

Physicochemical Investigations of Hydrogels Containing Gold Nanoparticles Designed for Biomedical Use

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Abstract

Currently, many investigations are being performed to develop dressing materials with a positive effect on the wound healing process. In general, innovative dressings should ensure wound exudate absorption, constitute an external barrier limiting the possibility of wound contamination and, importantly, also provide therapeutic properties. This work is focused on obtaining materials with potential use as dressings for treatment of difficult-to-heal wounds. The synthesis methodology of acrylic hydrogels modified with selected modifiers, i.e. arabic gum, nanogold, bee pollen and chamomile extract, was developed. Next, the sorption properties of the materials were determined as well as their behavior during the incubation in fluids imitating the environment of the human body. Additionally, the impact of such an incubation on their structure was evaluated by FT-IR spectroscopy. It was proved that the modifiers affected the sorption properties of hydrogels, i.e. samples with additives showed even approx. 2.5-fold lower swelling ability. In turn, incubation of hydrogels in simulated body fluids did not cause any rapid changes in pH, which may indicate the biocompatibility of the tested materials with the tested fluids. Thus, it may be concluded that the developed materials show great application potential for biomedical purposes and may be subjected to more advanced studies such as cytotoxicity assessments towards selected cell lines.

Keywords:

acrylic hydrogels, gold nanoparticles, sorption properties, incubation studies, FT-IR analysis

4. INTRODUCTION

In recent years, the application of hydrogels in various fields including medicine and related areas has been growing rapidly [1]. These materials are also widely used in agriculture [2], environmental protection [3] or cosmetology [4]. Growing interest in these polymers result from their unique properties including great water absorption capacity, flexibility, or the possibility of their modification with various active substances which may be further release to the application site [5]. Importantly, both hydrogels based on natural materials such as chitosan [6], gelatin [7] or arabic gum [8] and synthetic ones, i.e. poly(vinyl alcohol) [9] or acrylic acid [10] are widely applied.

Hydrogels based on acrylic acid are widely considered for application e.g. in controlled drug delivery systems [11], as wound dressings [12], in tissue engineering [13] or as biosensors [14]. For example, Mohamad et al. described hydrogels based on acrylic acid and bacterial cellulose obtained via

electron beam irradiation. They performed measurements as well as an analysis of the sorption properties of obtained materials. An interesting aspect of the research was the evaluation of the cytotoxicity of the prepared hydrogels toward L929 cell lines and *in vivo* experiments using Sprague Dawley rats. It was proved that the hydrogels did not exhibit cytotoxic activity. Furthermore, the application of hydrogels promoted more effective wound healing via the accelerated proliferation of fibroblasts compared to the control group [15]. Other investigations on the acrylic hydrogels have been conducted by Staneva et al. who modified these materials with cotton fabric and silver nanoparticles. Their studies aimed at determining the antibacterial activity of the composites prepared against *Escherichia coli* and *Acinetobacter johnsonii* and allowed them to demonstrate the antibacterial properties of the developed systems [16]. In turn, Kumar et al. proposed the hydrogels based on acrylic acid and tamarind kernel polysaccharides for application in regenerative medicinal treatment of the skeletal system. They proved that the presence of

the hydrogels accelerated the differentiation, mineralization and the expression of various osteogenic genes in Saos-2 cell lines which in the case of the bone injuries treatment may provide more effective osteogenesis process [17].

As mentioned previously, a significant feature of hydrogels is their potential modification with various materials that, in turn, increase the spectrum of their potential application [18]. Particularly important modifying agents are natural ones such as e.g. plant extracts [19]. However, substances of synthetic origin such as e.g. metallic nanoparticles are also widely applied [20]. For example, Park et al. developed alginate hydrogels modified with low molecular weight hyaluronate with potential use in cartilage regeneration. They proved that such hydrogels may successfully regulate the chondrogenic differentiation which contributes to the increase in the effectiveness of the cartilage regeneration [21]. Next, Derkach et al. conducted studies on determining the impact of anionic polysaccharide (κ -carrageenan) on the gelling process of gelatin-based hydrogels. They proved that the increase in the concentration of κ -carrageenan accelerated the mentioned process at the same time leading to the significant increase in the viscoelastic parameters of modified hydrogels [22]. In turn, the main purpose of the research of Makvandi et al. was to develop thermosensitive hydrogels based on hyaluronic acid and modified with corn silk and silver nanoparticles. Due to the good biocompatibility and antibacterial activity of these hydrogels, they have been determined as promising materials with great application potential for wound healing [23]. Next, Li et al. have developed hydrogels based on poly(ethylene glycol) and modified with gold nanoparticles (which acted as a radiosensitizer) and anticancer drug (doxorubicin). The drug release profiles *in vitro* and *in vivo* have been determined. Results of the skin safety investigations as well as the histologic observations of organs and the body weight changes indicated the biocompatibility of the developed materials. Furthermore, the inhibition of the growth and the proliferation of cancer cells has been observed which is a promising result in terms of the application of such materials in cancer treatment [24]. In turn, Ragab et al. developed an active multicomponent hydrogel for the treatment of chronic wounds. Such material has been synthesized using a methacrylated chitosan as a base and *Punica granatum* peel extract and ethyl acetate fraction as modifiers [25]. Moreover, the modification of hydrogel materials was also the research subject of Gallo et al. [26], Rask et al. [27] and Pankongadisak and Suwangton [28].

The main purpose of the investigations was to obtain and characterize the multicomponent hydrogel materials based on acrylic acid and arabic gum and modified additionally with gold nanoparticles, bee pollen and chamomile extract. Natural modifiers (bee pollen and chamomile extract) were selected due to their antibacterial and antioxidative properties beneficial for wound healing process [29, 30]. In turn, gold nanoparticles showing the large specific surface may constitute effective carriers for the selective and targeted delivery of active substances [31]. Additionally, they also increase drug stability [32]. Importantly, in appropriate concentrations these nanomaterials are biocompatible while at the same time maintaining antibacterial properties,

a particularly important factor in the case of the application of such modified hydrogels as dressing materials [33, 34]. Due to the potential application of these polymers for biomedical purposes, incubation investigations have been performed to evaluate their behavior in liquids simulating environments occurring in the human body. Additionally, the sorption properties of the obtained hydrogels have also been verified in these environments. After the incubation studies, the impact of such an immersion on the chemical structure of hydrogels has been evaluated via Fourier transform infrared (FT-IR) spectroscopy. The studies performed also included the characterization of gold nanoparticles which were used as modifiers of hydrogel matrix. Their size was determined via dynamic light scattering (DLS) wherein their optical properties were evaluated via UV-Vis spectrophotometry.

