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Original Article

Physicochemical Properties of Bromelain Adsorption on Magnetic Carbon Nanoparticles and in Vitro Cytotoxicity on Breast Cancer Cells

Karimian Rad F[1], Ramezani M[1]*, Mohammadgholi A[1]

[1]Department of Biology, Faculty of Science, Central Tehran Branch, Islamic Azad University, Tehran, Iran

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Abstract

Background and Aim: As a proteolytic enzyme extracted from the pineapple stalk, Bromelain (Br) is known as an anticancer agent. In the first stage of this research, we studied the physicochemical factors which influence the maximum adsorption of Br on magnetic carbone nanoparticles (MCNPs) and then the anticarcinogenic activity of Br enzyme alone. Moreover, they were evaluated in combination with these particles on MCF-7 breast cancer cells.

Materials and Methods: The operational determinants influencing Br adsorption such as pH, contact time (30, 60, 90, 120 and 180 min), adsorbent dosage (1 gr/L, 5 gr/L), initial Br concentration (50, 150 and 300 mg/L) and temperature (35 and 50°C) were studied in detail. Then cancer cells were exposed to various Br concentrations (0.1 µg/mL, 1 µg/mL, 10 µg/mL, and 100 µg/mL) and the cell viability was determined by methylthiazol tetrazolium (MTT) assay after 24, 48 and 72 h.

Results: The highest adsorption of Br on MCNPs was 44 mg/g and was achieved at pH 5, 35°C and 120 min with 50 mg/L initial Br concentration and 1g/L MCNPs. The adsorption used the Freundlich and pseudo first-order kinetic models. The results indicated that MCNPs could be a potential effective adsorbent for the removal of Br. MTT assay indicated that a 100 µg/mL concentration of Br alone (after 24 h) and in combination with MCNPs (after 72 h) could efficiently inhibit the MCF-7 breast cancer cells.

Conclusion: Although the dose of Bromelain on synthesized MCNPS is about 440 times less than Bromelain alone, it possesses a significant cytotoxicity ($P<0.001$). Moreover, synthesized MCNPS had a considerable advantage of slow delivery which is favorable for the treatment of cancer.

Keywords: Bromelain enzyme, Anticarcinogenic, Magnetic carbon nanoparticles, Breast cancer

***Corresponding Author:** Mina Ramezani**,** Department of Biology, Faculty of Science, Central Tehran Branch, Islamic Azad University, Ponak Square, Imam Hasan Street, Tehran, Iran. Email: mina.ramezani@gmail.com.

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Introduction

Breast cancer is the most common cause of mortality for woman worldwide and roughly 1.7 million new cases of breast cancer are diagnosed per year. While breast cancer death rates are decreasing in more developed countries, these rates are increasing in developing and developed regions. Almost 60% of deaths caused by breast cancer occur in developing countries (1). Despite various therapies such as surgery,

and radio as well as chemo therapy, more advances are still required in this issue. Chemical drugs have high rates of toxicity and are non-targeted directly to cancerous tissues which lead to various side effects and less patient compliance with the treatment (2).

Today, the use of magnetic nanoparticles as carriers of drug delivery has drawn much attention. Magnetic nanoparticles are considered as a type of nanoparticles that have been manipulated using magnetic fields. These particles usually consist of two components, a central magnetic material (iron oxide magnetic nanoparticles) and a functionally chemical component such as anticancer drugs (3). The functionalization of the surface of these particles with different groups and compounds is carried out to increase the solubility and biocompatibility as well as the conductivity of various substances. These structures can constitute a very effective drug delivery system due to the control and slowing down drug releasing which increases the therapeutic efficacy of the drug. Other mentioned benefits are protection of the drug molecule, particle size smaller than the cell, the ability to cross biological obstacles to deliver the drug to the target site, increased drug persistence in the bloodstream, and biocompatibility (4, 5).

