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Spectral manifestations of mechanisms of intermolecular interaction of maleimide-modified polyelectrolyte capsules used in the targeted therapy

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Methods of quantum-chemical modeling based on the density functional theory are used to study the mechanisms of intermolecular interaction between the elements of polyelectrolyte capsules of targeted delivery with maleimide, a substance that enhances the therapeutic effect of the capsule. Subjects of the study are molecules of layers of the polymer polyelectrolyte capsule: polyarginine and dextran sulfate, as well as the maleimide molecule. Based on the calculation of the structures of molecular complexes and their corresponding IR spectra, followed by an analysis of the parameters of the resulting hydrogen bonds, the presence of a quite strong hydrogen bonding was found between maleimide and arginine, which is part of the capsule. This suggests that the modification of arginine with maleimide promotes stronger hydrogen bonding with amino acids contained in the human body, which is confirmed by calculations, and makes it possible to use maleimide as an „anchor" that holds the capsule in the tissue.

Keywords: molecular modeling, density functional theory, IR spectra, intermolecular interaction, hydrogen bonding, maleimide, arginine, polyelectrolyte capsules.

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Introduction

Theranostics and targeted therapy are among the most extensively developing directions in biophysics and healthcare. In order to improve the therapeutic effect and reduce adverse side effects, many drugs are delivered directly to the target cells, almost without affecting other organs and prolonging the effect on the objects of treatment. There are various methods of the targeted drug delivery, among which the most popular are the following two directions: delivery using synthesized nanoparticles and nanocapsules [1–3] and delivery due to vesicles, micelles and liposomes formed in colloidal solutions and nanogels. Biodegradable and biocompatible carriers such as polyelectrolyte capsules are of particular interest [4,5].

The composition of biodegradable carriers may be different from case to case, depending on objectives of the therapy [6], at the same time the degree of biocompatibility is determined by the composition of materials of the capsule or liposome. This study considers the possibilities of intermolecular interaction of organic carriers based on amino acids. These include capsules composed of polyarginine and dextran sulfate [7].

It is worth to note that due to their composition, polyelectrolyte capsules based on polyarginine and dextran sulfate are completely biocompatible and non-toxic, because polyarginine is a polymer based on the basic amino acid of arginine, and dextran sulfate is a branched polymer of glucose of bacterial origin with part of hydroxyl groups in its glucose residues replaced by $-OSO_3$ sulfate groups.

Polyelectrolyte capsules have a wide range of applications that is permanently expanding [8–13]. Studies have been carried out *in vivo* on the delivery conditions [8], the effect of capsules on blood flow [9], the possibility of their disruption using ultrasound [10]. The use of polyelectrolyte capsules has shown to be effective in the transport of neuromidase [5] and as a nerve growth factor [11], as well as in the targeted delivery of highly toxic anticancer drugs [12,13], such as doxorubicin and mitoxantrone.

An important role is played not only by delivery, but also by the retention of the capsule or vesicle for a more complete release of the drug. One of the ways to solve this problem is the modification of nanocapsules with maleimide. Maleimide is an unsaturated cyclic imide of great importance in organic synthesis and modification of biological objects [14].

Thanks to the possibility of the thiol-maleimide reaction under physiological conditions, polymer materials, nanocapsules and liposomes modified with maleimide functional groups show a high ability to adhere to mucous tissues [15,16].

Subjects of the study and methods of modeling

In this study the mechanisms of intermolecular interaction based on the hydrogen bonding of maleimide with elements of a biodegradable carrier in order to retain it inside the affected tissue are investigated.

