Asian Journal of Chemistry Vol. 22, No. 3 (2010), 2081-2089

Simultaneous Removal of Indomethacine, Papaverine and Allopurinol from Aqueous Solution by Using Submerged Aquatic Plant *Nasturtium officinale*

VEYSI OKUMUS*, ELIF ORAL†, DAVUT BASARAN‡ and AHMET ONAY‡
Department of Biology, Faculty of Art and Science, University of Siirt, TR-56100 Siirt, Turkey
Fax:(90)(484)2236631; Tel: (90)(484)2231224 E-mail: veysiok@hotmail.com

Simultaneous removal of indomethacine, papaverine and allopurinol from aqueous solution by using submerged aquatic plant *Nasturtium officinale* biomass in high performance thin layer chromatography (HPTLC) was studied. Optimum biosorption conditions were determined as a function of contact time, pH, removal capacity of the amount of biomass and initial dry concentration. Experiments were performed in batch conditions. Concentrations of the drugs in the remaining solutions were simultaneously analyzed by HPTLC. Langmuir and Freundlich models were applied to describe the biosorption isotherm of the drugs by aquatic plant *Nasturtium officinale* biomass. According to the results, optimum parameters were found as 2.0 g biomass, pH:5.0 and 60 min contact time. Obtained from plots of Langmuir and Freundlich adsorption models, the highest drug uptakes were calculated from Langmuir isotherm and found to be 43.10, 39.68 and 38.61 mg/g for indomethacine, papaverine and allopurinol, respectively.

Key Words: Indomethacine, Papaverine, Allopurinol, Biosorption, HPTLC, *Nasturtium officinale*, Submerged aquatic plant.

INTRODUCTION

Drugs have been widely used to treat the different health problems throughout the world. Generally, they were unconsciously consumed in undeveloped countries. So, it can be said that this situation has been a potential problem for human health and environment. In the recent years, the authorities are become aware of this problem. However, there is not enough data available on the occurrence, fate and effects of pharmaceutical chemicals in the environment and the associated risks for humans, animals and the environment[1]. The chemical structure and biodegradation ability of the drugs are effective in this process. If they contain one or more asymmetric carbon atom, risk will be increase because the enantiomers show different chemical reactivity in environment and metabolism.

†Department of Chemistry, Faculty of Art and Science, University of Dicle, TR-21280 Diyarbakir, Turkey.
‡Department of Biology, Faculty of Art and Science, University of Dicle, TR-21280 Diyarbakir, Turkey.

Nowadays, biosorption has been alternatively used to remove the pollutant from environment[2-4]. Biosorption can be defined as the removal of the organic and inorganic substances *via* various physico-chemical mechanisms including ion exchange, sorption, complexation, chelation by biological materials[5]. In the developing countries, the treatment systems are not enough to reduce the pollution. Economic biosorption is important for those countries since it requires easy steps according to the other treatment process. The method generally used to remove the inorganic pollutants[6-8]. However, its use for removing organic contaminants from polluted samples has received considerably less attention. For this reason, there is a requirement to develop biosorption methods to organic pollutant such as pesticides, drugs and dyes[1,2, 9-11].

Aquatic plants such as *Myriophyllum spicatum* and *Ceratophyllum demersum* were successfully used to remove the heavy metals from aqueous solutions[12-14]. Morever, *Emericella nidulans*, *Phanerochaete chrysosporium* and *Penicillium miczynskii* were also used to remove the 2,4-D[11,15]. In the recent years, the biomaterials such as bacteria, fungi, algae and submerged aquatic plants have been used to removal of pollutants from water. From this point of view submerged aquatic plant *Nasturtium officinale* was used to simultaneously removal the drugs include indomethacine, papaverine and allopurinol from aqueous solution. Indomethacine (IND) is a non-steroidal anti-inflammatory drug commonly used to reduce fever, pain, stiffness, and swelling. It works by inhibiting the COX1 and COX2 enzymes which responsible for the production of prostaglandins, molecules known to cause these symptoms[16]. Allopurinol (ALP) is used primarily to treat hyperuricemia (excess uric acid in blood plasma) and its complications, including chronic gout[17]. Papaverine (PPV) is an opium alkaloid used primarily in the treatment of visceral spasm, vasospasm (especially those involving the heart and the brain) and occasionally in the treatment of erectile disfunction[18,19]. The biosorption ability of the submerged aquatic plant *N. officinale* are well known[20-22].

