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Assessment of molecular recognition element for the quantification of human epidermal growth factor using surface plasmon resonance

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Abstract

Background: A method for the selection of suitable molecular recognition element (MRE) for the quantification of human epidermal growth factor (hEGF) using surface plasmon resonance (SPR) is presented. Two types of hEGF antibody, monoclonal and polyclonal, were immobilized on the surface of chip and validated for its characteristics and performance in the quantification of hEGF. Validation of this analytical procedure was to demonstrate the stability and suitability of antibody for the quantification of target protein.
Results: Specificity, accuracy and precision for all samples were within acceptable limit for both antibodies. The affinity and kinetic constant of antibodies-hEGF binding were evaluated using a 1:1 Langmuir interaction model. The model fitted well to all binding responses simultaneously. Polyclonal antibody (pAb) has better affinity (K_D = 7.39e^{-10} M) than monoclonal antibody (mAb) (K_D = 9.54e^{-9} M). Further evaluation of kinetic constant demonstrated that pAb has faster reaction rate during sample injection, slower dissociation rate during buffer injection and higher level of saturation state than mAb. Besides, pAb has longer shelf life and greater number of cycle run.
Conclusions: Thus, pAb was more suitable to be used as a stable MRE for further quantification works from the consideration of kinetic, binding rate and shelf life assessment.

Keywords: antibody; BIAcore; biosensors; epidermal growth factor; validation.

INTRODUCTION

Epidermal growth factor (EGF) is a single chain polypeptide that exhibits growth stimulating activity on various epidermal and epithelial tissues both *in vivo* and *in vitro* (Cohen, 1962). EGF has an isoelectric point of pH 4.6 (Razis et al. 2006) and molecular weight of 6045 Da (Cohen and Carpenter, 1975). Radioimmunoaassay (RIA) and enzyme-linked immunosorbent assay (ELISA) are the widely used methods for the quantification of EGF (Yamagata et al. 1989; Kim et al. 1992; Sivakesava et al. 1999; Wang et al. 2005; Chen et al. 2006; Razis et al. 2006).

Surface plasmon resonance (SPR) technique can be used to study the interaction of relevant biomolecules which provides more advantages as compared to ELISA and RIA. SPR technology provides label-free detection of biomolecular interactions in real time. High quality binding data can be obtained for a wide range of biomolecules, providing information on concentration, specificity, affinity and kinetics. Binding patterns are monitored with respect to time and hence, kinetic analysis can be

determined. SPR is widely used in the quantification of bioactive compounds using antigen-antibody interaction (Ramanan et al. 2010; Wong et al. 2010). The measurement using SPR can be entirely automated, allowing unattended operation, good accuracy and robustness (Besenicar et al. 2006; Chavane et al. 2008). Several studies have been performed to identify a better binding partner for the quantification of biopharmaceutical protein. Based on excellent reproducibility, its linearity and long lifetime, a high affinity antibody binder had been selected to be employed in the at-line assay for bioactive antibody concentration (Chavane et al. 2008). However, information on the assessment of antibody-antigen interaction using affinity and kinetics to identify a better quantifying partner is not available in the literature.

Biomolecular interaction analysis (BIA) using SPR involves immobilization of an interacting counterpart component, ligand on a sensor chip surface. Sensor chip is the signal transducer in the biosystem where analyte, interactant that present in solution will be interacted with ligand that attached covalently to the sensor chip surface (Bostrom-Caselunghe and Lindeberg, 2000). Sensor chip is consist of a glass support with a thin gold layer (Hahnefeld et al. 2004). Carboxymethyl (CM) is the most common general-purpose chip, with a very stable carboxymethylated dextran matrix that being tethered onto glass-deposited gold thin film (De Crescenzo et al. 2008). CM's dextran matrix have high surface binding capacity that supports a wide range of ligand immobilization chemistry for variety of biomolecules make it suitable for immobilization of any ligands (Pattnaik, 2005). Moreover, it minimizes any nonspecific binding to gold layer, allows covalent immobilization of biomolecules using well-characterised chemistry, and provides hydrophilic environment that suitable for a wide variety of protein interaction (Gao et al. 2008).

The objective of this work was to identify a suitable antibody to be used as a stable molecular recognition element (MRE) on chip for the quantification of hEGF from different fermentation broths based on parameters of affinity and kinetic constant. The study described evaluation of method to quantify hEGF using SPR technique. Two different antibodies, monoclonal and polyclonal, were immobilized onto CM5 chip. Suitable antibody for quantification has been chosen based on several criteria such as higher specificity, kinetics of antibody-antigen complex and the stability for long storage for detection under various conditions.

