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Influence of pH on cephalexin adsorption onto SBA-15 mesoporous silica: Theoretical and experimental study

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ABSTRACT

Cephalexin adsorption from aqueous solutions using SBA-15 mesoporous silica as adsorbent and the influence of pH solution on drug adsorption were studied. In order to have a better knowledge about the way the drug molecules interact with the inorganic matrix, the adsorption process was estimated by applying the computational chemistry software YAeHMOP (Yet Another extended Hückel Molecular Orbital Package). A strong correlation between the theoretical calculations and the experimental results was established, showing that the adsorbate–adsorbent interaction is pH dependent. Calculated cephalexin horizontal adsorption energy was almost 9 eV more stable than the one corresponding to vertical adsorption, and also the lowest enthalpy of contact and the maximum adsorption percent were found for the cationic cephalexin–silica system. Cephalexin adsorption through the NH_3^+ group is 8 eV stronger than the molecule adsorption through the COO^- group. In agreement with these theoretical predictions, experimental results indicate that the electrostatic attraction between CPX ions and the surface of mesoporous silica is favored at pH values between 2 and 2.56, the maximum being for cephalexin adsorption obtained at pH 2.3.

1. Introduction

Cephalosporins are lactam antibiotics with the same fundamental structural requirements as penicillin. They are used for the treatment of infections caused by gram-positive and gram-negative bacteria. They act by inhibiting the synthesis of essential structural components of bacterial cell wall. They are among the safest and the most effective broad-spectrum bactericidal antimicrobial agents, being the most prescribed of all antibiotics [1]. Cephalexin is a first-generation cephalosporin. The molecule (HCpx) contains NH_2, C(O)NH, and C(O)OH groups, so it is similar in structure and acid-base properties to dipeptide molecules. Depending on the pH, cephalexin molecules exist in aqueous solution as positively charged H_2Cpx^+ species, as $HCpx^-$ zwitterions, or as Cpx^-, anionic, negatively charged entities [2].

Besides, mesoporous silica materials have recently been proposed as hosts for stabilizing different pharmaceutical drugs [3–5]. As the average lifetime of cephalexin is about 0.9 h, its immobilization and stabilization in a matrix followed by a controlled release constitutes a very interesting challenge.

The most common and well-known ordered mesoporous framework, SBA-15 mesoporous silica, is a 2D hexagonal planar structure, having *p6mm* symmetry [6]. Silanol groups (Si—OH) are effectively present on the SBA-15 surface. The adsorption of molecules onto the external and pore surfaces of SBA-15 does not modify the mesoporous hexagonal planar ordering [7]. This fact, along with its relatively large pore size, suggests that this particular mesoporous structure could be an appropriate matrix for the loading and further release of drugs in a reversible way. Effectively, SBA-15 could adsorb organic molecules of different sizes, since the pore diameter of the silica matrix can be tailored in a broad meso range, with the extra bonus of high specific surface and the absence of microbial pathogens [8,9].

In previous work it was demonstrated that cephalexin adsorption on SBA-15 mesoporous silica depends on the nature

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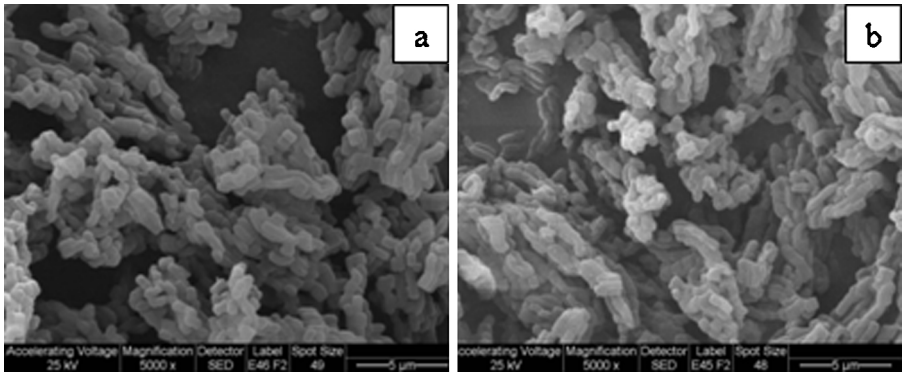

Fig. 1. SEM images of (a) SBA-15; (b) SBA-15/cpx.

of the functionalizing groups present on the silica surface [10–12].

