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# A new approach for pharmacokinetics of single-dose cetuximab in rhesus monkeys by surface plasmon resonance biosensor

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ABSTRACT

A novel assay method has been developed and validated, using surface plasmon resonance (SPR), for quantitation of cetuximab (C225) in monkey serum. By injecting non-labeled antibody samples onto a biosensor surface on which epidermal growth factor receptor (EGFR) was immobilized, the concentration of C225 can be accurately measured. This assay has a range of reliable response from 0.05 to 50 μg/ml C225 in monkey serum, which was well fitted with a sigmoidal model. The immobilized EGFR was found to be stable for at least 100 regeneration cycles at room temperature. Intra- and inter-assay CVs ranged from 3.20% to 8.89% and from 5.93% to 11.11%, accuracy from 92% to 107.52% and from 90% to 106.88%, respectively. Matrices such as 50% human serum, 50% Sprague Dawley rat serum, chimeric recombinant anti-CD20 monoclonal antibody, human γ-globulin and chimeric recombinant her2 antibody did not interfere with C225 analysis on the sensor surface. This is the first report on the quantitation of C225 in monkey serum by an optical biosensor technology. This method was used to characterize the pharmacokinetics of C225 in rhesus monkeys. After a single-dose of intravenous infusion administration of 7.5, 24 and 75 μg/kg, average $C_{max}$ ranged from 168 ± 28 to 1624 ± 113 μg/ml, and $AUC_{0-\infty}$ ranged from 15,739 ± 1059 to 295,017 ± 44,533 μg h/ml. C225 elimination followed a bi-exponential profile with $t_{1/2}$ ranging from 2.7 ± 0.7 to 6.7 ± 0.1 h. It was non-linear serum pharmacokinetics of C225 across the investigated dosage range in monkeys (7.5–75 mg/kg).



## 1. Introduction

Epidermal growth factor receptor (EGFR) is a protein tyrosine kinase which plays a crucial role in signal transduction pathways regulating key cellular functions such as survival and proliferation. Among the recent advances in the molecular targeted therapy of cancer, the applications centered on EGFR are currently the most promising and the most advanced at clinical level [1]. Considering the set of therapeutic tools targeting EGFR [2], there are at present two well-identified emerging categories of drugs with monoclonal antibodies (Mabs), on one hand, tyrosine kinase inhibitors (TKIs) and on the other hand, EGFR inhibitors. Cetuximab (C225) is a Mab (IgG1) directed at the extracellular domain of the receptor, and a novel biologic agent that has been shown both in vitro studies and in vivo animal xenograft models to have profound synergy when combined with either platinum drugs or with other chemotherapeutic agents or radiation therapy [3,4]. Cetuximab, a chimerized monoclonal antibody, was developed to target the EGFR. Cetuximab binds to the extracellular domain of the EGFR thereby preventing ligand activation of EGFR [5]. The resultant inhibition of EGFR signaling can lead to cell cycle arrest, cell death via apoptosis, and inhibition of cell invasion and angiogenesis.

Overexpression of EGFR mRNA and/or protein has been documented in a number of malignancies, including ovarian cancer. Approximately 35% to 70% of ovarian cancers expressed EGFR mRNA analyzed via reverse transcriptase–polymerase chain reaction and radioligand binding assays and 98% demonstrated EGFR protein expression by Western blot analysis [6–8]. High EGFR expression in ovarian cancer specimens has been associated with advanced stage, an aggressive phenotype, and poor clinical outcome [6,7,9,10]. In addition, high EGFR expression has been associated with chemotherapy resistance in human cancer cell lines. EGFR expression as well as the expression of EGFR-related proteins has been shown to become more intense and diffuse in tumor specimens obtained after treatment with cisplatin compared to the staining in matched pretreatment tumor specimens [11]. Therefore, the use of an EGFR inhibitor such as cetuximab to disrupt the EGFR signaling pathway could potentially inhibit the emergence of chemotherapy resistance.

In preclinical studies, cetuximab has been found to repress the growth of cultured A431 tumor cell lines and xenografts that express high levels of EGFR [12,13]. Cetuximab has also been

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shown to enhance the effects of a variety of chemotherapeutic agents, including platinum compounds, in a variety of human tumor cell lines that express the EGFR, including ovarian cancer cell lines [9,14,15]. These intriguing preclinical findings have been supported by the results of clinical trials that revealed that the addition of cetuximab plus cisplatin in patients with platinum resistant squamous cell carcinoma of the head and neck resulted in objective responses [16]. Furthermore, phase III clinical trials demonstrated that combination of cetuximab and chemotherapy yielded superior response rates and in some cases improved survival in patients with head and neck cancer and colorectal cancer [17,18].

