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# EFFECT OF SOME METAL β-DIKETONATES ON THE BACTERICIDAL ACTIVITY OF POLYMER FILMS AGAINST GRAM-POSITIVE MICROORGANISMS

It is shown that the β-diketonates inclusion in polystyrene and polymethylmethacrylate films through covalent binding allows obtaining polymeric materials with high bactericidal activity against gram-positive bacteria *S. aureus* and *M. luteus*. Thereat, the nature and quantity of the introduced metal and the polymer matrix play an important role.

**Keywords:** vinyl-β-diketonates, polymeric metal complexes, the bactericidal activity.

In the past decade, the synthesis of new polymeric biocides is a top trend in the polymer chemistry for special purposes. Metal containing polymer materials have a special place among of polymeric biocides. As a biocide fragments of compounds containing toxic metals, for example Hg, Pb, Sn, As, were used firstly [1-2]. Currently the possibility for the application of metals with lesser toxic effects – Ag, Zn, Cu, etc. is studied [3-6]. An important way to reduce the toxicity of the metal components of the biocides is to obtain polymers in which the metal is bonded to the macromolecule through the chemical bond and metal is not released during operation. This trend is the most promising.

Previously, authors studied the antibacterial properties of polystyrene with the β-diketonates fragments of certain metals against gram-positive microorganisms *P.aeruqinosa* and *E.coli* [7]. A significant effect of the metal nature as well as the macro chain nature and structure on bactericidal properties was demonstrated.

A continuation of above mentioned research is given work aimed at the study of polystyrene and polymethylmethacrylate containing chain β-diketonates of silver (I), cobalt (II), (III) and copper (II), covalently bonded to the macromolecule, the bactericidal activity of polymer coatings against the gram-positive microorganisms *S.aureus* and *M.luteus*.

## Materials and research methods

Vinyl-β-diketone 5-methyl-5-hexene-2,4-dione (MHD) was prepared as in [8], and its cobalt complex (MHD-Co (II)), according to [9]. Metal – containing polymers based on polystyrene (PS) and polymethylmethacrylate (PMMA) were synthesized by several methods (for details: see Table 1.). Before use, they were subjected to two-fold reprecipitation from benzene solution in cold ethanol. They were air dried to constant weight.

The metal content in the polymer was determined by atomic absorption spectrophotometer "Saturn". Sample of the polymer (0.05-0.10 g) was preliminary dissolved at the heating on a sand bath in 25-30 ml mixture of concentrated nitric and perchloric acid (5:3). The resulting solution was diluted to 10 ml with distilled water.

Standard solutions containing 0,5; 1,0; 2,0; 5,0; 10,0 mg / ml of metal were prepared through the subsequent dilution in 2-10 times of initial standard sample ((GSORM – Co, Cu, Ag) produced by Special Design Technological Bureau A.V. Bogatsky at the Physico-Chemical Institute of the National Academy of Sciences of Ukraine, Odessa) with a 1 mg/ml concentration of the corresponding metal. To prevent the possibility of errors due to the influence of mineral acids (nitric and perchloric) their content was balanced in the analyzed and standard solutions.

To study the bactericidal activity of samples the polymer films on the surface of standard slides were prepared from these samples. For this purpose the metal polymers were dissolved in toluene (~ 3%) and applied on a glass surface. Then samples were dried under an infrared lamp.

Determination of bactericidal properties of polymer coatings was performed as follows. The test samples were applied on the surface of standard slides, and then they were putted in a Petri dish and sterilized in dessicator. A cell suspensions of *Escherichia coli*, *Pseudomonas aeruginosa* provided by the Museum of Microorganism Cultures at the L.V. Gromashevsky Institute of Epidemiology and Infectious Diseases of the Academy of Medicinal Sciences of Ukraine were used as test objects. Preservation of the test-dies was performed on the surface of the die sloping plain agar (SPA) at 4ºC. 24 hour cultures grown in test-tubes on at 37ºC were used in research. Cultures of the test microorganisms grown in test-tubes on the SPA were washed with sterile saline. The concentration of the resulting suspension was determined using a calibration curve; optical density was measured with a spectrophotometer "Spekol-10" (Germany). The cell suspension was diluted with saline to a concentration of $5 \cdot 10^3$ cells / ml and was applied in an sterile plate with a test samples in Petri dishes. Plates were kept for 30 minutes, then they were filled with aseptic (MPA). Dishes with the samples were incubated in a thermostat at 37ºC during 24 hours. Whereupon the number of colony forming units (CFU) in the control and test samples were counted.

