PREPARATION AND CHARACTERIZATION OF BIO-HYBRID HYDROGEL MATERIALS

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Abstract

In recent decades, research has focused on the development of modern hydrogel dressings due to their open porous structure, moisture retention and good mechanical strength, which ensures an optimal environment for cell migration and proliferation. Active hydrogel dressings, currently available on the market, are not endowed with additional medicinal substances. In this work the authors attempted to introduce a carrier-drug system into the hydrogel matrix to improve the wound healing process and the tissue recovery. The main goal of the research was to obtain the bio-hybrid sodium alginate/poly(vinyl alcohol)/Aloe vera (SA/PVA/AV)-based hydrogel matrices modified with the thermosensitive polymeric carrier – the active substance (hydrocortisone) system. First, thermosensitive polymeric nanocarriers were obtained, then the encapsulation was conducted, using varied amounts of hydrocortisone (25 and 50 mg) to maintain the stability of the resulting emulsions. The last stage was preparing the bio-hybrid hydrogel matrices by the chemical cross-linking method. The non-invasive dynamic light scattering (DLS) technique was employed for the analysis of the average particle size of the polymeric carriers and the carrier-drug systems. Moreover, the studies also determined the swelling behaviour and the gel fraction of the obtained bio-hybrid hydrogel matrices modified with carrier-drug systems by the infrared spectroscopy (FT-IR). The presented research results constitute a good experimental basis for further modifications, the final effect of which is assumed to be a modern bio-hybrid 3rd generation dressing.

Keywords: *hydrogel matrix, drug delivery system, carrier-drug system, synthesis, hydrocortisone*

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Introduction

Hydrogels are one of the most promising materials widely used in medicine, due to the ability to control the water content and sensitivity to stimulus changes (e.g. temperature and pH). They are characterized by properties similar to biological tissues and they exhibit high biocompatibility [1,2]. Besides, various substances can be incorporated into the hydrogel matrix to induce a change in the initial physicochemical properties of the material. In the world literature here are many reports on hybrid hydrogel matrices modified with active substances or drugs [2-4].

In the pharmaceutical and medicine industries, hydrogels are used primarily in the production of active dressings, in tissue engineering and as systems for the controlled delivery of active substances [1,4-6]. In terms of the controlled and prolonged release of active substances, polymer nanoparticles enable effective penetration of active substances, proteins or DNA through cell membranes. They also demonstrate stability in the blood and they do not stimulate the immune system and inflammatory processes. The most commonly obtained structures exhibit nanometric sizes (range 100-500 nm). Polymer nanoparticles are stable, they have colloidal structures and exist in the form of nanospheres and nanocapsules. The materials used in nanopharmacology are mainly biodegradable polymers, e.g. chitosan, polylactic acid, gelatin, (N-(2-hydroxypropyl) methacrylamide) and their copolymers [7-9].

The pharmaceutical market offers a wide range of dermatological preparations, as even the smallest skin lesions may be associated with an allergic reaction or a serious disease. Patients use various types of ointments, gels or lotions containing active substances, such as salicylic acid, hydrocortisone and plant extracts. Ideally, a skin substitute scaffold should maintain the moist healing environment on the wound surface. It should also persist long enough so that the cells have enough time to migrate through the scaffold and build a new extracellular matrix responsible for the movement of keratinocytes and growth factors. Thus, the wound starts to be covered with a single epithelial layer. Therefore, the hydrogels properties, such as the ability to create a permanent moist medium in the wound and to absorb wound exudates, are a key factor to maintain cellular activity by means of the substances promoting the skin reconstruction [10-12].

A bio-hybrid hydrogel material well described in the literature is a hydrogel based on hydroxyl chitin enriched with tannic acid used to treat skin ulcers and burns. The hydrogel is endowed with strong antioxidant, antibacterial and hemostatic properties. Metal ions which are chelating sites with multiple galloyl groups of tannic acid form a stable complex [13,14]. Hybrid hydrogel matrices based on poly(vinyl alcohol), chitosan and silver nanoparticles are widely used in medicine due to the antibacterial properties of nanometric silver against many bacterial and fungal strains [15,16]. Also, in cancer therapies, a promising material is the chitosan/nanogold hybrid hydrogel used as a delivery system for drugs, e.g. doxorubicin (DOX). Studies have shown that the drug released from the hydrogel is biologically active but has lower cytotoxicity due to the controlled release in the expected location in the patient's body, such an approach is called targeted therapy [17].

