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Published on Web 12/11/2002

Toward a Glucose Biosensor Based on Surface-Enhanced Raman Scattering

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Abstract: This work presents the first step toward a glucose biosensor using surface-enhanced Raman spectroscopy (SERS). Historically, glucose has been extremely difficult to detect by SERS because it has a small normal Raman cross section and adsorbs weakly or not at all to bare silver surfaces. In this paper, we report the first systematic study of the direct detection of glucose using SERS. Glucose is partitioned into an alkanethiol monolayer adsorbed on a silver film over nanosphere (AgFON) surface and thereby, it is preconcentrated within the 0−4 nm thick zone of electromagnetic field enhancement. The experiments presented herein utilize leave-one-out partial least-squares (LOO−PLS) analysis to demonstrate quantitative glucose detection both over a large (0−250 mM) and clinically relevant (0−25 mM) concentration range. The root-mean-squared error of prediction (RMSEP) of 1.8 mM (33.1 mg/dL) in the clinical study is near that desired for medical applications (1 mM, 18 mg/dL). Future studies will advance toward true in vivo, real time, minimally invasive sensing.

Introduction

According to the National Institutes of Health, an estimated 17 million people have diabetes mellitus (type I and II) in the United States today, including 151 000 juvenile cases. In 1997, a total of $44 billion was spent diagnosing, monitoring, and treating diabetes.[1] In diabetes mellitus, the body either fails to produce or to respond to insulin, which regulates glucose metabolism, resulting in large fluctuations in glucose levels. These fluctuations can cause a range of secondary complications, including kidney disease, heart disease, blindness, nerve damage, and gangrene. Current treatment of diabetes consists of self-regulation of blood glucose levels through frequent monitoring and a combination of diet, medication, and insulin injection, depending on the type of diabetes. Most patients measure their glucose levels by withdrawing small samples of blood using a "finger-stick" apparatus, followed by indirect electrochemical detection of hydrogen peroxide produced by enzymatic oxidation of glucose with glucose oxidase. Sampling in this manner is both painful and inconvenient; however, the electrochemical detection is currently the best technology available for frequent use. As a result, many patients fail to adequately monitor their glucose levels, risking secondary complications. A faster, easier, and less painful method for frequently measuring glucose levels would be of great individual, clinical, and societal benefit. Continuous monitoring of blood glucose would open the door to feedback control of implanted insulin pumps. In fact, the development of a reliable and robust sensor technology is the single stumbling block to the realization of an artificial pancreas.

Because of the importance of this issue, many groups are researching methods for minimally invasive, biologically compatible, quantitative glucose detection.[2,3] Mid-infrared absorption, one of the more promising techniques, is sensitive to temperature, pH, and competing absorption by water. Current mid-infrared absorption studies utilize an indwelling probe to minimize complicating factors.[4] In laser polarimetry, another approach being developed, polarized light is rotated by chiral molecules, such as glucose, while passing through the aqueous humor of the eye. This technique is capable of detecting glucose concentrations as low as 20 mg/dL (∼1.0 mM) in vitro; however, the optical activity of the other constituents of the aqueous humor, such as ascorbate and albumin, as well as the birefringence of the cornea, make this approach extremely difficult.[5] Indirect detection of glucose is also done using fluorescence or other optical techniques.[6,7] These techniques rely on the enzymatic reaction of glucose to produce the detected byproduct. Biomolecules similar to the analyte can interfere with this multistep process, giving false positives.

One technique capable of addressing the major weaknesses of the aforementioned methods (interfering water absorption, overlapping signals from competing analytes, and indirect

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10.1021/ja028255v CCC: $25.00

