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Determining Surface Plasmon Resonance Response Factors for Deposition onto Three-Dimensional Surfaces

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Abstract

Intrinsic sorption rates of ligand/receptor binding have been measured by surface plasmon resonance (SPR) using response factors for deposition of proteins or smaller molecules on planar surfaces. In this study generalized expressions for SPR response factor and effective refractive index are developed to measure rates of analyte sorption onto 3-D surfaces. The expressions are specialized for two limiting cases of immediate practical interest and broad applicability: analyte deposition onto a homogeneous anisotropic porous media and deposition onto close-packed solid spheres adjacent to the sensor surface. These new equations specify media capacity, characteristic size and analyte concentration that are necessary to obtain identifiable responses from interaction with anisotropic porous media or chromatographic resin. These developments are illustrated by comparing response factors for Adenovirus Type 5 on planar surfaces, porous media and adsorptive spheres.

Keywords

Response factor; adenovirus; surface plasmon resonance; porous media

1. Introduction

Adsorption of complex molecular structures such as protein or virus is important to developing biosensors, biocompatible materials, bioseparations, medical devices and pharmaceuticals (Roper and Lightfoot, 1995; Shabram et al., 1997; Lee et al., 1999; Abad et al., 2002; Yang and Etzel, 2003). Measuring macromolecular adsorption rates can lead to optimization of surface structures, charge and chemistries of novel resins, membranes or biomaterials (Faegerstam et al., 1992; Morrill et al., 2003; Howerton and McGuffin, 2004). Surface plasmon resonance (SPR) quantitatively measures adsorption rates (Power et al., 1998; Kim et al., 2002; Roy et al., 2002; Choe et al., 2003) as does Total Internal Reflectance Fluorescence (Lok et al., 1983; Ho et al., 1996; Buijs and Hlady, 1997; Britt et al., 1998), Quartz Crystal Microbalance/Dissipation (Murray and Cros, 1998), calorimetry (Morin and Freire, 1991; Todd and Gomez, 2001), ellipsometry (Nasir and McGuire, 1998), voltammetry (Rouhana et al., 1997; Giacomelli et al., 1999) and nuclear magnetic resonance spectroscopy (Scholten, 1990). SPR has the advantage of providing direct, unambiguous measurements to evaluate effects of binding site and concentration (Rich and Myszk, 2002) down to subnanomolar analyte levels without using labels or secondary reagents for signal enhancement (Roper and Purdom, 2004). Intrinsic sorption rates measured by SPR provide information about specificity, affinity and kinetics.

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Intrinsic sorption rates are measured by fitting two-compartment or effective rate models to SPR sensorgrams. Sensorgrams are obtained using response factors that relate shifts in wavelength or angle of the minimum in reflected light intensity to time-dependent changes in volume fractions and refractive indices of analyte, sorptive surface and solvent (Jung et al., 1998). Regressing SPR binding curves against bulk concentration data to estimate binding constants underestimates intrinsic sorption rates when diffusive mass transfer is relatively slow (Lok, 1983; Yarmush, 1997) as is the case for large structures like Ad5 that have low molecular diffusivities. Binding constants between influenza virus and lipid membranes measured by SPR did not distinguish intrinsic sorptive rates from virus diffusion (Critchley 2004).

Response factors and intrinsic sorption rates have only been determined by SPR for small-molecule interactions with planar surfaces that lack heterogeneity or geometric effects present in commercial 3-D materials (Rich and Myszk, 2003). The planar SPR surfaces used have been derivatized with receptors at concentrations low enough to prevent slow mass transfer from masking sorption rates (Rich and Myszk, 2002). Examples of sorption rate measurements on planar surfaces include: (1) affinity interaction between human papillomavirus major capsid protein and nickel immobilized on an expanded bed adsorbent measured by SPR was used to screen buffer conditions to maximize capacity and identify imadazole eluent content that optimized purity and yield (Choe et al., 2003); (2) interactions between bovine serum albumin and reversed-phase surfaces prepared on silver using self-assembled and mixed self-assembled monolayers of n-alkylthiols measured by SPR showed optimum alkyl chain length (C2 to C18) and buffer composition to adsorb BSA (Power et al., 1998); and (3) non-specific adsorption of human serum albumin to thin poly(vinyl alcohol) (PVA) films prepared on nonporous, poly(styrene) support matrices determined by SPR correlated to the degree of surface coverage which was influenced by concentration, molecular weight, and degree of hydrolysis of the PVA (Barrett et al., 2001). Moghimi et al. (2003) monitored protein-poloxamine accumulation on three-dimensional 95-nm polystyrene nanoparticle interfaces using SPR, but did not estimate rate constants. Intrinsic sorption rates and response factors for complex molecular structures larger than proteins, sorption onto 3-D synthetic surfaces, and sorption onto high-capacity surfaces have not been determined.

