, dedicated HPLC systems have become widely available, as have simple, highly automatable immunoassays [16]. Immunoassays present great promise in point-of-care diagnostics. The immunoassay-based methods for HbA1c determination use monoclonal or polyclonal antibodies that typically recognize amino acids at the amino terminus of the β -chain. The performance of modern automated immunoassays is at least as good as, if not better than, HPLC, and a number of such immunoassays are thus extensively used for measurements in clinical laboratories. A reported drawback of some of the immunoassays is that the specificities of the respective antibodies and thus the overall approaches show substantive variance [17].

While not used as extensively as for HbA1c, many of these methods are also employed for fructosamine measurement, including affinity chromatography [18], HPLC of glycosylated lysine residues following hydrolysis of the glycosylated proteins and immunoassay-related techniques (e.g., enzyme-linked immunosorbent assays or radio-immunoassays) [19]. Several reagent-specific colorimetric methods, notably featuring thiobarbituric acid [20] and nitroblue tetrazolium [21], have also been used, but their high degree of non-specificity and interference from free glucose renders the less attractive for routine usage [22]. A number of the existing approaches were broadly used for glycosylated protein (or fructosamine) measurements and it is only recently that a significant thrust to develop and to standardize glycosylated albumin assays has taken place [14]. The advance in monoclonal antibodies specific to glycosylated albumin [23], while beneficial in principle, requires considerable sample preparation and has not increased the availability of commercial glycosylated albumin assays to date.

The respective limitations of these approaches led to innovative efforts in recent years to develop accurate methods for diagnosis of these important glycemic markers, preferably in a rapid, reagent-free manner. Ideally, one could envision the substantive advantages of such an approach that could measure both these analytes simultaneously. This would provide a more comprehensive indicator of glycemic history and avoid the pitfalls of determination and interpretation of a single marker due to specific pathological conditions.

4. Emergence of optical sensing approaches

In this milieu, optical and (direct) molecular spectroscopic approaches offer a novel approach to bridge the gaps in the existing landscape. Powered by their intrinsic molecular specificity and multiplexing capability, molecular spectroscopy, in particular, has emerged as an intriguing possibility for translating molecular markers into serum assays for accurate disease screening. Optical methods, including Raman spectroscopy, near-infrared (NIR) and mid-infrared (MIR) absorption spectroscopy, optical coherence tomography (OCT), photoacoustic spectroscopy and polarization spectroscopy, have provided promising results for non-invasive blood-glucose measurements – an outstanding problem in continuous diabetes monitoring [2b,2c,2e, 24]. These investigations also paved the way for probing

these glycemic markers in serum and blood samples and in some cases even in a non-invasive manner.

4.1. Optical sensing of HbA1c

The advances in photonics technologies combined with the widespread availability of cheaper instrumentation have led to a significant rise in interest in the characterization, the identification and the quantification of HbA1c. As an illustrative example, NIR absorption spectroscopy, which probes the overtone and combination vibrational transitions of the molecules, has been employed to quantify Hb and absolute HbA1c content simultaneously, thereby allowing indirect measurement of HbA1c in hemolyte samples [25]. This result is consistent with Kamath and co-workers' findings that underline a strong correlation between the glycated hemoglobin values obtained by the spectroscopic method and those obtained by the standard HPLC method [26]. Despite the IR spectrum encoding information on the structure of the protein and its modification by adding the glycan moiety, application of traditional IR spectroscopy is probably limited in its use as a glycation probe because spectral congestion among many overlapping features washes out the information of interest. Further, the implementation of NIR spectroscopic methods has fundamental limitations due to the competing absorption by water, which, though smaller than in the MIR region, is not absent. The application of multivariate calibration models presents a possible remedy for the determination of quantitative analyte-specific information. Here, the chemometrics challenge is not only to determine chemical markers that identify the different forms of hemoglobin (principally, native and glycated) but also to quantify molecular composition in clinical samples. While the former aim is critical for molecular identification and providing the scientific basis of these investigations, the latter objective is of larger biomedical interest and involves prediction in complex mixtures of stochastically varying compositions of molecules. However, the chemometric modeling of NIR spectra for quantification is not a panacea, as detailed by Arnold et al. [2c]. The possibility of spurious correlations diminishes the clinical viability of the NIR absorption method for detecting glycated hemoglobin.

