Project Report

Antimicrobial Activity of Silver, Copper, and Zinc Ions/Poly(Acrylate/Itaconic Acid) Hydrogel Matrices

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Abstract: The design and use of new potent and specific antimicrobial systems are of crucial importance in the medical field. This will help relieve, fight, and eradicate infections and thus improve human health. The use of metals in various forms as antimicrobial therapeutics has been known since ancient times. In this sense, polymeric hydrogel matrices as multifunctional materials and in combination with various metal forms can be a great alternative to conventional treatments for infections. Hydrogels possess high hydrophilicity, specific three-dimensional networks, fine biocompatibility, and cell adhesion and are therefore suitable as materials for the loading of active antimicrobial agents and acting in antimicrobial areas. The biocompatible nature of hydrogels’ matrices makes them a convenient starting platform to develop biocompatible, selective, active controlled-release antimicrobial materials. Hydrogels based on acrylate and itaconic acid were synthesized and loaded with silver (Ag⁺), copper (Cu²⁺), and zinc (Zn²⁺) ions as a controlled release and antimicrobial system to test release properties and antimicrobial activity in contact with microbes. The metal ions/hydrogel systems exhibited favorable biocompatibility, release profiles, and antimicrobial activity against methicillin-sensitive Staphylococcus aureus (MSSA), methicillin-resistant Staphylococcus aureus (MRSA), Staphylococcus aureus, Escherichia coli, and Candida albicans microbes, and have shown that they have the capacity to “fight” with the life-threatening infections. Antimicrobial activity depends on types of metal ions, the composition of polymeric matrices, as well as the types of microbes. Designed metal ions/poly(acrylate/itaconic acid) antimicrobial systems have shown to have good potential as antimicrobial therapeutics and suitable biomaterials for medical applications.

Keywords: metal ions; poly(acrylate/itaconic acid) hydrogels; biocompatibility; controlled release of metal ions; antimicrobial activity; medical applications

1. Introduction

Metals and their various forms have been used as antimicrobial agents since ancient times [1,2]. Their medical applications are very beneficial. Silver, copper and zinc have been used to heal infections. Silver is used as an agent to relieve burns. Composite of mercury with copper, silver and tin has an application in dentistry [3,4].

The planet’s crust and the ecosphere are enriched in metals. According to the Great Oxidation Event (GOE), which occurred 2.3–2.4 billion years ago, there was a wide exposure of metal ions to microbes. There has been an increase in the oxygen content of the atmosphere so that various oxidized forms of metal compounds are found in and around the earth’s crust. Metals such as copper, iron, and zinc were used for redox reactions using enzymes. Metals are vital for life processes, so cells need a homeostasis mechanism to take care of intracellular concentration. Zinc and copper are closely related to the pathogen-killing mechanism in eukaryotes, where oxidative stress is used to kill the affected microbes. Metals such as gold, silver and mercury can have a beneficial effect on microbial growth control [5].
Nowadays, there is a great need to create effective antimicrobial systems that can be applied either locally or directly to places that are abundant with microbes, to overcome the problem of microbial resistance to antibiotics and therapeutics. For these purposes, hydrogels have emerged as very favorable biomaterials whose properties can be easily adjusted [6–11]. Hydrogels are given great attention due to their favorable properties (biocompatibility, hydrophilic nature, absorptivity, permeability, and the ability to load antimicrobial agents into their polymeric network), the possibility of easy production, and the ability to achieve smart behavior. Hydrogels can be loaded with antimicrobials and be an alternative and amenable solution to traditional antibiotic treatments. Controlled therapeutic release, local administration, stimulated switch on-off release, and improved biocompatibility are all important advantages that a broad diversity of hydrogels can provide and that is exactly what antimicrobial biomaterials currently require [8–13]. Antimicrobial hydrogels can be widely applied in the field of wound dressings, urinary tract coatings, catheter-associated infections, gastrointestinal infections, osteomyelitis, and contact lens [8–11].

Medical technology requires sterility as the first and most important principle, yet microbial infections still cause millions of deaths every year in the world [14–16]. The “bidding” between humans and microbes escalated mainly due to therapeutic agents’ resistance to microbes [17–19]. Such resistance has developed on a very large scale over time, greatly reducing the effectiveness of therapeutic agents, and is an ever-growing problem. Drug-resistant microbial infections result in many negative effects on the health system and society, such as treatment with higher doses of therapeutics, longer hospital stays and increased mortality, leading to a greater desire for new antimicrobial technologies and materials for medical use. To address these health and social problems, a new generation of antimicrobial systems are being explored [12,13,20]. Surprisingly, metal ions have also found applications in lots of advanced antimicrobial systems [21]. Metal ions of silver (Ag) [22], zinc (Zn) [21,23], copper (Cu) [24], along with their oxides, could kill or inhibit the growth of microbes through multiple mechanisms, thereby making the development of resistance unlikely.

