



## Oxytetracycline antibiotic adsorption capacity of $\beta$ -cyclodextrin/carboxymethylcellulose hydrogel films

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### Abstract

Oxytetracycline (OTC) is an effective antibiotics used in aquaculture for a long time. However, OTC may cause severe environmental contamination and has aroused concerning about its impact on public health. For this reason, this article aims to removal OTC from aqueous solution by using the  $\beta$ -cyclodextrin /carboxymethylcellulose ( $\beta$ -CD/CMC) hydrogel films as adsorbent. The hydrogel film were prepared by esterification-crosslinking method. The films were evaluated for active  $\beta$ -CD content, swelling ratio and adsorption capacity. The adsorption of OTC have been studied in terms of pseudo-first- and -second-order kinetics. The Langmuir and Freundlich adsorption models have also been used to the equilibrium adsorption data. The results obtained from the study indicated that the adsorption capacity of OTC mainly depends on  $\beta$ -cyclodextrin content.

**Keywords:** Oxytetracycline,  $\beta$ -cyclodextrin /carboxymethylcellulose, adsorption capacity, antibiotic

### Introduction

Cultivation of shrimps have become one of large aquaculture businesses since humans have generally consumed shrimps as an important source of protein [1]. Among the countries in Southeast Asia, Thailand became the world leader and exporter of shrimp in the early 1990s, and frozen shrimp was the country's leading food export in earnings overall by 1995 due to the physiography of tropical coastal areas [2].

In the past, cultivation of shrimps in Thailand initially operated with extensive culture and then changed to intensive culture in order to increase density and harvest size of shrimp and to reduce virus populations present in ponds [3][4][5]. Currently, recirculating aquaculture system (RAS) has been utilized in cultivation of shrimps instead of either extensive or intensive system as it saves water consumption, decreases environmental impact and also supports the growth of shrimps. Nevertheless, viral infection of shrimps in RAS still exists. Shrimp farmers have to reduce viral disease in shrimps by using antibiotics in the system.

Oxytetracycline (OTC), a kind of tetracycline antibiotics, is widely used against viral disease in shrimp aquaculture and has to be removed from the system without accumulation in the ponds and muscle of shrimps because OTC affects to human health. Recently, there are several ways to eliminate OTC, which are biological processes, filtration, sedimentation, advanced oxidation processes, membrane processes and adsorption [6]. Adsorptive materials have been extensively used for the conditioning, remediation and removal of antibiotic because of low-cost technology and easy operation. Activated carbons and graphene polymers combined with and without  $\beta$ -cyclodextrin ( $\beta$ -CD) are promising materials that can remove OTC in the shrimp farming since  $\beta$ -CD can absorb large molecules with a hydrophobic inner cavity and a hydrophilic exterior as well as a remarkable capacity to form inclusion complexes with various molecules through host-guest interactions [7].

In this work,  $\beta$ -CD/carboxymethylcellulose hydrogel films were used as adsorbent to remove OTC. The experimental equilibrium data were then fit to Langmuir and Freundlich isotherms. In addition, pseudo-first- and -second-order kinetic models were discussed in details to determine the characteristics of the adsorption process. These analytical data can be further applied to reduce OTC in shrimp cultivation.

## Materials and methods

### Materials

Oxytetracycline hydrochloride was supplied by K 3545 Pharma-Chemical Co. Ltd. (Thailand), sodium carboxymethylcellulose (degree of substitution: 0.83) was purchased from Wealthy Chemical Industry (Suzhou) Co. Ltd,  $\beta$ -cyclodextrin was supplied by N&R Bio Industries Inc. and citric acid monohydrate was purchased from Loba Chemie Pvt. Ltd.

### Methods

#### Preparation of $\beta$ -CD/CMC hydrogel films

$\beta$ -CD/CMC hydrogel films were prepared by modifying the method used by Ghorpade et al. [8]. NaCMC (2%w/v) aqueous solutions containing 1.4 g citric acid and  $\beta$ -CD (0, 0.6, 1.4 % w/v) were prepared by using magnetic stirrer at room temperature. The solutions were kept overnight at room temperature to remove air bubbles. The clear solutions were coated on the square plate (6x6 cm) and dried in a hot air oven at 45 °C for 12 h. The dried films were cured at 145 °C for 5 min. The cured hydrogel films were washed by distilled water and isopropanol for 1 h in order to remove the unreacted components. Thereafter, the hydrogel films were dried in a hot air oven at 45 °C for 3 h and stored in a desiccator.

