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RESEARCH OF COMPLEX OF *CALENDULA OFFICINALIS* EXTRACT–HYDROGEL FOR APPLICATION IN COSMECEUTICALS

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The complexes *Calendula officinalis* extract–hydrogel were prepared and investigated, which are intended for the use in cosmeceuticals. The hydrogel matrix was fabricated based on the copolymers of polyvinylpyrrolidone (PVP) with 2-hydroxyethyl methacrylate (HEMA) with different content of monomeric blocks, namely at the weight ratio of HEMA:PVP=60:40, 70:30, 80:20, and 90:10. The physicochemical characteristics of the obtained hydrogels were determined as follows: water content 48–69%, the degree of swelling 1.19–1.35, conditional porosity 53–84%, elasticity 82–89%, and pure plasticity 11–18%. Ethanol extract of *Calendula officinalis* was obtained by infusion, and the content of flavonoids was determined. The sorption capacity was measured and the kinetics of hydrogel swelling and extracts desorption from the complex was studied. The effects of hydrogel composition and thickness on the complex sorption capacity were studied. It was stated that the degree of swelling increases with increasing the PVP content in the original composition, therefore the hydrogel with the composition HEMA:PVP=80:20 seems to be the most optimal sample with enhanced sorption and desorption properties. The rate of release also depends on the content of PVP in the original composition and the content of pores, which are remained after washing. It was established that the best sorption of the extract and faster desorption are observed at the hydrogel matrix thickness of 1 mm. The study of flavonoid content in the obtained complexes and sorption/desorption processes revealed rapid sorption and desorption within 24 h, which is sufficient for the development of cosmeceutical mask on the basis of the investigated complexes.

Keywords: hydrogel, extraction, hydrogel–extract complex, flavonoids, kinetics, *Calendula officinalis.*

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Introduction

Nowadays, hydrogels attract attention of experts in the field of cosmeceuticals, as they can effectively promote the penetration of active cosmeceuticals components and significantly increase their retention in the skin. The specially designed hydrogels can load different active plants components with different physical and chemical properties and realize the skin targeted transportation of these components, to enter target sites and target cells and achieve controlled release. For example, the hydrogels show a good application prospect to solve the skin problems, treatment of skin diseases and skin care [1].

The word «hydrogel» dates back to an article published in 1894 [2]. The described substance was not a hydrogel as we describe it today; indeed, it was a colloidal gel made of inorganic salts. It is important to note that the history of the term «hydrogel» is long [1,3]. The first cross-linked network material was a polyhydroxyethylmethacrylate (pHEMA), which was invented later, in 1960. Since then, the number of studies about hydrogels began to rise [3].

Hydrogels have been defined as two- or multicomponent systems consisting of a three-dimensional network of polymer chains and water that fills the space between macromolecules. Depending on the properties of the used polymer (polymers) as well as on the nature and density of the network joints, such structures in equilibrium can contain various quantities of water; typically in the swollen state, the mass fraction of water in a hydrogel being much higher than the mass fraction of polymer [4].

Hydrogels are mainly formed from biopolymers and/or polyelectrolytes. According to the source, hydrogels can be divided into those that were formed from natural polymers and those that were formed from synthetic polymers. Depending on the ionic charges on the bound groups, hydrogels may be cationic, anionic, or neutral. The types of cross-linking agents also can be the criteria for classification. Hydrogels can be physical, chemical, or biochemical. It is also possible to divide hydrogels into groups based on their structure: amorphous, semicrystalline, crystalline, and hydrocolloid aggregates [5].

The approaches used for the synthesis of polymers, in general, are chain-growth and step-growth polymerization techniques. In chain-growth polymerization, the polymer chain grows by activating the monomers. In addition, there are such methods as the polymerization in bulk, the polymerization in solution, the polymerization in suspension and emulsion polymerization. There are also non-conventional radical polymerization methods such as atom transfer radical polymerization and reversible addition fragmentation chain transfer polymerization. The basic strategy that combines different monomers is copolymerization. The common copolymerization techniques are random, alternating, and block copolymerization [6–8].