measurement complications) is vibrational Raman spectroscopy. It has been shown that normal Raman spectroscopy (NRS) can readily detect physiological concentrations of glucose in vitro from a simulated aqueous humor solution.[8] Using partial-least squares (PLS), Lambert et al. were able to predict glucose levels ranging from 50 mg/dL (2.8 mM, hypoglycemic) to 1300 mg/dL (72.2 mM, severe diabetic) with a standard error of 24.7 mg/dL (1.4 mM). Berger et al. were able to detect glucose concentrations with an accuracy of 26 mg/dL (1.4 mM) in serum and 79 mg/dL (4.4 mM) in whole blood using PLS.[9] Although these are promising results, the laser exposure in both experiments is significantly higher than is biologically permissible.[10] The high laser powers and long acquisition times are required due to the inherently small normal Raman scattering cross section of glucose, 5.6×10^{-30} cm^2 molecule^{-1} sr^{-1} according to McCreery and co-workers.[11] To establish the proper context, consider that benzene, a strong Raman scatterer, has a cross section of 2.8×10^{-29} cm^2 molecule^{-1} sr^{-1} and water, a weak Raman scatterer, has a cross section of 1.1×10^{-31} cm^2 molecule^{-1} sr^{-1}.[11] Note that the reported Raman cross section for glucose is only five times smaller than that of benzene and 50 times larger than that of water. From the one reported glucose Raman cross section, it is clear that normal Raman scattering should produce sufficiently large signals for effective quantitative analysis. Any approach that produces a stronger optical signal from glucose will only improve the feasibility of clinical measurements.

Raman optical activity spectroscopy and Raman difference spectroscopy are both examples of highly sensitive Raman techniques capable of detecting small differences in Raman cross section. In both of these techniques, however, the resultant difference signals are very small and long data acquisition times are required.[12,13] Such an approach is not desirable for a rapid, robust, clinical analysis method. One way to increase the Raman cross section is to exploit resonance Raman spectroscopy.[14] In the case of glucose, this would require excitation in the deep ultraviolet region ($\lambda \sim 200$ nm) of the spectrum. Ultraviolet excitation is unlikely to be appropriate for in vivo sensing due to photodamage of DNA. Alternatively, the Raman cross section can be amplified by surface-enhanced Raman spectroscopy (SERS). Using SERS should retain all of the advantages of normal Raman spectroscopy while achieving significantly stronger signal intensity.

SERS is a process whereby the Raman scattering signal is increased when a Raman-active molecule is spatially confined within range of the electromagnetic fields generated upon excitation of the localized surface plasmon resonance of nanostructured noble metal surfaces. The ensemble-averaged Raman signal increases by up to 8 orders of magnitude,[15] whereas the nonensemble-averaged Raman signal can increase by 14 or 15 orders of magnitude in special cases.[16,17] Both chemical and conformational information can be elucidated from SERS. Theoretical analysis suggests that molecules confined within the decay length of the electromagnetic fields, viz. 0−4 nm, will exhibit SER spectra even if they are not chemisorbed.[18] SERS possesses many desirable characteristics as a tool for the chemical analysis of in vivo molecular species including high specificity, attomole to high zeptomole mass sensitivity, micromolar to picomolar concentration sensitivity, and interfacial generality.[19]

The work presented in this paper represents the first step toward the development of a glucose biosensor using SERS. Silver film over nanospheres (AgFON) substrates are used in this investigation since they have been successfully used in electrochemical, ultrahigh vacuum, and ambient SERS experiments.[20−22] Previously, SERS has been used to detect a wide variety of analytes present at low concentrations, including, but not limited to, pollutants,[23] explosives,[24,25] chemical warfare agents,[26] and DNA.[27] However, only a few of the existing studies present quantitative results.[28,29] The success of chemometric techniques in the analysis of normal Raman data[30] suggests that this same approach may also benefit the quantitative analysis of SERS data. This work is an initial effort to explore the systematic response characteristics of this glucose biosensor by combining the strengths of SERS and PLS within a clinically relevant glucose concentration range.

Chemometric methods, such as PLS, are used when the spectrum of an analyte of interest is embedded within a complex background spectrum. Long data acquisition times, like those needed in Raman optical activity and Raman difference spectroscopy, are not required when PLS is used to analyze the SERS data. PLS was chosen for data analysis because it is a time efficient technique that requires only knowledge of analyte concentrations in order to verify predictions. The derived calibration vectors show the vibrational features of the analytes found, making it easy to verify that the desired analyte (glucose) is, in fact, the analyte recognized and analyzed by the PLS algorithm.

Furthermore, the results presented here demonstrate the utility of a stationary phase or partition layer in preconcentrating

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glucose within the zone of enhanced electromagnetic fields, enabling SERS detection and quantitation of glucose. A cross-validated PLS algorithm is used to predict glucose concentrations both within and outside of the physiologically relevant range. The achieved prediction error is near the desired accuracy for biomedical application.