In order to maximize the loading capacity of cephalexin in the silica matrix, it is necessary to know factors favoring matrix–drug interactions. Therefore, the main objective of this work was to analyze the effect of changes in the pH solution on cephalexin adsorption. In addition, a theoretical approach based on performing Hückel calculations was used to analyze cephalexin–surface interactions.

2. Experimental

2.1. Chemicals

The chemicals used in this study include triblock copolymer poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) (PEO) (Pluronic P123, MW: 5800, Aldrich), tetraethyl orthosilicate (TEOS) (98%, Aldrich) and cephalexin monohydrate (CPX) (Interlude Company S.A.).

2.2. Synthesis of mesoporous silica

Mesoporous silica SBA-15 was prepared according to the methodology described by Zhao [6], using TEOS as silica source and PEO as organic structure-directing agent. The molar composition used was 1TEOS:5HCl:0.0178PEO:160.91H_2O. The solid material obtained was washed with water, dried at 120 °C and calcined for 6 h at 550 °C.

2.3. Cephalexin adsorption

Batch equilibrium tests were carried out for CPX adsorption on the prepared SBA-15, under stirring for 5 h. Sample solutions were withdrawn at equilibrium to determine the residual concentration. After decanting and filtering, the concentration of CPX remaining in the solution was determined using UV–vis spectrophotometry, measuring the absorption at 262 nm (UV-1800 Shimadzu, Japan). The amount of CPX adsorbed at equilibrium, q_e (mg/g), was calculated by:

$$q_e = \frac{(C_o - C_e)V}{W}$$

The effect of pH on CPX removal was studied by varying the pH of the CPX solution from 2.3 to 7.3, where the pH values were adjusted by adding either 0.1 N HCl or 0.1 N NaOH and measured using an electrode pH meter. The CPX initial concentration was fixed at 1 mg/mL, whereas other parameters such as adsorbent dosage (0.2 g), stirring rate (120 rpm), solution volume (500 mL) and solution temperature (25 °C) remained constant.

2.4. Characterization

Scanning electron microscopy (SEM) was carried out on Philips 505 microscope. FT-IR spectra were collected by means of an FT-IR Spectrometer Spectrum 1000, Perkin Elmer, using KBr in the frequency range of 4000–400 cm^{-1}. Nitrogen adsorption–desorption isotherms were determined using a Micrometrics ASAP 2020 at 196 °C. Before the measurements, the samples were degassed at 100 °C for 700 min. The surface area was calculated according to the Brunauer–Emmett–Teller (BET) equation. The pore size was obtained by the BJH method.

2.5. Computational method

To simulate the silica structure, we modeled a cell of 155 atoms: 40 Si, 95 O atoms and 20 H atoms. In order to reproduce the surface, this cell was extended in two dimensions parallel to the surface. On the other hand, the starting point for isolated cephalexin molecule ($C_{16}H_{17}N_3O_4S$) was the 7-(α-amino-α-phenylacetamido)-3-methyl-3-cephem-4-carboxylic acid structure optimized with the Gaussian program [13].

To study the cephalexin–silica interaction, we performed calculations by using the YAeHMOP program (Yet Another extended Hückel Molecular Orbital Package), which makes a reasonable prediction of molecular and electronic structures based on a physical model of molecular and solid electronic charge density distribution functions [14]. During the calculations, the structures of both molecule and substrate were optimized at steps of 0.02 Å and convergence in energy of 0.01 eV. We analyzed the adsorption of cephalexin molecule that exists as a zwitterion, anionic and cationic chemical species depending on pH conditions.

3. Results and discussion

3.1. Characterization of SBA-15 before and after cephalexin adsorption

3.1.1. Scanning electron microscopy

The SEM micrographs of the mesoporous silica obtained reveal the typical form of SBA-15 as agglomerates with the form of wheat grains composed of small cylinders 5–6 μm in size. This shape is conserved after cephalexin adsorption (Fig. 1).