The infrared spectra of  $\beta$ -CD, CMC and hydrogel films were obtained by using FTIR spectrophotometer (Perkin Elmer/Spectrum one). The spectra were the range of 500–4000  $\text{cm}^{-1}$  at an average of 32 scans and resolution of 2  $\text{cm}^{-1}$ .

#### Determination of active CD content

The amount of active  $\beta$ -CD in the hydrogel films was determined by using phenolphthalein assay [9][10]. The standard solutions of  $\beta$ -CD in 0.05 M trisaminomethane hydrochloride (Tris HCl) buffer (pH = 7) were prepared in the concentration range of 50- 400 mg/L. One mL of these standard solutions was mixed with 4 mL of working phenolphthalein solution in the test tubes. The sample solutions were prepared in separate test tubes by adding 0.01 g of hydrogel films in the mixture of 1 mL of Tris HCl buffer and 4 mL of working phenolphthalein solution. All test tubes were vortexed and stored in dark for 2 h. The absorbance of the standard and sample solutions was measured at 550 nm using UV–vis spectrophotometer (U1900 UV/VIS, Hitachi). Percent decrease in the absorbance of solutions was calculated using equation (1) :

$$\% \text{ Decrease in adsorption} = \frac{\text{Control} - \text{Test}}{\text{Control}} \quad (1)$$

The mixture of 1 mL Tris HCl buffer and 4 mL working phenolphthalein solution was used as control for standard solutions whereas solution containing 0 % w/v (0.05 g) was used as control for sample solutions. The calibration curve (% decrease in absorbance vs  $\beta$ -CD concentration) for standard solutions was plotted and amount of active  $\beta$ -CD was determined as equation (2) :

$$\text{Active } \beta\text{CD content} = \frac{C_{\beta\text{CD}}}{1000 \times W_{\text{film}}} \quad (2)$$

where,  $C_{\beta\text{CD}}$  is the concentration of active  $\beta\text{CD}$  (mg/L),  $W_{\text{film}}$  is the weight of hydrogel film (g)

#### Determination of swelling ratio

The swelling ratio of the hydrogel films was determined by modifying method of Teow et al. [11]. The dry hydrogel films were immersed in deionized water, pH 7 at room temperature. The swollen hydrogel films were taken out from the deionized water after 15 min, superficially dried with tissue paper and weighted. The weight of the swollen hydrogel ( $W_s$ ) was measured. The absorbency was calculated using equation (3) :

$$\text{Swelling ratio} \left( \frac{\text{g}}{\text{g}} \right) = \frac{W_s - W_d}{W_d} \quad (3)$$

Where,  $W_d$  is the weight of dry hydrogel films. The hydrogel films were again immersed in the deionized water. The procedure was repeated at specific time intervals and swelling ratio was determined till 24 h. The measurements were performed in triplicate.

### Kinetic experiments

Adsorption kinetics experiments were conducted in a 125 ml beaker by dispersing 0.1 g of adsorbent into 50 mL of synthetic OTC solutions (500 mg/L). The solution has natural pH (pH 7) which was detected using a pH meter. Then, the mixture was vigorously shaken at a speed of 150 rpm with a temperature control at 30 °C. The samples were taken at desired time for filtration with 0.22 µm nylon filter membranes and then quantified by high performance liquid chromatography (HPLC). VertiSep<sup>TM</sup> AQS HPLC with C18 column (250 × 4.6 mm, 5 µm) at 30 °C. The mobile phase consisted in acetonitrile: methanol: 0.4% orthophosphoric acid adjusted to pH 2.7 with triethanolamine (20:10:70). The flow rate was 0.7 ml/min, with an injection volume of 10 µL, while the UV detector was set at 254 nm [12]. In control experiments, the OTC solutions without adsorbents were conducted at the same condition. The adsorption process order and the rate constant were estimated by applying the pseudo-first order and pseudo-second order kinetic models (Table 1), Models with high correlation coefficient ( $R^2$ ) were chosen.

