

Real-Time Glucose Sensing by Surface-Enhanced Raman Spectroscopy in Bovine Plasma Facilitated by a Mixed Decanethiol/Mercaptohexanol Partition Layer

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A new, mixed decanethiol (DT)/mercaptohexanol (MH) partition layer with dramatically improved properties has been developed for glucose sensing by surface-enhanced Raman spectroscopy. This work represents significant progress toward our long-term goal of a minimally invasive, continuous, reusable glucose sensor. The DT/MH-functionalized surface has greater temporal stability, demonstrates rapid, reversible partitioning and departitioning, and is simpler to control compared to the tri-(ethylene glycol) monolayer used previously. The data herein show that this DT/MH-functionalized surface is stable for at least 10 days in bovine plasma. Reversibility is demonstrated by exposing the sensor alternately to 0 and 100 mM aqueous glucose solutions (pH ~7). The difference spectra show that complete partitioning and departitioning occur. Furthermore, physiological levels of glucose in two complex media were quantified using multivariate analysis. In the first system, the sensor is exposed to a solution consisting of water with 1 mM lactate and 2.5 mM urea. The root-mean-squared error of prediction (RMSEP) is 92.17 mg/dL (5.12 mM) with 87% of the validation points falling within the A and B range of the Clarke error grid. In the second, more complex system, glucose is measured in the presence of bovine plasma. The RMSEP is 83.16 mg/dL (4.62 mM) with 85% of the validation points falling within the A and B range of the Clarke error grid. Finally, to evaluate the real-time response of the sensor, the 1/e time constant for glucose partitioning and departitioning in the bovine plasma environment was calculated. The time constant is 28 s for partitioning and 25 s for departitioning, indicating the rapid interaction between the SAM and glucose that is essential for continuous sensing.

Diabetes mellitus is a chronic disorder that requires careful regulation of glucose levels in order to maintain the health of

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diabetic patients. Failure to regulate these levels within tight limits leads to severe secondary health complications to the diabetic patient's retina, kidneys, nerves, and circulatory system.^{1,2} Most commonly, diabetics measure glucose levels four to six times per day with the electrochemically based finger-stick method. The finger-stick method is not capable of continuous monitoring and suffers from low patient compliance due to the pain and discomfort associated with blood sampling from the capillaries. Such intermittent testing can fail to detect significant fluctuations in blood glucose levels and place the patient in dangerously hypo- or hyperglycemic conditions. The development of a continuous monitoring device for glucose with as low a degree of invasiveness as possible will clearly have an enormous impact on long-term human health.

In recent years, the development of continuous, implantable glucose sensors has been the focus of various research groups. The most advanced glucose sensors measure glucose indirectly by electrochemical detection methods. Glucose is enzymatically reduced by glucose oxidase in the presence of oxygen, resulting in production of hydrogen peroxide.^{3,4} While hydrogen peroxide is detected, other interfering analytes such as ascorbic acid, uric acid, and acetaminophen can give false positive signals.⁵ Clearly, specificity is very important in the development of glucose sensors.

Infrared (IR) and Raman spectroscopies have recently been explored as useful techniques for glucose detection. The unique vibrational signatures of each molecule allow direct and selective identification of glucose. Arnold and co-workers have demonstrated the detection of glucose both in aqueous solution⁶ and in whole blood in the near-IR region.⁷ However, due to low spectral resolution and overlap with second overtone peaks of water, glucose detection using near-IR spectroscopy is difficult. On the other hand, Raman spectroscopy has higher spectral resolution and significantly reduced interference from water. Moreover, Raman spectroscopy has been able to detect glucose in the

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physiological range in vitro and in aqueous humor.⁸ Due to the inherently weak Raman signals of glucose, however, high power and long acquisition times are required for quantitative detection.⁹ This problem can be overcome by utilizing surface-enhanced Raman spectroscopy (SERS).^{10–12} Enhancement is observed when molecules are brought into proximity to nanoscale roughened metal surfaces.¹³ The intensified local electromagnetic field generated by the nanoscale structures is able to enhance the observed Raman scattering by 10^6 – 10^8 ,^{14,15} and in exceptional cases as much as 10^{14} times.^{16,17}

In our previous work, development of a sensing platform for glucose detection using SERS was demonstrated.^{10–12} The metal film over nanospheres (MFON) SERS-active surface was functionalized with alkanethiol or an alkanethiol derivative self-assembled monolayer (SAM) to partition and departition glucose closer to the surface. Quantitative measurements were performed using the SAM-functionalized SERS surface in phosphate-buffered saline (pH 7.4) and in the presence of bovine serum albumin.¹¹ In addition, SAM stability on the AgFON and AuFON was demonstrated for 3 and 10 days, respectively.¹² Reversibility of glucose partitioning was also demonstrated on these sensors.