3.1.2. Nitrogen adsorption/desorption isotherms

According to the IUPAC classification, before and after drug adsorption the silica materials showed typical type IV isotherms presenting an H1 hysteresis loop. These results are associated with the presence of pores in the range of mesopores. As an example, the N_2 isotherms of SBA-15 and SBA-15/cpx at pH 2.3 are shown

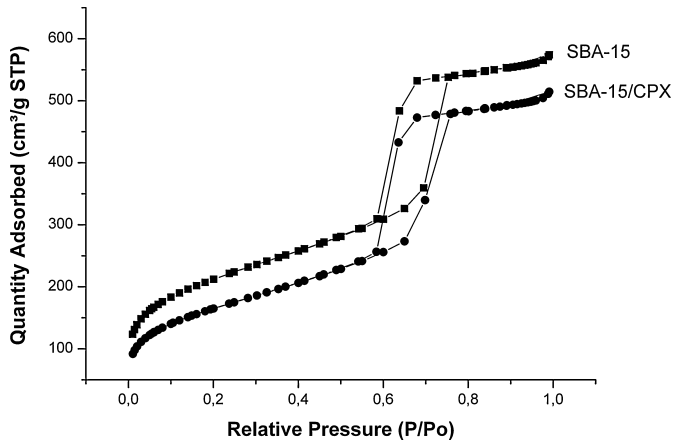

Fig. 2. Nitrogen adsorption and desorption isotherms.

Table 1
Textural properties of SBA-15 and SBA-15/cpx.

Sample	pH	S: BET (m^2/g)	Pore volume (cm^3/g)
SBA-15	–	750	0.72
SBA-15/cpx	2.3	469	0.58
	3.3	479	0.605
	4.3	480	0.602
	5.3	482	0.62
	6.3	489	0.63
	7.3	497	0.65

in Fig. 2. Additionally, the P/P_0 position of the inflection range from 0.60 to 0.80 confirms the presence of mesopores.

The textural characteristics of the samples studied are summarized in Table 1. It was observed that the incorporation of cephalexin into the matrix produces a decrease of the surface area. The 750 m^2/g area measured for the starting SBA-15 diminishes when the pH increases, being the smallest value observed for the lowest pH tested. Similarly, the pore volume decreases from the value of 0.72 cm^3/g for SBA-15 to 0.58 cm^3/g for the SBA-15/cpx sample obtained at pH = 2.3. From the decrease of the mentioned textural parameters, it was inferred that cephalexin was successfully loaded into the pores of the silica matrix.

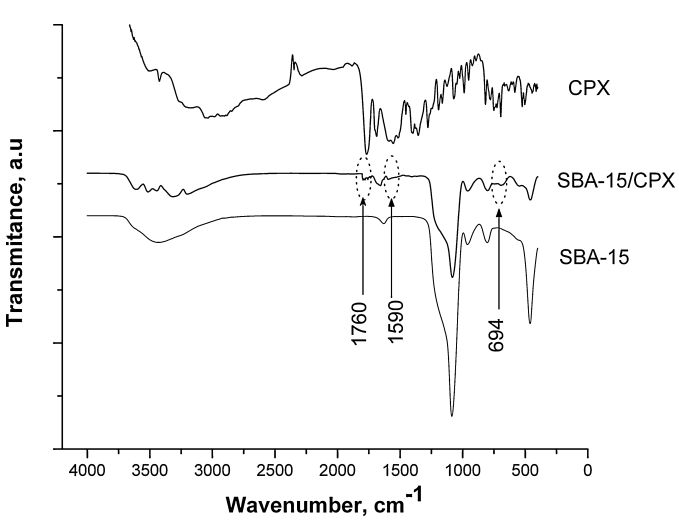

Fig. 3. FTIR spectra of cephalexin, SBA-15, and SBA-15 after cephalexin adsorption at pH = 2.3.

