【Original Article】

# Adsorption of Coomassi Brilliant Blue R-250 on the Rice Bran

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**Summary**

Coomassi Brilliant Blue R-250(CBB) has been used to stain proteins in the gel electrophoresis. In the gel electrophoresis, the destaining step is important to avoid the raise of background by dye and thereby, the fast and efficient gel destaining is needed. We studied whether the rice bran could adsorb CBB in the dye solution. As a result, higher adsorption of CBB on the rice bran was observed between pH 2.5 to 3.5. Moreover, adsorption of CBB increased with increasing the rice bran dosage and constant adsorption observed at dosage rate of 2.5 g/l and higher. On the other hand, adsorption of CBB on the rice bran was occurred rapidly in the early period and 75% of CBB was adsorbed in 1 min of agitation. Our results showed that the rice bran could quickly adsorb CBB and was an effective adsorbent for CBB.

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**Key words:** Rice bran, Adsorption, CBB, Gel electrophoresis

## Introduction

The gel electrophoresis has been used in the wide range of fields such as biochemistry, pharmacy, medicine and so on, and is one of the most important methods to analyze the purity and molecular weight of proteins[1~3]. In the gel electrophoresis, the destaining step is important to avoid the raise of background by dye and thereby, the fast and efficient gel destaining is needed. In general, Coomassi Brilliant Blue R-250 (CBB) has been used as dye to stain proteins in the gel. In order to achieve the fast and efficient destaining, the ion exchange resin or activated carbon is utilized to remove the dye molecule in solution. Nishikawa et. al. have been reported that Okara was an effective adsorbent for CBB dye molecule in solution and its addition enabled them to obtain a clear solution[4]. We found that the rice bran was an effective adsorbent for the removal of CBB dye molecule in solution, as well as Okara. In this investigation, we describe adsorption of CBB on the rice bran from the dye solution.

## Materials and Methods

### Adsorbent and reagents

The adsorbent, the rice bran was obtained from a rice shop. CBB was purchased from Wako Company and other chemical reagents used were analytical grade.

### Adsorption studies

Adsorption experiments were conducted according to Nishikawa's method [4] with a slight modification. Batch adsorption experiments were carried out by mixing 50 ml of the CBB solution (containing 25% of methanol) of a particular concentration with a pre-weighted amount of the rice bran in 100 ml of erlenmeyer flasks. The pH of the mixture was adjusted by adding acetic acid or 0.1 M NaOH solution. The erlenmeyer

flasks were transferred to a water bath shaker at 30 ℃ and were agitated for a pre-determined time interval at 120 rpm. The parameters such as the rice bran amount, agitation time and pH were controlled during adsorption experiments. When adsorption of CBB was completed, the mixture was filtrated with a filter paper and the amount of unadsorbed CBB in the filtrate was estimated spectrophotometrically (Wavelength: 550 nm). The percentage of CBB adsorption was calculated using the following equation:

Percentage adsorption = $\{(C_0 - Cf) / C_0\} \times 100$

where $C_0$ and Cf are the initial and final concentrations (mg/l).

## Results

### Effect of pH on adsorption of CBB.

Adsorption experiments were conducted with the rice bran at the constant dosage (10 g/l) and the CBB concentration (30 mg/l) for 2 hrs by varying pHs. Figure 1 shows the effect of pH on CBB adsorption on the rice bran. Higher CBB adsorption was observed between pH 2.5 to 3.5, whereas lower adsorption of CBB was obtained in other pHs. Consequently, we chose pH 3 as an optimum value.