Intrinsic sorption rates measured on uniform planar surfaces do not unambiguously extend to heterogeneous structures or 3-D geometries. For example, sorption rates measured with a standard SPR cell were 1.2 to 5.8 times higher than estimates from chromatogram fits (Morrill et al., 2003). Differences may have arisen from misattributed phase partitioning in chromatogram fits or innate mass-transfer rate differences between protein sorption on ideal planar surfaces compared to sorption in chromatographic resin. Response factors for analyte interactions with 3-D surfaces could allow fast estimates of interaction rates for these systems with minimal amounts of analyte. Protein equilibrium constants can be measured on planar SPR systems up to 15 times faster using 130-fold less protein by SPR than by fitting chromatographic profiles (Morrill et al., 2003).

The purpose of this study is to develop and analyze generalized expressions for SPR response factor and effective refractive index for discontinuous distributions of analyte that accurately reflect deposition in three-dimensional systems for all planar Kretschmann-type devices. Without these expressions, sorption rates of complex molecular structures directly on most 3-D adsorptive surfaces cannot be accurately interpreted. Existing expressions are based on continuous refractive-index distributions which misattribute thermodynamic analyte partitioning to system-specific SPR sensitivity. Specific equations are derived for two limiting cases of immediate practical interest and broad applicability to biomolecular adsorption of protein or virus: (1) analyte deposition onto a homogeneous anisotropic porous media; and (2) analyte deposition onto close-packed solid spheres adjacent to the sensor surface. Nearly all adsorptive media used to analyze, recover or purify complex biomolecular structures can be

classified as close-packed solid spheres (Coffman et al., 1994) or homogeneous anisotropic porous media (Roper and Lightfoot, 1995). A response factor for planar adsorption is used to analyze deposition of Adenovirus Type 5 onto a planar surface. The new expressions are applied to calculate media capacity, resin size and analyte concentrations required to obtain an SPR response from adsorption of two widely-varying complex biomolecular structures onto anisotropic porous media or a chromatographic resin.

2. Materials and Methods

2.1. Cell Culture and Propagation

Methods for cell culture and adenovirus infection and propagation were adapted from those published by Graham and Prevec (1995). All chemicals were from Sigma (St. Louis, MO, USA) unless otherwise noted. Briefly, human embryonic kidney cells (P/N 293 HEK; ATCC, Rockville, MD) at a concentration of 1×10^6 cells/mL were inoculated in 5 mL of Dulbecco's Modified Eagles Medium. The medium was supplemented with 0.1 g/L alanine, 0.110 g/L sodium pyruvate, 1 g/L glucose, 0.584 g/L L-glutamine, 37 g/L sodium bicarbonate and 10 mL/L Penicillin (10,000 units/mL)-Streptomycin (10,000 ug/mL)-Glutamine (29.2 mg/mL) (100 $\times$) from Gibco (Auckland, N.Z.), pH 7.8. Cells were incubated in T-flasks from Corning (Corning, NY, USA) at 37°C and 5% CO₂ for 48–96 hours. Flasks with cells at 90% confluence were either split 1:5 into additional T-flasks to propagate the cell line or infected with Ad5.

Cells at 90% confluence were infected with Ad5 by adding a 1:100 dilution of Ad5 from ATCC (Rockville, MD, USA) to the T-flasks. Infected flasks were incubated 1 hr at 37°C and 5% CO₂, then supplemented with additional culture medium and incubated again. The cytopathic effect was observed after 48–72 hours and virus was harvested by agitating T-flasks to resuspend infected cells. Cells suspended in 50 mL polypropylene tubes were centrifuged (Sorvall RTH-750 rotor; 3,000 $\times$ g; 5 min) to separate cells from supernatant. Supernatant was removed and combined with 10% glycerol before storage at –70°C for future infection. Recovered cell pellets were resuspended in equal volumes of Tris buffer, pH 7.8, + 1 mM CsCl and frozen at –70°C then thawed (repeated three times) to release Ad5. Cell debris was centrifuged out at previous conditions and supernatants were treated with 150 units/mL Benzonase™ Nuclease for 30 minutes at 25°C to digest nucleic acid. Digested supernatants were ultracentrifuged (Beckman SW28 rotor; 20,000 rpm; 12 hrs) atop light (1.2 g/mL) and heavy (1.45 g/mL) CsCl bands to purify viral capsids.

