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(出版者 / Publisher)
法政大学大学院理工学研究科
(雑誌名 / Journal or Publication Title)
法政大学大学院紀要. 理工学研究科編
(巻 / Volume)
65
(開始ページ / Start Page)
1
(終了ページ / End Page)
2
(発行年 / Year)
2024-03-24
(URL)
https://doi.org/10.15002/00030680

Measurement of endotoxin adsorption capacity and the applicability of adsorption isotherms to nonwoven fabric with Polymyxin B

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The maximum amount of endotoxin adsorption by a newly developed adsorbent, ST059 (Japan Hemotech Co.), was measured by repeating solution exchanges. The interaction between endotoxins and two adsorbents, Toraymyxin® (Toray Ind., Inc.) and ST059 was examined using a method to create endotoxin adsorption isotherms. Results were analyzed using the Langmuir adsorption isotherm, and the applicability of the Langmuir adsorption isotherm was examined. Both Toraymyxin® and ST059 exhibited a strong affinity for endotoxins; however, the results with Toraymyxin® may not be well fitted by the Langmuir adsorption isotherm, whereas those with ST059 were well explained by the Langmuir plot, suggesting high applicability of the Langmuir adsorption isotherm.

Key Words *: Endotoxin, Adsorption, Langmuir adsorption isotherm.*

1. Introduction

Endotoxins (Et), released from gram-negative bacteria, trigger immune responses and can cause severe symptoms, even in small doses. Polymyxin B, a cyclic antibiotic, can neutralize Et but has limitations due to its nephrotoxicity and neurotoxicity. Toraymyxin®, using immobilized polymyxin B on nonwoven fabric, reduces side effects but is highly expensive. ST059, an alternative with different nonwoven materials, may lower manufacturing costs by reducing the use of polymyxin B, potentially affecting adsorption efficiency.

This study aims to evaluate the endotoxin adsorption performance of these two adsorbents and identify the key factors that influence adsorption, thereby elucidating the adsorption mechanism.

2. Experimental procedures

(1) Endotoxin adsorption experiment

Et (Control Standard Endotoxin, Japanese Pharmacopoeia Endotoxin RS) was dissolved in sterile water to prepare a test solution. The adsorbent (Toraymyxin®, ST059) was immersed in the solution as described. Then, the solution was heated at 37°C in the thermostatic chamber.

(2) Measurement of absorbance

The required number of vials containing reconstituted LAL reagent (Endospecy® ES-24S Kit) was prepared and immersed in an ice-water bath. Each of the 200 μL samples, standard solutions (Endospecy® ES-24S Kit), or the blank were transferred into the vials containing reconstituted LAL reagent using pipette tips. The test tube mixer stirred the mixture for approximately 2 seconds. LAL reagent vials were incubated in the thermostatic chamber at 37°C for 30 minutes. Once the assay incubation was completed, PyroColor Diazo Reagents DIA60-STV were added to each tube and vortexed for 2 seconds to mix. Absorbance was measured by the spectro-photometer at 545 nm using distilled water as a reference.

3. Theoretical

Assuming that the adsorbate molecules are adsorbed onto the adsorbent surface as a monolayer, the following Langmuir adsorption equation holds at steady state, i.e.,

$$W = \frac{aW_S C}{1 + aC} \tag{1}$$

where C represents the equilibrium concentration of the adsorbate, W_S represents the saturation adsorption capacity, and a represents the adsorption equilibrium constant. Just by taking the reciprocals both sides, we have,

$$\frac{1}{W} = \frac{1}{W_S} + \frac{1}{aW_S} \cdot \frac{1}{C} \tag{2}$$

Alternately multiplying C both sides of Eq. (2), we have,

$$\frac{C}{W} = \frac{1}{aW_S} + \frac{1}{W_S} \cdot C \tag{3}$$

Determination of W_s and a value may be possible either by plotting $1/C$ versus $1/W$ according to Eq. (2), or by plotting C versus C/W according to Eq. (3), and the superiority may be discussed by examining the linearity of the resulting plots.

4. Results and Discussion

(1) Measurement of Et adsorption capacity of ST059

Fig.1 shows the time-dependent cumulative adsorption of Et by ST059. In this experiment, the Et solution was replaced every 24 hours. Results reveal that ST059 adsorbed over 99% of Et in 0-144 hours, with a gradual decrease to about 5% at 240 hours, indicating saturation with a capacity of 1653 EU/mg.