In dermatology, the transdermal drug delivery of the hydrophobic active substances, such as: hydrocortisone, fluocinolone acetonide, tazarotene, is an ongoing challenge. Hydrocortisone is a glucocorticoid steroid hormone commonly used in the topical treatment of dermatological diseases, such as: atopic dermatitis, psoriasis, mycosis and acne. In general, this active substance is characterized by anti-inflammatory and anti-allergic activities [18], it can be used also for adrenal replacement therapy. However, as hydrocortisone is highly hydrophobic, it is difficult to synthesize it and later – to develop its efficient application procedures. Therefore, it seems beneficial to combine hydrocortisone with a hydrogel matrix used as a carrier. The studies so far have indicated that hydrocortisone may be incorporated into hydrogels based on chitosan, gelatin, methylcellulose or carboxymethylcellulose [19,20], which allows to obtain better therapeutic effects within the significantly shortened treatment time.

The main objective of the research presented in this paper was to prepare the bio-hybrid SA/PVA/*AV* based hydrogel matrices modified with the thermosensitive polymeric carrier and to characterize the active substance (hydrocortisone) system in terms of the basic physicochemical and structural properties.

Materials and Methods

N-isopropylacrylamide, N, N'-methylene bisacrylamide (NMBA), hydrocortisone, sodium alginate and poly(ethylene glycol) diacrylate (PEGDA) M_n = 700 g/mol (used as a cross-linking agent) were purchased from Sigma – Aldrich (Germany). Poly(vinyl alcohol) (M_n = 72 000 g/mol), ammonium persulphate employed as an initiator and glycerine were acquired from POCH SA (Poland). *Aloe vera* lyophilisate was purchased from a shop with cosmetics and herbal raw materials - Zrob sobie krem, Poland. All the applied chemical reagents were of high purity.

The first research stage consisted in the synthesis of thermosensitive polymeric carriers with the radical polymerization reaction, using N-isopropylacrylamide, N, N'-methylene bisacrylamide and ammonium persulfate, according to the literature data with some modifications [21]. After that, the active substance (hydrocortisone) was introduced into the polymeric carriers - the drug amount was 25 or 50 mg – based on the commercially available ointments. The emulsion mixing was performed for 10 min, 3 h, 5 h and 24 h. The obtained samples were analyzed using the dynamic light scattering (DLS) technique and afterwards they were lyophilized prior to being introduced into the hydrogel structure. In the last stage the bio-hybrid hydrogel matrices were synthesized via the chemical cross-linking method developed in the authors' previously published research which is currently the subject of a patent application [22]. In order to obtain the SA/PVA/*AV* hydrogels, the following solutions were prepared: a 5% solution of poly(vinyl alcohol), 2% solution of sodium alginate, 2% solution of *Aloe vera* extract and 1% solution of ammonium persulfate. Next, the proper amounts of the polymers solutions in the ratio 1:1 and the constant amounts of poly(ethylene glycol) diacrylate (7.5%) and glycerin (1.7%) were mixed. A slight addition of glycerin ensured transparency of the material and had a positive effect on the membrane flexibility. Subsequently, the mixtures were heated to 70°C and the 4.4% (v/v) ammonium persulfate was added. After that, all the specimens were poured into Petri dishes and placed on a heating plate with a temperature of 80°C for 1.5 h.

The non-invasive dynamic light scattering (DLS) technique was used to analyze the average particle size of the polymer carriers and the carrier-drug systems. The conducted research also included determining the swelling behaviour and the gel fraction. It also determined the chemical structure of the resultant bio-hybrid hydrogel matrices modified with carrier-drug systems via the infrared spectroscopy (FT-IR).

The average particle size measurements were performed using Zetasizer Nano ZS device produced by Malvern Instruments Ltd., which allows to assess the particle sizes in the range from 0.6 to 6000 nm. Each sample was measured three times, with each measurement consisting of several runs in order to determine the mean values precisely. The measurements were carried out at 25°C.

The swelling behaviour (absorption capacity) ratio was evaluated by immersing the samples in the isotonic buffer used in biological application (PBS, phosphate-buffered saline, pH = 7.4) and the distilled water at 37°C. The dried and weighted (W_d) hydrogel samples were soaked in the immersion fluids. The swollen samples were taken out and weighted (W_s) after 1 and 24 h. The water uptake of all tested hydrogel samples was determined using the following equation: absorption capacity [g/g] = $(W_d-W_s)/W_d$.