**Table 1** The adsorption kinetics models and adsorption isotherm models

Model	Linear equation	Comment	Reference
Pseudo-first order kinetic	$\log(q_e - q_t) = \log q_e - \frac{k}{2.303} \cdot t$ (4)	$K$ and $q_e$ values were calculated from the plot of $\log(q_e - q_t)$ versus $t$	[13]
Pseudo-second order kinetic	$\frac{t}{q_t} = \frac{1}{k \cdot q_e^2} + \frac{1}{q_e} \cdot t$ (5)	$K$ and $q_e$ values were calculated from the plot of $t/q_t$ versus $t$	[14]
Langmuir isotherms	$\frac{1}{q_e} = \frac{1}{q_m} + \frac{1}{b q_m C_e}$ (6)	$Q_m$ values was calculated from the plot of $1/q_e$ versus $1/C_e$	[15]
Freundlich isotherms	$\log q_e = \log K_f + \frac{1}{n} \log C_e$ (7)	$K_f$ values was calculated from the plot of $\log q_e$ versus $\log C_e$	[16]

### Adsorption isotherm

The working solutions in a certain range of concentration (10–1000 mg/L) containing deionized water. Batch adsorption experiments with 50 mL of OTC solutions were carried out in 125 mL flasks with screw caps. The pH of solution was adjusted pH 7 with hydrochloric acid or sodium hydroxide. Then, 0.1 g adsorbents were added to flasks and shaken at 150 rpm at 30 °C for 3 h. The flasks were wrapped in aluminum foil to prevent photocatalysis reaction. In all sets of experiments, each test was conducted in duplicate, and the mean values were recorded. In control experiments, the OTC solutions without adsorbents were conducted at the same conditions. The Langmuir and Freundlich isotherm parameters are significant for predicting the maximum adsorption capacity and describing the surface properties and affinity of the adsorbent (Table 1). The isotherms are selected to analyze the equilibrium experimental data for the adsorption of OTC onto hydrogel films.

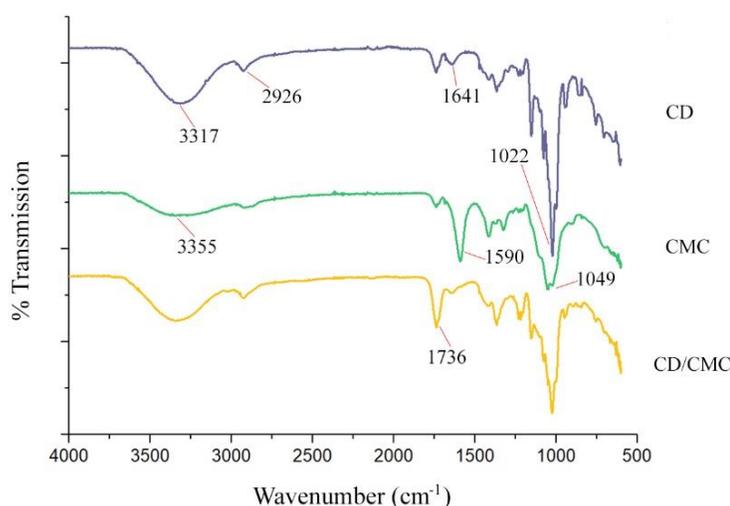
### Results and discussion

The  $\beta$ -CD content were found to be dependent upon the concentration of  $\beta$ -CD in the feed. These molecules can easy access to the phenolphthalein dianion within their cavities and decolorize the solution [9]. CMC exhibits poor crosslinking when used alone. From total  $\beta$ -CD present in the hydrogel films, only active  $\beta$ -CD molecules can form an inclusion complex with hydrophobic molecule. In this study was found that the 70% citric acid brought about sufficient crosslinking between the CMC chains. This leads to the linking between hydroxyl groups of the same polymer chain (intra polymer crosslinking) [17][18]. Therefore, an increase in the concentration of  $\beta$ -CD may improve the  $\beta$ -CD/CMC crosslinking and increase the active  $\beta$ -CD content of the hydrogel films (Table 2) [8].

**Table 2** Composition, active  $\beta$ -CD content of hydrogel films

Sample	CA (g)	$\beta$ -CD (g)	Active CD (mg/g)
HF1	1.4 (70 % w/w of polymer)	0 (0 % w/w of polymer)	0
HF2	1.4 (70 % w/w of polymer)	0.6 (30 % w/w of polymer)	73.82
HF3	1.4 (70 % w/w of polymer)	1.4 (70 % w/w of polymer)	120.51