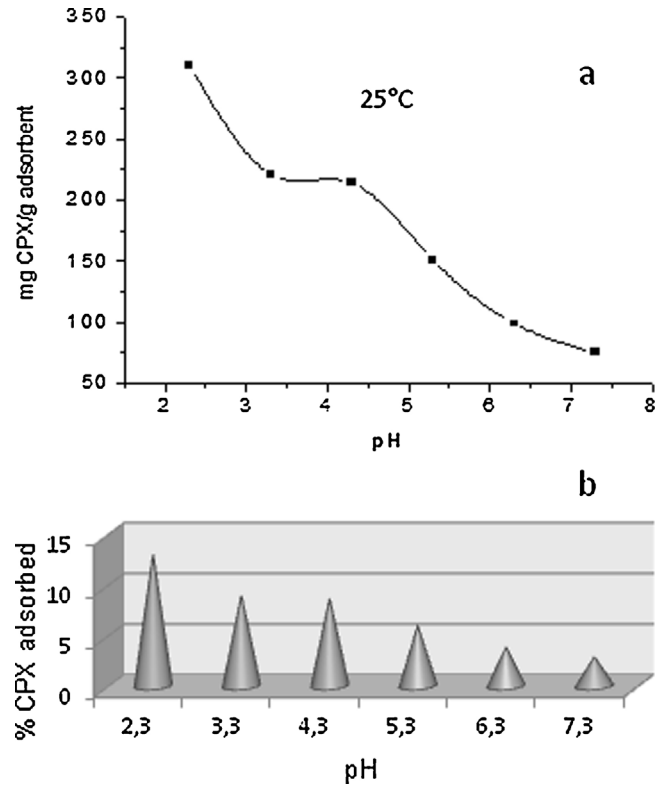

Fig. 4. CPX loading on SBA-15. (a) q_e vs. solution pH and (b) adsorption percentages of CPX vs. solution pH.

3.1.3. FTIR spectroscopy

Fig. 3 illustrates the FTIR spectra of SBA-15, SBA-15/cpx obtained at pH = 2.3 and cephalexin.

In the case of SBA-15 material, the spectrum shows the bands around 1220, 1070, 794 and 471 cm^{-1} assigned to the typical Si—O—Si stretching and bending vibrations of condensed silica network and the peaks around 960 cm^{-1} corresponding to non-condensed Si—OH groups [15,16]. The broad band around 3400 cm^{-1} and the strong peak around 1630 cm^{-1} are due to the stretching and bending vibrations of adsorbed H_2O.

Cephalexin showed a characteristic band at 3438 cm^{-1} due to amide N—H stretching vibrations. The bands at 3271 and 3209 cm^{-1} are due to intermolecular hydrogen-bonded amine groups, while a band at 3042 cm^{-1} is due to acidic hydroxyl groups. Characteristic bands appearing at 1758 and 1690 cm^{-1} are due to four-membered lactam carbonyl and secondary amide carbonyl groups, respectively. The bands at 1591 and 2919 cm^{-1} are due to N—H bending vibrations and C—H stretching vibrations, respectively. The bands appearing at 1455, 1406, and 1350 cm^{-1} are due to C—H bending vibrations. The C—N stretching vibrations are observed at 1283 cm^{-1}, whereas C—O stretching vibrations are observed at 1073 cm^{-1}. Characteristic peaks due to monosubstituted phenyl groups appeared at 745 and 696 cm^{-1}, whereas the peak due to C—S stretching vibrations merged at 745 cm^{-1} [17]. Peaks appearing at 1758, 1591, 1410 and 696 cm^{-1} for cephalexin also appeared in the drug-loaded matrix, indicating the chemical stability of cephalexin after entrapment. Similar results were also observed in our previous studies [11,12].