2. EXPERIMENTAL PART

2.1. Materials

Potassium hydroxide (pure, p.a.), diacrylate poly(ethylene glycol) (crosslinking agent, PEGDA 700, average $M_n = 700$ g/mol; $d = 1.120$ g/mL), 2-hydroxy-2-methylpropiophenone (photoinitiator, $d = 1.077$ g/mL), arabic gum (pure, p.a., powder) were bought in Avantor Performance Materials Poland S.A. Acrylic acid (99%, anhydrous, contains 200 ppm hydroquinone monomethyl ether as inhibitor) was supplied by Merck. The bee pollen and chamomile were purchased in Herbapol (Lublin, Poland). Gold nanoparticles were obtained according to the procedure described in [35].

2.2. Synthesis of hydrogels

In order to obtain hydrogel materials, the first step was to neutralize 13.5 mL of acrylic acid with 15 mL of 40% KOH solution. Due to the exothermic character of this reaction, the process was performed in a cooling medium (tap water). After cooling to room temperature, arabic gum, an adequate amount of the selected modifier (chamomile extract or bee pollen suspension), a crosslinking agent (PEGDA 700) and a photoinitiator (2-hydroxy-2-methylpropiophenone) were added to the mixture. Such prepared reaction mixtures were subsequently treated with UV radiation for 3 min (as a source of radiation, an EMITA VP-60 lamp was used, power: 180 W, $\lambda = 320$ nm). A row of syntheses was conducted using various amounts of selected modifiers. Compositions of the hydrogels obtained are presented in Table 1 (hydrogels modified with bee pollen and gold nanoparticles) and in Table 2 (materials with chamomile extract and gold nanoparticles).

After the synthesis, the prepared hydrogels were dried at room temperature and subjected to studies aimed at characterizing their physicochemical properties. The experiments performed included incubation studies determining the behavior of obtained materials in liquids simulating environments occurring in the human body. Furthermore, the sorption properties of hydrogels were also evaluated. Additionally, gold nanoparticles used as one of the modifying agents were characterized using dynamic light scattering (DLS) and UV-Vis spectrophotometry.

Table 1
Compositions of hydrogels modified with bee pollen and gold nanoparticles

| Series | Acrylic acid [mL] | KOH [mL] | Crosslinking agent [mL] | Photoinitiator [mL] | Arabic gum [g] | Bee pollen* [v/v] | Gold nanoparticles suspension [mL] |
|--------|-------------------|----------|-------------------------|---------------------|----------------|-------------------|------------------------------------|
| I | 13.5 | 15.0 | 0.03 | 0.13 | 5 | 0 | - |
| | | | | | | 10 | |
| | | | | | | 20 | |
| | | | | | | 30 | |
| II | 13.5 | 15.0 | 0.03 | 0.13 | 5 | 0 | 5 |
| | | | | | | 10 | |
| | | | | | | 20 | |
| | | | | | | 30 | |

* bee pollen suspension – prepared via introducing 1 g of this substance into 100 mL of distilled water

Table 2
Compositions of hydrogels modified with chamomile extract and gold nanoparticles

| Series | Acrylic acid [mL] | KOH [mL] | Crosslinking agent [mL] | Photoinitiator [mL] | Arabic gum [g] | Chamomile extract** [v/v] | Gold nanoparticles suspension [mL] |
|--------|-------------------|----------|-------------------------|---------------------|----------------|---------------------------|------------------------------------|
| III | 13.5 | 15.0 | 0.03 | 0.13 | 5 | 0 | - |
| | | | | | | 10 | |
| | | | | | | 20 | |
| | | | | | | 30 | |
| IV | 13.5 | 15.0 | 0.03 | 0.13 | 5 | 0 | 5 |
| | | | | | | 10 | |
| | | | | | | 20 | |
| | | | | | | 30 | |

** chamomile extract – prepared via introducing 1 g of this substance into 100 mL of distilled water (water temperature 96°C, brewing time 10 min)

2.3. Characteristic of gold nanoparticles using the DLS technique

The size of gold nanoparticles was determined via the dynamic light scattering (DLS) technique. The measurement was performed for gold nanoparticles suspension before its introduction into the polymer matrix and using Zetasizer Nano ZS Malvern. The analysis was conducted at room temperature. In order to characterize the gold nanoparticles more precisely, the optical properties of their suspension were determined via UV-Vis spectrophotometry. The study was carried out using a Thermo Scientific Evolution 220 (the measurement range: 350–700 nm) and also at room temperature.

2.4. Investigation on the sorption properties

One of the basic analysis performed for hydrogel materials is the analysis of their sorption ability. This is particularly important in terms of their potential application as wound dressings, one of the most essential features of which is a capacity for wound exudate absorption. The sorption properties of

hydrogels was evaluated using 2% citric acid, Ringer liquid (liquid isotonic to the human blood), SBF (simulated body fluid, isotonic to the human blood plasma), Tyrode solution (isotonic to the interstitial fluid), Hank solution (buffer used in the cell culture media to maintain optimal physiological pH) and distilled water (used as a reference liquid). The study was conducted for all obtained hydrogels. For this purpose, hydrogel samples were introduced into the sterile vessels containing 50 mL of the tested solution. After 1 h samples were separated from the liquids, excess water was removed using a paper towel and weighed again. The sorption capacity of the materials was defined using swelling ratios α [g/g] which were determined using the following Equation (1):

$$\alpha = \frac{(m - m_0)}{m_0} \quad (1)$$

where:

- α – swelling ratio [g/g],
- m – mass of a swollen hydrogel [g],
- m_0 – mass of a dry hydrogel (before swelling) [g].

