Microfluidic chip for fast bioassays—evaluation of binding parameters

Jakub Štěpánek, Michal Příbyl,^{a)} Dalimil Šnita, and Miloš Marek

Department of Chemical Engineering, Institute of Chemical Technology, Prague, Technická 5, 166 28 Praha 6, Czech Republic

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A seven channel polystyrene (PS) microchip has been constructed using a micro-milling machine and a high-temperature assembling. Protein A (PA) has been immobilized by a passive sorption on the microchannel walls. Two bioaffinity assays with human immunoglobulin G (hIgG) as a ligand have been carried out. (i) PA as the receptor and fluorescently labeled hIgG (FITC-hIgG) as the ligand, (ii) PA as the receptor with hIgG as the quantified ligand and fluorescently labeled goat anti-human IgG (FITC-gIgG) as the secondary ligand. One incubation step of the assays took only 5 min instead of hours typical for enzyme-linked immunosorbent assay applications. Calibration curves of the dependence of a fluorescence signal on the hIgG concentration in a sample have been obtained in one step due to a parallel arrangement of microchannels. A mathematical model of the PA-FITC-hIgG complex formation in the chip has been developed. The values of the kinetic constant of the PA-FITC-hIgG binding ($k_{\text{on}}=5.5 \text{ m}^3 \text{ mol}^{-1} \text{ s}^{-1}$) and the equilibrium dissociation constant of the formed complex ($K_d \leq 3 \times 10^{-6} \text{ mol m}^{-3}$) have been obtained by fitting to experimental data. The proposed microchip enables fast evaluation of kinetic and equilibrium constants of ligand-receptor bioaffinity pairs and the ligand quantification. As the use of microfluidic chips for immunoassays is often limited by price, we used procedures and chemicals that allow for an inexpensive construction and operation of the microdevice, e.g., temperature assembling as a fabrication technique, detection via an ordinary digital camera, nonspecific polystyrene as a substrate, passive sorption of biomolecules as an immobilization technique, etc.

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I. INTRODUCTION

Recently, considerable effort has been devoted to studies, development and modeling of microfluidic and microreaction systems¹ for bioaffinity assays. Biomicrofluidics is probably the most promising area of microengineering because of advantages due to small characteristic dimensions of microchips, such as low consumption of bioanalytes, faster performance of bioassays, high degree of integration of unit operations on a single chip, possible construction of mobile devices, easy temperature control, electrokinetic control of dosing, etc. Protein microsystems on a chip can be divided into two groups (i) microarrays and (ii) microfluidic chips.²

The microarray systems are mostly used for qualitative or semiquantitative detection of various proteins and haptens, e.g., proteins related to autoimmune diseases, cancer and cardiac markers, drugs, etc. The development of protein microarrays consists of many the following steps: a proper choice of the substrate, chip surface modification, receptor deposition, sample application, incubation, and detection.³ The technology of the protein microarray construction mostly adopted from the technology of DNA chips often fails because of the complexity of protein structures,

^{a)} Author to whom correspondence should be addressed. Electronic mail: Michal.Pribyl@vscht.cz; Phone: +420 2 2044 3168; Fax: +420 2 2044 4320.

FIG. 1. (a) Structure of IgG. The molecule consists of two Fab and one Fc fragments. The Fc fragment binds to PA. The Fab fragments generally bind specific parts (epitopes) of the corresponding antigen. (b) Scheme of the one-step assay PA-FITC-hIgG. (c) Scheme of the sandwich assay PA-hIgG-FITC-gIgG.

variations in the antigen-antibody affinities, cross reactivity, etc.⁴ Nevertheless, a number of commercial protein microarrays for specific applications is available on the market.⁵

The protein reaction systems based on the microfluidic technology are used less than those based on microarrays. However, they enable a variety of important applications such as measurement of diffusion coefficients, determination of binding constants, immunoassays, isoelectrical focusing, cell manipulation, etc.² Microfluidic devices are often small and easily transportable, which can be important, e.g., for detection of chemical and biological weapons⁶ or for diagnostics of dangerous diseases in the developing world.⁷ Yager *et al.*⁷ introduced a portable microfluidic diagnostic card that integrates sample conditioning, analyte dosing, mixing, immunoassay, and detection.

