
**Separation Science and Technology**
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713708471

# Kinetic Studies of Phytosterol Adsorption on Zeolite

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**To cite this Article** Chuang, Yu-Hsuan , Ju, Yi-Hsu and Widjaja, Arief(2007) 'Kinetic Studies of Phytosterol Adsorption on Zeolite', Separation Science and Technology, 42: 3, 611 — 624
**To link to this Article: DOI:** 10.1080/01496390601070182
**URL:** http://dx.doi.org/10.1080/01496390601070182

*Separation Science and Technology*, 42: 611–624, 2007

ISSN 0149-6395 print/1520-5754 online
DOI: 10.1080/01496390601070182



# Kinetic Studies of Phytosterol Adsorption on Zeolite

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**Abstract:** Equilibrium isotherms of sterol adsorption on zeolite show the characteristics of irreversible equilibrium adsorption. First order and second order surface adsorption control mechanisms as well as micropore diffusion control model failed to satisfactorily describe the kinetics of sterol adsorption on zeolite. From the analysis of adsorption data, it was found that macropore diffusion control model satisfactorily describes the kinetics of sterol adsorption on zeolite. The effect of temperature on the diffusivity during adsorption was found to conform to the Eyring equation. It was shown that a change in temperature has a negligible effect on the selectivity of sterol adsorption on zeolite.

**Keywords:** Adsorption, campesterol, kinetic model, $\beta$-sitosterol, zeolite

## INTRODUCTION

Phytosterols are important structural components of plant membranes, and free phytosterols serve to stabilize phospholipids bilayers in plant cell membranes just as cholesterol does in animal cell membranes. Their function appears to be to control membrane fluidity and permeability, and signal transduction (1).

Received 30 May 2006, Accepted 25 September 2006
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Phytosterols are also important precursors of steroids which are important pharmaceuticals. Estradiol, pregnenolone, progesterone, aldosterone, testosterone, and cortisol that function as human hormone are all synthesized from different sterols. Recently, phytosteryl ester was found to be effective in lowering plasma cholesterol concentration by inhibiting the absorption of cholesterol from small intestine. This physiological activity has led to the development of functional foods, such as salad oil, dressing with added sterol, and margarine blended with fatty acid sterol ester (FASE). Because FASE and oil (TAG) are completely soluble, a lot of attention is being focused on the addition of sterol ester (SE) in oil-related foods (2).

Sterols can be synthesized chemically, but the process is usually complex and costly. Pure sterol can be obtained by the separation of sterol mixture obtained from natural sources. Natural sterols have similar physical and chemical properties due to their similarity in chemical structures. Hence the separation of sterol from natural sources is difficult.



Many reports on the separation and purification of sterol are available in the literature, such as solvent crystallization (3), molecular distillation (4), enzyme-catalyzed reaction (5), high speed countercurrent chromatography (6, 7), and zeolite adsorption (8). Relatively few kinetic studies on the separation of sterols were reported. Berezin et al. (8) studied the adsorption kinetics of sterols on NaY type zeolite for the selective separation of campesterol over $\beta$-sitosterol from a hexane solution. From molecular modeling and the analysis of the molecular size of sterols and zeolite pore dimensions, they hypothesized that size selectivity was the principal mechanism for separation. This was consistent with the observed trend that the adsorption rate decreased with a decreasing sterol diameter. Separation and purification of sterols with saturated side chain ($\beta$-sitosterol and campesterol) from commercial sterol mixture (5% brassicasterol, 30% campesterol, 15% stigmasterol, 50% $\beta$-sitosterol) were carried out by Chuang et al. (9). Their process involved solvent crystallization to obtain a campesterol and $\beta$-sitosterol fraction, followed by zeolite adsorption and desorption to separate campesterol and $\beta$-sitosterol. This work aims to clarify the kinetics of sterol adsorption on zeolite and to study the effect of temperature on the selectivity of sterol adsorption.