2.5. Investigations on the behavior of hydrogel in simulated body fluids

In order to determine the behavior of obtained hydrogels in liquids simulating environments occurring in the human body incubation studies have been performed. Studies were conducted in SBF, artificial saliva and Ringer liquid. Approx. 1 g hydrogel samples were introduced into sterile vessels containing 50 mL of the liquid. Next, solutions with immersed samples were incubated at 37°C. During a four-week incubation period, the pH values of the solutions were measured and pH changes were also checked for the tested liquids without the immersed hydrogels (such liquids were treated as reference liquids).

2.6. Analysis of the chemical structure of hydrogels via Fourier transform infrared (FT-IR) spectroscopy

To determine potential changes in the structure of hydrogels caused by immersion in the simulated body fluids, FT-IR analysis was performed. The study was conducted for hydrogels containing 10% and 30% of the additive both before and after the incubation in Ringer liquid, SBF and artificial saliva. For this purpose, a Spectrum 65 (Perkin Elmer) spectrophotometer equipped with an attenuated total reflectance (ATR) attachment with a diamond/ZnSe crystal was used. FT-IR spectra were recorded within the range: 4000–600 cm^{-1} (32 scans, resolution 4.0 cm^{-1}) at room temperature.

3. RESULTS AND DISCUSSION

3.1. Characteristic of gold nanoparticles

In the research, the size of the particles obtained was verified using DLS technique wherein their optical properties were determined via UV-Vis spectrophotometry. Results of the performed investigations are shown below in Figure 1.

According to the literature reports, the term “nanoparticles” refers to the particles with at least one dimension ranging from 1 nm to 100 nm [36]. Based on the DLS analysis performed it was observed that the tested suspension contained particles having a size of approx. 30 nm. Thus it may be concluded that the particle suspension used a modifying agent of the polymer matrix contain nanoparticles. Importantly, the narrow particle size distribution was shown to indicate that the suspension with an uniform particle size and probably without the agglomerates of particles was introduced into the reaction mixture.

Figure 1b shows the results of UV-vis spectrophotometric analysis. Suspension of gold nanoparticles is characterized by an intense ruby color which is caused by the surface plasmon resonance (SPR). This, in turn, is a result of the collective oscillations of free conduction electrons caused by the electromagnetic field [37–39]. Thus the presence of gold nanoparticles in a tested suspension may be confirmed via confirmed UV-Vis spectrophotometry wherein the SPR absorption peak characteristic for nanogold occurs within the wavelength range 515–570 nm [40]. What is more, the

position of the absorption band is strictly related to such factors as e.g. the size and the shape of the particles, their interactions with the medium in which they are suspended or the refractive index. Thus it is assumed that a single SPR band in the absorption spectra indicates the presence of spherical nanoparticles while in the case of anisotropic nanoparticles two or more SPR bands depending on their shape are observed [37, 41]. In the case of the tested suspension, maximum absorbance observed on the UV-Vis spectra was at approx. 515 nm which proves the presence of gold nanoparticles in tested medium. The maximum absorbance characteristic for gold nanoparticles in the similar range was also reported by Ngo et al. [42], Duan et al. [43] and Amendola and Meneghetti [44]. Next, due to the presence of a single absorption band on the UV-Vis spectrum obtained, it may also be concluded that spherical-shape nanoparticles occur in the analyzed suspension.

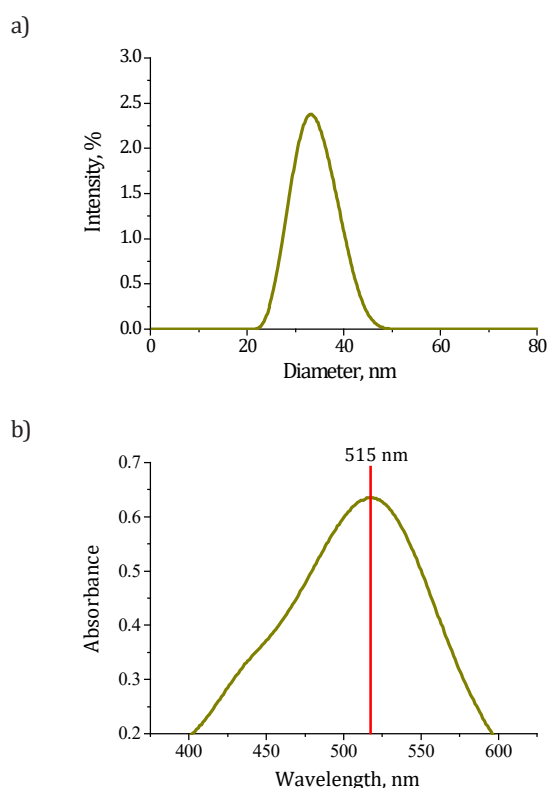


Fig. 1. Results of DLS analysis (a) and UV-Vis spectrum of gold nanoparticles suspension (b)

3.2. Investigations on the sorption properties of hydrogels

Considering the potential applications of the developed materials in biomedical fields such as pharmacy, an essential aspect of the research was to determine the sorption ability of the obtained hydrogels. Swelling properties are particularly important in the case of the use of such materials as dressings, where one of the major tasks is to absorb the wound exudate, or in drug delivery systems where swelling of the material proceeds in parallel to the release of the active substance present in the polymer matrix.

Characteristics of the sorption of prepared materials in selected liquids is presented in Figure 2.

The swelling of hydrogel materials is due to the hydration of hydrophilic functional groups present in the structure of these polymers. Diffusion of water or other fluids is based on the migration of this fluid into pre-existing or dynamically forming spaces between polymer chains. After the introduction of the hydrogel matrix into the tested liquid, processes of dissociation and solvation of functional groups present in the polymer structure take place. As a result of the electrostatic interactions, the repulsion of similar ones (with the same charge) is observed, which in turn leads to the formation of free spaces between polymer chains and finally to the loosening of the polymer structure. As a result, water molecules easily penetrate such spaces [45, 46].