MATERIALS AND METHODS

Materials

The experiment was conducted using SPR, Biacore 3000 (GE Healthcare) with Biacore 3000 control software (ver. 4.1). CM5 chip and amine coupling kit [containing 1-ethyl-3-(3'-dimethylaminopropyle) carbodiimide (EDC), N-hydroxysuccinimide (NHS) and 1 M ethanolamine hydrochloride (pH8.5)] were purchased from GE Healthcare (Uppsala, Sweden). Anti-EGF monoclonal antibody (mAb) and polyclonal antibody (pAb) were purchased from Calbiochem, Merck, Germany. Standard human EGF (hEGF) was obtained from Peprotech, USA. All other chemicals were purchased from Merck (USA). HEPES buffer [10 mM 4-(2hydroxyethyl)piperazine-1-ethane-sulfonic acid (HEPES)], 150 mM NaCl, 3 mM ethylenediaminetetraacetic acid (EDTA), 0.005% Tween 20 (pH 7.4), sodium acetate buffers (10 mM sodium acetate, with pH 4.0 to 5.5 adjusted by adding 1 M HCl) and glycine buffer (10 mM, pH 2.5) used for regeneration, is filtered and degassed prior to use.

Surface preparation

The scouting for the buffer pH, ranging from 2.5 to 5.5, was carried out to find the optimum conditions for the immobilization of anti-EGF monoclonal (mAb) and anti-EGF polyclonal (pAb) antibody. Immobilization was carried out using amine coupling onto CM5 chip. Prior to activation, the system was equilibrated with running buffer (HEPES buffer, pH 7.4) at a flow rate of 5 µL/min. Equal volume of 100 mM N-hydroxysuccinimide (NHS) and 400 mM 1-ethyl-3-(3'-dimethylaminopropyl) carbodiimide (EDC) mixture was injected to activate the carboxymethylated dextran surface of the CM5 chip, followed by immobilization of both antibodies. Both anti-EGF antibodies were diluted to 10 µg/mL in 10 mM sodium acetate buffer at optimum pH and injected for immobilization at a flow rate of 5 µL/min for 30 min. Deactivating solution, 1 M ethanolamine (pH 8.5) was injected to deactivate unreacted carboxymethylated dextran.

Standard and sample preparation

Standard hEGF at a concentration ranging from 3.9 to 2000 ng/mL was prepared in HEPES buffer. Prior to injection of hEGF standard, the surface of the chip was prepared by flushing the buffer through the chip for 60 sec across the chip surface. The measurement of the binding response was taken after 100 sec of the injection. All the responses were calculated after subtracting the equivalent reference response. Deactivated surface has been used as the reference surface in order to subtract the value of non-specific binding on the chip. After each measurement, the surface was regenerated with one min pulse of 10 mM glycine (pH 2.5) at 5 µL/min. Samples of protein from recombinant *E. coli* and *S. cerevisiae* strains (without hEGF gene) were extracted through glass bead shaking (Ramanan et al. 2008). After the separation of cell debris by centrifugation (Rotor model 1189, Universal 22R centrifuge, Hettich AG, Switzerland), the supernatant was mixed with HEPES buffer at 1:1 ratio and the resultant solution was passed to the surfaces similar to the standards.

Validation parameters

Several validation parameters, which include specificity, range, repeatability, precision, recovery, dilution paradox, sensitivity and stability were determined according to International Conference on Harmonization (ICH) guideline (ICH, 1996).

Specificity. Ultrapure water, BSA, culture medium (LB and BMGY media) and crude extracts (*E. coli* and *P. pastoris*) were assessed to analyze the effect of binding due to the presence of other components in the samples. Crude extracts of *E. coli* and *P. pastoris* were prepared using glass bead shaking method (Ramanan et al. 2008). All samples were mixed with HEPES buffer at a ratio of 1:1 while HEPES buffer alone was used as a reference.

Range. Different concentrations of hEGF were prepared in HEPES buffer, ranging from 3.9 ng/mL to 2000 ng/mL. 4 parameters fit equation was used to generate a standard curve (Equation 1).

$$Y = Rhi - \frac{Rhi - Rlo}{1 + \left(\frac{x}{b}\right)^{c}}$$

[Equation 1]

(Y: Response unit (RU); Rhi: Response values as the concentration tends to infinity; Rlo: Response values as the concentration tends to zero; X: Analyte concentration; b: inflection point; c: slope of curve).

Repeatability and intermediate precision: A series of sample (30-120 ng/mL) were prepared by different analyses (n = 3) and being analyzed on two separate days (n = 2).

Recovery. Crude extracts of *E. coli* and *P. pastoris* were used to prepare 3 samples spikes with known concentration of hEGF. All samples for hEGF recovery were spiked at the level of 30-120 ng/mL.

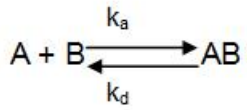
Dilution paradox. hEGF samples were serially diluted in HEPES buffer up to three fold and being quantified in three different days. The samples are being tested to analyze any error due to dilution. This is called “Dilution paradox”.