Quantitative detection of glucose was demonstrated using tri-(ethylene glycol)-terminated alkanethiol (EG3) and decanethiol (DT) SAMs on the SERS-active surface. Although EG3 SAM is known to be biocompatible,^{18–20} its synthesis is challenging, and therefore, its availability is limited. DT, on the other hand, is hydrophobic and not compatible with an aqueous environment. In this work, a new mixed SAM, based on two commercially available components, DT and mercaptohexanol (MH), was explored to attain a real-time, quantitative glucose monitoring device. Self-assembled monolayers have been the focus of much research.²¹ The exact mechanism of mixed SAM formation has not been well characterized. However, according to a space-filling computer model, combining the longer DT component with the shorter MH component creates a void, which will be referred to as a pocket in this paper. Further, it is hypothesized that this pocket improves glucose partitioning, bringing glucose even closer to the SERS-active surface than was possible with EG3. In addition,

the DT/MH SAM has dual hydrophobic/hydrophilic functionality analogous to EG3. Based on the stability and reversibility parameters, we have experimentally confirmed that the DT/MH mixed SAM has better performance as a partitioning layer than the DT and EG3 SAMs used previously.

Herein, we demonstrate (1) the stability of the DT/MH partition layer on an AgFON surface, (2) partitioning and departitioning of glucose on a DT/MH-functionalized AgFON surface, (3) quantitative detection of glucose in aqueous solution with interfering analytes and in bovine plasma, and (4) real-time kinetics of glucose partitioning and departitioning.

EXPERIMENTAL SECTION

Materials. All the chemicals were reagent grade or better and used as purchased. Silver pellets (99.99%) were purchased from Kurt J. Lesker Co. (Clairton, PA). Oxygen-free high-conductivity copper was obtained from McMaster-Carr (Chicago, IL) and cut into 18-mm-diameter disks. To clean substrates, NH_4OH , H_2O_2 , and $\text{CH}_3\text{CH}_2\text{OH}$ from Fisher Scientific (Fairlawn, VA) were used. Surfactant-free, white carboxyl-substituted latex polystyrene nanosphere suspensions (390 ± 19.5 -nm diameter, 4% solid) were purchased from Duke Scientific Corp. (Palo Alto, CA). Ultrapure water ($18.2 \text{ M}\Omega \text{ cm}^{-1}$) from a Millipore system (Marlborough, MA) was used for substrate and solution preparation. Bovine plasma was obtained from Hemostat Laboratories (Dixon, CA). Glucose, lactate, and urea were purchased from Sigma (St. Louis, MO). Decanethiol ($\text{CH}_3(\text{CH}_2)_9\text{SH}$), and 6-mercapto-1-hexanol ($\text{HS}(\text{CH}_2)_6\text{OH}$) were purchased from Aldrich (Milwaukee, WI). Disposable filters, pore size $0.45 \mu\text{m}$, were acquired from Gelman Sciences (Ann Arbor, MI).

AgFON Fabrication and Incubation Procedure. The copper substrates were cleaned by sonicating in 5:1:1 $\text{H}_2\text{O}/30\% \text{H}_2\text{O}_2/\text{NH}_4\text{OH}$. Approximately $10 \mu\text{L}$ of nanosphere solution was drop-coated onto a clean copper substrate and allowed to dry at room temperature. Then, 200-nm-thick Ag films were deposited onto and through the nanosphere mask using the Kurt J. Lesker electron beam deposition system (Clairton, PA) to form AgFON substrates. The mass thickness and deposition rate ($2 \text{ \AA}/\text{s}$) of the Ag metal were measured by 6-MHz gold-plated quartz crystal microbalance purchased from Sigma Instruments (Fort Collins, CO). AgFON substrates were first incubated in 1 mM DT in ethanol for 45 min and then transferred to 1 mM MH in ethanol for at least 12 h (Figure 1). Then the SAM-functionalized surfaces were mounted into a small-volume flow cell for SER spectra collection.