Considering the results of the performed investigations, it was proven that the highest swelling ratios were calculated for hydrogels immersed in distilled water. On the other hand, samples tested in SBF, Hank solution and Tyrode solution showed significantly lower swelling ability. This phenomenon may be caused by the presence of such ions as Ca^{2+} , Mg^{2+} or Na^+ in these solutions which may affect the crosslinking density of the hydrogel matrix. For example, due to the presence of divalent ions, i.e. Ca^{2+} and Mg^{2+} , additional crosslinks between polymer chains in the polymer structure may be formed. As a result, an increase in the crosslinking density of such polymer followed by the limitation of free spaces available for absorbed liquid takes place. The presence of Na^+ ions may also reduce the sorption capacity of hydrogel materials. When the hydrogel swells in a medium containing sodium

ions, the ion exchange including the hydrogen ions formed as a result of dissociation of functional groups occurring in the polymer structure and the sodium ions from absorbed liquids may take place. Such neutralization, in turn, may reduce the hydrophilic character of the carboxyl group leading finally to the lower swelling properties of such a structure. These conclusions are consistent with the conclusions of studies concerning the impact of various ions on the swelling process performed by Zhu et al. [47].

Low values of the swelling ratios were also calculated for samples swelling in 2% citric acid solution. The low swelling ability of hydrogels in low pH solutions is related to the high concentration of hydrogen ions in the presence of which the protonation of carboxylic anions may occur. As a result, the main anion - anion repulsive forces are eliminated. This, in turn, leads to the reduction in the formation of free spaces between polymer chains and, finally, to the reduction in the swelling capacity of such material. Similar results were presented by Al-Anbakey et al. who also observed that hydrogel materials placed in low pH solutions exhibited low sorption capacity [48]. Thus, it was proved that sorption properties of hydrogels depend on the type of fluid in which such a study is performed.

The presence of various modifying agents in the hydrogel matrix may also affect sorption properties. In the case of all the tested samples, the highest swelling ratios were observed for unmodified hydrogels. As the modifier content increased, swelling ratios decreased, e.g. $\alpha = 17.69$ g/g for samples containing 10% bee pollen and $\alpha = 8.38$ g/g for a sample containing 30% bee pollen (swelling ratios in distilled water).

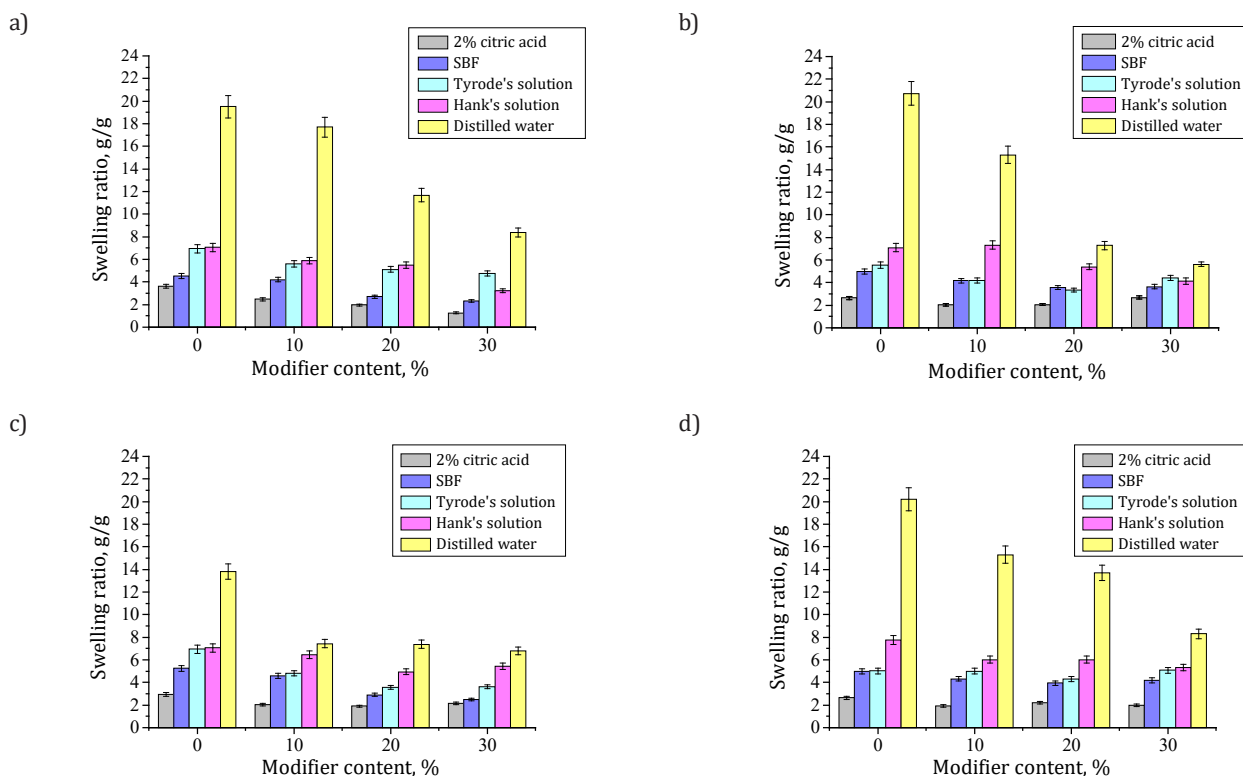


Fig. 2. Results of the swelling studies of obtained hydrogels: a) series I (modified with bee pollen); b) series II (modified with bee pollen and gold nanoparticles); c) series III (modified with chamomile extract); d) series IV (modified with chamomile extract and gold nanoparticles)

The greatest decrease in the swelling was observed in the case of samples tested in distilled water. Higher sorption properties of unmodified hydrogels may stem from the fact that more free spaces occur in the matrix of these polymers compared to the matrix of modified materials in which spaces between polymer chains are partially filled by modifiers. Thus, the modifying agents significantly limit the space which might be filled by the absorbed liquid. Therefore, the reduction in the swelling capacity of such materials is observed. Moreover, additives such as bee pollen or chamomile contain a number of chemical compounds, including those with aromatic rings. These structures cause the so-called steric effect which may also limit the penetration of fluids between polymers. In the case of the presence of gold nanoparticles, any significant changes in swelling properties of the materials modified additionally with these additives was not noticed. Only in the case of hydrogels containing both chamomile extract and gold nanoparticles was an increase in the swelling ratios observed. This is probably a result of the release of some amounts of the modifying agents into the tested liquid and thus forming free spaces previously occupied by the modifiers which may be filled with absorbed liquid.

