

REMOVAL OF ANTIBIOTICS FROM WASTEWATER BY HYDROXYAPATITE OBTAINED FROM BIOWASTE OF SHELLFISH

Zlate Veličković^{1*}, Zoran Bajić¹, Vladica Stojanović¹, Bogdan Vujičić¹, Ljubomir Gigović¹,
Radovan Karkalić¹, Dragana Mitov¹, Aleksandar Marinković²

¹University of Defence in Belgrade, Military Academy, Veljka Lukića Kurjaka 33, 11000 Belgrade,
Republic of Serbia

²Faculty of Technology and Metallurgy, University of Belgrade, Karnegijeva 4, 11000 Belgrade,
Republic of Serbia

Abstract

Addressing the problem of microbial resistance to antibiotics is one of the main priorities of the World Health Organization (WHO). One way to fight this is to reduce and control the use of antibiotics in the world. However, the COVID 19 pandemic has led to sudden and uncontrolled consumption of antibiotics because they are used as standard medical prophylaxis despite the fact that they do not act on the Corona virus. Another way to fight microbial resistance is to reduce the contact of antibiotics with bacteria. Traces of antibiotics in wastewater cause resistance of bacteria that are in them and have a detrimental effect on humans and the environment due to the limited removal of antibiotics by traditional wastewater treatment. In this study, we investigated the possibility of using as an adsorbent to remove the antibiotic hydroxyapatite obtained from shells of river shells (NHAP/U) from the family Unionidae, a material that accumulates in large quantities on the banks of large rivers as waste. The resulting adsorbent NHAP/U was characterized and tested for removal from wastewater of the most commonly used antibiotic during the COVID-19 pandemic amoxicillin (AMOX). In order to predict the optimal adsorption conditions using the Response Surface Method (RSM), the influence of variable factors (adsorption conditions), pH values, adsorbent dose, contact time and temperature on the adsorbent capacity was investigated. Maximum removal of 97.8% of AMOX was achieved under defined conditions. The best adsorption of AMOX was achieved for pH between 5.0 and 7.0. Adsorption data for amoxicillin at 25, 35 and 45 °C were compared with isothermal models Langmuir, Freundlich, Dubinin-Radushkevich and Temkin, as well as for modelling adsorption kinetics using pseudo-first order, pseudo-second order and Elovich kinetic model. The results showed that the adsorption of AMOX on NHAP/U followed a pseudo-second kinetic model and a Freundlich isothermal model. Thermodynamic parameters indicate the endothermic, feasible and spontaneous nature of the adsorption process.

Keywords: antibiotic, antimicrobial resistance, adsorbent, hydroxyapatite, wastewater

1. INTRODUCTION

Antibiotics are chemical compounds with a wide range of applications in medicine and veterinary medicine. They are used to treat diseases caused by various bacterial infections. In addition to being used to treat diseases, they are very widely used to prevent disease and promote growth in animal breeding. Most of the administered doses (30–90%) are excreted as active forms from the organism without metabolism (Khachatourians 1998; Hirsch et al. 1999). These active antibiotic residues are found in the environment (surface water, groundwater and soil) as a result of domestic, agricultural and industrial wastewater runoff. Widespread use of antibiotics alters microbiological ecosystems, exerts selective pressure on susceptible bacteria and leads to the survival of resistant strains, as well as the development of antimicrobial resistance, making existing antibiotics ineffective in treating various emerging infectious diseases (Vogt and Jackson 2001., Aarestrup and Wegener, 1999., Blanco et al. 1997, Schwarz et al. 2001). In addition to long-term effects, antibiotics can cause allergic reactions in some people and disrupt the original microbiological system when they enter the human body through the food chain and drinking water (Khaniki, 2007., Kümmerer, 2008.).

Conventional water and wastewater treatment technologies based on biological treatment, filtration, coagulation, flocculation and sedimentation, are not efficient enough to completely remove antibiotics and can only achieve their partial removal (Batt et al. 2006., Gulkowska, et al. 2008., Watkinson et al. 2007). Advanced oxidation technologies that produce $\bullet$ OH radicals for active and non-selective decomposition of pollutants, achieve complete removal of antibiotics (Alsager et al. 2018a., Alsager et al. 2018b, Rivera-Utrilla et al. 2013.). However, the main limitations of these methods are related to high costs and end secondary products (Ikehata et al. 2006., Ganiyu et al. 2015) . These findings have focused researchers' attention on developing new alternative technologies that are simple and effective for removing antibiotics from water.

Adsorption provides various advantages over other purification technologies, such as small initial investment, simpler construction, simple operation, and the ability to adapt to be selective or nonselective in nature (Ahmed et al. 2018., Akhtar et al 2016.). So far, various adsorbents have been used to remove antibiotics from water, such as carbon nanotubes (Patiño, Y. et al. 2015), activated carbon (Rakić et al. 2015.), various minerals such as zeolite, clay, and metal oxides (Ridder et al. 2012., Lozano-Morales et al. 2018.). Special attention has recently been paid to various biomaterials in the field of adsorption, either due to the possibilities of easy modification and biocompatibility, or due to good adsorption properties.

Among these adsorbents, special attention is paid to the development of biowaste-based adsorbents (Baccar et al. 2012.,), because in this way two problems in the environment are solved. Waste that has a detrimental effect on the environment is used to solve other environmental problems such as water pollution. Shellfish biowaste burdens the shores of many rivers, lakes and seas. This waste, which consists mainly of different forms of CaCO_3 (calcite and aragonite) is very suitable for different ways of conversion to hydroxyapatite, which in previous research showed good adsorption properties for different pollutants, and is environmentally friendly due to its biocompatibility, easy storage of spent adsorbent, easy modifications and low price.

2. MATERIALS AND METHODS

2.1. Materials

A large number of different chemicals were used during the research. Bearing in mind that the properties of the adsorbent, as well as the research results largely depend on the purity of the reagent, high purity chemicals were used:

- 5% hydrogen peroxide solution - H_2O_2 (Sigma-Aldrich, PA),
- concentrated nitric acid HNO_3 (Fluka, ultrapure) - used for digestion of shells in order to determine the elemental composition and adjust the pH level,
- concentrated phosphoric acid H_3PO_4 (Sigma-Aldrich, PA),
- sodium hydroxide - NaOH (Sigma Aldrich, PA) - used to adjust the pH of the solution in the adsorption process and titration during the synthesis of adsorbents,
- 96% ethyl alcohol (Sigma Aldrich, PA) - used in the washing of adsorbents,
- deionized water (resistance of $18 \text{ M}\Omega \text{ cm}$) - used for sample preparation and dilution of the solution,
- amoxicillin (Hemofarm A.D) – used for performing an adsorption experiment (as 10 mg dm^{-3} concentration solution),
- biowaste of shellfish from the family Unionidae, collected from the banks of the Tisza River.