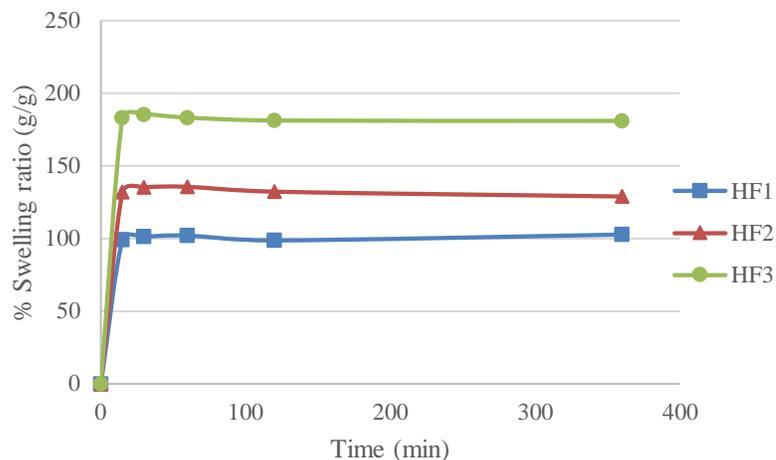
The samples included cyclodextrin (CD), carboxymethylcellulose (CMC), and  $\beta$ -CD/CMC hydrogel film were analyzed by FTIR in order to determine the change of wave numbers that imply the existence crosslinks between CD and CMC. The results as shown in Figure 1 demonstrate that the spectrum of CD show the dominant peaks at around  $3300\text{ cm}^{-1}$  (O-H stretching),  $2926\text{ cm}^{-1}$  (C-H stretching),  $1641\text{ cm}^{-1}$  (H-O-H bending) and  $1022\text{ cm}^{-1}$  (C-O-C stretching). Besides, the spectrum of CMC express a broad band at  $3355\text{ cm}^{-1}$  (O-H stretching),  $1590\text{ cm}^{-1}$  (C=O stretching), and  $1049\text{ cm}^{-1}$  (C-O-C stretching). After a hydrogel was formed, the spectrum could show the dominant peaks at around  $1736\text{ cm}^{-1}$  due to the crosslink reaction. The crosslink reaction between CD and CMC could occur after citric acid was added, which led to the esterification reaction. Afterwards, the ester group (C=O at  $1736\text{ cm}^{-1}$ ) become dominant expression in FTIR when compared to CD and CMC chromatograms.

**Figure 1** FTIR chromatogram of three samples; cyclodextrin (blue), carboxymethylcellulose (green), and cyclodextrin /carboxymethylcellulose hydrogel film (yellow)

### Swelling studies

The time profile of swelling capacity of the adsorbent was investigated. We found that the swelling percentage increases drastically and reaches the equilibrium after 1 h (Figure 2) due to the slow diffusion of water molecules to the polymer network. The hydrogel films without  $\beta$ -CD show the lowest equilibrium swelling ratio of  $101.28 \pm 2.48\text{ g/g}$ . The swelling ratio in hydrogel films with  $\beta$ -CD increases with increasing  $\beta$ -CD content. Since  $\beta$ -CD has a hydrophilic exterior in nature, an increase of  $\beta$ -CD content in the hydrogel films could enhance their

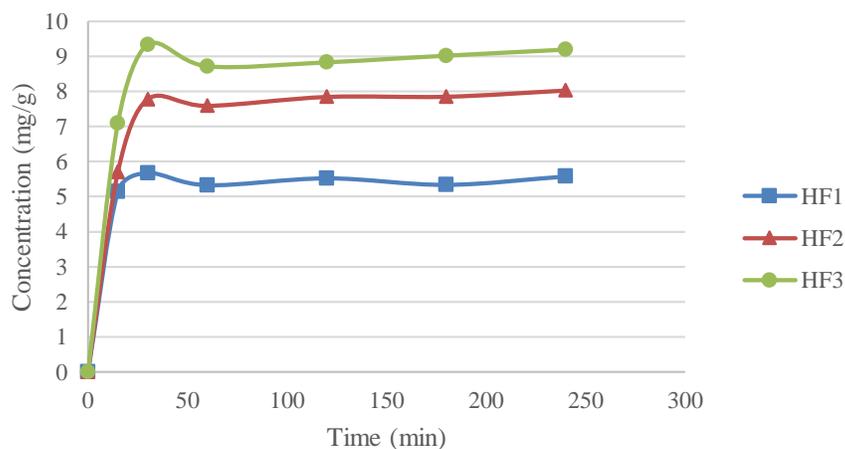
hydration. This may create loose polymer network and increase the average distance between the polymer chains resulting in the increase of water retention capacity of the hydrogel films [8].