3.2. Effect of pH on CPX adsorption

The pH influence on CPX loading is shown in Fig. 4. At pH values around 2.3, the amount of CPX adsorbed is higher than in the pH range from 3 to 8. This fact can be explained by the different CPX

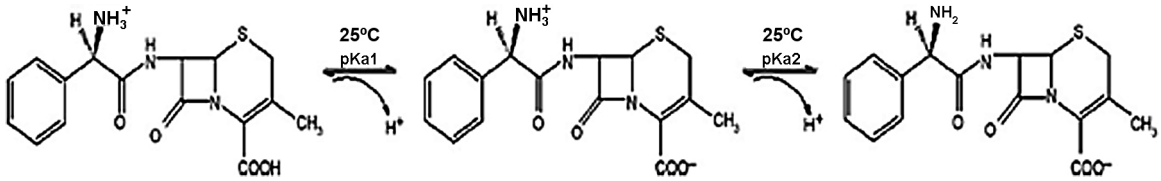

Fig. 5. Cephalexin ionization states: p*K*a1 = 2.56, p*K*a2 = 6.88, isoelectric point = 4.5.

species present at different pH values. Cephalexin is a zwitterionic molecule with pKa values of 2.56 and 6.88 [18], its ionic state being determined by the pH of the solution, as shown in Fig. 5. At pH 6.88 and above, CPX is anionic charged, at pH values below 2.56 it is cationic, existing as a zwitterion species between both p*K*a values. The net charge of silica surface is positive at pH values below it PZC = 2 and negative at higher pH values. Hence, the electrostatic attraction between CPX ions and the surface of the mesoporous silica is favored at pH values between 2 and 2.56. Then, as was observed experimentally, the adsorption capacity decreased with increasing pH.

3.3. Molecular modeling

In addition, to elucidate the nature of and the factors influencing drug adsorption, a theoretical approach based on performing Hückel calculations was applied to analyze cephalexin–silica surface interactions.

It is known that CPX molecule exists as a zwitterion, anionic and cationic chemical species depending on pH conditions. The basic and/or acidic groups of the molecule facilitate electrostatic interactions with the silica surface and determine the adsorption geometry of cephalexin species. Calculations show that the major molecule–surface interactions are produced when a vertical adsorption of cephalexin on the silica surface takes place through the carboxyl group of cephalexin (Fig. 6(a)), and a quasi-horizontal adsorption arises through the cephalexin amino group (Fig. 6(b)). Then, we analyzed the most favorable adsorption geometries cited for the zwitterion, anionic and cationic species. The importance of silica as a drug carrier is based on the ability of the silanol groups situated on the mesopore walls to adsorb the molecule of pharmacological interest. Si—OH groups lead to an increased interaction between the solid and the drug, contributing to an improved adsorption capacity.

Fig. 7 shows the energy mapping for anionic cephalexin adsorption on the silica surface. We can see that the horizontal adsorption is a more exothermic process than the vertical one. The molecule adsorption through the NH_2 group is almost 6 eV more negative (more stable) than the anionic species adsorption through the COO^- group. However, it is important to emphasize that both curves do not present an absolute minimum energy showing a weak adsorption process. The species is located 4 Å and 2.5 Å from the surface when the horizontal and the vertical adsorption, respectively, take place on the silica and this is because the steric effects produce more repulsion during the horizontal adsorption.

Fig. 8 shows the energy mapping for zwitterion cephalexin adsorption. In this case, cephalexin adsorption through the NH_3^+ group is 8 eV stronger than the molecule adsorption through the COO^- group. The zwitterion species is located 4 Å from the silica surface when the horizontal adsorption occurs and only 5 Å below it when the vertical adsorption takes place. It is evident that the competition between acid and basic groups is present during zwitterion adsorption.

Fig. 9 shows the energy mapping for cationic cephalexin adsorption. Similar to previous analyses, the molecule locates nearest the silica surface when the adsorption occurs via the COOH group (2.5 Å vs. 4 Å); therefore the horizontal adsorption is almost 9 eV more stable because the NH_3^+ group of cephalexin interacts better with OH groups of the silanol surface.

In general, it would appear that the presence of the amino groups of the molecule leads to better interaction with Si—OH groups; therefore, the adsorption of cephalexin by the common silica is not particularly high (all the curves do not present absolute minimums of energy). This could be related to the low cephalexin adsorption reported in the experimental studies. On the other hand, considering all the chemical cephalexin structures, the cationic cephalexin–silica system presents the lowest enthalpy of contact during adsorption and this could be indicative of the maximum adsorption percent reported for cationic cephalexin compared with anionic and zwitterion species.

