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# Adsorptive removal of methylene blue by agar: effects of NaCl and ethanol

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## Abstract

Adsorption of methylene blue (MB) on agar was investigated as a function of temperature (308-328 K), different concentrations of NaCl and HCl and various weight percentages of binary mixtures of ethanol with water. It was observed that the maximum experimental adsorption capacity, $q_{m, \exp}$, in water is up to 50 mg g$^{-1}$ and decreases with increase in weight percentage of ethanol and NaCl and HCl concentration compared to that of water. Analysis of data using ARIAN model showed that MB adsorbs as monomer and dimer on the surface of agar. Binding constants of MB to agar were calculated using the Temkin isotherm. The process is exothermic in water and other solutions. The mean adsorption energy ($E$) value indicated binding of MB to agar is chemical adsorption. Kinetics of this interaction obeys from the pseudo-second-order model and diffusion of the MB molecules into the agar is the main rate-controlling step.

## 1. Background

Dyes are important pollutants, causing environmental and health problems to human being and aquatic animals. Wastewater containing dyes presents a serious environmental problem because of its high toxicity and possible accumulation in the environment. Therefore, their removal from industrial effluents before discharging into the environment is extremely important. Conventional methods for the removal of dyes in effluents include physical, chemical, and biological processes. Adsorption methods employing solid adsorbents are widely used to remove certain classes of chemical pollutants from wastewater. Many researchers have investigated the use of cheap and efficient adsorbents to remove dyes from wastewater [1-4]. In this work, we investigated adsorption of MB on agar. Agar is a gelatinous substance derived from a polysaccharide that accumulates in the cell walls of agarophyte red algae [5,6] and results of this work also show the role of agar in accumulation of dye (a polluant) in agarophyte red algae. Agar can be used as gelling agents for spread foods, soft-texture confectionery, as a fat replacer [7] and widely used in a number of preparations in biomedical, food, cosmetics and pharmaceutical industries [8]. MB is used as a redox indicator, as a stain for bacteriology [9], for treating malaria and a number of diseases [10,11] and as a treatment for fungal infections of fishes and fish eggs [12]. MB commonly employed in textile industries and contributes to the pollution of water discharges [13]. In this work, we studied effects of temperature, NaCl and ethanol on adsorption of methylene blue on agar. We can use these effects to treat wastewater and also recycle adsorbent and adsorbate.

## 2. Experimental

### 2.1. Materials

Methylene blue chloride (C.I. 52015), NaCl, ethanol (99.9%) and agarose were purchased from Merck and agar was obtained from Fluka. They were used without further purification. Agar consists of a mixture of agarose and agaropectin. Agarose is a linear polymer, made up of the repeating monomeric unit of agarobiose and agarobiose is a disaccharide made up of D-galactopyranose and 3,6-anhydro-L-galactopyranose, linked by a glycosidic bonding, Figure 1(a). Agaropectin is a heterogeneous mixture of smaller molecules that occur in lesser amounts. Their structures are similar but slightly branched and sulfated (3% to 10% sulfate), and they may have methyl and pyruvic acid ketal substituents [14], Figure 1(b). Agarose normally represents at least two-thirds of the natural agar.

Methylene blue (C.I: Basic Blue 9) is one of the basic dyes [15], Figures 1(c) and 1(d).

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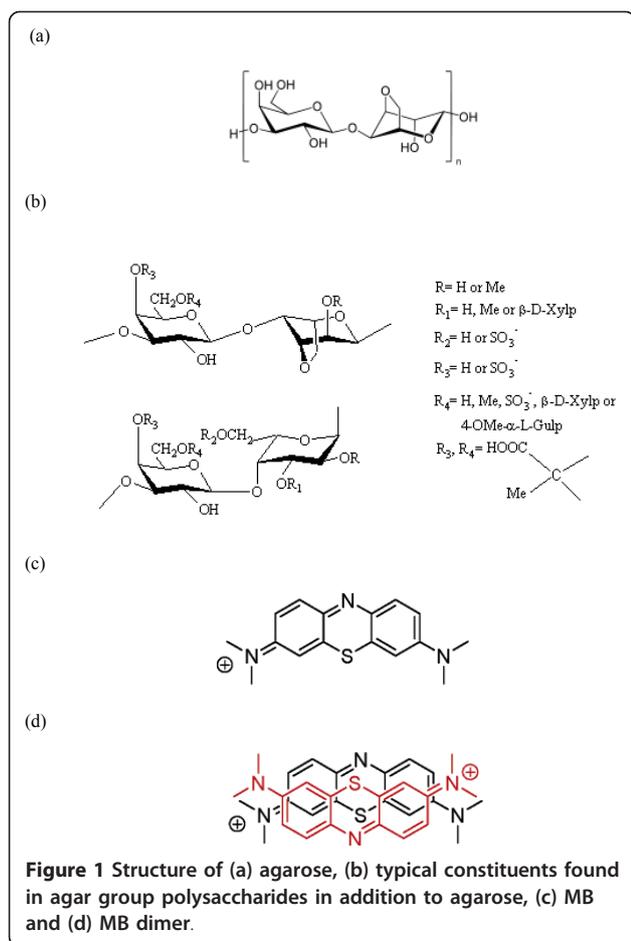
**Figure 1 Structure of (a) agarose, (b) typical constituents found in agar group polysaccharides in addition to agarose, (c) MB and (d) MB dimer**.

## 2.2. Methods

Ten ml of MB solution of different initial concentrations was transferred to a series of 15-ml glass stoppered bottles, each containing 0.034 g of agar sample. The solutions were shaken at 120 rpm in a temperature controlled shaking water bath (Fater electronic Co., Persian Gulf model) at 308, 318 and 328 K within ± 0.1 K for 20 h to reach equilibrium under experimental conditions.

The initial concentrations of MB were in the range of $2 \times 10^{-5}$-$4 \times 10^{-4}$ M. After adsorption, the contents of MB in the residual solutions were determined by spectrometry (UV-Vis 160, Shimadzu) at) at their $\lambda_{max}$ = 665 nm. Adsorption of MB on agarose was studied by above-mentioned method at 306 K. The agar and agrose FTIR spectra were determined using an infrared spectrophotometer with Fourier transformation (FTIR-8400 S, Shimadzu).

The relation between equilibrium adsorption capacity $q_e$ (mg g$^{-1}$) and dye equilibrium concentration $c_e$ (M) has been studied by some equations. One of these equations is the Temkin isotherm [16] and is represented by

$$q_e = c_1 \ln c_2 c_e \tag{1}$$

where $c_1$ (mg g$^{-1}$) is a measure of adsorption capacity and $c_2$ is adsorption equilibrium constant (M$^{-1}$).

The Langmuir equation [17] in linearized form is given as

$$\frac{c_e}{q_e} = \frac{1}{q_m K} + \frac{c_e}{q_m} \tag{2}$$

where $K$ is the Langmuir adsorption constant and $q_m$ (mg g$^{-1}$) is the maximum adsorption capacity of surface. The Dubinin-Radushkevich equation [18] is given by

$$\ln q_e = \ln q_D - B_D \left( RT \ln \left( 1 + \frac{1}{c_e} \right) \right)^2 \tag{3}$$

where $B_D$ is related to the free energy of adsorption per mole of adsorbate (mol$^2$ J$^{-2}$) and $q_D$ (mg g$^{-1}$) is the maximum theoretical monolayer saturation capacity. The apparent energy of adsorption from Dubinin-Radushkevich isotherm, $E$, (J mol$^{-1}$) that gives information about chemical and physical adsorption can be computed using the relationship