## MODEL DEVELOPMENT

Solute adsorption onto active sites of an adsorbent from the bulk solution can be divided into three stages:

1. solute transports from bulk solution through fluid film to the external surface of adsorbent, film mass transfer resistance occurs at this stage;
2. solute diffuses to the inside active site through micropores of zeolite crystals and through intercrystalline macropores, micropore diffusion resistance and macropore diffusion resistance exist at this stage;

3. after diffusion to the internal surface, solute is absorbed onto active sites, internal surface adsorption resistance exists at this stage.

External film mass transfer resistance can be neglected due to vigorous stirring usually applied during adsorption. Equations describing liquid-solid adsorption based on various models are given below.

## Internal Surface Adsorption Control Model

In this model, both micropore diffusion and macropore diffusion resistances are neglected. The adsorption rate depends on the solute concentration and adsorbent capacity. Assuming that solute is evenly adsorbed onto active sites during adsorption, and that each site can hold only one adsorbate (solute) molecule, then the irreversible adsorption of solute can be described by (10)

$$\frac{dC}{dt} = -k_a C^n \left[ S_T - \frac{V}{w}(C_0 - C) \right], \tag{1}$$

Let $S_T - (V/w)C_0 = a$, and $V/w = b$, the relation between adsorbate concentration ($C$) and time can be obtained by integrating Eq. (1) with initial condition $C = C_0$ at t = 0.
If n = 0, the rate equation becomes

$$-k_0 t = \frac{1}{b} \ln \frac{C + a/b}{C_0 + a/b}, \tag{2}$$

If n = 1, the rate equation becomes

$$-k_1 t = \frac{1}{a} \ln \frac{C(a/b + C_0)}{C_0(a/b + C)} \tag{3}$$

## Micropore Diffusion Control Model

The simplest case to consider in this model is a single (hypothetical) isotropic spherical adsorbent particle, in contact with sorbate with an initial concentration of $C_0$. The solution for the uptake curve is given by (11)

$$\frac{C}{C_0} = \frac{\alpha}{1 + \alpha} \left( 1 + \sum_{n=1}^{\infty} \frac{6(1 + \alpha) e^{-D_c q_n^2 t / r_c^2}}{9 + 9\alpha + q_n^2 \alpha^2} \right) \tag{4}$$

where $q_n$ is the nth root of $\tan q = 3q/(3 + \alpha q)$ and $\alpha$ is related to bulk solbate concentration by noting that as $t \to \infty$, $C_\infty/C_0 = \alpha/(1 + \alpha)$. Eq. (4) converges rapidly at large time. For fractional uptakes greater than 70%,

only the first term in Eq. (4) is needed and Eq. (4) reduces to

$$\frac{C}{C_0} = \frac{C_\infty}{C_0} + \frac{6\alpha}{9 + 9\alpha + q_1^2\alpha^2} \exp\left(\frac{-D_c q_1^2 t}{r_c^2}\right).$$

After some algebraic manipulation, the above equation can be rewritten as

$$1 - \frac{m_t}{m_\infty} = \frac{6\alpha(1+\alpha)}{9 + 9\alpha + q_1^2\alpha^2} \exp\left(\frac{-D_c q_1^2 t}{r_c^2}\right), \tag{5}$$

## Macropore Diffusion Control in Irreversible System

In a macroporous pellet, even if most of the adsorption capacity is in the micropores, it is possible that intercrystalline macropore diffusion is the dominant mass transfer resistance. For systems with irreversible equilibrium (rectangular isotherm), the "shrinking core" model can be applied. Under such conditions, adsorption occurs with an adsorption front, that is, a sorbate-free core in the adsorbent that diminishes with time. In analyzing macropore diffusion, it is usually assumed that transport occurs only through pores and mass flux through solid can be neglected. The uptake curve is given by (12)

$$\frac{t}{\tau} = 1 + 2\left(1 - \frac{m_t}{m_\infty}\right) - 3\left(1 - \frac{m_t}{m_\infty}\right)^{2/3}, \tag{6}$$

where

$$\tau = \frac{1}{6}\frac{R_p^2}{\varepsilon_p D_p}\left(\frac{q_s}{C_0}\right); \tag{7}$$