Hydrogels contain polymer chains. Therefore, the properties of the polymer govern the properties of the hydrogel. Since the absorption of water is the main feature of a hydrogel, not all polymers are useful in the preparation of hydrogels. In hydrogels, the polymer chains are connected via crosslinks to form a three-dimensional network. Cross-linking between polymer chains affects their physical properties: elasticity, viscosity, solubility, strength and melting point, to name a few [6].

Natural hydrogels can be divided into three classes: polypeptide- and protein-based (collagen, fibrin, fibrinogen, gelatin, silk, elastin, myosin, keratin, and actin); polysaccharide-based (chitin, chitosan, alginate, hyaluronic acid, cellulose, agarose, dextran and glycosaminoglycans); polynucleotide-based (DNA, linear plasmid DNA, and RNA) [7,9].

Hydrogels are optimal candidates for medical use, they can provide direct mechanical support and control cell differentiation in intervertebral disk. In addition, the application of hydrogels includes drug delivery and cell delivery systems. Hydrogels are also being explored for their ability to coat and improve the biocompatibility of a host of different medical implants and devices. These coatings are becoming increasingly multifunctional and they are very significant for next-generation biosensors [10–12]. The prospects of its use for cosmetic purposes, namely for cosmetic masks, are also being discussed. The design of cosmeceuticals masks is an interesting area, in which we can use hydrogels because they have a large surface area with respect to the volume. It helps them to increase the absorption of filler solutions and their release. The porous structure and pore size are enough to prevent the entry of microorganisms, and also permit to proceed of cellular respiration and gas exchange, inhibit skin dehydration, are flexible and compatible with the skin, protect against infections etc. The type of polymer is also an important point to create such composition.

Bioactive compounds are present in different plants extracts and exhibit biological activity [13–15]. For example, the leaves of *Calendula officinalis* contain triterpenoid esters, flavoxanthin, auroxanthin, carotenoids (lutein, zeaxanthin, and beta-carotene). The flowers of *Calendula officinalis* contain flavonol glycosides, triterpene oligoglycosides, oleanane-type triterpene glycosides, saponins, and a sesquiterpene glucoside. Plant extracts are also widely used in cosmetics, presumably due to the presence of such compounds as flavonoids, saponins, resins, and essential oils. Plant pharmacological studies have suggested that Calendula extracts may have anti-viral, anti-genotoxic, and anti-inflammatory, anti-bacterial and fungicidal properties. That is why there is a possibility to saturate the hydrogels with *Calendula officinalis* extracts and to receive biologically active materials, which can be used in cosmeceutical to design cosmetic masks with moisturizing, anti-inflammatory and healing properties.

The aim of this work was to determine the composition and thickness of the hydrogel with the optimal sorption and desorption of *Calendula officinalis* extract to create hydrogel cosmeceutical masks based on them. Flavonoids in the complex were also determined and the equilibrium time was determined when desorption of the extract was stopped. Hydrogels were obtained by polymerization, extracts of *Calendula officinalis* were prepared by infusion, and hydrogel-extract complexes were obtained by saturation in volume.

Experimental

The hydrogels based on copolymers of polyvinylpyrrolidone (PVP) with 2-hydroxyethyl methacrylate (HEMA) were used for the study. The polymer composition in HEMA:PVP=60:40, 70:30, 80:20, and 90:10% (wt.) was provided by alternating dissolution of PVP and $K_2S_2O_8$ in an aqueous monomer mixture, the solution was deaerated by using a water-jet pump. The prepared composition was slowly poured into a mold by using a capillary funnel. The filled mold was placed in a dry-air thermostat with the precision of temperature control (±1°C). The polymerization was conducted by supporting the following step mode: I – heating to 55°C; II – holding at 55°C for 3 h; III – heating to 70°C; IV – holding at 70°C for 1.5 h; V – heating to 85°C; VI – holding at 85°C for 1 h; VII – cooling in a thermostat to 50°C; removing the mold from the thermostat and cooling to the room temperature in a heating bath. The mold was opened and the formed film was removed. After that, the film was irradiated by ultraviolet rays for 0.25 hours; backwashed in 1.5% sodium bicarbonate solution at the temperature of 45–50°C and then at the same temperature in distilled water. The resulting film was transferred to distilled water for stabilization for at least 2 hours. The film is kept in distilled water in an airtight container until its application [13,16].