$$E = \frac{1}{\frac{1}{(2B_D)^{\frac{1}{2}}}} \tag{4}$$

In this work, the results are studied by "*adsorption isotherm regional analysis model*" or abbreviated as ARIAN model [19]. This model is introduced for studying adsorption isotherms up to four regions. In ARIAN model which is explained briefly, it is assumed that depending on the used concentration range, different interactions may occur between adsorbate and adsorbent. The concentration range related to each kind of interactions is called a "*region*" and data of various regions are interpreted by different adsorption isotherms. In each region, the adsorbate concentration range that satisfies locally in an adsorption isotherm or small plateau, is called a "*section*". A region may include one or more sections. The sections would be symbolized by large English alphabets. Region 1 obeys Henry's law and its slope is approximately one, it means that adsorption increases linearly with concentration. Region 2 includes only formation of monolayer surface aggregates and can be studied by an appropriate isotherm. In region 3, new surface aggregates form. Data of region 3 are analyzed by the bilayer equation and its derived ones [19] and equilibrium constants of monolayer and bilayer adsorption of adsorbate molecules are obtained. The bilayer isotherm is given as [19]

$$\frac{c_e}{q_e} = \frac{1 + c_e K_{sa} + x c_e^2 K_{sa}}{q_{mon} K_{sa} + 2 q_{mon} x c_e K_{sa}} \tag{5}$$

where $q_{mon}$ is the monolayer adsorption capacity and $K_{sa}$ and $x$ are the adsorption equilibrium constants of adsorbed molecules in surface aggregates and in the second layer, respectively. If adsorbed molecules adsorb mostly on the first layer, equation (5) can be written as

$$\frac{c_e}{q_e} = \frac{1}{q_{mon}K_{sa}} + \frac{c_e}{q_{mon}} + \frac{xc_e^2}{q_{mon}} \qquad (6)$$

On the other hand, if the adsorption process is monolayer, equation (5) can be reduced to

$$\frac{c_e}{q_e} = \frac{1}{q_{mon}K_{sa}} + \frac{c_e}{q_{mon}} \qquad (7)$$

where equation (7) is a Langmuir-type equation.

Region 4 is studied by the reverse desorption equation [19] and is as plateau or curve goes down.

## 3. Results and discussion

### 3.1. Adsorption of MB on agar

Figure 2 shows the adsorption isotherms of MB on agar at 308-328 K. These isotherms are classified as S2 [9]. Analysis of data shows that these adsorption isotherms are two-portion.

The initial concentration range of the first portion is up to $1.8 \times 10^{-4}$ M MB (or $c_e \approx 8 \times 10^{-6}$ M) and data of these portions fit in the Temkin equation and not to the Langmuir isotherm and can be considered as region 2 (here named as section 2A), Table 1. The initial concentration of second portion starts from $1.8 \times 10^{-4}$ M MB and like the first portion its data fit to the Temkin isotherm and does not satisfy the bilayer isotherm and its derived ones [19], such as the Langmuir-type equation and thus according to ARIAN model can not be considered as the third region (because MB bilayers do not form) but the region 2 and is named section 2B, Table 1. In these sections, MB adsorbs on agar as monolayer and in spite of increase in dye concentration, due to



**Figure 2 Variation of $q_e$ vs. $c_e$ for adsorption of MB on agar and agarose from water**.

alternately repeating 3,6-anhydro-L-galactopyranose units, MB molecules can not form surface aggregates on agar. Dyes aggregate as a function of concentration and in the commonly used concentration range ($10^{-6}$-$10^{-3}$ M) the main equilibrium is a monomer-dimer reaction [20]. In the used concentration range of MB, its dimer aggregates form [15], Figure 1(d) and in sections 2A and 2B, MB molecules adsorb on agar as monomer and dimer, respectively. On the other hand, we studied adsorption of MB on pure agarose at 306 K. Binding constant (obtained from the Temkin isotherm), maximum experimental adsorption capacity ($q_{m,\ exp}$) and mean adsorption energy ($E$) values (obtained from the Dubinin-Radushkevich equation) for adsorption of MB on agarose are 496946 $M^{-1}$, 9.78 mg $g^{-1}$ and 10.1 kJ $mol^{-1}$, respectively that are similar to those of section 2A for adsorption of MB monomers on agar at 308 K, Table 1. This observation shows important role of negatively charged of agar on adsorption of MB dimers on its surface, section 2B. FTIR spectra of agarose and MB-adsorbed agarose are shown in Figures 3 and 4.

The characteristic absorption peaks of agarose were observed at 3481 $cm^{-1}$ (-OH stretching of the hydroxyl group), 1078 $cm^{-1}$ (glycosidic bonding) [21] and 930 $cm^{-1}$ (vibration of C-O-C bridge of 3,6-anhydro-L-galactopyranose) [22] Figure 3. As shown in Figure 4, the characteristic absorption peak of MB-adsorbed agarose at 3446 $cm^{-1}$ is due to interaction of oxygen atom of -OH groups of D-galactopyranose units with $= N\,(CH_3)_2^+$ group of MB molecules. The absorption peaks at 1070 and 931 $cm^{-1}$ show that glycosidic bonding and oxygen atom of 3,6-anhydro-L-galactopyranose do not interact with MB respectively, Figure 4. On the other hand, the characteristic absorption peak of agar at 3497 $cm^{-1}$ (-OH stretching of the hydroxyl group) was transferred to 3448 $cm^{-1}$ which shows interaction between MB and -OH groups of agar and agar peaks in 1647 $cm^{-1}$ (C = O stretch peak) [23], 1072 $cm^{-1}$ (glycosidic bonding), 932 $cm^{-1}$ (C-O-C bending of 3,6-anhydro-L-galactopyranose), 1250 $cm^{-1}$ (S = O of sulfate esters) [21] and 864 $cm^{-1}$ (L-galactopyranose-6-sulfate shoulder) [9] did not change in MB-adsorbed agar FTIR spectrum, Figures 5 and 6.

Presence of constituents like sulfate hemiesters and methyl ethers decreases 3,6-anhydro-L-galactopyranose content of agar. As previously found [9], there is an inverse relationship between sulfate and 3,6-anhydro-L-galactopyranose content of agar and as reported, agar is a polyanionic molecule with zeta potential $\approx$ -20 mV [24]. Due to decrease in 3,6-anhydro-L-galactopyranose content of agar and its negatively charged surface, interaction of MB with -OH groups of agar surface results in a greater $q_{m,\ exp}$ value compared to that of interaction of MB with -OH groups of agarose, Table 1.
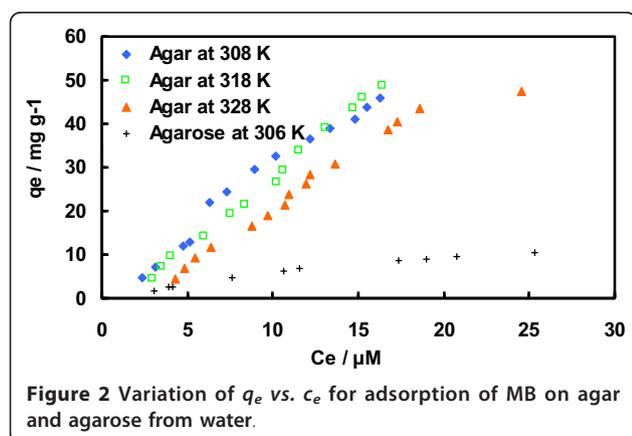
**Table 1 Parameters obtained from the Temkin isotherm and maximum experimental capacity of adsorption values, $q_{m,\ exp}$, for sections 2A and 2B of adsorption of MB on agar in water and different concentrations of NaCl at 308-328 K**

| [NaCl](M) | Section 2A | | | | Section 2B | | | |
|---|---|---|---|---|---|---|---|---|
| | $q_{m,\ exp}$ (mg g$^{-1}$) | $c_1$ (mg g$^{-1}$) | $c_2$ (M$^{-1}$) | $R^2$ | $q_{m,\ exp}$ (mg g$^{-1}$) | $c_1$ (mg g$^{-1}$) | $c_2$ (M$^{-1}$) | $R^2$ |
| | T = 308 K | | | | | | | |
| 0.0 | 12.89 | 139.66 | 675821 | 0.997 | 45.93 | 303.03 | 399806 | 0.995 |
| 0.01 | 14.07 | 169.05 | 180821 | 0.987 | 33.19 | 564.47 | 84368 | 0.986 |
| 0.03 | 14.74 | 200.49 | 90892 | 0.944 | 28.84 | 574.38 | 55314 | 0.997 |
| | T = 318 K | | | | | | | |
| 0.0 | 21.64 | 204.88 | 454648 | 0.991 | 48.75 | 514.35 | 192568 | 0.990 |
| 0.01 | 13.67 | 174.22 | 143533 | 0.990 | 27.26 | 472.86 | 76245 | 0.991 |
| 0.03 | 14.12 | 189.73 | 79864 | 0.979 | 30.33 | 470.48 | 29543 | 0.985 |
| | T = 328 K | | | | | | | |
| 0.0 | 21.52 | 220.79 | 309683 | 0.995 | 47.53 | 454.13 | 170397 | 0.990 |
| 0.01 | 22.99 | 174.25 | 134102 | 0.999 | 32.63 | 369.22 | 48913 | 0.989 |
| 0.03 | 19.90 | 234.48 | 75471 | 0.996 | 31.50 | 502.89 | 27409 | 0.994 |