The life cycle of chip was evaluated by monitoring the reduction in response cycles to ensure the validity of the analytical procedure was maintained whenever used.

Data analysis

For the determination of kinetic rate constant of mAb, all data sets were analyzed globally to a simple 1:1 Langmuir binding interaction model using BIAevaluation Biacore 3000 control software (ver. 4.1).

The same model was applied to pAb to find equivalent kinetic and affinity constants to identify the most suitable MRE to be used in hEGF quantification purpose.



[Equation 2]

k_a, $\frac{1}{Ms}$ is an association rate constant which represents the rate of AB complex formation per second in 1 M solution of A and B. k_d, $\frac{1}{s}$ is a dissociation rate constant, which represent dissociation of AB complexes per second. Dissociation equilibrium constant (K_D) or affinity constant, can be calculated by ratio of rate constants, $\frac{k_d}{k_a}$.

RESULTS AND DISCUSSION

Surface preparation

The sensorgram for anti-EGF monoclonal antibody (mAb) and anti-EGF polyclonal antibody (pAb) immobilization on the sensor surface (CM5) at acidic pH (3.5 and 4 respectively) is shown in Figure 1. The similar amount (~12300 RU) of both antibodies were immobilized on the respective surface of the chip. The surface concentration of the immobilized antibody on sensor surface is 12.3 ng/mm^2 (according to the correlation of SPR response with surface protein concentration, 1 kRU = 1 ng/mm^2) (Stenberg et al. 1991). High density of the ligand was chosen to provide a mass transfer limitation so that the response to different concentrations of hEGF would be significant (Karlsson et al. 1993). Maximum immobilization is required to increase the sensitivity of measurement to enable the quantification of very low analyte concentrations (Wong et al. 2010).

Regeneration using glycine buffer (10 mM, pH 2.5) was performed to remove the bound analytes and to retain the similar activity for the subsequent sample injection. Regeneration solution should not give much changes in the binding capacity of the MRE (Andersson et al. 1999). Regeneration solution washes analyte from capturing agent by breaking non-covalent bonds between the antibody paratope and the antigen epitope. The changes in pH value of buffer could be the prime choice to regenerate the surface. However, the use of high pH would cause removal of MRE attached on the surface (Ramanan et al. 2010). In many cases, HCl at concentration ranging from 10 to 100 mM or 10 mM glycine at pH ranging from 1.7 to 2.2 were used as regeneration solution (Andersson et al. 1999). Glycine buffer (10 mM, pH 2.5) was identified as a preferred regeneration buffer and selected for subsequent experiments.

Validation parameters

The performances of both anti-EGF as MRE in capturing hEGF were characterized to determine whether mAb or pAb would be the most suitable sensor MRE for the quantification of unknown amount of hEGF.

Specificity

The specificity of interaction between different substances and immobilized antibodies (mAb and pAb) are shown in Table 1. A less cross-interaction (RU < 10) with both MRE was evidenced for all the samples. On the other hand, hEGF sample shows high cross-interaction with both immobilized ligands proved the ability of both MRE to ensure the identity of the analyte. The detection of hEGF produced signal responses that varied depending on the mass and analyte being bound due to changes occurred

in the SPR signal for a given change in mass concentration at the surface layer of sensor chip (Hahnefeld et al. 2004). Hence, it is important to consider the ability of MRE to discriminate between target analyte and non-target analyte to have accurate signal from its own analyte.

Table 1. Specificity of anti-EGF monoclonal antibody (mAb) and anti-EGF polyclonal antibody (pAb) used in biosensor chip.

Sample	Protein concentration (mg/mL)	Response (RU)	
		mAb	pAb
Ultra pure water	0	2.94 (0.64)	-14.24 (2.57)
HBS-EP buffer	0	-0.02 (1.21)	1.60 (1.04)
BSA	0.5	2.81(0.26)	-0.98 (1.98)
BSA	5	5.28 (0.49)	2.60 (0.24)
culture medium (LB)	ND	3.23 (0.99)	6.24 (0.89)
culture medium (BMGY)	ND	4.30 (1.05)	7.31 (0.12)
crude extract *(E. coli)* no hEGF	ND	0.27 (0.35)	-1.54 (0.24)
crude extract *(P. pastoris*) no hEGF	ND	9.29 (0.35)	3.88 (2.21)
hEGF	2.5e-4	81.41(0.43)	153.79 (1.37)

* ND: Not determined. Samples were analyzed in triplicates. Their means (n = 3) and standard deviation (in parentheses) are reported.

