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Research Article

# APPLICATION OF ISOLATED AGAROSE AS A NEW ADSORBENT IN ANALYSIS OF TARTRAZINE BY A VISIBLE-SPECTROPHOTOMETRY METHOD

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**ABSTRACT**

This study aims to isolate agarose from agar and use isolated agarose as an adsorbent in the analysis of tartrazine by the vis-spectrophotometry method. Isolation of agarose from agar was conducted with the mixture of propylene glycol and distilled water (19:1) as solvent at 105°C, agarose was then precipitated from solution by addition of isopropyl alcohol and the mixture allowed at a temperature of 10°C. The adsorption and desorption capacity experiment of agarose to tartrazine was carried out with variations in pH, contact time and concentration of tartrazine. Adsorbed or released tartrazine by agarose was quantified by spectrophotometry method at a wavelength of 427 nm. The adsorption process of agarose to tartrazine was found optimal at the appropriate experimental condition, namely at acidic milieu (pH 4) and contact time for 60 minutes, meanwhile the desorption process of isolated agarose was optimal at basic condition (pH 9) and contact time for 60 minutes. Adsorption and desorption process of 0.25 g of agarose was optimal in an experiment used 25 ml of 5.6 µg/ml tartrazine solution as adsorbate, which gave adsorption and desorption capacity of 0.1108 (± 0.0014) and 0.1083 ± (0.0014) mg/g, respectively. It could be concluded that agarose can be used and developed as a new adsorbent in the analysis of tartrazine.

**Keywords**: adsorbent capacity, agarose, agar, spectrophotometry method, tartrazine

## INTRODUCTION

Agar-producing seaweed (agarophyte) comes from various genera, such as *Gelidium* (Gelidiaceae), *Gracilaria* (Gracilariaceae), *Pterocladia* (Gelidiaceae) and *Ahnfeltia* (Phyllophoraceae) were found in some countries[1]. *Gracilaria gigas* (Glacilariaeceae) is the main species that is cultivated as an agar-producing commodity in Indonesia; two main hydrocolloid components in agar are agarose and agaropectin. Agarose is the major fraction of agar. Structurally, agarose is a linear polymer consisting of repeating unit shown in Figure 1, containing both α- and β-glycosidic bond. The two monosaccharides present are β-D-galactose and 3,6-anhydro-α-L-galactose, linked by glycosidic bonds β(1–4) between β-D-galactose and 3,6-anhydro-α-L-galactose, giving the disaccharide basic unit called neoagarobiose and α(1–3) between 3,6-anhydro-α-L-galactose and β-D-galactose, giving the disaccharide basic unit called agarobiose[2]. Agar and agarose have important applications in the fields of biotechnology, pharmaceuticals, cosmetics and the food industry[3].

Tartrazine ($C_{16}H_9N_4Na_3O_9S_2$) has a molecular weight of 534.36 g/mol, the melting point of 300 °C, absorption of maximum at 425 nm. The chemical name this compound is 4,5-dihydro-5-oxo-1-(4-sulfophenyl)-4-[(4-sulfophenyl) azo] salt -1H-pyrazole-3-trisodium carboxylic acid[4]. Tartrazine with another name E102 (EFSA-European Food Safety Authority), FD and C Yellow 5 (FDA-US Food and Drug Administration) or C.I. 19140 (Color Index International), is certified as FDA coloring for its use in food, medicine and cosmetics[5].

JECFA (Joint FAO / WHO Expert Committee on Food Additives and the European SCF (Scientific Committee for Food) have set Acceptable Daily Intake (ADI) tartrazine from 0-7 mg/kg body weight/day in the latest security evaluation in 2016 to be 0-10 mg/kg body weight/day, on the basis of no convincing evidence of adverse effects to the highest dose level tested (10 mg/kg body weight/day) in long-term studies. Exposure to food for children aged 1-10 years below the ADI limit does not cause health problems[6]. Excessive consumption of tartrazine can trigger childhood asthma, urticaria, hyper-active disorders. Studies have found tartrazine can interfere with chromosomal synthesis and induce DNA damage. Research in mice found that high concentrations of tartrazine can increase sperm malformations and cause symptoms such as anxiety[7]. Therefore, the content of tartrazine in food and other products must be controlled for use to ensure safety for consumers.

In this study, agarose was isolated from agar powder by modification of Provonchee method[8]. Evaluation of isolated agaroses such as its sulfate content[9], gel formation point and a melting point of gel[10] and gel strength were conducted by a standard method. Agarose is used as an adsorbent of tartrazine in solution and the adsorption capacity and desorption of agarose to tartrazine dye stuff with variations in pH, contact time and the concentration of the tartrazine solution were determined in this study. Adsorption and desorption were analyzed quantitatively by the visible-spectrophotometry method.

## MATERIALS AND METHOD

Agar powder from *Gracilaria gigas* seaweed produced by PT. Satelit Sriti (from East Java), tartrazine dyestuff (Wako Pure Chemical Industries) propylene glycol and isopropanol (Brataco), potassium bi phthalate, boric acid and ethanol pa (Merck, Germany).

