

Antibacterial Activity of Benzyl Penicillin Water Solutions With Silver

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ABSTRACT

It is established that combination of silver and penicillin allows solving the vital tasks of improving the antibiotic's efficiency and creating a medication, to which bacteria would not adapt at certain concentrations of silver in the solution. The preparations with silver nano-particles and benzyl penicillin have lower antibacterial activity in comparison with the ionic form silver complexes, but the antimicrobial activity of preparations with silver nanoparticles becomes less dependent on the substrate's concentration and nature. Taking into account the low toxicity and the marginal impact of the substrate on such preparations' activity, the application of benzyl penicillin – silver nano-particles complexes is feasible.

Keywords: benzyl penicillin water solutions, antibacterial activity, silver

1. INTRODUCTION

Penicillin is a pharmaceutical product used for treating diseases, caused by cocci and certain anaerobic rod bacteria. The advantage of penicillin consists in the fact that it is one of the least toxic antibiotics, used in medical practice. It will suffice to mention that of several thousands of patients, which were given penicillin, only a few had toxic responses: temperature response of four patients, neurotoxic responses from the central nervous system of two patients and the local response from oral musoca of one patient [1]. At the same time, there are observed cases of allergic reactions to the repeated administration of penicillin or its derivatives. The extreme example of allergy is anaphylactic shock after intramuscular injection of penicillin, which sometimes causes death. Apparently, the application of penicillin

group medications has not yet exhausted its potential, especially in combination with other chemotherapeutic preparations.

The penicillin group is produced by various species of penicillium mold fungi (Penicilliumchrysogenum, Penicilliumnotatum). As a result of fungi's life activity various forms of penicillin can be obtained, where **R** is a radical of various natures (1–6):

Radicals (1–6) are notated with letters *G, X, V, F, K, O*, and the medications are called respectively benzylpenicillin, *p*-hydroxybenzyl-penicillin, phenoxymethylpenicillin 2-pentenyl-penicillin, *n*-heptyl-penicillin, allylmercaptomethylpenicillin.

The antimicrobial range of benzylpenicillin is presented in Table 1 [2]. By its pharmacology type penicillin is a bacteriostatic, and in certain concentrations a bactericidal antibiotic. Various types of penicillins have various degree of bioactivity.

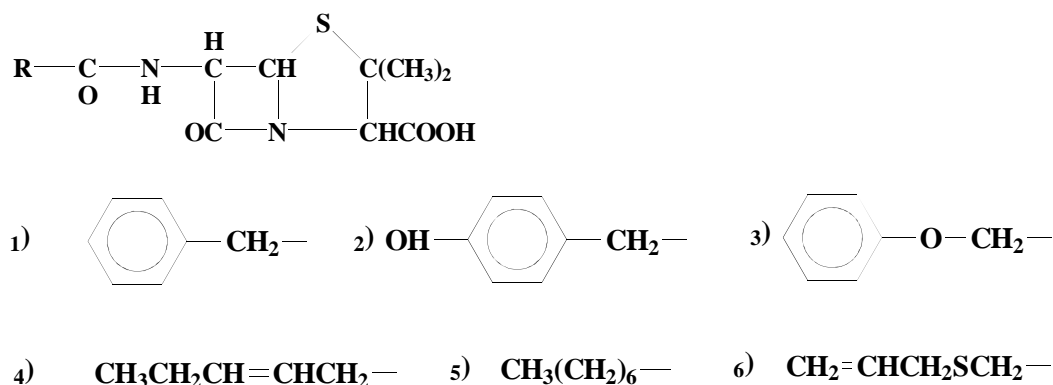


Figure 1 Chemical diagrams

Table 1 Antimicrobial range of benzylpenicillin

Microorganism	Minimal inhibitory concentration, mg/l
<i>Streptococcus pyogenes</i>	0.004–0.03
<i>S. pneumoniae</i>	0.006–0.06
<i>Staphylococcus aureus</i>	0.006–0.30
<i>Clostridium tetani</i>	0.02–0.30
<i>Corynebacterium diphtheriae</i>	0.02–0.30
<i>Streptomyces israeli</i>	0.06–0.30
<i>Nocardia asteroides</i>	30–100
<i>Escherichia coli</i>	20
<i>Klebsiella pneumoniae</i>	100

The penicillin-sensitive microorganisms rather quickly and easily develop resistance to this antibiotic. Thus, *S. aureus* stops its growth at the penicillin concentration equal to 0.06 mg/l at average, but after 20 sequential subculturings with gradually increasing concentrations of the antibiotic the resistance of staphylococcus increases by 700 times. To stop the bacteria's growth the penicillin concentration equal to 42 mg/l is required, but after 40 subculturings their resistance increases by over 5500 times. The resistance of microorganisms' strains to the penicillin group is explained by their ability to produce specific enzymes – beta-lactamase (penicillinase), which hydrolyzes the beta-lactam ring of penicillins, depriving them of their antibacterial activity. Microorganisms, which have developed resistance to a certain type of penicillin, are usually resistant to other types of penicillin as well. The penicillin resistance of bacteria is accompanied with the ability to form such enzyme as penicillinase. Sometimes microorganisms lose their virulence simultaneously with obtaining penicillin resistance. But their virulence is restored after several passages through animals, and their resistance to the antibiotic stays as well. So, of considerable scientific and practical interest are such research works, which study the antibacterial range of several medications at once, for example, silver and antibiotics. The application of silver is explained by the fact that despite its long usage the resistance of bacteria to silver is virtually not observed.