Some reports on clinical pharmacokinetic study showed that serum concentrations of cetuximab were measured using ELISA [19–22]. However, there is no report in literature on the preclinical pharmacokinetic data.

Radioimmunoassay and ELSA method are traditionally used for quantitative analyses of biotechnology derived drugs in biomatrics. Biomolecular interaction analysis (BIA) from Biacore uses the optical phenomenon of surface plasmon resonance (SPR) to monitor biomolecular interactions in real time without labeling. SPR measures change in refractive index of the solution close to the senor surface, resulting from changes in the mass concentration of molecules in the solution [23]. The Biacore system has some advantages in immunoassays over the conventional ELISA or RI methods. First, SPR technology can be used to measure complex formation without labeling the reactants. Second, complex formation can be monitored in real time, providing detailed information about the reaction kinetics, and equilibrium dissociation constants (affinities). Third, crude samples may be analyzed without sample preparation. Nowadays, researches using Biacore focused on thermodynamics, kinetics and affinity studies. To concentration analysis, there are some reports on determination of substance residues in food or environments [24–27] and few reports on drug quantitation in biomatrics for pharmacokinetic studies using Biacore. Kikuchi et al. [28] first reported Biacore application for pharmacokinetic study. However, there was no validation of the method. In addition, sensitivity of the method was low. The company, which developed cetuximab first, has conducted the SPR measurements for quantitation of C225 [29], but there was no report on the assay method.

Although it has good clinical effect, the price of cetuximab is too high, more than $15,000 per month, to afford for common patients especially patients in developing countries. The company in China manufactured cetuximab by itself and the price is less expensive. In our study, using the Biacore system, we developed a method to measure the concentration of C225 donated by the Chinese company in monkey serum. Here we show the characteristics and advantages of this assay method and its application for pharmacokinetic studies. There is the first report on the quantitation of C225 in monkey serum using Biacore.

## 2. Materials and methods

### 2.1. Materials

- Biacore 3000 biosensor instrument, amine coupling kit, sensor chip CM5 and P-20 Surfacant (BIACORE, Uppsala, Sweden)
- Running buffer–HBS buffer (HEPES buffered saline): 10 mM HEPES with 0.15 M NaCl, 3.4 mM EDTA and 0.05% P-20 surface, pH 7.4
- Sample diluent: HBS containing 1 mg/ml carboxymethyl dextran (Fluka Chemical Corp., Ronkonkoma, NY)
- Regeneration solution: 10 mM HCl + 1 M NaCl
- Pooled normal monkey serum from Laboratory Animal Center of the Academy of Military Medical Sciences
- EGFR (170 kD) was purchased from Sigma Corporation.
- C225 was donated by Huabei Pharmaceutical Factory, Hebei Province, China.

### 2.2. Animal

Rhesus monkeys were supplied by the Laboratory Animal Center of the Academy of Military Medical Sciences. The animals were individually housed in stainless steel cages in a room with controlled temperature ($25 \pm 1^{\circ}$C) and humidity ($55 \pm 5\%$) and a 12 h light/dark cycle.

The animals were fed with standard diet and had free access to water. All procedures involving animals and their care were carried out according to the guidelines of the Institutional Ethical Committee for Care and Use of Laboratory Animal of Academy of Military Medical Sciences in accordance with the governmental guidelines on animal experimentation, National Institutes of Health "Principles of Laboratory Animal Care".

### 2.3. Biacore quantitative assay for C225 in monkey serum

EGFR was selected for C225 analysis and immobilized onto a flow cell of CM5 sensor chip using the amine coupling kit according to the procedure described by the manufacturer. During immobilization, HBS was used as a mobile phase at a flow rate of 10 μl/min. The carboxymethyl dextran matrix of the sensor chip surface was first activated with an injection of 70 μl of the EDC/NHS reagent mixture. Then, 70 μl of EGFR (1 μg/ml in 10 mM NaOAc (pH 5.7)) was injected and allowed to covalently couple to the sensor surface. Finally, the unreacted sites were blocked by injection of 70 μl of 1 M ethanolamine (pH 8.5). The samples were assayed over the immobilized EGFR sensor surface at room temperature. The mobile phase was HBS at a flow rate of 10 μl/min. Biacore response of about 5500 RU was achieved after immobilization. For concentration measurements, an untreated surface was used as the reference cell. During analysis, 10 μl of C225 standard sample and five times diluted serum sample was injected and passed over the reference and the EGFR immobilized surface. Data from the reference flow cell were subtracted to remove the effects of non-specific binding. Regeneration of the sensor surface was achieved by injecting 10 μl of 10 mM HCl plus 1 M NaCl, followed by a return to HBS.