Another budding domain of research is non-invasive determination of skin advanced glycation end products (AGEs) *in vivo* by measurement of skin intrinsic fluorescence (SIF)²⁷. Based on the prior reports of the strong correlation between SIF and AGEs assayed directly from skin biopsies, Felipe et al. evaluated the association of SIF with HbA1c and human glycation index in a cohort of children with Type I diabetes [28]. More recent work conducted in an at-risk population of 479 previously undiagnosed subjects from nine US centers suggested "skin fluorescence spectroscopy has similar screening performance to fasting plasma glucose (FPG) and A1C and is a viable approach for detection of abnormal glucose tolerance" [29]. However, the specificity of tissue autofluorescence raise significant concerns about its universal applicability for determination of HbA1c as opposed to an aggregate of skin AGEs. Preliminary studies featuring refractive index-based measurement techniques, including optical coherence tomography and Abbe refractometry, have also been presented [30]. These investigations are based on the underlying changes in optical properties caused by glycation of proteins, but they are limited in their applicability to dilute solutions of hemoglobin and its glycated counterparts.

Of the photonic probes, Raman spectroscopy and its derivatives appear to be the most promising with regard to use in the clinic, based on the recent work in identifying and quantifying hemoglobin glycation. This molecular spectroscopic tool is based on the underlying inelastic scattering principle where energy exchange between incident photon and a molecule takes place [31]. The process of inelastic scattering is a reflection of vibrational, rotational and other low-frequency modes in the molecule, so it provides a highly specific probe with unique chemical fingerprinting capability. Notwithstanding its ability to offer quantitative, label-free specimen characterization, spontaneous Raman spectroscopy is limited by its sensitivity since approximately one-in-a-million photons is subjected to inelastic scattering.

To address the sensitivity issue, variations of Raman spectroscopy, such as resonance Raman spectroscopy, surface-enhanced Raman spectroscopy (SERS) [32], stimulated Raman scattering (SRS) and coherent anti-Stokes Raman scattering (CARS) have been developed. Notably, Ishikawa and co-workers have exploited the surface-enhancement resonance Raman spectroscopy (SERRS) technique, which couples both surface enhancement and resonance enhancement, for sensitive, specific detection of HbA1c [33]. In this article, they used silver nanoparticles (AgNPs) of 60 nm diameter to enhance the intrinsically weak Raman signal. Both hemoglobin A (HbA) and HbA1c were incubated with AgNPs, following which the colloidal solutions were deposited on the slides via spin-coating. Fig. 2 shows that HbA1c exhibits a characteristic SERRS band in the range 770–830 cm^{-1} (predominantly at 827 cm^{-1}) in addition to the Raman bands at 1403 cm^{-1} and 1652 cm^{-1} present in HbA SERRS spectrum. This observation that 827 cm^{-1} specifically originates from the HbA1c was corroborated by incubation of glucose with HbAm, which also showed this band. Interestingly, different aggregation patterns were observed when HbA and HbA1c were incubated with AgNPs.

This SERRS-based approach is promising and novel. However, precise quantification of HbA1c using it is difficult as a result of longstanding concerns about aggregate reproducibility and the spurious background of the SERRS spectrum. Another Raman spectroscopic route, namely drop-coating-deposition Raman (DCDR) spectroscopy, was proposed by our laboratories in view of its easy, label-free detection capability with routine instrumentation [34]. In DCDR, the solution of interest (e.g., hemolysate solution obtained from a whole-blood sample) is microdeposited (or microprinted) on a compatible substrate, followed by solvent evaporation and backscattering Raman spectroscopic detection. This process provides substantive signal amplification by simple pre-concentration of the analytes [35], which can be ascribed to the formation of the “coffee ring” pattern due to the interplay between contact line pinning, solvent evaporation and capillary flow [36]. Critically, even though the DCDR-preconcentration process produces protein deposits that are in a solid-like state, multiple studies have shown that the deposits remain well hydrated and as such retain their solution conformation. A study on the physico-chemical principles of the phenomena has hypothesized an important role for the glassy-skin formation at the droplet surface that can be responsible for long-term preservation of “solution-gel-like” conditions of the deposited biomolecules [37]. Fig. 3(A) shows a representative composite photograph showing the drop-coating ring pattern produced by air-drying Hb and HbA1c