3.3. Results of the investigations on the behavior of hydrogels in simulated body fluids

Incubation studies were aimed at determining the behavior of the obtained hydrogels in liquids simulating environments occurring in the human body. These investigations are particularly important in terms of the potential

application of the developed materials for biomedical purposes. The behavior of hydrogels presented as pH changes in the course of their four-week incubation in selected liquids is presented below in Figures 3–5.

In the case of all hydrogels immersed in various types of fluids, an analogous course of the curves presenting pH changes was observed. Firstly, i.e. after the first week of the immersion, a drop in pH value in all tested environments was reported. Such a pH decrease may be due to the interactions that probably occurred between the dry hydrogel sample and the incubation medium. It is also likely that such a change in pH may be a result of the beginning of the degradation of the immersed samples.

At the beginning of the immersion, the tested liquid penetrates the interior of the material and a dry hydrogel begins to swell. Next, the elution of unreacted or non-crosslinked reagents present in the polymer matrix probably takes place.

Additionally, during the first days of the immersion liquid penetrating the material, being incubated causes the elution of the additives, i.e. bee pollen or chamomile extract. All these processes may contribute to the pH changes observed at the beginning of the investigation. In the next weeks of pH measurements, stabilization of the pH values may be observed. Any changes in pH values were not observed and its constant value maintained over the next days of the study which may indicate the buffering properties of the hydrogels.

Slight greater changes in pH may be noticed in the case of modified hydrogels. In the case of samples with various modifying agents, a slightly larger pH decrease was observed compared to this change reported for unmodified materials.

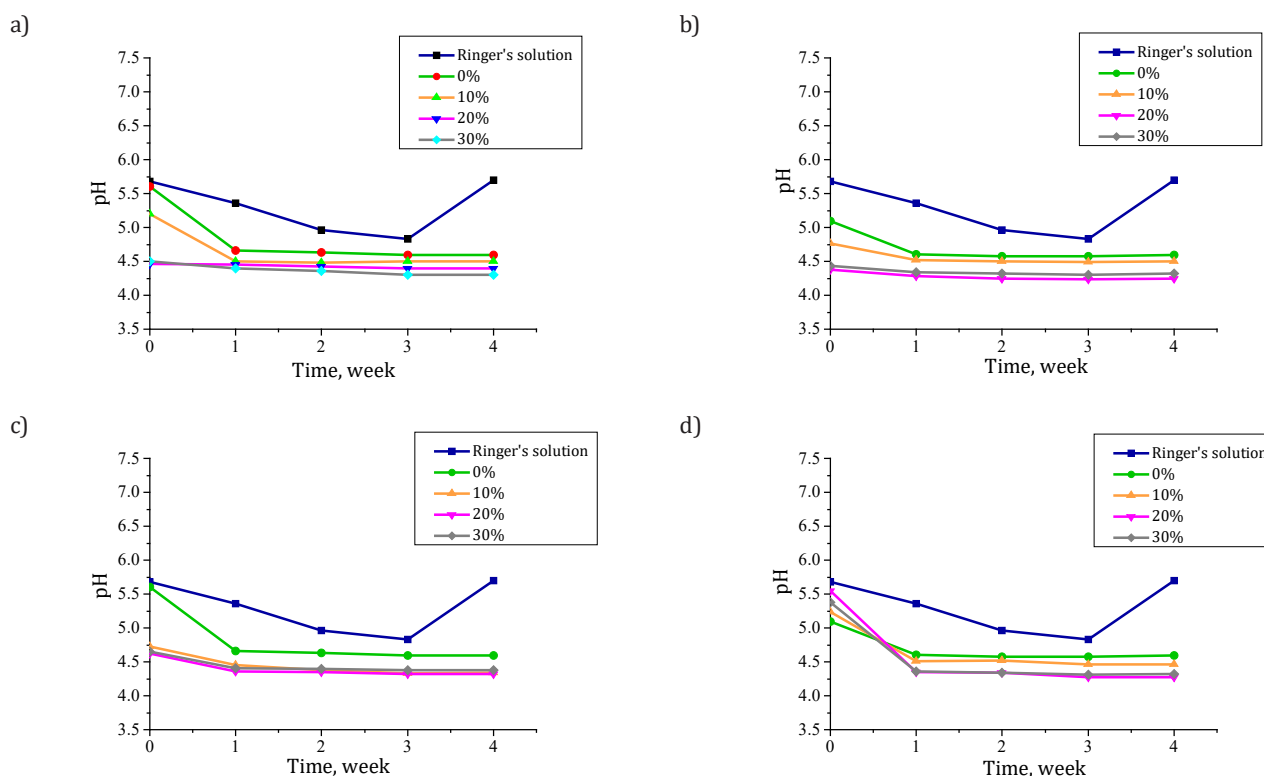


Fig. 3. pH changes measured during the incubation of hydrogels in Ringer liquid: a) series I (modified with bee pollen); b) series II (modified with bee pollen and gold nanoparticles); c) series III (modified with chamomile extract); d) series IV (modified with chamomile extract and gold nanoparticles)