## Results and discussion

The samples of polymers containing fragments of MHD-M (M=Ag (I), Co (II), Co (III), Cu(II)) were obtained by different routes (Table 1).

Samples **2, 10** – are PS (sample **2**) and PMMA (sample **10**) containing chain fragments MHD – Co (II). They were obtained via the radical polymerization of the corresponding vinyl monomers in the presence of MHD – Co (II), which is both the initiator and comonomer in given process (Fig. 1) [10].

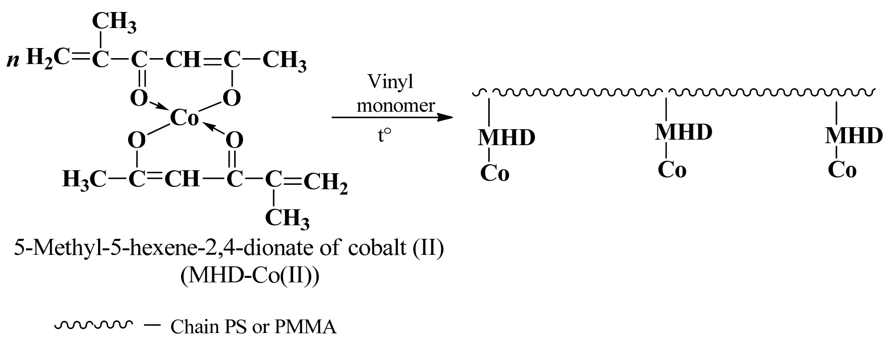


Fig.1. Scheme of the metal-containing synthesis via the copolymerization method

Metal-content in such samples is relatively low. This is due to the limited solubility of MHD-Co (II) in the monomers as well as due to the presence of certain limit concentration (~ $1\cdot10^{-2}$ mol/l), above which MHD – Co (II) behaves itself as an inhibitor of the radical polymerization [11].

Table 1

**Method, the conditions for obtaining of polymers samples and metal content in these samples**

| Sample № | Metal polymer | $w_M$, mas.% | Method of obtaining | Conditions |
|---|---|---|---|---|
| **1** | PS | – | Radical polymerization in mass | Initiator – PB. T= 85ºC. $C_{in}= 5\cdot10^{-3}$ mol/l |
| **2** | PS -Co(II) | 0,19 | The same | Initiator – MHD-Co(II). $C_{in}= 5\cdot10^{-3}$ mol/l. T= 85ºC |
| **3** | PS -Co(II) | 0,92 | Complexation of polymeric ligand with a metal salt | $m_{(CH3COO)2\ Co\ \cdot4H2O}$=0,031 g. T= 25ºC |
| **4** | PS -Co(III) | 0,53 | The same | $m_{(CH3COO)2Co\cdot4H2O}$=0,031 g. $m_{PB}$= 0,200 g. T= 50ºC |
| **5** | PS-Cu(II) | 1,90 | The same | $m_{(CH3COO)2Cu\cdot2H2O}$=0,025 g. T= 25ºC |
| **6** | PS-Ag(I) | 1,57 | The same | $m_{AgNO3}$=0,063 g. T= 25ºC |
| **7** | PS -Ag(I)-PS | 0,02 | Grafted radical polymerization of styrene on the sample 6 | Initiator – sample 6. $C_{in}$ = 0,2 mas.%. T=70ºC |
| **8** | PS -Ag(I)-PMMA | 0,05 | Grafted radical polymerization of MMA on the sample 6 | The same |
| **9** | PMMA | – | Radical polymerization in mass | Initiator – PB. T= 70ºC. $C_{in}= 10\cdot10^{-3}$ mol/l |
| **10** | PMMA-Co(II) | 0,23 | Radical polymerization in solution | Solvent – methylethylketon. Initiator – MHD-Co(II). $C_{in}= 5\cdot10^{-3}$ mol/l. T= 70ºC. |
| **11** | Control | – | – | The surface of the glass plate |

Two samples **1** and **9** – are the PS and PMMA respectively, obtained at the initiation of benzene by peroxide (PB). They contain no metal and were used as a reference samples, as well as to evaluate the effect of the polymer matrix on the bactericidal properties. Sample **11** was a clean glass plate and served as the control one.