Surface-Enhanced Raman Spectroscopy. A Spectra-Physics model Millennia Vs laser ($\lambda_{\text{ex}} = 532 \text{ nm}$) was used to excite a Spectra-Physics model 3900 Ti-sapphire laser to produce the 785-nm excitation wavelength (λ_{ex}); the laser spot size on the sample was less than 0.5 mm in diameter. This excitation wavelength was chosen to minimize autofluorescence of proteins.^{22,23} The SERS measurement system includes an interference filter, an edge filter (Semrock, Rochester, NY), a model VM-505 single-grating monochromator with the entrance slit set at $100 \mu\text{m}$ (Acton Research Corp., Acton, MA), and a LN_2 -cooled CCD detector (Roper

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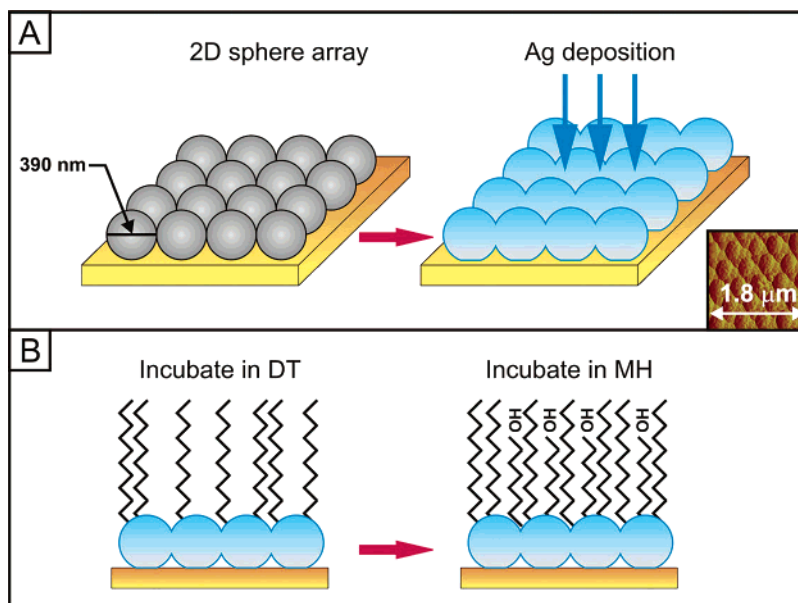


Figure 1. Schematic for fabricating DT/MH-functionalized AgFON. (A) Nanospheres (diameter 390 nm) were self-assembled to form a hexagonal close-packed 2D crystal. Metal (Ag) was then deposited via electron beam deposition. Inset shows an atomic force micrograph of an AgFON surface. The image is $1.8\ \mu\text{m} \times 1.8\ \mu\text{m}$. (B) The FON surface was incubated in a solution of 1 mM DT in ethanol for 45 min and then transferred to 1 mM MH in ethanol for at least 12 h. This DT/MH SAM creates a pocket for glucose to fit into, bringing glucose closer to the SERS-active surface.

Scientific, Trenton, NJ). A collection lens with magnification 5 was used to collect the scattered light. The small-volume flow cell was used to control the external environment of the AgFON surfaces throughout the SERS experiments.

Quantitative Multivariate Analysis. All data processing was performed using MATLAB (MathWorks, Inc., Natick, MA) and PLS_Toolbox (Eigenvector Research, Inc., Manson, WA). Prior to analysis, the spectra were smoothed using the Savitsky–Golay method with a second-order polynomial and window size of 9. Cosmic rays were removed from the spectra using a derivative filter. The slowly varying background, commonly seen in SERS experiments, was removed by subtracting a fourth-order polynomial fit. This method greatly reduced varying background levels with minimum effect on the SERS peaks. The chemometric analysis was performed using the partial least-squares (PLS) method and leave-one-out (LOO) cross-validation algorithm.