In order to determine the gel fraction (GF%), the obtained hydrogel materials were cut into 10 x 10 mm pieces, dried at 40°C for 24 h and weighted (W_0). Then dried hydrogel samples were soaked in the distilled water for 48 h up to an equilibrium swelling weight to remove the leachable or soluble parts from the matrices. Then, the gel materials were dried again in the same conditions and weighted (W_e). The gel fraction (GF%) was calculated by the following equation:

$$\%GF = W_e/W_0 \cdot 100\%$$

To identify the chemical structure of the hydrogels, the infrared spectroscopy was used with Thermo Scientific Nicolet iS5 FTIR spectrometer equipped with iD7 ATR accessory in the range of 4000 - 400 cm^{-1}.

Results and Discussions

The size of the drug carriers is an extremely important parameter. Therefore, in this study, the key analysis consisted in determining the average particle size of the thermosensitive polymeric carrier and the carrier - drug system. The average size of the unloaded (empty) polymeric carriers particles was 118 nm, but having removed the unreacted monomer via the dialysis method, this parameter decreased below 100 nm. In turn, the encapsulated carriers analysis showed that the presence of the drug increased the particle size. Additionally, the drug amount and the encapsulation time directly affected the average particle size, which is shown in TABLE 1.

TABLE 1. The average particle size of polymeric carrier - drug systems.

System number	Drug amount	Mixing time	Average particle size
1	25 mg	10 min	400 nm
2		3 h	391 nm
3		5 h	505 nm
4		24 h	1150 nm
5	50 mg	10 min	425 nm
6		3 h	617 nm
7		5 h	1049 nm
8		24 h	2285 nm

Comparing the systems containing the same drug amount but with the different mixing time, it can be concluded that the system prolonged mixing caused a significant increase in the particle size. The systems with the highest amount of drug displayed a much larger average particle size after the 24-hour mixing (2285 nm) than after the 3-hour mixing (617 nm). However, it was possible to obtain stable systems without the agglomeration effect with the average size below 500 nm. These thermosensitive carriers – the hydrocortisone systems were used for further research on developing the bio-hybrid hydrogel materials. FIG. 1 presents the photos of the obtained various systems.

The swelling tests results revealed that the obtained bio-hybrid hydrogel matrices had a slightly higher absorption capacity in the water environment than in the phosphate buffer (FIG. 2).

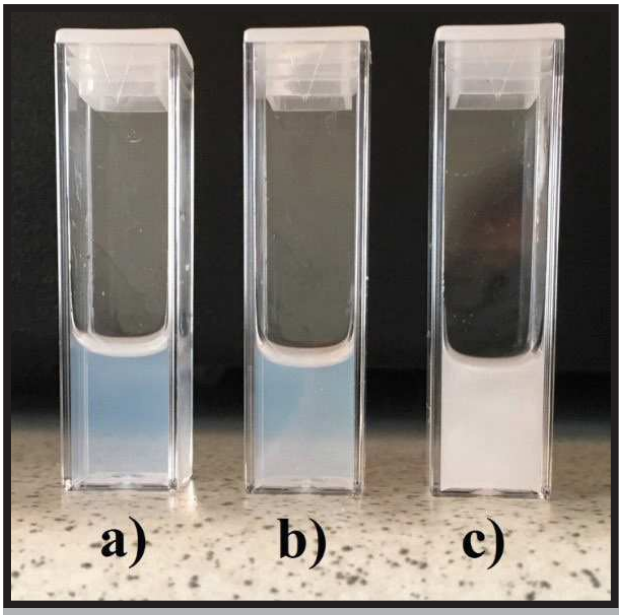

FIG. 1. Photos of the thermosensitive polymeric carrier – the hydrocortisone systems obtained after mixing for: a) 10 min, b) 3 h and c) 24 h.

The obtained results were very similar, however, the samples containing a higher drug concentration revealed a slightly higher swelling degree in each of the used media. This phenomenon resulted from the fact that the packing density of the chains in the hydrogel matrix decreased with adding new components to the system. When a higher concentration of the drug was released from the hydrogel matrix, additional gaps replacing the absorbed fluid were created. It was observed that the swelling degree decreased with time, probably due to the lack of free hydroxyl groups and/or spherical hindrance. As a result, no further hydration occurred. Based on the results, the tested bio-hybrid hydrogel matrices proved to be stable materials that did not change the shape during the incubation (they only slightly increased in size). Comparing the media used in the study, the samples in the PBS fluid showed the better stability and the more compact and flexible structure than the samples incubated in the distilled water.