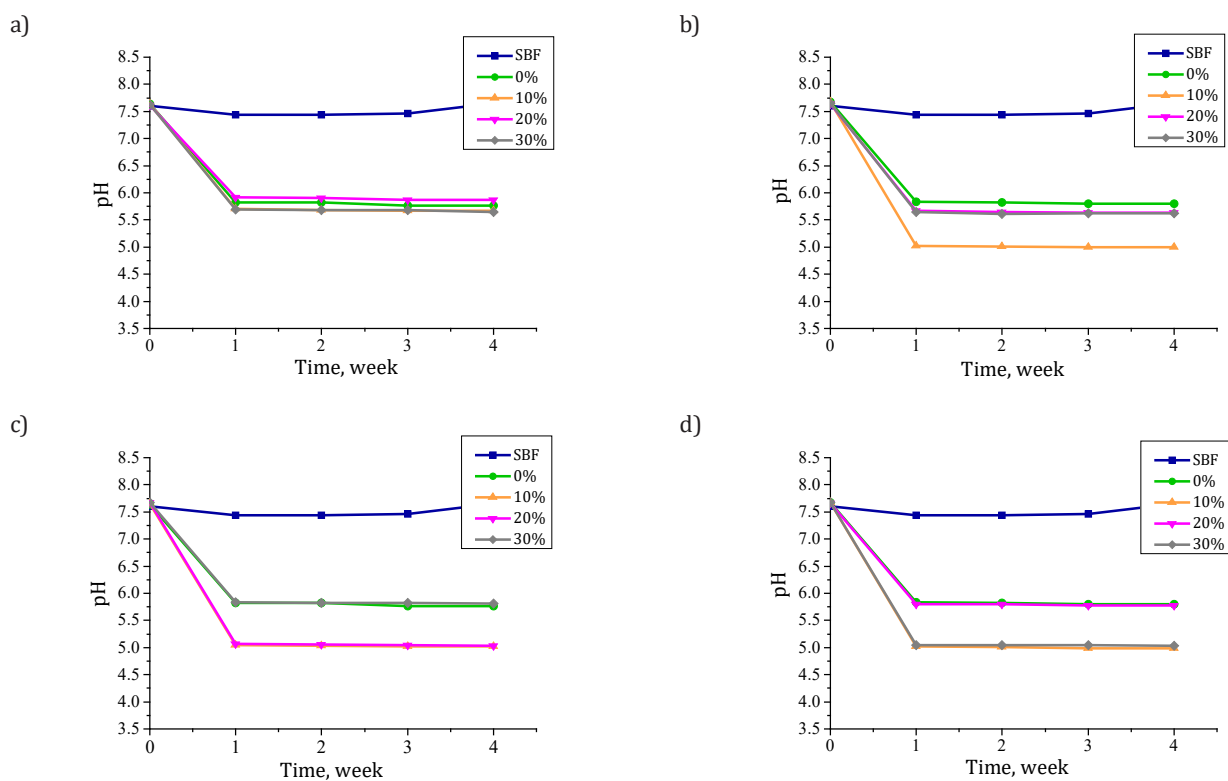


Fig. 4. pH changes measured during the incubation of hydrogels in SBF: a) series I (modified with bee pollen); b) series II (modified with bee pollen and gold nanoparticles); c) series III (modified with chamomile extract); d) series IV (modified with chamomile extract and gold nanoparticles)

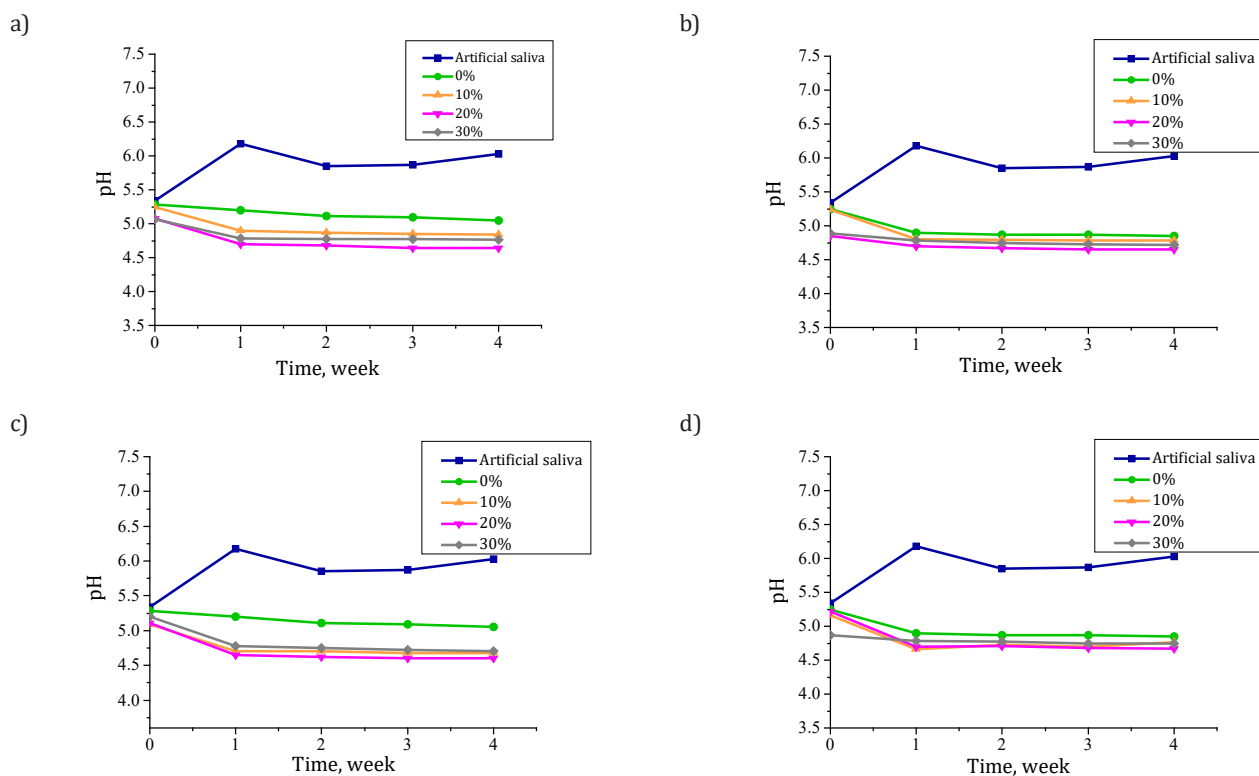


Fig. 5. pH changes measured during the incubation of hydrogels in artificial saliva: a) series I (modified with bee pollen); b) series II (modified with bee pollen and gold nanoparticles); c) series III (modified with chamomile extract); d) series IV (modified with chamomile extract and gold nanoparticles)

This probably results from the release of the modifying substance from the polymer matrix which, in turn, may be caused by the interactions of the hydrogel sample with the incubation liquid. The release of both bee pollen and chamomile extract may cause greater changes in pH of tested liquids due to the presence of various chemical compounds such as e.g. organic acids or different enzymes in these additives which may contribute to the additional acidification of the environment. Nonetheless, these changes are only observable during the first week of the incubation. During the next week of the study, the pH of the solutions stabilizes and the lack of any further changes may indicate the biocompatibility of the analyzed materials with respect to the applied liquids.