Some previous studies have indicated the anticarcinogenic activity of Br. Br is a 26 kD dietary proteolytic enzyme extracted from *Ananas comasus* plant, which has been associated with the therapeutic properties (6, 7). It has been used as a traditional remedy in Central and South America for centuries (8). Remarkable therapeutic properties such as immunomodulatory, anti-inflammatory, antimicrobial, antithrombotic and antimetastatic activities were attributed to Br (7, 9). Non-toxic nature of Br has made it a proper candidate for cancer treatment (10). It was reported that Br is absorbed into the human intestine without degradation and losing biological activities (11).

The most anti-carcinogenic study of Br on human cells has been limited to a few preclinical experiments (12). The antiproliferative effects of Br against human A431 epidermoid carcinoma and A375 melanoma cells were indicated by Bhui *et al.* (13). Paroulek *et al.* indicated the antitumor impact of Br against the GI101A breast cancer cell line through apoptosis (14). In 2013, Amini *et al*. could inhibit the proliferation of four gastrointestinal carcinoma cell lines by Br which promote apoptosis via caspase system and extranuclear p53 (15). Gani *et al.* reported the antiproliferative activity of fresh pineapple juices on ovarian and colon cancer cell lines (8). Moreover, recently the anticarcinogenic activity of Br against oral cancer cells was indicated by Lee *et al*. (16). However, it was reported that Br has remarkable anticancer effects and also indicated toxic effects towards normal cell lines (17). Therefore, the aim of this research was to investigate the factors effective on the maximum absorption of Br on synthesized MCNTs and to evaluate the effect of Br enzyme in combination with MCNPs in order to produce the same cytotoxicity with a lower concentration of Br against MCF-7 breast cancer cells.

Materials and Methods

Preparation of Samples

Bromelain was purchased from Salamat Parmoon Amin Company, and active carbone, N-Cetyl-N،N،N-trimethylammonium bromide, acetonitril (C_2H_3N), disodium hydrogen phosphate (Na_2HPO_4), ferric chloride (FeCl2), ferrous chloride (FeCl3), Fe(NO3)3.9H2O, (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) (MTT), trypsin-EDTA, methanol, fetal bovine serum (FBS), Dulbecco's Modified Eagle Medium (DMEM), penicillin, streptomycin, hydrochloric acid and sodium hydroxide were acquired from Merk (Germany). The MCF-7 cell line (Breast Cancer cells) was bought from Pasteur Institute (Iran) and cultured in DMEM medium (containing 10% FBS) and 100 U/ml penicillin and 100µg/ml streptomycin at 5% CO2 pressure at 37°C in 25 cm^3 flask. The cells were then cultured in 24-well culture plates (1.5×10^5 cells per milliliter). The cell counts were performed using a hemocytometre slide.

Synthesis of Magnetic Nano Particles

The magnetic absorbent was made through a chemical coprecipitation according to Liu *et al.* with some modifications (18). In this method, 0.5 g of activated carbon powder was diffused in 20 ml saturated nitric acid (65%) and homogenized for 3 h in an ultrasonic bath at 80°C. The activated carbon was used in order to adsorb high molecular weight substances such as Br. Subsequently, the sample was filtered and the resulting powder was added to 200 ml of aqueous solution with

0.4 g/l concentration containing iron nitrate. Modification of MCNPs using cityl trimethymethyl ammonium bromide was carried out to deposit Fe3O4 on carbon nanoparticles.

The solution was homogenized by an ultrasonic bath for 30 minutes. The final solution was filtered by Watman filter paper No. 41. In the next step, the sample was placed inside an electric furnace under nitrogen gas for 3 h at a temperature of 750°C. The synthesized adsorbent was washed several times with distilled water before use and separated in the vicinity of an outer magnet with power of 4.2 T. The resulting absorbent was dried in the oven at 105°C and stored.

Adsorption of Br on MCNPs

1 g of Anaheal as the source of Br was added to one liter of methanol to prepare the stock solution, and it was sonicated using an ultrasonic bath for 1 hour. In order to draw a calibration curve for spectrophotometry, the diluted solution and standard solutions at concentrations of 5 to 50 mg/L were prepared. The standard absorbance values for each sample were obtained using spectrophotometry. All adsorption experiments were carried out indoors and inside 25ml Erlenmeyer flasks containing 10 mL of Br solution and adsorbent (MCNPs) in various concentrations, temperatures and exposure times.