We have identified seven milestones to be achieved on the path to continuous, minimally invasive, quantitative, in vivo sensing of glucose in aqueous humor and interstitial fluid. The first, and most critical in terms of scientific feasibility, is the demonstration of quantitative sensing of glucose using SERS. This work accomplishes exactly that goal. The remaining six milestones require (1) optimization of the partition layer, (2) glucose sensing in mixtures, (3) study of nonspecific partitioning, (4) fabrication of a AgFON substrate on the tip of a fiber optic probe and in vivo testing of the SERS-active probe, (5) miniaturization of the implanted AgFON surface to micro/nanoscale dimensions, and (6) miniaturization of the detection apparatus.

Experimental Section

Materials. Ag (99.99%, 0.04" diameter) was purchased from D. F. Goldsmith (Evanston, IL). Glass substrates were 18 mm diameter, No. 2 coverslips from Fisher Scientific (Fairlawn, VA). Pretreatment of substrates required H_2SO_4, H_2O_2, and NH_4OH, all purchased from Fisher Scientific (Fairlawn, VA). Surfactant-free white carboxyl-substituted polystyrene latex nanospheres with diameters of 390 ± 19.5 nm were obtained from Duke Scientific Corporation (Palo Alto, CA). Tungsten vapor deposition boats were purchased from R. D. Mathis (Long Beach, CA). 4-aminothiophenol (90%), L-cystein (97%), 3-mercaptoproprionic acid (99+%), 11-mercaptoundecanoic acid (95%), 1-hexanethiol (95%), 1-octanethiol (98%), 1-decanethiol (96%), 1-hexadecanethiol (92%), 3-mercapto-1-propanesufonic acid (Na^+ salt, 90%), benzenethiol (99+%), cyclohexylmercaptan (97%), α-D-Glucose (ACS Reagent Grade) were purchased from Aldrich (Milwaukee, WI) and used as received. Poly-DL-lysine hydrobromide was purchased from Sigma (St. Louis, MO). Ethanol was purchased from Pharmco (Brookfield, CT). For all steps of substrate and solution preparation, ultrapure water (18.2 MΩ cm^{-1}) from a Millipore academic system (Marlborough, MA) was used.

AgFON Fabrication and Incubation Procedure. Borosilicate glass substrates were pretreated in two steps (1) piranha etch, 3:1 H_2SO_4:30% H_2O_2 at 80 °C for 1 h, was used to clean the substrate, and (2) base treatment, 5:1:1 H_2O:NH_4OH: 30% H_2O_2 with sonication for 1 h, was used to render the surface hydrophilic. Approximately 2 μL of undiluted nanosphere solution (4% solids) were drop coated onto each substrate and allowed to dry in ambient conditions. The metal films were deposited in a modified Consolidated Vacuum Corporation vapor deposition system[31] with a base pressure of 10^{-7} Torr. The mass thickness of Ag in all cases was 200 nm and deposition rates for each film (1 nm/sec) were measured using a Leybold Inficon XTM/2 quartz-crystal microbalance(QCM) (East Syracuse, NY). Fresh AgFON samples were incubated in 1 mM solutions of the partition layer self-assembled monolayers (SAMs) in ethanol for >12 h before being exposed to glucose solutions of the desired concentration. Each sample was dosed in a separate vial. The glucose incubation time was arbitrarily chosen to be between 10 min and 1 h. The effectiveness of the partition layer determines the required incubation time. Glucose solutions ranged in concentration from 0 to 250 mM in 80% ethanol:20% water.

Micro-SERS Apparatus. Spatially resolved SER spectra were measured using a modified Nikon Optiphot (Frier Company, Huntley, IL) confocal microscope with a 20x objective in backscattering geometry. The laser light from a Coherent (Santa Clara, CA) model 590 dye laser operating at λ_{ex} = 632.8 nm or a Spectra-Physics (Moutainview, CA) model Millenia Vs laser operating at λ_{ex} = 532.0 nm was coupled into a 200 μm core diameter fiber using a Thorlabs (Newton, NJ) fiber launch. Appropriate Edmund Scientific (Barrington, NJ) interference filters and Kaiser (Ann Arbor, MI) holographic notch filters were placed in the beam path. The backscattered light was collected by an output fiber optic coupled to an Acton (Acton, MA) VM-505 monochromator (entrance slit set at 250 μm) with a Roper Scientific (Trenton, NJ) Spec-10:400B liquid N_2-cooled CCD detector.