### Isolation of Agarose

A total of 20 g of agar powder was dissolved in a mixture of propylene glycol and distilled water (19:1), the mixture was heated using an electric stove to a temperature of 80-105°C. The solution was then allowed to cool until 40°C; 2000 ml of isopropanol was gently added by continuously stirring to get a homogenous solution. The mixture was placed in the refrigerator at 10ºC for 24 h until the precipitation of agarose was completely forming. The precipitate was separated from the solution by centrifuging process. The agarose precipitate was washed with isopropanol then allowed in the refrigerator for 24 h until the precipitate was completely formed. Re-purify agarose yields were performed by reheating agarose in propylene glycol and distilled water (950 ml and 50 ml) to a temperature of 105ºC for 1 h. The solution was cooled to 40°C, and 1000 ml of isopropanol was added and mixed well. The mixture then placed in the refrigerator at a temperature of ± 10°C for 24 h. The agarose precipitation was filtered *in vacuo*. The agarose was dried in a vacuum desiccator to ensure that agarose is free of water and remains dry. The dried agarose was then stored in a tight container for further evaluation.

### Determination of Sulfate Content

1 mg of isolated agarose was carefully ground in an agate mortar with 100 mg of KBr and compressed into thin discs or pellets (KBr pellet method). Then transmittance of the pellet was measured using FT-IR Spectrometer. The infrared absorbance band at 2920 $cm^{-1}$ describes C-H which is used as an index for the total amount of sugar content. The sulfate found in agarose is calculated using the ratio of absorbance at certain wave numbers. Total sulfate was = absorbance at 1250 /absorbance at 2920 $cm^{-1}$.

### Determination of Agarose Gel Strength

A total of 1.5 g of agarose was dissolved in 100 ml of distilled water so that the concentration was 1.5 % w/v, heated to a temperature of 60ºC for 15 min. Incubation at 10ºC for 17 ± 2 h. Subsequently measured gel strength using Brookfield's CT3 Texture Analyzer on a 6 mm probe diameter (r = 3 mm), probe speed of 0.5 mm /s with a depth of 25 mm with a contact area of 28.26 $mm^2$. The strength of the gel is expressed in units of $g/cm^2$.

### Determination of Gelling Point and Melting Point of Agarose Gel

A total of 1.5 g of agarose was weighed and dissolved in 100 ml of distilled water in a beaker glass so that the concentration of the solution was 1.5 %. The solution was boiled in a water bath for 5 min. Then 15 ml of solution was poured into a test tube with a diameter of 18 mm and a height of 150 mm. The reaction tube was placed on a shelf; then allowed in a water bath at 60ºC. Cold water was flowed into the water bath to reduce the temperature of 0.3-0.5ºC per minute. During the cooling process, the test tube is periodically tilted while observing the solution inside. If after tilting 45º does not flow, the thermometer was immediately inserted into the test tube. The observed temperature was recorded as a gel formation point. The test tube containing the gel from the measurement point of gel formation is allowed to stand for 1 hour until the gel was formed completely. On the surface of the gel was placed a tin ball, the test tube was then placed into a water bath with a temperature of 20ºC. Then the water bath was heated at a heating rate of about 1ºC per minute. When the ball sunk, the temperature of the water bath is recorded. The temperature of the melting point of the gel was obtained from the water bath temperature minus 5ºC.

### Tartrazine Standard Solution Preparation

Tartrazine stock solution was made at the concentration of 200 µg/ml in ethanol. Standard solutions were then prepared by dilution of the stock solution quantitatively with ethanol to get the standard solutions with a concentration of 6, 8, 10, 12, 14, 16, and 18 µg /ml, respectively.

### Measurement of Tartrazine Maximum Wavelength

The standard tartrazine solution with a concentration of 10 µg/ml was measured for its absorption maximum by scanning at a wavelength of 400-800 nm using a spectrophotometer

### Regression Equation and Correlation Coefficient (R)

The absorption of the tartrazine standard solution with a concentration of 6, 8, 10, 12, 14, 16, and 18 µg /ml (x1, x2, x3, x4, x5, x6, and x7) were measured at maximum wavelength, λ 427 nm to get absorbance of y1, y2, y3, y4, y5, y6 and y7. Data of concentrations and data of absorbance solutions were then processed with the Microsoft Excel program to obtain the regression equation (y = ax + b) and correlation coefficient (R).

### Limit of Detection (LOD) and Limit of Quantitation (LOQ)

LOD and LOQ of analysis were determined on the base of standard deviations of absorbance y1, y2, y3, y4, y5, y6, y7.

The standard deviation was calculated with the following equation

$$SDy = \sqrt{\frac{\sum(y - yi)^2}{n - 1}}$$

The LOD and LOQ analysis was calculated by the following equation.

$$\text{LOD} = \frac{3 \text{ x SDy}}{\text{slope b}} \qquad LOQ = \frac{10 \text{ x SDy}}{\text{slope b}}$$

### The Mobile Phase of TLC Separation of Tartrazine Solution

3 µl of the tartrazine stock solution was applied on a Silica gel TLC 254 plate with a size of 10 x 5 cm. The mobile phase used was a mixture of methanol and dichloromethane with various concentrations. Mobile phase development allowed with a distance of 8 cm. The mobile phase was selected which gave the Rf values of 0.4 to 0.6.

### Preparation of Phthalate Buffer (pH 4.0)

50 ml of 0.2 M of potassium phthalate solution was mixed with 0.1 ml of 0.2 N of HCl and dissolved with $CO_2$ free distilled water to 200 ml.

### Preparation of Borate Buffer Solution (pH 9.0)

50 ml of 0.2 M of the boric acid solution was mixed with 20.8 ml of 0.2 N of sodium hydroxide solution and dissolved with $CO_2$ free distilled water to 200 ml.