mixture solution on a quartz substrate. As can be seen in Fig. 3(A), the proteins are concentrated on the annular ring structure with little or no material left in the interior region.

Using the DCDR approach, our study established that the glycation of hemoglobin results in subtle, but highly reproducible, changes in the acquired Raman spectra. These spectral differences, presumably resulting from the reduction in the α -helix content [38] and weakening of the heme–globin interaction [39] in HbA1c, facilitate the accurate classification of glycated and non-glycated hemoglobin in hemolysate models using conventional multivariate techniques. Fig. 3(B) shows the principal component (PC) scores plot used for segmentation of the two analytes. Furthermore, the acquired Raman spectra revealed excellent reproducibility of spectral characteristics at different locations in the coffee-ring pattern. Partial least squares (PLS) analysis of the spectral responses illustrates a linear response between the intensity patterns and the analyte concentrations, with a limit of detection (LOD) nearly 15-fold smaller than the lowest physiological concentration of HbA1c encountered in clinical samples. Given the strong characteristic Raman signature of hemoglobin (both glycated and non-glycated forms) and the substantially lower concentration of other analytes (e.g., albumin, IgG and transferrin), it was conjectured that these findings would also translate to clinical samples collected from diabetic patients. Lin et al. have expanded on these ideas and performed similar experiments on whole-blood samples acquired from normal volunteers and diabetic patients [40]. With the aid of PC analysis (PCA) and linear discriminant analysis (LDA), they have shown a high degree of diagnostic sensitivity and specificity in distinguishing diabetic patients from healthy subjects, based on spectroscopic detection of the glycemic marker in blood samples. However, significant efforts need to be made to advance these preliminary findings in order to display the viability of the method as a potential therapy-monitoring and guidance tool.

4.2. Optical sensing of glycated albumin

In comparison to the arsenal of existing and emerging approaches for HbA1c, the domain of direct photonic sensing for fructosamine, especially glycated albumin, has been relatively underexplored. Most of the literature reports focus on the application of fluorescence spectroscopy for characterization of glycated albumin [41] and for studying the effects of glycation on changes in binding and oxidation properties of albumin [42]. For example, fundamental studies measuring the fluorescence lifetimes of native and glycated albumin proteins (human serum albumin and bovine serum albumin) have revealed that the shorter lifetime (when the decay curve is fitted by double and triple exponential functions) is sensitive to the onset and the progression of glycation [43]. Also, fluorescence spectroscopy in conjunction with HPLC has been used to assess the impaired drug-binding properties of *in vitro* and *in vivo* glycated albumin [44].

Despite simplicity and characterization ability, intrinsic fluorescence methods are inadequate for quantification of glycated albumin in complex biological matrices, due to the presence of broad spectral bands (i.e., lack of distinct analyte signatures) in the absence of targeted imaging agents. To address the lack of specificity, researchers have employed FTIR (Fourier-transform infrared) spectroscopy. As mentioned above, IR absorption spectroscopy is sensitive to alterations in secondary structure so it has been used for not only

