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Role of porosity in tuning the response range of microsphere-based glucose sensors

Saurabh Singh and Mike McShane*

Department of Biomedical Engineering, Texas A&M University, College Station, TX 77843, USA

Abstract

Luminescent microspheres encapsulating glucose oxidase have recently been developed as implantable glucose sensors. Previous work has shown that the response range and sensitivity can be tuned by varying the thickness and composition of transport-controlling nanofilm coatings. Nevertheless, the linear response range of these sensors falls significantly below the desired clinical range for *in vivo* monitoring. We report here an alternative means of tuning the response range by adjusting microsphere porosity. A reaction-diffusion model was first used to evaluate whether increased porosity would be expected to extend the response range by decreasing the flux of glucose relative to oxygen. Sensors exhibiting linear response ($R^2 > 0.90$) up to 600 mg/dL were then experimentally demonstrated by using amine-functionalized mesoporous silica microspheres and polyelectrolyte nanofilm coatings. The model was then used for sensor design, which led to the prediction that sensors constructed from ~12 μm microspheres having an effective porosity between 0.005 and 0.01 and ~65 nm transport-limiting coatings would respond over the entire physiological glucose range (up to 600 mg/dL) with maximized sensitivity.

Keywords

Glucose; biosensors; luminescence; microparticles; layer-by-layer self assembly; reaction-diffusion system

1. Introduction

Luminescent microsphere-based glucose sensors have been proposed and studied for prospective use in minimally-invasive continuous glucose monitoring—as so-called “smart tattoo” materials (Brown and McShane 2006; Brown et al. 2006; McShane 2002; Singh and McShane 2010; Stein et al. 2007; Stein et al. 2008). The goal of this sensing strategy is to implant the microbeads in the highly-vascularized dermis, where they can be optically interrogated with high efficiency, and the emission measurements (intensity or lifetime) may be used to predict interstitial glucose concentrations. Prototype sensors have comprised various compositions of microspheres loaded with an oxygen-sensitive luminophore and glucose oxidase, which is known to catalyze the oxidation of glucose to gluconic acid. These glucose-sensing beads are based on an indirect sensing mechanism: wherein the oxygen concentration inside the beads, which is indirectly proportional to glucose level present in

*Corresponding Author: Mike McShane, 335K Zachry Engineering Center, MS 3120, College Station, TX 77843-3120, Phone: (979) 845-7941, Fax: (979) 845-4450, mcshane@tamu.edu.

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the environment, is transduced via quenching of luminescence of a long-lifetime luminophore, such as a metal-porphine complex.

As these sensors rely on the direct measurement of oxygen, optimum sensor performance can only be attained when the ratio of glucose to oxygen concentration is less than 1. When this is not the case, such as *in vivo* (the normoglycemic glucose concentration is ~5.5 mM (120mg/dL), whereas the oxygen concentration can range from 90 μ M to 277 μ M (Stucker et al. 2002)), the lack of oxygen renders the reaction scheme oxygen-limited and the sensors cannot cover the entire physiological glucose range (up to ~33mM; 600 mg/dL). In general, this problem can be addressed by either decreasing the transport of glucose or increasing the oxygen transport. Typically, the former approach is taken: membranes that slow glucose diffusion are used (Gough et al. 1985; Rosenzweig and Kopelman 1996). Recently, perm-selective nanofilms (NFs) formed using layer-by-layer self-assembly (LbL) technique were used to extend the response range of microscale sensors (Stein et al. 2008). Key advantages of using this technique include: (1) the film characteristics such as thickness and diffusivity can be fine-tuned at the nanoscale by choosing the right material composition and polyelectrolyte deposition conditions and (2) the materials can be deposited on virtually any surface, regardless of shape and size. Such NFs have been reported to substantially reduce the transport of large molecules (e.g., glucose) without significantly affecting the transport of small molecules (e.g., oxygen) (Liu and Bruening 2004).

It has also been shown that by varying the thickness, material, and deposition conditions of NFs, the response range and sensitivity of microsphere-based glucose sensors can be tailored, to a degree (Stein et al. 2008). For example, sensors with 5, 10, and 15 bilayers of PAH/PSS (NF assembly performed in 0.2 M NaCl solution) exhibited response range of approximately 50, 100, and 150 mg/dL, respectively. Based on these observations, it is estimated that approximately 60 bilayers of PAH/PSS will be required, to obtain a linear response up to 600 mg/dL. This number of steps would be labor-intensive, time consuming, and would likely encounter efficiency and material waste problems due to colloidal stability issues. Therefore, there is a need to explore other alternatives for increasing the response range of these sensors.