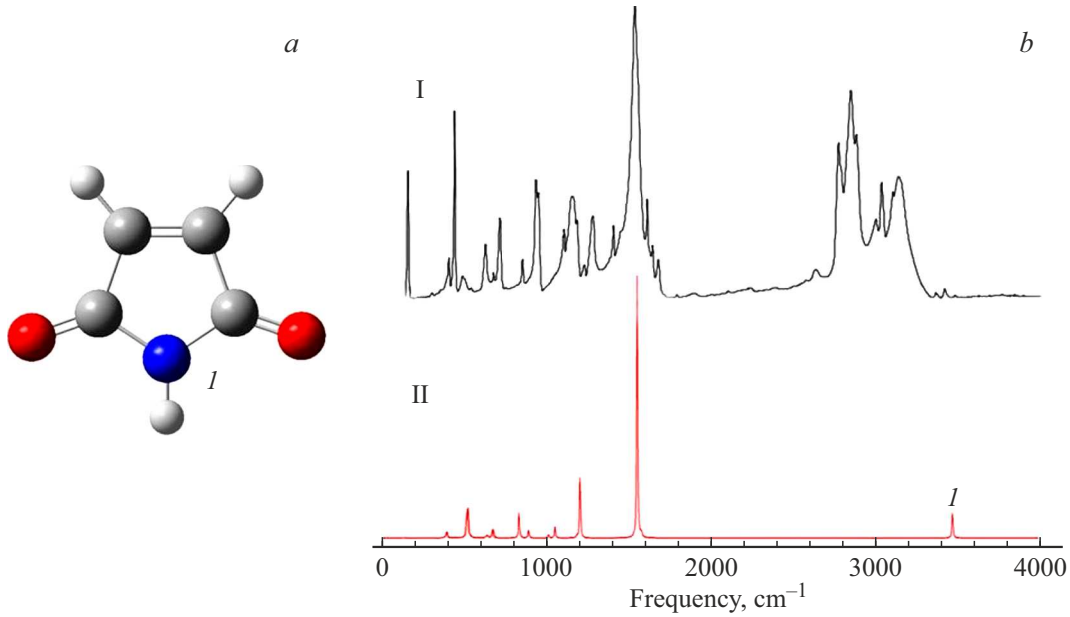

Figure 1. Calculated structure (*a*) and IR spectra of maleimide (*b*): (I) — experimental [22], (II) — calculated. Number *1* shows the −NH bond and its corresponding band in the IR spectrum of maleimide.

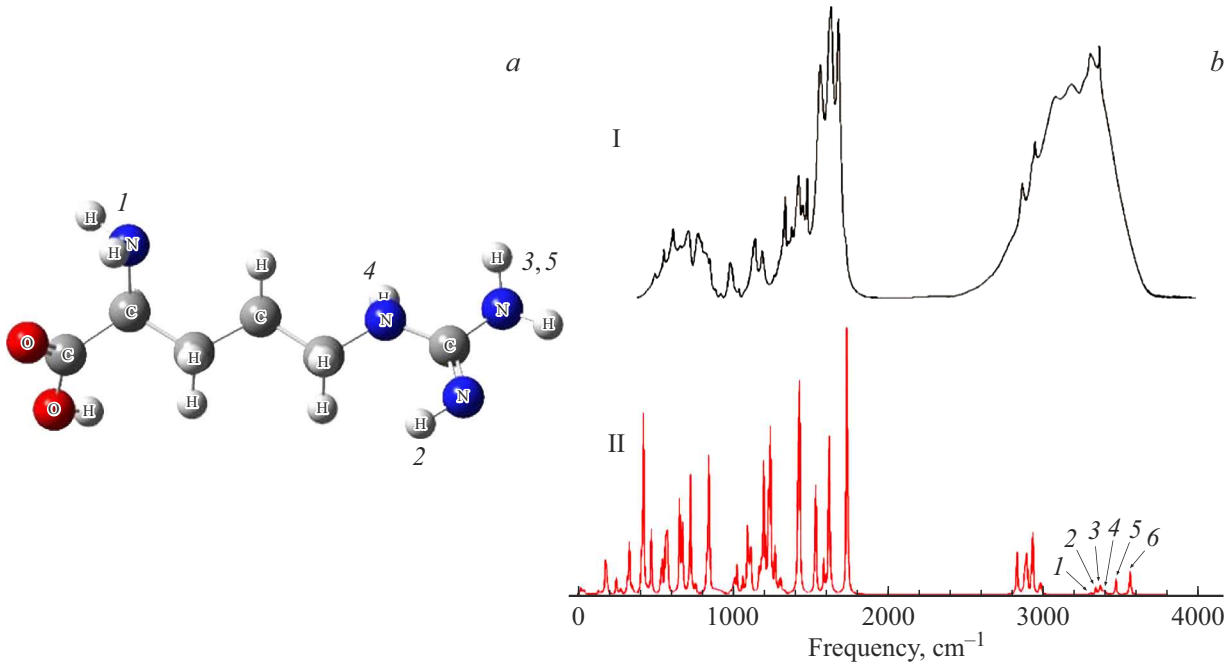

Figure 2. Calculated structure (*a*) and IR spectra arginine (*b*): (I) — experimental [23], (II) — calculated. Numbers show −NH and −OH bonds in arginine and their correspondent bands in the calculated IR spectrum.

Maleimide is produced as a result of dehydration of non-cyclic amide, which is formed by the reaction of maleic anhydride with amines [17]; it is widely used to modify biological objects [18]. For example, in [19] the synthesis of nanogels functionalized with maleimide is described. It has been found that maleimide-modified nanogels exhibit high mucoadhesive properties on conjunctival tissue *ex vivo* compared to the known mucoadhesive chitosan. These results confirm the potential of nanogels containing maleic acid imide as a new platform for sustainable drug delivery. An increase in mucoadhesive properties was also found in [10], where liposomes with maleimide groups demonstrated better retention *in vitro* on the bladder tissue, which is related to their ability to form bonds with thiols present in the mucosal tissue.

Molecular modeling of complexation, including the calculation of structures and spectra of molecules, as well as their complexes, was carried out on the basis of the density functional theory (DFT) method [20] with the use of the B3LYP functional and the 6-31G(d) basis set using the Gaussian software package [21]. The Avogadro and GaussView software programs were used for imaging of molecular structures.

Results and discussion

In the course of study, the structure and IR spectra of maleimide (Fig. 1), arginine (Fig. 2), dextran sulfate (Fig. 3) and glutamine molecules, as well as maleimide-arginine, maleimide-dextran sulfate, arginine-glutamine and arginine-glutamine-maleimide molecular complexes (Fig. 4–7) were calculated.

Let us consider in more detail the intermolecular interaction of layers of the polyelectrolyte capsule — polyarginine and dextran sulfate with maleimide.

Due to the fact that both polyarginine and dextran sulfate are long polymer molecules with repeating fragments, it makes sense to consider the intermolecular interaction of only one link of the polymer chain for a qualitative analysis of the complexation possibilities. This technique will significantly reduce the time and volume of calculations. The backbone of the polyarginine chain is arginine, a basic protein amino acid. IR spectra and the calculated structure of arginine are shown in Fig. 2. In the spectrum of arginine, stretching vibrations of bonds involving the −NH and −OH groups are manifested at frequencies of 3311 and 3372 cm^{-1} (Fig. 2, *b*; peaks *1, 3* — symmetric vibrations), 3342, 3403 and 3563 cm^{-1} (Fig. 2, *b*; *2, 4, 6*), 3472 cm^{-1} (Fig. 2, *b*; *5* — asymmetric vibrations).

IR spectra and the calculated structure of dextran sulfate are shown in Fig. 3. It can be seen that in the spectrum of dextran sulfate, the stretching vibration of the bond involving the −OH group is manifested at a frequency of 3468 cm^{-1} (Fig. 3, *b*; *1*).

To find out the effect of maleimide, numerical modeling of the intermolecular interaction and complexation of arginine with maleimide (Fig. 4, Table 1) and dextran sulfate with maleimide (Fig. 5, Table 2) was carried out.

Table 1–4 shows parameters of the formed bonds, where the bond energy was calculated using the Iogansen's empirical formula [25,26]

$$-\Delta H = 0.3\sqrt{\Delta\nu - 40}. \quad (1)$$

The strength of the formed hydrogen bonds was estimated in accordance with the classification given in [27], where hydrogen bonds are considered strong when their energy is 14.34–28.65 kkal/mol and the hydrogen bridge length is 2.2–2.5 Å, the medium bond energy is in the range of 3.82–14.43 kkal/mol, and the hydrogen bridge length is 2.5–3.2 Å, the energy of weak bonds is less than 2.87 kkal/mol, and the length of the hydrogen bridge is 3.2–4.0 Å.

Let us consider various options for attaching maleimide to polyelectrolyte capsule materials. There are four options for the attachment of arginine: 1) through the −OH group of arginine forming two bonds with −NH and C=O groups of maleimide, 2) through the −CO group of arginine and aminogroup of maleimide, 3) through maleimide and arginine aminogroups, 4) through the −OH group of arginine and −C=O of maleimide. Calculated structures and corresponding IR spectra for all attachment options are shown in Fig. 4. Parameters of hydrogen bonds are shown in Table 1.

As can be seen, in each variant, hydrogen bonds are formed that are classified as medium-strength hydrogen bonds (according to the classification of [27]). This is evidenced by rather large frequency shifts of 422, 263 and 232 cm^{-1} and high band intensities of 1797, 996 and 804 km/mol, respectively.

Thus, a conclusion can be made that arginine interacts quite strongly with maleimide forming stable molecular complexes.

Let us consider the variants of complexation of maleimide with dextran sulfate. Calculations have shown that there are two attachment options: 1) through the hydroxyl group of dextran sulfate and the −C=O group of maleimide, 2) through the −COH group of dextran sulfate and the amino group of maleimide (Fig. 5, Table 2).

With the interaction of maleimide and dextran sulfate two hydrogen bonds are formed (Table 2): 1) O−H···O with a weak strength and 2) N−H···O with a medium strength. This suggests that arginine has a much more pronounced complexation with maleimide compared to dextran sulfate, which leads to the need to form a capsule with an outer shell of polyarginine.

Let us consider the interaction between the outer layer of the capsule (polyarginine) modified with maleimide and the substance of the mucous membrane by way of the example of amino acid, which is most common in the human body and can exist in the free form, i.e. glutamine.

The effect of maleimide attachment to the arginine-glutamine molecular complex is shown in Fig. 6 and 7 and in Tables 3 and 4. Fig. 6 illustrates the calculated structure and IR spectrum of the arginine-glutamine molecular complex, and Fig. 7 shows the same molecular complex but after the maleimide is attached. Tables 3 and 4 show the hydrogen bond parameters of the arginine-glutamine (Table 3) and arginine-glutamine-maleimide (Table 4) complexes.

As the calculations have shown, glutamine and arginine have two options for the attachment: 1) through the amino group of arginine and the −C=O group of glutamine and 2) through −C=O group of arginine and −OH group of glutamine. Parameters of hydrogen bonds are shown in Table 3. Number *1* in Fig. 6 shows spectral peaks corresponding to the formed bond.

In the course of the analysis of hydrogen bond parameters, it has been found that the interaction of arginine with

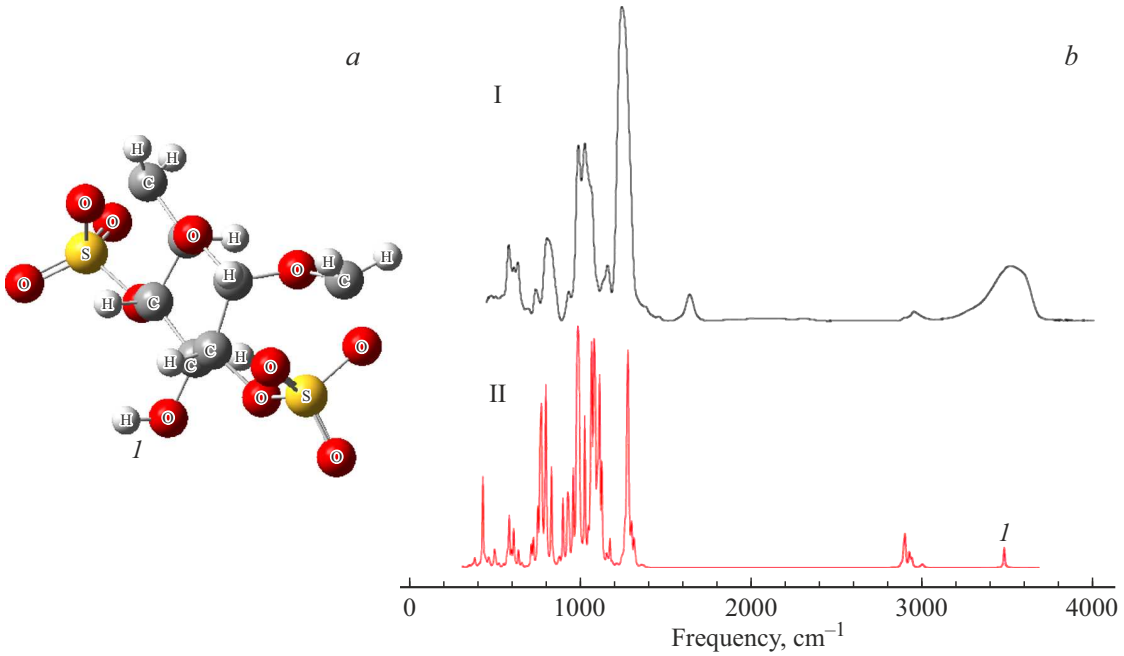

Figure 3. Calculated structure (a) and IR spectra of dextran sulfate (b): (I) — experimental [24], (II) — calculated. Number *1* shows the −OH bond and the band in the calculated IR spectrum corresponding to stretching vibrations of the −OH group.

Table 1. Calculated parameters of hydrogen bonds of the arginine-maleimide molecular complex

Number of variant	Bond type	Length of the H-bond R, Å	Length of the hydrogen bridge R_b, Å	Frequency ν, cm^{-1}	Frequency shift $\Delta\nu$, cm^{-1}	Bond energy $-\Delta H$, kkal/mol	Intensity I_{IR}, km/mol
1	O-H···O	1.84	2.83	3300	263	4.48	540
	N-H···O	2.17	3.19	3369	80	1.9	328
2	N-H···O	194	2.96	3268	181	3.56	996
3	N-H···N	1.86	2.89	3027	422	5.86	1797
4	O-H···O	1.83	2.82	3331	232	4.16	804

Table 2. Calculated parameters of hydrogen bonds of the dextran sulfate-maleimide molecular complex