In most research works, carried out in various spheres of medicine and dealing with the usage of silver, the metal and various chemotherapeutic agents are contradistinguished. As a rule, the researchers note the negative or positive characteristics of silver preparations – their wide antibacterial range, low concentration limits of biological action, no allergic reactions. Actually, contradistinction of silver medications and other chemotherapeutic agents is a methodologically wrong

approach, hindering the creation of new efficient medications for treating various diseases. Silver is a unique element of the periodic system, having unconventional biological activity. The special biological properties of silver are the result of its atoms' electron shell structure. The element can form stable compounds with functional groups, containing sulfur, nitrogen, phosphorus and oxygen. Strong chemical bonds and the ability to form complex compounds with proteins, enzymes and other substances, which influence the living organisms, are preconditions for regulating physical, chemical and biological properties of medical products. Combination of silver with various organic compounds, having biological properties, is probably the basis of creating new efficient medications. The successful achievements in antibacterial medications pharmacology can be already noted – such as using the combination of polyvinyl-pyrrolidone with silver, known under the trade name Poviargol [3–8].

There are research works in which attempts to eliminate the penicillin resistance of microorganisms have been taken by using combinations of silver ions with antibiotics [9]. The essential fault of such complexes is that they are quickly decomposed, losing their antibacterial activity. So, we have studied the antibacterial activity of solutions of silver nitrate and silver nano-particles with benzyl penicillin.

2. OBJECTS AND METHODS OF RESEARCH

To determine the antibacterial activity of combination medications made of silver and benzyl penicillin the photocolometric and potentiometric analysis methods were used. The experiments with benzyl penicillin and silver nitrate solutions were carried out with strains of *E.*

Coli. The antimicrobial range of benzyl penicillin at various concentrations of colloidal silver was studied in strains of *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *B. cereus*. The culture media were prepared in accordance with the methodology, laid out in the microbiological and virological research methods manual [10]. As a growth medium the meat-peptone broth, fish-peptone broth and agar-agar were used, pH 7.0–7.2. Microorganisms with concentration 50 units per 1 ml were placed into the nutrient solution, containing the preparation under study, and the optical density of the solution at wavelength 600 nm was measured. The solutions were thermo-stated at temperature 36 °C. The solutions' optical density alteration with time was studied automatically by means of «Specol» spectrophotometer. The silver particles in solutions were of average particle size 20–60 nm. The silver particles' size was determined by Heller's calibration curve. The particles were stabilized with polyvinyl-pyrrolidone of molecular weight 30000 r.u. in concentration 1 g/l. Potentiometric studies were carried out by means of a digital voltmeter V7/23 with input impedance no less than 1010 ohm. As a reference electrode a saturated silver-chloride electrode was used.

3. RESULTS AND DISCUSSION

In Tables 2–4 the alteration of the optical density of culture media with strains of *E. coli* at various concentrations of silver ions – electrolyte $AgNO_3$ – is presented. The dependence of silver ions' activity on the protein phase concentration in meat-peptone broth should be pointed out. Thus, at protein phase concentration 2 g/l, the minimal inhibitory concentration amounted to about 12 mg/l, and the bacteriostatic concentration – to 1–4 mg/l. At reducing the protein phase in the solutions under study up to 0.2 g/l the minimal inhibitory concentration and the bacteriostatic concentration reached 0.6–1 and 2 mg/l respectively. The high amount of protein in the solution reduces the activity of silver ions due to the formation of complex metal and protein compounds.

The antibacterial activity of metal depends on protein compounds' nature. In the fish nutrient broth the minimal inhibitory concentration of silver amounted to 0.8 mg/l. Probably, the meat-peptone broths contain a higher amount of organic sulfur compounds, which reduce the solution's activity.