It was hypothesized that the ratio of glucose to oxygen concentration can also be controlled by adjusting the porosity of microspheres, which would result in improved oxygen transport and, correspondingly, an extended response range. In this scenario, the effective transport of glucose would be minimally affected as it is mostly controlled by the outer NF coating. This concept can be explained by calculating the effective diffusivity for glucose and oxygen through a semi-infinite plate shown in Figure 1, which consists of two different regions of thickness d_1 and d_2 . In this case, Region 1 can be assumed as a perm-selective NF ($d_1=65$ nm), and Region 2 can be assumed as the sensor matrix ($d_2=3$ μ m), which could be either of extremely low porosity (0.005) or very high porosity (0.6) for the two cases to be compared (It has been assumed that both matrices have same tortuosity). For known diffusivities of substrates through the different regions, the effective diffusivity of a laminate material i ($D_{e,i}$) can be estimated using the equation (Crank 1980)

$$D_{e,i} = \frac{d_1 + d_2}{\frac{d_1}{D_{1,i}} + \frac{d_2}{D_{2,i}}} \quad (1)$$

where $D_{1,i}$ and $D_{2,i}$ are the diffusivities of substrate i in Region 1 and 2, respectively.

We considered glucose and oxygen as the substrates, and used the known diffusivities for glucose (9.87×10^{-10} cm²/s) and oxygen (2.52×10^{-7} cm²/s) in PAH/PSS nanofilms for

Region 1 (Liu and Bruening 2004; Singh and McShane 2010). For Region 2, assuming diffusion does not occur through the solid material of the matrix, but only through the hydrated pores, the diffusivities for glucose ($1.97 \times 10^{-8} \text{ cm}^2/\text{s}$) and oxygen ($1.00 \times 10^{-7} \text{ cm}^2/\text{s}$) were estimated by multiplying the diffusivity of substrates in water to the porosity of the matrix, which is 0.006 (Singh and McShane 2010). For the laminate with extremely low matrix porosity (left figure), the effective diffusivity of glucose and oxygen were estimated to be 1.4×10^{-8} and $1.01 \times 10^{-7} \text{ cm}^2/\text{s}$, respectively, using (1). In this case, the ratio of glucose to oxygen diffusivity is ~ 0.14 . In contrast, for the laminated matrix with high porosity (0.6), the effective diffusivities for glucose and oxygen were estimated to be 4.55×10^{-8} and $5.94 \times 10^{-6} \text{ cm}^2/\text{s}$, respectively. Therefore, the estimated ratio of glucose and oxygen diffusivity is ~ 0.0075 . In addition, it can be observed that increase in the porosity of the matrix from 0.006 to 0.6 resulted in *ca.* 59 times increase in the effective diffusivity of oxygen, whereas it only increased *ca.* 3 times for glucose. This shows in a simple way how the ratio of glucose to oxygen transport rate can be manipulated by varying the porosity of the sensor matrix. In this work, the effect of microsphere porosity on the predicted sensor response was studied using a mathematical model, followed by experimental validation, with the overall goal of designing glucose sensors with tailored microsphere porosity and coating properties to achieve a linear response up to 600 mg/dL.

2. Theory

The redox reaction of glucose and oxygen catalyzed by GOx may be expressed as

where G and O_2 are the GOx co-substrates, glucose and oxygen, respectively, E_{ox} and E_{red} are the oxidized and reduced forms of the enzymes, respectively, $E_{red}P_1$ and $E_{ox}P_2$ are the enzyme-substrate complexes, and k_1 , k_2 , k_3 , and k_4 are the rate constants of the respective reaction steps (Gibson et al. 1964).