In this study, *N. officinale* was used as a biomass to remove the drugs including indomethacine, papaverine and allopurinol from aqueous solution. Critical parameters such as time, pH, amount of biomass and concentration of drug affect the biosorption process were evaluated. To explain the biosorption mechanism, experimental results were applied to common isotherms and kinetic model.

EXPERIMENTAL

The structures of the studied drugs are shown in Fig. 1. IND, PPV (as hydrochloride salt) and ALP were obtained as individual standards from Sigma. Chromatographic grade organic solvents were supplied from Merck and Riedel. The double deionized water was used for preparation of all solutions. All solutions were passed through from 0.45 μm membrane filter (Sartorius Goettingen-Germany) before injection to the TLC plate. HPTLC (Camag) was used for the analysis of the drugs.

N. officinale was collected from a small-local lake in the vicinity of the tigris river. The plants were washed with diluted HCl solution (0.1 M) and distilled water before used.

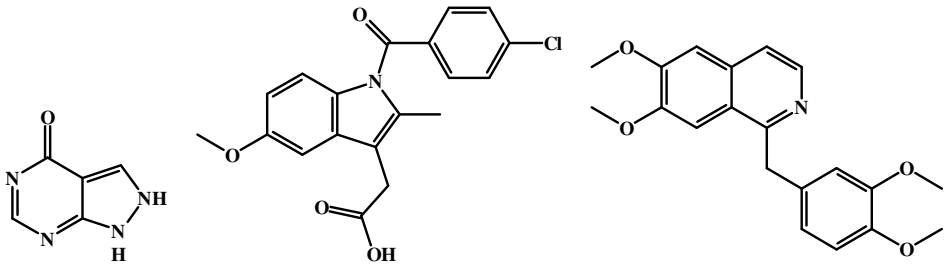

Fig. 1. Chemical structures of studied drugs

Chromatographic separation: The different concentrations of the IND, PPV and ALP were spotted on TLC aluminum plate pre-coated with silica gel 60 F_{254} obtained from Merck (layer thickness 0.2 mm) using Camag Linomat V and 100 µL syringe. The plates were pre-washed with methanol and activated at 50 ºC for 5 min prior to chromatography. The samples were streaked in the form of narrow bands of length 6 mm, 15 mm from the bottom and 15 mm from the margin using a nitrogen aspirator. Distances between the tracks were automatically selected. Camag twin trough chamber (10 cm × 10 cm) was saturated for 20 min. After the chamber saturation, the plates were developed to a distance of 9.5 cm with the development time being 20 min. A mixture of *n*-hexane-toluene-2-propanol (0.5:2.0:1.0, v/v/v) was used as the mobile phase. Then, the plates were dried in an oven at the 50 ºC. Spectrodensitometric analyses were carried out using scanner-III in the absorbance mode. The analysis was performed at the associate maximum wavelength of the drug mixture as 260 nm. The colorless bands for each of the drugs were scanned on TLC plate to obtain the UV-vis spectra. It was found that the wavelength selected was suitable for the analysis (Fig. 2). The parameters used were: slit dimension 6.00 mm × 0.45 mm, scanning speed 1 mm s^{-1}, data resolution 100 µm s^{-1}. The source of radiation was D2 and W lamps emitting continuous UV spectra. The chromatograms were integrated using winCATS evaluation software.

Biosorption experiments: The biosorption experiments were performed in 100 mL Erlenmeyer flask at 25 ºC using an orbital shaker in a constant temperature. The initial pH values of the solutions were about 6 during the batch experiments and pH adjustment was not done. Approximately 2 g wet weight of *N. officinale* was added to each flask and placed on the orbital shaker. The initial drug concentrations for the contact time experiments were 50 mg L^{-1} for each of the drugs and the incubation times ranged from 5 to 120 min. The volume of the solution was 50 mL. The data used to derive the Langmuir constants were obtained by using *N. officinale* biomass and different concentrations of drug (5, 10, 25, 50 and 100 mg L^{-1}). The contents of the flasks were filtered to separate the biomass from the solution. The filtrates were then analyzed with an HPTLC for simultaneous determination of the drugs concentrations in the samples by injecting 10 µL of sample solution to the

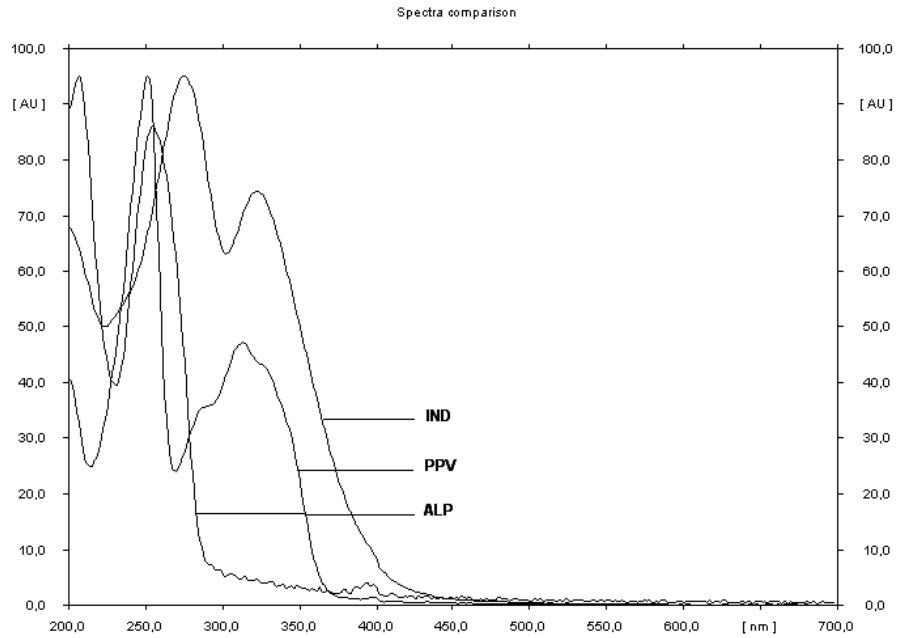

Fig. 2. UV spectra of standard IND, PPV and ALP, 100 ng each of drugs on TLC plate

TLC plate. The control experiments were performed for each of drugs to measure any adsorption onto the glassware. The results of drug analysis were used to calculate specific adsorption (mg drug adsorbed per g of biomass, dry weight). All the experiments were repeated four times and mean values were given.

RESULTS AND DISCUSSION

Calibration graphs of the drugs: Calibration graphs were found to be linear in the concentration range of 50-500 ng spot^{-1} for IND, PPV and ALP. The peak areas and concentrations were subjected to least square linear regression to calculate the calibration equations and correlation coefficients. The regression data as shown in Table-1 showed a good linear relationship over the low concentration range of 50-500 ng spot^{-1}. The three dimensional overlay chromatograms of the calibration of the drugs in the range of 50-500 ng spot^{-1} were given in Fig. 3. The linearity of calibration graphs were validated by high value of correlation coefficient and the standard deviation for intercept values was less than 3 %.

TABLE-1
THE CALIBRATION EQUATIONS FOR IND, PPV AND ALP USING HPTLC

Drug	Linearity[a]	r^2	Slope	Intercept
IND	50-500	0.9913	9.093	519.294
PPV	50-500	0.9927	18.630	841.477
ALP	50-500	0.9965	10.357	158.223

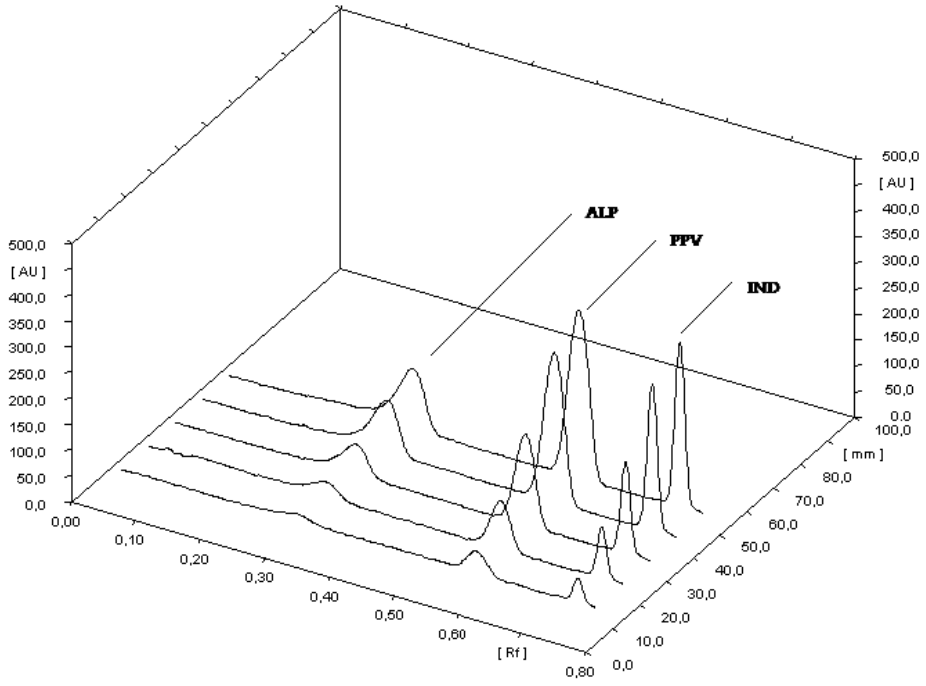

Fig. 3. Three dimensional overlay chromatograms of the IND, PPV and ALP in the range of 50-500 ng spot^{-1} at 260 nm

Effect of the contact time on the biosorption: It is well known that contact time between adsorbate and biomass is of great importance in biosorption, since it highly depends on the nature of the system used. From this point of view, the effect of the contact time on the biosorption of drugs on *N. officinale* was examined. The contact time was studied between 5-120 min. It was found that the adsorptive quantity of drugs on *N. officinale* increased when the contact time was increased. The biosorption of drugs by *N. officinale* was very rapid for 30 min and the equilibrium was nearly reached after 60 min (Fig. 4). Therefore, optimum contact time was selected as 60 min for further experiments.

Effect of pH on drug biosorption: The pH of the initial solution has a critical role in the biosorption of both metals and organic compounds[6-8]. The charge of the adsorbate and the adsorbent often depends on the pH of the solution[23]. So, in this type of experiments, the pHs of the initial solutions have to be adjusted. The pH values ranging from 2.0 to 7.0 were studied in the experimental run. The drugs concentrations of the remaining solution after 60 min were determined by HPTLC. It was found that the initial pHs of the solutions were effected by the equilibrium drug concentrations. It was found that the drugs studied were adsorbed completely on the biomass at nearly pH 5.0 (Fig. 5.) because the highest biosorption efficiencies were obtained at this pH value.

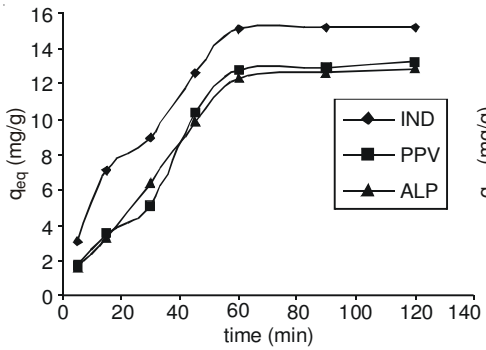

Fig. 4. Effect of contact time on the equilibrium bisorption capacity (initial drug concentrations (c_o) = 50 mg/L, pH = 6.0, temperature (T) = 25 ºC, biosorbent amount (m) = 2.0 g, stirring speed = 150 rpm)

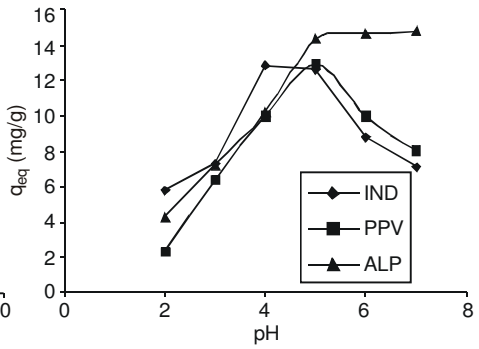

Fig. 5. Effect of pH on the equilibrium bisorption capacity (initial drug concentrations (c_o) = 50 mg/L, contact time = 60 min, temperature (T) = 25 ºC, biosorbent amount (m) = 2.0 g, stirring speed = 150 rpm)

Effect of the amount of biomass: The amount of biomass is another important parameter since it determines the capacity of a biosorbent for a given initial concentration. The effect of the biosorbent amount in equilibrium uptake was examined for the different weights as 0.5, 1.0, 1.5, 2.0 and 2.5 g. It was observed that a 2.0 g amount of biomass was enough for the biosorption of the drugs. The effect of amount of biomass an equilibrium biosorption capacity was shown in Fig. 6. The increasing in the biomass amount can be attributed to increase biosorbent surface areas and the availability of more adsorption sites.

Effect of initial drug concentration on the biosorption: The initial adsorbate concentration affects the equilibrium adsorbate uptake. The effect of initial drug concentrations on the biosorption capacity of *N. officinale* biomass is shown in Fig. 7. Then, the equilibrium biosorption efficiency increased with the increasing of the initial concentration of the drug concentrations.

Adsorption isotherms: The biosorption capacity of *N. officinale* as a biosorbent which is obtained from the mass balance on the sorbate in a system with solution volume V, is often used to acquire the experimental adsorption isotherms. Under the optimum conditions, the biosorption capacities of biosorbent for each of the drugs at equilibrium were calculated from the following equations:

$$q_{eq} = \frac{(c_o - c_{eq})V}{X} \quad (1)$$

where c_o is the initial concentrations of each of the drugs in solutions, c_{eq} is the concentrations of the each of the drugs in solutions at equilibrium, V is the volume of the solution, X is the mass of biosorbent.

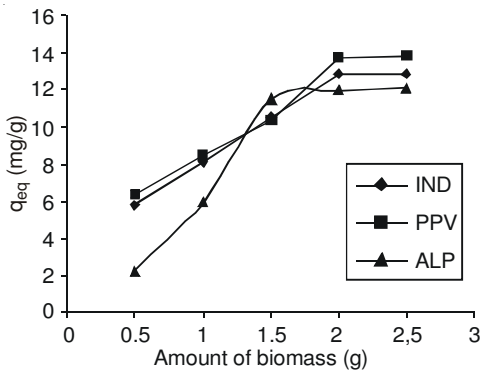

Fig. 6. Effect of amount of biomass on the equilibrium bisorption capacity (initial drug concentrations (c_o) = 50 mg/L, contact time = 60 min, pH:5.0, temperature (T) = 25 ºC, stirring speed = 150 rpm)

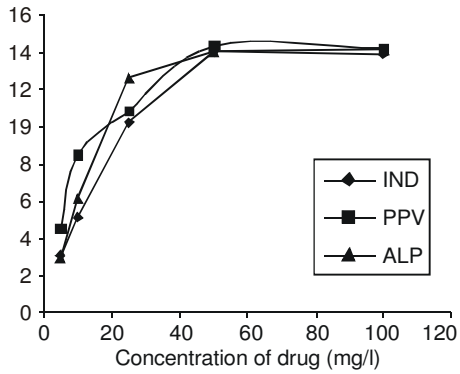

Fig. 7. Effect of concentrations of drugs on the equilibrium bisorption capacity (contact time = 60 min, pH:5.0, biosorbent amount 2 g, temperature (T) = 25 ºC, stirring speed = 150 rpm)

To understand the distribution of the solutes between the liquid and solid phases equilibrium conditions, the equilibrium concentration of the each of the drugs in the solution and the concentration sorbed onto the surface of the biomass were analyzed by the Langmuir (eqn. 2) and Freundlich (eqn. 3) sorption models. The Langmuir and Freundlich models are expressed as:

$$\frac{c_{eq}}{q_{eq}} = \frac{1}{K_b A_s} + \frac{c_{eq}}{A_s} \tag{2}$$

$$\text{In}\, q_{eq} = \ln K_F + \frac{1}{n} \ln c_{eq} \tag{3}$$

where q_{eq} and c_{eq} were described above. A_s, K_b, K_F and n are the adsorption isotherm parameters. A_s is the maximum amount of the drugs per unit weight of biosorbents to form a complete monolayer on the surface bound at high c_{eq} and K_b is a constant related to the affinity of the binding sites. A_s represents a practical limiting adsorption capacity when the surface is fully covered with drugs and assists in the comparison of adsorption performance, particularly in cases where the biosorbent did not reach its full saturation in the experiments[24,25]. The Langmuir equation is valid for monolayer sorption onto a homogeneous surface with a finite number of identical sites. According to the Langmuir equation, some assumptions are made as: the adsorption phenomenon is a reversible interaction, the properties of the adsorbed molecules do not change, there isn't lateral interaction between the adsorbed molecules and all of the adsorption sites have the same affinity for the sorbent[25]. The empirical Freundlich equation given above is based on a monolayer adsorption by the adsorbent with a heterogeneous energy distribution of active sites. K_F and n are indicators of