The results show that $q_{m,\ exp}$ values decrease with increase in temperature, Figure 2. Decrease in temperature, increases $q_e$ value of adsorption of MB molecules on agar and repulsion between these positively charged molecules decreases binding constant of MB to agar chains. Binding constants of MB to agar were calculated by the Temkin equation. At each temperature, binding constant of MB dimers to agar in section 2B is less than that of MB monomers to agar in section 2A of adsorption isotherm, Table 1. Interaction of MB molecule with agar is exothermic in both sections 2A and 2B, Table 2.

Ion-dipole interaction between MB and -OH groups of agar chains is chemical and the mean adsorption energy ($E$) values of process, obtained from the Dubinin-Radushkevich equation, at different temperatures are between 7.6-8.1 kJ mol$^{-1}$ in section 2A and 8.4-9.5
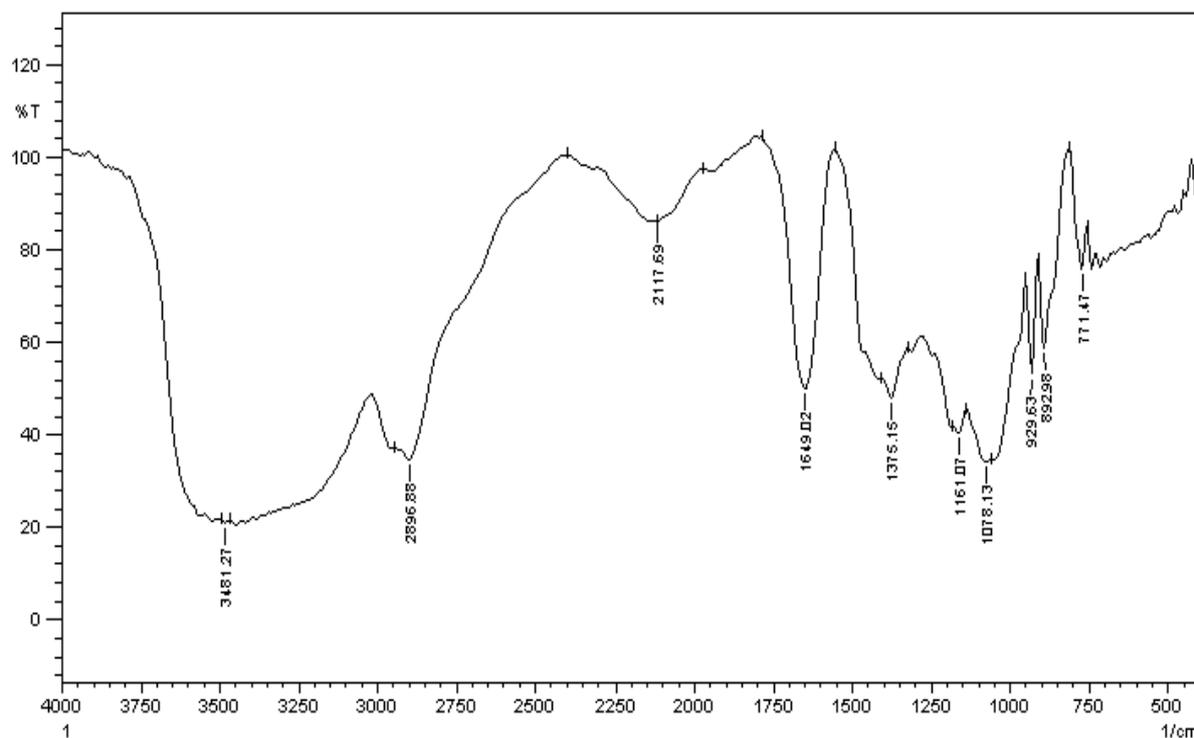


**Figure 3** FTIR spectrum of agarose.
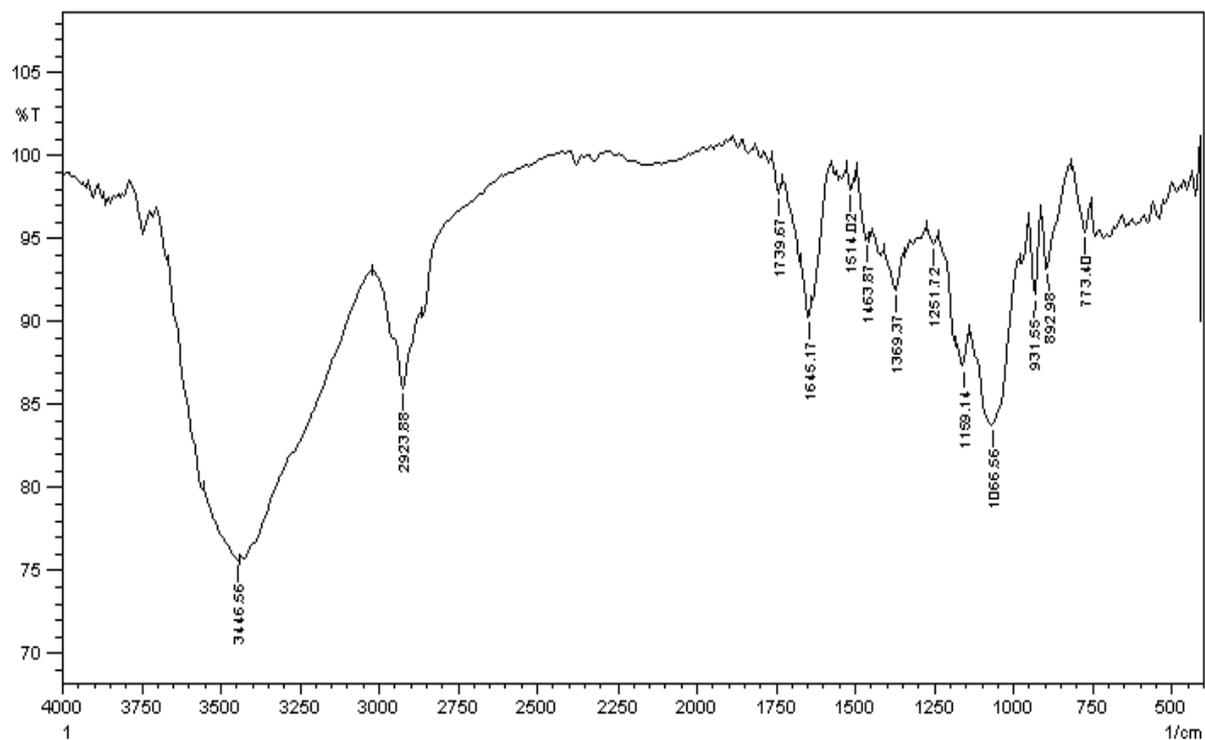
**Figure 4** FTIR spectrum of MB-adsorbed agarose.
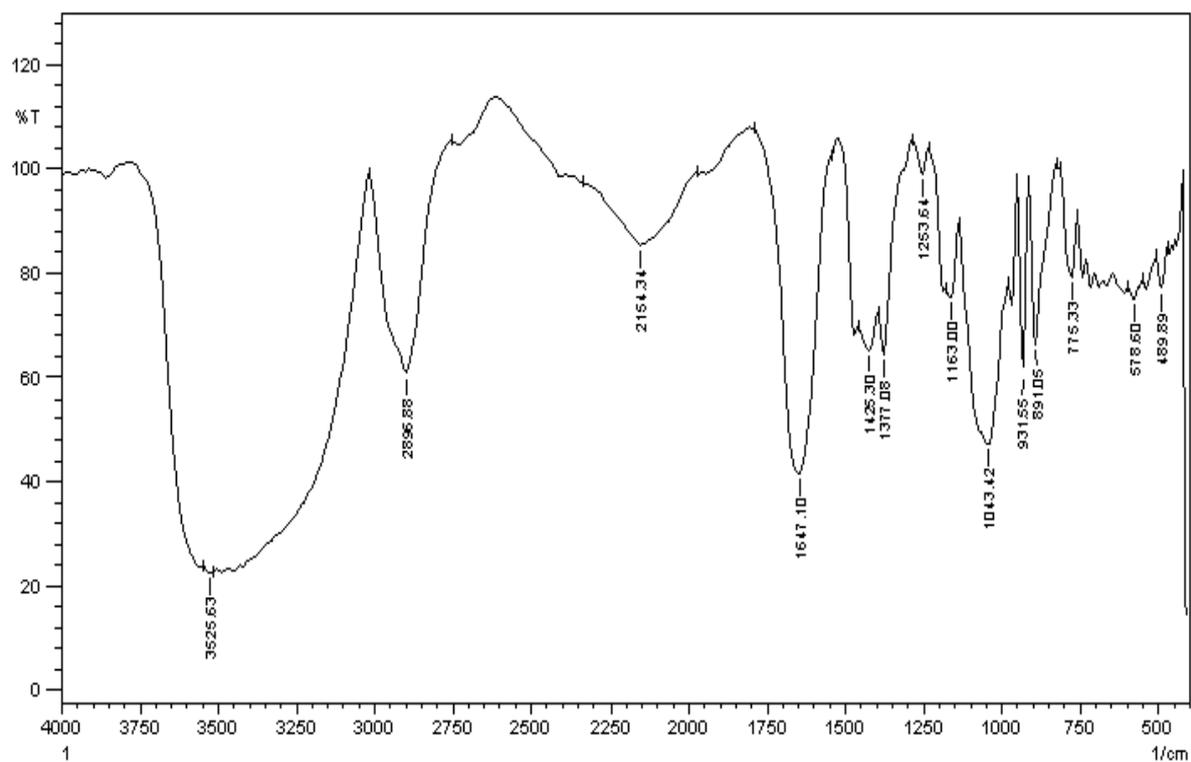