3.4. Results of Fourier transform infrared (FT-IR) spectroscopy

Results of the spectroscopic analyses performed to verify the impact of the incubation of hydrogels in simulated physiological liquids on their chemical structure are shown in Figures 6 and 7. Based on the literature data, absorption bands observed on the above-presented FT-IR spectra were assigned to the adequate functional groups presented in Table 3.

A broad band of relatively high intensity observed within the range of 3700–3300 cm^{-1} may be assigned to the –OH groups resulting probably from the presence of water molecules in the structure of the analyzed hydrogels. Among the absorption bands characteristic for acrylic hydrogels, bands in the following ranges may be observed: 3000–2900 cm^{-1} (stretching vibrations of –CH₂ groups), 1740–1650 cm^{-1}

(stretching vibrations of –C=O groups) and 1580–1520 cm^{-1} (stretching vibrations of –OH groups). Due to the possibility of the overlapping of bands deriving from groups characteristic for both polymer matrix and the compounds occurring in modifiers, it is difficult to clearly assign absorption bands visible on obtained FT-IR spectra for particular components. For example, an absorption band corresponding to the stretching vibrations of the carbonyl groups may derive both from –C=O bonds present in the hydrogel matrix and the conjugated esters from luteolin present in chamomile extract [49] or to phenolic compounds derived from bee pollen [50]. Furthermore, due to the numerous amino acids occurring in the compositions of the modifying agents, absorption bands within the range 1650–1500 cm^{-1} may be assigned to the amino groups while the absorption band at 1360–1000 cm^{-1} may be assigned to the stretching vibrations of C–N groups. Next, band visible within the 1050–1000 cm^{-1} range corresponds to the stretching vibrations of –CO groups and is characteristic for polysaccharides (included in the composition of modifying agents).

Analyzing the FT-IR spectra of samples before and after the incubation in simulated body fluids, it is important to determine the changes in the intensity of the absorption bands. The decrease in the intensity of particular bands or their complete disappearance may indicate changes occurring in the immersed material such as e.g. the degradation of incubated polymers or the release of the modifying agents present in the polymer matrix. The greatest changes in the intensity of particular bands have been marked in above-presented FT-IR spectra via the black frame.

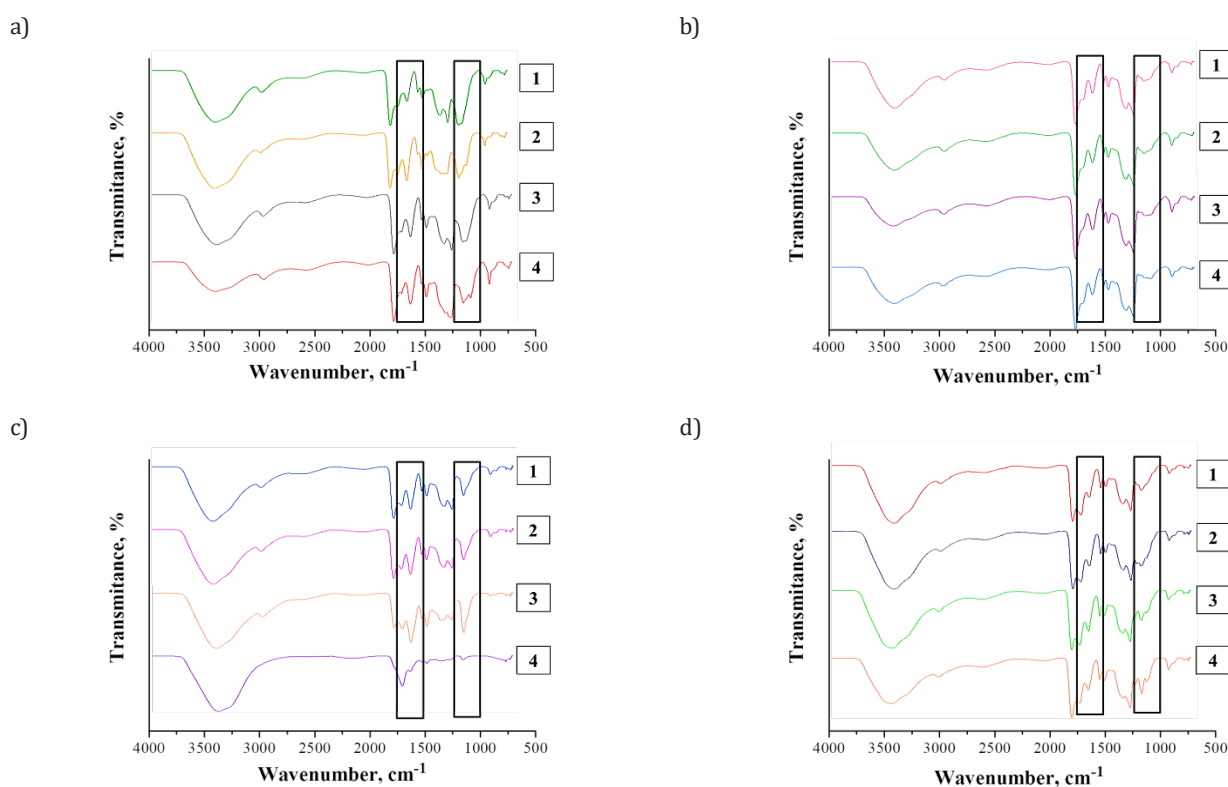


Fig. 6. FT-IR spectra of hydrogels containing: a) before incubation; b) Ringer incubation; c) SBF incubation; d) artificial saliva incubation; 1) 10% bee pollen; 2) 30% bee pollen; 3) 10% bee pollen and gold nanoparticles; 4) 30% bee pollen and gold nanoparticles