### 2.4. Validation of Biacore quantitative assay for C225 in monkey serum

Validation was based on the FDA guidelines for Bioanalytical Method Validation [29].

#### 2.4.1. Construction of C225 standard curve

A series of six standard samples from 50 to 0.05 μg/ml of C225 in monkey serum were prepared. Before assayed, the standard samples were diluted 1:5 into sample diluent. The relationship between Biacore response and C225 concentration was described by a four-parameter sigmoidal model:

$$R = \frac{R_{\max} + (R_{\min} - R_{\max})}{[1 + (C/\mathrm{EC}_{50})^{r}]} \quad (1)$$

where $R$ is Biacore response, $R_{\max}$ is the estimated maximum of the function, $R_{\min}$ is the estimated minimum of the function, $C$ is C225 concentration, $\mathrm{EC}_{50}$ is the estimated midpoint of the regression line, and $r$ is the slope of the apparent linear region of the curve. A logarithmic calibration plot was then constructed of the normalized binding response versus C225 concentration.

#### 2.4.2. Quantitation limit

The quantitation limit was evaluated by repeatedly assaying the lowest level standard of 0.05 μg/ml C225 in monkey serum and analyzing accuracy and the coefficient of variation (CV). Before assayed, the standard samples were diluted 1:5 into sample diluent.

#### 2.4.3. Baseline stability (regeneration)

For assaying the concentration of C225 in monkey serum sample, four different concentrations of the C225 were added to monkey serum and assayed at the beginning, middle and end of 100 cycles. Before assayed, the standard samples were diluted 1:5 into sample diluent.

#### 2.4.4. Precision and accuracy of the assay

To establish intra-assay precision, three samples of C225 were prepared in monkey serum at concentrations of 0.1, 1.00 and 10.00 μg/ml and assayed six times each. For inter-assay precision assessment, the samples were assayed on six different days. Estimates of precision were expressed as a CV relative to the overall mean observed concentration for all analytical runs at each concentration level. Estimates of accuracy were expressed as the percentage of the overall mean observed concentrations versus the corresponding actual concentration.

#### 2.4.5. Specificity of the assay

The specificity of the assay was tested by adding C225 to 50% human serum, to 50% Sprague Dawley rat serum, to 20% pooled monkey serum containing 5 μg/ml of chimeric recombinant anti-CD20 monoclonal antibody, human γ-globulin and chimeric recombinant her2 antibody, respectively, to test the effect on binding of C225 to the immobilized EGFR sensor surface.

### 2.5. Pharmacokinetic studies

#### 2.5.1. Experimental design and dosage groups

The developed method was used to evaluate the pharmacokinetics of C225 in rhesus monkeys. Nine male rhesus monkeys (weighing 4.3 ± 0.7 kg) were used in pharmacokinetics experiments. Monkeys were divided into three equal groups by a simple randomization method. 30 min intravenous infusion of 7.5, 24, or 75 mg/kg dose was administered to study the linear characteristics of pharmacokinetics.

The blood samples were drawn from the femoral veins of the animals using a puncture needle before dosing (0 h), at 10, 20, 30 min during intravenous infusion and 1, 4, 8, 12, 24, 72, 120, 168, 216, 264, 312, 360 and 456 h after dosing. Freshly collected whole blood were immediately transferred to heparinized tubes, followed by centrifugation (1800 × *g* for 15 min at 4 °C) to separate serum. Serum samples were collected and kept at −20 °C before analysis.

