

# Changes in the Structure of the Benzylpenicillin Sodium Salt Molecule under the Pulsed Magnetic Field

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**Abstract.** The study aims at the Fourier spectra of an antibiotic exposed to a high-intensity pulsed magnetic field. The applied frequency was  $f = 40$  kHz, with a number of pulses  $n = 1$ , at intensities of the pulsed magnetic field  $H = 0.09 \times 10^6$  A/m,  $H = 0.50 \times 10^6$  A/m,  $H = 0.82 \times 10^6$  A/m. It was assumed that under the conditions of the pulsed magnetic field there might be a change in the structure of the drug molecule. The research has revealed that an increase in the pulsed magnetic field power leads first to a decrease in the intensity of the band in the Fourier spectrum, and then to its increase. In addition, changes were recorded in the amide group of benzylpenicillin sodium salt molecules depending on the intensity of the pulsed magnetic field applied to the antibiotic. © 2021 Journal of Biomedical Photonics & Engineering.

**Keywords:** antibiotic; Fourier spectroscopy; magnetic field intensity; biological activity.

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## 1 Introduction

Currently, the question of the effectiveness of antibiotics has become a matter of great urgency. As a rule, antibiotics are used in the form of solutions, which results in a complex water-antibiotic-biosystem. Here, the problem of the interaction of a water molecule with a solid (powdered) antibiotic comes to the fore. In Ref. [1], it was noted that antibiotics in the form of solid samples (powders) are stable during storage, while those in the form of aqueous solutions quickly lose their biological activity. Therefore, it is important to find out the reason for the increase in the antibacterial activity of the benzylpenicillin sodium salt after being exposed to the pulsed magnetic field (PMF). The structure of a benzylpenicillin molecule (Fig. 1) demonstrates such characteristic features of penicillin antibiotics as the presence of a  $\beta$ -lactam ring, an amide group, and a thiazole cycle.

In Ref. [2], the potential of benzylpenicillin was calculated as an index of the reactivity of the antibiotic. It was shown that during the transition from a neutral penicillin molecule to its anion, the dipole moment

increases from 4.7 D to 18.2 D, and the benzylpenicillin anion molecule takes on a complex semispherical spatial structure. The authors showed that in this case, the carbonyl groups are located on the outer side.

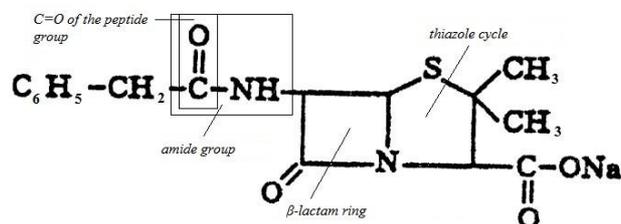


Fig. 1 Structural formula of benzylpenicillin sodium salt.

The authors of study [3] used quantum-chemical calculations to study the interaction of solid samples of benzylpenicillin with water and determined the location of water molecules during the formation of hydrate structures of the antibiotic. In this case, a three-dimensional grid of hydrogen bonds is formed, which includes all the functional groups of the antibiotic.

Jason Potticary et. al. [4] crystallized coronene (polyaromatic hydrocarbon) into another polymorph using the method of growing crystals under the conditions of the magnetic field action. Crystals, grown in a magnetic field with an induction of 1 T, differ in their appearance from crystals grown in a zero field, and retain their structure under environmental conditions. The researchers found the new form to be the most stable at low temperatures. As a result of the new supramolecular configuration, significant changes in the electronic, optical and mechanical properties were noted.

In another similar work, polymorphism during the crystallization of drugs by a magnetic field was investigated (Project №736899, UK, the Netherlands, Ireland). The European researchers have already established that it is possible to synthesize specific polymorphs of pharmaceutical substances, such as carbamazepine, indomethacin, and paracetamol. They found out [5] that concrete crystalline forms could exhibit unique properties that may not be shown by other forms of the same substance.

The researchers of the Kalinin Medical Institute [6] made an attempt to trace the action of the constant magnetic field on barbamil (sleeping substance). Three times, at different times of the year, the experimental batch of mice received a sleeping substance that had been previously exposed to a constant magnetic field. It was observed that under the influence of the “magnetized” sleeping pill the mice slept longer than the animals from the control group that received the same dose of the “non-magnetized” drug.