**Determination of the Optimum pH of Adsorption**

**Adsorption in acidic condition (pH4)**

10 ml standard solution of tartrazine with a concentration of 10 µg/ml was added with 15 ml of phthalate buffer solution (pH 4) and 0.25 g of isolated agarose was added to the solution. The mixture was then stirred at 200 rpm using a magnetic stirrer for 30 min. The solution was filtered to get the tartrazine adsorbed agarose and filtrate. The filtrate was poured into a 25 ml measuring flask and its volume was made up with 70 % ethanol to 25 ml. The absorbance of solution contained the rest of non-absorbed tartrazine was measured using spectrophotometer at a wavelength of 427 nm (N = 3).

**Adsorption in a neutral condition (pH 7)**

10 ml standard solution of tartrazine with a concentration of 10 µg/ml was added with 15 ml of 70 % ethanol and 0.25 g of isolated agarose was then added to the solution. The mixture was then stirred at 200 rpm using a magnetic stirrer for 30 min. The solution was filtered to get the tartrazine adsorbed agarose and filtrate. The filtrate was poured into a 25 ml measuring flask and its volume was made up with 70 % ethanol to 25 ml. The absorbance of solution contained the rest of non-adsorbed tartrazine was measured using spectrophotometer at a wavelength of 427 nm (N = 3).

**Adsorption in a basic condition (pH 9)**

10 ml standard solution of tartrazine with a concentration of 10 µg/ml was added with 15 ml of borate buffer solution (pH 9) and 0.25 g of isolated agarose was added to the solution. The mixture was then stirred at 200 rpm using a magnetic stirrer for 30 min. The solution was filtered to get tartrazine absorbed agarose and filtrate. The filtrate was poured into a 25 ml measuring flask and its volume was made up with 70 % ethanol to 25 ml. The absorbance of solution contained the rest of non-adsorbed tartrazine was measured using spectrophotometer at a wavelength of 427 nm (N = 3).

The adsorption capacity of adsorbent was calculated by the following equation:

$$Q = \frac{V\ (C_0 - C_t)}{m}$$

Explanation:
Q = adsorption capacity (mg/g)
V = volume of solution (l)
$C_0$ = tartrazine concentration before adsorption process (µg/ml)
$C_t$ = tartrazine concentration after adsorption process (µg/ml)
m = weight of agarose (g)

**Determination of the Optimum pH of Desorption Tartrazine by Agarose**

**Desorption in acidic condition (pH 4)**

10 ml of 70 % ethanol was added to 15 ml of phthalate buffer solution (pH 4) and 0.25 g of agarose that has adsorbed tartrazine in acidic condition was added gently to the solution. The mixture was then stirred at 200 rpm using a magnetic stirrer for 30 min. The mixture was then filtered to get the tartrazine desorbed agarose and the filtrate containing tartrazine released by agarose. The filtrate was poured into a 25 ml measuring flask and its volume was made up with 70 % ethanol to 25 ml. The absorbance of the solution containing tartrazine was measured using spectrophotometer at a wavelength of 427 nm (N = 3).

**Desorption in basic condition (pH 9)**

20 ml of 70 % ethanol was added to 5 ml of borate buffer solution (pH 9) in 50 ml Erlenmeyer and 0.25 g of agarose that has adsorbed tatrazine in basic condition was added gently to the solution. The mixture was then stirred at 200 rpm using a magnetic stirrer for 30 minutes. The mixture was then filtered to get the tartrazine desorbed agarose and the filtrate containing tartrazine released by agarose. The filtrate was poured into a 25 ml measuring flask and its volume was made up with 70 % ethanol to 25 ml. The absorbance of the solution containing tartrazine was measured using spectrophotometer at a wavelength of 427 nm (N = 3). To get quantitatively releasing of tartrazine from the adsorbent, to the same agarose was carried out three times desorption process.

Desorption capacity of adsorbent was calculated by following equation:

$$dQ = \frac{V\ (C_t - C_0)}{m}$$

Explanation:
dQ = desorption capacity (mg/g)
V = volume of solution (l)
$C_0$ = tartrazine concentration before adsorption process (µg/ml)
$C_t$ = tartrazine concentration after adsorption process (µg/ml)
m = weight of agarose (g)

**Determination of optimum contact time of adsorption in optimum pH (pH 4)**

3 of 50 ml Erlenmeyer were prepared for the experiment. To each of Erlenmeyer 10 ml of standard tartrazine solution with a concentration of 10 µg/ml was added and followed by adding 15 ml of a buffer solution of pH 4 and 0.25 g of isolated agarose. The mixture was then stirred at 200 rpm using a magnetic stirrer for 30, 60 and 90 min. The mixture was filtered to get the agarose that adsorbed tartrazine in various contact time and the filtrate contained the residual of tartrazine. Each filtrate was poured into a 25 ml volumetric flask; the volume was made up with 70 % ethanol to 25 ml. The absorbance of the solution was measured using a spectrophotometer at a wavelength of 427 nm (N = 3).

**Determination of maximum adsorption capacity of isolated agarose to various concentration of tartrazine at optimum pH and optimum contact time**

7 of 50 ml Erlenmeyer were prepared for the experiment. To each of Erlenmeyer 10 ml of standard tartrazine solution with a concentration of 6, 8, 10, 12, 14 µg/ml was added and followed by adding 15 ml of a phthalate buffer solution of pH 4 and 0.25 g of isolated agarose. The mixture was then stirred at 200 rpm using a magnetic stirrer for 60 min. The mixture was filtered to get the agarose that adsorbed tartrazine from the various concentration of the solution and the filtrate contained the residual of tartrazine. Each filtrate was poured into a 25 ml volumetric flask; the volume was made up with 70 % ethanol to 25 ml. The absorbance of the solution was measured using a spectrophotometer at a wavelength of 427 nm (N = 3).