#### 2.5.2. Pharmacokinetic analysis

Pharmacokinetic data analysis was performed by the non-compartmental method. The maximum serum concentration ($C_{max}$) and the time to $C_{max}$ ($T_{max}$) were determined from the observed serum concentrations of C225. The terminal elimination half-life ($t_{1/2}$) was calculated as $0.693/k_{el}$, where the $k_{el}$ was apparent elimination rate constant of C225 from serum. The area under the serum concentration–time curve ($AUC_{0-\infty}$) from zero to infinity was calculated as the sum of $AUC_{0-t} + AUC_{t-\infty}$. $AUC_{0-t}$ from zero to the last measurable time was calculated by trapezoidal rule and $AUC_{t-\infty}$ was calculated as $C_t/\lambda_n$, where $C_t$ was the last observed serum concentration after administration and $\lambda_n$ was elimination constant calculated from the slope of the terminal phase of the serum concentration curves. The apparent total clearance (CL) was calculated as dose/$AUC_{0-\infty}$. The volume of distribution at steady state ($V_{ss}$) and the mean residence time (MRT) were calculated by the non-compartmental method.

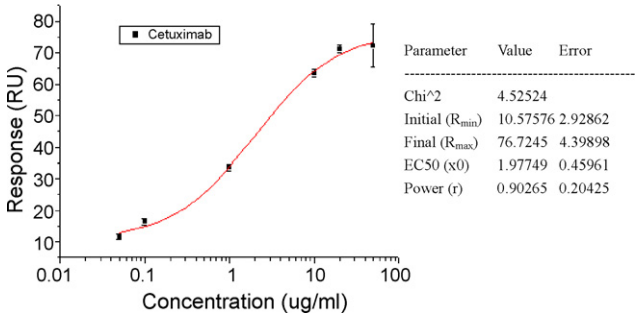


**Fig. 1.** The standard curve of C225 qunatitated by Biacore in 20% monkey serum. The fitted curve was based on the four-parameter sigmoidal model (average ± SD, $n = 6$).

#### 2.5.3. Statistical analysis

All parameters are expressed as mean ± SD unless noted. Dose proportionality after a single intravenous infusion administration of different dosages was evaluated by comparison of the dose normalized $C_{max}$ and $AUC_{0-\infty}$ across dosage levels using an ANOVA and linear regression analysis. Statistical analysis was performed using Origin 6.0 (Microcal Software Inc., USA). A *P*-value below 0.05 indicates significant difference between data means.

## 3. Results

### 3.1. Validation of Biacore quantitative assay for C225 in monkey serum

#### 3.1.1. Curve fitting for C225 standard curve

A series of dilutions from 50 to 0.05 μg/ml of C225 in monkey serum was prepared and assayed. A four-parameter sigmoidal model was used to fit the standard curve (Fig. 1). As shown in Table 1, at each C225 level, the value predicted by the model was close to the experimental data, which indicated the model fits well to the C225 standard curve over the concentration range.

#### 3.1.2. Determination of the lower limit of quantitation

To determine whether the lower concentration on the standard curve can be measured with acceptable accuracy and variability, C225 serum sample at 0.05 μg/ml was assayed six times. The results are presented in Table 2. The accuracy was 99.14% and precision 1.8%. However, CV of C225 serum sample at 0.025 μg/ml was 21.27% overrun the criteria for validation of analytical. Therefore, the concentration of 0.05 μg/ml, as a lower limit of quantitation of this assay, can be measured with good accuracy and precision according to the criteria for validation of analytical methods [29].

**Table 1**
Results of the C225 standard curve fitted with a four-parameter sigmoidal model.

| C225 (μg/ml) | Biacore response (RU) | | |
|---|---|---|---|
| | Experimental[a] | Predicted[b] | Residue[c] |
| 50 | 72.31 ± 6.85 | 73.3257 | −1.02 |
| 20 | 71.24 ± 1.31 | 69.43456 | 1.81 |
| 10 | 63.55 ± 1.32 | 64.28771 | −0.74 |
| 1 | 33.51 ± 0.98 | 33.78183 | −0.27 |
| 0.1 | 16.31 ± 1.14 | 14.76532 | 1.54 |
| 0.05 | 11.56 ± 0.96 | 12.88479 | −1.32 |

[a] Mean ± SD, $n = 6$.
[b] Based on Eq. (1).
[c] Residue was the value of the experimental response minus predicted response.