measurements of glycated albumin under static conditions but also evaluations of the dynamic build-up of glycated albumin when incubating albumin with glucose [45]. In particular, Chesne et al. studied the 1500–1700 cm^{-1} range in the absorption spectra acquired from the samples when albumin was incubated in the absence or the presence of physiological (5 mM) and pathological (25 mM) concentrations of glucose. Their analyses focused on two characteristic absorption features, corresponding to the region of amide-bound protein (NH-CO) [46]. The authors observed a decrease in the height of these peaks when albumin was incubated with glucose, indicating structural protein modifications. Specifically, glycation of albumin at pathological concentrations of glucose induced a significant reduction in the area of the amide I band (1649–1659 cm^{-1}), a marker of the α -helix structure. These findings are consistent with FTIR observations by Mantsch and co-workers on platelets isolated from the blood of diabetic patients [45a]. The data gathered in Mantsch's studies also accurately reflected the structural modifications in membrane proteins of platelets, indicating the potential of the technology for translation from the spectroscopists' workbench to the clinical laboratory.

Raman spectroscopy can be used to analyze aqueous solutions as it does not suffer from the large water-absorption effects associated with FTIR, and, generally, provides higher spectral detail (due to less cluttering of peaks). Encouraged by the findings of our DCDR study in glycated hemoglobin, we have performed proof-of-concept experiments in characterization of glycated albumin [47]. These experiments revealed that glycation in albumin induces subtle changes in the Raman spectral signatures. Several studies have suggested that glycation of albumin happens at multiple sites corresponding to the arginine, lysine and cysteine residues that can be attributed to their high nucleophile properties [13,48]. Since the prominent Raman features of albumin (e.g., 1655 cm^{-1} amide-I band, 1447 cm^{-1} CH_2 deformation band, 1002 cm^{-1} phenylalanine band and the tyrosine doublet at 828 cm^{-1} and 850 cm^{-1}) are not prominently associated with the above residues, it is not unexpected that the glycation-induced changes would be subtle. Importantly, though, with the aid of multivariate classification approaches, such as logistic regression based on PC scores, glycated albumin can be differentiated from its non-glycated counterpart with 100% accuracy. Appropriate control studies alleviate any possibility of the characterization of glycated albumin component based on spurious correlations with system or environmental parameters.

Also, Raman microscopy was pursued over a segment of the annular ring to characterize the reproducibility of the determinations. The absence of significant inhomogeneity in the drop-coated samples, especially at a given radial distance from the center of the ring, was noted via profilometry measurements (Fig. 4A). The richness of the Raman spectroscopic data also enabled concentration prediction with a relative error of prediction of 8.5% over the physiological range of glycated albumin (Fig. 4B). The precision profile of Fig. 4C, where the relative standard deviation is plotted as a function of the molecular concentration, reveals an LOD of 13.7 μM – nearly four times smaller than the lowest physiological concentration encountered in practice. Taken together, these observations underline the feasibility of clinical application of this proposed method. Finally, such translation to the clinic is likely to be facilitated by the relative abundance of albumin in serum relative to

other proteins and small molecules and its strong and characteristic Raman signature, which does not exhibit significant overlap with the other principal serum proteins.

Other techniques that have been espoused for this important diagnostic purpose include refractive index-based measurements and an intriguing aptamer-based surface-plasmon sensing approach [30b,49]. While none of these methods have been validated in an adequate number of clinical samples, the emergence of novel spectroscopic and imaging techniques is extremely encouraging and heralds the potential development of a truly label-free, glycated albumin-specific, high-throughput photonic assay.

5. Conclusions and future prospects

In addition to the ubiquitous need for blood-glucose estimation, we consider that comprehensive glycemic control information necessitates accurate detection of both glycated hemoglobin and albumin. The concomitant determination of this panel of biomarkers is warranted by not only the different lifetimes of these molecules but also the unique interferences faced in the determination of each. For example, if the values were not concordant, it would require further investigations before interpreting either value as a reflection of the true glycemic history. Currently, there is no low-cost, real-time measurement device or methodology for the multiplexed detection of this panel of glycemic markers and much less progress has been made on this front, even in the research domain, with most proposals focusing exclusively on their marker of choice.