2.2. Synthesis of adsorbents

The sherds of the river shell were thoroughly washed and rinsed in distilled water, air-dried for 24 h, ground in a steel mill for crushing sediments and sifted into a 0.5 -1 mm granulation powder. The shell powder was washed in vacuo with deionized water, ethyl alcohol and dried in vacuo for 24 h at 110 °C (mechanically prepared river shells - MPRSh).

In the stainless steel vessel of a planetary ball mill (Retsch PM100 CM), 10 g of MPRSh was mixed with 11.23 ml (18.92 g) of concentrated H_3PO_4 - $CaCO_3$ ratio: $H_3PO_4 \rightarrow Ca / P = 1.67$, zirconium beads were added in ratio 20: 1 of the bead mass to a sample and the mixture was treated in a ball mill for 10 h at 500 grp for further mechanosynthesis. After treatment in a ball mill, the obtained mixture was washed copiously in vacuo with deionized water to remove unreacted parts of the acid and dried in vacuo for 24 h at 110 °C to obtain a second NHAP/U adsorbent (hydroxyapatite).

2.3. Material characterization methods

The synthesized adsorbents were characterized by FTIR, SEM and EDX techniques. The elemental composition was determined by a chemical elemental analyser, the content of individual elements was determined by dissolving in acids and measuring the content on a plasma mass spectrometer with a plasma-coupled plasma ICP-MS system Agilent 7500C (Agilent Technologies, Inc.) and an atomic adsorption spectrometer. The concentration of amoxicillin before and after adsorption was determined using a Shimadzu UV-1800 spectrophotometer (Kyoto, Japan). The infrared Fourier transform spectrum (FTIR) was recorded in the transformation mode between 400 and 4000 cm^{-1} at a resolution of 4 cm^{-1} using an infrared (IR) spectrometer with Fourier transformation (FT) - Nicolet iS 50 manufactured by Thermo Scientific. The adsorbents morphology was observed using Tescan Mira 3 FEG scanning electron field microscopy (FESEM). The pH value of the zero charge point (pH_{PZC}) of adsorbents was determined by the "drift" method (Gao et al. 2009).

2.4. Amoxicillin adsorption research

Adsorption experiments were performed in a batch system where the initial concentration of amoxicillin solution was fixed $C_0 = 10.00 \text{ mg dm}^{-3}$, and the dose of adsorbent was varied from 135 to 1350 mg dm^{-3} . In order to examine the pH value influence on the adsorption process, the pH value was varied from 4.0 to 10.0. Thermodynamic and kinetic adsorption experiments were performed at temperatures of 25, 35 and 45 °C, and the adsorption process was monitored in a time interval of 5 to 90 minutes. The amount of adsorbed molecules was calculated as the difference between the initial and equilibrium concentration.

The adsorbent capacity was calculated according to the Eq. (1):

$$q = \frac{C_i - C_f}{m} V \quad (1)$$

Where q is adsorption capacity in mg g^{-1} , C_i and C_f are initial and final amoxicillin concentrations in mg dm^{-3} , V is volume of the solution in dm^3 , and m is the mass of adsorbent, expressed in g.

2.4.1. Kinetic studies

The study of kinetics provides an insight into the possible mechanism of adsorption along with the reaction pathways. The adsorption data were analysed by linear, non-linear least-squares and graphic method in the form of pseudo-first, pseudo-second-order (Lagergren) and second order model (Table 1) (Karanac et al., 2018).

Table 1. Kinetic model equations

Kinetic model	Nonlinear form	Linear form	Equation
Pseudo-first-order equation (<i>Lagergren</i>)	$q = q_e(1 - e^{-k_1 t})$	$\ln(q_e - q) = \ln q_e - k_1 t$	(2)
Pseudo-second order equation (<i>Lagergren</i>)	$q = \frac{t}{\frac{1}{k_2 q_e^2} + \frac{t}{q_e}}$	$\frac{t}{q} = \frac{1}{k_2 q_e^2} + \frac{1}{q_e} t$	(3)
Second order	$q = \frac{t}{\frac{1}{k_2 q_e^2} + \frac{t}{q_e}}$	$\frac{t}{C_t} = k_2 t + \frac{1}{C_0}$	(4)

Diffusion models as Weber-Morris, Dunwald-Wagner model and Homogenous Solid Diffusion Model (HSDM) were used for modelling diffusional processes/limiting step of overall process (Table 2) (Karanac et al., 2018, Budimirović et al., 2017; Taleb et al., 2015; Taleb et al., 2016).

Table 2. Equations of diffusion kinetic models

Kinetic model	Nonlinear form	Equation
Weber-Morris	$q = k\sqrt{t} + C$	(5)
Dunwald-Wagner model	$\frac{q}{q_e} = 1 - \frac{6}{\pi^2} \sum_{n=1}^{\infty} \frac{1}{n^2} \exp[-n^2 K t]$ $\log \left(1 - \left(\frac{q}{q_e} \right)^2 \right) = -\frac{K}{2.303} t$	(6)
Homogenous Solid Diffusion Model (HSDM)	$\frac{\partial q}{\partial t} = \frac{D_s}{r^2} \frac{\partial}{\partial r} \left(r^2 \frac{\partial q}{\partial r} \right)$ $\frac{q}{q_s} = 1 + \frac{2R}{\pi r} \sum_{n=1}^{\infty} \frac{(-1)^n}{n} \sin \frac{n\pi r}{R} \exp \left[\frac{-D_s t \pi^2 n^2}{R^2} \right]$	(7)

Activation energy for amoxicillin adsorption was calculated using Arrhenius (Eq. 8):

$$k' = k_0 \exp \left[\frac{-E_a}{RT} \right] \quad (8)$$

Where k' ($\text{g mg}^{-1} \text{ min}^{-1}$) is the pseudo-second order rate adsorption constant, k_0 ($\text{g mmol}^{-1} \text{ min}^{-1}$) is the temperature independent factor, E_a (kJ mol^{-1}) is the activation energy, R ($8.314 \text{ J mol}^{-1} \text{ K}^{-1}$) is the gas constant and T (K) is the adsorption absolute temperature. A plot of $\ln K'$ versus $1/T$ gave straight line with slope $-E_a/R$ from which activation energy was calculated.