It should be noted that the range of variation of the measured parameters, including temperature, contact times, as well as initial Br concentrations and dosage of MCNPs, were determined using the optimal range chosen by other researchers (19).

After stabilization of the conditions, the samples were placed in a mixer at 240 rpm for proper mixing of the adsorbent and adsorbed material. Subsequently, the adsorbent was separated from the solution using a magnet and nanometer filter. The residual concentrations of the solution were measured via the spectrophotometer calibration chart at the maximum absorbance wavelength of the Br.

The optimum pH of the solutions was adjusted using 0.1M hydrochloric acid and 0.1M sodium hydroxide.

The amount of Br adsorbed on the adsorbent and its removal efficiency is obtained using equations 1 and 2.

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

(Equation 1)

$$\text{Removal Efficiency } (\%) = \frac{(C_0 - C_e)}{C_0} \times 100$$

(Equation 2)

In Equation 1, q_e is the Br (mg) absorbed on the absorbent (g), C_e and C_0 are the equilibrium and initial concentrations of Br respectively, and M and V are the mass of adsorbent (g) and the volume of solution (L), respectively.

Contact Time Effect

The contact time was studied over a 180 min period using optimum pH 5, 50 mg/L Br concentration and 1g/L adsorbent (MCNPs) at room temperature, and then the equilibrium time was determined.

In order to prepare the sample solutions, 1 mg of Br and 10 mg of MCNPs were poured into 10 mL of methanol and sonicated in an ultrasonic bath for 1 hour. Then, the pH of the samples was adjusted to 5. The absorbance of Br on MCNPs was measured after 30, 60, 90, 120 and 180 min. The percentage of Br drug absorbed at different contact times and the optimum contact time (the maximum adsorption of the drug by the MCNPs) were obtained by drawing the diagram. It should be noted that the kinetic equations of adsorption were obtained from the results of these experiments.

Different Concentrations of Br and MCNPs

The effects of different concentrations of MCNPs (1 and 5g/L) and Br solution (50, 150 and 300 mg/L) were studied at the optimum pH and contact time and 35°C temperature. In other words, three samples were prepared with 1g/L MCNPs and 50, 150 and 300 mg/L of Br concentrations and three samples with 5 g/L MCNPs and 50, 150 and 300 mg/L of Br concentrations respectively. Subsequently, the equilibrium isotherms of the adsorption were investigated as previously explained.

Temperature Effect

The adsorption process was carried out at two temperatures of 35 and 50°C to determine the optimum temperature as well as the thermodynamic study. At each temperature, three doses of Br (50, 150 and 300 mg/L) aqueous solution under optimum pH, contact time and adsorbent concentration of 1g/L were used. Shaker-incubator was used to adjust the temperature and mixing speed. The Br-free adsorbent with the same concentration was used as a control. Then, the effect of temperature parameter on the adsorption of Br on MCNPs was determined as explained previously.

Adsorption Equilibria

At the equilibrium moment, the rate of absorption equals the rate of desorption, resulting in no change in the concentration of the adsorbed material on the adsorbent surface, which is a dynamic balance. In other words, the number of molecules that adhere to the surface is equal to the number of molecules that are separated from the surface.

Adsorption Isotherm

The equilibrium amounts of the adsorbed material usually increase with increasing its concentration in the solution. The relationship between the amounts of the matter adsorbed per unit mass of adsorbent (q_e) in terms of equilibrium concentration absorbed in the solution (C_e) at a specific temperature is called adsorption isotherms.