$\tau$ can be obtained from the slope of the straight line by plotting t against

$$1 + 2\left(1 - \frac{m_t}{m_\infty}\right) - 3\left(1 - \frac{m_t}{m_\infty}\right)^{2/3}.$$

## Eyring Equation

Eyring and his coworkers were the first to apply the "absolute reaction rate" model originated from chemical reaction rate to investigate the dynamic properties of the liquid transport process (13). The temperature dependence of the diffusivity can be expressed as

$$\mathrm{D_p} = \mathrm{D}^* e^{-E/RT}, \tag{8}$$

where $\mathrm{D}^*$ is the pre-exponential factor.

## MATERIALS AND METHODS

### Materials

Sterol obtained from diethyl ether crystallization in our previous work (9) was employed as the starting material for zeolite adsorption in this study. Its composition is 49% campesterol, 49% $\beta$-sitosterol, 1% brassicasterol, and 1% stigmasterol. Cholesterol (purity $> 99\%$) and $\beta$-sitosterol were obtained from Sigma-Aldrich (MO, USA) and MP Biomedicals (Eschwege, Germany), respectively. All solvents and reagents were either of HPLC grade or analytical reagent grade. All other chemicals used were obtained from commercial sources. Y type zeolite (sodium ion) was purchased from Sigma-Aldrich (MO, USA). The composition and physical properties of Y type zeolite (sodium ion) used are: pore diameter $= 7.4$ Å, surface area $= 920\ m^2/g$, porosity $= 0.3$, particle size $= 2$ microns. Its chemical composition is: 63.8% $SiO_2$, 22.9% $Al_2O_3$, 13.0% $Na_2O$, 0.13% $Fe_2O_3$, and 0.38% CaO. Fresh zeolite was activated at 500°C for 18 h before use.



### Batch Zeolite Adsorption of Sterols

Sterol that contained 49% campesterol and 49% $\beta$-sitosterol (2 mg − 20 mg) was dissolved in hexane (10 mL), then fresh zeolite (100 mg) was added with magnetic stirring at 400 rpm. The temperature was maintained at 30°C by using a water bath. A one hundred microliter sample was withdrawn at regular intervals and put in a micro centrifuge tube and 50 μL internal standards (10 mg squalene in 10 mL ethyl acetate) were added. After removing zeolite by centrifugation, the composition of sterol in the liquid phase was analyzed by gas chromatography. A sample was taken after 5 days to ensure that the system reached equilibrium. The equilibrium sterol concentration and the amount of sterol adsorbed were obtained using different initial sterol concentrations. Different kinetic models were employed to fit experimental data in order to determine the rate limiting step during zeolite adsorption. Experiments were carried out at different temperatures in order to study the effect of temperature on adsorption.

### Gas Chromatography Analysis

The sample was dissolved in ethyl acetate and 1 μL of this was injected into a Shimadzu GC-17A (Kyoto, Japan) GC equipped with flame ionization detector using DB-5HT (5%-phenyl)-methylpolysiloxane non-polar column (15 m × 0.32 mm i.d.; Agilent Tech. Palo Alto, California). Injection and detector temperatures both were 370°C. The initial column temperature was 240°C, and the column temperature was increased at 15°C/min until 300°C.

## RESULTS AND DISCUSSION

In liquid-solid adsorption, as in other adsorption process, information concerning the relevant adsorption equilibrium is essential for the analysis and set up of a kinetic model as well as for the design of an adsorption separation process (12).

Figure 1 shows the equilibrium isotherm curves of sterol adsorption on zeolite in hexane at 50°C. The equilibrium isotherm curves show that the adsorption of sterol on zeolite is of irreversible equilibrium form. The irreversibility of the process was evidenced from the fact that the desorption of campesterol cannot be achieved by contacting with fresh hexane. Desorption of campesterol can be accomplished by using ethanol as the solvent (9).

From Fig. 1, the maximum amount of cholesterol (MW = 386) adsorbed per unit zeolite, $S_T$ (mole/g zeolite), and the saturation cholesterol concentration on zeolite, $q_s$ (mole/L) can be obtained as $S_T = 0.000285$ and $q_s = 0.00285$, respectively.