2.2. Adenovirus Chromatography

The method for Ad5 chromatography was adapted from Shabram, et al (1997). Briefly, Resource Q™ is an anion exchange resin with terminal quaternary ammonium anion groups (Amersham Biosciences, Piscataway NJ, USA). Primary capsid proteins of Ad5 -- hexon, penton, fiber -- have isoelectric points <6.0, yielding a net negative charge on the capsid at physiological pH. CsCl-banded Ad5 adsorbed onto Resource Q™ was eluted in 10 mM HEPES buffer using a NaCl gradient from 40 to 600 mM at 1 mL/min. Peak detection was at A₂₅₄. Residual nucleic acid and viral or cell protein were undetectable by HPLC or spectrophotometric methods (Roitsch et al., 2001). Resource Q™ chromatography has a static capacity of $\sim 5 \times 10^{11}$ virus per milliliter, a detection limit $> 1 \times 10^8$ particles per milliliter, and a linear Ad5 virus particle/HPLC area ratio of 20,085 (Shabram et al., 1997).

2.3. Surface Plasmon Resonance Derivatization

Derivatization of the planar gold sensor with 11-mercaptopundecanoic acid (MUDA) and N,N-diethylethylenediamine (DEEDA) was adapted from protocols in previously published methods (Mrksich and Whitesides, 1996; Li et al., 2003). Chemicals were obtained from Sigma (St. Louis, MO, USA) unless otherwise noted. Briefly, the gold sensor surface was calibrated

to $\eta_{H_2O} = 1.333$ in 18 M Ω distilled, deionized water (Millipore Corp, Bedford, MA, USA). The surface was cleaned with 0.1 M NaOH and 1% Triton, then equilibrated in 10 mM MES buffer, pH 5.0 (3.0 mL/h). It was then equilibrated in degassed ethanol and exposed to 2.0 mM MUDA in degassed ethanol until refractive index stabilized (6–24 hrs; 0.1 mL/h). The self-assembled monolayer of MUDA was rinsed with ethanol (0.6 mL/h), then 10 mM MES buffer, pH 5.0, (3.0 mL/h) until refractive index stabilized. Freshly prepared solutions of 0.4 M N'-(ethylcarbonimidoyl)-N,N-dimethylpropane-1,3-diamine hydrochloride (EDAC) and 1.0 M N-hydroxysuccinimide (NHS) in 10 mM MES were mixed 1:1 and injected for 20 minutes (3.0 mL/h) to prepare the terminal carboxyl group for amide bond formation. 1.0 M DEEDA in 0.01 M MES buffer, pH 5, was added for 2 hrs (3.0 mL/h). The surface was rinsed first with 10 mM MES buffer, pH 5, then with 0.01 M MES buffer, pH 6.7 (6.0 mL/h).

2.4. Surface Plasmon Resonance Measurements

A surface plasmon resonance (SPR) integrated flow cell from Nomadics, Inc (Stillwater, OK, USA) was attached to a syringe pump from Cole Parmer, Inc (Vernon Hill, IL, USA), via a manual polyethyletherketone (PEEK) injection valve from Upchurch, Inc (Oak Harbor, WA, USA) using PEEK tubing and fittings. All binding assays were carried out at 25°C. Refractive index baselines were established by equilibrating the surface using a 10 mM HEPES, pH 7.5 running buffer with NaCl added at 14.4 mM. Ad5 analyte was diluted into the running buffer. At time zero, 1.6 pM Ad5 analyte in 10 mM HEPES pH 7.5 at 14.4 mM NaCl was injected onto the SPR system at 300 μ L/min by switching the injection valve to a second syringe pump for 5 minutes. The surface was then washed with running buffer for an equivalent period to evaluate dissociation of the analyte. The surface was regenerated to baseline response unit (RU) values between injections using 2 M NaCl followed by 0.04 M sodium dodecyl sulfate (SDS) (Roper and Nakra, 2006).

2.5. Estimating Response Factors for Ad5

Physical properties of Ad5 capsid ($r \sim 40$ nm; $MW = 1.65 \times 10^8$ Da) were applied to estimate maximum surface coverage, Q_{max} , of 54.4 ng Ad5 per mm^2 corresponding to an initial surface active site concentration, $R_T = 3.3 \times 10^{-12}$ moles Ad5 per dm^2 . The SPR response factor for Ad5 was determined as shown in Eq. 3, using the conversion factor of 10^6 plasmon resonant response units (RU) per refractive index unit (RIU) (Myszka et al., 1998). Substituting refractive index values of $\eta_A = 1.57$ for pure protein and $\eta_s = 1.333$ for water in Eq 3 gives an R_f value of 2.2×10^{10} RU per gram adenovirus per cm^2 .