2.4.2. Isotherm models

The equilibrium adsorption data were fitted by the isotherm models Langmuir, Freundlich, Temkin and Dubinin-Radushkevich isothermal models (Karanac et al. 2018). The Langmuir equation is based on assumption that a point of maximum adsorption corresponds to a saturated mono-layer of adsorbate molecules on the adsorbent surface - where the energy of adsorption remains constant and no transfer of the adsorbate in the plane of the surface occurs. The Freundlich sorption isotherm, widely and reliably utilized as a mathematical determining expression, allows for a calculation encompassing surface heterogeneity and exponential distribution of active sites as well as their respective energies

(Karanac et al., 2018). Temkin, conceived this equation for subcritical vapours in micropore solids where the adsorption process follows a pore filling mechanism onto energetically non-uniform surface. Temkin isotherm is based on the assumption that the decline of the heat of sorption as a function of temperature is linear rather than logarithmic. The Dubinin-Radushkevich model for subcritical vapours in micropore solids where the adsorption process follows a pore filling mechanism onto energetically non-uniform surface (Karanac et al., 2018). Equations of adsorption isotherms models presented in Table 3.

Table 3. Adsorption isotherms equations

Isotherms	Nonlinear form	Linear form	Equation
Langmuir	$q = \frac{q_m K_L C}{1 + K_L C}$	$\frac{C}{q} = \frac{1}{K_L q_m} + \frac{C}{q_m}$	(9)
Freundlich	$q = K_F C^{1/n}$	$\log q = \log K_F + \frac{1}{n} \log C$	(10)
Temkin	$q_e = \frac{RT}{b} \ln(AC_e)$	$q_e = \frac{RT}{b} \ln A + \frac{RT}{b} \ln C_e$	(11)
Dubinin-Radushkevich	$q_e = q_m \exp\left(-B(RT)^2 \left(\ln\left(1 + \frac{1}{C_e}\right)\right)^2\right)$	$\ln q_e = \ln q_m - B(RT)^2 \left(\ln\left(1 + \frac{1}{C_e}\right)\right)^2$	(12)

2.4.3. Thermodynamic studies

The feasibility of the experimental data obtained from the adsorption studies were analysed through the thermodynamic investigation. The parameters of free energy change (ΔG° , kJ/mol), enthalpy change (ΔH° , kJ/mol) and entropy change (ΔS° , J/mol/K) were calculated using the Van't Hoff equations (13) and (14) (Karanac et al., 2018):

$$\Delta G^\circ = -RT \ln(b) \quad (13)$$

$$\ln(b) = \frac{\Delta S^\circ}{R} - \frac{\Delta H^\circ}{(RT)} \quad (14)$$

Separation factor (R_L) is in relation to Langmuir isotherm and it is used to assess adsorption feasibility on the given adsorbent. It is calculated using next Eq (15):

$$R_L = \frac{1}{(1+bC_0)} \quad (15)$$

Where C_0 (mol dm⁻³) is initial adsorbate concentration, b (dm³ mol⁻¹) is Langmuir constant. Value of R_L points out to the isotherm type: irreversible ($R_L = 0$), favourable ($0 < R_L < 1$), linear ($R_L = 1$), unfavourable ($R_L > 1$).

2.5. Statistical analysis of experimental data

All adsorption experiments were repeated three times and mean values were taken for further processing and modelling. The obtained results were analysed using the normalized standard deviation Δq (%), which is calculated using the following equation:

$$\Delta q(\%) = \sqrt{\sum \frac{[(q_{exp} - q_{cal})/q_{exp}]^2}{N - 1}} \times 100 \quad (16)$$

where q_{exp} and q_{cal} are the experimental and calculated values of adsorbed amoxicillin, N is the number of data used in the analysis. The maximum deviation is <3%, which is an experimental error. Standard errors for isothermal parameters, kinetic and thermodynamic parameters were determined using the commercial software Microcal Origin 8.0 (Pantić et al. 2019).

In order to confirm the adsorption model that best corresponds to the experimental data, they were analyzed by ANOVA variance analysis, using the F value together with the values of the correlation coefficient (R) from the regression analysis. (Pantić et al. 2019, Bajić et al. 2019)

2.7. Optimization of experimental adsorption conditions

Optimization of adsorption conditions was performed using the RSM (Surface Response Methodology) method. Classical adsorption optimization usually involves examining the impact of each variable separately. However, it is difficult to predict optimal reaction conditions based on such results due to the possible interactions between the different independent variables involved in the adsorption reactions. (Pantić et al. 2019, Bajić et al. 2019) Recently, various statistical programs have been used that are useful to help establish the design of the experiment. Using response surface methodology (RSM) as a mathematical function, it is possible to examine the individual and interactive influences of different variables in relation to different predictors. In that way, we get the optimal conditions that are needed to get the best results. (Pantić et al. 2019) In addition, it has been proven that central composition design (CCD) and Bok-Behnken design (BBD) are efficient designs of RSM models in optimizing the adsorption process. (Pantić et al. 2019) In this study, the synthesized modified adsorbent NHAP/U was used to remove amoxicillin from water. The adsorption process is optimized by numerical and graphical optimization methods using Bok-Behnken design. It was used design with four factors (input variables) and three levels of values in which 29 experiments with five replications in the central point were performed. The capacity of the adsorbent was taken as a response. Extremely optimized conditions were confirmed by additional experimental testing. The conditions of the adsorption experiment are given in Table 4.

Table 4. An experimental amoxicillin adsorption plan was performed using a four-factor BBD design with three levels of value

Ordinal number	A Dose ads. (mg/L)	B t (min)	C T(°C)	D pH	Response q_e (mg g ⁻¹)
1.	745	90	25	7	13.57
2.	745	45	25	10	8.65
3.	745	45	25	7	10.87
4.	745	45	45	4	10.35
5.	1350	45	45	7	6.75
6.	1350	45	25	7	6.48
7.	745	90	45	7	14.15
8.	745	5	35	4	8.3
9.	135	90	35	7	35.77
10.	745	45	45	10	9.1
11.	745	45	25	4	9.76
12.	135	45	35	10	26.45
13.	1350	45	35	10	5.3
14.	745	5	35	10	7.33

15.	745	90	35	10	11.07
16.	135	45	45	7	6.75
17.	745	5	45	7	9.35
18.	135	5	35	7	26.8
19.	135	45	35	4	30.09
20.	745	45	35	7	11.1
21.	1350	5	35	7	6.22
22.	745	45	35	7	11.1
23.	1350	90	35	7	9.13
24.	745	90	35	4	12.54
25.	745	45	35	7	11.1
26.	135	45	25	7	30.65
27.	745	45	35	7	11.1
28.	745	45	35	7	11.1
29.	1350	45	35	4	6.01

3. RESULTS AND DISCUSSION

3.1. Physical and chemical characterization of adsorbents

The elemental composition of shellfish sherd is given in Table 5. This elemental composition is similar to that of other authors (Vei et al. 2018, Buasri et al. 2013) and indicates that shellfish sherd are mostly calcium carbonate-based mineral composition (calcite and aragonite) and the organic part of chitin that connects calcite structures. The composition of shells also includes various microelements - ions, which replace Ca^{2+} in the structure of calcium carbonate and are incorporated into the shell during its formation. The availability and rate of bioaccumulation of these ions is a function of environmental and biological factors. Thus, different habitats, contamination - the presence in the water of different ionic species, different stages of shell development can represent different patterns of metal incorporation.