**Figure 5** FTIR spectrum of agar.
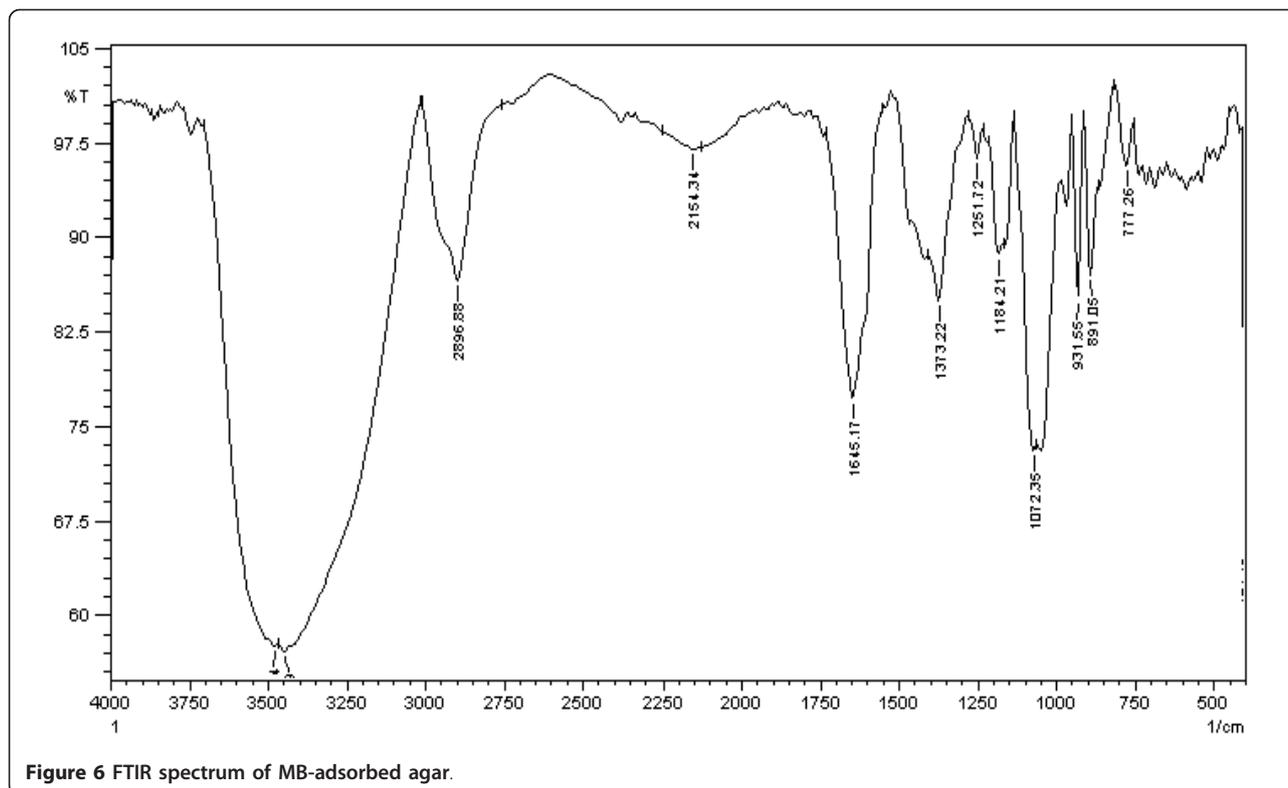
**Figure 6** FTIR spectrum of MB-adsorbed agar.

kJ mol$^{-1}$ in section 2B respectively. Finally, comparison of adsorption of MB on a series of adsorbents [25-32] shows high adsorption capacity of agar for MB, Table 3.

### 3.2. Effect of ionic strength on adsorption of MB

Effect of 0.03 and 0.1 M NaCl solutions on adsorption of MB on agar was studied. Figures 7(a) and 7(b) show adsorption isotherms of MB on agar from NaCl solutions at 308-328 K.

As seen in Table 1, adsorption process occurs in sections 2A and 2B and in the used concentration range of NaCl, $q_{m,\ exp}$ values are less than those values in water. At each temperature, with increase in NaCl concentration $q_{m,\ exp}$ values increase, Figures 7(a) and 7(b) and binding constant values of MB to agar decrease, Table 1. In the former case, solubility at low ionic strength increases with salt concentration (salting in). As found for adsorption of malachite green on silica gel [33] pairing salt ions with charged group of MB molecules

shields intermolecular repulsion and solubility of MB decreases at higher NaCl concentration. Thus, solvent activity toward solubility of hydrophobic solutes reduces and NaCl salts out MB molecules and increases MB adsorption. As shown in Figure 8, MB molecules aggregate and deposit in the presence of 0.3 M NaCl at 273 and 328 K and the weight of deposited MB increases with increase in temperature.

As shown in Table 2, in the presence of NaCl adsorption process in sections 2A and 2B is less exothermic and more disordered than that in water. These may be due to increase in hydrophobic interaction between MB molecules which results from shielding their intermolecular repulsion and slightly endothermic dissolution of NaCl in water. Also, $\Delta H$ and $\Delta S$ values of adsorption process in section 2B are less than those of section 2A, Table 2.

Also, at each temperature and NaCl concentration, binding constants of MB to agar, like those in water, decrease from section 2A to 2B.