Figure 2 depicts the model for the glucose sensing scheme. When sensors are exposed to bulk glucose and oxygen, these substrates diffuse inside the sensor matrix and trigger reaction (2). Within a few seconds, steady state is attained within the microspheres, after which the substrate average concentrations and spatial distributions depend on the delicate balance between reaction and diffusion rates. The reaction-diffusion model has been used to describe such phenomena (Pao 1993). As bulk oxygen is assumed to be fixed, the diffusion rate of glucose and, consequently, the consumption rate of oxygen inside the sensors primarily depend on bulk glucose concentrations. With increased glucose level, we expect to observe higher reaction rates, resulting in depleted oxygen levels inside microspheres; as the transduction mechanism for our sensors involves quenching of luminescence by oxygen, increased emission intensity will be observed for increased glucose levels.

For modeling purposes, reaction scheme (2) results in a system of six coupled partial differential equations (PDEs) that describe the behavior of the system in time and space for each individual particle. For this study, it was assumed that the boundary conditions for the sensor are constant and uniform throughout the surrounding and therefore the solution only depends on the radial coordinate (r). The PDE used to model this system can be written as

$$\frac{\partial C_i}{\partial t} + \frac{1}{r^2} \frac{\partial}{\partial r} \left(-D_i r^2 \frac{\partial C_i}{\partial r} \right) = R_i \quad (3)$$

where i is the subscript denoting one of the six reactive species, G , O_2 , E_{ox} , $E_{red}P_1$, E_{red} , $E_{ox}P_2$. D_i and R_i represent the diffusivity and reaction rates of the respective species involved in the reaction scheme. To convert the predicted oxygen level to an estimate of relative luminescence, the Stern-Volmer equation was applied:

$$\frac{F_0}{F} = \frac{\tau_0}{\tau} = 1 + K_{SV} \cdot [O_2] \quad (4)$$

In this equation, F_0 and τ_0 are the luminescence intensity and lifetime, respectively, in the absence of oxygen, F and τ are the luminescence intensity and lifetime in the presence of oxygen, $[O_2]$ is the oxygen concentration, and K_{SV} is the Stern-Volmer constant. For PtOEP immobilized in the algilica matrix, the previously determined value of $14,200 \text{ M}^{-1}$ (Stein et al. 2007) was used for K_{SV} . For PtP immobilized within silica microparticles, K_{SV} was experimentally determined to be $9,819 \text{ M}^{-1}$ (Figure S1). Percentage increase in luminescence at each glucose level was then estimated by taking luminescence at zero glucose as the base signal.

3. Experimental

3.1. Simulations

The sensor model was solved numerically using diffusion mode in COMSOL 3.5a (COMSOL, Inc, Burlington, MA), which estimates the approximate solution using the finite element method. The model was solved for glucose concentrations ranging from 0 to 33 mM (600 mg/dL), with oxygen concentration fixed at 277 μM . A detailed description of the model that was used to predict the response of sensors has been provided in the supplementary material. The output solution was plotted using COMSOL or was exported as a matrix file and imported into MATLAB (Mathworks, Natick, MA) workspace for further analysis. The model was used to investigate the general effects of porosity and microsphere size on sensor response. In addition, the response of sensors made specifically from algilica and porous silica were also simulated to allow direct comparison with experimental results.

3.2. Chemicals

Amine-functionalized silica microspheres (Zorbax[®]) with average diameter and porosity of 7 μm and 0.6, respectively, were obtained from Agilent. Pt(II) octaethylporphine (PtOEP, Frontier Scientific), Pt(II) meso-Tetra (4-carboxyphenyl) porphine (PtP, Frontier Scientific), dimethyl sulfoxide (DMSO, Aldrich), glucose oxidase (GOx, EC 232-601-0, Sigma), *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (EDC, Fluka), *N*-hydroxysulfosuccinimide sodium salt (NHSS, Toronto Research Chemicals Inc.), and sodium acetate (Sigma) were used to prepare PtOEP/GOx-doped "algilica" and PtP/GOx-doped silica microspheres (Stein et al. 2008). BCA[™] protein assay kit was acquired from Thermo Scientific to determine protein loading. Poly-(allylamine hydrochloride) (PAH, MW 70 kDa, Aldrich), poly-(sodium 4-styrenesulfonate) (PSS, MW 70 kDa, Aldrich), and sodium chloride (Sigma) were used during the deposition of multilayer thin films. Rhodamine B isothiocyanate (RITC, Aldrich) was conjugated to PAH (PAH-RITC), and used in LbL NF deposition to serve as a fluorescence intensity reference. β -D-glucose (MP Biomedicals, Inc.), oxygen and nitrogen gas (PraxAir), and PBS (Sigma) were used during dynamic testing. All necessary pH adjustments were performed using titrations of 1.0 M HCl and 1.0 M NaOH (Fluka). All chemicals listed above were reagent grade and used as

received. Ultrapure water with a resistivity greater than 18 M Ω -cm was used to prepare all aqueous solutions. All experiments were conducted at ~25 °C and the oxygen concentration in the glucose and buffer reservoirs was maintained at 277 μ M (air saturated) by aeration.