Immunoassays are often carried out in a heterogeneous arrangement, where a receptor (antigen or antibody) is immobilized on a solid phase. For a recent overview of such applications and their parameters see Bange *et al.*⁸ Particularly, Kakuta *et al.*⁹ developed a microfluidic chip with a pressure driven control for detection of a brain peptide. The microchip contains gel particles with an immobilized receptor. This heterogeneous immunoassay could be carried out within 8 min. Hayes *et al.*¹⁰ exploited coated solid paramagnetic particles for the heterogeneous immunodetection of the parathyroid hormone. A heterogeneous immunoassay for detection of bacterium related proteins with the receptor bound directly on microchannel walls was reported by Xiang *et al.*,¹¹ their microfluidic chip was controlled by an electric field.

Mathematical modeling of reaction-transport processes is an important tool for fast development of microfluidic devices. Heterogeneous bioaffinity interactions in a gel-like medium,¹² in a complex microchannel structure,¹³ and in an electrokinetically driven¹⁴ or pressure driven¹⁵ microcapillary reactors were recently reported. Gao *et al.*¹⁶ used a mathematical model of an electrokinetically driven H-shape microchip for estimation of operational parameters of a heterogeneous immunoassay. Mathematical modeling is also necessary when binding and equilibrium constants of bioaffinity systems are evaluated. Kinetic and equilibrium parameters can be determined by a variety of techniques. Liu *et al.*¹⁷ developed a fast capillary electrophoresis method for the evaluation of bovine serum albumin (BSA)-heparin binding parameters in a homogeneous arrangement. Elbs *et al.*¹⁸ determined the binding constant of a polypeptide on immobilized vancomycin in a microarray device with a confocal fluorescence detection. Another method in the heterogeneous arrangement with the use of an acoustic waveguide device was presented by Saha *et al.*¹⁹

In this article, a bioaffinity system coupling the protein A [(PA), the immobilized receptor] and the human immunoglobulin G [(hIgG), ligand] is studied. PA is derived from the cell wall of *Staphylococcus aureus*. The protein has a high affinity for the Fc part of immunoglobulins [Fig. 1(a)]. When conjugated with peroxidase or fluorescein, it may be used as an universal label for immunoassays, western blots, or immunocytochemistry.²⁰ PA is also often used in purification of IgGs or immobilization of IgGs.²¹

Construction of a simple capillary microchip, a procedure of the protein A immobilization,

FIG. 2. Microchip layout. (a) PS plate with the microchannel structure. (b) Assembled microchip.

formats of two bioassays based on the interaction PA-hIgG, and detection techniques are described in the experimental section. A mathematical model of the incubation phase of the PA-hIgG assay is derived in Sec. III. Evaluation of binding and equilibrium constants of the PA-hIgG interaction is also explained.

II. EXPERIMENTS

A. Microchip fabrication

The microchip consists of two polystyrene (PS) plates (nonspecific PS Anicrin). The seven-channel microcapillary structure is milled (micromilling machine STEP-FOUR PRECISE 760) in the bottom plate [Fig. 2(a)]. The depth and width of microchannels are $80 \pm 10 \mu\text{m}$ and $250 \mu\text{m}$, respectively. These dimensions are given by the precision of the micromilling machine in the z direction and by the radius of the drill, respectively. The microchannel length varied from 0.037 to 0.078 m. Thus, a typical internal volume of the channel was about $1 \times 10^{-9} \text{ m}^3$ ($1 \mu\text{l}$). Holes for tubing installation are drilled in the top plate. The PS plates are inserted between two glass slabs. This sandwich is assembled at 100°C within 15 min. The tubing is finally glued (Acrifix 192) to the microchip [Fig. 2(b)].