Moreover, many drug-resistant microbes have evolved because of the misuse of traditional antibiotics and other antimicrobial drugs. Therefore, fabricating antimicrobial hydrogels through the incorporation of antimicrobial agents will be the key to overcoming these limitations [25]. Antimicrobial hydrogels will finally be able to conquer the vast issues of traditional therapies. Antimicrobial biomaterials, their unique combinations, and the approaches currently being developed will provide a promising future for anti-infection treatment.

This report presents data for a series of polymeric hydrogel matrices based on acrylate and itaconic acid, with different content of itaconic acid. Valuable data were given concerning the biocompatibility, release and antimicrobial properties of this polymeric hydrogel system. Silver, copper and zinc metal ions are loaded into polymeric matrices to design release and antimicrobial platforms, where, in addition to the controlled release of ions, the antimicrobial activity of the matrices also takes place. Under precisely defined conditions, metal ions/hydrogel matrices are exposed to contact with several microbial cultures to assess the antimicrobial activity of these materials. The effect of itaconic acid, as well as metal ions, on biocompatibility, release and antimicrobial properties, is confirmed. So, our research is presented as a unified whole, a series of synthesized and tested samples, which confirms the medical application of these polymeric biomaterials—the process of the controlled release of ions and, at the same time, a newly added property arises, which is antimicrobial activity.

2. Results and Discussion

Biocompatibility of materials is a very valuable property for polymeric biomaterials that have potential medical applications [26,27]. In vitro biocompatibility is a specific property of a material obtained in contact with a specific cell line [28]. The biocompatible
behavior of poly(2-hydroxyethyl acrylate/itaconic acid) (A) hydrogel series and A hydrogel series loaded with silver, copper and zinc ions was examined in a test on normal human fibroblasts (MRC5). Considering the cell viability of fibroblasts that were in contact with the hydrogel extracts, the cytocompatibility slightly decreased with the increase of itaconic acid content and extract concentrations. Two hydrogel samples: a sample containing 7 mol% IA (A7) for 100% extract concentration and another sample loaded with zinc ions (ZnA7) for 100% extract concentration showed cell survival of less than 50% which is not acceptable for medical use (Figure 1). The highest IA content (7 mol%) in the hydrogel leads to localized acidity, which could cause increased cell death. All remaining tested hydrogel samples showed cell viability over 50%, which is a proper cytocompatibility for medical applications (Figure 1) [29–32].

Figure 1. Cell viability for hydrogel A series based on acrylate and itaconic acid and A series loaded with silver, copper and zinc ions.
Polymeric hydrogel systems based on 2-hydroxyethyl acrylate and itaconic acid are designed to support a controlled therapeutic-release process and are suitable for metal ions’ loading. Therefore, active metal ions which participate in metabolic processes can be loaded into them as therapeutic agents. In this way, newly designed polymeric platforms for the controlled release of active ions can (copper, silver and zinc) influence and accelerate tissue regeneration, because the ions are released at a projected and adjustable rate, to the targeted site in a defined time interval [29–32]. Decades of wide and uncontrolled use of antibiotics led to the development of microbial resistance, thus dictating the discovery of novel antimicrobial materials [33]. Cu$^{2+}$, Zn$^{2+}$, and Ag$^+$ are important for many biological processes in the human body and have been used in different formulations for curing many diseases since ancient times [34].

Data for entrapment efficiency EE (%) revealed the influence of hydrogels’ composition on their ability to absorb Cu$^{2+}$, Zn$^{2+}$, and Ag$^+$ ions. The increase of entrapment efficiency with the increase of IA implies the involvement of COO$^-$ groups in the incorporation of ions. The electrostatic interaction between positively charged Cu$^{2+}$, Zn$^{2+}$, and Ag$^+$ and negative ionized carboxyl groups implies the quantity of incorporated metal ions. Thus, higher amounts of carboxyl groups will attract more metal ions [29–32]. EE values for the sample without IA is about 15% for all ions, and a linear increase from 60–80% as IA content increases from 2–7 mol% for all ions.