### Internal Surface Adsorption Control

If adsorption on the surface active site is the rate limiting step in the irreversible adsorption of sterol on zeolite, then the relation between sterol concentration and time can be described by Eq. (1). Data for the adsorption of cholesterol on zeolite at 30°C were arranged according to Eqs. (2) and (3), presented in Table 1, and plotted in Fig. 2 and Fig. 3 for n = 0 and n = 1, respectively. The results show that both the first order and the second order

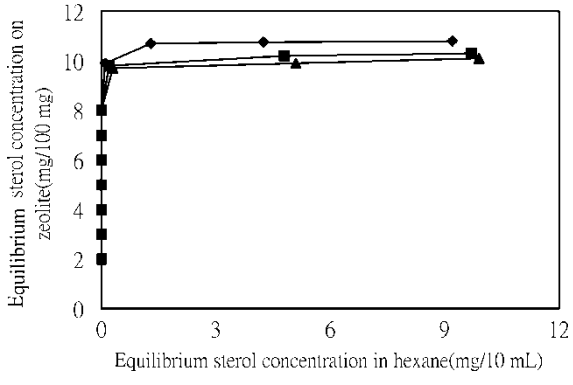


***Figure 1.*** Adsorption isotherm curve of different sterols at 50°C. (◆) cholesterol (■) campesterol (▲) β-sitosterol. Zeolite 100 mg, hexane 10 mL, magnetic stirrer speed 400 rpm. Equilibrium concentration was measured after 5 days of adsorption. The amount of sterol adsorbed on zeolite was determined as the difference between the initial weight of sterol and the amount of sterols left in hexane.

***Table 1.*** Cholesterol adsorption data[a] at 30°C

| Time (h) | Cholesterol fraction | Cholesterol conc. (mmole/L) | $-\frac{1}{b}\ln\frac{C_t+\frac{a}{b}}{C_0+\frac{a}{b}}$ | $-\frac{1}{a}\ln\frac{C_t\left(\frac{a}{b}+C_0\right)}{C_0+\left(\frac{a}{b}+C_t\right)}\times10^{-3}$ | $m_t$ ($10^{-2}$ mmole) | $1-\frac{m_t}{m_\infty}$ | $1+2\left(1-\frac{m_t}{m_\infty}\right)-3\left(1-\frac{m_t}{m_\infty}\right)^{2/3}$ |
|---|---|---|---|---|---|---|---|
| 0 | 1.00 | 5.18 | 0 | 0 | 0 | 1.00 | 0 |
| 0.50 | 0.830 | 4.30 | 3.35 | 0.711 | 0.880 | 0.830 | 0.0104 |
| 1.0 | 0.790 | 4.13 | 4.04 | 0.874 | 1.04 | 0.798 | 0.0148 |
| 2.0 | 0.780 | 4.04 | 4.45 | 0.974 | 1.13 | 0.780 | 0.0178 |
| 3.0 | 0.740 | 3.84 | 5.34 | 1.20 | 1.33 | 0.741 | 0.0252 |
| 5.0 | 0.690 | 3.58 | 6.55 | 1.53 | 1.59 | 0.692 | 0.0368 |
| 9.0 | 0.600 | 3.13 | 8.87 | 2.22 | 2.04 | 0.605 | 0.0637 |
| 17 | 0.510 | 2.67 | 11.5 | 3.15 | 2.50 | 0.516 | 0.101 |
| 30 | 0.410 | 2.12 | 15.3 | 4.76 | 3.06 | 0.409 | 0.164 |

[a]Cholesterol 20 mg, zeolite 200 mg, hexane 10 mL, 400 rpm magnetic stirring, temperature 30°C. The value of a ($=S_T-(VCo/w)$) is 2.59E-05, and b ($=V/w$) is 0.05. $m_\infty$ can be calculated to be 0.0518. $m_t$ was calculated from difference between $m_\infty$ and cholesterol concentration at time t.