Chemometrics Method. All data processing was performed using MATLAB (MathWorks, Inc., Natick, MA) and PLS_Toolbox (Eigenvector Research, Inc., Manson, WA). Prior to analysis, cosmic rays were removed from the spectra using a derivative filter and the slowly varying background, commonly seen in SERS experiments, was removed by subtracting a fourth-order polynomial. The data were then mean-centered. Data analysis was performed using partial least-squares (PLS) leave-one-out (LOO) analysis.

PLS was chosen from among the many chemometric techniques available because it only requires knowledge of the concentrations of the analyte of interest during calibration.[32,33] Other techniques, such as classical least-squares require knowledge of all of the chemicals present in the sample. Although the precise amount of glucose added to each sample is known in the presented experiments, the knowledge of the other chemicals in the background (e.g., polystyrene from substrate preparation or impurities in the partition layers) was less certain. PLS has previously been used to analyze data for similar studies.[9]

Whenever a chemometric technique is used, proper validation is essential to obtain meaningful results. Usually, two separate data sets are used, one for calibration and one for validation. Because of the limited number of samples in the data set, LOO was chosen as the cross-validation technique.[34] In LOO analysis, one sample at a time is left out of the calibration set. The PLS model is developed using the remaining data and then applied to the lone sample. The predicted concentration of this sample is then compared to the actual concentration and used to evaluate the quality of the model. The process is then repeated, leaving each sample out, one at a time, to build up a set of validation results. LOO cross-validation enables evaluation of a new technique despite a relatively small data set.

Prediction error in the calibration and validation sets was determined by calculating the root-mean-squared error of prediction (RMSEP)

$$\mathrm{RMSEP} = \sqrt{\frac{(\mathrm{conc}_1 - \mathrm{pred}_1)^2 + (\mathrm{conc}_2 - \mathrm{pred}_2)^2 + \ldots + (\mathrm{conc}_n - \mathrm{pred}_n)^2}{n}} \quad (1)$$

In this equation, conc represents the actual concentration of a sample, pred represents the predicted concentration for that sample, and n is the total number of samples. The choice of the number of loading vectors to use in the PLS results discussed here was determined by the number of loading vectors needed for the root-mean-squared error of calibration (RMSEC) to stabilize at a minimum value.

Results and Discussion

All attempts to observe glucose on AgFON surfaces using SERS without a partition layer were unsuccessful. This result is in agreement with all previous attempts to measure glucose using SERS that are known to us, except one. The only published SER spectrum of glucose we are aware of uses a two-step surface preparation technique using electrochemically roughened electrodes and colloidal nanoparticles.[35] Although

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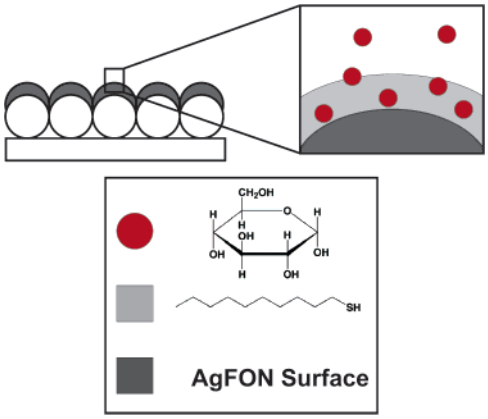

Figure 1. Schematic showing hypothetical glucose concentration gradient created by 1-decanethiol partition layer.