Table 5. Concentrations of major, minor and trace elements obtained in this study using ICP-AES and ICP-MS method

Method		ICP-AES				
Element	Ca (wt %)	Fe (wt %)	Mg (wt %)	Si (wt%)	Na (wt%)	Mn ($\mu\text{g g}^{-1}$)
Content	34.1	0.003	0.104	0.004	0.182	30.4
Method		ICP-MS				
Element	Cd ($\mu\text{g g}^{-1}$)	Co ($\mu\text{g g}^{-1}$)	Cu ($\mu\text{g g}^{-1}$)	Ni ($\mu\text{g g}^{-1}$)	Pb ($\mu\text{g g}^{-1}$)	Zn ($\mu\text{g g}^{-1}$)
Content	0.094	0.079	31.2	2.01	3.09	21.5

The analysed sherds of the river shell are composed of two polymorphs of calcium carbonate: calcite and aragonite, with calcite being the dominant form. Recent studies have found that the dominant

CaCO_3 polymorph may be temperature dependent, aragonite deposition is at a high temperature and calcite deposition is at a low temperature (Kuklinski and Taylor 2009; Ramajo et al. 2015; Krzeminska et al. 2016). Confirmation of the composition of the shells can also be seen on the spectrum of energy dispersive spectrometry (Figure 1) where the main building blocks are observed before (a) and after the modification of the shell (b). After modification, we notice the presence of phosphorus in the adsorbent, which confirms the transition of calcium carbonate to hydroxyapatite.

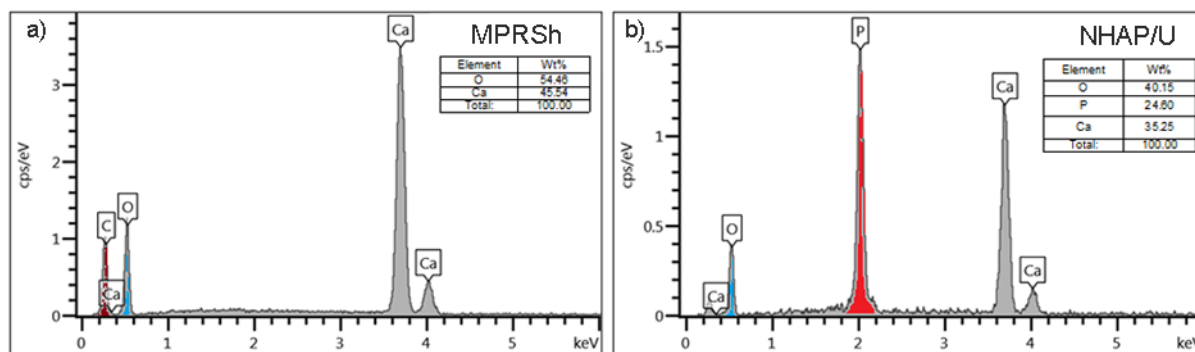


Fig. 1. EDS spectrum of shellfish powder before (a) and after modification (b)

In the photographs of scanning electron microscopy (Figure 2) with different magnifications, we can clearly see the lamellar structure of the shell. The lamellae consist of materials based on calcium carbonate (calcite and aragonite) with a thickness of about 1 μm and with cavities between the lamellae with a diameter of about 50 to 100 nm, they are interconnected by organic polymer chitin, nanopores are observed on the lamellae surface.

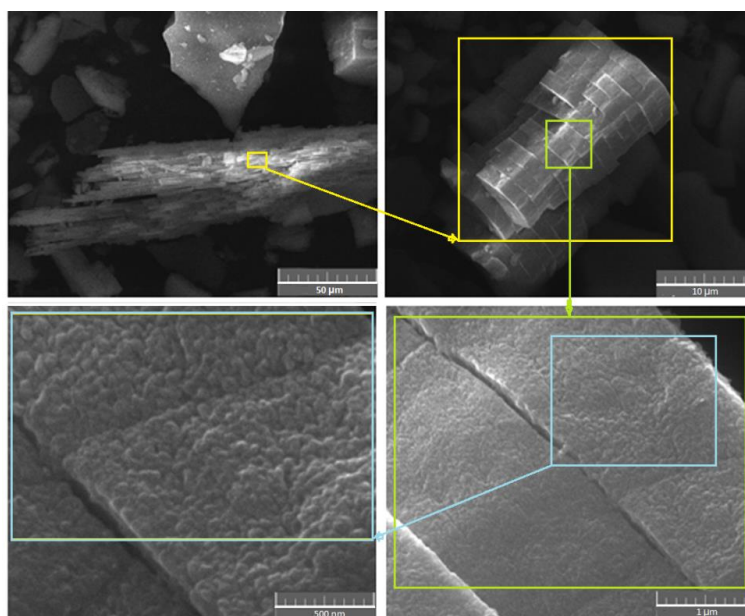


Fig. 2. SEM representation of mechanically prepared shells of shells at different magnifications

After mechanosynthesis, the lamellar structure derived from calcium carbonate is lost and we get the granular morphology of hydroxyapatite, which is presented in Figure 3. Electron scanning microscopy images showed the presence of rounded HAp microparticles, in isolated and agglomerated forms.

Based on observations, HAP particles can be considered as microspheres whose crystal size is well below 1 μm .

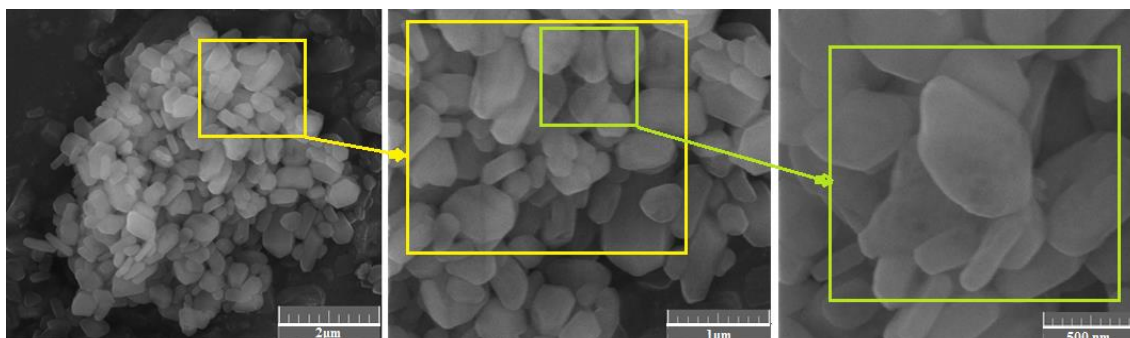


Fig. 3. SEM representation of mechanosynthetic modified shells of shells at different magnifications

The physical properties of the adsorbent, specific surface area, and zero charge point are given in Table 6. The change in pH_{PZC} value occurred under the influence of the change in the surface properties of the adsorbent due to modification (Table 6). At $\text{pH} < \text{pH}_{\text{PZC}}$, negatively charged species participate in electrostatic attraction with a positively charged adsorbent surface and vice versa, at $\text{pH} > \text{pH}_{\text{PZC}}$, electrostatic repulsion is a major factor leading to low adsorption efficiency.

Table 6. Physical properties of MPRSh and RMHAp adsorbents

Adsorbent	Specific surface area ($\text{m}^2 \text{g}^{-1}$)	pH_{PZC}
MPRSh	2,58	7,2
NHAP/U	1,95	7,05

Figure 4 shows the FTIR spectra of both adsorbents (MPRSh and NHAP/U) before and after adsorption of amoxicillin from aqueous solution. On the spectrum a the characteristic peaks 710, 856 and 1460 cm^{-1} indicate the carbonate group in the sample which confirm that the sample contains CaCO_3 . In addition, small infrared absorption spectra are shown at ~ 1790 , and $\sim 2874 \text{ cm}^{-1}$ and have been attributed to regimens of combining different ranges of CO_3^{2-} (Khiri et al. 2016). The spectrum at $\sim 1083 \text{ cm}^{-1}$ is related to the C – O tensile vibrations as CO_2 adsorbed on the CaO surface (Khiri et al. 2016).

The FTIR spectrum (c) of the NHAP/U adsorbent showed pronounced peaks at 560 cm^{-1} corresponding to the symmetrical bending regime of PO_4^{3-} and 1064 cm^{-1} corresponding to the asymmetric stretching regime of the PO_4^{3-} group corresponding to the vibrational structures of hydroxyapatite (Khiri et al. 2016). The large peak at Figure 4 in the spectrum a and the smaller peak in the spectrum c at 1460 cm^{-1} represent carbonate (CO_3), which is more pronounced before mechanosynthesis, i.e. before the conversion of calcium carbonate into hydroxyapatite.

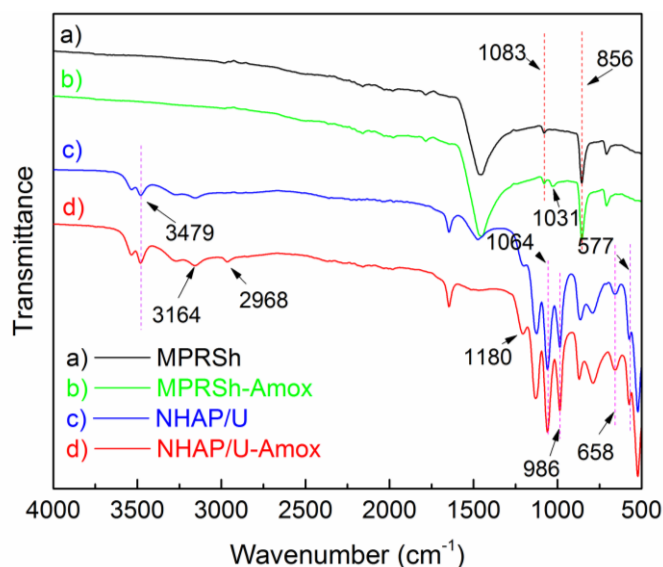


Fig. 4. FTIR spectrum of shellfish sherds and modified sherds before and after adsorption of amoxicillin from aqueous solution

The characteristic vibration peaks of shell dust before and after modification with a comparative review by other researchers are given in Table 7.

Table 7. FTIR shell powder vibration mode before and after mechanosynthesis (MPRSh and NHAP/U) and references

	Vibration frequency (cm ⁻¹)			
	FTIR measurement	Khiri et al. (2016)	Salma et al. (2010)	Islam et al. (2013)
Symmetrical deformation CO₃²⁻	710	708		706
Asymmetric deformations CO₃²⁻	856, 1460	855, 1454		857, 1455
Symmetric stretching vibration CO₃²⁻	1083	1082		1082
CO₃²⁻ deformations	1790	1786		1794
PO₄³⁻ bending	560	565	560, 599	
PO₄³⁻ stretching	1064	1024	1046	
CO₃²⁻ group	1460	1454	1424	

After sorption of amoxicillin (spectra b and d) on both adsorbents, changes were observed in the appearance of new peaks, decrease in intensity, disappearance and displacement of peaks.

3.2. Influence of solution pH on adsorption

The influence of pH value on the system is manifested through surface tension, surface properties, degree of ionization of groups present on the surface of the adsorbent, as well as on the speciation of ions in aqueous solution at a certain pH value. Due to the amphoteric properties of amoxicillin

molecules, their pH is determined by the ionization state of a solution (Ali et al. 2020). Thus, at lower pHs due to protonation, the adsorbent has a positive charge and, on the other hand, the amoxicillin molecule has a carboxyl group that approaches the zero point pH to become carboxylate which has a negative charge and is easily absorbed. Thus, as shown in Fig. 5, pH 6 was selected as the optimum value for the continuation of adsorption experiments given its proximity to the isoelectric point of the adsorbent, which shows that the electrostatic processes in the adsorption of amoxicillin on the adsorbent were predominant (Ali et al. 2020) this is in line with previous research (Ali et al. 2020).