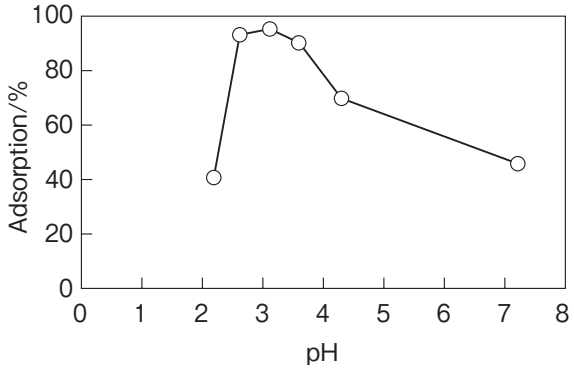


**Fig. 1 Effect of pH on adsorption of CBB.**

### Effect of the dosage on adsorption of CBB.

Adsorption experiments were carried out in the erlenmeyer flask at constant concentration (30 mg/l), pH (3.0) and agitation time (1 hr). In this experimental condition, adsorption of CBB increased with increasing the rice bran dosage and constant adsorption observed at dosage rate of 2.5 g/l and higher as shown in Fig.2. We selected 2.5 g/l as an optimum dosage.

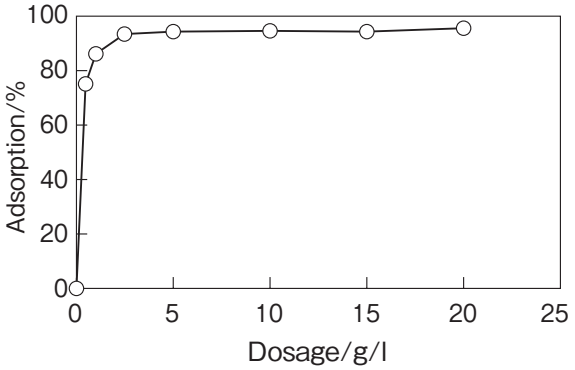


**Fig. 2 Effect of the dosage on adsorption of CBB.**

### Effect of agitation time on adsorption of CBB.

The effect of agitation time on adsorption of CBB from the dye solution was investigated under conditions of pH 3, 30 mg/l of CBB concentration and 2.5 g/l of the rice bran. Adsorption of CBB on the rice bran was occurred rapidly in the early period and then gradual adsorption was observed. Since adsorption curve reached plateau in agitation time greater than 20 min, we chose 40 min of agitation as an optimum contact time.

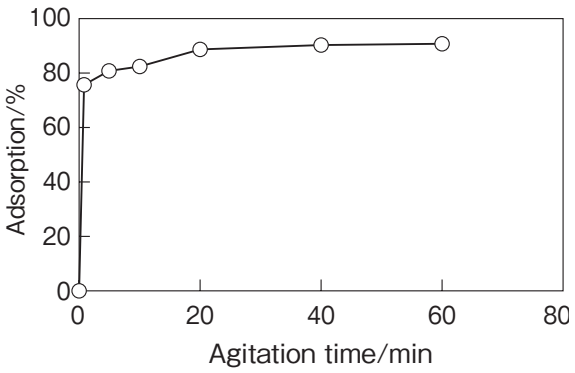


**Fig. 3 Effect of agitation time on adsorption of CBB.**

Table 1 shows the optimum condition for adsorption of CBB from the dye solution.

**Table 1 The optimum condition of adsorption of CBB from the dye solution.**

| | |
|---|---|
| pH | 3.0 |
| Dosage (g/l) | 2.5 |
| Agitation time (min) | 40 |

Adsorption of CBB from the dye solution was conducted under the optimum condition shown in Table 1. Figure 4 shows the photograph of the CBB adsorption test.

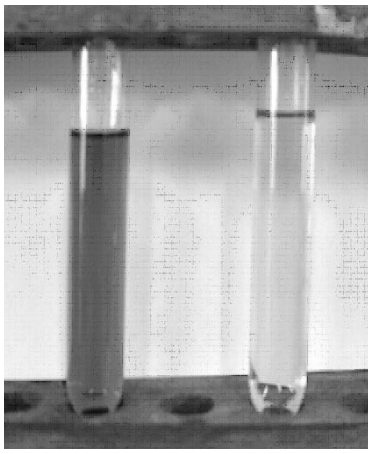

**Fig. 4 The photograph of the CBB adsorption test. Left: CBB solution, Right: after removal of CBB**

The rice bran significantly adsorbed CBB from the dye solution and enabled it to obtain a clear solution after filtrating the reaction mixture with a filter paper.