this substrate has potential for future work in glucose detection, substrate stability remains to be demonstrated. Electrochemically roughened electrodes are known to have metastable nanostructures; their enhancement factors are strongly potential dependent and, at sufficiently negative potentials, experience irreversible loss of SERS-activity. Also, colloidal nanoparticles aggregate when exposed to media with high ionic strength such as would be encountered in glucose sensing. Excellent stability of SERS-activity has been unambiguously demonstrated for bare AgFON surfaces over potential ranging from Ag oxidation to H_2 evolution[20] and at high temperatures ($< T_s = 500$ K) in ultrahigh vacuum.[21] Similarly, the self-assembled monolayers (SAMs)[36] used in this work are known to be extremely stable by themselves and as adsorbates on AgFON surfaces.[22,37] The historic difficulty of SERS detection of glucose must be attributable to its weak or nonexistent binding to bare silver surfaces since its normal Raman cross section should provide sufficient signal. In the experiment performed by Weaver and co-workers, the glucose, apparently, must be trapped in the junction between the roughened electrode and the colloidal nanoparticle. To increase glucose interaction with the AgFON surface, a SAM can be formed on its surface to preconcentrate the analyte of interest (Figure 1), in a manner analogous to that used to create the stationary phase in high performance liquid chromatography (HPLC).[22,36,38−40] Implementing a partition layer has three advantages: (1) the SAM stabilizes the Ag surface against oxidation; (2) the SAM is exceedingly stable; and (3) preconcentration functionality is built in and tailorable by synthetic control of the partition layer.

It is instructive to consider two limiting cases that provide useful estimates of the time response that is potentially achievable. Consider the following kinetic scheme for the SERS-based glucose sensor

$$G_{\text{bulk}} + S \underset{k_{\text{des}}}{\overset{k_{\text{ads}}}{\rightleftharpoons}} G_{\text{ads}} \underset{k_{-\text{p}}}{\overset{k_{\text{p}}}{\rightleftharpoons}} G_{\text{part}} \qquad (2)$$

In general, glucose must diffuse from bulk solution, G_{bulk}, to the solution/alkanethiol SAM, S, interface where it is adsorbed, G_{ads}, and then partitioned, G_{part}, into the SAM. D is the glucose diffusion coefficient, k_{ads} is the bimolecular rate constant for adsorption of glucose at the solution/SAM interface, k_{des} is the unimolecular rate constant for desorption of glucose from the solution/SAM interface, k_{p} is the rate constant for partitioning of glucose into the SAM, and $k_{-\text{p}}$ is the rate constant for departitioning of glucose. If diffusion is the rate-limiting step, one can calculate the time, τ, for $^1/_2$ monolayer of glucose to accumulate at the solution/SAM interface[41]

$$\tau = \frac{\pi \Gamma_{\text{max}}^2}{16\, C^2 D} \qquad (3)$$

where Γ_{max} is the packing density for a full monolayer and C is the bulk concentration of glucose. Substituting $D = 6.8 \times 10^{-6}$ cm^2 s^{-1}, $\Gamma_{\text{max}} = 4.1 \times 10^{-10}$ mole cm^{-2}, and C = 1mM = 1.0×10^{-6} mole cm^{-3}, the value of τ is 4.8×10^{-3} s. Under these conditions, the SERS-based glucose sensor would be expected to achieve ~10 millisecond time response. Now consider the case where the kinetics of adsorption/desorption and partitioning/departitioning are rate determining. This situation has been examined by Harris and co-workers[42,43] in their study of solute retention kinetics at reversed-phase chromatographic surfaces. From a detailed analysis of a temperature-jump relaxation experiment, Harris determined that $k_{\text{ads}} = (12.7 \pm 3) \times 10^8$ M^{-1} s^{-1}, $k_{\text{des}} = (1.07 \pm 0.15) \times 10^6$ s^{-1}, $k_{\text{p}} = 1.8 \times 10^5$ s^{-1}, and $k_{-\text{p}} = 1.3 \times 10^5$ s^{-1} for the system in which 1-anilino-8-naphthalene sulfonate (ANS) is the solute, C4-silica is the chromatographic surface, and 0.4 M NaCl in 50/50 CH_3OH/H_2O is the solution. The rate constants measured for other systems including ANS/C18-silica and a neutral solute, N-phenyl-1-naphthylamine (1-NPN), at C4 silica were of similar magnitude. Harris' results allow us to conclude, at least qualitatively, that the rate limiting step in the transport of glucose from bulk solution to the interior of the alkanethiol SAM partition layer where it is detected at the AgFON surface by SERS is diffusion, rather than adsorption/desorption or partitioning/departioning. Consequently, we anticipate the future development of SERS-based glucose sensors with real-time response.