In this study, we used the models proposed by Langmuir and Freundlich isotherm to study and analyze the experimental data and to explicate the equilibrium state in the adsorption between the solid and liquid phases. The Langmuir isotherm model represents a single layer and uniform adsorption of adsorbed material with the same energy on all the adsorbent surfaces. It also states that all adsorption sites have the same affinity for the adsorbent molecules, and there is no transition process of adsorbed materials on the adsorbent surface. On the other hand, in the Freundlich isotherm equation, the adsorption is considered as multi-layered, non-uniform and heterogeneous. The linear equations of the Langmuir and Freundlich equilibrium isotherms have been shown in equations 3 and 4, respectively.

$$\frac{C_e}{q_e} = \frac{1}{k_L Q_M} + \frac{C_e}{Q_M}$$

(Equation 3)

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

(Equation 4)

In equation 3, C_e stands for the equilibrium concentration of Br, q_e is the amount of Br absorbed at equilibrium, Q_M is the maximum adsorption capacity, k_L (L/mg) is Langmuir adsorption constant, and k_f and n are Freundlich constants dependent on the adsorption capacity and intensity, with n values less than 1 indicating weak adsorption and values between 1 to 2 and 2 to 10 indicating moderate and favorable adsorption, respectively. Parameters n and k_L are set using the slope and y intercept of the source ln q_e linear graph against ln Ce, respectively.

The desirability of the adsorption process in the Langmuir model can be determined using the RL dimensionless factor shown in Equation 5.

$$R_L = \frac{1}{1+k_L C_0}$$

(Equation 5)

R_L values greater than 1 indicate undesirable adsorption, equal to 1 specify linear adsorption, equal to 0 shows irreversible adsorption, and values between 0 and 1 are desirable adsorption (20).

Kinetic Models of Adsorption Systems

Two kinetic equations, i.e. pseudo-first-order and pseudo-second-order, were used in order to find the best-fitted model for the adsorption mechanism. The pseudo-first-order kinetic equation is based on the capacity of the adsorbent and is applicable when surface adsorption occurs from within a boundary layer via a diffusion mechanism (21), whereas the pseudo-second-order kinetic equation shows that chemical adsorption is the dominant and controlling mechanism in the process of surface adsorption, which is based on solid-phase adsorption (22).

$$\ln(q_e - q_t) = \ln q_e - k_1 t$$

Equation 6)

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

(Equation 7)

In Equation 6, q_e and q_t (mg/g) are the absorption capacities at equilibrium time and a given time t (min), respectively and k_1 (1/min) is the pseudo-first-order rate constant. The values of q_e and k_1 were obtained from the intercept and slope of the ln (q_e-q_t) linear graph versus t, respectively.

In Equation 7, k_2 (g/mg.min) is the pseudo-second-order rate constant. q_e and k_2 values were calculated from the intercept and slope of the t/q_t linear graph against t, respectively.

In Vitro Cytotoxicity

The potential cytotoxicity of Br enzyme alone and in combination with MCNPs (44 mg/1g) on MCF-7 cells was examined by methylthiazole Tetrazolium (MTT) assay. In this method, 100 µl of culture medium containing 10000 MCF-7 cells were added to 96- well plates and incubated for 24 hours. On the second day, 100 µl of pure Br solution and MCNPs containing Br with different concentrations (0.1, 1, 10 and 100 µg

/mL) were added to the plates and incubated for another 24-72 hours. Medium, untreated cultured cells and Br standard were served as the respective control. Then, 20 µl of MTT solution was added to all plates and incubated in the dark for 4 hours. The medium containing MTT was withdrawn, and formazan crystals were dissolved in 150 µl of acidic isopropanol and incubated for 15 minutes at room temperature. Finally, the optical density (OD) of the solution was measured at 570 nm. The viability was specified as the ratio between viable treated cells against untreated control cells.

Statistical Analysis

Statistical analysis was conducted by the SPSS software version 26. All the experiments were carried out in triplicate. We used one-way ANOVA followed by the Tukey-Kramer post hoc test to analyze the data. The significant level was considered $P < 0.05$.