Thus, the effect of drugs on the organism may depend on the characteristics of drugs: physical properties, chemical structure, dose (concentration), as well as on the medicinal form of the drug. The influence of physical factors on the drug can enhance its biological effect in the organism [7].

In our previous work [8], we found a 12–24% increase in the antibacterial effect of benzylpenicillin sodium salt processed by a pulsed magnetic field with the field intensity ranging from  $0.09 \times 10^6$  A/m to  $1.23 \times 10^6$  A/m at the frequency  $f = 40$  kHz (single-turn inductor) and a number of pulses  $n = 1$ . The pulsed magnetic field action was carried out on a powdered pharmaceutical preparation. Fig. 2 presents the scheme of the PMF action on the drug: inductor 1, pulse current generator 2, PMF sensor 3, and oscilloscope 4. The PMF sensor is connected to the oscilloscope 4. A vial 5 filled with about 4 mm of benzylpenicillin is placed in the middle of inductor 1, after which the pulsed magnetic field treatment is performed.

To determine sensitivity of the bacterium *Escherichia coli* (*E. coli*.) to benzylpenicillin, antibacterial discs were used. The standard disc-diffusion method with sterile dilutions was developed in late 1960s to early 1970s [9] and since then has not undergone any significant changes from a methodological point of view. This method can be used for individual antibacterial therapy to treat the concrete infection in patients, to monitor the spread of antibiotic resistance in a certain area, as well as to study

new chemical compounds for the presence of antibacterial activity [10].

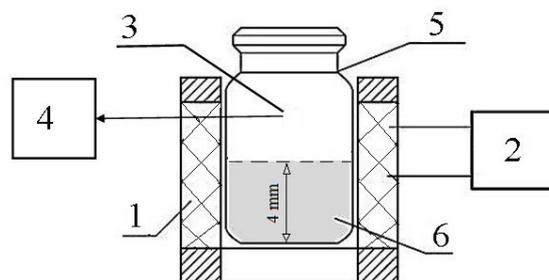


Fig. 2 Scheme of the pulsed magnetic field action on benzylpenicillin.

The study [11] of benzylpenicillin sodium salt in the powdered form (1 g) revealed that after the exposure of benzylpenicillin sodium salt to the pulsed magnetic field, the antibiotic obtained the concentration of 0.01 g/ml. The action of benzylpenicillin was performed on *E. coli* cells with the bacterial culture *E. coli* seeded on the meat-peptone agar. The discs were distributed on the surface of the sown agar by tweezers and then soaked in a solution of benzylpenicillin of 10  $\mu$ l per disc. The cups were then incubated in a thermostat at 37 °C for 18 h.

The antibacterial activity was evaluated by measuring the average diameter of the lysis zone. The measurement was carried out as follows: the cups were placed upside down on a dark matte surface so that the light fell on them at an angle of 45° (accounting for the reflected light), after which the diameter of growth retardation zones was measured using calipers (the measurement error is  $\pm 0.1$  mm). The results obtained during the experiment were checked for reliability using the Student’s criterion (the level of statistical significance  $< 0.05$ ). The diameter of each lysis zone was measured at least 10 times, after which the average diameter was calculated. To increase the reliability of the results, independent series of repeated experiments were conducted.

The increase in antibacterial activity of benzylpenicillin sodium salt after PMF processing discovered in of this study can be explained by the transition of the PMF-processed molecule to an excited state when its conformation changes slightly. It was also found that the biological effect of the PMF action on drugs decreases with time, being maximally manifested within the day. PMF treatment of benzylpenicillin sodium was not accompanied by an increase in its acute toxicity or emergence of free radicals.

The aim of the work is to study the structural changes in a molecule of benzylpenicillin sodium salt after exposure to a pulsed magnetic field.

## 2 Experimental Procedure

The study of benzylpenicillin sodium salt was carried out at the PMF parameters (Table 1) with the frequency of  $f = 40$  kHz. The exposure time of the test drug to the PMF was 0.0002–0.005 s [12, 13].

Table 1 Parameters of the PMF action on benzylpenicillin sodium salt.