Several SAMs were tested to determine their effectiveness as a partition layer. The twelve SAMs tested in this work were 4-aminothiophenol, L-cystein, 3-mercaptopropionic acid, 11-mercaptoundecanoic acid, 1-hexanethiol, 1-octanethiol, 1-decanethiol (1-DT), 1-hexadecanethiol, poly-DL-lysine, 3-mercapto-1-propanesufonic acid, benzenethiol, and cyclohexylmercaptan. Of these, only the straight chain alkanethiols were found to be effective partition layers, especially 1-decanethiol (which forms a monolayer on silver ~1.9 nm thick).[44] It is interesting to note that 1-decanethiol almost completely fills the theoretical first decay length of the electromagnetic fields from the SERS substrate.[18] The decay length has been estimated at ~1.5 nm in multiple studies as well.[45−47] Figure 2 shows example spectra from the different stages of assembly of the glucose/1-DT/

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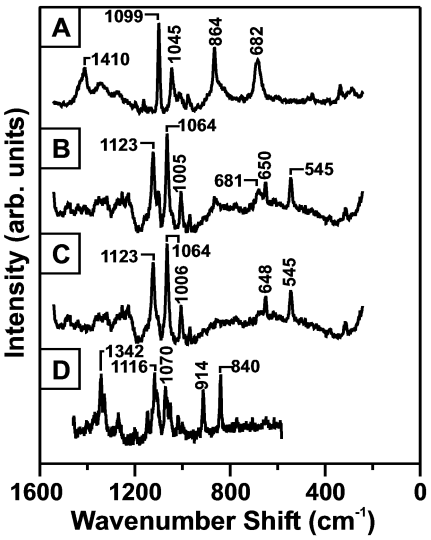

Figure 2. Spectra used in quantitative analysis. (A) 1-DT monolayer on AgFON substrate, $\lambda_{ex} = 532$ nm, $P = 1.25$ mW, acquisition time = 30 s. (B) Mixture of 1-DT monolayer and glucose partitioned from a 100 mM solution, $\lambda_{ex} = 532$ nm, $P = 1.25$ mW, acquisition time = 30 s. (C) Residual glucose spectrum produced by subtracting (A) from (B). (D) Normal Raman spectrum of crystalline glucose for comparison, $\lambda_{ex} = 632.8$ nm, $P = 5$ mW, acquisition time = 30 s.

AgFON surface. Figure 2A shows the SER spectrum of 1-DT on a AgFON surface. After 10 min incubation in 100 mM glucose solution, the SER spectrum in Figure 2B was observed. This spectrum is the superposition of the SER spectra for the partition layer and glucose. Figure 2B clearly shows vibrational features from both the analyte glucose (1123 and 1064 cm^{-1}) and 1-DT (1099, 864, and 681 cm^{-1}) constituents. The SERS difference spectrum resulting from subtraction of spectrum 2A from spectrum 2B is shown in Figure 2C. The difference spectrum can be compared directly to the normal Raman spectrum of crystalline glucose shown in Figure 2D. The vibrational bands seen at 914 and 840 cm^{-1} in the crystalline glucose spectrum (Figure 2D) are not observed in the spectra shown in Figure 2B and 2C because these bands are strongest in crystalline glucose; this phenomenon has been previously observed.[35]

In the initial quantitative experiment, AgFON surfaces with a monolayer of 1-DT were incubated for 10 min in a solution containing glucose concentrations ranging from 0 to 250 mM. SER spectra were then measured from each sample using $\lambda_{ex} = 632.8$ nm ($P_{laser} = 4.7$ mW, 90s). In all 36 cases, the measurements were made on samples in dry, ambient conditions. Upon performing LOO−PLS analysis, 21 loading vectors were found to minimize the root-mean-squared error of calibration (RMSEC), see inset of Figure 3. The resulting cross-validated glucose concentration predictions, using 21 loading vectors, can be seen in Figure 3. The corresponding error of prediction is 3.3 mM. This result has been repeated with multiple, similar data sets.