Table 2 Optical density alteration of the studied solutions of *E. coli* strains at various silver concentrations. Protein phase and other components content in the meat-peptone broth is 2 g/l

N	Ag ⁺ , mg/l	Time, hours; alteration of optical density, ΔD				
		0	21	46	90	120
1	0.00	0.00	0.30	0.38	0.58	0.64
2	1.0	0.00	0.20	0.30	0.36	0.36
3	2.0	0.00	0.16	0.22	0.29	0.32
4	4.0	0.00	0.16	0.20	0.25	0.30
5	6.0	0.00	0.14	0.18	0.23	0.28
6	12	0.00	0.00	0.00	0.00	0.00

Table 3 Optical density alteration of the studied solutions of *E. coli* strains at various silver concentrations. Protein phase and other components content in the meat-peptone broth is 2 g/l

N	Ag ⁺ , mg/l	Time, hours; alteration of optical density, ΔD				
		0	21	46	90	120
1	0.00	0.00	0.10	0.16	0.18	0.24
2	0.60	0.00	0.00	0.14	0.18	0.23
3	1.00	0.00	0.00	0.08	0.09	0.16
4	1.80	0.00	0.00	0.00	0.00	0.00
5	2.60	0.00	0.00	0.00	0.00	0.00
6	4.00	0.00	0.00	0.00	0.00	0.00

Table 4 Optical density alteration of the studied solutions of *E. coli* strains at various silver concentrations. Protein phase content in the fish nutrient broth is 1 g/l.

N	Ag ⁺ , mg/l	Time, hours; alteration of optical density, ΔD				
		0	21	46	90	120
1	0.00	0.00	0.10	0.26	0.28	0.30
2	0.20	0.00	0.00	0.24	0.30	0.38
3	0.40	0.00	0.00	0.20	0.39	0.42
4	0.80	0.00	0.00	0.00	0.00	0.03
5	1.60	0.00	0.00	0.00	0.00	0.00
6	2.00	0.00	0.00	0.00	0.00	0.00

The carried-out research allows making a conclusion that the antibacterial activity of both silver ions and the medications under study depends on the composition of the solution. The dependence of the ions' antibacterial activity on composition and concentration of the solution components is one of the drawbacks of silver in its ionic form.

Establishing concentrations of silver in its ionic form for preparations under study was of interest. Potentiometric method of registering complex silver and antibiotic compounds is rather simple and highly sensitive. Thus, the potential of a silver electrode depends on the activity of silver ions C : $\varphi = \varphi^{\circ} + 0.059 \lg C$. Taking into account that the standard potential φ° is equal to 0.799 V, we can rewrite the Nernst equation as follows: $\varphi = 0.799 + 0.059 \lg C$. According to the results in Fig. 1 we can calculate the equilibrium constants of silver and antibiotic complex

compounds, as well as calculate thermodynamic parameters (alteration of entropy, Gibbs free energy, and enthalpy) by the dependence of equilibrium constant of silver and antibiotic complex on the temperature.

The interaction of silver solutions and antibiotics is complicated, which is noted in the work [9]. Probably, for preparations of the penicillin group at the first stage the associated complexes of silver with antibiotic molecules are formed, Figure 2.

This bond is loose, but gradually silver sulphides are formed, stabilized by organic compounds. The complex compounds are registered by the potentiometric method using a silver electrode. After 60–80 hours, depending on the reacting substances' concentrations, a colloidal solution is formed, containing the mix of silver sulphide and firm complex organic compounds, the structure of which requires detailed study and specification.

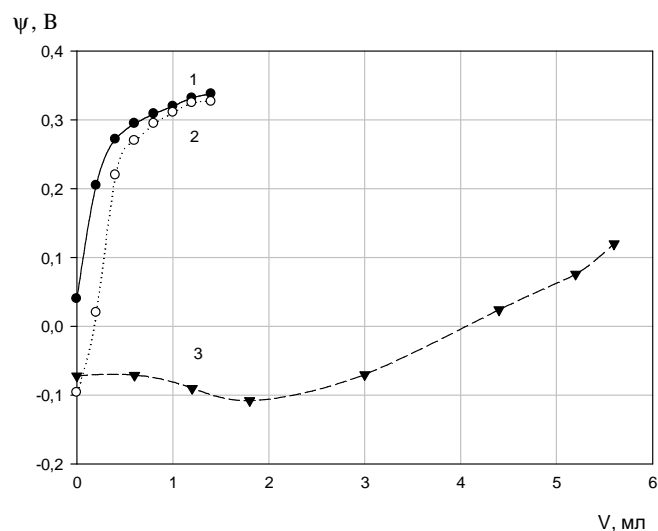


Figure 2 Titration of 20 cm³ of benzylpenicillin (2.5 · 10⁻³ mol/l) with silver nitrate solution (4 · 10⁻³ mol/l). Potentials with respect to the saturated silver-chloride electrode. 1 – AgNO₃ – H₂O; 2 – benzylpenicillin – AgNO₃, T=293 K; 3 – benzylpenicillin – AgNO₃, T=353 K

The microbiological activity of benzyl penicillin to the studied strains of *E.coli* makes up for the minimal inhibitory concentration and for bacteriostatic concentration 200...300, 60...90 mg/l respectively. Comparing these ranges with the data, given in other sources [1], one may note the discrepancy of these values. Probably, the difference is caused by the different resistance of strains under study to the preparation.