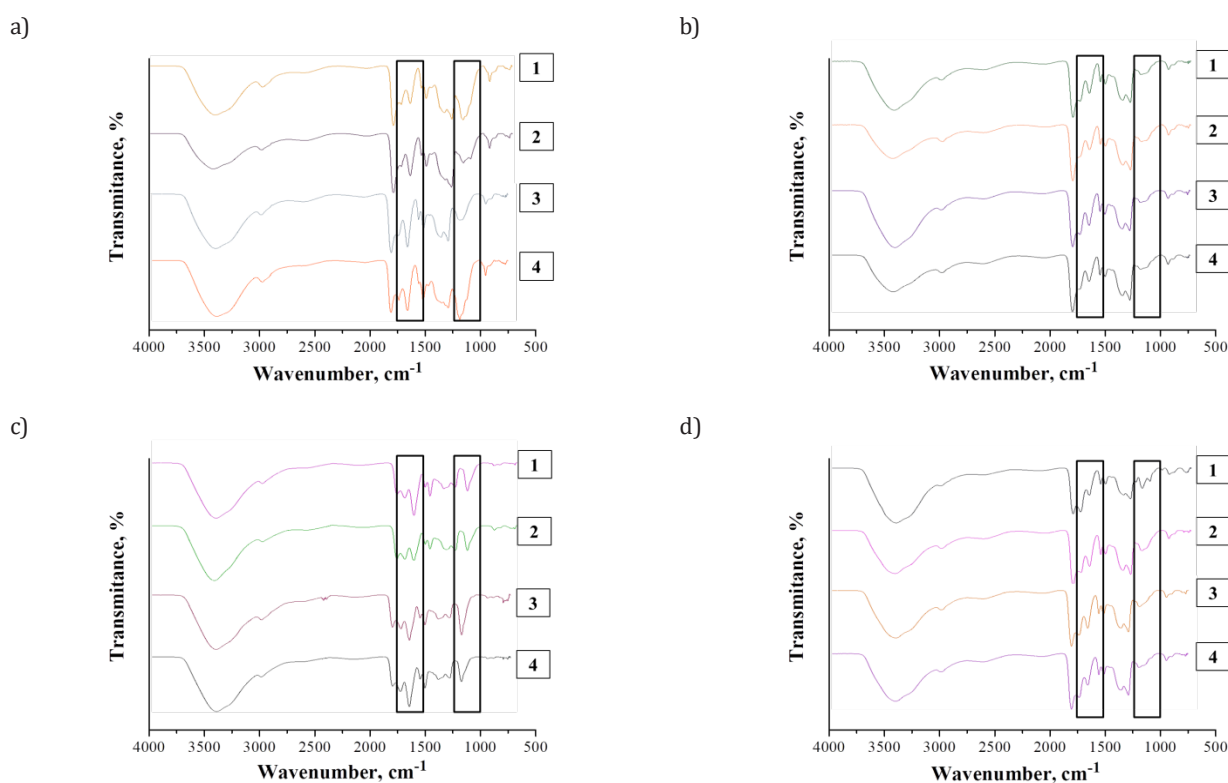


Fig. 7. FT-IR spectra of hydrogels containing: a) before incubation; b) Ringer incubation; c) SBF incubation; d) artificial saliva incubation; 1) 10% chamomile extract; 2) 30% chamomile extract; 3) 10% chamomile extract and gold nanoparticles; 4) 30% chamomile extract and gold nanoparticles

Table 3
Vibrations observed on FT-IR spectra and corresponding functional groups

| Wavelength range [cm ⁻¹] | Functional group | Type of vibration |
|--------------------------------------|------------------|-------------------|
| 3700–3300 | OH | stretching |
| 3000–2900 | CH ₂ | stretching |
| 1740–1650 | C=O | stretching |
| 1650–1500 | N-H | deformation |
| 1580–1520 | COOH | stretching |
| 1360–1000 | C-N | stretching |
| 1050–1000 | C-O | stretching |

The changes concern the absorption bands which may be attributed to the vibrations of groups characteristic for compounds present in the compositions of modifiers applied. Thus, it may be concluded that the decrease in the intensity of these bands indicates the release of certain amounts of the modifiers to the incubation medium. These conclusions are consistent with the results of pH changes observed during the incubation studies which indicated the acidification of the incubation environment. Considering different incubation fluids, the highest changes in the intensity of observed bands were observed in SBF and, importantly, in this solution the greatest pH decrease was reported during the incubation period. Therefore, it may be concluded that in this incubation medium the greatest amount of the additive is released or that the degradation occurs most intensively.

4. CONCLUSIONS

Based on the results presented above, the following conclusions and observations were formulated:

- The DLS analysis showed that the suspension introduced into the hydrogel matrix contained gold particles having a size of approx. 30 nm and so gold nanoparticles. Moreover, the occurrence of a single narrow peak on UV-Vis spectrum indicates that a suspension with particles of a uniform size was used.
- The highest swelling capacity was shown by hydrogel materials in distilled water. Ions such as Ca²⁺, Na⁺ or Mg²⁺ in absorbed liquids reduce the sorption properties of hydrogels which results from the formation of additional crosslinks between these ions and hydrophilic functional groups present in polymer chains of the hydrogel network.
- Modified hydrogels showed a lower sorption capacity than unmodified ones. This may result from the fact that additives present in the matrix fill the spaces between the polymer chains thus limiting the space available for liquids.
- Incubation of tested hydrogels in simulated body fluids did not cause any rapid changes in their pH, which may indicate the biocompatibility of materials with the fluids used.
- During spectroscopic analysis, changes in intensity of selected absorption bands on FT-IR spectra of samples before and after the incubation were observed. This probably resulted from the partial degradation of the materials being incubated or from the release of modifiers present in the polymer matrix into the incubation medium.

- The analyses did not show any significant influence of the addition of nano-gold on the sorption properties of hydrogels or on the course of their incubation process. However, the introduction of this additive into the polymer matrix is important because of the antibacterial effect of gold nanoparticles, which is beneficial due to the potential use of these materials as dressings.
- The presence of additives in the polymer matrix affects selected physicochemical properties of these materials. It was proved that selected additives, i.e. bee pollen or chamomile, reduce the sorption capacity of hydrogels. However, due to their therapeutic effect, such a modification allows materials to be designed with new properties which may be beneficial in terms of their use as dressing materials.

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