Number of variant	Bond type	Length of the H-bond R, Å	Length of the hydrogen bridge R_b, Å	Frequency ν, cm^{-1}	Frequency shift $\Delta\nu$, cm^{-1}	Bond energy $-\Delta H$, kkal/mol	Intensity I_{IR}, km/mol
1	O-H···O	1.93	2.91	3389	79	1.87	484
2	N-H···O	1.98	3	3300	149	3.13	497

Table 3. Calculated parameters of hydrogen bonds of the glutamine-arginine molecular complex

Number of variant	Bond type	Length of the H-bond R, Å	Length of the hydrogen bridge R_b, Å	Frequency ν, cm^{-1}	Frequency shift $\Delta\nu$, cm^{-1}	Bond energy $-\Delta H$, kkal/mol	Intensity I_{IR}, km/mol
1	N-H···O	2.08	3.09	3333	52	1.04	437
2	N-H···O	1.9	2.91	3242	130	2.85	900
3	N-H···O	2.14	3.15	3341	44	0.6	188
4	O-H···O	1.78	2.76	3062	242	4.3	790

Table 4. Calculated parameters of hydrogen bonds of the glutamine-arginine-maleimide molecular complex

Number of variant/ bond number	Bond type	Length of the H-bond R, Å	Length of the hydrogen bridge R_b, Å	Frequency ν, cm^{-1}	Frequency shift $\Delta\nu$, cm^{-1}	Bond energy $-\Delta H$, kkal/mol	Intensity I_{IR}, km/mol
1/1	N-H···O	1.98	2.99	3265	120	2.7	835
1/2	N-H···O	1.92	2.93	3301	148	3.12	602
2/1	N-H···N	1.83	2.84	2854	595	7	1311
2/2	O-H···O	1.74	2.72	2976	328	5	1782

