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Calibration-free concentration analysis for an analyte prone to self-association

Hiroshi Imamura, Shinya Honda*

Biomedical Research Institute, National Institute of Advanced Industrial Science and Technology, 1-1-1, Higashi, Tsukuba, Ibaraki 305-8566, Japan

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ABSTRACT

Calibration-free concentration analysis (CFCA) based on surface plasmon resonance uses the diffusion coefficient of an analyte to determine the concentration of that analyte in a bulk solution. In general, CFCA is avoided when investigating analytes prone to self-association, as the heterogeneous diffusion coefficient results in a loss of precision. The derivation for self-association of the analyte was presented here. By using the diffusion coefficient for the monomeric state, CFCA provides the lowest possible concentration even though the analyte is self-associated.

Introduction

Calibration-free concentration analysis (CFCA) based on surface plasmon resonance (SPR) extracts the concentration of the active molecules in a mixture of active and inactive (denatured) molecules [1–6]. Recently, the CFCA has been increasingly applied to the studies [7–16]. The CFCA uses the known relation between the diffusion coefficient and the molecular weight of the analyte. Thus, the molecular weight of the analyte must be known, and obtaining accurate results for analytes prone to self-association is difficult. In addition, lack of sufficient knowledge about the association state of the analyte can result in misinterpretation of the calculated concentration. The present paper describes a formula for estimating the effect of self-association of the analyte on the SPR response and subsequent CFCA results.

Basic theory of calibration-free concentration analysis

The process of 1:1 binding between a ligand A and analyte B can be described as:

$$A_{bulk} \underset{k_c}{\overset{k_c}{\rightleftarrows}} A_{surface} + B \underset{k_d}{\overset{k_a}{\rightleftarrows}} AB \quad (1)$$

where A_{bulk} is the analyte in the bulk, $A_{surface}$ is the analyte on the surface of a gold sensor tip, B is the ligand immobilized on the tip, AB is the analyte-ligand complex, k_c is the mass transport coefficient, k_a is the association rate constant, and k_d is the dissociation rate constant. The basic equations for the SPR analysis are:

$$\frac{d\left[A_{surface}\right]}{dt} = \frac{k_c\left([A_{bulk}] - \left[A_{surface}\right]\right) - k_a\left[A_{surface}\right][B] + k_d[AB]}{h_{diff}} \quad (2)$$

and

$$\frac{d[AB]}{dt} = k_a\left[A_{surface}\right][B] - k_d[AB] \quad (3)$$

where h_{diff} is the characteristic height of the diffusion layer that depends on the shape of the flow cell, the flow rate, and the diffusion coefficient of the analyte D [2,4]. In a situation in which mass transport (diffusion into the surface of the tip with the ligand) is rate-limiting, the concentration of $A_{surface}$ does not depend on time, as shown by:

Abbreviations: SPR, surface plasmon resonance; CFCA, calibration-free concentration analysis.

* Corresponding author.
E-mail address: s.honda@aist.go.jp (S. Honda).

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$$\frac{d\left[A_{surface}\right]}{dt} = 0 \tag{4}$$

During the initial association phase, dissociation can be neglected, as shown by:

$$k_d = 0 \tag{5}$$

For SPR, the relation between the response signal R and [AB] is expressed as:

$$R = MG[AB] \tag{6}$$

where M is the molecular weight of the analyte and G is a factor converting concentration to an R value.

Using Eqs. (4)–(6), Eqs. (2) and (3) can be rewritten as:

$$\frac{dR}{dt} = \frac{MGk_c k_r[A_{bulk}]}{k_c + k_r} \tag{7}$$

where k_r is k_a[B]. When diffusion (mass transport) is rate-limiting, $k_c \ll k_r$ can be assumed. Thus, $k_c k_r/(k_c + k_r)$ in Eq. (7) can be replaced by k_c, which gives:

$$\frac{dR}{dt} = MGk_c[A_{bulk}] \tag{8}$$

Eq. (7) or (8) can be used for CFCA [2,4,17].