Time Constant Analysis. The data were processed using PeakFit 4.12 software (Systat Software Inc, Richmond, CA). To remove the varying background in SER spectra, a fourth-order polynomial was subtracted from the baseline using MATLAB software. The spectra were further preprocessed in PeakFit with linear best-fit baseline correction and Savitsky–Golay smoothing. The amplitude of the Raman bands was obtained by fitting the data to the superposition of the Lorentzian amplitude line shapes.

RESULTS AND DISCUSSION

The results presented below show significant advancement toward an implantable, real-time continuous SERS-based glucose sensor. Our previous work demonstrated the ability to detect glucose with SERS using decanethiol as the partition layer.¹⁰ Subsequently, EG3 was used as the partition layer because it is biocompatible and has the ability to resist nonspecific binding of proteins due to its hydrophilic properties.¹¹ Moreover, stability, reversibility, and resistance to serum protein interference of the

EG3-functionalized glucose sensor were demonstrated. Finally, the SERS-based glucose sensor was optimized for NIR laser excitation wavelength with the EG3 partition layer to reduce photodamage of tissue and optimize signal on Au surfaces.¹² This development showed enhanced spectral stability and gave more accurate measurements. However, due to the intricate synthesis, availability of EG3 is scarce. In the present work, a new mixed SAM layer, consisting of DT and MH, has been developed. We also demonstrate (1) long-term stability of the DT/MH-functionalized AgFON surface, (2) reversibility of the sensor, (3) quantitative measurement of glucose, and (4) real-time partitioning and departitioning of the glucose sensor.

Temporal Stability of DT/MH-Modified Substrate. An implantable glucose sensor must be stable for at least 3 days.²⁴ In our previous work, we demonstrated that SAM-functionalized AgFON substrates were stable for at least 3 days in phosphate-buffered saline by electrochemical and SERS measurements.¹² Herein, we demonstrate the stability of the DT/MH-functionalized AgFON surface for 10 days in bovine plasma (Figure 2). SER spectra were captured every 24 h from three different samples and three spots on each sample ($\lambda_{\text{ex}} = 785\ \text{nm}$, $t = 2\ \text{min}$). Figure 2A represents the DT/MH spectrum acquired on day 2. Figure 2B shows the average intensity of the 1119-cm^{-1} peak for DT/MH on the AgFON for each day as a function of time. The 1119-cm^{-1} band corresponds to a symmetric stretching vibration of a C–C bond.²⁵ Only a 2% change in intensity of the 1119-cm^{-1} peak was observed from the first day to the last day with a standard deviation (STDV) of 1216 counts, indicating that it did not vary significantly during the 10-day period. This small change in intensity can be attributed to the rearrangement of the SAM during the incubation in bovine plasma.²⁶ The temporal stability

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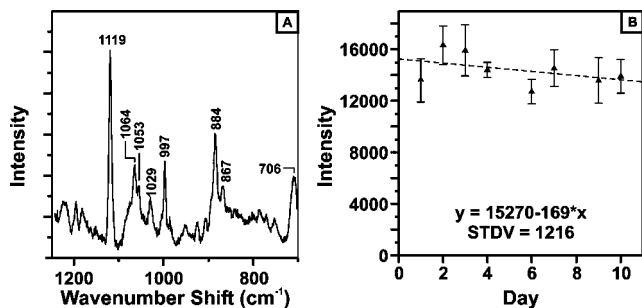


Figure 2. Stability of the DT/MH-functionalized FON. (A) SERS spectrum of DT/MH-functionalized FON (day 2) (B) Time course of intensity of 1119-cm⁻¹ peak. Signal intensities stayed stable over a 10-day period with STDV = 1216 counts, $\lambda_{\text{ex}} = 785$ nm, $P_{\text{laser}} = 55$ mW, and $t = 2$ min.