Since mAb recognizes only one epitope on the antigen, they are more specific in detecting the target analyte with less likely to cross-react with other proteins. Because of their specificity, mAb are excellent as the primary antibody in an assay and often give significantly less background signals than pAb. On the other hand, pAb recognizes multiple epitopes on the antigen that lead to an increase in the signal produced. In this case, both immobilized anti-EGF antibodies are approved specifically enough to be used as MRE as the entire samples gave less cross-interaction with both MRE. Hence, both immobilized antibodies are specific for target protein binding.

According to ICH guidelines, specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. The binding of analyte to MRE should not affect with other components such as impurities, degraded compounds and resins by competing the same binding site with the target protein. Substances purity needed is not so strict as long as the contaminants do not interrupt the response (De Crescenzo et al. 2008). Moreover, analyte/ligands interactions should not interfere significantly with buffer/medium used to prepare the analyte (Chavane et al. 2008). Maximum specificity is required to minimize non-specific binding to obtain accurate responses for a given assay since nonspecific adsorption can lead to background signals that are able to interrupt signals for interactions (Taylor et al. 2008).

Range

The assay response to the increment in hEGF concentration was not linear. Hence, 4 parameters fit equation was used to generate a standard curve. The responses of pAb and mAb with respect to different concentrations of hEGF (0 to 2000 ng/mL) are shown in Figure 2.

The range of an assay is the interval between (and including) the upper and lower limits of analyte quantitation with acceptable precision and accuracy. Maximum response to the particular concentration of hEGF was different for both antibodies, demonstrating the different intervals between lower and upper limit quantitation of hEGF for both MRE. Different maximum responses between these two antibodies were observed, suggesting their differences in the kinetics and affinity constants. The pAb approached a steady-state response at 250 ng/mL while mAb approached a steady-state response at 500 ng/mL. The response approached a steady state when high concentration of analyte was used due to equilibrium kinetics. This is the point where the active ligand surface is reduced due to the occupation of analyte (hEGF) to the ligand. Further increase of analyte concentration does not affect the response. It is important to note that the response of pAb (154 RU) to 250 ng/mL was higher than the response of mAb (103 RU) to 500 ng/mL. Higher response (RU) was achieved for pAb at particular

concentration might be due to the recognization of multiple epitopes on the antigen that lead to an increase in the signal produced.

Repeatability and Intermediate precision

Figure 3 shows the response of interaction with respect to the concentration. Repeatability and precision were evaluated to obtain reproducible results each time biosensor was reused over time with different analysts. Repeatability, which is also referred as intra-assay precision express precision of the assay under same operating conditions over a short period of time. Intermediate precision (inter-assay precision) is the precision within laboratory over different operators in different occasions. Both MRE were reproducible and have good intermediate precision. Precision (intra-assay and inter-assay) are within acceptance criteria since % CV (Coefficient of Variation) fell in range < 17 for both antibodies.

Recovery and dilution paradox

The suitable antibody will be used as a stable MRE on chip for the quantification of hEGF from different fermentation samples. Hence, spiking of analytes with appropriate levels of different impurities was performed to establish discrimination of analytes with the availability of impurities. Samples were prepared by diluting hEGF standard in impurities solution; crude extract of *E. coli* and *P. pastoris* and the recovery were monitored. Table 2 shows the average recovery at different spiking levels in different protein extracts containing hEGF. Acceptable recovery should be fall within 80% to 120% and all samples tested showed that the percentage recovery of expected value is more than 87%. Hence, it was considered as significant since high recovery indicated the presence of impurities that did not affect the assay result and the measurements were due to the presence of hEGF. Meanwhile, a series of samples were prepared in buffer by serial dilution and then quantified in three different days (Table 3). The result indicated that almost 90% hEGF was recovered using both antibodies as MRE.

Table 2. The average recovery at different spiking levels in different protein extracts containing hEGF.

Type of antibody	Source of protein extract	Expected value (ng/mL)	Observed value (ng/mL)	Percentage recovery of expected value (%)
anti-EGF monoclonal antibody (mAb)	*E. coli*	30	32.67 (0.32)	108.89
		60	70.33 (0.29)	117.21
	P. pastoris	30	33.66 (1.92)	112.20
		60	66.16 (0.67)	110.26
anti-EGF polyclonal antibody (pAb)	*E. coli*	30	26.26 (0.58)	87.53
		60	54.35 (0.57)	90.58
	P. pastoris	30	34.32 (0.52)	114.40
		60	65.19 (0.83)	108.66

Samples were analyzed in triplicates. Their means (n = 3) and standard deviation (in parentheses) are reported.