Results and Discussion

Effect of pH on Br Absorption Efficacy on MCNPs

It was found that in pH 5 which is close to the pH of the Br isoelectric point, the highest adsorption (88%) was occurred. By increasing the pH from 3 to 5, the absorption efficiency increases and as the pH increases further, the absorption efficiency decreases. Isoelectric points for Fe_3O_4 nanoparticles and Br are 6.81 and 4.6 respectively. At a pH of 5 that is closer to the pH of the isoelectric point, there was the highest absorption efficiency. Therefore, pH 5 was considered as optimum pH. In this case, Br molecules are absorbed in a passive state on magnetic nanoparticles. Hence, the predominant mechanism of absorption in the aquatic environment has been electrostatic reactions. An increase in absorption efficiency at pH between 3 and 5 may be due to changes in the acidic groups of Br enzyme molecules that increase the electrostatic attraction between the anionic molecules of Br and the positively charged surface of carbon nanoparticles and therefore the absorption efficiency increases (23). Decreased removal efficiency in the pH 5 to 9 is more likely due to increased hydroxide formation and their high competition with anions in the Br enzyme for adsorption on active sites of synthesized carbon magnetic nanoparticles (23).

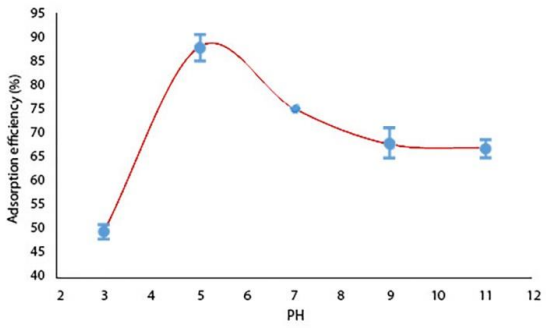

Figure 1. Effect of solvents on the extraction efficiency.

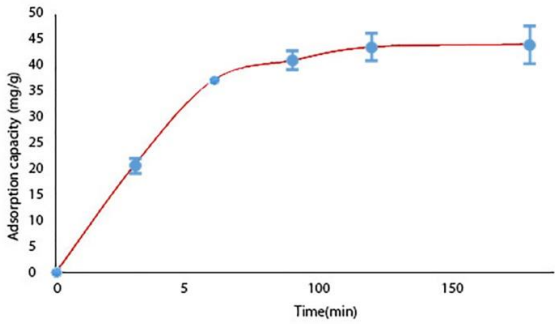

Figure 2. Effect of contact time on absorption of Br from aqueous solution on MCNPs (Br concentration 50 mg/L, MCNPs concentration 1g/L, pH 5 and temperature 35°C).

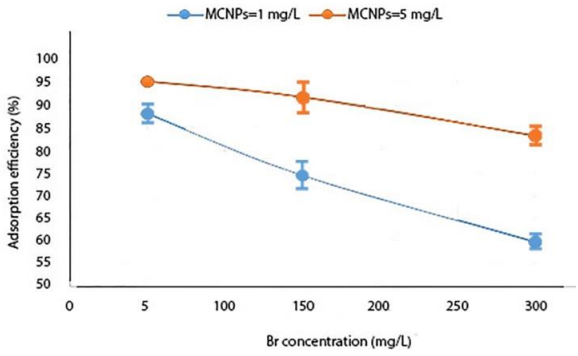

Figure 3. Effect of Br and MCNPs concentrations on adsorption efficiency (pH 5 and 35°C temperature).

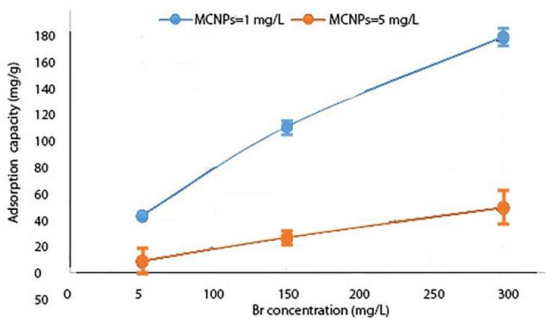

Figure 4. Effect of Br and MCNPs concentrations on adsorption capacity (pH 5 and 35°C temperature).

