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BSA adsorption onto commercial activated carbon modified by microwave assisted chemical activation

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In the study, effects of pH and temperature on the equilibrium and the kinetics of bovine serum albumin (BSA) adsorption onto commercial and modified activated carbon were investigated. Activated carbon was modified firstly by treatment with Na_2CO_3, secondly thermally treated by microwave technique, and finally subjected to treatment with HCl. The modified activated carbon samples were characterized by Fourier Transform Infrared (FTIR) and BET analyses. The results indicated that it was possible to increase the surface area of the commercial activated carbon (CAC) after the modification process (MAC) with an enhancement percentage of 59%, compared to initial untreated CAC sample. The adsorption capacities decreased at pH values lower and higher than the isoelectric point value; and increased with increasing temperature for both activated carbon samples. Moreover, adsorption isotherm and kinetic data were well explained by the Langmuir isotherm and pseudo-second order kinetic models, respectively.

Keywords: Bovine serum albumin, activated carbon, microwave treatment, modification, adsorption, isotherm models, kinetics.

INTRODUCTION

The adsorption of proteins from solution onto solid surfaces is a vital and very active research field because of its application in protein purification, drug delivery systems, biosensors, design of food processing equipment and artificial implants. This process is complicated and involves various interactions. Most proteins are known as amphipathic molecules and this property makes them surface-active molecules. Hence, different results are obtained from the adsorption process because of the characteristic features of surfaces such as hydrophobicity and hydrophilicity. The adsorption process results in a change in physicochemical properties. The affinity of a protein for an interface is affected by the structural stability of the protein, the ionic strength and pH of the solution, and the surface properties of the sorbent [1-4].

Bovine serum albumin (BSA) is a globular blood plasma protein. It has a high conformational adaptability and is often used as a reference protein for adsorption experiments. Sustainability of the osmotic pressure and pH of blood, and transport of various compounds are the most important physiological functions of serum albumin. The isoelectric point of BSA is about pH 4.5-5.0 [5-8].

Activated carbons (AC) are widely used as an effective adsorbent for the removal of organic pollutants such as dyes and heavy metals from aqueous solutions due to their large porous surface area, controllable pore structure, thermo stability and low acid/base reactivity. The physical and chemical properties of AC depend on their internal pore structure and surface functional groups. The commercial activated carbons are generally produced from wood and coconut shells. However, recently, other lignocellulosic materials, which are generally considered as a waste, have been used as precursors in the synthesis of this adsorbent for specific applications. The utilization of lignocellulosic biomass like rice hulls, bran, sawdust and fibers is very important for the economy, and simultaneously brings a solution to the waste problem [9-11].

In literature, the protein adsorption has been a subject of interest for various scientists and many studies have evaluated the adsorption of proteins on various adsorbents. In the present study, modified and non-modified activated carbons were used as adsorbents. For evaluation of the protein adsorption ability of the activated carbon samples, bovine serum albumin (BSA) was chosen as the target protein. The aims of this study were: i) to modify the surface properties of commercially available activated carbon; ii) to investigate the effect of pH and temperature on the adsorption of BSA from

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aqueous solution; iii) to perform modelling studies to represent the adsorption isotherms and kinetics.

MATERIALS AND METHODS

Materials

Commercial activated carbon was obtained from Altek A.Ş., Istanbul. Bovine serum albumin and other chemicals of analytical grade were purchased from Sigma Aldrich.

Modification of activated carbon

Commercially available activated carbon Acticarbone-CPW (produced from marine pine wood by chemical activation with phosphoric acid) was used in this study. Commercial activated carbon (CAC) was modified using the method described by Jiang et al. [10] with some modifications. Firstly, CAC was impregnated with a solution of 10% Na_2CO_3 at a ratio of 5:1 (weight of impregnation reagent/weight of activated carbon) and kept at room temperature for 24 h. Afterwards, the mixture was subjected to microwave treatment for 7 min at 540 W of microwave output power. The mixture was cooled to room temperature and then was boiled with 10% HCl solution for 30 min. After the boiling procedure, the mixture was washed several times with distilled water; and was dried at 100°C for 24 h. The modified activated carbon sample obtained from this treatment was named MAC.