Formula for an analyte in equilibrium between a monomer and dimer

When the analyte is a mixture of a monomer (A1) and a dimer (A2), each of which has a one-to-one binding with B, R is expressed as:

$$R = R_{A1} + R_{A2} \tag{9}$$

where $R_{A1} = M_{A1}G$[A1B] and $R_{A2} = M_{A2}G$[A2B], and the A1 and A2 subscripts designate the monomer and dimer, respectively. The time dependence of R is given by:

$$\frac{dR}{dt} = \frac{dR_{A1}}{dt} + \frac{dR_{A2}}{dt} \tag{10}$$

where $dR_{A1}/dt = M_{A1}Gk_{c,A1}[A1_{bulk}]$ and $dR_{A2}/dt = M_{A2}Gk_{c,A2}[A2_{bulk}]$. When the analyte in the bulk has reached equilibrium between the monomer and dimer, and a fraction (α) of the total-monomer-molar concentration (c_A) is converted into the dimer, the concentration of the monomer and the dimer can be expressed as:

$$[A1_{bulk}] = c_A(1-\alpha) \tag{11}$$

and

$$[A2_{bulk}] = c_A\alpha/2 \tag{12}$$

Typically, $[A1_{bulk}]$ and $[A2_{bulk}]$ are expressed as molar concentrations, such as nmol/mm^3. Here, the molar concentration was converted into a weight concentration (ng/mm^3):

$$[A1_{bulk}]_W = M_{A1}[A1_{bulk}] = M_{A1}c_A(1-\alpha) \tag{11a}$$

and, according to $M_{A2} = 2M_{A1}$:

$$[A2_{bulk}]_W = M_{A2}[A2_{bulk}] = 2M_{A1}[A2_{bulk}] = 2M_{A1}c_A\alpha/2 = M_{A1}c_A\alpha \tag{12a}$$

where the subscripted W indicates the weight concentration.

Eq. (10) can be rewritten as:

$$\frac{dR}{dt} = GM_{A1}c_A\left[k_{c,A\,1}(1-\alpha) + k_{c,A\,2}\alpha\right] \tag{13}$$

The value of k_c depends on the diffusion coefficient of the analyte [2,4].

$$k_{c,A1} = mD_{A1}^{2/3} \tag{14}$$

or

$$k_{c,A2} = mD_{A2}^{2/3} \tag{15}$$

where m is a constant depending on the flow rate, the size of the flow chamber and the detection area. For CFCA, the diffusion coefficient D is estimated by molecular weight *via* Stoke's law and the Einstein-Sutherland equation [2].

$$D = \frac{k_B T}{6\pi\eta(f/f_0)\sqrt[3]{3Mv/(4\pi N_A)}} = \frac{s_0}{(f/f_0)v^{1/3}}M^{-1/3} = s_0 s M^{-1/3} \tag{16}$$

where k_B is Boltzmann's constant, T is the thermodynamic temperature, η is the viscosity, f/f_0 is the friction factor for the analyte relative to a sphere of the same size, v is the specific volume, N_A is Avogadro's number, and s_0 is $k_BT/\{6\pi\eta(3/(4\pi N_A))^{1/3}\}$, considered to be a constant. Here, s is defined as:

$$s = (f/f_0)^{-1}v^{-1/3}. \tag{17}$$

Eqs. (14) and (15) can be rewritten as:

$$k_{c,A1} = m'M_{A1}^{-2/9} \tag{18}$$

and

$$k_{c,A2} = m'M_{A2}^{-2/9} \tag{19}$$

where m' is $m(s_0 s)^{2/3}$. Then, Eq. (13) can be rewritten as:

$$\frac{dR}{dt} = GM_{A1}c_A m'\left[M_{A1}^{-2/9}(1-\alpha) + (2M_{A1})^{-2/9}\alpha\right] \tag{20}$$

Case study for an analyte that forms 100% dimer: deviation in the CFCA concentration assuming 100% monomer from the exact concentration

A typical CFCA protocol assumes that the analyte is 100% monomeric, and the active concentration is determined from the SPR response $(dR/dt)_{exp}$, where the subscript indicates an experimental value. When the fraction of dimeric analyte is 0% [*i.e.*, $\alpha = 0$ in Eq. (13)], then:

$$c_{A,\,\alpha=0} = (dR/dt)_{exp}\Big/(GM_{A1}k_{c,A1}) \tag{21}$$

where $c_{A,\alpha\,=\,0}$ is c_A when $\alpha = 0$.

However, if the analyte forms 100% dimer [*i.e.*, $\alpha = 1$ in Eq. (13)], the concentration, c_A, is given by: