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Albumin nanoparticles as a powerful diazinon adsorbent

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ABSTRACT

The aim of this study was to evaluate the diazinon adsorption by bovine serum albumin nanoparticles (BSA NPs) at different concentrations of BSA NPs (0.1, 0.01, and 0.001 g/ml), concentrations of diazinon (1%, 0.5%, 0.3%, and 0.1% v/v), ionic strengths (0, 0.1%, and 1% v/v), temperatures (4, 25, and 37 ºC), and pHs (2, 7, and 13). This study showed that the increase of concentration of BSA NPs, diazinon, and temperature led to increase of adsorption rate of diazinon. But, with the increase of pH and ionic strength, the decrease of adsorption rate was observed.

Key words: Diazinon, Nanoparticles, Adsorption, Bovine serum albumin

INTRODUCTION

Diazinon is an organophosphate insecticide, and inhibits acetylcholinesterase (AChE). This pesticide has adverse effects on the different human systems including nervous system, respiratory system, cardiovascular system, etc [1]. AChE decomposes acetylcholine (ACh) neurotransmitter into cholin and acetate. The inhibition of AChE leads to accumulation of ACh in the synaptic cleft, and leads various adverse effects [2]. Diazinon enters to human body by different pathways, such as skin, mucosa, respiratory, and gastrointestinal system. Diazinon changes to diaxozon while enters to body by various enzymes, and this compound is more toxic than diazinon. Although mammalian liver cells can degrade diaxozon by hydrolase enzymes located in liver microsomes, but this degradation do not occur in insect cells [3, 4]. In spite of hydrolysis in microsomes, diazinon has adverse effects on the genes and cell cycle machinery, and induces cell death and apoptosis [5].

Since diazinon has high stability at various environmental conditions, it can survive in the soil and on the plants at a long time [3]. To adsorb diazinon from soil and water, different materials have been proposed, such as TiO_2/SiO_2 nanocatalyst [6], alumina [7], MCM-41, and MCM-48 [8]. Unfortunately, all of them are toxic or release heavy metal ions. We think that the use of natural and non-toxic adsorbents is the key of this problem. Albumin is an important protein in blood, and regulates osmotic pressure. This protein has three homologous domains, and forms a heart-shaped structure. Albumin is an adsorbent biomolecule which can bind to wide range of molecules including cations, anions, hormones, bilirubin, fatty acids, and some pharmaceutics [9, 10]. We guess that diazinon may attach to albumin via molecular interactions. Here, we presented bovine serum albumins NPs (BSA NPs) to adsorb diazinon. BSA NPs have a natural base, and no cytotoxicity of them has been reported. Thus, the aim of this study was to evaluate diazinon adsorption property of BSA NPs at different conditions.

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MATERIALS AND METHODS

Materials

BSA was purchased from Sigma-Aldrich Chemical Co., (St Louis, MO). Diazinon (60% v/v) was obtained from Insecticide Company, India. Whatman filters No. 42 were provided from Whatman Ltd, USA. Other chemicals such as ethyl alcohol (70% v/v), nitric acid (1 M), and formaldehyde (10% v/v) were purchased from Zyst Fannavar Shargh Company, Yazd, Iran.

Synthesis and characterization of BSA NPs

To synthesize BSA NPs, the method of Juna et al was used with some modifications [11]. In the first step, 25 mL of ethyl alcohol (70% v/v) and 100 µL of formaldeide (10% v/v) were gently added to 50 ml of BSA at concentration of 200 mg/mL, shacked for 20 minutes at room temperature, and then incubated 30 minutes at 37 ºC. In the next step, synthesized BSA NPs were centrifuged at 10000 rpm, and washed three times with distilled water (DW). The shape and structure of BSA NPs were characterized by scanning electron microscopy (SEM) (Hitachi S-2400, Japan). Briefly, BSA NPs (0.001 g/ml) were dried on aluminum surface, sputtered with gold, and examined with SEM at 15 KV. Also, the size distribution of NPs was analyzed by a dynamic light scattering (DLS) (Malvern Instruments, Italy).

The effect of different parameters on the adsorption rate

The serial concentrations of diazinon (1%, 0.5%, 0.3%, 0.1% v/v) were separately added to BSA NPs, and incubated for 5 minutes. This experiment was separately done at different concentrations of BSA NPs (0.1, 0.01, and 0.001 g/ml), temperatures (4, 25, and 37 ºC), pHs (2, 7, and 13), and ionic strengths (0, 0.1%, and 1% v/v). To evaluate the effects of each variable, other parameters were constant. The detail of experiments was held in caption of Fig. 2, Fig. 3, Fig. 4, and Fig. 5.

After incubation, all tubes were centrifuged at 10000 rpm, supernatant of each tube was isolated, and the optical density (OD) of each sample was read by UV-visible spectroscopy (ELICO, India) at 340 nm [12, 13]. The adsorption rate of each sample was calculated according to formula 1.

Formula 1. The adsorption rate = (A-B)×100/A

A= The OD of diazinon before adsorption

B= The OD of diazinon after adsorption

Statistical analysis

All tests were carried out triplicate, and results were shown as mean ± standard deviation (SD). SPSS software (V.16.0 for Windows; SPSS Inc.) was used for data analyzing, and One- way ANOVA was applied to measure significant differences. P-values <0.05 were considered as significant difference.

RESULTS

Characterization of BSA NPs

The SEM image and the size distribution of BSA NPs are shown in Fig. 1a and Fig. 1b, respectively. As shown, the shape of NPs was approximately spherical, and the agglomeration was not observed. Also, the size distribution of BSA NPs was near 30-50 nm.

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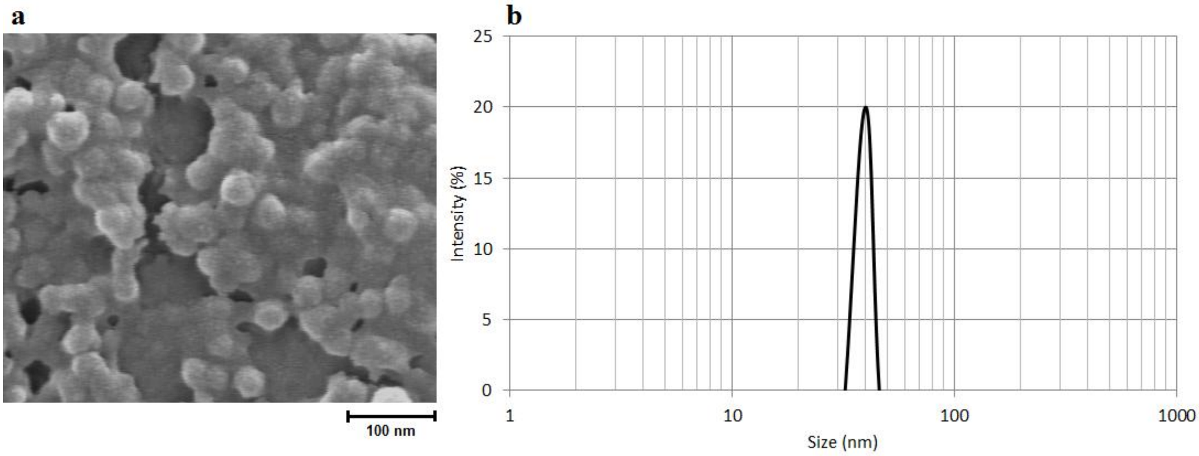

Fig. 1. The characteristics of BSA NPs. The SEM image (a) and the size distribution (b) of BSA NPs.

Adsorption rate at different conditions

The effect of concentration of BSA NPs, pH, temperature, and ionic strength is shown in Fig. 2, Fig. 3, Fig. 4, and Fig. 5, respectively. As shown in Fig. 2 and Fig. 4, with increase of concentration of BSA NPs and temperature, the increase of adsorption rate was seen. As seen in Fig. 3 and Fig. 5, with increase of pH value and ionic strength, the decrease of adsorption rate was observed. As another finding, the adsorption rate was dos-dependent, i.e., the less concentration, the less adsorption.

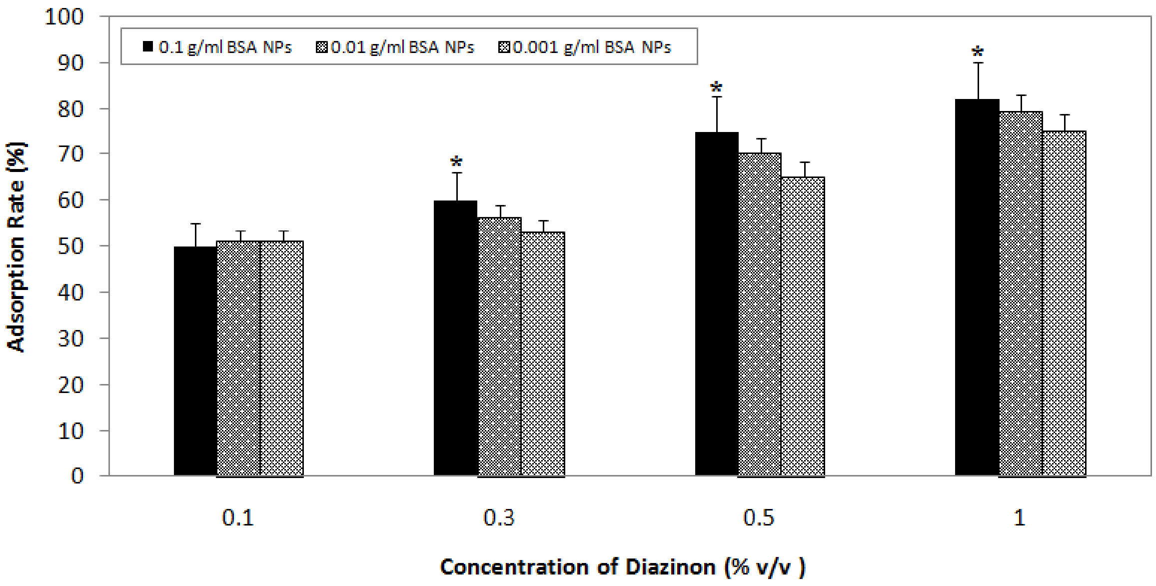

Fig. 2. The effect of concentrations of BSA NPs on the adsorption rate. n=3, *P<0.05 compared with the adsorption rate of BSA NPs at concentration of 0.001 g/ml. In this experiment, pH=7, temperature=25 ºC, ionic strength=0.1%.

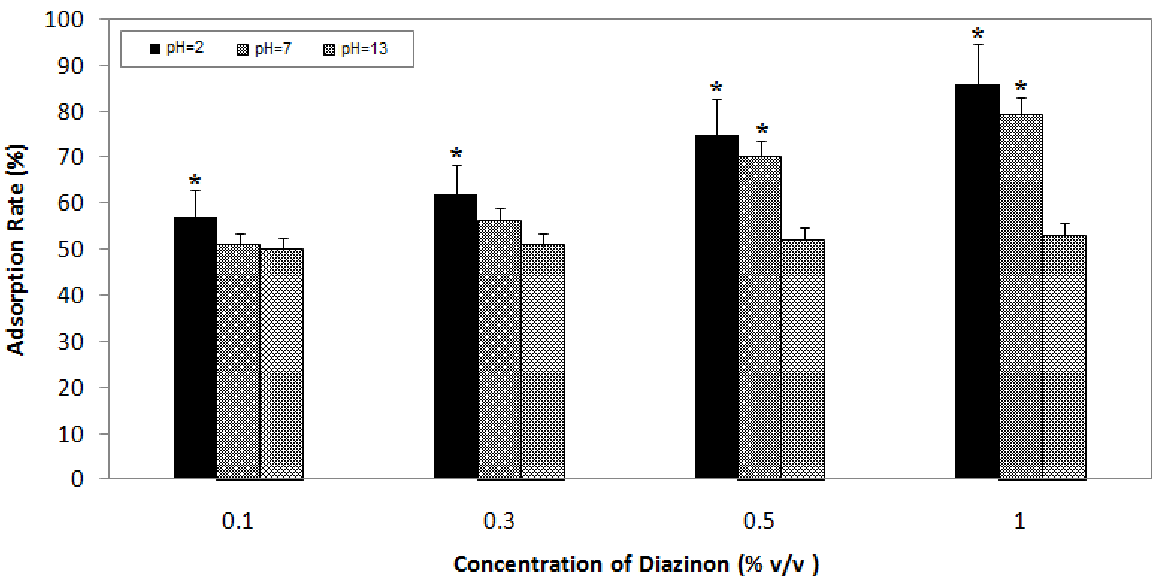

Fig. 3. The effect of pH on the adsorption rate. n=3, *P<0.05 compared with the adsorption rate at pH=13. In this experiment, concentrations of BSA NPs=0.01 g/ml, temperature=25 ºC, ionic strength=0.1%.

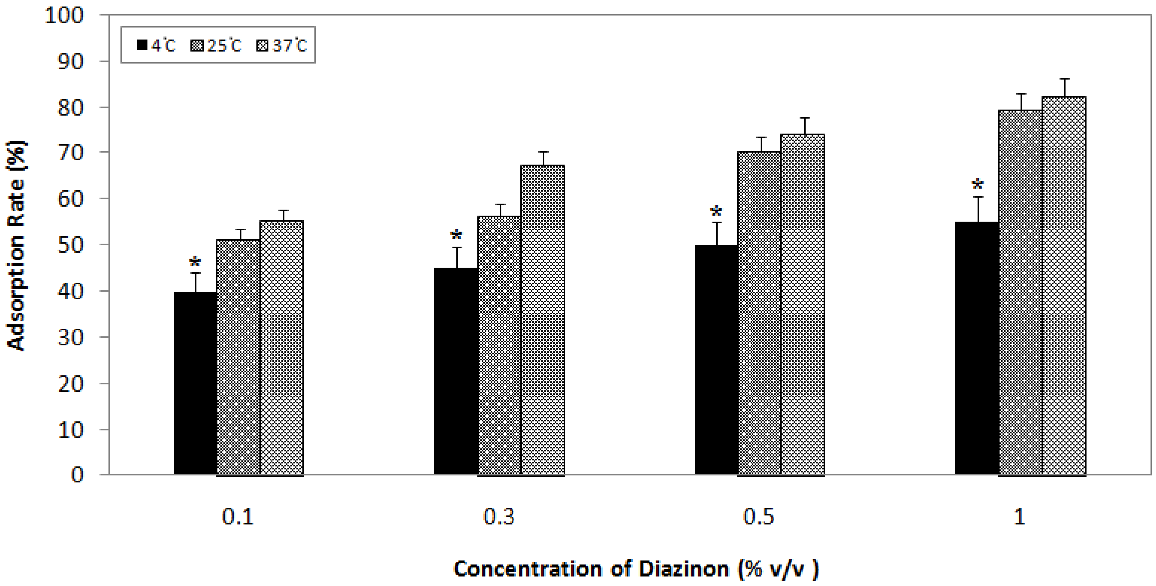

Fig. 4. The effect of temperature on the adsorption rate. n=3, *P<0.05 compared with the adsorption rate at 25 ºC and 37 ºC. In this experiment, concentrations of BSA NPs=0.01 g/ml, pH=7, ionic strength=0.1%.