**Determination of maximum desorption capacity at optimum pH on agarose adsorbed various concentrations of tartrazine solution at optimum pH and optimum contact time**

For desorption experiment 7 of 50 ml of Erlenmeyer were prepared, to each Erlenmeyer were 20 ml of 70 % ethanol and 5 ml of borate buffer (pH 9) added, following by adding agarose that adsorbed tartrazine solution concentration of 6, 8, 10, 12, and 14 µg/ml from experiment above (Adsorption in acidic

condition). The mixture was then stirred at 200 rpm using a magnetic stirrer for 60 min. The mixture was filtered to get the agarose that has released tartrazine and the filtrate contained a various concentration of tartrazine. Each filtrate was poured into a 25 ml volumetric flask; the volume was made up with 70 % ethanol to 25 ml. The absorbance of the solution was measured using a spectrophotometer at a wavelength of 427 nm (N = 3). To ensure that adsorbed tartrazine was completely released by agarose, the desorption process at pH 9 was repeated up to 2 times, and followed by monitoring the desorption solution by TLC method on the presence of residual tartrazine.

## RESULTS AND DISCUSSION

### Agarose Isolation and Determine its Physical Properties

Separation of agarose from agaropectin was designed base on difference polarity of two compounds. By heating agar in propylene glycol and distilled water (95:5) at 105°C, both agarose and agaropectin will be dissolved completely. The polarity of the solvent mixture will be reduced by adding a second solvent namely, isopropyl alcohol. By the cooling process at 10°C, agarose that relative more lipophilic will start to precipitate. Agarose precipitation was separated by centrifugation and filtration and then was dried in a vacuum desiccator. Agarose powder from isolation, was re-dissolved, precipitated and dried with solvent and the same method explained above to obtain agarose in the form of white powder with a yield of 66.05 %. Physical properties of isolated agarose were determined by standard methods and its 1.5 % gel will give a gelling point of 37°C, melting point of 87°C, the gel strength of 1000.8 g/cm$^2$. The total sulfate content of isolated agarose was found to be 0.13 %.

### Analysis of Tartrazine Standard and Validation of Analysis Method

Determined of the maximum wavelength of 10 µg/ml of tartrazine standard in 70 % ethanol with spectrophotometer gave a maximum at 427 nm (ε 21.481). TLC analysis of 200 µg/ml of tartrazine standard in ethanol on stationary phase Silica gel F254 plate (Merck) and used the mixture of dichloromethane with methanol (7:3) and 5 drops of glacial acetic acid as the mobile phase. TLC plate was developed with the mobile phase for 10 cm distance after the drying process tartrazine will appear as a single spot with Rf 0.52. Each solution tartrazine standard with a concentration of 6, 8, 10, 12, 14, 16, and 18 µg /ml were measured its absorption at maximum wavelength, λ 427 nm to get absorbance of 0.252, 0.327, 0.410, 0.492, 0.574, 0.655, and 0.737. Data of concentrations and data of absorbance solutions were then processed with the Microsoft Excel program to obtain the regression equation, y = 0.040 x + 0.004 and correlation coefficient (R) 0.999. Limit of detection (LOD) and Limit of Quantitation (LOQ) was found 0.72 and 2.4 µg/ml, respectively. Determined of the maximum wavelength of 10 µg/ml of tartrazine standard in 70 % ethanol with spectrophotometer gave a maximum at 427 nm (ε 21.481). TLC analysis of 200 µg/ml of tartrazine standard in ethanol on stationary phase Silica gel F254 plate (Merck) and used the mixture of dichloromethane with methanol (7:3) and 5 drops of glacial acetic acid as the mobile phase. TLC plate was developed with the mobile phase for 10 cm distance after the drying process tartrazine will appear as a single spot with Rf 0.52. Each solution tartrazine standard with a concentration of 6, 8, 10, 12, 14, 16, and 18 µg /ml were measured its absorption at maximum wavelength, λ 427 nm to get an absorbance of 0.252, 0.327, 0.410, 0.492, 0.574, 0.655, and 0.737. Data of concentrations and data of absorbance solutions were then processed with the Microsoft Excel program to obtain the regression equation, y = 0.040 x + 0.004 and correlation coefficient (R) 0.999. Limit of detection (LOD) and Limit of Quantitation (LOQ) was found 0.72 and 2.4 µg/ml, respectively.

In reference was found that S. Pereira, *et al*. in 2007 used HPLC with UV-DAD detector to the analysis of tartrazine from juice fruit. With ODS as the stationary phase and the mixture of methanol and ammonium acetate 0.08 mol/l (45:55) they found LOD and LOQ analysis were 0.05 and 0.14 µg/ml and correlation coefficient, R was 0.999[11]. Meanwhile, M. P. Urquiza in 2000 reported a rapid method based on capillary zone electrophoresis coupled with photodiode-array detection has been developed to determine Tartrazine E-102 and some dyestuffs in beverages. The separation was performed by using a Bare CElect-FS75 CE column, using a 10 mM phosphate buffer at pH 11.0. They found that tartrazine standard solution gave LOD and LOQ of analysis of 1.3 and 4.4 µg/ml with coefficient correlation, R 0.995[12]. It can be seen that the capillary zone electrophoresis method for tartrazine analysis is more sensitive than HPLC and spectrophotometry method.

### pH Optimum of the Adsorption Process

To find out an optimum of pH solution in the adsorption process, a solution of 4 µg/ml of tartrazine in 25 ml of volumetric was adsorbed with 0.25 g of isolated agarose for 30 min with a variety of pH value. The absorbance of the residual tartrazine solution was measured by visible spectrophotometer at a wavelength, λ 427 nm. Concentration adsorbed tartrazine was calculated by regression equation of tartrazine standard, the experimental data were presented in Table 1.