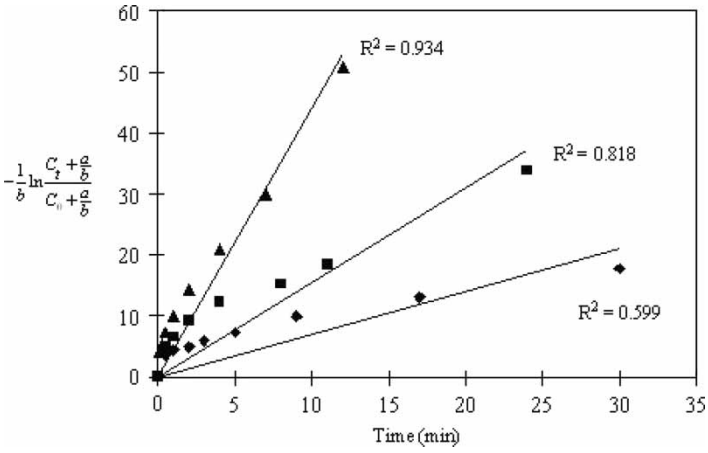


***Figure 2.*** Integral uptake curves of cholesterol assuming zero order surface adsorption control. Cholesterol 20 mg, zeolite 200 mg, hexane 10 mL, stirring speed 400 rpm. Temperature: (◆) 30°C (■) 40°C (▲) 50°C. Data were calculated according to Eq. (2) as shown in Table 1.

surface adsorption control model do not satisfactorily fit the experimental data of cholesterol adsorption on zeolite at 30°C. Similar conclusions were obtained for experimental data of cholesterol adsorption on zeolite at 40°C and 50°C (data not shown). The results obtained in this study are contradictory to those of Berezin et al. (8). Their sterol adsorption data were well fitted by the first order surface adsorption control model.

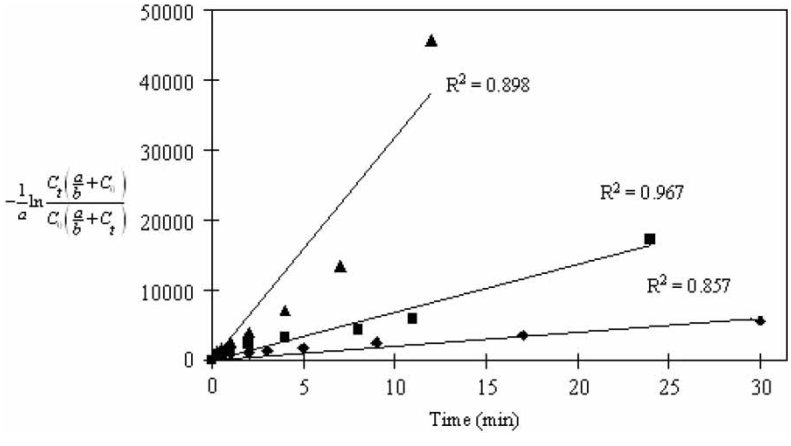


***Figure 3.*** Integral uptake curves of cholesterol assuming 1st order surface adsorption control. Reaction conditions are the same as in Figure 2. Temperature: (◆) 30°C (■) 40°C (▲) 50°C. Data were calculated according to Eq. (3) as shown in Table 1.

## Micropore Diffusion Control

As shown in Table 1 for a typical adsorption experiment, $C_\infty/C_0 = 2.12/5.18$, so $\alpha = C_\infty/(C_0 - C_\infty) = 0.693$. The first root of $\tan q = 3q/(3 + 0.693q)$ was obtained by trial and error as $q_1 = 4.28$. Eq. (6) becomes $1 - m_t/m_\infty = 0.293\exp(-D_c q_1^2\, t/r_c^2)$.

This equation is usually employed for describing the adsorption behavior of the micropore particle at large time. Plot of $\ln(1 - m_t/m_\infty)$ against $t$ should result in a straight line with slope $= -D_c q_1^2/r_c^2$ and intercept $= \ln(0.293)$. From Fig. 4 it is clear that at large time, when fraction uptake is high, data fall on a straight line in accordance with Eq. (5). However, intercepts of all 3 straight lines are all higher than ln(0.293). Thus the micropore diffusion control model fails to describe zeolite adsorption of sterol satisfactorily.