2.6 SPR response factors relate to refractive index of analyte, sorptive surface and solute

Jung et al. (1998) established a quantitative formalism consistent with Maxwell's equations that relates SPR response to adsorbate refractive index, film thickness and coverage on planar SPR surfaces. This formalism is valid for SPR measurements in all systems that use the Kretschmann geometry. It includes a system-specific sensitivity factor that is evaluated separately for different planar configurations. The formalism considers nonuniform 1-D coverage by analytes much smaller than the exponentially decaying evanescent field length, but SPR responses to adsorption on 3-D surfaces or to discontinuous distribution of analyte perpendicular to a planar SPR surface were not discussed. Ramsden et al. (1994) used a similar formalism to evaluate optical waveguide sensor response due to adsorbed cells of complex shapes. The present study extends the quantitative formalism to characterize SPR response from adsorption on 3-D structures and from discontinuous distribution of analyte perpendicular to the planar SPR surface.

Elements of the formalism are briefly reviewed to provide structure for the development. SPR response, R_f , occurs as a shift in wavelength or angle of the minimum in reflected light intensity

that corresponds to a time-dependent change in effective refractive index of the medium adjacent to the metal sensor surface, $\eta_{\text{eff}}(t)$ relative to an initial (solvent) refractive index, η_s :

$$R_f(t) = m_1(\eta_{\text{eff}}(t) - \eta_s) + m_2(\eta_{\text{eff}}(t) - \eta_s)^2 \quad (1)$$

where m_1 is a system-dependent sensitivity factor that varies from ~ 3100 to 8800 nm/RIU for different types of commercially-available or home-built planar, Kretschmann-type SPR spectrometers and m_2 is a fitted coefficient for a quadratic term which is negligible for small changes in refractive index.

The effective refractive index, $\eta_{\text{eff}}(t)$, in Eq. 1 consists of refractive indices of adsorbing analyte, η_A , and solvent, η_s in the sensing region weighted by changing surface coverage, $\theta(t)$, and term arising from the SPR signal source. SPR signal is generated by an evanescent electromagnetic field that decays away exponentially into the medium in the z -direction perpendicular to the sensor surface and exhibits a characteristic decay length, l_d . The measured refractive index perpendicular to the sensor surface is therefore also weighted by the light intensity, or square of the field strength: $\exp(-2z/l_d)$. The effective index of refraction is then determined by integrating the intensity-weighted refractive index over the depth of the interrogated field,

$$\eta_{\text{eff}}(t) = (2/l_d) \int_0^\infty \eta(t, z) \exp(-2z/l_d) dz \quad (2)$$

Jung et al. (1998) analyzed only continuous Heaviside functions of $\eta(t, z)$, such as monolayer adsorption of analyte, A , with radius r on a planar surface at a particular time. Planar monolayer adsorption yields a bilayer refractive index: $\eta(z) = \eta_A$ for $0 \leq z \leq 2r$ and $\eta(z) = \eta_s$ for $z > 2r$, that is, for distances greater than analyte diameter. This expression for the bilayer refractive index may be substituted into Eq. 2 and the result substituted into Eq. 1 to obtain the SPR response:

$$R_f = m_1(\eta_A - \eta_s)[1 - \exp(-4r/l_d)] \quad (3)$$

The system-dependent sensitivity factor, m_1 , in Eq. 3 may be calibrated for different instruments or analytes. In one Kretschmann-type device it was evaluated by scaling a conversion factor of 10^6 SPR response units (RU) per refractive index unit (RIU) by the maximum surface coverage, Q_{max} , to give $10^6/Q_{\text{max}}$ (Chinowsky et al., 2003). Another Kretschmann-type device uses the product of two values to represent sensitivity: a factor of 1000 RU per angle change of $\sim 0.1^\circ$ and a factor for unit angle change per RIU (Biacore, 2004). This product may be similarly calibrated by maximum surface coverage of a particular analyte.

Eqn 3 allows the analyte thickness, $2r$, for *continuous layers* to be estimated using experimental values of SPR response, R_f , and estimates of sensitivity factor m_1 , characteristic decay length l_d , and refractive indices for analyte and solvent, respectively. We now proceed to derive more general expressions that allow *discontinuous* distributions of heterogeneous refractive index to be analyzed via SPR.

3. Results and Discussion

3.1. Estimating intrinsic sorption rates with SPR using models for adsorption

Accurate values of intrinsic sorption rates are obtained by fitting SPR sensorgrams to two-compartment (Myszka et al., 1998) or effective rate (Goldstein et al., 1999; Mason et al., 1999) models that distinguish intrinsic sorption rates from diffusive mass transport of analyte to the sensor surface. A sensorgram plots the SPR response versus the time of exposure to an analyte suspension or solution. The SPR response corresponds to a shift in wavelength or angle of the minimum in reflected light intensity that has been related to a time-dependent change in effective refractive index and volume fraction of the medium adjacent to the metal sensor surface by a response factor.