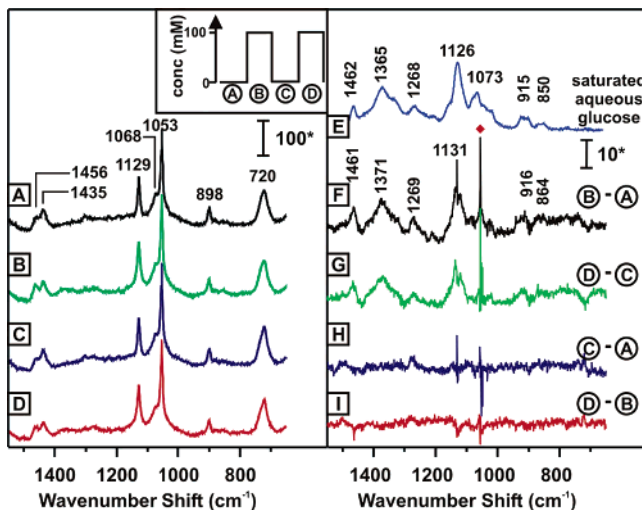


Figure 3. Glucose pulsing sequence on the SAM-modified AgFON surface (inset). SER spectra of the sample cycled between 0 and 100 mM aqueous glucose solutions (A–D), $\lambda_{\text{ex}} = 532$ nm, $P_{\text{laser}} = 10$ mW, $t = 20$ min, and pH ~ 7 . Normal Raman spectrum of aqueous saturated glucose solution (E). Difference spectra showing partitioning/departitioning of glucose (F–I). (◆) Imperfect subtraction of the narrow band at 1053 cm⁻¹ due to nitrate results in a very sharp peak in the difference spectra. An asterisk (*) denotes $\text{adu} \cdot \text{mW}^{-1} \cdot \text{min}^{-1}$.

of the 1119-cm⁻¹ peak intensity indicates that the DT/MH SAM was intact and well ordered, making this SAM-functionalized surface a potential candidate for an implantable sensor.

Reversible Glucose Sensing. An implantable glucose sensor must also be reversible in order to successfully monitor fluctuation in glucose concentration throughout the day. To demonstrate the reversibility of the sensor, the DT/MH-modified AgFON sensor was exposed to cycles of 0 and 100 mM aqueous glucose solutions (pH ~ 7) without flushing the sensor between measurements to simulate real-time sensing (Figure 3 inset). Nitrate was used as an internal standard in all the experiments (1053-cm⁻¹ peak) to minimize effective laser power fluctuations. The 1053-cm⁻¹ band corresponds to a symmetric stretching vibration of NO₃⁻ and was used to normalize the spectra.²⁷ SER spectra were collected for each step ($\lambda_{\text{ex}} = 532$ nm, $P = 10$ mW, $t = 20$ min) (Figure 3A–D).

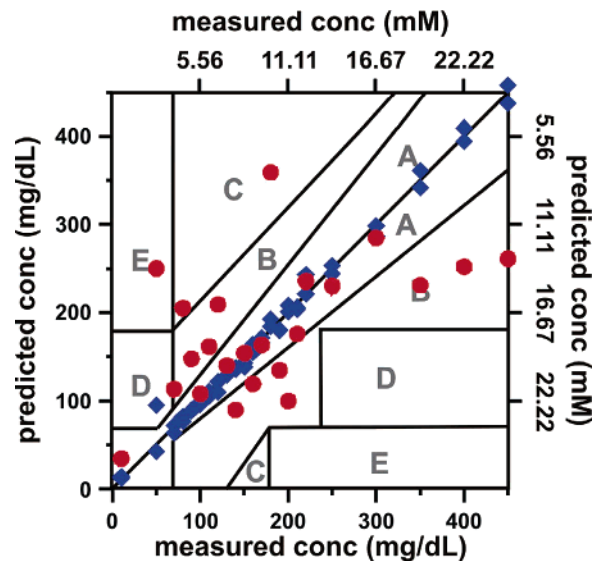


Figure 4. Calibration (◆) and validation (●) plots using two substrates and multiple spots. PLS calibration plot was constructed using 23 data points taken over a range of glucose concentrations (10–450 mg/dL) in 1 mM lactate and 2.5 mM urea at pH ~ 7 . RMSEC = 9.89 mg/dL (0.55 mM) and RMSEP = 92.17 mg/dL (5.12 mM). $\lambda_{\text{ex}} = 785$ nm, $P_{\text{laser}} = 8.4$ mW, and $t = 2$ min.