Samples **3-6** are PS containing chain fragments MHD-M (M = Co (II), Co (III), Cu (II), Ag (I)). These samples were obtained through the method of complexation. Schematically, the process is depicted in Figure 2. First, the polymer ligand (Stage I, Figure 2) was obtained at 80 ºC through the styrene radical copolymerization with MHD (monomer ratio was 30:1, w/w %) in the presence of PB as an initiator ($C_{PB}=1\cdot10^{-2}$ mol/l). Then polymer metal complexes (Stage II, Figure 2) were obtained through the complexation of this macroligand (1% solution in dioxane) with the salt of the corresponding metal. The use of this method has allowed to increase (in 5-10 fold) the metal content in the polymers.

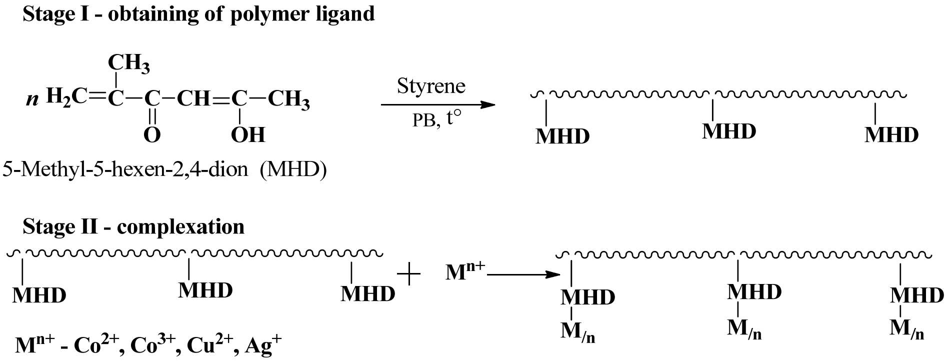


Fig. 2. Scheme the metal-containg synthesis via the method the complexation method.

Samples **7** and **8** were obtained through the grafting of styrene radical polymerization and MMA, respectively, on silver containing polymer (sample **6**), which was taken as a macroinitiator (Fig. 3). Due to the method of obtaining, these polymers have the grafted side chains and are branched molecules by structure that differ them from the initial macroinitiator, however the metal content is low, that is due to the significant increase in weight of either grafted PS or PMMA.

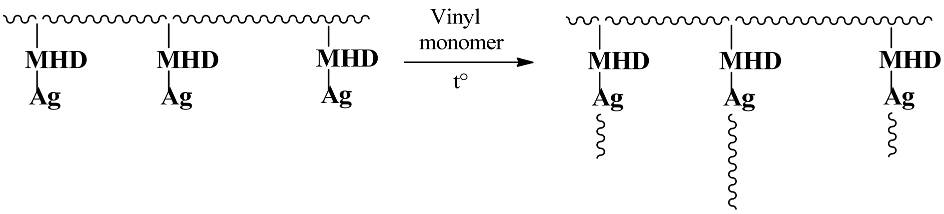


Fig. 3. Scheme for the synthesis of metal polymers with the branched structure

The bactericidal properties of polymer films relatively to the gram-positive bacteria *S. aureus* and *M. luteus* are given in the Table 2.

The features observed for *S. aureus* are considered first. As can be seen from the data of the Table 2, metal-free matrix PS and PMMA themselves have a low bactericidal activity as compared to the control. In this case, PMMA has a little higher activity as compared to the PC. This is confirmed by the results of the comparison of samples **2, 10**, which were obtained by the same method, they have close content of cobalt and differ only by the nature of the polymer matrix.

The data in the Table 2, indicate that the nature of metal in the polystyrene strongly influences its bactericidal activity against S.aureus. Polystyrene with β-diketonate copper fragments (sample 5) has the highest bactericidal activity. It is in 34 times more active than the metal-free PC and in 46 times higher than the control sample.

Table 2

**Bactericidal properties of metal polymer films relatively to the gram-positive bacteria *S. aureus* and *M. luteus***

| Sample | *Staphylococcus aureus* | | *Micrococcus luteus* | |
|---|---|---|---|---|
| | Number of colony forming units (CFU) | Colony diameter,mm | Number of colony forming units (CFU) | Colony diameter,mm |
| **1** | 340±18 | 1-3 | 130±10 | 1-2 |
| **2** | 260±8 | 1-4 | 250±21 | 1-2 |
| **3** | 200±11 | 2-3 | 130±14 | 1-2 |
| **4** | 230±14 | 1-3 | 130±10 | 1-2 |
| **5** | 10±2 | 2-3 | 130±24 | 1-2 |
| **6** | 45±5 | 1-2 | 100±8 | 1-2 |
| **7** | 450±12 | 2-3 | 160±26 | 1-2 |
| **8** | 300±26 | 1-2 | 20±4 | 1-2 |
| **9** | 250±29 | 2-3 | 120±11 | 1-2 |
| **10** | 120±13 | 1-2 | 40±9 | 1-2 |
| **11** | 460±47 | 2-3 | 265±17 | 2-3 |