Fig. 1a relates a sensorgram and SPR response to a two-compartment model of adsorption in an SPR sensor cell. Briefly, in an open channel, analyte at uniform concentration C_T in an outer compartment flows at a rate F parallel to the sensor surface. A uniform analyte concentration C in an inner compartment increases at a rate k_m due to mass transfer from the outer compartment and at a rate k_r due to desorption from the sensor surface. Simultaneously, it also decreases at a rate k_f due to adsorption to available sites on the surface. The intensity of light reflected at a specific angle from a conducting gold film adjacent to the flow channel is decreased by resonant interaction with delocalized, coherently oscillating surface plasmons, forming a minimum. The angle at which the minimum, or “dip” occurs shifts in proportion to the change in refractive index adjacent to the glass surface as analyte binds in the shaded cuboid sensor region at concentration Q . Graphing the shift in angle due to analyte sorption over time yields the SPR sensorgram.

Sensorgrams give an average measure of concentration of bound analyte over the length and width of the flow-cell sensor surface. Fitting partial differential equations derived from the continuity equation to the sensorgram data is impractical because spatial variations in concentration are not captured.

Accurate intrinsic sorption rate constants can be estimated from fitting two-compartment or effective rate models to SPR sensorgrams if the underlying data conform to two implicit model criteria. First, SPR responses must be accurately related to effective volume fractions and refractive indices of analyte, sorptive surface and solvent, respectively, distributed normal to the surface within the cuboid sensor region (Ramsden et al., 1994). Second, aggregate concentrations of free and bound analyte normal to the sensor surface within the cuboid sensor region must be uniform. Otherwise, effective rate coefficients that account for boundary layer height relative to mean free path are required (Wofsy and Goldstein, 2002).

3.2. Accounting for interaction of SPR signal decay and discontinuous distal adsorption on 3-D surfaces

We now extend the formalism relating SPR response to parameters of planar SPR surfaces reviewed in Methods Section 2.3 to analyte adsorption on 3-D surfaces within the sensing region and discontinuous distribution of analyte normal to the sensor surface. As with Eqs 1–3, the new expressions that follow are valid for SPR measurements in all systems that use the Kretschmann configuration. A system-dependent sensitivity coefficient, m_1 , which is a parameter within the response factor, is determined independently by calibrating different Kretschmann-type systems. In any plane parallel to the sensor surface at distance z within the cuboid sensing region, contributions to spatially varying refractive index, $\eta(t, z)$, from analyte, $\eta_A(z)$, solid particles, $\eta_p(z)$ or solvent, $\eta_s(z)$, are proportional to the area fraction of analyte, $\theta(t, z)$, solid $\Phi(t, z)$ or solvent $(1 - \theta(t, z) - \Phi(t, z))$ in that plane, respectively, viz:

$$\eta(t, z) = \eta_A(z)\theta(t, z) + \eta_P(z)\Phi(t, z) + (1 - \theta(t, z) - \Phi(t, z))\eta_S(z) \quad (4)$$

Discretizing $\eta(t, z)$ into planar coordinates x and y is inordinate unless performing SPR imaging, since SPR response constitutes an average obtained from the cuboid sensing region. Refractive index variations in x and y dimensions are projected onto the z -axis in Eq 4 to be consistent with the x - and y - averaging in the physical system. Using the present approach, Eq 4 permits *discontinuous* functions of $\eta(t, z)$ normal to the sensor surface to be analyzed, for the first time. This will be illustrated in the cases that follow.

The time-varying expression in Eq 4 may be substituted into Eq 2 to obtain the measurable effective refractive index of the cuboid sensing region relative to an initial refractive index that consists of *either* solvent or solid, *or* both solvent and solid. The refractive index change is then inserted into Eq 3 to relate SPR responses to area fractions and refractive indices of analyte, sorptive solid and solvent, respectively, in 3-D analyte distribution and absorption. Two cases of immediate practical interest are examined: (1) analyte deposition onto a homogeneous anisotropic porous adsorbent adjacent to the sensor surface; and (2) analyte deposition onto close-packed solid spheres adjacent to the sensor surface. Response factors from these cases are applicable to a variety of porous media that may be used to obtain SPR adsorption-rate measurements. Porous membranes formed by sintering granular polymer beads, for example, form layers of micron-scale particles arranged homogeneously at the membrane surface.

Development of these two cases neglects weak variations in decay length due to local changes in $\eta_{\text{eff}}(t)$ and nonidealities in SPR response related to incorporating media such as increased dip-angle width and secondary minima. Dip-angle width is minimized and secondary minima eliminated by forming a homogeneous, close-packed solid structure that also enhances minimum percent reflectivity (Davies and Faulker, 1996). Homogeneous deposition of monodisperse polystyrene spheres on a sensor has produced minor surface roughness and unimodal SPR profiles (Moghimi et al., 2003). Variations in decay length can also be minimized by homogeneous deposition, small surface coverages or adlayer thicknesses, or matched refractive index values of solid and solvent (Jung et al., 1998).