B. Immobilization procedure

PA (ZYMED) was immobilized in the polystyrene channels by a passive sorption. The mass concentration of PA in Tris buffer (50 mM, pH=8, SIGMA) was equal to 50 g m^{-3} and the immobilization time was 20 min. Solution of 1% bovine serum albumin (BSA) in Tris buffer was used as a blocking agent to eliminate nonspecific sorption of antibodies directly on the PS surface. The effect of the BSA blocking on the immobilization is shown in Fig. 3. The top channel represents our standard experimental configuration, i.e., PA immobilization, BSA blocking, bioaffinity reaction of PA with FITC-hIgG (SIGMA), washing with TRIS-Tween20 buffer (SIGMA), and detection of fluorescence intensity. No PA was introduced into the central channel. BSA then blocks the entire PS surface and no FITC-hIgG molecules can be immobilized on the microchannel walls. After washing with Tris-Tween20 buffer, no fluorescent signal is emitted. The blocking step was omitted in the bottom channel. The signal is slightly more intensive than in the top channel because the FITC-hIgG molecules are bound on the immobilized PA receptors and also directly adsorbed on the unblocked PS surface.

FIG. 3. The effect of the BSA blocking.

Stability of the PA immobilization in microchannels was also studied. The microchannels with the immobilized PA were filled by Tris buffer. FITC-hIgG solution was then introduced in a selected microcapillary at various times after the PA immobilization. The fluorescence signal related to the surface concentration of the PA–FITC-hIgG complex was finally observed. The PA layer was found to be stable for at least several hours.

C. Assay formats

Two noncompetitive heterogeneous bioaffinity assays with hIgG as a ligand were proposed. PA was used as the immobilized receptor in the assays. FITC-hIgG is the ligand in the one-step assay [Fig. 1(b)]. The incubation step started when a microchannel was filled with a ligand sample. The duration of the incubation step involving formation of the PA-FITC-hIgG complex was set to 5 min. This time is sufficiently longer than the diffusion transport time of antibodies from the center of the capillary to the PA binding sites (about 92 s). After the incubation step, the capillaries were washed and the fluorescence emission proportional to the concentration of the formed PA-FITC-hIgG complex was recorded.

In the sandwich assay, hIgG (SIGMA) was used as the quantified primary ligand. The amount of the formed PA-hIgG complex was indirectly determined in the second incubation step with a surplus of the fluorescently labeled secondary ligand, anti-human FITC-gIgG (SIGMA). The fluorescent emission was proportional to the concentration of the PA-hIgG-FITC-gIgG complex and thus to the concentration of hIgG in the sample [Fig. 1(c)]. The duration of each incubation step was again set to 5 min.

The biochemicals were diluted in Tris buffer (50 mM, pH=8) that was also used as the washing solution. Always before the detection, the microcapillaries were washed by Tris-Tween20 buffer (50 mM, pH=8) to break nonspecific bonds. All used solutions were dosed by a mechanical micropipette. All experiments were carried out at room temperature (25 °C).

D. Fluorescence signal quantification

The quantification of bioaffinity complexes is based on fluorescence techniques. Olympus BX51WI fluorescence microscope together with Olympus Camedia C 5050 digital camera was used to record the fluorescence images. The representative 80×80 pixel sample of each channel was selected from the obtained image, and the average value of the green color component of the image (GCCl value) in the range from 0 to 256 was evaluated. Representative samples were selected from the center of the microchannels because the fluorescence signal at the sidewalls is affected by light interference. The distribution of fluorescence signals in the microchannels is not ideally uniform due to surface roughness given by the microfabrication technique. Hence, the sample should cover a larger (representative) microchannel area (6400 pixels in this study).

FIG. 4. The one-step assay. Fluorescence image of the microfluidic chip. The mass concentrations of FITC-hIgG in the samples dosed into the capillaries w_{Ab}^{in} are from the top to the bottom equal to 0, 10, 50, 100, 200, 500, and 1000 g m⁻³, i.e., C_{Ab}^{in} is equal to 0, 0.0817, 0.409, 0.817, 1.63, 4.09, and 8.17 according to Eq. (1).

E. Experimental results—one step assay

Each capillary was filled with a solution of FITC-hIgG at the start of the experiment. After the incubation step and the washing out, the fluorescence image of the microcapillary field was obtained (Fig. 4) and analyzed.