3.3. Sensor preparation and characterization

Hybrid alginate-silica “algilica” microspheres were synthesized using a protocol detailed elsewhere (Stein et al. 2007). Dried microspheres of both algilica and silica were sent to an external lab for surface area and pore size analysis (Delta Lab, North Huntingdon, PA). A particle size analyzer (ElZone 540, Micromeritics®) was used to measure the concentration and size distribution of the microspheres. PtOEP, which is extremely hydrophobic, was immobilized within algilica microspheres using insolubility-induced precipitation. Subsequently, GOx was electrostatically absorbed into the PtOEP-doped alginate matrix, following which amines of GOx were covalently attached to algilica using standard EDC/NHSS coupling. Finally, PtOEP- and GOx-doped microspheres were coated with nanofilms comprising [PAH-RITC/PSS]₂-[PAH/PSS]₂₃-PAH NFs using LbL technique (Stein et al. 2008).

To prepare glucose sensors using amine-functionalized silica microspheres, particles were suspended in a phosphate-buffered solution (pH = 9.0) containing 50 mg/mL of both EDC and NHSS. PtP in DMSO was added to a final concentration of 50 μ M and the mixture was vortexed for ~2 hours. Dye-labeled microspheres were rinsed twice in deionized (DI) water, and then suspended in EDC/NHSS solution (50 mg/mL) in acetate buffer (pH = 5) to activate the free carboxylate moieties of PtP. Subsequently, GOx (35 mg/mL) dissolved in bicarbonate buffer was added to the dye-labeled microspheres and the solution was vortexed for one hour, which led to the coupling of amines of GOx to the activated carboxylate moieties of PtP. Finally, the microspheres were rinsed with DI water and coated with [PAH-RITC/PSS]₂-[PAH/PSS]₂₃-PAH NFs using the LbL technique (Stein et al. 2008). Thus, the two batches of sensors to be compared were comprised of slightly different sizes of microspheres, significantly different porosity, and identical nanofilm coatings.

Separate batches of indicator (i.e., PtOEP or PtP) and GOx-loaded algilica and silica microspheres were prepared without NF coating to determine the concentration of immobilized GOx using BCA protein assay (Smith et al. 1985). In addition, the distribution of GOx and indicator dye in the microspheres was determined via confocal microscopy (Leica TCS SP5) using a 63X oil objective (N.A=1.4) and pinhole setting of 1 Airy.

The sensor response was experimentally determined using a flow-through system controlled via a custom-designed software suite (LabVIEW, National Instruments) described previously (Stein et al. 2007). Briefly, ~10⁷ microspheres were immobilized using double-sided pressure-sensitive adhesive attached to a glass slide, which was mounted inside a reaction chamber made from Delrin. A green LED with emission peak at ~518 nm was used as an excitation light source. A bifurcated optical fiber bundle was coupled into the bottom of the reaction chamber to excite the sensors and collect the emitted fluorescence. Fluorescence signals were monitored using a charge-coupled device array detector (USB 2000, OceanOptics). Emission intensity was recorded at 580 and 645 nm, corresponding to the emission maxima for RITC and PtOEP, respectively. The reaction chamber was placed inside an incubator at 25°C and all solutions were prepared in phosphate-buffered saline (PBS) to maintain physiological conditions.

The procedures used to convert experimental measurements of intensity versus time to a response profile, from which sensitivity and range may be calculated, have also been detailed elsewhere (Stein et al. 2008). Sensitivity and linear range were estimated similarly for the theoretically predicted data.