Optical sensing approaches provide promising tools in the multiplexed analysis of markers of dysglycemia, at concentrations below the levels encountered in human pathophysiology. A wide array of methods has been designed based on differential markers in native and glycated proteins. Of these methods, vibrational spectroscopy, due to its label-free, real-time detection capabilities, shows the greatest promise, although significant work still needs to be done in order to reduce the cost and the size of instrumentation. Research efforts can be fruitfully directed at incorporating judicious processing steps prior to spectroscopic/photonic sensing, such as ultrafiltration or suitable microfluidic separation of analyte molecules depending on their respective diffusion coefficients. Ultimately, the goal is to design and to develop a table-top or even a hand-held miniaturized spectrometer to be used in conjunction with lab-on-chip devices that can together offer multiplexed, high-throughput, clinically viable detection of the glycemic marker panel.

Although only a few of the emerging optical methods have been tested in clinical patient samples, they have demonstrated the feasibility of clinical application by offering high levels of accuracy and precision for physiologically relevant levels of these biomarkers. Evidently, extensive trials will be necessary on patient samples, such as serum and whole blood, to ascertain analytical reliability, and clinical sensitivity and selectivity. The challenge in reaching that level from the current stage is substantial – but so is the pay-off, given the unprecedented increase in diabetes incidence globally. The broader area of non-invasive and minimally invasive diabetes diagnostics, often referred to as the “Holy Grail of biophotonics”, truly presents a shining opportunity for technological innovations.

Acknowledgments

Financial support

This research was supported by the National Institute of Biomedical Imaging and Bioengineering, Bethesda, MD, USA (9P41EB015871-26A1) and the JHU Whiting School of Engineering Startup Funds, Baltimore, MD, USA.

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HIGHLIGHTS

- Long-term glycemic markers give information for diabetes screening and monitoring
- Emerging spectroscopic methods offer reagent-free, multiplexed quantitation
- Photonics for rapid assessment of glycoprotein-based biopharmaceutical synthesis

Fig. 1.

(a) Formation mechanism of HbA1c. In the first step, the glucose molecule adds to the N-terminal valine of the beta chain forming the Schiff base, and, in the subsequent step, this Schiff base undergoes an irreversible Amadori rearrangement resulting in the formation of stable glycated hemoglobin. (b) The esterification reaction between glycated HbA1c and thiophene-3-boronic acid self-assembled monolayer-covered gold electrodes. {Reproduced with permission from [10]}.

Fig. 2.

Surface-enhanced resonance Raman spectra of HbA and HbA1c. The characteristic band around 770–830 cm⁻¹ is only present in the SERRS spectrum of HbA1c and forms the basis for the selective detection of HbA1c. {Reprinted with permission from [33]}.

(a)

(b)

Fig. 3.

(a) Photograph illustrating the drop-coating ring pattern produced by air-drying Hb and HbA1c mixture solution on a quartz substrate. The proteins are observed to be concentrated on the ring with little or no material left in the interior region, ring width is $\sim 300 \mu\text{m}$ and the ring diameter is 3.5 mm. (b) Score plots corresponding to principal components 3, 4, and 5 for the spectral dataset acquired from the single-protein Hb and HbA1c drop-coated rings. Red circles and blue squares represent Hb and HbA1c samples respectively. {Reproduced with permission from [34]}.

Fig. 4.

(a) 2D spatial Raman mapping of a drop-coated glycated albumin ring (analyte concentration = 31.25 µM). 2D spatial Raman mapping-based concentration prediction results for a representative glycated albumin drop-coated ring. The profile along the radial direction (X-axis) illustrates an approximately symmetric shape with a steeper descending outer part (i.e., over pixels 8, 9 and 10) as compared to the more gradual descent in the inner part of the ring (i.e., over pixels 3, 2 and 1). (b) PLS prediction results of glycated albumin samples. The solid line denotes $y = x$ values. (c) Relative standard deviation plot of precision for glycated albumin determination. The red circle represents the computed values from the experimental measurements, whereas the solid black curve is the best-fit exponential curve. {Reprinted with permission from [47]}.