The GCCI value obtained for a blind experiment (concentration of FITC-hIgG was equal to zero) was subtracted from the other values measured in the capillary field. Thus, the calibration curves of the dependence of the normalized GCCI intensity (proportional to the concentration of the formed complex) on the concentration of the antibody in the sample were constructed. The calibration curve corresponding to Fig. 4 is shown in Fig. 5 (dots). Instead of the GCCI value, a spatially averaged dimensionless concentration of the formed PA-FITC-hIgG complex $\bar{C}_C$ is plotted. The dimensionless concentration $\bar{C}_C$ is defined as the concentration ratio of the formed bioaffinity complex and the total amount of PA binding sites. The relation between the GCCI value and $\bar{C}_C$ is assumed to be linear and the proportionality constant is evaluated on the basis of a mathematical model (see Sec. III). The dimensionless molar concentration of the ligand in the sample C_{Ab}^{in} is related to the mass concentration of the ligand in the sample w_{Ab}^{in} via

FIG. 5. Calibration curve of the one-step assay, experimental points (dots), obtained from the mathematical model ($Da = 1.72$, $\kappa_d = 3.75 \times 10^{-3}$, and $k = 97.7$) (line).

TABLE I. Model parameters.

Parameter	Description	Unit
c_{Ag}^{tot}	total surface concentration of the PA binding sites (Refs. 24 and 25)	$1 \times 10^{-7} \text{ mol m}^{-2}$
D_{Ab}	hIgG diffusivity (Ref. 28)	$4 \times 10^{-11} \text{ m}^2 \text{ s}^{-1}$
$2L_1$	microchannel width	$2.5 \times 10^{-4} \text{ m}$
$2L_2$	microchannel depth	$8 \times 10^{-5} \text{ m}$

$$C_{Ab}^{in} \equiv c_{Ab}^{in} L_1 / c_{Ag}^{tot} = w_{Ab}^{in} L_1 / (M_{Ab} c_{Ag}^{tot}), \quad (1)$$

where c_{Ab}^{in} , L_1 , M_{Ab} , and c_{Ag}^{tot} are the molar concentration of the ligand in the sample, one half of the microchannel width (see Table I), the molar weight of the hIgG molecules²² ($M_{Ab} \approx 1.53 \times 10^5 \text{ g mol}^{-1}$), and the total concentration of the PA binding sites, respectively.

The calibration curves of the one-step assay were obtained also for the ranges w_{Ab}^{in} 0–100 g m^{-3} and 0–300 g m^{-3} . These dependencies are almost linear because the free PA binding sites are in the excess with respect to the reacting ligand.

F. Experimental results—sandwich assay

The microchannels were filled with hIgG samples of known concentrations in the first incubation step. After washing, the secondary ligand FITC-gIgG was introduced to each channel (the same concentration was used in all the channels) and the second incubation step was carried out. The microchannels were then washed out and the fluorescence image of the capillary array was recorded.

PA firmly captures the Fc domain of hIgG. The PA-gIgG binding is weak but not negligible. Hence, two blind experiments (i.e., the concentration of hIgG in the sample is equal to zero) have been carried out on the chip (i) PA was not immobilized in the channel and (ii) PA was immobilized in the channel. The GCCI values of the blind experiments were subtracted from all the GCCI values in the microcapillary array and the calibration curves were constructed (Fig. 6).

Blind experiment (i) provided no fluorescence emission because the microchannel surface was completely blocked by BSA and no PA binding sites were available. Blind experiment (ii) resulted

FIG. 6. Calibration curves of the sandwich assay after the subtraction of blind experiments. PA was immobilized in the blind experiment (circles), PA was not immobilized in the blind experiment (triangles). The lines only connect the experimental points. The hIgG concentrations in the samples dosed into capillaries are 0, 10, 50, 100, 200, and 300 g m^{-3} , i.e., C_{Ab}^{in} is equal to 0, 0.0817, 0.409, 0.817, 1.63, and 2.45 according to Eq. (1).

FIG. 7. Geometry of the microchannel cross section. Microchannel width $2L_1$ and depth $2L_2$ are equal to 250 and 80 μm , respectively.

in a low intensity fluorescence emission corresponding to the binding of FITC-gIgG on the immobilized PA layer free of hIgG molecules. When the concentration of hIgG in the samples is low, the difference in the fluorescence intensity between the calibration curves in Fig. 6 is given by the fluorescence contribution of the interaction of the FITC-gIgG molecules with the PA binding sites, i.e., the bottom calibration curve in Fig. 6 is valid, and therefore the GCCI value of the blind experiment (ii) should be subtracted. There are almost no free binding sites left for FITC-gIgG if a high concentration of hIgG in the samples is used, i.e., the top calibration curve in Fig. 6 is then relevant and hence the GCCI value of the blind experiment (i) should be subtracted.