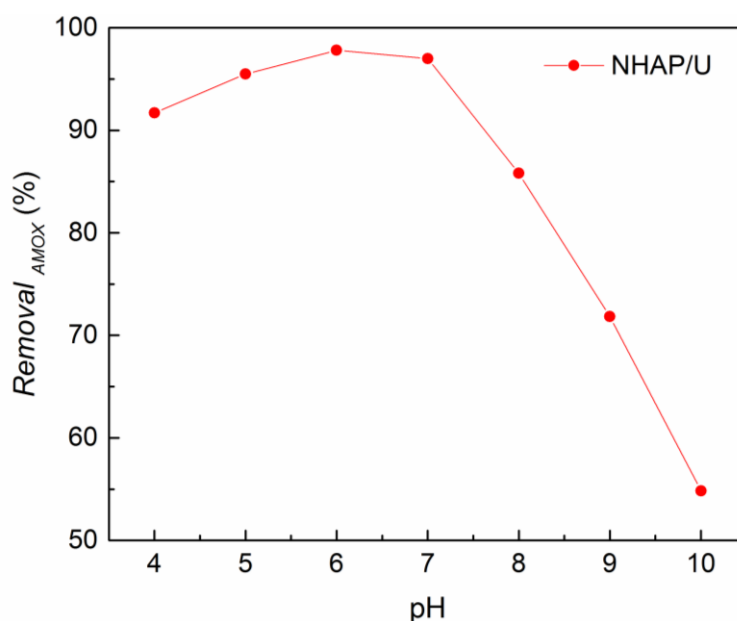


Fig. 5. Influence of pH value of the initial solution on amoxicillin removal on NHAP/U adsorbents ($C_{i[\text{amoxicillin}]} = 10,00 \text{ mg L}^{-1}$, $t=90 \text{ min}$, $m/V = 135 \text{ mg L}^{-1}$, $T = 25 \text{ }^{\circ}\text{C}$)

3.3. Adsorption kinetics

The effect of time on amoxicillin adsorption was monitored in the range of 5 to 90 minutes. The final equilibrium was established after 120 minutes but since the difference in the removal of *amoxicillin* from 90 to 120 minutes ranged from 3 % in order to speed up the process as the final time was taken 90 minutes.

In order to determine the kinetic model that accompanies adsorption in order to interpret the adsorption mechanism, we used pseudo-first, pseudo-second-order and second-order models (Table 1). Table 8 shows the kinetic parameters for amoxicillin absorption on NHAP/U adsorbents.

Table 8. The kinetic parameters for amoxicillin adsorption on NHAP/U adsorbents ($C_{i[\text{amoxicillin}]} = 10.00 \text{ mg L}^{-1}$, $\text{pH} = 6$; $m/V = 135 \text{ mg L}^{-1}$, $T = 25 \text{ }^{\circ}\text{C}$)

Model parameters	Pseudo-first	Pseudo-second	Second-order
q_e	21.09	35.098	35.098
$k (k_1, k_2)$	0.0497	0.00314	0.0006
R^2	0.936	0.995	0.817

The results shown in Table 8, according to the regression coefficient (R^2) and standard error for all model parameters, indicate that the kinetics for all adsorbents are best described using a pseudo-second order model.

The rate constants of diffusion kinetic models, intra-particle diffusion, Weber-Morris, Dunwald-Wagner and homogeneous solid diffusion models for amoxicillin adsorption on NHAP/U adsorbents under the same experimental conditions are presented in Table 9.

The complex nature of the kinetics of adsorption processes can be described by observing the adsorption of all ions adsorbed on the adsorbent as a single step, as described by a pseudo-second order equation, but can also be described by consecutive / competitive steps.

Table 9. Parameters of diffusion kinetic models ($C_{i[\text{amoxicillin}]} = 10.00 \text{ mg L}^{-1}$, $\text{pH} = 6$; $m/V = 135 \text{ mg L}^{-1}$, $T = 25^\circ\text{C}$)

Model	Model parameters	Values
Weber-Morris Step 1 (Intra-particle diffusion)	$k_{p1} (\text{mg g}^{-1} \text{ min}^{-0.5})$	3.4997
	$C (\text{mg g}^{-1})$	6.634
	R^2	0.998
Weber-Morris Step 2 (equilibrium)	$k_{p2} (\text{mg g}^{-1} \text{ min}^{-0.5})$	0.2643
	$C (\text{mg g}^{-1})$	29.016
	R^2	0.979
Dunwald-Wagner model	K	0.0191
	R^2	0.868
Homogenous Solid Diffusion Model (HSDM)	D_s	$2.28 \cdot 10^{-11}$
	R^2	0.861

The Weber-Morris model reveals two linear steps that describe the adsorption process: fast kinetics in the first step and slower in the second. The first linear part describes the external mass transfer to the adsorbent surface, while the second part describes the process of material transfer into the porous structure of the adsorbent, and strictly depends on the size and shape of the pores as well as the density of their network on NHAP/U adsorbents. Intra-particle and film diffusion slow down the transport of adsorbates. In the final phase of the process, adsorption takes place slowly until saturation is achieved on the entire available surface of the adsorbent.

3.5. Adsorption activation energy

In relation to the results of kinetic research performed at temperatures of 298, 308 and 318 K, it is possible to determine the activation energy using the Arrhenius equation (Table 10). Linear form of the Arrhenius equation (19):

$$\ln K' = -\frac{E_a}{RT} + \ln A \quad (19)$$

where K' is the reaction rate constant at a certain temperature, E_a shows the activation energy, R is the universal gas constant (8.314), T is the temperature in K and A is the Arrhenius factor (frequency for a given reaction).

Table 10. Pseudo-second model parameters for amoxicillin adsorption on NHAP/U adsorbents
($C_{i[\text{amoxicillin}]} = 10.00 \text{ mg L}^{-1}$, $\text{pH} = 6$; $m/V = 135 \text{ mg L}^{-1}$)

Temperature	$q_e (\text{mg g}^{-1})$	$k_2 (\text{g (mg min)}^{-1})$	R^2
298 K	35.097	0.003138	0.995
308K	36.735	0.004009	0.998
318 K	39.156	0.004699	0.998

Physisorption or physical adsorption generally possesses energy up to 40 kJ mol^{-1} , while chemisorption requires higher energy and activation energy over 40 kJ mol^{-1} (Karanac et al. 2018). Based on the obtained results where E_a for NHAP/U is $15.94 \text{ kJ mol}^{-1}$ we can conclude that the main mechanism of adsorption is physical adsorption.

3.6. Adsorption isotherms

The state of interactions / bonds on the surface of the adsorbate / adsorbent can be observed by fitting the experimental data with different adsorption isotherms. The normalized correlation coefficient and standard deviation were used to estimate the fit of the adsorption data. The experimental data were compared with the Langmuir, Freundlich, Temkin, and Dubinin-Radushkevich isotherm models already discussed, the parameters of which are shown in Table 3. By analysing the experimental data on the adsorption of amoxicillin molecules on the tested adsorbents, the best fit for both adsorbents is given by the Freundlich isothermal model. The results of modeling the adsorption of amoxicillin on the tested adsorbents are given in Table 11 (linear modeling) and Figure 6 (nonlinear modeling). According to the Freundlich isotherm, the mechanism of ion adsorption on MRM and RMHAp can be described as heterogeneous adsorption, where the adsorbed ions / molecules have different enthalpies and adsorption activation energies. The value of n from the Freundlich isotherm is a measure of adsorption intensity or surface heterogeneity.