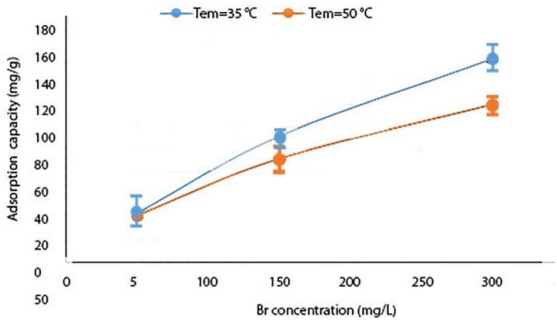

Figure 5. Effect of temperature on adsorption capacity of Br from aqueous solutions (MCNPs 1g/L and pH 5).

Effect of Contact Time on Br Adsorption on MCNPs

Figure 2 shows that 1 g of nanoparticles can absorb 43.5 g of Br after 120 minutes and the final concentration of aqueous solution reaches 6.5 mg/L. With increasing contact time from 120 to 180 min, the adsorption capacity increased only about 0.5 mg/L and reached to 44 mg/g. Therefore, 120 minutes was considered as the equilibrium time for further studies. The amount of Br adsorbed on MCNPs in the first 30 minutes is much higher and faster than subsequent times (Figure 2). It may be due to the high levels of synthesized nanoparticles which have been able to provide a large number of active sites for bromelain molecules. Regarding the contact time effect, it was found that rapid uptake of Br at the initial contact time may be due to the high level of MCNPs that have been able to provide many active sites for drug molecules. Omidvar also observed that the equilibrium time for

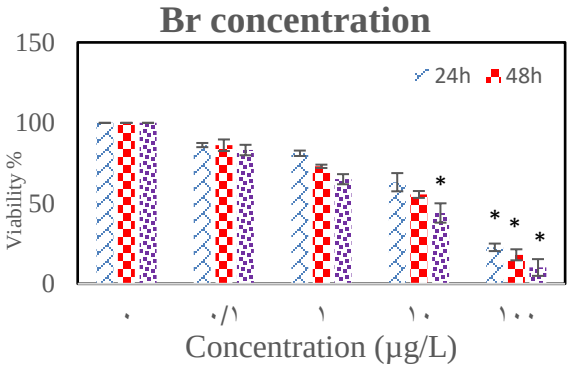

Figure 6. Percent of cells viability at different concentrations of Br after 24, 48 and 72 hours by MTT assay * (P<0.001).

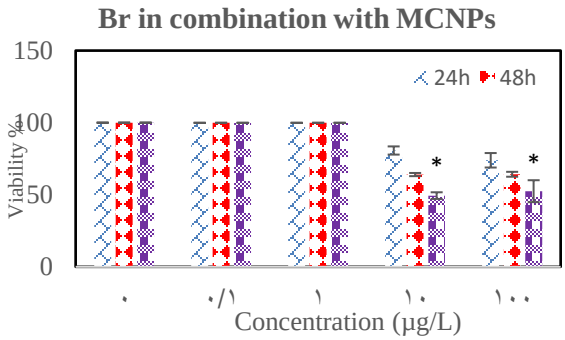

Figure 7. Percent of cells viability at different concentrations of Br in combination with MCNPs after 24, 48 and 72 hours by MTT assay * (P <0.001).

removal of 98% of amoxicillin from aqueous solution at 50 mg/L concentration was approximately 100 minutes (24).

Effect of Initial Br Concentration and dose of Adsorbent

In Figures 3 and 4, the effects of different concentrations of MCNPs and Br solution have been indicated on the adsorption efficiency and adsorption capacity, respectively.

As it has shown, by increasing the dosage of MCNPs from 1 to 5 g/L, the adsorption efficiency increased, and the adsorption capacity decreased significantly (P<0.001).

The results indicated that by increasing the initial concentration of Br drug from 50 to 300 mg/L at 1 g/L dosage of MCNPs, the adsorption capacity increased from 44 to 179.4 mg/g, while the adsorption efficiency decreased from 88 to 59.97 percent. Moreover, at 5 g/L dosage of MCNPs, the adsorption capacity increased from 9.52 to 49.92 mg/g, while the adsorption

Table 1: Br adsorption equilibrium isotherm parameters on MCNPs (MCNPs 1g/L and pH 5).