adsorption capacity and adsorption intensity, respectively. The Freundlich isotherm is also more widely used but provides no information on the monolayer adsorption capacity, in contrast to the Langmuir model[24,25].

The Langmuir and Freundlich adsorption isotherms of drugs obtained at 25 ºC were given in Figs. 8 and 9. The adsorption constants and correlation coefficients obtained from the Langmuir and Freundlich isotherms at 25 ºC were also given in Table-2. As seen from this table, high regression correlation coefficients (> 0.99) were obtained and the results showed that the adsorption equilibrium data have fitted very well Langmuir adsorption models in the concentration ranges studied. The uptake values obtained in this study are comparable with those values. The applicability of Langmuir isotherm to the drug biosorptions express that monolayer adsorption on the surface of adsorbent conditions exist under the experimental conditions employed.

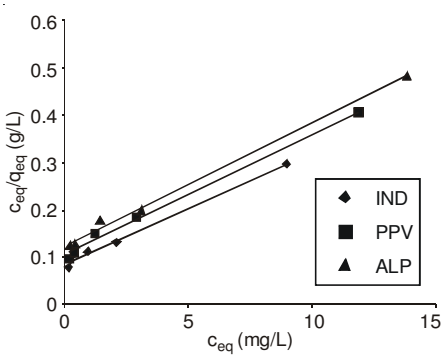

Fig. 8. Langmuir adsorption isotherms of the drugs adsorption on *N. officinale*

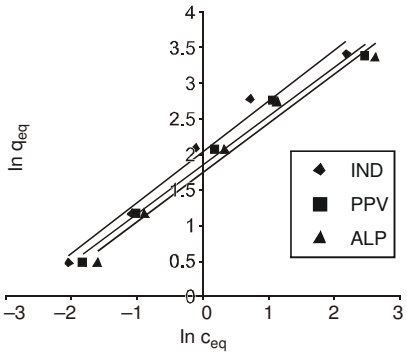

Fig. 9. Freundlich adsorption isotherms of the drugs adsorption on *N. officinale*

TABLE-2
ADSORPTION ISOTHERM PARAMETERS FOR IND, PPV AND ALP USING *N. officinale* AS BIOSORBENT

Compound	Langmuir isotherm			Freundlich isotherm		
	A_s (mg/g)	K_b (L/mg)	r^2	K_F	n	r^2
IND	43.10	0.27	0.9917	7.57	1.40	0.9800
PPV	39.68	0.24	0.9939	6.36	1.46	0.9852
ALP	38.61	0.21	0.9955	5.69	1.45	0.9767

Conclusion

The biosorption of drugs from aqueous solution by *N. officinale* was investigated by using HPTLC. As an alternative to the bacterial biosorbent, submerged aquatic plant *N. officinale* was used to remove the IND, PPV and ALP from aqueous solution. The results obtained from the biosorption of the studied drugs showed

that *N. officinale* would adsorb IND, PPV and ALP based on the Langmuir coefficients. The maximum biosorption capacitiy of *N. officinale* was 43.10 mg/g for IND, 39.68 mg/g for PPV and 38.61 mg/g for ALP. The adsorption equilibrium data fitted well to the Langmuir isotherm. The approach is suitable for routine monitoring as information on the acidic herbicides are necessary to understand the environmental rate of these compounds. *N. officinale* can be used as a potential biosorbent in removal of drugs from aqueous solutions. This natural growing material is easily available and economic for treatment of industrial wastewater.

ACKNOWLEDGEMENT

The authors would like to thank to Ersin KILINC, Researcher Assistant, Chemistry Department, University of Dicle, for the analysis of the drugs with HPTLC and evaluating the text.

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(*Received*: 29 May 2009; *Accepted*: 27 November 2009) AJC-8097