Data in Table 1 showed that adsorption of tartrazine at pH value of 4, 7, and 9 gave an adsorption capacity (Q) of 0.0267, 0.0083 and 0.0033 mg/g, respectively. From this experiment, it has been proven that the adsorption process of tartrazine by agarose will be maximal in acid condition (pH 4).

### pH Optimum of the Desorption Process

In the experiment that use of an adsorbent to isolate any dyes from food or pharmaceutical preparation samples, an adsorbent not only to have a high adsorption capacity but must also be able to re-release adsorbed dyestuffs into the test solution completely so that it can be quantified by various methods of analysis. In this experiment, the agarose which has adsorbed the tartrazine solution in an acidic condition was continuously used as a desorption experiment subject that was carried out in an acidic and alkaline condition. The experiment results were presented in Table 2.

Data in Table 2 showed that the adsorption process of 4 µg/ml of tartrazine solution for 60 minutes at pH 4 and pH 9 will give desorption capacity of 0.0033 (± 0.0014) and 0.0241 (± 0.0014) mg/g, respectively. It can be concluded that tartrazine desorption process from agarose is higher in basic than in acidic condition.

### Contact Time Optimum of the Adsorption Process

To find out the optimal contact time for the adsorption process of tartrazine by isolated agarose, hence adsorption experiments were carried out with variations in contact time. Where in this experiment, the adsorption process of 0.25 g isolated agarose was carried out on 4 µg/ml tartrazine solution with variations of contact time of 30, 60 and 90 minutes. The experimental results are displayed in Table 3.

**Table 1**: **Determination of adsorption capacity of agarose to 4 µg/ml of tartrazine solution at various pH value**

| pH | Co (µg/ml) | Absorbance after adsorption | Ct (µg/ml) | Co-Ct (µg/ml) | Q (mg/g) | Average Q ± SD (mg/g) |
|---|---|---|---|---|---|---|
| Acidic (pH 4) | 4 | 0.153 | 3.725 | 0.275 | 0.0275 | 0.0267±0.0014 |
| | | 0.154 | 3.750 | 0.250 | 0.0250 | |
| | | 0.154 | 3.725 | 0.275 | 0.0275 | |
| Neutral (pH 7) | | 0.160 | 3.900 | 0.100 | 0.0100 | 0.0083±0.0014 |
| | | 0.161 | 3.925 | 0.075 | 0.0075 | |
| | | 0.161 | 3.925 | 0.075 | 0.0075 | |
| Basic (pH 9) | | 0.163 | 3.975 | 0.025 | 0.0025 | 0.0033±0.0014 |
| | | 0.162 | 3.950 | 0.050 | 0.0050 | |
| | | 0.163 | 3.975 | 0.025 | 0.0025 | |

$C_0$ = tartrazine concentration before adsorption, Ct = tartrazine concentration after adsorption, Q = adsorption capacity

**Table 2: Determination of agarose desorption capacity at various pH condition on the agarose that adsorbed 4µg/ml of tartrazine solution at pH 4 for 60 minutes**

| pH | Co (µg/ml) | Absorbance after desorption | Ct (µg/ml) | Ct - Co (µg/ml) | dQ (mg/g) | Average desorption capacity ± SD (mg/g) |
|---|---|---|---|---|---|---|
| Acidic (pH 4) | 0 | 0.005 | 0.025 | 0.025 | 0.0025 | 0.0033±0.0014 |
| | | 0.006 | 0.050 | 0.050 | 0.0050 | |
| | | 0.005 | 0.025 | 0.025 | 0.0025 | |
| Basic (pH 9) | | 0.014 | 0.250 | 0.250 | 0.0250 | 0.0241±0.0014 |
| | | 0.014 | 0.250 | 0.250 | 0.0250 | |
| | | 0.013 | 0.225 | 0.225 | 0.0225 | |

$C_0$ = tartrazine concentration before desorption, Ct = tartrazine concentration after desorption, dQ = desorption capacity

**Table 3: Determination of the adsorption capacity of agarose to 4 µg/ml of tartrazine solution at optimum condition (pH 4) for various contact time**

| Contact time (min) | Co (µg/ml) | Absorbance after adsorption | Ct (µg/ml) | Co-Ct (µg/ml) | Q (mg/g) | Average Q ± SD (mg/g) |
|---|---|---|---|---|---|---|
| 30 | 4 | 0.153 | 3.725 | 0.275 | 0.0275 | 0.0267±0.0014 |
| | | 0.154 | 3.750 | 0.250 | 0.0250 | |
| | | 0.154 | 3.725 | 0.275 | 0.0275 | |
| 60 | | 0.139 | 3.375 | 0.625 | 0.0625 | 0.0617±0.0014 |
| | | 0.140 | 3.400 | 0.600 | 0.0600 | |
| | | 0.139 | 3.375 | 0.625 | 0.0625 | |
| 90 | | 0.160 | 3.900 | 0.100 | 0.0100 | 0.0125±0.0025 |
| | | 0.159 | 3.875 | 0.125 | 0.0125 | |
| | | 0.158 | 3.850 | 0.150 | 0.0150 | |

$C_0$ = tartrazine concentration before adsorption, Ct = tartrazine concentration after adsorption, Q = adsorption capacity