**Table 2 ΔH and ΔS values of sections 2A and 2B of adsorption of MB on agar in water and various concentrations of NaCl at 308-328 K**

| System | ΔH (kJ mol$^{-1}$) | ΔS (J mol$^{-1}$ K$^{-1}$) | System | ΔH (kJ mol$^{-1}$) | ΔS (J mol$^{-1}$ K$^{-1}$) |
|---|---|---|---|---|---|
| | **Section 2A** | | | **Section 2B** | |
| Water | -32.8 | 5.2 | Water | -36.1 | -10.7 |
| NaCl (0.01 M) | -12.6 | 59.5 | NaCl (0.01 M) | -22.7 | 21.0 |
| NaCl (0.03 M) | -7.8 | 69.4 | NaCl (0.03 M) | -29.7 | -6.4 |

**Table 3 Comparison of adsorption of MB on different adsorbents**

| Adsorbent | $q_{max}$ (mg g$^{-1}$) | W$_{adsorbent}$ (g) | pH | Isotherm | T (K) | [MB] (mg/L) | Volume(ml) | Reference |
|---|---|---|---|---|---|---|---|---|
| pumpkin seed hull | 141.92 | 0.3 | neutral | Langmuir | 303 | 25-300 | 200 | [24] |
| fuel oil fly ash | 47 | 7 | 7 | experimental | 298 | 0-1000 | 50 | [25] |
| sepiolite | 59.81 | 5 | 9 | kinetics | room temp. | 384 | 24 | [26] |
| silica nano-sheets | 9.7 | 0.23 | 5.5 | kinetics | 293 | 22.39 | 90 | [27] |
| beer brewery waste | 4.92 | 0.5 | 7 | kinetics | 298 | 2.5 | 2000 | [28] |
| activated carbon | 378.56 | 0.01 | neutral | Langmuir | 305 | 0-1000 | 50 | [29] |
| SDS-modified zeolite | 12.32 | 0.25 | 6-9 | experimental | 298 | 25 | 25 | [30] |
| KNbO$_3$ | 1.48 | 0.25 | 1 | Langmuir | room temp. | 2-10 | 250 | [31] |
| Agar | 47.53 | 0.034 | neutral | experimental | 328 | 12.8-121.5 | 10 | this study |

The mean adsorption energy (*E*) value, obtained from the Dubinin-Radushkevich equation, in different concentrations of NaCl at 308-328 K varies in the range of 6.0-7.7 and 5.7-7.8 kJ mol$^{-1}$ in sections 2A and 2B respectively which are less than those values in water.

### 3.3. Effect of organic solvent on adsorption of MB
Effect of different weight percentages of binary mixtures of ethanol with water up to 10% on adsorption of MB on agar was studied at 308-328 K. As shown in Figures 9, 10, 11 and 12 in the used concentration range of ethanol, $q_e$ (and thus $q_{m, exp}$) values are less than those

values in water and the used concentrations of NaCl and ethanol.

As given in Figures 9, 10, 11 and 12, at each certain temperature with increase in ethanol weight percentage, due to hydrogen binding and hydrophobic interaction of MB with alcohol molecules, solubility of MB increases and thus $q_e$ (and thus $q_{m, exp}$) values decrease which is according to the trend of changes in their dielectric constant values [34]. As results show at each certain temperature and section, binding constants of MB to agar decreased with increasing weight percentage of ethanol, Table 4.

As previously studied, adsorption of sudan I, acid orange 7, acid red 27, acid red 112 on fibrous activated carbon in ethanol-water mixtures [35] and adsorption of malachite green on silica gel in 2-propanol-water mixtures [33] decreased with increase in organic solvent weight percentage.

It is known experimentally that (1) the dissolution of hydrocarbon in water is exothermic and the entropy of the system decreases [36] and increase in weight percentage of ethanol in the used mixtures results in more negative $\Delta H$ values of the adsorption process and (2) hydrophobic interaction is endothermic and entropy of system increases [36]. The latter effect increases $\Delta H$



**Figure 7 Variation of $q_e$ *vs.* $c_e$ for adsorption of MB on agar in (a) 0.01 and (b) 0.03 M NaCl.**



**Figure 8 Deposition of MB *vs.* time in 0.3 M NaCl.** Initial concentration of MB is $8 \times 10^{-4}$ M in ten ml of solution.

**Figure 9 Variation of $q_e$ vs. $c_e$ for adsorption of MB on agar from 2.5 weight percentage of ethanol in water.**



**Figure 11 Variation of $q_e$ vs. $c_e$ for adsorption of MB on agar from 7.5 weight percentage of ethanol in water.**

values of adsorption process in 5% ethanol in section 2A and 2.5% ethanol in section 2B and with more increase in ethanol weight percentage $\Delta H$ values of process in sections 2A and 2B decrease due to the former effect, Table 5.

Results show that the mean adsorption energy ($E$) values in sections 2A and 2B at 308-328 K, obtained from the Dubinin-Radushkevich equation at various weight percentages of ethanol, vary in the range of 5.1-8.1 and 7.4-12.9 kJ mol$^{-1}$ in sections 2A and 2B, respectively.

### 3.4. Effect of pH on adsorption of MB

Effect of different weight percentages of binary mixtures of ethanol with water in pH values 1.5 and 3 on adsorption of MB on agar was studied at 308-328 K. As shown in Figures 13(a) and 13(b), $q_{m, \text{exp}}$ values are less than those values in water and the used concentrations of NaCl and ethanol.

FTIR spectra of agarose and H$^+$-adsorbed agarose are shown in Figure 14. The characteristic absorption peak of agar at 3497 cm$^{-1}$ (-OH stretching of the hydroxyl

group) was transferred to 3419 cm$^{-1}$ which shows interaction between H$^+$ ions and -OH groups of agar and agar peaks in 1250 cm$^{-1}$ (S = O of sulfate esters) [21] and 864 cm$^{-1}$ (L-galactopyranose-6-sulfate shoulder) [9] disappeared in H$^+$-adsorbed agar FTIR spectrum, Figure 14. Thus, interaction of H$^+$ ions with anionic groups of agaropectin chain, such as sulfate ester groups, decreases the negative charge of agar. Both ionic strength of HCl and decrease in negative charge of agar result in a decrease in $q_e$ and adsorption binding constant values of process in the presence of HCl, Table 6, compared to those of in the presence of NaCl. MB has not pKa value and does not react with H$^+$. $\Delta H$ and $\Delta S$ values of process were calculated using adsorption binding constants obtained the Temkin equation and are the same order of their values in the presence of NaCl, Table 7. The mean adsorption energy ($E$) values, obtained from the Dubinin-Radushkevich equation, at different temperatures are between 5.0-6.7 kJ mol$^{-1}$ in section 2A and 7.1-9.4 kJ mol$^{-1}$ in section 2B. MB degrades in pH values above 12 [37,38] and thus we did not carry out experiments under alkaline conditions.



**Figure 10 Variation of $q_e$ vs. $c_e$ for adsorption of MB on agar from 5 weight percentage of ethanol in water.**



**Figure 12 Variation of $q_e$ vs. $c_e$ for adsorption of MB on agar from 10 weight percentage of ethanol in water.**
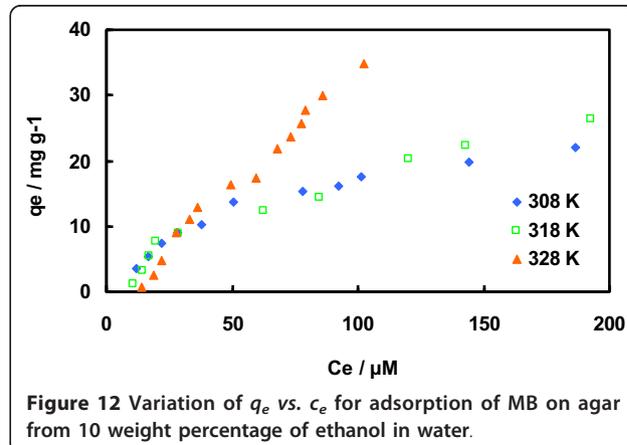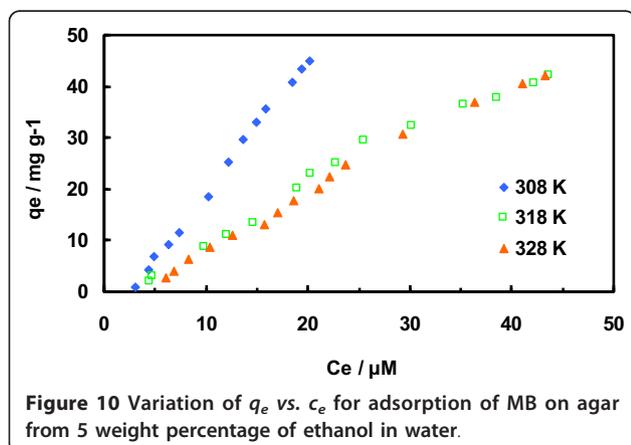
**Table 4 Parameters obtained from the Temkin isotherm and maximum experimental capacity of adsorption values, $q_{m, exp}$, for sections 2A and 2B of adsorption of MB on agar in different weight percentages of ethanol-water binary mixtures at 308-328 K**