## Macropore Diffusion Control in Irreversible Equilibrium System

If the adsorption of sterol on zeolite can be described by macropore diffusion control in irreversible equilibrium system, then plot of $1 + 2(1 - m_t/m_\infty) - 3(1 - m_t/m_\infty)^{2/3}$ against time should yield a straight line. Cholesterol adsorption data at 30°C were arranged according to Eq. (6) in Table 1 and plotted in Fig. 5. Cholesterol adsorption data at other temperatures were calculated in the same way (data not shown). Figures 6 and 7 show the corresponding results for zeolite adsorption of campesterol and $\beta$-sitosterol, respectively (adsorption data not shown). All experiments were carried out under the following conditions: pure sterol 20 mg, zeolite 200 mg, hexane 10 mL, magnetic stirrer speed 400 rpm.

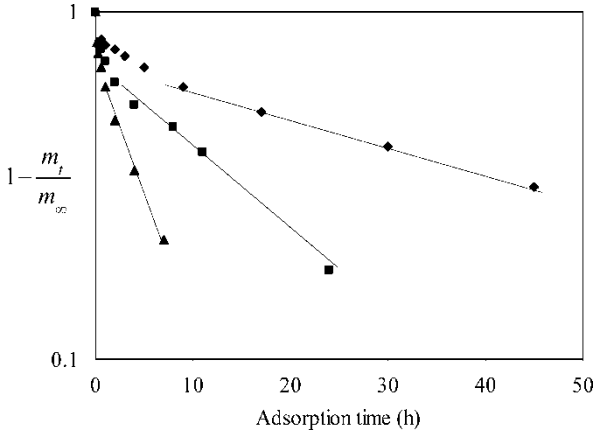


***Figure 4.*** Integral uptake curves of cholesterol in micropore diffusion control. Reaction conditions are the same as in Figure 2. Temperature: (◆) 30°C (■) 40°C (▲) 50°C. Data were calculated according to Eq. (5) as shown in Table 1.

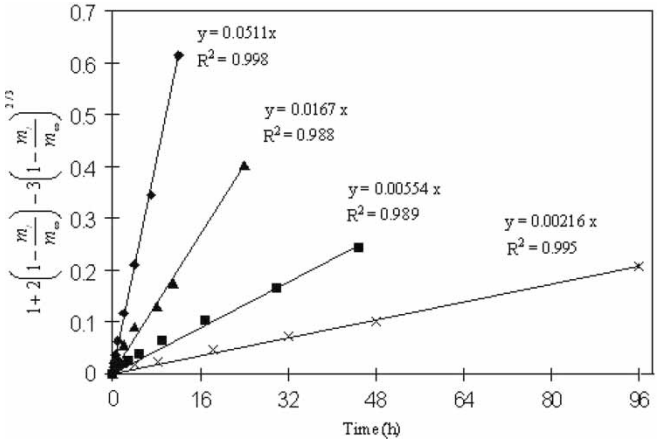


***Figure 5.*** Integral uptake curves of cholesterol in irreversible equilibrium model. Reaction conditions are the same as in Figure 2. (×) 20°C (■) 30°C (▲) 40°C (◆) 50°C. Data were calculated according to Eq. (6) as shown in Table 1.



Figures 5–7 show that the macropore diffusion control model satisfactorily fits the experimental data of sterol adsorption on zeolite. From the slope $(1/\tau)$ at different temperatures, where $\tau = (1/6(R_p^2/\varepsilon_p\ D_p)(q_s/C_0))$, the pore diffusivity $(D_p)$ can be calculated and the results are shown in Table 2.