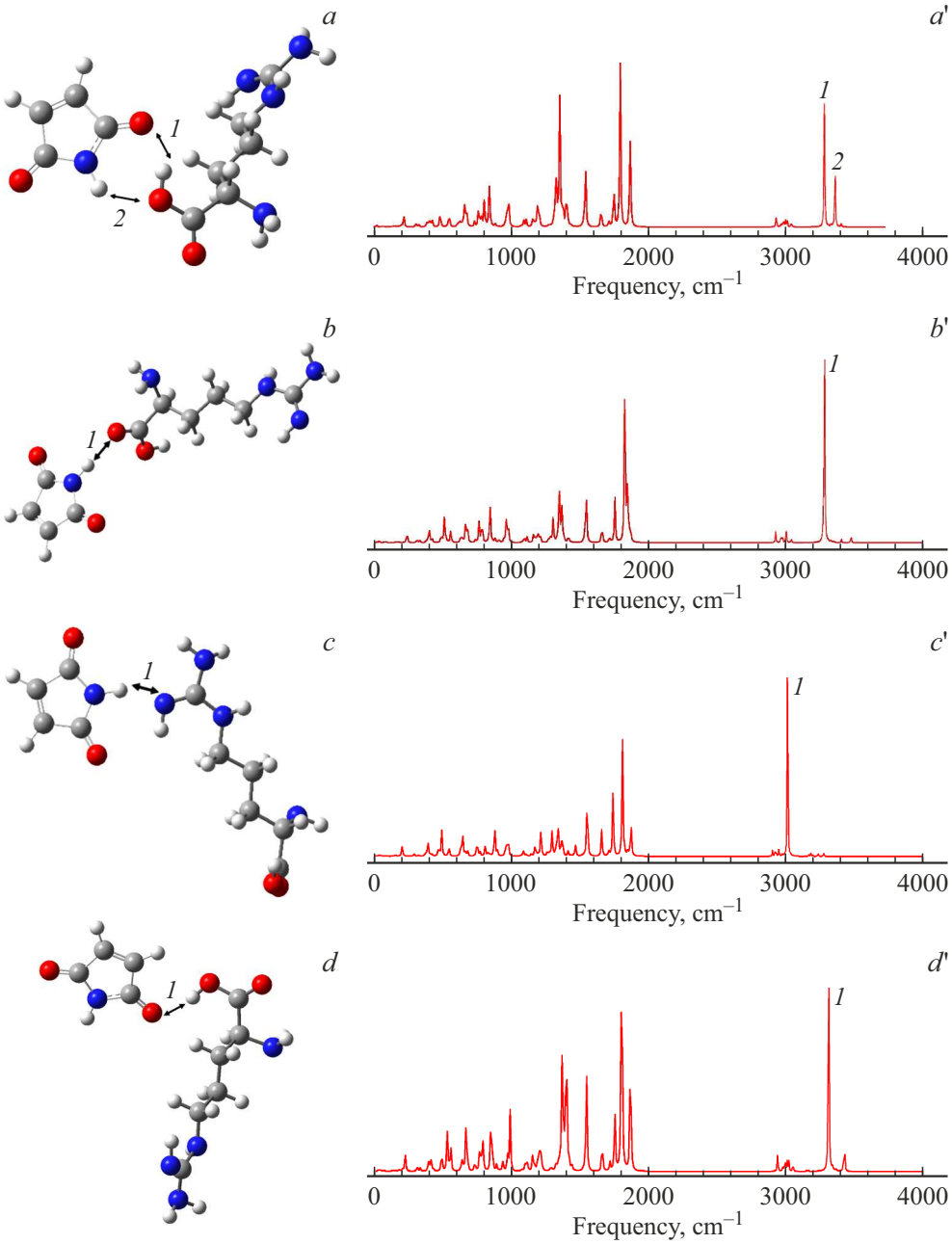

Figure 4. Variants of calculated structures (a, b, c, d) and their corresponding IR spectra of the maleimide-arginine molecular complex (a', b', c', d'). Numbers *1* and *2* near the curves show the formed hydrogen bonds and the bands corresponding to them in the IR spectrum of the complexes.

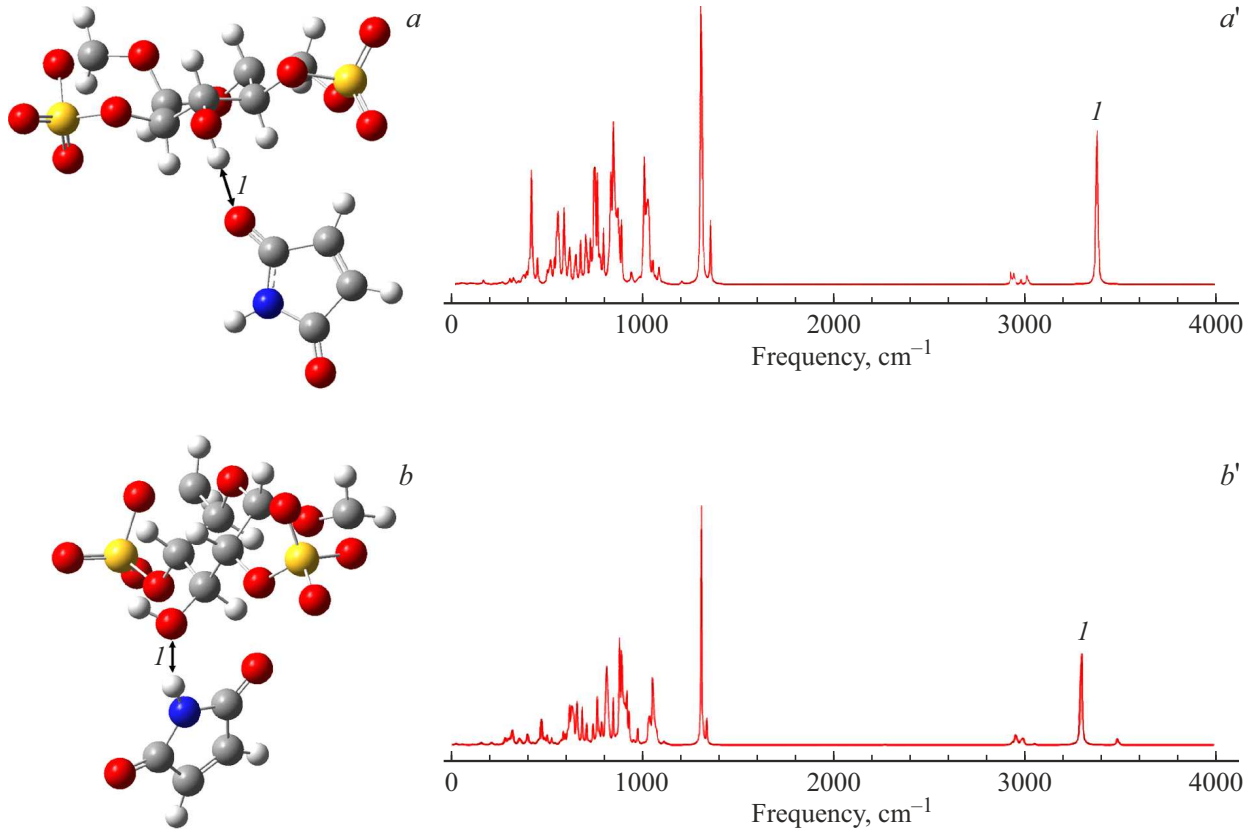

Figure 5. Calculated structures (a, b) and IR spectra of the maleimide-dextran sulfate molecular complex (a', b'). Number *1* shows the formed hydrogen bonds and their corresponding bands in the IR spectrum of the complexes.

glutamine forms medium-strength bonds with energies of 2.85 and 4.3 kkal/mol.