Table 11. Parameters of adsorption isotherms of amoxicillin adsorption on NHAP/U

Isothermal models and parameters		Temperature		
		25 °C	35 °C	45 °C
Langmuir isotherm	$q_m (\text{mg g}^{-1})$	32.613	35.326	38.538
	$K_L (\text{L mg}^{-1})$	4.19243	4.26244	4.31710
	$K_L (\text{L mol}^{-1})$	1531912	1557495	1577488
	R^2	0.970	0.972	0.973
Freundlich isotherm	$K_F (\text{mg g}^{-1}) (\text{dm}^3 \text{mg}^{-1})^{1/n}$	22.160	24.014	26.238
	n	3.86	3.65	3.42
	R^2	0.992	0.994	0.994
Temkin isotherm	$A_T (\text{dm}^3 \text{g}^{-1})$	261.96	224.90	196.22
	b_T	4.09	4.55	5.08
	R^2	0.902	0.903	0.900
Dubinin-Radushkevich isotherm	$q_m (\text{mg g}^{-1})$	21.07	22.44	24.01
	$K_{ad} (\text{mol}^2 \text{kJ}^{-2})$	9.76	9.70	9.63
	$E_a (\text{kJ mol}^{-1})$	7.157	7.180	7.205
	R^2	0.802	0.807	0.811

Values of n near zero indicate a highly heterogeneous surface. Values of $n < 1$ (Table 11) imply a chemisorption process, and higher values indicate combined adsorption, e.g. physisorption and chemisorption with different process contributions at different system balancing steps. The values in Table 16 indicate that the adsorption was combined in all cases.

The calculation of the separation factor (R_L) according to equation (20) which is based on the parameter b of the Langmuir isotherm indicates the feasibility of adsorption on a given adsorbent. It is calculated using the following equation:

$$R_L = \frac{1}{(1 + bC_0)} \quad (20)$$

where C_0 (mol L^{-1}) is the initial adsorbate concentration and b (L mol^{-1}) is the Langmuir constant. The value of R_L indicates the feasibility of the adsorption process: irreversible ($R_L = 0$), favourable ($0 < R_L < 1$), linear ($R_L = 1$), unfavourable ($R_L > 1$). The R_L for adsorption of amoxicillin on NHAP/U ranges from 0.023 to 0.192 indicating that the adsorption process is favourable.

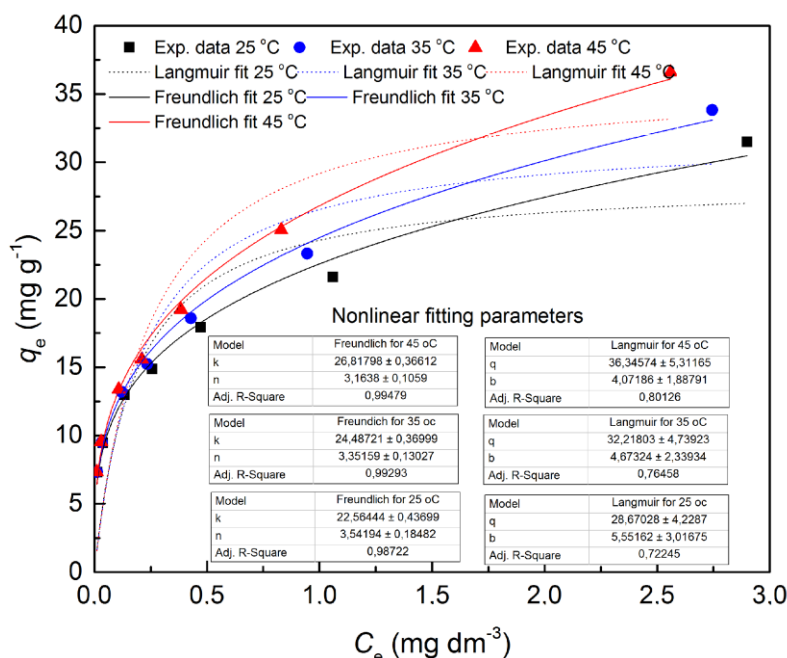


Fig. 6. Review of the results of adsorption experiments with the nonlinear fitting models of isotherms for the removal of amoxicillin on adsorbent NHAP/U

3.7. Thermodynamic studies

Gibbs free energy (ΔG^0), enthalpy (ΔH^0) and entropy (ΔS^0) were calculated by Van't Hoff equation (21) and (22):

$$\Delta G^0 = -RT \ln(b) \quad (21)$$

$$\ln(b) = \frac{\Delta S^0}{R} - \frac{\Delta H^0}{(RT)} \quad (22)$$

where T is the absolute temperature in K , R is the universal gas constant ($8.314 \text{ mol}^{-1} \text{ K}^{-1}$) and the adsorption constant b is calculated using the Langmuir isotherm (Table 12). ΔH^0 and ΔS^0 were calculated from the slope and sections in the diagram $\ln(b) - T^{-1}$, assuming that the adsorption kinetics are stationary. The calculated thermodynamic parameters are shown in Table 12.

Table 12. Calculated Gibbs free adsorption energy, enthalpy and entropy for amoxicillin adsorption on MRM, and RMHAp at 25, 35 and 45 °C

ΔG° (kJ mol ⁻¹)			ΔH°	ΔS°	R^2
25 °C	35 °C	45 °C	(kJ mol ⁻¹)	(J mol ⁻¹ K ⁻¹)	
-45.26	-46.82	-48.37	1.16	157.68	0.997

Negative values of Gibbs free energy (ΔG°) and positive values of entropy (ΔS°) at all temperatures indicate that reactions in the adsorption process take place spontaneously. A decrease in the Gibbs free energy (ΔG°) with an increase in temperature also indicates that the spontaneity of the reaction increases.

Positive values of ΔS° indicate a tendency of greater disorder of the NHAP/U surface systems and amoxicillin solution. In Table 12 we can see that the Gibbs free energy values (ΔG°) for both adsorbents are approximate, and the positive entropy values (ΔS°) at all temperatures, while the positive enthalpy values (ΔH°) for NHAP/U are noticeable, which indicates endothermic process. In general, the exchange of free energy in the case of physisorption is somewhere between -20 and 0 kJ mol⁻¹, for simultaneous chemisorption and physisorption between -20 and -80 kJ mol⁻¹, and chemisorption less than -80 kJ mol⁻¹. The obtained results indicate that in these cases, chemisorption and physisorption are present at the same time.