W (energy), kJ	0.45	2.49	4.11
H (intensity), 10 <sup>6</sup> A/m	0.09	0.50	0.82
f (frequency), kHz	40		

Control samples of the drug were not exposed to the PMF. To register the spectra, the powder was thoroughly ground with KBr in an agate mortar at the ratio of 1:100. The test mixture was placed in a cuvette in a special holder, after which the diffuse reflection spectra were recorded.

As is well-known, the most informative regions of the Fourier spectra are the medium-frequency (1000–1970 cm<sup>-1</sup>) and high-frequency (3200–3800 cm<sup>-1</sup>) regions. The spectra were recorded using a FSM-2201 Fourier spectrophotometer (Infaspec, Russia) in the wavenumber range from 470 cm<sup>-1</sup> to 5000 cm<sup>-1</sup>.

The studied spectra were recorded with a spectral resolution *Fspec* software (*Monitoring*, Russia). The limit of the permissible absolute error of the wavenumber scale on the device is ±0.05 cm<sup>-1</sup>, the error of measuring the absorption intensity is 0.05%.

### 3 Results and Discussion

Figs. 3 and 4 present spectra of benzylpenicillin as a result of the PMF action for two regions of the spectrum. In the region of N–H valence oscillations, the following bands are observed: 3320 cm<sup>-1</sup>, 3355 cm<sup>-1</sup>, 3369 cm<sup>-1</sup> (Fig. 3).

Table 2 presents the changes recorded in the Fourier spectrum when the antibiotic was exposed to the PMF. The intensities of the bands 3320 cm<sup>-1</sup> and 3369 cm<sup>-1</sup> decrease in proportion to the magnetic field intensity. The intensity of the 3355 cm<sup>-1</sup> band first decreases slightly (by about 3%) and then returns to its original state.

Fig. 4 presents the bands of absorption of the pharmacy antibiotic in the carbonyl region.

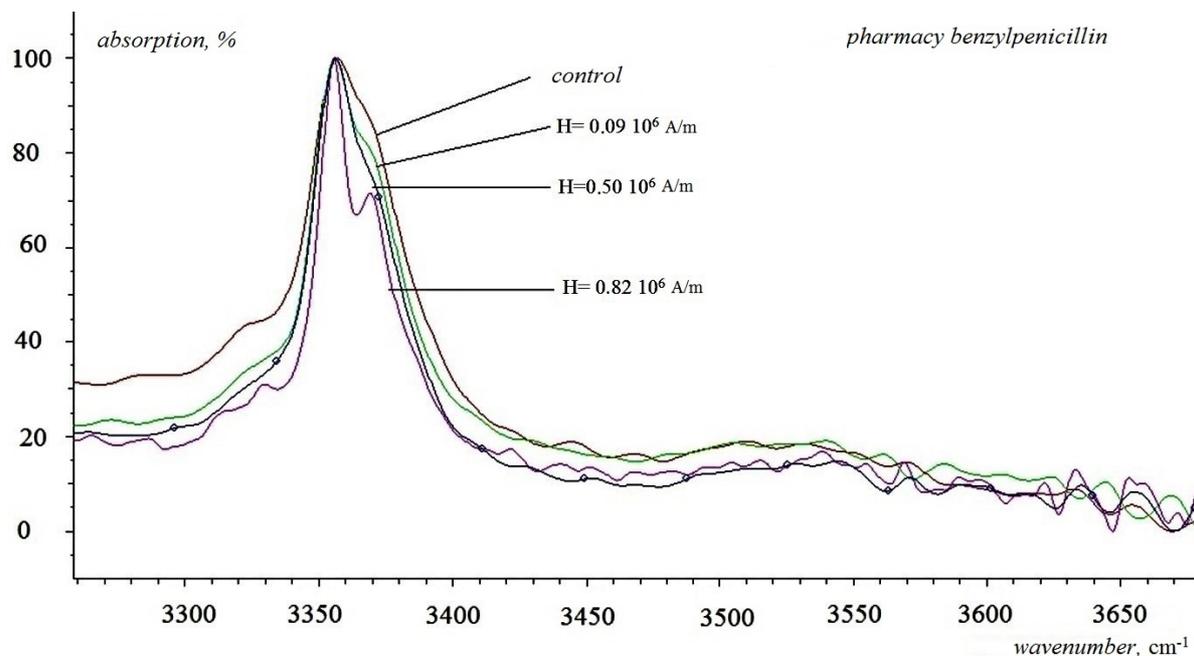


Fig. 3 Fourier spectra of the antibiotic after the PMF processing.