4. Results and Discussion

Particle characterization revealed approximately uniform distribution of PtP and RITC-GOx in the silica microspheres, similar to what was previously observed for PtOEP and GOx in algilica microspheres (Stein et al. 2007). The average diameter of porous silica microspheres was determined to be $7\pm0.5\text{ }\mu\text{m}$, and the concentration of immobilized GOx was found to be 1 mM. The average size and porosity of algilica microspheres was determined to be $12\pm3\text{ }\mu\text{m}$ and 0.005 via electric sensing zone and BET methods, respectively, and the concentration of GOx immobilized in algilica microspheres was determined to be 0.2 mM. These values were used in modeling the response of specific sensors.

It is important to consider that algilica and the amine-modified silica microspheres may have different hydrophobicities, which could affect the effective transport of oxygen and glucose within the microspheres and thereby affect the response of such sensors. However, after the immobilization of GOx within the pores and on the surface of such microspheres, we expect both matrices to be hydrophilic, since hydrophobicity/hydrophilicity is a surface characteristic. Therefore, as most of the surface in both matrices is covered with GOx, we do not expect a significant difference between the hydrophobicity/hydrophilicity of the two matrices.

Another noteworthy point is that the modeling used here predicted the response of monodisperse (uniform, single size) microsphere sensors, whereas in reality both types of particles used exhibit some polydispersity. However, we note that the size (diameter) distribution for both types of microspheres is normal, and we have confirmed from additional modeling with particles of the same size range (10 of microns) that the response predicted for a single microsphere with size equal to the number average of a normal distribution is equivalent to the weighted response estimated for microspheres of different sizes sampled from a normal distribution with the same mean. Therefore, the number average size of such distributions (12 and $7\text{ }\mu\text{m}$ for algilica and porous silica) was used to predict the response of two types of sensors.

Figure 3 (top) contains a graph of the theoretically-predicted response of $12\text{ }\mu\text{m}$ microsphere sensors, with varying microsphere porosity, coated with 65 nm thick NFs. The predicted linear response range for microspheres with porosity of 0.001, 0.005, and 0.01 was estimated to be *ca.* 60, 150, and 225 mg/dL, respectively. The microspheres with porosity higher than 0.1 were predicted to exhibit a linear response in the entire physiological glucose range (0–600 mg/dL). The increased porosity results in an expected increase in the response range accompanied by reduced sensitivity, a trend attributed to increasing oxygen transport. Algilica microspheres, for which the average porosity was experimentally determined to be 0.005, are expected to exhibit a linear response only up to approximately 200 mg/dL when PSS/PAH NFs are employed. In contrast, the improved oxygen transport expected for microspheres of the same size with higher porosities yields a predicted linear response over a much wider range—up to 600 mg/dL. It is evident, though, that the penalty for achieving a higher range is greatly reduced overall sensitivity. The ratio of sensitivities of sensors made from microspheres of porosity 0.005 to the sensors made from microspheres of porosity 0.6 is approximately 18. These data lead to the inference that microspheres with porosity somewhere in between 0.01 and 0.1 can be used to make sensors that exhibit linear response up to 600 mg/dL with maximized sensitivity.

The effects of varying microsphere size on the sensor response, assuming microsphere porosity and NF thickness are held constant at 0.005 and 65 nm, respectively, are shown in Figure 3 (bottom). With other properties held constant, increased particle size is expected to result in an improved sensitivity, as well as an increased response range. The predicted

linear response range for microspheres with 6, 12, and 24 μm diameter was estimated to be *ca.* 125, 150, and 230 mg/dL, respectively. In smaller particles, lower consumption of oxygen and glucose is expected; therefore, it is reasonable to expect an increase in sensitivity with increasing size of the microspheres, as sensitivity is directly proportional to the difference between the average oxygen concentration in the bulk and inside the microspheres. In addition, the increase in particle size results in an increase in net transport resistance to glucose relative to oxygen (Table S1), which further leads to an increased response range. Using identical materials and coatings, the sensitivity of sensors comprising 12 μm spheres is estimated to be approximately 1.5X of the sensors comprising of 6 μm spheres.

It can also be observed that the shape of the response profile transforms from an inverse exponential to a sigmoid as the size of the sphere increases. We infer that the shape of the response profile depends on the Thiele modulus, which is the ratio of potential reaction rate to the potential mass transport rate (Rosen 1976). The same behavior is observed with an increase in the concentration of immobilized GOx (Singh 2010). With an increase in GOx concentration, potential reaction rate increases, whereas the potential diffusion rate remains constant, resulting in an increase in the Thiele modulus. For increasing particle size, there is a decrease in the potential mass transport rate with an increase in the sphere size, and the potential reaction rate remains constant due to fixed immobilized GOx concentration. Therefore, with everything else held constant, Thiele modulus increases with increasing sphere size. We infer that there is a certain threshold value for the Thiele modulus, below which the response profile is inverse exponential; once the Thiele modulus exceeds this critical value the response profile becomes sigmoidal.