Temperature (°C)	Adsorption Isotherm Models					
	Langmuir Model			Freundlich Model		
	Q_M	k_L	R^2	k_f	n	R^2
35	222.2	0.0336	0.987	19.53	2.11	0.997
50	126.58	0.0343	0.9611	17.33	2.43	1

Q_M: Maximum adsorption capacity (mg/g), k_L: Langmuir isotherm constant (L/mg), n: Intensity of the adsorption, k_F: Freundlich isotherm constant (mg/g) $(L/mg)^{1/n}$), R^2: Correlation coefficient

efficiency decreased from 95.2 to 83.2 percent. It is clear that a rise in the amount of adsorbent is associated with higher efficiency in the removal process. This may be due to the increase of the adsorbent area, which leads the Br molecules to reach the active sites of adsorbent without competition. On the other hand, a rise in the amount of adsorbent had a negative effect on the adsorption capacity, which was probably due to the lack of active adsorbent surfaces during the Br adsorption process.

Temperature Effect

The effect of temperature (35 and 50°C) on Br adsorption of different concentrations of Br solution (50, 150 and 300 mg/L) was investigated at optimum pH 5, contact time 120 min and adsorbent concentration 1g/L. The adsorption capacity decreased with increasing temperature from 35 to 50°C which is detectable only at high concentrations of the aqueous solution containing Br (Figure 5). For example, at a dose of 50 mg//L, by increasing temperature there was no significant change in the amount of drug absorbed on the nanoparticles.

At the initial concentration of aqueous solution containing 150 mg/L of Br with increasing temperature, the adsorption capacity decreased from 111.9 to 91.8 mg/g, and at an initial concentration of 300 mg/L by increasing the temperature from 35 to 50°C, it was observed that the reduction of adsorption capacity decreased from 179.9 to 139.8 mg/g.

Adsorption Isotherms

The C_e/q_e parameter is plotted in terms of C_e at 35°C and 50°C to determine the Langmuir model parameters. Moreover, to determine Freundlich adsorption model, parameters lnq_e is plotted in terms of lnC_e at the same temperatures (Table 1). The data in table 1 shows that the adsorption behavior on MCNPs is more consistent with the Freundlich model than the Langmuir model. The correlation coefficient for the Freundlich model at 35°C is greater than 0.99 and at 50°C is equal to 1 which represents the Br absorption behavior which is based on the Freundlich model. Furthermore, the values obtained for the parameter n in the Freundlich model for both temperatures were above two, which indicates the desired adsorption of Br onto the MCNPs.

The maximum adsorption capacity based on the Langmuir model was 222.2 mg/L, which was obtained at 35°C. However, the values of k_f at 35 and 50°C were 19.53 and 17.33 mg/g, respectively, both of which indicate favorable adsorption of Br onto MCNPs.

Adsorption Kinetics

The drug adsorption data at different contact times on MCNPs were used to investigate the kinetic behavior of Br adsorption at 35°C and pH 5. The parameters for the

Table 2: Kinetic parameters of the Br adsorption process on MCNPs (temperature 35°C, MCNPs concentration 1g/L, pH 5).

q_e (experimental)	Kinetic Models					
	pseudo-first-order			pseudo-second-order		
	q_e Cal (mg/g)	K_1	R^2	q_e	K_2	R^2
44	49.89	0.0337	0.9893	54.644	0.0005	0.9748

K_1: First-order rate constant (1/min), K2: Second-order rate constant (g/mg.min), q_e: Adsorption capacity at equilibrium (mg/g), R^2: Correlation coefficient

pseudo-first and second orders were calculated. For this purpose, the parameters of ln (q_e-q_t) and t/q_t were plotted in terms of t, and linear regression was performed (Table 2).

The correlation coefficient values of the kinetic models indicate that the Br adsorption process on MCNPs follows the pseudo-first-order kinetic model. The experimental data obtained from the adsorption experiments are in better agreement with the pseudo-first-order kinetic equation. At equilibrium in a solution containing 50 mg/mL of Br and 1 g/L MCNPs at 35°C, the amount of adsorbed Br was 44 mg. This number is very close to the number predicted by the pseudo-first-order kinetic model (49.89 mg). Hence, it can be concluded that the dominant mechanism in the Br adsorption process on MCNPs is physical adsorption.