**Table 2**
Evaluation of the limit of quantitation.

| Add concentration ($\mu g\,ml^{-1}$) | Found concentration ($\mu g\,ml^{-1}$) | Accuracy (%) | CV (%) |
|---|---|---|---|
| 0.05 | 0.04<br>0.04<br>0.05<br>0.04<br>0.05<br>0.03 | 99.14 | 1.8 |

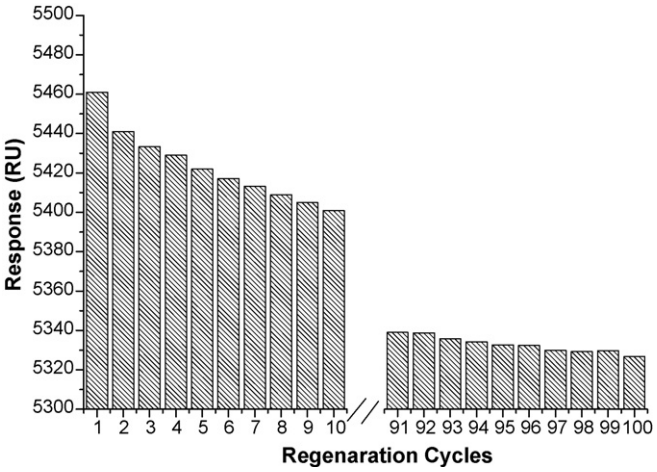


**Fig. 2.** EGFR was immobilized onto the sensorchip surface. After C225 addition, surface was regenerated with 10 mM HCl plus 1 M NaCl. The response of immobilized EGFR surface were obtained after each regeneration for 100 cycles.

### 3.1.3. Regeneration

Since the immobilized EGFR can be neutralized in this assay system, it is imperative to determine how many times it can be used and regenerated without significant loss of assay sensitivity or accuracy. As shown in Fig. 2, it was demonstrated that EGFR immobilized onto the sensorchip could withstand at least 100 regeneration cycles without loss of activity or significant change in baseline (accuracy 98.40%, CV 0.69%) using 10 mM HCl + 1 M NaCl. In Fig. 3, the binding capacity of four different concentrations of C225 was shown. Binding capacity changed less than 5% from the beginning to the end of 100 regenerations.

### 3.1.4. Precision and accuracy of the assay

To intra-assay, the CV values for six injections at each concentration level were all below 8.89% (Table 3). Each mean value calculated was from 92% to 107.52% of the actual value. These results confirm that the assay has good intra-assay precision and accuracy. For inter-assay reproducibility assessment, the samples were assayed on six different days. The CV values at each level were all below 11.11% (Table 3). All mean accuracy values were from 90% to 106.88%. Therefore, this assay also has good inter-assay precision and accuracy.

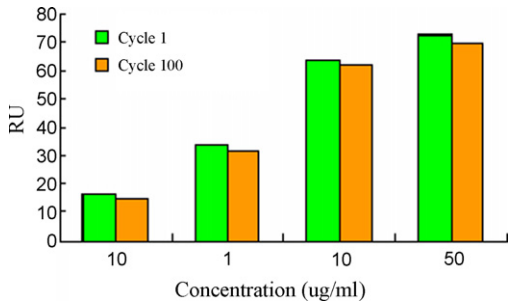


**Fig. 3.** The binding capacity of immobilized EGFR was evaluated throughout 100 regeneration cycles using 10 mM HCl + 1 M NaCl. Four different concentrations of C225 diluted into 20% monkey serum were injected and the amount bound to the EGFR was measured initially and after 100 regeneration cycles.

**Table 3**
Determination of the accuracy and precision of intra-assay and inter-assay.

| | C225 Concentration ($\mu g/ml$) | | |
|---|---|---|---|
| | 0.1 | 1 | 10 |
| Intra-assay ($n = 6$) | | | |
| Observed mean[a] ($\mu g/ml$) | 0.92 | 0.96 | 10.75 |
| SD ($\mu g/ml$) | 0.029 | 0.08 | 0.96 |
| CV (%) | 3.20 | 8.37 | 8.89 |
| Accuracy (%) | 92.00 | 96.00 | 107.52 |
| Inter-assay ($n = 6$) | | | |
| Observed mean[b] ($\mu g/ml$) | 0.09 | 0.97 | 10.69 |
| SD ($\mu g/ml$) | 0.01 | 0.06 | 1.02 |
| CV (%) | 11.11 | 5.93 | 9.52 |
| Accuracy (%) | 90.00 | 96.60 | 106.88 |

[a] From six replicates.
[b] From six independent assays, each in triplicate.