**Figure 2** Swelling behaviour of the adsorbent with time

### Adsorption kinetics and adsorption isotherm

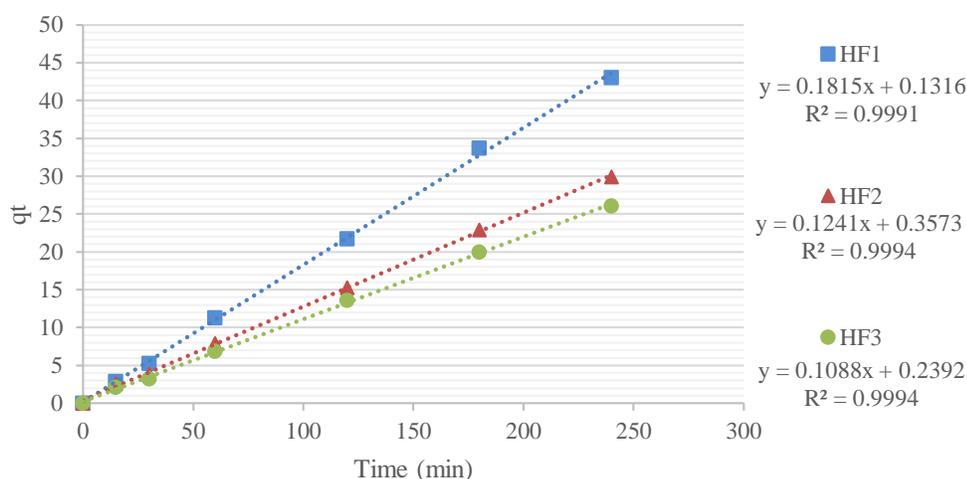
Kinetic study provides important information about the mechanism of oxytetracycline adsorption onto hydrogel films. The significant decreased of OTC concentration occurred in the first 30 min, and then this trend became slow until equilibrium was achieved (Figure 3). From the  $R^2$  values (as shown in Table 3) are indicated to be a pseudo-second-order kinetic equation rather than pseudo-first-order kinetic equation fit well with the adsorption data of OTC. The results are shown in Figure 4. The pseudo-second order kinetic model is expressed by equation (5). The theoretical predicted values  $q_{e,theory}$  and is more consistent with the actual calculated from experiment value  $q_{e,exp}$  (Table 3). It demonstrates that pseudo-second order kinetic model can reflect the adsorption mechanism appropriately and covers all of the adsorption process.



**Figure 3** Effect of contact time on adsorption of hydrogel films

**Table 3** Adsorption kinetic of OTC on hydrogel films

Hydrogel films	Pseudo-first order kinetic model			Pseudo-second order kinetic model			
	$k_{pf}$ (min <sup>-1</sup> )	$q_e$ (mg/g)	$R_1^2$	$k_{ps}$	$q_{e,theory}$ (mg/g)	$q_{e,exp}$ (mg/g)	$R_2^2$
HF1	0.0182	1.83	0.5577	0.25	5.51	5.46	0.9991
HF2	0.0157	1.67	0.5018	0.04	8.06	7.94	0.9994
HF3	0.0182	3.21	0.7522	0.05	9.19	9.11	0.9994