The extract of *Calendula officinalis* was received by infusion at the room temperature for 7 days in a dark place in a ratio of 1:10 (10 g of dry crushed materials and 100 ml of 70% ethanol solutions). The obtained extracts were filtered through a folded filter to exclude parts of plants. The hydrogels in the form of square (1 cmr1 cm) were filled with the extract. The degree of swelling, elasticity, solidity, plasticity of the samples, we have analyzed for an hour, 5 hours and 1 day.

Kinetics of hydrogels swelling α, g(extract)/g(n) and extract desorption D, g(extract)/g(n) was determined in the following way. The hydrogel was filled with ethanol plant extract and it was weighed after some time. After that, it was dried and the degree of desorption was analyzed. The degree of swelling was determined by the formula: $\alpha_1=(m_{t(s)}-m_o)/m_o$, where $m_{t(n)}$ is the mass of swollen hydrogel and m_o is the mass of dry copolymer. The degree of extract desorption was determined by the formula: $D=(m_{t(c)}-m_o)/m_o$, where $m_{t(c)}$ is the mass of the dried sample at a certain point in time, g.

The total flavonoids content of the plants extracts was determined by aluminum complex formation with flavonoids. 0.5 ml of 2% $AlCl_3$-ethanol solution was added to 0.5 ml of extract. The solution was incubated at the room temperature for one hour. The absorbance of the reaction mixtures was measured at 420 nm wavelength in the cuvette with a layer thickness of 10 mm by using the Ulab108UV spectrophotometer. In parallel, the absorbance of a standard quercetin sample solution was measured by using a spectrophotometer under the same conditions. The total flavonoid content was calculated from the quercetin concentration (mg g^{-1}) by using the equation based on the calibration curve.

The kinetics of extract desorption from the complex hydrogel-extract was determined in the following way. The hydrogel filled with an extract was put into water and the flavonoids content in an aqueous solution was analyzed at certain intervals (1, 2, 3, 4, 5 hours and 1, 2, 5 days). The aluminum chloride colorimetric method was used for the determination of the total flavonoid content.

Results and discussions

Hydrogel masks, which can be used in cosmeceuticals, must have a unique porous structure, hydrophilic functional groups, that will provide swelling of the matrix and capacity to absorb, retain bioactive components and be compatible with biological systems. Therefore, it was suggested to prepare a hydrogel matrix based on copolymers of polyvinylpyrrolidone with 2-hydroxyethyl methacrylate (GEMA). This hydrogel base of pGEMA-pr-PVP is formed by two monomeric blocks of HEMA and PVP and contains functional groups: hydroxyl and carbonyl (HEMA) and amide (PVP). The sorption capacity depends on the quantity of these groups and also on the porosity of hydrogel. Sorption capacity is very important because it determines the quantity of extract with which we saturate the hydrogel. The features of saturation the hydrogel with extracts are related to the technology of hydrogel production and the composition of the material [16].

The received results showed that the synthesized hydrogels exhibit high sorption capacity, elasticity, shape stability in water/solvents and biotolerance. Such characteristics provide the possibility to saturate the copolymers of HEMA & PVP (pHEMA-pr-PVP) with medicinal herbs extracts and receive materials, which can be used for prolonged enrichment of the skin with biologically active components. Physical and chemical properties of the received hydrogels are as follows: water content 48–69%, the degree of swelling 1.19–1.35, conditional porosity 53–84%, elasticity coefficient 82–89%, and plasticity coefficient 11–18% [14].