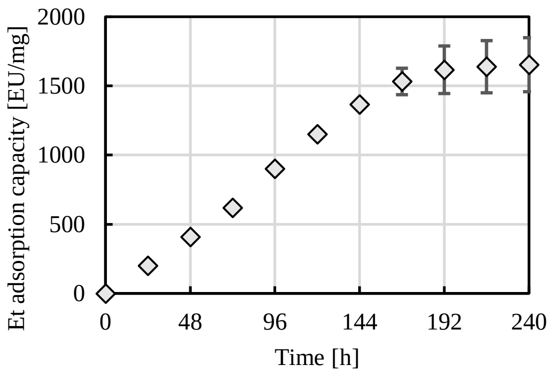

Fig.1 Time course of Et adsorption capacity using ST059.

(2) Langmuir adsorption isotherm

Figures 2 and 3 show the Langmuir adsorption isotherm in Toraymyxin® and ST059, respectively. Based on the data distribution and the determination coefficients of the two graphs in Fig.2, the adsorption of Et aligns more with Eq. (3) in Toraymyxin®. However, the negative adsorption equilibrium constant (a) was found for Toraymyxin® (Table 1), suggesting a deviation from the Langmuir isotherm model.

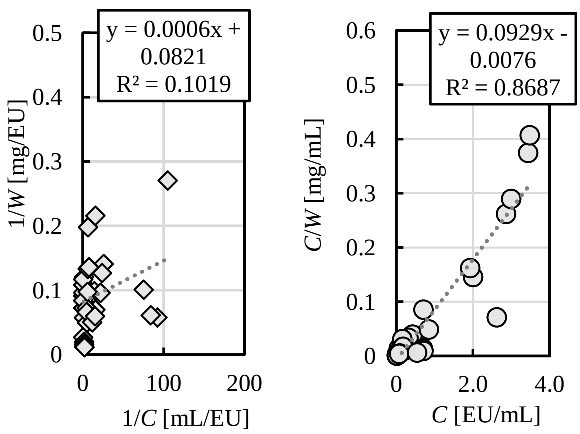

Fig.2 Langmuir adsorption isotherm of Et in Toraymyxin®.
(Right: Eq. (2); Left: Eq. (3))

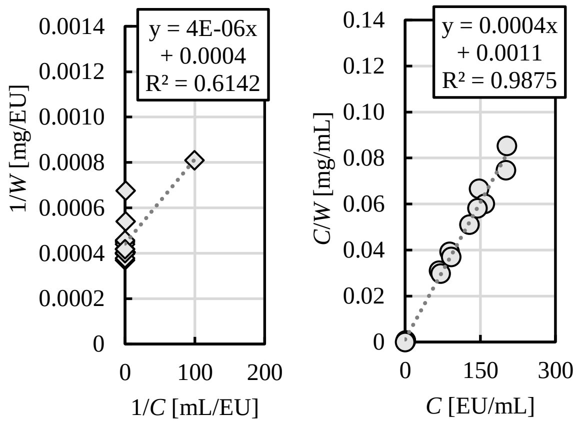

Fig.3 Langmuir adsorption isotherm of Et in ST059.
(Right: Eq. (2); Left: Eq. (3))

Table 1 W_S and a values determined by Eq.(3)

Adsorbent	W_S [EU/mg]	a [mL/EU]
Toraymyxin®	10.76	-12.16
ST059	2517.17	0.36

The data distribution and determination coefficients in Fig.3, however, indicated that adsorption of Et aligns with Eq. (3) in ST059. The saturated amount of Et adsorption (W_S =2517 EU/mg, Table 1) by ST059 was well correlated with the value determined by the repeated method (1653 EU/mg) which also supports the applicability of the Langmuir isotherm.

5. Conclusions

The adsorption experiments showed that Toraymyxin® exhibited a strong affinity for endotoxins; however, its adsorption mechanism may not be explained by the Langmuir isotherm. The maximum amount of Et adsorption in ST059 was 1653 EU/mg by repeatedly changing the solution, which corresponded well with the saturated adsorption value of 2517 EU/mg that was determined by the well-fitted Langmuir isotherm.

Although both adsorbents include polymyxin B, adsorption mechanisms may not be identical.

References

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