Table 2 Changes in the Fourier spectrum under the PMF action on benzylpenicillin.

Band, cm <sup>-1</sup>	Control	Processing at intensity H = 0.09 × 10 <sup>6</sup> A/m	Processing at intensity H = 0.50 × 10 <sup>6</sup> A/m	Processing at intensity H = 0.82 × 10 <sup>6</sup> A/m
3355	100	96.7	96.7	100
3369	85.6	80.6	73.8	70.4
3320	42.6	33.5	29.6	27

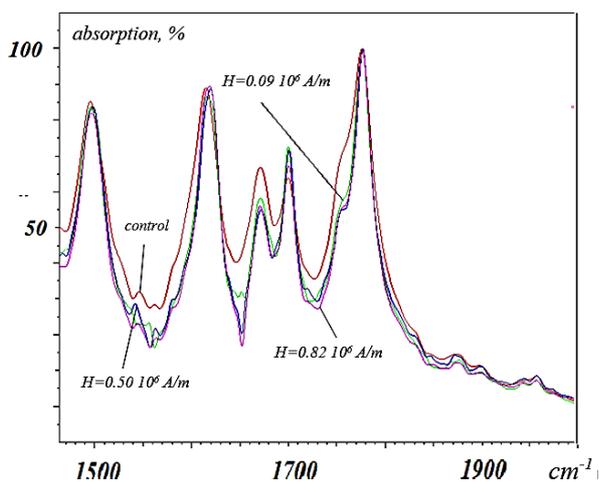


Fig. 4 Absorption bands in the carbonyl region of the Fourier spectrum of pharmacy antibiotic.

The  $1700\text{ cm}^{-1}$  region displays the bands that correspond to valence oscillations  $\text{C}=\text{O}$  of the peptide group ( $1698\text{ cm}^{-1}$ ), oscillations  $\text{C}=\text{O}$  of the group attached to the  $\beta$ -lactam ring (the  $1773\text{ cm}^{-1}$  band), the  $1672\text{ cm}^{-1}$  band belongs to the complex oscillation of the amide group.

The following conclusions can be drawn from the given spectra:

1. Intensity of the  $1773\text{ cm}^{-1}$  band does not depend on the PMF processing of the drug.
2. Intensities of the  $1672\text{ cm}^{-1}$  and the  $1699\text{ cm}^{-1}$  are redistributed depending on the power of the PMF action: the intensity of the high-frequency band increases, and the intensity of the low-frequency band decreases.

In our previous work [14], we found that the enhancement of the biological effect from the PMF action on the antibiotic disappears after about 24 hours. The Fourier spectra were studied for the effect of the repeated exposure to the PMF.

The spectra of the powdered benzylpenicillin in potassium bromide were studied under the following experimental conditions:

- sample 1 was exposed to the PMF once, i.e. the spectra were recorded after 24 hours;
- sample 2 was exposed to the PMF twice with a 24 h interval, i.e. the spectra were recorded after 48 h;
- sample 3 was exposed to the PMF three times with an interval of 24 h, i.e. the spectra were recorded after 72 h.

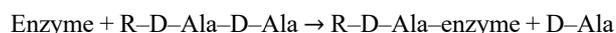
When processing the powdered antibiotic by the pulsed magnetic field with the intensity  $H = 0.09 \times 10^6\text{ A/m}$ , it was noticed that in the region of  $\text{N}-\text{H}$  valence oscillations, the band intensity in the  $3400\text{ cm}^{-1}$  region decreases 24 and 48 h after the PMF action on the same drug compared to the unprocessed drug, and the maxima become more distinct. In this case, the band of  $3470\text{ cm}^{-1}$  decreases in intensity more substantially at PMF intensities  $H = 0.09 \times 10^6\text{ A/m}$  and  $H = 0.50 \times 10^6\text{ A/m}$ . Apparently, there is a break in the

hydrogen bonds with the water molecules contained in KBr. In the low-frequency region of the spectrum, no changes are observed. At intensity  $H = 0.82 \times 10^6\text{ A/m}$ , a decrease in intensity of bands of  $3335\text{ cm}^{-1}$  and  $3370\text{ cm}^{-1}$  was observed in the region of oscillations of the carbonyl group [15, 16].