| $W_{EtOH}$% | Section 2A | | | | Section 2B | | | |
|---|---|---|---|---|---|---|---|---|
| | $q_{m, exp}$ (mg g$^{-1}$) | $c_1$ (mg g$^{-1}$) | $c_2$ (M$^{-1}$) | $R^2$ | $q_{m, exp}$ (mg g$^{-1}$) | $c_1$ (mg g$^{-1}$) | $c_2$ (M$^{-1}$) | $R^2$ |
| | | | | T = 308 K | | | | |
| 0.0% | 12.89 | 139.66 | 675821 | 0.997 | 45.93 | 303.03 | 399806 | 0.995 |
| 2.5% | 13.89 | 107.62 | 498376 | 0.992 | 44.77 | 386.78 | 148424 | 0.974 |
| 5.0% | 11.78 | 157.64 | 350988 | 0.988 | 45.20 | 460.79 | 159595 | 0.999 |
| 7.5% | 19.61 | 279.42 | 213849 | 0.994 | 34.45 | 359.95 | 160740 | 0.991 |
| 10.0% | 10.34 | 70.53 | 160879 | 0.995 | 22.08 | 76.66 | 154787 | 0.962 |
| | | | | T = 318 K | | | | |
| 0.0% | 21.64 | 204.88 | 454648 | 0.991 | 48.75 | 514.35 | 92568 | 0.990 |
| 2.5% | 16.41 | 163.78 | 302494 | 0.960 | 46.53 | 281.13 | 200848 | 0.977 |
| 5.0% | 13.34 | 113.92 | 285825 | 0.994 | 42.25 | 290.63 | 123161 | 0.994 |
| 7.5% | 19.01 | 238.80 | 137308 | 0.997 | 35.51 | 266.06 | 130419 | 0.991 |
| 10.0% | 7.71 | 120.46 | 102685 | 0.968 | 26.43 | 104.21 | 73747 | 0.922 |
| | | | | T = 328 K | | | | |
| 0.0% | 21.52 | 220.79 | 309683 | 0.995 | 47.53 | 454.13 | 170397 | 0.990 |
| 2.5% | 21.07 | 225.10 | 225667 | 0.993 | 46.15 | 256.85 | 275633 | 0.988 |
| 5.0% | 13.10 | 133.48 | 217722 | 0.998 | 42.30 | 333.37 | 99317 | 0.998 |
| 7.5% | 16.96 | 187.31 | 109602 | 0.984 | 32.21 | 345.68 | 51340 | 0.986 |
| 10.0% | 16.55 | 146.56 | 71996 | 0.973 | 34.90 | 334.97 | 28921 | 0.991 |

### 3.5. Kinetics of adsorption of MB on agar

Experiments were carried out in $1 \times 10^{-4}$ M (portion 2A) and $3 \times 10^{-4}$ M (portion 2B) MB that are in the portions 2A and 2B of adsorption isotherms of MB on agar in water at 318 K, respectively.

In both cases, reaction kinetics is two-stage. As given in Figure 15, we observed a rapid adsorption step in 3-5 minutes after beginning reaction so that in the third (for $1 \times 10^{-4}$ M MB) and fifth (for $3 \times 10^{-4}$ M MB) minutes $q_t$ is approximately equal to $\frac{q_e}{2}$ and then the adsorption rate decreases and equilibrium attains after 680 and 580 minutes in $1 \times 10^{-4}$ M and $3 \times 10^{-4}$ M MB, respectively.

This observation shows that at first due to negatively charged of agar, MB adsorbs rapidly on the -OH groups of anionic agaropectin chains and then adsorption occurs slowly on -OH groups of agarose chains of agar.

Data obtained from kinetics of MB adsorption on pure agarose in 318 K verified that adsorption of MB on agarose chains of agar occurs in the slower step of adsorption kinetics, Figure 15. The kinetics of sorption of MB on agar and pure agarose was investigated using the pseudo-first-order, pseudo-second-order and pore-diffusion models. The linearized form of pseudo-first-order model [39] is given by:

$$\ln \left( \frac{q_{e,1} - q_t}{q_{e,1}} \right) = -k_1 t \tag{8}$$

where $q_{e,1}$ sand $q_t$ are the adsorption capacity at equilibrium obtained from the pseudo-first-order model and at time $t$, respectively (mg g$^{-1}$). $k_1$ is the rate constant of pseudo-first-order adsorption (min$^{-1}$). The pseudo-second-order kinetic model [40] is expressed as:

**Table 5 ΔH and ΔS values of sections 2A and 2B of adsorption of MB on agar in different weight percentages of ethanol and 2-propanol aqueous binary mixtures at 308-328 K**

| System | ΔH (kJ mol$^{-1}$) | ΔS (J mol$^{-1}$ K$^{-1}$) | System | ΔH (kJ mol$^{-1}$) | ΔS (J mol$^{-1}$ K$^{-1}$) |
|---|---|---|---|---|---|
| | Section 2A | | | Section 2B | |
| Water | -32.8 | 5.2 | Water | -36.1 | -10.7 |
| EtOH (2.5%) | -33.4 | 0.5 | EtOH (2.5%) | 26.0 | 183.3 |
| EtOH (5.0%) | -20.0 | 41.3 | EtOH (5.0%) | -19.9 | 34.9 |
| EtOH (7.5%) | -28.2 | 10.4 | EtOH (7.5%) | -47.6 | -53.8 |
| EtOH (10.0%) | -33.8 | -10.2 | EtOH (10.0%) | -70.3 | -128.7 |

**Figure 13 Variation of $q_e$ vs. $c_e$ for adsorption of MB on agar in (a) 0.001 and (b) 0.032 M HCl**.
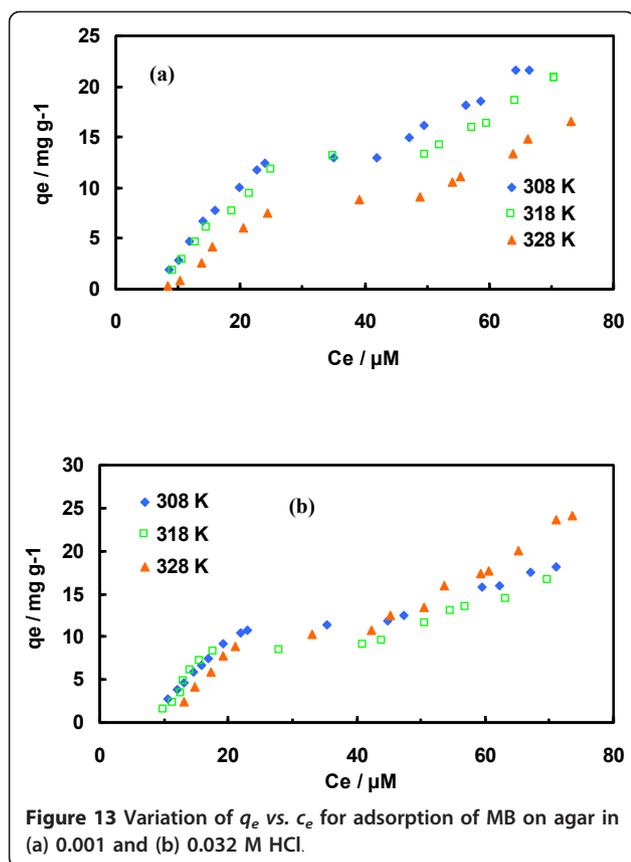
$$\frac{t}{q_t} = \frac{1}{k_2 q_{e,2}^2} + \frac{t}{q_{e,2}} \tag{9}$$

Where $k_2$ is the rate constant of pseudo-second-order adsorption (g mg$^{-1}$ min$^{-1}$) and $q_{e,2}$ is the adsorption capacity at equilibrium obtained from the pseudo-second-order kinetic model (mg g$^{-1}$). In order to quantitatively compare the applicability of these two models, $\Delta q_t$, normalized standard deviations of $q_t$, in relation to the experimental and calculated values of $q_t$, are given as

$$\Delta q_t = 100 \times \sqrt{\frac{\sum \left[ (q_{t,exp} - q_{t,cal}) / q_{t,exp} \right]^2}{(n-1)}} \tag{10}$$

where $n$ is the number of data points and $q_{t,\,exp}$ and $q_{t,\,cal}$ are experimental and calculated adsorption capacity of cellulose at a given time $t$, respectively. Data of kinetics of adsorption of MB on agarose (the first stage) and agaropectin (the second stage) chains of agar and pure agarose fitted in the pseudo-second-order model better than pseudo-first-order model and thus kinetics of interaction obeys from the pseudo-second-order model, Table 8.