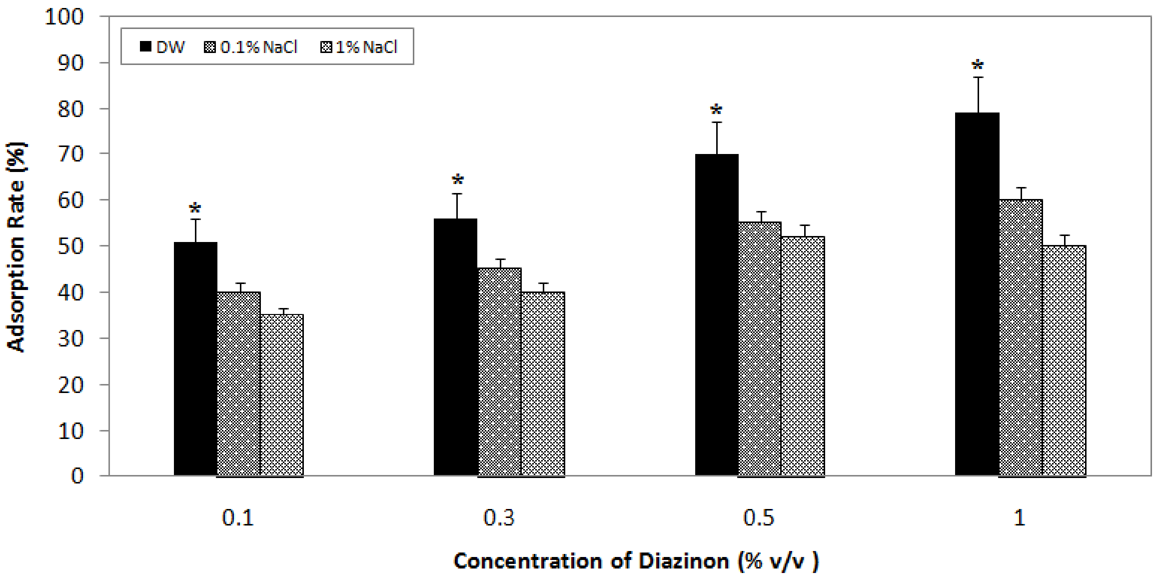

Fig. 5. The effect of concentration of salt ions on the adsorption rate. n=3, *P<0.05 compared with the adsorption rate when the concentration of NaCl is 0.1% and 1% NaCl. In this experiment, concentrations of BSA NPs=0.01 g/ml, pH=7, temperature=25 ºC.

DISCUSSION

Diazinon is an organophosphate insecticide which acts by acetyl cholinesterase inhibition. This toxin has adverse effects on human nervous system, respiratory system, and cardiovascular system. Since diazinon is one of the occupational risks among farmers, especially in developing countries [2-5], the introducing and developing of materials which can adsorb it is very important. The aim of this study was to evaluate diazinon adsorption by BSA NPs.

This study showed that BSA NPs had higher adsorption at acidic condition (pH=2) than neutral and basic conditions. In general, the increase of pH had adverse effect on adsorption. These finding reveals pH-dependent pattern of adsorption rate. The authors hypothesize that the reason of increase of adsorption with decrease of pH is due to induction of positive charge of BSA NPs by H+ atoms.

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We found that the zeta potential of BSA NPs at pH 2 was +36.5, which confirmed this hypothesis. Fig. 6 shows the schematic images of diazinon, emulsifier, emulsified diazinon, BSA molecule, and BSA NPs. We know that diazinon is a hydrophobic molecule, and is emulsified with emulsifier for agricultural application. Commonly, the emulsifiers of diazinon have negative charge head and hydrophobic tail, and interact with diazinon and water molecules, to form micelle or liposome. We explain BSA NPs with positive charge interact with negative charge of emulsifier, and actually micelles and liposome are adsorbed. It seems that electrostatic force is the main mechanism of adsorption of BSA NPs and diazinon. It must be mentioned that BSA NPs can adsorb diazinon at neutral and basic condition, too. This reveals that other non-covalent forces may be included to describe adsorption. This study showed that the adsorption rate was increased with increase of concentration of BSA NPs and diazinon, which is a very important finding. We think that the reason of high adsorption rate of high concentration of BSA NPs is due to high molecular interaction between BSA NPs and emulsified diazinon. The quantity of active parts of BSA NPs which interact with emulsified diazinon is increased by increase of concentration of BSA NPs.

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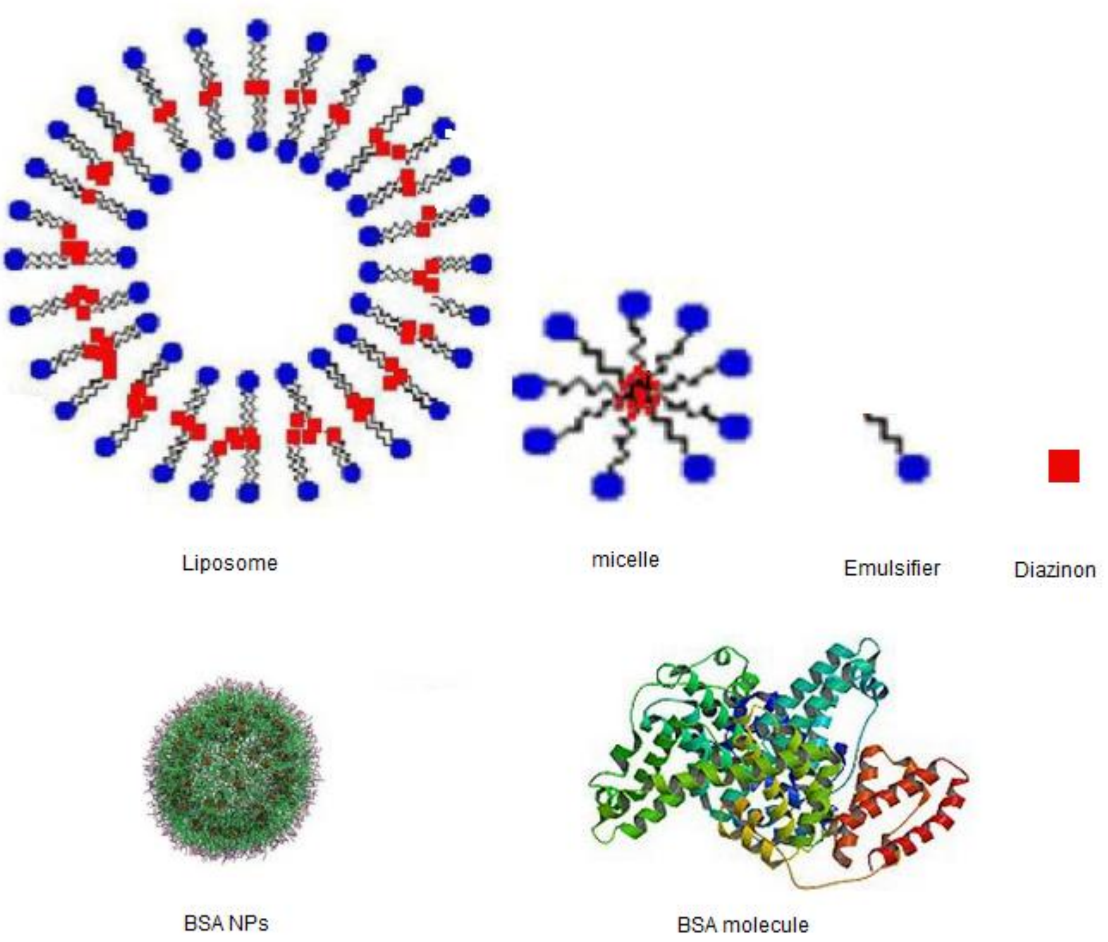