Figure 4 (top) contains the theoretically-predicted response of our prototype algilica (diameter = 12 μm , porosity = 0.005) and silica (diameter = 7 μm , porosity = 0.6) microsphere sensors. The linear response range of algilica-based sensors was predicted to be *ca.* 150 mg/dL, whereas porous silica-based sensors were predicted to exhibit a linear response up to 600 mg/dL. From these predictions, the algilica-based sensors are expected to exhibit a response range of approximately 200 mg/dL, while the porous silica-based sensors are predicted to demonstrate a highly linear response even up to 600 mg/dL. However, the estimated sensitivity of porous silica-based sensors is also approximately 24 times lower than the sensitivity of algilica-based sensors.

Figure 4 (bottom) is a graph of the experimentally-determined response profile of both sensor types. The linear response range for algilica-based sensors was determined to be *ca.* 125 mg/dL, and silica-based sensors were found to exhibit a linear response up to 600 mg/dL ($R^2 > 0.90$). The trends in range and sensitivity match very well with the theoretical predictions (top); however, the absolute values for experimental sensitivity were higher than those predicted from modeling. This discrepancy is explained in part by the experimental measurements being performed on an entire population of sensors which are in close proximity to one another. In this situation, the sensors themselves impose a barrier to the supply of oxygen, and the sensors buried deep within the population see reduced oxygen levels in comparison to the sensor that are located on the surface. Furthermore, when glucose flux is high, oxygen is depleted in the area adjacent to the particles, such that closely-packed particles do not act independently. Therefore, in reality, some of the particles contributing signals in the measurements are exposed to average oxygen levels lower than 277 μM . Since that level was used as the surface boundary condition in the modeling, the actual depletion of oxygen is expected to be even higher than the depletion predicted via modeling. This behavior has been confirmed via modeling using arrays with small numbers of particles. However, while the magnitudes did not agree, the shapes of the theoretical and experimental curves match closely. The key point is that the experimental response

determined for sensors made from highly porous silica microspheres exhibited a linear correlation with glucose ($R^2 > 0.90$) up to 600 mg/dL, which was predicted via modeling. To improve the sensitivity, larger particles with slightly higher porosity may be used.

5. Conclusion

We have found that, in addition to changing the properties of transport-controlling coatings (Brown and McShane 2006; Stein et al. 2007; Stein et al. 2008), the porosity and dimensions of the matrix can also be varied to tailor the response range and sensitivity of enzymatic glucose sensors. These provide additional options for sensor fabrication, because surface-functionalized microspheres with user-specified size distribution, porosity, and pore volume can be produced using standard sol-gel methods (Katiyar et al. 2006). From the modeling we predict that sensors constructed from $\sim 12 \mu\text{m}$ microspheres having an effective porosity between 0.01 and 0.1 with $\sim 65 \text{ nm}$ PAH/PSS LbL coatings would respond over the entire physiological glucose range with maximized sensitivity.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.

Semi-infinite plate model (2D) for a low-porosity matrix (porosity = 0.005) of thickness, d_2 , with PAH/PSS NF of thickness, d_1 , on its left side (left). Semi-infinite plate model for a high porosity matrix (porosity=0.6) with similar dimensions and NF on its left side (right). White space represents voids/pores in the matrix through which diffusion can occur. No transport can occur through the solid blocks.

Figure 2.
Schematic of microsphere sensors with dimensions used in the model.

Figure 3. Theoretical predictions on the effect of porosity on the response of 12 μm particles (top), and on the effect of particle size on the response of microspheres with average porosity of 0.005 (bottom).

Figure 4. Theoretical (top) and experimental (bottom) response of algilica and porous silica microspheres coated with 65 nm PAH/PSS NF. The error bars indicate one standard deviation obtained from three replicate measurements at different time points. Note that the response of silica-based sensors has been scaled by a factor of seven to more clearly show the profile on the same scale as the larger particles.