The attachment of maleimide gives an additional peak in the IR spectrum of the formed molecular complex (Fig. 7). It can be seen from the analysis of the calculated structure and IR spectrum that there are two options for the formation of the ternary molecular complex: 1) through amino groups of glutamine and maleimide combined with −C=O group of maleimide and arginine group, respectively, 2) through the bond between the amino groups of arginine and maleimide and the −C=O group of maleimide and −O-H group of glutamine. Table 4 shows parameters of the formed hydrogen bonds.

As can be seen from Table 4, when maleimide is added to the glutamine-arginine molecular complex, it can be seen that the hydrogen bonding of glutamine and arginine in the presence of maleimide is significantly higher than that in the glutamine-arginine complex.

It should be noted that hydrogen bonding is more pronounced when two amino groups are combined and when −O-H groups are combined with −C=O groups. It was found that the strongest were bonds that caused frequency shifts in 595 and 328 cm^{-1} bands with energies of 7 and 5 kkal/mol, related to medium bonds close to strong, and to bonds of medium strength, respectively [27].

When comparing parameters of hydrogen bonding of the glutamine-arginine and glutamine-arginine-maleimide complexes, it can be concluded that the addition of maleimide will promote stronger hydrogen bonding of arginine with glutamine.

Conclusions

As a result of molecular modeling, it was found that maleimide forms hydrogen bonds with elements of the polyelectrolyte capsule and glutamine, an amino acid contained in the human body. In the case of the interaction between maleimide and dextran sulfate, all possible variants of complex formation were considered, in which one medium-strength hydrogen bond and one weak bond were formed, while the interaction with arginine resulted in the formation of three medium-strength hydrogen bonds, one weak and one strong hydrogen bond.

Thus, maleimide has a higher degree of the complexation based on the hydrogen bonding when interacting with arginine than with dextran sulfate. Based on this, it can be concluded that when modifying capsules with maleimide, it is necessary to build the structure of polyelectrolyte capsules with an outer layer consisting of polyarginine for the best fixation of maleimide on the capsule shell.