As a filler, we used ethanol extract of *Calendula officinalis*, which contains active compounds, such as carotenoids, flavonoids, salicylic acid, saponins, and phytoncides. In cosmetics, calendula flower extract is commonly used for antiseptic, soothing and emollient properties. The research of the antibacterial properties of hydrogels saturated with ethanol plant extract showed positive results, namely high antibacterial activity [14].

Different samples of hydrogel (GEMA:PVP, parts by weight: 60:40, 70:30, 80:20, and 90:10) were used to design a hydrogel material with ethanol extract of *Calendula officinalis*. The swelling kinetics of the test samples is shown in Fig. 1. Hydrogels with lower PVP content and, consequently, with higher HEMA content, have a higher degree of swelling. That is why it is possible to control the degree of swelling by changing the polymer composite.

The degree of swelling increases with increasing the PVP content in the original composition, when there is a higher quantity of hydrophilic groups. The absolute degree of hydrogel swelling occurs under normal conditions after 24 hours.

An important technological parameter of hydrogel materials is the quickness of the filler release.

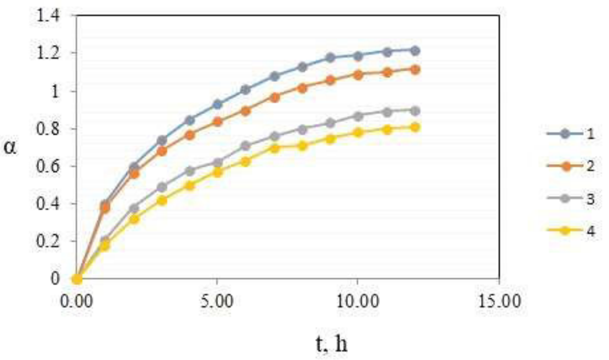

Fig. 1. Kinetics of hydrogel samples swelling depending on the composition. The composition of the hydrogel GEMA:PVP, parts by weight: 1 – 60:40; 2 – 70:30; 3 – 80:20; and 4 – 90:10

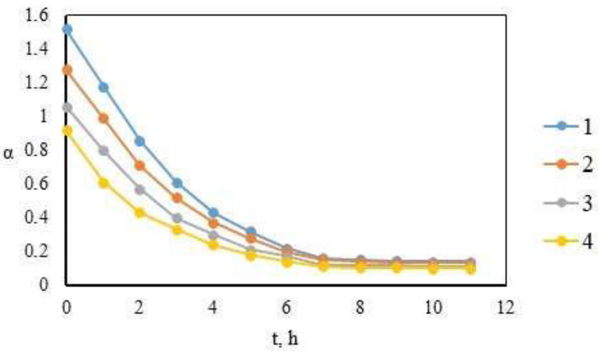

Fig. 2. Kinetics of extracts desorption from the hydrogel depending on the composition. The composition of the hydrogel GEMA:PVP, parts by weight: 1 – 60:40; 2 – 70:30; 3 – 80:20; and 4 – 90:10

The kinetics of *Calendula officinalis* extract desorption from different hydrogel samples is shown in Fig. 2.

According to the obtained results, the quickness of the filler release depends on the PVP content in the original composition and the content of pores, which are remained after washing. The extract release time is about 8–10 hours. The hydrogels are light brown, which indicates that not all quantity of the extract is released, the part of it persists in the hydrogel.