The gel fraction value (%GF) represents the insoluble gel fraction as a result of inter-molecules crosslinking formation. The obtained bio-hybrid hydrogel matrices with the carrier-drug system were characterized by higher levels of crosslinking (over 67%) (FIG. 3). The content of the gel fraction reached the value of 66-67%, while the addition of the drug slightly increased this parameter. Also, the most important fact is that the thermosensitive polymer carrier - hydrocortisone did not reduce the degree of matrix cross-linking and it was even slightly higher. Moreover, with the cross-linking increase, the mechanical properties of the hydrogel increased too and thus the mechanical resistance of the material.

The chemical structure of the thermosensitive carrier – hydrocortisone systems and the bio-hybrid hydrogel matrices are presented in FIG. 4 and 5, respectively.

In the case of the modified thermosensitive carrier – drug system (FIG. 5), a much more intense band was observed in the range of 3500-3000 cm^{-1} which originated from the stretching vibrations of the O-H group the N-H group vibrations and most likely - from the strong hydrogen interactions that occurred between individual components.

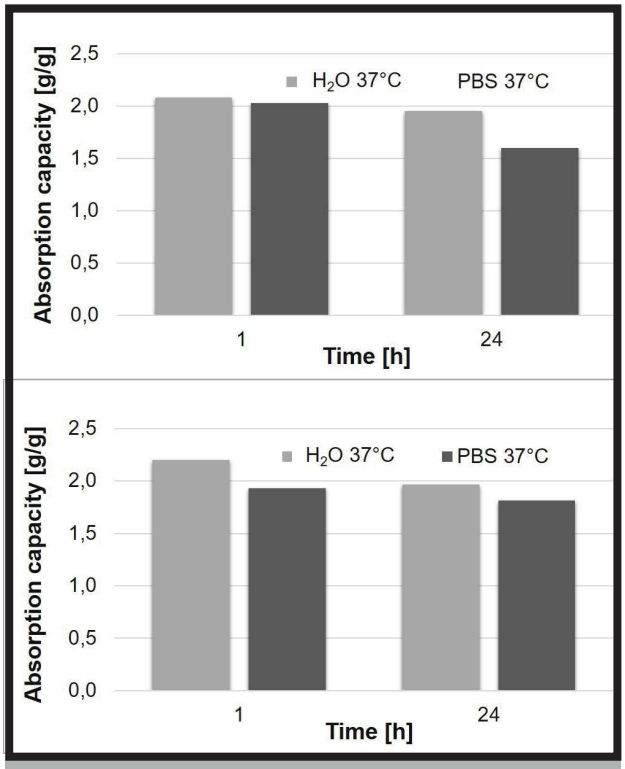

FIG. 2. The absorption capacity of the bio-hybrid hydrogel matrix modified with the thermosensitive polymeric carrier - hydrocortisone at varied drug concentrations in different fluids: a) 25 mg of the drug, b) 50 mg of the drug.

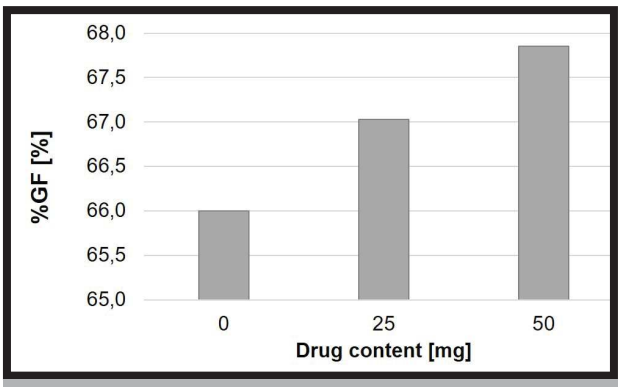

FIG. 3. The gel fraction of hydrogel material and bio-hybrid hydrogel matrices modified with the thermosensitive polymeric carrier - hydrocortisone at varied drug concentrations.