Case I: Analyte deposition onto a homogeneous anisotropic porous media with solids fraction Φ , total capacity R_{tot} , and uniform boundary layer fluid-phase concentration—Total capacity is calculated as $R_{\text{tot}} = R\delta$, where R and δ represent effective volumetric receptor concentration and receptor layer thickness, respectively. To obtain uniform boundary layer fluid-phase concentration, diffusion time to the surface must be less than diffusion time to adsorptive sites. Methods to achieve uniform boundary layer concentration are considered elsewhere (Roper 2004). In this case, any plane a vertical distance, z , from the sensor surface has analyte area $\theta(z, t)Lb$ and solids area $\Phi(z)Lb$, where L and b are sensor length and width, respectively, as shown in Fig 1a. The factor $\theta(z, t)$ corresponds to the probability that analyte adsorbs at vertical distance z and time t . The fraction of fixed, homogeneous solid media in this case is time-invariant. For homogeneous anisotropic porous media, solid area on every plane may be projected onto a sub-plane of area ΦLb , Φ being independent of z . Similarly, analyte adsorbing on every plane at any moment from inception to equilibrium may be projected completely onto a sub-plane of area $\theta(t)Lb$. The effective refractive index is then the sum of each of these projected areas weighted by their respective refractive indices and by the exponentially decaying light intensity. Substitution into Eq. 4 and rearranging gives:

$$\eta_{\text{eff}} - \eta_S = (\eta_A - \eta_S)\theta(t) + (\eta_P - \eta_S)\Phi \quad (5)$$

after integrating the exponential weight factor from $z=0$ at the sensor surface to $z=\infty$ to get $l_d/2$, since Φ and $\theta(t)$ are independent of z .

The SPR signal is then proportional to the appropriate refractive-index difference: either $(\eta_{\text{eff}} - \eta_s)$ after depositing the homogeneous anisotropic porous media onto a clean gold sensor surface with $\theta(t)=0$; or $(\eta_{\text{eff}} - \eta_s - (\eta_p - \eta_s)\Phi)$ after exposing analyte to the porous media adjacent to the gold sensor surface. The latter result yields

$$R_f(t) = m_1(\eta_A - \eta_{H_2O})\theta(t) \quad (6)$$

The SPR signal in Eq. 6 is proportional to the uniform partition coefficient $\theta(t)$ and is not decreased by $[1 - \exp(-2d/l_d)]$ as was the case in Eq. 3 for adsorption directly onto a planar, possibly derivatized, gold sensor surface. This expression is applicable to ligand interacting with receptors derivatized onto polymer matrices when analyte diffusion time to surface is less than its diffusion time to an adsorptive site on the polymer. Eq. 6 reveals that the physical basis for improved SPR response reported for analyte sorption onto polymer matrices on SPR sensor surfaces is a thermodynamic, time-dependent analyte partitioning within the interrogated zone, which is often misattributed to improved system-specific sensitivity, m_1 . Analyte area fraction $\theta(z,t)$ may be related geometrically to solids area fraction $\Phi(z)$ as will be shown in Case II.

Case II: Analyte of radius r deposited from a uniform boundary layer fluid-phase concentration onto solid spheres of radius R that are hexagonally close-packed (HCP) onto the surface—Area void at the radius of the HCP spheres is $\varepsilon = 0.0931$. A uniform boundary-layer fluid-phase concentration implies diffusion time to the surface is less than diffusion time to sphere adsorptive sites. Fig. 1b illustrates Case II, where analyte deposition onto sensor area Lb is prevented, for example, by an electrostatically-repulsive SAM. Define dimensionless distance $\chi = z/R$ normal to the sensor surface, dimensionless analyte-to-adsorbent ratio $\zeta = 2r/R$ and dimensionless weighting factor $v = 2R/l_d$. Geometrical considerations show that for small ζ and small $\zeta v/4$, any plane a distance χ from the sensor surface has analyte area fraction $\theta(\chi,t) = (1 - \varepsilon)\zeta(2 + \zeta)f(t)$ and solids area fraction $\Phi(\chi) = (1 - \varepsilon)(2\chi - \chi^2)$. Time-dependent analyte deposition, $f(t)$ varies from 0 to approximately the hard-sphere random sequential adsorption jamming limit of 0.546 (Viot et al., 1992). Substituting these terms into Eq. 4 and Eq. 2, respectively, then integrating and combining terms gives:

$$\eta_{\text{eff}}(t) - \eta_s = \frac{2}{v}(1 - \varepsilon)\left(1 - \frac{1}{v}\right)(\eta_p - \eta_s) + (1 - \varepsilon)\zeta(2 + \zeta)f(t)(\eta_A - \eta_s) \quad (7)$$