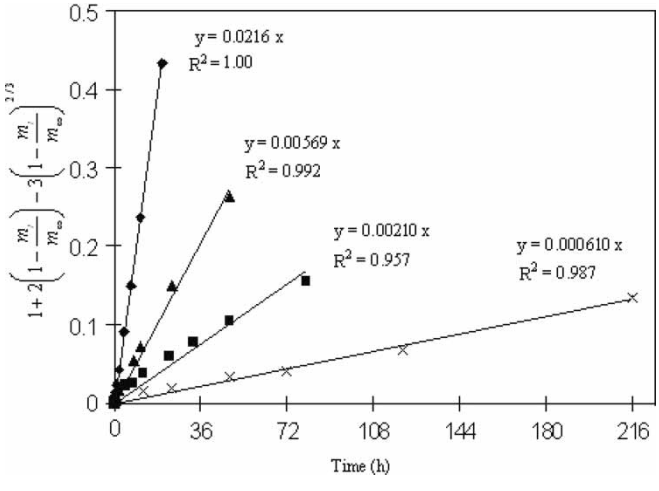


***Figure 6.*** Integral uptake curves of campesterol in irreversible equilibrium model. Campesterol 20 mg, zeolite 200 mg, hexane 10 mL, stirring speed 400 rpm. (×) 20°C (■) 30°C (▲) 40°C (◆) 50°C. Data were calculated in the same manner as cholesterol in Table 1.

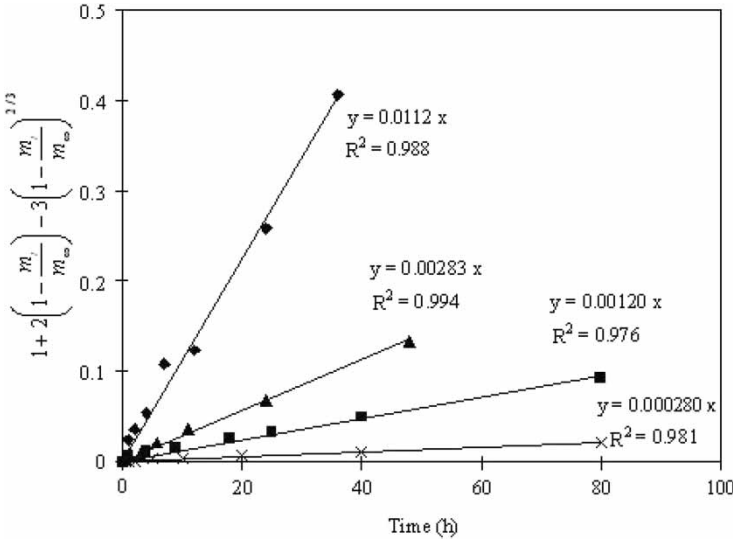


***Figure 7.*** Integral uptake curves of $\beta$-sitosterol in irreversible equilibrium model. $\beta$-sitosterol 20 mg, zeolite 200 mg, hexane 10 mL, stirring speed 400 rpm. (×) 20°C (■) 30°C (▲) 40°C (◆) 50°C. Data were calculated in the same manner as cholesterol in Table 1.

Eq. (6) should be applicable only when sorbate concentration is subjected to a step change at the boundary, which is different from the actual experimental condition in which the outer boundary of the adsorbant was in contact with a solution which sorbate concentration changed with time. Wen (14) obtained an equation similar to Eq. (6) for describing shrinking core model under pseudo steady state assumption. Since the rate of adsorption was actually quite slow as can be seen from Table 1, a pseudo steady state can be assumed except when time is small.

Data showing the dependence of $D_p$ on temperature for zeolite adsorption of sterol were plotted in Fig. 8 according to Eq. (8). Similar slopes of the 3

***Table 2.*** Diffusivity of sterols at different temperatures

| | Cholesterol | | Campesterol | | $\beta$-sitosterol | |
|---|---|---|---|---|---|---|
| T (K) | $1/\tau \times 10^3$ | Dp ($10^{-19}$ m$^2$/s) | $1/\tau \times 10^4$ | Dp ($10^{-19}$ m$^2$/s) | $1/\tau \times 10^4$ | Dp ($10^{-19}$ m$^2$/s) |
| 293 | 2.16 | 3.40 | 6.10 | 0.960 | 2.80 | 0.440 |
| 303 | 5.54 | 8.72 | 21.0 | 3.31 | 12.0 | 1.88 |
| 313 | 16.7 | 26.4 | 56.9 | 8.96 | 28.3 | 4.45 |
| 323 | 51.1 | 80.4 | 216 | 34.0 | 112 | 17.6 |