Release profiles for Ag$^+$ ions are presented in Figure 2. Hydrogels exhibited an initial faster range of release, followed by a slower phase. The initial rapid release occurred most likely due to the release of Ag$^+$ ions from the hydrogels’ surface. In the first 12 h, 93.98%, 80.64%; 85.89%; 83.85%; and 90.00% of Ag$^+$ ions were released from A0; A2; A3.5; A5; and A7, respectively. The hydrogels reached a maximum of released Ag$^+$ ions approximately after 48 h of immersion, which was followed by the equilibrium and constantly released concentrations. It was noticed that the release of Ag$^+$ is related to hydrogels’ composition (itaconic acid content). Ag$^+$ ions were released slightly faster for the A0 sample, compared to the copolymeric A2; A3.5; A5; and A7 matrices. The release rate was increased with the increase of itaconic acid content, and the higher amounts of the incorporated silver(I) ions were released faster [31].

The transport mechanism of Ag$^+$ ions for the controlled release process was analyzed using three models: Ritger-Peppas, Peppas-Sahlin, and Peppas-Sahlin when $m = 0.5$ [35–39]. All three applied equations proved to be adequate for the analysis of Ag$^+$ ions’ transport mechanism. According to obtained AIC values, the Peppas-Sahlin model was the most appropriate for these hydrogels. Based on obtained values of diffusion kinetic constant ($K_1$) and macromolecular relaxation kinetic constant ($K_2$), the transport of Ag$^+$ ions for the release is controlled both by the diffusion and relaxation of polymeric chains [31].

The controlled release profiles of Cu$^{2+}$ ions that were obtained are shown in Figure 2. The initial rapid release in the first stage can be interpreted as a consequence of the release of surface-bonded copper ions. Immediately after the first hour of immersion, an abrupt increase of released copper ions was observed. In the first 12 h, 92.42%, and 86.31% of Cu$^{2+}$ was released from A0, and A7. Tested matrices reached a maximum of copper released after approximately 48 h of immersion, and further the concentration of released Cu$^{2+}$ remained constant until the end of the study. A0 showed a fast initial release and reached the maximum release faster than the copolymeric hydrogel. The release rate for the A7 sample was slower compared to A0, and following the IA content, the rate of release was highest for the sample with the highest IA content [29].

To define the transport mechanism and release kinetics, three models were used: Ritger-Peppas, Peppas-Sahlin, and Peppas-Sahlin when $m = 0.5$ [35–39]. All three applied equations proved suitable for the analysis of Cu$^{2+}$ release studies. $R^2$ values confirmed the good fitting of experimental data. According to obtained AIC values, the Peppas-Sahlin model was the most convenient for all tested hydrogels. As can be noticed, the macromolecular relaxation kinetic constant ($K_2$) had lower values compared with the
diffusion kinetic constant \( (K_1) \), indicating Fickian diffusion had a dominant influence on Cu\(^{2+}\) transport mechanism [32].

**Figure 2.** In vitro release profiles of silver, copper and zinc ions from A series hydrogel matrices.

Controlled release profiles of the Zn\(^{2+}\) ions are presented in Figure 2. The fast initial release of Zn\(^{2+}\) ions in the first 12 h characterizes the first phase of release, whereby 73.00\% (A0); 61.77\% (A2); 64.97\% (A3.5); 69.86\% (A5); and 75.87\% (A7) of total released zinc ions were detected in the release medium. Afterwards, a slower release was noticed, while the concentration of released zinc reached the maximum. After approximately 48 h of immersion in the release medium, the plateau was reached and the released concentration remained constant. The release rate was slower for copolymeric hydrogels compared to homopolymeric A0, confirming the influence of hydrogel composition on release properties. The release rate was increased with the increase of itaconic acid content, and higher amounts of the incorporated Zn\(^{2+}\) ions were released faster [32].

The transport mechanism of Zn\(^{2+}\) ions during the controlled release was studied using three models: Ritger-Peppas, Peppas-Sahlin, and Peppas-Sahlin when \( m = 0.5 \) [35–39]. By interpreting the values of characteristic parameters of each applied model it was noticed that all of them are suitable for analysis of Zn\(^{2+}\) ions transport mechanism. Based on the obtained values of diffusion kinetic constant \( (K_1) \) and macromolecular relaxation kinetic constant \( (K_2) \), the transport of Zn\(^{2+}\) ions during the release was controlled both by diffusion and relaxation of polymer chains [32].
Biocompatible poly(acrylate/itaconic acid) hydrogel matrices are obtained by gelation of 2-hydroxyethyl acrylate and itaconic acid, varying itaconic acid fraction. Subsequently, metal ions are loaded in the polymeric network. Further, these matrices were exposed to microbes during the controlled release process of metal ions to assess the antimicrobial activity of designed materials (Scheme 1).