Figure 3E shows the normal Raman spectrum of a saturated aqueous glucose solution for comparison. In the normal Raman spectrum of a saturated aqueous glucose solution, peaks at 1462, 1365, 1268, 1126, 915, and 850 cm⁻¹ correspond to crystalline glucose peaks.²⁸ The difference spectra (Figure 3F, G) represent partitioning of glucose in DT/MH SAM, which clearly show the glucose features at 1461, 1371, 1269, 1131, 916, and 864 cm⁻¹. This corresponds to the peaks in the normal Raman spectrum of glucose in aqueous solution (Figure 3E). The literature has shown that SERS bands can shift up to 25 cm⁻¹ when compared to normal Raman bands of the same analyte.²⁹ The sharp peak seen in all of the difference spectra at 1053 cm⁻¹ represents imperfect subtraction of the nitrate internal standard. The absence of glucose spectral features in the difference spectra (Figure 3H, I) represents complete departitioning of glucose. The DT/MH mixed SAM presents a completely reversible sensing surface for optimal partitioning and departitioning of glucose.

Quantitative Detection of Glucose Using the DT/MH Partition Layer. In order for a glucose sensor to be viable, it should be able to detect glucose in the clinically relevant range 10–450 mg/dL (0.56–25 mM), under physiological pH and in the presence of interfering analytes (Figure 4). The data are presented in the Clarke error grid, a standard for evaluating the reliability of glucose sensors in the clinically relevant concentration range (0–450 mg/dL).³⁰ Data points that fall in the A and B range are acceptable values. Values outside the A and B range result in

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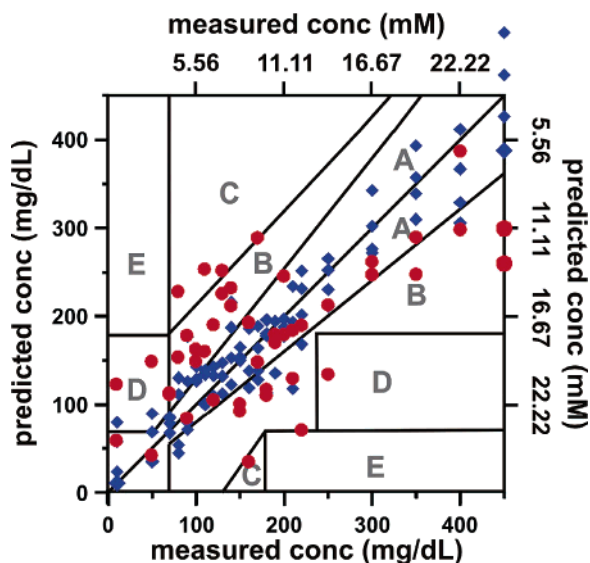


Figure 5. Calibration (◆) and validation (●) plots using three substrates and multiple spots acquired in 2 days. PLS calibration plot was constructed using 92 data points. The validation plot was constructed using 46 data points taken over a range of glucose concentrations (10–450 mg/dL) in bovine plasma. RMSEC = 34.3 mg/dL (1.9 mM) and RMSEP = 83.16 mg/dL (4.62 mM). $\lambda_{\text{ex}} = 785$ nm, $P_{\text{laser}} = 10\text{--}30$ mW, and $t = 2$ min.

potential failure to detect blood glucose levels outside of the target range and erroneous diagnosis. DT/MH-functionalized AgFON samples were placed in a flow cell containing water (pH ~ 7) with lactate (1 mM) and urea (2.5 mM) in physiological concentrations, which are potential interferences for glucose detection. Glucose solutions ranging from 10 to 450 mg/dL with lactate and urea were then randomly introduced in the cell and incubated for 2 min to ensure complete partitioning. SER spectra were collected using two substrates and multiple spots with a near-infrared laser source ($\lambda_{\text{ex}} = 785$ nm, $P = 8.4$ mW, $t = 2$ min). A calibration model was constructed using partial least-squares leave-one-out (PLS-LOO) analysis with 46 randomly chosen independent spectral measurements of known glucose concentrations. The calibration model was based upon seven latent variables which take into account variation in laser power, the environment in the laboratory, and SERS enhancement at different locations. The PLS analysis results in a root-mean-square error of calibration (RMSEC) of 9.89 mg/dL (0.549 mM). This RMSEC value is lower than that reported in our previous work using the EG3-modified AgFON.