The sample **6**, containing silver, demonstrates quite good bactericide ability as well. It is in 8 times more active than the PS containing no metal. However, the reduction of silver content in the polymer as a result of grafting (samples **7, 8**) dramatically decreases its bactericidal properties. Cobalt macrocomplexes though they have a somewhat higher activity than the reference samples, but in comparison with polymers containing the copper- and silver- their bactericidal activity can not be referred as a high. Thus, as can be seen from the Table 2, method of obtaining as well as the metal valence in practice does not affect the number of CFU.

In the case of *M.luteus* as for *S.aureus* and PS, and PMMA matrix have a weak bactericidal activity, but in 2 times higher than the control sample. However, the influence of the matrix associated with of β-diketonates fragments for *M.luteus* is manifested in an interesting way.

If the inclusion of the metal atoms in PS-film, irrespective of their nature and quantity, has almost no effect on their antibacterial properties, then for PMMA pattern it is completely different. Inclusion of MHD-M fragments into the PMMA chain increases bactericidal activity of polymer films essentially. Thus for PMMA containing just only

0,23 wt.% of Cobalt (sample **9**), it bactericidal activity is increased in 3 times as compared to the metal-free PMMA.

The fact that it is namely the combination of PMMA-M leads to the improved bactericidal activity and the fact that polystyrene (sample **6**), containing 1,57 wt.% of silver has CFU =100, which is slightly different from the reference sample (CFU = 130). Grafting of PMMA to this sample leads to a drastic reduction in the amount of silver in the polymer (0,05 wt.%), but it increases significantly its bactericidal activity. It is in 5 times higher than the initial macroinitiator (sample **6**) and in 8 times higher than the same amount for the sample **7**, which is grafted by PS.

Our studies allow to suggest, that varying the nature and quantity of the metal as well as the nature of the polymer matrix, which is bonded to metal chelates, then universal polymeric materials, possessing bactericidal action against several microorganisms, could be obtained. Another way for the creation of such materials is combination of several metal complexes used for immobilization. For example, to obtain coating with high bactericidal activity against to both microorganisms studied in given report, then it could be the PMMA film, containing both copper and silver β-diketonates in its composition. These assumptions need to be clarified in this research direction and, above all, the development of approaches to the synthesis of heterometallic polymer materials is necessary.

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## ВПЛИВ β-ДИКЕТОНАТІВ ДЕЯКИХ МЕТАЛІВ НА БАКТЕРИЦИДНУ АКТИВНІСТЬ ПОЛІМЕРНИХ ПЛІВОК ПО ВІДНОШЕННЮ ДО ГРАМПОЗИТИВНИХ МІКРООРГАНІЗМІВ

**Резюме**
Показано, що введення β-дикетонатів металів в полістирольні та поліметилметакрилатні плівки шляхом ковалентного зв'язування, дозволяє одержувати полімерні матерали, які мають бактерицидну активність по відношенню до грампозитивних бактерій S. aureus и M. luteus. При цьому важливу роль мають як природа та кількість металу, що вводиться, так і полімерна матриця.
**Ключові слова:** вініл-β-дикетонати, полімерні металокомплекси, бактерицидна активність.

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## ВЛИЯНИЕ β-ДИКЕТОНАТОВ НЕКОТОРЫХ МЕТАЛЛОВ НА БАКТЕРИЦИДНУЮ АКТИВНОСТЬ ПОЛИМЕРНЫХ ПЛЕНОК ПО ОТНОШЕНИЮ К ГРАМПОЛОЖИТЕЛЬНЫМ МИКРООРГАНИЗМАМ

**Резюме**
Показано, что введение β-дикетонатов металлов в полистирольные и полиметилметакрилатные пленки, путем ковалентного связывания, позволяет получить полимерные материалы, обладающие высокой бактерицидной активностью по отношению к грамположительным бактериям S. aureus и M. luteus. При этом существенную роль играют как природа и количество вводимого металла, так и полимерная матрица.
**Ключевые слова:** винил-β-дикетонаты, полимерные металлокомплексы, бактерицидная активность.