Characterization of the modified activated carbon

In order to determine the physical and chemical changes of activated carbon after the modification treatment, the modified activated carbon (MAC) was characterized by Fourier Transform Infrared (FTIR) and BET analyses. Pore size distribution of CAC and MAC samples was analysed by the BJH desorption method. While FTIR was used to identify the chemical structure, surface structure was examined by BET analysis. FTIR spectra of the samples were collected using a Perkin Elmer Pyris1 FT-IR spectrometer. The KBr pellets were prepared containing 1/2 g of the activated carbon sample.

Protein adsorption studies

Adsorption experiments were carried out to evaluate the adsorption capacities and isotherms of activated carbon samples at different pH (4-7) and temperature (25-40°C) values. The experiment was initiated by the addition of adsorbent to 50 mL of BSA solution at the desired pH and temperature. Buffer solutions were used to keep the pH value steady. The mixture was shaken for 60 min at 100 rpm in a shaking incubator. During the processing time of 60 min, the samples were taken from the mixture at timed intervals and centrifuged to remove the particles. The protein concentration in the supernatant was determined by the Lowry method [12]. The amount of protein adsorbed on the adsorbent was calculated by the mass balance before and after adsorption. For each sample, the assay was carried out in triplicate and the average was taken. In this study, all experiments were carried out at least in duplicate and the reproducibility between trials was within ±5%. Adsorption capacities were calculated by using the following equation:

$$q_e = \frac{(C_0 - C_e) \cdot V}{m}$$

where q_e is the equilibrium adsorbed concentration (mg/g), C_0 and C_e are the initial and equilibrium protein concentrations (mg/L), respectively. V is the volume of the solutions and m is the weight of adsorbent (g).

Adsorption isotherm models

Langmuir, Freundlich and Temkin isotherm models were used to evaluate the data obtained from the BSA adsorption experiments:

Langmuir model:

$$\frac{1}{q_e} = \frac{1}{Q*b*C_e} + \frac{1}{Q}$$

where q_e is the equilibrium adsorbed concentration (mg g^{-1}), C_e is the equilibrium protein concentration (mg L^{-1}), Q is the maximum sorption capacity (mg g^{-1}) and b is the adsorption equilibrium constant.

Freundlich model:

$$\ln q_e = \ln K_F + \frac{1}{n}\ln C_e$$

where K_F is the Freundlich affinity coefficient, n is the Freundlich exponential coefficient.

Temkin model:

$$q_e = \frac{RT}{b_T}\ln A_T + \frac{RT}{b_T}\ln C_e$$

where A_T and b_T are the Temkin coefficients.

Adsorption kinetics of protein

Several methods are available to study the adsorption mechanism. In this study, in order to determine the adsorption kinetics, the data obtained from the BSA adsorption process were analysed with the pseudo-first order, pseudo-second order, Elovich and intra particle diffusion models.

Pseudo-first order model:

$$\log\frac{q_e - q_t}{q_e} = \frac{k_1 t}{2.303}$$

where q_e is the adsorbed amount at equilibrium (mg g^{-1}); q_t is the adsorbed amount at time t (mg/g); k_1 is the pseudo-first order adsorption kinetic parameter (min^{-1})

Pseudo-second order model:

$$\frac{t}{q_t} = \frac{1}{k_2 {q_e}^2} + \frac{t}{q_e}$$

where k_2 is the pseudo-second order adsorption kinetic parameter (g mg^{-1} min^{-1}).

Elovich model:

The Elovich equation is valid for chemisorption kinetics and systems in which the surface is heterogenous.

$$q_t = \frac{1}{\beta}\ln(\alpha\beta) + \frac{1}{\beta}\ln t$$

where α is the initial adsorption rate (mg g^{-1} min); β is a constant related to the extent of surface coverage and activation energy consumption (g mg^{-1})

Intra particle diffusion model:

$$q_t = k_i t^{0.5} + C_i$$

where k_i is the intra particle diffusion kinetic parameter (mg g^{-1} min^{-2}); C_i is a constant related to layer thickness (mg g^{-1}).