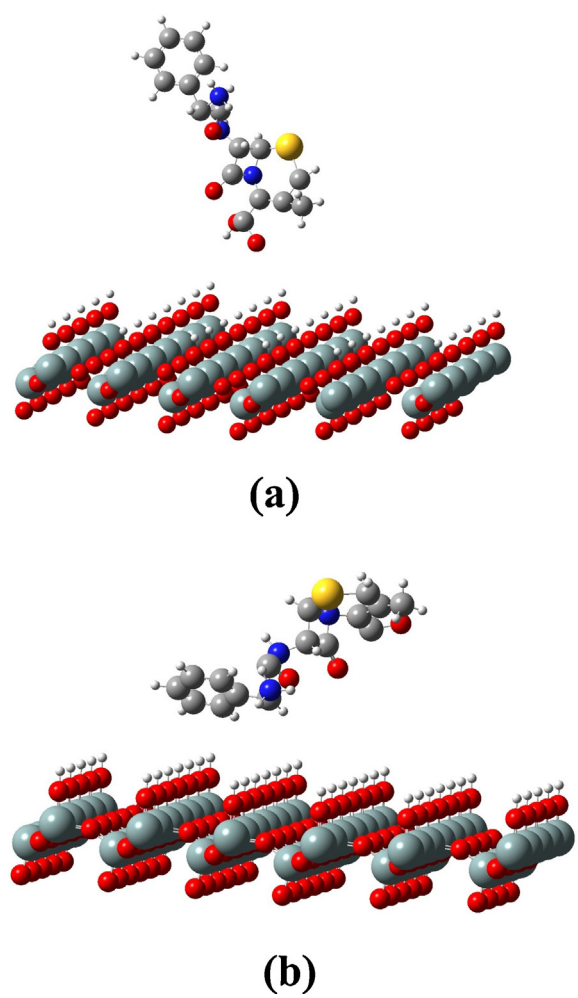

Fig. 6. Schematic view of cephalexin (a) vertical and (b) horizontal adsorption geometries.

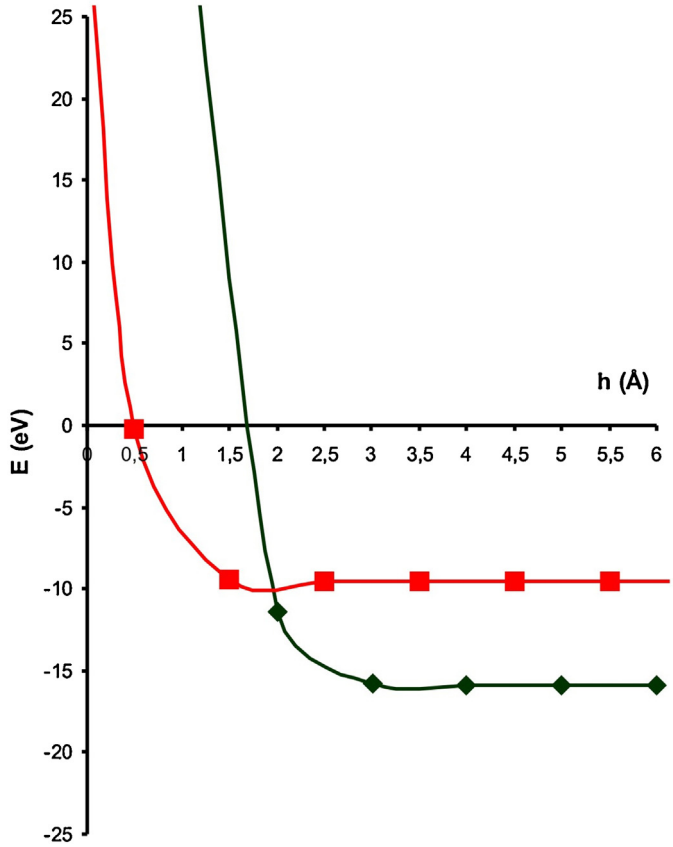

Fig. 7. Adiabatic total energy curves for the (■) vertical and (♦) horizontal adsorption of cephalexin anionic species.