**Table 4: Determination of maximum adsorption capacity of isolated agarose to various concentration of tartrazine at pH 4 for 60 minutes**

| Co (µg/ml) | Absorbance after adsorption | Ct (µg/ml) | Q (mg/g) | Co-Ct (µg/ml) | Average Q ± SD (mg/g) |
|---|---|---|---|---|---|
| 2.4 | 0.093 | 2.225 | 0.0175 | 0.175 | 0.0167±0.0028 |
| | 0.094 | 2.250 | 0.0150 | 0.150 | |
| | 0.093 | 2.225 | 0.0175 | 0.175 | |
| 3.2 | 0.123 | 2.975 | 0.0225 | 0.225 | 0.0225±0.0003 |
| | 0.123 | 2.975 | 0.0225 | 0.225 | |
| | 0.123 | 2.975 | 0.0225 | 0.225 | |
| 4 | 0.139 | 3.375 | 0.0625 | 0.625 | 0.0617±0.0014 |
| | 0.140 | 3.400 | 0.0600 | 0.600 | |
| | 0.139 | 3.375 | 0.0625 | 0.625 | |
| 4.8 | 0.160 | 3.900 | 0.0900 | 0.900 | 0.0908±0.0014 |
| | 0.159 | 3.875 | 0.0925 | 0.925 | |
| | 0.160 | 3.900 | 0.0900 | 0.900 | |
| 5.6 | 0.183 | 4.475 | 0.1125 | 1.125 | 0.1108±0.0014 |
| | 0.184 | 4.500 | 0.1100 | 1.100 | |
| | 0.184 | 4.500 | 0.1100 | 1,100 | |
| 6.4 | 0.240 | 5.900 | 0.0500 | 0.500 | 0.0492±0.0014 |
| | 0.240 | 5.900 | 0.0500 | 0.500 | |
| | 0.241 | 5.925 | 0.0475 | 0.475 | |
| 7.2 | 0.266 | 6.550 | 0.0650 | 0.650 | 0.0625 ± 0.0025 |
| | 0.268 | 6.600 | 0.0600 | 0.600 | |
| | 0.267 | 6.575 | 0.0625 | 0.625 | |

$C_0$ = tartrazine concentration before adsorption, Ct = tartrazine concentration after adsorption, Q = adsorption capacity

**Table 5. Determination of desorption capacity at pH 9 on agarose adsorbed various concentrations of tartrazine solution at pH 4 for 60 minutes**

| Co (µg/ml) | A1 | Ct1 (µg/ml) | A2 | Ct2 (µg/ml) | Ct total (µg/ml) | Ct total – Co (µg/ml) | dQ (mg/g) | Average dQ± SD (mg/g) |
|---|---|---|---|---|---|---|---|---|
| 0 | 0.011 | 0.175 | 0.004 | 0 | 0.175 | 0.175 | 0.0175 | 0.0166± 0.0014 |
| | 0.010 | 0.150 | 0.004 | 0 | 0.150 | 0.150 | 0.0150 | |
| | 0.011 | 0.175 | 0.004 | 0 | 0.175 | 0.175 | 0.0175 | |
| 0 | 0.013 | 0.225 | 0.004 | 0 | 0.225 | 0.225 | 0.0225 | 0.0216 ± 0.0014 |
| | 0.012 | 0.200 | 0.005 | 0.025 | 0.225 | 0.225 | 0.0225 | |
| | 0.012 | 0.200 | 0.004 | 0 | 0.200 | 0.200 | 0.0200 | |
| 0 | 0.027 | 0.575 | 0.006 | 0.050 | 0.625 | 0.625 | 0.0625 | 0.0608 ± 0.0028 |
| | 0.026 | 0.550 | 0.005 | 0.025 | 0.575 | 0.575 | 0.0575 | |
| | 0.027 | 0.575 | 0.006 | 0.050 | 0.625 | 0.625 | 0.0625 | |
| 0 | 0.038 | 0.850 | 0.007 | 0.075 | 0.925 | 0.925 | 0.0925 | 0.0898 ± 0.0025 |
| | 0.037 | 0.825 | 0.007 | 0.075 | 0.895 | 0.895 | 0.0895 | |
| | 0.037 | 0.825 | 0.006 | 0.050 | 0.875 | 0.875 | 0.0875 | |
| 0 | 0.043 | 0.975 | 0.009 | 0.125 | 1.100 | 1.100 | 0.1100 | 0.1083 ± 0.0014 |
| | 0.043 | 0.975 | 0.008 | 0.100 | 1.075 | 1.075 | 0.1075 | |
| | 0.042 | 0.950 | 0.009 | 0.125 | 1.075 | 1.075 | 0.1075 | |
| 0 | 0.020 | 0.400 | 0.006 | 0.050 | 0.450 | 0.450 | 0.0450 | 0.0450 |
| | 0.022 | 0.450 | 0.006 | 0.050 | 0.450 | 0.450 | 0.0450 | |
| | 0.022 | 0.450 | 0.006 | 0.050 | 0.450 | 0.450 | 0.0450 | |
| 0 | 0.027 | 0.575 | 0.005 | 0.025 | 0.595 | 0.595 | 0.0595 | 0.0623 ± 0.0027 |
| | 0.027 | 0.575 | 0.006 | 0.050 | 0.625 | 0.625 | 0.0625 | |
| | 0.028 | 0.600 | 0.006 | 0.050 | 0.650 | 0.650 | 0.0650 | |

$C_0$ = tartrazine concentration before desorption, A1 = Absorbance after first desorption, A2 = Absorbance after second desorption, Ct 1= tartrazine concentration after first desorption, Ct2 = tartrazine concentration after second desorption dQ = desorption capacity