Cytotoxicity

The cytotoxicity levels of Br and Br in combination with MNCTs (44 mg/1g) against MCF-7 cells were investigated using MTT assay. Br has a potent cytotoxicity at high concentrations (100 µg/ml) as shown in Figure 6. It was observed that even after 24 h, the cells were severely vacuolated and a significant decrease ($P<0.001$) occurred in the number of living cells relative to the control sample. As the results shows in all cases, cytotoxicity of Br alone is higher than in combination. Br in combination with MNCTs indicated a significant decrease ($P<0.001$) in cell viabilities only after 72 h (Figure 7), which shows a gradual release of the drug over time. On the other hand, the concentration of Br in combination with MCNPs is 440 times less than Br but it still has the same cytotoxicity effect.

All in all, our results showed that the effectiveness of combination of MCNPs with Br. Br is a mixture of thiol proteases and non-proteasome constituents. It has been said that the anti-cancer property of Br is mainly related to its protease components (6). In the present study, it was attempted to examine the anti-cancer properties of Br enzyme alone and also in combination with MCNPs.

Chen *et al.* functionalized SWCNTs using an ultrasonic DSPE-PEG2000-Amoni amine system. The results of this test showed that the encapsulating Br enzyme in SWCNTs has a significant effect on decreasing the growth and progression of breast cancer disease (25). It was observed that a high Br concentration can cause nanotube aggregation. Therefore, adsorption of Br on the Magnetic carbone nanotube is strongly related to the protein concentration (26).

Bhatnagar *et al*. synthesized a nanoparticle containing the Br enzyme and showed that it had a higher level of anticancer activity than the Br due to the increased release of the Br enzyme (27). In 2016, they developed a hyaluronic acid grafted PLGA copolymer capable of targeting cancer cells as well as higher cellular uptake and cytotoxicity (28). Wei *et al*. decorated nanoparticles based on lactobionic acid- conjugated chitosan with Br to prepare tumor targeted nanoparticles (29). In 2017, Nasiri *et al*. reported a successful conjugation of Br to the SPIONS (superparamagnetic iron oxide nanoparticles) and demonstrated the targeted delivery of Br using SPIONS against cancer cells in vivo and in vitro (17).

Conclusion

The data of this study indicated that the optimized experimental parameters for Br adsorption were pH (5), adsorbent concentration (1g/L), temperature (35°C), contact time (120 min) and initial Br concentration (50mg/L). The adsorption followed the Freundlich model better than Langmuir and Freundlich's values indicating the favorable adsorption of Br onto MCNPs. Moreover, the adsorption followed first-order kinetics. Therefore, the predominant mechanism in the adsorption process on MCNPs is physical adsorption. The reduction in adsorption capacity with increasing temperature suggested the exothermic nature of adsorption.

The results indicated that MCNPs could be a potential effective adsorbent for the removal of Br from the solution. Br alone and in combination with MCNPs at 100 µg/ml concentration exhibited severe cytotoxicity effect on MCF-7 cells. However, the dose of Br on synthesized MCNPS was approximately 440 times less than Br alone, but it indicated a significant cytotoxicity on these cells ($P<0.001$). Another advantage of using MCNPs may be due to the slower delivery of encapsulated Br which is favorable for cancer treatment. Nevertheless, in this study, releasing amount of the Br was not directly measured and inferred from cell viabilities changes over time. Further trials,

particularly in vivo experiments, need to be performed to obtain more definite results.

Acknowledgment

The present study was conducted in the laboratory of the Central Tehran Branch of Islamic Azad University as a M.Sc. project. The project was designed and executed by Ramezani. M and Mohammadgholi. A. Karimian R conducted the experiments and analyzed the data. All the authors have read and approved the manuscript.

Conflict of Interest

The authors declare that they have no conflict of interest.

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