3.8. Optimization of adsorption conditions

Using the response surface methodology (RSM) as a mathematical function by commercial software design Expert 9 tests, the individual interactive and the impact of various variables in relation to the different predictors. The mutual influence of the input variables was analysed by analysing the variances of ANOVA using the quadratic model of the equation shown in Table 13.

Table 13. ANOVA variance analysis for a square response surface model for the removal of amoxicillin from water using NHAP/U adsorbent

Source	Sum of square	df	Mean Square	F Value	p-value Prob > F	
Model	1698.412	14	121.3151	7.317285	0.0003	significant
A-dose	1143.115	1	1143.115	68.94852	< 0.0001	
B-t	50.8015	1	50.8015	3.06416	0.1019	
C-T	44.75376	1	44.75376	2.699383	0.1226	
D-pH	7.064674	1	7.064674	0.426115	0.5245	
AB	10.74707	1	10.74707	0.648224	0.4342	
AC	146.8245	1	146.8245	8.855915	0.0100	
AD	2.1541	1	2.1541	0.129927	0.7239	
BC	1.554995	1	1.554995	0.093792	0.7639	
BD	0.053782	1	0.053782	0.003244	0.9554	
CD	0.0049	1	0.0049	0.000296	0.9865	
A^2	203.3648	1	203.3648	12.26622	0.0035	
B^2	15.59554	1	15.59554	0.940666	0.3486	

C ²	21.03158	1	21.03158	1.268548	0.2790
D ²	4.474291	1	4.474291	0.269873	0.6115
Residual	232.1096	14	16.57925		
Lack of Fit	232.1096	10	23.21096		significant
Pure Error	0	4	0		
Cor Total	1930.521	28			

A graph of the optimal conditions with respect to the input variables for removing amoxicillin from water using NHAP/U is shown in Figure 7 and 8. After optimization of pH value (6) and temperature (45 °C), other parameters were optimized to obtain a satisfactory capacity above 30 mg g⁻¹ (yellow field).

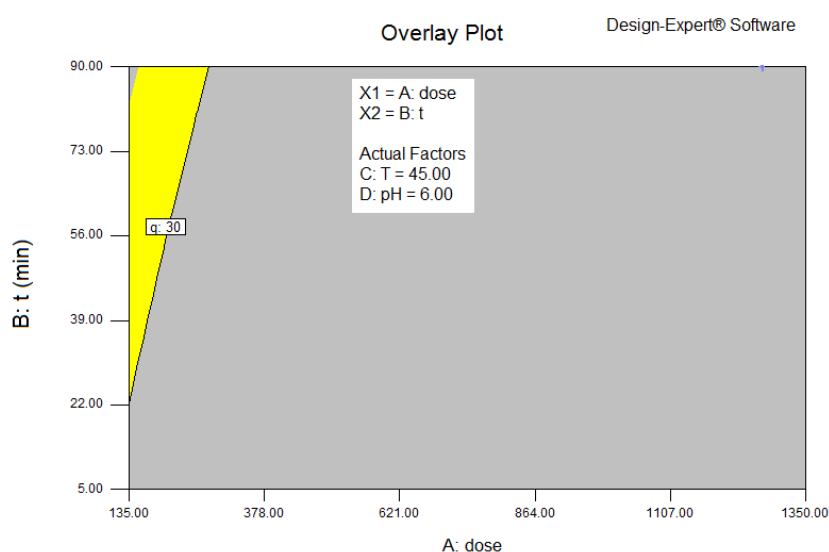


Figure 7. Optimization of input parameters in relation to the maximum capacity of the adsorbent

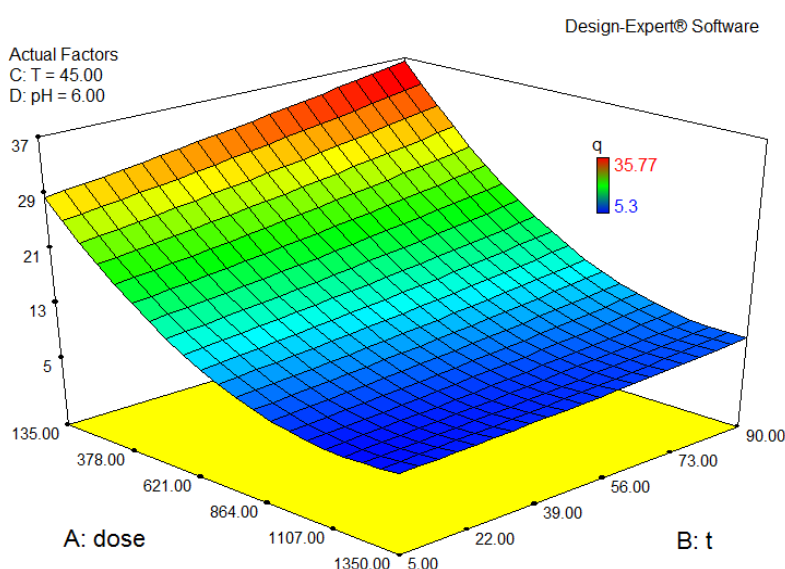


Figure 8. 3D diagram of mutual interactions of dependence of significant input variables (adsorbent dose and time)

4. CONCLUSION

NHAP showed excellent amoxicillin removal performance. The results of isothermal, kinetic and thermodynamic studies suggested simultaneous physisorption and chemisorption between amoxicillin molecules and the surface of NHAP/U adsorbent during the adsorption process. The optimal parameters for maximum amoxicillin adsorption were: system pH value - 6, adsorbent dose - 135 mg L⁻¹, adsorption time - 90 minutes and temperature - 45 °C. Adsorption was spontaneous and endothermic as described by thermodynamic parameters. Bok-Behnken's design within the response surface method has been successfully used in the optimization of experimental adsorption conditions, the goal of optimization is to determine the optimal adsorption conditions with a smaller number of experiments. Optimization methods are maximally harmonized with the principles of environmental protection because it reduces: the number of experiments, the amount of consumed expensive and environmentally harmful chemicals and the generation of waste. Errors and predicted response values, derived from a mathematical model, showed acceptable results and confirmed the favourable effect of the studied factors on amoxicillin adsorption using NHAP/U. This paper investigates the sustainable use of biowaste for the treatment of water contaminated with antibiotics, whereby the biowaste that burdens the banks of our rivers is used to remove water pollutants, thus obtaining a double benefit for the environment.

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