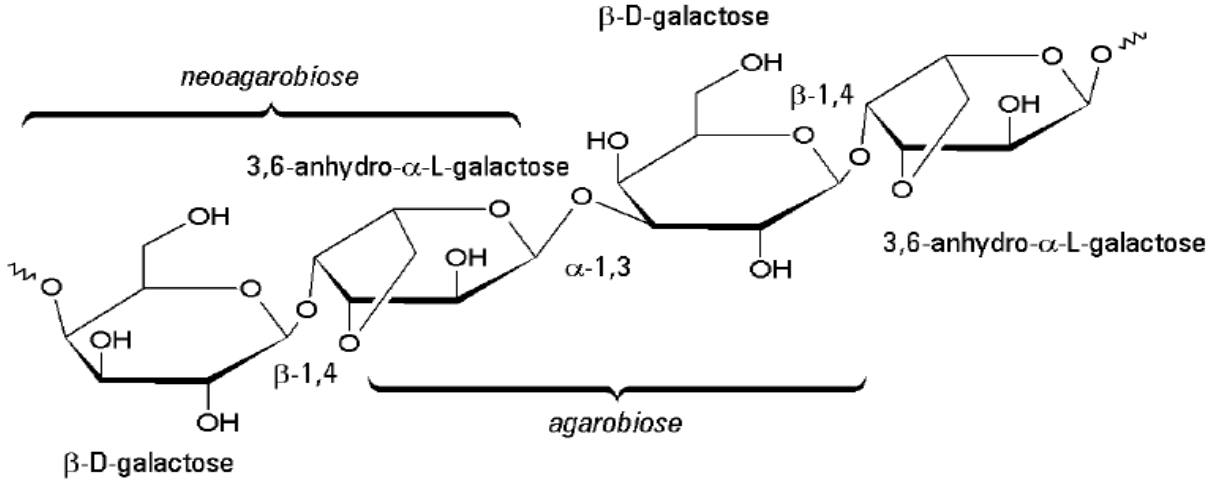


**Figure 1: Backbone structure of agarose**

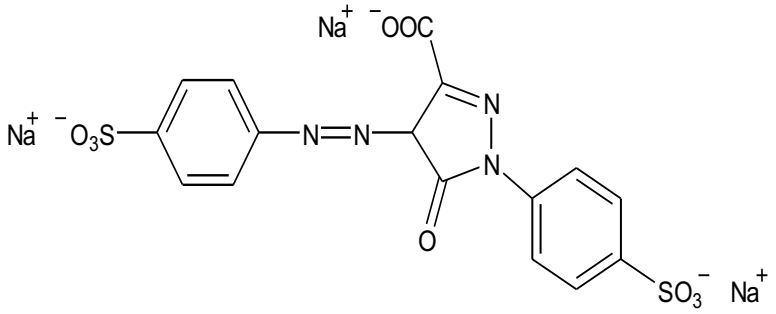


**Figure 2: Chemical structure of tartrazine**

From data in Table 3 above can be seen that adsorption process of 4 µg/ml of tartrazine with contact time for 60, 30, and 90 min give an adsorbent capacity of agarose of 0.0617 (± 0.0014), 0.267 (± 0.0014), and 0.0125 (± 0.0025) mg/g respectively. It can be resumed that adsorption process of tartrazine solution at pH 4 by agarose will be optimum when contact time adjusted for 60 min.

**Determination of Maximum Adsorption Capacity of Isolated Agarose to Various Concentration of Tartrazine at Optimum pH and Optimum Contact Time**

After it was found that the adsorption process of 4 µg/ml of tartrazine solution by 0.25 g of agarose showed an optimal adsorption capacity at pH 4 and contact time for 60 minutes, then to obtain an ideal ratio between adsorbent and adsorbate and to get appropriate adsorption capacity value, the experiment was continued by conducting adsorption process of 0.25 g of isolated agarose against various concentration of tartrazine. Experiment result was presented in Table 4.

Data of Table 4 showed that adsorption experiment 0.25 g of isolated agarose at pH 4 with contact time for 60 min toward various concentrations of 25 ml of tartrazine, namely 2.4, 3.2, 4.0, 4.8, 5.6, 6.4, and 7.2 µg/ml will give adsorption capacity of 0.0167 (± 0.0028), 0.0225 (± 0.0003), 0.0617 (± 0.0014), 0.0908 (± 0.0014), 0.1108 (± 0.0014), 0.0492 (± 0.0014), 0.0625 (± 0.0025) mg/g, respectively. If the data of Table 4 was analyzed more deeply, it can be seen, that the independent variable, the concentration of tartrazine versus dependent variable, an adsorption capacity, will apparently give a parabolic curve relationship, with the maximum value of tartrazine concentration at 5.6 µg/ml which gives an adsorption capacity of 0.1108 (± 0.0014) mg/g. R. Shiralipour and A. Larkin in 2016 reported work about pre-concentration and determination of tartrazine dye from aqueous solutions using modified cellulose nanosponges and quantified by the spectrophotometric method. They found that tartrazine standard solution in the concentration level of 2–300 ng/ml will give a linear correlation between concentration and absorbance, Limit of Detection was 0.15 ng/ml and the maximum adsorption capacity was 180 mg/g[13]. We need to increase an adsorption capacity of isolated agarose by developing its physical properties such as the diameter of the particle, water content, and also to optimize the experiment condition, such as solvent, temperature and contact time.

**Determination of maximum desorption capacity at optimum pH on agarose adsorbed various concentrations of tartrazine solution at optimum pH and optimum contact time**

Furthermore, on agarose which has adsorbed the tartrazine concentration solution of 2-7 µg/ml at pH 4 for 60 min, as shown in Table 5, a desorption tartrazine experiment was carried out at pH 9 (borate buffer) with 70 % ethanol as a solvent. The results of the experiment were presented in Table 5.