## Discussion

Nishikawa et. al. have been reported that Okara was a good adsorbent for CBB adsorption [4] from the dye solution. In the paper, they described that CBB was adsorbed in acidic conditions, thereby CBB was attached to residual protein in Okara. As shown in Fig.1, adsorption of CBB on the rice bran was also observed in acidic conditions as same as Okara. This suggests that the $-SO_3H$ group in the CBB molecule and the $-NH_2$ group in protein remaining in the rice bran are related to adsorption of CBB on the rice bran[4].

As shown in Fig.2, although agitation time of 20 min and higher was needed to reach the equilibrium state, 75% of CBB was adsorbed rapidly on the rice bran in 1 min of agitation. This result strongly suggested that the rice bran could adsorb CBB quickly and it was one of the most efficient adsorbent for CBB.

On the other hand, the maximum CBB adsorption capacity of the rice bran was calculated from the Langmuir isotherm. The Langmuir isotherm is the most widely used equation for calculating the adsorption of dye molecules from liquid solutions[5,6]. The linear Langmuir equation is shown as follows:

$$1/q = (1/A \cdot Qm \cdot Cf) + 1/Qm$$

where Cf (mg/l) is the concentration of the CBB solution at equilibrium, q (mg/g) is the amount of CBB adsorbed at equilibrium, Qm (mg/g) is the maximum adsorption capacity and A is Langmuir constant. The plot of 1/q as a function of 1/Cf gives a straight line with an intercept of 1/Qm and a slope of 1/(A·Qm). Figure 5 shows the Langmuir plot.

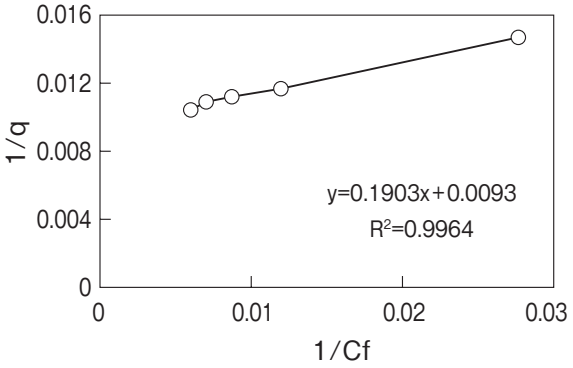


**Fig. 5 Langmuir adsorption isotherm.**

The maximum adsorption capacity of the rice bran, Qm was found to be 108 mg/g from the intercept of the straight line in Fig.5. Nisikawa et. al. have been reported that Qm values of Okara and the commercial active carbon are 197 mg/g and 3 mg/g, respectively [4]. Although the Qm value of the rice bran was smaller than that of Okara, it was 36 times larger in comparison with that of the

active carbon.

Consequently, the rice bran could quickly adsorb CBB and was found to be an effective adsorbent for CBB.

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# クーマシーブリリアントブルー R-250 のヌカへの吸着

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**要　旨**

クーマシーブリリアントブルー R-250 は、ゲル電気泳動法においてタンパク質を染色するのに使用されている。このゲル電気泳動法において、脱色段階で色素によるバックグランドの上昇を避けることは重要であり、それ故、迅速かつ効率の良い脱色法が必要とされている。我々は、染色溶液中の CBB をヌカを用いて吸着除去できないかどうか検討した。その結果、pH2.5 から 3.5 の間でヌカへの高い CBB の吸着が観察された。さらに、CBB の吸着はヌカ使用量の増加とともに上昇し 2.5 g/l 以上で一定となった。一方、ヌカへの CBB の吸着は撹拌時間初期の段階で急激に起こり、75% の CBB が 1 分以内に吸着した。従って、ヌカは CBB を急速に吸着することができ、CBB の吸着剤として有効であることが明らかとなった。

**キーワード**：ヌカ、吸着、クーマシーブリリアントブルー、ゲル電気泳動

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