Fig. 6. The schematic images of diazinon, emulsifier, emulsified diazinon, BSA molecule, and BSA NPs.

The reason of high adsorption rate with high concentration of diazinon is unclear, but the authors hypothesize when concentration of diazinon is increased, the molecular interactions of diazinon and BSA NPs are raised, too. Another parameter which affected the results of present study was temperature. In this study, the adsorption rate at room temperature, 4 ºC, and 37 ºC was studied, and the highest adsorption was seen at 37 ºC. we explain that the molecular interaction of diazinon and BSA NPs are increased at higher temperatures. The range of temperature between 0-40 ºC (which is common environmental temperature) must be evaluated in future study. As another finding, we found that increase of concentration of NaCl salt decreased the adsorption rate. We hypothesize that decrease of electrostatic forces by ions leads to decrease of adsorption rate. In the present study, we used BSA NPs with size about 30-50 nm, but it must be noted the size of NPs may affect the adsorption rate, and this parameter must be also studied in future researches.

Although there is no report on diazinon adsorption by BSA NPs, some studies have been reported on the adsorption of diazinon by other materials. For example, Aberoomand Azar et al. showed photo catalytic degradation of diazinon by TiO_2/SiO_2 nano-catalyst [6]. They indicated that degradation of diazinon was seen within 105 minutes after NP treatments. Also, Armaghan et al. demonstrated the adsorption of diazinon and fenitrothion by alumina NPs [7]. In another work, Armaghan et al. showed that MCM-41 and MCM-48 are an good adsorbent of diazinon and fenitothion [8]. Moussavi et al. revealed diazinon adsorption on NH4Cl-induced activated carbon [14]. Compared with these diazinon adsorbents, BSA NPs have natural base with no cytotoxicity. The limitation of this study was the diazinon evaluation assay. As described in materials and methods section, we used UV spectroscopy at 340 nm. Although diazinon have adoption at UV wavelength, and can be used for detection of diazinon at high concentration, but high performance liquid chromatography is the gold standard to quantify diazinon at low concentration. Taken together, BSA NPs could adsorb diazinon, and the adsorption was related to concentration of BSA NPs and diazinon, pH, temperature, and salt ions. We suggest that BSA NPs should be used in the actual field as a coating agent on the air filter. This product may be useful for farmer or the worker in diazinon manufactures.

5. CONCLUSIONS

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This study showed that BSA NPs could adsorb diazinon. We found that the increase of concentration of BSA NPs, diazinon, and temperature led to increase of the adsorption rate, but the decrease of adsorption rate was seen after increase of pH and salt ions.

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CONFLICT OF INTEREST

There was no conflict of interest in this study.

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