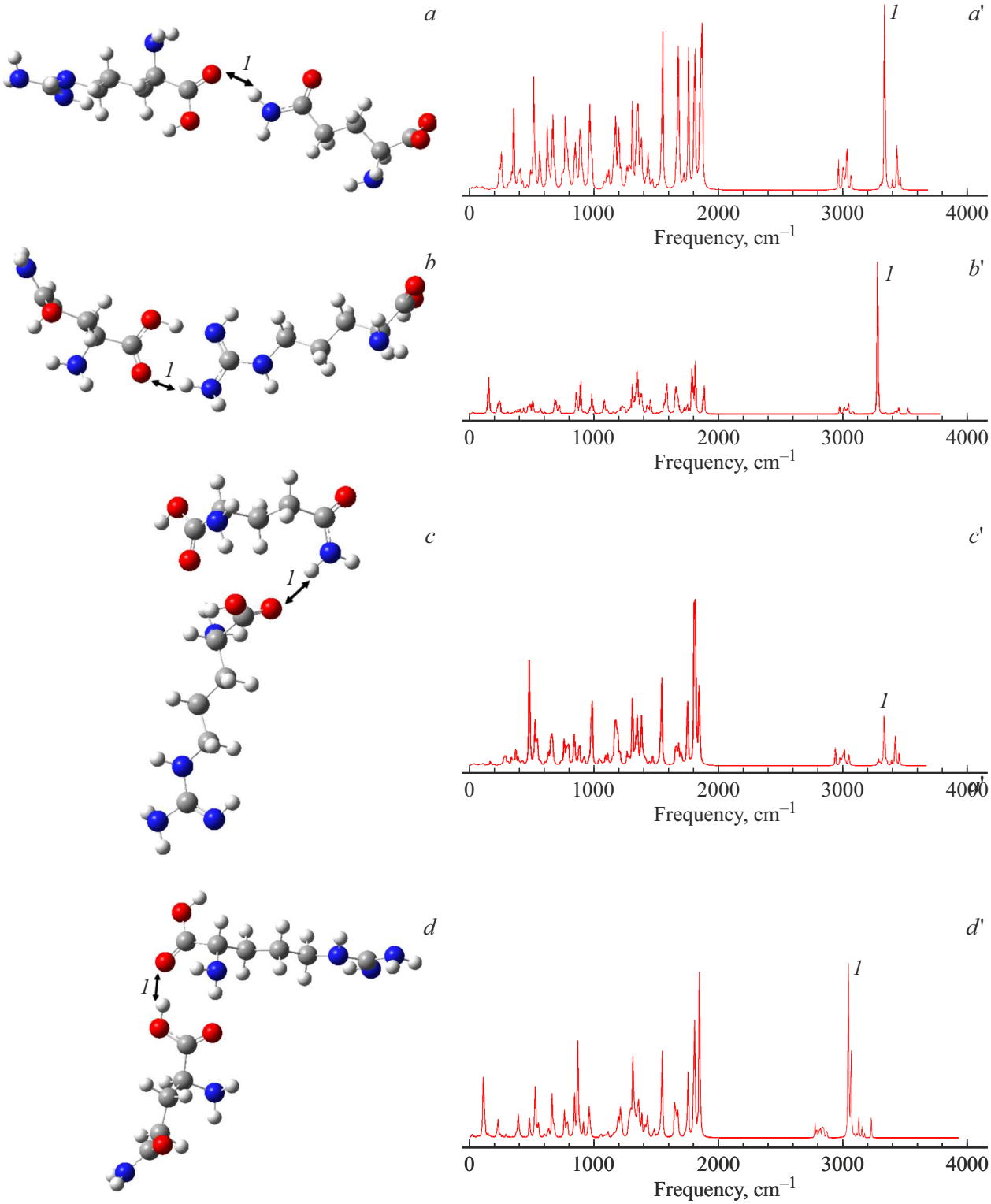

Figure 6. Variants of calculated structures (a, b, c, d) and IR spectra of the glutamine-arginine molecular complex (a', b', c', d'). Number *1* shows the formed hydrogen bonds and their corresponding bands in the IR spectrum of the complexes.

Calculations have shown that the interaction of arginine with glutamine results in the formation of medium-strength hydrogen bonds. After the introduction of the maleimide molecule into the arginine-glutamine molecular complex, the strength of the resulting hydrogen bonds increases significantly. Thus, it may be concluded that the modification of capsules with maleimide is a very effective way of increasing their therapeutic activity.

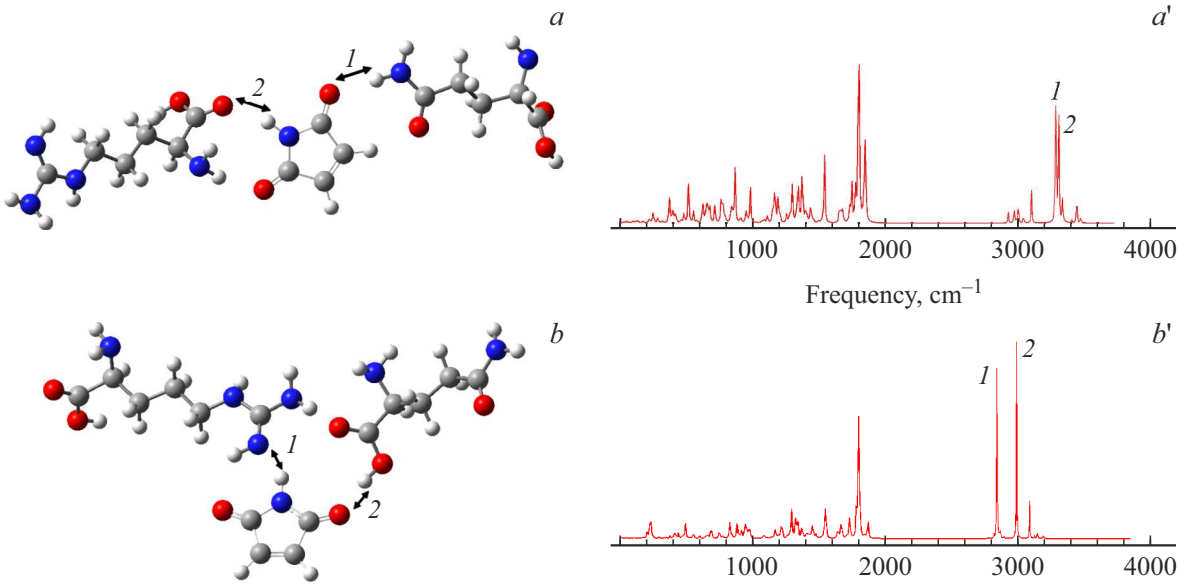

Figure 7. Variants of calculated structures (a, b) and IR spectra of the glutamine-arginine-maleimide molecular complex (a', b'). Numbers *1* and *2* show the formed hydrogen bonds and the bands corresponding to them in the IR spectrum of the complexes.

Compliance with ethical standards

All procedures performed in the study involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the Declaration of Helsinki (1964) and its later amendments or comparable ethical standards. Informed voluntary consent was obtained from each study participant.

Conflict of interest

The authors declare that they have no conflict of interest.

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