The undesirable interaction of the secondary antibody with the PA binding sites can be avoided, e.g., by the use of a chicken IgG that does not contain the Fc domain. However, the observed dependence of GCCI on hIgG concentration is monotonic and, in principle, can be used for the quantification of hIgG.

From the comparison of Fig. 5 and Fig. 6, it follows that the full saturation of the PA binding sites in the sandwich assay occurs for lower hIgG concentrations than in the one-step assay. One possible explanation is that this phenomenon is caused by a steric hindrance of the IgG interactions. The used FITC-gIgG is Fab specific. It means that one hIgG molecule can bind at least two FITC-gIgG molecules. However, this is possible only for low surface concentrations of hIgG. As the surface concentration of the captured hIgG grows, some of the Fab fragments of hIgG cannot be occupied by FITC-gIgG because of the space restriction above the bioaffinity layer given by the size of the antibody molecules and by the overlap of the adjacent hIgG molecules. For example, Matson and Little²³ observed that a steric hindrance created by crowding of adjacent antibody molecules immobilized via an oriented coupling mechanism (the PA-hIgG complex in our work) leads to a decrease in antigen binding efficiency of the protein layer (binding of the secondary ligand FITC-gIgG in our work).

III. MATHEMATICAL MODELING

A. Mathematical model of the incubation

In order to evaluate kinetic and equilibrium parameters of the PA-hIgG heterogeneous interaction, a mathematical model of the incubation phase of the one-step assay was constructed. The cross section of the fabricated microcapillaries has a rectangular shape (Fig. 7). The width and depth of the channel are $2L_1$ and $2L_2$, respectively. The reaction-transport processes were modeled in one quarter of the microchannel because of axial symmetry. The boundaries of the system are indexed as B1-4 (Fig. 7). During the incubation phase, hIgG molecules (Ab) are transported by diffusion to free PA binding sites (Ag) immobilized on the capillary walls and a PA-hIgG complex (C) is formed

The complex formation can be described as

$$\frac{\partial c_C}{\partial t} = k_{\text{on}} c_{A_g} c_{Ab}|_{B1, B2} - k_{\text{off}} c_C, \quad (2)$$

where c_C , c_{A_g} , c_{Ab} , k_{on} , k_{off} , and t are the surface molar concentration of the complex, the surface molar concentration of PA, the volume molar concentration of hIgG (here at the boundaries $B1$ and $B2$), the kinetic constant of the complex formation, the kinetic constant of the complex dissociation, and time, respectively. The reaction equilibrium is characterized by the equilibrium dissociation constant $K_d = k_{\text{off}}/k_{\text{on}}$. The total concentration of the PA binding sites $c_{A_g}^{\text{tot}}$ is expressed as the sum of the surface concentrations of the free PA binding sites and the PA-hIgG complex

$$c_{A_g}^{\text{tot}} = c_{A_g} + c_C. \quad (3)$$

The value of the parameter $c_{A_g}^{\text{tot}}$ is estimated^{24,25} as $1 \times 10^{-7} \text{ mol m}^{-2}$. The surface concentration of PA was found to be equal to $8.3 \times 10^{-8} \text{ mol m}^{-2}$ in a PA monolayer investigated by Dubrovsky *et al.*²⁵ However, one PA molecule can bind two IgG molecules and a fraction of the immobilized PA remains inactive. Dubrovsky *et al.*²⁵ then used the PA monolayer for immobilization of IgGs and found that the surface density of antibodies varied from 6.8 to $11.21 \times 10^{-8} \text{ mol m}^{-2}$, which approximately corresponds to the concentration of the free PA binding sites.