In addition to having a low RMSEC, it is important to use an independent validation set to test the calibration model.³¹ For this model, a set of 23 data points was used to validate the model. The root-mean-square error of prediction (RMSEP) was calculated to be 92.17 mg/dL (5.12 mM). Figure 4 depicts that 98% of the calibration points and 87% of the validation points fall in the A and B range of the Clarke error grid. The RMSEP can be improved by increasing the number of data points in the calibration set.

To transition from the in vitro sensor to an in vivo sensor, the sensor should also demonstrate quantitative detection in a more complex medium. Bovine plasma was used to simulate the in vivo

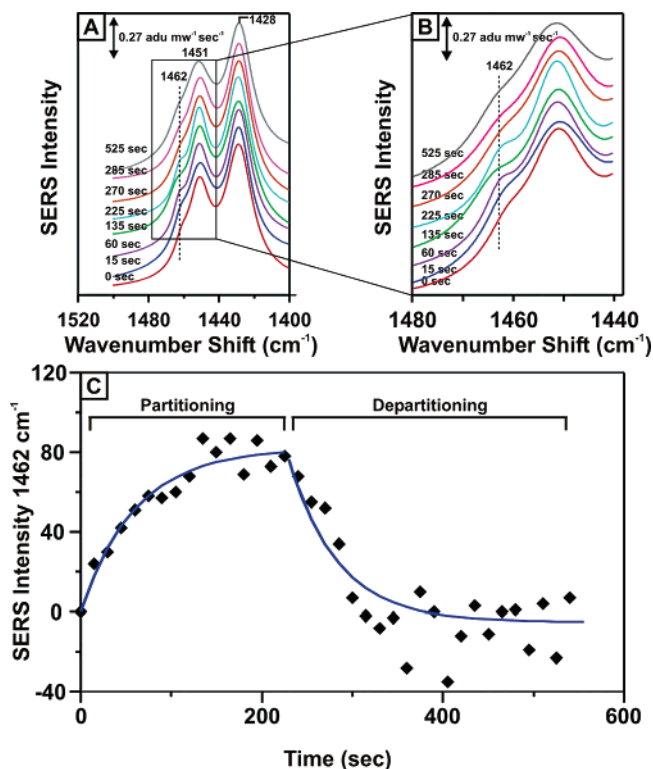


Figure 6. Real-time SERS response to a step change in glucose concentration in bovine plasma. (A) SER spectra of the SAM and glucose at various times. Peaks at 1451 and 1428 cm^{-1} are features of SAM and 1462 cm^{-1} indicates glucose. Glucose was injected at $t = 0$ s, and the cell was flushed with bovine plasma at $t = 225$ s. (B) Expanded scale version of (A) from 1480 to 1440 cm^{-1} . (C) Partitioning and departitioning of glucose. $\lambda_{\text{ex}} = 785$ nm, $P_{\text{laser}} = 100$ mW, and $t = 15$ s. The $1/e$ time constants were calculated to be 28 s for partitioning and 25 s for departitioning.

environment of an implantable glucose sensor, which will eventually be implanted under the skin in the interstitial fluid. Prior to use, bovine plasma was passed through a 0.45- μm -diameter pore size filter. The filtered plasma was then spiked with glucose concentrations ranging from 10 to 450 mg/dL. DT/MH-functionalized AgFON substrates were placed in the flow cell and exposed to glucose-spiked bovine plasma. SER spectra were collected at each concentration using multiple samples and multiple spots in random order to construct a robust calibration model ($\lambda_{\text{ex}} = 785$ nm, $P = 10\text{--}30$ mW, $t = 2$ min). Calibration was constructed using PLS-LOO analysis described above using seven latent variables and presented in a Clarke error grid (Figure 5). To construct the calibration, 92 randomly chosen data points were used, resulting in an RMSEC of 34.3 mg/dL (1.90 mM). For the validation, 46 data points were used with an RMSEP of 83.16 mg/dL (4.62 mM). In the Clarke error grid, 98% for calibration and 85% for validation fall in the A and B range. The errors in both experiments can be reduced by using more data points for the calibration. In addition, error can also be attributed to variation in SERS enhancement at different spots and different substrates.¹⁴ The results show that the DT/MH-modified AgFON glucose sensor is capable of making accurate glucose measurements in the presence of many interfering analytes.