Although it is important that quantitative SERS detection is demonstrated in the aforementioned data set, a clinically relevant concentration range is of the highest priority. Accordingly,

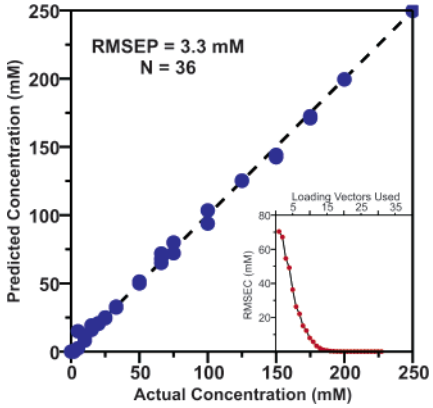

Figure 3. Plot of PLS predicted glucose concentrations versus actual glucose concentrations using leave-one-out cross-validation (21 loading vectors). Ag FON samples were made ($D = 390$ nm, dm = 200 nm), incubated for 17 h in 1 mM 1-DT solution, and dosed in glucose solution (range: 0−250 mM) for 10 min. Each micro-SERS measurement was made under ambient conditions, using $\lambda_{ex} = 632.8$ nm (4.7 mW, 90 s). Dashed line is not a fit, but rather represents perfect prediction. Inset shows the root-mean-squared error of calibration as a function of number of loading vectors used in the PLS algorithm.

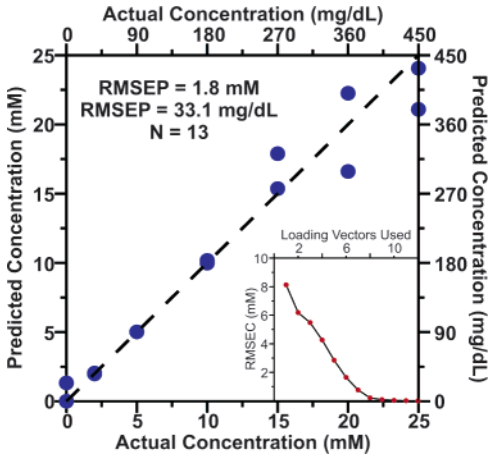

Figure 4. Plot of PLS predicted physiologically relevant glucose concentrations versus actual glucose concentrations using leave-one-out cross-validation (10 loading vectors). AgFON samples were made ($D = 390$ nm, $d_m = 200$ nm), incubated for 19 h in 1 mM 1-DT solution, and dosed in glucose solution (range: 0−25 mM) for 1 h. Each micro-SERS measurement was made, whereas samples were in an environmental control cell filled with glucose solution, using $\lambda_{ex} = 632.8$ nm (3.25 mW, 30 s). Dashed line is not a fit, but rather represents perfect prediction. Inset shows the root-mean-squared error of calibration as a function of number of loading vectors used in the PLS algorithm.

AgFONs with a monolayer of 1-DT were incubated for an hour in glucose solutions diluted by a factor of 10 (0−25 mM, 0−450 mg/dL). SER spectra were then measured from each sample using $\lambda_{ex} = 632.8$ nm ($P_{laser} = 3.25$ mW, 30s). In all 13 cases, the measurements were made on samples in a simple environmentally controlled cell, bathed in the corresponding glucose solution. Upon performing LOO−PLS analysis, 10 loading vectors were found to minimize the root-mean-squared error of calibration (RMSEC), see inset of Figure 4. The resulting cross-validated glucose concentration predictions, using 10 loading vectors, can be seen in Figure 4. The corresponding error of prediction is 1.8 mM. Fewer loading vectors and a lower RMSEP in the smaller concentration range experiment may be attributable to the onset of a nonlinear signal versus glucose concentration relationship (i.e., the nonlinear portion of the

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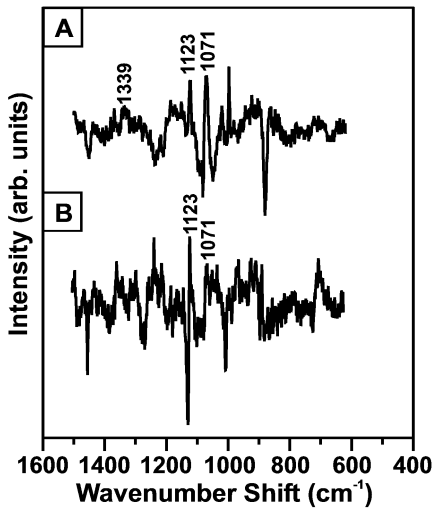

Figure 5. Calibration vectors used to produce PLS predictions shown in Figures 3 and 4, respectively.

partition isotherm) as higher concentrations are partitioned. Future experiments will explore this phenomenon in detail.