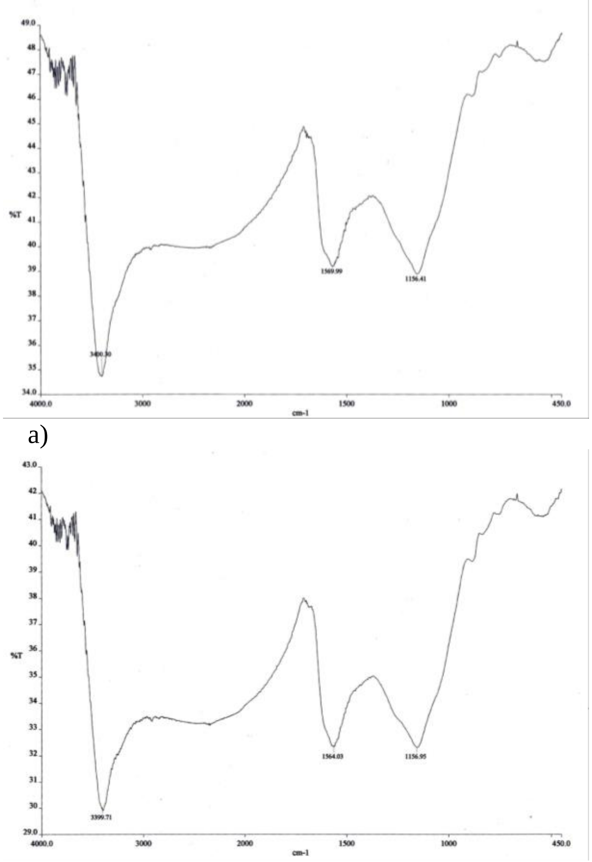
RESULTS AND DISCUSSION

Characterization of the modified activated carbon

The FTIR results of CAC and MAC are shown in Fig. 1 (a-b). As can be seen from these figures, there is no significant difference between the FTIR spectra of these two activated carbon samples. This could be explained with the neutralizing effect between Na_2CO_3 and HCl used for modification of the activated carbon. According to literature, the peaks observed around 3400 cm^{-1} were stretches arisen from –OH groups [11, 13-15]. When two spectra of activated carbon were compared, it was observed that the –OH stretching intensity of MAC sample slightly decreased due to the treatment with Na_2CO_3.

On the other hand, in both spectra, peaks were present around 1155 cm^{-1}, which pointed to C-O stretching [15]. Moreover, it was considered that the observed peaks around 1565 cm^{-1} contained multi groups and these groups were referred to as C=C and –COOH groups, as stated in the literature [11, 15-17].

The results of surface structure analyses of CAC and MAC are given in Table 1. Pore size distribution of CAC and MAC samples analysed by the BJH desorption method is presented in Figure 2 (a,b). Generally, the surface areas of these two activated carbon samples were high because of numerous micropores on the surface structure. The findings showed that the surface area of modified AC increased up to 2492 m^2/g compared to the initial untreated AC sample surface area of 1572 m^2/g. On the other hand, it was found that the mesoporous surface area increased from 1014 to 1653 m^2/g with an enhancement rate of 1.63.



a)

b)

Fig. 1. FT-IR spectra of a) CAC b) MAC.

Table 1 BET surface analysis results of CAC and MAC samples

Sample	S_{BET} (m^2/g)	S_{mes} (m^2/g)	V_{tot} (cm^3/g)	V_{mic} (cm^3/g)	V_{mes} (cm^3/g)	R_{avg} (nm)
CAC	1572	1014	1.608	0.256	1551	2.026
MAC	2492	1653	2.427	0.402	2296	1.948