Real-Time Study of Partitioning and Departitioning of Glucose. In addition to reversibility, which is an important characteristic for a viable sensor, the sensor should be able to

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partition and departition glucose on a reasonable time scale. The real-time response was examined in a system with bovine plasma simulating the in vivo environment. To evaluate the real-time response of the sensor, the $1/e$ time constant for partitioning and departitioning was calculated.

A DT/MH-functionalized AgFON was placed in bovine plasma for ~ 5 h. The AgFON surface was then placed in a flow cell. SER spectra were collected continuously ($\lambda_{\text{ex}} = 785$ nm) with a 15-s integration time. To observe partitioning, 50 mM glucose solution in bovine plasma was injected at $t = 0$. At $t = 225$ s, 0 mM glucose solution in bovine plasma was injected into the flow cell to evaluate the departitioning of glucose. An excitation wavelength of 785 nm was used to reduce autofluorescence caused by proteins.^{22,23} The amplitude was then plotted versus time as shown in Figure 6C. The $1/e$ time constant was calculated from the exponential curve fitted to the data points. The spectra shown in Figure 6A and B demonstrate real-time amplitude changes in the 1462-cm^{-1} peak as the glucose concentration varies. The amplitude of the 1462-cm^{-1} peak was obtained by fitting the data to the superposition of three Lorentzian line shapes using PeakFit. The $1/e$ time constant is 28 s for partitioning and 25 s for departitioning, calculated from the exponential fit (Figure 6C). These experiments demonstrate that partitioning and departitioning occur rapidly making the SERS-based glucose sensor a potential candidate for implantable, continuous sensing.

CONCLUSIONS

This work demonstrates significant progress toward developing a real-time, continuous, quantitative SERS glucose sensor. A new mixed SAM was explored to partition and departition glucose efficiently. The new SAM consists of DT and MH providing the appropriate balance of hydrophobic and hydrophilic groups. The DT/MH-functionalized SERS surface showed temporal stability for at least 10 days in bovine plasma, making it a potential candidate for implantable sensing. Furthermore, quantitative glucose measurements, in the physiological concentration range, in a mixture of interfering analytes and in bovine plasma were also demonstrated. Finally, the DT/MH-functionalized SERS surface also partitioned and departitioned glucose in less than 1 min, which indicates that the sensor can be used for real-time, continuous sensing.

The temporal stability of the DT/MH-functionalized AgFON surface is evident from the fact that the intensity of a SERS band

did not change for a period of 10 days when incubated in bovine plasma. The intensity of the 1119-cm^{-1} SAM peak varied by only 2% during a period of 10 days with an STDV of 1216. This fluctuation can be attributed to molecular rearrangement of the SAM.²⁶

Moreover, reversibility of the DT/MH-functionalized glucose sensor was demonstrated by injecting cycles of 0 and 100 mM glucose in water (pH ~ 7). The difference spectra demonstrate complete partitioning and departitioning of glucose. In addition, the time constant for glucose partitioning and departitioning is less than 1 min, indicating rapid interaction between the SAM and glucose essential for continuous sensing.

The ability for DT/MH-functionalized AgFON to measure glucose concentrations accurately was evaluated using the Clarke error grid. The sensor was exposed to various concentrations of glucose in an aqueous solution (pH ~ 7), consisting of 1 mM lactate and 2.5 mM urea. The cross-validation error is 92.17 mg/dL, and 87% of the validation data fall in the *A* and *B* range of the Clarke error grid. Furthermore, a more complex media, bovine plasma, was used to demonstrate accuracy of the glucose sensor. In this case, the cross-validation error is 83.16 mg/dL and 85% of the validation data fall in the *A* and *B* range of the Clarke error grid.

Significant progress toward developing a continuous, real-time, quantitative glucose sensor is reported. In future work, the SERS glucose sensor will be implanted in the interstitium in an animal to demonstrate in vivo glucose sensing, and cross-calibrated with qualified hospital laboratory equipment. Ultraminiature spectrometers are under development that will enable portable glucose sensing.

ACKNOWLEDGMENT

This work was supported by the National Institutes of Health (DK066990-01A1), the U.S. Army Medical Research and Materiel Command (W81XWH-04-1-0630), the National Science Foundation (CHE0414554), and the Air Force Office of Scientific Research MURI program (F49620-02-1-0381).

Received for review July 29, 2005. Accepted August 25, 2005.

AC051357U