Only diffusion transport of hIgG is considered because dosing before the incubation and washing after the incubation are carried out always very fast (within 1 s). The molar balance of hIgG in microchannels can be then written as

$$\frac{\partial c_{Ab}}{\partial t} = D_{Ab} \left(\frac{\partial^2 c_{Ab}}{\partial x^2} + \frac{\partial^2 c_{Ab}}{\partial y^2} \right), \quad (4)$$

where D_{Ab} is the antibody diffusivity in the aqueous solution. The hIgG binding to the microchannel walls can be described by the Robin boundary conditions,

$$-D_{Ab} \frac{\partial c_{Ab}}{\partial x} \bigg|_{B1} = k_{\text{on}} c_{A_g} c_{Ab}|_{B1} - k_{\text{off}} c_C, \quad -D_{Ab} \frac{\partial c_{Ab}}{\partial y} \bigg|_{B2} = k_{\text{on}} c_{A_g} c_{Ab}|_{B2} - k_{\text{off}} c_C. \quad (5)$$

The Neumann boundary conditions are considered in the axes of symmetry

$$\frac{\partial c_{Ab}}{\partial y} \bigg|_{B3} = 0, \quad \frac{\partial c_{Ab}}{\partial x} \bigg|_{B4} = 0. \quad (6)$$

At the start of the experiment, the hIgG concentration is approximately uniform in the entire microcapillary and equal to the molar concentration in the sample c_{Ab}^{in} . All the PA binding sites are free of ligand

$$c_{Ab}(t=0, x, y) = c_{Ab}^{\text{in}}, \quad c_C(t=0, x, y) = 0. \quad (7)$$

B. Dimensionless model equation

The model equations were transformed into a dimensionless form in the following way: The volume concentration of the hIgG molecules and the surface concentration of the PA-hIgG complex are scaled by the factors $c_{A_g}^{\text{tot}}/L_1$ and $c_{A_g}^{\text{tot}}$, respectively. The time and spatial coordinates are scaled by the diffusion transport time L_1^2/D_{Ab} and by the characteristic dimension of the channel L_1 , respectively.

The dimensionless form of Eqs. (2) and (4)–(7) is then written as

$$\frac{\partial C_C}{\partial \tau} = \text{Da} [C_{Ab}|_{B1, B2} (1 - C_C) - \kappa_d C_C], \quad (8)$$

$$\frac{\partial C_{Ab}}{\partial \tau} = \frac{\partial^2 C_{Ab}}{\partial X^2} + \frac{\partial^2 C_{Ab}}{\partial Y^2}, \quad (9)$$

$$-\left. \frac{\partial C_{Ab}}{\partial X} \right|_{B1} = \text{Da}[C_{Ab}|_{B1}(1 - C_C) - \kappa_d C_C], \quad -\left. \frac{\partial C_{Ab}}{\partial Y} \right|_{B2} = \text{Da}[C_{Ab}|_{B2}(1 - C_C) - \kappa_d C_C], \quad (10)$$

$$\left. \frac{\partial C_{Ab}}{\partial X} \right|_{B4} = 0, \quad \left. \frac{\partial C_{Ab}}{\partial Y} \right|_{B3} = 0, \quad (11)$$

$$C_C(\tau=0, X, Y) = 0, \quad C_{Ab}(\tau=0, X, Y) = C_{Ab}^{\text{in}}, \quad (12)$$

where C_C and C_{Ab} are the dimensionless concentrations of the complex and the hIgG molecules, τ is the dimensionless time, and X, Y are the dimensionless spatial coordinates. There are three dimensionless groups, the Damköhler number Da defined as $\text{Da} \equiv L_1 k_{\text{on}} C_{Ag}^{\text{tot}} / D_{Ab}$, the dimensionless molar concentration of hIgG in the sample C_{Ab}^{in} defined by Eq. (1), and the dimensionless equilibrium dissociation constant $\kappa_d \equiv K_d L_1 / c_{Ag}^{\text{tot}}$. The Damköhler number is a ratio of the characteristic diffusion time of the antibody and a characteristic reaction time of the complex formation. The dimensionless concentration C_{Ab}^{in} is proportional to the ratio of the total amount of hIgG molecules in the sample and the total amount of PA binding sites.

Dynamical analysis of the model Eqs. (8)–(12) was carried out using a finite element software Comsol. The fixed model parameters used in the numerical analysis are summarized in Table I.