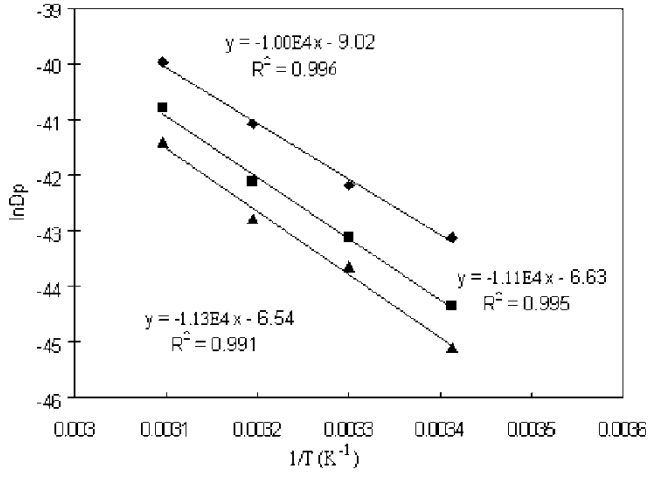


***Figure 8.*** Eyring plot showing temperature dependence of diffusivity for sterols in NaY zeolite. Sterol 20 mg, zeolite 200 mg, hexane 10 mL, stirring speed 400 rpm. (◆) Cholesterol (■) campesterol (▲) $\beta$-sitosterol.



lines in Fig. 8 indicate that temperature has a negligible effect on the adsorption selectivity of zeolite for the three sterols studied. This conclusion is consistent with the results of our previous study (9).

## CONCLUSION

The kinetics of sterol adsorption onto zeolite could not be described by the micropore diffusion control model, the first order and the second order surface adsorption control models. Instead, the experimental data of sterol adsorption on zeolite were well fitted by a macropore diffusion control model. From the Eyring plot showing the temperature dependence of diffusivity for sterol diffusion in NaY zeolite, it was found that temperature has a negligible effect on the selectivity of sterol adsorption on zeolite. The kinetic model proposed in this study, together with the separation process developed in our previous study (9), should be valuable for process designing for the separation of phytosterol.

## NOMENCLATURE

| | |
|---|---|
| $C$ | Adsorbate concentration in liquid [M] |
| $C_0$ | Initial adsorbate concentration in liquid [M] |
| $D_c$ | Intracrystalline diffusivity [$m^2 \cdot s^{-1}$] |

| | |
|---|---|
| $D_p$ | Pore diffusivity [$m^2 \cdot s^{-1}$] |
| $E$ | Diffusional activation energy [$J \cdot (kg\ mole)^{-1}$] |
| $k_a$ | N-th order adsorption rate constant [$g \cdot L^{-1} \cdot s^{-1} \cdot M^{-n}$] |
| $k_0$ | Zero order adsorption reaction rate constant [$g \cdot L^{-1} \cdot s^{-1}$] |
| $k_1$ | First order adsorption reaction rate constant [$g \cdot L^{-1} \cdot s^{-1} \cdot M^{-1}$] |
| $m_t$ | Amount of sterol adsorbed on zeolite at time $t$ [mole] |
| $m_\infty$ | Amount of sterol adsorbed on zeolite at time $t \to \infty$ [mole] |
| $r_c$ | Microparticle radius [m] |
| $R_p$ | Radius of saturated shell [m] |
| $q_s$ | Saturation limit of sorbate concentration on zeolite [M] |
| $S_T$ | Maximum adsorbability of adsorbent [$mole \cdot g^{-1}$] |
| $V$ | Solution volume [L] |
| $w$ | Adsorbent amount [g] |
| $\varepsilon_p$ | Porosity of adsorbent particle |



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