The hydrogel with the composition HEMA:PVP=80:20 was chosen as the most optimum sample with high sorption and desorption properties, which can be used to create a hydrogel mask. The hydrogels of such composition was obtained with different thickness (4 mm, 3 mm, 2 mm, and 1 mm), because the quickness of absorption and the extract release also depends on the thickness of the hydrogel. All samples were used to design a hydrogel complex with ethanol solution of *Calendula officinalis*. The

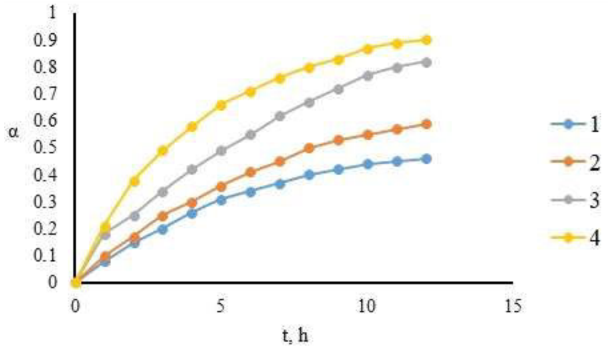

Fig. 3. Kinetics of hydrogel samples swelling and achieving equilibrium swelling depending on the thickness of the hydrogel. The composition of the hydrogel HEMA:PVP=80:20 (parts by weight). The thickness: 1 – 4 mm; 2 – 3 mm; 3 – 2 mm; and 4 – 1 mm

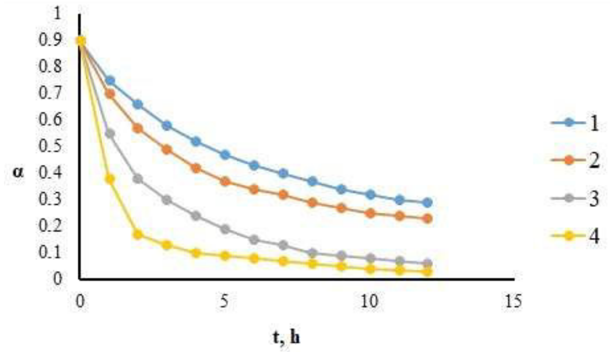

Fig. 4. Kinetics of extracts desorption from the hydrogel and achievement of final drying depending on hydrogel thickness. The composition of the hydrogel HEMA:PVP=80:20 (parts by weight). The thickness: 1 – 4 mm; 2 – 3 mm; 3 – 2 mm; and 4 – 1 mm

The concentration of flavonoids in extracts of *Calendula officinalis* and complexes, expressed in quartcetin equivalent

Parameter	Concentration, mg Q / g of extract *							
	1 h	2 h	3 h	4 h	5 h	1 d	2 d	5 d
in solution after desorption	40.84±0.505	39.12±0.392	37.56±0.530	37.08±0.479	36.95±0.657	36.94±0.368	36.94±±0.509	36.94±±0.313
remaining quantity in complexes	1.48±0.489	3.20±0.263	4.76±0.539	5.24±0.437	5.37±0.792	5.38±0.215	5.38±0.497	5.38±0.105

Note: Each value is the mean of the three measurements±standard deviation.

results of the research are presented in Fig. 3.

It is observed that the thinner the hydrogel, the better is the extract sorption, and also the faster is desorption (Fig. 4). The thicker the hydrogel, the slower is the sorption, and the longer is desorption. Given these facts, such hydrogels are proposed for designing cosmetics masks. The desorption time up to 24 hours is enough, that is why the hydrogel with the thickness of 1 mm is the most optimal variant for using as a matrix to create a complex of hydrogel-extract *Calendula officinalis.*

Therefore, the composition of GEMA:PEG=80:20 (wt.) and the thickness of 1 mm is the most optimal sample of hydrogel.

The total flavonoids content in extracts and in the received hydrogel–extract complexes was determined by spectrophotometric analysis based on aluminum complex formation with flavonoids. The original concentration of flavonoids in the ethanol extract of *Calendula officinalis* was 42.32±0.521 mg KV/g of extract. The results regarding the determination of the time dependences of remaining quantity of extract in the hydrogel by flavonoids by the difference between the origin quantity and after desorption are given in Table.