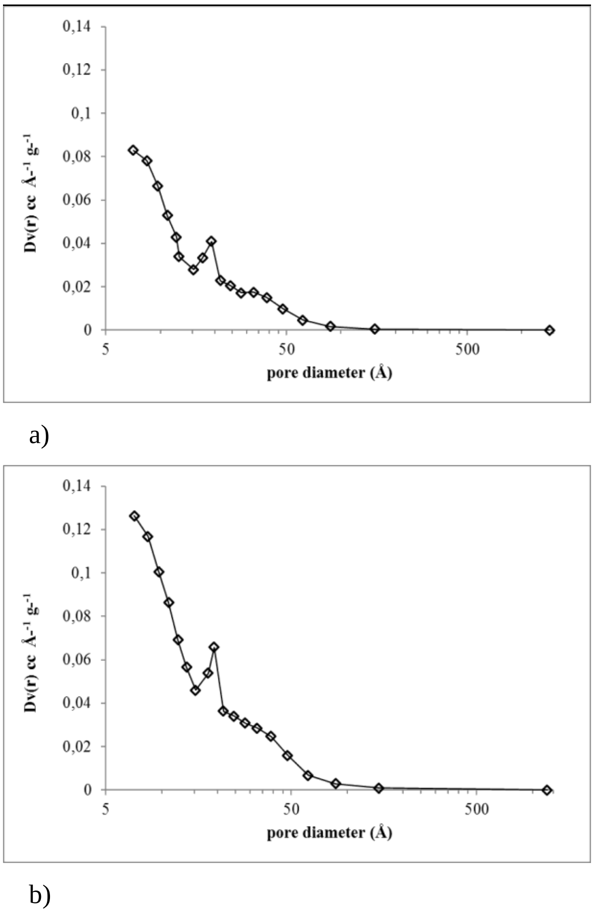


Fig. 2. Pore size distribution in a) CAC b) MAC.

Moreover, as a result of micropore intensity increase in the MAC sample, it was observed that the average pore diameter decreased from 2.026 nm to 1.948 nm. According to the literature, pores with a diameter of 2<r<50 nm are defined as mesopores. The increase in the average pore diameter of MAC samples (mesopores) was similar to that of CAC samples. This obviously showed that although diameters of mesopores were r>2 nm, these values were very small to increase the pore diameter. In other words, the diameter of the obtained mesopores was similar to that of micropores.

Adsorption of BSA on the commercial (CAC) and modified (MAC) activated carbon

Effect of pH on BSA adsorption

pH is one of the critical and most important parameters for determining the adsorbent's surface characteristics and the adsorption equilibrium in the adsorption process. It affects the chemical speciation of ions and the ionization of active functional sites on the sorbent surface. The effect of pH on the adsorption of BSA onto commercial (CAC) and modified (MAC) activated carbon samples was assessed in the pH range from 4 to 7 and the pH dependence of BSA adsorption is presented in Figure 3. As can be seen from this figure, maximum adsorption capacities are observed at pH 5 for both activated carbon samples, which is near to the isoelectric point of BSA. The adsorbed amount of BSA decreased at pH values lower and higher than the isoelectric point value. This decrease could be explained by the bilateral electrostatic repulsion of protein molecules since they are with the same electric charge. Protein molecules at the pI are natural, and the repulsion force between BSA molecules becomes almost zero [2,18,19]. Therefore, according to these results, the optimum pH value was found as 5.0 for BSA adsorption.

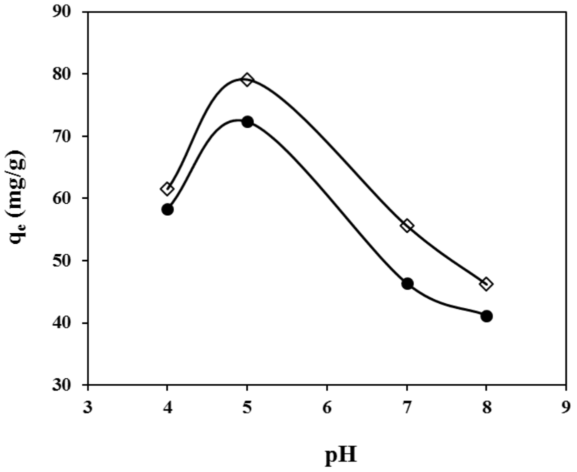


Fig. 3. Effect of pH on the BSA adsorption capacity of activated carbon samples (● CAC; ◊ MAC).