Sensitivity and stability

Determination of detection limit (DL) was performed to establish the sensitivity level for the detection of analyte. On the other hand, quantification limit (QL) was used to determine the minimum level of analyte could be quantified with acceptable accuracy and precision. The DL and QL values for mAb and pAb are shown in Table 4. The DL for pAb and mAb was 2.7 ng/mL and 3 ng/mL respectively. The QL for mAb (9.15 ng/mL) was slightly higher than the QL for pAb (8.3 ng/mL), which suggesting pAb can quantified lower concentration of analyte with acceptable accuracy and precision compare to mAb.

Stability is important to ensure the validity of the analytical procedure is maintained whenever used. Sensor performance was monitored along the usage in order to evaluate the stability of the chip (Table 5). The result showed that the binding capacity of mAb was reduced at every ~300 run cycles while the binding capacity of pAb could be maintained more than 1000 cycles for quantification of the target analyte. Chip storage and applications under harsh environment may affect the stability of MRE and hence, storage at 4ºC may keep the MRE stable. Preparation of samples using running buffer at mild pH may also prevent the degradation of MRE. This allows the chip to be used repeatedly in many cycles. Sensor surfaces need to be recyclable in order to reduce cost and time of detection assays

concomitant with the advantages of using optical biosensor (Strehlitz et al. 2008; Taylor et al. 2008). Convenient methodology is crucially needed especially for the determination of product concentration at time intervals during the production.

Table 3. Comparison of the use of different antibodies as MRE on the dilution paradox for the detection of hEGF using SPR.

Sample ID	Expected value, ng/mL	Observed value, ng/mL				Percentage recovery of expected value (%)			
		Day 1	Day 3	Day 7	% CV	Day 1	Day 3	Day 7	Overall
Anti-EGF monoclonal antibody (mAb)									
3X	30	31.88 (0.63)	33.26 (0.16)	31.74 (0.25)	2.19	106.28	110.88	105.81	107.65
2X	60	65.95 (0.06)	73.46 (0.28)	66.58 (0.52)	4.67	109.92	122.44	110.97	114.44
1X	120	118.19 (0.16)	112.46 (0.2)	115.81 (0.78)	5.83	98.49	93.72	96.51	96.24
Anti-EGF polyclonal antibody (pAb)									
3X	30	26.44 (1.42)	25.81 (0.55)	28.36 (1.03)	4.94	88.13	86.02	94.52	89.56
2X	60	50.57 (1.04)	57.59 (0.11)	59.94 (0.28)	8.70	84.28	95.98	99.90	93.39
1X	120	117.70 (0.13)	132.50 (0.28)	135.29 (0.22)	7.36	98.09	110.41	112.74	107.08

Samples were analyzed in triplicates. Their means (n = 3) and standard deviation (in parentheses) are reported.

Table 4. Parameters of standard curve.

	Anti-EGF monoclonal antibody (mAb)	Anti-EGF polyclonal antibody (pAb)
Rhi	139.80	179.10
Rlo	3.42	5.10
b	199.20	54.87
c	1.15	1.28
DL	3.02	2.74
QL	9.15	8.30

Table 5. Stability of the chip.

	Anti-EGF monoclonal antibody (mAb)		Anti-EGF polyclonal antibody (pAb)	
	Response Unit (RU)	Total no of cycles	Response Unit (RU)	Total no of cycles
1	51.03	288	135.20	213
2	22.71	394	120.35	1416

*at concentration 125 ng/mL.

Evaluation of kinetics and equilibrium constants

SPR provide advantages in the estimation of association and dissociation equilibrium constant (affinity), distinct association and dissociation rate constant separately since it is a real time procedure (Hahnefeld et al. 2004). Reaction rate constant will determine the rate of antibody binding when an analyte is allowed to react with immobilized ligand (Karlsson et al. 1993).

Table 6 represents values of rate constant and affinity for both antibodies using antigen binding data. The association and dissociation rate of both antibodies fall in the range of most rate constant reported in the literature (Karlsson, 1999). Quantitatively, pAb shows faster association rate ($1.76e^{6}$ M^{-1} s^{-1}) as compared to mAb ($7.48e^{5}$ M^{-1} s^{-1}). There is a difference in affinity between the weaker binder, mAb (K_D = 9.54 nM) and the tightest binder, pAb (K_D = 0.739 nM). The dissociation equilibrium constant, K_D can be calculated from the ratio of k_d and k_a.