Adding Ag^+ in amount of 0.5 mg/l results in bacteriostatic effect at benzyl penicillin concentration of 10 mg/l and the microorganisms' growth is totally suppressed at 25 mg/l of benzyl penicillin (The protein phase content in the solution is 2 g/l). What is important, the subculturing of strains up to 10 times doesn't cause the adaptation of strains to the combination medications based on silver and benzyl penicillin.

Microbiological activity is demonstrated not only by the associated complexes of silver and benzyl penicillin, but also by decomposition products of these substances. As pointed out above, after storing the solutions for 60-80 hours or after heating them up to 70-90 °C a colloidal

solution is formed stabilized with decomposition products of the antibiotic. At the components ratio 1:1 the minimal inhibitory concentration of the colloidal solution is 60 mg/l, and its bacteriostatic concentration is 15 mg/l.

The usage of solutions, containing Ag^+ ions, is not always feasible due to several reasons. Firstly, silver and penicillin preparations are unstable and after some time become decomposed. Secondly, the preparation activity depends on the substrate concentration, particularly on the protein phase content in the solution, on its ion composition. The above-mentioned drawbacks are eliminated by using colloidal solutions of silver.

The stabilized mixture of benzyl penicillin and silver nano-particles is stable within 2-3 weeks and demonstrates antimicrobial activity. The minimal inhibitory concentration of the antibiotic at silver content 1 mg/l is equal to 20-30 mg/l; the bacteriostatic concentration is 10 mg/l. The subculturing of strains up to 10 times does not increase the resistance of bacteria.

The research findings with various strains are presented in Table 5.

Table 5 Antimicrobial range of benzylpenicillin at various concentrations of colloidal silver

Microorganism	Silver nanoparticles content, mg/l	Minimal inhibitory concentration, mg/l
Staphylococcus aureus	5	0.01-0.10
Escherichia coli	5	1-8
Klebsiellapneumoniae	2	10-20
B. cereus	10	2-10

4. CONCLUSION

- The combination of silver and penicillin allows solving the vital tasks of improving the antibiotic's efficiency and creating a medication, to which bacteria would not adapt at certain concentrations of this metal in the solution.

- The antimicrobial activity of preparations becomes less dependent on the substrate's concentration and nature.

- The preparations with silver nanoparticles have lower antibacterial activity, but taking into account the low toxicity and the marginal impact of the substrate on the preparations' activity, the application of benzyl penicillin – silver nano-particles complexes is feasible.

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REFERENCES

- [1] N.S. Egorov, Basics of antibiotics science, MSU publ. office, Moscow, 1994.
- [2] V.P. Baskakova, Pharmacology, Vysha shkola, Kiev, 1981.
- [3] G.E. Afinogenov, V.V. Kopeykin, Factors, influencing the bacteriostatic activity of silver colloids, *Applicat. of silver preparat. in med.* 3 (1994) 51-53. CII SB RAMS, Novosibirsk.
- [4] K.M. Krylov, V.D. Badikov, V.V. Kopeykin, Clinical-bacteriological efficiency evaluation of a new antiseptic – Poviargol, *Applicat. of silver preparat. in med.* 3 (1994) 54-55. CII SB RAMS, Novosibirsk.
- [5] A.G. Afinogenova, A.V. Rusak, G.E. Afinogenov et al., Eye drops on the basis of a new silver-containing antiseptic – Poviargol, *Silver in med., boil. and technol.* 5 (1996) 214-218. CII SB RAMS, Novosibirsk.
- [6] A.M. Gnetnev, B.Ya. Pozdnyakova, I.A. Emelyanova et al., Research findings of the bactericidal action of Poviargol in comparison with other detergents,

- Applicat. of silver preparat. in med. 3 (1994) 56–63. Clin. Immunol. Instit. of SB RAMS, Novosibirsk.
- [7] R.D. Liberzon, A.M. Gnetnev, B.Ya. Pozdnyakova, Experience of using Poviargol and ultrasound for anaerobic wound infection treatment, *Silver in med. and technol.* 4 (1995) 105–108. CII SB RAMS, Novosibirsk.
- [8] A.M. Gnetnev, A.S. Kolmykova, Prospects of integrated use of Poviargol and ozone for fighting hospital-acquired infections in surgical hospitals, *Silver in med. and technol.* 5 (1996) 80–83. CII SB RAMS, Novosibirsk.
- [9] A.N. Lopanov, *Silver. Physical-chemical properties. Bioactivity*, Agat, St. Petersburg, 2005.
- [10] O.M. Birger (ed.), *Microbiological and virological research methods manual*, Medicina publ. house, Moscow, 1982.