C. Determination of parameter values

Calibration curves at $t=5$ min after the start of incubation were computed in a space of the following three parameters: The Damköhler number Da , the dimensionless equilibrium dissociation constant κ_d , and a proportionality constant k (see below). The calibration curves were expressed as the dependences of the spatially averaged dimensionless concentration of the ligand-receptor complex ($\bar{C}_C$) on the dimensionless concentration of immunoglobulins C_{Ab}^{in} in the sample. The spatially averaged dimensionless concentration is defined as

$$\bar{C}_C \equiv \int_0^1 C_C(t D_{Ab} / L_1^2, X, L_2 / L_1) dX. \quad (13)$$

The fluorescence signal intensity observed experimentally (expressed as the GCCI value) is linearly dependent on the $\bar{C}_C$ value according to $\text{GCCI} = k \bar{C}_C$, where k is the proportionality constant that depends on the parameters of the fluorescence signal recording (exposition time, sensitivity, etc.) and has to be evaluated for each experiment.

The three independent parameters (Da , κ_d and k) were evaluated by an optimization technique. A physically relevant (*optimal*) set of these parameters was identified in the three-dimensional parameter space by minimizing of a penalty function defined as the sum of squares of deviations between the experimentally and numerically obtained points on the calibration curves (Fig. 5). In the k – Da plane, the optimum was found at $\text{Da}=1.72$ and $k=97.7$ (Fig. 8). In the κ_d – Da plane, the optimum is not represented by a single point, but it forms a *valley* for $\text{Da}=1.72$ and $\kappa_d \leq 3.75 \times 10^{-3}$ (Fig. 9). The calibration curve computed for the optimum ($\text{Da}=1.72$, $k=97.7$, and $\kappa_d \leq 3.75 \times 10^{-3}$) fits satisfactorily the experimental data (Fig. 5).

The kinetic constant of the complex formation can be evaluated from the definition of Damköhler number, $k_{\text{on}}=5.5 \text{ m}^3 \text{ mol}^{-1} \text{ s}^{-1}$. This value is comparable with the values $k_{\text{on}}=3.952 \text{ m}^3 \text{ mol}^{-1} \text{ s}^{-1}$, $k_{\text{on}}=8.02 \text{ m}^3 \text{ mol}^{-1} \text{ s}^{-1}$, and $k_{\text{on}}=12 \text{ m}^3 \text{ mol}^{-1} \text{ s}^{-1}$ obtained for the same bio-affinity system with the use of different detection techniques on glass,²⁴ gold,¹⁹ and porous SiO_2 layer,²⁶ respectively. The determined value of the equilibrium dissociation constant

FIG. 8. Location of the optimum in the parameter plane of the proportionality constant of the fluorescence signal k vs Damköhler number Da , $\kappa_d = 3.75 \times 10^{-3}$.

$K_d \leq 3 \times 10^{-6} \text{ mol m}^{-3}$ of the PA-hIgG complex (computed for $\kappa_d \leq 3.75 \times 10^{-3}$) is lower than the data reported for PA immobilized on other substrates^{19,26} ($3.4 \times 10^{-5} \text{ mol m}^{-3}$ and $1.8 \times 10^{-5} \text{ mol m}^{-3}$).

IV. CONCLUSIONS

Both the experimental and modeling parts of this work demonstrate a simple technique for a fast quantification of biological ligands (the sandwich assay) and the evaluation of kinetic and equilibrium reaction constants of ligand-receptor affinity pairs (the one-step assay) in the heterogeneous arrangement.

The proposed polystyrene capillary microchip enables a quantitative determination of hIgG concentration in an aqueous sample. The studied assay was carried out in two different formats (i) the one-step and (ii) the sandwich. The incubation time was set to 5 min, which leads to a substantial reduction of the time demands of the method if compared with standard enzyme-linked immunosorbent assays.

Kinetic and equilibrium parameters of the PA-hIgG system were determined using a mathematical model of the incubation phase and an optimization technique. It is expected that the same procedure can be applied to various heterogeneous ligand-receptor systems. If a microarray chip

FIG. 9. Location of the optimum in the parameter plane of the dimensionless equilibrium dissociation constant κ_d vs Damköhler number Da , $k = 97.7$.

were employed,¹⁸ a large number of binding and equilibrium characteristics could be quickly determined. This method can also be important, e.g., in the modeling of signal transmission in cellular systems,²⁷ etc.