Table 6. Kinetic and affinities constant of two different antibodies.

	k_a (M^{-1} s^{-1})	k_d (s^{-1})	K_D (M)
Anti-EGF monoclonal antibody (mAb)	$7.48e^{5}$	$7.14e^{-3}$	$9.54e^{-9}$
Anti-EGF polyclonal antibody (pAb)	$1.76e^{6}$	$1.3e^{-3}$	$7.39e^{-10}$

The analyzed data and fitted data for mAb and pAb in a series of hEGF concentrations are shown in Figure 4. All responses were fit globally (graphically and numerically) to a 1:1 Langmuir binding interaction model using BIAcore. There are not many differences deviations between experimental data (solid lines) and overlaid fitted data (dashed line). This gives a good indication of the reliability and validity of the fitting. Figure 5 shows a plot of residual from the entire set data for mAb and pAb respectively. The residual plot assesses the quality of the fit to the reaction model by depicting the deviation of the experimental data from the theoretical fit. Points in the residual plot are scattered within a band. For pAb, points in the residual plot are scattered within a band (low level of noise) and shows less systematic differences between fitted and experimental data. These results illustrate that it is possible to interpret accurately equivalent binding responses for pAb. On the other hand, the residual plot for mAb shows a little systematic differences between fitted and experimental data with low level of noise in association data. However, it is still considered fitted since for typical sensorgram, the noise level should not exceed 1 RU and in the global analysis, the noise level might be higher. While, dissociation data are independent of the concentration of analyte, hence residuals in the dissociation phase are less significant (Karlsson and Larsson, 2004). The data will poorly fit the model if the residuals vary systematically, or if their magnitude exceeds machine noise (Kuziemko et al. 1996).

A two-dimensional distribution kinetic plot of the kinetic binding constant can also be used to visualize affinities distribution of both antibodies tested in this study (Figure 6). This distribution kinetic plot is constructed by plotting the association rates versus the dissociation rates for each compound. The diagonal lines represent equilibrium isotherms to help with visualization of affinity distribution which higher affinities complexes appear in the upper right-hand corner. The data point further up on the y-axis represent slower dissociation rates while faster residuals in association rates points to the right on the x axis. Hence, pAb appears to have better characteristic in term of affinity and reaction rate as compared to mAb. This interpretation is consistent with the quantitative data presented in Table 6.

The properties of good bio-recognition elements are high specificity, high affinity of antibody-antigen complex and have the stability for long storage and for detection under various conditions (Hahnefeld et al. 2004; Taylor et al. 2008). Based on good reproducibility achieved with pAb immobilized surfaces, pAb surfaces were then further employed to measure unknown concentration of hEGF content in the culture samples. On a similar note, high affinity antibody was selected to monitor the production of monoclonal antibody by hybridoma cells based on its excellent reproducibility within initial rate reaction, antibody accumulation at the end of the injection and surface life time (number of cycles) (Chavane et al. 2008).

CONCLUDING REMARKS

A method to select a better MRE for quantification of hEGF using SPR was presented. Several parameters such as specificity, range, repeatability, precision, recovery, dilution paradox, sensitivity and stability were determined accordingly for the validation of biosensor method for hEGF quantification. Accurate results were achieved as determined according ICH guidelines. Repeatability and intermediate precision values within laboratory (intra-assay and inter-assay), limit of detection, quantification limit and measurement range demonstrated the ability of SPR system to quantify our target molecule.

Specificity, accuracy and precision for all samples are within acceptable limit and hence, it is suitable to be applied for rapid quantification method. Polyclonal antibody (pAb) with better affinity reveals that analyte will bind to pAb stronger/tighter than mAb. pAb also have higher level of steady state and faster reaction rate as compared to monoclonal antibody (mAb). Furthermore, pAb has longer shelf life and greater cycle can be run. Hence, pAb is more suitable to be used as stable molecular recognition element from the kinetics and shelf life assessment aspects.

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Figures

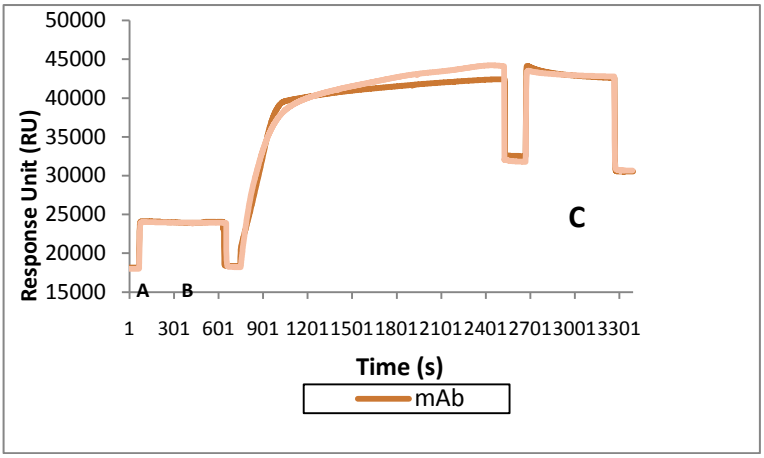

Fig. 1 The sensorgram shows the procedure of mAb and pAb immobilization on the sensor surface. During immobilization, a constant flow of HEPES buffer of 5 µl/min was maintained. (A) Activation; NHS-EDC mixture was injected to activate the carboxymethylated dextran surface of the CM5 chip; (B) Immobilization; both mAb and pAb (10 µg/mL) in 10 mM sodium acetate buffer (pH4.5) were injected; (C) Deactivation; 1 M ethanolamine (pH 8.5) was injected to deactivate unreacted carboxymethylated dextran.