Pathogenic Staphylococcus aureus (S. aureus) can cause numerous human diseases [40]. This serious situation is predominantly due to antibiotic-resistant strains [41]. Methicillin-resistant S. aureus (MRSA) is one of the foremost causes of hospital- and community-associated infections. Resistance to the whole category of β-lactam antibiotics makes MRSA infections difficult to cure [42]. The methicillin-sensitive (MSSA) and methicillin-resistant form (MRSA), a dominant infective pathogen agent responsible for most superficial skin infections, lead to enhanced morbidity, mortality, and extraordinary health care costs [43].

Silver(I) ions were loaded in poly(2-hydroxyethyl acrylate/itaconic acid) hydrogel matrices (tags AgA0–AgA7). Silver ions bind to the COO\(^-\) groups of itaconic acid in a buffer of pH 7.40 and it turns out that the silver ions content increased as the itaconic acid (IA) content increased [32]. The data obtained from the antimicrobial study revealed the antimicrobial activity of the silver(I) ions loaded hydrogels against MSSA and MRSA strains shown in Figure 3. The samples showed higher activity for MSSA than for MRSA. When analyzing the composition, samples containing IA of 0–5 mol% (correspondingly rising silver ion content) the antimicrobial activity according to MSSA was almost the same (about 40%). Only for the sample with the highest content of IA (with the highest content of silver ions), the activity reached up to over 80%. When considering the behavior of samples according to MRSA, it can be seen that the sample that did not contain silver ions was inactive according to MRSA. The antimicrobial activity reached over 80% for the sample with the highest silver ion content.

Inhibition of microbial growth was influenced by the composition of the hydrogels. By increasing the content of silver ions, the inhibition of bacterial growth was more pronounced, so the best results were shown by hydrogels with the highest silver ions’ content for both MSSA and MRSA. The mechanism of action of silver on microorganisms is based on the hypothesis that the silver ion (Ag\(^+\)) can bind to the bacterial cell membrane through the interaction between Ag\(^+\) and the thiol group in proteins on the cell membrane, thus affecting the bacterial cells’ viability by inhibiting the replication of DNA [44].

Copper(II) ions were embedded in poly(2-hydroxyethyl acrylate/itaconic acid) hydrogel matrices (tags CuA0, CuA7). Copper ions bind to the COO\(^-\) groups of itaconic acid in a buffer of pH 7.40, and the copper ions content increased as the IA content increased [30].
Antimicrobial activity of CuA0 and CuA7 hydrogel matrices were tested for three strains: Gram-positive bacteria *S. aureus*, Gram-negative bacteria *E. coli*, and fungus *C. albicans*, and monitored for 72 h. The data obtained (Figure 4) indicated the strong antimicrobial activity of the hydrogels loaded with Cu$^{2+}$ ions. The inhibition of microbial growth was near 100% for all three strains: Gram-positive bacteria *S. aureus*, Gram-negative bacteria *E. coli*, and fungus *C. albicans*. Even the microbial reduction was high, a slight decrease in antimicrobial activity was noticed with regards to the selected time points of Cu$^{2+}$ release. The maximum was detected after the first hour and the minimum for 72 h, owing to the quantity of residual copper, which interacts with microbial cells. From the obtained data, it follows that, by adjusting the content of IA in hydrogels, and thus the incorporated copper ions, antimicrobial activity can be precisely projected. Antimicrobial copper(II)/poly(acrylate/itaconic acid) hydrogels showed antimicrobial activity for 72 h.

Microbes are killed quickly when they come in contact with various forms of copper, and copper ions released from the surface are said to play an important role in the killing process. In research studies, it was shown that many microbial species, such as *E. coli*, *S. aureus*, *Salmonella enterica*, *Campylobacter jejuni*, *Clostridium difficile*, *Listeria monocytogenes*, and *Mycobacterium tuberculosis*, are efficiently killed on copper or copper alloy surfaces [45].