### 3.1.5. Specificity of the assay

From Table 4, when 5 μg/ml of C225 was added to each mixture, the assay values by the Biacore were mainly from C225 because the concentration values of the samples were very close to each other. This result indicates that addition of these matrices did not affect the binding of C225 to the immobilized EGFR. Therefore, these results have shown this assay has good specificity for C225 and no significant interference from several types of matrices. These results further suggest this assay would be very useful in quantitating C225 in samples from several kinds of studies such as bioassays, protein stability tests, and excipient compatibility evaluations.

## 3.2. Pharmacokinetic studies

Serum concentration–time profiles of C225 in rhesus monkeys following intravenous infusion at 7.5, 24 and 75 mg/kg were shown in Fig. 4, and corresponding mean pharmacokinetic parameters were listed in Table 5.

C225 reached peak serum concentration rapidly following intravenous infusion administration with a $T_{max}$ of 0.5 h for all three doses. Average $C_{max}$ ranged from $168 \pm 28$ to $1624 \pm 113$ μg/ml, and $AUC_{0-\infty}$ ranged from $15739 \pm 1059$ to $295017 \pm 44533$ μg h/ml.

**Table 4**
The effect of different matrices on binding of C225 to the immobilized EGFR sensor surface (mean ± SD, $n = 3$).

| Matrix | Obtained mean concentration (5 μg/ml) | CV (%) | Accuracy (%) |
|---|---|---|---|
| 50% human serum | 4.9 ± 0.3 | 7.1 | 98 |
| 50% Sprague Dawley rat serum | 5.1 ± 0.5 | 9.8 | 102 |
| 20% pooled monkey serum containing 5 μg/ml of chimeric recombinant anti-CD20 monoclonal antibody | 4.5 ± 0.4 | 8.8 | 90 |
| 20% pooled monkey serum containing 5 μg/ml of human γ-globulin | 5.3 ± 0.6± | 11.8 | 106 |
| 20% pooled monkey serum containing 5 μg/ml of chimeric recombinant her2 antibody | 5.0 ± 0.2 | 3.7 | 100 |

**Table 5**
Pharmacokinetic parameter of C225 in rhesus monkeys after intravenous infusion ($n = 3$).

| Parameter | Low-dose 7.5 mg kg$^{-1}$ | Mid-dose 24 mg kg$^{-1}$ | High-dose 75 mg kg$^{-1}$ |
|---|---|---|---|
| $AUC_{0-t}$ (μg h ml$^{-1}$) | 15605 ± 1078 | 108908 ± 12407 | 254775 ± 35343 |
| $AUC_{0-\infty}$ (μg h ml$^{-1}$) | 15739 ± 1059 | 114090 ± 12788 | 295017 ± 44533 |
| $AUC_{t-\infty}$ (μg h ml$^{-1}$) | 135 ± 73 | 5182 ± 1032 | 40241 ± 9243 |
| MRT (h) | 98 ± 6 | 142 ± 1 | 161 ± 2 |
| $CL_S$ (ml h$^{-1}$ kg$^{-1}$) | 0.48 ± 0.03 | 0.26 ± 0.02 | 0.21 ± 0.04 |
| $V_{SS}$ (ml kg$^{-1}$) | 47 ± 6 | 30 ± 3 | 41.6 ± 5.6 |
| $K_{el}$ (h$^{-1}$) | 0.0110 ± 0.0025 | 0.0074 ± 0.0008 | 0.0043 ± 0.0001 |
| $t_{1/2}$ (h) | 2.7 ± 0.7 | 3.9 ± 0.5 | 6.7 ± 0.1 |
| $C_{max}$ (μg ml$^{-1}$) | 169 ± 28 | 966 ± 165 | 1624 ± 113 |

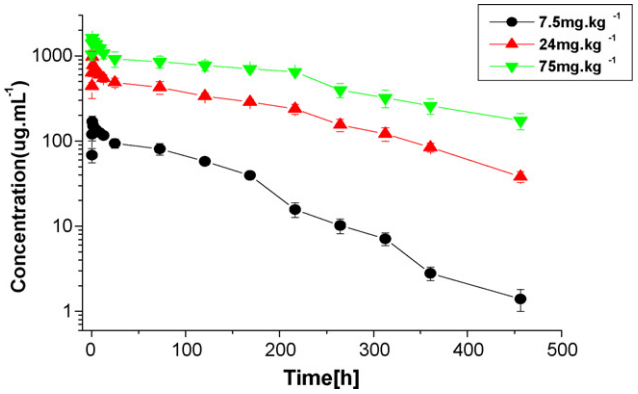


**Fig. 4.** The serum concentration–time profiles of C225 in rhesus monkeys during and after intravenous infusion administration of 7.5, 24 and 75 μg/kg. Symbols represented the observed data (mean ± SD).