When the drug was re-processed with the PMF on the second day, a change in the intensity of the bands of the Fourier spectra was observed, and when the PMF was applied to the antibiotic twice for a period less than 48 hours, the band intensity also decreased. Thus it can be assumed that the repeated PMF treatment of the same drug has an effect on the intensity of oscillations. For example, when comparing the  $3335\text{ cm}^{-1}$  band without the PMF action to a two-time exposure, the intensity decreases by about 13%. It is assumed that after the high-intensity PMF action, the charge accumulates in the drug, and after the elimination of the magnetic field, the effect gradually disappears since, as mentioned above, the time of retention of the PMF effect is about 24 h.

According to the results obtained, the changes in the spectra of benzylpenicillin sodium salt suggest that the hydrogen bonds play a decisive role in the change in biological activity. The recorded changes in the functional groupings of the antibiotic may be due to the fact that when the drug molecule is placed in the pulsed magnetic field, the orientation of the molecule changes relative to the direction of the applied PMF.

It is well-known that penicillins cause the death of microorganisms by blocking the inclusion of acetylmuram peptides in the growing cell walls of bacteria acting mainly on the enzyme D-D-transpeptidase, which cleaves the terminal D-alanine from N-phcilmuramylpentapeptide and catalyzes the formation of cross-links. Therefore, the antibacterial activity of  $\beta$ -lactam antibiotics is primarily associated with the suppression of the activity of transpeptidase. D-alanyl-D-alanine plays the role of an identification mark for the enzyme. Another factor is acylation of transpeptidase by peptidoglycan, accompanied by the loss of one D-alanine molecule:



Then transpeptidase is regenerated binding the terminal residue of D-alanine of the peptidoglycan molecule to a chain of four glycine molecules (connected to the lysine residue), the second molecule of peptidoglycan. Penicillins having groupings similar to D-alanyl-D-Alanine in the region of the  $\beta$ -lactam ring (Fig. 5) irreversibly inhibit transpeptidase, which causes the death of *E. coli* cells.

Therefore, changes in the valence angles of the amide grouping near the  $\beta$ -lactam ring after exposure to the pulsed magnetic field can cause minor changes in the structural conformation of the benzylpenicillin molecule and contribute to an increase in the affinity to the active centre of the enzyme, thereby increasing the antibacterial activity of the drug.

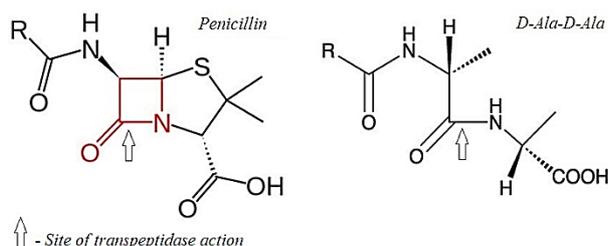


Fig. 5 Comparison of the molecular structure of penicillin and D-alanyl-D-alanine.

#### 4 Conclusion

An increase in power of the magnetic field action on benzylpenicillin sodium salt leads to a decrease in the intensity of the  $3320\text{ cm}^{-1}$  and the  $3369\text{ cm}^{-1}$  bands. Changes in the amide group were recorded depending on the application of the PMF to the antibiotic. Perhaps, the changes are produced by the geometry of the molecule.

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Changes in the structural conformation of the benzylpenicillin sodium salt molecule, processed by the pulsed magnetic field, could contribute to an increase in the affinity to the active centre of the enzyme of transpeptidase, thereby possibly increasing the antibacterial activity of the drug. The observed changes in the spectra of benzylpenicillin sodium salt after PMF processing are small, so further studies are required to study the PMF action on the structure of the antibiotic and its antibacterial activity.

#### Disclosures

All authors declare that there is no conflict of interests in this paper.

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