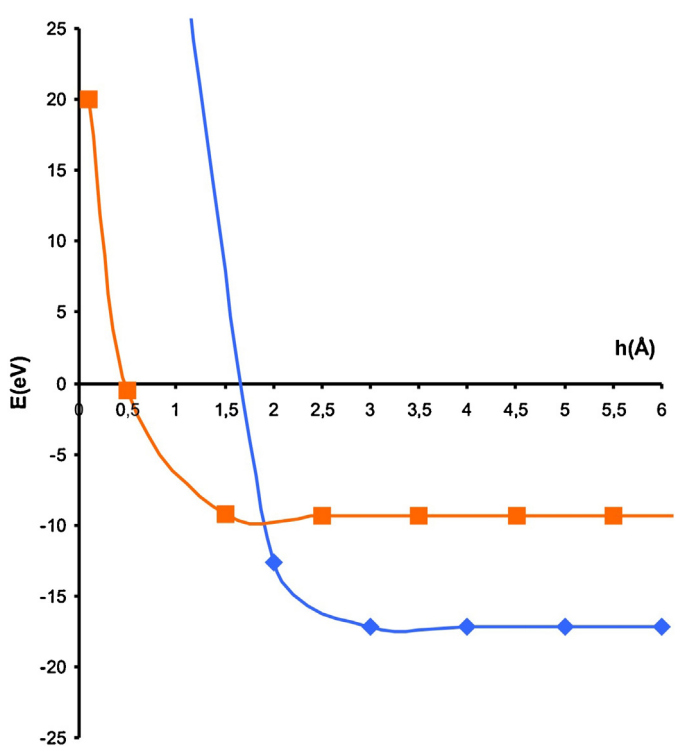

Fig. 8. Adiabatic total energy curves for the (■) vertical and (♦) horizontal adsorption of cephalexin zwitterion species.

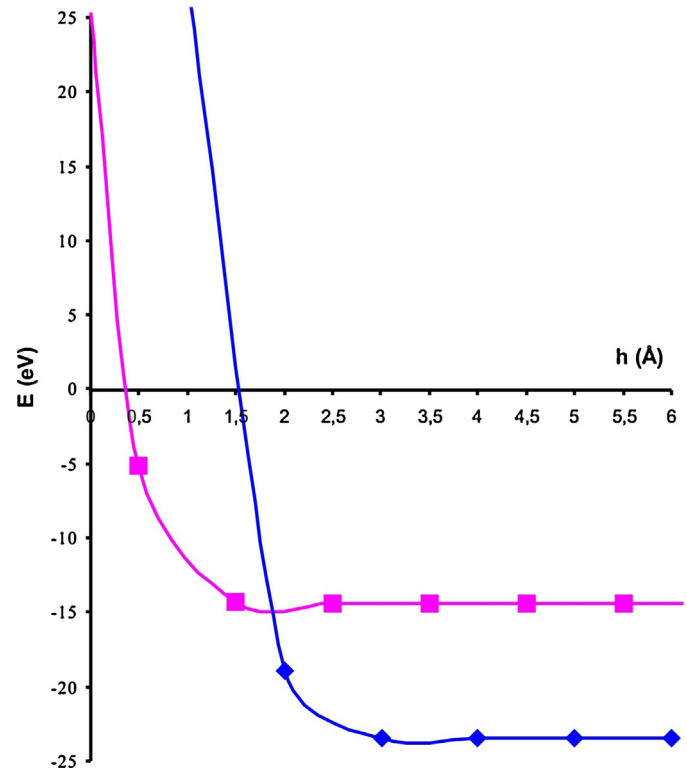

Fig. 9. Adiabatic total energy curves for the (■) vertical and (♦) horizontal adsorption of cephalexin cationic species.

4. Conclusions

The adsorption of cephalexin on SBA-15 was investigated. The effect of pH solution on drug adsorption was of special interest.

It was proved that there is a good correlation between the theoretical studies carried out in this work and the experimental results. Molecular modeling and docking calculations demonstrated that the higher adsorption capacity occurs when cephalexin is cationic at pH below 2.56. Horizontal adsorption is more stable because the NH_3^+ group of cephalexin interacts better with OH groups of the silanol surface.

Acknowledgments

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