Effect of temperature on BSA adsorption

In order to investigate the effect of temperature on the adsorption of BSA onto CAC and MAC samples, the experiments were performed in the temperature range from 25 to 40°C. The BSA adsorption capacity of CAC and MAC in respect to temperature is presented in Fig. 4. The results showed that the temperature of solution extremely influenced the BSA adsorption. The adsorption capacity of all samples increased with increasing temperature. This increase might be due to the increase of the diffusion on adsorbent surfaces with increasing protein activity. The same result was stated in the studies of BSA adsorption o on TiO_2 [5], BSA adsorption on SBA-15 [20] and BSA adsorption on monodispersed hollow silica nanospheres [18].

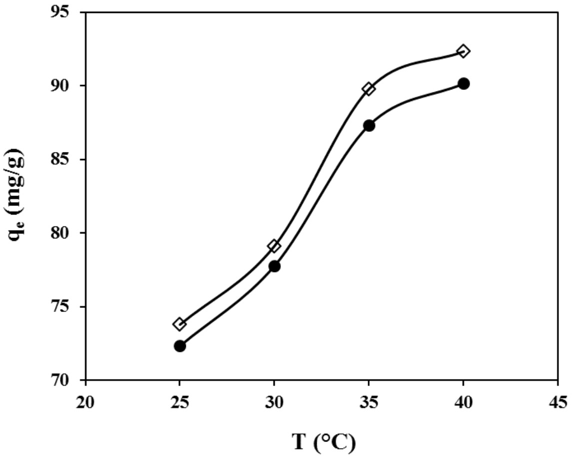


Fig. 4. Effect of temperature on the BSA adsorption capacity of activated carbon samples (● CAC; ◊ MAC)

Adsorption isotherm modelling

Three classic adsorption models (Langmuir, Freundlich and Temkin isotherm models) were used to describe the equilibrium adsorption data of the BSA onto activated carbon samples. Among these isotherm models, Langmuir isotherm model was determined as the most appropriate one for the BSA adsorption data with high values of the correlation coefficient (R^2) and low values of the standard error (σ). This result indicates that BSA adsorption occurs as a monolayer onto the homogenous adsorbent surface. The estimated parameters of these models are presented in Table 2.

As can be seen in Table 2, the maximum adsorption capacity of CAC and MAC for BSA adsorption was found as 134.77 mg/g and 136 mg/g, respectively, at 40°C and pH 5. It was observed that the adsorption capacity decreased at pH 7 and pH 8. This showed that the maximum adsorption occurred at pH 5 near its isoelectric point, where it is neutral. This could be explained with the hydrophobic interactions between BSA and activated carbon. Furthermore, it was known that BSA was negatively charged at pH 7.5. So, above this pH value, the adsorption was limited because of the electrostatic repulsions between the negatively charged activated carbon and BSA molecules [21].

On the other hand, the maximum adsorption capacity values of MAC were slightly higher than those of the CAC samples. This slight increase was explained with the large molecular size of BSA to enter the micropores. As explained in the previous section, the mesopore diameter of the MAC sample was similar to that of the micropores. So, these mesopores were not sufficient to increase the adsorption capacity of the MAC sample. The same behaviour was observed in the adsorption of BSA onto materials with different pore size in the literature [22-23].

Additionally, the mineral residue remaining on the surface of the activated carbon increased the hydrophilicity of the MAC sample, which could prevent the increase of the adsorption capacity [24].

Table 2. Estimated parameters and statistical values of isotherm models for BSA adsorption onto CAC and MAC samples (pH= 5.0, T= 40°C).