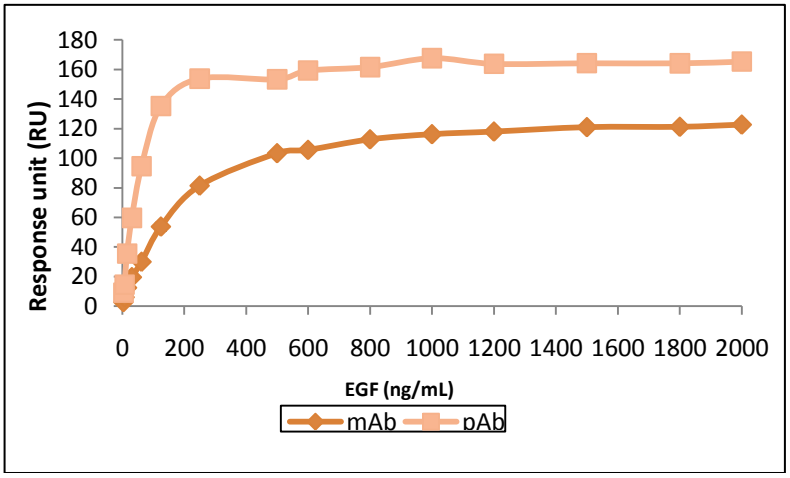

Fig. 2 Comparison of the use of different antibodies as MRE on the accuracy of the detection of hEGF using SPR. Different concentrations of hEGF ranging from 3.9 ng/mL to 2000 ng/mL formed a non-linear curve. At some point, the responses approached a steady-state due to equilibrium kinetics.

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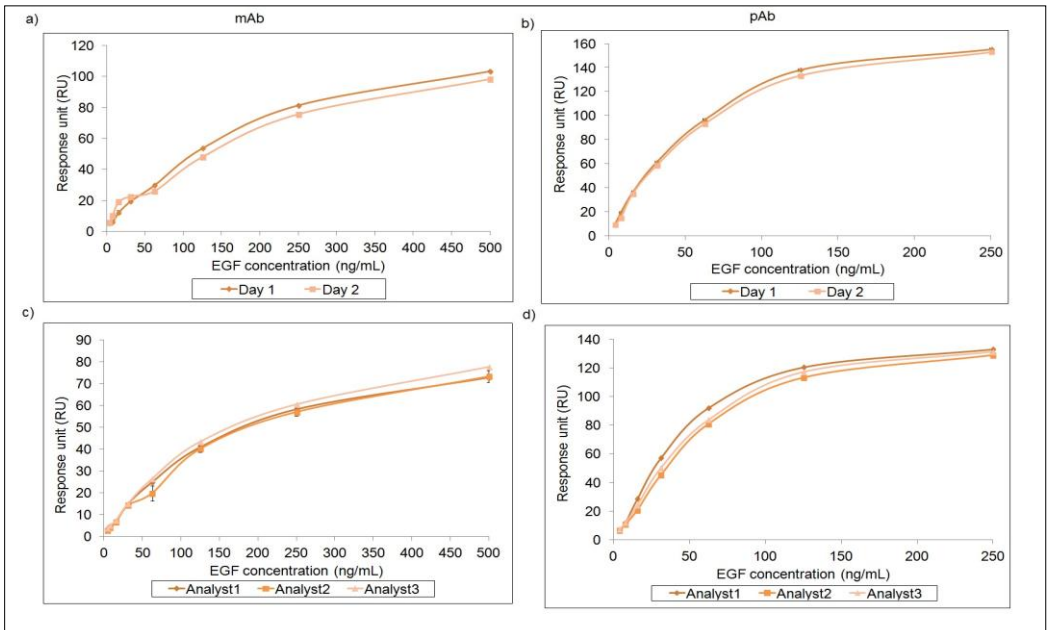

Fig. 3 Profile of repeatability (a and b) and intermediate precision (c and d) for hEGF quantification using different antibodies as MRE.