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- ¹ V. Hessel, S. Hardt, and H. Lowe, *Chemical Micro Process Engineering Fundamentals, Modelling and Reactions* (Wiley-VCH, Weinheim, 2004).
- ² A. M. Dupuy, S. Lehmann, and J. P. Cristol, *Clin. Chem. Lab. Med.* **43**, 1291 (2005).
- ³ J. Petrik, *Transfus. Med.* **16**, 233 (2006).
- ⁴ R. Barry and M. Soloviev, *Proteomics* **4**, 3717 (2004).
- ⁵ M. F. Templin, D. Stoll, J. Bachmann, and T. O. Joos, *Comb. Chem. High Throughput Screening* **7**, 223 (2004).
- ⁶ J. Wang, *Anal. Chim. Acta* **507**, 3 (2004).
- ⁷ P. Yager, T. Edwards, E. Fu, K. Helton, K. Nelson, M. R. Tam, and B. H. Weigl, *Nature* **442**, 412 (2006).
- ⁸ A. Bange, H. B. Halsall, and W. R. Heineman, *Biosens. Bioelectron.* **20**, 2488 (2005).
- ⁹ M. Kakuta, H. Takahashi, S. Kazuno, K. Murayama, T. Ueno, and M. Tokeshi, *Meas. Sci. Technol.* **17**, 3189 (2006).
- ¹⁰ M. A. Hayes, N. A. Polson, A. N. Phayre, and A. A. Garcia, *Anal. Chem.* **73**, 5896 (2001).
- ¹¹ Q. Xiang, G. Q. Hu, Y. L. Gao, and D. Q. Li, *Biosensors* **21**, 2006 (2006).
- ¹² M. Pribyl, D. Snita, P. Hasal, and M. Marek, *Chem. Eng. J.* **101**, 303 (2004).
- ¹³ M. Pribyl, V. Knapkova, D. Snita, and M. Marek, *Chem. Pap.* **59**, 434 (2005).
- ¹⁴ M. Pribyl, V. Knapkova, D. Snita, and M. Marek, *Microelectron. Eng.* **83**, 1660 (2006).
- ¹⁵ M. Zimmermann, E. Delamarche, M. Wolf, and P. Hunziker, *Biomed. Microdevices* **7**, 99 (2005).
- ¹⁶ Y. L. Gao, G. Q. Hu, F. Y. H. Lin, P. M. Sherman, and D. Q. Li, *Biomed. Microdevices* **7**, 301 (2005).
- ¹⁷ X. J. Liu, X. Liu, A. Y. Liang, Z. Shen, Y. Zhang, Z. P. Dai, B. H. Xiong, and B. C. Lin, *Electrophoresis* **27**, 3125 (2006).
- ¹⁸ M. Elbs and R. Brock, *Anal. Chem.* **75**, 4793 (2003).
- ¹⁹ K. Saha, F. Bender, and E. Gizeli, *Anal. Chem.* **75**, 835 (2003).
- ²⁰ D. Wild, *The Immunoassay Handbook*, Third ed. (Elsevier, Oxford, 2005).
- ²¹ T. K. Christopoulos and E. P. Diamandis, *Immunoassay* (Academic, San Diego, 1996).
- ²² D. P. McCormic, R. P. Wenzel, J. A. Davies, and W. E. Beam, *Infect. Immun.* **6**, 282 (1972).
- ²³ R. S. Matson and M. C. Little, *J. Chromatogr.* **458**, 67 (1988).
- ²⁴ A. Dodge, K. Fluri, E. Verpoorte, and N. F. de Rooij, *Anal. Chem.* **73**, 3400 (2001).
- ²⁵ T. Dubrovsky, A. Tronin, S. Dubrovskaya, S. Vakula, and C. Nicolini, *Sens. Actuators B* **23**, 1 (1995).
- ²⁶ M. P. Schwartz, S. D. Alvarez, and M. J. Sailor, *Anal. Chem.* **79**, 327 (2007).
- ²⁷ M. Pribyl, C. B. Muratov, and S. Y. Shvartsman, *Biophys. J.* **84**, 3624 (2003).
- ²⁸ T. Jossang, J. Feder, and E. Rosenqvist, *J. Protein Chem.* **7**, 165 (1988).