CAC Sample			
Langmuir Isotherm			
Q (mg/g)	b (L/mg)	σ	R^2
134.77	0.02703	0.00077	0.9359
Freundlich Isotherm			
K_F ($mg^{1-1/n}L^{1/n}g^{-1}$)	n	σ	R^2
41.99	5.7060	0.07896	0.9172
Temkin Isotherm			
A_T (L/g)	b_T (J/mol)	σ	R^2
1.1737	131.9671	10.8043	0.8842
MAC Sample			
Langmuir Isotherm			
Q (mg/g)	b (L/mg)	σ	R^2
136.00	0.01884	0.00057	0.9433
Freundlich Isotherm			
K_F ($mg^{1-1/n}L^{1/n}g^{-1}$)	n	σ	R^2
36.50	5.1647	0.06207	0.9437
Temkin Isotherm			
A_T (L/g)	b_T (J/mol)	σ	R^2
0.6562	123.2266	8.1491	0.9213

Adsorption kinetics modelling

Evaluation of the adsorption kinetics and adsorption equilibrium is very important to plan and control the adsorption process. In order to describe the adsorption kinetics, the pseudo-first order, the pseudo-second order, Elovich and the intra particle diffusion models were used for the evaluation of kinetic data obtained from the adsorption experiments. The adsorption kinetics experiments were carried out at different temperature and pH values. The estimated parameters and statistical data of these models are presented in Tables 3 and 4.

As can be seen from the tables, among these models, the second order kinetic model was found as the most appropriate one for all experimental data with high values of the coefficient of determination and low standard error values. Therefore, because of the fact that the pseudo-second order kinetic model suggests that the adsorption process incorporates chemisorption mechanism, it may be concluded that adsorption of BSA molecules onto activated carbon samples consists of chemical adsorption. The result obtained was in agreement with the studies published in the literature, and several authors have stated that the pseudo-second order kinetic model was better for BSA adsorption onto monodispersed hollow silica nanospheres [18] and Fe_3O_4 magnetic nanoparticles [19].

A summary of the BSA adsorption capacity of several adsorbents studied by several authors is given in Table 5. The adsorption capacities of CAC and MAC were higher than the results proposed in the literature for BSA adsorption except for silica gel, sepiolite and activated carbon.

Table 3. Statistical values of adsorption models for BSA adsorption onto CAC and MAC samples for various temperature values (pH= 5.0).

CAC Sample								
	Pseudo-first order		Pseudo-second order		Elovich		Intraparticle	
T (°C)	R^2	σ	R^2	σ	R^2	σ	R^2	σ
25	0.9662	5.9986	0.9937	0.0287	0.9280	8.6661	0.8432	12.3238
30	0.9716	5.0766	0.9927	0.0318	0.9215	8.3377	0.8887	9.8437
35	0.9856	4.1337	0.9978	0.0150	0.9749	5.4351	0.8450	13.0579
40	0.9837	4.5692	0.9976	0.0148	0.9621	6.9312	0.8732	12.3885
MAC Sample								
	Pseudo-first order		Pseudo-second order		Elovich		Intraparticle	
T (°C)	R^2	σ	R^2	σ	R^2	σ	R^2	σ
25	0.9757	4.5804	0.9975	0.0186	0.9638	5.5739	0.8685	10.3576
30	0.9739	4.9770	0.9941	0.0278	0.9373	7.6453	0.8769	10.5424
35	0.9797	5.0747	0.9974	0.0157	0.9542	7.5709	0.8794	12.0425
40	0.9810	5.0075	0.9980	0.0135	0.9653	6.7349	0.8737	12.5541

Table 4. Statistical values of adsorption models for BSA adsorption onto CAC and MAC samples for various pH values (T= 40°C).

CAC Sample								
	Pseudo-first order		Pseudo-second order		Elovich		Intraparticle	
pH	R^2	σ	R^2	σ	R^2	σ	R^2	σ
4	0.9522	5.5917	0.9787	0.0684	0.8556	9.4456	0.8976	8.0669
5	0.9837	4.5692	0.9976	0.0148	0.9621	6.9312	0.8732	12.3885
7	0.9378	4.8705	0.9671	0.1236	0.8562	7.2486	0.8067	8.2916
8	0.9357	3.9330	0.9829	0.0929	0.9071	4.6951	0.8939	4.9982
MAC Sample								
	Pseudo-first order		Pseudo-second order		Elovich		Intraparticle	
pH	R^2	σ	R^2	σ	R^2	σ	R^2	σ
4	0.9861	2.7174	0.9973	0.0254	0.9767	3.5281	0.8132	9.5382
5	0.9810	5.0075	0.9980	0.0135	0.9653	6.7349	0.8737	12.5541
7	0.8638	8.6977	0.9409	0.1297	0.7757	10.8938	0.8233	9.7983
8	0.9410	3.0856	0.9836	0.1190	0.9073	3.8342	0.8512	4.7872