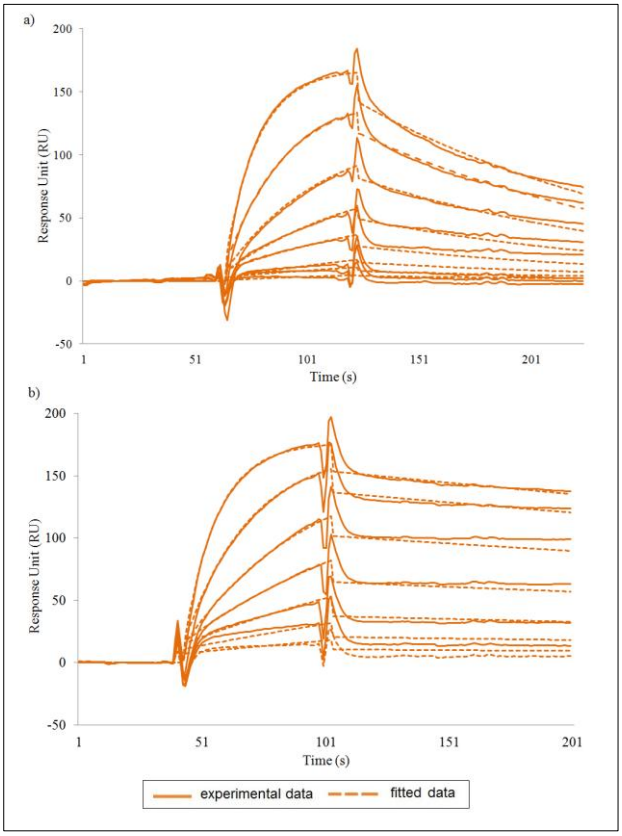

Fig. 4 Global analysis of protein-antibody interaction. hEGF responses and their fitted were overlaid in a series of hEGF concentrations; a) mAb: 3.9 to 500 ng/mL b) pAb: 3.9 to 500 ng/mL. All responses were fit globally to a 1:1 Langmuir binding interaction model using BIACORE.

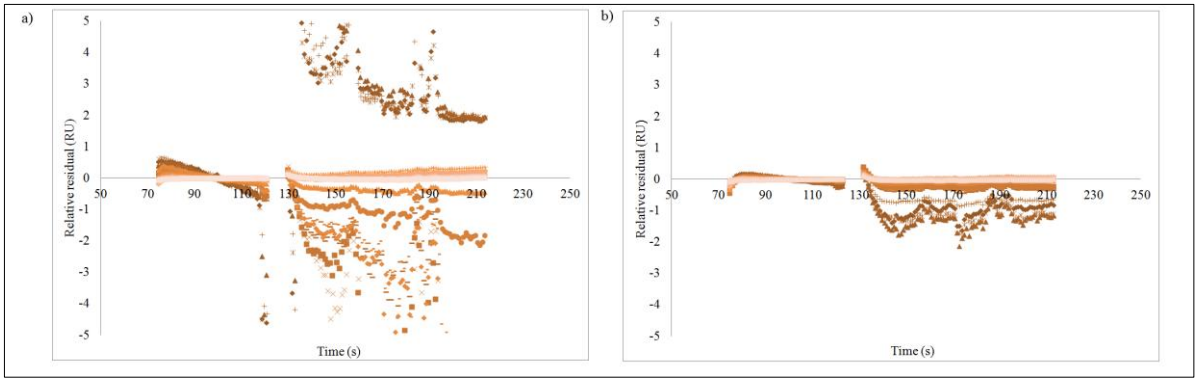

Fig. 5 Residual plot obtained in data analysis within the concentration range using 1:1 Langmuir binding interaction model for a) Anti-EGF monoclonal antibody (mAb); b) Anti-EGF polyclonal antibody (pAb).

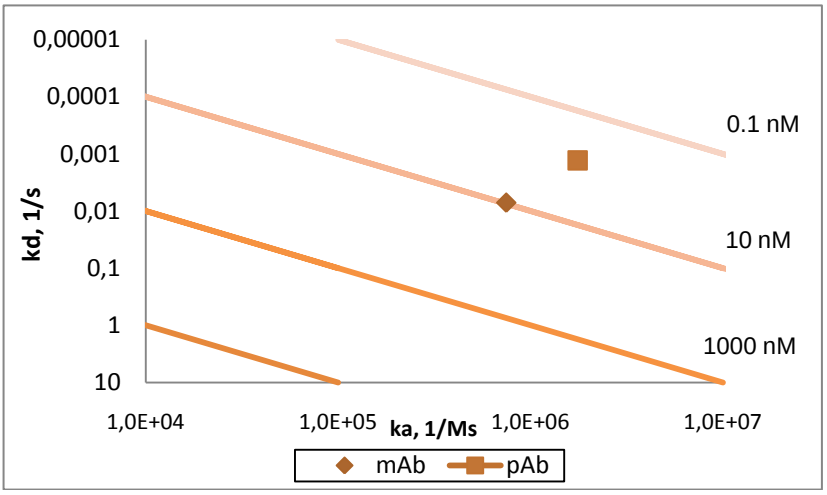

Fig. 6 Kinetic distribution plot of two different antibodies. Rate constants were determined from BIACORE. The diagonal lines represent equilibrium binding isotherms.

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