In the calibration vectors (Figure 5A and B) used to generate the prediction plots seen in Figures 3 and 4, the characteristic vibrational bands of glucose are clearly visible at 1123 and 1071 cm^{-1}. These calibration vectors represent the portions of glucose that do not overlap with bands of the partition layer or analytes present in the background. Accordingly, some glucose features are absent, while others represent the portion of the glucose band not overlapping with those bands of 1-DT. Clearly, 1-DT is not an ideal partition layer from the perspective of spectral band position. An active research program exploring other potential partition layers that have larger partition coefficients and more favorable vibrational characteristics is underway.

Conclusions

Glucose is extremely difficult to detect by surface-enhanced Raman spectroscopy (SERS) because it has both a modest normal Raman cross section and it adsorbs weakly or not at all to bare silver surfaces. In this paper, we report the first systematic detection of glucose using SERS. The SER bands observed clearly at 1123 and 1064 cm^{-1} demonstrate the vibrational features of glucose in solution. The adsorption problem has been circumvented by partitioning glucose into an alkanethiol monolayer adsorbed on the silver surface thereby preconcentrating it within the zone of electromagnetic field enhancement. Of the 12 partition layers studied, only straight chain alkanethiols were found to be effective. Consequently, 1-decanethiol was chosen as the partition layer for all the studies presented here.

Two data sets are presented to support our assertion of quantitative detection of glucose using SERS. The first probes the quantitative prediction of glucose over a large concentration range (0−250 mM), demonstrating a root-mean-squared error of prediction (RMSEP) of 3.3 mM. The second covers the clinically relevant concentration range (0−25 mM/0−450 mg/dL), performed in a liquid environment with short (viz. 30 s) data acquisition times. This data set is effectively treated using LOO−PLS and displays a RMSEP of 1.8 mM (33.1 mg/dL), near that desired for medical applications. The calibration vectors derived in both experiments using the PLS algorithm show the characteristic vibrational features of glucose.

Although the apparatus used to perform SERS measurements in the presented work is quite expensive, less expensive alternatives exist for future clinical work. Today, it is possible to purchase a fully packaged, self-contained instrument that costs a factor of 10 less than a laboratory-scale instrument and weighs less than 15 lbs.[48] Clearly, it is desirable to make another order of magnitude reduction in cost and weight. The rapid progress in the development of miniature of UV−vis absorption,[49] IR absorption,[50,51] and ultimately Raman[52] spectrometers using micro and nanophotonics[53,54] approaches are likely to make this possible.

This work demonstrates that SERS has significant potential for biomedical applications requiring quantitative glucose detection. Although the experiments described here present only proof-of-concept results, future studies will undoubtedly make progress toward true in vivo, real time, minimally invasive sensing. In the long run, it is our intent that the SERS substrate will be miniaturized to a microscale or even nanoscale device that can either be implanted subcutaneously or can be incorporated as a component of a prosthetic lens in the eye with little or no discomfort to diabetic individuals. Instead of using a visible red excitation source as done here, we anticipate that it will be advantageous to explore near infrared excitation within the "therapeutic window", between 700 and 1200 nm, where absorption by skin is at its minimum. Designer surfaces with large enhancement factors in this range are accessible using nanosphere lithography and the principles of nanoparticle optics.[20,31] As the partition layer is optimized for maximal preconcentration of glucose near the SERS substrate, the laser power density can be concomitantly reduced to meet the American National Standards Institute guidelines for human exposure (<2.5 mW cm^{-2} for 0.25 s, $\lambda = 633$ nm, directed at the eye).[10] Experiments performed concurrently with those detailed here have shown linear predictive capability using SERS for other physiological analytes (i.e., urea). Future investigations will also focus on multi-analyte detection and detection of glucose within a complex mixture of biomolecules.

Acknowledgment. The first two authors, K.E.S.P. and C.L.H., contributed equally to this work. The authors acknowledge support from the Institute for Bioengineering and Nanoscience in Advanced Medicine at Northwestern University, National Institutes of Health (EY13002 and EY13015), National Science Foundation (EEC-0118025 and DMR-0076097), the Air Force Office of Scientific Research MURI program (F49620-02-1-0381), an ACS Division of Analytical Chemistry fellowship sponsored by GlaxoSmithKline (C.L.H.), and a Northwestern University Presidential Fellowship (C.L.H.).

JA028255V

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