Table 5. Adsorption capacities of various adsorbents compared with the present study.

Adsorbents	Experimental Conditions		Adsorption capacity (mg/g)	Ref.
	pH	Temp. (°C)		
Sepiolite	7.0	25	219.4	[1]
Kaolinite	7.0	25	29.1	[1]
Expanded perlite	7.0	25	2.3	[1]
Unexpanded perlite	7.0	25	1.1	[1]
Activated carbon	7.0	23	13.4	[9]
Silica coated $MnFe_2O_4$	3.8	25	100	[14]
Activated carbon	7.0	40	3.2	[26]
Activated carbon	5.7	25	312	[27]
Activated carbon	5.3	25	27.3	[28]
Silk fibroin microspheres	7.4	37	16	[29]
TiO_2	5.0	40	82	[30]
Silica gel	7.0	25	228	[31]
Hydroxyapatite	6.8	30	49	[32]
Commercial activated carbon	5.0	40	134.8	Present study
Modified activated carbon	5.0	40	136	Present study

CONCLUSIONS

Commercially available AC powder was modified by treating with chemical activation agents such as Na_2CO_3 and HCl, followed by heat treatment applying microwave technique. After the modification process, the surface area of the modified AC increased up to 2492 m^2/g compared to the initial untreated AC sample surface area of 1572 m^2/g.

Adsorption experiments were carried out using adsorption equilibrium and kinetics to investigate the adsorption ability of the commercial and modified activated carbon samples.

The maximum adsorption capacities for CAC and MAC samples were obtained at pH 5, near to pI of the protein where the repulsion force between adsorbed proteins is minimal. The amount of adsorbed BSA increased with increasing temperature for CAC and MAC samples, which indicates that the adsorption of BSA on activated carbon samples is an endothermic process. The isotherm data for BSA adsorption on activated carbon fitted well the Langmuir isotherm model. The maximum adsorption capacity of CAC and MAC for BSA adsorption was found as 134.77 mg/g and 136 mg/g, respectively, at 40°C and pH 5. Furthermore, kinetic data fitted the pseudo-second order kinetic model. Hence, it can be concluded that activated carbon samples can be employed as effective adsorbents for adsorption of proteins.

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АДСОРБЦИЯ НА АЛБУМИН ОТ ГОВЕЖДИ СЕРУМ (BSA) ВЪРХУ ТЪРГОВСКИ АКТИВЕН ВЪГЛЕН И МОДИФИЦИРАН С ХИМИЧНО АКТИВИРАНЕ С МИКРОВЪЛНОВО ВЪЗДЕЙСТВИЕ

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(Резюме)

В настоящата работа са изследвани ефектите на pH и температурата върху равновесието и кинетиката на адсорбция на албумин от говежди серум (BSA) върху търговски и модифициран активен въглен. Активният въглен беше модифициран с микровълнова техника със следващо третиране със солна киселина. Модифицираният активен въглен беше охарактеризиран с Фуриерова ИЧ-спектрометрия (FTIR) и БЕТ анализи. Резултатите показват, че по този начин е възможно да се повиши повърхността на търговския активен въглен (CAC) след модификацията до (MAC) с 59%, в сравнение с контролната проба (CAC). Адсорбционният капацитет намалява при ниски pH, но е по-висок отколкото в изо-електричната точка. Той нараства с температурата са двата изследвани въглена. Освен това, адсорбционната изотерма и кинетичните данни се обясняват добре с изотермата на Langmuir и кинетично уравнение от псевдо-втори порядък.