The SPR signal is then proportional to the appropriate refractive-index difference given by Eq. 7. This difference is $(\eta_{\text{eff}} - \eta_s)$ after adding solid particulate to a clean gold sensor surface with $f(t)=0$ which renders 2nd term on the right-hand-side of Eq. 7 zero. Or it is $[\eta_{\text{eff}} - \eta_s - (2/v)(1 - \varepsilon)(1 - 1/v)(\eta_p - \eta_s)]$ after exposing analyte to fixed solid spheres adjacent to the gold sensor surface. The dimensionless analyte/adsorbent ratio ζ varies from about 0.08 for sorption of 40-nm virus on 1-micron-scale sintered membranes to ~ 0.0004 for small protein adsorption on 10-micron beads.

3.3. Measuring adsorption of adenovirus and cytochrome c on planar surface, membrane and resin

Adenovirus Type 5 (Ad5) is a non-enveloped, double-stranded DNA viral vector used in gene therapy to treat diseases such as cancer, diabetes, hemophilia, cystic fibrosis, heart disease and musculoskeletal disorders that have an underlying genetic basis (Edelstein, 2004). Ad5 has a

capsid radius of about 40 nanometers, a molecular mass of 165×10^6 daltons and a molecular diffusivity of $4.5 \times 10^{-8} \text{ cm}^2 \text{ s}^{-1}$ (Russell, 2000). Ad5 vectors for gene therapy are prepared by adsorption onto ion-exchange chromatographic resin, for which kinetic adsorption rates are desired. Cytochrome c is a globular, well-characterized biological redox protein with a molecular mass of 12,400 daltons, a diameter of 3.1 nanometers and a molecular diffusivity of $1.6 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$ (Margoliash and Schejter, 1996).

First consider monolayer adsorption of analyte on a planar sensor surface. Substituting pure protein refractive index of $n_A = 1.57$, a decay length of $l_d = 240 \text{ nm}$ (Jung et al., 1998) into Eq. 3 and applying physical properties of Ad5 yields an R_f value of 2.2×10^{10} RU per gram adenovirus per cm^2 . An R_f value of 1.4×10^{10} RU per gram cytochrome c per cm^2 calculated in the same manner is consistent with a surface coverage of $\sim 1 \text{ pg/mm}^2$ of protein on a two-dimensional surface that corresponds to a unit change in refractive index (RIU) of 10^{-6} (Chinowsky et al., 2003). Fig. 2a shows SPR sensorgrams of 1.6 pM Ad5 (14.4 mM NaCl) deposition onto a planar sensor derivatized with DEAE-SAM obtained using this response factor. Also shown is an Ad5-free control injection. Refractive index increases linearly to 43.6 RU during the 5-minute adsorption period and remains near this value during the subsequent 5-min equilibration period with running buffer.

Now consider analyte deposition onto homogeneous anisotropic porous media from a uniform-concentration boundary layer without adsorption onto the planar surface of the sensor. The porous media could be a polymer layer or highly permeable monodisperse or bidisperse media. Comparing Eq. 6 and Eq. 3 shows SPR response to this deposition is $\theta(t)/(1 - \exp(4r/l_d))$ times the SPR response to monolayer surface adsorption. Let porous media of $\Phi = 0.2$ extend to the decay length of the evanescent wave and assume $\theta = 0.05$, which corresponds to a modest capacity of about 50 mg/mL surface-associated analyte in the media, and set operating conditions for uniform concentration. Eq. 6 yields R_f values of 2.7×10^{10} RU per gram cytochrome c per cm^2 and 2.2×10^9 RU per gram adenovirus per cm^2 , respectively. These values indicate SPR response to sorption increases 2-fold for deposition of cytochrome c, but decreases by about 90% for deposition of Ad5 on porous media compared with R_f values for monolayer adsorption of these analytes. For 1.6 pM Ad5 this would be at the level of noise that is shown in Fig 2a. ResourceQTM chromatography media has 100-nanometer pores that exclude Ad5 capsid from intraparticle adsorption, limiting static capacity for Ad5 to 0.14 mg/mL (Shabram et al., 1997). This value of static capacity corresponds to monolayer Ad5 adsorption on the exterior surface of the resin sphere. If ResourceQTM were considered a homogeneous anisotropic porous media, static capacity (or Ad5 concentration of 1.6 pM) would need to increase >500-fold to distinguish an SPR response from Ad5 adsorption.