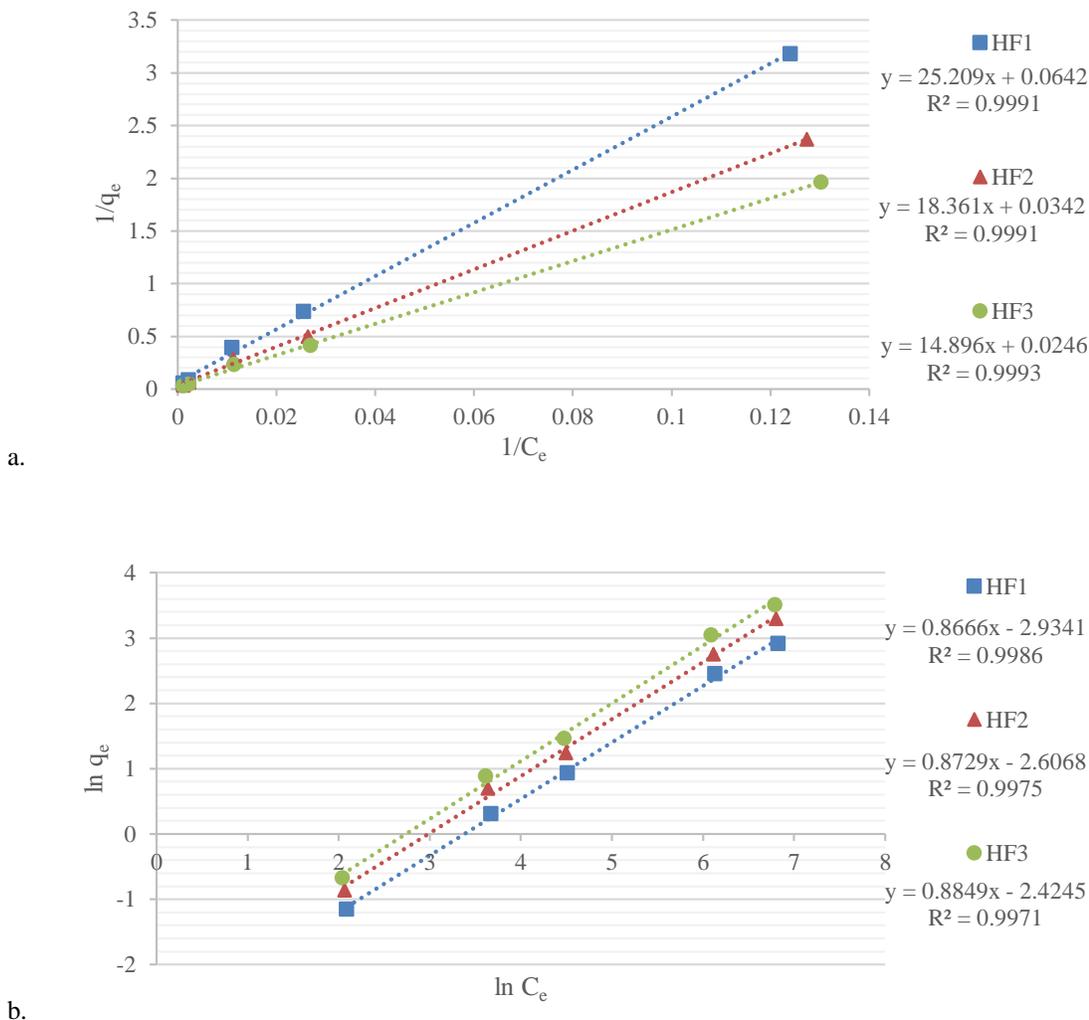


**Figure 4** Pseudo-second order adsorption kinetic

Adsorption isotherm is important for determining the adsorption behavior of an adsorbent. Table 4 shows the adsorption parameters of OTC on hydrogel films fit by Freundlich and Langmuir models. Figure 5 shows the adsorption isotherm at contact time 180 min. It seems that Langmuir isotherm model fits the experimental data more precisely and can describe the process of  $\beta$ -CD/CMC hydrogel films adsorption system. The maximum adsorption capacity  $Q_m$  of OCT on  $\beta$ -CD/CMC hydrogel films (HF1, HF2, HF3) from Langmuir model are 15.58, 29.24 and 40.65 mg g<sup>-1</sup> respectively. Meanwhile, the value of  $1/n$  is also found to be greater than 1. We consider that the hydrophobic cavity of  $\beta$ -CD may pack molecules to form stable host-guest molecular.

**Table 4** Adsorption parameters of OTC on hydrogel films fit by Freundlich and Langmuir models

Hydrogel film	Freundlich model			Langmuir model		
	K	n	$R^2$	$q_{max}$ (mg/g)	b	$R^2$
HF1	0.0011	1.15	0.9986	15.58	0.0026	0.9991
HF2	0.0025	1.14	0.9975	29.24	0.0019	0.9991
HF3	0.0038	1.13	0.9971	40.65	0.0017	0.9993



**Figure 5** The isotherm of adsorption for 180 min (a. Langmuir isotherm model; b Freundlich isotherm model)

## Conclusion

In summary, the  $\beta$ -CD/CMC hydrogel films was prepared successfully. An increase in the concentration of  $\beta$ -CD in the feed increases the active  $\beta$ -CD content. The hydrogel films with  $\beta$ -CD showed better swell ability than those hydrogel films without  $\beta$ -CD. The pseudo-second order kinetic model fit well with the experimental adsorption. The maximum adsorption capacity of the hydrogel films (HF1, HF2, HF3) obtained from Langmuir isotherm model are 15.58, 29.24 and 40.65 mg g<sup>-1</sup> respectively. These results illustrate that  $\beta$ -CD/CMC hydrogel films can be used as adsorbents in removing oxytetracycline from water.

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