The experimental results indicate that the extract desorption from the complex occurs before the establishment of a certain equilibrium for 5 days, after that desorption process is stopped. While the flavonoids content in the solution is determined in 2 and 5 days, its amount is not changed. The remaining quantity of flavonoids in the hydrogel–*Calendula officinalis* extract complex was determined by the difference between the contents in the original extract and in the solution after desorption.

The comparison between the received results and the previously published data [14,16], showed that the selection of copolymers of HEMA, PVP and hydrogel sample thickness allows preparing the hydrogel materials that exhibit improved physical and mechanical properties and can be used as a matrix to create hydrogel–extract complexes. These results showed that the complexes based on HEMA:PVP contain flavonoids that have biological activity and can be used as cosmetic masks.

Conclusions

This research revealed the specifics, which can be used to create the hydrogel complexes with *Calendula officinalis* extract for different samples of hydrogels with the composition GEMA:PVP=60:40, 70:30, 80:20, and 90:10 (wt.) and with different thicknesses from 1 to 4 mm. The formation of hydrogel–extract complexes was established and the kinetics of plant extract sorption and desorption was studied. The HEMA:PVP=80:20 (wt.) is the most optimal composition for the creation of the hydrogel–extract complex. The study of flavonoid content in the received complexes and sorption/desorption processes revealed rapid sorption and desorption within 24 h, which is sufficient for cosmeceutical masks. The results indicate the prospects for the development of hydrogel cosmetic masks with moisturizing, anti-inflammatory and wound-healing properties.

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ДОСЛІДЖЕННЯ КОМПЛЕКСУ ЕКСТРАКТ *CALENDULA OFFICINALIS*–ГІДРОГЕЛЬ ДЛЯ ЗАСТОСУВАННЯ В КОСМЕЦЕВТИЦІ

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У даній роботі одержано та досліджено комплекси гідрогель–екстракт *Calendula officinalis* для застосування в космецевтиці. Гідрогелеву матрицю одержано на основі кополімерів полівінілпіролідону (ПВП) з 2-гідроксиетил-метакрилатом (ГЕМА) з різним вмістом мономерних блоків, а саме співвідношенням ГЕМА:ПВП 60:40, 70:30, 80:20 і 90:10 (мас. ч.). Фізико-хімічні характеристики одержаних гідрогелів наступні: водовміст 48–69%, коефіцієнт набрякання 1,19–1,35, умовна пористість 53–84%, число пружності 82–89%, чисто пластичності 11–18%. Етанольний екстракт *Calendula officinalis* одержано методом настоювання, в ньому визначено вміст флавоноїдів. Визначено сорбційну ємність та досліджено кінетику набрякання гідрогелів і десорбцію екстрактів з комплексу. Досліджено вплив складу композиції та товщини гідрогелю на сорбційну ємність комплексу. Встановлено, що ступінь набрякання зростає зі збільшенням вмісту у вихідній композиції ПВП, тому найоптимальнішим зразком з високими сорбційними і десорційними властивостями є гідрогель зі складом композиції ГЕМА:ПВП=80:20. Швидкість вивільнення також залежить від вмісту ПВП у вихідній композиції і від вмісту пор, що залишаються після вимивання. Встановлено, що краща сорбція екстракту і швидша десорбція спостерігаються при товщині гідрогелевої матриці 1 мм. Дослідження вмісту флавоноїдів у одержаних комплексах та процесів сорбції/десорбції виявило швидку сорбцію та десорбцію протягом 24 год, що є достатнім для розробки космецевтичних масок на основі комплексів.

Ключові слова: гідрогель, екстракція, комплекс гідро гель–екстракт, флавоноїди, кінетика, Calendula officinalis.

RESEARCH OF COMPLEX OF *CALENDULA OFFICINALIS* EXTRACT–HYDROGEL FOR APPLICATION IN COSMECEUTICALS

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Keywords: hydrogel; extraction; hydrogel–extract complex; flavonoids; kinetics; Calendula officinalis.

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