Zinc(II) ions were loaded in poly(2-hydroxyethyl acrylate/itaconic acid) hydrogel matrices (tags ZnA0–ZnA7). Zinc ions bind to the COO$^-$ groups of itaconic acid in a buffer pH of 7.40, and the zinc ions’ content rose as the IA content increased [32]. Antimicrobial activity for *E. coli* microbe was monitored for 72 h. The data obtained showed that the inhibition of *E. coli* growth reached almost 100% when the microbial strains were exposed to all tested Zn(II)/poly(2-hydroxyethyl acrylate/itaconic acid) hydrogels (Figure 5). With regards to the selected time points, the antimicrobial activity decreased. Therefore, it can be said that the maximum microbial inhibition capacity of hydrogels was at the beginning of the release process, and the lowest was at the end of the release study. This behavior is related to the activity of residual zinc in hydrogels after the release started. The obtained results indicated that by adjusting the content of IA in hydrogels, and thus the incorporated zinc ions, antimicrobial activity can be modulated. Antimicrobial zinc(II)/poly(acrylate/itaconic acid) showed antimicrobial activities for 72 h during the release process. Antimicrobial action can be explained by the positively charged zinc ions attacking the negatively charged cell walls of bacteria, which leads to cell wall leakage and the death of the bacteria [46].

![Figure 3. Antimicrobial activity of silver(I) ions in poly(acrylate/itaconic acid) matrices in contact with methicillin sensitive (MSSA) and methicillin resistant *Staphylococcus aureus* (MRSA).](image-url)
Louis, methacrylate and \( \text{CuSO}_4 \cdot \text{H}_2\text{O} \) (Sigma-Aldrich, St. Louis, MI, USA). Ethylene glycol dimethacrylate (EGDMA) (Aldrich, USA), potassium persulfate (PPS) (Sigma-Aldrich, St. Louis, MI, USA) and \( N,N',N''-\text{tetramethylethylene diamine (TEMED)} \) (Sigma-Aldrich, St. Louis, MI, USA) were used as gelation agents in syntheses. The source of silver(I), copper(II) and zinc(II) ions were solutions of silver nitrate (AgNO\(_3\)) (Sigma-Aldrich, St. Louis, MI, USA), copper sulfate pentahydrate (CuSO\(_4\)·5H\(_2\)O) (Sigma-Aldrich, St. Louis, MI, USA),

3. Materials and Methods

The reactants used were 2-hydroxyethyl acrylate (HEA) (Sigma-Aldrich, St. Louis, MI, USA) and itaconic acid (IA) (Sigma-Aldrich, St. Louis, MI, USA). Ethylene glycol dimethacrylate (EGDMA) (Aldrich, USA), potassium persulfate (PPS) (Sigma-Aldrich, St. Louis, MI, USA) and \( N,N',N''-\text{tetramethylethylene diamine (TEMED)} \) (Sigma-Aldrich, St. Louis, MI, USA) were used as gelation agents in syntheses. The source of silver(I), copper(II) and zinc(II) ions were solutions of silver nitrate (AgNO\(_3\)) (Sigma-Aldrich, St. Louis, MI, USA), copper sulfate pentahydrate (CuSO\(_4\)·5H\(_2\)O) (Sigma-Aldrich, St. Louis, MI, USA),

Figure 4. Antimicrobial activity of copper(II) ions in poly(acrylate/itaconic acid) matrices in contact with \( C. \text{albicans} \), \( S. \text{aureus} \), and \( E. \text{coli} \).

Figure 5. Antimicrobial activity of zinc(II) ions in poly(acrylate/itaconic acid) matrices in contact with \( E. \text{coli} \).
MI, USA) and zinc acetate dihydrate (Zn(CH$_3$CO$_2$)$_2$·2H$_2$O) (Sigma-Aldrich, St. Louis, MI, USA), respectively. Phosphate buffers were used in experiments.

3.1. Preparation of Hydrogels

Hydrogels were prepared using gelation of HEA and IA monomers. IA content was varied (0, 2, 3.5, 5 and 7 mol%), which resulted in hydrogel samples marked as A0, A2, A3.5, A5 and A7, respectively. All reactants were dissolved in a water/ethanol mixture, and the reaction mixture was subsequently degassed in a nitrogen atmosphere, to eliminate oxygen, and later placed between two glass plates sealed with a rubber spacer. Gelation was carried out at 55 °C, for 24 h. After gelation, hydrogels were cut into the form of discs. The samples were later immersed in deionized water, for seven days, to remove unreacted components. The discs were dried at room temperature.