This was also confirmed by the determined %GF which was the highest for the bio-hybrid matrices containing the thermosensitive carrier – drug system, thus indicating a significant matrix cross-linking. Moreover, this broadest band in the range 3500-3000 cm^{-1} corresponded to the O-H groups stretching vibrations coming from SA, PVA and *Aloe vera*. Later, at a wavenumber of 2940 cm^{-1} the characteristic band appeared, which can be assigned to the C-H group stretching vibrations. The band at 1350-1330 cm^{-1} could be attributed to the bending vibrations of C-H and O-H. Additionally, all the FT-IR spectra showed a marked vibration band centered at 1730 cm^{-1} - exhibiting the presence of an ester group characteristic of poly(ethylene glycol) diacrylate (PEGDA). The absorption peaks of PEGDA were also seen at 1164, 1190, 1035 cm^{-1} for the C-O-C stretching [23].

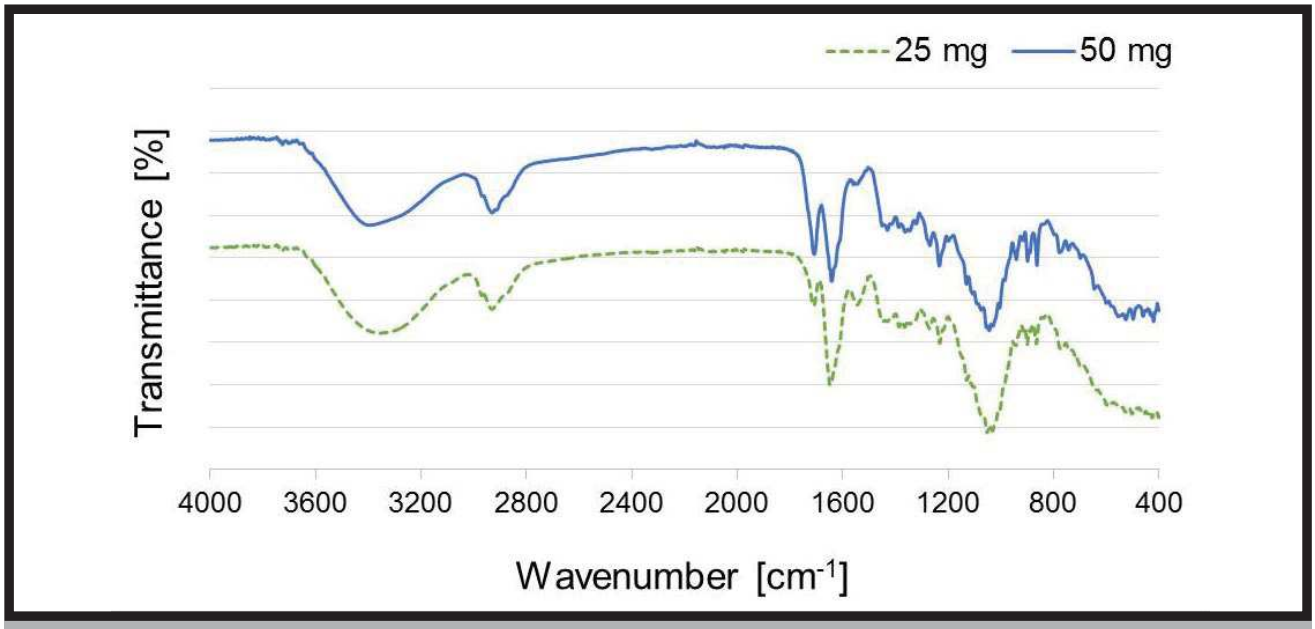

FIG. 4. The FT-IR spectra of thermosensitive polymeric carrier - hydrocortisone at varied drug concentrations.