Mckay and Poots [41] proposed a pore-diffusion equation, given by

$$q_t = k_{dif} t^{0.5} + I \tag{11}$$



**Figure 14 FTIR spectrum of H$^+$-adsorbed agar**.
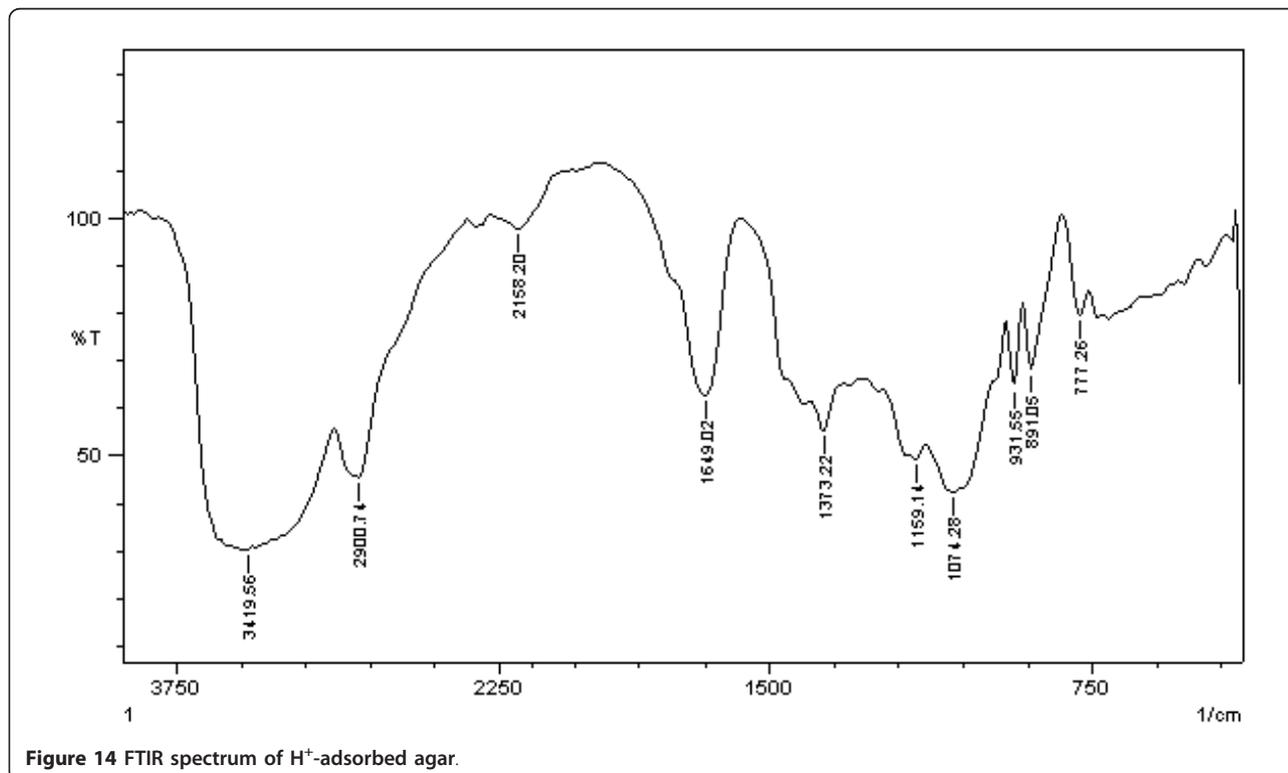
**Table 6 Parameters obtained from the Temkin isotherm and maximum experimental capacity of adsorption values, $q_{m, exp}$, for sections 2A and 2B of adsorption of MB on agar in water and different concentrations of HCl at 308-328 K**

| [HCl](M) | Section 2A | | | | Section 2B | | | |
|---|---|---|---|---|---|---|---|---|
| | $q_{m, exp}$ (mg g$^{-1}$) | $c_1$ (mg g$^{-1}$) | $c_2$ (M$^{-1}$) | $R^2$ | $q_{m, exp}$ (mg g$^{-1}$) | $c_1$ (mg g$^{-1}$) | $c_2$ (M$^{-1}$) | $R^2$ |
| | | | | T = 308 K | | | | |
| 0.0 | 12.89 | 139.66 | 675821 | 0.997 | 45.93 | 303.03 | 399806 | 0.995 |
| 0.001 | 12.52 | 121.89 | 138175 | 0.995 | 21.74 | 206.99 | 46497 | 0.983 |
| 0.032 | 10.92 | 12.58 | 121097 | 0.996 | 18.21 | 148.73 | 53190 | 0.995 |
| | | | | T = 318 K | | | | |
| 0.0 | 21.64 | 204.88 | 454648 | 0.991 | 48.75 | 514.35 | 192568 | 0.990 |
| 0.001 | 11.83 | 112.26 | 129443 | 0.986 | 20.92 | 230.70 | 36358 | 0.987 |
| 0.032 | 8.26 | 147.58 | 110745 | 0.971 | 16.63 | 156.46 | 43918 | 0.992 |
| | | | | T = 328 K | | | | |
| 0.0 | 21.52 | 220.79 | 309683 | 0.995 | 47.53 | 454.13 | 170397 | 0.990 |
| 0.001 | 7.58 | 80.45 | 119275 | 0.978 | 16.64 | 196.03 | 32842 | 0.989 |
| 0.032 | 8.94 | 152.03 | 92994 | 0.995 | 24.20 | 251.69 | 36180 | 0.969 |

where $k_{dif}$ (mg g$^{-1}$ min$^{-0.5}$) is the pore-diffusion rate constant and $I$ (mg g$^{-1}$) is proportional to the boundary layer diffusion effects. As shown in Table 8, kinetics of interaction of MB with agarose (the first stage) and agaropectin (the second stage) chains of agar and pure agarose obey from the pore-diffusion model.

For the adsorption of the adsorbates onto the adsorbent in aqueous solution, two diffusion steps are absolutely necessary: (1) mass transfer from water to the adsorbent surface across the boundary layer (film diffusion) and (2) diffusion of adsorbate molecules within the pores of material, binding the pores and capillary spaces (intraparticle diffusion) [42].

The values of the intercepts give an idea about the boundary layer thickness: the greater the intercept, the greater the boundary layer effect. The boundary-layer resistance is affected by the rate of adsorption and increase in contact time, which reduces the resistance and increases the mobility of dye during adsorption. As seen in Table 8, the intercept of the first stage of 3 × 10$^{-4}$ M MB is positive and in this case due to greater concentration of MB compared to 1 × 10$^{-4}$ M MB there is boundary layer effect. However, the negative values of the intercepts of the pure agarose, the first stage of 1 × 10$^{-4}$ M MB and the second stages of 1 × 10$^{-4}$ and 3 × 10$^{-4}$ M MB of adsorption kinetics of MB on agar, shown in Table 8, suggest that the boundary-layer effect is

close to minimum [42] and diffusion of the MB molecules into the agarose and agaropectin chains of agar and pure agarose is the main rate-controlling step. As seen in Table 8, due to negative charge of agar, $k_{dif}$ values of process on agarose chains of agar are greater than those on pure agarose. Also, in each stage of kinetics of process on agar, the greater MB concentration, the greater $k_{dif}$ values of process, Table 8. Kinetic results show that -OH groups of agar adsorb MB molecules and thus formation and adsorption of MB dimers results in appearance section 2B.