3.2. In Vitro Biocompatibility Assay

Biocompatibility of materials was expressed through cellular viability measured for MRC5 cells (human lung fibroblast, obtained from ATCC, LGC Standards Sp. z o.o., Lomianki, Poland) using a standard MTT assay (MTT assay is used to measure cellular metabolic activity as an indicator of cell viability, proliferation and cytotoxicity—this colorimetric assay is based on the reduction of a yellow tetrazolium salt (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide or MTT) and methods suitable for materials testing [27,47,48].

3.3. Metal Ions Loading in Hydrogel’s Matrices

Metal ions (silver(I), copper(II) and zinc(II)) were loaded into hydrogels’ matrices by xerogel disk immersion in metal source solutions and left for 24 h to swell and imbibe metal ions. Afterwards, samples were dried at room temperature. Samples were designated as the first two letters from the metal name (Ag, Cu and Zn) and the third letter (A0, 2, 3.5, 5, and 7) from acrylate and number from an itaconic acid fraction (example AgA0).

3.4. In Vitro Controlled Release Study

The study of metal ions released from the hydrogels was performed in vitro, in phosphate buffer (pH 7.40) at 37 °C. The volume of buffer was constant (50 mL) for all the experiments and the stirring speed was adjusted to 50 rpm. The hydrogels were placed in the buffer and, at the predetermined time intervals, a known quantity of the buffer’s sample was withdrawn and the concentration of released metal ions was determined by atomic absorption spectrophotometer (Phillips PYE UNICAM SP9, Boston, MA, USA). The measurements were performed in triplicate.

3.5. Antimicrobial Assay

To evaluate the antimicrobial activity of the hydrogels, *Staphylococcus aureus* (MSSA) (ATCC25923) and *Staphylococcus aureus* (MRSA) (ATCC43300) indicator strains were used [49,50]. The time of incubation was 24 h at 37 °C. A microtiter plate-based antimicrobial assay was performed in triplicate and microbe growth was monitored by measuring the absorbance of the samples at 600 nm (OD600) (Multiskan FC, Thermo Fisher Scientific, Vantaa, Finland).

Microbial strains of Gram-negative bacteria *Escherichia coli* (ATCC 25922), Gram-positive bacteria *Staphylococcus aureus* (ATCC 25923) and fungus *Candida albicans* (ATCC 10259) were also used for antimicrobial activity test. Decimal dilution of fresh overnight broth culture (Tryptone soy broth with 0.6% yeast extract-TSYB, Torlak Institute, Belgrade, Serbia) was prepared in saline to obtain the initial number of cells of ca. 10$^5$ CFU/mL in samples (adjusted using McFarland turbidity standard). A quantitative test was performed [51] with some modifications. The appropriate hydrogel sample was challenged to 1 mL saline with an indicator strain prepared to obtain ca. 10$^5$ CFU/mL. After the appropriate time of incubation (24 h) at 37 °C, 9 mL of saline was added, and after vigorously stirring the 100 µL aliquots were diluted in sterile saline. From all dilutions, the
adequate aliquots were placed in sterile Petri dishes, covered with tryptone soy agar (Torlak Institute, Belgrade, Serbia) and incubated 24 h at 37 °C. Afterwards, the colony-forming units (CFU) of each plate was determined and the percentage of viable cell reduction (R(\%)) was calculated using:

\[
R(\%) = \frac{C_0 - C}{C_0} \times 100
\]

where \(C_0\) is the number of microorganisms in the control sample and \(C\) is the number of microorganism colonies of the sample with metal ions. The procedure was performed in triplicate.

4. Conclusions

This report provides valuable data from a series of hydrogel matrices based on acrylate and itaconic acid. Their biocompatibility, ability to incorporate metal ions, and realization of the process of controlled releases, where at the same time there is antimicrobial action of these materials, were significant achievements of the research. These hydrogel matrices as antimicrobial materials can be an alternative and viable solution to traditional antibiotic treatments. We designed smart silver(I), copper(II) and zinc(II)/poly(acrylate/itaconic acid) hydrogel platforms with antimicrobial activity during a controlled release process. It was shown that the composition of hydrogels, the type of incorporated ions, the type of microbes and the observed time of release and exposure to microbes affected the antimicrobial activity of the tested materials. Obtained antimicrobial materials, unique combinations of organic polymers and inorganic agents-metal ions, can provide a promising prospect for anti-infection treatment.

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