The numerical value in this analysis were calculated using the m_1 value obtained in Section 2.5 for a commercially-available Kretschmann-type SpreetaTM sensor. Different values may be calculated using an m_1 value obtained by calibrating any Kretschmann-type sensor (Biacore, home-built, etc). The magnitude of the response will scale in proportion to m_1 , but any linear system-specific sensitivity will produce *identical trends* in sorbent capacity and analyte concentration required to obtain distinguishable SPR responses.

Finally, consider analyte deposition onto monodisperse 10- μm nonporous spheres hexagonally close packed onto the sensor surface from a uniform-concentration boundary layer without adsorption onto the sensor surface. Equations 1 and 7 show the SPR response from analyte deposition is proportional to $10^6/Q_{\text{max}}$ and $(1 - \epsilon)\zeta f(t)[2 + \zeta](\eta_a - \eta_s)$. The dimensionless analyte-to-adsorbent ratio, ζ , is 0.008 for Ad5 and 0.00062 for cytochrome c. Substitution yields R_f values of about 3.4×10^8 RU per gram per cm^2 for both cytochrome c and adenovirus. These values are about 2% of comparable results for monolayer adsorption on a planar sensor. No distinguishable SPR response would be expected from 1.6 pM Ad5 adsorption on 10- μm

ResourceQ™ beads. This result is consistent with the fact that a value of $f(t)=0.546$ has been used and that a calculation shows surface area on 10- μm spheres available for sorption within the active sensor region is ~4% that of the underlying sensor surface. Replacing the 10- μm resin with a membrane formed by sintering polymer beads of 1- μm length scale, as illustrated in Fig. 2b, would increase these R_f values by a factor of about 10 and raise the SPR response for 1.6 pM Ad5 to a distinguishable level.

4. Conclusions

Response factors for analyte sorption onto 3-D structures in the cuboid sensor region or from discontinuous distribution of analyte normal to the sensor surface have been analyzed. Novel response factors that account for interactions between exponentially decaying evanescent signal interacting with distal adsorption on any planar Kretschmann-type device have been derived. A new general method was developed to calculate response factors that are *discontinuous* functions of refractive index for any geometry. Existing methods rely on continuous functions of refractive index. The method was applied to determine response factors for deposition of adenovirus and cytochrome c onto planar surfaces, homogeneous anisotropic porous media and hexagonally close-packed spheres. Deposition on anisotropic porous media with modest capacity increased the magnitude of SPR response for protein, but decreased it for virus relative to planar deposition. SPR response to deposition on the spherical resin was proportional to the available surface area and capacity in the cuboid sensor region.

The utility of the method was illustrated by identifying porous capacity and sphere size that would maintain SPR response to adenovirus deposition on 3-D surfaces relative to values obtained on a planar sensor. Derived expressions can calculate values of solids volume, surface capacity and analyte size or concentration for *any system* of homogeneous porous media or packed spheres that would allow measurable response factors. Fitting SPR sensorgrams obtained using response factors that account for interaction of exponential decay with distal adsorption as well as non-uniform analyte concentration in the boundary layer to a two-compartment model are expected to rapidly produce accurate values of intrinsic sorption rates on anisotropic porous media or spherical chromatographic resin.

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Fig. 1a

Fig. 1b

Direct SPR of Intact Adsorptive Resin

Fig. 1.

Conventional open-channel surface plasmon resonance (SPR) compared with incorporating solids into the flow cell. (a) The SPR sensorgram corresponds to analyte sorption, described by a two-compartment model (adapted from Biacore, 2004). Section 3.1 of the text contains a description of the drawing and defines the parameters. (b) Deposition of virus or protein on a solid sphere incorporated into the flow cell is illustrated (not to scale). A self-adsorbed monolayer (SAM) a few nanometers thick is formed on the surface with terminal moieties that electrostatically repel analyte adsorption. Operating at a Reynolds number (Re) of 0.6 in a bed of 10- μm spheres yields a diffusive boundary layer thickness, δ , for Ad5 (radius=40 nm) of 250 nm. Electromagnetic field that generates the SPR signal decays exponentially with a length constant of about 240 nm.

Fig. 2a

Fig. 2b

Fig. 2.

(a) SPR sensorgrams showing 1.6 pM Ad5 interaction with DEAE-SAM at 14.4 mM NaCl. Duplicate experiments are shown (hollow squares; hollow diamonds). A control injection containing no Ad5 gave a baseline SPR response (filled squares). Details of the experiments are listed in the Methods Section 2.4. (b) A scanning electron microscope image of a membrane formed by sintering polymer beads approximately 1 μm in diameter. The bar is 5 μm . The membrane surface appears on the left-hand-side and the interior on the right-hand-side.