In reference found that G.L. Dotto *et al* in 2012 studied tartrazine adsorption onto chitin and chitosan at various pH that quantified by spectrophotometer at λ425 nm. They found an optimum adsorption process at pH 3 where chitin and chitosan gave adsorption capacity of 30 and 350 mg/g[14].

Data of Table 5 showed that desorption experiment at pH 9 on 0.25 g of agarose that adsorbed various concentrations of 25 ml of tartrazine at pH 4 with total contact time for 60 min, namely 2.4, 3.2, 4.0, 4.8, 5.6, 6.4, and 7.2 µg/ml will give desorption capacity of 0.0166 (± 0.0014), 0.0216 (± 0.0014), 0.0608 (± 0.0028), 0.0898 (± 0.0025), 0.1083 (± 0.0014), 0.0450, 0.0623 (± 0.0027) mg/g, respectively. The data of Table 5 showed that the independent parameters, the concentration of tartrazine versus dependent parameters, desorption capacity, will also apparently give a parabolic curve relationship, with the maximum value of tartrazine concentration at 5.6 µg/ml which gives desorption capacity of 0.1083 ± (0.0014) mg/g. Meanwhile, the adsorption capacity value of agarose to tartrazine is only slightly higher, namely 0.1108 (± 0.0014) mg /g. If desorption capacity value of agarose to tartrazine was compared to its adsorption capacity, it will give a high percentage, namely 97.74 %. It means if agarose is used as an adsorbent in the analysis of tartrazine from a real sample solution, then in the desorption process step; it can release tartrazine in a quantitative amount.

## CONCLUSION

With a combination of propylene glycol, distilled water, and isopropyl alcohol as the solvent and by increasing and decreasing the temperature of the experiment, from agar was isolated agarose with a yield 67 %, where its physical properties close to the agarose standard. The adsorption capacity of isolated agarose to tartrazine was optimal at the appropriate experimental condition, namely at acidic milieu (pH 4), and contact time for 60 minutes, meanwhile the desorption capacity of isolated agarose was optimal at basic condition (pH 9) and contact time for 60 minutes. Adsorption and desorption process of 0.25 g of agarose was optimal in an experiment used 25 ml of 5.6 µg/ml tartrazine solution as adsorbate, which gave adsorption and desorption capacity of 0.1108 (± 0.0014) and 0.1083 ± (0.0014) mg/g, respectively. Desorption capacity value of agarose to tartrazine was close to its adsorption capacity value, namely 97.74 %, it means if agarose used as an adsorbent in the analysis of tartrazine, it will give a quantitative measurement. It has never been reported in reference to previous research results about the use of agarose as an adsorbent in the analysis of tartrazine.

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## REFERENCES

1. McHugh DJ. A guide to the seaweed industry. FAO Fisheries Technical Paper. Food and Agriculture Organization of the United Nations.Rome; 2003. p. 441-448.
2. Zucca P, Fernandez R, Sanjust E. Agarose and its derivatives as supports for enzyme immobilization. Molecules; 2016. p. 1–25.
3. Wang TP, Chang LL, Chang SN, Wang EC, Hwang LC, Chen YH, Wang YM. Successful Preparation and Characterization of Biotechnological Grade Agarose from Indigenous Gelidium amansii of Taiwan. Process Biochemistry 2012; 47(3): 550–554.
4. Raymond CR, Sheskey PJ, Owen SC. Handbook of Pharmaceutical Excipients. Fifth Edition. London: Royal Pharmaceutical Society of Great Britain; 2006.
5. Bhatt D, Vyas K, Singh S, John PJ, Soni I. Tartrazine induced neurobiochemical alterations in rat brain sub-regions. Food and Chemical Toxicology 2018; 113: 322–327.
6. Bastaki M, Farrell T, Bhusari S, Pant K, Kulkarni R. Lack of genotoxicity *in vivo* for food color additive tartrazine. Food and Chemical Toxicology 2017; 105: 308–314.
7. Zhao X, Liu Y, Zuo J, Zhang J, Zhu L. Rapid and sensitive determination of tartrazine using a molecularly imprinted copolymer modified carbon electrode. Journal of Electroanalytical Chemistry 2017; 785: 90–95.

8. Provonchee RB. Agarose purification method using glycol. The United States Patent No. 4.9990.611. Philadelphia: FMC Corporation; 1991.
9. Rochas C, Lahaye M, Yaphe W. Sulfate content of carrageenan and agar determined by infrared spectroscopy. Botanica Marina 1986; 29(4): 335–340.
10. Guiseley KB, Kirkpatrick FH, Provonchee RB, Dumais MM, Nochumson S. Further fractionation of agarose. Hydrobiologia; 1993. p. 505-511.
11. Alves SP, Brum DM, de Andrade ECB, Netto ADP. Determination of synthetic dyes in selected foodstuffs by high-performance liquid chromatography with UV-DAD detection. Food Chemistry 2008; 107(1): 489-496.
12. Perez Urquiza M, Beltran JL. Determination of dyes in food stuffs by capillary zone electrophoresis. Journal of Chromatography A 2000; 898: 271–275.
13. Shiralipour R, Larkin A. Pre-concentration and determination of tartrazine dye from aqueous solutions using modified cellulose nanosponges. Ecotoxicology and Environmental Safety 2017; 135: 123-129.
14. Dotto GL, Vieira ML, Pinto LA. Kinetics and mechanism of tartrazine adsorption onto chitin and chitosan. Industrial and Engineering Chemistry Research 2012; 51: 6862-6868.

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