## 4. Conclusion

Adsorption isotherms of MB on agar in the presence of NaCl and ethanol were analyzed by ARIAN model. In the used concentration range of MB, it adsorbs as monomer and dimer on agar in sections 2A and 2B, respectively. Data obtained from IR spectroscopy show that MB interacts with -OH groups of agarose and agaropectin chains of agar.

Binding constants of MB to agar surface in the section 2A are greater than those of section 2B. Adsorption process in water and other solutions is exothermic.

Due to salting in, adsorption capacity of surface decreases with increase in NaCl and HCl concentration. With increase in NaCl concentration, MB deposits from solution.

**Table 7 ΔH and ΔS values of sections 2A and 2B of adsorption of MB on agar in water and various concentrations of HCl at 308-328 K**

| System | ΔH (kJ mol$^{-1}$) | ΔS (J mol$^{-1}$ K$^{-1}$) | System | ΔH (kJ mol$^{-1}$) | ΔS (J mol$^{-1}$ K$^{-1}$) |
|---|---|---|---|---|---|
| | Section 2A | | | Section 2B | |
| Water | -32.8 | 5.2 | Water | -36.1 | -10.7 |
| HCl (0.001 M) | -6.2 | 78.4 | HCl (0.001 M) | -14.7 | 41.6 |
| HCl (0.032 M) | -11.0 | 61.9 | HCl (0.032 M) | -16.2 | 38.0 |
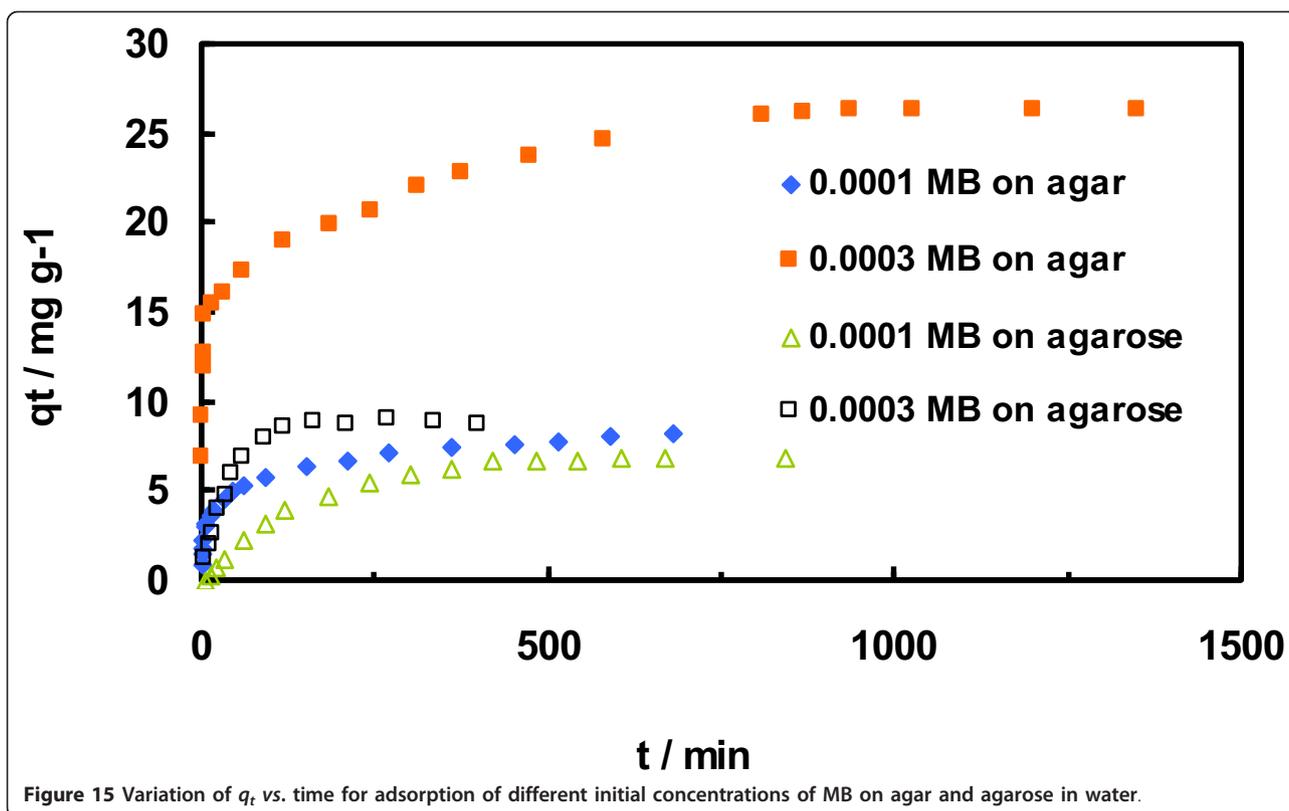
**Figure 15 Variation of $q_t$ vs. time for adsorption of different initial concentrations of MB on agar and agarose in water.**

**Table 8 Kinetic parameters of the first and second stage of adsorption of MB on agar and those of on pure agarose obtained from the pseudo-first-order, pseudo-second-order and pore-diffusion kinetic models at 318 K**

| [MB](M) | pseudo-first-order | | | pseudo-second-order | | | pore-diffusion | | |
|---|---|---|---|---|---|---|---|---|---|
| | $q_{e,1}$ (mg g$^{-1}$) | $k_1$ (min$^{-1}$) | $\Delta q_t$ | $q_{e,2}$ (mg g$^{-1}$) | $k_2$(*) | $\Delta q_t$ | $k_{dif}$ (*) | $I$ (mg g$^{-1}$) | $R^2$ |
| | | | First stage of process on agar | | | | | | |
| 0.0001 | 2.71 | 1.625 | 9.14 | 3.89 | 0.329 | 3.58 | 2.487 | -0.278 | 0.990 |
| 0.0003 | 13.87 | 1.144 | 6.79 | 17.24 | 0.069 | 4.49 | 5.046 | 3.950 | 0.969 |
| | | | Second stage of process on agar | | | | | | |
| 0.0001 | 4.75 | 0.0106 | 29.53 | 5.52 | 0.0026 | 20.79 | 0.322 | -0.106 | 0.998 |
| 0.0003 | 11.80 | 0.0031 | 16.89 | 14.93 | 0.0002 | 7.96 | 0.435 | -0.743 | 0.996 |
| | | | Pure agarose | | | | | | |
| 0.0001 | 7.04 | 0.0066 | 1.85 | 9.52 | 0.0006 | 2.35 | 0.337 | -0.091 | 0.971 |
| 0.0003 | 9.10 | 0.0234 | 6.58 | 12.35 | 0.0016 | 3.23 | 0.893 | -0.572 | 0.979 |

* Dimensions of $k_{dif}$ and $k_2$ are in mg g$^{-1}$ min$^{-0.5}$ and g mg$^{-1}$ min$^{-1}$, respectively.

Adsorption of MB on agar decreases with increasing weight percentage of ethanol in water-ethanol mixtures. This is mostly due to hydrogen bonding and hydrophobic interaction between ethanol molecules with MB which increases solubility of MB and thus decreases its $q_{m, \text{exp}}$ values in these solutions. The mean adsorption energy ($E$) values showed that binding of MB to agar surface is due to chemical adsorption. Kinetics of this interaction obeys from the pseudo-second-order and pore-diffusion models. Also kinetic data showed that in the beginning adsorption process, MB adsorbs rapidly on anionic agaropectin chains of agar and then slowly on its agarose chains.

**Authors' contributions**
This project was based on the ideas of BS and carried out under his guidance and consultation. FA carried out experimental work. Authors read and approved the final manuscript.

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