C225 elimination followed a bi-exponential profile with a $t_{1/2}$ ranging from 2.7 ± 0.7 to 6.7 ± 0.1 h.

Based on the weighted regression analysis of the pooled data, $C_{max}$ and $AUC_{0-\infty}$ values increased as the dose increased in a linear manner but not proportionally. When the dose of C225 increased in a ratio of 1:3:10, the $AUC_{0-\infty}$ values increased in a ratio of 1:7.2:18.7 and the $C_{max}$ values increased in a ratio of 1:5.72:9.61. This coincided with a decrease in total clearance ($P < 0.001$) and an increased elimination half-life ($P < 0.001$), consistent with non-linear disposition. Therefore, our results support non-linear rather than linear serum pharmacokinetics of C225 across the investigated dosage range in monkeys (7.5–75 mg/kg).

## 4. Discussions

The analysis methods of biotech drugs are of concern by scientists. Traditional assay methods, radioimmunoassay and ELISA, for pharmacokinetic studies of antibody drugs have drawbacks: time and labor consuming. The biosensor-based assay of antibody represents a significant improvement over the traditional assay methods for assaying concentration of the antibody in biomatrices for pharmacokinetic study. Not only is there a dramatic reduction in the amount of sample required to perform analyse, but in real time to obtain the results.

The baseline stability of the covalently immobilized EGFR after multiple cycles of regeneration represents an issue that is significant to biosensor-based assays but not a factor in ELISAs, since ELISAs only utilize an immobilized plate for one assay and the plate is then discarded. It is important to assess whether the baseline can return to pre-sample levels upon regeneration since accumulation on the sensor chip surface can reduce the total binding capacity. In addition, the capacity to bind a sample must also be evaluated. The sensitivity of this assay as determined by the limit of quantitation is 0.05 μg/ml in 20% monkey serum. The sensitivity of the method was comparable with ELISA. The experiments to test the specificity of this assay provide further evidence that the assay is specific for quantitation in the presence of other antibodies. Since this assay incorporates binding to covalently immobilized EGFR, only C225 that are capable of binding to EGFR are quantitated. The residues obtained between the value predicted by the model and the experimental data illustrate the goodness of the fit of the four-parameter equation employed. The method has a wide assay range from 0.05 to 50 μg/ml.

Cetuximab is expensive and the amount of drug given to me by Huabei Company is little. Therefore, the amount of sample used in the SPR assay was less. In our experiment, the response was low, because the injection time of sample was short (only 1 min), so the amount of injection sample was less than saturating value. And the binding capacity of the surface assayed in our experiment was not of saturating binding capacity.

Two of the most common parameters examined during assay validation are precision and accuracy. The acceptance criteria for this assay were that results should be quantitated within 15% coefficient of variation as a measure of precision and also that results should be within 15% of the target value as a measure of accuracy. In order to test these two parameters, serum samples were prepared with various amounts of C225 added. Aliquots of these samples were prepared and these aliquots were assayed multiple times on multiple days. The precision parameters of this assay, both intra-assay and inter-assay precision, were within the predetermined acceptable 15% range. In addition, all of the samples were within 15% of target. The results obtained for precision and accuracy were within the acceptance criteria, and were comparable with other immunoassays such as enzyme immunoassays or radioimmunoassays.

## 5. Conclusions and future work

We developed an assay method using the Biacore system that allows the quantitative measurement of C225 in biomatrix for pharmacokinetic studies with a single injection and the method was successfully applied to preclinical pharmacokinetic study of C225 in rhesus monkeys. There were some reports [21–27] on clinical pharmacokinetic profile of cetuximab (Erbituxe) by ELISA and there is no report on the quantitation of C225 in monkey serum. This is the first report on the quantitation of C225 in monkey serum by an optical biosensor technology. In future we expect that the method developed here can be expanded for use in studies of pharmacokinetic studies of other biotech drugs and that it offers a new tool of the pharmacokinetic study.

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