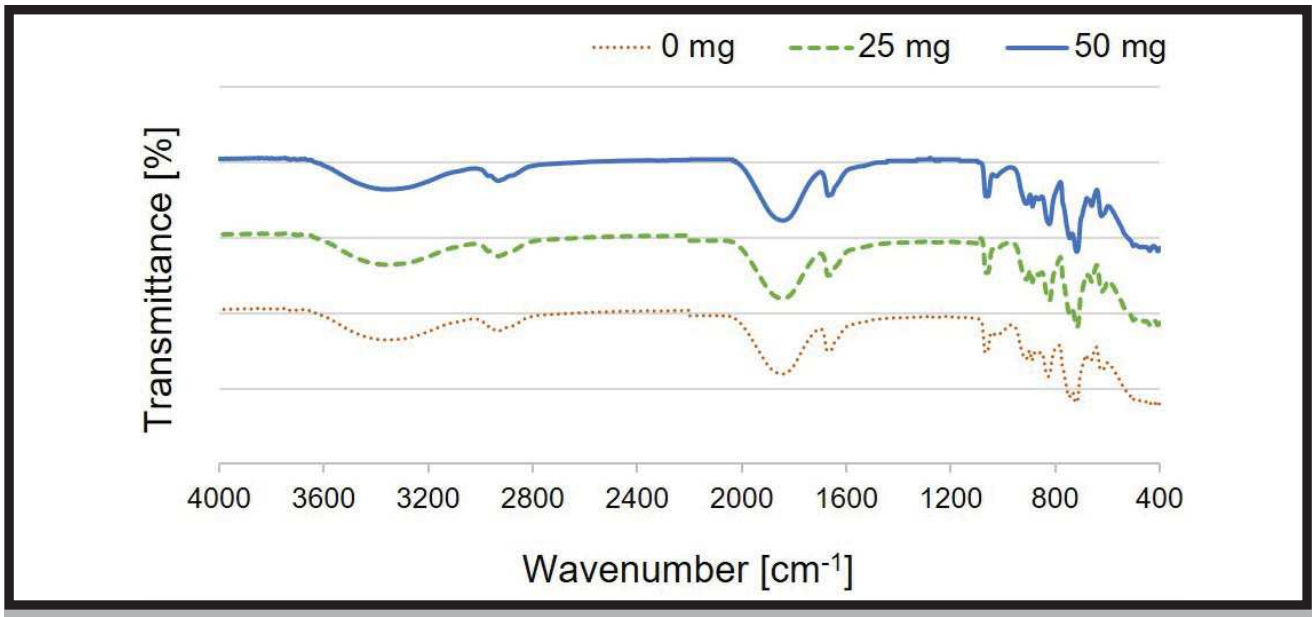

FIG. 5. The FT-IR spectra of the bio-hybrid hydrogel material modified with the thermosensitive polymeric carrier - hydrocortisone at varied drug concentrations.

In the case of the sample with 50 mg hydrocortisone (FIG. 4), there was a significantly more intense absorption band in the range of 1870-1600 cm^{-1}, coming from the C=O stretching vibrations of the drug structure. The presence of the intense absorption band in the range of 1150-1100 cm^{-1} proved the valence vibrations from the C-N group in the systems. This was confirmed by the presence of N-H and C-N groups of poly(N-isopropylacrylamide). However, the peaks at 1607 and 1450 cm^{-1} corresponded to the asymmetric and symmetric stretching vibrations of carboxylate anion (COO^-), respectively. Moreover, there were bands located at 985 and 810 cm^{-1} assigned to the COH out-of-plane bending and -CH_2 twisting [24-25].

The characteristic absorption bands for the -C-H (3000-2850 cm^{-1}), -C-C (1452-1402 cm^{-1}) and -C-O (1200-1000 cm^{-1}) groups were also evident both in the bio-hybrid hydrogel materials and in the thermosensitive carrier - hydrocortisone and probably they overlapped [23,26].

Conclusions

The application of thermosensitive polymers as drug carriers may increase the therapeutic value of the drugs used by modifying their solubility, retention time and crossing biological barriers. This contributes to the reduction of side effects that result from the prolonged use of the medicament and higher effectiveness of the therapy.

The analysis of the encapsulated polymeric carriers showed that the presence of the drug, its amount and the encapsulation time increased the average size of particles. It was possible to obtain the time-stable empty thermosensitive carrier with the average particle size below 100 nm and the encapsulated system - containing hydrocortisone introduced into hydrogel structure - with the average size below 500 nm.

Based on the results and observations, it can be concluded that the bio-hybrid hydrogel matrices are stable materials and the presence of an additional component i.e. the thermosensitive carrier – the hydrocortisone system - does not reduce the degree of matrix cross-linking or its swelling ability. Moreover, the swelling tests results indicated that the systems containing the higher drug concentration had the slightly higher sorption capacity in both the tested immersion media. The FT-IR spectra of the bio-hybrid systems confirmed no changes in the structure associated with the presence of encapsulated polymeric carriers.

The conducted research is a good experimental basis for further investigations aimed at developing a novel third-generation bio-hybrid dressing system. The main goal of the project will be preparing the bio-hybrid hydrogel materials enhanced with the nanocarrier-drug system with the controlled drug release as an innovative way of treating *Psoriasis* - a serious skin disease with autoimmune background. The expected positive results, especially regarding non-toxicity, will enable the selection of a prototype and further work on the novel bio-hybrid hydrogel materials to improve wound healing.

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